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Opening Remark and speech by Rector Dr Aung Win
University of Myitkyina

Good Morning…,

Chief Minister of Kachin State, speakers from Japan, Malaysia, Vietnam and Myanmar Pro-rectors of Myitkyina University, Professors, University Teachers and students from Myitkyina University, invited guests and all participants.

I am very pleased to welcome all of you to the first Myanmar International Science and Culture Conference 2019 (MISCC 2019) organized by Myitkyina University. The main aim of this conference is to promote, strengthen and expand the networking among local researchers and international researchers. First of all, I wish all of you to be in health of body and peace of mind. I would like to extend a welcome to every to this special occasion. Firstly, I'd like to express the brief history of Myitkyina University. The Aged University of Myitkyina was first opened on the 14th July, 1958. Myitkyina College was changed into the regional college of Myitkyina on 23rd of May 1977. Myitkyina College was upgraded into Myitkyina Degree College on 12th February, 1986 and Myitkyina Degree College was developed into Myitkyina University in 1998. The area of the campus is 220.788 acres. There are 14 academics departments, 294 teaching staff and 260 office staff at Myitkyina University. The courses offered by Myitkyina University are B.A, B.Sc., M.A and M.Sc. Degrees.

Secondly, as you all know, Today's world is called a global village and IT age. I am glad to hear there are over 200 papers that are going to be presented to MISCC 2019. It is hoped that participants who are going to present their respective papers related with Natural Science, Life Science and Social Science, and Culture and Linguistics will contribute a lot of benefits to our society. Because research is the life blood of Universities. Finally, I's like to say "No research, no good result at University Campus". I am sure this conference will make us beneficial to some extend so I would like to remind all the participants to take part in this conference actively.

Thank you very much.

Plant-based bioplastics for sustainable development: recent progress and advancement

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Using biomass as starting material for chemicals and plastics contributes to global sustainability without depletion of scarce resources, because of their large potential to substitute petrochemical derivatives to bio-based ones in industries. Poly(L-lactic acid) (PLLA) is one of the most typical plant-based bioplastics (biomass plastics) showing good biodegradability. However its demerits such as low physical properties and slow molding process have restricted the development of industrial applications of PLA. The present talk deals with recent progress and advancement of biomass plastics applications.

We have focused biomass products such as plant oils and cellulose for preparation of functional bio-based materials. For example, natural plant oil sources are found in abundance in the world; and hence, are expected as an ideal alternative chemical feedstock. Inexpensive triglyceride natural oils have been utilized extensively for coatings, inks, plasticizers, lubricants, resins and agrochemicals in addition to their applications in food industry.

We have developed biocomposites from epoxidized plant oils and cellulose fibers. Cellulose nanofibers reinforced oil polymer-based polymers to provide good mechanical and thermal properties of the composites. Green nanocomposite coatings were prepared by an acid-catalyzed curing of epoxidized plant oils with 3 glycidoxypropyltrimethoxysilane. The nanocomposite showed excellent film properties; the hardness and mechanical strength improved by incorporating the silica network into the organic polymer matrix and the good flexibility was observed. The dynamic viscoelasticity analysis clearly showed reinforcement effect by the inorganic network.

We have synthesized branched bio-based polyesters from lactic acid and a plant oil and examined their applications to improve properties of PLLA. Castor oil was used as initiator for synthesis of a branched bio-based polymer by ring-opening polymerization of L-lactide or polycondensation of L-lactic acid. The addition of only a small amount of the branched polymer to PLLA improved the strain at break, showing good plasticization effect of the branched polymer for PLLA. The terminal hydroxyl group in this branched polymer was modified by phthalic anhydride. The product with potassium salt form greatly induced the crystallization of PLLA to improve the thermal and mechanical properties of PLLA.

Eucommia ulmoides accumulates trans-1,4-polyisoprene (EuTPI) in the leaves, bark, root, and fruit coatings, which exhibits higher modulus and less flexibility than cis-1,4-polyisoprene. EuTPI can be regarded as a new class of high-performance bio-based elastomers. The blending of EuTPI with PLLA significantly improved the toughness and impact resistance of PLA.

A Glimpse on Enchanting Kachin State

Prof. Dr. Toe Hla

Scholars, Collages, Ladies & Gentlemen

It's a great honor to me for being invited as a keynote speaker in this international symposium held on this very auspicious day of 30 November 2019 and in this grandiose university of Myitkyina, Kachin state, When I recall my memory, I am still remembering the date of my first arrival at Myitkyina Intermediate College which was then newly open for welfare of the youths living in this Kachin State. It was 9 September 1963 that I reported my first university service duty as a tutor in History Department.

A saying has it that one should not forget the first place where one has overcome difficulties encountered in one's life. I started my university service carrier in this University (then an Intermediate College enrolled less than a hundred students) on 9 September 1963; It is now over 50 years past. Indeed, Myitkyina is an unforgettable place in my life for two amazing event. One is that I started my service life here and another is that I got imarried a beautiful girl of Myitkyina native.

Ladies and Genetlemen

Myanmar or the Republic of the Union of Myanmar is a fairly big nation on the main land Southeast Asia. It was on 4 January 1948 that Myanmar reemerged as a Union together with its old family members. There are seen States incorporated to form to form a Union; they are the Chin State, the Kachin State, the Kavah State, the Kayin State, the Mon State, the Rakhine State and the Shan state. They all had been parts of Myanmar Kingdom since the Bagan Period. One can come across in Bagan Inscriptions a racial name of Kachin. Inscriptions mention a Kachin race as Jingphaw (Theinphaw). Among the ethnic races more commonplace are the Chins, Kachins, Kayins, Mons, Rakhines and the Shans respectively. Among them I would like to present some points of the Kachin State history very briefly.

Geographically, Ka,chin State in the northern part of the Union bordering from the northeast with the People's Republic of China, It is a hilly region in the middle of which lies the Mogaung-Monyin Plain. Rice and sugar cane cultivation are commonplace in this state. Moreover, it has nature resources that could raise their standard of living to a greater extent had they been under good government with many technical know-how and skill workers. At least, half of the State is covered with dense forests underneath of which there lies invaluable deposits of mineral ores. Kachin State is known worldwide as a Land of the best Jades. The most valuable jades occur in this state and nowhere else as far as we know. What we urgently need in the Kachin State is to have an able and responsible government that could efficiently manage the mining business and extracting forest products. Firstly peace and tranquility should prevail in the State. Without peace how can people enjoy better life? How can they manage production of natural resources and economic prosperity without good governance? Scholars in this symposium could I do hope enhance the audience their scholarly views on social development plans. We also need to educate the youths to be free from narcotic drug abuse. It is because the drug abuse could ruin the society and enslave the State as Chinese people had been dearly suffered by the British imperialists in the 19 and early 20 centuries. And what matters most is the loss of one's characters.

Building an Academic Atmosphere

Professor Than Tun had once said that a University without any implementation of research work could not live long. A university always enhances not only new subjects to acquire but also latest innovative information and findings. Anthropologists and historians as well should dig up all of forgotten customs, traditions and events that led to changes of any form, of the people in the State irrespective of then race and religion... We have no authentic ethnic histories so far to rely on for national history, Scholars mostly do researches only on Bamar history. What we now need is to write ethnic histories using all available source materials. We have to foster ethnic or equipped with research techniques and writing systems or inspired and interested in ethnology, anthology, old traditions and form of changes taken place in the State they could better write own histories all available and reliable source materials collected from every nook and cranny.

In harmony with the political, social and economic developments of the Kachin State, some changes in education could also be now observed a little bit. But many challenges are ahead of us to overcome. We the educators as well as university professors are responsible to do for their best in order to carry out development schemes for the State and also for the Union.

Evaluation of Some Physiochemical Properties and Antioxidant Activities of Ginger Wines

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Toe Toe Khaing, Associate Professor, Dr, Department of Engineering Chemistry, Mandalay Technological University
Thida Tin, Associate Professor, Dr, Department of Engineering Chemistry, Mandalay Technological University
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Abstract

Wine is one of the functional fermented foods that have many health benefits. Wine can act as a nutrient supplement for seasonal fruits and vegetables throughout the year. Using fruits and vegetables having medicinal and nutritional value as a substrate for wine production, the health benefits of them can be improved widely. [Nandagopal, G., & Nair, P. S. (2013). Production of wine from ginger and indian gooseberry and a comparative study of them over commercial wine. American Journal of Engineering Research, 2(5), 19-38.] Ginger which is known for its high medicinal and nutritional value is used as the substrate. The wine produced resembled the commercial wine in terms of its composition, taste and aroma. During the fermentation period, the wines were analyzed for pH, alcohol, sugar, total dissolved solid, total suspend solid, turbidity, titratable acids and specific gravity. Biomass was also determined. pH show a decreased trend then attains minima and then increased. As the fermentation days proceed, the specific gravity increased and the alcohol percentage increased gradually. Three types of ginger wine were prepared, only ginger (G), with yeast (GY) and with pectinase enzyme (GE) with three sugar concentrations. After one, two and three month, the physical properties of wine were determined. The antioxidant activities of all types of wine were examined by using DPPH assay when wine age reaches three month.

Keywords— Ginger wine, yeast, pectinase enzyme, fermentation period, antioxidant

Introduction

Wine is one of the functional fermented foods that have many health benefits. The quality of the wine is influenced by the choice of the substrate, climate, soil type, sugar content, strain of yeast and storage conditions (Jones and Davis, 2000). Large numbers of distilled and un-distilled alcoholic products are enjoyed in different geographical regions throughout the world (Jones, 1985). Wine is the end product of partial or complete alcoholic fermentation of the juice of grape (Prescott and Dunn, 1987). It is also made from a variety of fruits, such as grapes, peaches, plums or apricots etc. and saps of different palm tree (Okafor, 1978). Wine is an un-distilled beverages having 6-20% ethanol by volume (Pearson, 1971). Wine represents a safe and healthful beverage. It also provides calories and vitamins. Generally wine is made from grapes. The grapes are crushed to squeeze out the juice and then are left for some time to ferment (Amerine *et al*., 1980). Wine can act asthma health supplement attributed to polyphenols, flavonoids, minerals etc. extracted from fruits and vegetables during the process of fermentation. Ginger (*Zingiber officinale* Rosc.) is an important commercially grown crop for its aromatic rhizomes which are used as aspice, condiment and as a medicine. Ginger has many biological and medicinal properties. It is used in pharmacology because of low toxicity. Ginger is antitumor, antioxidant, anti-inflammatory, ant apoptotic, cytotoxic, anti-proliferative and antiplatelet activities (Sekiwaet *al*., 2000; Wei *et al*., 2005). Ginger rhizome contains phenolic ketones like gingerols as well as the shogaols. The chemical composition of fresh ginger varies with geographical locations. The rhizomes have on average 84.37% moisture, 1.17% protein, 0.86% fat, 0.93% fiber, 0.96% ashes, 0.34% sugars and 11.42% starch (% wet basis) (Leonel M., 2005; Adebowate B.O., 2014). In this research work, three types of ginger wines were prepared with yeast and pectinase enzyme. The physical and chemical properties and antioxidant activity of were determined.

Material and Methods

Sampling

The sample of Ginger was obtained from Lai Mwae, Kyaing Tong, Eastern Shan State. Sugar and yeast were collected from Myoma Market, Kyaing Tone. Pectinase enzyme was also purchased from Chemeco Chemical shop, Yangon.

Figure (1) Used materials in Ginger Wine; Ginger, Brown sugar, Yeast and Enzyme

Sample Preparation

Ginger was washed with tab water and peeled. The peeled Ginger was sliced into small pieces and boil at 100°C for 15 min. After cooling each 25.0 g of samples was placed in wine making pot. The 825 g of sugar was dissolved in 500 ml of distilled water and stirred until all sugar is melted. Three sugar concentrations were prepared such as 825.0g/3L, 825.0 g/3.5L and 825.0 g/4.0L. The sugar solutions were added into the wine making pot, respectively. The 1 g/L concentration of yeast and 1.0 g/L concentration of enzyme were prepared and put it into respective pot. After that the pot was covered and set-up air locks on. The

air pipe was imbedded into lime water. Different nine types of wine were obtained. All wine types were tabulating the following Table (1).

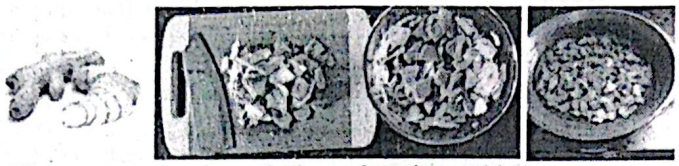


Figure (2) Preparation of Ginger for wine making

Table (1) Type of wines and its constituents

Type	Constituents				Sub-type of wine Destilled water (l)		
	Ginger (g)	Sugar (g)	Yeast (g/L)	Enzyme (g/L)	I	II	III
G	25	825.0			3.0	3.5	4.0
GY	25	825.0	1.0		3.0	3.5	4.0
GE	25	825.0		1.0	3.0	3.5	4.0

Determination of Some Wine Properties

(i) Monthly Monitoring

The pH content of the prepared ginger wine was directly measured by using pH meter, Schott Gerate CG 712 pH meter (Germany). The alcohol content of the prepared ginger wine was directly measured by using alcohol meter. The sugar content was measured by using refractometer. The TDS content of the prepared wine was measured by using TDS tester. The TSS content was directly measured by using photometer at l max 430 nm. The turbidity content was measured by using photometer at l max 530 nm. Estimation of titratable acids was done by titrimeteric method using 0.1M NaOH in terms of tartaric acid. Biomass was determined by dry weight method in g/ml. Specific gravity of wine was also determined.

(ii) Determination of Antioxidant Activity

In this experiment, 1-1 diphenyl -2- picryl hradrazyl (DPPH) powder (stable free radical), Ascorbic acid (standard antioxidant) and Analar grade Ethanol (solvent) were used. The absorbance was determined at 517 nm wavelength. DPPH free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This free radical is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The use of DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometer. Three solution such as DPPH solution, standard (Ascorbic acid) solution and sample solution were prepared.

Results and Discussions

The reference pH range of common wine was found to be 3-4. The pH of all types of prepared wines was fall in reference pH range of wine. When yeast and enzyme were used in wine making process, the pH range of wine is suitable within wine age one month. After one month, G and GE-type of wine has no alcohol content. But, GY-type of wines was found to be 5-8% of alcohol within one month. After two months, G-type of wine has no alcohol content. Alcohol content of GY-type increase to 6-9% and GE-type of wine started to found about alcohol 2-4%. Three month later, the alcohol content of G-type of wine becomes 3-4 %. GY-type reach 10-12% of alcohol and GE-type of wine became 6-9%. Most wines around the world contain 9-16 % of alcohol. After three month, the sugar content in G type of wine remains 6-10%. But GY and GE types contain small amount of sugar. The total dissolved solid (TDS) content of wine increase when their lives increase except. In contrasts, total suspended solid (TSS) content in all types of wine decrease when their aging times increase. Three month later TSS content of wines was found to be gradually decreased. It means that the suspend solid in all wine were settle down as the precipitate at the bottom of the wine making pot. The turbidity content of the prepared ginger wine was directly measured by using photometer. Turbidity of wine significantly reduces with wine age. Three months later, all wines become clear and transparent. Determination of titratable acidity of wine with indicator is based on neutralization of all acids and their acid salts, by sodium hydroxide solution. According to consumption of the base solution, titratable acidity was calculated. Sodium hydroxide is used for neutralization of all substances in wine that have acid character, so the amount of total acids in wine is expressed as the mass of tartaric acid, as for the wine quality this acid is one of the most important. The total amount of acid in wine were found to be GE type> GY type > G type. The biomass of wine, making without enzyme and yeast has highest. After three month, antioxidant activity of G-III, GY-III, GE-III & Commercial wine were determined by using DPPH assay. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the change in colorization from violet to pale yellow and subsequent fall in absorbance of the stable radical DPPH was measured at 517nm for various concentrations i.e. 6.25-100 µg/ml and result were presented in Table 3. The electron scavenging potential of wines were found to be GE-III > commercial wine > GY-III > G-III. The IC_{50} value for each wine sample was defined as the concentration of extract causing 50% inhibition of absorbance, since IC_{50} is a measure of inhibitory concentration, a lower IC_{50} value would express higher antioxidant activity of the sample. The greater the bleaching action the higher the antioxidant activity AEAC value (Ascorbic acid equivalent to antioxidant capacity), and this is reflected in lower IC_{50} value (Lim et al., 2006). The same action was found in GE-III having the lowest IC_{50} value 17.98 $\pm$ 0.03 µg/ml. The IC_{50} values were found to be 17.98+0.03 mg/ml for GE-III, 20.17+0.13 g/ml for commercial wine, 22.31+0.14 mg/ml for GY-III and 24.85+0.07 mg/ml for G-III, respectively. These results showed that the electron capture activity of GE-III is nearly the same with the standard ascorbic acid (15.51+0.04 µg/ml).

Table (2) Monthly monitoring of prepared ginger wines & commercial wine

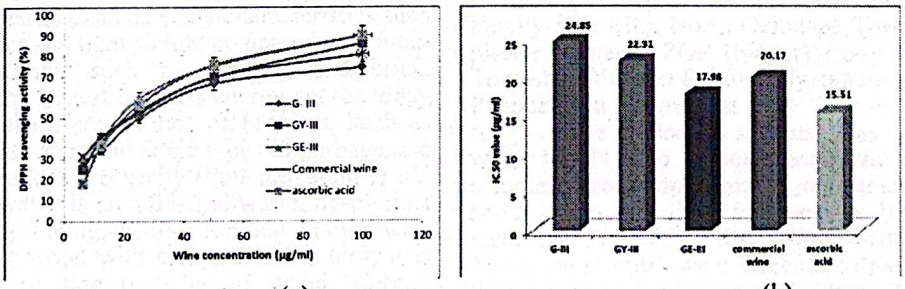
Properties	Aging Time (month)	Type of Wine									
		G Type			GY Type			GE Type			Commercial Wine
		I	II	III	I	II	III	I	II	III	
pH	One	3.81	3.47	3.66	3.20	3.62	3.43	3.21	3.68	3.20	3.16
	Two	3.81	3.33	3.81	3.30	3.41	3.33	3.33	3.40	3.30	
	Three	4.01	3.56	3.94	3.23	3.59	3.55	3.25	3.34	3.30	
Alcohol Content (%)	One	0.0	0.0	0.0	5.0	8.0	8.0	4.0	2.0	2.0	16.20
	Two	2.0	1.0	1.0	9.0	8.0	6.0	7.0	8.0	4.0	
	Three	4.0	3.0	2.0	12.0	10.0	10.0	12.0	11.0	9.0	
Sugar content (°Brix)	One	17.7	16.0	11.8	4.8	4.8	4.5	8.3	6.0	7.2	3.5
	Two	15.8	13.3	8.6	7.3	4.9	2.6	6.5	3.0	2.4	
	Three	9.9	7.0	6.3	3.3	2.4	2.4	3.1	2.1	1.5	
TDS (mg/ml)	One	441	528	442	657	448	672	450	418	372	720
	Two	694	612	316	816	528	788	424	532	464	
	Three	761	702	680	889	608	821	636	777	983	
TSS (mg/ml)	334	334	447	387	85	58	71	220	218	170	40
	177	177	176	180	22	32	21	58	120	37	
	102	102	120	98	19	19	15	15	51	58	
Turbidity (FAU)	One	270	300	277	73	49	49	103	121	112	25
	Two	258	281	115	49	39	41	61	58	72	
	Three	120	177	94	23	23	23	27	24	24	
Titratable Acid (g/tartaric acid)	One	5.55	4.56	4.68	6.45	6.41	4.53	6.45	4.00	6.43	8.25
	Two	5.78	6.23	3.78	5.28	6.12	6.38	5.28	6.05	5.25	
	Three	5.43	6.26	6.98	7.81	7.62	7.20	7.50	7.45	7.38	
Specific gravity	One	1.188	1.170	1.151	1.167	1.178	1.175	1.170	1.096	1.111	1.044
	Two	1.174	1.149	1.142	1.159	1.154	1.147	1.161	1.061	1.101	
	Three	1.158	1.123	1.124	1.136	1.131	1.123	1.131	1.038	1.016	
Biomass (g/ml)	Three	0.367	0.358	0.371	0.018	0.016	0.016	0.015	0.022	0.011	0.012

**Values are the average of duplicate experiments*

Table (3) Free radical scavenging activity of various types of wine against DPPH radical

Wine Type	Concentration (mg/mL) and DPPH scavenging activity (%)					IC_{50}
	100	50	25	12.5	6.25	
G-III	74.44±0.01	66.64±0.05	50.14±0.08	34.28±0.04	29.61±0.18	24.85±0.07
GY-III	86.14±0.11	69.66±0.21	53.58±0.16	39.22±0.15	25.27±0.08	22.31±0.14
GE-III	89.91±0.02	75.28±0.03	59.61±0.08	40.19±0.02	31.33±0.02	17.98±0.03
Commercial Wine	80.88±0.10	69.81±0.15	52.34±0.11	40.96±0.16	31.27±0.11	20.17±0.13
Ascorbic Acid	90.05±0.01	75.68±0.03	56.42±0.01	36.57±0.01	18.09±0.02	15.51±0.04

**Values are the average of duplicate experiments and represented as mean ± standard deviation*



(a) (b)

Figure (3-a) DPPH scavenging activity of different types of wines and standard Ascorbic acid
(3-b) IC $_{50}$ value of test samples and standard ascorbic acid

Conclusion

This study focused on the process monitoring of homemade wine during its fermentation period. The experimental investigation was aimed to study the variation in each parameter during the fermentation period. The final analysis of wine of various parameters-pH, alcohol content, sugar content, TDS, TSS, turbidity, titratable acid vale, specific gravity and biomass were conducted. These studies were compared with that of commercially available wine. IC_{50} value of GE-III type of wine was found to be as 17.98±0.03 µg/ml equivalent to that of 15.51±0.04µg/ml of standard ascorbic acid. According to the experimental data, wine prepared enzyme is better than others. The activity is also dose dependent because it shows better activity when increasing the dose. Homemade wines have relatively low alcohol content than the commercially available wine and there is no usage of either any preservative or any additives, so homemade wines are not harmful for health and are acceptable for daily usage. The results of process monitoring and final analysis can refer the results to develop a small scale wine industry.

Acknowledgements

I would like to express our profound gratitude to Rector, Technological University (Myintkyina) and Deputy Editors and all members of Editorial Board for their giving permission to publish journals. We also would like gratitude to Dr. Kyaw Thar Htun, Pro-rector of TU (Kyaing-Tong) for helping and supporting everything we want.

References

Jones G.V. and Davis R.E (2000), "Climate influences on grapevine phenology, grape composition, and wine production and quality for Bordeaux, France", American Journal of Enology and Viticulture, 51(3), p-249-261.

Jones, K.C.(1985), "Adsorptio of Fermentative Organisms to Alcoholic Environments. In: "Alcoholic Beverages"", G.G. Brich and MG. Lindldy (Eds). Elsevier Appliedn Sc. Publication, p-171

Prescott, S.C. and Dunn, C.G. (1987). "Industrial microbiology", 3rd edn. McGraw-Hill Book Co., NewYork.,

Okafor, N. (1978), "Microbiology and Chemsitry Oil Palm-Wine. Advance Applied microbiology", Academic Press, London 2, 273-256.

Pederson, C.S. (1971), "Microbiology of Food Fermentation", The AVI Publishing Co. Inc. pp 133-161

Amerine, M.A., Berg, H.W., and Cruess, W.V. (1980), "The Technology of Wine Making", The AVI publishing Co., INC., Westport, Connecticut.

Sekiwa, Y., Kubota, K., Kobayashi, A. (2000) "Isolation of novel glucosides related to gingerdiol from ginger andtheir antioxidative activities", J. Agric. Food Chem.,48:373–377.

Wei Q.Y., Ma J.P., Cai Y.J., Yang L., Liu Z.L. (2005), "Cytotoxic and apoptotic activities of diarylheptanoids and gingerol-related compounds from the rhizome of Chineseginger", J Ethnopharmacol.,102:177–184.

Leonel M, Sarmento SBS, Ferrari TB. (2005), "Low quality ginger as a source of starch. Raízes e Amidos Tropicais". ;1:9-18. 9.

Adebowate BO, Gbenga BL, YewandeF. (2014), "Morphology, functional and pasting properties of ginger starches prepared by four different drying methods", British Journal of Pharmaceutical Research. 4:1439-1450.

Lim Yau Yan, Lim Theng Teng and Tee Jing Jhi. 2006. Antioxidant properties of guava fruit: Comparision with some local fruits. Sunway Academic J. 3: 9-20

Study on Production of Plasterboards from Biomass Waste (Sawdust)

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Abstract

Using the biomass waste material to produce plasterboards can prevent environmental pollution. This work mainly concerned with the production of plasterboards from sawdust (SD). Plaster of paris (POP) was used as binder. The physicochemical properties and characterization of sawdust and plaster of paris were also conducted. Six types of plasterboards (SDPOP) were produced by mixing sawdust (70 g) and various proportions of plaster of paris (70 g, 105 g, 140 g, 175 g, 210 g and 245 g) by cold compressing molding method. The produced plasterboards were characterized according to physicochemical and physicomechanical properties such as modulus of rupture (MOR), thickness, density, water absorption, swelling thickness as well as hardness. Based on the physicomechanical properties, SDPOP 6 plasterboard was quality grade plasterboard among the six types of produced plasterboards. It has modulus of rupture of 1849.84 psi, thickness of 0.77 cm, density of 1.2257 g cm^{-3}, water absorption of 23.84 %, swelling thickness of 20.11 % and hardness of 89 Shore D. The surface morphology of SDPOP 6 plasterboard showed that for the sawdust with plaster of paris, the presences of dispersed particles in the size range of approximately 30 μm are observed. Good adhesion between the sawdust and plaster of paris was also observed. The heat characteristic of SDPOP 6plasterboard was also studied by TG-DTA analysis. The heat resistance of SDPOP 6 plasterboard remained unchanged until at 600 °C. According to physicomechanical properties, produced plasterboards can be used for decoration such as walls and ceilings.

Keywords: POP, sawdust, plasterboards, physicochemical, physicomechanical properties

Introduction

Nowadays, different types of residential, commercial and industrial buildings are required for the development of Myanmar. Ceiling sheets are essential components for the hot climatic building to insulate thermal effect.

Plasterboard is a popular building product used to construct ceiling and interior walls. Standard plasterboard is made by sandwiching a layer of gypsum plaster between two thick sheets of paper (Dias, 1994).

In the production of plasterboards, the two main component involved are binder (POP) and the other is a fibrous material (Sawdust). The binder plays an important role to obtain good strength of the board and also for low cost products. The use of plaster in building construction is growing due to its different application such as renders, pieces for decoration, plates, dry wall and blocks. The advantages of using commercial plaster are excellent performance, attractive appearance, easy application and its healthful contribution to living conditions (Paulo and Camarini, 2012). Wood is a unique material that has many characteristics such as aesthetics and light weight compared with man-made materials such as concrete and brick. Remains the largest biomass energy source today and several different kinds of biomass, such as wood chips, corn, and some types of garbage, are used to produce electricity (Puvanasvaran, *et al.*, 2011). Sawdust is an industrial waste, by-product of cutting, grinding, drilling, sanding, or otherwise pulverizing wood with a saw or other tool; it is composed of fine particles of wood. Certain animals, birds and insects which live in wood, such as the woodpecker and carpenter ant are also responsible for producing the sawdust (Tilak, *et al.*, 2018). Sawdust is the main component of plasterboard. A major use of sawdust is for plasterboard; coarse sawdust may be used for wood pulp (Tomas, 2014).

The high availability and low cost of the sawdust, it can be more useful and valuable by mixing sawdust with polymer in order to improve the mechanical properties composites. The main application area of sawdust filled composites is the building and automotive industry, but they are also applied for the preparation of various household articles, furniture, office appliances (Muhammad, *et al.*, 2011).

Materials and Methods

All necessary research facilities were provided by the polymer Department, Department of Research and Innovation, (DRI). The apparatus consists of conventional Lab ware, glassware and modern equipments. Some of the instruments used in the experiments are E-Mettler balance (210 ± 0.1 mg) (LA – 310 S), Muffle furnace (Range 100-1100 °C Gallenkamp, England), TG-DTA (Hi-TGA 2950, DTG-60 H Thermo gravimetric analyser), Mixer machine (Henschel Mischer, Germany), Hydraulic hot press (Apex Construction Ltd., Gravesend England), Electro-hydraulic tensile tester (Thwing-Albert Instrument Company Philadelphia, USA), Hardness tester (H.W Wallace and Co. Ltd., England), Veneer clipper (or) screw gauge, Specific gravity balance (Wallace Test Equipment), Sieves (US Series Equivalent, The Tyler Std. Screen Scale), Scanning Electron Microscope (No. JSM-5610, JEOL Ltd., Japan), Thermal control status oven (H 053, 240 V, England), EDX-700 spectrometer and X-ray diffractomer.

Collection of Samples

In the experiments, sawdust was collected from Family Saw Mill, North Okkalapa Township and plaster of paris, POP (binder) from Kyauktada Township, Yangon Region, Myanmar.

Preparation of Sawdust

The collected sawdust was soaked in water for 24 h to reduce the amount of water-soluble sugars and tannins and then cleaned sawdust was solar dried for five days. It was dried again in at 115 °C for 1 h and cooled in desiccators. The dried samples were screened to pass through the sieve aperture of 25 mesh (0.5 mm). The sieved (25 mesh) material was then stored in an airtight plastic bag for further experiment.

Determination of Physicochemical Properties of Sawdust and Plaster of Paris

The physicochemical properties (moisture content, ash content, solid content, bulk density, and pH) of the prepared samples were determined by conventional methods (Table 1).

Characterization of Plaster of Paris by EDXRF Analysis

The relative abundance of elements present in plaster of paris was obtained with the help of EDX-700 spectrometer (Figure 1) and the description data (Table 2).

Characterization of Sawdust and Plaster of Paris by SEM Analysis

The scanning electron micrographs of sawdust and plaster of paris were obtained with the help of a Scanning Electron Microscope (JSM-5610, JEOL Ltd., Japan) (Figures 2 and 3).

Characterization of Sawdust and Plaster of Paris by TG-DTA Analysis

Thermal analysis of the samples were determined by a DTA-60H (Hi-TGA 2950) thermal analyzer. The TG-DTA thermogram (Figures 4 and 5) and the description data (Tables 3 and 4) are presented.

Characterization of Plaster of Paris by XRD Analysis

The nature of the plaster of paris was determined by X-ray diffractometer. The XRD spectrum of plaster of paris (Figure 6) and the description data (Table 5) are given.

Production of Plasterboards

In this research, all of the plasterboards were produced by cold compressing molding method.

Effect of proportion of plaster of paris (POP) on the production of plasterboards

Each sawdust (70 g) was dipped in 20 °C water for 3 h, and then mixed with (70, 105, 140, 175, 210 and 245 g) of plaster of paris by Henschel mixer for 10 min. The mixture was then laid in mold. Care must be taken to get uniform surface layer in cold press section. Later, this mat was carefully transferred to the hydraulic press machine. Excess water was squeezed out by compression at 2800 psi pressure for 24 h. The boards were kept at room temperature for 1-2 weeks and then the edges and both sides of the board were trimmed and sanded (15.24 cm x 15.24 cm).

Determination of the Physicochemical and Physicomechanical Properties of Plasterboards

The physicochemical and physico-mechanical properties (moisture content, modulus of rupture, thickness, water absorption, swelling thickness, density, and hardness) of the produced sawdust-plaster of paris plasterboards were determined by the conventional method and modern techniques. The physicochemical and physico-mechanical properties of the produced plasterboards (Figures 7, 8 and 9) and the description data (Table 6) are presented.

Characterization of SDPOP 6 Plasterboards

SEM analysis

The morphology of the prepared samples was studied by using scanning electron micrograph for analysing micro and macro pores present on the surface of samples. The scanning electron photomicrograph of plasterboard SDPOP 6 (Figure 10) is given with the help of Scanning Electron Microscope (JSM-5160, JEOL Ltd., J

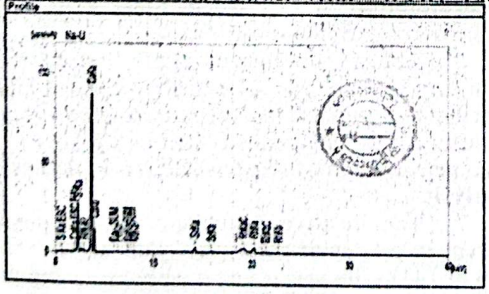


Figure 1. EDXRF spectrum of plaster of paris

TG-DTA analysis

Thermal analysis of the sample was determined by a DTA-60H (Hi-TGA 2950) thermal analyzer. The sample (*ca.* 5 mg) was required and measured in the temperature range 0~600 °C at 20.00 °C/min and nitrogen gas at 50.00 mL/min. The TG-DTA thermogram (Figure11) and the description data (Table 7) are given.

Determination of Heat Resistance of SDPOP 6 Plasterboard

Plasterboards were cut into (2.54 cm x 2.54 cm) pieces are weighed. These pieces of plasterboards were heated at different temperatures (100, 200, 300, 400, 500, 600° C) for 5 h. Weight loss percentages were determined after. The results are presented in Table 8 and Figure 12. The photograph is shown in Figure 13.

Application of Sawdust-Plaster of Paris Plasterboard

The photographs of SDPOP plasterboards are shown in Figure14.The resistance of produce plasterboards were tested by screw and nail. These are shown in Figure 15.

Results and Discussion

Table 1 shows that the physicochemical properties of the sawdust and plaster of paris determined by the conventional method. Plaster of paris was used as a binder for making plasterboard and the results of physicochemical are given (Table 1).

Table 1. Determination of the Physicochemical Properties of Sawdust and Cement

No.	Physicochemical Properties	Sawdust	Paris of Plaster
1.	Moisture content (%)	20.11	0.97
2.	Ash content (%)	11.25	14.25
3.	Solid content (%)	79.89	99.03
4.	Bulk density (g mL^{-1})	23.10	80.47
5.	pH	6.32	7.86

Characterization of Plaster of Paris by EDXRF Analysis

In the present work, relative abundance of elements presents in plaster of paris (POP) (Table 2) and the EDXRF spectrum (Figure 1) are given.

Table 2. Relative Abundance Element in Plaster of Paris

No.	Element	Relative Abundance (%)
1.	Ca	58.367
2.	S	41.267
3.	P	0.149
4.	Sr	0.136
5.	Fe	0.036
6.	Cu	0.025

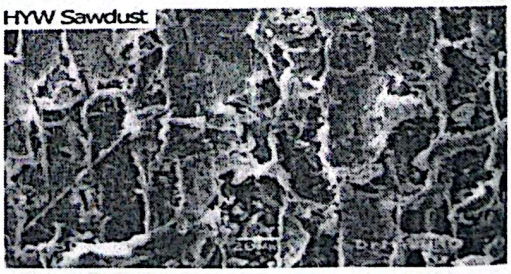


Figure 2. SEM photomicrograph of sawdus

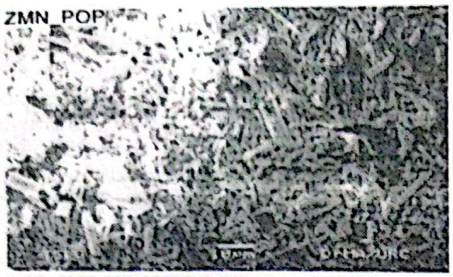


Figure 3. SEM photomicrograph of plaster of paris

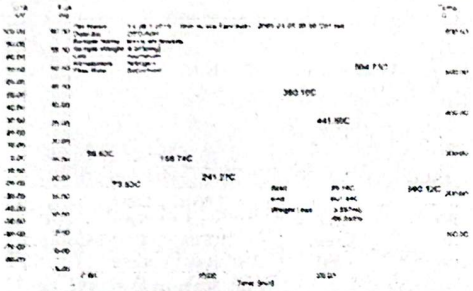


Figure 4. TG-DTA thermogram of sawdust

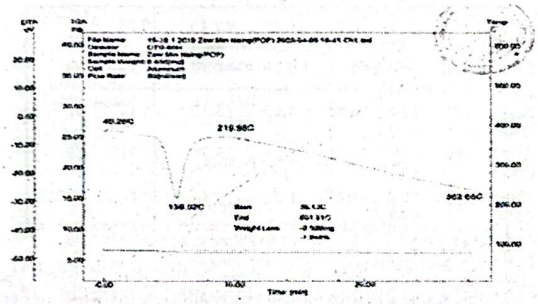


Figure 5. XRD spectrum of plaster of paris

Characterization of Sawdust and Plaster of Paris by SEM Analysis

Surface morphology of sawdust and plaster of paris are given (Figures 2 and 3). It is obviously seen that lamellar structure, as they are typical form on the surface of the sawdust. In this image, stick like nature of POP is not similar in size.

Characterization of Sawdust and Plaster of Paris by TG-DTA Analysis

Thermal stability of sawdust and plaster of paris are (Figures 4 and 5) and the interpretation (Tables 3 and 4) are given.

Thermal decomposition of sawdust occurs at the temperature range from 40 °C to 600 °C. On the basis of the thermogram of sawdust, the initial region of the TG profile shows a change in temperature profile at about 73.83 °C. It may be dehydration process and found to have weight loss percent about 25 % at the temperature of 39 °C to 324 °C. The major stage of the loss of about 50 % occurs from 324 °C to 400 °C. The exothermic peak was observed by DTA around 390 °C. It may be attributed to decomposition of small segments from cellulose and lignin. The exothermic peak was found at 504 °C from DTA with the weight loss percent about 20 %. It means to point out decomposition of cellulose backbones and formation on char, residual weight 5 %.

On the basis of the thermogram of plaster of paris, the weight loss % about 9.44 % was found within the temperature range 40 °C to 140 °C. This is due to the dehydration of moisture and absorbed water, indicated by the endothermic peak at 139.02 °C. The residual weight was 90.56 %.

Table 3. Thermal Analysis Data of Sawdust

Sample	TG		DTA		Remarks
	Break in Temperature(°C)	Weight loss (%)	Peak Temperature (°C)	Nature of peak	
	39-324	25	73.83	endo	Dehydration due to surface water
SD	324-400	50	390.16	exo	Decomposition of small segments from cellulose and lignin
	400-510	20	504.73	exo	Decomposition of backbones of cellulose and burn into char, residual weight 5 %

Table 4. TG-DTA Data of Plaster of Paris

Sample	TG		DTA		Remarks
	Break in Temperature (°C)	Weight loss (%)	Peak Temperature (°C)	Nature of peak	
POP	40-140	9.44	139.02	endo	Dehydration due to moisture and absorbed water

Characterization of Plaster of Paris by XRD Analysis

In the X- ray diffraction profile of plaster of paris, a big hump is observed at nearly 2 θ value of 30°. Corrected FWHM, θ is the diffraction angle and K is a constant close to unity. Figure 6 shows XRD spectrum of plaster of paris. The crystallite size of plaster of paris is presented (Table 5).

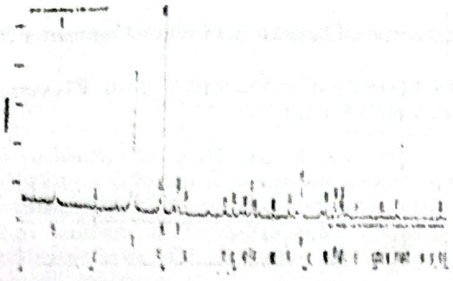


Figure 6. XRD spectrum of plaster of paris

Table 5. XRD Data of Plaster of Paris

No.	Miller	FWHM	2 Theta	Crystallite
1.	1 0 0	0.121	14.733	74.732
2.	1 1 0	0.202	25.662	42.704
3.	2 0 0	0.139	29.733	62.277
4.	1 2 2	0.192	38.434	45.292
				Average Crystallite Size

On the Aspect of the Production of Plasterboards

For the production of plasterboard, sawdust mixed with various proportion of plaster of paris (POP) under the pressure 2800 psi.

For all of the produced plasterboards, physicochemical and physicomechanical parameters such as thickness, swelling thickness, density, water absorption, modulus of rupture, and hardness were determined. Among these parameters, modulus of rupture is more specific than other for determining plasterboards quality.

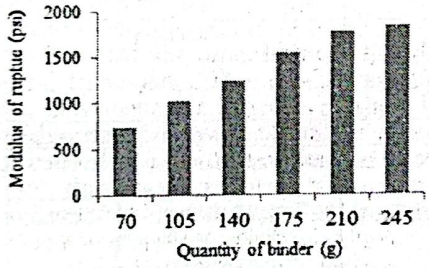
Effect of proportion of plaster of paris on the production of plasterboards

SDPOP 1, SDPOP 2, SDPOP 3, SDPOP 4, SDPOP 5 and SDPOPC 6 plasterboards were produced sawdust (70 g) with various proportions of plaster of paris (70, 105, 140, 175, 210 and 245 g) used. These plasterboards were made at 2800 psi of pressure. The results of the physicochemical and physicomechanical properties plasterboards are presented (Table 6 and Figures 7, 8 and 9). It was found that the plasterboard SDPOP 6 made with sawdust (70 g) and 245 g of plaster of paris has the highest modulus of rupture among them. Moreover, the water absorption tests of the plasterboards are also found to be satisfactory. The plasterboard SDPOP 6 has the lowest water absorption percentage among them (Table 6). Therefore, plasterboard SDPOP 6 was chosen to make the most suitable plasterboards.

Table 6. Physicochemical and Physicomechanical Properties of Plasterboards with Various Proportions of Plaster of Paris (POP)

Plasterboards	POP (g)	MOR (psi)	Thickness (cm)	Density (g/cm³)	*Water Absorption (%)	*Swelling Thickness (%)	Hardness Shore (D)
SDP OP 1	70	740.25	0.57	0.52 82	59.05	67.17	58
SDP OP 2	105	103 0.01	0.65	0.78 71	49.13	40.88	65
SDP OP 3	140	124 5.84	0.65	0.80 64	40.43	38.19	74
SDP OP4	175	154 5.47	0.68	0.95 14	28.32	35.17	82
SDP OP5	210	178 1.67	0.70	1.05 14	25.75	25.95	89
SDP OP 6	245	184 9.84	0.77	1.22 57	23.84	20.11	89

*After 24 h — Applied pressure - 2800 psi
POP = plaster of paris — Pressing time - 24 h
SDPOP 1 = SD 70 g with 70 g POP, SDPOP 4 = SD 70 g with 175 g POP
SDPOP 2 = SD 70 g with 105 g POP, SDPOP 5 = SD 70 g with 210 g POP
SDPOP 3 = SD70 g with 140 g POP, SDPOP 6 = SD70 g with 245 g POP



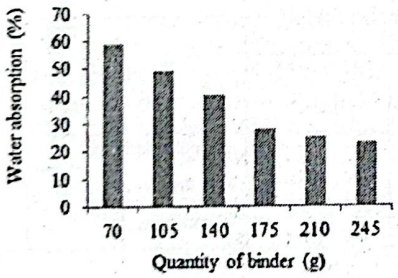


Figure 8. Water absorption of SDPOP plasterboards as a function of quantity of binder

Characterization of SDPOP 6 Plasterboard

SEM analysis

The surface morphology of plasterboard (SDPOP 6) shows that for the sawdust with plaster of paris, the presence of dispersed particles in the size range of approximately 30 μm was observed. Good adhesion between the sawdust and plaster of paris was also observed. Thus, there is mutual reinforcement between sawdust and binder (POP) in SDPOP 6 plasterboard which contributes to the improvement of modulus of rupture. SEM

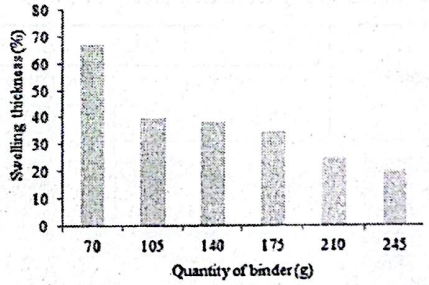


Figure 9. Swelling thickness of SDPOP plasterboards as a function of quantity of binder

photograph of SDPOP 6 plasterboard is shown in Figure10.

TG- DTA ANALYSIS

Thermal stability of plasterboard (SDPOP) was investigated by TG-DTA analysis (Figure 11). On the basis of the thermal analysis data in Table 7,

depicts the break in temperature and decomposition temperature of plasterboard. The first stage occurred from 120 to 180 °C. At this stage the board had to withstand the temperature up to 180 °C. The loss weight is 16.12 %. This is due to dehydration of moisture and absorbed water. The second stage has taken place about 13.98 % was observed. This loss in weight can be attributed to decomposition of cellulose and lignin. The third stage has taken place about 9.32 % was observed. This loss in weight can be attributed to decomposition of plaster of paris. This finding can be correlated with the exotherm as shown by TG-DTA thermogram of plasterboard.

Figure 10. SEM photomicrograph of SDPOP 6 plasterboard

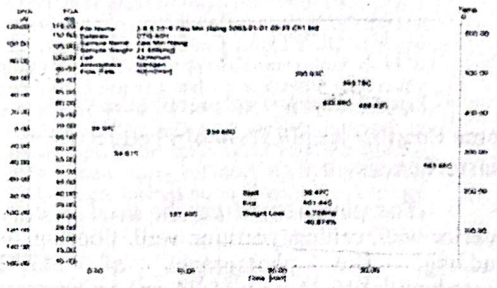


Figure 11. TG-DTA thermogram of SDPOP 6 plasterboard

Table 7. TG-DTA Data of SDPOP 6 Plasterboard

Heat Resistance of SDPOP 6 Plasterboards at Different Temperatures

Sample	TG		DTA		Remarks
	Break in Temperature (°C)	Weight loss(%)	Peak Temperature (°C)	Nature of peak	
Plasterboard	120-180	16.12	157.40	endo	Dehydration of moisture and absorbed water
	180-400	13.98	390.03	exo	Decomposition of cellulose and lignin
	400-600	9.32	463.76	exo	Decomposition of plaster of paris

Table 8 and Figure 12 show the heat resistant property of the test samples. The characteristic heat resistance shows the insulating property of plasterboards towards heat and fire. When SDPOP 6 plasterboard was heated in the muffle furnace at different temperatures (500°C, 600°C, 700°C, 800°C and 900°C) changes of the surface colour of plasterboards were occurred. It can be seen that the colour of plasterboards did not change at 600°C. It was found that the heat resistance of SDPOP 6 plasterboards are remained until 600°C. At 900°C, surface of the plasterboard became light brown. This was due to burning at the temperature. Photographs of the heat resistance of SDPOP 6 is shown in Figure 13.

Table 8. Determination of the Heat Resistance of SDPOP 6 Plasterboards at Different Temperatures

Temperature (°C)	Before heating weight(g)	After heating weight (g)	Weight Loss (%)	Observation
500	4.6845	3.0102	35.7412	no colour change
600	4.8141	2.7737	42.3838	no colour change
700	5.0600	2.9150	42.3910	pale pink colour
800	5.9866	3.4457	42.4431	pink colour
900	5.4654	2.9079	46.7943	light brown colour

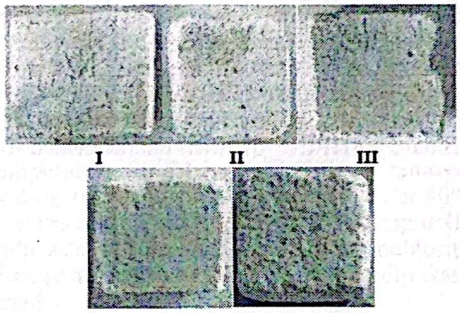


Figure 12. Photographs of the heat resistance of SDPOP 6 plasterboards at different temperatures

I = SDPOP 6 after heated at 500 °C
II = SDPOP 6 after heated at 600 °C
III = SDPOP 6 after heated at 700 °C
IV = SDPOP 6 after heated at 800 °C
V = SDPOP 6 after heated at 900 °C

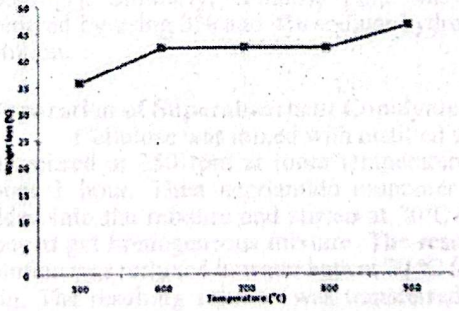


Figure 13. Weight loss (%) of SDPOP 6 plasterboards as a function of different temperatures

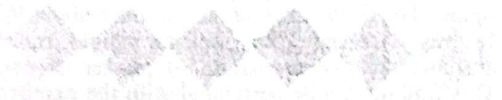


Figure 14. Photographs of produced plasterboards

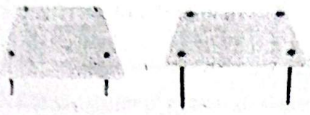


Figure 15. Screwed and nailed Plasterboards

Some Possible Application of Produced Plasterboards

The plasterboard can be used in eaves, exterior wall, ceiling, partition wall, flooring, and cladding. The photographs of SDPOP plasterboards (15.24 cm x 15.24 cm) are presented in Figure 14. Figure15 shows screwed and nailed plasterboards.

Conclusions

The plasterboards were fabricated by mixing sawdust (70 g) with various proportions of plaster of paris (70, 105, 140, 175, 210 and 245 g) by cold compressing molding method. The influencing factors on the physicomechanical properties of produced plasterboards (SDPOP 1, 2, 3, 4, 5, and 6) such as modulus of rupture, thickness, water absorption, swelling thickness and hardness were examined.

From the experimental results, among the plasterboards using sawdust (70 g) with plaster of paris (POP) 245 g, (namely SDPOP 6) was found to be a quality grade plasterboard possessing 1849.84 psi of modulus of rupture, 0.77 cm of thickness, 1.2257 g cm^{-3} of density, 23.84 % of water absorption, 20.11 % of swelling thickness, and 89 Shore D of hardness. The produced plasterboard (SDPOP 6) was characterized by SEM and TG-DTA analysis and heat resistance experiment. From the results, the heat resistance of SDPOP 6 plasterboard is remained until at 600 °C.

Sawdust-cement plasterboards produced by simple hand lay-up process provide an opportunity of replacing exist-ing materials with a higher strength, low cost alternative that is environmentally friendly. Plasterboards made of wastes can change harm to benefit, by being the "green" material capable of natural degradation.

Acknowledgements

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References

Dias,A.M.N.(1994) "Building Plaster: Characterization of powder, Paste and Motor and Application Rendering", MSc Thesis, Department of Engineering Chemistry, University of Sao Paulo, Portugal,15-50

Muhammad, A.M.M.I., Hamdan, S., Rahman, M.R. and Isalm, M.S. (2011). "Treated Tropical Wood Sawdust-Polypropylene Polymer Composite: Mechanical and Morphological Study", J. of Biomater and Nano-biotechnol., 2, 453-444

Paulo, S.B. and Camarini,G.. (2012). "Recycled Plaster: Physical and Mechanical Properties", J. Adv. Mater. Res., 4, 1307-1310

Puvanasvaran, A.P., Hisham S. and Sued, K.M. (2011). "Investigation on the Mechanical Characteristics of Sawdust and Chip wood Filled Epoxy", J. Mech. Eng.and Technol., 3, 71-77

Tilak, L.N.,Santhosh, K.M.B., Singh, M. .and Niranjan, T. (2018). "Use of Sawdust as Fine Aggregate in Concrete Mixture", Inter. Res. J. Eng. and Technol., 5, 1249-1253

Tomas, U.G..J. (2014). "Effect of Sawdust as Fine Aggregate in Concrete mixture for Building Construction", Inter. J. Adv. Sci and Technol., 63, 73-82

Synthesis and Swelling Capacity of Superabsorbent, Rice Straw Cellulose Graft Copolymer

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Abstract

In this research, rice straw was used as cellulose sources for synthesizing a cellulose polyacrylamide superabsorbent by graft polymerization. The cellulose from rice straw was prepared by soda process using different concentrations of sodium hydroxide. The rice straw cellulose superabsorbent had been successfully synthesized using acrylamide monomer as grafting agent and N,N-methylene-bis acrylamide (NNMBA) as cross linker and potassium persulphate (KPS) as initiator. Then it was characterized using FT-IR, SEM and XRD. The result of FT-IR spectrum shows that acrylamide has been grafted onto rice straw cellulose. SEM data indicate that the fibrous morphology of rice straw cellulose disappears and gel aggregate with many microporous holes are formed. Crystal size of cellulose analyzed by XRD was 29.3 nm indicated the nanocrystal structure and superabsorbent as amorphous structure. The effect of varying cellulose to acrylamide ratios on grafting efficiency and swelling degree of superabsorbent in water was investigated. The maximum swelling degree of superabsorbent was 1029 % in water using cellulose to acrylamide ratio (1:2.5), 0.5 wt % KPS and 0.1 wt % NNMBA. This result shows the highest water absorbency. The rice straw cellulose superabsorbent can be applied to absorb and control slow release of water and fertilizer in agriculture.

Keywords— Rice straw cellulose, Superabsorbent, Swelling degree

Introduction

Myanmar is the world's sixth largest rice producing country. Rice is the country's most important crop and is grown on over 8 million ha. Therefore rice straw (RS) is the most abundant agricultural waste. The agricultural wastes such as wheat straw, rice straw, corn straw, cassava dregs and others can be used as a more attractive alternative to get cellulose [6].

The cellulose can be isolated from rice straw which contains 43-49% cellulose, 25-28% hemicellulose, 15.8% lignin, 12.5% silica, resin, gum, protein and mineral compound [9]. Natural polymer such as cellulose is possible to develop as superabsorbent polymer through grafting modification to the main backbone of cellulose. Superabsorbent is a kind of polymer that is able to absorb and hold large amount of water [8]. The use of superabsorbent in agriculture aims to reduce irrigation water consumption and plant mortality rates, increase the availability of water in the soil that allows plants to last long , reducing the tendency of soil compaction, prevent erosion, increase the fertilizer efficiency and bind heavy metals [2].

Superabsorbent polymer or hydrogel is a three dimensional network formed from hydrophilic polymer cross link. Hydrophilic properties of hydrogel are the factors that determine the ability to absorb and retain large amounts of water. Hydrogels are sensitive to environment changes such as changes in pH, temperature and concentration of the environment [7].

In this research, the cellulose is extracted from rice straw, and will be used to synthesize the superabsorbent with acrylamide monomer by free radical polymerization method. The aim of this research is to produce superabsorbent copolymer from rice straw cellulose for improvement in agricultural productivity and to recycle the agricultural waste into useful product.

Materials and methods

Rice straw was collected from Lun-Taung village, Madayar Township, Mandalay region, Myanmar. Rice straw was used as raw material. Sodium hydroxide (NaOH) was used to extract cellulose from rice straw. Potassium hydroxide (KOH) and hydrogen peroxide (H_2O_2) were used to remove impurities from cellulose. Sodium hypochlorite (NaOCl) was used to bleach. Potassium persulphate (KPS) as initiator and N, N-methylene-bis-acrylamide (NNMBA) as cross linker, acetic acid and ethanol were used.

Preparation of Cellulose Pulp from Rice Straw

$$\text{Swelling Degree} = \frac{Wss - Wd}{Wd} \times 100\% \qquad (2)$$

The rice straw was washed by distilled water and sundried. The dried straw was cut into small pieces and grinded with grinder. The rice straw powder was sieved by using 100 mesh size sieves. The cellulose pulp was prepared employing three different concentrations of sodium hydroxide 2%, 3% and 4%. 30 g of sifted raw rice straw powder was mixed with 300 mL of 2% sodium hydroxide and refluxed in water bath at 100°C for 2 hours and then filtered [11]. Hemicellulose is removed by adding 300 mL of 5% (w/v) potassium hydroxide at room temperature for 24 hours. Lignin is removed by adding 10 % (v/v) hydrogen peroxide at 40°C for 8 hours and then filtered. The resulting cellulose pulp was bleached with sodium hypochlorite and filtered and washed. Then bleached pulp was oven dried.

Ten grams of the bleached pulp was treated with 50mL of 17.8% sodium hydroxide solution for about 25 minutes at 20°C. The material was filtered and washed with a liter of water. The residue was rinsed with 100mL of 10% acetic acid. Finally, the acid was completely removed by washing the pulp with hot water. The pulp obtained was dried at room temperature for 24 hours followed by oven dry at 100°C for 2 hours [1]. Similarly, cellulose pulp was also prepared by using 3% and 4% sodium hydroxide solution.

Preparation of Superabsorbent Copolymer

Cellulose was mixed with distilled water and stirred at 350 rpm at room temperature for about 1 hour. Then acrylamide monomer was added into the mixture and stirred at 70°C for 1 hour to get homogeneous mixture. The resulting solution was refluxed in water bath at 70 °C for 30 min. The resulting solution was transferred into the beaker containing certain amounts of NNMBA and KPS which was kept stirring with magnetic stirrer at70 °C until pasty product was

obtained and then the product was cut into a small piece. Then the samples were dewatered with ethanol and dried 65 °C to constant weight. Experiments were carried out with a variation on acrylamide, potassium persulphate and N, N-methylene-bis-acrylamide [9].

Scanning Electron Microscope (SEM) Analysis of Cellulose and Superabsorbent Copolymer

Scaning electron microscopy was used to study the morphological structures of cellulose and grafted copolymer by detecting the presence of connected microporosity. Original rice straw cellulose and grafted copolymers were qualitatively analysed by SEM method. The morphological structures of cellulose and grafted copolymer were measured at Department of Chemistry in Yadanabon University, Mandalay, Myanmar.

Determination of Grafting Efficiency

The efficiency of the grafting is the ratio the mass grafted of monomer on cellulose with the initial monomers.The grafting efficiency was determined by using the following equation 1 [3].

Determination of Swelling Degree

The Japanese Industrial Standard K 8150 method has been used to measure the swelling. For determination of swelling degree, superabsorbent was immersed in deionized water for 48 hours at room temperature occasionally stirring. After swelling, the swollen superabsorbent was filtered and the swelling degree was calculated as shown in equation 2 [3].

Where Wss is the weight of absorption of the copolymer after swelling and W_d is the weight of dry copolymer.

FT-IR Analysis

Table 1 Effect of Alkali Concentration on Yield of Rice Straw Cellulose Pulp

3		4	8.50	

The Fourier Transform Infrared (FT-IR) Spectroscopy was used to identify the functional group of the active compounds based on the peak value in the region of infrared radiation. The FTIR spectra of the original rice straw cellulose and grafted copolymer were recorded by FTIR spectrophotometer (PerkinElmer, UK, L1600400) in the wave number range 400-4000 cm^{-1} .The FTIR spectrum of rice straw cellulose and the grafted copolymer were measured at Department of Chemistry in Monywa University, Sagaing, Myanmar.

Crystallization Analyzed by XRD

The crystallization of rice straw cellulose and superabsorbent copolymer were examined by XRD measurement performed on a multiplex 2 kW using Cu K-alpha at 40 kV and 50 mA.

Scherrer equation was used for calculating crystallite size in plane

Where λ is wavelength of X-ray tube, λ = 1.54056 A°, β is the pure integral of width of the reflection at half maximum height (FWHM, full width at half maximum) of 012 peak at 2θ about 22° and 0.89 is the Scherrer constant. Percentage crystallinity (%Cr) was calculated by equation 4 [9]. XRD analysis was measured at University Research Centre (URC) Yangon, Myanmar.

Where $I_{crystallinity}$ is the intensity at maximum of crystalline peak ($22°<2\theta<23°$) and $I_{noncrystallinity}$ is the intensity at minimum ($16°<2\theta<17°$).

Water Retention Test for Superabsorbent Copolymer

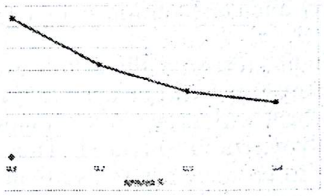
The used soil was firstly dried in oven for two days at 60 °C to remove moisture from soil. The superabsorbent was mixed in container with 300 g of soil. The mixture was irrigated with 200 ml of water, and the container was weighed at different set intervals. This measurement was carried out at room temperature. The weight loss of the mixtures against time was calculated at every interval time and so water retention capacity of superabsorbent was obtained. Controlled experiment without superabsorbent was also performed as reference. Water retention (WR) percentage of soil treated with superabsorbent was determined as the following equation 3 [4].

Where, M_1 is the weight of wet soil at certain interval time and M_2 is the weight of oven dry soil.

Results and discussion

Effect of Alkali Concentration on Yield of Rice Straw Cellulose Pulp

Three different concentrations of alkali were used for the experiment and the results are shown in the following Table. 1. According to the results, when 2% and 3% concentration of alkali were used, the resulting pulp obtained high yield (33%) and (30%). But it was hard and colored. When 4% concentration of alkali was used, the yield of pulps were considerably low but quality of pulp was found to be better and could be bleached easily.



fect of Cross Linker Content on swelling Degree

Effect of Cr ; Degree

The inker on the swelling capacity of rice straw cellulose superabsorbent was investigated and the results are shown in Figure (1). The amount of NNMBA was varied from 0.1 to 0.4 wt%. It is obvious that cross-linker content at 0.1 wt%, showed the maximum swelling degree. The swelling degree decreased with increase in cross-linker

concentration from 0.2 to 0.4wt%. The content of cross-linker is a crucial factor influencing the cross-linking density of the copolymer. Increasing the cross-linked density made rigid structure and failed to hold a large quantity of water on swell degree [9].

Effect of Initiator Content on Swelling Degree

The effect of the content of initiator on the swelling capacity of rice straw cellulose superabsorbent was investigated and the results are shown in Figure (2). The amount of KPS varied from 0.5 to 2.0 wt%. The maximum swelling degree was observed at 0.5% KPS, because the polymerization was initiated by the free radicals generated from KPS under heat. More initiators would produce more free radicals that can enhance the grafting efficiency. The swelling degree decreased with increase in initiator concentration. Too much initiator would produce too many free radicals, thus increasing the collision probability between free radicals, eventually terminating the reaction.

The generated short graft chains would not form a three dimensional network easily, thus decreasing the swelling capacity [12].

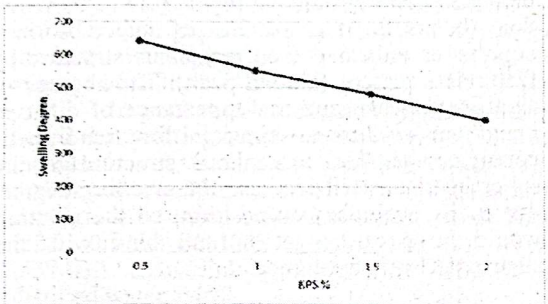


Fig. 2 Effect of Initiator Content on Swelling Degree

Effect of Cellulose to Acrylamide Ratio on Grafting Efficiency

Grafting efficiency was investigated with different cellulose to acrylamide ratio. The results are shown in Table (2).

Cellulose : Acrylamide	Grafting Efficiency
1:1	79
1:1.5	80
1:2	82
1:2.5	86
1:3	91

Table 2 Effect of Cellulose to Acrylamide Ratio on Grafting Efficiency
0.5wt% KPS, 0.1 wt% NNMBA

According to the results, grafting efficiency increased with increasing monomer concentration. Higher in monomer ratio made free radical come closer than lower ratio of monomer and tends to form more crosslink [7].

Effect of Cellulose to Acrylamide Ratio on Swelling Degree

The swelling characteristics of the superabsorbent were measured by gravimetric analysis. The sequence of swelling influencing factors in water and urea are NNMBA > AM>AA>KPS. NNMBA crosslinking agent is the most important factor, followed by the monomer AM and AA then the KPS initiator [6]. Swelling degree was investigated with different cellulose to acrylamide ratio. The results are shown in Table (3).

Table 3 Effect of Cellulose to Acrylamide Ratio on Swelling Degree 0.5 wt% KPS, 0.1 wt%NNMBA

Cellulose: Acrylamide	Swelling Degree (%)
1:1	240
1:1.5	390
1:2	640
1:2.5	1029
1:3	800

Swelling degree increased with increasing monomer concentration at cellulose: acrylamide (1:1) to (1:2.5). At cellulose: acrylamide (1:3) swelling degree decreased. These could be explained that the increasing monomer concentration enhances the free radical on the cellulose chain and therefore form more cross-linking between cellulose chains. Higher in crosslink density reduces the free volume available for swelling by increasing the tightness of network structure [3].

For agricultural application, swelling behavior of the copolymer network is more important than other factors. The maximum swelling degree of copolymer in water was 1029% at cellulose to acrylamide ratio (1:2.5), 0.5 wt% KPS and 0.1 wt% NNMBA.

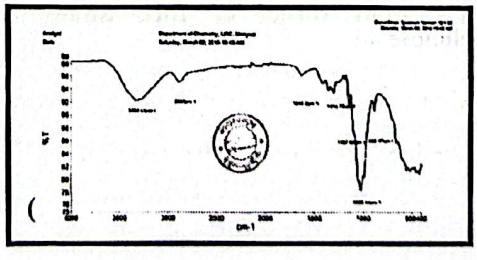
Characterization of Cellulose and Superabsorbent Copolymer Using FT-IR

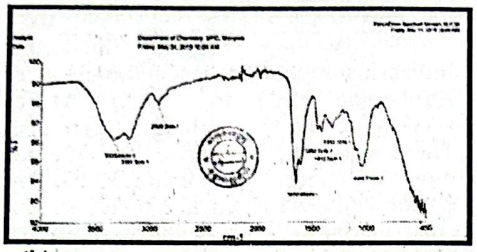
In this study KPS was used for initiating polymerization of acrylamide and graft copolymerization of acrylamide was triggered from hydroxyl groups on the chain of RS simultaneously.

In Figure (3,a), the absorption at 3332.42 cm^{-1} is assigned to stretching of OH groups which was diminished after graft copolymerization. It can be explained that the partial hydrogen bond of cellulose is destroyed enhancing the new formation of cross-links with acrylamide monomer.

The change spectra can be seen with the peaks at 3333.64 cm^{-1} and 3187.3 cm^{-1} in Figure (3,b). Those two bands indicate the unsymmetric and symmetric stretching of NH_2 groups which are characteristics of the $-CONH_2$ group present in the acrylamide monomer [10].

The absorption bands at 1650.96 cm^{-1} in Figure (3,b) is assigned to the stretching of the C=O which indicated the presence of i amide in the grafted copolymer. This absorption band is not observed in cellulose. These changes provided strong evidence of the grafting of acrylamide onto cellulose. The peak at 1026.44 cm^{-1} in Figure (3,a) indicates the C-O-C stretching of cellulose. The bands observed in at 1450.7 cm^{-1} in Figure (3,b) is assigned the stretching CN of grafted copolymer [10].





(b)

Fig.3 FTIR Spectrum of Rice Straw Cellulose and (b) FITR Spectrum of Superabsorbent Copolymer using Cellulose: Acrylamide (1:2.5)

Scanning Electron Microscopy of Rice Straw Cellulose and Grafted Copolymer

The morphological structure including average breadth of fibres in rice straw cellulose pulp was determined using Scanning Electron Microscope (SEM). The SEM analysis of the pulps using alkali concentration 4% is shown in Figure (4).According to the SEM results, it was found that average breadths of fibres varied between 3.7μm to 9.4μm. This indicated that the average breadth of the fibres obtained from soda pulping process showed satisfactory conditions to be used as a cellulose backbone for graft copolymerization process [1].

The morphology of grafted copolymer was examined by SEM for the aim of detecting qualitatively the presence of connected microporosity. The SEM image of grafted copolymer is shown in Figure (5).

According to the SEM analysis, it can be seen that microporosity seems to be present after grafting of acrylamide monomer onto the cellulose backbone. The pores provided more available regions for the diffusion of water molecules and thus the superabsorbent may demonstrate a higher water absorption capacity.

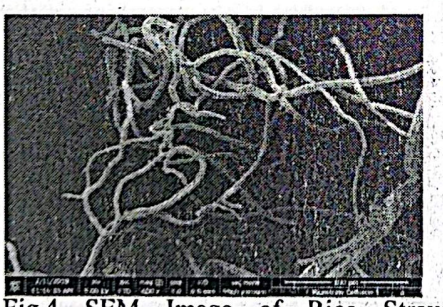


Fig.4 SEM Image of Rice Straw Cellulose

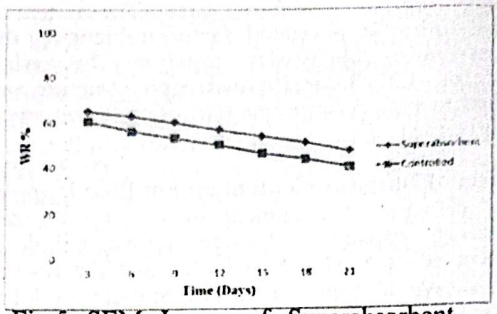


Fig.5 SEM Image of Superabsorbent Copolymer

Characterization of Rice Straw Cellulose and Superabsorbent Copolymer Using XRD

X-ray diffractograms of rice straw cellulose and superabsorbent copolymer are presented in Figure (6,a) and (6,b). In Figure (6,a), it can be seen the diffraction pattern of rice straw cellulose with sharp peak at 22.3° as intensity of crystalline. The peak at 16.7° called intensity of amorphous [6]. The percent crystallinity of cellulose is 33.3%, means cellulose is semi-crystalline.The crystalline size of cellulose calculated by Scherrer's law was 29.3 nm, which indicated nanocrystal size [6]. Figure (6,b) shows that the diffraction pattern of superabsorbent copolymer indicated the amorphous structure by featureless pattern (abroad peak). The absence of significant peaks and the appearance of diffused maximum indicates typical for amorphous copolymer [5].The amorphous structure of the superabsorbent will increase the swelling degree, due to its irregular composition, so the solution would be easy to get in and bind with the superabsorbent copolymer.

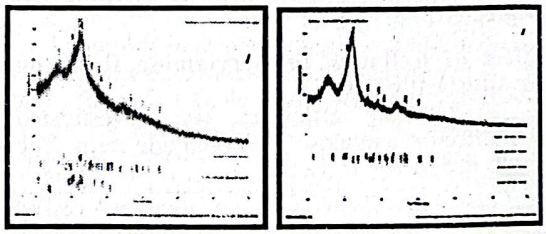


Fig.6. (a) XRD Diffractogram of Rice Straw Cellulose and (b) XRD Diffractogram of Superabsorbent Copolymer

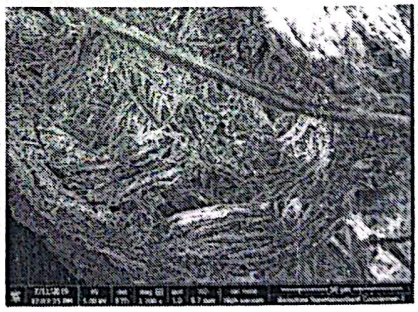


Fig. 7 Water Retention Test in Soil

Water Retention in Soil

The addition of micro-porous superabsorbent in soils can markedly increase water holding abilities by absorbing and retaining large amount of water which indirectly increase the nutrient availability in plant roots. Water retention test in soils with and without the addition of

superabsorbent was carried out under room temperature and results are shown in Figure (7).

According to the results, water retention in soil decreased as the time was prolonged and soil containing superabsorbent possessed higher water retention over controlled one. This result indicates that superabsorbent can enhance the water retention capacity in soil.

Conclusion

In this research work, there were mainly three parts ; production of cellulose from rice straw, synthesis of superabsorbent copolymer with acrylamide and rice straw cellulose, characterization of the rice straw cellulose and superabsorbent copolymer.

Rice straw cellulose was prepared by soda process using different alkali concentration (2%, 3% and 4%). The experiment carried out using 4% alkali concentration at 100°C was found to be the satisfactory condition for quality of pulp. According to the SEM analysis of fibres in rice straw pulp sample, it was found that there were enough quality for required properties of fibres such as paper making and graft copolymerization as a biopolymer. The average breadth of fibres varied between 3.7μm to 9.4μm. According to the literature, the suitable quality of fibres, which are to be used in paper production and value added products, is ranging between 4μm to 10μm in average breadth.

In graft copolymerization process, it was found that grafting efficiency and swelling degree increased with increasing monomer ratio. The maximum swelling degree for superabsorbent copolymer by using cellulose to acrylamide ratio 1:2.5, 0.5 wt % KPS initiator and 0.1 wt % NNMBA cross linking agent was 1029 % in distilled water.

The SEM analysis of grafted copolymer showed that the pores were found after grafting of cellulose with acrylamide and this result indicated that cross links between monomer and cellulose backbone were grafted well.

In FTIR analysis, the results also showed that acrylamide had been grafted onto rice straw cellulose backbone successfully. The diffraction pattern of XRD indicated that cellulose was nanocrystal with the size of 29.3 nm and the superabsorbent copolymer as the amorphous structure, Based on water retention test, it was found that superabsorbent copolymer had good water retention ability. The superabsorbent copolymer should be used for agriculture to increase plant growth and crops yield percent.

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References

[1] Bhaskra, R.M.P and Seshadri, T.R. (1940)."Paper Pulps from Annual Crops", Department of Chemical Technology, Andhra University, Waltair.

[2] Buchholz, Fredric L; Graham, Andrew T, eds. (1997). Modern Superabsorbent polymers Technology,1ed. John Wiley & Sons.I SBN 978-0-471-19411-8.

[3] D.Swantomo, Rochmadi, Basuki K.T, Sudiyo. R,(2013). "Synthesis and characterization of Graft Copolymer Rice Straw Cellulose – Acrylamide Hydrogels using Gamma Radiation Atom Indonesia " Vol.39, No.2, (57-64).

[4] Francesco F. Montesano, Angelo Parente, Pietro Santamaria, Alessandro Sannino, Francesco Serio.(2015)." Biodegrradeble Superabsorbent Hydrogel Increases Water Retention Properties of Growing Media and Plant Growth"; Agriculture and Agricultural Science Procedia 4,481-458.

[5] Hadman, H., Muhid, M.N.M., Endud, E., Listiorili and Ramli, Z. (1997). "Si MASMMR, XRD AND FESEM Studies of Rice Husk Silica for the Synthesis of Zeolites". J. Non-Crystalline Solids, Vol.211,pp.126-131

[6] Helmiyati, A Fiyriyani and f Meyanti, (2017). " The copolymerization synthesis And Swelling Capacity of Cellulose – Poly Superabsorbent (acrylic acid-Co-acrylamide) based on rice straw ", IOP Publishing.

[7] Helmiyati, AsepSaefumillah and Wina Yulianti, (2014)."Synthesis and Swelling Kinetics of Superabsorbent Rice straw Cellulose Graft Copolymer", *Asian Journal of Chemistry.*

[8] J.mohammad,Z.mehr and K.Kabiri. (2008). Iran.Polym.J.,17,451

[9] ShuPing Jin, Jian – Ying MAO, (2015). "A slow Degradable Superabsorbent Hydrogel for Agriculture from Raw corn straw", Chemistry and Chemical Engineering, Hexi University, People's Republic of China.

[10] Silverstein, R.M., Website, F.X and Kiemle, D.J (2005),"Spectrometric Identification of Organic Compound" New York. 7th Edition, John Wiley & Sons, Inc.

[11] Thurein Kyaw and Khin Khin Lay, (2016). " Effect of Gamma Radiation Grafting of Rice straw Cellulose-Acrylandmide Monomer", *International Journal of Advances in Agricultural and Environmental Engineering* Volume 3, Issue 2 ISSN 2349- 1523 EISSN 2349- 1531.

[12] Wei-Ming Cheng[1], Xiang-Ming Hu[2], De-Ming Wang[3] and Guo-Hua Liu[4], (2015), "Preparation and Characteristics of Corn Straw-Co-AMPS-CO-AA Superabsorbent Hydrogel", *Journal of polymer*, 7, 2431-2445.

Green Synthesis of Copper Oxide Nanoparticles Using *Carcia Papaya* and *Azadirachta indica* Leaf Ethanol Extract and Their Characterization

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Moe Myat Myat, Associate Professor, Department of Engineering Chemistry, Myanmar Aerospace Engineering University
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Abstract

Green Technology is one of the most advanced and applied technologies in the global world. From this research, Myanmar traditional medicinal plants namely *Carica papaya* and *Azadirachta indica* were selected for the study. The synthesis of copper oxide nanoparticles was performed by using copper nitrate as a precursor with the help of ethanol leaves extract as an reducing agent by stirring at room temperature. The preliminary detection of phytochemical compounds, present in leaves of selected plants were carried out. The elemental analysis of leaves were also determined by using Energy Dispersive X- ray Fluorescence spectroscopy. The synthesized copper oxide nanoparticles were identified by using Fourier Transform Infrared Spectroscopy. The crystalline size and lattice of copper oxide nanoparticles were also investigated by X Rays Diffraction method. From XRD result, the average crystallite size of copper oxide nanoparticle from the ethanol leaves extract of *Carica papaya* and *Azadirachta indica* were found 24.1 and 37.4 nm respectively. The synthesized copper oxide nanoparticles were found to be cubic crystalline structure.

Keywords: copper oxide nanoparticles, Green synthesis, *Carica papaya* and *Azadirachta indica* leaf extract

Introduction

Nanotechnology is rapidly increasing field that making an impact on human life such as pharmaceutical, health, food, electronic, chemical industry, energy science, cosmetics, environmental science and space industries etc (Dinker Pawar *et al*., 2015).

Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. Nanoparticles often possess unexpected optical properties as they are small enough to confine their electrons and produce quantum effects (Website 1).Metallic nanoparticles have been prepared by several physical and chemical approaches depending on the accessibility and viability of procedures to attain the essential applications (Mohanpuria, *et al*, 2008).The physical approaches for the synthesis of nanoparticles comprise laser ablation, electric arc discharge, flame pyrolysis, and so on.

The chemical synthesis of nanoparticles can be simply changed into green synthesis by the appropriate choice of reducing agents, solvents, and stabilizers (Punnoose, *et al*., 2014). The green synthesis for the synthesis of metal and metal oxide nanoparticles also makes use of numerous approaches, electrochemical, supercritical liquids, extracts from natural sources and environmentally-friendly circumstances (Vollmer, 2010).Among these approaches for the synthesis of nanoparticles, utilizing plant extracts has enticed substantial consideration, due to easy sampling and cost efficiency enabling the large scale biosynthesis of nanoparticles (Khan, M.; 2014).In green synthesis method, the plant extract has been used as reducing agent for the synthesis of copper nanoparticles (DinkerPawar, *et al* 2015). Plant extracts comprise of a wide range of naturally occurring chemical compounds, which are generally recognized as natural products. These natural products possess varieties of biological activities due to their exceptional variety in their chemical structures (Harvey., 2008).

Copper is most widely used material in the world due to their electrical, optical, catalytic, biomedical and antifungal, antibacterial applications among various metal particles such as gold, silver, iron, palladium, zinc etc. Copper nanoparticles act as antimicrobial agent in various fields. The copper is highly toxic to microorganism such as bacteria and non-toxic to animal cells, due to these phenomena it is considered to be an effective bactericidal metal. Nanoparticles were synthesized from all the parts of the plant separately like seed, stem, flower, and leaf (DinkerPawar, *et al* 2015).Among various metal oxide NPs, copper nanoparticles, due to their excellent physical and chemical properties and low cost of preparation, have been of great interest. Copper nanoparticles have wide applications as heat transfer systems, antimicrobial materials, super strong materials, sensors and catalyst. Copper nanoparticles are very reactive because of their high surface to volume ratio and can easily interact with other particles and increase their antimicrobial efficiency (Ansilin , e*t al*., 2016). The aim of the present study is to determine the synthesis of copper oxide nanoparticles by using two kinds of leaves ethanol extract and their characterization.

Botanical Description

Botanical Description of *Carica papaya* Linn. (Thinbaw)

Family name – Caricaceae
Botanical name –*Carica papaya* Linn
English name–Papaya
Myanmar name –Thin baw
Parts used–Leaves

Figure (1) The leaves of *Carica papaya* Linn. (Thinbaw)

Botanical Description of *Azadirachta indica* A. Juss(Tamar)

Family name – Meliaceae
Botanical name – *Azadirachta indica* A.Juss
English name – Neem
Myanmar name – Tamar
Part used – Leaves

Figure (2)The leaves of *Azadirachta indica* A. Juss (Tamar)

Materials and methods

Collection of Sample

Two traditional medicinal plants such as *Corica papaya* Linn (Thin baw) and *Azadirachta indica* A. Juss (Tamar) leaves were collected from Mahar Aungmyae township, Mandalay Region.

Preliminary Phytochemical Test for Leaf Samples

The phytochemical test for leaves of *Corica papaya* and *Azadirachta indica* . The quantitative phytochemical analysis of this species exhibited the presence of alkaloids, flavonoids, glycosides, saponins, polyphenol, Tannin, Steroid, reducing sugar and phenolic compounds. (Harborne J., 1973).

Preparations of Leaves Extract

The collected samples were washed with distilled water. These samples were cut into small pieces and allowed to air dry. The dried samples were grounded into fine powder with the aid of hand grinder. The dried powder sample (70 g) was percolated with 95% ethanol (800 mL) for one month. The ethanol extract was filtered with Whatman No.1 paper. The solution was stored in refrigerator at 16°C.

Synthesis of Copper Oxide Nanoparticles from ethanol extract by Stirring

30 mL of 50 mM copper nitrate solution were mixed with 120 mL of plant leaf ethanol extract. This mixture was stirred on a magnetic stirrer with 500 rpm. This reaction mixture was maintained at 50°C for the duration of reaction times 8 hours to reduce of copper ions. The mixture was centrifuged with 6000 rpm for 30 mins. The precipitates of copper oxide nanoparticles were obtained. The resultant copper oxide nanoparticles were washed with distilled water until, it reached then with pH 7 and acetone. And then dried in a petridish.

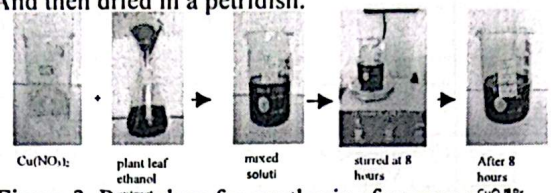

Figure 3. Procedure for synthesis of copper oxide nanoparticles

Results and Discussion

Elemental Analysis of Two Kinds of Leaves Powder

The elements that contain in two kinds of leaves sample were analyzed by using EDXRF (Energy Dispersive X-ray Fluorescence) Spectroscopy at Department of Chemistry, Monywa University.

Table 1 Elemental Analysis for Leaves of Two Kinds of Samples Powder

No.	Element	Symbol	Relative Abundance (%)	
			Tamar	Thinbaw
1	Potassium	K	1.097	0.891
2	Calcium	Ca	0.405	0.884
3	Silicon	Si	0.365	0.926
4	Sulfur	S	0.330	0.266
5	Magnesium	Mg		1.311
6	Iron	Fe	0.034	0.038
7	Aluminum	AL		0.345
8	Phosphorus	P	0.171	0.102
9	Manganese	Mn	0.002	0.004
10	Zinc	Zn	0.002	0.002
11	Copper	Cu	0.002	0.002
12	Titanium	Ti	0.003	0.004

From the experimental results, the higher amount of calcium and potassium in tamar and magnesium in thinbaw were found the leaves of two samples. Moreover, sulphur, iron, phosphorus, manganese, zinc and copper were also found in that order. These mineral contents indicate that the leaves of two samples powder were rich source of mineral.

Phytochemical Test for Two Kinds of Leaves Sample

Preliminary phytochemical analysis was performed in order to know different types of chemical constituents present in the leaves sample.

Elemental Analysis of Leaves Samples

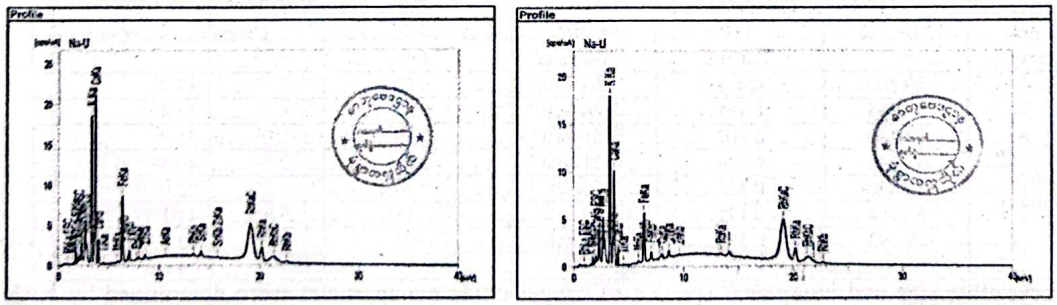

Figure 4. Elemental analysis of (*Carica papaya* and *Azadirachta indica*) leaves sample by EDXRF method

No.	Tests	Reagents	Observation	Results	
				I	II
1	Alkaloid	Wagner's solution Dragendroff's solution	Reddish brown ppt Pale orange ppt	+ +	+ +
2	Flavonoid	Mg, Conc HCl, Conc H_2SO_4	Brown colour solution	+	+
3	Phenolic	10 % $FeCl_3$	Brown colour solution	+	+
4	Terpene	Acetic anhydride $CHCl_3$, Conc H_2SO_4	No Pink color solution	–	–
5	Reducing sugar	Benedict's solution	Greenish yellow ppt	+	+
6	Saponin	Distilled water	Forth	+	+
7	Steroid	Acetic anhydride, Conc H_2SO_4	Blue green colour solution	+	+
8	Polyphenol	1 % $FeCl_3$ +1%$K_3[Fe(CN)_6$	Greenish blue colour solution	+	+
9	Glycoside	Lead acetate	Pale yellow ppt	+	+
10	Tannin	10% $FeCl_3$, dil H_2SO_4	Yellowish brown ppt	+	
11	Lipophenol	0.5 N KOH, 4 drops of NaOH	No Deep color solution	–	–

(+) = presence of constituents (-) = absence of constituents
I = *Corica papaya*, II = *Azadirachta indica*

The results of the amount of copper oxide nanoparticles from ethanol leaf extract samples and copper nitrate solution are shown in Table (3).

Table 3 The Results of the Amount of Copper oxide Nanoparticles from Two Kinds of Leaves Extract

Sample	$Cu(NO_3)_2$:Sample	Time (hr)	Weight of CuONPs (g)	Yield percent (%)
Carica papaya	1:4	8	0.24	25.60
Azadirachta indica	1:4	8	0.15	16.01

volume ratio → Ethanol Leaves extract : $Cu(NO_3)_2$ = 1 : 4

The result of the amount of copper oxide nanoparticles from selected leaf extract samples and 30mL of 50 mM copper nitrate solution and also 120 mL of leaf ethanol extract.

FT-IR Measurement

The FT-IR spectrum Figure reported the characteristic peaks of synthesized of CuO from the *Azadirachta indica* and *Carica Papaya*. Two extract leaves, was observed in the range 500 – 825 cm^{-1} (Padil V. V., *et al.*, 2013).

The broad absorption peak at around 3363 – 3393 cm^{-1} is caused by absorbed water molecules since the nano crystalline materials exhibit a high surface to volume ratio and thus absorb moisture (Radhakrishnan, *et al.*, 2014).

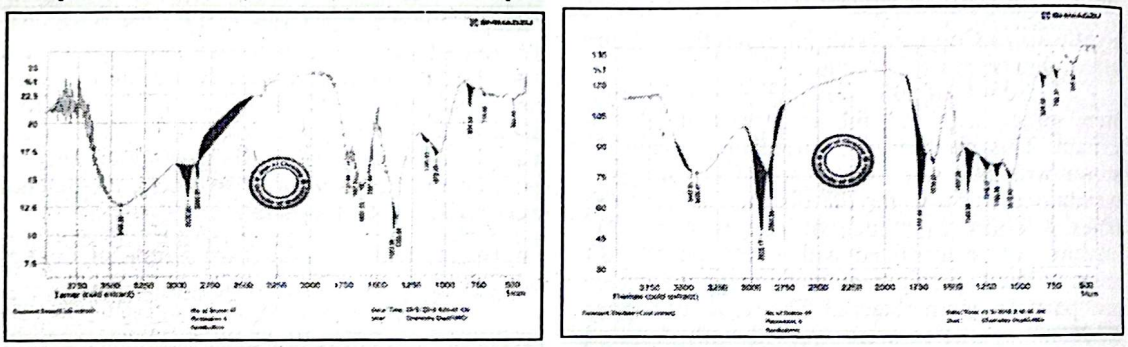

Figure 5 FT-IR spectrum of (*Azadirachta indica* and *Carica papaya*) leaves extract sample

XRD Analysis of Copper Oxide Nanoparticles

Sample name	2 θ value	FWHM radians	β (radian)	d-spacing (nm)	Crystalline size (nm)
Azadirachta indica (Tamar)	29.700	0.094	1.64×10^{-3}	0.270	83.7
	36.565	0.485	8.46×10^{-3}	0.221	15.4
	42.380	0.207	3.61×10^{-3}	0.192	35.9
	43.292	0.184	3.21×10^{-3}	0.188	40.3
	50.450	0.326	5.69×10^{-3}	0.163	22.0
	61.594	0.171	2.99×10^{-3}	0.135	41.1
	69.793	0.273	4.76×10^{-3}	0.121	23.6
	Average				37.4 nm

The crystallite size and interplanar spacing of copper oxide nanoparticles were determined by XRD analysis.

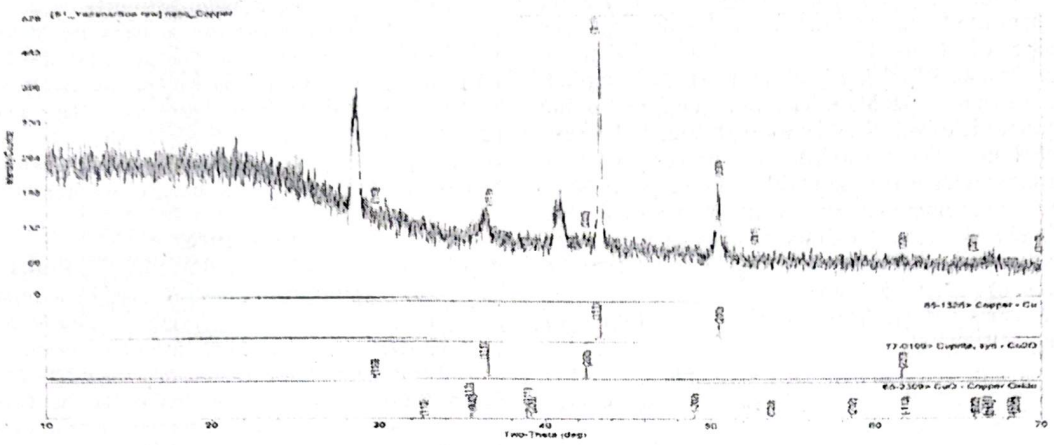

Figure 6 XRD Results of Average Crystallite Size of copper oxide nanoparticles by using *Azadirachta indica* (Tamar) Leaves Extract

Table 4 XRD Results of Average Crystallite Size of copper (I) oxide Nanoparticles by using *Azadirachta indica* (Tamar) Leaves Extract

Sample name	2 θ value	FWHM radians	β (radian)	d-spacing (nm)	Crystalline size (nm)
Azadirachta indica (Tamar)	29.700	0.094	1.64×10^{-3}	0.270	83.7
	36.565	0.485	8.46×10^{-3}	0.221	15.4
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	69.793	0.273	4.76×10^{-3}	0.121	23.6
	Average				37.4 nm

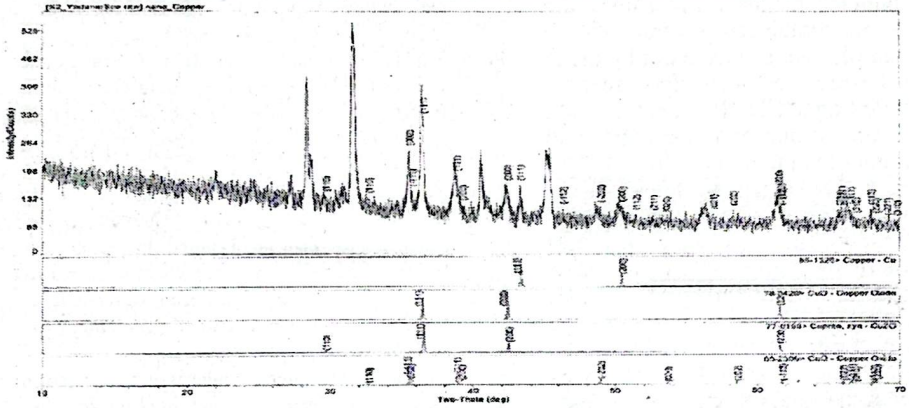

Figure.7.XRD Results of Average Crystallite Size of copper oxide Nanoparticles by using *Carica papaya* (Thinbaw) Leaves Extract

Table 5 XRD Results of Average Crystallite Size of copper oxide Nanoparticles by using *Carica papaya* (Thinbaw) Leaves Extract

Sample name	2 θ value	FWHM radians	β (radian)	d-spacing (nm)	Crystalline size (nm)
Carica papaya (Thinbaw)	29.668	0.287	2.14×10^{-3}	0.271	26.8
	32.655	0.290	5.06×10^{-3}	0.247	26.1
	35.508	0.215	3.75×10^{-3}	0.227	34.7
	36.521	0.305	5.32×10^{-3}	0.221	24.7
	38.833	0.528	9.21×10^{-3}	0.208	14.2
	39.247	0.289	5.04×10^{-3}	0.210	25.6
	42.371	0.418	7.29×10^{-3}	0.192	17.7
	43.298	0.189	3.29×10^{-3}	0.188	38.9
	46.294	0.330	5.76×10^{-3}	0.176	22.1
	61.509	0.382	6.66×10^{-3}	0.136	17.8
	Average				24.1

According to XRD results, the crystallite size of copper oxide nanoparticles was found within the range of 15.4- 83.7 nm prepared by using *Azadirachta indica* (Tamar)leaves extract. Interplanar spacing between copper oxide nanoparticles was found within the range of 0.859-0.968 nm. The crystallite size of copper oxide nanoparticles was found within the range of 14.2-38.9 nm prepared by using *Carica papaya* (Thinbaw) leaves extract. Interplanar spacing between silver nanoparticles was found within the range of 0.821-0.967 nm.

Scherer's Equation for Estimation of Crystalline Size

$$D = \frac{K\lambda}{\beta \cos\theta} \text{ A}^\circ, \quad d = \frac{K\lambda}{2\sin\theta}$$

Where, D =the average crystalline size in A°

K = the shape factor

λ = the wavelength of X-ray (1.5406 A°)

β =full-width at half maximum (FWHM) of different peaks (radian)

θ = the Bragg angle

d = interplanar spacing

Conclusion

In this investigation, the phytochemical compounds present in two different leaves were examined by phytochemical tests which informs the presence of alkaloid, flavonoid, phenolic, polyphenol, steroid, saponin, glycoside, reducing sugar and tannin respectively. Therefore, these samples contain valuable phytochemical constituents for human's health. Furthermore, the determinations of elemental composition of two different leaves samples were carried out by using EDXRF (Energy Dispersive X-ray Fluorescence) spectroscopy. According to EDXRF spectroscopy, it was found that the amounts of potassium and magnesium are higher than that of others.

Copper oxide nanoparticles were synthesized by using copper nitrate solution and alcohol leaves extract of *Carica papaya* and *Azadirachta indica* as reducing agent. The resulting samples were characterized by FT-IR spectrum of these ethanol leaves extract showed the characteristic absorption peak of copper oxide nanoparticles was observed at 500-825 cm^{-1} and some other related functional groups.

The crystallite size of nanoparticles were determined by using x-ray diffractometer. According to XRD, the average crystallite size of CuO nanoparticle from the leaves extract of *Carica Papaya* and *Azadirachta indica* were found 37.4 and 24.9 nm respectively. XRD results thus confirm the formation of CuO nanoparticles of diameter less than 100 nm. According to XRD peak search report of CuONPs, the crystallite size of the CuO NPs was found to be 24.1 nm (*Carica papaya*) and 37.4 nm (*Azadirachta indic).*

Acknowledgements

The author would like to thank the Myanmar International Science and Culture Conference (MISCC 2019) for allowing to present this paper and Professor and Head Dr Yi Yi Myint, Department of Chemistry, University of Mandalay and Professor and Head Dr Myat Myat Mon, Department of Engineering Chemistry, Mandalay Technological University for their strong encouragements.

References

Ansilin S, Kavya Nair.J, Aswathy C., Roma V., Peter J., (2016), " Green Synthesis and Characterization of Copper Oxide Nanoparticles using *Azodirachta Indca* (Neem) Leaf Aqueous Extract". *Journal of Nanosicence and Technology*, Vol.2 (5), P.221-223.

Dinker Pawar, Shahista Shaikh, Deshmukh Shulakasana, Rohinikanawade, (2015). "Green Synthesis of Copper Nanoparticles Using Gloriosa Superbal Leaf Extract" *B.G.P Sahyadri Junior college, Department of Biology, Sangamner*-422605.

Harborne J, Phytochemical methods , London: Chapman & Hall, (1973) pp. 49-88.

Harvey, A.L.(2008) *Natural products in drug discovery.* Drug Discov. Vol. 13, pp.894–901.

Khan. M., Kuniyil. M., Adil. SE., Al-Warthan. A, Alkhathlan. HZ, Tremel. W, Tahir. MN, Siddiqui. MR,(2014), "Biogenic synthesis of palladium nanoparticles using Pulicaria glutinosa extract and their catalytic activity towards the Suzuki coupling reaction". *International Journal of Nanomedicine* Dalton Trans., Vol.43, P. 9026–9031.

Mohanpuria, P., Rana, N.K., and Yadav, S.K., (2007) "Biosynthesis of Nanoparticle: Technological concept and Future Application". *Journal of Nanoparticle Research*, vol.7, pp .9275- 9280.

Padil, V.V.T. and Cernik, M. (2013) "Green Synthesis of Copper Oxide Nanoparticles using Gum Karaya as a Biotemplate and their Antibacterial Application". *International Journal of Nanomedicine*, vol. 8, pp. 889-898.

Punnoose, A., Dodge, K., Rasmussen, J.W., Chess, J., Wingett, D., and Anders, C.,(2014) "Cytotoxicity of ZnO Nanoparticles can be Tailored by Modifying their Surface Structure": A green chemistry approach for safer nanomaterials. *American Chemical Society Sustainable Chemistry and Engineering* , Vol. 2, pp.1666–1673.

Siavash, Iravani 2011, "Green Synthesis of metal nanoparticles using plants", *The royal society of chemistry*, Vol.13, P. 2638-2650.

Ved Prakash, R K Diwan and U K Niyogi (2015), "Characterization of synthesized copper oxide nanopowders and their use in nanofluids for enhancement of thermal conductivity", *Indian Journal of Pure and Applied Physics*, Vol. 53, pp. 753-75

Vollmer. C, Redel. E, Abu-Shandi. K, Thomann. R, Manyar. H, Hardacre. C, Janiak. C, (2010) "Microwave Irradiation for the Facile Synthesis of Transition-Metal Nanoparticles (NPs) in Ionic Liquids (ILs) from Metal–Carbonyl Precursors and Ru-, Rh-, and Ir-NP/IL Dispersions as Biphasic Liquid–Liquid Hydrogenation Nanocatalysts for Cyclohexene", *Journal of Applied Electrochemistry*, Vol. 16, P. 3849–3858.

Green Synthesis of Copper Nanoparticles using Leaf of Selected Myanmar Traditional Medicinal Plant

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Abstract

In this research work, the leaf of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale) was collected from Mahaaungmyay Township, Mandalay region. Preliminary detection of phytochemical compounds present in this leaf was investigated. The elemental compositions of the leaf was also determined by using EDXRF (Energy Dispersive X-ray fluorescence) Spectroscopy. Copper/Copper Oxide nanoparticles were synthesized by using green synthesis method from an aqueous leaves extract. The crystalline size of synthesized copper /copper oxide nanoparticles were characterized by X-ray diffraction (XRD) method and by using Fourier Transform Infrared (FT-IR)Spectrometer. Antimicrobial activities of synthesized copper/copper oxide nanoparticles were examined by agar well diffusion method. The synthesis of nanoparticles using the plant extract is an enormous things method for cost and reduce the environmental pollution.

Keywords : Phytochemical, nanoparticles, aqueous solution, antimicrobial, X-Ray diffraction

Introduction

Nanoparticles can be synthesized by several methods, including physical, chemical and biological synthesis. Various plants were used for the synthesis of nanoparticles using green synthesis method. Nanoparticles were synthesized from all the parts of the plant separately like seed, stem, flower, leaf and skin of the fruits. Many researchers used green synthesis methods for different metal nanoparticles due to their growing need of eco-friendly properties (Christopher L, Kitchens, Douglas E, *et al.*, 2010).

Green synthesis method was found to be the best method when compared to the other method such as chemical reduction, photochemical reduction, electrochemical reduction, heat evaporation etc., (Geoprincy G, Vidhyasrr BN, *et al.*, 2013). In this method, the plant extract has been used as capping and reducing agent for the synthesis of nanoparticles due to their reducing properties present in the leaf extract (Umesh B. Jagtap, *et al.*, 2013). The nanoparticles synthesized from plant extract werefound to be covered by the medicinal properties of plant extract which could be used in drug, targeted drug delivery and cosmetic applications (Mallikarjunaa K, Narasimhab G, *et al.*, 2011).

The limitations of physical synthesis includes, high cost, energy consumption and time etc. Sol-gel method, wet chemical techniques are widely used chemical synthesis methods. Problems associated with chemical synthesis are the eco-toxicity, health hazard to humans and animals (Syed N, *et al.*, 2012).

Green synthesis is based on redox reaction, in which metal ions are reduced to stable nanoparticle by the components of an organism or its extract. Green synthesis method have several advantages over other methods namely cost effectiveness, simplicity, use of less temperature, the usage of less toxic materials, moreover it is compatible for medical and food applications (Chandrakant K, Tagad, *et al.*, 2013).

Copper is most widely used material in the world due to their electrical, optical, catalytic, biomedical and antifungal/antibacterial applications among various metal particles such as gold, silver, iron, palladium, zinc and quantum dots (Konghu Tian, Cailin Liu, *et al.*, 2012). It can give more yields and reaction rate in mild reaction conditions when compared to other traditional catalysts (Nguyen Thi Phuong Phong, *et al.*, 2011).

Copper nanoparticles act as antimicrobial agent in various fields. The copper is highly toxic to microorganism such as bacteria (*E-coli*, *Staphylococcus Aureus*, *Pseudomonas aeruginosa*). It is consider to be safe for human beings such as food package application and in water treatment applications (Kalimuthu K, Babu RS, *et al.*, 2008).

In this present research, *Sphagneticola calcendulacea* (L.) Rruski leaf extract has been used. This plant extract was used as reducing agent for the synthesis of copper nanoparticle. The current investigation focused on the aqueous leaf extract to synthesize copper nanoparticle and to be used as an antimicrobial agent.

Botanical name- *Sphagneticola calcendulacea* (L.)
Family name-Asteraceae
Genus -Sphagneticola
Common name -Nay-kyar-gale
Parts used -Leaves

Figure (1) The Leaves of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale)

Materials and Methods

Sample Collection and Preparation

The medicinal plant *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar gale) leaf was selected for synthesis of copper nanoparticles. The leaf sample was washed with water to removal impurities from surface and cut into small pieces and dried at room temperature.

Preliminary Phytochemical Test for Leaves Samples

The preliminary phytochemical tests were carried out to detect the presence or absence of organic constituents in the *Sphagneticola calcendulacea* (L.)Pruski (Nay- kyar –gale). (Harborne J, 1973)

Determination of Elemental Analysis

The elemental analysis of leaf of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale) was determined by using EDXRF (Energy Dispersive X-ray Fluorescence) Spectroscopy.

Preparation of Plant Leaves Extract

The leaf of the sample was washed with distilled water and cut into small pieces were dried at room temperature. The dry pieces samples were grounded into fine powder with the aid of hand grinder. 10g of powdered sample were boiled with 200 ml of deionized water for 30 minutes. It was cooled and filtrated. The filtrated extracts used for further studies. (Saranyaadevi K, *et al.*, 2014)

Synthesis of Copper/Copper Oxide Nanoparticle using 50 mM of $Cu(NO_3)_2$ Solution and *Sphagneticola calcendulacea* (L.) Leave Extract (1:4)

In this research, water extract of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar -gale) leaf was used for the synthesis of copper/ copper oxide nanoparticles.

About 0.93786 g of $Cu(NO_3)_2$ was weighted accurately and dissolved in 100 mL of distilled water and stored. The 20 mL of this solution were mixed with 80 mL of plant leaf extract. This mixture was stirred on a magnetic stirrer with 700 rpm. This reaction mixture was maintained at 80° C for reaction times 5 hrs for the reduction of copper ions. The mixture was centrifuged with 3000 rpm for 30 mins. The precipitates of copper nanoparticles were obtained. The resultant copper nanoparticle were washed with distilled water .And then dried in pertidish. (Lee, *et al.*,2011 and Yallapa, *et al.*, 2013)

Synthesis of Copper/Copper Oxide Nanoparticle using 30 mM of $Cu(NO_3)_2$ Solution and *Sphagneticola calcendulacea* (L.) Pruski Leaf Extract (1:4)

About 0.564 g of $Cu(NO_3)_2$ was weighted accurately and dissolved in 100 mL of distilled water and stored. The 20 mL of this solution were mixed with 80ml of plant leaf extract. This mixture was stirred on a magnetic stirrer with 700 rpm. This reaction mixture was maintained at 80° C for reaction times 5 hrs for the reduction of copper ions. The mixture was centrifuged with 3000 rpm for 30 mins. The precipitates of copper nanoparticles were obtained. The resultant copper nanoparticle were washed with distilled water .And then dried in pertidish.

Synthesis of Copper/Copper Oxide Nanoparticle using 10 mM of $Cu(NO_3)_2$ Solution and *Sphagneticola calcendulacea* (L.) Pruski Leaf Extract (1:4)

About 0.188 g of $Cu(NO_3)_2$ was weighted accurately and dissolved in 100 mL of distilled water and stored. The 20ml of this solution were mixed with 80ml of plant leaf extract. This mixture was stirred on a magnetic stirrer with 700 rpm. This reaction mixture was maintained at 80° C for reaction times 5 hrs for the reduction of copper ions. The mixture was centrifuged with 3000 rpm for 30 mins. The precipitates of copper nanoparticles were obtained. The resultant copper nanoparticle were washed with distilled water .And then dried in petridish.

Characterization of Copper/Copper Oxide Nanoparticles

The size of nano crystallites were determined by X-ray diffraction (XRD) at University Research Centre, Yangon. Estimation of crystallites size was carried out by using Debye-Scherrer's equation (Debye, *et al.*, 2010).

Functional Group Determination of Copper/ Copper Oxide Nanoparticles

Functional groups contained in synthesized copper/copper oxide nanoparticles were measured by FT-IR spectroscopic method.

Determination of Antimicrobial Activities of Synthesized Copper/Copper Oxide Nanoparticles using Leaves Extract of *Sphagneticola calcendulacea* (L.) Pruski

For the measurement of antimicrobial activities, the synthesized Copper/Copper Oxide Nanoparticles from the leaf extract was determined by Agar well diffusion method. Seven types of microorganisms were used to screen the antimicrobial activities.

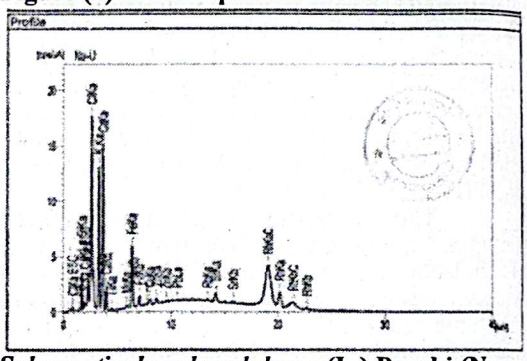
Results and Discussion

Determination of Phytochemical Test for Leaves of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale)

Sphagneticola calcendulacea (L.) Pruski (Nay-kyar-gale) leaf was tested by phytochemical screening. According to phytochemical result, alkaloid, glycoside, phenolic, reducing sugar, tannin, saponin, polyphenol, flavonoid, steroid, were present in *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale) leaf.

Determination of Elemental Content of Leaves of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale)

Figure (2) EDXRF pattern of Leaves of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale)



The results of elemental content for leaf of Nay-kyar-gale was determined by using EDXRF method. This result was shown in Table (1).

Table (1) The Result of Elemental Content for Leaves of *Sphagneticola calcendulacea* (L.)

No	Element	Symbol	Relative Abundance %
			Sample I
1	Chlorine	Cl	1.734
2	Silicon	Si	1.581
3	Potassium	K	1.109
4	Calcium	Ca	0.837
5	Sulfur	S	0.314
6	Manganese	Mn	0.004
7	Iron	Fe	0.053
8	Barium	Ba	-
9	Titanium	Ti	0.006
10	Copper	Cu	0.003
11	Zinc	Zn	0.002
12	Strontium	Sr	
13	Aluminum	Al	0.274
14	Phosphorous	P	0.112

Pruski (Nay-kyar-gale)

According to this table, the higher amount of chlorine and silicon were found in leaves of Nay-kyar-gale. Moreover, potassium, calcium, sulfur, aluminum, iron, phosphorous, titanium, manganese, copper, zinc were also found .

The Result of the Amount of Copper/Copper Oxide Nanoparticles from Leaves Extract of (Nay-kyar-gale) at Different Concentration

Table (2) The Result of the Amount of Copper/Copper Oxide Nanoparticles from Leaves Extract of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale)

Sample (10)g	Concentration Of $Cu(NO_3)_2$mM	Yield	
		g	%
Nay-kyar-gale	50	0.52	5.2
	30	0.34	3.4
	10	0.19	1.9

According to this table (2) copper/copper oxide nanoparticle were determined by using different concentrations intervals. It was found that, the amount of copper/copper oxide nanoparticles depend on the contact concentration. When concentration increase, the amount of copper/copper oxide nanoparticle also increase.

XRD Analysis of Copper/Copper Oxide Nanoparticles by using *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale) Leaf Extract

The crystalline size and interplanar spacing of copper/copper oxide nanoparticles were determined by XRD analysis.

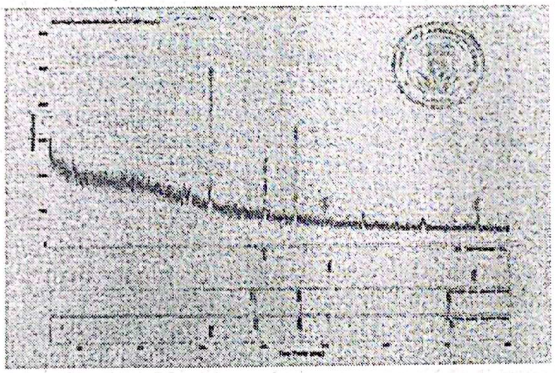


Figure (3) XRD Spectrum of Copper/ Copper Oxide Nanoparticles using 50mM of $Cu(NO_3)_2$ Solution and Leaves Extract of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale)

Table (3) XRD Results of Crystallite Size of Copper/Copper Oxide Nanoparticles by using 50 mM of $Cu(NO_3)_2$ Solution and Leaves Extract of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale)

Sample	Bragg angle 2θ	Miller Indices (hkl)	FWHM	β(radius)	d-spacing (nm)	Crystalline Size (D) nm
Nay-kyar-gale	36.658	110	0.147	2.5×10^{-3}	2.4509	54.0
	42.387	200	0.329	5.7×10^{-3}	2.1307	24.1
	43.335	111	0.224	4.2×10^{-3}	2.0862	32.5
	50.659	200	0.105	1.8×10^{-3}	1.8005	75.6
	62.337	220	0.266	4.6×10^{-3}	1.4883	29.8
Average						43.2

According to XRD results, the crystallite size of copper/copper oxide nanoparticles using leaves extract of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale) were found to be 43.2nm.

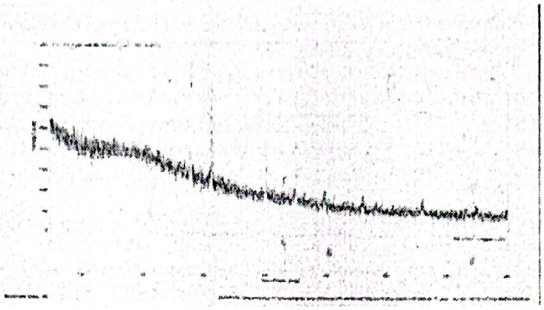


Figure (4) XRD Spectrum of Copper/ Copper Oxide Nanoparticles using 30mM of $Cu(NO_3)_2$ Solution and Leaves Extract of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale)

Table (4) XRD Results of Crystallite Size of Copper/Copper Oxide Nanoparticles by using 30 mM of $Cu(NO_3)_2$ Solution and Leaves Extract of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale)

Bragg's angle 2θ	Miller Indices (hkl)	FWHM	β radius	d-spacing (nm)	Crystallite size (D)nm
43.078	111	0.371	6.4×10^{-3}	0.20981	21.4

According to XRD results, the crystallite size of copper/copper oxide nanoparticles using leaves extract of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale) were found to be 21.4nm.

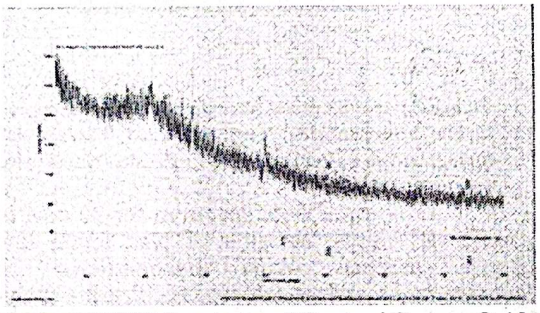


Figure (5) XRD Spectrum of Copper/ Copper Oxide Nanoparticles using 10mM of $Cu(NO_3)_2$ Solution and Leaves Extract of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale)

Table (5) XRD Results of Crystallite Size of Copper/Copper Oxide Nanoparticles by Using 10 mM of $Cu(NO_3)_2$ Solution and Leaf Extract of *Sphagneticola calcendulacea* (L.)Pruski (Nay-kyar-gale)

Bragg's angle 2θ	Miller Indices (hkl)	FWHM	β radius	d-spacing (nm)	Crystallite size (D)nm
50.498	200	0.153	2.6×10^{-3}	0.18051	52
73.862	220	0.179	3.1×10^{-3}	0.12820	44.6
Average					48.3

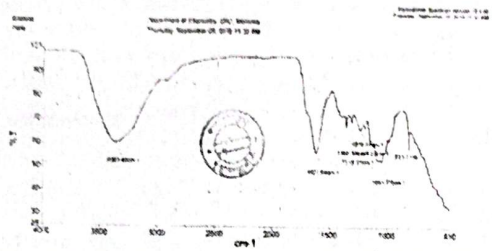
According to XRD results, the crystallite size of copper/copper oxide nanoparticles using leaves extract of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale) were found to be 48.3 nm.

FT-IR Assignments of Copper /Copper Oxide Nanoparticles

To identify the functional groups, the FT-IR spectrums were measured at Department of Chemistry,

Monywa University. The infrared spectrum (Figure 6) informs at 400-865cm^{-1}, which can be assigned to the vibration of copper oxide nanoparticle (Pricilla J, *et al.*, 2017).

FT-IR Analysis of Copper /Copper Oxide Nanoparticles from *calcendulacea* (L.)Pruski (Nay-kyar-gale) Leaves Extract



Figure(6) FT-IR Spectrum of Copper /Copper Oxide Nanoparticles from the *Sphagneticola calcendulacea* (L.)Pruski (Nay-kyar-gale) Leaves Extract

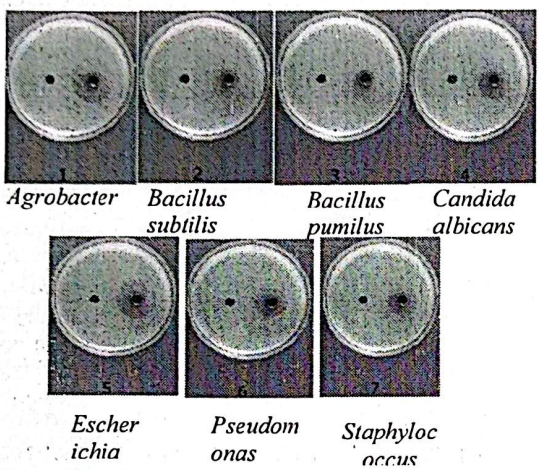


Figure (7) Antimicrobial Activities of Copper/Copper Oxide Nanoparticles from the Leaf Extract of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale)

From the FT-IR spectrum for 30 mM of copper (II) nitrate solution, it was found that copper /copper oxide nanoparticles of *Sphagneticola calcendulacea* (L.)Pruski (Nay-kyar-gale) leaves extract contain O-H or N-H stretching vibration of hydroxyl or amine group, C = C stretching vibration of alkene group, O-H in plane bending vibration of alcohol group, Cu-O stretching vibration.

Table (6) FT-IR Assignment of Copper /Copper Oxide Nanoparticles from the *Sphagneticola calcendulacea* (L.)Pruski (Nay-kyar-gale) Leaf Extract

No.	Wave number (cm^{-1})	Assignment of functional group
1	3363.40	O-H or N-H stretching vibration of hydroxyl or amine group
2	1621.54	C = C stretching vibration of alkene group
3	1362.38	O-H in plane bending vibration of alcohol group
4	1318.31,1194.23,1051.77	C-O stretching vibration ether group
5	865.1	Cu-O stretching vibration

Determination of Antimicrobial Activities of Copper/Copper Oxide Nanoparticles by using Leaf Extract of (Nay-kyar-gale)

The study of antimicrobial activities of copper/copper oxide nanoparticles from the leaf extract of *Sphagneticola calcendulacea* (L.) Pruski (Nay-kyar-gale) was performed by agar-well diffusion method on seven microorganisms.

Table (7) Antimicrobial Activities of Copper/Copper Oxide Nanoparticles from the Leaf Extract of *Sphagneticola calcendulacea* (L.)Pruski (Nay-kyar-gale)

Sample	Solvent	Inhibition zone(mm)						
		(1)	(2)	(3)	(4)	(5)	(6)	(7)
CuNPs (Nay-kyar-gale)	Distilled water	25.07 +++	10.02 +	10.19 +	20.13 +++	22.30 +++	19.51 +++	26.2 +++

Agar –well-8mm
8mm ~ 12mm(+)
13mm ~ 17mm(++)
18mm above(+++)

Organisms
(1) *Agrobacterium tumefaciens*
(2) *Bacillus subtilis*
(3) *Bacillus pumilus*
(4) *Candida albicans*
(5) *Escherichia coli*
(6) *Pseudomonas fluorescens*
(7) *Staphylococcus aureus*

From antimicrobial activity of synthesized copper/copper oxide nanoparticle from the leaf extract of *Sphagneticola calcendulacea* (L.)Pruski (Nay-kyar-gale) can inhibit seven types of microorganisms. It shows the highest activity on *Agrobacterium tumefaciens, Candida albicans, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus* than *Bacillus subtilis* and *Bacillus pumilus* bacteria.

Conclusion

In this research work, Copper/Copper oxide nanoparticles were synthesized by using Copper (II) nitrate solution and aqueous leaf extract of *Sphagneticola calendulacea* (L.) Pruski (Nay-kyar-gale) as reducing agent. The different concentrations (50 mM,30 mM, 10 mM) of Copper (II) nitrate solution were determined in this investigation.

Firstly, phytochemical screening of aqueous leaves extract were analysed. According to the preliminary phytochemical screening results, Nay-kyar -gale leaves contained alkaloids, glycosides, phenolic compounds, reducing sugar, tannins, saponins, polyphenol, flavonoids and steroids respectively.From the result of elemental composition, chlorine (Cl), silicon(Si) and potassium (K) were found to be higher than the other elements in Nay-kyar-gale leaf.

The yield % of the synthesized copper/copper oxide nanoparticle from the leaves extract of Nay-kyar-gale by using 50 mM, 30 mM, 10 mM of $Cu(NO_3)_2$ solution were founded to be 5.2%,3.4% and 1.9% respectively.

From XRD result , the average crystalline size of synthesized copper/copper oxide nanoparticles were found to be 43.2nm, 21.4 nm and 48.3 nm for different concentration of 50 mM, 30 mM, 10 mM $Cu(NO_3)_2$ solution using Nay-kyar-gale leaf extract. The synthesized particles were observed within the nano size range. The peak in XRD patterns, using leaves extract of Nay-kyar-gale contain metallic copper peak and other impurity peaks were detected. XRD analysis confirms the possibility of secondary materials that bind the copper nanoparticles in clusters.

In FT-IR spectroscopy, the absorption peaks of the copper/copper oxide nanoparticles using Nay-kyar-gale leaves extract consist of hydroxyl or amine group at 3163.40 cm^{-1}, C=C stretching vibration of alkene group at 1621.54 cm^{-1}, O-H in plane bending vibration of alcohol group at 1362.38 cm^{-1} and ether group at 1318.31cm^{-1},1194.23cm^{-1}and 1051.77 cm^{-1}. The

characteristic absorption peak of copper oxide nanoparticles was observed at 865.1 cm^{-1} .

The antimicrobial activities of the copper/copper oxide nanoparticles from the leaf extract of Nay-kyar-gale were examined by agar well diffusion method. It was found that the leaf extract of copper/ copper oxide nanoparticles can inhibit seven types of microorganisms. It shows the highest activity on *Agrobacterium tumefaciens, Candida albicans, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus* than *Bacillus subtilis* and *Bacillus pumilus* bacteria.Copper nanoparticles can inhibit the seven types of microorganisms. The biosynthesized nanoparticles have more effective antimicrobial activity to the pathogens.

The synthesis of copper nanoparticles using the plant extracts is an enormous method for cost and to reduce the environmental pollution and have effective antibacterial activity.

References

Chandrakant K, Tagad, Sreekantha Reddy Dugasanic, Rohini Aiyer, Sungha Parke, Atul Kulkarni, Sushma Sabharwal. (2013)."Green synthesis of silver nanoparticles and their application for the development of optical fiber based hydrogen peroxide sensor". *Sensors and Actuators* B; 183: 144– 149.

Christopher L, Kitchens, Douglas E, Hirt, Scott M, Husson, Alexey A, Vertegel. (2010)," Synthesis, Stabilization, and Characterization of Metal Nanoparticles",The Graduate School of Clemson University.

Dubye, S.P., Lahtinen, M., & Sillanpaa, M.(2010)." Tansy fruit mediated greener synthesis of silver and gold nanoparticles." *Process biochemistry*,45,1065-1071.

Geoprincy G, Vidhyasrr BN, Poonguzhali U, Nagendra Gandhi N, Renganathan S. (2013). "A review on green synthesis of silver nano particles." *Asian.J.Pharma.Clini.res.* 6(1): 8 –12.

Harbone J,Phytochemical methods (1973),London:Chapman & Hal,(PP.49-88)

Irshad N, Avijit M, and Chakraborthy GS (2013), *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, P-353

Kalimuthu K, Babu RS, Venkataraman D, Bilal M, Gurunathan S. (2008),"Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. "Colloids Surf B. 65(1): 150-153.

Konghu Tian, Cailin Liu, Haijun Yang, Xianyan Ren. (2012)."In situ synthesis of copper nanoparticles/polystyrene composite" Colloids and Surfaces A: *Physicochem. Eng. Aspects* 397 2012; 12– 15.

Mallikarjunaa K, Narasimhab G, Dillipa GR, Praveenb B, Shreedharc B, Sree Lakshmic C (2011),"Green Synthesis of Silver Nanoparticles Using *Ocimum*Leaf Extract and Their Characterization. Digest.J.Nanomat. "Biostruct. ,6(1): 181–186.

Nguyen Thi Phuong Phong, Vo Quoc Khuong, Tran Duc Tho, Cao Van Du, Ngo Hoang Minh.(2011),"Green synthesis of copper nanoparticles colloidal solutions and used as pink disease treatment trug for rubber tree", Proceedings of IWNA, November 10-12.

Pricilla J, Ramakrishnan A, Nithyanandhi Y, Renuka S (2017), "Structural and Antibacterial Activity of Copper Oxide Nanoparticles" *Journal of Applied Physics* PP 57-63

Saranyaadevik., Subha V, Ernest Ravindran R.S, Renganathan S (2014)"Synthesis and Characterization of Copper Nanoparticle using *Copparis Zeylanica* leaf Extract", *International Jouranl of Chem Tech Research*, 1 (10), P.4533-4541

Syed, N and Yah , L X (2012) Impact of High Performance Human Resource management practies on Employee Job Satisfaction Empirical Analysis Interdisciplinary.Journal of contemporary Research in Business 17(1),86-105.

Umesh B. Jagtap, Vishwas A. Bapat.(2013)." Green synthesis of silver nanoparticles using Artocarpus heterophyllus Lam. Seed extract and its antibacterial activity.", Industrial Crops and Products.; 46: 132– 137.

Determination of Some Physicochemical Properties of Chewing Gum

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Abstract

In this research work, a survey of chewing gum was carried out to choose research sample in Mandalay. During the survey, brands and price were recorded. The moisture content of the sample was investigated byAOAC method. The ash and the ash insoluble in dilute hydrochloric acid of the sample were investigated by recommended methods. pH of the chewing gum sample was measured by using pH meter. Elemental analysis of chewing gum sample was performed by EDXRF (Energy Dispersive X-ray Fluorescence) spectrophotometer. The fat was extracted from sample with petroleum ether by using Soxhlet extraction method. The nitrogen and protein contents were estimated by micro-Kjeldhal method. Reducing sugar content was determined by Iodometric method.

Keywords: chewing gum, EDXRF, micro-Kjeldhal, pH, Soxhlet extraction

Introduction

Chewing gum is a soft, cohesive substance designed to be chewed without being swallowed. Modern chewing gum is composed of gum base, sweeteners, softeners, plasticizers, flavors, colors, and, typically, a hard or powdered polyol coating. Its texture is reminiscent of rubber because of the physical-chemical properties of its polymer, plasticizer, and resin components, which contribute to its elastic-plastic, sticky, chewy characteristics. *(Estruch, 2008)*

Gum base is made of polymers, plasticizers, and resins. Polymers, including elastomers, are responsible for the stretchy and sticky nature of chewing gum. Plasticizers improve flexibility and reduce brittleness, contributing to the plastic and elastic nature of gum. The interactions of plasticizers within gum base are governed by solubility parameters, molecular weight, and chemical structure. Resins compose the hydrophobic portion of the gum base, responsible for its chewiness. Although the exact ingredients and proportions used in each brand's gum base are trade secrets within the gum industry. (Wikipedia)

Many of the studies investigating the effects of chewing gum on attention have reported positive results for attention, but are inconsistent as to which attributes of attention are affected. Studies have assessed different attributes of attention including vigilance, sustained attention, selective attention, and mood including alertness. Studies have also assessed the effects of chewing gum on mood, assuming that positive mood, including alertness, enhances attention which facilitates learning (Hirano & Onozuka, 2015).

The physical and chemical properties of chewing gum impact all aspects of this product, from manufacturing to sensory perception during mastication. Chewing gum, a kind of sham feeding alternative to early feeding, which is expected to stimulate the cephalic-vagal reflex to increase hormone secretion and then enhance intestinal motility, may produce a positive effect on postoperative ileus by reducing postoperative inflammation. (Su, B.U., 2015)

Previous meta-analyses have suggested that patients who had gum chewing after surgery might recover intestinal function earlier; however, the sample sizes of the randomized controlled trials (RCTs) included in these analyses were small and an increasing body of relevant research recently have been reported, making it worth reconsidering the evidence on this issue.(Ho,Y.M) In the present study, chewing gum sample was selected for determination of physicochemical properties.

Aim and Objectives

Aim

Aim of this research work is to determine the physicochemical properties of chewing gum sample.

Objectives

- To survey the chewing gum samples
- To determine the moisture and pH contents of the chewing gum sample
- To determine the ash insoluble in dil HCl and moisture contents of the chewing gum sample
- To investigate the elemental contents in the chewing gum sample by EDXRF method
- To extract the fat from sample with petroleum ether by using Soxhlet extraction method.
- To estimate the nitrogen and protein content by micro-Kjeldhal method
- To study the reducing sugar content in the selected sample by Iodometric method

Experimental

Product Survey on the Stores

A survey of chewing gum sample was carried out to choose research samples. During the survey, brands, amount, price and amount of chewing gum were recorded and these results were shown in Table (1).

Sample Collection

Based on the survey, chewing gum samples were selected for analysis. One brand of chewing gum sample was purchased from Yadanabon Market, Mandalay. Chewing gum samples were cut into pieces and used for analysis throughout the experiment.

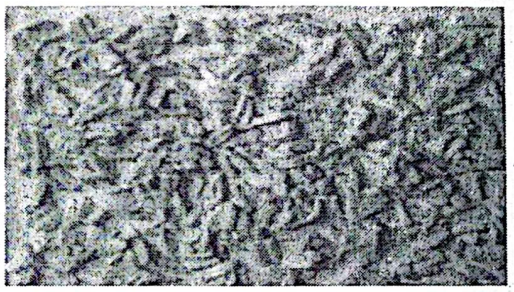

Figure 1 Chewing gum sample

Determination of Moisture Content (AOAC,1990)

Moisture Content of sample was determined by the oven method at 100±5 °C.

$$\text{Acid insoluble ash, percent by mass} = \frac{W_1 \times 100}{W_2}$$

Where, W_1 = weight in gram of the acid insoluble ash

W_2 = weight in gram of the prepared ash

$$\text{Moisture (\%)} = \frac{\text{loss in weight (g)}}{\text{weight of sample (g)}} \times 100$$

Accurately weighed sample (1 g) was added to 10 mL crucible. It was then heated for 1hr in drying oven at 100±5 °C. After heating, the crucible was removed from the oven and placed in a dessicator for cooling and weighing was repeated until a constant weight was obtained. The moisture percent was represented by the loss in weight. The moisture content can be calculated by the following formula.

Determination of pH of Chewing Gum (AOAC, 2000)

The basic principle of electrometric pH is determination of the activity of the hydrogen ions by potentiometric measurement using a glass electrode and reference electrode. The various pH indicators exhibit a specific solution can be measured by a pH meter or by pH indicators. pH meter is an apparatus with electrodes sensitive to hydrogen (hydronium ions). This instrument measures the small voltage produced by the presence of hydrogen ions and reads out the pH.

Sample (1 g) was put into the beaker. 100 mL of distilled water was added into the beaker and stirred with magnetic stirrer for 30 min at room temperature at a rate of 1600 rpm. It was allowed to stand for 30 min .Then, pH was determined with a pH meter.

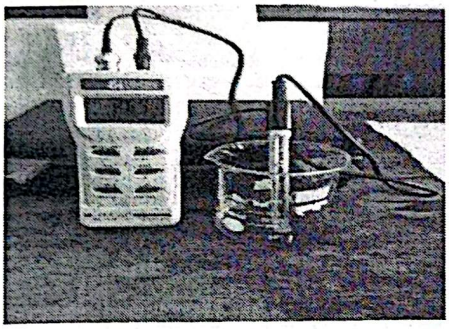

Figure 2 Determination of pH

Determination of Ash Insoluble in Dilute HCl (Woodman, A.G., 1941)

The sample (10 g) were burnt about 10 hour in fire stove at 800-1000 °C until to obtain white-gray colour ash. The resulting ash was sieved by using the 150 mesh size sieve and stored in plastic bottle for futher uses. The yield percentage (%) of chewing gum ash was calculated.

About 1g of ash was accurately weighed in a clean and dry platinum basin of 100mL capacity. Hydrochloric acid (25 mL) was added to the ash and covered with a watch glass and heated on a small flame of a burner to near boiling. It was allowed to cool and filtered through preweighed filter paper. The filter paper was washed with hot water until the washing was free from chlorides (To check this, few drops of 2 M Nitric acid 0.1 M Silver nitrate solution was added to the filtrated obtained. No precipitate or milky turbidity should occur in the solution, if it was chloride free.) The filter paper and the residue were kept in an air oven maintained at 105±2°C for about three hours and then weighed. The yield percent of acid insoluble ash was calculated by the following formula.

Calculation

$$\text{yield of ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of wheat straw}} \times 100$$

Determination of Elemental Composition of the Selected Sample

X-Ray fluorescence analysis using ED-XRF spectrometers is a commonly used technique for the identification and quantification of elements in a substance. SPECTRO's stationary ED-XRF spectrometers are based on the energy-dispersive-X-ray-fluorescence analysis method. The atoms in the sample material, which could be any solid, powder or liquid are excited by X-Rays emitted from an X-Ray tube or radioisotope. For increasing sensitivity, the primary excitation radiation can be polarized by using specific targets between the X-Ray tube and the sample (ED-P(polarization)-XRF). All elements specific X-Ray fluorescence signals emitted by the atoms after the photoelectric ionization are measured simultaneously in a fixed mounted semi-conductor detector or sealed gas-proportional counter. The radiation intensity of each element signal, which is proportional to the concentration of the element in the sample, is recalculated internally from a stored set of calibration curves and can be shown directly in concentration units.

Mineral contents of the chewing gum were measured at Department of Physics, University of Mandalay, by using EDXRF (Energy X-ray Fluorescence) Spectroscopy.

Determination of Fat Content of Chewing gum (Mishra, A., 2017)

About 50 g of sample accurately weighted was introduced into a thimble and a piece of cotton wool was placed in the open end of the thimble. The thimble containing sample was then placed in a Soxhlet apparatus. Then the apparatus was fixed with round-bottom flask (500 mL) containing petroleum ether (300 mL). The extraction flask was heated on the heating mantel for 6 hours at boiling point of petroleum ether.

After the extraction was completed, most of the ether extract was distilled off. The content in the flask were carefully transferred to a weighted specimen tube. The remaining ether in the specimen tube was vapourized about 100°C until constant weight was obtained.

$$\text{Fat (\%)} = \frac{\text{weight of fat (g)}}{\text{weight of sample(g)}} \times 100$$

Determination of Nitrogen and Protein Content by Using Kjeldahl's Method (Wybraniec, S., 2013)

Digestion

About 5 g of sample was weighed and placed in the Keldahl's digesting flask. About 5 g of annular sodium sulphate, 0.25g of anhydrous copper II sulphate and 12.5 mL of 98% sulphuric acid were added into it in such a way as to wash down any solid adhering to the neck. The flask was shaken until the contents were thoroughly mixed and it was heated till the mixture became colorless. The digestion was continued for half an hour to make sure that all nitrogen in the sample was converted to ammonium sulphate. It was allowed to cool and 5mL of distilled water was carefully added with frequent shaking.

Distillation

The Kjeldah's distillation apparatus was setup, taking care that the tip of the condenser extended below the surface of the standard sulphuric acid solution 50 mL in the receiver .The digested solution was poured into the flask

$$\text{Nitrogen \%} = \frac{(V_2 - V_1) \times N_A \times 0.01401 \times 100}{W}$$

together with 100mL of 40% sodium hydroxide to make mixture strongly alkaline .The evolved ammonia was distilled off .

Titration

The distillate was titrated with standard sodium hydroxide solution, using methyl orange as an indicator. A blank determination was carried out without sample using all the reagents as in the case of sample.

Calculation

The nitrogen content of sample can be calculated by using following formula.

Where,

V_2= the volume of acid used in the test (in milliliter)

V_1 = the volume of acid used in the blank (in milliliter)

N_A= the concentration of acid used (in normality)

W = the weight of sample (in gram)

Protein % = Nitrogen content ×6.25

Where, 6.25 = a factor of protein nitrogen conversion

Determination of Reducing Sugar Content by Iodometric Titration Method Using Sodium Hydroxide (Browe,C.A., 1912)

Sample (1 g) was dissolved in 100 mL distilled water in the volumetric flask. 1 mL of sample solution was placed in the flask. 2 mL of iodine solution and 4.5 mL of 0.1 mL sodium hydroxide solution were added into the flask and closed the flask and left the flask in the dark place for 15 minutes. 0.6 mL of 1M hydrochloric acid solution was added to mixture in the flask. The mixture solution was titrated 0.05 M sodium thiosulphate solution in the burette. When the liquid become straw color, 1mL of starch indicator was added. The solution became dark blue again and 0.05 M sodium thiosulphate solution was added until the colorless solution was reached and end point was obtained. From the experimental data, the reducing sugar content can be calculated.

Results and discussion

Result of Survey in the Yadanabon Market

A survey of chewing gum sample was carried out and recorded brands and price. The results are shown in Table (1).

Table 1 Result of survey in Yadanabon market

No.	Brand	Amount (g)	Price(Kyats)	Country
1.	SUGUS	300	2000	Thailand
2.	MENTOSE	300	2500	Vietnam
3.	BELO	150	950	Thailand
4.	KI KI	120	500	Myanmar
5.	FEIO	135	550	Myanmar

Moisture Content of Chewing Gum Sample

The moisture content of the sample was investigated by recommended method. The results are shown in Table (2)

Table 2 Results of moisture content for chewing gum sample

NO. of experiment	Moisture Content (%)
1.	1.26
2.	1.25
3.	1.26

The moisture content of chewing gum sample was found to be 1.25-1.26%.

pH Content of the Chewing Gum Sample

pH values of the chewing gum sample were determined and the results are described in Table (3).

Table 3 Results of pH for chewing gum sample

NO. of experiment	pH of Sample
1.	7.8
2.	7.9
3.	7.9

pH content of the chewing gum sample was found to be 7.8-7.9.

Ash Content and Acid Insoluble Ash Content of Chewing Gum Sample

The ash content and ash insoluble in dil HCl of the sample were investigated by recommended methods. The results are shown in Table (4) and (5).

Table 4 Results of ash content for chewing gum sample

No. of experiment	Weight of Sample (g)	Weight of ash (g)	Ash Content (%)
1.	10	0.151	1.51
2.	10	0.148	1.48
3.	10	0.153	1.53

Table 5 Results of acid insoluble ash content for chewing gum ash sample

No. of experiment	Weight of Ash (g)	Weight of acid insoluble ash (g)	Ash Content (%)
1.	1	0.0556	5.56
2.	1	0.0606	6.06
3.	1	0.0578	5.78

The ash content of chewing gum was found to be 1.48-1.53%. The ash insoluble in dilute HCl was found to be 5.56-6.06%.

Elements Present in Chewing Gum Ash by EDXRF Analysis

The elements that contain in chewing gum ash were analyzed by using EDXRF at Department of Physics, University of Mandalay. These results are shown in Table (6).

Table 6 The Results of element content in chewing gum ash

No	Symbol	Element	Content
1	Si	Silicon	6.8310%
2	Cl	Chlorine	4.4920%
3	Ca	Calcium	3.3660%
4	K	Potassium	2.2770%
5	Fe	Iron	1.1440%
6	S	Sulfur	0.6359%
7	Al	Aluminum	0.4300%
8	P	Phosphorus	0.2032%
9	Ti	Titanium	0.0411%
10	Ba	Barium	0.0241%
11	Sr	Strontium	0.0195%
12	Cu	Copper	0.0194%

Fat , Nitrogen and Protein Content of Chewing Gum Sample

The fat was extracted from sample with petroleum ether by using Soxhlet extraction method. The nitrogen and protein content were estimated by micro-Kjeldhal method. The results are shown in Table (7).

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Table 7 The results of fat, nitrogen and protein content in chewing gum

NO. of experiment	Fat Content(%)	Nitrogen Content(%)	Protein Content(%)
1.	0.30	0.039	0.24

Reducing Sugar Content of Chewing Gum Sample

The reducing sugar content was determined by Iodometric method and the results are shown in Table (8).

Table 8 The Results of reducing sugar content in chewing gum sample

No. of experiment	Reducing sugar Content($mmolmL^{-1}$)	Reducing sugar Content(g/g)
1.	0.0250	0.4500
2.	0.0253	0.4553
3.	0.0251	0.4518

Conclusion

In this research work, a survey of chewing gum was carried out to choose research sample. During the survey, brands and price were recorded. The moisture of the sample was investigated by recommended method. The moisture content of the sample was found to be 1.25-1.26%. So this food product is sufficiently dry and the packaging method of choice has a reasonable condition. pH value of the chewing gum sample was measured by using pH meter. The pH values of the sample were 7.8-7.9 and this indicated that the sample was slightly alkaline.

The ash content and the ash insoluble in dil HCl of the sample were investigated by recommended methods. The ash content was found to be 1.48-1.53% based on sample weight and the ash content insoluble in dil HCl was found to be 5.56-6.06 % based on ash weight. Acid insoluble ash is a part of total ash and the higher is the impurity, the higher is the acid insoluble ash.

Elemental analysis of Chewing gum sample was performed by EDXRF (Energy Dispersive X-ray Fluorescence) Spectrophotometer. Chewing gum sample contains the more amount of silicon (6.8310%), chlorine (4.4920%) and calcium (3.3660%) than other elements. Silicon is an important element for the synthesis of collagen which leads to improving skin strength and elasticity. Chlorine compound are widely used in the food industry to kill the bacteria and disinfect. A certain amount of calcium need to be consumed to build and maintain strong bones and healthy communication between the brain and other parts of the body.

The fat was extracted from sample with petroleum ether by using Soxhlet extraction method. The fat content was found to be 0.3 %.The nitrogen and protein content were estimated by micro-Kjeldhal method and the nitrogen content was 0.039% and protein percent was 0.24%. Reducing sugar content was determined by Iodometric method and reducing sugar content was found to be 0.0250-0.0255mmolmL^{-1}(0.4500-0.4553g/g). It was found that chewing gum contains small amount of fat and large amount of reducing sugar.

Acknowledgements

We would like to express our heartfelt gratitude to Rector, Dr Thida Win, University of Mandalay for encouragements on our research work. We deeply thank to Dr Yi Yi Myint, Head of Department of Chemistry, University of Mandalay for her guideline and encouragement on our research work.

References

AOAC. (1990). Official Methods of Analysis. 15th Edition. Association of Analytical Chemists, Washington, DC., USA, pp: 200-220

AOAC. (1990). Official Methods of Analysis. 15th Edition. Association of Analytical Chemists, Washington, DC., USA

Browe, C.A. (1912). " Handbook of Sugar analysis", John Wilcy and Sons, New York

Estruch, R.A. (2008). "Gum base, In Fitz, D. Formulation and Production of Chewing and Bubble Gum". 2nd edition. Essex: Kennedy's Publications Ltd. Pp: 93–118.

Hirano, Y., & Onozuka, M. (2015). "Chewing and attention: a positive effect on sustained

attention"., *BioMed Research International*, 1-6. DOI: 10.1155

Ho, Y. M., Smith, S. R., Pockney, P., Lim, P., and Attia, J. (2014). "A meta-analysis on the effect of sham feeding following colectomy: should gum chewing be included in enhanced recovery after surgery protocols?" Diseases of the Colon and Rectum, vol. 57, no. 1, pp: 115–126.

Mishra, A., (2017). "Soxhlet Extraction Method- Estimation of Fat in Food", Discover Food Tech.Com. Your Guide to Food Technology

Su, B. U. and Hill. A. G. (2015). "Perioperative use of chewing gum affects the inflammatory response and reduces postoperative ileus following major colorectal surgery," Evidence-Based Medicine, vol. 20, no. 5, pp: 185-186.

Wikipedia. (2016). "Chewing gum" - Wikipedia.html., For other uses, see Chewing gum (disambiguation)., "CFR - Code of Federal Regulations Title 21". *www.accessdata.fda.gov*. Retrieved pp:12-15.

Woodman. A.G., (1941). "Food Analnysis", Typical Methods and the Interpretation of Results, Mc Graw-Hill Book Co., Inc., New York

Wybraniec, S., Michalowski, T., and Asuero, A.G. (2013). "An Overview of the Kjeldahl Method of Nitrogen Determination. Part II. Sample Preparation, Working Scale, Instrumental Finish, and Quality Control", Critical Reviews in Analytical Chemistry · DOI: 10.1080/ 10408347.2012.751787

Purification of Natural Dyestuff Extracted From Mango Bark (*Mangifera Indica*) for the Application on Fabric Cotton

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Abstract

This paper concerns with developmental process for the purification of natural dyestuff extracted from an abundantly occurring plant namely *Mangifera indica* L. (Mango Tree), available almost everywhere in Myanmar. The aim of the study is to produce the purified natural dyestuff extracted from mango bark, to investigate colour of dyed fabric cotton using various dyeing methods, and to study the fastness properties of dyed sample materials. The compounds that the most important colouring ingredients present in mango bark were determined by phytochemical tests besides modern techniques: UV and FT IR. Optimization of dye purification condition was done by varying solvent types, time and temperature. These purified natural dye extracts were applied in fabric cotton sample by using mordants with various dyeing conditions: pre, simultaneous and post mordanting method. The colour fastness tests of dyed samples for light, washing fastness, and rubbing were carried out and these properties were standardized with Blue Wool Scale method and Grey Scale method respectively. In light fastness properties, dyed fabric cotton sample of purified dyestuff from ethanol-water (PEW), 2:3 and water (PW) dye extracts with different mordants in various dyeing conditions showed the same resistance grade (4). In wash fastness test, PW dye extract (except post with $CuSO_4$) was better than PEW and found to be resistance grade (3), and staining grade (5). In addition, PW dye extract was also the best and revealed staining grade (4) in dry rubbing test and staining grade (3) in wet rubbing test. This paper performed different fashion hues obtained from the same dye extract using different mordants. The current study can contribute to support students' job opportunities and a good chance in Small and Medium Enterprises (SME) of our country.

Keywords- mango bark, colourfastness, mordants, dyestuff

Introduction

Review on Dye

A dye is a coloured substance which can be made to adhere to fabrics such as cotton, silk or linen (Adeel, *et.al,* 2009). Natural dyes are obtained from flowers, trees, shrubs, berries, leaves, insects and minerals. These dyes have been used for centuries to produce colors for fabrics, yarns, leather, foods, etc. Natural dyes can give subtle and soft colours through to the brightest colour to the yarns and fabrics (Chanayatht, *et.al,* 2013). Primitive dyeing techniques included sticking plants to fabric or rubbing crushed pigments into cloth. The methods became more sophisticated with time and techniques using natural dyes from crushed fruits, berries and other plants, which were boiled into the fabric, were developed (Haji, 2010). The natural dye extraction methods and dyeing methods described by traditional dyers are incomplete. Therefore, there is an absolute necessity to develop and standardize newer methods for the extraction and purification of natural dyes (Harbone, 1984). The process of extraction, purification and dyeing of colours has been evolved to obtain the maximum colouring compounds in the final dye powder.

Materials and Methods

The bark of mango tree (*Mangifera indica* L.) was selected to extract the purified natural dyestuff. Mango tree, Tha-yet in Myanmar name (Figure 1) derived from anacardiaceae family (Kress, *et.al,* 2003) is grown everywhere in Myanmar. 96 % ethanol-water (2:3) v/v and water were used as the solvent to extract the colouring matter from mango bark. Various commonly used solvents and laboratory chemicals, laboratory equipment were used. Fabric cotton was dyed with purified dyestuff and crude dyestuff to compare the colour of the dyed textile substances. Copper (II) sulphate and chrome were used as mordant. ISO/TC38/No.3 washing machine, GOTECH/GT-7020/Sr.No.8900426/ Abrasion type rubbing fastness tester and No.740 Acme Type fade light fastness tester were used for colour fastness test of dyed materials at the Textile Mill, Thamaing, Yangon Region, Myanmar. FT IR spectroscopy was used to assign the functional group of mango bark, and double bonded conjugation in dye was checked with UV spectroscopy.

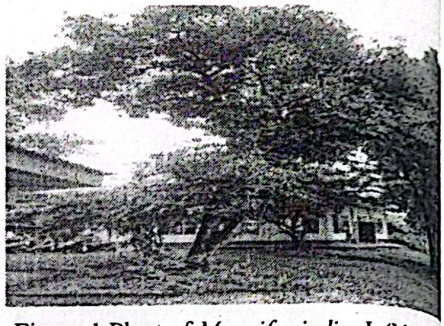
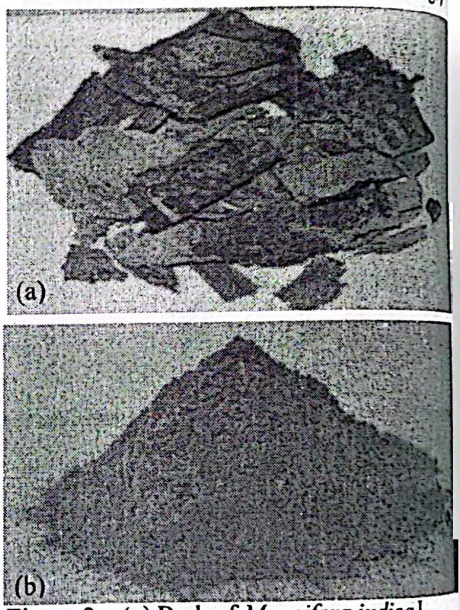


Figure 1 Plant of *Mangifer indica* L.(Mango)



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The raw materials of mango bark (Figure 2 a) were cut from the trunk of mango tree and the were cleaned with water by brushing to remove impurities. They were dried at room temperature and ground into powder form in a grinder (Figure

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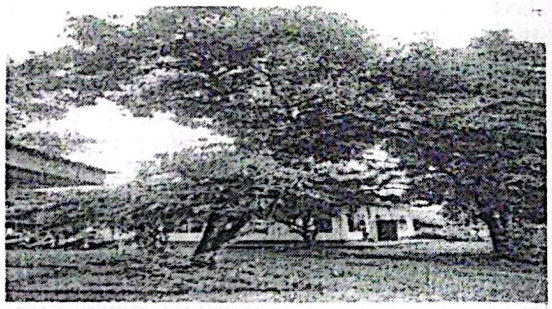

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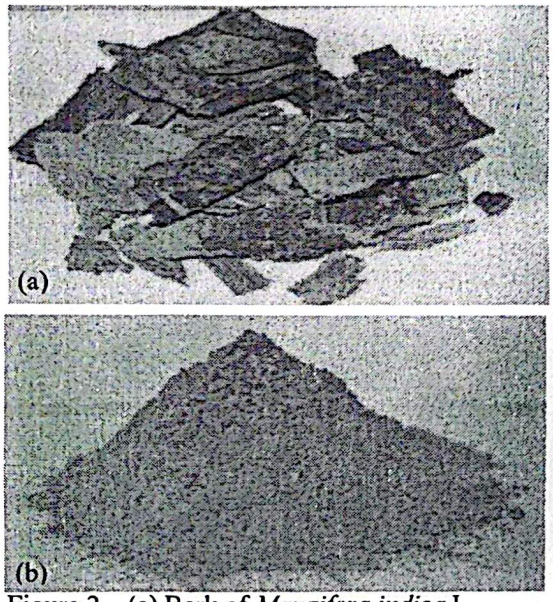

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2 b). Before commencing the extraction and purification of the natural dyestuff, the presence of major types of chemical compound in the mango bark was determined by using preliminary phytochemical tests besides confirmed by modern spectroscopic method such as FT IR and UV.

Optimization of the Condition of Dye Purification

100 g of mango bark powder was placed in a round bottomed flask and 500 mL ethanol-water (2:3) v/v was added in it. The flask was heated in water bath at 60 °C for one h. After extracting the dye, the extracted solution was filtered off and ethanol-water was recovered by using distillation method. After distillation, the concentrated dye solution is dried in the oven at 60 °C. In this way, crude dyestuff was extracted as powder form. Similarly, crude dyestuff from water extract was prepared as the above procedure. Table 1 shows the conditions of crude dyestuff extraction from ethanol-water and water. Since the purified dye yield depends on several parameters such as solvent types, solid to liquid ratio, time and temperature, the optimum conditions for purification of natural dyes were determined. Firstly, 100 g of mango bark powder refluxed with the same procedure as the extraction of crude dyestuff. And then, this extracted solution was purified with different conditions. The optimum dye purification conditions were shown in Table 2. After extracting the dye solution, it was filtered and the volume the solution was reduced by distillation to get the 1/3 of the dye solution. And then this solution was kept overnight at room temperature for precipitation. The precipitate both in the ethanol-water and water solution were obtained with decantation. These active particles, purified dyes, were dried in the oven at 60 °C.

Table 1 Conditions for Extraction of Crude Dyestuff

Solvent (v/v)	Solid to Liq. ratio	Temp (°C)	Time (h)	Yield (%)
ethanol-water (2:3)	1:5	60	1	33.84
Water	1:5	60	1	34.91

Table 2 Optimum Conditions for Purification of Natural Dyestuff

Solvent (v/v)	Temperature (°C)	Time	Solvent	Temperature (°C)	Time	Yield (%)
ethanol-water (2:3)	60 °C	1 h	Ethanol-water	Room Temperature	overnight	30.46
Water	60 °C	1 h	water	Room Temperature	overnight	31.23

Mordanting the textile Material (Fabric cotton)

Mordanting was a process to fix the dyestuff to the textile materials. Before mordanting and dyeing, the fabric cotton was soaked in water for one hour. 0.2 g of $CuSO_4$ was dissolved with 100 mL of deionized water, the $CuSO_4$ mordant solution was obtained. $K_2Cr_2O_7$ mordant solution (chrome) was prepared as the same as procedure of copper (II) sulphate solution ($CuSO_4$).

Dyeing the textile Material (Fabric cotton)

In this study, the three dyeing methods namely, pre-mordanting, post-mordanting and simultaneous mordanting and dyeing were carried out. In pre-mordanting method, mordanting process was done before dyeing operation. In simultaneous mordanting and dyeing method, mordant, dye and fabric cotton were added in a pot and treated together. In post-mordanting method, mordanting process was done after dyeing operation. The conditions for each dyeing method are shown in Table 3.

Table 3 Conditions for Dyeing

Type of Textile & Type of Mordant	Type of Dye	Dye conc. (%)	Temp (°C)	Time (min)	Mordant : Liq. (v:v)
Fabric cotton & Copper (II) sulphate	Crude	7	80	60	1:5
	Pure	5	80	60	1:5
Fabric cotton & Chrome	Crude	7	80	60	1:5
	Pure	5	80	60	1:5

Surveying in Myanmar Yanant Textile

Dye economy and quality maintenance of the present study were surveyed in Myanmar Yanant Textile which is situated in Bogyoke Market, Yangon shown in Figure 3.

Figure 3 Myanmar Yanant Textile at Bogyoke Market in Yangon, Myanmar

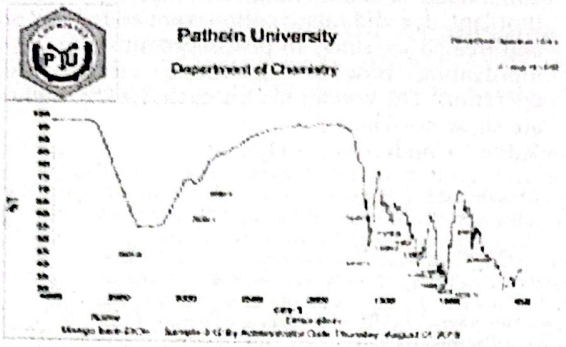


Figure 4 FT IR spectrum of purified dyestuff from ethanol-water extract

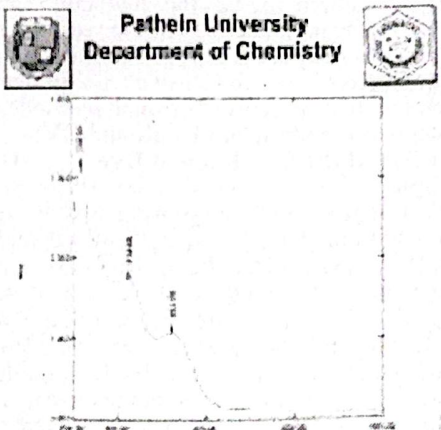
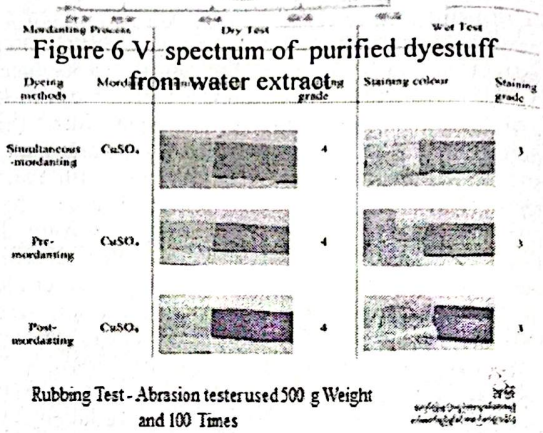


Figure 6 V- spectrum of purified dyestuff from water extract



Results and Discussion

Phytochemical Test and Spectroscopic Analysis of Mango Bark Dyestuff

Phytochemical tests and modern spectroscopic methods: (FT IR and UV) confirmed the presence of the following compounds in the dyestuff. They were alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins and terpenoids. According to phytochcemical tests, the dyestuff contains alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins and terpenoids. However, cyanogenic glycosides were not found in it. Among these compounds, glycosides, tannins, flavonoids and phenolic compounds are the substances which can give the colour. Tannins are the most important ingredients which are necessary for dyeing with natural dyes. Moreover, most of the colouring matters are also found in flavonoids and phenolic compounds. So the compounds that do not give colour were removed to obtain purified, it contained glycosides, tannins, flavonoids and phenolic compounds. In this study, the crude and purified natural dyestuffs were applied on fabric cotton to compare the colour and their fastness properties. As for the FT IR spectrums shown in Figure 4 & 5, 3364 cm^{-1} mainly showed stretching vibration of –OH group for phenolic compounds besides 1647 cm^{-1} and 1617 cm^{-1} described C=C stretching vibration contained in mango bark (ethanol-water extract) purified dyestuff. FT IR spectrum of water extract purified dyestuff revealed at 3301 cm^{-1} for stretching vibration of –OH group for phenolic compounds and 1611 cm^{-1} for stretching vibration of aromatic group of flavonoids. In addition, the maximum wavelength at 258 nm, 314 nm and 362 nm showed that double bond conjugated system as well as non-bonding electrons contained in the water extract purified dyestuff assessed by UV spectrum as shown in Figure 6.

Testing the Colour Fastness of Dyed Fabric Cotton

The colour development on dyed fabric cotton was determined by fastness to light, washing and rubbing and thus carried out to study the fastness properties of natural dyestuff on fabric cotton standardized with Blue Wool Scale method and Grey Scale method respectively. The results of pure dyestuff of mango bark were shown in Figure 7, 8, 9, 10, 11 and 12 besides comparatively described in Table 4, 5 and 6. In Tables, good light fastness with the resistance grade, 4 could be observed in all samples pure dye and crude dye extract from mango bark, by using all dyeing methods at each dyeing concentration. In the case of washing test, it was found that both purified dye and crude dye showed slightly staining and even no staining grade, 5 on the adjacent undyed cotton. Especially pure dyestuff from water extract (PW) was the more effective than pure dyestuff from ethanol-water extract (PEW). Good rubbing fastness could be observed in fabric cotton with purified dye and crude dye using three dyeing methods. In rubbing fastness for dyed fabric cotton, the dry rubbing fastness ratings showed good (slighting staining, 4) in purified dye except PW with post- mordanting $CuSO_4$ whereas the wet rubbing fastness ratings showed fair in the crude dye of water extract.

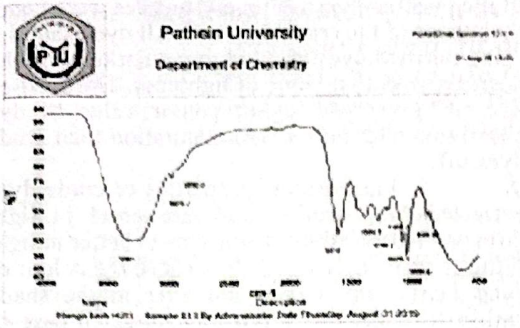
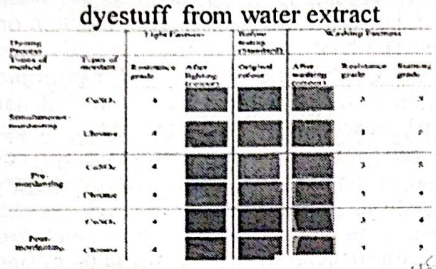
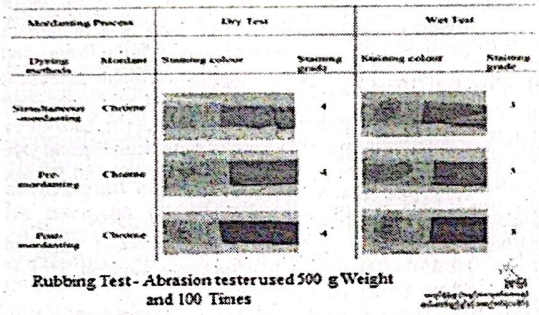


Figure 5 FT IR spectrum of purified dyestuff from water extract



Wash Test - ISO/TC38/No.3

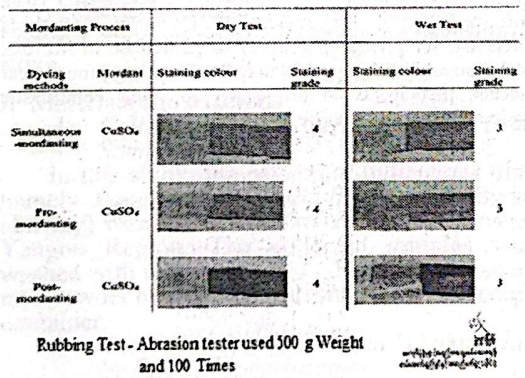
Figure .7. ight and washing fastness properties for three dyeing methods using different mordants with pure dyestuffs of ethanol-water extract dyes



Rubbing Test - Abrasion tester used 500 g Weight and 100 Times

Figure 8 .Light and washing fastness properties for three dyeing methods using different mordants with pure dyestuffs of water extract dyes

Figure 9 Rubbing fastness properties for three dyeing methods using copper (II) sulphate mordant with pure dyestuffs of ethanol-water extract dyes



Rubbing Test - Abrasion tester used 500 g Weight and 100 Times

Figure 10 Rubbing fastness properties for three dyeing methods using chrome mordant with pure dyestuffs of ethanol-water extract dyes

Figure .11.Rubbing fastness properties for three dyeing methods using copper II sulphate mordant with pure dyestuffs of water extract dyes

Figure.12.Rubbing fastness properties for three dyeing methods using chrome mordant with pure dyestuffs of water extract dye

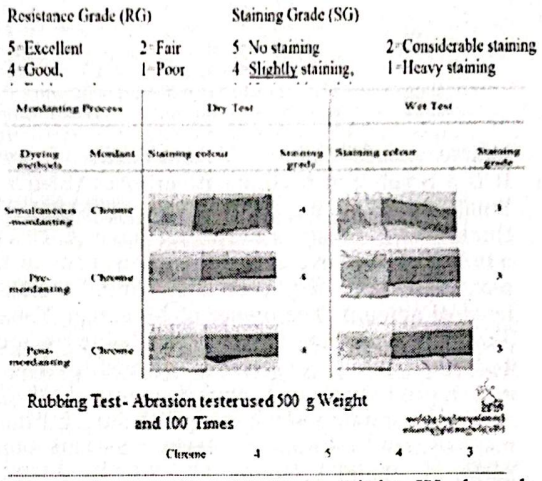
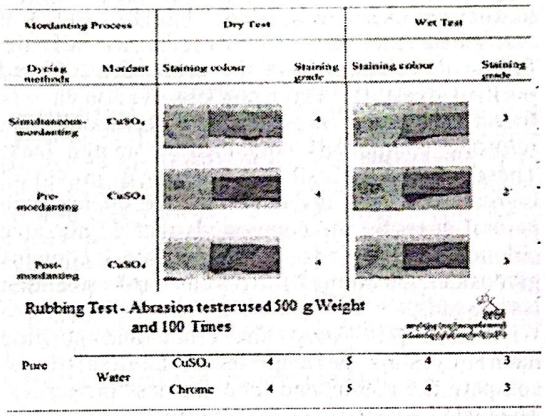


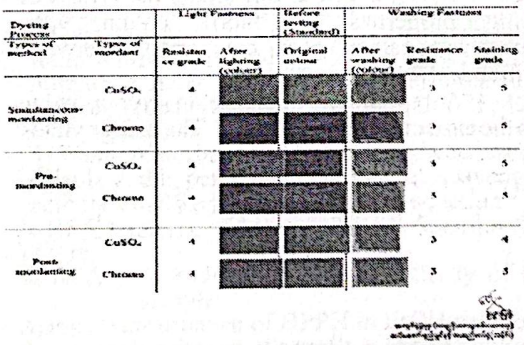
Table 4 Comparative Study on Light, Wash and Rubbing Fastness Test of Pre-mordanting Fabric Cotton with Ethanol-water and Water Extract Dyes (Crude and Pure Dyes)

Table 5.Comparative Study on Light, Wash and



Rubbing Fastness Test of Simultaneous Mordanting Fabric Cotton with Ethanol-water and Water Extract Dyes (Crude and Pure Dyes)

Table 6 Comparative Study on Light, Wash and Rubbing Fastness Test of Post-mordanting Fabric Cotton with Ethanol-water and Water Extract Dyes (Crude and Pure Dyes)



Surveying in Myanmar Yanant Textile

The present study was lead to approach the job opportunity for the students who join in dye industry after University. So this study goal was supported by surveying dye economy and quality

Type of dye	Mordant	Resistance Grade (RG) 5 Excellent 4 Good, 3 Leafy good 2 Fair 1 Poor	Staining Grade (SG) 5 No staining 4 Slightly staining, 3 Noticeable staining. 2 Considerable staining 1 Heavy staining			Light fastness SG
Crude	Ethanol-water	$CuSO_4$	4	4	4	3
		Chrome	4	5	4	3
	Water	$CuSO_4$	4	4	3	2
		Chrome	4	5	4	2
Pure	Ethanol-water	$CuSO_4$	4	4	4	3
		Chrome	4	5	4	3
	Water	$CuSO_4$	4	4	4	3
		Chrome	4	5	4	3

maintenance in Myanmar Yanant Textile, Yangon. It is a Small and Medium Enterprises (SME) or Small and Medium-sized Businesses (SMBs). The small and medium enterprises have a certain number of employees and the investment in the plant and machinery cost falls within a certain level of amount. The owner of Myanmar Yanant Textile provided sharing the knowledge for local and international experience of textile dye in nature, and explained how to grow SME by recruiting, training, retaining, promoting, business management, information technique and high-performing employees.

Conclusion

In this work, bark of *M. indica* was collected from Hinthada Township, Ayeyarwady Region, Myanmar and then phytochemical tests besides modern techniques: FT IR and UV confirmed the presence or absence of the following compounds in the crude dyestuff and purified dyestuff. From the observation, it was found that, glycosides, tannins, flavonoids and phenolic compounds contained in mango bark. These phytochemicals are the most important ingredients which are necessary for dyeing with natural dyes. So the compounds that do not give colour were removed to obtain purified, it contains glycosides, tannins, flavonoids and phenolic compounds.

In this study, the crude and purified natural dyestuff were applied on fabric cotton to compare the colour and their fastness properties. The yield percent of purified dyestuff was less than those of crude dyestuff since purified dye consists of only colour compounds. In the application of purified dyestuff and crude dyestuff extracted from mango bark, purified dyestuff gives better fastness results and more attractive colours than crude dyestuff. On the other hand, the cost of extracting purified dyestuff may be greater than that of crude dyestuff. In this study, the effects of dyeing properties on fabric cotton were emphasized rather cost comparison between purified and crude dye.

A remarkable increase in colour value was the case of purified dyestuff. The colour yields of the purified dyestuff on all samples were better than those of the crude dyestuff. All dyed samples using purified dyestuff tend to get darker with the decrease in colour value of lightness. The purified dyestuff gives the higher colour value of dye absorption and more colour saturation than crude dyestuff.

Colour *fastness* properties of crude dyes extracted from mango bark are good in light fastness. The purified dyestuff gives better in light fastness than crude dyestuff because the colour of dyed fabric cotton does not alter in the shade within 10 hours of dyed testing time. In case of dyed fabric cotton, both purified dye and crude dye show exceptionally good fastness to washing. There were slightly stain and even no stain on the adjacent undyed cotton in washing. By making reference to the results obtained, the dye improved the rubbing fastness remarkably.Purified natural dyestuff, especially (PW), extracted from mango bark gave subtle and soft colours to fabric cotton. Since purified natural dyestuff was provided in powder form, it could be easily used on cottage industry level and mass production level dyeing. In addition, this study can contribute to get the students' job opportunity after Universities besides benefit to local economy of our country.

References

Adeel, S., Ali, S., Bhatti L. A. and Zsila, F. (2009). "Dyeing Of Cotton Fabric Using Pomegranate (Punica Granatum) Aqueous Extract". *Asian J.Chem*, **21**(5), 3493-3499

Chanayatht, A.I., Sorasak, L. and Suree, P. (2013). "Pigment Extraction Techniques from the Leaves of Indigofera tinctoria Linn. and BaphkacanthusCusia Brem. and Chemical Structure Analysis other Major Chemical Components". *International Journal of Engineering Sciences & Research Technology*, **2**(10), 2667-2669

Haji, A. (2010). "Functional Dyeing of Wool with Natural Dye Extracted from Berberis Vulgaris Wood and Rumex Hymenosepolus Root As

Biomordant" *Iran J.Chem. Chem. Eng.* **29**(3), 55-59

Harbone, J.B. (1984). *Phytochemical Methods-A guide to modern techniques of plant analysis.* London: 2nd Ed., Chapman and Hall, 701-710

Kress, W.J., Defilipps, R.A., Farr, E. and Daw Yin Yin Kyi. (2003). *A check list of the Trees, Shrubs, Herbs, and Climber of Myanmar*. Washington, DC; 1st Ed., The United State Department of griculture, 217-219

Acknowledgements

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Study on Medicinal Benefits Such as Antimicrobial and Antioxidant Activities of *Pandanus amaryllifollius* Roxb (Swan-Hmway)

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Abstract

In this study, *Pandanus amaryllifollius* Roxb (Swan-Hmway), belonging to the family Pandanaceae, has been chosen to investigate elemental composition and antimicrobial and antioxidant activities according to its well-known properties used in food coloring and flavoring. The elemental composition of plant sample was examined by EDXRF spectrometry. The antimicrobial activity of crude extracts such as pet-ether, ethyl-acetate, ethanol and watery extracts were investigated by Agar Well Diffusion method on six microorganisms: *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli*. It was found that all extracts showed the antimicrobial activity on all tested microorganisms with inhibition zone diameter ranged between 11 mm and 17 mm. Investigation of antioxidant activity of ethanol and watery extracts of the leaves sample was performed by DPPH free radical scavenging assay observing that ethanol extract ((IC_{50} = 76.458 µg/ml) is more potent in antioxidant activity than watery extract ((IC_{50} = 134.685 µg/ml) but weaker than standard BHT (IC_{50} = 20.94 µg/ml).

Keywords Pandanus amaryllifollius Roxb., elemental analysis, antimicrobial activity, antioxidant activity

I. Introduction

Green leafy vegetable consumption associates with decreased risk of persistent metabolic diseases. So, one of the green leafy vegetable, *Pandanus amaryllifollius* Roxb., belonging to the family Pandanaceae, has been chosen to study some medicinal benefits. It is a tropical plant which is widely cultivated in several countries, including Malaysia, Indonesia, Chinese and Bangladesh. This green plant has fan-shaped, blade-like leaves and very rare flowers. It is used mostly as a flavoring agent for certain rice and bread recipe [4]. In Myanmar, pandan leaves impart not only flavor, but also green color for food. It is widely used in pudding for bread, and in rice noodles for sago or Shwe-Yin-Aye.

Like other green leafy vegetables, pandan leaves are also known as potential source of several lipophilic antioxidant e.g. β-carotene, vitamin E, phenolic compounds, ascorbic acid. Besides the lipophilic antioxidants, it also contains quercetin, alkaloids, fatty acids and ester. They possess antioxidant activity and thus have the potential to be used as cheap natural sources for reducing cellular oxidative damage and reduce degenerative conditions such as cardiovascular diseases and cancers [1].

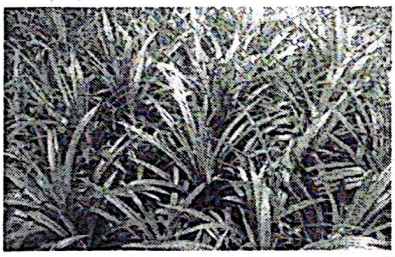

Figure.1 Photograph of *Pandanus amaryllifollius* Roxb.

II Materials and Methods

A. *Collection and Preparation of Plant Samples*

In this study, the Myanmar indigenous plant namely *Pandanus amaryllifollius* Roxb. (Swan-Hmway) was collected from Yan-Kin Township, Yangon Region. The collected samples were washed with water and aired - dried and then made into powder by grinding mill and stored in air-tight container.

B. *Determination of Elemental Composition by EDXRF spectroscopy*

Semi-quantitative estimation of metals in the selected plant sample was carried out by X-ray fluorescence spectrometer.

C. *Investigation of Antimicrobial Activity by Agar Well Diffusion Method*

Antimicrobial activities of different crude extracts such as pet ether (PE), ethyl acetate (EtOAc), ethanol (EtOH), and watery (H_2O) extracts were investigated on six microorganisms: *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* by Agar Well Diffusion method [5] at the Myanma Pharmaceutical and Industrial Research Department (MPIRD), Ministry of Industry, Yangon.

The solutions of different crude extracts, nutrient agar medium (made by mixing agar powder, sodium chloride, peptone and yeast extract) and inoculum were prepared by the procedure described by Nicklin *et al.*, [3].

The crude extracts of pet ether (PE), ethyl acetate (EtOAc), ethanol (EtOH) and water (1 g each) were dissolved in 1cm^3 of their respective solvent. 1 ml of each of bacteria suspension of 24 hours of nutrient agar was streaked evenly onto the surface of trypticase soy agar plates with sterile cotton swab. Immediately after hardening of the agar, the wells were made with a 10 mm sterile cork borer from each sedded agar. After removing the agar, the wells were filled with the drug extract to be tested. The plates were incubated at 37°C for 18-24 hours. The diameters of the inhibition zone were measured and recorded in mm.

D. *Determination of Antioxidant Activity of Crude Extracts by DPPH Assay*

The free radical scavenging activity of the leaves of *P. amaryllifollius* (Swan-Hmway) was measured by using DPPH ((2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay [2].

The control solution, prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of ethanol, and the sample solution, prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of test sample solution, were incubated at room temperature and shaken on shaker for 30 minutes. After incubation, the absorbance of different concentrations (200, 100, 50, 25, 12.5, 6.25 µg/mL) of tested sample was measured at 517 nm. Absorbance measurements were used to calculate the percentage of radical scavenging activity (% RSA) by the following equation:

$$\% RSA = [A_{DPPH} - (A_{Sample} - A_{blank})/ A_{DPPH}] \times 100$$

Where,

% RSA = % radical scavenging activity of test sample

A_{DPPH} = absorbance of DPPH in EtOH solution

A_{Sample} = absorbance of sample + DPPH solution

A_{Blank} = absorbance of sample + EtOH solution

The antioxidant power (IC_{50}) is expressed as the test substances concentration (µg/ml) that results in a 50% reduction of initial absorbance of DPPH solution and that allows to determine the concentration. IC_{50} (50% inhibitory

concentration) values were calculated by linear regressive excel program.

III. Results and Discussion

A. Qualitative Elemental Analysis by Energy Dispersive X-ray Fluorescence (EDXRF) Spectrophotometer

The relative abundance of elements presents in leaves of *P. amaryllifollius* was determined by EDXRF spectrometer observing that K, Ca, Cl, Fe, S, Mn, Br, Rb, Zn, Sr and Cu were present. Among them, K has the highest content and which is predominant in the leaves of *P. amaryllifollius*. So, the potassium rich sample, *P. amaryllifollius* may help to reduce blood pressure, regulate fluid balance, muscle contractions and nerve signals and also protect against stroke and prevent osteoporosis and kidney stones.

Table 1. Relative abundance of elements in leaves of *pandanus amarylifollius* by ed-xrf spectroscopy

Element	Relative Abundance (%)
K	45.894
Ca	27.984
Cl	21.845
Fe	1.182
S	1.124
Mn	0.775
Br	0.341
Rb	0.300
Zn	0.298
Sr	0.143
Cu	0.112

B. Screening of Antimicrobial Activity of Crude Extracts of Pandanus amaryllifollius Roxb. *by Agar Well Diffusion Method*

Antimicrobial activity of the crude extracts of *P. amaryllifollius* sample was screened by Agar Well Diffusion method on six tested micro-organisms. The measurable zone diameter, including the well diameter, shows the degree of antimicrobial activity. The larger the inhibition zone diameter, the greater the activity is on the organisms.

It was found that all extracts showed antimicrobial activity on all tested microorganisms with inhibition zone diameter between 11 mm and 17 mm. The highest inhibition zone was observed 17 mm on *S. aureus* and *E.coli* by EtOAc extract and 17 mm on *E.coli* by EtOH extract. Therefore, EtOH and EtOAc extracts of *P. amaryllifollius* exhibited more potent antimicrobial activity than the other extracts.

Table.2 Inhibition zone diameters (mm) of the crude extracts of *pandanus amaryllifollius against* tested microorganisms

Organisms	Inhibition Zone Diameter of Crude Extracts (mm)			
	PE	EtOAc	EtOH	H_2O
B. subtilis	13(+)	16(++)	16(++)	12(+)
S. aureus	13(+)	17(++)	15(++)	11(+)
P. aeruginosa	11(+)	12(+)	12(+)	11(+)
B. pumilus	13(+)	15(++)	16(++)	12(+)
C. albicans	14(+)	15(++)	15(++)	12(+)
E. coli	13(+)	17(++)	17(++)	15(++)

Diameter of agar well = 10 mm

Sample	IC_{50} (µg/ml)
Ethanol extract	76.46
Watery extract	134.69
Standard BHT	20.94

10 mm ~ 14 mm = (+),20 mm above = (+++)

15 mm ~ 19 mm = (++),No activity = (-)

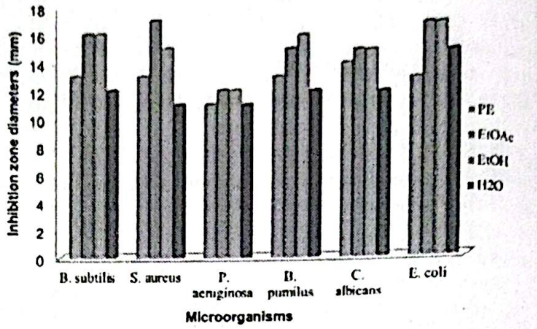

Fig. 2 Comparison of inhibition zone diameters (mm) of different crude extracts from *P. amaryllifollius* leaves on six microorganisms

C. Screening of Antioxidant Activity by DPPH Assay Method on Ethanol and Watery Extracts of Pandanus amaryllifollius Roxb.

The antioxidant activity of ethanol and watery extracts of *P. amaryllifollius* were determined by DPPH free radical scavenging assay which is an easy, rapid and sensitive way to survey the antioxidants activity of the plant extracts. The antioxidant activity was expressed as % RSA and IC_{50} (50% inhibitory concentration). It was found that increasing the concentration of the sample, the % RSA was also increased. These results were shown in Table III and Fig. 3.

The IC_{50} values of ethanol extract and watery extract were found to be 76.46 µg/ml and 134.69 µg/ml, respectively. Since IC_{50} value of ethanol extract is lower than watery extract, ethanol extract is more potent in antioxidant activity than watery extract but these showed less antioxidant activity by comparing with BHT (IC_{50} = 20.94 µg/ml). However, we can say that the selected plant, *P. amaryllifollius* possessed the mild antioxidant property.

Table.3 % oxidative inhibition of different concentrations of *pandanus amaryllifollius and* standard bht

Sample	%RSA ± SD at Different Concentrations (µg/mL)					
	6.25	12.5	25	50	100	200
EtOH	25.70 ± 0.04	29.08 ± 0.02	31.99 ± 0.04	43.25 ± 0.04	56.00 ± 0.02	79.17 ±
H_2O	12.01 ± 0.02	15.95 ± 0.00	19.61 ±	39.21 ± 0.02	45.87 ± 0.03	57.79 ± 0.02
BHT	17.01 ± 0.01	31.16 ± 0.00	59.06 ± 0.02	67.09 ± 0.01	.48 ± 0.00	87.28 ± 0.00

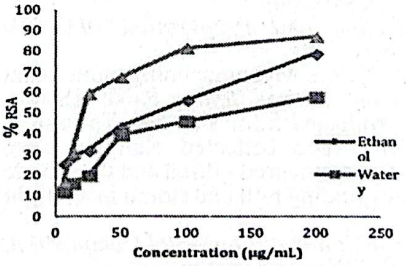

Fig. 3% RSA of ethanol and watery extracts of the leaves of *P. amaryllifollius* and BHT on antioxidant activity

Table.4 Ic_{50} values of ethanol and watery extracts of leaves of *pandanus amaryllifollius and* standard bht

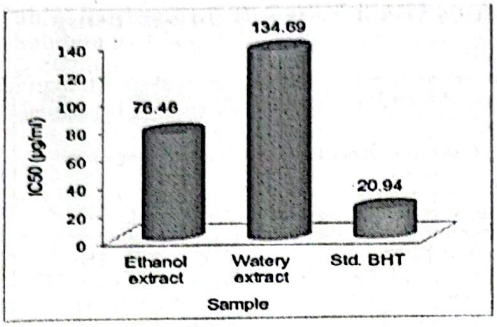

Fig. 4 IC_{50} values of crude extracts of *Pandanus amaryllifollius Roxb* and standard BHT

IV Conclusion

This study gives the valuable observations about the elemental composition, antimicrobial and antioxidant activities of the leaves of *Pandanus amaryllifollius* Roxb.

The relative abundance of elements presents in leaves of *P. amaryllifollius* was found to possess K, Ca, Cl, Fe, S, Mn, Br, Rb, Zn, Sr and Cu. Among them, the three elements such as K, Ca and Cl were present with the highest content. The crude extracts of ethyl acetate and ethanol, exhibit antimicrobial activity on all tested microorganisms, especially on *S. aureus* and *E.coli* by EtOAc extract and *E.coli* by EtOH extract that informs the medicinal values of selected sample for the treatment of skin diseases, wound infections, diarrhea, dysentery and other diseases infected by the microorganisms. Moreover, the leaves of *P. amaryllifollius* contributes to mild potency in free radical scavenging activity that points out the effectiveness of medicinal uses of this plant.

Acknowledgment

The author would like to thank the members of JMEST for allowing to present this paper, and also thanks to Dr Ni Ni Than (Professor and Head), Department of Chemistry, University of Yangon, for her kind encouragement.

References

[1] A. M. Mahmoud, A. B. Mohamad, A. H. Kadhum and N. A. Ludin, "Natural dye extracted from *Pandanus amaryllifollius* leaves as sensitizer in fabrication of dye-sensitized solar cells". *Int. J. Electrochem. Sci.*, vol. 12, 2017, pp. 747-761

[2] G. Marinova, and V. Batchvarov, "Evalution of the Methods for Determination of the Free Radical Scavenging Activity by DPPH". *Bulgarian Journal of Agriculatural Science*, **17**(1), 2011, pp. 11-24

[3] J. Nicklin, K. Gracme Cook, T. Paget and R. A. Killington, "Instant Notes in Microtriology". 1st Ed., Viva Books Private Limited, 1999, pp. 714

[4] N. Andriati, and S. Matthias, "Pandan leaves: Vanilla of the East as potential natural food ingredient". *Agro Food industry Hi Tech.*, vol. 25, No.(3), 2014, pp. 57-61

[5] R. Cruickshank, J. P. Duguid, B. R. Marimion and R. H. Swain,. *The Practice of Medical Microbiology*. 12th Ed., Churchill Livingstone Ltd, London, vol. 11, 1975, pp. 812-825

[6]

Adsorptive Removal of Dye from Aqueous Dyed Waste Solution using Geosorbent

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Abstract

Dyes and pigments are widely utilized in the textiles, paper, plastics, leather, food-stuff, cosmetic industries as well as natural and artificial fibers to color products. In this research work, the treatment of dyed solutions was carried out by adsorption using geosorbent. The quicklime powder was chosen as geosorbent. The elemental composition of quicklime powder was studied by EDXRF (Energy Dispersive X-ray Fluorescence) spectroscopy. Firstly, the color removal of known concentration of chemical dye solution (congo red dye solution) was carried out using quicklime powder. The adsorption isotherms were also studied. The isothermal data fit the Langmuir and Freundlich isotherms. Then, the natural dyed waste solutions, (Sappan wood and Djenkol shell dyed waste solutions) were collected from Saunder Weaving and Vocational Institute, Amarapura Township in Mandalay Region. These dyed waste solutions were treated using quicklime powder by shaking and standing method. Both methods have high efficiency for dye adsorption by geosorbent. In shaking method, the contact time effect and dosage effect were performed. From the experimental data, the shaking method was better than the standing method for the treatment of dyed waste solutions. The experimental results show that geosorbents have great potential for application to treat dyed solution and this research can also help in waste management and keeping environmental clean.

Keywords : geosorbent, adsorption, congo red, Sappan wood, Djenkol shell

Introduction

Use of the dyes along with lots of water in textile industries has resulted in the production of polluted and coloured waters being discharged into the environment (near by land or rivers) without any treatment because the conventional treatment methods are not effective. These coloured compounds are aesthetically objectionable and inhibiting sunlight penetration into these waters (e.g. streams, rivers etc). Ground-water are also affected by these pollutants because of leaching from the soil.[1]

The major constituents of textile effluent are dyes. Some dyes are reported to cause allergic dermatitis, skin irritation, cancer and mutation in humans. The toxicity and carcinogenic nature of these dyes and their precursors pose a threat to the environment. Moreover, their degradation often leads to the formation of highly carcinogenic aromatic amines, for recycling coloured wastewaters must be there are proper treatment for this polluted water.[1]

There are many various physical and chemical techniques have been used to eliminate dyes from wastewaters, like adsorption, reverse osmosis, coagulation, flocculation, membrane technology, and biological treatments.

Adsorption is the phenomenon of accumulation of large number of molecular species at the surface of liquid or solid phase in comparison to the bulk. Adsorption process involves two components, adsorbent and adsorbate. Adsorbent is the substance on the surface of which adsorption takes place. Adsorbate is the substance which is being adsorbed on the surface of adsorbent.[2] In the present study, treatment of dyed solutions was carried out by adsorption using quicklime powder as geosorbent.

Materials and Methods

Sampling

The Djenkol shell dye waste (Myanmar name- Da-nyin) and Sappan wood dye waste (Myanmar name- Tein-nyet) were collected from Saunder Wearing and Vocational Institute, Amarapura Township in Mandalay Region. The geosorbent, quicklime powder were collected from Htone-bo Village, Patheingyi Township, Mandalay Region. The quicklime powder were sieved by using 100 and 120 mesh size sieves and stored in plastic bag and used throughout the research. The Congo red dye was purchased from chemical shop.

Determination of Maximum Wavelength of Dye Solutions

The maximum wavelength of natural dyed waste solutions, Sappan wood dye and Djenkol shell dye, were determined using photocolorimeter. The maximum wavelength was found to be at 400nm for Sappan wood dye solution and at 490nm for Djenkol shell dye solution. Similarly, the maximum wavelength of congo red dye solution was determined by spectrophotometer, and the value was 497nm.

Preparation of Standard Curve of Congo Red Aqueous Solution

Congo red powder (0.01 g) was dissolved in 1000 ml of distilled water to prepare stock solution (10 mg/L). Then, the solution was diluted to the concentration of 5, 4, 3, 2, 1mg/L of congo red aqueous solution. The absorbance of each solution was measured at the maximum wavelength (497 nm). The standard curve was constructed by plotting the concentration against the absorbance. This curve was used to determine concentration of congo red dye solution after adsorption process.

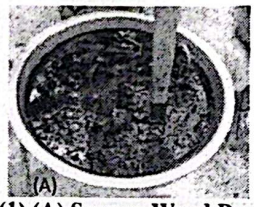


Figure (1) (A) Sappan Wood Dye Waste

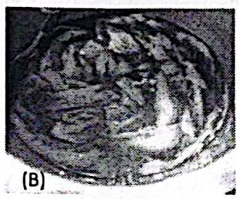


Figure (1) (B) Djenkolshell Dye Waste

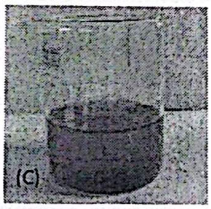


Figure (1) (C) Congo Red Dye Solution

Table (1) Absorbances of Congo Red Aqueous Solution by Using UV-visible pectrophotometer at 497 nm

No.	Concentration (mg/L.)	Absorbance
1.	1.00	0.012
2.	2.00	0.023
3.	3.00	0.034
4.	4.00	0.046
5.	5.00	0.057

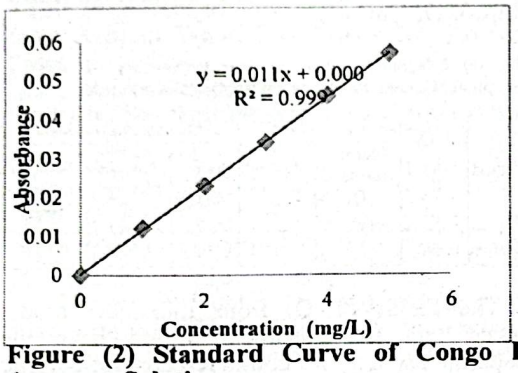


Figure (2) Standard Curve of Congo Red Aqueous Solution

Determination of Elemental Composition of Quicklime Powder

The elemental composition of quicklime powder was measured at Department of Physics, University of Mandalay, by applying EDXRF (Energy Dispersive X-ray Fluorescence Spectroscopy).

Adsorptive Removal of Congo Red Dye from Aqueous Solution by Adsorption Using Quicklime Powder

Standing Method

100 ml of (5 mg/L) aqueous congo red dye solution was added into in a conical flask containing 1 g of quicklime powder. The mixture was allowed to stand for 2 hr. Then, the adsorbent was removed by centrifugation at 1000 rpm for 5 minute. The absorbance of supernatant was measured by UV visible spectrophotometer at 497 nm wavelength. Then, the equilibrium concentration of dye solution was determined by applying standard curve of congo red solution. The percent color removal was also calculated by the following equation.

$$\%\text{removal} = \frac{C_0 - C_e}{C_0} \times 100$$

Where C_o is the concentration of aqueous dye solutions before adsorption and C_e is the concentration of aqueous dye solutions after adsorption.Similarly, the procedure was repeated for standing time 4 hr, 6 hr, 8 hr, 10 hr respectively.

Shaking Method

100 ml of (5 mg/L) aqueous congo red dye solution was added into in a conical flask containing 1 g of quicklime powder. The mixture was shaken for 30 min at 180 rpm. Then, the adsorbent was removed by centrifugation at 1000 rpm for 5 minute. The absorbance of supernatant was measured by UV visible spectrophotometer at 497 nm wavelength. Then, the equilibrium concentration of dye solution was determined. The percent color removal was also calculated by the following equation.

$$\%\text{removal} = \frac{C_0 - C_e}{C_0} \times 100$$

Where C_o is the concentration of aqueous dye solutions before adsorption and C_e is the concentration of aqueous dye solutions after adsorption.Similarly, the procedure was repeated for shaking time 60 min, 90min, 120 min, 150 min respectively. From the C_o and C_e, the Langmuir adsorption isotherm and the Freundlich adsorption isotherm were studied.

Adsorptive Removal of Dye from Sappan Wood and Djenkol Shell Dye Waste Solutions by Adsorption Using Quicklime Powder

Adsorption experiments were performed by standing and shaking method using quicklime as adsorbent to remove dye from aqueous solution under various conditions of agitation time and adsorbent dose. Then, adsorbent was removed by centrifugation. The absorbance of the supernatant was measured by photocolorimeter at their maximum wavelength (400nm for Sappan wood dye and 490nm for Djenko shell dye). The percent color removal was calculated.

$$\%\text{ color removal} = \frac{A_0 - A_e}{A_0} \times 100$$

Where, A_0 and A_e are the absorbance of dye waste solution before and after adsorption.

Results and Discussion

Results of Elemental Composition of Quicklime Powder

The elemental composition of quicklime powder was measured by applying EDXRF (Energy Dispersive X-ray Fluorescence Spectroscopy). The results were shown in Table (2). The result showed that calcium was the highest percent (34.18 %) in quicklime powder.

Table (2) Elemental Composition of Quicklime Powder

No.	Element	Relative Abandance (%)
1.	Calcium	34.1800
2.	Silicon	0.0888
3.	Iron	0.0644
4.	Aluminum	0.0623
5.	Chlorine	0.0335
6.	Potassium	0.0239
7.	Phosphorus	0.0221
8.	Strontium	0.0135
9.	Titanium	0.0098

Adsorptive Removal of Congo Red Dye by Adsorption Using Quicklime Powder

Removal of congo red from aqueous solution was measured on the effect of contact time by standing and shaking methods. The results were shown in Table (3) and Table (4).

Table (3) Effect of Contact Time on Aqueous Solution of Congo Red Dye by (1g) Quicklime Powder (Standing Method)

No.	Contact time (standing) (hr)	Initial conc: C_o (mgL^{-1})	Equilibrium conc: C_e(mgL^{-1})	Percent removal (%)
1.	2.00	5.00	1.84	63.20
2.	4.00	5.00	1.58	68.40

3.	6.00	5.00	1.36	72.80
4.	8.00	5.00	1.23	75.40
5.	10.00	5.00	0.88	82.40

Table (4) Effect of Contact Time on Aqueous Solution of Congo Red Dye by (1g) Quicklime Powder (Shaking Method)

No.	Mass of sample (g)	Contact time (min)	Initial conc. C_o (mgL^{-1})	Equilibrium conc. C_e (mgL^{-1})	Percent removal (%)
1.	1.00	30	5.00	1.05	79.00
2.	1.00	60	5.00	0.88	82.40
3.	1.00	90	5.00	0.53	89.40
4.	1.00	120	5.00	0.26	94.80
5.	1.00	150	5.00	0.18	96.40

The adsorption capacity of quicklime in both methods shows that the contact time between the adsorbent and adsorbate increases, the adsorption rate increases. Almost all the color of dye was removed by quicklime.By using these data, Langmuir and Freundlich isotherm were studied.

Study on Adsorption Isotherms

The adsorption equilibrium models often provide insight into the sorption mechanism, surface properties and affinity of adsorbent. The most common used equilibrium models are Langmuir and Freundlich[7].

The Langmuir adsorption isotherm is represented by the following equation:

$$C_e/q_e = 1/Q_0\, b + C_e / Q_0$$

Where, C_e (mg/L) is the equilibrium concentration of dye, q_e (mg/g) is the amount of dye adsorbed at equilibrium, Q_0 is the monolayer adsorption capacity (mg/g) and b is the constant related to the free energy of adsorption. Hence a plot of C_e/q_e versus C_e yield a straight line with Q_0 calculated from the slope and the value of b as its intercept. The Freundlich isotherm is an exponential equation and can be written as:

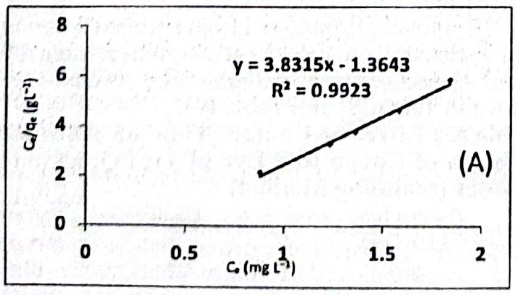
$$q_e = K_f C_e^{1/n}$$

Where, K_f is the constant indicative of the relative adsorption capacity of adsorbent (mg/g), and 1/n is the constant indicative of the intensity of adsorption.

Table (5), Table (6) and Figure (3) showed the data for Langmuir and Freundlich isotherms.

Table (5) Adsorption of Congo Red Dye onto Quicklime Powder (Standing Method)

No.	Contact time (hour)	C_e (mgL^{-1})	q_e (mgg^{-1})	C_e/q_e (gL^{-1})	log [$C_e(mgL^{-1})$]	log [$q_e(mgg^{-1})$]
1.	2.00	1.8400	0.3160	5.8228	0.2648	−0.5003
2.	4.00	1.5800	0.3420	4.6199	0.1987	−0.4660
3.	6.00	1.3600	0.3640	3.7363	0.1335	−0.4388
4.	8.00	1.2300	0.3770	3.2626	0.0899	−0.4237
5.	10.00	0.8800	0.4120	2.1359	−0.0555	−0.3851



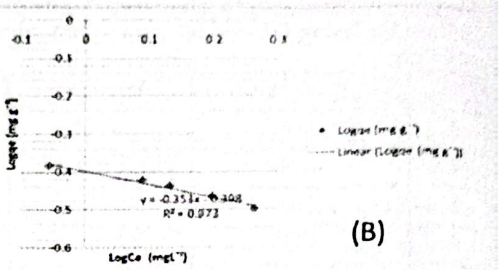


Figure 3 (A) Langmuir Isotherm (B) Freundlich Isotherm

Table (6) Langmuir and Freundlich Parameters for the Adsorption of Congo Red Dye onto the Quicklime Powder

Method	Langmuir parameters			Freundlich parameters		
	R^2	Monolayer coverage Q_0 (mgg^{-1})	Langmuir constant, b	R^2	Freundlich constant, n	Sorption capacity K (mgg^{-1})
standing	0.9923	0.2610	2.8982	0.973	2.5125	0.1495

The result of Q_0 from Langmuir mode showed that the adsorbent would have th acceptable capacity for congo red adsorption. Th values of sorption intensity in the Freundlic model indicated a high tendency toward adsorbin the dye onto the adsorbent. The results proved tha the surface of the adsorbent is heterogeneous an the adsorption of congo red dye on the adsorbe is multilayer.

Adsorptive Removal of Dye from Sappan Wood Dye Waste Solution by Quicklime Powder

Removal of Sappan wood dye from aqueou dyed solution was measured on the effect o contact time and effect of dosage by standing an shaking methods using quicklime as geosorbent The results were shown in Table (7) and Table (8 and Table (9).

Table (7) Effect of Contact Time on Removal Dye from Sappan Wood Dye Waste by (1g) Quicklime Powder (Standing method)

No.	Contact time (standing) (hr)	Initial absorbance A_o	Equilibrium absorbance A_e	Percent color removal (%)
1	2	1.38	0.96	31.16
2	4	1.38	0.70	49.28
3	6	1.38	0.44	68.12
4	8	1.38	0.27	73.19
5	10	1.38	0.57	58.70
6	12	1.38	0.41	70.30
7	14	1.38	0.30	78.26
8	16	1.38	0.24	82.61
9	18	1.38	0.24	82.61
1	20	1.38	0.40	71.01

The result showed that the contact time between the adsorbent and adsorbate increase, the adsorption rate increase. It was observed that on standing time 10 hour the color of dye become desorption.

Table (8) Effect of Contact Time on Removal of Dye from Sappan Wood Dye Waste by (1g) Quicklime Powder (Shaking method)

No.	Contact time (shaking) (min)	Initial absorbance A_0	Equilibrium absorbance A_e	color removal (%)
1.	30	1.38	0.95	31.16
2.	60	1.38	0.87	36.96
3.	90	1.38	0.65	52.90
4.	120	1.38	0.59	57.25
5.	150	1.38	0.54	60.87

Table (9) Effect of Dosage on Removal of Dye from Sappan Wood Dye Waste by Quicklime Powder

No.	Mass of Adsorbent (g)	Contact time (hr)	Initial absorbance A_0	Equilibrium absorbance A_e	color removal (%)
1.	0.20	1.00	1.38	1.28	7.25
2.	0.40	1.00	1.38	1.27	7.97
3.	0.60	1.00	1.38	1.23	10.87
4.	0.80	1.00	1.38	1.00	27.54
5.	1.00	1.00	1.38	0.87	36.96

The percent color removal increases with increasing of adsorbent due to increasing of adsorption sites. The optimum time for Sappan wood dye waste by standing was found to be 16 hours for contact time.

Adsorptive Removal of Dye from Djenkol Dye Waste by Quicklime Powder

'Removal of Djenkol dye from aqueous dyed solution was measured on the time effect and dosage effect by standing and shaking methods. The results were shown in Table (10), Table (11) and Table (12).

Table (10) Effect of Contact Time on Removal of Dye from Djenkol Dye Waste by (1g) Quicklime Powder (Standing method)

No.	Contact time (hr)	Initial absorbance A_0	Equilibrium absorbance A_e	:olor remova (%)
1.	2	1.27	0.85	33.07
2.	4	1.27	0.08	93.70
3.	6	1.27	0.04	96.85
4.	8	1.27	0.10	92.13
5.	10	1.27	0.11	91.34
6.	12	1.27	0.04	96.85
7.	14	1.27	0.03	97.64
8.	16	1.27	0.03	97.64

Table (11) Effect of Contact Time on Removal of Dye from Djenkol Dye Waste by (1g) Quicklime Powder (Shaking method)

No.	Contact time (minute)	Initial absorbance A_0	Equilibrium absorbance A_e	color removal (%)
1.	30	1.27	0.05	96.06
2.	60	1.27	0.03	97.64
3.	90	1.27	0.01	99.21
4.	120	1.27	0.01	99.21
5.	150	1.27	0.01	99.21

The results show that the dye is rapid adsorption at the first time 30 min and then the process gradually becomes slowly. The percent color removal increases with increasing of adsorbent due to increasing of surface area. The optimum dose was 0.6 g for Djenkol dye waste solution.

Table (12) The Effect of Dosage on Color Removal of Djenkol Dye Waste by Quicklime Powder (shaking method)

No.	Mass of Adsorbent (g)	Contact time (hr)	Initial absorbance A_0	Equilibrium absorbance A_e	color removal (%)
1	0.20	1.00	1.27	0.07	94.49
2	0.40	1.00	1.27	0.04	96.85
3	0.60	1.00	1.27	0.03	97.64
4	0.80	1.00	1.27	0.03	97.64
5	1.00	1.00	1.27	0.03	97.64

Conclusion

In this research work, the dyed solutions were treated by chemical adsorption method. The dye waste solutions were collected from Saunder Weaving and Vocational Institute, Amarapura Township, Mandalay Region. The congo red dye solution was prepared in laboratory. The quicklime powder was chosen as the adsorbent for the adsorption study. The elemental composition of quicklime powder was determined by EDXRF spectroscopy. The quicklime powder mainly contained calcium.The colour intensity of the congo red aqueous dye solution (chemical dye) was reduced using quicklime powder as adsorbent by standing and shaking methods. It was observed that congo red dye adsorbed on quicklime powder. From the study of the adsorption isotherm, the Q_0 from the Langmuir model showed that the adsorbent would have an acceptable capacity for congo red dye adsorption. The value of sorption intensity in the Freundlich model indicated a high tendency toward adsorbing the dye onto the adsorbent. So, the isothermal data fitted the Langmuir and Freundlich model. The removal color of (natural dyes) Sappan wood dye waste and Djenkol shell dye waste solutions were also carried out by applying quicklime powder. In shaking method, time effect and dosage effect were also studied. The results of percent color removal in time effect show that the contact time increased, the adsorption rate increased. In dosage effect, the percent color removal increases with increasing adsorbent dose. From the experimental data, the shaking method is more efficient than the standing method for the treatment of dye wastes solutions. Therefore, the geosorbent, quicklime is considered both useful and affordable to treat dye waste solutions for reducing environmental pollution.

References

[1] Salman. J.M; Amrin.A.R; Hassan. F.M; Jouda. S.A Removal of congo red dye from aqueous solution by using natural materials Mesopotama Environmental Journal, Vol. 1, No. 2: 82-89. Mesop.environ. J-2015.

[2] Wong, Jin Shyan, *et al.*, "Acute anuric renal failure following jering bean ingestion", Asian Journal of Surgery 30.1, 2007, 80-81.

[3] Larson, JAMESL., and BICHARDF. Clark. "Plant toxins in the tropics". Tropical Infectious Diseases (Second Edition), 2006, 102-19

[4] Beer, Johnj. The Emergence of the German Dye Industry, 1959.

[5] Hunger, K, ed., Industrial Dyes. Chemistry, Properties, Applications.Weinheim: Wiley-VCH, 2003.

[6] P.Somasundaran, Encyclopedia of Surface and Colloid Science, 2006, 2rd Edition Taylar& Francis, New York.

[7] T.Santhi, S.Manonmani, Removal of Methylene Blue From Aqueous Solution By Bioadsorption onto RicinuscommunisEpicarp Activated Carbon," Department of Chemistry, Karpagam University, Coimbatore-641021, India, Department of Chemistry, PSG College of Arts and Science, Coimbatore-641014, India, 2009.

Isolation and Characterization of Cellulose Microfiber from Betel Nut Husk Wastes

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Abstract

The possibility of using betel nut fibers from native betel nut plant is a source of natural cellulose fibers for the production of cellulose microfiber. Cellulose microfiber is from betel nut fibers were isolated. The isolation was performed sequentially using acid (hydrochloric acid) and alkali treatment (sodium hydroxide) and hydrogen peroxide bleaching under different conditions. Natural cellulose fibers isolated from betel nut fibers have been characterized for their composition, structure and properties. Some physicochemical properties of betel nut fibers were determined such as moisture content, ash content, water absorption and pH. The chemical analysis indicated an increase in alpha cellulose content and decrease in lignin and hemicellulose for the cellulose microfiber. Betel nut fibers were treated with of sodium hydroxide. Betel nut fibers were bleached with hydrogen peroxide for the removal of lignin and whiteness. The whiteness of cellulose fibers were characterized by Reflection Transmission Color Densitometer. Cellulose microfibers were characterized by different techniques such as FT IR, TG-DTA, SEM, and XRD.

Keywords: betel nut fibers, cellulose microfiber, isolation, characterization

Introduction

Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundred to many thousands of β (1-4) linked D-glucose units (Figure 1) (Khalil et al., 2014). Cellulose is an important structural component of the primary cell wall of green plants. Cellulose is the most abundant renewable carbon resource on earth.

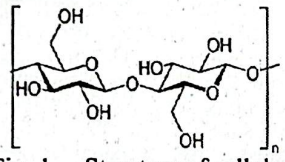


Fig. 1 Structure of cellulose

Cellulose is the most abundant polymer in the world. Cellulose provides strength and stability to the plant cell walls and the fiber. Additionally, these polymers are cheap and inexpensive. It is a sources renewable and biodegradable organic material (Dufresne, 2006 and Hansson *et al.*, 2009).

The amount of celluloses depends on the fiber's properties. These properties influence economics of the fiber production and the utility of the fiber in various applications (Redyy and Yang, 2005). Cellulose fibers can be used in many areas. They are reinforce base for biodegradable starch films (Anglès and Dufresne, 2000 and Babaee *et al.*, and Ashori, 2015), biomedical field (Costa *et al.*, 2013), and tissue engineering (Nasri-Nasrabadi *et al.*, 2014). cellulose micro and nanofibers having a bright future in various applications including papermaking additives, thickening agents for food and cosmetic products, and various medical applications (Dinand *et al.*, 1999 and Turbak *et al.*, 1983).

The physical and chemical properties of cellulose and its chemical reactivity and behavior are strongly influenced due to the arrangement of the hydroxyl group and hydrogen bonding with the relation to each other and to the fiber axis. Native cellulose is arranged by sequence like amorphous region and crystalline region. The amorphous region could get wrecked and the crystalline region isolated (Gardner *et al.*, 2008).

Among the various natural fibers, betel nut fiber is characterized as extremely strong and low weight. The fibers are predominantly composed of cellulose and varying proportions of hemicellulose, lignin, pectin and other materials. These fibers have special advantages in comparison to other synthetic fiber in that they are abundantly available from a renewable resource and also biodegradable. The palms have been cultivated as gardens and hedges in Tanintharyi Region, Mon State, Kayin State and Rakhine State in the Myanmar (Mg Saw Win, 2018).

Betel nut is the fruit of Areca palm tree (Areca catechu), a species of palm, which is native of Myanmar and widely grows across Asia, Taiwan and India. Betel nut is often chewed with betel leaf and lime, an ancient practice which has been carried throughout generation. The fruit is in round or oval shape, with the color of golden yellow to orange. The betel nut (BNH) is the fibrous part of the fruit, which equals to approximately 60-80% of total volume and weight of betel nut. The part is the outer cover of the areca fruit (Yusriah *et al.*, 2012).

It is a all slender palm, thin, with a smooth whitish unbranched stem, reaching a height of 40-60 ft. The trunk grows remarkably straight, 40-50 ft high. The stem is surrounded by a crown of pinnate are confluent. The lower portion of the petiole is expanded into a broad, tough, shealth-like growth (Theingi Tun, 2015).

The fruit is generally ovoid, about 1 to 2 inches in width, 2 $\frac{1}{2}$ inches in length and is brightly orange or scarlet when fully ripe. The pericarp (65%) is hard and fibrous, and the kernel (speed, 35%) called the areca nuts vary greatly in size and shape (Figure 2). The hardness and astringency of the nuts also show considerable variations. Some nuts are large, flat and are almost bitter. Others are conical or spherical, and so bland in taste (Thandar Win *et al.*, 2000).

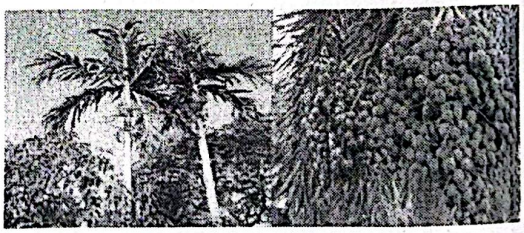


Fig. 2 The plant and fruit of betel nut

II. Materials and Methods

Materials

All necessary research facilities were provided by Department of Chemistry. All specific chemicals used were of reagent grade and products of BDH chemicals Ltd Poole England.

Collection and Isolation of Betel Nut Fibers

Betel nut husk waste samples were collected from Dawei Township, Tanintharyi Region. Betel nut husk wastes were soaked in water at room temperature for 5 days to loosen the fiber. The

betel nut fibers were separated manually from the nut part by hand stripping method and washed thoroughly with distilled water before drying in oven at 60 °C for 24 h. The dried betel nut fibers were kept in desiccators to keep the fibers from the moisture from the temperature. The isolation of cellulose microfiber was performed by classical chemical treatment such as alkali treatment, bleaching processes and acid hydrolysis.

Some Physicochemical Properties of Betel Nut Fibers

The moisture content determined by balancing methods and the ash content was analyzed by Muffle furnace at 600 °C for 5 h. Water absorption was determined after immersion of the samples in water at room temperature for 24 h. The betel nut fibers were soaked in distilled water and then pH of betel nut fibers was determined by pH meter.

Isolation of the Cellulose Microfibers from Betel Nut Fibers

The sample were dried in an oven 24 h and then cut into small pieces before delignification with NaOH solution. Betel nut fibers were immersed in various NaOH solution concentrations (0.5, 1, 1.5 and 2) for 24 h and then heated and stirred at 70 °C for 4 h to produce fibrils. After treated, the fibers were cooled in air, filtered quantitatively and then washed with distilled water until the pH of 7. The alkali treated fibers were undergone bleaching treatment using hydrogen peroxide and sodium silicate. The alkali treated fibers were bleached with 10, 15, 20, 25, 30, 35 and 40 % of w/w H_2O_2 in 30 % of w/w sodium silicate solution at 50 °C for 1 h. This bleaching step was treated twice. The bleached pulps were cooled, filtered and then washed. They were dried with air in room temperature and then transferred into the desiccator. The bleached fibers were treated with 2 M hydrochloric acid and were stirred for 90 min. The suspension was washed with distilled water until the pH become natural and then it was filtered. And then the cellulose microfiber was dried with air in room temperature.

Characterization of Raw, Alkali, Bleached and Acid Hydrolyzed Betel Nut Fibers

Fourier Transform Infra-red (FT IR) spectroscopy was used in examination of changes in the chemical composition of the cellulose fibers after chemical treatment. The untreated betel nut fibers were derived from the aqueous suspension of treated betel nut fibers was analyzed. The cellulose samples that were prepared using the different reaction conditions were also evaluated by FT IR spectroscopic technique. Thermogravimetric-Differential Thermal Analysis (TG-DTA) was measured at the Universities' Research Centre (URC). Thermogravimetric analysis was performed for the study of the thermal characteristics of the raw and acid hydrolyzed fibers of betel nut fibers. Bleaching of betel nut fibers were examined by using Reflection Transmission Color Densitometer at the Universities' Research Centre (URC). The surface morphology of the sample was analyzed by using a scanning electron microscope at the accelerating voltage of 15 KV. Raw and acid hydrolyzed betel nut fibers were qualitatively analyzed by X-ray diffractometer at the Universities' Research Center (URC). The average crystalline size was calculated by Debye Scherrer equation.

III. Results and Discussion

Some Physicochemical Properties of Betel Nut Fibers

Moisture content of natural fiber determines the dimensional stability, electrical resistivity, tensile strength and swelling behavior of natural fiber reinforced composites. Therefore, low moisture content of dried betel nut fibers is favorable in fabricating betel nut fibers reinforced polymer composites with high dimensional stability (Yusriah *et al.*, 2012). The moisture content of betel nut fibers was found to be 8.44. Ash is the solid remains of fires. Specially, it refers to all non-gaseous, non-liquid residues after a complete combustion. In this study, ash contents in betel nut fibers were found to be 1.53 %. The cellulose content in the fiber also influences water absorption in natural fiber where water absorption increased with the increase in cellulose content due to the increase in amount of free hydroxyl groups (Yusriah *et al.*, 2012). In this study, Water absorption of betel nut fibers were found to be 297.35 %. The pH of betel nut fibers was studied. The pH of betel nut fibers was found to be 7.55 (Table 1 and Figure 3).

Table 1. Some Physiochemical Properties of Betel Nut Fibers

Physicochemical Properties	Content Percent (%)
Moisture Content	8.44
Ash Content	1.53
Water Absorption	297.35
pH	7.55

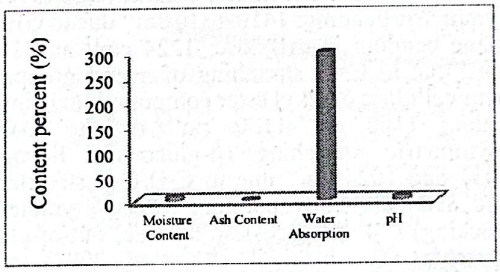


Fig. 3 Some physicochemical properties of betel nut fibers

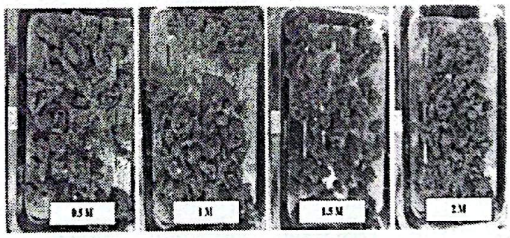


Fig. 4 Alkali treated betel nut fibers

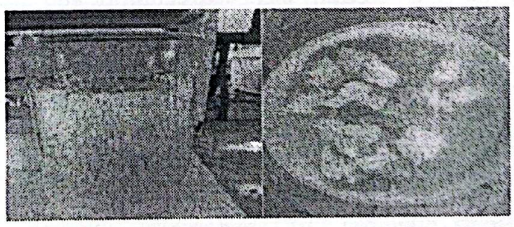


Fig. 5 Betel nut fibers bleached with 30% of w/w hydrogen peroxide in 30 % of w/w sodium silicate solution

Fig. 6 Acid hydrolyzed betel nut fibers with hydrochloric acid

FT IR Measurement of Raw and Alkali Treated, Bleached and Acid Hydrolyzed Betel Nut Fibers

Fourier Transform Infrared spectroscopy (FT IR) was used to analyze the presence of functional groups that included in raw, alkali treated, bleached and acid hydrolyzed of betel nut fibers. The cellulose, hemicellulose and lignin of the infrared spectra are especially composed of different oxygen containing functional groups such as esters, ketones and alcohols (Erdtman, 2003). FT IR spectral data of raw, alkali treated, bleached and acid hydrolyzed fibers of betel nut fibers were shown in Figure 7.

FTIR spectra showed the following characteristic vibration peaks of cellulose: 3600-3200 cm^{-1} region related to O-H stretching vibration of the hydroxyl groups in cellulose molecules of the fibers; 2905-2875 cm^{-1} due to CH and CH_2 stretching; 1718 cm^{-1} is due to stretching of the acetyl ester and carbonyl aldehyde group (C=O) of hemicellulose and lignin; 1680- 1620 cm^{-1} corresponding to OH from absorbed water; 1600-1500 cm^{-1}, which due to C=C aromatic skeletal vibrations of lignin; 1420 cm^{-1} due to CH_2 symmetric bending; 1410-1310 cm^{-1} due to OH in-plane bending in cellulose; 1224 cm^{-1} and 1236 cm^{-1} due to C=O stretching of acetyl groups in hemicellulose or alkyl ester compounds existing in lignin; 1160 cm^{-1}-1130 cm^{-1} due to C-O-C asymmetric stretching (β-glucosidic linkage); 1031 and 1024 cm^{-1} due to C-O/C-C stretching; and 898 cm^{-1} corresponding to the asymmetric (rocking) C-1 (β-glycosidic linkage) out-of plane stretching vibrations (Carrillo *et. al.*, 2004). In all FT IR spectra, the band observed at round 3336 cm^{-1} is attributed to O-H stretching vibration of the hydroxyl groups in cellulose molecules of the fibers and at around 1369 cm^{-1} OH bending vibration of cellulose. The presence of a peak around 2917, 1718, 1570 cm^{-1}, which could be assigned to C-H stretching vibration from the – CH_2 group of cellulose and hemicelluloses, stretching of the acetyl ester and carbonyl aldehyde group (C=O) of hemicellulose and lignin and C=C aromatic skeletal vibrations of lignin, respectively. (Alemdar and Sain, 2008). The intensity of these bands decreased in the IR spectra of the alkali treated, bleached cellulose and acid hydrolyzed fibers. These results was due to the amount of cellulose was high, as confirmed by the presence of many bands characterizing these materials, hemicellulose were completely removed from the extracted fibers and the absence of the majority of characteristics lignin peaks in the spectra of acid/alkaline treated fibers, as well as for the cellulose microfiber, which was believed to be due to the removal of lignin after the chemical treatments.

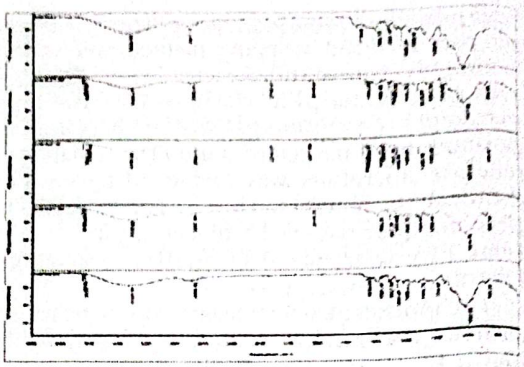
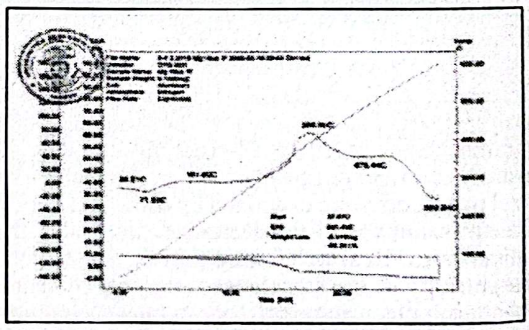


Fig. 7 FT IR measurement of raw and alkali treated, bleached and acid hydrolyzed betel nut fibers

TG-DTA Measurement of Raw Betel Nut and Acid Hydrolyzed Cellulose Fiber

TG-DTA thermogram of raw betel nut fiber and acid hydrolyzed cellulose fiber were shown in Figure 8. In the first stage, temperature ranges between about the temperature range is 38 °C to 172 °C with 11.27 % of weight loss in raw and 38 °C to 278 °C with 17.67 % of weight loss in acid hydrolyzed cellulose fibers are due to the loss of moisture. In the second, the temperature range between 172 °C to 400 °C with 55.86 % of weight loss in raw and 278 °C to 375 °C with 60.24 % of weight loss in acid hydrolyzed cellulose fibers are due to the degradation of hemicellulose and lignin. The third, temperature range between 400 °C to 600 °C with 27.39 % of weight loss in raw and 375 °C to 600°C with 18.07 % of weight loss in acid hydrolyzed cellulose fibers are due to the degradation of cellulose. The total weight loss percent in raw and acid hydrolyzed cellulose observed 93.241 % and 96.466 %, respectively.

Due to the removal of lignin and hemicellulose the weight loss of cellulose microfiber is lower than that of crude fibers at temperature between 375 °C and 448 °C. The onset temperature of degradation of crude fiber of betel nut fibers was 589 °C, whereas that of the cellulose microfiber of betel nut fibers decreased up to 448 °C. The residue of raw betel nut fibers and cellulose microfiber was 27.39 % and 18.07 %, respectively (Figure 9). The higher amount of residual content of crude fibers is mainly due to the lignin and hemicellulose content of the fibers. Removal of hemicellulose and lignin after bleaching and alkali treatment caused a lower amount of residue for cellulose microfibers.



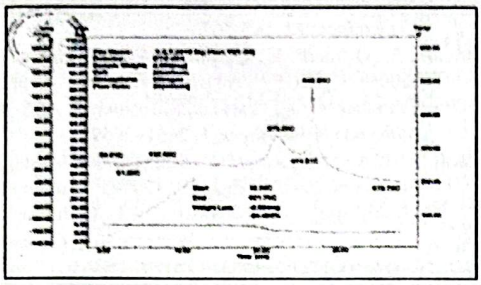


Fig. 8 TG-DTA thermogram of raw betel nut fibers and acid hydrolyzed betel nut fibers

Reflection Transmission Color Densitometer Measurements of Bleached Betel Nut fibers

The alkali treated betel nut fibers were bleached with (10, 15, 20, 25, 30, 35 and 40 %) volume by volume of hydrogen peroxide (H_2O_2) and (30 %) volume by volume of sodium silicate. The bleach pulps of betel nut fibers were characterized on the color (Figure 9). The suitable condition for bleaching should be the one that can remove higher amount of lignin from the fiber and yield high whiteness value. Although, the high concentration of hydrogen peroxide (30 %) contributed to the low whiteness value.

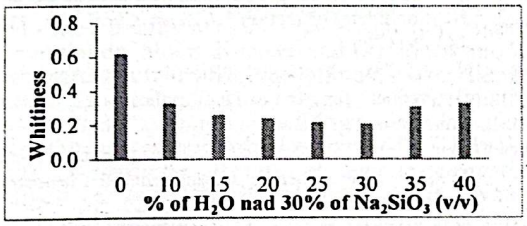


Fig. 9 Color density of betel nut fibers

Scanning Electron Microscopy (SEM) Examination on the Morphology of Raw and Acid Hydrolyzed Betel Nut Fibers

Morphological features of the raw and acid hydrolyzed betel nut fibers were examined by Scanning Electron Microscopy (SEM) as shown in Figure 10. The SEM microphotographs of the raw betel nut fibers were observed amorphous nature. The SEM microphotographs of acid hydrolyzed betel nut fibers were found that needle shape nature of surface morphology.

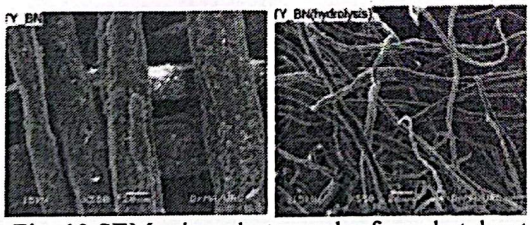


Fig. 10 SEM microphotograph of raw betel nut fiber and acid hydrolyzed betel nut fibers

X-Ray Diffraction (XRD) Analysis of Raw and Acid Hydrolyzed Fibers

XRD analysis was used to investigate the image of the crystalline behaviour of cellulose fibers. Figure 11 show the corresponding X-ray diffractograms of raw fibers and cellulose microfiber. The diffractogram of raw betel nut fibers shows peaks at 2θ values around 14°, 16° and 22°, which correspond with the peaks mentioned for cellulose I structure, that indicated the crystal integrity had been maintained (Nishiyama, *et al.*, 2003). The acid hydrolyzed fibers can be seen peaks at 2θ values around 20.03°, 22.28°, 26.10°, 26.60°, 28.13°, 30.72°, 36.07° which correspond with the peaks mentioned for cellulose II (Langan, *et al.*, 2001). This indicates that the cellulose I (raw fiber) completely transformed into cellulose II (cellulose microfiber) through alkali treatment, bleaching and acid hydrolysis process. The acid hydrolyzed fibers of betel nut fibers of amorphous region were disappeared and the crystalline peaks were appeared. The average crystallite size of the cellulose microfiber was calculated by using Debye-Scherrer equation and by using XRD software. The average crystalline size of cellulose microfibers were found to be 0.031 μm (by using Debye-Scherrer equation) and 0.038 μm (by XRD software), respectively.

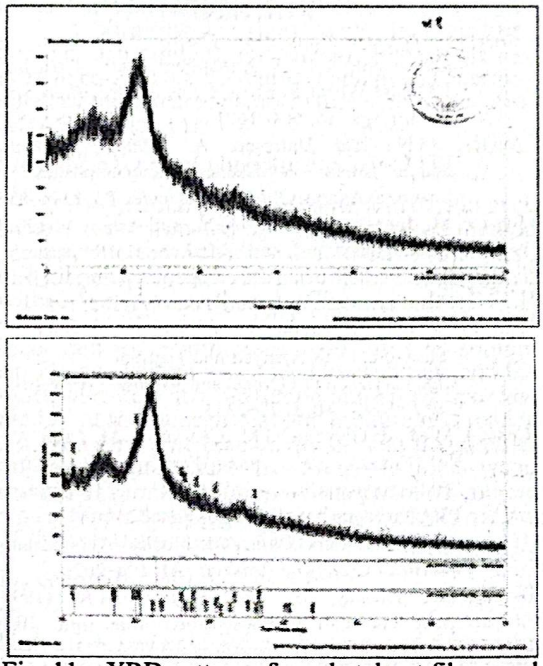


Fig. 11 XRD pattern of raw betel nut fibers and acid hydrolyzed betel nut fibers

Conclusion

Cellulose microfibers were isolated from betel nut fibers. Some physicochemical properties of betel nut fibers were determined such as moisture content, ash content, water absorption and pH. The betel nut fibers were treated with sodium hydroxide. 2 M of sodium hydroxide solution is the suitable concentration due to the fiber that can be separated into many fibrils than others. Alkali treated fibers was bleached with 30% volume by volume of hydrogen peroxide in 30% volume by volume of sodium silicate. And then bleached fibers were hydrolyzed with 2 M of hydrochloric acid. The resultant of acid hydrolyzed fibers of betel nut fibers were characterized by FT IR, TG DTA, EDXRF, XRD and SEM techniques. According to FT IR spectral data of acid hydrolyzed betel nut fibers, it can be noted that there is an absorption band at 1730 cm^{-1}, 1594 cm^{-1}, 1507 cm^{-1}, and 1236 cm^{-1} for the raw betel nut fibers, which disappeared in the cellulose microfiber. It was observed that C-H stretching vibration of alkyl group, C=O stretching vibration of carbonyl group and C=C aromatic skeletal vibration were disappeared in these spectra because hemicellulose and lignin were removed. In TG DTA thermogram of raw and acid hydrolyzed of betel nut fibers, the total weight loss percent was observed 93.241 % and 96.466 %.

SEM images of betel nut fibers and cellulose microfibers indicated that the nature of fibers. XRD diffractograms of raw samples of betel nut fibers, it was found that amorphous cellulose and acid hydrolyzed betel nut fibers observed the crystalline shapes. The crystallite sizes of betel nut fibers were observed 0.031 μm by using Debye-Scherrer equation and 0.038 μm by XRD software.

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References

Alemdar, A and Sain, M. (2008) " Isolation and Characterization of Nanofibers from Agricultural Residues-Wheat Straw and Soy Hulls," *Bioresource Technology*., **99**, 664- 1671.

Anglès, M.N., and Dufresne, A. (2000). "Plasticized Starch/Tunicin Whiskers Nanocomposites. 1. Structural Analysis," *Macromolecules*, **33**, 8344–8353.

Babaee, M., Jonoobi, M., Hamzeh, Y., and Ashori, A. (2015). "Biodegradability and Mechanical Properties of Reinforced Starch Nanocomposites Using Cellulose Nanofibers," *Carbohydrate Polymers*, **132**, 1–8.

Carrillo, F, Colom, X, Suñol, J.J, and Saurina, J. (2004). "Structural FTIR Analysis and Thermal Characterization of Lyocel and Viscose-Type Fibers," *Eur Polym J.*, **40**, 2229–2234.

Costa, L.M.M., de Olyveira, G.M., Cherian, B. M., Leão, A.L., de Souza, S.F., and Ferreira, M. (2013). "Bionanocomposites from Electrospun PVA/Pineapplenanofibers/Stryphnodendron Adstringens Bark Extract for Medical Applications," *Industrial Crops and Products.*, **41**, 198–202.

Dinand, E., Chanzy, H., and Vignon, M.R. (1999). "Suspensions of Cellulose Microfibrils from Sugar Beet Pulp," *Food Hydrocolloid*, **13**, 275–283.

Dufresne, A. (2006). "Comparing the Mechanical Properties of High Performances Polymer Nanocomposites from Biological Sources," *Journal of Nanoscience and Nanotechnology*, **6**, 322–330.

Erdtman, H. (2003). "Lignins: Occurrence, Formation, Structure and Reactions," *J Polym Sci Pol Lett.*, **10**, 228–230.

Gardner, D.J., Oporto, G.S., Mills, R., and Azzi Samir, M.A.S. (2008). "Adhesion and Surface Issues in Cellulose and Nanocellulose," *J. of Adhesion Science Technology*, **22**, 545-567.

Hansson, S., Östmark, E., Carlmark, A., and Malmstr (2009). "ARGET ATRP Forversatile Graf Cellulose Using Various Monomers," *ACS Materials & Interfaces*, **1**, 2651–2659.

Khalil, H.P.S.A., Davoudpour, Y., Islam, M.N., Mustap Sudesh, K., and Dungani,R. (2014). "Produc Modification of Nanofibrillated Cellulose Various Mechanical Processes," *Carboh Polymers*, **99**, 649–665

Langan, P., Nishiyama, Y., and Chanzy. H. (2001). "X-ra Structure of Mercerized Cellulose II at 1 Å Resolution," *Biomacromolecules.*, **2**, 410–416.

Mg Saw Win. (2018). "A Study on The Preparation Performance of A Particleboard Derived from Nut Fiber Treated with Cashew Nut Shell L Yangon: PhD(Thesis), Department of Chem University of Yangon.

Nasri-Nasrabadi, B., Mehrasa, M., Rafienia, M., Bonakda Behzad, T., and Gavanji, S. (2014). "P Starch/Cellulose Nanofibers Composite Prepared Salt Leaching Technique for Tissue Engine *Carbohydrate Polymers.*, **108**, 232–238.

Nishiyama, Y., Sugiyama, J., Chanzy, H., and Langan, (2003). "Crystal Structure and Hydrogen Bon System in Cellulose I, a from Synchrotron X-ra Neutron Fiber Diffraction," *J. Amer. Chem. Soc.*, 14300-14306.

Redyy, N., and Yang, Y., (2005). "Bio-fiber from Agric By-product for Industrial Applications," *Tre Biotechnol*, **23**, 22-27.

Thandar Win. (2000). "The Analysis of Metals from Betel N Yangon, MSc (Thesis), Department of Chemis University of Yangon.

Theingi Tun. (2015). "Preparation Cellulose Nanofibers from *COCOS NUCIFERA* (Coconut Coir Fibers) and *ZEA MAYS L.* (Corn Husk Yangon, MSc (Thesis), Department of Chemis University of Yangon.

Turbak, A.F., Snyder, F.W., and Sandberg, K.R.J. (19 "Microfibrillated Cellulose, a New Cellulose Prod Properties, Uses and Commercial Potential," *J Ap Polym Sci*, **37**, 815–827.

Yusriah, L., Sapcan, S.M., Zainudin, E.S., and Mariatti, M (2012). "Exploring the Potential of Betel Nut Fiber Reinforcement in Polymer Composites; Effect of Fi Maturity". *Procedia Chemistry*, **4**, 87-94.

Investigation of Some Biological Activities of *Anthocephalus* Candamba Roxb. (Ma U) Bark

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Abstract

Anthocephalus cadamba Roxb. (Ma u) is one of the important medicinal plants. This research aims to investigate nutritional value, total phenolic contents and some biological activities such antioxidant, antimicrobial activities and cytotoxic effect. Determination of nutritional values were 9.05 % of moisture, 10.33 % of ash, 3.58 % of protein, 34.81 % of fiber, 1.04 % of fat, 41.19 % of carbohydrate and energy value of 188.44 Kcal /100 g on the basis of dried sample. The total phenolic content of watery and ethanol extracts were found to be (28.01 ± 0.016) and (36.98 ± 0.013) µg GAE/mg respectively. In the screening of the antioxidant activity, IC_{50} values of ethanol extract 3.33 µg / mL was found to be more potent than watery extract 7. 21 µg/mL. The antimicrobial activities of ethyl acetate, methanol and ethanol extracts were determined against six strains of microorganisms with the inhibition zone diameters in the range between 12-23 mm. The cytotoxicity of watery and ethanol extracts of bark of *A. cadamba* Roxb. was free from cytotoxic effect until 1000 µg/mL concentration.

Keywords : *Anthocephalus cadamba* Roxb., total phenol contents, antioxidant activity, cytotoxicity

Introduction

Anthocephalus cadamba Roxb. (Ma u) is one of the important medicinal plants in India, belonging to the Rubiaceae family. *A. cadamba* are commonly known as *Cadamba* and *Kadamba* (Sanskrit and Hindi), Bur-flower tree (England), sako (Laos), laran (Malaysia) and ma u or ye ma u (Myanmar). It is an ever green tree found in different parts of India, Nepal, Laos, Cambodia, China, Malaysia and Myanmar [1]. *A. cadamba* Roxb. is widely distributed thoursoughout the greater part of India and is used as a folk medicine in the treatment of fever, anemia, uterine complaints, blood diseases and skin diseases. It is also treatment of various aliments like diabetes mellitus, diarrhea, fever, inflammation, hemoptysis , cough, vomiting, wounds, ulcers, debility and antimicrobial activity [2].The bark of the *A.cadamba* Roxb. is anti-inflammatory, digestive, and diuretic property and is given to treat and inflammation of eyes. *A.cadamba* bark is used in the treatment of hoarseness of thoursoat. In traditional medicine in Indian, bark and roots of the tree are used for fevers, colic, coughs and muscular pains, burning sensations in the stomach, poisoning and female problem [3]. This research work was to investigate the determination of total phenol contents; total flavonoid contents and some biological activities such as, antioxidant, antimicrobial activities and cytotoxic effect.

Fig. 1 Photograph of plant and bark of *A. cadamba* Roxb.

Materials and Methods

In this research, bark of *A.cadamba* Roxb. (Ma u) were collected from Ayeyarwady Region, Myanmar. Dried powdered sample (100g) was weighed and placed in two different conical flasks. The 500 mL of ethanol and distilled water was added into each flask respectively and stopped with a cork. The sample was allowed to macerate and placed in a shaker, shaken continuously for about 6 hour and the suspension was allowed to stand for 18 hr. The extract was filtered rapidly taking care not to lose any solvent, and transferred to porcelain basin and evaporated to dryness on water bath. The dried filtrate was then placed in oven, maintained till constant weight, at 105°C. The crude extracts were used to investigate for their chemical and biological activities.

Determination of Nutritional Values

Nutritional values such as moisture content, ash content, fat content, fiber content, protein content, carbohydrate content and energy value of the selected sample were determined by AOAC method [4].

Procedure for total phenolic content (TPC)

The total phenolic content (TPC) in each sample was estimated by Folin-Ciocalteu method according to the procedure described by [5]. First, 0.5 mL of prepared extract solution was mixed with 0.5 mL of methanol. Then, 5 mL of FCR reagent (1:10) was added to the mixture and incubated for 5 minutes. 4 mL of 1 M sodium carbonate solution was added to each tube and the tubes were kept at room temperature for 2 hour and the UV absorbance of reaction mixture was read at λ_{max} 765 nm. The blank solution was prepared as the above procedure by using distilled water instead of sample solution. Total phenolic content was estimated as µg gallic acid equivalents per mg of different extract (mg GAE / mL).

Procedure for antioxidant activity

The free radical scavenging activity of crude of bark of *A. cadamba* Roxb. was measured by using DPPH free radical scavenging assay [6]. DPPH free radical scavenging activity of ethanol and water extracts of bark of *A.cadamba* Roxb. was determined by UV-visible spectrophotometer [7]. The control solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of ethanol in the brown bottle. The sample solution was also prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of test sample solution. These bottles were incubated at room temperature and were shaken on shaker for 30 minutes. After 30 minutes, the absorbance of different concentrations (1.562, 3.125, 6.25, 12.5, 25 and 50 µg / mL) of tested sample was measured at 517 nm using UV-7504 spectrophotometer. Absorbance measurements were done in three times for each concentration and the mean value so obtained were used to calculate percentage of radical scavenging activity (% RSA) by the following equation. % RSA of crude extracts of bark of *A. cadamba* Roxb. and standard ascorbic acid .

$$\% \text{ RSA} = [A_{DPPH} - (A_{Sample} - A_{Blank}) / A_{DPPH}] \times 100$$

Where,

% RSA = % radical scavenging activity of test sample

A_{DPPH} = absorbance of DPPH in EtOH solution

A_{Sample} = absorbance of sample + DPPH solution

A_{Blank} = absorbance of sample + EtOH solution

The antioxidant power (IC_{50}) is expressed as the test substances concentration (µg / mL) that result in a 50 % reduction of initial absorbance of

DPPH solution and that allows to determine the concentration. IC_{50} (50 % inhibition concentration) values were calculated by linear regressive excel program.

Procedure for Antimicrobial Activity

The antimicrobial activity of four crude extracts such as petroleum ether, ethyl acetate, ethanol and water of bark of *A. cadamba* Roxb. (Ma u) was determined against six strains of microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* by employing agar well diffusion method [9].

Procedure for determination of cytotoxicity

The brine shrimp (*Artemia salina*) was used in this study for cytotoxicity bioassay [8]. Test solution (0.5 mL) was mixed with 4.5 mL artificial sea water and placed in the chamber of ice cup. Alive brine shrimp (10 napullis) were taken with Pasteur pipette and placed into each chamber which was kept at room temperature for about 24 hours. After 24 hours incubation, the number of survival brine shrimp was counted and 50 % lethality dose (LD_{50}) was calculated. The control solution was prepared as the above procedure by using distilled water instead of sample solution [10].

Result And Discussion

Nutritional values such as moisture, ash, protein, fiber, carbohydrate, fat and energy values in the bark of (Ma u) were determined. It contains moisture (9.05 %), ash (10.33 %), fiber (34.81 %), fat (1.04 %), protein (3.58 %), carbohydrate (41.19 %) and energy value is (188.44) kilocalories in 100 g of sample. The relatively high moisture content of the plant indicates a possible reduction in plant shelf life. The ash percent is a measure of the quality of food. The smaller the ash content is the better the food quality. Low fat foods are known to reduce cholesterol. Higher fibre content in food causes intestinal irritation and lower bioavailability. The carbohydrate content of these sample even though appear to be high, they have many medicinal properties. According to the results, the presence of the important nutrients like fat, fibre, protein, carbohydrate and the physical properties like moisture and ash mean the selected sample could be used as a nutritionally valuable and healthy ingredient to improve traditional medicinal formulation and to treat many diseases.

Total Phenol Contents

The total phenol content (TPC) of watery and ethanolic extracts of bark of *A. cadamba* Roxb. is shown in Table 1. According to the results, the higher TPC (µg GAE / mL) was detected in ethanol (36.98 µg GAE / mL) than watery (28.01 µg GAE / mL) extracts. This means that phenolic compounds are more soluble in ethanol. Comparison of TPC in watery and ethanol extracts of bark of *A. cadamba* Roxb. is represented in Figure 2.

TABLE 1. Total Phenol Contents in Watery and Ethanol Extracts of Bark of *A.cadamba* Roxb.

Samples	Total Phenolic Contents (mg of GAE/g of extract)
Watery	28.01 ± 0.016
Ethanol	36.98 ± 0.013

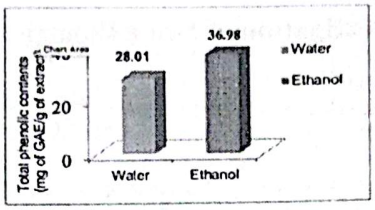

Fig. 2. Total phenolic contents of watery and ethanol extracts of bark of *A. cadamba* Roxb

Antioxidant Activity

The antioxidant activity of watery and ethanolic extracts of bark of *A. cadamba* was evaluated by DPPH (2, 2-diphenyl 1-picrylhydrazyl) radical scavenging assay. Ascorbic acid was used as standard. It was found that as the concentration were increased, the absorbance values were decreased. The larger the % RSA indicates the higher antioxidant activity. In contrast, the lower the IC_{50} indicates the more effective antioxidant activity. The radical scavenging activity of *A. cadamba* bark ethanol extract (IC_{50} = 3.33 µg / mL) was found to be higher antioxidant activity than water extract (IC_{50} = 7.21 µg / mL). However it was found to be lower than that of standard ascorbic acid (IC_{50} = 1.71 µg / mL) in antioxidant activity. The result of IC_{50} value of two extracts and standard ascorbic acid are tabulated in Table 2 and showed in Figures 3.

TABLE 2. . IC_{50} value of ethanol and watery extracts and ascorbic acid (Std.)

Samples	IC_{50} (µg / mL)
95 % ethanol	3.33
Water	7.21
Ascorbic acid (Std.)	1.71

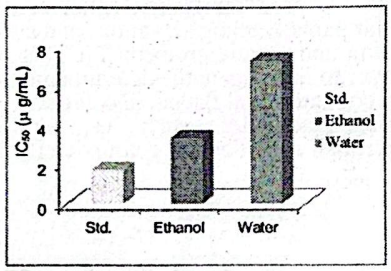

Fig. 3. IC_{50} value of ethanol and watery extracts and ascorbic acid (Std.)

Antimicrobial Activity

Antimicrobial activity of five crude extracts (water, ethanol, methanol, ethylacetate, petether) obtained from bark of *Anthocephalus cadamba* Roxb. (Ma u) were investigated on 6 different strains of microorganisms such as *Bacillus subtilis, Staphylococus aureus, Pseudomonus aeruginosa, Bacillus pumilus, Candida albicans,* and *Escherichia coli* and by agar well diffusion method. The measurable zone diameter, including the well diameter, shows the degree of antimicrobial activity. The well diameter is 10 mm in this experiment. The larger zone diameter, the more activity on the test organisms.The results of antimicrobial activity were shown in Table 3. It was found that pet-ether and water extracts of (Ma u) bark did not showed any antimicrobial activity against all of the microorganisms tested. Ethanol and methanol extracts exhibited also microorganisms in the range between 12 ~ 15 mm. Ethyl acetate extract showed highest antimicrobial activity with the inhibition zone diameters range 18 ~ 23 mm against all six

microorganisms test. From this investigation, it can be deduced that (Ma u) bark may be slightly effective for the treatment of some diseases such as arthritis, skin rashes, kidney failure or neurological problems, infected by the test microorganisms.

TABLE 3. Inhibition Zone Diameter of Various Extracts of Bark of *A. cadamba* Roxb. (Ma u)

Organisms	Inhibition Zone Diameter of Extracts (mm)				
	PE	MeOH	EtOAc	EtOH	H_2O
Bacilus subtilis	-	12	23	15	-
Staphylococcus aureus	-	13	18	13	-
Pseudomonas aeruginosa	-	12	18	12	-
Bacillus pumilus	-	12	23	14	-
Candida albicans	-	12	20	13	-
Escherichia coli	-	12	20	13	-

Diameter of agar well=10mm, 10mm ~ 14mm = resistant, 15mm ~ 19mm =intermediate, 20mm above = susceptible, No activity = (-)

Cytotoxic Effect

The cytotoxicity of crude extracts was expressed in terms of means ± SEM (standard error mean) and LD_{50} (50 % Lethality Dose) and the results are shown in Table 3. In this experiment, potassium dichromate ($K_2Cr_2O_7$) and caffeine were used as standard. Potassium dichromate is generally used as the positive control for this brine shrimp bioassay and caffeine, a natural product, DMSO and artificial sea water, as negative control. The nauplli were counted against a lighted background after 24h initiation of test. Nauplli were considered death at each dose and as the control were determined. From these results, LD_{50} values of watery and ethanol extracts showed (>1000 µg / mL). Standard caffeine did not show cytotoxicity until 1000 µg / mL concentrations whereas LD_{50} of standard $K_2Cr_2O_7$ was 1.5 µg / mL.

Conclusion

The bark of *A. cadamba* (Ma u) are rich sources of fibre and carbohydrate. The total phenolic content of ethanol extract was found to be higher than that of watery extracts. And then the bark of *A. cadamba* exhibited promising antioxidant and antimicrobial activities. The effects of cytotoxicity of both extracts were not found the maximum dose of 1000 µg / mL.

Acknowledgment

The authors feel indebted to Professor Dr. Ni Ni Than, University of Yangon, Yangon, Myanmar, for her stimulating suggestions.

References

[1] A. Dubey, S. Nayak and D. C. Goupale, "*Anthocephalus cadamba*: A Review", Pharmacog. J., vol. 2, pp. 71-76, January 2011.

[2] Vartak, V. D., M. S. Kumbhojkar and D. S. Nipunage, "Sacred Groves of Tribal Areas Along the Western Ghat Treasure Trove of Medicinal Plants". Bull Med. Ethnobot Res., vol. 8, pp. 77-84, 1987.

[3] P. M. Ram and S. Liza, "Antibacterial Properties of *Anthocephalus cadamba* Fruits", Asian Journal of Plant Science and Research, vol.1(2), pp.1-7, 2011.

[4] K. Helrich, "Official Methods of Analysis of the association of official Analytical Chemists", United State of America: 15th Ed., Association of Official Analytical Chemists, Inc., 1990, pp. 77-80.

[5] M. Saxena, J. Saxena, R. Nema, D. Singh and A. Gupta, "Phytochemistry of Medicinal Plants", Journal of Pharmacognosy and Phytochemistry, vol. 1(6), pp. 168-182, 2013.

[6] K. Balaji, N. A. Sangeetha, D. Tassha, S. Mukesh and F.Shivkanya, "Phytochemical analysis and in *vitro* antioxidant activity of *Parkia speciosa*", Pharmaceutical chemistry unit, faculty of pharmacy, vol. 46, pp. 81-8, 2015.

[7] G. B. Marini-Bettolo, M. Nicolettic and M. Patamia, "Plant Screening by Chemical and Chromatographic Procedure Under Field Conditions". J. Choursomato., vol. 45, pp. 121-123, 1981.

[8] N. Ali, U. Allen, S. W. A. Shaha, I. Shaha, M. Junaid, G. Ahmed, W. Ali and M. Ghias, "Acute Toxicity, Brine Shrimp Cytotoxicity, Anthelminitic and Relaxant of Fruits of Rubus Fruiticosis Agg". BMC Complement. Alter. Med., vol. 13, pp.138-143, 2013.

[9] S. M Finegold, W. J. Martin and E. G. Scott, "Diagnostic Microbiology" London: Mos by Co. Ltd., 1976, pp. 586-588.

[10] Dockery, M. and S. Tomkins. *Brine Shrimp Ecology*. London: 1st ed., The British Ecology Society, 2000, pp. 92-93.

Encapsulation of Natural Food Colourant Extracted from Red Cabbage and Determination of Some Physical Properties

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Abstract

Today consumers are increasingly associating natural food colorants to health benefits. The natural food colorant are used inplace of synthetic food dyes. Anthocyanins as a natural food colourant are rich source of red cabbage. The phytochemical screening of red cabbage was determined. The anthocyanin was extracted from the red cabbage using distilled water, acidified ethanol and 2% citric acid solution. The different pH values of extracted anthocyanin solutions were utilized in coloring of foods. Total anthocyanin content of 2 % citric acid extract was determined by pH differential method. Some qualitative tests of anthocyanin such as ferric chloride test, dilute hydrochloric test and dilute sulphuric acid test were carried out. Anthocyanin extract was encapsulated, using the mixture of gum arabic and maltodextrin at a 1:1 ratio as wall materials by microwave - assisted drying at 1100 W. Tween – 80 was used as emulsifying agent. The encapsulated powder was analysed for pH, moisture and solubility in water by using the powder characterization technique.

Keywords : Anthocyanin, red cabbage, encaspulated, maltodextrin, tween – 80

Introduction

Color is one of the most important quality attributes affecting the consumer's acceptance of food since it gives the first impression of food quality. Food colorants are either natural or synthetic depending on source; Natural colorants are extracted from renewable sources such as plant materials, insects, algae etc, while the synthetic colorants are manufactured chemically and are the most commonly used dye in the food pharmaceutical and cosmetic industries. The chemicals may have harmful side effect and could cause health problems in adults on children [5].

Due to perceived safety and physiological advantage of the natural colorants over synthetic ones, interest are being geared into search of new natural colorants and the verification of the safety of existing ones. Anthocyanin, as with most natural food colorants, suffer from inherent instability [5].

But natural colorants are generally more sensitive to light, temperature, pH and redox agents. There are several techniques and wall materials that are available for encapsulation of natural food colorants to overcome their instability, solubility and handling problems [1].

The red cabbage extract is rich in anthocyanins and could be used as a good source for producing a brilliant red color for many foods. The red cabbage includes many vitamin C, vitamin K, potassium, and folate. While cardiovascular protection and decreased risk of type 2 diabetes have been areas of increased research interest with respect to red cabbage intake. The other health benefit of this plant is in cancer prevention. Red cabbages have the highest antioxidant activity compared to the other cabbages.

In this research, anthocyanin as a natural food colorant was extracted from red cabbage using distilled water, acidified ethanol and 2% citric acid. And then, it was applied in coloring of foods and encapsulated with a mixture of gum arabic, maltodextrin and Tween-80. This research is that the encapsulated anthocyanin powder should give an increase its stability and possibilities for the use of colouring.

Materials and Method

Sample Collection

Red Cabbage was purchased from Ocean Supermarket, Chan Aye Tharzan Township, Mandalay, Mandalay Region, Myanmar. The encapsulating agents were: Maltodextrin (DE 4-17), gum arabic and Tween-80 (Atlas Chemical centre and Able Chemical centre). All reagents were analytical grade.

Phytochemical Investigation on Red Cabbage

Phytochemical investigation on red cabbage was carried out according to the reported methods [3].

Preparation of the Anthocyanin Extracts from Red Cabbage

Each 100g of red cabbage was cut into small pieces and grinded in grinder. And then they were dissolved in 300ml of distilled water, 300 ml of 2% citric acid and 300 ml of acidified ethanol.

They were put on the magnetic stirrer for about 30 minutes at room temperature and then placed into the refrigerator overnight. They were then filtered using filter papers [2]. The same procedure was followed to extract until become colorless and then the final volume (1000 ml) was obtained. The extracts were kept in refrigerator until further analysis.

Choice of Solvent for Extraction of Anthocyanin

In order to known the stability of anthocyanin pigments of water and 2% citric acid extracts, they were left in sunlight and noted [4]

Determination of Total Monomeric Anthocyanins

Before the determination of anthocyanin content, maximum absorption of anthocyanin extract was detected by UV-VIS spectrophotometer. Thus results are shown in Figure (3). Total anthocyanin content of 2% citric acid extract was determined using the spectrophotometric pH-differential method described by (Wang and Xu., 2007) using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 mol/L) and sodium acetate buffer, pH 4.5 (0.4 mol/L). The absorbance was measured at 520 and 700 nm with distilled water as blank using a UV-VIS spectrophotometer. The absorbance (A) difference between the pH 1.0 and pH 4.5 samples was calculated.

Absorbance (A) = (A_{520nm} pH 1.0 — A_{700nm} pH1.0) – (A_{520nm} pH4.5 - A_{700nm} pH4.5)

The total anthocyanin content was calculated as cyanidin-3-glucoside according to the following equation:

$$\text{Total anthocyanin content} = \frac{A \times MW \times DF \times 10^{3}}{\varepsilon \times l}$$

Where, (molecular weight) = 449.2 $gmol^{-1}$ for cyanidin-3-glucoside;

DF = the dilution factor
l = path length
A = Absorbance

= (Molar absorptivity of cyanidin-3-glucoside) 26900, M^{-1} cm^{-1}

Qualitative Tests for Detection of Anthocyanins in 2% Citric Acid Extract

The presence of anthocyanins in red cabbage extracts was tested by using three common reagents: (i) sulphuric acid (ii) ferric chloride and (iii) dilute hydrochloric acid [1].

Dilute Sulphuric Test

A little dilute cold sulphuric acid was added to extracted solution and gently warmed. Then, it was cooled and shaken with amyl alcohol. Red color of anthocyanin was appeared in alcohol layer which indicates the presence of anthocyanin in extract.

Ferric Chloride Test

The red cabbage was extracted with amyl alcohol. Then, the solution was filtered. A little sodium acetate and a small quantity of ferric chloride solution (5%) were added to the extract. Grayish green colour was appeared in the layer. It indicates the presence of cyanidin and malvidin in the water extract.

Dilute Hydrochloric Acid Test

The dilute hydrochloric acid (2%) was added to a few ml of the extract solution. Red color was produced due to oxoniun salt of anthocyanin which indicates the presence of pelargonidin. The dilute sodium hydroxide (5%)was added to the above solution. The colour was changed to green which indicates the presence of cyanidin. Then the solution was acidified, the red color was appeared.

Application of Anthocyanin Solutions Extracted from the Red Cabbage

3g of jelly powder was added to 100 ml of acidified ethanol and heated at 70°C and stirred for about 10 minutes to reach the desired condition. After that it was transferred into steel bowl and allowed to cool in water bath.It is shown in Figure (6)

100 g of rice with 200 ml of acidified ethanol extracted solution were added in a rice cooker and it was cooked. It was then transferred to a plate.It is shown in Figure (7).

50g of sago seeds was added to 100 ml of 2%citric acid extracted anthocyanin solution and stirred constantly at 70°C. After it reached to the desired condition, it was cooled and transferred to a steel bowl. It is shown in Figure (8).

Preparation of Encapsulated Anthocyanins Powder

Anthocyanins extract (180 ml) was added to the mixture (120 ml) of 40 per cent gum arabic solution and 40 per cent maltodextrin solution obtaining total volume of 300 ml. This immiscible mixture was emulsified at 1000 rpm for 10 min by using magnetic stirrer until the anthocyanins dispersed completely. Two drops of Tween-80 was added to aid the emulsification process [6]. The anthocyanin emulsion was concentrated in low pressure rotatory evaporation at 70°C. The concentrated solution was transferred to crucible and placed in a microwave domestic oven. It was treated for up to 10 minutes at microwave power intensities (1100W). It was ground into powder and immediately placed in brown bottle and stored in refrigerator.

Determination of Some Physical Properties of Encapsulated Powder

Some physical properties such as pH, moisture content and solubility in water were determined by reported method.

Determination of pH

The pH of samples (1 % solution) was measured by using pH meter.

Moisture Content

The sample were accurately weighed (1 g, W_0) and dried by loss on dying measurement (W_1) as adapted from [7]. The temperature of drying was 105°C. The weights of the sample before and after drying were calculated for the moisture contents. All samples were performed in triplicate. The moisture content was determined as the percentage of moisture in the powder using the following equation.

$$\text{Moisture content} = \frac{W_0 - W_1}{W_0} \times 100$$

Solubility

Solubility was measured according to the method of Anderson and Frances (2004). One gram of sample was dispensed into 20 mL of distilled water while taking care to break up any lumps using a glass rod. After 30 min of stirring, centrifuged for 10 min. The supernatant was then decanted and the weight of its solid content determined after it had been evaporated to a constant weight. The Water Solubility Index (WSI) was then calculated as:

$$\text{WSI} = \frac{\text{Wt : of dissolved solids in supernatant}}{\text{Wt : of dry powder sample}}$$

Results and discussion

Preliminary Phytochemical Screening of Red Cabbage

According to the phytochemical examination alkaloid, flavonoid, phenol, polyphenol, terpene, steroid, reducing sugar, saponin, tannin, glycoside and carbohyrate were found to the present in the sample. Among these, phytochemicals, flavonoid plays an important role in food colour.

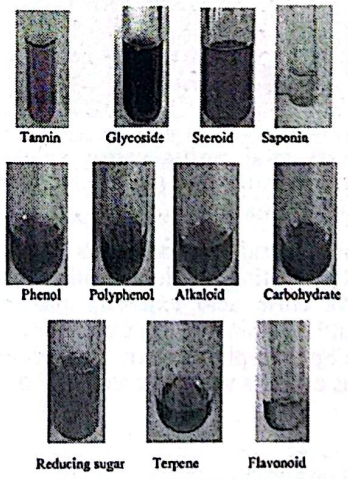


Figure 1. The results of phytohemical screening of red cabbage

Extraction of Anthocyanun from Red Cabbage

Anthocyanin was extracted from red cabbage using three solvents; distilled water, acidified

ethanol and 2% citric acid.Material to solvent ratio was1:10 w/v (Figure 2). The pH values were found to be 5.2 for distilled water extract, 4.3 for acidified ethanol extract and 3.2 for 2% citric acid extract.

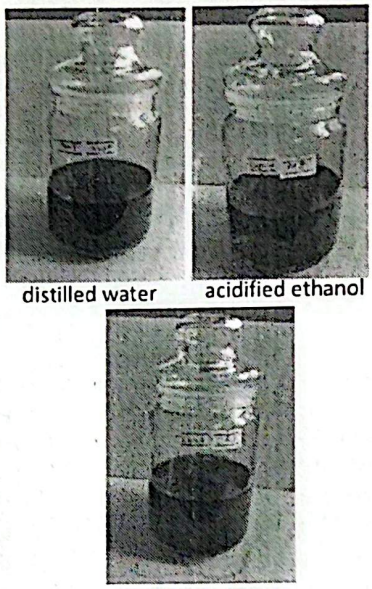
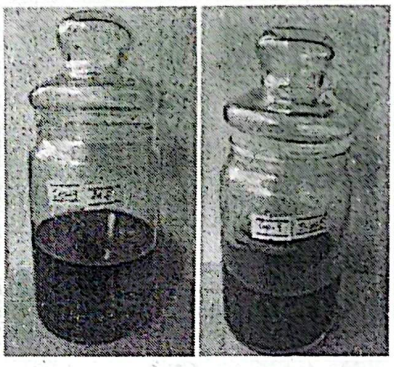


Figure 2. Extracted of anthocyanin

Choice of Solvent for Extraction of Anthocyanins

In order to known the stability of anthocyanin pigments of water and 2 % citric acid extracts, they were left in sunlight. The changes of color are shown in Figure (3). After five days, water extract was changed the color to darken purple but 2 % citric acid extracts color was little changed.



(a) (b)

Figure 3. Extracted anthocyanin solutions using (a) distilled water and (b) 2% citric acid as solvents(after 5 days)

Determination of Total Anthocyanins Pigment

Before determination of total anthocyanins pigment in 2% citric acid extracts, maximum absorption of anthocyanin extracts was determined by UV-Visible Spectro-photometer. The spectrum of anthocyanins extracts was presented in Fig. 4.

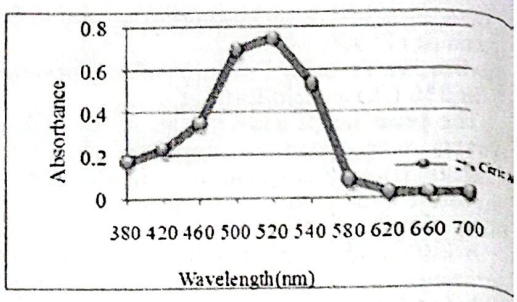


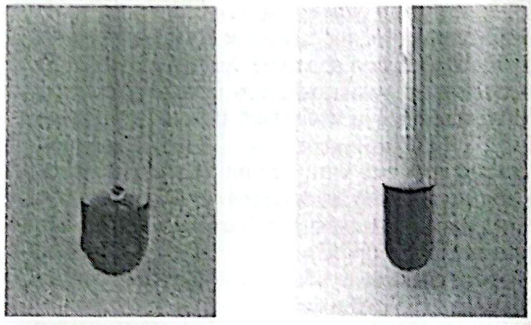
Figure 4. Absorption spectrum of anthocyanin extract in citric acid

According to the spectrum, maximum absorption was found to be 520 nm for citric acid extracts. So, its value was used for the calculation of anthocyanins content in pH differential method. The yield pigments of 2% citric acid solution was found to be 78.50(mg/100g).

Qualitative Tests for Anthocyanins in 2 % Citric Acid Extract

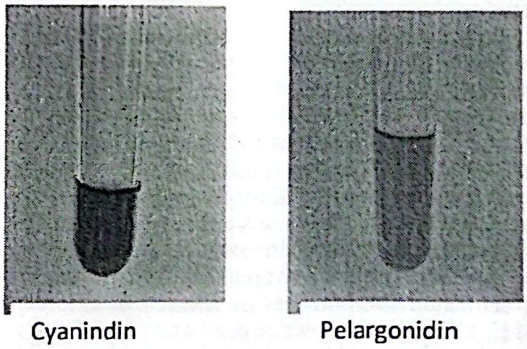
The presence of anthocyanin in red cabbage was tested by using three tests;(1) sulphuric acid test,(2) ferric chloride solution test and (3) dilute hydrochloric acid test.According to the tests pelargonidin,cyannidin and malvidin were found to be present in the extract.They are shown in Figure 5.

(i) Sulphuric acid test (ii) Ferric chloride solution test



Anthocyanin Malvidin

(iii) Dilute hydrochloric acid test



Cyanindin Pelargonidin

Figure 5. Qualitative Tests for Anthocyanin

Application of Anthocyanin Solutions Extracted from the Red Cabbage

The acidified ethanol extract and 2%citric acid extract were utilized in coloring of foods. It was found that the acidified ethanol extract gives purple color and the 2% citric acid extract gives reddish pink color depending on their different pH values.

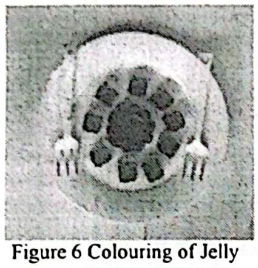


Figure 6 Colouring of Jelly

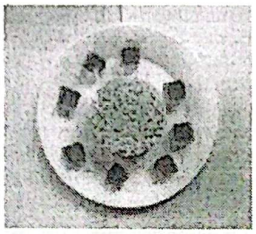


Figure 7. Colouring of Rice

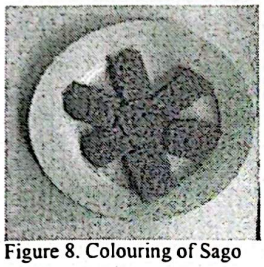


Figure 8. Colouring of Sago Kyo

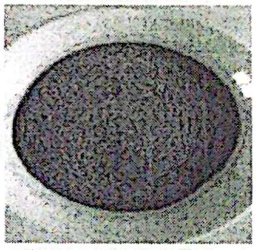


Figure 8. Encapsulated anthocyanins powder

Encapsulation of 2% Citric Acid Anthocyanin Powder

To increase the stability of anthocyanins and protect from light, high temperature, pH and oxidation, encapsulation was carried out. In this research, anthocyanins pigment (citric acid extract) for core material, a mixture of gum arabic and maltodextrin for wall materials and Tween-80 as emulsifying agent were used.

Some Physical Properties of Anthocyanin Powder

Table 1 Some Physical Properties of Encapsulated Powder

No.	Parameters	Results
1	pH	3.8
2	Moisture (%)	3.48
4	Solubility in water (%)	93

Conclusion

An attempt has been made to extract the anthocyanins as natural food colorant from red cabbage using 2% citric acid solution and to prepare encapsulated powder and to determine some physical properties. Anthocyanin was extracted from red cabbage using three different solvents, such as distilled water, acidified ethanol and citric acid. Their pH values were found to be 5.2 for distilled water extract, 4.3 for acidified ethanol extract and 3.2 for 2% citric acid extract. Total anthocyanin content was determined by pH differential method. The yield of anthocyanins expressed as cyanidin-3-glucoside was found to be (78.50 mg/100 g) in red cabbage extract. Anthocyanin extract was encapsulated by using the mixture of gum arabic and maltodextrin as wall materials and emulsifier Tween-80. In some physicochemical properties, such as pH 3.8, moisture content 3.48%, and solubility of water 93 % were found. The tentative results of this research indicated that the encapsulated anthocyanins powder can be used as natural colorant instead of synthetic ones.

Acknowledgements

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References

[1] A. Buzarbarua, "A Texbook of Practical Plant Chemistry", S.Chand & Company Ltd.Ram Nargar, New Delhi. pp.165, 2000.

[2] G. Spagna, R.N. Barbagallo, A. Todaro, M.J. Durantel and P.G. Piffer, "A Method for Anthocyanin Extraction from Fresh Grape Skin", *Italian Journal of food Sciences*, vol. 3, pp. 337-346, 2003.

[3] Harbone , J.B., (1984). Phytochemical Method; " A Guide to Modern Techniques of Plant Analysis", 2 nd Ed., Charpman and Hall Ltd., U.S.A.

[4] K. Bronnum-Hansen, F. Jacobsen and M. J. Flink, "Anthocyanin Colourants from Elderberry (*Sambucus nigra*, L.), 1. Process Considerations for Production of Liquid Extract", J. Food Technol, vol. 20, pp. 703-711, 1985.

[5] Mazza , G and Brouillard , R., (1987) " Colour stability and structural transformations of cyaniding 3,5-diglycoside and four 3- deoxyanthocyanins in aqueous solutions". J. Agric .food chem. , 35; 322-426

[6] S. Krishnan, R. Bhosale and R.S. Singhal, "Microen capsulation of Cardamom Oleoresin: Evaluation of Blends Gum Arabic, Maltodextrin and a Modified Starch as Wall Materials", Carbohydrate Polymers, vol. 61, pp. 95-102, 2005.

[7] Soradech, S., Nunthanid, J., Limmatvapirat, S., & Luangtana-anana, M. (2012). "Anapprach for the enchancement of the mechhanical properties and film coating efficiency of shellac by the formation of composite films based on shellac and gelatin". *Journal of food Engineering*, 108:94-102.

Efficacy of Compost Biofertilizer with Bioinoculant *Trichoderma* on *Capsicum annuum* L.

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Abstract

This research work concerns with the study on the effect of *Trichoderma* inoculated compost biofertilizer on the growth and yield of Chilli (*Capsicum annuum* L.) at Water Utilization Research Section, Department of Agricultural Research, Yezin, Myanmar. In this study, compost biofertilizer was prepared by inoculation of *Trichoderma* substrate (Yezin Isolate) based on farm waste materials such as cow dung, rice straw and rice bran. Some physicochemical properties, nutrients and relative elemental composition of the waste materials and prepared compost biofertilizer were determined by modern and conventional methods. Biological properties and germination percent of compost biofertilizer were also determined. Field experiment was laid out in Randomized Complete Block (RCB) design with four treatments and five replications. Four treatments were T1 (NPK), T2 (30% *Trichoderma* compost biofertilizer), T3 (NPK + 30% *Trichoderma* compost biofertilizer) and T4 (control). Parameters of soil samples were analyzed before sowing and after harvesting. The growth parameter including plant height was highly significant ($p < 0.01$). Among these, the highest yield of T3 (NPK + 30% *Trichoderma* compost biofertilizer) was 1625.77 kg/ha. The lowest yield of T4 (control) was 951.16 kg/ha whereas T2 (30% *Trichoderma* compost biofertilizer) treatment produced the yield of chilli (1605.08 kg/ha). According to these findings, *Trichoderma* inoculated compost biofertilizer had positive impact on the plant growth and yield of chilli and could reduce excessive chemical fertilizer for crop production.

Keywords: *Trichoderma* inoculated compost biofertilizer, chilli, growth, yield

Introduction

Fertilizers are increasingly important to improve crop yields needed to feed a growing world population. Soil management strategies today are mainly dependent on inorganic chemical-based fertilizers, which cause a serious threat to human health and the environment. Biofertilizer has been identified as an alternative for increasing soil fertility and crop production in sustainable farming (Itelima *et al.*, 2018).

A biofertilizer is simply a substance which contains living microorganisms which when applied to the soil, a seed or plant surface colonizes the rhizosphere and promotes growth by increasing the supply or availability of nutrients to the host plant. The application of biofertilizer to the soil increase the biodiversity which constitutes all kinds of useful bacteria and fungi including the arbuscular mycorrhiza fungi (AMF) called plant growth promoting rhizobacteria (PGPR), composting accelerator (*Trichoderma*) and nitrogen fixers. There are so many microorganisms thriving in the soil, especially in the rhizosphere of plant. A considerable number of these microorganisms possess a functional relationship and constitute a holistic system with plants. They have beneficial effects on plant growth (Vessey, 2003).

Composting have agricultural and other biowaste is the most widely adapted process for their recycling into the soil for replenishing with the scavenged nutrients, particularly the organic matter and micronutrients (Densilin *et al.*, 2010). Filamentous fungi of the genus *Trichoderma* are common rhizosphere inhabitants. They have been widely studied due to their capacity to parasitize other fungi (mycoparasitism) and to compete with deleterious plant microorganisms. Consequently, when the root system is colonized, the association is potentiated, providing protection in this zone against pathogenic microorganisms, and also a robust root system is developed improving nutrient and water uptake (Harman *et al.*, 2004). In this research work, compost biofertilizer was prepared by using *Trichoderma* substrate (Yezin Isolate).

Chilli (Capsicum annum L.)is one of the mo valuable commercial spice crops and rich sour of ascorbic acid (Densilin *et al.*, 2010). Hence study was undertaken to find out the effect c chemical fertilizer, *Trichoderma* compo biofertilizer and combined application of chemic fertilizer and *Trichoderma* compost biofertiliz on the growth and yield of chilli.

Materials and Methods

Sample Collection and Preparation

Farm waste materials such as cow dung, ric straw and rice bran were collected from Sein Sa Pin Village, Maeutaw village group, Zaeyarthi Township, Nay Pyi Taw. Cow dung, rice straw rice bran, bioinoculant *Trichoderma* substrat (10^7 cfu/g) (200:40:4:1) and water (18L) were t be used in composting process for preparation o biofertilizer by open heap layering metho *Trichoderma* substrate was provided by Plan Pathology Research section, DAR, Yezin. Size o box used in composting was 8 ft x 4 ft x 2.5 ft.

Field Experiment

The field experiment of chilli was grown in research field at Water Utilization Researc Section, Department of Agricultural Researc (DAR), Yezin, Nay Pyi Taw from Decembe 2015 to March, 2016. Chilli seeds (Mi Thar St *Capsicum annuum* L.) were provided fro Horticulture Research Section, DAR, Yezir After seedlings of chilli were transplanted in twenty plots of equal size (6×3) feet square There were 3 rows in each plot. The experimen was carried out in a randomized complete block design plots with four treatments consisting o chemical fertilizer (46N- 46P- 90K) (T1), 30% *Trichoderma* inoculated compost biofertilize (T2), chemical fertilizer with 30% *Trichoderma* inoculated compost biofertilizer (T3) and unfertilized plots as control (T4) with five replications.

Recommended rate of fertilizer for chilli (DAR) = Urea (83 kg/ha) + T-super (37 kg/ha) + Potash (64 kg/ha)

Weekly plant height measurements were taken from two weeks after transplanting and the shoot fresh and dry weight, root fresh and dry weight

and fruity-yield per plant were recorded after the harvest.

Methods

All samples were subjected to physical and chemical analyses using conventional and modern techniques. And also four treatments were analyzed before sowing and after harvesting. Standard methods of analyses were followed. Some part of this research work was done in Water Utilization Research Section, Department of Agricultural Research, Yezin, Nay Pyi Taw. Soil texture was determined by international pipette method. Measurement of moisture content was determined by oven drying method and soil pH was measured with a glass electrode using a 1:2.5 soil to water ratio. Organic carbon was determined Tyurin's method, electrical conductivity was determined by conductivity meter, available nitrogen content was determined by alkaline permanganate method and available phosphorous content by Olsen's method. Available potassium, exchangeable calcium, magnesium and potassium were determined by AAS. In the analytical procedures of the experiments, recommended methods and techniques were applied (FAO, 2008 and AOAC, 2009). The microbes from heap sample were isolated by dilution series method in Plant Pathology Research Section, DAR, Yezin. Germination test of chilli was conducted for compost maturity. Statistical analysis was carried out using International Rice Research Institute (IRRISTAT version 5.0) in this study.

Results and Discussion

Nutrients of Raw Materials and Compost Biofertilizer

Table 1 represents moisture, bulk density, pH and organic carbon of raw materials. Rice bran has higher bulk density and organic carbon than that of cow dung and rice straw. It indicated that the pH values of cow dung and rice straw are suitable to prepare compost biofertilizer. The nutrient contents of the raw materials (cow dung, rice straw and rice bran) are presented in Table 2. Cow dung contains 1.79 % of N, 1.43 % of P, 0.26 % of K, rice straw contains 1.02 % of N, 0.33 % of P, 0.13 % of K and rice bran contains 2.03 % of N, 3.43 % of P, 1.58 % of K. The most common element contents in cow dung, rice straw and rice bran were iron, manganese, copper and zinc. The sufficient amount of N, P, K and micronutrients were found to be present in raw materials.

Biological Properties and Germination Test

Types of microorganism (*Trichoderma* spp., *Aspergillus* spp. and *Aspergillus niger*) were the common genera observed in biocompost after composting (Table 3).Germination % of chilli seeds was found to be 82% for compost maturity and stability after 70 days and thus ready to be used for chilli cultivation.

Physicochemical Properties and Nutrients of Compost Biofertilizer

The physicochemical characteristics such as moisture content (4.28%), bulk density (0.45 g/mL), water holding capacity (57.00%), pH value (7.20), organic carbon (18.17%), organic matter (31.33), C/N ratio (17.47) and electrical conductivity (3.88 dS/m) were found in prepared biofertilizer (Table 4). Organic carbon plays a very important and sometimes spectacular role in the maintenance and improvement of soil properties. The prepared biofertilizer was found to contain 1.04 % of N, 0.20% of P, 0.86 % of K. The most common trace elements in biofertilizer were iron, manganese and zinc. The contents of NPK are essential to maintain and sustain the soil fertility. So, the sufficient amount of N, P, K and macro and micronutrients were present in compost biofertilizer (Table 5). Toxic metals of the biofertilizer were described in Table 5. No toxic metals analysed by AAS were found in prepared biofertilizer.

Table 1 Some Physicochemical Properties of Raw Materials

Properties	Cow Dung	Rice Straw	Rice Bran
Moisture (%)	15.64	10.37	12.29
Bulk Density (g/mL)	0.43	0.18	0.47
pH	7.63	7.27	5.51
Organic Carbon (%)	12.42	46.82	53.05

Table 2 Nutrients of Raw Materials

Macro and Micronutrients	Cow Dung	Rice Straw	Rice Bran
Total N (%)	1.79	1.02	2.03
Total P_2O_5 (%)	1.43	0.33	3.43
Total K_2O (%)	0.26	0.13	1.58
Total Ca (%)	1.32	0.48	1.20
Total Mg (%)	0.71	0.03	0.68
Total S (%)	0.03	0.04	0.04
Fe (ppm)	967.50	133.40	152.30
Mn (ppm)	798.40	914.00	117.00
Cu (ppm)	20.02	16.38	14.46
Zn (ppm)	68.23	28.33	73.32

Table 3 Determination of Microorganism in Biocompost

Biocompost	Population of Microorganism (CFU)*		
	Trichoderma spp.	*Aspergillus spp.*	*Aspergillus niger*
30 Days	5.0×10^5	6.0×10^5	1.0×10^6
50 Days	3.0×10^6	8.0×10^5	8.0×10^6
70 Days	1.2×10^6	8.0×10^5	1.4×10^6

* CFU – Colony Forming Unit

Table 4 Physicochemical Properties of Compost Biofertilizer

Properties	Content
Moisture (%)	4.28
Bulk Density (g mL^{-1})	0.45
Water Holding Capacity (%)	57.00
pH	7.20
Organic Carbon (%)	18.17
Organic Matter (%)	31.33
C/N ratio	17.47
Electrical Conductivity	3.88

Table 5 Macro and Micronutrients in Prepared Biofertilizer

Macro and Micronutrients	Composition (%)
Total N	1.04
Total P_2O_5	0.20
Total K_2O	0.86
Total Ca	0.69
Total Mg	0.15
Total S	0.05
Fe	0.0908
Mn	0.0074
Zn	0.0098
Cu	ND

ND = Not Detected

Table 6 Toxic Metal Contents in Compost Biofertilizer by AAS

Toxic Metals	Content (ppm)	Concentration limits (ppm)*	German Standard ppm)**
Lead	ND	300	150
Nickel	ND	420	50
Cadmium	ND	35	3
Chromium	ND	-	150
Arsenic	ND	41	-
Mercury	ND	7.8	3

* (Ohio Enviromental Protection Agency, 2012) ND-Not Detected

** (European Commission Orbit Association, 2008)

Analysis of Physicochemical Parameters of Soil

Table 7 shows the physical parameters and chemical compositions of farm soil media before sowing. The category of this type of soil falls in the sandy loam. Soil pH may influence nutrient absorption and plant growth. The pH values of treated soils were found to be in the range of 6.34 and

6.44, it can be considered as a slightly acidic type of soil. It has been found that the electrical conductivity of all soil samples were ranged from 0.59 dS/m to 1.07dS/m, and thus they were salt free soils. If there is plentiful supply of humus or organic carbon, the decay of these by microorganisms liberates carbon dioxide, which may be converted to increase the biomass (Teakle, 1958).The humus content of treated soil were higher than that of original control soil. The content of available nitrogen in the farm soil was 42.00 ppm. The available phosphorous and potassium in the farm soil were 43.70 ppm and 150.90 ppm, respectively. On the context of what has been described above, the value of low N, P and K contents were found in the farm soil. Hence, the fertilizer treated soils would have more pronounced effect on the plant growth.

Analysis data of all soil samples after harvesting chilli were presented in Table 8. All types of soil are sandy loam and it cannot be any changed before and after harvesting. pH value of soils at all stages were 7.04 to 7.23. In addition, in the field work study, the pH of the soil (after harvesting) was found to increase than before the transplanting stage. Soil organic matter can be improved by adding biofertilizer, vermicompost and biocomposted organic wastes or biodegradable products(Kannahi *et al.*, 2015). The highest organic carbon (1.49 %) and humus content (2.57%) were found in T2 (30% biofertilizer). The nutrient contents in all soil samples were reduced after harvesting stage because frequently most of them were removed from the soil permanently by the crop produced.

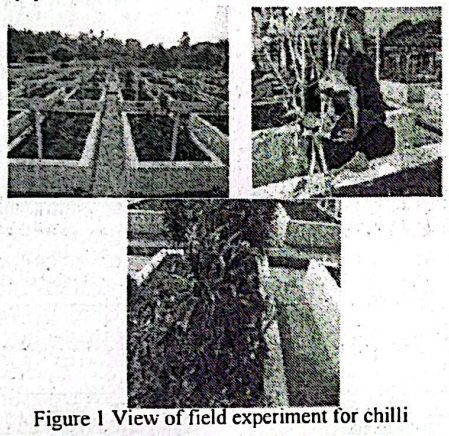
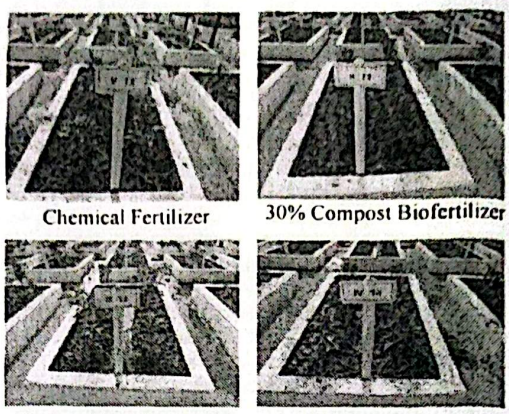


Figure 1 View of field experiment for chilli

Table 7 Analysis Data of the Soil Using for Chilli before Sowing

Analytical Item	T1	T2	T3	T4
Texture-Sand (%)	69.40	69.64	70.28	70.92
Silt (%)	16.92	16.44	17.32	16.04
Clay (%)	13.68	13.92	12.36	13.00
Moisture (%)	1.52	0.58	0.40	1.01
pH	6.40	6.34	6.39	6.44
Electrical Conductivity (dS/m)	0.78	0.64	1.07	0.59
Organic Carbon (%)	1.08	1.15	1.14	1.06
Humus (%)	1.86	1.98	1.96	1.83
Total N (%)	0.25	0.19	0.21	0.17
C/N ratio	4.32	6.05	5.43	6.24
Available N (ppm)	45.78	44.03	52.30	42.00
Available P (ppm)	48.23	34.90	34.92	43.70
Available K (ppm)	228.25	225.28	230.43	150.93
Exchangeable Ca (meq/100g)	8.97	8.23	9.95	7.50
Exchangeable Mg (meq/100g)	0.67	1.25	0.93	1.38
Exchangeable K (meq/100g)	0.24	0.32	0.39	0.29

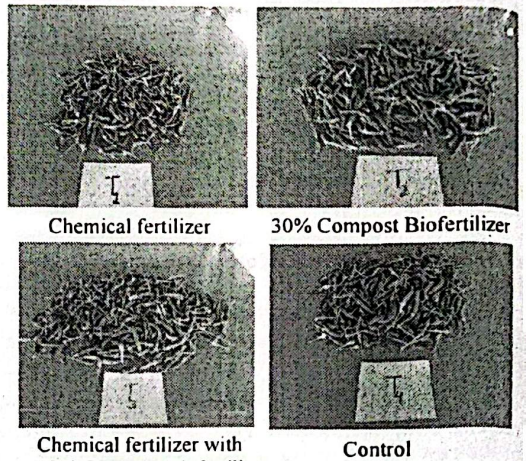
T1 = Chemical fertilizer,
T2 = 30% compost (biofertilizer),
T3 = Chemical fertilizer + 30% compost (biofertilizer),
T4 = Control



Chemical Fertilizer | 30% Compost Biofertilizer

Chemical Fertilizer with 30% Compost Biofertilizer (T3) | Control (T4)

Figure 2 Vegetative growth stage of chilli



Chemical fertilizer | 30% Compost Biofertilizer

Chemical fertilizer with 30% Compost Biofertilizer | Control

Figure 3 Maturity stage of chilli

Table 8 Analysis Data of the Soil Using for Chill after Harvesting

Analytical Item	T1	T2	T3	T4
Texture-Sand (%)	65.08	69.20	69.49	65.1
Silt (%)	20.16	14.65	14.31	19.8
Clay (%)	14.76	16.15	16.20	15.0
Moisture (%)	0.34	0.61	0.14	0.43
pH	7.23	7.04	7.22	7.20
Electrical Conductivity (dS/m)	0.62	0.48	0.92	0.53
Organic Carbon (%)	1.31	1.49	1.29	1.26
Humus (%)	2.26	2.57	2.22	2.17
Total N (%)	0.08	0.18	0.14	0.13
C/N ratio	16.38	8.28	9.21	9.69
Available N (ppm)	40.71	34.59	41.60	39.6
Available P (ppm)	26.20	26.18	17.45	13.1
Available K (ppm)	97.50	177.90	129.70	145.0
Exchangeable Ca (meq/100g)	8.28	8.19	8.86	6.89
Exchangeable Mg (meq/100g)	0.65	1.21	0.85	1.31
Exchangeable K (meq/100g)	0.22	0.27	0.33	0.24

T1 = Chemical fertilizer,
T2 = 30% compost (biofertilizer),
T3 = Chemical fertilizer + 30% compost (biofertilizer),
T4=Control

Effect of Chemical Fertilizer and Biofertilizer on Growth and Yield of Chilli

The effect of fertilizers on the growth and yield of chilli was studied and compared with control in Table 8. During cultivation, daily

determinations of plant height were carried out. In this work, plant height was highly significance ($p< 0.01$) and shoot fresh weight was significance ($p<0.05$) for chilli. There was not significance in root fresh weight and root dry weight, shoot dry weight, number of total fruit per plant and fruit weight per plant for chilli($p>0.05$).

The maximum total yield of about 1625.77 kg/ha for chilli was obtained using chemical fertilizer with compost biofertilizer (T3). The second highest yield (1605.08 kg/ha) was found in Treatment 2 (30% compost biofertilizer). Therefore, in present study the combined treatments enhanced efficient utilization of nutrients than the others which expedited crop growth and yield.

Table 9 Effect of Chemical Fertilizer and Biofertilizer on Growth and Yield of Chilli

Treatment	Plant height (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Number of total fruits plant^{-1}	Fruit weight plant^{-1}	Total yield (kg/ha)
T1	64.90	161.57	10.67	61.04	6.55	76.90	75.31	1350.44
T2	60.26	110.12	11.44	45.44	6.92	93.70	89.51	1605.08
T3	71.62	190.22	13.46	68.36	6.95	96.07	90.66	1625.77
T4	59.24	86.71	8.49	41.32	6.45	61.00	53.04	951.16
F Test	**	*	ns	ns	ns	ns	ns	ns
LSD(5%)	6.24	76.41	5.95	22.29	1.92	61.08	55.86	1001.71
CV(%)	7.10	40.40	39.20	29.90	20.80	54.10	52.60	52.60

T1=Chemical Fertilizer, T2=30% Compost Biofertilizer, T3=Chemical Fertilizer with Compost Biofertilizer, T4=Control

** ($p<0.01$), * ($p<0.05$), ns = non significant

Conclusion

In this present study, *Trichoderma* inoculated compost biofertilizer was prepared at Department of Agricultural research, Yezin, Nay Pyi Taw. The physicochemical parameters and nutrients of compost biofertilizer were found to suit for plant growth and it has no detectable toxic metals. From the field experiment, the combined application of chemical fertilizer and compost biofertilizer improved the physical parameters (plant height, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight and number of total fruits). The maximum 1625.77 kg/ha yield increase over control of chilli was noticed in T3, which was 951.16 kg/ha in T4 (control). It may be concluded that application of *Trichoderma* inoculated compost biofertilizer along with chemical fertilizer could save at least 30% compost biofertilizer giving higher yields in chilli compared to T1 (chemical fertilizer) and hence could be useful for different crop production instead of chemical fertilizer.

Acknowledgement

The authors wish to thank Dr Aung Win (Rector), Dr Aye Aye Ko and Dr Soe Myint Aye Pro-Rectors of University of Myitkyina for permitting the opportunity to present this paper. We wish to express our gratitude to Professor and Head Dr Ni Ni Aung and Professor Dr Nant Si Si Htay, Department of Chemistry, University of Myitkyina for their permission and encouragement to carry out this research. We would like to express their profound gratitude to the Members of MISCC – 2019 (1 st Myanmar International Science and Culture Conference, 2019), University of Myitkyina and Department of Higher Education, Ministry of Education, Myanmar, for provision of opportunity to do this research.

References

AOAC. (2009). "Methods of Analysis". Washington: 3rd Ed., Benijamin Franlin Station, pp. 530-543.

Densilin, D.M, Srinivasan, S., Manju, P and Sudha, S. (2011). "Effect of Individual and Combined Application of Biofertilizers, Inorganic Fertilizer and Vermicompost on the Biochemical constituents of Chilli (Ns-1701)". *Journal of Biofertilizers & Biopesticides*, vol. 2(1), pp. 103-106.

FAO. (2008). "Guide to Laboratory Establishment for Plant Nutrient Analysis". Rome: FAO, pp. 30-44.

Harman ,G.E., Howell, R.C., Viterbo, A., Chet, I and Lorito, M. (2004). "*Trichoderma* species opportunistic, Avirulent Plants Symbionts". *Nature Reviews (Microbiology)*, vol. 2, pp. 43-56.

Itelima, J.U., Bang, W.J and Onyimba, I.A. (2018). "A Review: Biofertilizer – A key player in enhancingsoil fertility and crop productivity". *J Microbiol Biotechnol Rep*, vol. 2(1), pp. 22-28.

Kannahi, M and Ramya, R. (2015). " Effect of Biofertilizer, Vermicompost, Biocompost and Chemical Fertilizer on Different Morphological and Phytochemical Parameters of *Lycopersicum Esculentum* L.". *World Journal of Pharmacy and Pharmaceutical Sciences*, vol. 4(09), pp.1460-1469.

Teakle, L.J.H and Boyle, R.A. (1958). "Fertilizers for the Farm and Garden". London: Angus and Robertson. 1st Ed., pp. 24-26.

Vessey, J.K. (2003). "Plant Growth Promoting Rhizobacteria as Bio-fertilizers". *Plant and Soil*, vol. 255, pp. 571-586.

Chemical Composition and Bioactivity of the Essential Oil from the Peel of *Citrus medica* L.

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Yuzana Hmwe, Candidate, MRes, Department of Chemistry, University of Yangon

Abstract

This research focuses on the investigation of the chemical compounds and antimicrobial activity of *Citrus medica* L. peels' essential oil. The essential oil is extracted from the fresh peels of lemon by water distillation method. The chemical constituents in the oil were analyzed by GC- MS, and the antimicrobial activity of the oil was screened by agar well diffusion method. The major components of the essential oil were nerol and linalool. The essential oil showed high activity against all the tested microorganisms.

Keywords: *Citrus medica* L., peel, essential oil, GC-MS, antimicrobial activity

Introduction

Citrus medica L. commonly known as citron, is a small tree, having large fruit in shape. The citron was the first citrus fruit cultivated in the Mediterranean region and especially in Crete according to Protopapadakis, 1987 and Isaac, 1959. The citron rind is popular as a dessert, essence in perfumery (Andrews, 1961 and Fleisher, 1996). The most important part of the citron is the peel which is a fairly important article in international trade. In Bangladesh, India, Indonesia, citron peel is eaten raw with rice. Chinese and Japanese people prize the citron for its fragrance and it is a common practice in central and northern China to carry a ripe fruit in the hand or place the fruit in a dish on a table to perfume the air of a room. The dried fruits are put with stored clothing to repel months. In India, the peel is a remedy for dysentery and is eaten to overcome halitosis. Emmanuel *et al.* reported that *C. medica* essential oil showed fungi toxicity against some fungi. *C. medica* is relevant to treatment of diabetes and alzheimer's disease. The peel contains citroflavonoids consisting of a mixture of hesperidoside, naringoside and ecryodietyoside. Essential oil and vitamin C are also found in addition to glycosides hesperidin (vitamin P) and rutin [3-5]. The peel oil of *C. medica* contained limonene, γ-terpinene, (Z)-citral and (E)-citral. Limonene was the major constituent in the oil of peel, while the content of the other constituents varied. In addition, *C.medica* essential oil contains d-limonene, α-terpinene, valencene, mircene, α-pinene, linalool, geraniol, citronellol and α-terpineol. So, the present study is to characterize the chemical composition and antimicrobial activity of the peel of the essential oil from *Citrus medica* L.(Bhuiyan *et al.*, 2009).

Materials & Methods

Plant material

The *Citrus medica* fruits were collected from environs of High Education Department (Yangon), Lower Myanmar. The collected fruits were peeled and the fresh peels were cut into small pieces in the extraction made in the present research work.

Essential oil isolation

Small pieces of *Citrus medica* peels (60 g) were placed in the 500 mL round-bottomed flask and distilled water (250 cm^3) was added. The water distillation apparatus was set up by placing the water distillation unit between the round-bottomed flask and a condenser in Figure 1. The round-bottomed flask was placed in a heating mantle and heated to boil for 10 hours when no more essential oil distilled out. The condensed oil and water (oil-water) mixture coming out from condenser collected in the receiver. It was taken out the way of the outlet at the bottom of the receiver. The oil was extracted from the oil-water mixture by shaking with petroleum ether in a separating funnel. The petroleum ether extracted was distilled to get very small amount of essential oil.

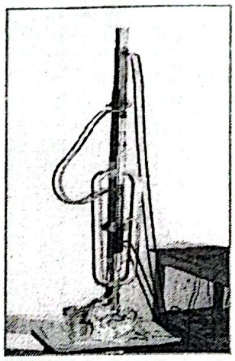

Figure 1 Water distillation set up for extraction essential oil from *Citrus medica*

Identification of the compound

Some constituents of essential oil were identified by GC-MS at Universities' Research Centre. GC-MS, Perkin Elmer, Auto system XI, Gas chromatograph and Perkin Elmer,Turbo Mass Gold Mass spectrometer was utilized. In GC, the column is packed with Quartz wool and Helium gas is applied as mobile phase.

Antimicrobial activity of essential oil

Essential oil (1 g) was dissolved in petroleum ether (1mL). All bacteriological media tested were prepared by using aspectic condition. Agar well diffusion method was employed for determing antimicrobial activity of the extracts. Two small holes of 10 mm diameter each were cut out in the inoculated agar to place samples to be tested. The volume of each sampled placed in each hole was 0.1 cm^3. Two samples, namely petroleum ether and essential oil were tested. The petri- dish was then incubated at 37°C for 48 hours, and the diameters of clear inhibition zone around the hole, if appeared were measured (Priestman, 1953).

Results and Discussion

GC-MS analysis of essential oil

In the gas chromatogram (Figure 2) of the extracted essential oil, one outstanding major peak was eluted at the retention time of 7.155 min, four other prominent peaks were eluted at retention time of 6.676, 6.785, 6.981 and 7.460 min, the remaining small peaks number about ten in Table 1. In the mass spectrometric analysis of the eluted compounds, using the computer matching facility with standard spectral library, the single most prominent compound eluted at retention time 7.155 min was found to be (+/-) linalool, i.e, (+/-) -3,7- dimethyl-1,6- octadiene-3-ol. In the mass spectrum, the small molecular in the peak at m/z 154, M-CH_3 at m/z 139, M-H_2O at m/z 136 and the

base peak at m/z 69 resulted from cleavage allylic to the non terminal olefinic group in the linalool can clearly be seen in Figure 6. By a similar method, the other four prominent peaks correspond to 4-methyl-1-(1-methyl ethyl)-3-cyclohexene for the peak with retention time 6.676 min in Figure 3; (+)-α- terpineol for the peak with retention time 6.785 min in Figure 4; nerol, i.e, (Z)-3,7-dimethyl-2,6-octadien-1-ol for the peak with retention time 6.981 min in Figure 5; and thymol for the peak with retention time 7.460 min in Figure 7.

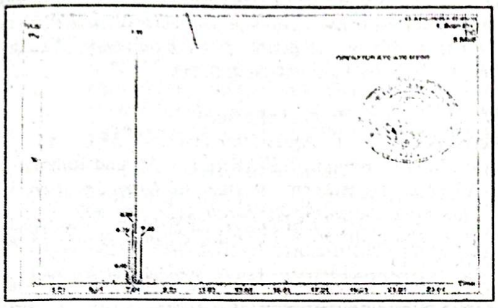

Figure 2 Gas chromatogram of water distillation essential oil from *C.medica* fruit peels

Table 1 Constituents of Peel Essential Oil from *Citrus medica* L.

No.	Name of Components	Retention Time (min)
1	1, 3-Alpha Pinene	5.60
2	1,6-acatadine - 3-ol, 3,7-dimethyl	5.93
3	3-cyclohexen- 1-ol , 4 methyl - 1-(1-methyl ethyl)	6.676
4	Alpha-terpineol (P-menth-1-en-8-ol)	6.678
5	2,6-octadien-1-ol, 3,7-dimethyl (or) nerol	6.981
6	1,6-octadien-3-ol, 3,7-dimethyl (or) linalool	7.155
7	Thymol	7.460

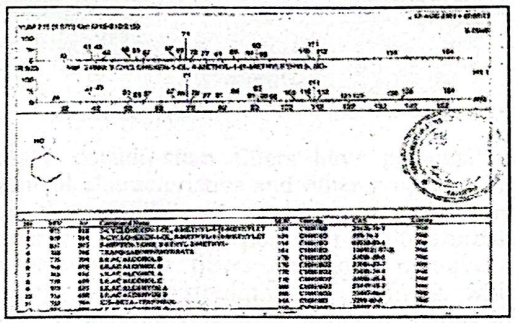

Figure 3 Mass spectrum of compound eluted with RT 6.676 min and library mass spectrum of 4 methyl-1-(-1-methyl ethyl) 3-cyclohexen-1-ol

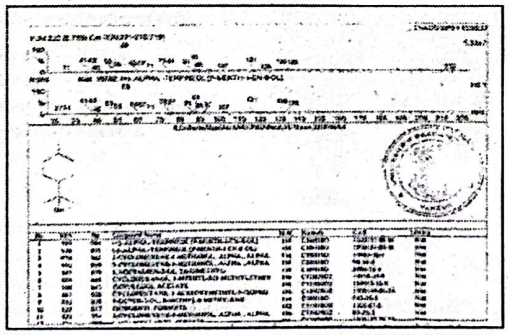

Figure 4 Mass spectrum of compound eluted with RT 6.678 min and library mass spectrum of (+) - α – terpineol

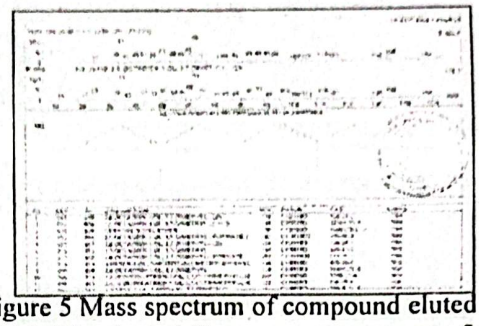

Figure 5 Mass spectrum of compound eluted with RT 6.981 min and library mass spectrum of nerol (or) (Z)-3, 7-dimethyl-1-ol-2, 6-octadiene

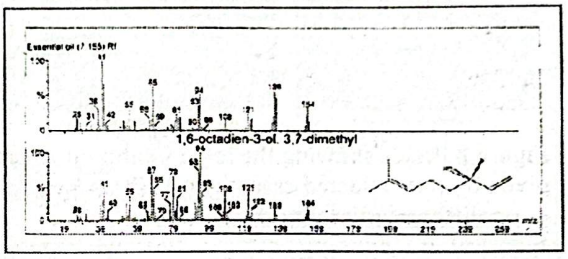

Figure 6 Mass spectrum of compound eluted with RT 7.155 min and library mass spectrum of 1,6-octadien-3-ol, 3,7-dimethyl or linalool

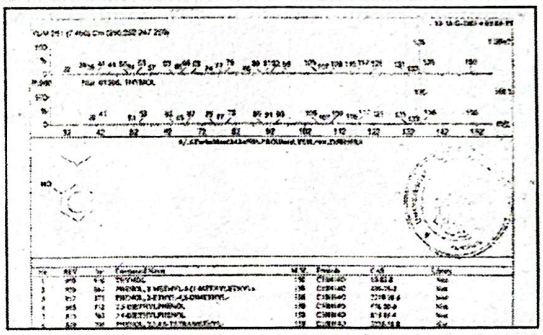

Figure 7. Mass spectrum of compound eluted with RT 7.460 min and library mass spectrum of thymol

Screening of antimicrobial activity of essential oil

In the antimicrobial screening of essential oil by agar well diffusion method on *Bacillus subtilis, Staphylococcus aureus, Pseudomonus aeruginosa, Bacillus pumilus, Candida albicans* and *Mycobacterium species* in Figure 8, it was observed that essential oil was highly active (38-60 mm inhibition zone diameters) on all the tested microorganisms in Table 2.

Table 2 Qualitative Estimation of Bioactivity for Essential Oil Extract

No.	Microorganisms	Diameter of Zone of Inhibition (mm)
1	*Bacillus subtilis*	45 mm (+++)
2	*Staphylococcus aureus*	38 mm (+++)
3	*Pseudomonas aeruginosa*	60 mm (+++)
4	*Bacillus pumilus*	45 mm (+++)
5	*Candida albicans*	45 mm (+++)
6	*Mycobacterium species*	60 mm (+++)

(+++) = highest activity

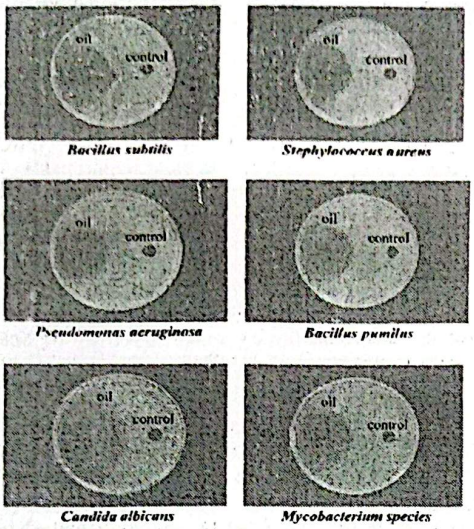

Figure 8 Plates showing the large inhibition zones given by the extracted essential oil *Citrus medica* L. on different microorganism

Conclusion

The chemical constituents of the essential oil of *Citrus medica* L. peel obtained by water distillation method were analyzed by gas chromatography- mass spectroscopy (GC-MS). The peel oil contains nerol and linalool as major constituents. The essential oil exhibited antimicrobial activity by agar well diffu method on the six tested microorganisms, nam *Bacillus subtilis, Staphylococcus aure Pseudomonas aeruginosa, Bacillus pumi Candida albicans* and *Mycobacterium speci* Therefore, the essential oil from *Citrus medi* peels, with its good antimicrobial activity, ha good potential to be used in the formulation o drug for certain infections.

Acknowledgements

The author would like to thank for the allowing to pres this paper and Professor and Head,Dr Amar Yi and Profes Dr U Myint Ngwe, Department of Chemistry, Taung University, for their kind encouragement.

References

Andrews, A.C. (1961). "Agriculture History". **35 (1)**, 35.

Bhuiyan, M.N.I., Begum, P.K., Sardand, M. and Rahman, (2009). "Constituent of Peel of *Citrus medica* L. *Journal of Scientific Research*, **1**(2), 387-392.

Fleisher, Z. and Fleisher, A. (1996)."Perfumer and Flavorist **21** (6), 11.

Isaac, E. (1959). "Economic Geography". **35 (1)**, 71. do 10.2307/1420803.

Protopapadakis, E. (1987)." A Study on Glutamic Oxaloacetate Transaminase Isoenzymes of Citr Cultivars" *J. Amer. Soc. Hort.* **62 (3)**, 413-419.

Priestman, J. and Edwards, F.C.G. (1953). *Aids to Qualitative Pharmaceutical Analysis,* Pag and Bros (Narwish) Ltd., London, 98.

Preparation and Comparison of potential for Different Agricultural Materials Sponges and Conventional Sponges

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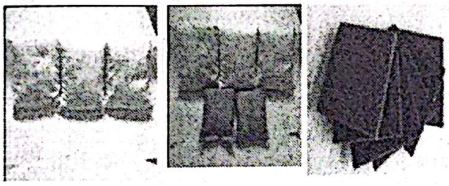
Abstract

In this research paper, kitchen sponge using banana stem (*Musaacuminata*), water hyacinth (*Eichhorina crassipies*) and spong gourd (*Luffa acgyptiaca*) were prepared. And then, the fragment lemongrasses (*Cymbopogon citrates*) were mixed each agricultural materials. Because it removes odor and gives good smell. The agricultural materials have unit morphological features. The anatomical structures of these materials were determined by Scanning Electron Micrograph (SEM). The results indicated that cellulose and holocellulose contents are high. Moreover these materials were analyzed by Fourier Transform Infrared (FTIR). It was used to study the absorption peaks of agricultural materials. The appearance of absorption peaks was due to the presence of some functional groups. The biodegradability of these materials were tested by soil bacterial. And then, the comparison of conventional sponges and agricultural material sponges of potential such as absorption property and biodegradable property. The purpose of this research, the agricultural material sponges were fully decomposed and help to reduce agricultural wastes. The conventional sponges were source of much waste, cannot be recycle and disposable and full of chemical.

Keywords: SEM, FT-IR, Biodegradable and absorption property

Introduction

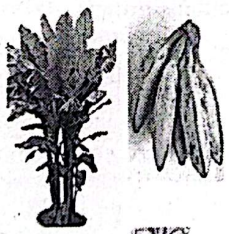
Conventional sponges are source of much waste and cannot be recycled or composed. They are disposable and full of chemicals. These effects are global warming. Agricultural materials such as banana stem, water hyacinth and luffa sponge gourd that are used as kitchen sponges. Agricultural material sponges are biodegradable and full composed. They help to reduce agricultural waste and save our earth. Many environmentally conscious consumers appreciate that luffa products are biodegradable, natural and a renewable resource. In many other countries luffa is also used to make household cleaning products for scrubbing pots, pans, barbecue grills, tires and many other surface that are not harmed by the abrasive fibers.



Banana pseudo-stem fibers have physical and chemical characteristics and other properties that make them good quality fiber. In terms of physical properties, it has been reported in the literature that the banana stem fibers has good modulus of elasticity, tensile strength, and stiffness, which makes if a promising fiber material. It has low density and strong moisture absorption quality. Its absorbance and release of moisture are quite fast.

Botanical description of banana plant

Family name - Musaceae
Botanical name - *Musaacuminata*
Myanmar name - Nget pyaw
English name - Banana plant
Part used - Trunk



Family name - Potederiaceae
Botanical name - *Eichhornia crassipies*
Myanmar name - Beda- pin
English name - Water hyacinth
Part used - Stem

Water hyacinth is a free-floating plant, which grows up to three feet in height. It has thick, waxy, rounded, glossy leaves, which rise well above the water surface on the stalks.

Material and Methods

Materials

Sample Collection

Agricultural materials such as banana stem, water hyacinth, luffa sponge gourd and lemongrass were collected from Myitkyina Township, Kachin State. These materials were chopped and dried in shady place. And then the dried samples were grinded into pieces.

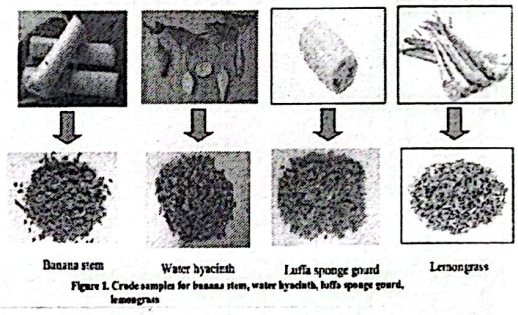


Figure 1. Crude samples for banana stem, water hyacinth, luffa sponge gourd, lemongrass

Methods

Determination of Scanning Electron Micrograph

SEM is a total that can form the image of the specimen surface microscopically. The interaction of the electron beam with the specimen produce some phenomena items, namely backscattering electron beam, X-rays, secondary electrons and electron absorption. SEM technique is the investigation and analysis of surface.

Determination of Functional Group

Additionally, the physico-chemical properties of the agricultural materials fiber were also studied. The Infrared (IR) spectroscopy is probably one of the most widely used instrumental methods for investigating physico-chemical properties of textile materials. When a sample of organic compound is passed by infrared, certain frequencies are absorbed while others are transmitted through the sample.

Biodegradability Test

The study of biodegradability of agricultural materials can be done by burying them in ground (one feet depth). While buried in the ground, the growth of microorganism plays a major role during the degradation process of fiber cellulose, which results in loss of tenacity. Based on the soil burial test, there was found that the agricultural fiber loses strength rapidly when buried in the ground.

Observation for Water Absorption Test

The absorption efficiency between agricultural material sponges (AMS) with conventional sponges were compared by lab-experiment. Agricultural materials can absorb water and more than conventional sponge.

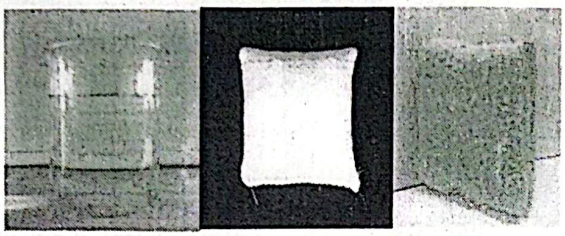
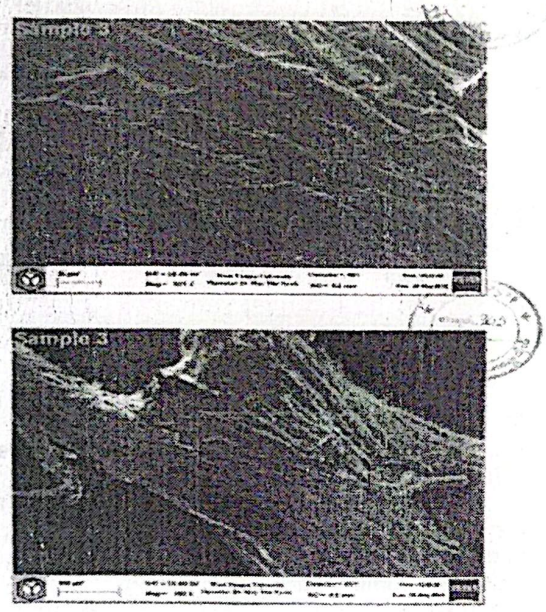


Figure 2. Water absorption test

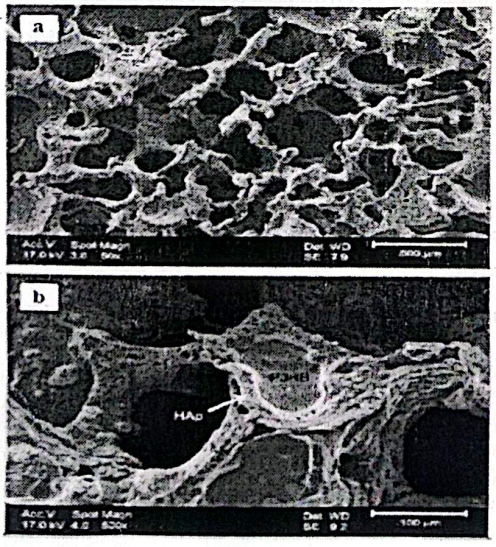
Results and Discussions

Results of SEM Spectrum

An analysis of the anatomical structure of banana stem, water hyacinth and Luffa gourd sponge was carried out using Scanning Electron Microscopy (SEM). The chemical analysis indicated there is a high holocellulose content and low ligning content in these agricultural materials. These results demonstrate that the agricultural materials have a potential value for pulping.



(c)



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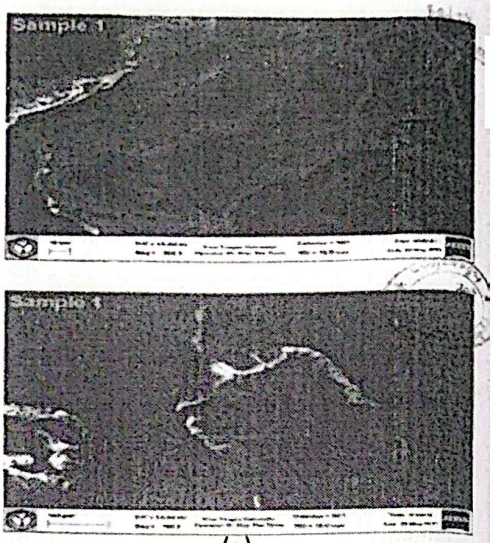
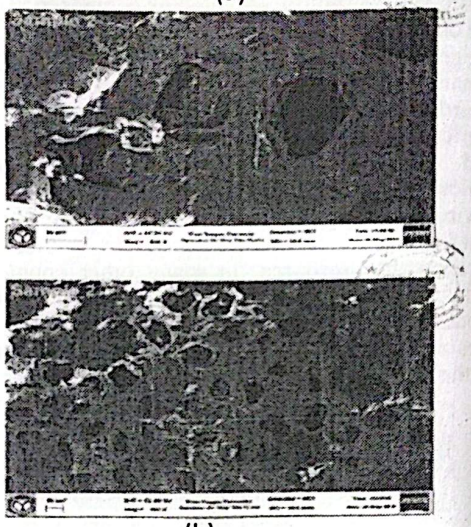


(a)



(b)

Figure 3. SEM micrograph of (a) water hyacinth, (b) banana stem (c) luffa sponge gourd (d) conversional sponge

Analysis Data of FTIR Spectrum

The IR spectrum is obtained by plotting the percentage of absorbance or percentage transmittance values against the frequencies. The Fourier Transform Infrared spectroscopy (FTIR) was used to study the absorption peaks of agricultural materials. The appearance of absorption peaks was due to the presence of some functional groups.

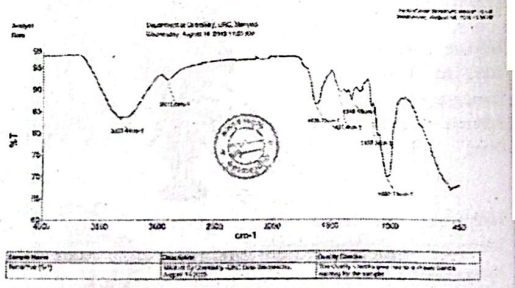


Figure 4. FTIR spectrum of water hyacinth

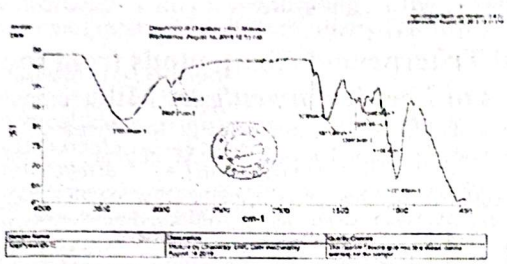


Figure 5. FTIR spectrum of banana stem

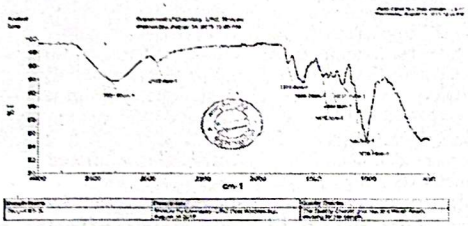


Figure 6. FTIR spectrum of luffa sponge gourd

Table 1. FTIR Spectral Assignment for Agricultural Materials

Sample	Observed	References	Characteristic vibration
Water hyacinth	3336.95 cm^{-1}	3650-3200 cm^{-1}	υ_{OH} Stretching
	1625.79 cm^{-1}	1600-1700cm^{-1}	υ_{CO} Stretching
Banana stem	3333.44 cm^{-1}	3650-3200 cm^{-1}	υ_{OH} Stretching
	1730cm^{-1}	1600-1700cm^{-1}	υ_{CO} Stretching
Luffa sponge gourd	3331.05 cm^{-1}	3650-3200 cm^{-1}	υ_{OH} Stretching
	1737.4cm^{-1}	1600-1700cm^{-1}	υ_{CO} Stretching

Experimental Results for Biodegradability Test

In this work, biodegradation of AMS were tested by soil burial test. They show this process of AMS for 3 months in Figure .

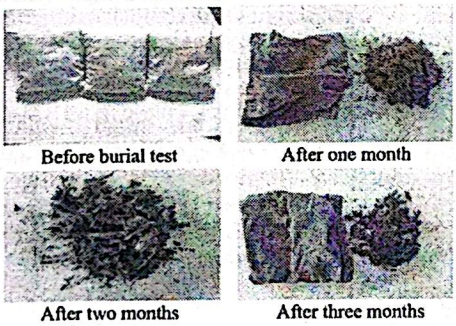


Figure 7. Biodegradability Tests

Lab experiment of water absorption for agricultural and convensional sponge

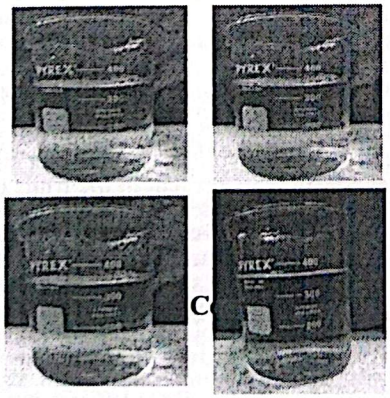


Figure 8. Water absorption test

In this research paper, the properties of cleaning sponges made of agricultural materials have been studied. Firstly, the agricultural material sponges were prepared by using banana stem, water hyacinth and luffa sponge gourd. And then the lemon grasses was used as a filler. It removes odors and gives good smell. These materials were used as a kitchen sponge. The anatomical structure agricultural material stems were analyzed by scanning electron microscopy (SEM). The results indicated that the cellulose and holocellulose contents are high. The fiber of their stem are long and slender and the helicoidal fiber structure and film structure are found by SEM. So, these materials have unite morphological features. These results demonstrate that the agricultural materials have potential values for pulping. And then these fibers have water absorbing property due to the hydroxyl groups in the chemical structures of cellulose. Moreover, the fourier transform infrared spectroscopy (FTIR) was analyzed to study the absorption peaks of agricultural materials. The appearance of absorption peaks were due to the presence of absorption peaks were due to the presence of hydroxyl functional groups. That is, the handmade sponges have strong moisture absorption quality. They absorb as well as release moisture very fast. According to biodegradability test, these agricultural material sponges are biodegradable and have no negative effect on environment and thus can be categorized as ecofriendly fibers. Finally, the absorption efficiency between agricultural material sponges (AMS) with conventional sponges were compared by lab-experiment. AMS are biodegradable, full composed and help to reduce agricultural waste conversional sponges are source of much waste, cannot be recycled, disposable and full of chemicals. So, the conversional sponges are not good to us.

Acknowledgement

We wish to express our thanks Rector Dr Aung Win, Pro-rectors Dr Soe Myint Aye and Dr Aye Aye Ko.Special thanks are extended to Professor and Head Dr Ni Ni Aung and Professor Dr Nant Si Si Htay, Department of Chemistry, Myitkyina University for their kind encouragement.

References

Abed S.A., Ibnsouda S. K., Lattache H. and Hamadi F., (2007). "Scanning Electron Microscopy (SEM) and Enviromental SEM: Suitable Tools for Study of Adhesion State and Biofilm Formation". www. Intechopen.com.pp. 717-730.

Davis J.M., (1994). "Luffa sponge gourd production particles for temperature climates". Hort Scinence 29 (4): 263-266.

Dhake J.D, and Sapkal R.S., (1983)."Utilization of banana (Musa cavendishit) stems and leaves in paper and board making", Indian Plup and Paper 37(4), 5-8.

Dr. Soepadmo E., (ed.). (1998). "The Encyclopaedioa of Malaysia: Plants", Aquatic Flowering Plants by Cheksum Supiah Tawan, Editions Didier Millet.

Naryan R., *et.al.*, (1999)."Testing forbiodegradable plastic",US patent 5.

Isolation of Some Phenylpropenoid and Triterpenoid Compounds from the Fruits of Petroleum Ether Extract of *Foeniculum vulgare* Mill.

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HkawYung, Dr, Assistant Lecturer, Department of Chemistry, University of Myitkyina

Abstract

This research work concern with the study of chemical constituents present in the fruits of *Foeniculumvulgare*Mill. calledSamon-saba in Myanmar name, one of the Myanmar indigenous medicinal plant. Phytochemical investigations on the fruits of *Foeniculumvulgare*Mill.showed that it contains alkaloids, a-amino acids, flavonoids, glycosides, polyphenols, reducing sugars, saponins, steroids,terpenes, and tannins. Antimicrobial activities of ethanol and petroleum ether extractswere examined by Agar well diffusion method and tested organisms were *Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas fluorescens*and *Staphylococcus aureus* respectively. Then antioxidant activity of *Foeniculumvulgare*Mill.was investigated by DPPH assay method. Threecompounds, compound I (0.11%) [anethole, phenyl propenoid compound], compound II (0.041 %) [□-amyrin acetate, triterpenoid compound] and compound III (0.039 %) [□-amyrin, triterpenoid compound] fromthe fruits of petroleum ether extract of *Foeniculumvulgare*Mill.were isolated. These isolated compounds were identified by Thin Layer Chromatography (TLC), Ultraviolet (UV), Fourier Transform Infrared Spectrometry(FT-IR) and Gas Chromatography Mass Spectrometry (GC-MS) methods.

Keywords: Phytochemical tests, phenyl propenoid and triterpenoidcompounds, Fourier Transform Infrared Spectrometry(FT-IR), Gas Chromatography Mass Spectrometry (GC-MS)

Introduction

A large proportion of populations living in rural areas, rely on the indigenous medicinal system, largely based on the use of roots, leaves, barks, seeds, fruits and flowers. These plants may save many lives if they are used correctly. During the past decade, traditional systems 0 medic ne have become topics of global interest. Ii is estimated that 80% of the world's population utilize traditional medicine for the treatment of various diseases. Although modem medicine is well developed in the world, large sections of the population in developing countries still rely on the traditional practitioners, medicinal plants and herbal medicines for their primary care. Moreover, during the past decades, public interest in natural therapies has increased greatly in industrialized countries with expending use of medicinal plants and herbal medicine.

The term terpenoids comprise the large group of natural plant products and over twenty thousand such structures have been described from plant sources. They are all derived biogenetically from the 5-carbon precursor isoprene and, hence, are also known as isoprenoids. Monoterpenoids are commonly strong smelling; colourless oily substances of boiling point 140-180°C, although a few (e.g.camphor) are crystalline. They are usually separated and identified by combined gas chromatography-mass spectrometry (GC-MS), but thin layer chromatography is a convenient means of preliminary detection (Harbone and Baxter, 1993).

Fennels have smaller amounts of many other nutrients. On a weight basis, fennels contain more calcium (49 mg/100 g) as compared with apples (7.14 mg/100 g), bananas(3.88 mg/100 g), dates (25.0 mg/100 g), grapes (10.86 mg/100 g), orange (40.25 mg/100 g), prunes (18.0 mg/100 g), raisins (40.0 mg/100 g), and strawberries (14.01 mg/100 g). Phenolics are an important constituent of fruit quality because of their contribution to the taste, colour, and nutritional properties of fruit. Amongst the phenolics analyzed in the fruit of this plant are neochlorogenic acid (1.40%), chlorogenic acid (2.98%), gallic acid (0.169%), chlorogenic acid(6.873%), caffeic acid (2.960%), *p*-coumaric acid (4.325%),ferulic acid-7-o-glucoside (5.223%), quercetin-7-o-glucoside3.219%), ferulic acid (3.555%), 1,5 dicaffeoylquinic acid(4.095%), hesperidin (0.203%), cinnamic acid (0.131%), ros-marinic acid (14.998%), quercetin (17.097%), and apigenin(12.558%)(Roby,2013).

Phytochemical research carried out on *Foeniculum vulgare* has led to the isolation of fatty acids, phenolic components, hydrocarbons, volatile components, and few other classes of secondary metabolites from its different parts. Mostly these phytochemicals are found in essential oil. Some of the phytoconstituents of *Vulgare* were find application as coloring and antiaging agents(Grace, 1974 and Rasul, 2012).

Guillen and Manzanos investigatedtheyield andcomposition of the volatile components found in the pentaneextracts of leaves, stems, and seeds ofF. vulgare.Theyidentified a total of 37 volatile compounds from pentaneextracts of above mentioned parts of fennel by using gaschromatography (GC) and gas chromatography-mass spectrometry (GC-MS) techniques. (Guillen,1996).

A total of 78 compounds were identified from theactive antimycobacterial fraction of *vulgare*with thehelp of gas chromatography-mass spectra (GC-MS). Outof these, twenty compounds were tested against one sensitive and three MDR strains ofMycobacterium tuberculosisusing the Alamar Blue microassay. Compounds that showedsome degree of antimycobacterial activity against all strainstested were the following linoleic acid (MIC 100□g/mL),oleic acid (MIC 100□g/mL), 1,3-benzenediol (MIC 100-200□g/mL), undecanal (MIC 50–200□g/mL), and 2,4-undecadienal (MIC 25–50□g/mL). 2,4-Undecadienal was the most active compound against multidrugresistant *M.tuberculosis*species. Thus, the dietary intake off. Vulgare may lower the risk of *M. tuberculosis* infection (Esquivel-Ferrino,2012).

Foeniculum vulgare Mill. which is known as Samon-saba in Myanmar name is one of the Myanmar indigenous medicinal plants. In Myanmar fruits of *Foeniculum vulgare* Mill. is used orally with drinking water or warm water for the treatment of indigestion, cough, wind colic and worm infestation. The fruits contain phytoconstituents such as volatile oils (mono- and diterpenoid compounds). triterpenoids (□-amyrin), steroid compounds (□-sitosterol), and flavonoid compounds. The main constituent of fruit is anethole, monoterpenoid compound. The aim of the research work is to examine the phytochemical constituents present in the fruits of *Foeniculum vulgare* Mill. by test tube methods. In addition, extraction of the fruits of *Foeniculum vulgare* Mill. have to perform by using solvents having different polarly such as n-hexane and then ethyl acetate. To obtain various phenypropenoid and terpenoid compounds isolation of petroleum ether extracts are carried out by chrumatographic methods. The isolated compounds are also identified by thin layer chromatograph using appropriate spray reagents and spectroscopic methods such as ultraviolet and infrared

spectroscopy and confirming by Gas Chromatography Mass Spectrometry (GC-MS).

Botanical Description

Myanmar name - Samon-saba
Botanical name - *Foeniculum vulgare* Mill.
English name - Fennel
Family name - Umbelliferae

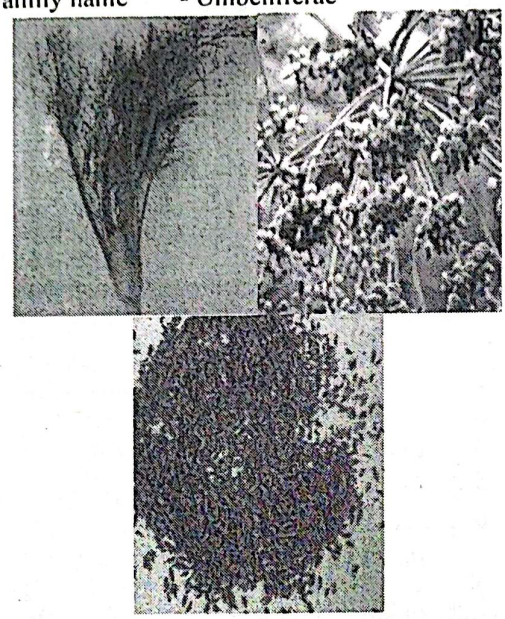


Figure 1. Leave, fruit and seed of *Foeniculum vulgare* Mill.

Materials and Methods

Sampling of Fruits of *Foeniculum vulgare* Mill

Fruits of *Foeniculum vulgare* Mill. used in this experimental section were collected from Mandalay Division. Fruits were air dried and made to powder by Using grinding machine. Dry powders of fruits were stored in air-tight container to prevent moisture changes and contamination. Dry powders of fruits were used to examine phytochemical test by general methods and to extract with various solvents and then to isolate to obtain monoterpenes and triterpenoid compounds.

Phytochemical Examination of Fruits of *Foeniculum vulgare* Mill

Phytochemical examination of plant material is one of the important experiments because it provides classification of groups of chemical substances present in it. Phytochemical examination of fruits of *Foeniculum vulgare* Mill. wascarried out by general methods.

Screening of Antimicrobial Properties of Fruits of *Foeniculum vulgare* Mill.

Determination of antimicrobial activity of fruits of *Foeniculum vulgare* Mill. were measured by applying seven organisms at the Department of Chemistry, Meikhtila University.

Examination of Antioxidant Activity of Fruits of *Foeniculum vulgare* Mill.

Antioxidant Activity of Fruits of *Foeniculum vulgare* Mill. was determined at Medical Biotechnology Laboratory, Biotechnology Research Department, Kyaukse, Mandalay Region.

Extraction of Fruits of *Foeniculum vulgare* Mill. with Petroleum Ether

Dried powdered fruits sample (about 100 g) of fruits of *Foeniculum vulgare* Mill. were weighed and placed in a container, and added petroleum ether (boiling point 60°-80°C) 500 cm^3. To get terpenoid compounds, extraction with petroleum ether was carried out at room temperature for 2 days with occasional shaking. After filtration, the petroleum ether solution was evaporated to dryness to afford petroleum ether extract (2.3 g, 2.3 %).

Isolation of Chemical Constituents from Petroleum Ether Extract

Isolation of Chemical Constituents was performed by column chromatography using silica gel and n-hexane only and n-hexane-ethyl acetate (19:1 and 9: 1) as solvent systems. After preparing the column, the tap was opened and fractions were collected at the rate of one drop per second. A small and dried bottle was placed under the tap of the column to collect the eluent about 2 cm^3 for each fraction. All these fractions were checked by TLC and totally 50 fractions were collected. It was found that bottle number 4-6 showed the same feature on TLC and evaporated to dryness to give Compound I as yellow solid (0.11 g, 0.11%). By evaporating the combined fractions of bottle number 9-15. which showed the same TLC feature, also yield Compound II as white solid (0.041g. 0.041%). Similarly. 'since fractions of' bottle number 40-45were found the same feature on TLC these were combined and then evaporated to afford white solid Compound III (0.039g. 0.039%).

Identification of Isolated Compounds from Petroleum Ether

Compound I was obtained from petroleum ether extract of fruits of *Foeniculum vulgare* Mill. and identified by thin layer chromatography using n-hexane only as solvent system. R_f value of compound I was measured and Vanillin-H_2-SO_4 spray reagent was used to observe the colour. Ultraviolet spectrum was taken in methanol solvent and wavelength of maximum absorption ($\square_{max}$) in nm was measured to determine the presence or absence of conjugation system in compound I. Infrared spectrum was taken and characteristic absorption bands in wave number (cm^{-1}) were determined to indicate the presence of functional groups in compound I. Compound II was obtained from petroleum ether extract of fruits of *Foeniculum vulgare* Mill.and identified by thin layer chromatography using n-hexane-AcOEt (1 9: 1) solvent system. R value of compound II was measured and Liebermann-Burchard spray reagent was used to observe the colour. Ultraviolet spectrum was taken in methanol solvent and wavelength of maximum absorption ($\square_{max}$)in urn was measured to determine the presence or absence of conjugation system in compound II. Infrared spectrum was taken and characteristic absorption bands in wave number (cm') were determined to indicate the presence of functional groups in compound II. Compound III was obtained from petroleum ether extract of fruits of *Foeniculum vulgare* Mill.and identified by thin layer chromatography using n-hexane-AcOEt (9:1) solvent system. R_f value of compound ITT was measured and Liebermann-Burchard spray reagent was used to observe the colour. Ultraviolet spectrum was taken in methanol solvent and wavelength of maximum absorption ($\square_{max}$) in nm was measured to determine the presence or absence of conjugation system in compound III. Infrared spectrum was taken and characteristic absorption bands in wave number (cm^{-1}) were determined to indicate the presence of functional

groups in compound III. Finally these isolated compounds were identified by Gas Chromatography and Mass Spectrometry (GC-MS).

Results and Discussion

Phytochemical Examination of Fruits of *Foeniculum vulgare* Mill.

Phytochemical tests were done on the fruits of *Foeniculum vulgare* Mill. to determine the presence or absence of organic compounds in it. The results are shown in Table 1. It was found that the Fruits of *Foeniculum vulgare* Mill. contains alkaloids, □-amino acids, flavonoids, glycosides, polyphenols, reducing sugars. saponins, steroids, terpnes, and tannins.

Table 1.Results of Phytochemical Examination on Fruits of *Foeniculum vulgare* Mill.

No.	Test	Extract	Reagent	Observation	Result
1.	Alkaloids	95 % ethanol	Mayer' reagent Wagner's reagent Dragendroff's solution	White ppt White ppt Orange ppt	+ + +
2.	α-amino acids	water	Ninhydrin solution	Pink spot	+
3.	Flavonoids	95% ethanol	Conc:HCl/Mg ribbon	Pink colour	+
4.	Glycosides	water	10 % lead acetate solution	White ppt	+
5.	Polyphenols	95 % ethanol	1% $FeCl_3$ solution	Black solution	+
6.	Reducing sugars	water	Benedict's solution	Red ppt.	+
7.	Saponin	water	Distilled water	Formation of frothing	+
8.	Steroids/ Terpenes	n-hexane	Acetic anhydride/Conc: H_2SO_4	Green solution	+
9.	Tannins	water	1 % gelatin solution	White ppt.	+

(+) presence (-) absence

Antimicrobial Screening of Fruits of *Foeniculum vulgare* Mill.

Antimicrobial properties of *Foeniculum vulgare* Mill. were shown in the Figure 2. Better responses of ethanol extract than petroleum ether extract were observed with the organisms *Agrobacterium tumefaciens, Bacillus subtilis, Candida albicans* and *Staphylococcus aureus* (Table 2).

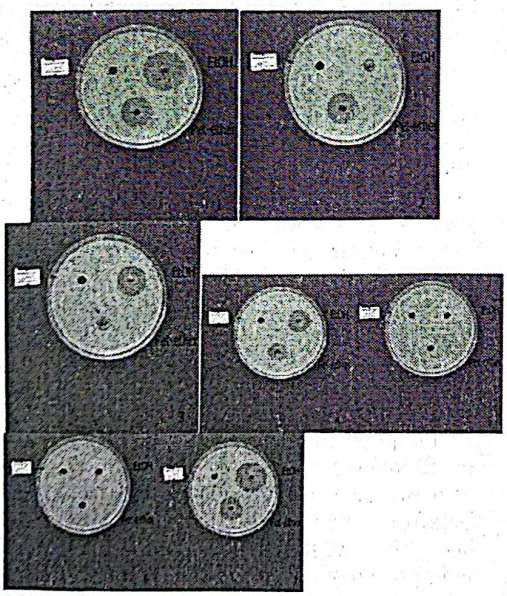


Figure 2. Examination of antimicrobial properties of fruits of *Foeniculum vulgare* Mill.

Table 3.Antioxidant Activity of *Foeniculum vulgare* Mill.

Samon-saba sample (concentration mg/mL)	1000	500	250	125	62.5	31.25	15.63	IC_{50} (mg/mL) ± SD	Me
DPPH Scavenging (%) ± SD	71.17±1.67	54.97±2.85	39.78±9.72	25.50±1.56	22.39±3.28	14.97±2.54	2.75±5.30	358.83±5.82	DPPH Radical Scaveng Assay

Identification of Isolated Compounds

Table 2. Antimicrobial Activity of Fruits *Foeniculum vulgare* Mill.

No	Microorganisms	EtOH	P et
1.	*Agrobacteriumtumefaciens*	+++	+
2	*Bacillus pumilus*	+	+
3	*Bacillus subtilis*	+++	+
4	*Candida albicans*	+++	+
5	*Escherichia coli*	–	–
6	*Pseudomonas fluorescens*	–	–
7	*Staphylococcus aureus*	+++	+

8 mm – 12 mm (+), 13 mm – 17 mm (++), 18 m above (+++), Agar well-8 mm

Antioxidant Activity of Fruits of *Foeniculu vulgare* Mill

The antioxidant activity of *Foeniculu vulgare* Mill. was measured by DPPH radic scavenging method. The IC_{50} values of fr sample was found to be 358.83 µg/mL in Table Lower antioxidant activity than IC_{50} with 8. µg/mL of the standard ascorbic acid was found.

Compound I

Compound I was obtained from petroleu ether extract of fruits of *Foeniculum vulgare* Mi R_f value of compound I was found to be 0.63 wit n-hexane only as solvent system and pink colou was observed with Vanillin-H_2SO_4 spray reagen Litter heating. Thin layer chromatogram c compound I was described in Figure 3. Therefor compound I may be phenylpropenoid compoun R_f value, solvent system, and colour observed wit spray reagent were listed in Table 4. Ultraviol spectrum was taken in methanol solvent an indicates the presence or absence of conjugate system in it. Ultraviolet spectrum of compound was shown in Figure 4. The wavelength o maximum absorption (□max) in nm was found t the 220 and 258nm, respectively. Infrare spectrum was shown in Figure 5. an characteristic absorption bands in wave numbe (cm^{-1}) were determined to indicate the presence o functional groups in compound I. The assignment of characteristic absorption hands for compound could be done as follows: an absorption band a 3010 cm revealed that the C-H stretching o aromatic system. The -CR stretching of sp aliphatic hydrocarbon was found at 2924 an 2853cm^{-1}, respectively. The absorption bands found at 1636 and 1459 cm^{-1} indicated the presence of -CC- stretching of aromatic system The -CH bending of sp^3 aliphatic hydrocarbon was found at 1384 cm^{-1}. the absorption bands at 121

and 1090 cm^{-1} indicated the -C-O-C stretching of aryl alkyl ether. The =C–H out of plane bending of aromatic system was found at 885 cm^{-1}. From the results obtained from thin layer chromatography, ultraviolet and infrared spectroscopy the isolated compound I could be assigned as a phenyl propenoid compound, anethole.

Figure 4.Ultraviolet spectrum of Compound I in methanol

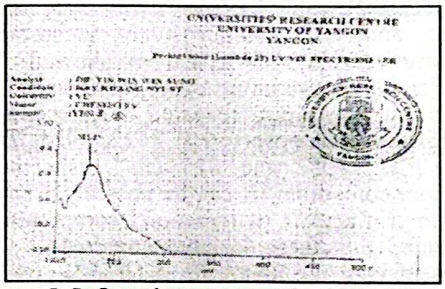
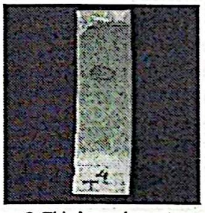
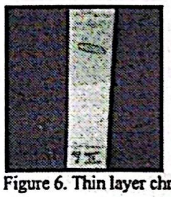


Figure 5. Infrared spectrum of Compound I



Adsorbent	Silica gel plate GF_{254}
R_f 0.63	
Solvent system	n-hexane only
Colour pink	
Spray reagent	Vanillin-H_2SO_4

Figure 3. Thin layer chromatogram of Compound I



Adsorbent	Silica gel plate GF_{254}
R_f	0.75
Solvent system	n-hexane –AcOEt 19:1
Colour	grey
Spray reagent	Liebermann-Burchard

Figure 6. Thin layer chromatogram of Compound II

Table 4. R_f Value, Solvent System, Colour Observed with Spray Reagent of Isolated Compounds

Compound	R_f value	Solvent system	Colour
I	0.63	n-hexane only	Pink (Vanillin-H_2SO_4)
II	0.75	n-hexane – AcOEt (19:1)	Grey (Liebermann-Burchard)
III	0.40	n-hexane – AcOEt (9:1)	Grey (Liebermann-Burchard)

Compound II

Compound II was obtained from petroleum ether extract of fruits of *Foeniculum vulgare* Mill. and R_f value was 0.75 using n-hexane-AcOEt (19:1) solvent system. Compound II also gave grey colour with Liebermann-Burchard spray reagent after heating. Therefore, compound II may also be terpenoid compound. Thin layer chromatogram of compound II was shown in Figure 6. Ultraviolet spectrum of compound II in methanol solvent was shown in Figure 7. and wavelength of maximum absorption (λ_{max}) in nm was found to be 222 nm, respectively. From this ultraviolet spectrum it was found that the absence of conjugated system in compound II. Infrared spectrum was described in Figure 8. and characteristic absorption bands in wave number (cm^{-1}) observed indicate the presence of functional groups in compound II. The assignment of characteristic absorption band for compound II could be done as follows: an absorption hand at 3020 cm^{-1} revealed that the C-H stretching of aromatic system. The -CH stretching of sp^3 aliphatic hydrocarbon was found at 2940 and 2822 cm^{-1} respectively. A sharp absorption band at 1727 indicated the presence of carbonyl group of acetyl moiety. The absorption bands found at 1647, 1503 and 1448 cm^{-1} indicated the presence of –C=C– stretching of aromatic system. The -CR bending of sp^3 aliphatic hydrocarbon was observed at 1380 and 1370 cm^{-1}. The absorption bands at 1240 and 1053 cm^{-1} indicated the -C-O- stretching of ester moiety. The =C-H out of plane bending of sp3 a moiety was found at 880 cm^{-1}. From the results obtained from thin layer chromatography, ultraviolet and infrared spectroscopy the isolated compound II could he assigned as a triterpenoid compound, □- amyrin acetate.

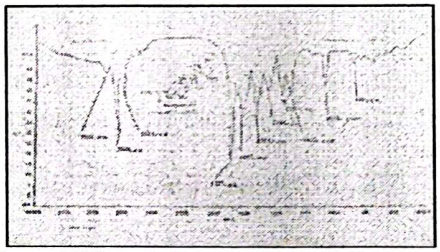


Figure 7.Ultraviolet spectrum of Compound II in methanol

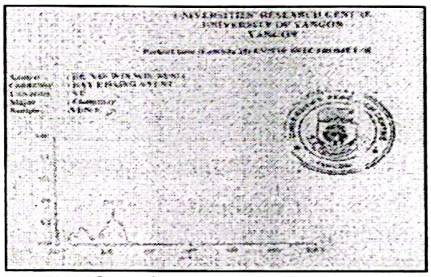
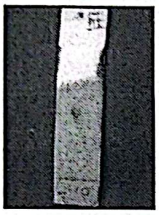


Figure 8. Infrared spectrum of compound II

Compound III

Compound III was obtained from petroleum ether extract of fruits of *Foeniculum vulgare* Mill. R_f value was found to be 0.40 with n-hexane-AcOEt (9:1) solvent system. Compound III showed grey colour with Liebermann-Burchard spray reagent after heating. Thin layer chromatogram of Compound III was shown in Figure 9. The Compound III may be terpenoid compound. Ultraviolet spectrum of compound III in methanol solvent was described in Figure 10. and wavelength of maximum absorption (λ_{max}) in

nm was found to be 222 nm, respectively. λ_{max} values indicate compound III may contain no conjugated system. Infrared spectrum of compound III was shown in Figure 11. and characteristic absorption bands in wave number (cm^{-1}) indicate the presence of functional groups in compound III. The assignment of characteristic absorption bands for compound III could be done as follows: an absorption band at 3422 cm^{-1}revealed that the -O-H stretching of alcohol. The -CH stretching of sp^3 aliphatic hydrocarbon was found at 2924 and 2852 cm^{-1}, respectively. The absorption hands found at 1627 cm indicated the presence of –C=C- stretching of olefin moiety. The -CH bending of sp^3 aliphatic hydrocarbon was observed at 1382 $^{-1}$.The absorption band at 1052 cm indicated the -C-O- stretching of alcohol group. The C-H out of plane bending of olefinic system was found at 825cm^{-1}. From the results obtained from thin layer chromatography, ultraviolet and infrared spectroscopy the isolated compound I could be assigned as a triterpenoid compound, □-amyrin.



Adsorbent	Silica gel plate GF_{254}
R_f	0.740
Solvent system	n-hexane –AcOEt 19:1
Colour	grey
Spray reagent	Liebermann-Burchard

Figure 9. Thin layer chromatogram of Compound III

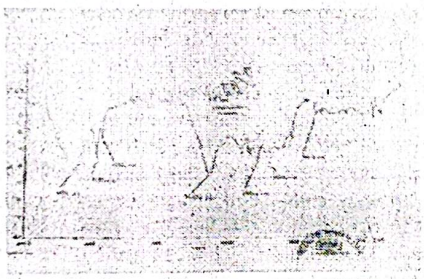


Figure 10.Ultraviolet spectrum of Compound III in Methanol

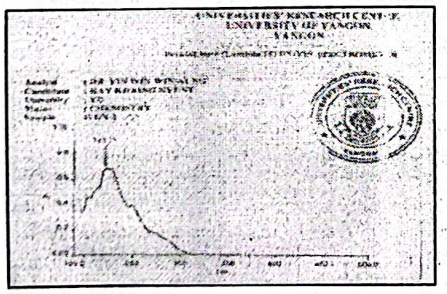


Figure 11. Infrared spectrum of Compound III

Conclusion

Phytochemical investigation of fruits of *Foeniculum vulgare* Mill. indicated that the fruits of *Foeniculum vulgare* Mill. contains alkaloids, □-amino acids, flavonoids, glycosides, polyphenols. reducing sugars, saponins, steroids, terpenes, and tannins. Extraction with petroleum ether gave the extract (2.3 g. 2.3%). Isolation of chemical constituents from petroleum ether extract achieved three compounds, one phenylpropenoid and two triterpenoids which were identified by thin layer chromatography and spectroscopic methods. Compound I was obtained as oily substance (0. 11 %). The compound I showed R_f value 0.63 on thin layer chromatogram with n-hexane only solvent system and pink colour with Vanillin-H_2SO_4 reagent. UV spectrum showe λ_{max} (wavelength of maximum absorption) value 220 and 258 urn. Infrared spectrum indicated tha the presence of functional groups found at 301(2924, 2853 1636, 1459, 1384. 1213, 1090, 885 cm $^{-1}$. Therefore compound I can be possibly assigne as anethole (phenyl propenoid compound Compound II was obtained as white soli (0.041%). The compound II showed R_f value 0.7 on thin layer chromatogram with n-hexane-AcOE (19:1) solvent system and grey colour wit Liebermann-Burchard reagent. The spectrur showed λ_{max} (wavelength of maximur absorption) value 222 nm. Infrared spectrur revealed the functional groups present at 302(2940, 2822.1 727, 1647, 1503, 1448, 1380, 124(1053 and 880 cm^{-1}). Therefore, compound II ca be possibly assigned as □-amyrin acetat (triterpenoid compound). Compound III wa obtained as white solid (0.039%). The compoun III showed R_f value 0.40 on thin laye chromatogram with n-hexane-AcOEt (9:1) solven system and grey colour with Liebermann Burchard reagent. UV spectrum showed □ma (wavelength of maximum absorption) value 22 nm. Infrared spectrum revealed the presence o functional groups observed at 3422, 2924, 2852 1627, 1382, 1052 and 825 cm^{-1} respectively Therefore compound III can be possibly assigne as □-amyrin acetate (trilerpenoicl compound). I was found that these isolated compounds wer consistent with the literature of GC-MS data. I this research, three phytochemical constituent were successfully isolated from petroleum ethe extract and could be possibly assigned.

Acknowledgements

I owe a great deal of gratitude to Dr Aung Win, Rector, Dr Aye Aye Ko and Dr SoeMyint Aye, Prorectors, Myitkyina University for their encouragements. Then we would like to express our gratitude to Dr Ni Ni Aung, Professor and Head Dr Nant Si Si Htay, Professor, Department of Chemistry Myitkyina University for their invaluable advice, kin guidance and encouragement for this results.

References

Badgujiar, S.B. 2014. *Foeniculum vulgare* Mill: A Review of Its Botany, Phytochemistry, Pharmacology, Contemporary Application, and Toxicology, BioMed Research International Volume

Chopra. R. N.: Nayar. S. L.: and Chopra. I. C. 1956. Glossary of India Medicinal Plant. India Publication trading Corp. New Delhi.

Esquivel-Ferrino, P.C. 2012. Antimicrobial Activity of Constituents from *Foeniculumvulgare*Var. Dulce Grown in Mexico, Molecules, vol. 17, no. 7, pp. 8471-8472

Farnsworth. N. 1966. Biological and Phytochemical Screening of Plants.Journal of Pharmaceutical Sciences.vol 55. pp 225-276.

Fieser, L. F. and Fieser. M. 1957. Organic Chemistry, Third Edition. Reinhold Publishing Corporation. New York.,pp 961.

Grae, I. 1974.Nature's Colors-Dyes from Plants, MacMillan, New York, NY, USA

Rasul, A. and Akhtar, N. 2012. Formulation development of a cream containing fennel extract: in vivo evaluation for antiaging effects, Pharmazie, vol. 67, no. 1, pp. 54-58

Guillen,M.D. and Manzanos, M. J. 1996. A Study of Several Parts of the Plant as a Source of Compounds with Industrial Inteest, Food Research International, vol. 29, no. 1, pp. 85-88

Harborne, J. B. and Baxter. H: 1993. Phytochemical Dictionary. A Handbook of Bioactive Compounds

from Plants. Taylor and Francis Ltd.. Washington DC.
Lambert J.B. 1987.Introduction to Organic Spectroscopy. Macmillan Publishing Company, New York.

Harborne. J. B. 1984. PhytochernicalMethods.A Guide to Modern Techniques of Plant Analysis.Chapman I lall Ltd. Ne N'urk.

Marini-Bettolo, G. B., Nicoletti..M., Patamia, M. 1981. Plant Screening by Chemical and Chromatographic Procedures Under Field Conditions. Journal of Chromatography.2j: pp 113-1 37.

MyaBwint and SeinGwan. 1952. Burmese Indigenous Medicinal Plants. Burma Medicinal Research Council Special Reports Series. No. 8.Yangon.pp 105.

Rastogi, R.P. 1990. Vol. 1.Compendium of India Medicinal Plants Central Drug Research Institute of Science Communication. New Delhi.

Rastogi, R.P. 1991. Vol. 11.Compendium of India Medicinal Plants.Central Drug Research Institute of Science Communication. New Delhi.

Rastogi, R.P. 1995. Vol. V. Compendium of India Medicinal Plants.Central Drug Research Institute of Science Communication. New Delhi. Raymond, E. 1982.Encyclopedia of Chemical Technology. Reinhold Pub. Corp. New York. no. 13, pp 705.

Rastogi. R.I. 1995. Vol. IV.Compendium of India .YledicinalPlants.Central Drug Research Institute of Science Communication. New Delhi.

Rastogi.R.P. 1993. Vol. III. Compendium of India Medicinal Plants.Central Drug Research Institute of Science Communication. New Delhi.

Roby, M. H. H., Sarhan, M. A., Selim, K. A. and Khalel, K. I. 2013. Antioxidant and Antimicrobial Activities of Essential Oil and Extracts of Fennel (*Foeniculumvulgare*L.) and Chamomile(Matricariachamomilla L.), Industrial Crops and Products, vol 44, pp. 437-445

Silverstein. R. M and Webster. F. X. 1998. Spetrometricdenhiheann of Organic Compounds.6th Edition.John Wile and Sons.Inc. New York.

Wealth of India. 1948. A Dictionary of Indian Raw Materials and Industrial Products Vol. I. Council of Scientific and Industrial Research. New Delhi. India.

Evaluation of Antimicrobial Activities and Qualitative Determination of Fatty Acid Composition of *Carica papaya* L. (Papaya) Seed Oil

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Abstract

Carica Papaya L. (Papaya) belongs to Caricaceae family. The qualitative phytochemical screening of papaya seed was performed using standard methods. The mineral elements of papaya seeds were analyzed by using Energy Dispersive X-ray Fluorescence (EDXRF) spectrometry. Moreover, papaya seed oil was extracted by Soxhlet method using pet-ether solvent. Antimicrobial activities of papaya seed oil were tested by Agar well diffusion method on six selected organisms. The chemical constituents of papaya seed oil were determined by Gas chromatography mass spectrometry, GC-MS.

Keywords: Papaya seed oil, EDXRF, preliminary phytochemical test, GC-MS

Introduction

Papaya (*Carica papaya* L.) is a plant that grows wild in many parts of the tropics. The seed of papaya fruits are generally discarded. However, in order to make a more efficient use of papaya, it is worth investigating the use of the seeds as a source of oil. A compound present in crushed papaya seed that is believed to have activity against helminths intestinal parasites, benzyl isothiocyanate, has been shown to have an effect on vascular contraction using a canine carotid artery in vitro model. Papaya seed oil utilized in high amount such oils could lead to reduce risk of coronary heart disease. In addition, high oleic oil has sufficiently stable to be used in demanding application such as frying. Area of spray oil for snacks, crackers, cereal dried fruit, bakery products where the oil is used to maintain product quality and to increase palatability. Papaya seed oil can be considered as high oleic oil and hence viewed as a healthy alternative to many other vegetable oils (Corbett P., 2003).The extraction and use of vegetable oils has for centuries played an important role in the manufacture of a large number of industrial products and food items. Currently, two main processes for the extraction of oil from seeds are of industrial importance: the hydraulic process and further purification and the chemical process using organic solvents (Marfa, & Afolabi, 1986). This latter process for extracting oil from seeds, although giving high oil extraction recovery, requires expensive capital investment and operational costs and causes undesirable effects on the quality of end products because of the high temperature used in the process (Christie W.W., 991). There is also on order process of aqueous extraction that is advantageous, because it presents no risks of fire or explosion, is non-toxic and the mild processing ensures high quality products. The operation is also more flexible with less initial investment and operational costs. The main disadvantages, as compared to conventional technologies, are lower efficiency of oil extraction and the reduction of product stability, leading to easier microbial contamination. Aqueous extraction, on the other hand, yields not more than 35% of the oil content of the seed (Tano–Debrah and Ohta 1994). In this research work, the total lipid analysis was determined by Soxhlet method using pet–ether solvent.

Botanical Aspects of Papaya Seed

Family name	: Caricaceae
Genus	: *Carica*
Species	: *Papaya*
Botanical name	: *Carica papaya* L.
English name	: Papaya
Myanmar name	: Thin-Baw
Part used	: seeds

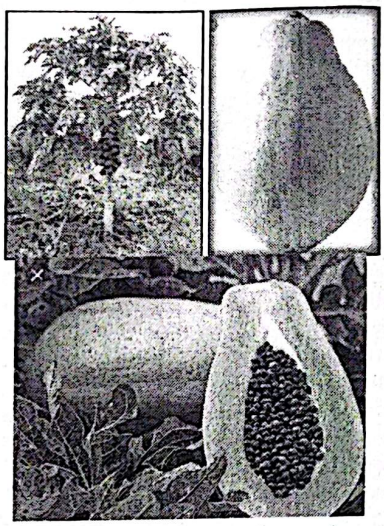


Figure 1. Plant, fruit and seeds of papaya

Health Benefits of Papaya Seeds (Krishna, KI et al., 2008)

Liver health
- papaya seed contain vital nutrients th help heal cirrhosis of the liver.

Kidney health
- papaya seed can be used to impro kidney health and preventing rena failure.

Anti-inflammatory properties
- papaya seeds are anti-inflammator making them great for alleviatin arthritis, joint disease, swelling, pain, an redness.

Anti-bacterial and anti-viral properties
- a small amount of papaya seeds has bee found to kill harmful bacteria like *E. col staph* and *salmonella.*

Arresting cancer
- papaya seeds contain isothiocyanat which works well for colon, breast, lung leukemia and prostate cancer.

Destroys parasites
- The seeds of papaya contain an alkaloi called "Carpaine" that kills intestina worms and amoeba parasites.

Natural contraceptive
- Papaya seeds are excellent nature contraceptives for both men and women without any side effects.

Aids digestion
- Both unripe papaya and papaya seeds are high in the enzyme papain. Papain is beneficial aid to protein digestion bu

best avoided for women currently or trying to get pregnant.

Skin problems

- Papaya seeds will help skin to be wrinkle-free and prevent aging.

Cure eye disorder

- Papaya seeds can cure disease related to our eyes as well.

Culinary Uses of Papaya

The ripe fruit of the papaya is usually eaten raw, without skin or seeds. The unripe green fruit can be eaten cooked, usually in curries, salads, and stews. The unripe green fruits and young leaves are boiled for use as part of lablab salad, while the flower buds are stir-fried with chilies and green tomatoes. Papayas have a relatively high amount of pectin, which can be used to make jellies. The papaya seeds are edible and have a sharp, spicy taste. They are ground and used as a substitute for black pepper or crushed in salad dressings, milk or honey.

Allergies and Side Effects

Green Papaya releases a latex fluid, possibly causing irritation and an allergic reaction in some people. Consumption of very large amounts of papaya may cause carotenemia.

Materials and Methods

Sample Collection and Preparation

Mature papaya fruits were collected from Nansan state, Kathar Township, Sagaing region, Myanmar. The seeds were manually removed from the fruits and dried at room temperature. The seeds were ground to powder and stored in air-tight container. It was used throughout the experiment.

Phytochemical Investigation on Papaya Seeds

Phytochemical investigation on papaya seeds were carried out according to the reported methods. (Harborne, J.B., 1984)

Determination of Mineral Contents from the Papaya Seeds

Mineral contents of defatted seed of the papaya seeds were measured at the Department of Chemistry, Monywa University, Myanmar by applying EDXRF (Energy Dispersive X- ray fluorescence Spectroscopy).

Extraction of Oil from Papaya Seed

The oil was determined by Soxhlet extraction method by using pet-ether. (Pearson, D., 1981)

Base Catalyzed Transesterification of Papaya Seed Oil

About papaya seed oil (1 g) was placed in 20 mL test tube and 20 mL of hexane was added and shake for a minute. Then 2 mL of 2N methanolic KOH solution was added and put in the cup and shake vigorously for 5 minutes to become two separate layers. The upper layer containing fatty acid methyl ester was drawn out with a syringe. The resulting fatty acid methyl ester layer was washed with distilled water to remove excess methanol and the traces of catalyst. The purified product was further subjected to high vacuum to remove the last traces of solvent to yield pure fatty acid methyl ester (Christie W.W., 2003). The resulting fatty acid methyl ester was weighed and the yield percent of fatty acid methyl ester was calculated. The result was indicated in Table (5). The upper layer is fatty acid methyl ester and the lower layer containing glycerin catalyst, soap and impurities were shown in Figure (2). The washing step for fatty acid methyl ester was indicated in figure (3).

Figure 2 Two Separate layers for base

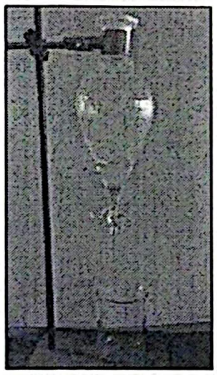
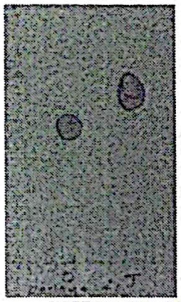


Figure 3 Purification of fatty acid methyl catalyzed reaction ester

TLC Analysis for Fatty Acid Methyl Ester

The composition of oil and the products of trans-esterification reaction were determined by TLC. The plates were then developed using a mixture of hexane and ethyl acetate (4:1) volumetric ratio in a developing chamber. When the developing solvents reached near the top of the plate, the plate was removed and air dried. Spots were visualized by UV light and in an iodine chamber. TLC for transesterification of fatty acid methyl ester in iodine developer and UV lamp were shown in Figure (4).



O = hexane extract
T = transesterification of fatty acid methyl ester

Figure 4 TLC for hexane extract and fatty acid methyl ester

Determination of the Composition of Fatty Acid Methyl Ester by Gas Chromatography- Mass Spectrometry Method

The compositions of fatty acid methyl ester were analyzed by Gas-Chromatography-Mass Spectrometry in the Department of Chemistry, University of Mandalay.

Determination of Antimicrobial Activities of Papaya seeds Oil

The antimicrobial activities of papaya seeds oil were tested by using Agar well diffusion method

at Department of Chemistry, Meiktila University. The results are shown in Figure (13) and Table (7).

Results and Discussion

Phytochemical Screening of Papaya Seeds

The results of preliminary phytochemical test of papaya seed were listed in Table (1).

Table 1. Results of Phytochemical Tests for the Papaya Seed

No	Test	Extract	Reagents	Observation	Remarks
1	Alkaloids	HCl	Dragendroff's Wagner	Orange ppt Yellow ppt	+ +
2	Flavonoids	EtOH	Mg coil, Con HCl	Pink color solution	+
3	Glycoside	D / W	10% lead acetate	White ppt	+
4	Terpenes	EtOH	$(CH_3CO)_2O$, Conc. H_2SO_4, $CHCl_3$	Deep red color	+
5	Saponins	D / W	Shaken	Froth	+
6	Polyphenols	EtOH	1% $FeCl_3$, 1% K[Fe (CN)$_6$]	Blue black color solution	+
7	Phenolic compound	EtOH	10% $FeCl_3$	Greenish blue color solution	+
8	Steroids	Pet - ether	$(CH_3CO)_2O$, con H_2SO_4, $CHCl_3$	Green color solution	+
9	Lipophilic compound	D / W	KOH	Deep colour solution	+
10	Reducing sugar	dil: H_2SO_4	Benedict's solution	No brick red ppt	-

(+) = Presence of constituent (-) = Absence of constituent

According to the table (1), phytochemical screening test of papaya seed consists of flavonoids, alkaloid, glycosides, saponins, terpenes, polyphenols, phenol, steroids and lipophilic compound respectively. However, reducing sugar was not detected.

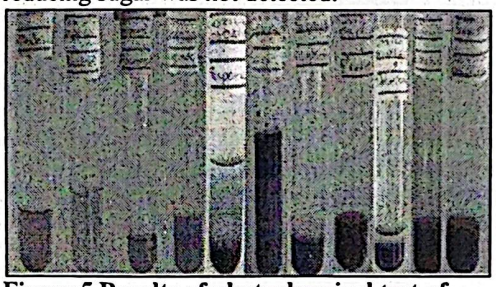


Figure 5 Results of phytochemical test of papaya seed

Determination of Mineral Content from Defatted Papaya Seed

As described in experimental section, the mineral contents of defatted papaya seed were determined by EDXRF method. The observed data were listed in Table (2).

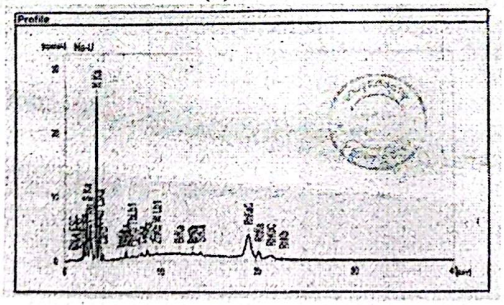


Figure 6 EDXRF spectrum of defatted papaya see

Table 2. Results of Mineral Contents of Defatted Papaya Seeds

No	Mineral	Content (%)
1	Potassium	1.788
2	Sulfur	0.588
3	Phosphorus	0.541
4	Calcium	0.334
5	Iron	0.007
6	Zinc	0.004
7	Manganese	0.003
8	Copper	0.002
9	Silicon	0.001

According to the Table (2), the defat papaya seed contain not only macrominerals, s as potassium, sulfur, phosphorus, and calcium also microminerals such as iron, zinc, mangan and copper. Among them, potassium (K) is major macromineral, followed by sulfur(phosphorus (P), calcium (Ca), iron (Fe), zinc (Z and manganese (Mn) present in defatted pap seed. The other elements were also observed trace amount in selected sample.

Determination of Oil from Papaya Seed

The oil analysis was determined by Soxh extraction method by using pet-ether. The met was shown in section (3.6) and the result v indicated in Table (3).

Table 3 Result of Oil from Papaya Seed

Sample name	Oil yield (g)	Percentage of oil (%
Papaya seed	12.90	25.59

The oil of papaya seed was found to be 25.59 High fat content was observed in this sample.

Determinations of Transesterification Papaya Seed

The extracted papaya seed oil was tra esterified by alkali catalyst to give fatty ac methyl ester (FAME). The catalyzed percenta yield was shown in Table (4).

Table 4 Result of Fatty Acid Methyl Ester Yie from Papaya Seed

Sample	Fatty Acid Methyl Ester yield (g)	Percentage of Fatty Acid Methyl Ester (%
Papaya seed	0.96	48

From the above Table, it can be seen that th papaya seed oil gives the maximum fatty ac methyl ester of 48%.

Determination of Fatty Acid Methyl Est (FAME) in Papaya Seed Oil by GC-M Analysis

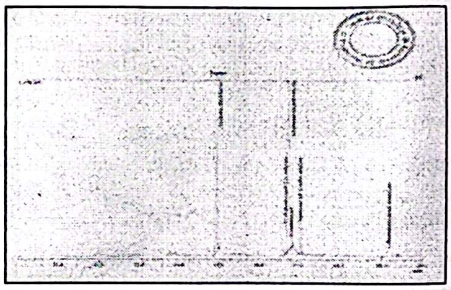


Figure 7 GC-MS spectrum of fatty acid methy ester (FAME) in papaya seed oil

Peak identification of fatty acid in the analyze sample were carried out by comparing with th retention time and molecular mass of mass spectr of standard obtained from library of the GC-MS instrument. (Harvey, T., J. Chan & A. H. Ronald 1978)

Table 5 Identified Fatty Acid Composition from Papaya Seed Oil.

No.	RT	MF	MW	Compound
1.	14.931	$C_{17}H_{34}O_2$	270	Palmitic acid
2.	16.699	$C_{19}H_{34}O_2$	294	Linoleic acid
3.	16.775	$C_{19}H_{36}O_2$	296	Oleic aid
4.	17.060	$C_{19}H_{38}O_2$	298	Stearic acid
5.	19.179	$C_{24}H_{38}O_4$	390	Phthalic acid

The GC chromatogram showed the presence of five compounds with retention times at 14.931, 16.699, 16.775, 17.060 and 19.179. They were palmitic acid, linoleic acid, oleic acid, stearic acid and phthalic acid.

Palmitic Acid Methyl Ester (1)

The mass spectrum of the compound (1) showed the molecular ion peak at m/z=270 corresponding to the molecular formula $C_{17}H_{34}O_2$ of saturated fatty acid.

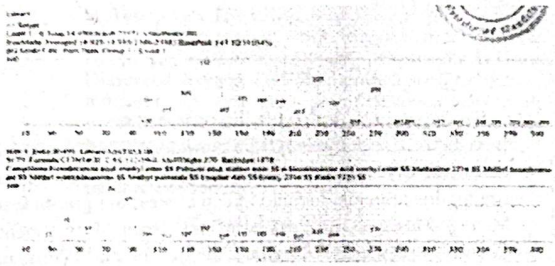


Figure 8 Mass spectrum of palmitic acid methyl ester from papaya seed

Linoleic Acid Methyl Ester (2)

The mass spectrum of the compound (2) showed the molecular ion peak at m/z=294 corresponding to the molecular formula $C_{19}H_{34}O_2$ indicating two degree.

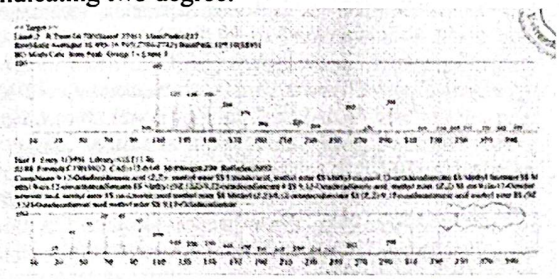


Figure 9 Mass spectrum of linoleic acid methyl ester from papaya seed

Oleic Acid Methyl Ester (3)

The mass spectrum of the compound (3) showed the molecular ion peak at m/z=296 corresponding to the molecular formula $C_{19}H_{36}O_2$ indicating one degree.

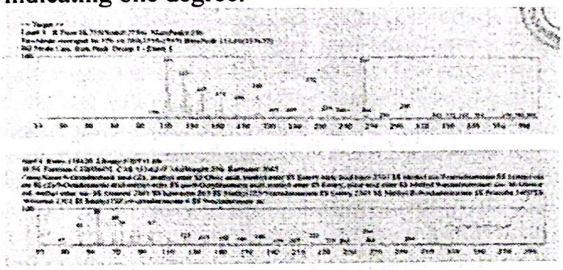


Figure 10 Mass spectrum of oleic acid methyl ester from papaya seed

Stearic Acid Methyl Ester (4)

The mass spectrum of the compound (4) showed the molecular ion peak at m/z=298 corresponding to the molecular formula $C_{19}H_{38}O_2$ of saturated fatty acid.

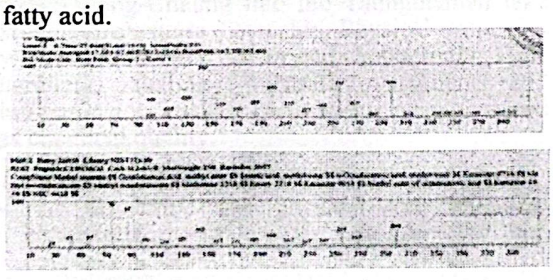


Figure 11 Mass spectrum of stearic acid methyl ester from papaya seed

Phthalic acid Methyl Ester (5)

The mass spectrum of the compound (5) showed the molecular ion peak at m/z=390 corresponding to the molecular formula $C_{24}H_{38}O_4$.

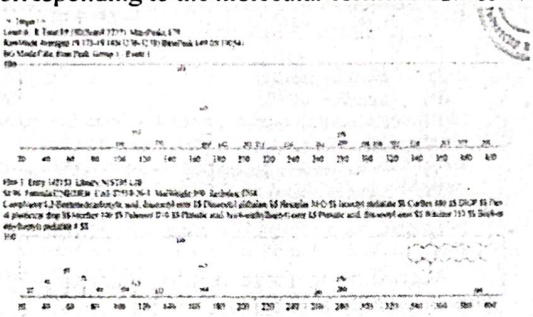


Figure 12 Mass spectrum of phthalic acid methyl ester from papaya seed

According to the GC-MS spectrum it was found that Oleic acid (71.09%) as main compound, palmitic acid (15.26%), stearic acid (4.58%), phthalic acid (4.08%), and linoleic acid (4.00%) respectively. In papaya seed oil, the most prominent unsaturated fatty acid are oleic acid (omega 9 fatty acid) and linoleic acid (omega 6 fatty acid).

Antimicrobial Activities of papaya seed oils

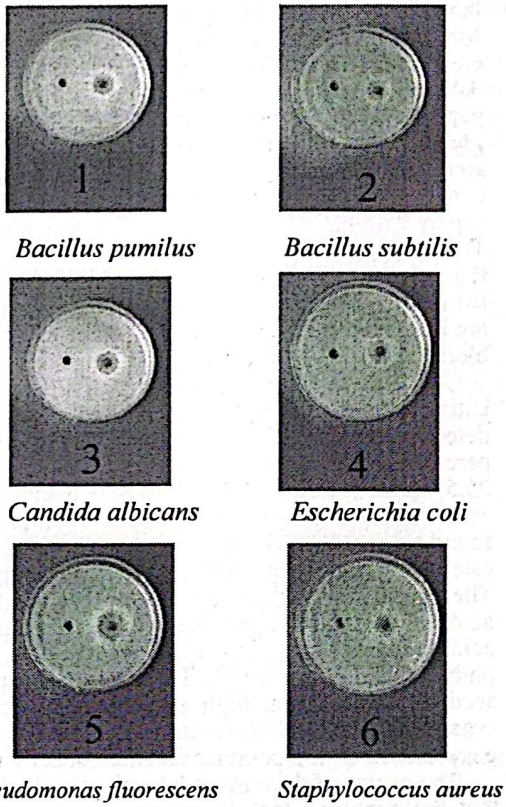


Figure (13) Antimicrobial Activities of Papaya Seed Oil

Table (6) Antimicrobial activities of papaya seed oils

Sample	Solvents	Inhibition Zone					
		I	II	III	IV	V	VI
Papaya seed	Pet ether	+++	+++	+++	+	+++	+++

Organisms

Agar well -10mm

(I) *Bacillus pumilus*
(II) *Bacillus subtilis*
(III) *Candida albicans*
(IV) *Escherichia coli*
(V) *Pseudomonas fluorescens*
(VI) *Staphylococcus aureus*

10mm~ 14mm (+)
15mm~19mm (++)
20mm above (+++)

According to these results, papaya seed oils show high activities on all tested organisms except *E. Coli*. The antimicrobial activities of papaya seed oil demonstrate that folk medicine could be as effective as modern medicine to combat pathogenic microorganisms.

Conclusion

Papaya (*Carica papaya* L.) is commonly known for its food and nutritional values throughout the world. The medicinal properties of papaya fruits and other plant are also well known in traditional system of medicine. The seeds are often thrown away but have some fantastic health benefits. In this research, papaya seed was selected for determination of fatty acid composition, elemental composition and phytoconstituents. According to the preliminary phytochemical tests, papaya seed contain alkaloids, flavonoids, glycosides, terpenes, saponins, polyphenol, steroids, phenolic compounds and lipophilic compounds, but show the absence of reducing sugars. Nine minerals are found in papaya seeds. They are potassium (K), sulfur(S), phosphorous (P), calcium (Ca), Iron (Fe), Zinc (Zn), manganese (Mn), copper (Cu) and silicon (Si). These minerals are important for building strong bones and teeth, blood, skin, hair, nerve function and muscle.

As described in experimental section, the extraction of papaya seed with pet ether was determined by Soxhlet method. The yield percentage of papaya seed oil was found to be 25.59%. Conversion of the oil to fatty acid methyl ester by base catalyzed transesterification was found to be 48%. The isolated fatty acid methyl ester was checked by TLC and GC-MS analysis. The chromatography results show that the fatty acid, identified in papaya seed oil were palmitic acid, linoleic acid, oleic acid, stearic acid and phthalic acid respectively. The extracted papaya seed oil showed that high activity on all tested organisms expect *Escherichia coli*. It shows that papaya seed oil is a source of medicinal activities.

The results of the current investigation showed that the analyzed sample, papaya seed contains rich source of oil, bioactive compound, essential minerals and nutrients respectively. And then, papaya seed oil contains the prominent unsaturated fatty acid such as oleic acid (omega 9 fatty acid) and linoleic acid (omega 6 fatty acid). These fatty acids are very important for the prevention of coronary heart disease, cancer and hypertension. Consequently, papaya seed is widely used in pharmaceutical and cosmetics. Moreover, papaya seed oil can aid in the relief of itchy, scaly, irritated skin conditions such as eczema and psoriasis.

Acknowledgements

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References

Apama, B., (2000), "A Textbook of Practical Plant Chemis Department of Botany, College, Guwahati, S. Chand Company Ltd, 36l, Ram Nagar, New Delhi 110055 m 100-101

Christie W.W., (2003), "Isolation, Separation, Identifica and Structural Analysis of Lipids", The Oily Pr Bridgwater, England

Cocks, L.V and C. Van Rede, (1966), "Laboratory and bc for oil and fat analysis", Academic press Inc., Lond New York, pp-97, 110, 123,168.

Corbett P., (2003), "It's time for an oil change, opportunities high oleic vegetable oils", inform 003;14:12: pp-62-7

Harborne, J.B., (1984), "Phytochemical Methods", "A Gui to Modern Technique of Plant Analysis", Chapman a Hall, London, New York, pp-37-222.

Harvey, T., J. Chan, and A.H. Ronald, (1978), "Composition Papaya Seeds", Food Sci. **43**, pp-255-256

Krishna, KL, *et al.*, (2008), "Review on Nutritional Medi and Pharmacological properties of Papaya (*Car papaya* L.)", Institute of Pharmacy, Ahmedabad 382481, Gujarat, India

Marfa, E.K., O.L. Oke, O.A. Afolabi, (1986), "Chemic composition of papaya (*carica papaya* L) seeds", Foc chem. **22**, 259-266

Pearson, D., (1981), "The Chemical Analysis of Food" 8th Edi Churchill Livingstone. New York, pp-7-31

Tano-Debrah, K.and Y. Ohta, (1994), "Enzyme-assiste aqueous extraction of fat from kernals of the shea tree butyrospermunparkii, J.Am. Oil chem. Soc. **71**, pp-97 983

Assessment of Water Quality of Taung-thaman Lake near the Water Flows from Shwe Taung Chaung to the Lake

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Abstract

In this research paper, studies on physical and chemical characteristics of water sample from Taung-thaman Lake at Amarapura Township, Mandalay Region have been carried out two seasons (September 2018 and December 2018). In the present work, physical properties such as pH, color, conductivity and turbidity were examined. Alkalinity, hardness, calcium, magnesium, chloride and sulphate were analysed as chemical constituents. Dissolved oxygen and biochemical oxygen demand of the water samples were determined as the pollution indicator. The determination of radio isotopes in the water samples were performed. The elemental analysis of water samples were determined by EDX-700. Moreover, heavy toxic metals such as Pb and Cd of the water samples were also determined by Atomic Absorption Spectrometry (AAS).

Keywords : AAS, biological determination, chemical characteristics, EDX-700, physical parameters, radio isotopes

Introduction

Water is the most important oxygen containing compound. Water is ever present in streams and lakes as vapour in the atmosphere in Landmasses and in all living organism. Expect oxygen, water is the important substance known to man.

Water is a basic nutrient of the human body and is critical to human life. It supports the digestion of food, adsorption, transportation and use of nutrients and the elimination of toxic and wastes form the body. Water is a clear, odourless, tasteless, inexpensive and versatile liquid made by combining two parts hydrogen and one part oxygen. Water is a principal component of every fruit and vegetable as well as making up a large portion of living things.

Chemically pure water is a combination of two elements hydrogen and oxygen, with a chemical symbol H_2O and chemical name as hydrogen monoxide. The study of water is known as hydrology, and is divided into a number of sub categories. Limnology if the branch of the science dealing with the characteristics of fresh water, including biological properties as well as chemical and physical properties. Oceanography is the science of the ocean and its properties.

For drinking purpose, water needs not however be absolutely pure and may have small quantities of salts dissolved in it. Water for domestic use should be free from harmful salty, free from objectionable minerals: iron, manganese, lead, arsenic and other poisonous metals and free from radioactive substances. Water should not contain impurities in hazardous concentrations and it must be free from diseases producing organism and poisons. Some of the heavy metals are among the most harmful of the elemental pollutants. Clean quality water means that it must be free from objectional bacteria, other micro-organisms, chemicals industrial wastes and sewages.

In the present work, the water sample was collected form the place that water flows form Shwe-Taung-Chaung into the Taungthaman lake around Amarapura Township. Physical properties were examined pH, colour and conductivity. Total hardness, alkalinity, metallic constituents and inorganic non-metallic constituents were analyzed as chemical quality.

1.1 Aim and Objectives

- To determine the physical and chemical characteristics of water sample for the cultivation.
- To examine the physical properties of water sample.
- To analyze the chemical constituent of water sample
- To compare the physical and chemical properties of water sample with WHO standard value.

Material and Methods

Sample Collection

Water sample were collected from the place that water flows from Shwe-Taung Chaung into the Taung-thaman lake on September 2018 and December 2018.

For the sample collection the plastic bottles of capacity 1 liter were used. Before filling, the sample bottle was rinsed with tap water and two or three times with the water which is to be collected.

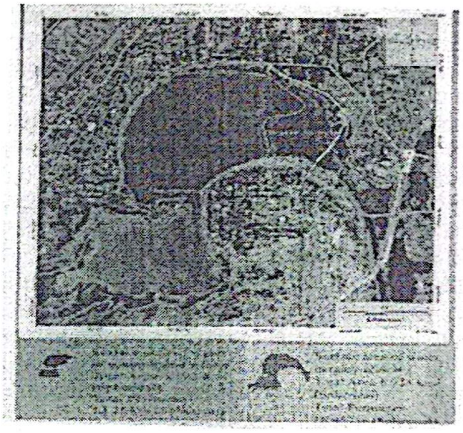

Figure (1) Area of Taung-thaman Lake according to various seasons per annual
(source: Google Map, 2018 & calculated with MapInfo software)

Selection of Method

To examine the water quality, two or more methods are available from the manuals for a parameter. However, although a method may be suitable for a type of water (e.g surface water) it may not applicable to another type (e.g waste water) due to their fixed detectable concentration range. Therefore, selection of method is important in water analysis. The methods used for this investigation were show in table (1).

Table (1) Methods used in the analysis of water

No	Parameter	Method	Determination (by)
1	pH	Direct measurement	pH meter
2	Color	Pt Co standard	Spectophotometer
3	Conductivity	Direct measurement	Spectrophotometer,
4	Turbidity	Absorptometric	Spectrophotometer,
5	Hardness, Calcium, Sulphate	EDTA Titrimetric	Titration
6	Magnesium	Calculation	
7	Alkalinity	Titrimetric	Titration
8	Chloride	Argentometric	Titration
9	Iron	Phenonthroline	Spectrophotometer
10	Manganese	Persulphate	Spectrophotometer
11	Oxygen dissolved	Winkler's Method	Titration
12	BOD	Iodometric	Titration

Physical Examination

Estimation of pH Value : (Direct Measurement by pH meter)

Principle: The basic principle of electrometric pH is determination of the activity of the hydrogen ions by potentiometer measurement using a glass electrode and a reference electrode.

Apparatus : (a) pH meter consisting of potentiometer, a glass electrode, a reference electrode and a temperature compensating device. (b) Beakers (c) Stirrer

Procedure : Electrodes were rinsed with distilled water and dried by gently blotting with a soft tissue. The instrument was standardized with electrodes immersed in a buffer solution of pH 4. The accuracy was checked with another buffer solution of pH 7. The pH of the sample was measured by dipping electrodes after cleaning into well-stirred for one minute.

Estimation of Color Apparent : (APHA Platinum Cabolt Standard Method.)

Principle : Color is determined by comparison of the sample with known concentration of colored solutions. The platinum-cobalt method of measuring color is the standard method, the unit of color being that produced by 1mg platinum in the form of the chloroplatinate ion. The color of water is extremely pH dependent and invariably increases as the pH of the water is raised.

Apparatus : Each Spectrophotometer, DREL/5.

Procedure : 25 mL sample was placed in the sample cell and the color was determined at 455 nm against 25mL demineralized water blank.

Estimation of Conductivity : (Direct Measurement method)

Principle : The reciprocal of resistance is conductance. It measures the ability to conduct a current and expressed in reciprocal ohms. Specific resistance is the resistance of cube 1cm on an edge. Practical electrodes measure a given fraction of the specific resistance, the fraction being the cell constant, C:

$$C = \frac{\text{Measured resistance, } R_m}{\text{specific resistance, } R_s}$$

When the cell constant is known and applied, the measured conductance is converted to the specific conduct once or conductivity, k_s, reciprocal of the specific resistance.

$$k_s = \frac{1}{R_s} = \frac{C}{R_m}$$

Apparatus : Each Conductivity Meter (range to 20,000 V mhosl cm)

Procedure : The conductivity of the sample w directly determined by the conductivity meter.

Estimation of Turbidity : (Absorptomet Method)

Principle : This method is based on a comparis of the intensity of light scattered by the sam under defined condition with the intensity of li scattered by a standard reference suspension un the same conditions. The higher the intensity scattered light, the higher turbidity Forma polymer is used as the reference suspension. T turbidity of a specified concentration of forma suspension is defined as 40 nephlometric u (NTU). This suspension has an approxim turbidity of 40 Jackson units (JTU) when measur on the candle trubidimeter.

Apparatus : Each spectrophotometer, DREL/5.

Procedure : The turbidity of 25 mL sample w directly measured at 450 nm against colorle distilled water blank.

Chemical Examination

Estimation of Hardness, Total : (EDT Micrometric Method)

Principle : EDTA can form a soluble chelat complex with certain metalions. If a small amou of dye such as Eriochrome Black T or calmagite added to an aqueous solution containing calciu and magnesium ions at a pH of 10.0 ± 0.1, th solution becomes wine red. If EDTA titrant added, the calcium and magnesium will b complexed, and when all of the calcium an magnesium has been complexed, the solution tur from wine red to blue. Magnesium ion must b present to yield a satisfactory end point. To insu this a small amount of complex metrically neutr magnesium salt of EDTA is added to the buff solution.

Apparatus : (a) Burette (b) Pipettes (c) Conica flasks

Reagents : (a) Buffer solution

16.9 g of NH_4Cl was dissolved in 143 ml concentrated NH_4OH, 1.179 g of EDTA and 0.78 of $MgSO_4.7H_2O$ or 0.44g of $MgCl_2.6H_2O$ wer dissolved in 50 mL distilled water. These tw solutions were mixed and diluted to 250 mL wit distilled water.

(b) Solid indicator mixture : 0.5g of Ferrochrom Black T dye and 100 g NaCl were ground.

(c) EDTA titrant, 0.01 N

Procedure : 25 mL sample was diluted to 50 mL distilled water, 2mL of buffer solution and 0.2g solid indicator mixture were added to the sample and 50mL distilled water blank. 2 or 3 drops of EDTA titrant were added to the blank until the purplish color turns to bright blue. The sample was titrated with EDTA titrant until the color changed as the blank.

Calculation

$$\text{Hardness as mg } CaCO_3/L = \frac{(A \times B) \times 100}{\text{mL sample}} \text{ (or) Calcium as mg } Ca/L = \frac{(A \times B) \times 400.8}{\text{mL sample}}$$

where, A = volume (mL) of titrant for sample,

B = mg $CaCO_3$ equivalent to 1.00 mL EDTA titrant.

Estimation of Calcium : (EDTA Titrimetric Method).

Principle : When EDTA (ethylenediamine tetraacetic acid or its salt) is added to water containing both calcium and magnesium, it combines first with calcium. Calcium can be determined directly, with EDTA, when the pH is made sufficiently high that the magnesium is largely precipitated as the hydroxide and an indicator is used that combines with the calcium only. Several indicators give a color change when all the calcium has been complexed by the EDTA at a pH of 12 to 13.

Apparatus : (a) Burette (b) Pipettes (c) Conical Flasks

Reagents : (a) Sodium hydroxide solution, 1N (b) Solid indicator mixture: 100g NaCl and 0.2 g murexide were ground to 40 to 50 mesh. (c) EDTA titrant 0.01 M (3.723 g dry disodium ethylenediamine tetracetate dihydrate per 1L distilled water).

Procedure : 25 mL distilled water was added to 25 mL sample. 1 mL NaOH and 0.2 g indicator mixture were added to the sample and the 50 mL distilled water blank. To the blank, 1 or 2 or 3 drops of EDTA titrated were added until red color turns to orchid purple color. The sample was titrant with DETA until the color changed as the blank.

Calculation

$$\text{Calcium as mg } CaCO_3/\text{ L} = \frac{(A - B) \times 100}{\text{mL sample}}$$

where, A = mL titrant for sample , B = mL titrant for blank (mg $CaCO_3$ equivalent to 1.00 mL EDTA titrant at the calcium indicator end point).

Estimation of Magnesium : (Calculation Method)

Magnesium concentration in the sample was calculated using the following relationship. mg/L Mg =[Total hardness (as mg/L $CaCO_3$) – Calcium hardness (as mg/L $CaCO_3$) × 0.244]

Determination of Inorganic Non-Metallic Constituents

Estimation of Alkalinity

Method : Titrimetric Method

Principle : Alkalinity exists in three forms: as normal carbonate, as bicorbonate and hydroxyl or caustic alkalinity. It is determined by tirating the sample with the standard solution of a strong mineral acid. Phenolphthalein indicator enables the measurement of that alkalinity fraction contributed by the hydroxide and half of the carbonate. Indicators responding in the pH ranges 4.5 are used, to measure the alkalinity contributed by hydroxide, carbonate, and bicarbonate.

Carbonate alkalinity is present when the phenolphthalein alkalinity is not zero but is less than total alkalinity. Hydroxide alkalinity is present if the phenolphthalein alkalinity is more than half the total alkalinity. Bicarbonate alkalinity is present if the phenolphthalein alkalinity is less than half the total alkalinity.

Apparatus : (a) Burette (b) Pipettes (c) Conical flasks

Reagents : (a) Standard Sodium Carbonate Solution (b) Standard Sulphuric acid Solution (0.02 N) (c) Phenolphthalein indicator (d) Methyl orange indicator

Procedure : 25 mL of sample was pipetted in a conical flask and 2-3 drops of phenolphthalein indicator was added. The pink color developed in the sample and the sample was titrated with 0.02 N sulphuric acid until pink color disappear i.e, pH value was 8.3. The volume of sulphuric acid was noted. 2-3 drops of methyl orange was added to the same flask, and titration was continued to the color of solution turned from yellow to orange indicating pH value come down to (4.5-4.4). The total volume of 0.02 N sulphuric acid was noted.

Calculation

$$\text{phenolphthalein alkalinity (P), mg/L } CaCO_3 = \frac{A \times 1000}{\text{mL sample}}$$

$$\text{Methyl orange alkalinity (M.O), mg/L } CaCO_3 = \frac{B \times 1000}{\text{mL sample}}$$

$$\text{Total alkalinity (T), mg/L } CaCO_3 = \frac{(A + B) \times 1000}{\text{mL sample}}$$

Total (T), phenolphthalein (P) and methyl orange (M.O) alkalinity were calculated as follows:
A = volume of 0.02 N H_2SO_4 solution for phenolphthalein indicator
B = volume of 0.02 N H_2SO_4 solution for methyl orange indicator.

Estimation of Sulphate : (Gravimetric Method)

Principle : Sulphate is precipitated in sulphuric acid as barium sulphate by the addition of barium chloride. The precipitation is carried out near the boiling temperature, and after a period of digestion the precipitate is filtered, washed, ignited or dried and weiged as $BaSO_4$.

Apparatus : (a) Steam bath (b) Drying oven (c) Muffle furnace (d) Analytical balance (e) Filter (f) Desiccators

Reagents : (a) Standard EDTA solution (b) Buffer solution (c) Indicator solution (d) Standard barium chloride solution

Procedure : 50 mL of sample was boiled to expel carbondioxide. To this boiling sample 5 mL of barium chloride standard solution was added and allowed to cool at room temperature. Then it was dilted to 150 mL, mixed and allowed precipitate to settle. Withdraw 50 mL of clear supernatant, 0.5 to 1.0 mL of buffer solution and several drop of indicator solution were added. It was titrated with standard EDTA solution to produce a blue color which did not change by addition of further drops of titrant.

Calculation : It was assumed that a water has a hardness of (x)mg/L and that 5 mL of barium chloride solution were added to a 50 mL of sample. If no sulphate is present, the titration would require (x) mL of hardness and 5 mL of barium chloride; i.e, a total of (x+5) mL of titrating solution. If the actual titration of an unknown sample would require (Y) mL, the loss due to the presence of sulphate was (x + 5) – (Y) mL = (A) mL sample examined, i.e, to 20 × (A× 0.96) mg of SO_4 per solution.

Estimation of Chloride: (Argentometric Method)

Principle : In a neutral or slightly alkaline solution, potassium chromate can indicate the end point of the silver nitrate titration of chloride. Silver chloride is precipitated quantitatively before red silver chromate is formed.

Apparatus : (a) Burette (b) Pipettes (c) Conical flask

Reagents : (a) Potassium Chromate indicator (b) Standard Sodium chloride solution, 0.0141 N. (c) Standard Silver nitrate titrant, 0.0141 N.

Procedure : To 100 mL sample 1.0 mL K_2CrO_4 solution was added, titrated with standard $AgNO_3$ solution to a pinkish yellow end point. A reagent blank was prepared and titrated as outlined above.

Calculation

$$mg\ Cl/L = \frac{(A - B) \times N \times 35450}{mL\ sample}$$

where A = mL titrant for sample , B = mL titrant for blank and

N = normality of $AgNO_3$ solution

Determination of Dissolved Oxygen (DO) and Biochemical Oxygen Demand (BOD)

Estimation of Dissolved Oxygen (DO) and Biochemical Oxygen Demand (BOD)

Determination : The samples were sent to Mandalay City Development committee, water and sanitation Department, water laboratory and determined the values of DO (by Winkler's Method) and BOD (by Iodometric Method) .

Bacteriological Examination of Water

The routine tests generally used in bacteriological examination of water are:

1. A quantitative test for all coliform bacilli know as presumptive coliform count.

2. A differential tests of typical coliform bacilli (Each coli) known as the differential coliform test.

The samples were sent to the Public Health Laboratory Mandalay and determinated the bacteriological examination of water.

Elemental Analysis of Water Sample by EDXRF

What man filter paper No. 1 were chosen and cut into 30 mm diameter circles. They are dried in an oven at 101°C for two hours and cooled at room temperature 10 drops of water sample I (collected on September 2018) will put into the paper by using graduated pipette (1 drop = 0.035 mL), dried by using drier and stored in a plastic bag for elemental analysis.]

The blank filter paper (without sample) and the filter paper with sample will sent to Universities Research Center, Yangon for element analysis by EDXRF. The results will describe in table (6).

Heavy Metal Analysis of Water sample by Atomic Absorption Spectrometry (AAS)

The trace amount of harmful toxic heavy metals in water sample will determined by AAS at University Research Center, Yangon. The research will describe in Table (7).

Determination of Radio-isotopes in Water Sample

The radio-isotopes in water sample will determine at Department of Nuclear Physics, Yadanabon University. The results was shown in figure 5.

Results and Discussions

Expression of Results

For the present work water sample was collected from location in Amarapura Township. Depth of water sample is shown in table (2).

Table (2) Depth of water sample

Sample	Collection area	depth
1	Taungthaman	3ft

The experimental results for physical, chemical and bacteriological parameter of water sample collected form the Taung-thaman Lake on September 2018 and December 2018 are given in Table (3).

Table (3) Results of the water sample from Taung-thaman lake near the place that w flows from Shwe Taung Chaung to the lake (September 2018 and Decem 2018)

No.	Test	Unit	Results (Sep-2018)	Result (Dec-2018)	Highest desirable level	Maximum permissible lev
	Physical Examination					
1.	pH	scale	7.1	7.0-8.5	7.0-8.5	6.5-9.2
2.	Color	units	>50	5	5	50
3.	Conductivity	micrombos/ cm	330	600	800	4000
4.	Turbidity	N.T.U	0.96	5	5	25
	Chemical analysis					
1.	Total Hardness as $CaCO_3$	mg/L	200	254	100	500
2.	Calcium as Ca	mg/L	16	26	75	200
3.	Magnesium as Mg	mg/L	39	46	30	150
4.	Total alkalinity as $CaCO_3$	mg/L	168	270	200	500
5	Sulphate as SO4	mg/L	<200	<200	200	400
6.	Chloride as Cl	mg/L	20	42	200	600

The total hardness, pH and the amount o magnesium of the water samples were within th standard limits. The color of the water samples ar larger than the maximum permissible level. Th conductivity, the amount of calcium, sulphate an chloride of the water samples are smaller than th highest desirable level The turbidity of water sample (September 2018) was less than the highes desirable level although that of water sample (December 2018) was higher than the maximum permissible level. The total alkalinity of water samples (September 2018) was smaller than the highest desirable level. D.O and B.O.D of the water samples was determined and the results were presented in table (4).

Table (4) DO and BOD value of water sample

Parameters	Result (Sep, 2018)	Result (Dec,2018)	Highest desirable level	Maximum permissible level
D.O (mg/L)	2.80	3.02	Above-5	-
B.O.D(mg/ L)	20.11	22.9	Less than-5	2

According to table (4), DO values for the water sample of September-2018 has 2.80 mg/L and December -2018 has 3.02 mg/L. DO values of water sample (September 2018 and December 2018) is above with W.H.O standard desirable level. So, the water sample has adequate dissolve oxygen in distribution system for drinking water. BOD value for the water sample (September-2018) has 20.11 mg/L and December 2018 has 22.9 mg/L. Higher value of water sample indicates the water is polluted. The bacteriologcal examination of water sample (September 2018) was done at Public Health Laboratory, Mandalay. The results were described in table (5).

Table (5) The result of bacteriological examination of water sample (September 2018)

Parameters	Result
Probable Coliform Count	5/5
Escherichia Coli Count	Isolated

According to table (5) *Escherichia* coli count was isolated and probable coliform count was observed. Therefore, from the point of view of bacteria, the water sample is found to be unsatisfactory. The elemental analysis of water sample September -2018 was performed and the results were shown in Table (6).

Table (6) Determination of the elements Analysis

No.	Element	Units	Result
1	Potassium	ppm	468.520
2	Calcium	ppm	*35.446*
3	Iron	ppm	*6.559*
4	Copper	ppm	1.838
5	Zinc	ppm	2.286

According to Table (6) the water sample contains the highest amount of potassium. The amount of calcium in water sample was the second largest and the content of Irons as higher than the others except potassium and calcium. The amount of zinc and copper was found to be 2.286 ppm and 1.838 ppm.

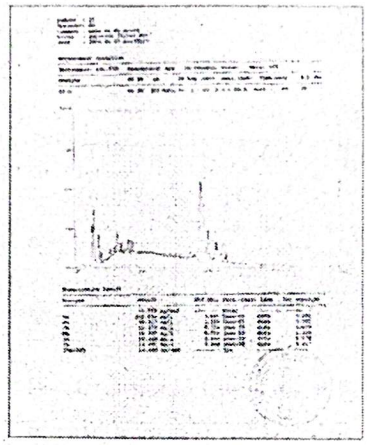

Figure (2) EDXRF Spectrum of water sample (standard)

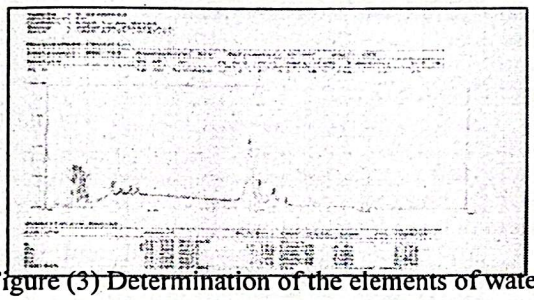

Figure (3) Determination of the elements of water sample by using EDXRF Analysis

The trace amount of heavy toxic metals such as lead an cadmium in water sample (September 2018) were determined by AAS and the results were described in Table (7).

Table (7) Determination of the toxic elements present in water sample by using AAS

No	Element	Result (ppm)	Upper limit concentration (ppm)
1	Lead	1.260	0.1
2	Cadmium	0.484	0.01

According to Table (7), the amount of Lead and Cadmium in water sample were larger than the upper limit concentration.

The determination of radio isotopes in the water sample (September 2018) was carried out in the results were shown in figure (4) and (5).

According to figure (4) and (5), it is clear that the water sample does not contain any radio isotopes because the background spectrum and sample spectrum are identical.

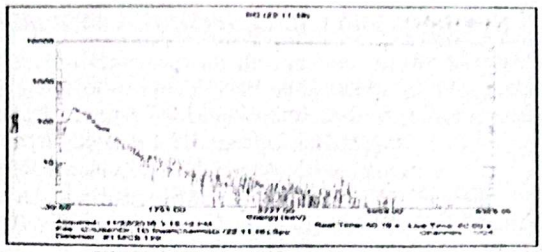

Figure (4) Background spectrum

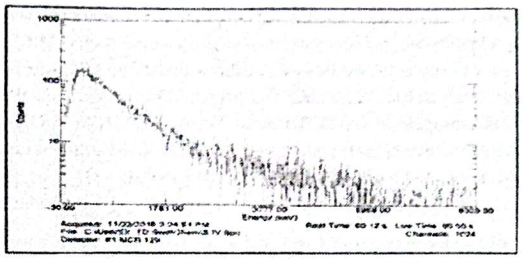

Figure (5) Spectrum of water sample

Conclusion

Water samples were collected from the lake, that water flows from Shwe Taung Chaung to the Taung-thaman lake on September 2018 and December 2018. From the data of water samples (September 2018 and December 2018), the total hardness, pH and the amount of magnesium of the water samples are larger than the maximum permissible level. The conductivity, the amount of calcium, sulphate and chloride of the water are smaller than the highest desirable level. The turbidity of water sample (September 2018) was less than the highest desirable level although that of water sample (December 2018) was highest than the maximum permissible level. The total alkalinity of water sample (September 2018) was smaller than the highest desirable level. The water samples of DO and BOD are higher than desirable level. E-coli count was isolated and probable coli form count was observed from the water samples. From the point of view of bacteria, the water sample is found to be unsatisfactory. There are no radioactive pollutants in water samples. According to EDXRF, the largest amount of potassium was found in the water sample. The toxic metals such as Pb, Cd were found in the water sample. According to the observed data, the water sample from Taung-thaman lake was polluted in some extent. It is concluded that the Taung-thaman Lake water is a good classes for irrigation but it is not suitable for drinking and domestic use.

Acknowledgment

We would like to express my deepest gratitude to Dr Thida Win, Rector, Dr Tin Tun Aung and Dr Myint Zu Min, pro-rector, University of Mandalay for their interest and encouragement on our research paper. We also wish to express our thanks to Dr Yi Yi Myint, Professor and Head, Department of Chemistry, University of Mandalay for her kind help and invaluable guidance for this research work.

References

Drinking Water and Health Report e-book, Volume III. Nicholls, L. Aid to Tropical Hypiece

Quick, F. J Introductory College Chemistry, Macmillan Publishing Co, mc, New York (1965).

WHO Guidelines for Drinking-Water Quality, Volume-i, Recommendation, second edition, World Health Organization, Geneva (1993).

WHO Guidelines for Drinking Water Quality, volume 2, Health Criteria and other Supportion Information (1994).

WMO Manual on water Quality Monitoring. WMO Operational Hydrology Report, No.27, World Meteorological Organization, Geneva (1998).

World Health Organization, European Standard for Drinking Water (1970).

World Health Organization, International Standards for Drinking Water (second Edition, Geneva, 1963).

Synthesis and Characterization of Biodegradable Starch-based Bioplastic from Potato Peel

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Abstract

This research is to prepare bioplastic from a combination of biopolymer of the waste material. This study also intended to show the bioplastic from combination of organic waste has potential to become alternative resources in plastic making industries. In the present work, physico-mechanical properties such as thickness, tensile strength, elongation at break, tear strength and solubility test of bioplastic were also measured. Biodegradability of bioplastic was determined by soil burial test. Bioplastic of potato peel was easily soluble in water. The prepared bioplastic was characterized by using Fourier Transform Infrared Spectroscopy (FT-IR) Thermogrivimetry-Differential Thermal Analysis (TG-DTA) and Scanning Electron Microscope (SEM). The decomposition temperatures of potato peel bioplastic obtained from Thermogravimetric analysis was found to be 450.96 °C. Many efforts have been exerted to develop starch-based bioplastic for conserving the petrochemical resources and reducing environmental impact.

Keywords : Starch-based bioplastic, biodegradability, FT-IR, TG-DTA, SEM

Introduction

Plastic is one of the few new chemical materials which pose environmental problem, polyethylene, polyvinyl-chloride, polystyrene and polyethylene terephthalate are largely used in the manufacture of plastics. Plastic plays the villain right from the stage of its production. The major chemicals that go into the making of plastic are highly toxic and pose serious threat to living being of all species on earth [1]. Some of the constituents of plastic such as benzene are known to cause cancer. Plastics resins themselves are flammable and have contributed considerably to several accidents worldwide. Synthetic macromolecular substances are mostly originating from petroleum and usually are non-biodegradable. The production of plastic has been significantly intensified to keep up with the demand of world rapidly growing population [2] and [3]. There are two kinds of plastic (i) starch-based bioplastic and (ii) petroleum-based plastic [4]. Plastic can cause damage to the environmental. Plastics are not easily broken down by micro-organisms and therefore most are not biodegradable. This leads to waste disposal problems. When plastics burns, they can produce toxic gases such as carbon monoxide, hydrogen cyanide and hydrogen chloride [5].

Bioplastics are plastics derived from renewable biomass sources, such as vegetable fats and oils, starch, straw, woodchips, food waste, etc. Bioplastic can be made from agricultural by products and also from used plastic bottles and other containers using microorganisms [6]. The bioplastic obtained will be environmentally friendly, trendy, user friendly and degradation tractable properties.

Materials and method

Sample Collection

Potatoes and tapioca were purchased from Yadanabon market, Mandalay.

Extraction of Cassava Starch

About 100 g of tapioca and 100 mL of distilled water were grinded in blender and then 100 mL of distilled water was added at grinded next times. The water was decanted and was dried leaving the starch behind.

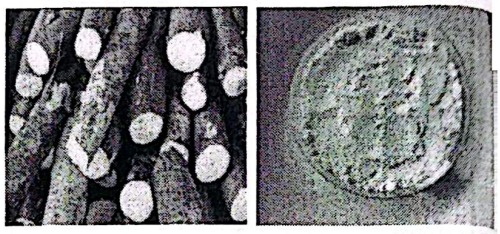


Figure 1 Tapioca and cassava starch

Qualitative Tests for Cassava Starch

Preparation of iodine solution

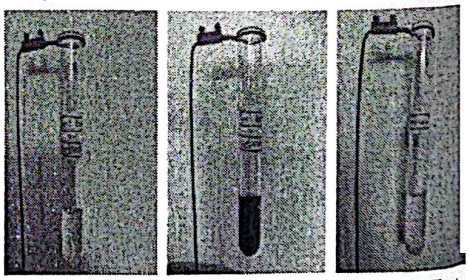
Potassium iodide 10 g was dissolved in abou 30 mL of distilled water. Iodine 5 g was added an heated gently with constant mixing until iodine wa dissolved. It was diluted to 100 mL with distille water.

Iodine solution test

2 mL of starch solution was taken and place in the test tube. 2-3 drops of iodine solution wa added into starch solution. Dark blue color wa changed and starch was present.

Alcohol test

An equal volume of ethanol and starch solution were taken and placed in the test tube. A few-minute later starch was precipitated from solution and starch was present.



Starch Solution Iodine Test Ethanol Test

Figure 2 Identification of starch

Preparation of Potatoes Peels

The peels of potato were removed from the fresh by using a stainless knife. Approximately 10 g of peels were dipped in 100 mL of acetic acid fo 30 min, it was boiled with 250 mL of water in the beaker for 30 min and dried at room temperatur for 30 min. The water was decanted off and the peels were left to dry 30 min at room temperature The peels were then placed in a clean beaker.

Preparation of Bioplastic Films

The peels of potato were grinded using the blender. 25 mL of the paste was placed in a 250 mL beaker. 3 mL of 0.5 M HCl was added and the mixture was stirred using a spatula. Then 2 mL of 1% glycerol solution was added and the mixture was stirred. A 3 mL of 1% cassava starch was then added as co-biopolymer and the mixture was stirred again. Then, a 3 mL of 0.5 M NaOH was added to the mixture and stirred. The mixture was poured into a mould and dried at room temperature about 3 days [6].

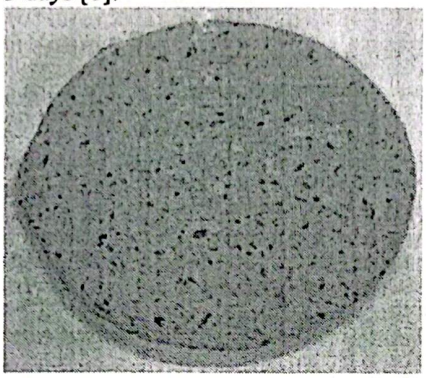


Figure 3 Bioplastic film of potato peel

Determination of Solubility of Bioplastic films

0.02 g of bioplastic films and 20 mL of distilled water were put into the beaker. The solubility of water of bioplastic films was investigated at various time intervals (60-300 mins).

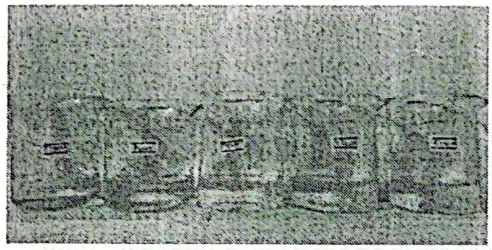


Figure 4 Solubility test of bioplastic films

Biodegradation Test (Soil Burial Test)

The biodegradability of the prepared bioplastic films were carried out by soil burial test. Biodegradation of the prepared films were determined daily from 1 to 12 days.

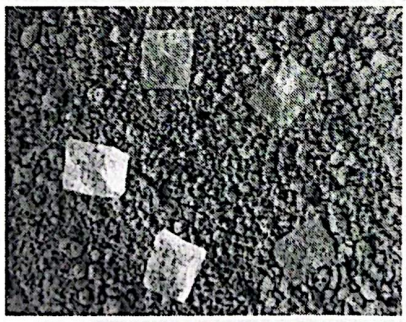


Figure 5 Biodegradation of bioplastic films

Determination of Physico-mechanical Properties

Physico-mechanical parameters of bioplastic film were determined such as thickness, tensile strength, elongation at break and tear strength were tested at Development Center of Rubber Technology, Yangon.

Determination of Functional Groups

FT-IR spectroscopy was used to characterize the functional group that present in the bioplastic film to predict the type of synthesized bioplastics in this study. FT-IR was determined at Department of Chemistry, University of Monywa.

Determination of Thermal Stability

Thermal stability of the bioplastic film was studied using thermal analyzer SDT-600 under nitrogen atmosphere rate increase of 20°C/min. Thermogravimetric Analysis was determined at Department of Chemistry, University of Mandalay.

Determination of Surface Morphology by Scanning Electron Microsopy

The morphology of the bioplastic film was examined at University Research Centre, Yangon.

Results and discussion

Determination of Solubility of Bioplastic Film

The solubilities of bioplastic film in water were investigated at various time intervals (60 to 300 mins) and results shows in Table 1.

Table 1 Results of Solubility of Bioplastic Film

	Potato film	1 hour	2 hours	3 hours	4 hours	5 hours
Weight of sample (g)	0.200	0.173	0.168	0.150	0.135	0.121
Weight loss (g)	0	0.027	0.032	0.050	0.065	0.079

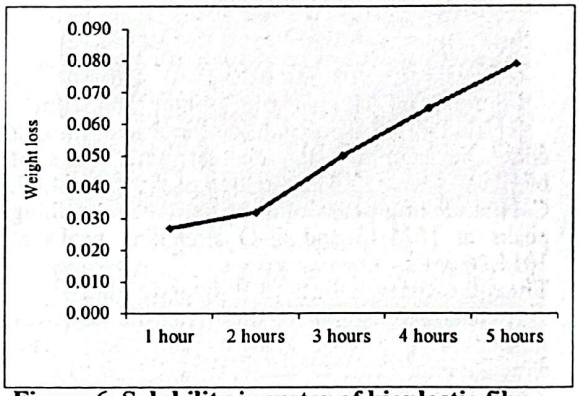


Figure 6 Solubility in water of bioplastic film

Biodegradation of Bioplastic Film

From soil burial test, the weight loss and degradation of bioplastic film were determined. They indicated the amount of degradation in natural environment by action of microorganisms. The starch content consumed by soil microorganisms, soil fracture the polymer chain thus cause the biodegradation.

Table 2 Results of Biodegradation of Bioplastic Film

	1 Day	3 Days	6 Days	9 Days	12 Days
Potato Film					Completely degraded

Physico-mechanical Properties of Bioplastic Film

The results of physico-mechanical properties of bioplastic film were tabulated in Table 3.

Table 3 Results of Physico-mechanical Properties

Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)	Tear strength (kN/m)
0.13	2.1	12	5.4

FT-IR Assignments of Bioplastic Film

The infrared spectrum of bioplastic film was carried out by FT-IR instrument at Department of Chemistry, University of Monywa. The result obtained was illustrated in Figure 7.

Table 4 Thermal Analysis Data of Bioplastic Film

No.	Temperature range (°C)	Weight loss (%)	Peak nature	Remark
1	40-290	24.9	A small exothermic peak at 296.90 °C	Loss of surface water and lower molecular weight compounds of film
2	290-450	38	An exothermic peak at 450.96 °C	Complete degradation of the polymer film

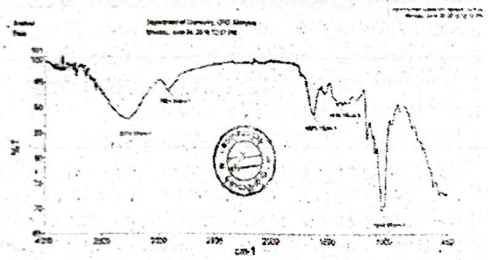


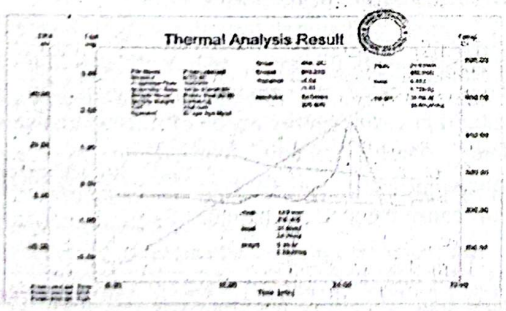
Figure 7 FT-IR spectrum of bioplastic film

The FT-IR spectrum of bioplastic film, it could be seen that the characteristic peaks of bioplastics were O–H stretching peaks at 3274.07, C–H stretching peaks at 2921.83, C=C stretching peaks at 1625.15 and C–O stretching peaks at 1014.86 cm^{-1}.

Thermal Analysis Data of Bioplastic Film

Thermal stability of the bioplastic film was studied using thermal analyzer and results data were listed in Table 4.

Figure 8 TG-DTA assignment of bioplastic film Scanning Electron Microscopy (SEM)



Surface morphology of bioplastic film was visualized using scanning electron microscope under normal atmospheric conditions. The sample was analysed in SEM at high vacuum in varying magnifications.

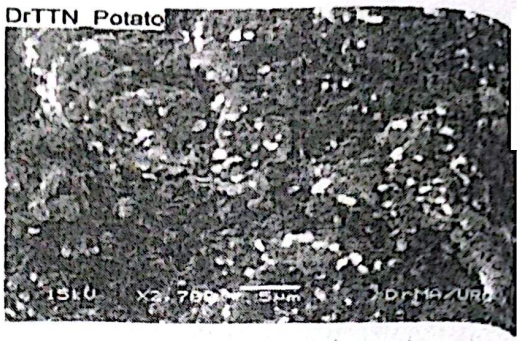


Figure 9 SEM image of bioplastic film

Conclusion

From this study, that starch-based bioplastic from potato peels with cassava starch were performed. This product has high solubility, biodegradable properties with decent thermal and mechanical properties and makes them a suitable alternative for the existing conventional plastics. The hydrophilic nature caused bioplastic to be degraded more easily, making bioplastics an eco-friendly packaging material. Many efforts have been exerted to develop starch based polymer for conserving the petrochemical resources and reducing environmental impact. Furthermore, starch is a renewable resource, cheap and can be easily modified. The means somedays it is unnecessary to rely on petroleum to prepare polymers, people may make biopolymers of suitable performances from the earth. This research can performed for reduce the environmental plastic pollution no longer as severe as today.

Acknowledgements

We would like to express my deepest gratitude to Dr Thida Win, Rector, University of Mandalay for her interest and encouragement on my research paper. We also wish to express thanks to Dr Yi YiMyint, Professor and Head, Department of Chemistry, University of Mandalay and Dr Hla Myoe Min, Professor, Department of Chemistry, University of Mandalay for their kind help and invaluable guidance for this research work.

References

[1] Jambeck J.R., *et al*, (2015). "Plastic Waste Inputs from Land into the Ocean", Science (80), Vol. 347 (6223), pp. 768-770

[2] Mooney B.P., (2009). "Production of Plant-based Biodegradable Plastics", *Biochem J.* Vol. 418, pp. 219-232

[3] Agustin MB, Ahmmod, B, Alonzo SMM, Patriana AM, (2014). "Bioplastic based on Starch and Cellulose Nanocrystal, from Rice Straw", *J. Reint Past Compos*, Vol. 33, pp. 2205-2213

[4] Gironi, F., and V. Piemonte, (2011). "Bioplastics and Petroleum-based Plastics: Strengths and Weaknesses", *J. Environmental Effects*, Vol. 33(21), pp. 1949-1959

[5] Teuten J., *et al*., (2009). "Transport and Release of Chemicals from Plastics to the Environment and to Wildlife", https:doi.org/10.1098/rstb.2008.0284

[6] Noor Fodimah Karder Suttan, Wan Lutti Wan Johari, (2017). "The Development of Banana Peel/Corn Starch Bioplastic Film: A Preliminary Study", BSTR, Vol. 5(1), pp. 12-17.

Physicochemical Analysis and Heavy Metals Concentration in Waste Water from Textile Industrial Zone, Meiktila Township in Mandalay Region

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Ni Ni Aung[3], Dr,Professorand Head,Department of Chemistry, University of Myitkyina

Abstract

This research work concerns with the investigation of physicochemical properties of waste water samples collected from textile industrial zone, Meiktila Township, Mandalay Region. The physical parameters such as temperature, pH, turbidity, conductivity, total dissolved solids and total suspended solids were determined. The chemical parameters such as total alkalinity, total hardness, chloride, sulfate, calcium, magnesium and manganese were examined. Dissolved oxygen(DO), biochemical oxygen demand(BOD), chemical oxygen demand (COD) were also measured.BOD and COD values of control and all waste water samples were very much higher than those of EPA standard. Heavy metals such as cadmium, chromium, copper, arsenic, lead, mercury and iron of waste water were investigated by Atomic Absorption Spectroscopic (AAS) method. The sewage and textile waste water were discharged into the paddy field near the textile industrial zone. Therefore the heavy metal contents of paddy plant samples were detected by AAS.

Keywords: Physicochemical properties, heavy metals , textile, waste water

Introduction

Wastewater from manufacturing or chemical processes in industries contributes to water pollution. Industrial waste water usually contains specific and readily identifiable chemical compounds. Water pollution is concentrated within a few subsectors, mainly in the form of toxic wastes and organic pollutants (Ren, X., 2000). Polluted water is unsuitable for drinking, recreation, agriculture, and industry. More seriously, contaminated water destroys aquatic life and reduces its reproductive ability. Eventually, it is a hazard to human health (Kumar and Kakrani., 2000).

As the world population increases, demands of clothing and apparel increase with the improving sense of fashion and lifestyle thus textiles are manufactured to meet the growing demands. However, this has brought both consequences to such countries either in a positive way which is an improvement of economy or in a negative way attributed to environmental pollution (Babu, *et al.*, 2007). Wastewater has serious negative impact not only on underground and surface water bodies and land in the surrounding area but also has an adverse effect on the aquatic ecological system (Contruvo., 1989).

Environmental problems of the textile industry are mainly caused by discharges of wastewater. Textile processing employs a variety of chemicals, depending on the nature of the raw material and product. Some of these chemicals are different enzymes, detergents, dyes, acids, sodas and salts. (Dr. Klaus Hunger., 2003). Industrial processes generate wastewater containing heavy metal contaminants. These could pose threats to public health and affect the aesthetic quality of potable water (Linus and Erik., 2007). Textile dyeing and finishing industry uses large amounts of water, mainly because of washing operation. Therefore, processing wastewaters have high flow rates. Textile wastewater contains substantial pollution loads in terms of chemical oxygen demand, biochemical oxygen demand, total dissolved solid, total suspended solid and heavy metals (Savin and Butnaru., 2008).

Materials and Methods

In this research work, all the investigations, the methods and techniques involved both conventional and modern instrumental techniques. In all methods, the techniques and analyses were carried out by standard recommended procedures (Vogel, 1968 and Sadhana, & Anand, 2014) and specific catalogues. Instruments employed in this work consist of glassware, labware and other supporting facilities mentioned in each experimental section.

Sample Collection

The three sampling sites and control (tube well) of water samples were collected from different locations of the textile industrial zone, Meiktila Township.

Textile industrial zone lies in east of Meiktila downtown. Industrial zone is far about 9 miles from Meiktila downtown. Control (tube well) and sampling site-1 is located in 3rd street of textile industrial zone. Sampling site-2 is situated in between 1st and 2nd street of textile industrial zone. Sampling site-3 lied junction of textile industrial waste water effluents.

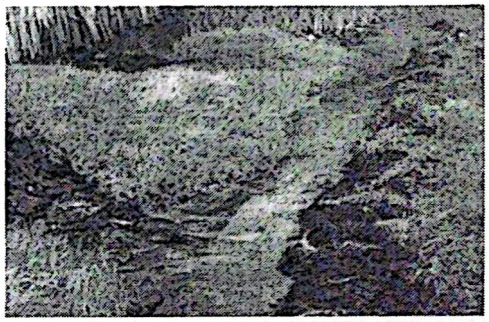


Figure (1). Sampling site-1

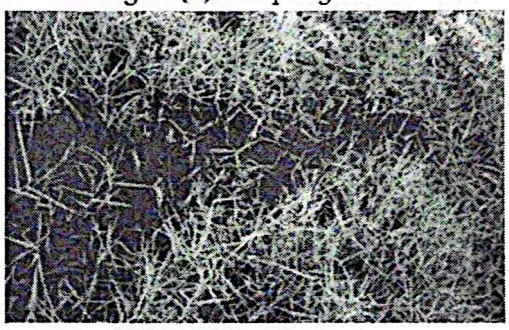


Figure (2). Sampling site-2

Figure (3). Sampling site-3

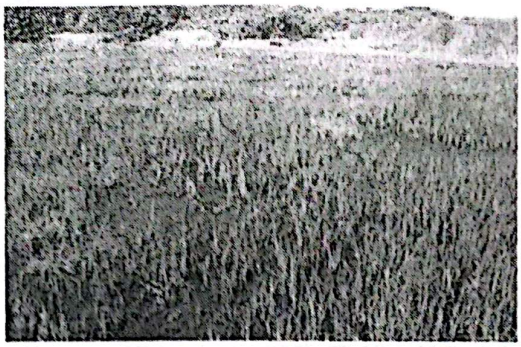


Figure (4). The paddy plants grown in the field

Waste Water Analysis

Water analysis for physicochemical parameters was carried out in the Water and Sanitation Department (Malun, Mandalay) and the Chemical Lab of Research Unit, Department of Fisheries. Different parameters; pH, temperature, total alkalinity, total hardness, chloride, sulphite, total dissolved solid, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand were investigated.

Analysis for heavy metals was undertaken at the laboratory in University of Research Center (URC),

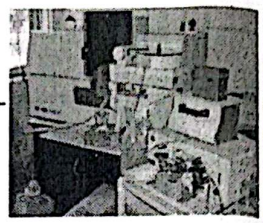
Yangon University. The composition of heavy metals such as copper, arsenic, lead, chromium, cadmium, iron, calcium, manganese and magnesium were determined by an Atomic Absorption Spectrophotometer. Specific hollow cathode lamps were employed for the determination of metals and air acetylene flame was used.

Results and Discussion

The physicochemical and heavy metals properties regarding the quality of waste water from control and three sampling sites in textile industrial zone are recorded in Tables and corresponding histograms are shown in Figures.

Determination of heavy metals in waste water (AAS-Analyst 800 PerkinElmer)

- Copper (Cu)
- Arsenic (As)
- Chromium (Cr)
- Cadmium (Cd)
- Iron (Fe)
- Calcium (Ca)
- Magnesium (Mg)
- Mercury (Hg)
- Lead (Pb)

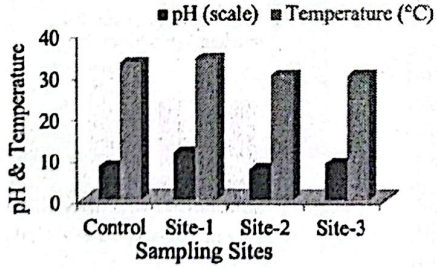


Methods used in the physicochemical analysis and heavy metals of waste water samples

Parameters	Methods/Instruments
pH (scale)	Direct Measurement/pH meter
Temperature (°C)	Direct Measurement/Thermometer
Turbidity (NTU)	Compare with Std Kaolin
Conductivity (μ mohs/cm)	Direct Measurement/Hach Conductivity Meter
Total Alkalinity (ppm)	Titration
Total Hardness (ppm)	Titration (EDTA)
Total Dissolved Solids (ppm)	Gravimetric
Total Suspended Solids (ppm)	Gravimetric
Chloride (ppm)	Titration (Argentometric)
Sulphate (ppm)	Titration (EDTA)
Dissolved Oxygen (ppm)	Winkler Titration
BOD (ppm)	Incubating
COD (ppm)	Permanganate Titration
As, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Hg, Pb (ppm)	AAS method

Table 1. pH, temperature, turbidity and conductivity values in control and waste water samples from textile industrial zone

No.	Parameters	Control	Site-1	Site-2	Site-3	EPA Standard (2008)
1.	pH (scale)	8.1	11.5	7.5	8.8	6.8 - 8.5
2.	Temperature (°C)	33	34	30	30	-
3.	Turbidity (NTU)	80	2000	1000	1400	5 - 25
4.	Conductivity (μmohs/cm)	2985	5333	4000	4000	100-1000



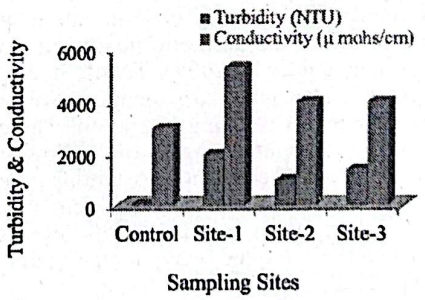


Table 3. TDS, TSS and DO values in control and waste water samples from textile industrial zone

Table 2. Total alkalinity, total hardness, chloride and sulphate values in control and waste water samples from textile industrial zone

No.	Parameters (ppm)	Control	Site-1	Site-2	Site-3	EPA Standard (2008)
1.	Total alkalinity	270	380	260	295	-
2.	Total hardness	220	325	230	245	251-350(Hard)
3.	Chloride	12.23	19.99	14.99	12.49	250
4.	Sulphate	300	900	740	980	250
5.	Calcium (Ca)	8.133	9.814	13.33	7.094	-
6.	Magnesium (Mg)	9.286	9.534	9.521	9.115	-

No.	Parameters (ppm)	Control	Site-1	Site-2	Site-3	EPA Standard (2008)
1.	Total Dissolved Solid	2000	4000	3000	3000	< 600
2.	Total Suspended Solid	600	1200	800	800	150
3.	Dissolved Oxygen	2.74	0.16	0.18	0.15	4 - 6

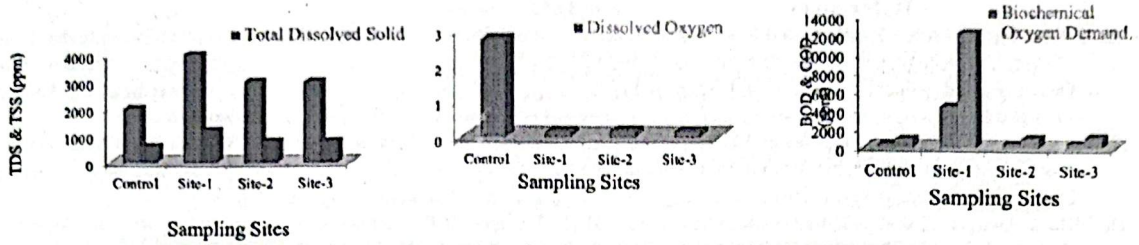


Table 4. BOD and COD values in control and waste water samples from textile industrial zone

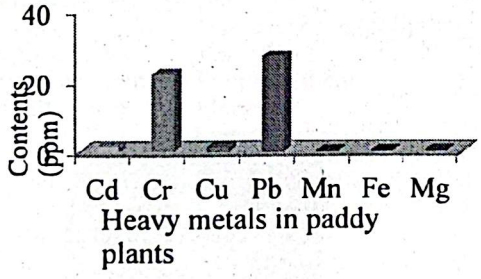
No.	Parameters (ppm)	Control	Site-1	Site-2	Site-3	EPA Standard (2008)
1.	Biochemical Oxygen Demand	380	4414	440	460	5
2.	Chemical Oxygen Demand	950	12284	1080	1150	10

Table 5. Heavy metals contents in control and waste water samples from textile industrial zone

No.	Heavy metals (ppm)	Control	Site-1	Site-2	Site-3	EPA Standard (2008)
1.	Cadmium (Cd)	0.207	0.138	0.132	0.138	0.005
2.	Mercury (Hg)	0.144	0.215	0.196	0.203	0.002
3.	Iron (Fe)	0.042	0.303	0.150	0.876	0.05-0.50
4.	Manganese (Mn)	0.096	0.120	0.113	0.105	0.5
5.	Arsenic (As)	ND	0.01	ND	0.02	0.05
6.	Chromium (Cr)	ND	0.06	ND	ND	0.005
7.	Copper (Cu)	ND	0.391	ND	0.007	0.10
8.	Lead (Pb)	ND	ND	ND	ND	0.05

Table 6. Some Heavy Metals Contents in Paddy Plant Samples

Heavy Metals	Contents (ppm)	Critical concentration of various heavy metals in plants (Sauerbeck, 1982)
Cd	1.53	5 - 10
Cr	22.04	1 - 2
Cu	1.37	15 - 20
Pb	27.09	10 - 20
Mn	0.20	< 20
Fe	0.24	< 50
Mg	0.29	< 50



Conclusion

In this research work, the physicochemical parameters and heavy metals concentration in waste water from textile industrial zone were investigated. The results related to physicochemical and heavy metals characteristics of textile effluents are evaluated. The pH, total hardness, turbidity, conductivity, sulphate, TDS, TSS, BOD and COD values were found to be higher than EPA standard. The results of DO values were observed less than EPA standard. Heavy metals contents were determined by Atomic Absorption Spectrophotometer. The cadmium, and mercury values were higher than EPA standard. From the overall analysis waste water from textile industrial zone may be impact to environment. There is a need to treat the effluent by central effluent treatment plant before discharge. According to investigation of heavy metals in plants, it was found that chromium and lead contents exceed above the range of limit value. It was surprising that Cr and Pb contents in the whole plants which is compared with that value in water samples. This may not be due to the effect of textile effluent but may be due to the effect of soil or fuel used in transportation because that paddy field is situated nearby highway road. Therefore, further research is needed to examine why concentrations of Cr and Pb are high in paddy plant samples grown in that area.

Acknowledgements

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References

Babu B.R., Parande A.K., Raghu S. and Kumar T.P., (2007). "Textile Technology, Cotton Textile Processing: Waste Generation and Effluent Treatment". J. Cotton Sci. 11, 141-153.

Contruvo, J.A., (1989). "Drinking Water Standards and Risk Assessment", Journal of the Institution of Water and Environmental Management, 3, 6-12.

Dr. Klaus Hunger (Editor) D-65779 Kelkheim., (2003), "Industrial Dyes" Chemistry, Properties, Applications Germany formerly Hoechst AG, Frankfurt, Germany.

Kumar and Kakrani., (2000). "Water Environment and Pollution", Department of Biology, Tolani College of Arts and Science, India.

Linus Hagberg and Erik Löfgren., (2007). "Soil and plant contamination by textile industries at ZFILM, Managua".

Ren, X., (2000). "Development of environmental performance indicators for textile process and product", Journal of Cleaner Production 8, 473-481.

Sadhana C., and Anand D.G., (2014). "Hand Book of Water, Air and Soil Analysis (A Lab Manual)", Department of Energy & Environment, Faculty of Science and Environment MGGV, Chitrakkot, satna MP, 485331, India.

Sauerbeck, D., (1982). "Welche Schwermetallgehalte in Pflanzen dürfen nicht überschritten werden, um Wachstumsbeeinträchtigungen zu vermeiden", Landw. Forsch. Sonderheft, Kongressband 39: 108-129.

Savin, I. and Butnaru, R., (2008). "Waste water Characteristics in Textile Finishing Mills", Environmental Engineering and Management Journal 7, 859-864.

USEPA. U.S., (2008). "Environmental Protection Agency", Sample Preservation Methods for Chemical Analysis of Water and Wastes.

Vogel, A. I., (1968). "A Text-Book of Quantitative Inorganic Analysis. London", 3rd Ed., The English Language Book Society.

Synthesis and Characterization of Zinc oxide Nanoparticles by Using *Murraya koenigii* Spreng Leaf (Pyindaw -thein)

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Abstract

In modern science, nanotechnology is an ablaze field for the researchers. In recent year nanotechnology has had numerous applications in different fields such as industry, biotechnology, energy, environment and etc. In this study, zinc oxide nanoparticles were prepared using aqueous solution of zinc acetate and watery extract of leaves of *Murraya koenigii* Spreng by precipitation method. Watery extract of leaves of *Murraya koenigii* Spreng used as reducing and stabilizing agent. The elemental composition of *Murraya koenigii* Spreng leaves were examined by Energy Dispersive X-ray Infrared (EDXRF) spectroscopy. The amount of calcium and potassium were higher than other elements in the leaves of *Murraya koenigii* Spreng. Moreover, the leaves have no toxic elements. The phytochemical composition of *Murraya koenigii* Spreng leaves was performed. The leaves contain valuable phytochemical compounds. The synthesized zinc oxide nanoparticles was characterized by Scanning Electron Microscopy (SEM), X-ray diffraction (XRD), Fourier Transform Infrared (FT,IR) Spectroscopy. The SEM image shows that synthesized zinc oxide nanoparticles have nanocrystalline nature. The crystallite size of synthesized zinc oxide nanoparticles were found within the nanoscale (16- 36 nm) by examination of XRD.

Keywords: Synthesized, *Murraya koenigii* leaves, ZnO nanoparticles, FT-IR and SEM.

Introduction

A particle is defined as a small particle which is between 1 and 100 nanometers in size. Nanotechnology has tremendous applications in diagnostic devices, drug delivery, tissue engineering, environmental chemistry, water filtration, quantum computers and producing ecofriendly energy production system (5). The main advantages of nanoparticles synthesis at room temperature and from leave extracts are partly full fill the green synthesis (2). The metal and metal oxide nanoparticles are synthesized by physical, biological, chemical and very recent green approaches. Zinc oxide nanoparticles have gained immense popularity in the scientific world due to their distinctive and fascinating properties (1). Zinc Oxide nanoparticles are most important, as there are used in bio medicinal, gas sensor, cosmetic and agriculture etc. Zinc oxide nanoparticles have environmental applications as it can out as catalyst which is helpful in reduction or elimination of the toxic hazardous chemicals from the environment (7). The botanical name of Pyindaw-thein is *Murraya koenigii* Spreng and the family name is Rutaceae. *Murraya koenigii* Spreng of leaves are used in falk veterinary medicine to treat wounds burns, poisoning and disorders of digestive system. In this research work, zinc oxide nanoparticles was synthesized using watery extract of leaves *Murraya koenigii* Spreng as reducing agent.

Botanical Description

Botanical Name - *Murraya koenigii* Spreng
English Name - Curry-leaf
Myanmar Name - Pyindaw-thein
Family Name - *Rutaceae*
Part use - Leaves

Figure (1) Pyindaw-thein leaves

Material and Methods

Collection of Sample

Fresh leaves of *Murraya koenigii* Spreng were collected from Mandalay Technological University Campus, Mandalay region. The fresh sample was cleaned by washing with water and air-dried. After that the dried sample were cut into small pieces then the sample were stored in a beaker and used for analysis. Zinc acetate was purchased from chemical shop.

The phytochemical analysis of leaves of *Murraya koenigii* Spreng

Phytochemical tests of the organic compounds present in *Murraya koenigii* Spreng leaves were performed by standard Phytochemical tests.(6)

The elemental analysis of leaves of *Murraya koenigii* Spreng

The elemental analysis of leaves of *Murraya koenigii* Spreng was performed by EDXRF (Energy Dispersive X-ray Fluorescence Spectroscopy) at Department of Chemistry, Monywa University.

Preparation aqueous leaves of *Murraya koenigii* Spreng

Murraya koenigii Spreng leaves were collected and washed with tap water and then distilled water. 30 g of cleaned leaves were cut into small pieces and they were taken in a 500 mL beaker, 400 mL of distilled water was added into the beaker. Then, the mixtures were heated at 80°C for 20 mins, the brown color of aqueous leaves extract was obtained. The extract was cooled at room temperature and filtered through what man paper No.1. Finally, watery extract of leaves of *Murraya koenigii* Spreng was obtained. The leaves extracte matter can be applied the capping agent as well as the protective agent for the synthesis of zinc oxide nanoparticles.

Synthesis of ZnO nanoparticles

50 mL of 1 M Zinc acetate aqueous solution (9.17 g Zinc acetate was dissolved in 50 mL distilled water) is placed in a beaker. 100 mL of *Murraya koenigii* Spreng leaves extract was added to the zinc acetate solution. The mixture was stirred magnetic using stirre for 6 hrs. ZnO nanoparticles were precipitated. The precipitate was separated from the reaction mixture by centrifugation at 6000 rpm for 15 mins and the superantant poured out. The brown paste was obtained which was redispersed in deionized water to remove excess biological molecules. The process of centrifugation and redispersion in deionized water was repeated three times to completely purify the nanoparticles. The brown paste was collected in petridish and was dried for 5 hrs using oven. Zinc oxide nanoparticles were obtained.

Characterization of synthesized ZnO nanoparticles

The synthesized ZnO nanoparticles were characterized by FT.IR (Fourier Transform Infrared), XRD (X-ray diffraction), SEM (Scanning Electron Microscopy).

Results and Discussions

Results of phytochemical tests of leaves of *Murraya koenigii* Spreng

The phytochemical screening of leaves of *Murraya koenigii* Spreng was determined by standard methods. The results were shown in Table (1).

Table (1) the results of phytochemical tests for the organic compounds present in *Murraya koenigii* Spreng leaves

No.	Constituent	Extract	Reagent used	Observation	Result
1.	Alkaloids	1% HCl	Wagner' reagents	Reddish brown ppt	+
			Dragen droff's reagents	Orange ppt	+
2.	Flavonoid	95 % ethanol	Con: HCl,Mg	Pink color solution	+
3.	Glycoside	Distilled water	10 % lead acetate	White ppt	+
4.	Phenolic	Distilled water	10 % $FeCl_3$	Purplish color solution	+
5.	Reducing sugar	Distilled water	Benedict's solution	Green color	+
6.	Saponin	Distilled water	Distilled water	Forth like comb	+
7.	Polyphenol	95 % ethanol	1 % $FeCl_3$,1% $K_3[Fe(CN)_6]$	Green blue color solution	+
8.	Steroid	95 % ethanol	CH_3Cl,acetic anhydride, conc:H_2SO_4	Green color solution	+
9.	Tannin	95 % ethanol	10% $FeCl_3$, dil:H_2SO_4	Yellowish brown ppt	+
10.	Terpene	Pet-ether	CH_3Cl,acetic anhydride, conc:H_2SO_4	Pink color solution	+

(+) = presence of Constituent
(-) = absence of Constituent

According to the table (1), the *Murraya koenigii* Spreng leaves contained Alkaloids, flavonoid, glycoside, phenolic, reducing sugar, saponin, polyphenol, steroid, tannin and terpene.

Results of Elemental Analysis of leaves of *Murraya koenigii* Spreng

The elemental analysis of leaves of *Murraya koenigii* Spreng was carried out and they were shown in Figure (2) and Table (2).

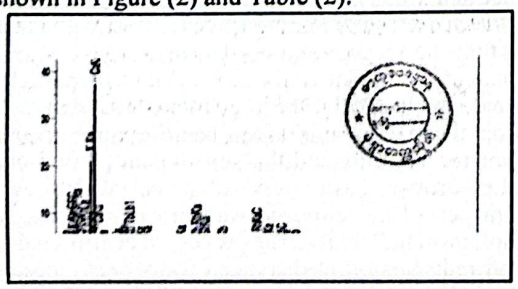

Figure (2) The EDXR Spectrum of synthesized Zinc Oxide Nanoparticles

Table (2) the results of elemental Analysis of leaves of *Murraya koenigii* Spreng.

	Element	
3.	ilicon	0.477
9.	opper	0.002

According to the Table (2), the *Murraya koenigii* Spreng leaves contained higher amount of calcium and potassium than other elements. Human body needs calcium to build and maintain strong bones. Heart muscles and nerves also need calcium to function properly. Some studies suggest that calcium, along with vitamin D, may have benefits beyond bone health; perhaps protecting against cancer, diabetes and high blood pressure (3). Potassium is one of the most important minerals in the body. High potassium diet may help reduce blood pressure and water retention, protect against stroke and prevent osteoporosis and kidney stones. (4)

Yield percent of Zinc Oxide nanoparticles

The yield percent of Zinc Oxide nanoparticles was calculated and shown in table (3).

Table (3) The results of the amount of Zinc Oxide nanoparticles .

Zinc acetat Leaves extı sample	Time (hr)	ight of coxide ıoparticl g)
extract sample	1 : 2	

Characterization of Synthesized Zinc Oxide nanoparticles

FT-IR spectrum of synthesized zinc oxide nanoparticles was shown in Figure (3) and assignment of the FT-IR spectrum of zinc oxide nanoparticles were illustrated in Table (4).

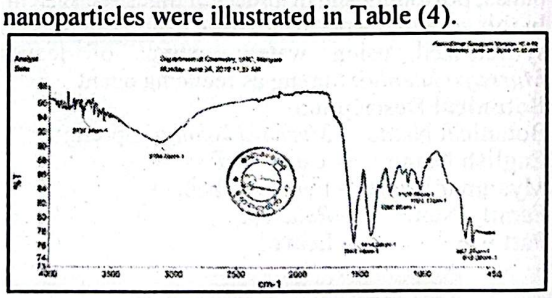

Figure (3) The FT-IR Spectrum of synthesized Zinc Oxide Nanoparticles

Table (4) Characteristic Absorption of FT-IR spectrum of synthesized ZnO Nanoparticles.

	-1	
4.	12 10	) – C stretching ation

From the FT.IR spectrum, amine group, ether group are found. These groups could be watery

extract leaves of *Murraya koenigii* Spreng covered on the surface of zinc oxide nanoparticle.

The surface morphology of synthesized zinc oxide nanoparticles was characterized by Scanning Electron Microscopy (SEM) was shown in figure (4).

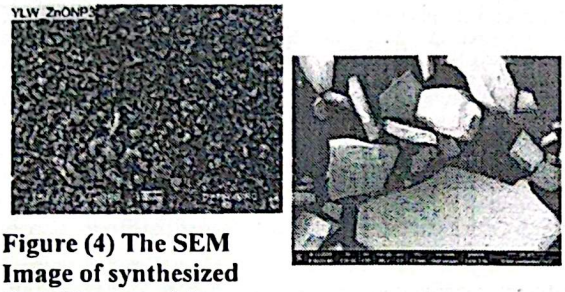

Figure (4) The SEM Image of synthesized ZnO Nanoparticles.

The crystallite size and inter planar spacing of synthesized zinc oxide nanoparticles were determined by XRD analysis. The crystallite size of nanoparticles was calculated using Debye-Scherrer's formula (8).

$$D = \frac{K\lambda}{\beta \cos\theta} \qquad d = \frac{K\lambda}{2 \sin\theta}$$

Where K is the Scherrer's constant, λ is the X-ray wavelength, β is the peak width of half maximum, and θ is the Bragg diffraction angle. The average crystalline size of ZnO is estimated by XRD data and it was obtained around 25.21 nm.The XRD photograph and the XRD results of synthesized zinc oxide nanoparticles were shown in Figure (5) and Table (5).

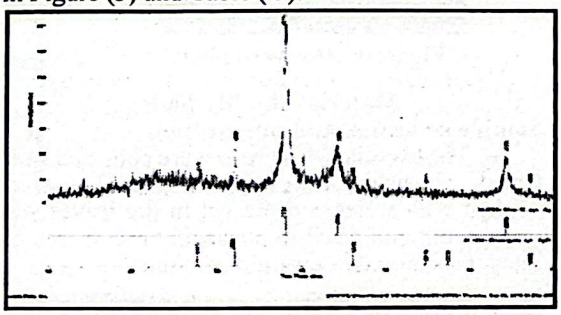

Figure (5) The XRD Sepectrum of Synthesized ZnO Nanoparticles

Table (5) The XRD Results of Crystallite Sizes of Synthesized zinc oxide Nanoparticles

Sr No.	Bragg's angle (2θ)	Miller Indices (hkl)	FWHM of peak (β)	Crystal lite size (L) nm	d-spacing (nm)
1	27.763	(111)	7.069×10^{-3}	20.69	0.2889
2	32.117	(200)	4.189×10^{-3}	34.66	0.2506
3	38.093	(111)	9.199×10^{-3}	16.12	0.2124
4	44.313	(200)	9.495×10^{-3}	15.93	0.1838
5	45.954	(220)	4.014×10^{-3}	38.51	0.1775
6	54.729	(311)	7.104×10^{-3}	21.33	0.1508
7	64.310	(220)	9.129×10^{-3}	18.00	0.1302
8	67.290	(400)	4.660×10^{-3}	36.48	0.1251
			Average	25.21	

According to XRD results, the crystallite sizes of synthesized zinc oxide nanoparticles were found to be within the range of 16-36 nm. The XRD results also showed that the structures of crystals were hexagonal.

Conclusion

In this research work, the leaves of *Murraya koenigii* Spreng, common name, Curry-leaf, Myanmar name, Pyindaw-thein, were selected for the synthesized of zinc oxide nanoparticles.

The phytochemical tests of *Murraya koenigii* Spreng leaves gave positive for alkaloid, flavonoid, glycoside, phenolic, reducing sugar, saponin, polyphenol, steroid, tannin and terpene respectively. The phytochemical analysis indicated that the leaves sample contained the valuable phytochemical constituents. The results of elemental analysis show that the leaves have no toxic metal. From the FT.IR spectrum, it could be seen that the characteristic peak of zinc oxide nanoparticles of leave extract of *Murraya koenigii* Spreng were (3124, 1553.1, 1414.09, 1260,1022, 667.37,615.06 cm^{-1}) found amine and ether group.The SEM analysis reviles that the synthesized zinc oxide crystal are found to be nanocrystalline nature.

According to XRD results, the crystallite sizes of synthesized zinc oxide nanoparticles were found to be within the range of 16-36 nm. The XRD results also showed that the structures of crystals were hexagonal.

Acknowledgment

The author would like to express my grateful appreciation to Professor Dr Myat Myat Mon, Head of Department, Department of Engineering Chemistry, Mandalay Technological University for her kind attentions, helpful advices, and invaluable suggestions. I also with to express my profound gratitude to my research director, Dr Thida Tin and Dr Toe Toe Khing, Associate Professor, Department of Engineering Chemistry, Mandalay Technological University, for her close supervision numerous invaluable suggestions, encouragement and comments without which this work would not have been completed.

References

1. Agnieszka Koladziejczak- Radzimska and Teofil Jesionowskic (2014), Zinc Oxide-From Synthesis to Application: A Review materials 2833-2881.
2. Cui, Y. Lieber, C.M. (2001). "Functional nanoscale electronic devices assembled nanowire block", Science, 29:185-853.
3. *https://www.mayoclinic.org.hu-depth*
4. *https://www.healthline.com.nutrition*
5. Jones, M. R (2011). "Templated Techniques for the Synthesis and Assembly of Plasmanic Nanostructures" chemical Reviews, **111**:3736-3827.
6. J.B. Harbone, "Phytochemical methods, A Guide to modern techniques of plant analysis", Chapman and Hall Ltd. U.S.A,1984.
7. S.Gunalan, R. Sivaraj.V. Rjendran, Green synthesized zinc oxide nanoparticles against bacterial and fungal pathogens, Prog. Nat sci, **22** (2012) **693-700** do; : 10.1016/j-pnsc 2012.11.015.
8. (Navaza, J, 1979) " Ageometrical approach to solving crystal structure" .

Estimtion of The Nutritional Values of Fresh *Aloe Vera* Gel and Antioxidant Activity Analysis

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Abstract

A popular medicinal plant, *Aloe Vera* L. (Sha-zaung-let-pat) is not only used in traditional medicine but also in beverage and cosmetic .The present study intended to investigate the nutritional composition and antioxidant activity of the gel of *Aloe vera* leaves. The preliminary phytochemical analysis of gel sample showed the presence of alkaloids, glycosides, steroids, reducing sugars, flavonoid, polyphenols, phenolic compounds, tannins, saponins, lipophenols and carbohydrates, respectively. The mineral content of the sample was determined by EDXRF spectroscopy. The amount of potassium was highest (3.885%) compared to other minerals in this sample. Then, the nutritional composition was investigated. From the experimental results of nutritional composition, (0.003 %) fat, (0.155%) fiber, (0.063%) protein, (1.274%) carbohydrate and (0.56 %) reducing sugar were found to be present in the gel sample. Vitamin C content (0.0034%) was also determined by iodometric titration. The antioxidant activity of the fresh sample was determined by DPPH assay. The IC_{50} value of fresh sample was found to be 625µg/mL.

Keywords: *Aloe vera*, phytochemicals, nutritional composition, antioxidant activity, DPPH assay

Introduction

Aloe vera L. is a perennial, drought resisting succulent plant belonging to the Asphodelaceae Family. (Manvitha.K & B. Bidya, 2014). It is originated in tropical Africa. Now it is widely cultivated in the warm climate region of Asia, Europe and America.(Zadeh.J.B and Moradikor N.,2014) *Aloe vera* leaves contain a diverse array of compounds, including anthraquinones, anthrones and their glycosides, chromones, carbohydrates, proteins, glycoproteins, amino acids, organic acids, lipids, sugars, vitamins, and minerals. (Sahu P.K, *et al.*, 2013) Many of the health benefits associated with *Aloe vera* have been attributed to the polysaccharides contain in the gel of the leaves. .These biological activities include promoting of wound healing, antifungal activity, hypoglycemic or antidiabetic effects, anti-inflammatory, anticancer, immune-modulatory, and gastroprotective properties. (Hamman J.H, 2008, Efterpi V. Christaki & Panagiota C. Florou-Paneri, 2010) *Aloe vera* gel contains many antioxidants which fight the free radical found in human bodies and it also increases the body metabolism. The extracted majority carbohydrate of the Aloe gel is generally denominated Acemannan whose important therapeutic properties are attributed .The Acemannan or Carrysin™, available commercially, is an acetylated polymer of mannose that is isolated from *A.vera* gel.(Elena Rodriguez.2010) *Aloe vera* has number of uses and mainly they are served in many food, drinks and medicine. Commercially, *Aloe vera* can be found in pills sprays, ointments, lotions, drinks, jellies, and creams.(Akev .N. *et al.*,2015) Currently, the used of *Aloe vera* has become popular because of herbal movement initiated by naturopaths, yoga gurus, alternative medicine promoters and holistic healers.(Ahlawat K.S & B.S Khatkar,2011) *Aloe vera* has been approved by USFDA as food flavoring agent in accordance with good manufacturing practices.(Akev.N.2015) In Myanmar, *Aloe vera* jam with Jaggery is used for health benefits especially for women. In the current study, one of the medicinal plants, *Aloe vera* L. was chosen for investigation of phytochemical constituents, nutritional values, and antioxidant activity.

Botanical Description

Botanical name - *Aloe vera* Linn.
English name - Barbados Aloe
Family name - Asphodelaceae
Myanmar name - Sha zaung let pat
Medicinal uses - purgative, carminative, eye diseases,anthelmintic, arthritis, diabetes, asthma, strangury and skin diseases

Figure 1. *Aloe vera* plant

Material and Methods

Sample collection and preparation

The leaves of *Aloe vera* were collected from Chauk Township, Magway Region. They were washed with water and the gel in the leaves was scraped out and dried in air at room temperature. They were stored in air-tight container

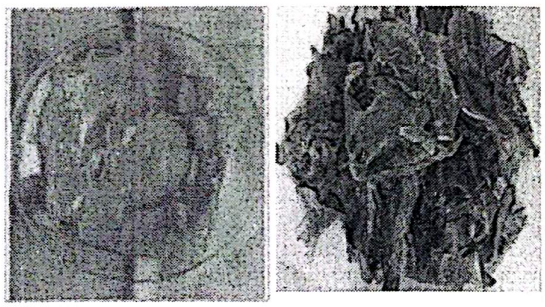

Figure 2. Fresh and dry

Phytochemical Screening

The various solvents extracts of sample were prepared to analyse certain phytochemicals such as alkaloids, glycosides, steroids, reducing sugar, flavonoids, terpene, polyphenol, phenolic compounds, tannins, sponins, lipophenols, and carbohydrates.(Herbone J.B-1973,Pandey O.P-2010)

Determination of Nutritional Composition

The determination of the nutritional composition of sample such as moisture, ash, mineral, oil, crude fibre, nitrogen, protein, carbohydrate, reducing sugar, and vitamin C was performed.

Table 1. Determination of Nutritional Composition of Sample

No.	Nutritional Content	Methods / Instruments
1.	Water	Oven drying method
2.	Ash	Muffle furnace
3.	Mineral	EDXRF Spectroscopy
4.	Fat	Soxhlet Apparatus
5.	Crude fibre	Muffle furnace
6.	Nitrogen and protein	Micro Kjeldahl's method
7.	Carbohydrate	Calculation
8.	Reducing Sugar	Iodometric titration
9.	Vitamin C	Iodometric titration

Determination of nutritional composition was carried out by the methods expressed in AACC-2000, AOAC-1999, FAO-2003 and Chowdhury.M.S-2016.

Determination of Antioxidant Activity of fresh

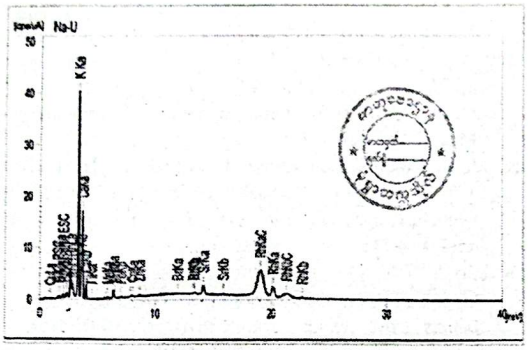

Figure 3. EDXRF Spectrum

Sample

DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of sample. In this study, the fresh sample was used. The DPPH radical-scavenging assay measures the ability of the compound to donate hydrogen to DPn5ywPH radical. The DPPH radical-scavenging activity of sample was also determined based on median

Table 2. Result for preliminary phytochemical screening

No	Test	Reagent used	Observation	Result
1.	Alkaloid	(i) Wagner's reagent,1%HCl (ii) Dragendorff's reagent,1 % HCl	(i) Reddish brown solution (ii) Orange ppt	+ +
2.	Glycoside	10 % Lead acetate , 10 mL D.W	White ppt	+
3.	Steroid	Acetic anhydride, 10 mL EtOH Conc : H_2SO_4	Green solution	+
4.	Reducing Sugar	Benedict's solution , 10 mL D.W	brick red ppt	+
5.	Flavonoid	10 mL EtOH , conc:HCl , Mg and conc:H_2SO_4	Red colour solution	+
6.	Terpene	10 mL EtOH ,0.3mL acetic anhydride and 0.5mL chloroform,conc: H_2SO_4	No change	-
7.	Polyphenol	10 mL EtOH , 1% $FeCl_3$ and 0.1% K_3 [Fe (CN)$_6$	Greenish blue solution	+
8.	Phenolic compound	10 mL D.W, 10% FeCl	Greenish blue solution	+
9.	Tannin	10 mL D.W, 10 % $FeCl_3$ and dil:H_2SO_4	Yellowish brown ppt	+
10.	Saponin	10 mL D.W, $NaHCO_3$	Froth	+
11.	Lipophenol	10 mL D.W, 0.5 M KOH and NaOH	Deep color solution	+
12.	Carbohydrate	Fehling A + B solution and 10 mL EtOH	Yellow ppt	+

(+) = presence of constituents; (-) = absence constituent

inhibition concentration (IC_{50}). In this experiment, ascorbic acid was used as standard antioxidant.

Results and Discussion

Phytochemical Constituents of Sample

Preliminary phytochemical analysis was performed in order to know different types of organic compounds present in sample. These phytochemicals are known to exhibit medicinal as well as physiological activities. The results are shown in Table 2.

According to this table, alkaloids, glycosides, steroids, reducing sugars, flavonoids, polyphenols, phenolics compounds, tannins, saponins, lipophenols and carbohydrates are detected in the sample.

Elemental Composition

The elemental composition was examined by EDXRF spectroscopy at the Department of Chemistry, Monywa University and the results were presented in Table (3)

Table 3. The Results of Elemental Composition

No.	Elements	Symbols	Results (%)
1.	Potassium	K	3.885
2.	Chloride	Cl	1.132
3.	Calcium	Ca	0.686
4.	Silicon	Si	0.125
5.	Sulphur	S	0.125
6.	Phosphorus	P	0.102
7.	Iron	Fe	0.031
8.	Zinc	Zn	0.008
9.	Stronium	Sr	0.003
10.	Manganese	Mn	0.003
11.	Titanium	Ti	0.003
12.	Copper	Cu	0.002
13.	Rubidium	Rb	0.002
14.	Bromodes	Br	0.001
15.	Hydrocarbon	CH	93.893

These results informed that the macrominerals such as potassium, chloride, calcium, silicon, sulphur, and phosphorus and microminerals such as iron, copper, zinc, strontium, manganese, titanium and rubidium are present in *Aloe vera* gel. The presence of heavy toxic metals was not detected. Because of the noticeable amount of potassium it will help in regulating the body fluid, promoting relaxation of muscles, and improve the activities of all cells in the bvody.

Nutritional Composition of the Sample

Some nutritional values present in the *Aloe vera* gel were measured by the methods mentioned in AACC(2000), AOAC(1999) and FAO(2003). The results are shown in Table (4).

Table 4. Results of Nutritional Composition of the sample

No.	Nutritional Compositions	Percent (%)
1.	Water	98.33
2.	Ash	0.175
3.	Fat	0.003
4.	Fiber	0.155
5.	Protein	0.063
6.	Carbohydrate	1.274
7.	Reducing sugar	0.560
8.	Vitamin C	0.0034

Antioxidant Activity of Fresh Sample

The half maximal inhibitory concentration value for the sample (IC_{50}= 625 µg/mL) was higher than that of standard ascorbic acid (IC_{50} = 0.125µg/mL). Therefore, the sample has lower antioxidant activity than standard ascorbic acid.

Table 5. The results of antioxidant activity of fresh juice of *Aloe vera*

Concentration (µg/mL)	DPPH Absorbance	Absorbance of Sample	% inhibition	IC_{50}
2200	2.6761	1.2867	51.91	
1100	2.6761	1.308	51.12	
550	2.6761	1.3461	49.69	625
275	2.6761	1.3982	47.75	

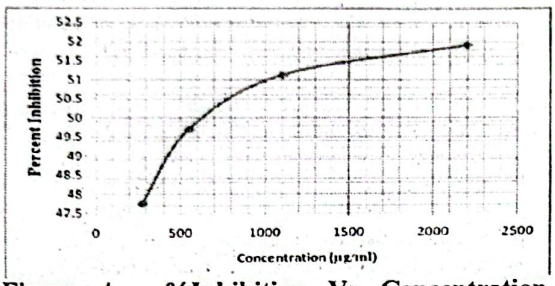

Figure 4. %Inhibition Vs Concentration (µg/mL) of Fresh Sample

Table 6. The Result of antioxidant activity of Ascorbic Acid.

Tested	Concentration	%DPPH	IC_{50}
Ascorbic acid	0.0625	41.7	0.125
	0.125	50.622	
	0.25	55.45	
	0.5	57.47	
	1	58.03	
	2	59.94	

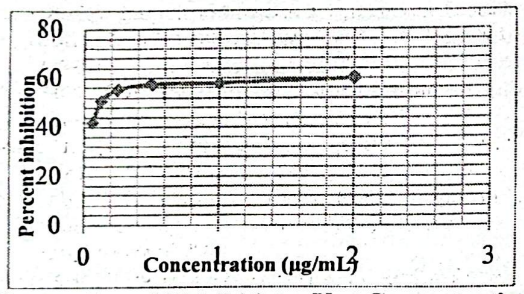

Figure 5. %Inhibition Vs Concentration (µg/mL) of Ascorbic Acid

Conclusion

The secondary metabolites alkaloids, glycosides, terpenes, polyphenols, phenolics, tannins, lipophenols which can improve defense system and the primary metabolite carbohydrates and proteins are present. The potassium, major electrolyte is the highest (3.885%) among other minerals in this sample. Other macro and micro minerals constituents such as phosphorus, sulfur, calcium, iron and zinc are also present. The nutritional compositions such as fat (0.003%), fiber (0.155%), protein (0.063%),carbohydrate (1.274%) reducing sugar content (0.56%) and vitamin C content (0.0034%) based on the fresh sample were also detected .So, consuming *Aloe vera* gel would benefit for human health. IC_{50}value of fresh juice was found to be (625µg/mL). By comparing the IC_{50} value of standard ascorbic acid (0.125µg/mL) gel of *Aloe vera* showed mild antioxidant activity. According to the experimental data, *Aloe vera* gel could provide human health benefits.

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References

AACC., (2000). "Approved methods of the American Association of Cereal Chemists", vol.1 (Method No. 30-25, 4415A). USA: American Association of Cereal Chemists.

AOAC., (1999). "Official Methods of Analysis of Association of Official Analytical Chemists", 15th ed., Arlington Va, USA: AOAC, 1990,1-50

Ahlawat.K.S. and Khatkar B.S., (2011). "Processing, Food application and safety of *Aloe vera* products", A review; Journal of Food Science and Technology-Mysore 45 (5)525-533

Akev. N.,A., Can, N., Sutlupinar, E., Candiken, N., Ozsoy, T.Y., Ozen, R., and Yanardag, E.Uzen., (2015). Twenty years of research on *Aloe vera*"; J.Fac. Pharm. Istanbul 45(2)191-215

Chowdhury.M.S, (2016). "Determination of amount of Vitamin C (Ascorbic Acid) from supplied commercial tablets by using Iodometric titration", 8.25. International Islamic University Chittagong

Efterpi V., Christaki & Panagiota C., and Florou-Paneri., (2010). "*Aloe vera*: A plant for many uses", Journal of Food, Agriculture & Environment 8 (2) 245-249

Elena Rodriguez Rjacinto Martin and Carlos Diaz Romero., (2010). "*Aloe vera* as a functional Ingredient in Foods", Critical Reviews in Food Science and Nutrition, 50(4) 305-326

FAO., (2003). "Food and Nutrition", paper 77, Food energy-methods of analysis and conversion factors.

Hamman J.H., (2008). "Composition and Application of *Aloe vera* Leaf Gel", Molecules 2008.13

Harbone J.B., (1973. "Phytochemical Methods": A guide to modern techniques of plant analysis, Chapman and Hall, New York, 279

Manvitha.K and B.Bidya (2014) Aloe Vera: a wounderful plant its history, cultivation and medicinal uses; Journal of Pharmacognosy and Phytochemistry, 2(5)85-88.

Pandey O.P, D.N. Bajpai and S.Giri, (1972). "Practical Chemistry (for B.Sc. I, II, III Year Students of All Indian Universities". Reprint 2010

Sahu P.K., Giri D.D, Ritu S., Pandey P., Gupta S., Shrivastava A. K., Kumar A., and Pandey K.D., (2013). "Therapeutic and Medicinal Uses of *Aloe vera*": A Review. Pharmacology & Pharmacy 4.599-610

Sharma.P., Admit C., Kharkwai, Harsha Kharkwai, Abdin M.Z., and Varma A., (2014). "A Riew on Pharmacological Properties of *Aloe vera*", Int. J. Pharm. Sci, 29(2) 31-37.

Zadeh.J.B and Moradikor N., (2014). "Component and Application *Aloe vera* Plant in Medicine", International Journal of Advanced €biological and Biomedical Research 2(5) 1876-1882

Extraction of Eco-Friendly Natural Dye Collected from Onion Skins and its Application on Silk Fabric

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Abstract

The aim of the study was to evaluate the performance of dyes extracted from onion skins in silk dyeing. Extraction medium was optimized by extracting dyes from fixed quantity of onion skins under pH values from 3 to 12. The maximum color strength of the extracted dye liquor was found to be at pH 10. The optimum dye extraction conditions i.e., temperature, time, and material-to-liquor ratio were found to be 90°C, 60 min and 1:10, respectively. An attempt was also made on the dyeing of silk fabrics with natural dyes using different mordants such as zinc sulphate, potassium dichromate, ferric sulphate and alum. Among these mordants, potassium dichromate demonstrated relatively more attractive colors and acceptable fastness on silk fabric in all dyeing methods as indicated. Moreover, fastness tests especially for rubbing, washing and light fastness on dyed silk fabrics were carried out. It can also be said that onion skins have good potentiality for dyeing of silk fabric.

Keywords: natural dye, mordant, silk fabric, dyeing, fastness properties

Introduction

Natural dyes are dyes or colorants derived from plants, invertebrates, or minerals. The majority of natural dyes are vegetable dyes from plant sources – roots, berries, bark, leaves and wood – and other organic sources such as fungi and lichens

Although chemical dyes have largely taken over around the world, interest in the traditional craft of natural dyeing has increased lately and naturally dyed materials are once more in demand (Marjo Moeyes, 1993).

The use of non-allergic, non-toxic and eco-friendly natural dyes on textile become a matter of significant importance due to the increased environmental awareness in order to avoid some hazardous synthetic dyes (Indian Journal of Fiber & Textile Research, 2009).

Silk has been known as the "queen of fibers" since its discovery. Clothes made from silk are luxurious and have many excellent qualities including the material's luster, light weight, superior mechanical performance, fine and smooth texture, excellent moisture transportation, and excellent draping quality (Cai et al.2001).

Natural dyes are capable of producing unique, uncommon, alleviating and soft shades as compared to synthetic dyes as well as Onion (*Allium cepa* L) is a vegetable used in our daily life for cooking food and widely cultivated around the world. Most onions cultivated contain about 89% water, 4% sugar, 1% protein, 2% fiber. Onion also contains different types of Vitamins like Vitamin B1, Vitamin B3, and Vitamin C; it also contains compounds such as phenolic and flavonoids as well as elements like iron, calcium, magnesium, manganese, and zinc (Onal, 1996). The skin of onion is not edible and considered as wastage. However, it contains coloring pigment called "Pelargonidin" (3, 5, 7, 4 tetrahydroxyantocyanidol). The amount of this coloring pigment is found to be 2.25% (ShaliniChandravanshi and Santosh, 2013).

The aim of the research was to evaluate the performance of dyes extracted from onion skin in silk dyeing. The specific objectives were to study the color produced on silk fabrics with various dyeing methods, to study the fastness properties of dyed silk fabrics, to solve the ecological and environmental problems related to the use of synthetic dyes and to substitute the imported product of the high quality and reasonable cost.

Materials and Methods

Selection, Collection and Preparation of Raw Materials

Natural dyestuff was obtained from many sources such as vegetables, fruits, roots, bulbs, trees and insects. In this study, one of the vegetable sources such as onion skin (*Allium cepa* L.) was used as raw material for the extraction of natural dyestuff. Moreover, they can be abundantly collected in Myanmar and easily obtained in any season and every time. In this research, the raw materials were collected from Monywa Township, Sagaing Region.

Firstly, the foreign matters contained in the raw materials were picked out and removed because they could affect the purity of dye solution. After that the raw materials were rinsed thoroughly with cold water to remove the dirt which were attached to the onion skins and then dried at room temperature. Finally, the dried onion skins were cut into very tiny pieces and they were stored in plastic bags for future use.

Silk fabrics were purchased from local market and mordants used for the zinc sulphate, potassium dichromate, ferric sulphate and alum of commercial grades were purchased from local market.

Preliminary Phytochemical Tests on Onion Skin

In this research, preliminary phytochemical tests were carried out to study the main compounds in plants which were present or absent in the onion skin. The following phytochemical investigations (Methods and Procedures expressed in the phytochemical Bulletin of Botanical Society of America) on the onion skin were carried out and the results of all phytochemical tests were shown in Table 2.

Testing the Color Fastness of Dyed Silk Fabric

In this study washing fastness, rubbing fastness and light fastness were carried out to determine the color fastness of dyed silk fabric. According to the ISO 105:3 test was used for washing fastness and the assessment of color fastness to rubbing was done by ISO 105:12 test method.

Extraction Process of Onion

Extraction is usually used to recover a component either from a solid or liquid. The outer skin of onion could be extracted with the water by heating. About (3, 5, 7, 9) g of pieces onion skin were put into a steel pot containing 400 ml of water. Then, the pot was boiled for one hour at 90°C. The solution was cooled and filtered with filter cloth. Finally, 200 ml each of dye solution was obtained.

Mordanting Process

In this research, three types of mordanting methods were used in dyeing operation. They are pre-mordanting, post-mordanting and simultaneous mordanting and dyeing methods. In this experiment, each of 3% zinc sulphate,

potassium dichromate, ferric sulphate and alum were used as a mordant.

Degumming of Silk Fabric

Silk fiber contains natural impurities called gum or sericin. This sericin was near about 20% of weight compared to the whole weight of silk fiber. Besides this silk fiber comprises some wax and natural colors. In this research, degumming had used to remove these materials by treating the substrate with soda ash. Raw silk fabric was degummed in according to the following recipe which was shown in Table 1, and the degummed fabric was washed with 2g/l detergent at 65°C for 10 minutes.

Table 1 Recipe for Degumming of Silk Fabric

Sr. No	Factors	Amount
1.	Detergent	5 g/l
2.	Soda Ash	3 g/l
3.	Sequestering Agent	1 g/l
4.	Wetting Agent	1 g/l
5.	Material: Liquid	1:20
6.	Temperature	90°C
7.	Time	60 minutes
8.	pH	9

Dyeing Process

In the preparation of dyed silk fabrics, (3, 5, 7, 9) g pieces of dried onion skins were placed in a stainless steel pot containing 400 ml of water and boiled at 90°C for one hour. Then the dye solution was cooled and filtered with filter cloth.

In pre-mordanting method, the dye liquid 200 ml was heated at 90°C and the mordanted fabrics 10 g were simmered in that solution for 30 min, 45 min and 60 min. After that, the fabrics were removed from the dye bath and allowed to cool. Finally, the dyed fabric were rinsed with water and dried at room temperature. In this research, the optimum dyeing condition was 90°C and one hour.

In post-mordanting method, the dye liquid 200 ml was heated at 90°C and the cleaned fabrics 10 g were simmered in that solution for 30 min, 45 min and 60 min. During the dyeing process, the fabric was frequently stirred to obtain a good dye penetration into the fabric. Then, the fabric was rinsed with water. Then the mordant solution was heated at 90°C and the dyed fabric was simmered in that solution for about 10 min. After that the fabric was rinsed with water and dried at room temperature.

In simultaneous-mordanting and dyeing method, the dye liquid 200 ml and 3 g of mordant was heated at 90°C and the cleaned fabric 10 g was simmered in that solution for 30 min, 45 min and 60 min. During the dyeing and mordanting process, the fabric was frequently stirred to obtain a good dye penetration of mordant into the fabric. Then, the fabric was rinsed with water and dried at room temperature.

Results and Discussion

Preliminary phytochemical investigation was carried out on the dried onion skin to determine the presence or absence of tannin, glycoside, saponin, phenolic compounds, flavonoid and polyphenol. The results of phytochemical tests were shown in Table 2. According to the results obtained, it was found that tannin, glycoside, saponin, phenolic compounds, flavonoid and polyphenol were present in the dried onion skin.

In this study, natural dye was extracted from dried onion skin (*Allium cepa* L.) and silk fabric was dyed by direct dyeing without mordant and various dyeing methods with mordants such as zinc sulphate, potassium dichromate, ferric sulphate and alum.

Effect of mordant on changes in color fastness of silk fabrics dyed with onion skin (3,5,7,9) g using pre-mordanting, post-mordanting and simultaneous-mordanting and dyeing methods at dyeing time 30 min were studied. According to these results, post-mordanting and simultaneous-mordanting and dyeing methods with potassium dichromate gave better color fastness than direct dyeing method without mordant and dyeing methods with mordant. Direct dyeing method gave better color fastness than various dyeing methods with ferric sulphate mordant because the fastness value of direct dyeing method without mordant was (3,3,3,3) and the various dyeing method with ferric sulphate mordant was (2-3,3,2-3,3). But it gave pale color than the other dyeing methods with mordant. Ferric sulphate mordant was less color fastness than zinc sulphate and alum mordants.

Similarly, effect of mordant on changes in color fastness of silk fabrics dyed with onion skin using pre-mordanting, post-mordanting and simultaneous-mordanting and dyeing methods at dyeing time 45 min were also studied. From these results, potassium dichromate mordant was given good color fastness by using post-mordanting and simultaneous-mordanting and dyeing methods. Three dyeing method with zinc sulphate mordant had better color fastness than ferric sulphate mordant and less color fastness than alum mordant.

In addition, effect of mordant on changes in color fastness of silk fabrics dyed with onion skin using pre-mordanting, post-mordanting and simultaneous-mordanting and dyeing methods at dyeing time 60 min were shown in Tables 3, 4 and 5. It was noted that post-mordanting and simultaneous-mordanting and dyeing methods with potassium dichromate gave better color fastness than the other mordants and pre-mordanting method. Zinc sulphate and alum mordants gave nearly the same color fastness.

Comparing the results, simultaneous-mordanting and dyeing methods with potassium dichromate mordant at the dyeing time 60 min gave the best color fastness. From the economic point of view, simultaneous-mordanting and dyeing method with potassium dichromate mordant at the dyeing time 60 min was the most suitable condition because it could save time and cost.

Table 2. Results of Phytochemical Test on Onion Skin

Sr.	Test	Types of	Reagent	Observation	Inference
1.	Glycoside	H_2O	10% Lead acetate solution	Red ppt.	+
2.	Saponin	H_2O	H_2O	Frothing	+
3.	Tannin	Ethanol	1% Gelatin	Jelly block	+
4.	Flavonoid	Ethanol	Dilute HCl + Mg ribbon	Pink	+
5.	Phenolic	H_2O	10% Ferric chloride solution	Deep blue	+
6.	Polyphenol	Ethanol	1% $FeCl_3$ and 1% $K_3[Fe(CN)_6]$	Greenish blue	+

Table 3. Effect of Mordant on Changes in Color Fastness of Silk Fabrics Dyed with Onion Skin Using Pre-mordanting Method

Mordanting temp. = 90°C | Mordanting time = 10 min
Dyeing temp. = 90°C | Dyeing time = 60 min
Material (Fabric) = 10g (12in × 12in) | Weight of mordant = 3g

Sr. No	Types of Mordant	Sample Code	Rubbing Fastness 500g,100 times		Washing Fastness ISO Test No.3,60°C,30 min		Light Fastness
			Dry	Wet	Change of Shade	Staining on Silk Fabric	
1.	No Mordant	FD_1	4	3	1	4	3
		FD_2	3-4	3	1	4	3
		FD_3	4	3	2	4	3
		FD_4	3	3	1	4	3
2.	Zinc Sulphate	FZ_1	4	3	1	4	4
		FZ_2	4	3	1	4	4
		FZ_3	3-4	3	1	4	4
		FZ_4	3-4	3	1	4	4
*3.	Potassium Dichromate	FP_1	4	4	1	4	4
		FP_2	4	4	1	4	4
		FP_3	4	4	1	4	4
		FP_4	4	4	1	4	4
4.	Ferric Sulphate	FF_1	3-4	3	1	4	4
		FF_2	3-4	2-3	1	4	4
		FF_3	3	2-3	1	4	4
		FF_4	3	2-3	1	4	4
5.	Alum	FA_1	4	3	1	4	4
		FA_2	4	3	1	4	4
		FA_3	4	3	1	4	4
		FA_4	4	3	2	4	4

*The most suitable condition
Note : 1= very poor, 2= poor, 3= fair, 4=good, 5= excellent

Table 4. Effect of Mordant on Changes in Color Fastness of Silk Fabrics Dyed with Onion Skin Using Post-mordanting Method

Mordanting temp. = 90°C | Mordanting time = 10 min
Dyeing temp. = 90°C | Dyeing time = 60 min
Material (Fabric) = 10g (12in × 12in) | Weight of mordant = 3g

Sr. No	Types of Mordant	Sample Code	Rubbing Fastness 500g,100		Washing Fastness ISO Test		Light Fastness
			Dry	Wet	Change of Shade	Staining on Silk	
1.	No Mordant	FD_1	4	3	2	4	3
		FD_2	3-4	3	2	4	3
		FD_3	4	3	2	4	3
		FD_4	3	3	2	4	3
2.	Zinc Sulphate	FZ_5	4	3-4	2	4	4
		FZ_6	4	3-4	2	4	4
		FZ_7	4	3-4	2	4	4
		FZ_8	4	3-4	2	4	4
*3.	Potassium Dichromate	FP_5	4	4	2	4	4
		FP_6	4	4	2	4	4
		FP_7	4	4	2	4	4
		FP_8	4	4	2	4	4
4.	Ferric Sulphate	FF_5	4	3	1	4	4
		FF_6	4	3	1	4	4
		FF_7	4	3	1	4	4
		FF_8	4	3	1	4	4
5.	Alum	FA_5	4	3	2	4	4
		FA_6	4	3	2	4	4
		FA_7	4	2-3	2	4	4
		FA_8	4	3	2	4	4

*The most suitable condition
Note : 1= very poor, 2= poor, 3= fair, 4=good, 5= excellent

Table 5. Effect of Mordant on Changes in Color Fastness of Silk Fabrics Dyed with Onion Skin Using Simultaneous-mordanting and Dyeing Method

Mordanting temp. = 90°C Mordanting time = 10 min
Dyeing temp. = 90°C Dyeing time = 60 min
Material (Fabric) = 10g (12in ×12in) Weight of mordant = 3g

Sr. No	Types of Mordant	Sample Code	Rubbing Fastness 500g,100 times		Washing Fastness ISO Test No.3,60°C,30 min		Light Fastness
			Dry	Wet	Change of Shade	Staining on Silk Fabric	
1.	No Mordant	FD_1	4	3	1	4	3
		FD_2	3-4	3	1	4	3
		FD_3	4	3	2	4	3
		FD_4	3	3	1	4	3
2.	Zinc Sulphate	FZ_9	4	3	1	4	4
		FZ_{10}	4	3	1	4	4
		FZ_{11}	3-4	3	1	4	4
		FZ_{12}	3-4	3	1	4	4
*3.	Potassium Dichromate	FP_9	4	4	3	4	5
		FP_{10}	4	4	3	4	5
		FP_{11}	4	4	3	4	5
		FP_{12}	4	4	3	4	5
4.	Ferric Sulphate	FF_9	3-4	2-3	1	4	4
		FF_{10}	3-4	2	1	4	4
		FF_{11}	3-4	3	1	4	4
		FF_{12}	3-4	3	1	4	4
5.	Alum	FA_9	4	3	1	4	4
		FA_{10}	4	2-3	1	4	4
		FA_{11}	4	3	1	4	4
		FA_{12}	4	2-3	1	4	4

*The most suitable condition

Note : 1= very poor, 2= poor, 3= fair, 4=good, 5= excellent
These experiments were conducted at Development Centre for Textile Technology in Ministry of Industry, Yangon Region.

Conclusion

In the application of natural dye on silk fabric, mordanting with potassium dichromate in all dyeing method could produce attractive color and acceptable fastness on silk fabric. It could also be concluded that simultaneous-mordanting and dyeing method was the best for color fastness. From the economic point of view, simultaneous-mordanting and dyeing method was more saving cost and time than the other two methods. According to grey scale results, all dyeing methods gave good grading in staining on silk fabric. So, this research would be implemented in any way for the development of Myanmar Traditional Textile Industries.

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References

Abrahart, E.N, (1962), "Dyes and their Intermediates", "Encyclopedia-Britannica", Volume 6.

Cai, Z, Jiang, G, &Yang, S (2001), "Chemical Finishing of Silk Fabric Color Technology", 117,161.

Grae, D. (1974), "Natures Colours: Dyes from Plant. New York", Macmillan Publishing Co. Inc., London, Collier Macmillan Publisher.

Harwood, R.J., and Bramhall, A.T. (1987), "Dyeing and Printing of Cotton and Polyester/Cotton Fabrics". Vol. 1, Scottish College of Textiles.

Indian Journal of Fiber & Textile Research, (2009), Vol. 34.

Kirk, R.E., and Othmer, D.F. (1950), "Encyclopedia of Chemical Technology". Vol. 5 New York: The Interscience Encyclopedia, Inc.

Lyle, (1977), "Performance of Textiles", United States of America.

Marjo Moeyes, (1993), "Natural Dyeing in Thailand", White Lotus Co., Ltd, Printed in Thailand.

Mary Jacob, (1992), "Natural Dyes- Classification and Sources", Convection on Natural Dyes, Book of Papers, Department of Textile Technology, IIT Delhi.

Onal, A., (1996) "Extraction of dyestuff from Onion (*Allium cepa* L) and its application in the Dyeing of Wool, Feathered-Leather and Cotton", Tr. J. of Chemistry, P-20, 194-203.

ShaliniChandravanshi and Santosh K. Upadhyay, (2013) "Interaction of Natural Dye (*Allium cepa* L) with Ionic Surfactant", Journal of Chemistry.

Trotman, E.R., (1950), "Dyeing and Chemical Technology of Textile Fibers", 4th.ed., London: Charles Griffin Co. Ltd.

Elemental Analysis, Antioxidant and Antimicrobial Activities of *Malvastrum coromandelianum* (L.) Garcke

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Abstract

In this research work, *Malvastrum coromandelianum* (L.) Garcke was chosen for chemical analysis. The preliminary phytochemical tests were performed by standard methods. It was found that *Malvastrum coromandelianum* (L.) Garcke contain alkaloids, glycosides, flavonoids, phenolic compounds, reducing sugar, saponins, steroids, polyphenols and lipophenols, respectively. The mineral contents of the *Malvastrum coromandelianum* (L.) Garcke were measured by EDXRF method. The highest amount of calcium (1.547%) and potassium (1.451%) were found in the sample. The antimicrobial activities of three extracts of sample (ethanol extract, n-hexane extract and ethyl acetate extract) were determined by Agar-well diffusion method. Ethyl acetate extract showed high activities on against *Pseudomonas aeruginosa*, *Candida albican* and *Escherichia coli*. In addition, the antioxidant activity of the sample was determined by DPPH method. The IC_{50} value of ethanol extract of sample was found to be 120 µg/mL.

Keywords : *Malvastrum coromandelianum* (L.) Garcke, antimicrobial activity, antioxidant activity, DPPH, EDXRF

Introduction

The use of plant-based drugs all over the world is increasing. Medicinal herbs or plants have been known to be an important potential source of therapeutics or curative aids. The use of medicinal plants has attained a commonding role in health system all over the world. This involves the use of medicinal plants not only for the treatment of diseases but also as potential material for maintaining good health and conditions.

Malvastrum coromandelianum (L.) Garcke is a common weed of gardens, cultivated fields and waste lands. It is one of the medicinal plants scientifically investigated[5]. It is used in traditional medicine as an anti-inflammatory, analgesic, antidysenteric plant and in the treatment of jaundice and ulcer[8].

Pharmacological screening showed various activities for this plant like antinociceptive and antimicrobial activity. Various parts of this plant are used by numerous tribal populations throughout the world. Ethnobotanical survey revealed that it is used to treat various disorders[1]. Pharmacological screening revealed that the plant possess antinociceptive, anti-inflammatory, analgesic, and antimicrobial activities[4].

Malvastrum coromandelianum (L.) Garcke, commonly known as false mallow, broom weed, and clock plant. It is widely used in traditional system of medicine for the cure of different aliments. Genus *Malvastrum* derives its name from *Malva*-the mallow. It belongs to the family Malvaceae and closely resembles to genus *Sida* Linn of the same family. The species has been described as a species of *coromandelianum* Linn, Malva-M genus (Flora of Madras). *Malvastrum coromandelinaum* possess three small family are well known for their antimicrobial and anifungal activities due to the presence of alkaloids, essential oils and phenolic[7].

Materials and methods

Sample Collection

The plant sample was collected from Zeyar Quarter, Sagaing Region. The aerial parts of the sample were dried and cut into small pieces. Then it was percolated with ethanol in air-tight bottle and stored for further analysis.

Preliminary Phytochemical Test

Phytochemical analysis was determined by using standard methods.

Determination of Minerals Contents

The mineral contents of the sample were determined by EDXRF spectroscopy. EDXRF measurement was carried out at the department of Chemistry, Monywa University.

Determination of Antimicrobial Activities

Three extracts of sample, n-hexane extract, ethanol extract and ethyl acetate extract were prepared. Then the antimicrobial activities were determined by Agar-well diffusion method at Pharmaceutical Research Department, Insein, Yangon.

Determination of Antioxidant Activity

DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of plant materials.

Preparation of Crude Extracts

Ethanol extract of Taw-pilaw sample was prepared by the following procedure.

The dried powder sample 400 g was extracted with 95% ethanol for two month and filtered. Evaporation of the solvent was provided ethanol crude extract of Taw-pilaw sample.

Preparation of Test Sample Solution

0.005 g of the crude extract to be tested was dissolved in 95% ethanol (10 mL) under vigorous shaking. After filtration, the filtrate was used as a stock solution (500 µg/ml). Desired concentration (500, 250, 125, 62.5, 31.25 µg/mL) of sample solutions were prepared from this stock solution by dilution with appropriate amount of 95% ethanol.

Preparation of 60µM DPPH Solution

DPPH 0.0024 g was thoroughly dissolved in 100 mL ethanol (95%). This solution was freshly prepared in the brown colored flask and kept in refrigerator for no longer than 24 hours.

Preparation of Standard Solution

Ascorbic acid 0.01 g was dissolved in 100 ml of 95% ethanol and it was used as a standard solution.

Procedure

(i) Control solution was prepared by mixing 60µM DPPH solutions (2.0 ml) and 95% ethanol (2.0 ml).
(ii) Similarly, the blank solution was prepared by mixing test solution (2.0 ml) and 95% ethanol (2.0 ml).
(iii) The sample solution was also prepared by mixing the test solution (2.0 ml) with 60µM DPPH solution (2.0 ml).

All these solutions were allowed to stand at room temperature for 30 minutes. Then, the absorbance was measured at 517nm using PD-303 UV spectrophotometer.

Results and discussion

Preliminary Phytochemical Screening

According to the phytochemical examination *Malvastrum coromandelianum* (L.) Garcke contains alkaloid, glycoside, flavonoid, phenolic, reducing sugar, saponin, steroid, polyphenol and lipophenol.

Determination of Mineral Contents

The mineral contents in *Malvastrum coromandelianum* (L.) Garcke (Taw-pilaw) was determined in this study. The observed elements are described in Table 1.

Table 1 Results of Mineral Contents in Malvastrum coromandelianum (L.) Garcke (Taw-pilaw)

Symbol	Elements	Concentration of Taw-pilaw %
Ca	Calcium	1.547
K	Potassium	1.451
Si	Silicon	0.803
Mg	Magnesium	0.676
S	Sulphur	0.524
P	Phosphorus	0.274
Fe	Iron	0.052
Ti	Titanium	0.006
Sr	Strontium	0.006
Mn	Manganese	0.005
Cu	Copper	0.003
Zn	Zinc	0.003
Cr	Chromium	0.001
Rb	Rubidium	0.001

According to this table, the highest amount of calcium (1.547 %) and potassium (1.451 %) were found in *Malvastrum coromandelianum* (L.). Moreover, it contains silicon, magnesium, sulphur, phosphorus, iron, titanium, strontium, manganese, copper, zinc, chromium and rubidium. The result showed that *Malvastrum coromandelianum* (L.) is a rich source of minerals.

Determination of Antimicrobial Activities

Bioactivities of crude extract from *Malvastrum coromandelianum* (L.) Garcke were determined by applying Agar-well diffusion method in various solvent systems (n-hexane, ethylacetate and ethanol) on six selected organisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilis*, *Candida albican* and *E-coli*. The results are shown in Table 2.

Table 2 Results of the Determination of Antimicrobial Activities of *Malvastrum coromandelianum* (L.) Garcke

Sample	Solvents	Organisms					
		I	II	III	IV	V	VI
Malvastrum coromandelianum	Pet-ether	-	-	-	-	-	-
	EtOAc	-	-	+++ (30 mm)	-	+++ 20 mm)	+++ 20 mm)
	EtOH	-	-	-	-	-	-

Organisms
(I) *Bacillus subtilis* (N.C.T.C-8236)
(II) *Staphylococcus aureus* (N.C.P.C-6371)
(III) *Pseudomonas aeruginosa* (6749)
(IV) *Bacillus pumilis* (N.C.I.B-8982)
(V) *Candida albicas*
(VI) *Escherichia coli* (N.C.I.B-8134)

Agar well - 10 mm
(+) 10 mm ~ 14 mm
(++) 15 mm ~ 19 mm
(+++) 20 mm above

Table 3 Absorbance, % Inhibition of Various Concentration of Ethanol Extract and IC_{50} Value

Extract	Concentration (µg/ml)	Mean Absorbance	Mean % Inhibition	IC_{50} (µg/ml)
Ethanol	500	0.215	80.72	120
	250	0.217	79.61	
	125	0.290	51.79	
	62.5	0.319	41.32	
	31.25	0.340	31.40	

According to this table, it was found that n-hexane and ethanol extracts showed no activity against all organisms. Ethyl acetate extract showed high activity on against *Pseudomonas aeruginosa*, *Candida albican*, and *Escherichia coli*.Thus, the sample, Taw-pilaw may be used as a source for the diseases caused by *Pseudomonas aeruginosa*, *Candida albican*, and *Escherichia coli* .

Antioxidant Activity of *Malvastrum coromandelianum* (L.) Garcke (Taw-pilaw)

To thoroughly examine the antioxidant activities of Taw-pilaw, in vitro radical scavenging activity screening was carried out by using DPPH assay. This method based on the capability of test sample to inhibit stable free radical (DPPH) that was followed by decrease in absorbance at 517 nm. Low absorbance value of test sample indicates high % inhibition against DPPH radical which in turn reveals high radical scavenging activity of that sample at constant concentration.

On the basis of absorbance value, % inhibition of sample extract at different concentration was calculated and from which IC_{50} values were derived and reported at Tables (3) and(4). The plots of concentration versus absorbance of sample extract and standard ascorbic acid were shown in Figures (2) and (4). The plots of concentration versus % inhibition of sample extract and standard ascorbic acid were shown in Figures (3) and (5). In addition, by comparison of radical scavenging activity (IC_{50} values) of ethanol extract of sample and standard ascorbic acid, it was found that the *Malvastrum coromandelianum* (L.)

Table 4 Absorbance, % Inhibition of Various Concentration of Ascorbic acid and IC_{50} Value

Extract	Concentration (µg/ml)	Mean Absorbance	Mean % Inhibition	IC_{50} (µg/ml)
Ascorbic acid	2	0.245	25.08	4.5
	4	0.148	54.74	
	6	0.089	72.78	
	8	0.073	77.68	
	10	0.063	80.73	

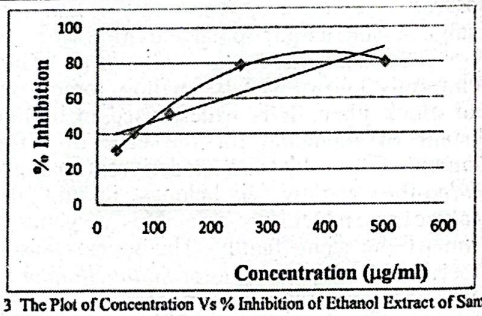

Figure 3 The Plot of Concentration Vs % Inhibition of Ethanol Extract of Sample

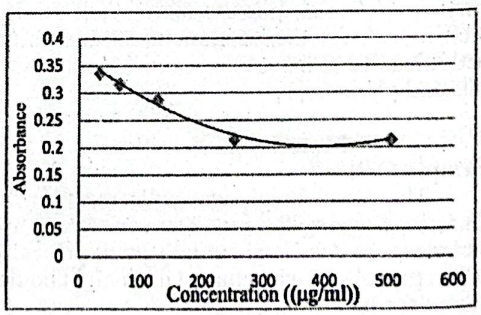

Figure 2 The Plot of Concentration Vs Absorbance of Ethanol Extract of Sample

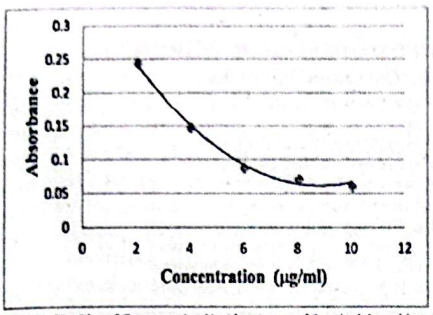

Figure 4 The Plot of Concentration Vs Absorbance of Standard Ascorbic acid

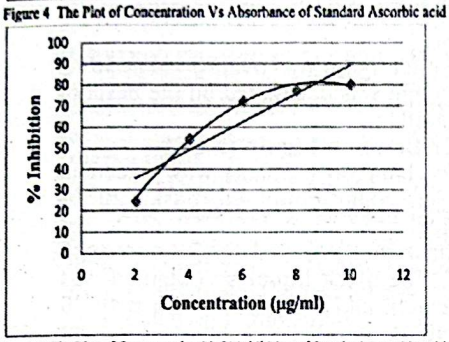

Figure 5 The Plot of Concentration Vs % Inhibition of Standard Ascorbic acid

Conclusion

In this research work, according to the phytochemical results of *Malvastrum coromandelianum contains alkaloid, flavonoid, glycoside, steroid, lipophenol, polyphenol, phenolic, saponin and* reducing sugar. From EDXRF analysis the contents of calcium and potassium were found to be higher than the other elements. Moreover, *Malvastrum coromandelianum* includes silicon, magnesium, sulphur, phosphorus, iron, titanium, strontium, manganese, copper, zinc, chromium and rubidium. Therefore, it is a rich source of mineral. The antimicrobial activities were found that n-hexane and ethanol extracts showed no activity against all organisms. Ethyl acetate extract showed high activity on against *Pseudomonas aeruginosa*, *Candida albican* and *E-coli*. The *Malvastrum coromandelianum* (L.) Garcke contains potent bioactive compounds and the plant is a good source for antimicrobial compounds. According to the study of antioxidant activity, the IC_{50} value of ethanol extract of sample was found to be 120 µg/ml. From the results obtained in the present study, it is concluded that the *Malvastrum coromandelianum* (L.) Garcke is good source of minerals and natural antioxidant.

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References

[1] Caceres, A., (1987). "Diuretic activity of plants used for the treatment of urinary ailments in Guatemala", *J. Ethnopharmacol*, 19:233-243

[2] David, K. and Holdsworth,(1992). " *International Journal of Pharmacognosy*" , 30(1), 27-32

[3] Karou, D., (2005). "Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso", *Afr. J. Biotechnol*, 4:823-828

[4] Reddy, Y., S.Venkatesh and B. Suresh,(2001). "Antinociceptive activity of *Malvastrum coromandelianum*", *Fitoterapia*, 72: 278-80

[5] Alam, M.S., (1996). "A new lactone from *Malvastrum coromandelianum*", *Indian journal of Chemistry section B-organic Chemistry Inciuding Medicinal Chemistry*, 35(12): p.1354-1355

[6] Andrade-cetto, A. and M. Heinrich, (2005). "Mexican plants with hypoglycaemic effect used in the treatment of diabetes", *J Ethnopharmacol*, 99:325-48

[7] Berry, T.N., (2013). "Mallow scrub-Hairstreak on Threelobe False Mallow (*Malvastrum coromandelianum*) (mallow family) in Starr County, American Batterflies", 21(1): p.33-35

[8] Dahanukar, S.A., R.A. Kulkarni and N.N. Rege, (2000). "Pharmacology of medicine plants and natural products", *Indian. J. Phamacol*, 32:81-118

Isolation Of Ligins From Culms Of Bamboo (*Dendrocalamus Gignanteus* Munro)

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Abstract

Lignins were first used in leather tanning and dye baths in the 1880s. Lignin can serve for several purposes such as binder, dispersant, emulsifier and sequestrant. In this research five types of bamboo lignins (**K.bL, Al.bL, S.bL, S.Hp.bL** and **Org.bL**) were isolated from bamboo culms of *Dendrocalamus gignanteus* Munro (Wabo-wa in Myanmar) and were characterized by UV, FT-IR and TG-DTA analyses. The purity, yield, colour, solubility, melting point and molecular structure of isolated bamboo lignins were found to be different from each other with respect to isolation method. Klason method provided the highest yield (**K.bL**, 32.64 %) in which lignin was isolated as insoluble residue after being treated extractive-free bamboo sample with 72 % sulfuric acid at room temperature for 5 days. Relatively higher yield of bamboo lignin (**S.bL**, 18.56 %) was provided by optimized soda extraction method thereby extraction was carried out at 100 °C for 7 h by using 20.0 % (w/v) of NaOH solution as an extractant and 1/10 of extractive-free bamboo to extractant ratio.

Keywords: *Dendrocalamus gignanteus* Munro, lignin, Klason lignin, soda lignin

Introduction

Lignin is a chemical compound that is most commonly derived from wood. It is an integral part of the cell walls of plants. It is one of the most abundant organic macromolecular compounds on earth after cellulose and chitin (Zuman & Rupp, 2001). Lignin is the principal carrier of the methoxy content of wood. The chemical structure, molecular weight, molecular weight distribution and the cross-linking grade depend on factor such as the age, type of the plant and the part of the plant from which the lignin is isolated (Singh *et al.*, 2005). Its basic chemical structural unit is methoxy-substituted propylphenyl moiety, bonded in an irregular pattern of ether and carbon–carbon linkages. Lignin and lignin derivatives, such as lignosulphonate are major by-products of the paper industry and are well known for their ability to bind heavy metal ions. Most lignin waste is burned to generate energy for pulp mills. However, based on its interesting functionalities and properties, lignins offer perspective for higher added value application in renewable product (Gosselink *et al.*, 2004). Lignins are also used as raw material for several chemicals such as vanillin, DMSO, ethanol, torula yeast, xylitol sugar and humic acid. Both alkali and sulphonate lignin are used as dispersants and foaming agent in gypsum board.

Materials and Methods

Three-year-old bamboo culms of *Dendrocalamus gignanteus* Munro. (Wabo-wa) were collected for isolation of lignins.

Preparation of Extractive-Free Bamboo Sample

Oven-dried bamboo powder (25.0 g) was taken in a cloth bag. It was placed in Soxhlet extractor and extracted with ethanol-toluene, 2:1 (v/v) (250 mL), for 8 hours. The material was then air dried and extracted for further 2 hours with water at 60 °C. The extractive-free bamboo powder thus obtained was dried in oven and stored in airtight container. It was used for isolation bamboo lignins.

Isolation of Klason Bamboo Lignin (K.bL)

Extractive-free bamboo (20.0 g) was placed in beaker. Into the beaker was slowly added 72 % sulphuric acid (v/v) (200 mL) with stirring to avoid the formation of lumps. Stirring was continued until gelatinization had completed and kept for 5 days at room temperature with occasional stirring. It was then diluted with distilled water (400 mL) and filtered onto a hardened filter paper and washed with 3 % sulphuric acid (100 mL). The lignin was then suspended in 3 % sulphuric acid (200 mL) and refluxed for 4 hours in order to remove the combined sulphuric acid and to hydrolyse any remaining pentosans. The lignin, which changed colour from almost black to brown, was filtered and washed free of acid. The resultant lignin was oven-dried at 50 °C for 24 h and the yield was calculated on the basis of extractive free bamboo.

Isolation of Soda Bamboo Lignin (S.bL)

Extractive-free bamboo (25.0 g) was placed in 500 mL two-neck round-bottomed flask. 20 % Sodium hydroxide solution (w/v) (250 mL) was added into the flask and refluxed for 7 hours with constant stirring. The black liquor so obtained was filtered under vacuum and then neutralized with 20 % sulphuric acid solution (v/v) under constant stirring to precipitate lignin. Precipitation completed at about pH 7. The resultant mixture was filtered under vacuum and washed repeatedly with hot distilled water till neutral. The lignin cake thus obtained was oven-dried at 50 °C for 24 h and stored in airtight container.

Isolation of Alkali Bamboo Lignin (Al.bL) by Extracting with 1.5 % NaOH Solution

Isolation of alkali bamboo lignin was done according to the procedure reported by Fang and Ma (2006). Extractive-free bamboo (10.0 g) was treated with 1.5 % NaOH aqueous solution (400 mL) at room temperature for 6 days under frequently stirring. The slurry thus obtained was filtered under suction. The supernatant was acidified to pH 5.5 using 20 % HCl solution. Then, 4 volumes of ethanol were added to remove crude hemicelluloses and hemicellulose-lignin complex. After filtration, the supernatant was concentrated under reduced pressure at 50 °C. The concentrated solution was re-precipitated at pH 1.5 by addition of 20 % HCl solution. The precipitated lignin was filtered and washed with pH 2.0 water and dried in oven at 50 °C for 24 h.

Isolation of Lignin by Alkaline Peroxide Treatment (S.Hp.bL)

Bamboo lignin was isolated by alkaline peroxide treatment as procedure reported by Abbot & Peterson (1985). **S.Hp.bL** lignin was also isolated by heating extractive-free bamboo (10.0 g) in 512 mL of 1 % H_2O_2 (pH 11) at 80 °C for 60 min under vigorous stirring. Then, solid residue was removed by vacuum filtration. The supernatant was acidified to pH 5.5 with 20 % HCl aqueous solution, precipitated with 4 volumes of ethanol to isolate crude hemicelluloses and hemicellulose-lignin complex. The supernatant was concentrated under reduced pressure at 50 °C to obtain the alkali-soluble lignin fraction and then re-precipitated at pH 1.5 with 20 % HCl solution. The obtained precipitate was filtered and rinsed with pH 2.0 water and then oven-dried at 50 °C for 24 h.

Isolation of Organosolv Lignin (Org.bL) by Refluxing with Ethanol:Water

The procedure adopted for isolation lignin was that proposed by Acemioglu *et al.* (2003).

Extractive-free bamboo (10.0 g), ethanol-water (3:2 v/v) (400 mL) and 0.02 N H_2SO_4 (0.8 mL) were refluxed at 75 °C for 2 h. The mixture thus obtained was diluted with deionized water and filtered under vacuum. The pulp residue was firstly washed with ethanol-water and then distilled water. The filtrate was concentrated under reduced pressure at 50 °C. Then, lignin in the solution was precipitated by addition of 20 % HCl solution to reach pH 1.5. The resultant lignin was filtered and washed with pH 2.0 water and oven-dried at 50 °C for 24 h.

Characterization of Isolated Bamboo Lignins

The identity of isolated bamboo lignins were characterized for solubility, melting point, maximum absorbance (λ_{max}), IR spectra and TG-DTA thermogram.

(i) Determination of Solubility

The solubility of isolated bamboo lignins was studied by using different solvents such as water, ethanol, methanol, 5 % NaOH and dioxane. Lignin (5 mg) was placed in test tube and solvent (100 mL) was added and shaken well. Then, the solubility of bamboo lignins in these solvents was noted.

(ii) Determination of Melting Point

The melting points of isolated bamboo lignins were measured in open capillary tube by using Gallenkamp melting point apparatus.

(ii) Determination of UV Spectrum

UV spectra of bamboo lignins were recorded on UV-vis spectrometer (UV-1800, Shimadzu) and the absorbance of individual lignin was recorded by scanning wavelength from 190 nm to 500 nm. Soda bamboo lignin (S.bL) and Klason bamboo lignins (K.bL) were dissolved in 5 % NaOH solution and their spectra were recorded.

Remaining three bamboo lignins (Al.bL, S.Hp.bL and Org.bL) were dissolved in ethanol and their spectra were recorded. After taking the spectrum, 2-3 drops of 5 % NaOH solution was added into individual solution and the spectra were taken again. The shift of λ_{max} due to the addition of NaOH was calculated.

(iv) Determination of FT-IR Spectrum

Functional groups present in bamboo lignins were analyzed by using FT-IR spectrometer at Department of Medical Research (DMR).

(v) Determination of TG-DTA Thermogram

Thermogravimetry was accomplished using TG-DTA analyzer (DTG-60 H) at the Universities' Research Centre, Yangon University. Data were then collected in the temperature range from ambient to 600°C, using a nitrogen gas flow rate of 50 mL min^{-1}. The mass of all samples was *ca.* 3 mg.

Investigation of Optimum Condition for Isolation of Soda Bamboo Lignin

(i) Effect of Sodium Hydroxide Concentration

Soda bamboo lignin was isolated from extractive-free bamboo by using various concentration of sodium hydroxide solution. The concentration of sodium hydroxide was varied as: 5.0, 10.0, 15.0, 20.0, 25.0, and 30.0 % w/v. Other parameters were kept at constant.

(ii) Effect of Volume of Sodium Hydroxide Solution

Soda bamboo lignin was isolated from 25.0 g of extractive-free bamboo by using different volume of 20 % sodium hydroxide (w/v). The volume of 20 % NaOH varied as 100, 150, 200, 250, 300 and 350 mL. The reaction mixture was refluxed for 7 hours. Other parameters were kept constant.

(iii) Effect of Extracting Time

Extractive-free bamboo (25.0 g) was treated (extracted) with 250 mL of 20 % sodium hydroxide (w/v) under reflux. The duration of extracting time was varied as 3.0, 4.0, 5.0, 6.0, 7.0, and 8 hours.

Investigation of Optimum Condition for Isolation of Klason Bamboo Lignin

The feasibility of isolation of Klason bamboo lignin was investigated by varying concentration of sulphuric acid, amount of acid solution, and extracting time.

Results and Discussion

Isolation of Lignins

Klason method is used to determine acid insoluble lignin content of herbaceous samples. The procedure of soda bamboo lignin isolation basically involved two steps, *viz.* alkaline extraction and acidified precipitation. The percentage recovery of soda lignin from black liquor is influenced by the precipitation conditions (precipitation pH and type of mineral acid).

The procedure of isolation of alkali lignin provided relatively pure lignin. Result highlighted that mild condition employed in this procedure was not capable to peel lignin from neighbouring polysaccharide moieties so as to release lignin as a dissolved substance into aqueous solution.

The advantages of lignin extraction with alkaline peroxides are low cost, accompanying strong bleaching effect as well as the recovery of the solubilized macromolecular hemicelluloses with a minimal degradation. This lignin preparation was white in colour and contained very small amounts of associated hemicellulose.

Organosolv processes, based on using mixtures of water and organic alcohols or acids and particularly, ethanol organosolv process, gained new relevance for biomass pretreatment in a biorefinery sense (Lignol process) as it allowed obtaining several high value products (cellulose, lignin, hemicellulose, extractives) under mild reaction conditions by a cost-effective process in which solvents were recovered and recycled at the end of the process.

Table 1 Yield of Isolated Bamboo Lignins at Different Isolation Conditions

Type of BambooLignin	*Yield (%)
K.bL	32.64
S.bL	18.56
Al.bL	2.00
S.Hp.bL	3.45
Org.bL	0.40

*Based on extractive free sample

Table 2 Solubility of Bamboo Lignins Isolated via Different Methods

Type of bamboo lignin	Test Solvent				
	Ethanol	Methanol	Water	5 % NaOH	Dioxane
K.bL	-	-	-	+	-
S.bL	-	-	-	+	-
Al.bL	+	+	-	+	-
S.Hp.bL	+	+	-	+	-
Org.bL	+	+	-	+	+

(+) = soluble (-) = insoluble

Most of the isolated bamboo lignins did not provide sharp melting point. The melting point seemed to be dependent on isolation method. Molecular weight and degree of hydrogen bonding generally influence the melting point of isolated lignin. Lignin isolated via organosolv process **(Org.bL)** has the lowest melting point. On the other hand, Klason lignin provided the highest melting point.

Table 3 Melting Point of the Bamboo Lignins Isolated via Different Methods

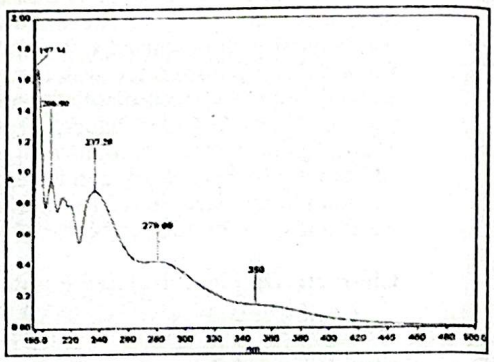
Type of bamboo	Melting point
K.bL	> 300
S.bL	193-198 (dec.)
Al.bL	203-204 (dec.)
S.Hp.bL	204-207 (dec.)
Org.bL	152-154 (dec.)

(dec.) = decomposition

UV spectra of soda and Klason bamboo lignins were measured by dissolving them in 5 % sodium hydroxide solution. In sodium hydroxide solution, the acidic ionisable functional groups of these lignins dissociated forming salt and become soluble. Accordingly, their λ_{max} shifted to longer wavelength due to the formation of phenolate ions (blue shift). Both lignins exhibited similar spectra as absorption maxima of "**K.bL**" at λ_{max} 244, 290 and 350 nm, and that of "**S.bL**" at 237, 280 and 350 nm, as shown in Figure 1.

Remaining bamboo lignins in ethanol exhibited three basic maxima of lignin to *ca.* 310, 280, and 230 nm. The absorption maximum around 280 nm originates from non-conjugated phenolic groups (aromatic ring). No direct correlation was seemed to establish between the absorption pattern and the presence of specific ring substituents as all lignins investigated here provided the peaks at 280 nm for "**Al.bL**", 282 nm for "**S.Hp.bL**", and 282 nm for "**Org.bL**". Specifically say, an absorbance peak at (280–283) nm, indicated the presence of the strong absorbing guaiacyl lignin.

In addition, the absorption maximum at 310 nm can be attributed to the absorption of the carbonyl moiety in ester bonds and indicating the presence of α- or α,β-unsaturated groups such as carbonyl or stilbene-like chromophores. All spectra curves show a shoulder between 310–320 nm, which can be linked to the presence of *p*-coumaroylation. Addition of NaOH bathochromic shifted in all spectra further confirmed the presence of phenolic hydroxyl groups in these lignins.



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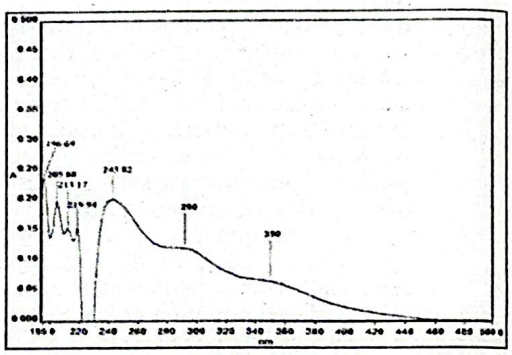

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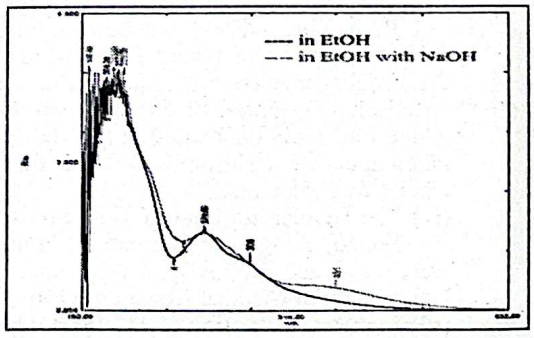
Figure 1 UV-visible spectra of (a) Klason bamboo lignin and (b) soda bamboo lignin (5 % NaOH)



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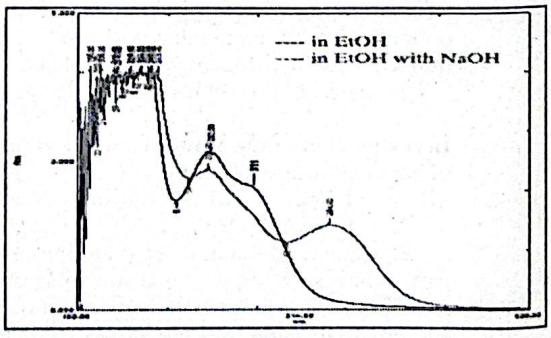

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Figure 2 UV-visible spectra of (a) alkali bamboo lignin and (b) S.Hp.bL lignin (ethanol with or without NaOH)

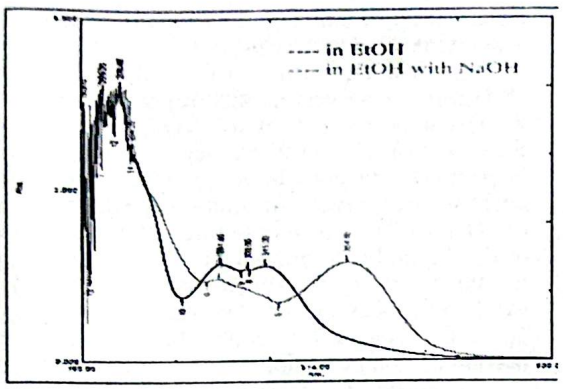


Figure 3 UV-visible spectrum of organosolvbamboo lignin (Org.bL) (ethanol with or without NaOH)

FT-IR spectrum of Klason bamboo lignin "**K.bL**" is showed in Figure 4(a). Strong and very broad band due to O–H stretching at 3650-2600 cm^{-1} and carbonyl stretching band at 1705 cm^{-1} indicated the presence of considerable content of carboxylic groups in this lignin providing that ester groups cleaved during 72 % sulphuric acid treatment forming carboxylic acid group. Spectrum provided C–H stretching vibration of aliphatic groups at 2939 cm^{-1} and C–H deformation at 1458 cm^{-1}. Band at 1605 cm^{-1} was attributed to C=C stretching vibration of aromatic ring. In addition, the presence of guaiacyl group in this lignin was confirmed from bands at 1211 cm^{-1} (ring breathing) and at 1103 cm^{-1} (in plane deformation of Aromatic C-H).

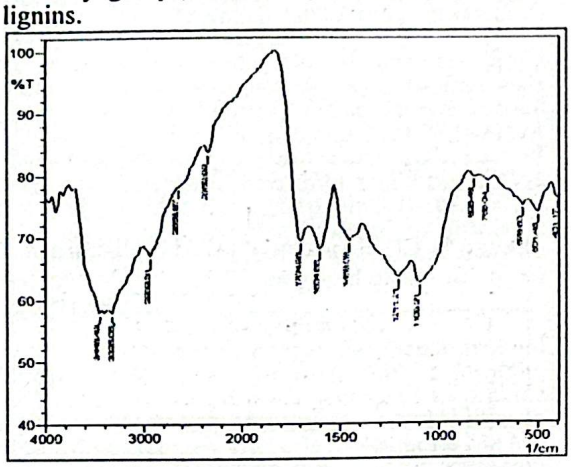
Figure 4(b) shows the IR spectrum of extracted bamboo lignin "**S.bL**". Strong and very broad band appeared between 3700-3000 cm^{-1}; another strong and broad band which centered 1041 cm^{-1}; and typical adsorption of hemicellulose backbone at 1643 cm^{-1} indicated the presence of significant amount of hemicellulose in "**S.bL**". Band at 3700-3000 cm^{-1} was due to the hydrogen bonded OH groups and the stretching frequency of the -O–H group as well as intramolecular and intermolecular hydrogen bonds. The OH bending of adsorbed water appeared at 1643 cm^{-1}. The bands between 1460 and 1320 cm^{-1} were provided from C-H, -OH or $-CH_2$ bendings. The band at 1041 cm^{-1} was due to $OCH–O–CH_2$ stretching (Kondo, 1997) of hemicellulose. In addition, aliphatic C–H stretching vibration was observed at 2893 cm^{-1}. Characteristic carbonyl peaks in lignin at (1715-1705) and (1675-1660) cm^{-1} were not distinctly observed in this spectrum.

In contrast to "**S.bL**", intense polysaccharide bands were not detected in FT-IR spectra of remaining four lignins (**Al.bL, SHp.bL** and **Org.bL**), suggesting a relatively low content of associated sugars in the samples.

As in the Figures 5 and 6, their spectra show minor changes in the peak intensities which confirmed that the core of the lignin structure did not change dramatically during the different periods of alkaline treatment processes. In these spectra, bands at (3441 and 3448) cm^{-1} were attributed to O–H stretching vibration which is characteristic of –OH groups or phenolic compounds.

The bands at 1655 cm^{-1} in "**Al.bL**" and 1655 cm^{-1} in "**Org.bL**" have been assigned to carbonyl stretching in conjugation with the aromatic ring while the bands at 1697 cm^{-1} have been attributed to carbonyl stretching in unconjugation with the aromatic ring.

From the analysis of IR-spectra of bamboo lignins, it may be inferred that ionisable (carboxylic acid and phenol groups) and polar functional groups (hydroxyl, carbonyl and methoxy groups) abundantly consist of bamboo lignins.



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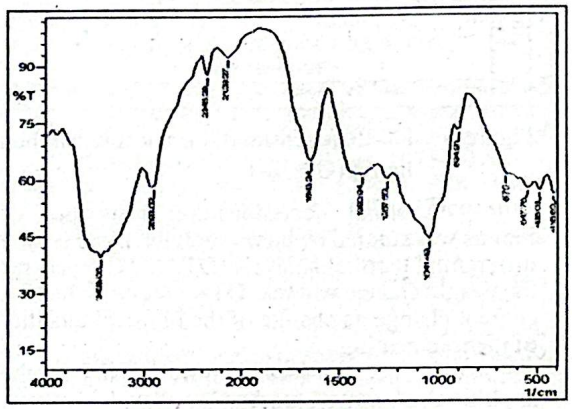

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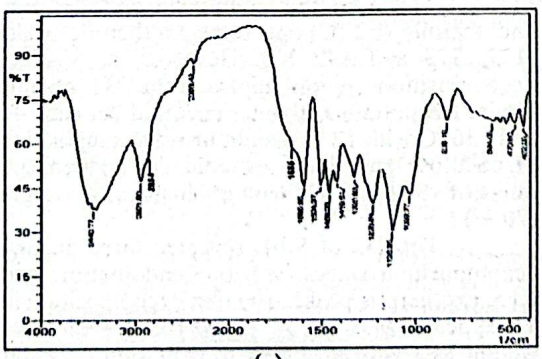
Figure 4 FT-IR spectra of (a) Klason bamboo lignin and soda bamboo lignin



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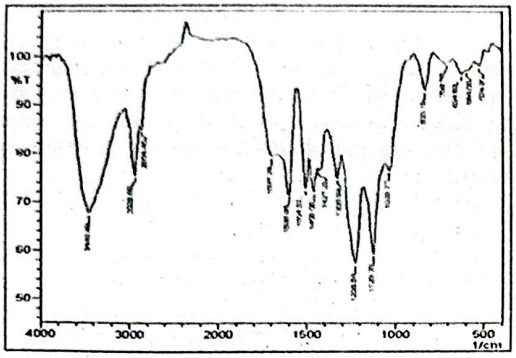

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Figure 5 FT-IR spectra of (a) Al.bL lignin and S.Hp.bL lignin

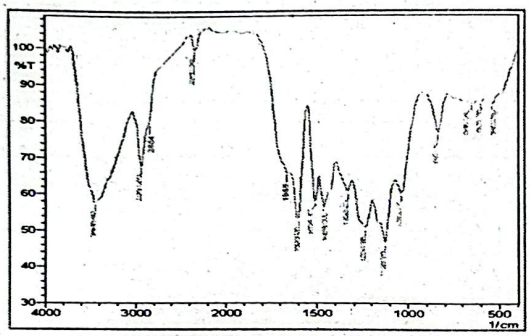


Figure 6 FT-IR spectrum of organosolv bamboo lignin (Org.bL)

Thermal decomposition behaviour of lignins was studied by thermogravimetry (TG) and differential thermal analysis (DTA). TG measures the weight change whereas DTA measures the heat content change as change of the physical quantity of them in heating.

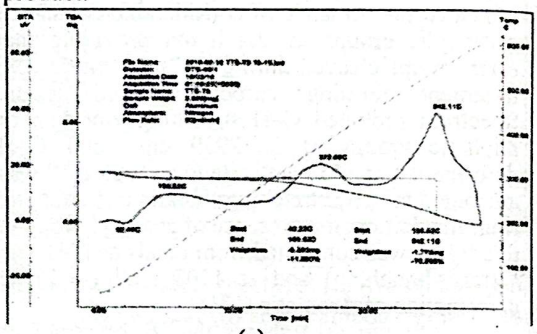
In TG, the losses of weight due to the evolution of water, carbon monoxide, carbon dioxide, and evaporation of other pyrolysis products, are collectively measured as a percentage of the original weight.

Thermogram of "**K.bL**" is shown in Figure 7. DTA of this compound provided one endothermic (62 °C) and three exothermic peaks (137, 372 and 542 °C). However, no distinct decomposition region appeared in TG of this lignin. Evaporation of water occurred between 40 and 136 °C with 12 % weight loss. Decomposition of cellulose and lignin covered the temperature range of 136 to 542 °C with gradual loss in weight (70 %).

The TG of **S.bL** revealed three distinct decomposition stages with one endothermic and three exothermic peaks. The first decomposition in TG appeared between 50 °C and 105 °C with 17 % weight loss was attributed to evolution of water which provided endothermic peak at 71 °C in DTA. Second stage which covered 276 to 340 °C with 44 % weight loss was due to decomposition of hemicellulose. Two exothermic peaks at 312 °C and 340 °C were found in this region. Decomposition process of lignin covered the range 340 to 460 °C with 27 % weight loss. In this region, an exothermic peak at 420 °C was observed. Above this temperature, no loss in weight was observed up to 600 °C. 11 % w/w of soda lignin was left as residual product.

Thermograms of "**Al.bL**" and "**S.Hp.bL**" were very similar as shown in Figures 8. Decomposition started around 30 °C and finished 570 °C with 97 % and 98 % weight loss respectively. No endothermic and four exothermic peaks were observed.. In addition, the thermogram of "**Org.bL**" is shown in Figure 9. Loss in weight of this lignin began around 30 °C and occurred up to 600 °C and 81 % weight loss. Two dominant exothermic peaks were observed at 131 °C and 561 °C. The latter could be attributed to decomposition of lignin.

Nada & Hassan (2000) showed that hemicellulose decompose between 200 and 260 °C, α-cellulose decompose between 240 and 350 °C with significant weight loss around 275 °C. The decomposition process of lignin covers a larger temperature range, between 280 and 500 °C, though at temperature lower than 400 °C only 30 % is decomposed rendering charcoal as the residue product.



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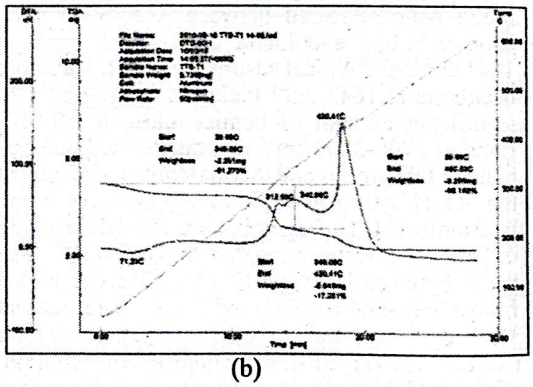

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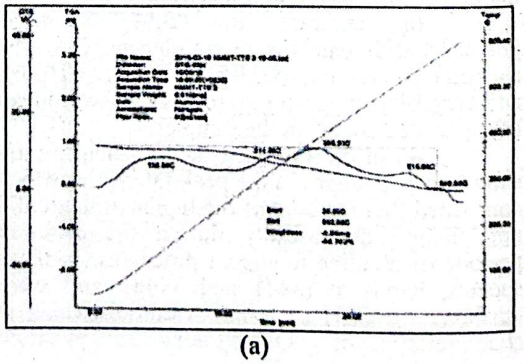
Figure 7 Thermograms of (a) Klason bamboo lignin and (b) soda bamboo lignin



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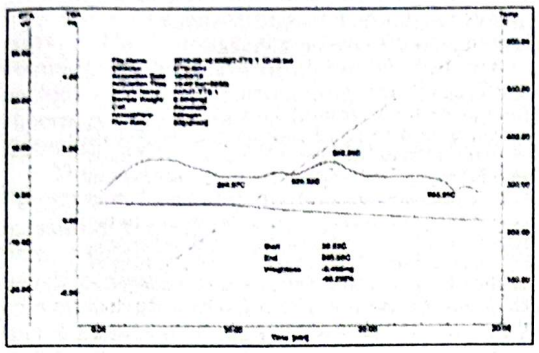

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Figure 8 Thermograms of alkali bamboo lignin and (b) S.Hp.bL lignin

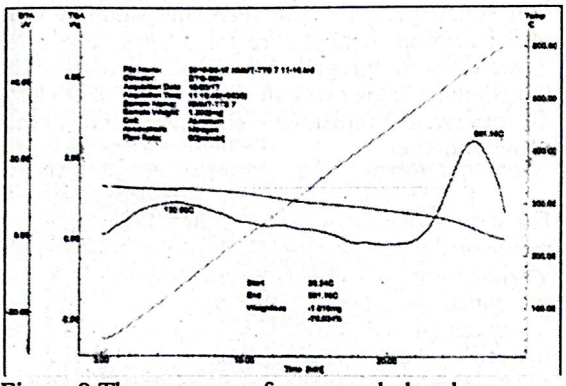

Figure 9 Thermogram of organosolv bamboo lignin (Org.bL)

Optimum Condition for Extraction of Klason and Soda Lignins

Results demonstrated that the optimum conditions elucidated for isolation of soda bamboo lignin were: 20.0 % (w/v) of sodium hydroxide solution, 10 mL sodium hydroxide solution per one gram of bamboo, 100 °C reaction temperature, and 7 h reaction time. The optimized conditions provided to isolate 18.56 % yield of soda lignin from extractive free bamboo sample via 20 % sulphuric acid precipitation.

The proper choice of sulphuric acid concentration was very important in the isolation of Klason lignin since low concentration of acid provided incomplete hydrolysis of cellulose and hemi-cellulose. On the other hand, high concentration led to formation of humic acids. For effective isolation, the ratio of solution to bamboo should be 10 mL per 1 gram. Lower ratio of solution did not provide homogenous hydrolysis and too much acid solution necessitated the handling of unnecessarily large volume of solution after the required dilution. In addition, hydrolysis for 5 days was enough to complete removal of polysaccharides. The gelatinization was not complete during the first 6 hours.

Conclusion

In this research, five types of bamboo lignins were isolated from *Dendrocalamus gignanteus* Munro. The yield, purity and physicochemical properties of isolated lignins were strongly affected by different methods employed for isolation.

UV and FT-IR analyses revealed that carboxylic acid and phenyl groups and polar functional groups (hydroxyl, carbonyl and methoxy groups) abundantly consist of bamboo lignins.

According to TG-DTA analysis all hemicellulose are substantially decomposed between 220 and 300 °C. Soda extraction method applied to isolate "**S.bL**" led to contaminate significant amount of hemicellulose and cellulose in lignin. In addition, the isolated soda lignin probably is relatively higher molecular weight when compared to other bamboo lignins such as Al.bL, S.Hp.bL and Org.bL.

Relatively higher yield of bamboo lignin (**S.bL**, 18.56 %) was provided by optimized soda extraction method thereby extraction was carried out at 100 °C for 7 h by using 20.0 % (w/v) of NaOH solution as an extractant and 1/10 of extractive-free bamboo to extractant ratio. Klason method provided the highest yield (**K.bL**, 32.64 %) in which lignin was isolated as insoluble residue after being treated extractive-free bamboo sample with 72 % sulfuric acid at room temperature for 5 days.

Acknowledgements

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References

Abbott, T. and Peterson, R. (1985). "Products of Alkaline Peroxide Attack on Wheat Straw, Oak, and Kenaf", *Biotechnology and Bioengineering*, vol. 27, pp. 1073-1076.

Fang, J.Y. and Ma, X.L. (2006). "In Vitro Simulation Studies of Silica Deposition Induced by Lignin from Rice", *J. Zhejiang Univ. Sci. B*, vol. 7 (4), pp. 267-271.

Gosselink, R.J.A., de Jong, E., Guran, B. and Abacherli, A. (2004). "Co-Ordination Network for Lignin-Standardization, Production and Applications Adapted to Market Requirements (EUROLIGNIN)", Industrial Crops and Products, *Int. J.*, vol. 20, pp. 121-129.

Singh, R., Singh, S., Trimukhe, K., Pandare, K., Bastawade, K., Gokhale, D. and VArma, A. (2005). "Lignin-Carbohydrate Complexes from Sugarcane Bagasse:Preparation, Purification, and Characterization", *Carbohy. Polym.*, vol. 62, pp. 57-66.

Zuman, P. and Rupp, E. (2001). "Lignin as Adsorbent and Detoxicant", *Int. J. Envi. Conscious Design & Manuf.*, vol. 10 (1), pp. 51-55.

Acemioğlu, B. (2004). "Removal of Fe (II) Ions from Aqueous Solution by Calabrian Pine Bark Wastes", *Bioresource Technol.*, vol. 93, pp. 99-102.

Nada, A.M.A. and Hassan, M.L. (2000). "Thermal Behavior of Cellulose and Some Cellulose Derivatives", *Polymer Degradation & Stability*, vol. 67, pp. 111-115.

Kondo, T. (1997). "The Assignment of IR Absorption Bands due to Free Hydroxyl Groups in Cellulose", *Cellulose*, vol. 4(4), pp. 281–292

Extraction and Characterization of Polyphenol Oxidase from Apple Fruits

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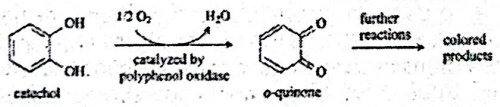
Abstract

The polyphenol oxidases (PPOs) enzyme was isolated from apple fruits obtained from Chin State. The nutritional values such as ash, moisture, fat, fiber and protein were determined according to the AOAC methods and Vitamin C content was determined by iodo-metric titration method. Polyphenol oxidase is present in majority of plant tissues and it is involved in adverse browning of plant products. Crude polyphenol oxidase from the pulp of apple fruits was extracted by homogenization with sodium phosphate buffer followed by precipitation with cold acetone. The spectroscopic method was used to assay the PPO activity by measuring the initial rate of benzoquinone. Therefore the enzyme activity was followed by measuring the absorbance at 400 nm. Enzymatic properties such as optimum pH, optimum temperature, effect of reaction time, effect of substrate concentration and enzyme concentration were studied by visible absorption spectrophotometry. From the experimental data, enzyme kinetic parameters such as maximum velocity (V_{max}), Michaelis-Menten constant (K_m), activation energy (E_a) and reaction order (n) were determined by using statistical and various graphical methods.

Keywords : Polyphenol oxidase, V_{max}, K_m, activation energy, methylene blue

Introduction

Apples, like other fruits, are an important source of polyphenol (phenolic compound) in the human diet. The fruit susceptibility to enzymatic browning is major problem for the fruit processing industry. Phenolic compounds are responsible for color, bitterness, flavor, and nutritional value in fruits and vegetables. Polyphenol oxidases (PPOs) have the ability to oxidize phenols and thus cause browning of plant extracts and bruised plant tissues. Polyphenol oxidase is present in plants, and fruits such as mushrooms, potato, sweet potato, apples, bananas, avocados, tea leaves, coffee beans, and tobacco leaves. PPO is important as it is responsible for eye, skin, inner ear and hair melanization, as well as browning in fruits and vegetables (Marín-Zamora *et al.*, 2005). Melanins, produced as a result of PPO activity, may exhibit antibacterial, antifungal, anticancer, and antioxidant properties. Plant PPOs have broad substrate specificities and are able to oxidize a variety of mono, di, or polyphenols. The proposed mechanism of PPO is based on its capacity to oxidize phenolic compounds. PPO (EC 1.10.3.1; *o*-diphenol oxidoreductase) is an oxidoreductase capable of oxidizing phenol compounds by employing oxygen as a hydrogen acceptor (Simpson, 2012). Catechol is oxidized initially to



o-quinone which can rapidly polymerize to form coloured compound as shown in figure 1.

Fig. 1 Catechol's oxidize and subsequent reactions

PPO is copper containing metalloenzyme predominantly located in the chloroplast thylakoid membrane (Valero and Garcia, 1998). Apples PPO were subjected to further characterization studies in terms of biochemical characteristics and potential for biotechnological applications. Apple contains a large amount of phenolic compounds which are readily oxidized during homogenization. After successive oxidation and polymerization of phenolic compounds in the apple extract, macromolecules of melanin are formed. PPO was purified and characterized by determining of reaction time, optimum pH, optimum temperature, substrate concentration and enzyme concentration using crude PPO.

Materials and Methods

Sample Collection

Apple fruits were purchased from Chap village, Mintut Townsphip, Chin State (Figure 2). This sample was identified at Botany Department, University of Yangon (Baker and Bakhuizen, 1963). The apple fruits washed tap water and cut into small pieces. And then, the samples were dried in room temperature for 5 days. The dried samples were grounded in electric motor. The powdered samples was stored in air-tight contains to prevent moisture changes and other contamination.

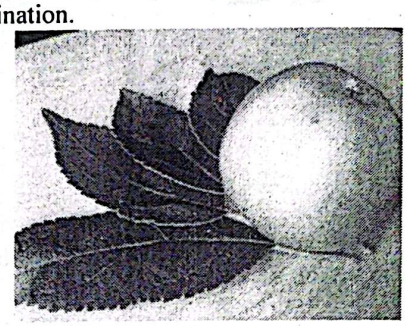


Fig. 2 Apple fruits from Chap village

Nutritional Analysis

Moisture content was determined based on the standard oven-drying method at 105°C for 24 hours. Ash content in apple fruits was measured by the muffle furnace method. Crude fiber content was determined by the fiber cap method. The Kjeldahl method, a chemical procedure for nitrogen, is still the basic method used for protein analysis. Protein content in apple fruits sample was calculated with conversion ratio N × 6.25. Fat content was determined by the Geber method. The ascorbic acid (vitamin C) content of fresh fruit was determined by the titrimetric method using potassium iodate.

Extraction of Polyphenol Oxidase from Apple Fruit

Firstly apple fruit were peeled. Secondly to prepare the crude extract, 150 g of sample was cut into small pieces and homogenized in 300 mL of 0.2 M phosphate buffer using blender for 1 minute at maximum speed. The crude extract sample was centrifuged at 3000 rpm for 45 minutes. The supernatant obtained was filtered under vacuum from a Buchner funnel containing Whatman No.1 filter paper and the filtrate was collected in a conical flask. Then, 150 mL of the filtrate was pipetted drop by drop into 300 mL of cold acetone for the formation of the precipitates. The precipitates, crude PPO separated by centrifugation at 3000 rpm for 45 minutes. The resultant light brown color precipitates was dried overnight at room temperature. The acetone powder that obtained was stored at -20 °C. The enzyme extraction from acetone powder (2 g) was

mixed with 100 mL of 0.2 M phosphate buffer (pH 6.8) and stirred for 1 hour at 4 °C with magnetic stirrer. The temperature was maintained by covering the beaker with aluminum foil and was enclosed with ice surrounding the beaker. The supernatant was used as crude PPO.

Determination of Polyphenol Oxidase Activity

The protein concentration was determined. Polyphenol oxidase activity was determined by measuring the increase in absorbance at 400 nm with spectrophotometer. The reaction mixture which contained 0.5 mL freshly prepared crude enzyme extract, 1.5 mL of 0.2 M phosphate buffer (pH 6.8) and 0.5 mL of 0.05 M catechol. PPO activity was assayed in triplicate. For blank solution, 1 mL of distilled water was used instead of 0.5 mL of enzyme solution.

Effect of Reaction Time for Polyphenol Oxidase

The PPO activity was determined in a different times interval of 1-10 minutes by using 0.2 M phosphate buffers (pH 6.8 and 30 °C. Each reaction was initiated by the addition of enzyme filtrate into the cuvette and the absorbance was recorded by using spectrophotometer.

Effect of Optimum pH and Optimum Temperature

The PPO activity was determined in a pH range of 5.8 to 7.6 by using 0.2 M phosphate buffer at 30 °C. In this buffering range, enzyme activity was measured spectrophotometrically using the procedure described for the PPO activity assay.

PPO activity was measured at different temperature in the range from 15 to 45 °C using catechol as a substrate (0.05 M). The standard mixture, without the enzyme, was heated to the appropriate temperature at 25 °C in a water bath. After equilibration of the reaction mixture at the selected temperature, the enzyme was added. The reaction mixture contained 1.5 mL of phosphate buffer (pH 6.8), 0.5 mL of 0.05 M substrate and 0.5 mL of crude PPO extract. The PPO activity was determined at the optimum temperature measured at 400 nm. Similarly the new set of the reaction mixtures were allowed to react at (15, 20, 30, 35, 40 and 45 °C). For blank solution, 0.5 mL of distilled water was used instead of 0.5 mL of enzyme solution.

Effect of Substrate Concentration and Enzyme Kinetic

For the determination of Michaelis constant (K_m) and maximum velocity (V_{max}), PPO activities were determined using substrate which include various catechol concentrations (0.01 M - 0.1 M). A 0.5 mL of catechol solutions (0.01, 0.03, 0.05, 0.07, 0.09 and 0.1 M) was added into six separate test tubes containing 1.5 mL of phosphate buffer solution (pH 6.8) and 0.5 mL of crude PPO extract and the absorbance was measured at 400 nm. For blank solution, 0.5 mL of distilled water was used instead of 0.5 mL of enzyme solution. K_m and V_{max} values of the PPO, for each substrate, were used from the plots of 1/V versus 1/[S] according to the Lineweaver-Burk methods (Line weaver and Burk, 1934).

Effect of Enzyme Concentration

The velocity of enzyme essay method was tested using different percent concentration of enzyme. The absorbance at 400 nm was measured.

Color Removal of Methylene Blue with Polyphenol Oxidase

PPO activity was measured at different time in the range from 1:30 to7:30 hour at 30°C. The reaction mixture contained 5 mL of enzyme solution and 25 mL of methylene blue 10 ppm solution and the absorbance was measured at 663 nm.

Results and Discussions

Nutritional Analysis of Apple Fruits

Results relative to apple samples are shown in table 1. The ash at food stuff is the inorganic residue remaining after the organic matter has been burnt away. In this research, the ash content was found to be 1.44 %. The moisture content was determined by oven drying to content weight and taking the loss in weight as water. The moisture content was found to be 19.18 %. The fat content was determined by Soxhlet extraction method using petroleum ether (B.P 60-80 °C). Fat can be also extracted by diethyl ether or benzene. The fat content was found to be 1.68 % .The crude fiber of foods depresses the digestibility of other organic nutrients. The fibrous constituents of plant foods are found in the cell walls enclosing the available nutrients. In the work, the fiber content was found to be 6.17 %. Protein is a nutrient that the body needs to grow and maintain itself. In plants, proteins are found in all living parts but usually in relatively small quantities except in the seed. In this work, the protein content was found to be 1.85 %. The carbohydrate contents of apple fruits to be 69.68 %. In conclusion, a defatted apple fruits is a good source of fiber. Thus study demonstrates that the nutritional value of protein from apple seed is relatively low.

Vitamin C (ascorbic acid) is an antioxidant that is essential for human nutrition. Vitamin C content present in apple fruit was determined by using iodometric titration method. The result of vitamin C content was found to be 5.4 mg/100 g.

Table 1. Average Nutrient Content in Apple per 100 g

No	Analyze	Content (%)
1.	Moisture	19.18
2.	Ash	1.44
3.	Protein	1.85
4.	Crude Fiber	6.17
5.	Crude Fat	1.68
6.	Carbohydrate	69.68

Extraction of Polyphenol Oxidase from Apple Fruits

A fruit extract is added to a solution of catechol and the rate of formation of colored compound is measured. It was found that the faster the rate of increase in absorbance of the reaction mixture, the greater the polyphenol oxidase activity of the fruit extract.

Polyphenol Oxidase Activities by Spectrophotometric Method

Polyphenol oxidase activity of apple fruits was determined by using spectrophotometer. Catechol was used as the substrate. The absorption of polyphenol oxidase was measured in the range from 340 to 460 nm .It was found that the maximum absorption of polyphenol oxidase was at 400 nm (Figure 3). One unit (U) of PPO activity was defined as the amount of the enzyme that increased the absorbance by 0.001 $min^{-1}1mL^{-1}$ (Oktay *et al.*, 1995).

$$\text{Polyphenol oxidae activity} = \frac{\text{Absorbance at 400 nm}}{0.001 \times \text{Volume of enzyme solution} \times \text{Reaction time}} \quad \text{----(1)}$$

The polyphenol oxidase activity (enzyme unit) was found to be 217.778 EU per gram of apple fruits.

Effect of Reaction Time on Concentration and Velocity of Polyphenol Oxidase-Catalyzed Reaction

Figure 4 shows the plot of velocity of polyphenol oxidase catalyzed reaction as a function of reaction time. At the beginning of the reaction (up to 3 minutes) the reaction was very fast and then velocity decreased steadily. In this research, reaction time at 3minutes was chosen for further kinetic studies.

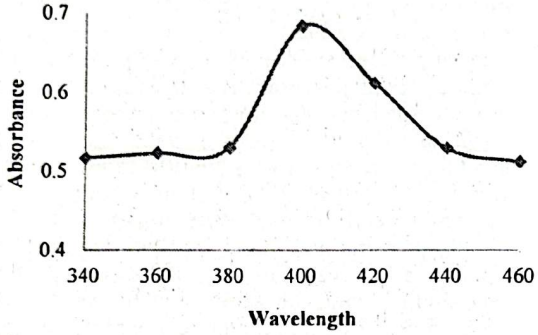
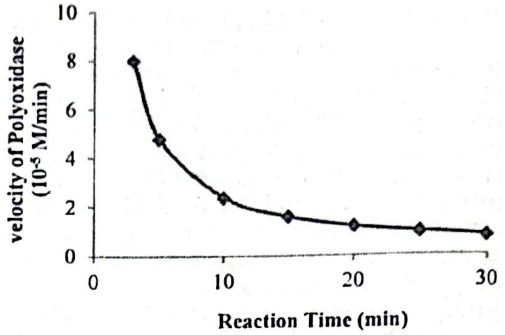


Fig. 3 Absorption spectrum of polyphenol oxidase

Optimum pH and Optimum Temperature of Polyphenol Oxidase Activity

Enzyme being mostly protein is affected by change in pH. The different buffers of pH values ranging from 5.8 to 7.6 were used to determine the activity of the prepared polyphenol oxidase sample. The solution was found immediately brown that was observed strongly at 400 nm. The pH was increased from slightly acidic to neutral, the enzyme activity increased and started to decline after pH 6.8. The optimum pH of polyphenol oxidase was found to be 6.8 (Figure 5). Over the optimum pH, the activities decreased rapidly due to the denaturing of enzyme protein.

The effect of temperature on PPO from apple fruits was tested. Enzyme activity is determined by the temperature of the medium, this is due to their protein nature, and since high temperature leads to protein denaturation thus a decreased enzyme



activity. As the temperature increases, the enzyme activities were found to drop at temperatures the activities were almost non-detectable.The optimum temperature of polyphenol oxidase was found to be 30 °C (Figure 6).

Figure 7 shows the graph for determination of activation energy. The activation energy of polyphenol oxidase-catalyzed reaction was calculated by using Arrhenius equation (Emis, 2002). The activation energy (E_a) was determined to be 6.7176 kcal mol^{-1} and 6.057 kcal mol^{-1} by statistical and graphical methods respectively for temperature between 15 °C to 30 °C.

Fig. 4 Plot of velocity of polyphenol oxidase catalyzed reaction as a function of reaction time

Effect of Substrate Specificity and Enzyme Kinetics

K_m and V_{max} values for PPO activity of apple fruit from different substrate concentration were used. In graphical methods, the values of K_m and V_{max} for PPO were determined by Michaelis-Menten, Lineweaver-Burk, Eadie-Hofstee, Hanes-Wilkinson, Eisenthal-Cornish Bowden plots (Figure 8-12). The statistical methods were used for obtaining V_{max} and K_m from experimental results. The comparison with V_{max} and K_m values obtained by Lineweaver-Burk, Eadie-Hofstee plot, Hanes-Wilkinson and Eisenthal-Cornish Bowden they were not much different from each other implying that they were comparable on the basic of quantitative aspects Michaelis-Menten plot which gave the approximate values. These values were shown in Table 2.

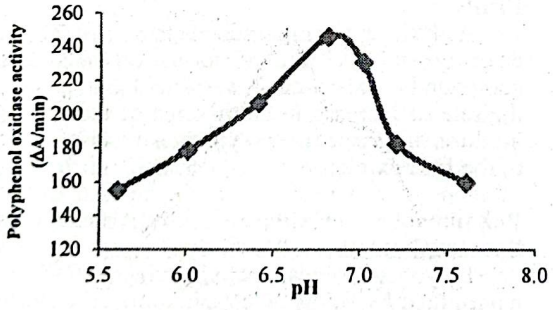


Fig. 5 Plot of polyphenol oxidase activity as a function of pH of the solution

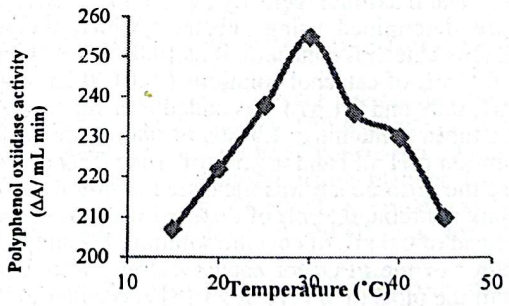


Fig. 6 Plot of polyphenol oxidase activity as a function of temperature of the solution

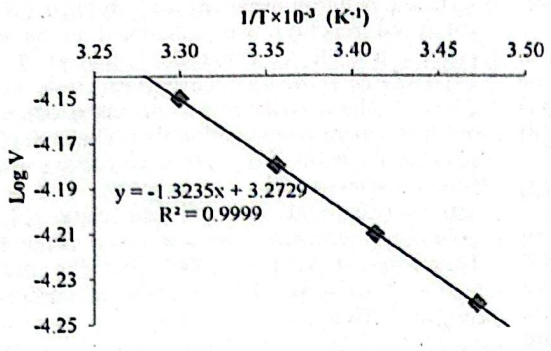


Fig. 7 Plot of log V as a function of 1/T

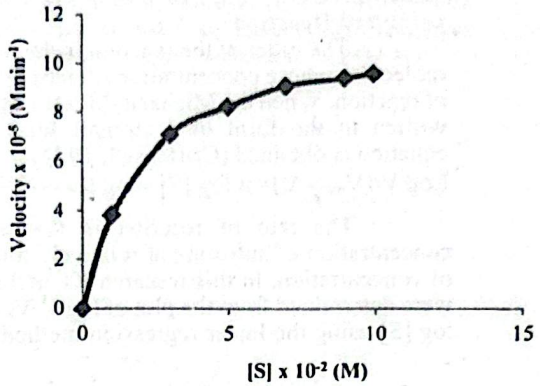


Fig. 8 Michaelis-Menten plot of velocity of polyphenol oxidase-catalyzed reaction as a function of substrate concentration

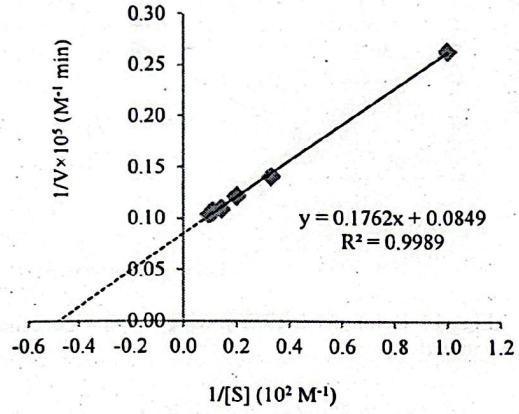


Fig. 9 Lineweaver-Burk plot of 1/V *vs.* 1/[S] used for graphic evaluation of V_{max} and K_m for polyphenol oxidase enzyme

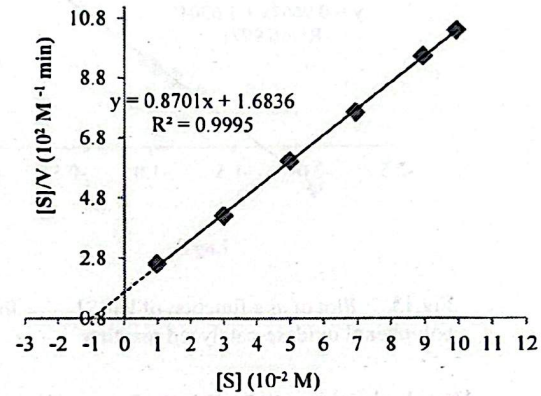


Fig. 10 Hanes-Wilkinson plot of [S]/ V *vs.* [S] used for graphic evaluation of V_{max} and K_m for polyphenol oxidase enzyme

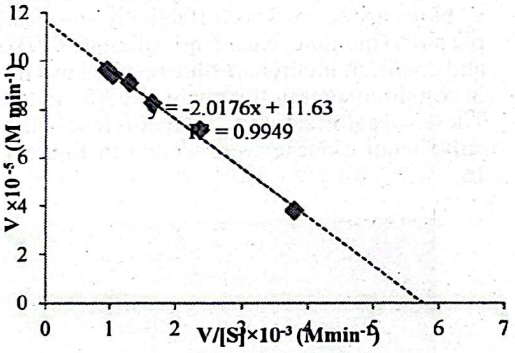


Fig.11 Eadie-Hofstee plot of V *vs.* V/[S] used for graphic evaluation of V_{max} and K_m for polyphenol oxidase enzyme

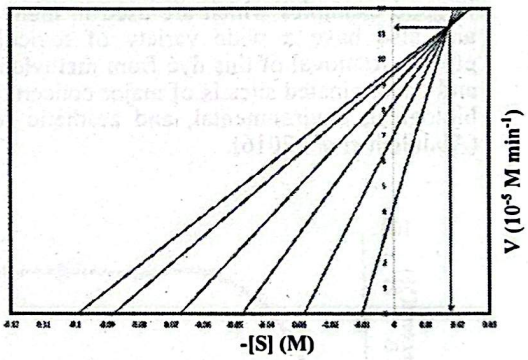


Fig. 12 Eisenthal-Cornish Bowden plot of V vs. -[S] used for graphic evaluation of V_{max} and K_m for polyphenol oxidase enzyme

Table 2 Comparison of V_{max} and K_m Values from Various Statistical and Graphical Methods

No.	Different Methods	Statistical method		Graphical method	
		Km (M)	Vmax	Km (M)	Vmax
1	Michaelis-Menten	-	-	0.0163	0.957
2	Lineweaver-Burk	0.0208	1.176	0.0202	1.159
3	Hanes-Wilkinson	0.0204	1.164	0.0192	1.321
4	Eadie-Hofstee	0.0202	1.163	0.0199	1.157
5	Eisenthal-Cornish Bowden	-	-	0.0178	1.123

Reaction Order (n) for Polyphenol Oxidase-catalyzed Reaction

The order of the reaction is the number of molecules whose concentrations determine the rate of reaction. When the Michaels-Menten equation is written in the form of a straight line, the Hill equation is obtained (Cristensen, 1974).

$$\text{Log } V/(V_{max}- V)= n \log [S] - \log K_m \text{ ----------(2)}$$

The rate of reaction is first order in concentration of substrate at relatively low values of concentration. In this research K_m and n values were determined from the plot of log V/V_{max}- V vs. log [S] using the linear regression method (Figure 13). The reaction order (n) for polyphenol oxidase catalyzed reaction was calculated to be 0.9679 proving that the reaction order is first order.

Effect of Enzyme Concentration

The activity of an enzyme is determined by the enzyme concentration. The velocity of enzyme essay method was tested using different percent concentration of enzyme. The enzyme activity was found to have linear relationship with volume of enzyme solution taken (Figure 14). Therefore, it was revealed that the prepared enzyme solution has significant polyphenol oxidase activity.

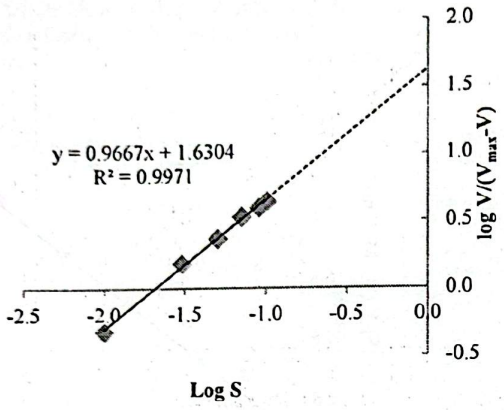


Fig.13 Plot of as a function of log[S] for polyphenol oxidase-catalyzed reaction

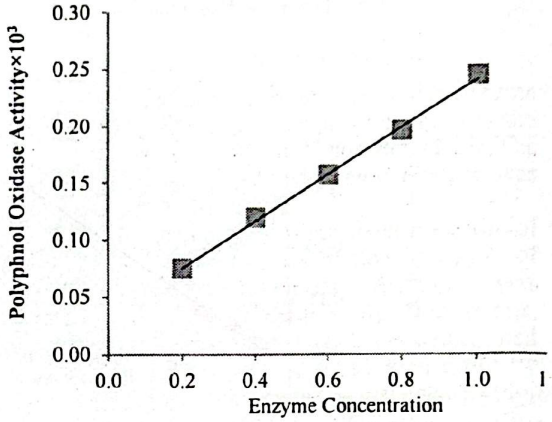


Fig. 14 Relations between with enzyme concentration and activity

Decolorization of Methylene Blue with Polyphenol Oxidase

Dyes are widely used in many fields, for instance in textile industry and biology in a number of biological staining procedures. Methylene blue is good examples which are used in many fields and also have a wide variety of toxicological effects. Removal of this dye from methylene blue and contaminated sites is of major concern due to biological, environmental, and aesthetic reasons (Abdullah *et al.*, 2016).

Decolorization of methylene blue dye was determined spectrophotometer. The absorbance was measured with spectrophotometer 663 nm. The experiment was incubated at 30 °C and inoculums concentration of 10 ppm. PPO extract as a biocatalyst worked effectively in catalyzing phenol to quinone: each 5 mL of crude PPO extract and 25 mL of methylene blue resulted in a decrease in colour content in the range of 63.02 to 86.61 %. These decolorizations of methylene blue with polyphenol oxidase were shown in figure 15 and 16.

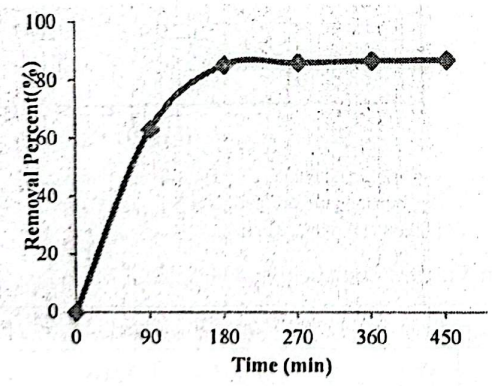


Fig. 15 Color removal percent with different time interval of polyphenol oxidase

Fig. 16 Color removal of methylene blue with polyphenol oxidase

Conclusion

The enzyme polyphenol oxidase plays a key role in plant defense system. In this research, the content of protein, ash, fat, moisture, fiber, and carbohydrate were found to be 1.85, 1.44, 1.68, 19.18, 6.17, and 69.68 % respectively. Vitamins C content was found to be 5.4 mg in apple. Ascorbic acid content depends on various stages of the fruit. From the above study, it may be deduced that fresh

apple is rich in nutritional values and vitamin C content.

The polyphenol oxidase was extracted from apple fruit using cold acetone and then crude enzyme powder 2 g /150 g was obtained. Polyphenol oxidase activity was well characterized by absorbance at 400 nm. The PPO activity was found to be 217.778 EU per gram of apple fruits. The optimum pH for polyphenol oxidase activity from apple fruit was found to be 6.8. These results indicated that PPO is unstable at acidic medium and relatively stable near neutral pH.The effect of optimum temperatures was found to be 30°C. This phenomenon may be associated with denaturation of the proteins at high temperatures, thus, lower temperatures favor protein (enzyme) activity.

The maximum velocity (V_{max}) and Michaelis-Menten Constant (K_m) values were obtained from the Lineweaver-Burk equation for apple fruit was found to be 1.176×10^{-4} $Mmin^{-1}$ and 2.077×10^{-2} M, respectively. The activation energy (E_a) of the polyphenol oxidase-catalyzed reaction was calculated to be 6.7176 kcal mol^{-1}. The reaction order of the polyphenol oxidase-catalyzed was determined by using linear regression and graphical method. The reaction order (n) for catalyzed reaction was calculated to be 0.9697 proving that the reaction order is first order.

The decolorization study was carried out for 1:30 hours and it resulted in a decrease in color of methylene blue content in the range of 63.02 to 86.61 %.

Acknowledgement

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References

Abdullah, T., Fawwaz A.L., and Absullah. M. (2016). "Decolorization of Methylene Blue and Malachite Green by Immobilized sp. Isolated with Polyphenol Oxidase". Environmental Science and Develoment, **7**, 95-100:

Baker, C.K., and Bakhuizen, R.C. (1963). "I Lord of Java", Auspieces of the Rukshervarium Leyen, **1**, 511-512 Netherlands:

Christense, H.N. (1974). "An Introduction to Biochemistry", English University Press Ltd., London:

Emis, Y. (2002). "Kinetic Studies with Crude Tomato Alcohol Dehydrogenase", Turk. J. Argic. Chem., **26**, 141-150:

Lineweaver, H., and Burk, D. (1934). "The Determination of Enzyme Sissociation Constants", J.Aim.Chem.Soc. **56**, 658-666:

Marín-Zamora, M.E., Rojas-Melgarejo, F., García-Cánovas, F., and García- Ruíz, P.A. (2005). "Cinnamic Ester of D-sorbitol for Immobilization of Mushroom Tyrosinase", Journal of Chemical Technology and Biotechnology, **80**, 1356-1364:

Oktay, M., Kufrevioglu, I., Kocacaliskan, I., and Sakiriroglu, H. (1995). "Polyphenol Oxidase from Amasya Apple", J Food Sci., **60**, 495-499:

Simpson, B.K. (2012). "Food Biochemistry and Food Biochemistry", 1st ed., A John Willey & Sons, Ltd. Publication., USA:

Valero, E., and Garicia-Carmona, F. (1998). "pH Dependent Effect of NaCl on Latent Grape Polyphenol Oxidase", J. Agric Food Chem., **46**, 2447-2451:

Phytoconstituents, Antioxidant Activity and Antitumor Activity of the Root of *Gentiana depressa* D. Don

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Abstract

In the present research, the root of *Gentiana depressa* D. Don was selected to investigate phytoconstituents, antioxidant and antitumor activities. By column chromatographic separation, four phytoconstituents: A (an aliphaticester),B(stigmasterol),C (a triterpenoid) and D (a flavanone) were isolated from EtOAc extract of *G. depressa*. The total phenolic contents (TPC) and antioxidant activity of ethyl acetate, ethanol and watery extracts of *G. depressa* were determined by Folin-Ciocalteu method and DPPH assay, respectively. The TPC was the highest in ethyl acetate extract (285.38 µg GAE/mg) but the lowest in watery extract. The highest antioxidant activity was found in ethyl acetate extract (IC_{50}: 2.89 µg/mL) whereas the lowest activity was recorded in watery extract. Among the isolated compounds, compound D (a flavonone) showed the highest antioxidant activity (IC_{50}: 6.55 µg/mL) but was lower than standard ascorbic acid. The antitumor activity of ethanol and watery extracts of *G. depressa* was screened by Potato Disc Assay method. Both extracts exhibited antitumor activity with the minimum concentration of 25 mg/mL in DMSO. A strong correlation was observed between antioxidant activity with TPC. The present research provided evidence that *G. depressa* could be a potential source of natural antioxidant and antitumor agent.

Keywords:*Gentiana depressa* D. Don, phytoconstituents, total phenolic content, antioxidant activity, antitumor activity

Introduction

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs have been reported to exhibit antioxidant activity and antitumor activity. Medicinal plants-derived natural antioxidants and antitumor agents are in the form of raw extracts or chemical constituents. Free radicals and other reactive oxygen species generated in human body by various endogenous systems cause oxidative stress. Oxidative stress can cause cellular and tissue damages, DNA mutation, cancer etc. (Rezaeian et al., 2015). The natural antioxidants are very efficient to block the process of oxidation.

Gentiana depressa D. Don (local name; Sao Kho) is belonging to the family Gentianaceae. Gentiana is the largest genus of Gentianaceae family with more than 400 species. It has been reported that Gentiana genus has interesting pharmacological and phytochemical properties. It has been used in traditional medicine and also has constituents in bitters and similar concoctions (Rosendal and Jan, 2002 and Prakash et al., 2017).

G. depressa is distributed in Sikkim of India, Bhutan, Nepal, Tibet, China and Northern Myanmar. It is spread on hill regions and growing at high altitudes of mountain ranges. It grows at 3000-4500 m altitude (Shabir et al., 2017 and Nampy et al., 2015).

Medicinal Uses of *Gentiana depressa*

The roots of *G. depressa* are known to be rich in medicinal values and nutritional values. *G. depressa* is used in traditional medicine in Asian countries in the treatments of liver disorders, anorexia, indigestion, malarial, fever, diarrhea, dysentery, laxative, inflammatory diseases and increases circulation (Lamichhane et al., 2014 and Yang et al., 2010). Gentiana species can inhibit tumor cell proliferation (Huang et al., 2013 and Matsukawa et al., 2016).

The main aim of the research work is to isolate some phytoconstituents and to screen some bioactivities such as *in vitro* antioxidant activity, *in vitro* antitumor activity and total phenolic contents of the root of *G. depressa*.

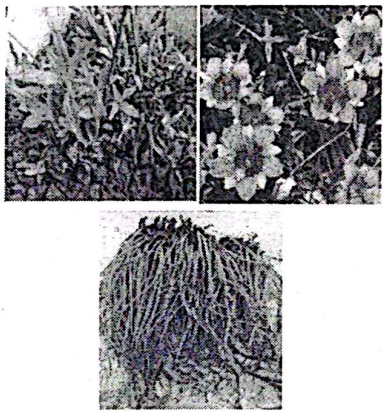


Figure 1 Photographs of the plants, flowers and roots of *Gentiana depressa* D. Don

Materials and Methods

Sampling of Plant Material and Identification

The roots of *Gentiana depressa* D. Don were collected from the mountain side of Pang War City, Chipwi Township in the Northern Kachin State of Myanmar. It was identified at the Department of Botany, Myitkyina University and then was air-dried at room temperature for two weeks and ground into powder and stored in air-tight containers.

Preparation of Various Crude Extracts

Dried powered sample of *G.depressa* (ca. 300 g) was percolated in 95 % EtOH (ca. 1 L) with occasional shaking for one week and filtered. This procedure was repeated three times. The combined filtrate was concentrated under vacuum evaporator to obtain EtOH crude extract. The crude extract was extracted with n- Hexane (ca. 500 mL) to obtain n-Hexane soluble fraction. The fraction was concentrated under vacuum evaporator to obtain n-Hexane crude extract. The defatted EtOH extract was partitioned between EtOAc and water. The EtOAc layer was concentrated by removing of solvent by using rotary evaporator to give EtOAc extract. In this way, n- Hexane extract and polar extracts (EtOAc, EtOH soluble and water soluble) from the root of *G.depressa* were obtained. The EtOAc crude extracts was kept for isolation of phytoorganic constituents.

Isolation of some Phytoorganic Constituents

About 3 g of EtOAc crude extract of the root of *G. depressa* was separated by column chromatography over silica gel adsorbent by eluting with(n-Hex: EtOAc, 40:1, 20:1 , 15:1, 10:1, 7:1, 5:1, 2:1, 1:1, 1:2, 1:3, 1:4 v/v and finally n-Hex:EtOAc: MeOH, 1:1:0.1 v/v). Successive fractions obtained were combined on the basic of their behaviour on TLC. Finally thirteen main fractions (F_1 to F_{13}) were collected. Fractions F_1, F_2, F_3, F_6, F_7, F_8, F_{10}, F_{11} and F_{13} were found as mixture.

Fraction F_4 was evaporated, washed with n-Hexane, and then n-Hex:EtOAc (40:1 v/v) and then recrystallized from MeOH, yielded 0.007 % (25 mg) of compound A as white amorphous. Fraction F_5 was evaporated, washed with n- Hexane, n-Hex: EtOAc (40:1 v/v) and then purified by recrystallization from MeOH, to give 0.013 % (50 mg) of compound B as colourless needle shaped crystals. After removal of the solvents, fraction F_9 provided white solid. The white solid were washed with n- Hexane, n-Hex:EtOAc (10:1 v/v) and then purified by recrystallization from MeOH, to give 0.011 % (40 mg) of compound C as white solid. Fraction F_{12} was evaporated, washed with n-Hexane, n-Hex: EtOAc (10:1 v/v) and then purified by recrystallization from MeOH, to give 0.005 % (20 mg) of compound D as yellow solid.

Characterization of Isolated Compounds

The isolated compounds were characterized by determination of their physical properties: melting point, R_f values, and some phytochemical reactions such as Liebermann-Burchard reagent, 5 % H_2SO_4, I_2 vapour, 5 % $FeCl_3$ and ethanolic hydroxylamine hydrchloride.

Identification of the Isolated Compounds

The isolated compounds were structurally identified by modern spectroscopic techniques such as UV-visible, FT IR and confirmed by comparison with the reported data and authentic compound.

Investigation of Some Bioactivities

Preparation of crude extracts by direct extraction method

Preparation of ethyl acetate extracts

The dried powdered sample (50 g) was extracted with 300 mL of EtOAc for one week and filtered. The filtrate was concentrated by removal of the solvent under reduced pressure to yield the respective ethyl acetate extract.

Preparation of 95 % ethanol extracts

The dried powdered sample (50 g) was extracted with 300 mL of 95 % EtOH in similar manner mentioned in above procedure to yield the respective ethanol extract.

Preparation of aqueous extracts

Watery extract of the dried powdered sample was prepared by boiling (50 g) of sample with 300 mL of distilled water for 6 h and filtered. The filtrate was concentrated by removal of water to give watery extract.

Each extracts were dried at normal pressure on a water bath and stored in refrigerator for screening some bioactivities.

(a) Determination of total phenol content of the root of *G. depressa* by Folin-Ciocalteu method

Total phenolic content (TPC) in EtOAc, 95 % EtOH and H_2O extracts of *G. depressa* were determined by Folin-Ciocalteu method (Rekha et al., 2012). The test sample solution (1 mg/mL) was prepared by dissolving 1 mg of respective crude extract in 0.5 mL each of distilled water and methanol. Each crude extract samples (0.5 mL) was added into 5 mL of 10 % FC reagent and incubated for 5 min at room temperature. To each tube, 4 mL of 1 M Na_2CO_3 was added and the tubes were kept at room temperature for 30 min and the absorbance of reaction mixture was read at 765 nm. Similarly, standard Gallic acid solutions with the concentration of (1000, 500, 250, 125, 62.5 and 31.25 µg/mL) were prepared and the absorbance of each reaction mixture was read at 765 nm. A standard curve was plotted by the absorbance against concentration of Gallic acid. The blank solution was prepared as above procedure by using distilled water instead of sample solution. Total phenolic content was estimated as µg Gallic acid equivalents per milligram (µg GAE/mg) of crude extract.

(b) Antioxidant Activity

The *in vitro* antioxidant activity of EtOAc, 95 % EtOH and H_2O extracts from the root of *G. depressa* as well as the isolated compounds was determined by DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay.

The sample solutions of the extracts were prepared by dissolving 2 mg of each extract in 10 mL of 95 % EtOH under vigorous shaking. The mixture solution was filtered and the stock solution was obtained. Desired concentrations (10.0, 5.0, 2.5, 1.25, 0.625 µg/mL) of extract sample solutions were prepared from this stock solution by serial dilution with appropriate amount of 95 % EtOH. Similarly, the sample solutions of isolated compounds were prepared in the concentrations of (60.0, 40.0, 20.0, 10.0, 5.0, 2.5 µg/mL). Ascorbic acid was used as standard in 0.625-10.0 µg/mL solutions. The control solution (1.5 mL of 60 µM DPPH in 1.5 mL of EtOH solvent), blank solution (1.5 mL of sample solution in 1.5 mL of EtOH) and test sample solution (1.5 mL of sample solution in 1.5 mL of 60 µM DPPH) were prepared and allowed to stand at room temperature for 30 min. After 30 min, the absorbances of these solutions were measured at 517 nm by using UV-1800 spectrophotometer. Absorbance measurements were done in triplicate for each solution and then mean values so obtained were used to calculate percent inhibition of oxidation by the following equation.

$$\% \text{ Oxidative Inhibition} = \frac{A_{DPPH} - (A_{Test\ sample} - A_{Blank})}{A_{DPPH}} \times 100$$

A_{DPPH} = absorbance of DPPH in 95 % EtOH solution

$A_{Test\ sample}$ = absorbance of (sample + DPPH) solution

A_{Blank} = absorbance of (sample+ 95 % EtOH) solution

(c) Antitumor Activity

The *in vitro* antitumor activity screening of 95% ethanol and watery extracts of *G.depressa* was carried on *Agrobacterium tumefacien* induced tumors on potato discs by Potato Crown Gall test or Potato Disc Assay method (Coker et al., 2003) at the Pharmaceutical Research Department, Ministry of Industry, Yangon, Myanmar.

Fresh, disease free potato tubers were used within 48 hours of transfer to the laboratory.

Tubers of moderate sizes were washed with water and surface- sterilized by immersion in 50 % sodium hypochlorite (Clorox) for 20 min. Both ends were removed and soaked for 10 min in another Clorox. A core of the tissue was extracted from each tuber by using surface- sterilized 1.0 cm wide cork borer. Then, 2 cm pieces were removed from each end and discarded. The remainder of the cylinder was cut into 0.5 cm thick discs with a surface- sterilized cutter. The discs were then transferred to 1.5 % agar plates (1.5 g of Difco agar was dissolved in 100 mL of distilled water, autoclaved and 20 mL poured into each petri dish). Each plate contained three discs. The procedure was done in the clean bench in the sterile room.

50 mg, 100 mg and 150 mg of each extract was separately dissolved in 2 mL of dimethyl sulphoxide (DMSO), i.e., the conentrations of each extracts at 25 mg mL^{-1}, 50 mg mL^{-1} and 75 mg mL^{-1} in DMSO were prepared. Each of the prepared solution was filtered through Millipole filters (0.22 μm) into a sterile tube. 0.5 mL of this solution was added to 1.5 mL of sterile distilled water and 2 mL of broth culture of *A. tumefaciens*strain (48 h culture containing $3\text{-}5\times10^{9}$cells/mL) were added aseptically. Controls were made in this way; 0.5 mL of DMSO and 1.5 mL of sterile distilled water were added to the tube containing 2 mL of broth culture of *A. tumefaciens* (from the same 48 h culture).

Using a sterile disposable pipette, 1 drop (0.05 mL) from these tubes was used to inoculate each potato disc, spreading it over the disc surface. The process of cutting the potatoes and incubation was conducted within 30 min. The plates were sealed with tape to minimize moisture loss and incubated at room temperature for one week. After incubation, the potato discs were stained with Lugol's solution (5 % I_2 and 10 % KI) for 30 min and the tumors were counted under a microscope and compared with control. The antitumor activity was examined by observation of tumor produced or not.

Results and Discussion

Identification of Isolated compounds

Compound-A (An aliphatic ester)

Compound A isolated as white amorphous in 0.007 % yield from EtOAc extract of the root of *G.depressa* has the melting point of 110-113°C. Its R_f value was found to be 0.41 in n-Hex: EtOAc (5:1 v/v) solvent system and no absorption was observed in readily accessible UV region. It gave pink spot on TLC chromatogram while spraying with 5 % H_2SO_4 followed by heating, a yellow spot with iodine vapour and a yellow spot while spraying with Liebermann-Burechard reagent and gave deep red colouration on testing with ethanolic hydroxylamine hydrochloride reagent which is the test for ester and so it could be classified as an ester.

FT IR : 2917 cm^{-1} and 2851 cm^{-1} (asym & sym. stretching vibration of $-CH_2$ and $-CH_3$), 1702 cm^{-1} (C=O stretching vibration of R-C(O)-O-R), 1459 cm^{-1} and 1369cm^{-1} (asymmetric and symmetric C-H bending vibration of $-CH_3$ group), 1287 $cm^{-1}$and 1072 cm^{-1} (C-C (=O)- O stretching vibration and O-C-C stretching vibration of ester), 963 cm^{-1} (=CH bending vibration), 752 cm^{-1}(C-H out of plane bending vitration of an alkene) (Silverstein, et al., 2005). All the results such as melting point, R_f value and chemical behavior on TLC, compound-A was classified as an aliphatic ester.

Compound-B (Stigmasterol)

Compound B was isolated as colourless needle shaped crystals in 0.013 % yield. Compound B gave brick-red spot on TLC chromatogram while spraying with 5 % H_2SO_4 followed by heating, a yellow spot with iodine vapour, green colouration when treated with Liebermann Burchard reagent and red colouration with Salkowski reaction. Therefore, compound B could be classified as a steroid. No absorption was observed in readily accessible UV region. The melting point of compound B (160-162 °C) was similar to that of authentic stigmasterol (160-164 °C). The R_f value of compound B was found at 0.50 in the solvent system of n-Hex:EtOAc (5:1v/v) and identical with that of authentic stigmasterol in any solvent system and they also give the same behavior on TLC. The Co-TLC of compound–B with authentic stigmasterol is shown in Figure 2.

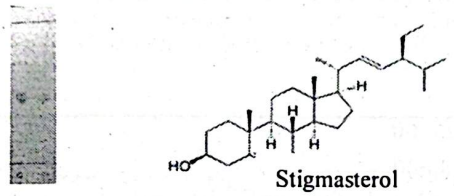
FT IR : 3393 cm^{-1} (stretching vibration of O-H group of alcohol), 3025 cm^{-1} (stretching vibration of =C-H of olefinic group), 2934 cm^{-1} and 2849 cm^{-1}(asy. and sym. C-H stretching vibration of $-CH_2$- and $-CH_3$ groups), 1667 cm^{-1} (C=C stretching vibration), 1463 cm^{-1} and 1377 cm^{-1}(asy. and sym. C-H bending vibration of CH_2 and CH_3), 1109 cm^{-1}(C-O stretching vibration of 2° alcohol), 1046 cm^{-1}(C-O stretching vibration of cyclic alcohol) (Silverstein, et al., 2005). The observed FT IR spectral data of compound B were also studied by comparing with those of reported stigmasterol (Jain and Bari, 2010) and are tabulated in Table 1.

Table 1. Comparison of FT IR Spectral Data of Isolated Compound-B and Stigmasterol*

Wave number (cm^{-1})		Band assignment
Compound-B	Stigmasterol*	
3339	3320	ν_{O-H} of alcoholic O-H
3025	3100-3010	$\nu_{=C-H}$ of olefinic group
2934,2849	2946,2854	ν_{C-H} (asym & sym) of $-CH_2$ and $-CH_3$ group
1667	1638	$\nu_{C=C}$ olefinic group
1463, 1377	1480, 1388	δ_{C-H} of (asym & sym) of $-CH_2$, $-CH_3$ group
1109	1189	ν_{C-O} of 2° alcohol
1046	1035	ν_{C-O} of cylic alcohol
957		$\delta_{=C-H}$ of trans-RCH=CHR' olefinic group
838, 800		$\delta_{=C-H}$ of RCH=CR'R" olefinic group

*Jain and Bari, 2010

From the physicochemical properties, melting point, R_f value and FT IR spectral data, isolated compound-B was identified as stigmasterol possessing the following molecular structure.



B Stigmasterol

Solvent system : Hex: EtOAc (5:1v/v)
Spraying agent : 5 % H_2SO_4, Δ
$R_f$value of compound -II : 0.5
R_f value of authentic stigmasteol : 0.5

Figure 2 Co- TLC of compound B with authentic stigmasterol

Compound –C (A triterpenoid)

Compound C isolated as white solid, 0.011 % yield, melting point: 240-243 °C, $R_f$value= 0.61 in n- Hex: EtOAc (1:1v/v) solvent system, gave purple spot on TLC chromatogram while spraying with 5 % H_2SO_4 followed by heating, a yellow spot with iodine vapour and purple spot with Lieberman-Burchard reagent. Thus, compound C was classified as a triterpenoid derivative. It is UV inactive.

FT IR: 3351 cm^{-1} (stretching vibration of O-H group of alcohol), 2931 cm^{-1} and 2866 cm^{-1} (asy. and sym. C-H stretching vibration of $-CH_2$- and $-CH_3$ groups), 1644 cm^{-1} (C=C stretching vibration), 1460 cm^{-1} and 1366 cm^{-1}(asy. and sym. C-H bending vibration of $-CH_2$ and $-CH_3$), 1126, 1036 cm^{-1} (C-O stretching vibration of 2° cyclic alcohol), 895 cm^{-1} (bending vibration of =CH) (Silverstein, et al., 2005). From the phytochemical colouring test and FT IR spectral data, the isolated compound C may be inferred as a triterpenoid derivative.

Compound D (A flavanone derivative)

Compound D was isolated as yellow solid, 0.005 % in yield, melting point:154-156 °C, R_f value= 0.59 in n-Hex:EtOAc:MeOH (1:1:0.1 V/V) solvent system. It was UV active and the absorbances λ_{max} at 234 nm, 282 nm and 347 nm indicated the presence of conjugated double bonds system (C=C-C=O). It gave brown spot on TLC chromatogram while spraying with 1 % $FeCl_3$ followed by heating, a yellow spot with iodine vapour and brown spot with 5 % H_2SO_4 and gave red colouration with Shinoda test. Thus, compound D was classified as a flavanone derivative.

FT IR: 3351 cm^{-1} (stretching vibration of O-H), 3046 cm^{-1} (C-H stretching vibration of aromatic ring), 2923 cm^{-1} and 2855 cm^{-1} (asy. and sym. C-H stretching vibration of $-CH_2$ and $-CH_3$ groups), 1702 cm^{-1} (C=O stretching vibration of carbonyl group), 1587 cm^{-1}, 1510 cm^{-1} and 1449 cm^{-1} (C=C ring skeletal stretching vibration of aromatic ring) 1377 cm^{-1} (asy. and sym. C-H bending vibration of $-CH_3$ group), 1213 cm^{-1} (O-H stretching vibration of phenolic –OH), 1161 cm^{-1} (=C-O-C stretching vibration of Ar-O-R), 968 $cm^{-1}$and 827 cm^{-1}(C-H out of plane bending vibration of Ar ring)(Silverstein, et al., 2005). All of above results indicated that compound D may be a flavanone.

Total Phenolic Content of Various Crude Extract of *G. depressa* by Folin-Ciocalteu (FC) Method

The total phenolic content in EtOAc, EtOH and watery extracts of *G. depressa* were estimated by Folin-Ciocalteu method. Gallic acid was used to construct standard calibration curve for total phenol. Total phenolic content (TPC) was expressed as microgram of Gallic acid equivalent per milligram of crude extract (µg GAE/mg). Standard calibration curve of Gallic acid was prepared by measuring the absorbance of various amount of Gallic acid in the range of 1000 to 31.25 µg/ml. As shown in Table 2 and Figure 3, the prepared Gallic acid standard curve gave a straight line, which obeyed Beer's Lambert law, with the linear regressive equation of $y = 0.0019x + 0.0576$, $R^2 = 0.9999$. From the standard curve it was found that with the increase in concentration of Gallic acid, the complexation would also increase and as a result increase in the absorbance is observed.

The results of total phenolic contents in the various crude extracts of *G. depressa* were presented in Table 3. From the result, TPC in EtOAc extract was found to be the highest (285.38 µg GAE/mg) followed by EtOH extract (213.34 µg GAE/mg) and the lowest TPC was recorded in watery extracts (83.58 µg GAE/mg).

Table 2. The Absorbance of Gallic Acid Standard Solution at λ_{max}765 nm

Concentration (µg/mL)	Absorbance at λ_{max} 765 nm
31.25	0.12
62.5	0.18
125	0.30
250	0.54
500	1.01
1000	1.99

Figure 3. A plot of Gallic acid standard curve

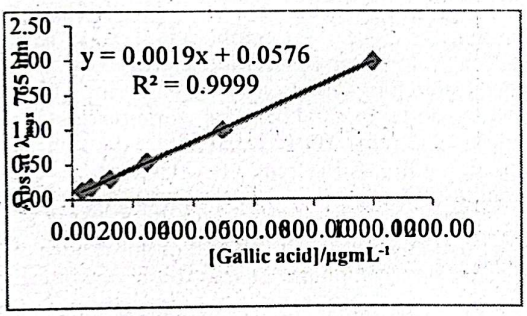


Table 3. Total Phenolic Content (TPC) of EtOAc, 95 % EtOH and watery extracts of the root of *G. depressa*

Samples	TPC (µgGAE/mg±SD)
EtOAc extract	285.38± 0.45
EtOH extract	213.34 ± 1.65
watery extract	83.58± 2.13

The antioxidant activity was expressed as 50 % oxidative inhibitory concentration (IC_{50}). The percent oxidative inhibition and IC_{50} values of all crude extracts and isolated compounds of *G.depressa* are summarized in Tables 4 and 5, respectively. Plots of percent oxidative inhibition Vs concentration of various crude extracts and isolated compounds of *G.depressa* were illustrate in Figure 4 and 5. From the results, the highest antioxidant activity was found in EtOAc extract of *G. depressa* (IC_{50}: 2.89 µg/mL) which was comparable to standard ascorbic acid (IC_{50}: 1.77 µg/mL) and the lowest activity in watery extract (IC_{50}: 8.99 µg/mL). The results suggest that the phenolic compounds contribute significantly to the antioxidant capacity of *G. depressa*.

Among the isolated compounds, compound D (a flavanone) showed the highest antioxidant activity (IC_{50}: 6.55µg/mL). All the isolated compounds exhibit lower antioxidant activity than standard ascorbic acid .

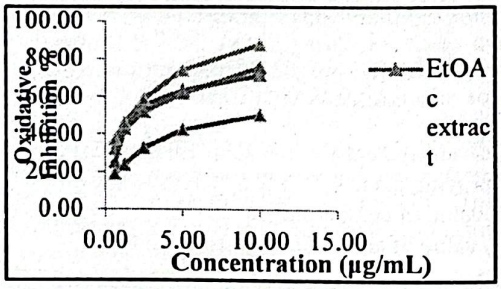


Figure 4 Plot of oxidative inhibition percent Vs concentration (µg/mL) of different crude extracts of *G. depressa* and standard ascorbic acid

Table 4. Percent Oxidative Inhibition and IC_{50} Values of Different Crude Extracts of *G.depressa* and Standard Ascorbic Acid

Extracts	Oxidative Inhibition (%) (mean ± SD) in different of Concentration (µg/mL)					IC_{50} (µg/mL)
	0.625	1.25	2.50	5.00	10.00	
EtOAc extract	32.12±1.06	45.44±2.62	55.31±0.23	63.28±2.95	71.43±1.01	2.89
EtOH extract	25.01±1.39	42.67±0.52	51.88±1.22	61.65±0.01	75.61±0.67	3.58
Watery extract	18.97±0.70	23.43±1.49	32.54±0.06	42.34±1.37	50.21±1.20	8.99
Ascorbic Acid	37.56±0.57	45.98±0.87	58.54±1.63	74.12±1.48	87.91±1.41	1.77

Table 5. Percent Oxidative Inhibition and IC_{50} Values of Isolated Compounds

Compounds	Oxidative Inhibition %(mean ± SD) in different conc: (µg/mL)							IC_{50} (µg/mL)
	0.625	1.25	2.50	5.00	10.00	20.00	40.00	
Compound A	19.55±1.01	24.57±1.97	28.53±1.06	34.68±1.06	38.33±0.06	45.15±0.78	50.36±0.97	33.79
Compound B	22.23±0.76	27.89±1.43	33.21±1.11	38.87±1.76	42.12±0.06	46.23±1.86	51.15±1.89	32.15
Compound C	23.44±1.45	27.56±2.01	34.12±1.06	39.09±1.78	44.23±1.16	48.81±1.98	52.23±1.56	29.48
Compound D	29.89±0.89	36.46±0.47	43.34±1.31	49.63±1.45	56.44±1.23	63.37±1.56	68.71±1.32	6.55

Figure 5Plot of oxidative inhibition percent Vs concentration (µg/mL) of isolated compounds from the roots of *G.depressa*

Antitumor Activity

The *in vitro* antitumor activity screening of different crude extracts viz., 95 % ethanol and watery extracts of *G.depressa* was carried out against *Agrobacterium tumefacien* induced tumors on potato discs by Potato Crown Gall test or Potato Disc Assay method. Crown gall is a neoplastic disease of plants characterized by the transformation of normal plant cells into autonomous tumor cell in a short period of time (Galsky et al., 1980). The causative agent of this disease is the Gram-negative bacterium *A. tumefaciens*. The ethanol and watery extracts of the root of *G. depressa* were tested for antitumorigenic activity against *A. tumefaciens* induced tumors on potato discs and antitumor activity was observed. The photographs illustrating the different concentration of plant crude extracts against *Agrobacterium tumefacien* are presented in Figure 6 and 7 and the observed antitumor activity of different crude extracts of *G. depressa* are summarized in Table 6. Both the 95 % ethanol and watery extracts of *G. depressa* exhibited antitumor activity against *Agrobacterium tumefaciens* induced tumors on potato discs with the minimum concentration of 25 mg mL^{-1} in DMSO at the observation period of day- 5 and day- 7.

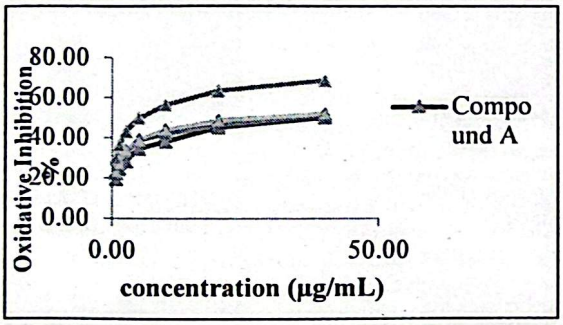


Table 6 Antitumor Activity of Different Crude Extracts of the Root of *G. depressa*

Samples	Concentration of extracts (mg mL^{-1}) in DMSO	Observation of tumor at	
		Day-5	Day-7
EtOH extract of *G. depressa*	25	-	-
	50	-	-
	75	-	-
Watery extract of *G. depressa*	25	-	-
	50	-	-
	75	-	-
Control(0.5 mL DMSO + 1.5 mL DW)	-	+	+

(-) = tumor negative
(+) = tumor positive
DMSO = dimethyl sulfoxide
DW = distilled water

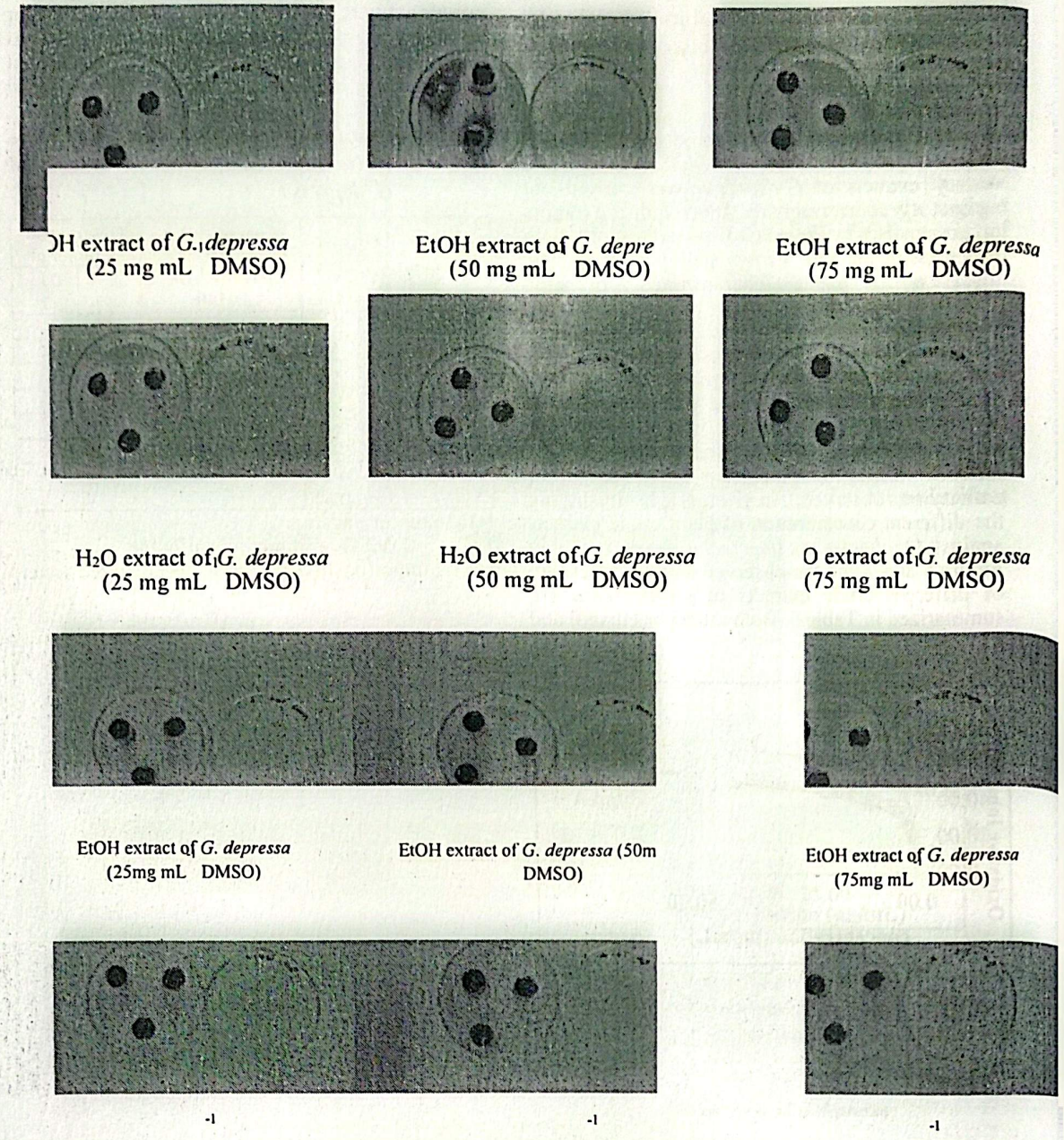


Figure 6. Photographs of observation of antitumor activity of different concentrations of EtOH and H_2O extracts of *G.depressa* on day-5

Figure 7 Photographs of observation of antitumor activity of different concentrations of EtOH and H_2O extracts of *G.depressa* on day-7

Conclusion

Plants belonging to genus *Gentiana* are very well-known for their pharmacological activities and reported as a potential source of antimicrobial, antioxidant, anti-inflammatory, and antitumor since long time.

The present study further confirmed the antioxidant and antitumor activities of *Gentiana depressa*. In the *in vitro* antioxidant activity screening of EtOAc, 95 % EtOH and watery extract of the root of *G. depressa* by DPPH free radical scavenging assay, EtOAc extracts was found to have the highest antioxidant activity (IC_{50} = 2.89 µg/mL) which was comparable to standard ascorbic acid (IC_{50} = 1.77 µg/mL). Among the isolated compounds, Compound–D (a flavanone) showed the highest antioxidant activity (IC_{50}: 6.55 µg/mL).

Total phenolic contents in EtOAc, EtOH and watery extracts of the root of *G. depressa* were screened by Folin-Ciocalteu Method. EtOAc extract of *G. depressa* was found to have the highest total phenolic content and the lowest TPC was found in watery extract. Correlation between total phenol content and antioxidant activity was very high suggesting that phenolic compounds are the most responsible compounds contributing to antioxidant activity.

The antitumor activity of 95 % EtOH and watery extracts of *G.depressa* was screened on *Agrobacterium tumefacien* by Potato Disc Assay Method. Both extracts exhibited antitumor activity against *A. tumefacien* induced tumors on potato discs with the minimum concentration of 25 mg/mL in DMSO, suggesting that the extracts may have potential to be further developed into therapeutic treatments.

Due to its high antioxidant activity, antitumor activity and high total phenolic content, *G.depressa* may be used to prevent the diseases related to oxidative stress such as cardiovascular disease, neurodegenerative diseases, diabetes, and rheumatism and also as an anticancer agent.

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References

Coker, P. S., Radecke, J., Guy, C. and Camper, N. D. (2003). "Potato Tumor Induction Assay: A Multiple Mode of Action Drug", *Phytomedicine*, **10**, 133-138

Galsky, A. G., Wilsey, J. P., Powell, R. G. (1980). "Crown Gall Tumor Disc Bioassay", *Plant Physiology*, **65**,184-185

Huang, S. H., Chen, E. C. F., Wu, C. T., Kho, C. L. and Tsay, H. S. (2013). "Comparative analysis among three Taiwan-specific Gentiana species and Chinese medicinal plant *Gentiana Scabra*," *Botanical Studies*, **54**(54); 1-7

Jain, P.S. and Bari, S.B. (2010). "Isolation of lupeol, stigmasterol and campesterol from petroleum ether of woody stem of *Wrightia Tinctoria*". *Asian Journal of Plant Sciences*, **9** (3), 163-167

Lamichhane, J., Chhetri, S.B., Bhandari, M., Pokhrel, S., Pokharel, A. and Sohng, J.K. (2014). "Ethnopharmacological survey, Phytochemical screening and antibacterial activity measurements of high altitude medicinal plants of Nepal; A bioprospecting approach," *Indian Journal of Traditional knowledge*, **13**(3), 496-507

Matsukawa, K., Ogata, M., Hikage, T., Minami, H., Shimotai, Y., Saitoh, Y., Yamashita, T., Ouchi, A., Tsutsumi, R., Fujioka, T., and Tsutsumi, K. I. (2006) "Antiproliferative Activity of Root Extract from Gentiana Plant (Gentiana triflora) on Cultured and Implanted Tumor Cells," *Biosci. Biotechnol. Biochem*, **70**(4); 1046-1048

Nampy, S., Shahina, P. M., Haseena T. and Ashwini H. S. (2015). "A taxanomic revision of *Swertia* L. (Gentianaceae) in Sourth India, with one new species and seven lectotypi fications," *Phytotaxa*, **195**(1); 031-052

Prakash, O., Singh, R., Kumar, S., Srivastava, S. and Ved, A. (2017). "*Gentiana Lutea Linn.* (Yellow Gentia); A comprehensive review," *Journal of Ayurvedic and Herbal Medicine*, **3**(3); 175-181

Rekha, C., and Poornima, M. (2012). "Ascorbic acid, total phenolic content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits". *International Journal of Pharmaceutical Research*, **1** (2), 303-310

Rezaeian, S., Pourianfar, H. R. and Janpoor, J. (2015). "Antioxidant properties of Several Medicinal Plants Growing Wild in Northeasten Iran", *Asia Journal of Plant Science and Research*, **5**(2): 63-68

Rosendal, J. S and Jan, S. (2002). "Chemotaxonomy and pharmacology of Gentianaceae," *Gentianaceae Systematics and Natural History*, **6**; 573-631

Shabir, M., Agnihotri, P., Husain, D., Tiwari, J. K. and Husa, T. (2017). "on the current status of the genus *Gentiana* L. (Gentianaceae) in India," *Pleione*, **11**(1); 16-24

Silverstein, R. M., F. X. Webster and D. J. Kiemle. (2005). Spectrometric Identification of Organic Compounds, 7th Ed, New York; John Willey & Son, INC., 72-126

Yang, J. L, Liu, L. L. and Shi, Y. P. (2010). "Phytochemicals and Biological Activities of Gentiana Species," *Natural Product Communications*, **5**(4); 649-664

Determination of Nutritional Values of Cat Food Based on Waste Fishes-Head and Fruits-Peel

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Abstract

The aim of this research was to prepare cat food based on the waste of fishes and fruits and to determine the nutritional values. Two types of fishes, *Stoleo phorus indicus* (van Hasselt, 1823) in myanmar (Nga-nitu) and *Coilia reynaldi* (Valenciennes,1848) (Mhee-tan-thwe) and two nutritive fruits, *Musa acuminata* L. (Maldaw-lat-hnyo) and *Citrus sinensis* L. (Lein-maw) were selected for the preparation of cat food. Then their waste heads and peels were dried and ground to get the powder samples. To examine the relative proportions of mineral constituents, Energy Dispersive X-ray Fluorescence (EDXRF) method was applied. Applied two ratios in the preparation of cat food based on natural resources (fishes-head: banana peel: orange peel: rice flour (or) wheat flour (or) tagu flour were (60 : 10 : 10 : 20) g and (40 : 10 : 10 : 40) g respectively. Examinations of nutritional values and determination of morphology structures by Scanning Electron Microscopic (SEM) method of the resulting cat food were carried out and compared the effectiness of the different compositions in this work. Cat food with effective nutritional values was prepared from the waste of animals and fruits for the local market successfully.

Keywords: nutritional values, Energy Dispersive X-ray Fluorescence (EDXRF), Scanning Electron Microscopic (SEM)

Introduction

The AAFCO Dog and Cat Food Nutrient Profiles have been criticized and faulted for not explicitly indicating the apparent nutrient digestibility, sometimes called nutrient availability or bioavailability, required to make the listed concentrations adequate for meeting the animal's daily requirements. When a minimum requirement has been established for a particular nutrient, the expected apparent digestibility to meet the minimum requirement for that nutrient at the recommended concentration listed in an AAFCO Nutrient Profile can be calculated using the formula: ((minimum requirement) x (its apparent digestibility in the diet(s) used to establish the minimum requirement) / (recommended concentration in the AAFCO Profile)) x 100.

In the above formula, the minimum requirement is expressed in the same units as in the AAFCO Nutrient Profile and digestibility is expressed in decimal equivalents. As an example, the NRC lists the minimum crude protein requirement for puppies to be met by formulas containing 18% crude protein on a dry matter basis with the digestibility of the protein sources estimated to be near 100%. The 2014 AAFCO Dog Food Nutrient Profile for Growth and Reproduction recommends the minimum crude protein concentration of dry matter to be 22.5%. Therefore, the expected apparent digestibility for crude protein in a diet formulated to meet the AAFCO Dog Food Nutrient Profile for Growth and Reproduction is at least 80% [(18 x (1.00)/22.5) x 100].

Therefore, when the energy density of the dog or cat food exceeds 4000 kcal ME/kg DM the nutrient concentrations should be corrected for caloric content before valid comparisons to the appropriate AAFCO Nutrient Profile are made.

Energy = (3.5 x g Crude Protein) + (8.5 x g Crude Fat) + (3.5 x g Nitrogen Free Extractb (CHO)) = (3.5 x 90) + (8.5 x 70) + (3.5 x 60) = 1120

% Nitrogen Free Extract = 100- (% Crude Protein + % Crude Fat + % Crude Fiber + % Moisture + % Ash)\

Energy = (3.5 x 90) + (8.5 x 70) + (3.5 x 60) = 1120

Cats are obligate carnivores and are metabolically designed to consume diets with the general composition: 1) animal (not plant)-based protein (>50% calories) 2) moderate fat (~20-40% calories) 3) very low carbohydrate (1-2% calories) 4) water-rich (~70%).

Keep this in mind when choosing food for cats but also understands that pet food ingredients are proof margin-driven. Protein is expensive. Fat and carbohydrate sources are cheap. This fact often results in the production of high fat/high carb/low protein diets as you will notice below. High-fat meat trimmings from the human market, not lean muscle meat, are what make their way into the pet food chain, and grains are less expensive than meat.

Fish: I do not favor feeding fish-based diets for several reasons: 1) allergy potential, 2) toxins/mercury levels, 3) PBDE levels (fire retardant chemicals with thyroid-disrupting properties), 4) often high in phosphorus and magnesium, 5) significant addiction issues - the cat will not eat anything else.

Composition: The caloric distribution of protein, fat, carbohydrate content of a diet. 100% of a diet's calories come from protein, fat, and carbohydrate. Therefore: % protein calories + % fat calories + % carbohydrate calories = 100% .This means that if, for example, a diet is appropriately low in carbohydrates, the diet will be higher in protein, fat, or both. It is like a three-way teeter totter - if one fraction goes up or down, one, or both, of the other two must increase or decrease accordingly to add up to 100%.

Carbohydrates: This is an important area of consideration – especially for diabetic patients. I often see diabetic cat caregivers taking this value far too literally…assuming that 6% vs 9% is statistically significant when it is not. Unfortunately, this macronutrient is not actually measured in a lab. Instead, it is calculated by subtraction. In other words, we add up the moisture + protein + fat + fiber + ash and subtract that value from 100% leaving us with the carbohydrate fraction. However, any error in those Big Five values will result in an error (usually an overage) in the carbohydrate value. Vitamins are organic compounds that take part in a wide range of metabolic activities. Vitamin deficiencies can cause a variety of health problems.

Even a value of 10% carbs could actually be closer to 0% carbs. Therefore, stick with the directive to stay below 10% carbohydrates but do not get caught up in whether a diet says 10%...or 5%....or 0% because these numbers are just not that accurate. Biological value of protein: Animal-based protein has a much higher biological value when compared to plant-based protein. Grains supply both protein and carbohydrate. Therefore, high carb diets often have much of their protein

coming from plants. Consider not only the amount of protein in a diet but also the source of the protein. Diets using whole grains or glutens (gluten = plant-based protein) have a portion of their protein coming from low biological value sources.

As carnivorous animals, cats derive most of their protein from meat, fish, and other animal products. Some animal-based protein is easier to than plant-based protein and is better suited to the cat's digestive system. Dietary protein contains 10 specific amino acids that neither cats nor dogs can make on their own. Known as essential amino acids, they provide the building blocks for many important biologically active compounds and proteins. In addition, they provide the carbon chains needed to make glucose for energy. High-quality proteins have a good balance of all of the essential amino acids.

Dietary fats, mainly derived from animal fats and the seed oils of various plants, provide the most concentrated source of energy in the diet. Fats contain more than twice much energy as protein and carbohydrates per gram. Dietary fats supply essential fatty acids that cannot be synthesized in the body and serve as carriers for important fat-soluble vitamins. Fatty acids play an important role in cell structure and function. Additionally, food fats tend to enhance the taste and texture of a cat's food. The maximum amount of fat in the cat's diet can be reasonably high without any known adverse effects. In many cat foods, 50% or more of the energy comes from fat. Studies indicate that cat foods containing even higher amounts of fat are safe. At a minimum, cat foods should have a fat content of about 9% of dry matter. Cats need a certain amount of energy to sustain the normal activities of their daily lives. Growth, pregnancy, lactation, and exercise all increase these normal energy requirements. Generally measured in terms of calories, energy comes from three major dietary components: carbohydrates, protein, and fats. While not essential in the diet, carbohydrates provide an abundant source of energy. The major sources of carbohydrates in commercial cat foods are cereals, legumes, and other plant foodstuffs. Because cats are carnivores, the short length of their long intestines limits their ability to ferment fibers that are found in many carbohydrates. Before weaning, kittens need 20–25 Calories for every 100 grams of body weight. Cat owners should start supplemental feedings from 2.5 to 4 weeks after birth, because mother's milk is no longer sufficient.In this research, nutritive cat food was prepared by applying fish heads, fruit peels and three kinds of flours not only as the source of carbohydrate but also as the additive and varying their composition ratios. Nutritional values, relative mineral constituents and the micro porous structures of the resulting food pallets were examined.

Botanical Description

Botanical Description of Coilia Fish

Myanmar name - Nga-nitu
Botanical name - *Stoleo phorus indicus* (van Hasselt, 1823)
English name - Coilia fish
Family name - Engrauli
Part used - Head fish

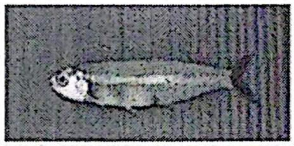

Figure 1. *Stoleo phorus indicus*

Botanical Description of Reynald's grenadier anchovy

Myanmar name - Mhee-tan-thwe
Botanical name - *Coilia reynaldi* (Valenciennes,1848)
English name - Reynald's grenadier anchovy fish
Family name - Engraulidae
Part used - Head fish

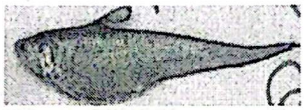

Figure 2. *Stoleo phorus indicus*

Botanical Description of banana

Myanmar name - Maldaw lat hnyo
Botanical name - *Musa acuminate*
English name - banana
Family name - Musaceae
Part used - Banana

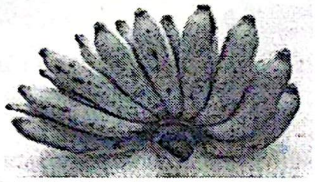

Figure 3. Fruits of *Musa acuminate*

Botanical Description of Orange

Myanmar name - Lein maw
Botanical name - *Musa acuminate*
English name - Orange fruit
Family name - Musaceae
Part used - Orange peel

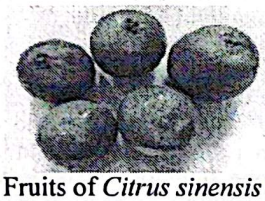

Figure 4. Fruits of *Citrus sinensis* L.

Botanical Description of Rice

Myanmar name - Manaw Hary
Botanical name - *Oryza sativa* L.
English name - Rice
Part used - Rice fluor

Figure 5. Rice of *Oryza sativa L*

Botanical Description of Wheat

Myanmar name - Jone
Botanical name - *Triticum* L.
English name - Wheat

Part Used - Wheat fluor

Figure 6. Wheat of *Triticum L*

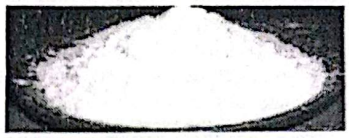

Figure 7. Tapioca of *Manihot esculenta*

Botanical Description of Tapioca
Myanmar name - Tagu
Botanical name - *Manihot esculenta*
English name - Tapioca
Part used - Tapioca fluor

Materials and Methods

Sampling of Raw Materials

Raw materials used in this research were collected from Myoma Market, Myitkyina, Kachin state. *Musa acuminata* (Maldaw lat hnyo) and *Citrus sinensis* L. (Lein maw) fruits peels were air dried in the oven at 60°C for 3 days and fish heads of *Stoleo phorus indicus* and *Coilia reynaldi* were air dried for 2 days and grains of *Oryza sativa* L. (rice), *Triticum* L.(wheat) and *Manihot esculenta* (tagu) were air dried for 1 day at 60°C. Then they were made to powder by using grinding machine. All dried powders were stored in air-tight container to prevent moisture changes and contamination and used in the preparation and analysis of cat food.

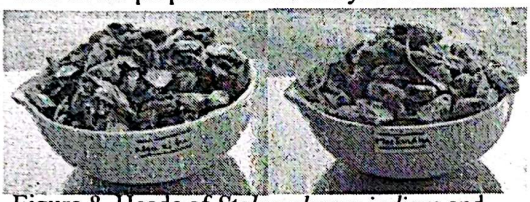

Figure 8. Heads of *Stoleo phorus indicus* and *Coilia reynald*

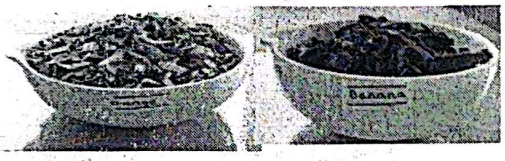

Figure 9. Peels of *Musa acuminate* and *Citrus sinensis* L.

Examination of Mineral Constituents

Determinations of the relative amount of mineral constituents present in all powdered samples were subjected at the department of Chemistry, Taung gyi University.

Preparation of Cat food

Dried fish heads powders of Nga-nitu and Mhee-tan-thwe were dried and stirred in the pan heated at 100°C sand for 5 mins and then added powder of fruit peels of banana and orange and finally added one flour out of three kinds (rice, wheat and targu) with continuous stirring. Then mixture constituents were removed from the furnace and poured into a container. 100 mL of distilled water was added into the mixture and mixed thoroughly. After mixing, the resulting paste was precast in the mold and dried in the oven at 60°C for one day to remove the moisture. Used ratio in the preparation was (60:10:10:20) g of the weight ratios of (Nga-nitu or Mhee-tan-thwe : Banana : Orange : Flour). By using flour at one time out of three kinds, there were obtained three formula ratios for each Nga-nitu and Mhee-tan-thwe and then totally 6 cat food samples were prepared.

Determination of Some Physical Properties of Cat Food Samples

Determination of moisture and ash contents of six prepared cat foods were performed by oven dried method (Oven, Model 10 Lab) and muffle furnace and their properties were shown in the Tables, 1, 3, 5, 7, 9 and 11respectively.

Examination of Nutritional Values of Cat Food Samples

Cat food samples obtained were measured their nutritional value such as protein, fat, fiber, carbohydrate and energy values at the Small Scale Industries Department, Ministry of Agriculture, Livestock and Irrigation, Yangon and the results were listed in the Tables, 2, 4, 6, 8, 10 and 12 respectively.

Determination of Morphological Structures of Cat Food Samples

Morphological structures of cat food samples were recorded at University Research Center, Yangon and the micrograms obtained were informed in the Figures 16 and 17.

Results and Discussion

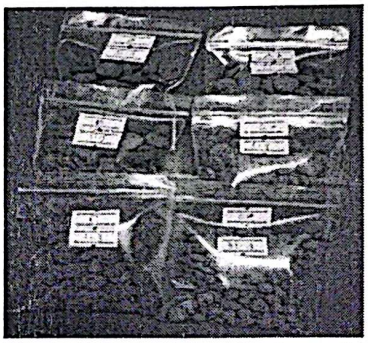

Figure 7. Prepared cat food samples

Cats foods prepared by the same ratio (60: 10: 10: 20) but with three kinds of flour were observed in fig. 7.

Table 1. Physical Properties of Cat Food Sample A

No.	Experiment	Present Chemical Analysis Results
1	Moisture (%)	9.04
2	Ash (%)	21.76

Table 2. Nutritional Values of Cat Food Sample A

No.	Experiment	Present Chemical Analysis Results
1	Protein (%)	54.31
2	Fat (%)	6.37
3	Fiber (%)	2.31
4	C/H (k cal)	6.21
5	Energy (k cal)	299.41

Table 3. Physical Properties of Cat Food Sample B

No.	Experiment	Present Chemical Analysis
1	Moisture (%)	6.84
2	Ash (%)	21.84

Table 4. Nutritional Values of Cat Food Sample B

No.	Experiment	Present Chemical Analysis Results
1	Protein (%)	54.48
2	Fat (%)	6.77
3	Fiber (%)	0.20
4	C/H (k cal)	9.87
5	Energy (k cal)	318.33

Table 5. Physical Properties of Cat Food Sample C

No.	Experiment	Present Chemical Analysis Results
1	Moisture (%)	6.48
2	Ash (%)	21.80

Table 6. Nutritional Values of Cat Food Sample

No.	Experiment	Present Chemical Analysis Results
1	Protein (%)	57.22
2	Fat (%)	6.09
3	Fiber (%)	3.51
4	C/H (k cal)	4.90
5	Energy (k cal)	303.29

Table 7. Physical Properties of Cat Food Sample A*

No.	Experiment	Present Chemical Analysis Results
1	Moisture (%)	7.72
2	Ash (%)	26.05

Table 8. Nutritional Values of Cat Food Sample A*

No.	Experiment	Present Chemical Analysis Results
1	Protein (%)	45.63
2	Fat (%)	6.27
3	Fiber (%)	2.61
4	C/H (k cal)	11.72
5	Energy (k cal)	285.83

Table 9. Physical Properties of Cat Food Sample B*

No.	Experiment	Present Chemical Analysis Results
1	Moisture (%)	5.71
2	Ash (%)	27.93

Table 10. Nutritional Values of Cat Food Sample B*

No.	Experiment	Present Chemical Analysis Results
1	Protein (%)	46.33
2	Fat (%)	5.98
3	Fiber (%)	2.16
4	C/H (k cal)	11.89
5	Energy (k cal)	286.70

Table 11. Physical Properties of Cat Food Sample C*

No.	Experiment	Present Chemical Analysis Results
1	Moisture (%)	5.24
2	Ash (%)	25.10

Table 12. Nutritional Values of Cat Food Sample C*

No.	Experiment	Present Chemical Analysis Results
1	Protein (%)	52.48
2	Fat (%)	5.76
3	Fiber (%)	0.36
4	C/H (k cal)	11.06
5	Energy (k cal)	306.00

All cat food samples range moisture (%) from 5.24% to 9.04%. The lowest ash (%) was given by sample A and the highest value was observed at sample B*. The resulting protein percentage of sample A, B and C were 54.31, 54.48 and 57.22 and sample A*, B* and C* were 45.63, 46.33 and 52.48 respectively. 50% or more of energy is come from fat and cat food should have minimum concentration of fat about 9% of dry matter. Cat's meal should not have more than 10% fibre. Much fibre in the diet can decrease the digestibility of other important nutrients. The lowest value of fibre present in sample B (0.2%) and the highest value can be found in sample C (3.51%). Generally measured in terms of calories, energy comes from three major dietary components: carbohydrates, protein, and fats. The daily requirement energy in cat has about 280-360 kilocalories. While not essential in the diet, carbohydrate provides an abundant source of energy.

Nga-nitu based cat food samples A, B and C showed their fat percentages as 6.37, 6.77 and 6.09. Mhee-tan-thwe based cat food samples were belonging to 6.27, 5.98 and 5.76 fat percentage. The cat's intestinal tract, including a relatively small colon and nonfunctional cecum, can not utilize dietary fibers like other animals. The diet of cat should be reasonable amount because much fiber in cat food can decrease the digestibility of other important nutrients.

Table 13. Relative Mineral Constituents of Flour Samples

No	Components	RF	WF	TF
1	Mn	74.0	76.3	0.007
2	Dy	7.16	12.1	ND
3	Hf	7.07	ND	ND
4	Ta	5.76	8.01	ND
5	Pt	1.53	ND	ND
6	Co	1.13	ND	ND
7	Zn	1.06	0.049	0.005
8	Br	0.563	0.613	ND
9	K	0.520	0.596	16.0
10	P	0.479	0.416	0.069
11	S	0.379	0.487	0.011
12	Cl	0.114	0.200	0.009
13	Ca	0.101	0.156	83.1
14	Al	0.042	0.045	ND
15	Si	0.039	0.022	0.009
16	Sn	0.008	ND	ND
17	Cu	ND	0.442	0.004
18	Rb	ND	0.001	ND
19	Fe	ND	ND	0.013
20	Ti	ND	ND	0.750
21	Sr	ND	0.062	0.002
22	W	ND	ND	0.004

RF- Rice flour
WF- Wheat flour
TF- Tagu flour
ND- Not detected.

Essential nutrients for cats are twelve minerals and they are calcium(Ca), phosphorus(P), magnesium(Mg), sodium(Na), potassium(K), chloride(Cl), iron(Fe), copper(Cu), zinc(Zn), manganese(Mn), selenium(Se) and iodine(I). It was found that all raw materials contained six essential minerals such as Ca, Cl, S, P, K and Zn.

Table 14. Relative Mineral Constituents of Used Banana and Orange

No.	Component	BNN	ORG
1	K	94.7	5.43
2	Ca	3.76	3.86
3	Cl	0.495	0.055
4	Si	0.282	0.054

5	P	0.202	0.330
6	Mg	0.155	ND
7	S	0.092	0.235

No.	Component	BNN	ORG
8	Fe	0.078	78.6
9	Al	0.064	0.059
10	Mn	0.031	0.188
11	Zn	0.029	0.476
12	Ti	0.024	ND
13	Sr	0.009	1.33
14	Rb	0.008	0.382
15	Dy	0.005	6.44
16	Cu	0.004	0.159
17	Br	0.004	0.032
18	Ta	0.003	0.562
19	Cr	0.003	ND
20	W	ND	ND
21	Hf	ND	1.74
22	Ni	ND	0.029

BNN = banana flour ORG = orange flourTable 15. Relative Mineral Constituents of Nga-nitu (NNT) and Mhee-tan-thwe (MTH)

No.	Component	NNT	MTH
1	Ca	81.6	84.9
2	K	10.8	6.43
3	Na	3.291	3.37
4	Cl	1.15	1.78
5	P	1.35	1.48
6	S	0.742	0.623
7	Fe	0.134	0.438
8	Si	0.059	0.212
9	Mg	0.189	0.196
10	Sr	0.069	0.183
11	Al	0.088	0.148
12	Zn	0.087	0.089
13	Ti	0.016	0.084
14	Br	0.034	0.036
15	Mn	0.041	0.023
16	Dy	0.011	0.012
17	Cu	0.004	0.004
18	Rb	ND	0.003
19	Au	0.002	0.003
20	Pb	ND	0.002
21	Cr	0.007	ND
22	V	0.005	ND

Morphological Structures of Cat Food Samples

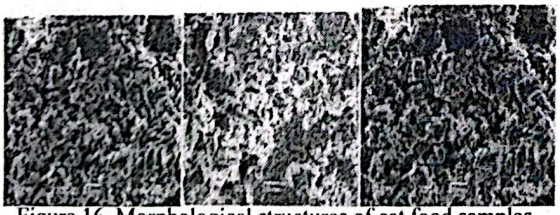

Figure 16. Morphological structures of cat food samples A, B and C

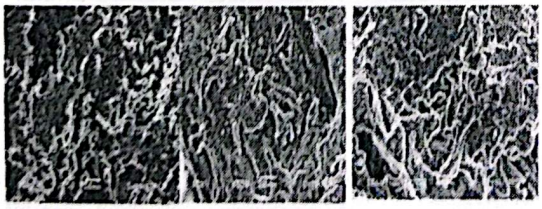

Figure 17. Morphological structures of cat food samples A*, B* and C*

All micro images of SEM data observed their similar adhesion within the constituents and they were shown in Figure 16 and 17.

Conclusion

Suitable moisture of most of prepared cat food can be obtained. Inorganic minerals residue, ash percentage of all cat food samples were 21.76% to 27.93%. Animal-based protein range 45.63% to 57.22% among the cat food samples. In the case of fat, there could be seen lesser concentration than suitable amount. Cats need small amount of fiber and carbohydrate in their meals. Calculated energy values were consistent with the requirement. Morphological structure for all sample are suitable. Most of prepared cat foods have suitable moisture to maintain. Inorganic minerals residue, ash percentage of six cat food samples varied from 21.76% to 27.93%. Animal-based protein ranged from 45.63% to 57.22% among the cat food samples. In the case of fat, there could be seen lesser concentration than the minimum amount (9% of dried matter). Cats need small amount of fiber and carbohydrate in their meals and the resulting nutritional values are consistent with these requirements. Calculated energy values were also closed with the literature values. Morphological structure for all sample are suitable. Cats need small amount of fiber and carbohydrate in their meals and the resulting nutritional values are consistent with this requirement. Calculated energy values were also closed with the literature values. Morphological structures of all cat food samples were similar and uniform adhesion within the chemical constituents present in it. As the contribution of this research work, the nutritive cat foods were produced by using animal and plant-waste successfully.

Acknowledgements

We owe a great deal of gratitude to Dr Aung Win, Rector, Dr Aye Aye Ko and Dr SoeMyint Aye, Prorectors, Myitkyina University for their encouragements. Then we would like to express our gratitude to Dr Ni Ni Aung, Professor and Head, Dr Nant Si Si Htay, Professor, Department of Chemistry, Myitkyina University for their invaluable advice, kind guidance and encouragement for this results.

References

National Research Council of the National Academies 2006. Your Cat's Nutritional Needs; A Science- Based Guide for Pet Owners.

Pierson, L.A.,Cat Food Nutritional Composition.2017,Typical nutrient analysis data provided by the respective companies.

Proposed Revisions Edited per Comments.2014, AAFCO Methods For Substantiating Nutritional Adequacy of Dog and Cat Foods.

Tony.B.D., How much protein does your need ,2017

Miller, K., How to read cat food labels.2009.,

Pierson, L.A., Cat food – Nutritional Composition.., 2012, Data provided by the respective companies.

Binky ,S., Dry Cat Food Nutritional Information ,2008.

Chemical Characterization of Sand Samples from Pamatti and Kyunpinthar Areas, Myitkyina Township, Kachin State, Myanmar

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Abstract

The aim of this research was to determine and characterize the chemical constituents of sands from Pamatti and Kyunpinthar areas, Myitkyina township, Kachin State, Myanmar and sand samples were collected and analysed in 2013. The major components of sands, silica (both soluble and insoluble)and aluminium (as Al2O3) were determined by HCl extract and ammonia method. Organic matter and soluble salts such as chloride (Cl-), carbonate (CO32-) ,bicarbonate (HCO3-) ,and sulphate as (SO42-)were examined by their respective titration methods. For the detection of heavy metals and other minerals such as arsenic (As) , chromium(Cr), cadmium(Cd), lead(Pb) ,iron(Fe), zinc(Zn), copper (Cu) and calcium(Ca), Atomic Absorption Spectrometer (AAS) was applied. This research work pointed out the percent composition of the chemical constituents of sands from Pamatti and Kyinpinthar and characterized systematically.

Keywords: sand, titration method, Atomic Absorption Specteometer.

Introduction

In general, sand may be classified according to their composition or according to their properties and the specific uses for which they are best adapted. The sand samples are collected from Pamatti and Kyunpinthar areas in Myitkyina region. This thesis attempts to describe the analysis of chemical characterization of sand samples from Pamatti and Kyunpinthar areas.The sands quantitative sizes have more significance. Sand can also be defined as a material made up of grains that are anywhere from 0.0625 millimeters to 2.0 millimeters in diameter. However, an exact grain size range for sands cannot be made from heavy minerals because heavy minerals are found in too many different sizes.The minerals that make up sand are also important when it comes to defining sand. Sand is made up of minerals. The most common mineral in sand is silicate.Furthermore, there are many different ways to analyze and study sand. For example, sand size may be examined by using the techniques of sieving, setting-velocity, and micros-copy. In addition, sand composition may be studied by using a high density liquid.

One of the first investigations of sand, specifically river and beach sand, was to determine mineral composition and chemical characterization.Today, the uses of sands are unlimited. Sand is used for abrasives and construction. It is added to plasters, concrete, structural day products and asphalt. Also, some of its con- stituents are very valuable. Sand is a naturally occurring granular material composed of finely divided rock and mineral particles. The composition of sand is highly variable, depending on the local rock sources and conditions, but the most common constituent of sand in inland continental set- tings and non-tropical coastal setting is silica (silicon dioxide, or SiO_2), usually in the form of quartz. The second most common form of sand is calcium carbonate, for example aragonite, which has mostly been created, over the past half billion years, by various forms of life, like coral and shellfish. It is, for example, the primary form of sand apparent in areas where reefs have dominated the ecosystem for millions of years like the Caribbean.

In terms of particle size as used by geologists, sand particles range in diameter from 0.0625 mm (or 1/16 mm) to 2 mm. An individual particle in this range size is termed a sand grain. Sand grains are between gravel(with particles ranging from 2 mm up to 64 mm) and silt (particles smaller than 0.0625 mm down to 0.004 mm). The size specification between sand and gravel has remained constant for more than a century, but particle diameters as small as 0.02 mm were considered sand under the Albert Atterberg standard in use during the early 20th century. A 1953 engineering standard published by the American Association of State Highway and Transportation Officials set the minimum sand size at 0.074 mm. A 1938 specification of the United States Department of Agriculture was 0.05 mm. Sand feels gritty when rubbed between the fingers; (silt, by composition, feels like flour).ISO 14688 grades sands as fine, medium and coarse with ranges 0.063mm to 0.2 mm to 0.63 mm to 2.0 mm. In the United States, sand is commonly diveded into five sub- categories based on size: very fine sand (1/16-1/8 mm diameter), fine sand (1/8 mm-1/4 mm), medium sand (1/4 mm-1/2 mm), coarse sand (1/2 mm-1 mm), and very coarse sand (1 mm-2 mm).

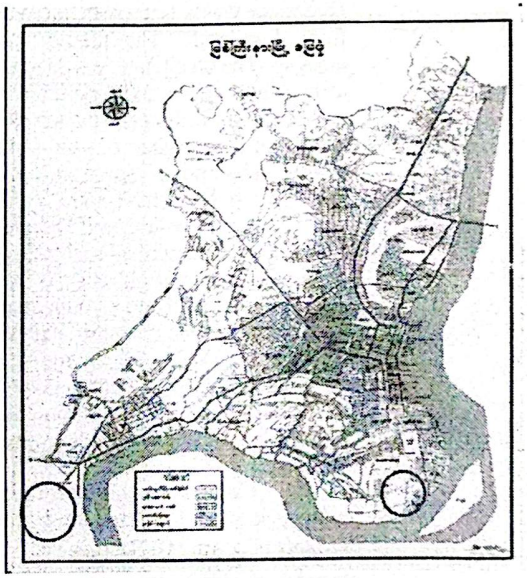

Figure 1.Map of Myitkyina Township and sampling site

Experimental

Collection and Preparation of Samples

The sand sample 1 were collected from Pamatti, Myitkyina Township, Kachin State in 2013.The sand sample 2 and 3 were collected from Kyinpinthar, Mytikyina Township, Kachin State in 2013.

Preparation of Sand Samples

The samples may be kept in bags, labeled suitably both outside and inside the bag. The sand should be shifted through a sieve with round holes 2mm in diameter. The sieving and crushing of this residue are repeated until all aggregate particles are fine enough to pass through the sieve and only stones and organic residues remain on it. Then the fine earth passing through 2mm sieve should be mixed and stored it in suitable bags and labeled them properly.

The sample is allowed to dry in an electric oven at 105°C cooled in a desiccator and weighed. From the loss in weigh the percentage of moisture of the sample is calculated.

Estimation of acid insoluble silica and soluble silica was presented by HCL extract method. For insoluble and soluble silica utilized and investigation percent composition was done by gravimetric analysis method.

To determine the concentration of sulphate present in the sand samples, 25 cm3 of water extract was pipetted into conical flask and the flask was gently warmed to expel carbondioxide, till its content began and 10 cm3 of 0.01M barium chloride solution was added. It was cooled to room temperature. Than 5 cm3 of ammonium buffer solution was added Eriochrome Black T was used as an indicator. It was titrated with 0.01M EDTA solution. The end point was blue.

Estimation of aluminum on sand samples was subjected by ammonia method, 10cm3 of the HCl extract obtained after removing silica was pipetted out into a 250 cm3 breaker and about 1g of ammonium chloride was added. The contents were brought to boil. After moving from the hotplate conc:ammonium hydroxide was added slowly stirring the contents, till the reaction was distinctly alkaline. Then the content were allowed to boil or couple of minutes and set aside for half an hour when the bulky precitate settled down leaving a clear supernatant liquid above. It was filtered and the filtrate was collected 500cm3 beaker. The precipitate was washed with hot water till the filtrate and free of chloride. The filtrate was kept for further analysis. The precipitate was dried, ignited and weighed to constant weight and reported as percentage of iron and alumina combined in the sample. Determination of elemental composition of sand samples were done by atomic absorption spectrometric method at the University of Research Centre, Yangon.

Results and Discussion

The work described in this thesis were the reports of the finding through analysis of the sand samples taken from Pamatti and Kyunpinthar areas.

The results of chemical analysis in this work were expressed in W/W % for moisture content, organic matter, carbonate, bicarbonate, chloride, sulphate, silica and aluminium. Calcium, chromium, iron, lead, copper, zinc, cadmium and arsenic were expressed in parts per million(ppm).

Comparison results of silica and aluminium contents in sand samples were shown in Table(2).

Table(1) Sample Collection

No;	Name	Collected Area	Colour
1	Sample-1	Pamatti	Black
2	Sample-2	Kyunpinthar (1)	Black
3	Sample-3	Kyunpinthar (2)	Black

Table(2) Results of Silica and Alumina

No;	Parameter	Sample-1	Sample-2	Sample-3
1	Silica	76.11 ppm	74.11 ppm	75.21 ppm
2	Alumina	21.81 ppm	20.83 ppm	21.57 ppm

Comparison results of carbonate, bicarbonate and iron contents in sand samples are shown in Table(3-2).

Table(3) Results of Carbonate, Bicarbonate and Iron

No.	Parameter	Sample-1	Sample-2	Sample-3
1	CO_3^{2-}	ND	ND	ND
2	HCO_3^-	0.02%	0.01%	0.02%
3	Fe	29.89 ppm	30.20 ppm	31.44 ppm

From the study of the Table(3) carbonate is not detected and bicarbonate is low value. These low content in sand samples can also be seen that iron content is higher than other metals. A high iron content gives red colour of the product after the baking.

Table(4)Metals content in sand samples of Pamatti and Kyunpinthar areas

	Content in ppm							
	Zn	Cu	Fe	Cd	Ca	Pb	As	Cr
Sample-1	0.853	0.450	29.89	-0.060	3.385	1.237	10.27	0.533
Sample-2	0.860	2.621	30.20	-0.065	2.372	1.244	9.466	0.515
Sample-3	1.150	1.897	31.44	-0.052	2.936	1.299	10.63	0.607

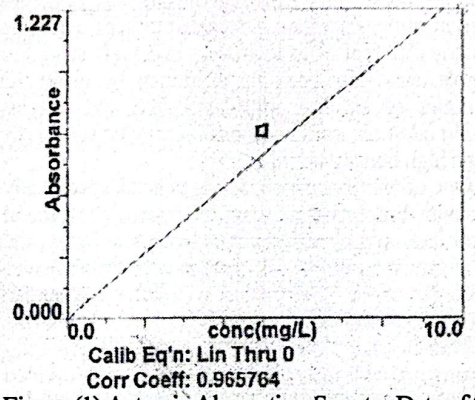

Figure (1) Automic Absorption Spectra Data of (Zn) Sample-1 = Pamatti, Sample-2 = Kyunpinthar (sample-1), Sample-3 = Kyunpinthar (sample-2)

Replicate Data : OhmmarAung (Zn) Sample-1

Repl #	SampleConc mg/L	StndConc mg/L	BlnkCorr Signal
1	0.855	0.855	0.110
2	0.852	0.852	0.110
3	0.852	0.852	0.110
Mean:	0.853	0.853	0.110
SD:	0.002	0.002	0.0002
%RSD:	0.206	0.206	0.21

Replicate Data : OhmmarAung (Zn) Sample-2

Repl	SampleConc	StndConc	BlnkCorr
1	0.862	0.862	0.111
2	0.856	0.856	0.110
3	0.860	0.860	0.111
Mean:	0.860	0.860	0.111
SD:	0.003	0.003	0.0004
%RSD:	0.360	0.360	0.36

Replicate Data : OhmmarAung (Zn) Sample-3

Repl	SampleConc	StndConc	BlnkCorr
1	1.148	1.148	0.148
2	1.146	1.146	0.148
3	1.164	1.164	0.150
Mean:	1.153	1.153	0.149
SD:	0.010	0.010	0.0012
%RSD	0.833	0.833	0.83

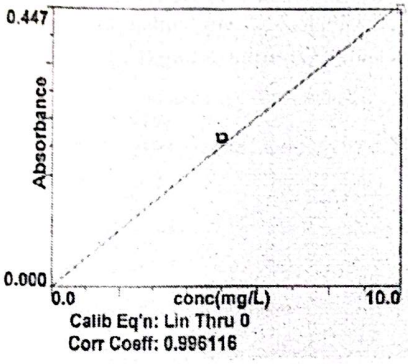

Figure (2) Automic Absorption Spectra Data of (Cu) Sample-1 = Pamatti, Sample-2 = Kyunpinthar (sample-1), Sample-3 = Kyunpinthar (sample-2)

Replicate Data :
OhmmarAung (Cu) Sample-1

Repl	SampleConc	StndConc	BlnkCorr
1	0.453	0.453	0.021
2	0.451	0.451	0.020
3	0.447	0.447	0.020
Mean:	0.450	0.450	0.020
SD:	0.003	0.003	0.0001
%RSD:	0.709	0.709	0.71

Replicate Data : OhmmarAung (Cu) Sample-2

Repl	SampleConc	StndConc	BlnkCorr
1	2.622	2.622	0.119
2	2.612	2.612	0.119
3	2.628	2.628	0.119
Mean:	2.621	2.621	0.119
SD:	0.008	0.008	0.0004
%RSD:	0.307	0.307	0.31

Replicate Data : OhmmarAung (Cu) Sample-3

Repl	SampleConc	StndConc	BlnkCorr
1	1.899	1.899	0.086
2	1.887	1.887	0.086
3	1.897	1.897	0.086
Mean:	1.894	1.894	0.086
SD:	0.006	0.006	0.0003
%RSD:	0.322	0.322	0.32

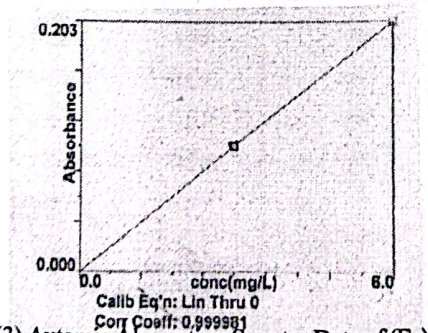

Figure (3) Automic Absorption Spectra Data of (Fe)

Sample-1 = Pamatti, Sample-2 = Kyunpinthar (sample-1), Sample-3 = Kyunpinthar (sample-2)

Replicate Data : OhmmarAung (Fe) Sample-1

Repl	SampleConc	StndConc	BlnkCorr
#	mg/L	mg/L	Signal
1	29.88	29.88	1.013

Sample concentration is greater than that of the highest standard.

2	29.91	29.91	1.015

Sample concentration is greater than that of the highest standard.

3	29.87	29.87	1.013

Sample concentration is greater than that of the highest standard.

Mean:	29.89	29.89	1.014
SD:	0.022	0.022	0.0008
%RSD:	0.075	0.075	0.08

Sample concentration is greater than that of the highest standard.

Replicate Data : OhmmarAung (Fe) Sample-2

Repl	SampleConc	StndConc	BlnkCorr
#	mg/L	mg/L	Signal
1	30.19	30.19	1.024

Sample concentration is greater than that of the highest standard.

2	30.19	30.19	1.024

Sample concentration is greater than that of the highest standard.

3	30.21	30.21	1.025

Sample concentration is greater than that of the highest standard. Mean:

	30.20	30.20	1.024
SD:	0.010	0.010	0.0003
%RSD:	0.034	0.034	0.03

Sample concentration is greater than that of the highest standard.

Replicate Data : OhmmarAung (Fe) Sample-3

Repl	SampleConc	StndConc	BlnkCorr
#	mg/L	mg/L	Signal
1	31.44	31.44	1.066

Sample concentration is greater than that of the highest standard.

2	31.44	31.44	1.066

Sample concentration is greater than that of the highest standard.

3	31.43	31.43	1.066

Sample concentration is greater than that of the highest standard. Mean:

	31.44	31.44	1.066
SD:	0.006	0.006	0.0002
%RSD:	0.018	0.018	0.02

Sample concentration is greater than that of the highest standard.

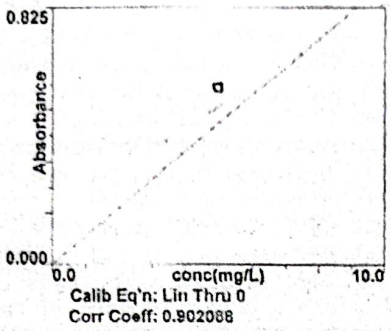

Figure (4) Automic Absorption Spectra Data of (Cd) Sample-1 = Pamatti, Sample-2 = Kyunpinthar (sample-1), Sample-3 = Kyunpinthar (sample-2)

Replicate Data : OhmmarAung (Cd) Sample-1

Repl #	SampleConc mg/L	StndConc mg/L	BlnkCorr Signal
1	-0.058	-0.058	-0.005
2	-0.062	-0.062	-0.006
3	-0.059	-0.059	-0.005
Mean: SD:	-0.060	-0.060	-0.005
	0.002	0.002	0.0002
%RSD:	3.638	3.638	3.64

Replicate Data : OhmmarAung (Cd) Sample-2

Repl #	SampleConc mg/L	StndConc mg/L	BlnkCorr Signal
1	-0.040	-0.040	-0.004
2	-0.079	-0.079	-0.007
3	-0.075	-0.075	-0.007
Mean:	-0.065	-0.065	-0.006
SD:	0.021	0.021	0.0019
%RSD:	33.12	33.12	33.12

Replicate Data : OhmmarAung (Cd) Sample-3

Repl	SampleConc	StndConc	BlnkCorr
1	-0.021	-0.021	-0.002
2	-0.072	-0.072	-0.006
3	-0.065	-0.065	-0.006
Mean:	-0.052	-0.052	-0.005
SD:	0.028	0.028	0.0025
%RSD:	52.49	52.49	52.49

Repl #	SampleConc mg/L	StndConc mg/L	BlnkCorr Signal
1	2.932	2.932	0.079
2	2.950	2.950	0.079
3	2.982	2.982	0.079
Mean:	2.936	2.936	0.079
SD:	0.012	0.012	0.0003
%RSD:	0.402	0.402	0.40

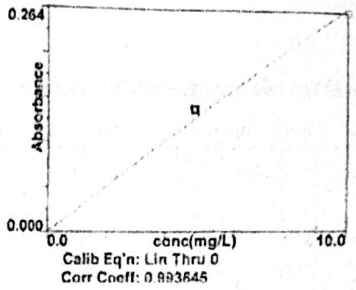

Figure (5) Automic Absorption Spectra Data of (Ca) Sample-1 = Pamatti, Sample-2 = Kyunpinthar (sample-1), Sample-3 = Kyunpinthar (sample-2)

Replicate Data : OhmmarAung (Ca) Sample-1

Repl #	SampleConc mg/L	StndConc mg/L	BlnkCorr Signal
1	3.406	3.406	0.092
2	3.371	3.371	0.091
3	3.377	3.377	0.091
Mean:	3.385	3.385	0.091
SD:	0.018	0.018	0.0005
%RSD:	0.539	0.539	0.54

Replicate Data : OhmmarAung (Ca) Sample-2

Repl #	SampleConc mg/L	StndConc mg/L	BlnkCorr Signal
1	2.370	2.370	0.064
2	2.371	2.371	0.064
3	2.375	2.375	0.064
Mean:	2.372	2.372	0.064
SD:	0.003	0.003	0.0001
%RSD:	0.110	0.110	0.11

Replicate Data : OhmmarAung (Ca) Sample-3

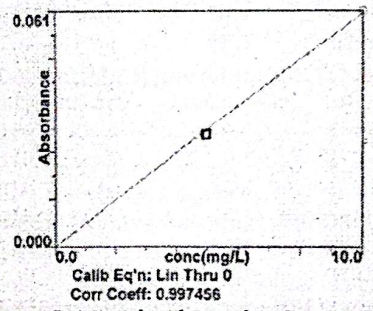

Figure (6) Automic Absorption Spectra Data of (Pb) Sample-1 = Pamatti, Sample-2 = Kyunpinthar (sample-1), Sample-3 = Kyunpinthar (sample-2)

Replicate Data : OhmmarAung (Pb) Sample-1

Repl #	SampleConc mg/L	StndConc mg/L	BlnkCorr Signal
1	1.237	1.237	0.007
2	1.241	1.241	0.007
3	1.232	1.232	0.007
Mean:	1.237	1.237	0.007
SD:	0.004	0.004	0.0000
%RSD:	0.347	0.347	0.35

Replicate Data : OhmmarAung (Pb) Sample-2

Repl #	SampleConc mg/L	StndConc mg/L	BlnkCorr Signal
1	1.227	1.227	0.007
2	1.219	1.219	0.007
3	1.287	1.287	0.008
Mean:	1.244	1.244	0.008
SD:	0.037	0.037	0.0002
%RSD:	2.989	2.989	2.99

Replicate Data : OhmmarAung (Pb) Sample-3

Repl	SampleConc	StndConc	BlnkCorr
1	1.302	1.302	0.008
2	1.304	1.304	0.008
3	1.292	1.292	0.008
Mean:	1.299	1.299	0.008
SD:	0.006	0.006	0.0000
%RSD:	0.494	0.494	0.49

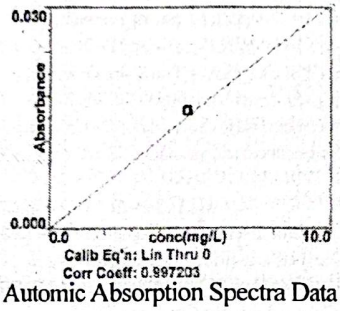

Figure (7) Automic Absorption Spectra Data of (As)

Sample-1 = Pamatti, Sample-2 = Kyunpinthar (sample-1), Sample-3 = Kyunpinthar (sample-2)

Replicate Data : OhmmarAung (As) Sample-1

Repl #	SampleConc mg/L	StndConc mg/L	BlnkCorr Signal
1	9.511	9.511	0.029
2	9.957	9.957	0.030
3	11.34	11.34	0.035

Sample concentration is greater than that of the highest standard.

Mean:	10.27	10.27	0.031
SD:	0.953	0.953	0.0029
%RSD:	9.276	9.276	9.28

Replicate Data : OhmmarAung (As) Sample-2

Repl #	SampleConc mg/L	StndConc mg/L	BlnkCorr Signal
1	8.757	8.757	0.027
2	9.835	9.835	0.030
3	9.806	9.806	0.030
Mean:	9.466	9.466	0.029
SD:	0.615	0.615	0.0019
%RSD:	6.493	6.493	6.49

Replicate Data : OhmmarAung (As) Sample-3

Repl #	SampleConc mg/L	StndConc mg/L	BlnkCorr Signal
1	10.87	10.87	0.033
2	10.31	10.31	0.031
3	10.71	10.71	0.033
Mean:	10.63	10.63	0.032
SD:	0.293	0.293	0.0009
%RSD:	2.754	2.754	2.75

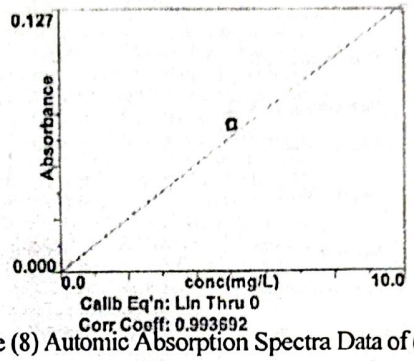

Figure (8) Automic Absorption Spectra Data of (Cr)

Sample-1 = Pamatti, Sample-2 = Kyunpinthar (sample-1), Sample-3 = Kyunpinthar (sample-2)

Replicate Data : OhmmarAung (Cr) Sample-1

Repl #	SampleCo mg/L	StndConc mg/L	BlnkCorr Signal
1	0.516	0.516	0.007
2	0.543	0.543	0.007
3	0.540	0.540	0.007
Mean:	0.533	0.533	0.007
SD:	0.015	0.015	0.0002
%RSD:	2.801	2.801	2.80

Replicate Data : OhmmarAung (Cr) Sample-2

Repl #	SampleConc mg/L	StndConc mg/L	BlnkCorr Signal
1	0.517	0.517	0.007
2	0.519	0.519	0.007
3	0.510	0.510	0.007
Mean:	0.515	0.515	0.007
SD:	0.005	0.005	0.0001
%RSD:	0.948	0.948	0.95

Replicate Data : OhmmarAung (Cr) Sample-3

Repl #	SampleConc mg/L	StndConc mg/L	BlnkCorr Signal
1	0.625	0.625	0.008
2	0.586	0.586	0.008
3	0.592	0.592	0.008
Mean:	0.601	0.601	0.008
SD:	0.021	0.021	0.0003
%RSD:	3.451	3.451	3.45

Table(5) Comparison Results of Pamatti and Kyunpinthar areas

No;	Parameter	Sample-1	Sample-2	Sample-3
1	Moisture	2.63%	2.52%	2.12%
2	Organic Matter	0.36%	0.34%	0.25%
3	Chloride as Cl^-	0.04%	0.03%	0.01%
4	Carbonate as CO^{2-}	ND	ND	ND
5	Bicarbonate as HCO^-	0.02%	0.01%	0.02%
6	Silica (Insoluble)	76.11%	74.11%	75.21%
7	Silica (Soluble)	0.35%	0.37%	0.48%
8	Sulphate as SO_4^{2-}	9.15%	8.15%	8.81%
9	Alumina as AL_2O_3	21.81%	20.83%	21.57%
10	Zinc as Zn	0.853 ppm	0.860 ppm	1.153 ppm
11	Copper as Cu	0.475 ppm	2.621 ppm	1.894 ppm
12	Iron as Fe	29.89 ppm	30.20 ppm	31.44 ppm
13	Cadmium as Cd	-0.060 ppm	-0.065 ppm	-0.052 ppm
14	Calcium as Ca	3.385 ppm	2.372 ppm	2.936 ppm
15	Lead as Pb	1.237 ppm	1.244 ppm	1.299 ppm
16	Arsenic as As	10.27 ppm	9.466 ppm	10.63 ppm
17	Cromium as Cr	0.533 ppm	0.515 ppm	0.601 ppm

According to the comparison data of the Pamatti and Kyunpinthar samples (Table 3-4) there are not much variation in the contents. It can be seen that the sand samples contain a large amount of silica and alumina. Therefore the sand and around these areas are generally heavy and marshy.

Conclusion

In this research, sand samples work were taken and analysed from Pamatti and Kyunpinthar areas which was done for the determination of chemical characterization of sand samples. The moisture content of sample I (2.63%) was found to be more than that of sample II (2.52%) and sample III (2.12%). The percentage of organic matter in the sample I, II and III were 0.36, 0.34 and 0.25.

According to the data, it was clearly seen that, the higher the content of iron, the lower the content of carbonate and bicarbonate and the suitable amount of silica and aluminium will the better quality of sand. The mineral such as Zn, Cu, Fe, Cd, Ca, Pb, As and Cr values were examined by AAS method.

One of the most impressive feats of nature is the erosion of massive mountains into sand by the powerful forces of wind and water. Individual sand grains are the size of table salt NaCl grains.

Therefore the sand around these areas are generally heavy and marshy.

Acknowledgements

I owe a great deal of gratitude to Dr Aung Win, Rector,Dr Aye Aye Ko and Dr Soe Myint Aye, Prorectors, University of Myitkyina, Dr Ni Ni Aung, Professor and Head, Dr Htay Htay Win, Professor, Department of Chemistry, University of Myitkyina for their invaluable advices, kind guidances and encouragements for this research work.

References

Aye, Y.Y., Chemical characterization of brick clay samples around Kyaukse and Myitnge areas

Mange, N. A., Maurer, H.F.W. 1992. Heavy Minerals in Colour. London: Chapman and Hall.

Pellant, C., Phillips,R. 1990.Rocks, Minerals and Fossils of the World.China:Little, Brown and Company.

Pettijohn,F.J.,Potter,P.E., Siever,R.1987,Sand and Sandstone. New York: Springer-Verlag.

Siever,R.1998,Sand. New York: Scientific American Library

Study on the Antimicrobial and Pesticidal Activities of Rhizomes of *Curcuma Amada* (Roxb.) (Mango-Ginger)

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Abstract

As the rhizomes of *Curcuma amada* (Roxb.) have a long history of medicinal use, this research deals with the investigation of some chemical constituents and screening of antimicrobial and pesticidial activities on the rhizomes of *Curcuma amada* (Roxb.). The *Curcuma amada* (Roxb.) was studied out for medical purpose from chemistry point of view. The elemental analysis of rhizomes determined by ED-XRF spectrometer indicated that the highest abundance percentage of K (4.628%) and P(0.436%) were observed for rhizomes. Nutritional values determined by AOAC method indicated that the percentage of moisture, ash, fiber, protein, fat and carbohydrate were 8.86%, 10.73%, 16.76%, 9.25%, 12.21% and 42.19% for rhizomes with the energy values of 315 Kcal/100g. Agar well diffusion method was used to determine in vitro antimicrobial activity on the different extract such as EtOAc, PE, EtOH and water extracts. It was found that EtOAc extract of rhizomes exhibited the highest activity (40-24mm) against *Bacillus subtilis*, *Bacillus pumilus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coil* and *Candida albicans*. The pesticidal activity of rhizomes of *Curcuma amada* (Roxb.) were determined by EtOH extract, EtOH only and water only. EtOH extract of *Curcuma amada* (Roxb.) is more active than EtOH only and water only.

Keywords: *Curcuma amada* (Roxb.) antimicrobial activity and pesticidal activity

I. Introduction

Curcuma amada (Roxb.) (mango ginger) is a plant of the ginger family Zingiberaceae and is closely related to turmeric. It is an important tree of pharmaceutical and trade value (Saji, *et al.*, 2004). The rhizomes are very similar to ginger. It is distributed in India, native of tropical Bangladesh, native of Thailand as well as in Myanmar. (Shankaracharya, *et al.*, 1982)

The rhizomes are used to treat stomach and chest pains, fever and debility. It is also useful against inflammations of mouth, ear, ulcers on the male sex organs, scabies, lumbago and stomatitis. Antioxidant, antimicrobial and pesticidal activities of the drug have already been reported. In vegetable and fruit production, insecticides are used to control pests.

Pesticides are an important but often controversial component of today's integrated pest management system. (Abbott, 1925). As pesticides are used to kill unwanted pests, weeds or diseases, there is concern that they may harm people, wildlife and the enviroment. This is why there are strict controls in place over their sale and use. Therefore, pesticide is a substance or mixture of substances intended for preventing, destroying or controlling any pest (Gupta, *et al.*, 2001). Pesticide products are some of the most widely tested chemicals with respect to toxicological harzard evaluation (e.g. multi-generation, developmental, and reproductive toxicology testing) and human health risk assessment for example., dietary, occupational (Holland and Greenhalgh, 1996). Pesticides have the potential to express toxicity to mammalian systems; including humans, aquatic organisms, plants and soil organisms. Pestisitance of a chemical is a determined by stability or length of life in soil, aquatic enviroments, and in animal and plant tissues. (Makanjuola, *et al.*,1989)

II. Materials and Methods

A. Collection Plant Sample

Curcuma amada (Roxb.) rhizome used in this study was collected from Pyay Township. Then the sample was identified at Botany Department, University of Yangon. The rhizome was peeled off and air dried at room temperature for two weeks. Then it was made into powdery by electric mortar-grinder and stored in air-tight container to prevent moisture changes and other contaminations.

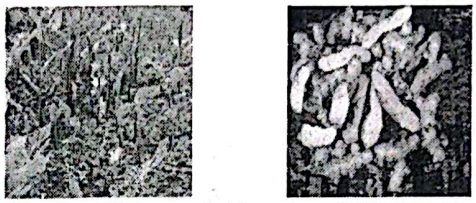


Fig. 1 Plants and rhizomes of *Curcuma amada* (Roxb) (Thayetkin)

B. Extraction of Plant Samples

Dried powdered sample (100g) was weighed and placed in six different conical flasks. 500 mL of different of solvent such as petroleum ether, ethyl acetate, ethanol, chloroform, methanol and distilled water was added into each flask respectively and stopped with a cork. The sample was allowed to macerate and placed in a shaker, shaken continuously for about 6 hr and the suspension was allowed to stand for 18 hr. The extract was filtered rapidly taking care not to lose any solvent, and transferred to preweighed porcelain basin and evaporated to dryness on water bath. The dried filtrate was then placed in oven, maintained till constant weight, at 105°C. The contents of extractable matter in milligram per gram of air-dried materials in different solvents.

C. Antimicrobial Activity of some Crude Extract of Curcuma amada (Roxb.) (Mango ginger) by Agar Well Diffusion Method

Screening of antimicrobial activity of crude extracts such as pet-ether. EtOAc, EtOH and watery extracts from *Curcuma amada* (Roxb.) were done by agar well diffusion method. In this investigation, the ezxtracts were tested in six microorganisms such as *B. subtillis*, *S. aureus*, *P. aeruginosa*, *B. pumilus*, *C. albicans and E. coli*. The inhibition zone diameter, including the agar well diffusion showed the degree of antimicrobial activity (Cruickshank, 1975). The lager the inhibition zone of crude extracts against six microorganisms tested were shown in Figure 3 and the observed data were summarized in Table 3.

D. Pesticidal Activity Crude extract

From crude extract 0.5g and 1g were accurately weighed and serially dissolved in 500 ml of ethanol. Then, it was made up to 20 ml by using ethanol. Thus from crude extract 1000 ppm and 2000 ppm concentration of test solutions was prepared. Test solution of each extracts were used for testing the black bean aphids (*Aphids* fabae) (Makanjuola *et al.*, 1989).

E. Screening of Pesticidal Activity of Rhizomes of Curcuma amada (Roxb.) by Disc method

The research deals with the investigation of some natural pesticides content and screening of pesticide activities of ethanol extract of rhizomes of mango ginger, EtOH only and water only. Among them, EtOH extract of mango ginger contain more pontent effect on pesticides.

III. Results and discussion

Table 1 Nutritional values of *C. amada* (Roxb.) (Mango giner)

No.	Parameters	Contents(%)
1.	Moisture	8.86
2.	Crude Protein	9.25
3.	Ash	10.73
4.	Crude Fat	12.21
5.	Crude fiber	16.76
6.	Carbohydrate	42.19
7.	Energy value (Kcal/100g)	315

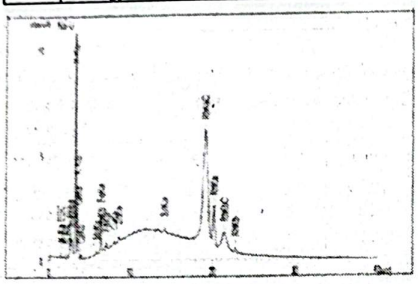
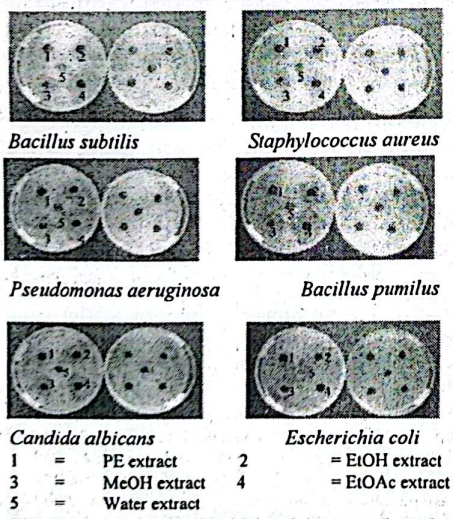


Figure 2.EDXRF Specturm of *Curcuma amada* (Roxb.) rhizomes powder

Table 2 Relative Abundance Elements in *Curcuma amada* (Roxb.) Rhizomes (Mango ginger)

No.	Element	Relative abundance(%)
1.	K	4.682
2.	P	0.436
3.	Ca	0.215
4.	S	0.154
5.	Fe	0.029
6.	Mn	0.004
7.	Zn	0.002
8.	Cu	0.001
9.	Sr	0.001
10.	COH	94.476



Bacillus subtilis *Staphylococcus aureus*

Pseudomonas aeruginosa *Bacillus pumilus*

Candida albicans *Escherichia coli*

1 = PE extract 2 = EtOH extract
3 = MeOH extract 4 = EtOAc extract
5 = Water extract

Figure 3. Antimicrobial activity screening of *Curcuma amada* (Roxb.) rhizomes

Table 3 Antimicrobial Activity of Curcuma amada (Roxb.) Rhizomes (mango ginger) Against Six Microorganisms

No.	Organisms	Inhibition Zone Diameter of Extracts (mm)				
		PE	Me OH	EtO Ac	EtOH	H_2O
1.	Bacillus subtilis	17 (++)	25 (+++)	30 (+++)	22 (+++)	12 (+)
2.	Bacillus pumilus	12 (+)	23 (+++)	24 (+++)	23 (+++)	12 (+)
3.	Staphylococcus aureus	15 (++)	24 (+++)	30 (+++)	23 (+++)	13 (+)
4.	Pseudomonas aeruginosa	13 (+)	23 (+++)	40 (+++)	20 (+++)	11 (+)
5.	Escherichia coli	17 (++)	24 (+++)	25 (+++)	20 (+++)	12 (+)
6.	Candida albicans	17 (+++)	23 (+++)	23 (+++)	36 (+++)	(-)

Diameter of agar well = 10mm
10mm~14mm = (+) (low)
15mm~ 19 mm = (++) (medium)
20mm above = (+++) (high)
No activity = (-)

Table 4 Mortality Percentages of *Aphids* Fabae Treated with Plant Extracts of Curcuma amada (Roxb.) by Disc Method

Plant extract and solvent	Conc. (ppm) and Vol. (mL)	No. of insects	Time (min)	Morlaity (%)
Ethanol extract	1000/ 20	15	60	86
	2000/ 20	15	45	93
Ethanol only	20 mL	15	60	73
Water only	20 mL	15	60	0
Control	-	15	-	-

Conclusion

From this study on *Curcuma amada* (Roxb.) of the following interence can be concluded. Agar well diffusion method was used to determine in the vitro antimicrobial activity on the extracted such as EtOAc, PE, MeOH, EtOH and water extracts.

It was that the EtoAc extract of *Curcuma amada* (Roxb.) rhizomes exhibited the highest activity (40-24) mm against *B. subtilis, S. aureus, P. aeruginosa. B. pumilus, C. albicans and E. coli.* The investigation of some natural pesticides content and screening of pesticidal activities of ethanol extract of mango ginger rhizomes, EtOH only and water only. Ethanol extracts more effective than other solvent. The present work reveals that *Curcuma amada* (Roxb.) rhizomes may be used as food source as well as medicine related to bacterial infections and pesticidal activity.

Acknowledgement

I would to express my sincere thanks to Dr Ni Ni Than, (Professor and Head), Department of Chemistry, University of Yangon, for her suggestions and permission to allow this research work.

References

[1] Abbott, W. (1925). "A method for Computing the Effectiveness of an Insecticide". Journal of economic entomology, **18**: 265-267
[2] Gupta, V. K. (2001). "The Wealth of India; First Supplemented Series (Raw Materials)", **2**, 259-260
[3] Holland, P. T and Greenhalgh, R. (1996). "Analysis of Pesticides Residues", Moye, H. A., (Ed)., John Wiely and Sons, New York, 167-193
[4] Makanjuola, W. G. (1989). "Evalution of Extracts of Azadirachtainndicafor the Control of Some Stored Product Pest". *Journal of stored product*, **25**: 231-237
[5] Saji, K. V. and Sasikumar B. (2004). "Mango ginger-endowed with Mango ginger and Tumeric Qualities." *Spice Ind.* **17**(9), 23-24
[6] Shankaracharya, N. B. (1982). "Mango ginger", India Coca, *Arecanut Species J.* **5**, 70-80

Synthesis and Characterization of ZnO-CuO Nanocomposites

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Abstract

ZnO nanoparticles, CuO nanoparticles and ZnO-CuO nanocomposite were successfully prepared by sol-gel method using zinc acetate dihydrate and copper(II) sulphate pentahydrate as precursors. X-ray diffraction (XRD) pattern revealed hexagonal structure of ZnO nanoparticles and monoclinic structure of CuO with average crystallite sizes of 25.8 nm and 23.0 nm respectively . ZnO-CuO nanocomposite having larger size (27.3 nm) than ZnO and CuO was indexed as hexagonal structure with the presence of only ZnO and CuO phases with no other impurities. Morphologies of ZnO nanoparticles, CuO nanoparticles and ZnO-CuO nanocomposite were investigated by SEM. FT IR analysis showed both characteristic vibration peaks of ZnO (470 cm^{-1}) and CuO (527 cm^{-1}) in FT IR spectrum of ZnO-CuO nanocomposite

Keywords: ZnO nanoparticles, CuO nanoparticles, ZnO-CuO nanocomposite, sol-gel method,

Introduction

Nanoparticles are particles ranging in size from 1 nm to 100 nm. Nanoparticles possess very high surface to volume ratios compared to their bulk materials. Nanoparticles can be applied in various fields like cosmetic, medicine and gas sensor (Akir *et al.*, 2016). Wastewater treatment using nanoparticles is one of the areas of concern in nanotechnology. Nanocomposites are composites in which at least one of the phases shows dimensions in the nanometre range (1 nm = 10^{-9} m) (Roy *et al.*, 1986). Properties of the nanocomposite differ markedly from that of the component materials.

ZnO is a wide band gap (3.37 eV) n-type semiconductor (Gao *et al.*, 2007). Its nanostructures are of particular interest for potential applications in sensors, catalysts, field emitters, light emitting diodes, solar cells, hydrogen storage, and information storage. CuO is an important narrow band gap (1.2 eV) p-type semiconductor with many applications in sensors, catalysis, batteries, high temperature superconductors, solar cells, and field emitters (Ghane *et al.*, 2010).

Composite nanostructures of these two wide and narrow band gap semiconductors may offer combined functionalities and open the way for new enhanced applications, such as photocatalysts. ZnO nanoparticles have widely been used as photocatalyst to degrade various organic dyes in wastewater. However, the light response of ZnO nanoparticles is limited since the high recombination of electron and hole of ZnO is very fast. Thus, CuO has been incorporated to suppress the electron–hole recombination, so that the photocatalytic efficiency of ZnO can be enhanced.Sol gel method is the simplest method for nanomaterial synthesis and has the ability to control the particle size and morphology through systematic monitoring of reaction parameter (Widiarti *et al.*, 2017). This method involves two stages of sol and gel formation. Many techniques could be applied in the sol-gel technology such as the changing of initial precursor, time of gel formation, type of catalyst, rate of solution formation, gel formation conditions and gel physical processing. Thus, the sol-gel process enables the formation of solid material through gelation from a solution (Owens *et al.*, 2016).The aim of the present work is to combine ZnO and CuO into a single composite and to characterize the as –synthesized ZnO - CuO composite.

Materials

Zinc acetate dihydrate, copper (II) sulphate pentahydrate, ethanol, sodium hydroxide, ethylene glycol and citric acid from BDH were used.

Synthesis of ZnO nanoparticles by sol-gel method

Zinc oxide nanoparticles were synthesized using the sol-gel method according to Widiarti *et al.* (2017) with slight modification. Firstly, 0.02 mol of zinc acetate dihydrate was dissolved in 20 mL of ethanol, magnetically stirred for an hour and kept for 48 hours at room temperature to form a gel. Then, the gel was washed with distilled water and ethanol for several times and dried in an oven at 120 °C for 24 hours. The sample was calcined at 500 °C for 4 hours to obtain the white coloured zinc oxide nanoparticles.

Synthesis of CuO nanoparticles by sol-gel method

Copper oxide nanoparticles were synthesized using the sol-gel method according to Widiarti *et al.* (2017) with slight modification. Firstly, 0.004 mol of copper (II) sulphate pentahydrate was dissolved in 20 mL of distilled water. Next, 0.4 g of citric acid was dissolved in 10 mL of distilled water. The two solutions were thoroughly mixed by stirring with a magnetic stirrer for 30 minutes. After that, 10 mL of 1 M sodium hydroxide solution was added drop-wise into the solution mixture and it was magnetically stirred for 1 hour. Then, it was kept for 48 hours at room temperature to get a gel. After the gel was washed with distilled water and ethanol for several times it was dried in an oven at 120 °C for 24 hours. The sample was then calcined at 500 °C for 4 hours to obtain the black coloured CuO nanoparticles.

Synthesis of ZnO-CuO nanocomposite by sol-gel method

ZnO-CuO nanocomposite was synthesized using the sol-gel method according to Widiarti *et al.* (2017) with slight modification. Firstly, 25 mL of ethylene glycol was mixed with 0.02 mol of zinc acetate dihydrate, 25 mL of distilled water and 6.3 g of citric acid with magnetically stirring for an hour. The suspension was heated at 60 °C and 0.002 mol of copper (II) sulphate pentahydrate was added to the suspension, mixed quickly and magnetically stirred for 3 hours. The solution was kept in the dark for 48 hours to get a gel. It was then washed with distilled water and ethanol for several times and dried at 120 °C for 24 hours.

The sample was then calcined at 500 °C for 4 hours to obtain the CuO-ZnO nanocomposite.

Characterization of ZnO nanoparticles, CuO nanoparticles and ZnO- CuO nanocomposite

Phase analysis and purity of synthesized ZnO nanoparticles, CuO nanoparticles and ZnO-CuO nanocomposite were investigated by X-ray analysis. X-ray diffraction patterns of the samples were recorded on X-ray diffractometer (Rigaku, Tokyo, Japan), using CuK_α radiation (λ= 1.54 Å) at 40 kV and 40 mA. The diffraction angle ranged from 10° to 70° of 2θ. The crystallite size was calculated by Scherrer method. The morphology of the samples was observed by scanning electron microscopy (JEOL-JSM 5610 LV, Japan). Fourier transform infrared (FT IR) spectra of the samples were recorded on a FT IR spectrometer (FT IR-8400 SHIMADZU, Japan) in a range of wavenumber from 4000 to 450 cm^{-1}.

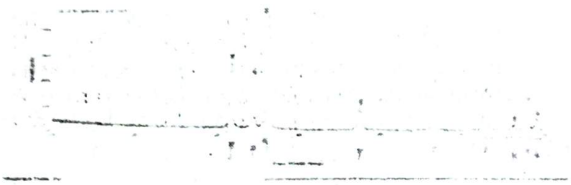


Figure 1 X-ray diffractogram of ZnO nanoparticles

Results and Discussion

Characterization by X-ray diffraction analysis

XRD pattern of ZnO shows well - resolved peaks and these peaks are well-matched with ZnO from standard library (Figure 1). The diffraction peaks appeared at 2θ values of 31.700°, 34.369 °, 36.171°, 47.499 °, 56.502 °, 62.809 °, 66.338°, 67.949° and 69.059 ° corresponding to Miller indices of (100), (002), (101),(110), (103), (200), (112), and (201). Only single phase of ZnO with no other phase was found in this XRD pattern indicating the purity of the ZnO sample (Table 1).

Table 1 Phase Identification of ZnO Nanoparticles

No.	Diffraction Angle '2θ' (°)	Interplanar Spacing 'd' (Å)	Miller Indices (hkl)	Phase ID
1	31.700	2.8202	100	ZnO
2	34.369	2.6072	002	ZnO
3	36.171	2.4813	101	ZnO
4	47.499	1.9126	102	ZnO
5	56.502	1.6274	110	ZnO
6	62.809	1.4782	103	ZnO
7	66.338	1.4079	200	ZnO
8	67.949	1.3784	112	ZnO
9	69.059	1.3589	201	ZnO

Crystallite size of ZnO nanoparticles was calculated by Scherrer equation,

$$\tau = \frac{0.9\lambda}{\beta cos\theta}$$

where , τ = the crystallite size (nm)

λ = the diffraction wavelength

θ = the diffraction angle (degree)

β = the full width at half maximum (FWHM) for the diffraction peak (radian).

Average crystallite size of ZnO nanoparticles was calculated to be 25.8 nm. Calculated data and the data obtained by XRD results (26.8 nm) are not much different (Table 2).

Table 2 Crystallite Size of ZnO Nanoparticles

No	Diffraction angle '2θ' (°)	Full Width at Half Maximum 'β'(°)	Full Width at Half Maximum 'β' (adian)	Calculated Crystallite Size (nm)	Crystallite Size from XRD Data (nm)
1	31.700	0.287	0.0050	28.7	30.7
2	34.369	0.271	0.0047	33.0	33.0
3	36.171	0.304	0.0053	27.4	29.1
4	47.499	0.411	0.0072	21.1	21.8
5	56.502	0.351	0.0061	25.6	26.8
6	62.809	0.382	0.0067	24.3	25.2
7	66.338	0.369	0.0065	25.7	26.7
8	67.949	0.415	0.0073	23.0	23.8
9	69.059	0.415	0.0073	23.2	23.9
Average crystallize size				25.8	26.8

Figure 2 shows the XRD pattern of CuO nanoparticles. The well-resolved characteristic peaks of CuO nanoparticles are observed in this XRD pattern. The XRD pattern of CuO is well-matched with copper oxide from standard library.Table 3 shows formation of single phase of CuO indicating the purity of CuO nanoparticles. Furthermore, crystallite size of CuO calculated by Scherrer formula was 23.0 nm and the data obtained by XRD results was 22.8 nm and found to be not much different (Table 4).

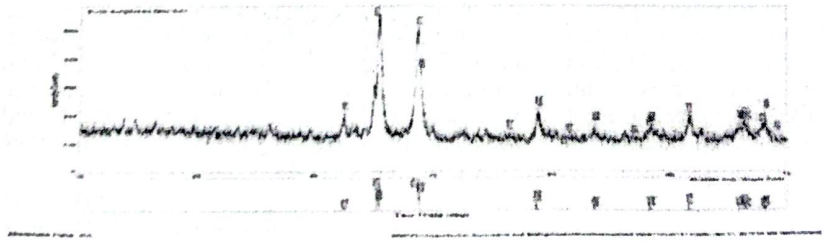


Figure 2 X-ray diffractogram of CuO nanoparticles

Table 3 Phase Identification of CuO Nanoparticles

No	Diffraction Angle '2θ' (°)	Interplanar Spacing 'd' (Å)	Miller Indices (hkl)	Phase ID
1	32.545	2.7489	110	CuO
2	35.587	2.5206	$\bar{1}11$	CuO
3	38.799	2.3191	111	CuO
4	38.998	2.3077	200	CuO
5	46.381	1.9561	$\bar{1}12$	CuO
6	48.852	1.8627	$\bar{2}02$	CuO
7	51.359	1.7775	112	CuO
8	53.617	1.7079	020	CuO
9	56.875	1.6176	021	CuO
10	58.209	1.5836	202	CuO
11	58.367	1.5797	202	CuO
12	61.535	1.5058	$\bar{1}13$	CuO

Figure 3 shows the XRD pattern of ZnO-CuO nanocomposite. Characteristic peaks of both ZnO and CuO are observed in the XRD pattern of ZnO-CuO nanocomposite. Phase identification by XRD analysis shows only ZnO phase and CuO phase with no other phases indicating ZnO-CuO nanocomposite was formed (Table 5). Crystallite size of the composite was calculated by Sherrer equation and found to be 27.3 nm which is not much different from the result given by XRD i.e., 30.4 nm (Table 6). Table 7 shows the comparison of average crystallite sizes of ZnO nanoparticles, CuO nanoparticles and ZnO-CuO nanocomposite. Crystallite Size of ZnO-CuO nanocomposite was larger than those of ZnO and CuO nanoparticles.

Table 4 Crystallite Size of CuO Nanoparticles

No	Diffraction angle '2θ' (°)	Full Width at Half Maximum 'β' (°)	Full Width at Half Maximum 'β' (radian)	Calculated Crystallite Size(nm)	Crystallite Size from XRD Data (nm)
1	32.545	0.285	0.0050	29.0	31.0
2	35.587	0.481	0.0084	17.3	17.7
3	38.799	0.483	0.0084	17.4	17.8
4	38.998	0.499	0.0087	16.8	17.2
5	46.381	0.173	0.0030	49.8	61.0
6	48.852	0.461	0.0080	18.9	19.4
7	51.359	0.261	0.0046	33.7	36.6
8	53.617	0.303	0.0053	29.3	31.1
9	56.875	0.307	0.0053	29.4	31.1
10	58.209	0.622	0.0109	14.6	14.8
11	58.367	0.490	0.0086	18.5	19.0
12	61.535	0.494	0.0087	18.7	19.1
13	65.931	0.404	0.0071	23.4	24.2
14	66.112	0.590	0.0103	16.0	16.3
15	66.332	0.634	0.0111	14.9	15.2
16	66.533	0.590	0.0103	16.1	16.3
17	67.777	0.539	0.0094	17.7	18.1
18	68.147	0.352	0.0062	27.2	28.4
19	69.105	0.340	0.0060	28.3	29.7
Average crystallite size				23.0	22.8

Table 5 Phase Identification of ZnO-CuO Nanocomposite

No.	Diffraction Angle '2θ' (°)	Interplanar Spacing 'd' (Å)	Miller Indices (hkl)	Phase ID
1	31.740	2.8169	100	ZnO
2	32.472	2.7550	110	CuO
3	34.390	2.6056	002	ZnO
4	35.374	2.5353	002	CuO
5	35.491	2.5273	$\bar{1}11$	CuO
6	36.191	2.4800	1	ZnO
7	38.670	2.3265	111	CuO
8	39.007	2.3072	200	CuO
9	46.217	1.9626	$\bar{1}12$	CuO
10	47.449	1.9145	102	ZnO
11	48.644	1.8702	$\bar{2}02$	CuO
12	51.372	1.7771	112	CuO
13	53.490	1.7117	020	CuO
14	56.560	1.6258	110	ZnO
15	56.708	1.6219	110	ZnO
16	56.767	1.6204	021	CuO
17	58.272	1.5821	202	CuO
18	61.527	1.5059	$\bar{1}13$	CuO
19	62.793	1.4786	103	ZnO

Table 5 Contd.

No	Diffraction Angle '2θ' (°)	Interplanar Spacing 'd' (Å)	Miller Indices (hkl)	Phase ID
20	65.682	1.4204	022	CuO
21	66.179	1.4109	$\bar{3}11$	CuO
22	66.270	1.4092	$\bar{3}11$	CuO
23	66.369	1.4073	200	ZnO
24	66.438	1.4060	310	CuO
25	67.832	1.3805	112	ZnO
26	68.020	1.3771	113	CuO
27	68.090	1.3759	220	CuO
28	68.922	1.3613	$\bar{2}21$	CuO
29	69.030	1.3594	201	ZnO

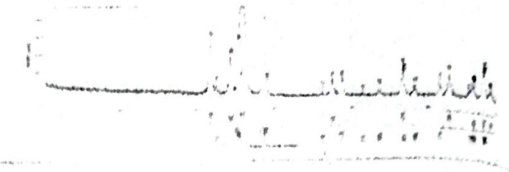


Figure 3 X-ray diffractogram of ZnO-CuO nanocomposite

Table 6 Crystallite Size of ZnO-Cu Nanocomposite

No	Diffraction angle '2θ' (°)	Full Width at Half Maximum 'β' (°)	Full Width at Half Maximum 'β' (radian)	Calculated Crystallite Size (nm)	Crystallite Size from XRD Data (nm)
1	31.740	0.326	0.0057	25.3	26.6
2	32.472	0.211	0.0034	39.1	44.5
3	34.390	0.323	0.0057	25.7	27.1
4	35.374	0.127	0.0022	65.5	100.0
5	35.491	0.199	0.0035	41.8	48.5
6	36.191	0.354	0.0062	23.6	24.6
7	38.670	0.330	0.0058	25.5	26.8
8	39.007	0.400	0.007	21.0	21.8
9	46.217	0.109	0.0020	79.3	100.0
10	47.449	0.414	0.0073	21.0	21.6
11	48.644	0.380	0.0067	22.8	23.8
12	51.372	0.253	0.0044	34.8	38.0
13	53.490	0.407	0.0071	21.8	22.6
14	56.560	0.399	0.0070	22.6	23.4
15	56.708	0.515	0.0090	17.5	17.9
16	56.767	0.288	0.0050	31.3	33.4
17	58.272	0.303	0.0053	29.9	31.8
18	61.527	0.302	0.0053	30.5	32.5
19	62.793	0.428	0.0075	21.7	22.4
20	65.682	0.530	0.0093	17.8	18.2
21	66.179	0.577	0..010 1	16.4	16.7
22	66.270	0.553	0.0097	17.1	17.4
23	66.369	0.503	0.0088	18.8	19.3
24	66.438	0.554	0.0097	17.1	17.4
25	67.832	0.513	0.0090	18.6	19.0
26	68.020	0.422	0.0074	22.7	23.4
27	68.090	0.492	0.0086	19.4	19.9
28	68.922	0.492	0.0086	19.5	20.0
29	69.030	0.425	0.0074	22.6	23.3
Average crystallite size				27.3	30.4

Table 7 Comparison of Crystallite Sizes of ZnO Nanoparticles, CuO Nanoparticles and ZnO-CuO Naocomposite

No	Sample	Crystallite size (nm)
1	ZnO Nanoparticles	25.8
2	CuO Nanoparticles	23.0
3	ZnO-CuO Nanocomposite	27.3

Table 8 shows lattice parameters of ZnO nanoparticles,CuO nanoparticles and ZnO-CuO nanocomposite. By XRD analysis ZnO nanoparticles was indexed as hexagonal with equal length of 'a' and 'b'(3.2562 Å) and longer 'c' (5.2117 Å).CuO nanoparticles was indexed as monoclinic with unequal lengths of 'a', 'b' and 'c' . ZnO-CuO nanocomposite was hexagonal with equal length of 'a' and 'b' (3.2383 Å) and longer 'c' (5.4953 Å).

Table 8 Lattice Parameters of ZnO Nanoparticles, CuO Nanoparticles and ZnO-CuO Nanocomposite

No	Sample	Axial length (Å)			Interaxial angle(°)		
		a	b	c	α	β	γ
1	ZnONanoparticles	3.2562	3.2562	5.2117	90	90	120
2	CuONanoparticles	4.6801	3.4223	5.1121	90	99.58	90
3	ZnO-CuO Nanocomposite	3.2383	3.2383	5.4953	90	90	120

Characterization by scanning electron microscopy

Figure 4 shows the SEM images of CuO nanoparticles, ZnO nanoparticles and ZnO-CuO nanocomposite with 1000 X magnification. CuO nanoparticles showed a large dense cluster of very small spherical particles.ZnO nanoparticles were observed as aggregate particles with nearly spherical shapes. After incorporation of CuO, morphology of the synthesized ZnO-CuO nanocomposite differed from morphologies of their individual constituents. It was found that the CuO nanoparticles were well-mixed with ZnO in the nanocomposites.

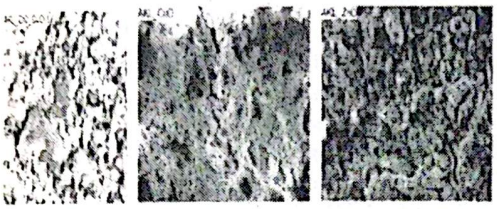
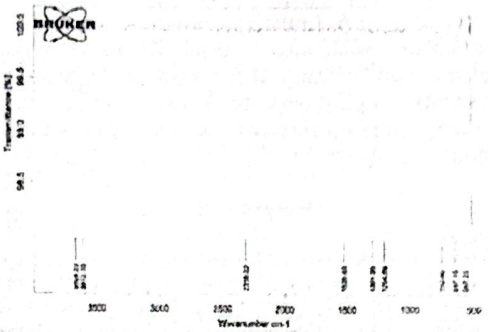


Figure 4 SEM images of
(a) CuO nanoparticles
(b) ZnO nanoparticles and
(c) ZnO-CuO nanocomposite
(magnificationn 1000 X)



Characterization by FT IR spectroscopy

Figures 5,6 and 7 show the FT IR spectra of ZnO nanoparticles, CuO nanoparticles and ZnO-CuO nanocomposite, respectively, and the corresponding FT IR spectral data are shown in Table 9. Zn-O stretching vibration was observed at 448 cm^{-1} in FT IR spectrum of ZnO nanoparticles. Zn-O stretching vibration was reported in the range of 400-460 cm^{-1} (Effendy *et al.*, 2017). Moreover, Cu-O stretching vibrations were observed at 648 and 567 cm^{-1} (Karthik *et al.*, 2011; Ansilin *et al.*,2016).The stretching vibration of Zn-O shifted to 470 cm^{-1} and that of Cu-O to 527 cm^{-1} in the ZnO-CuO nanocomposite.

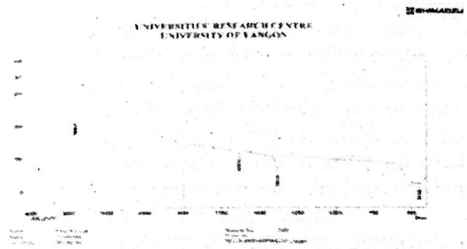


Figure 5 FT IR spectrum of ZnO nanoparticles

Figure 6 FTIR spectrum of CuO nanoparticles

Table 9 FT IR Spectral Data of ZnO Nanoparticles , CuO Nanoparticle and ZnO-CuO Nanocomposite

No	Observed wavelength (cm^{-1})			Reported value (cm^{-1})	Remark
	ZnO nanoparticles	CuO nanoparticles	ZnO-CuO nanocomposite		
1	-	648	679	400-700 *	Cu-O stretching
2	-	567	527	538**	Cu-O stretching
3	448	-	470	400-460 ***	Zn-O stretching

* Kumar and Rani, 2016; ** Ansilin *et al.*,2016; *** Effendy *et al.*, 2017

Conclusion

ZnO nanoparticles, CuO nanoparticles and ZnO-CuO nanocomposite were successfully prepared by sol-gel method using zinc acetate dihydrate and copper(II) sulphate pentahydrate as precusors.Phase purity, crystallite site and crystal structure of as-synthesized samples were characterized by X-ray diffraction analysis.Formation of single phase of ZnO in XRD pattern of ZnO was observed and the pattern was well-matched with the standard ZnO pattern.Crystallite site of ZnO was calculated by Scherrer formula and found to be 25.8 nm and its crystal structure was hexagonal with equal length of 'a' and 'b' (3.2562 Å) and longer 'c'(5.2117Å). XRD pattern of CuO indicated the characteristic peaks of CuO with no impurities. CuO was indexed as monoclinic with'a'= 4.6801 Å,'b' = 3.4223 Å and 'c'=5.1121Å and crystallite size was calculated to be 23.0 nm.XRD analysis exhibited presence of pure copper oxide and zinc oxide within the ZnO-CuO nanocomposite .Crystallite size of ZnO-CuO nanocomposite was 27.3 nm which was larger than those of ZnO and CuO . A large dense cluster of very small spherical particles of CuO nanoparticles and aggregate

particles with nearly spherical shapes of ZnO were observed. In ZnO-CuO nanocomposite CuO nanoparticles were well-mixed with ZnO nanoparticles. FT IR analysis showed the characteristic vibrations of Zn-O at 470 cm^{-1} and that of Cu-O at 527 cm^{-1} in the ZnO-CuO nanocomposite.

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References

Akir, S., Barrs, A., Coffinier, Y., Bououdina, M., Boukherroub, R. and Dakhlaoui- Omrani, A..(2016)."Eco-friendly Synthesis of ZnO Nanoparticles with Different Morphologies and their Visible Light Photocatalytic Performance for the Degradation of Rhodamine B". *Ceram. Int.*, **42**(8), 10259-10265

Ansilin, S. , Nair, J.K. , Aswathy, C. , Rama, V. , Peter, J. , Persis, J. J (2016)."Green Synthesis and Characterisation of Copper Oxide Nanoparticles using *Azadirachta indica* (Neem) Leaf Aqueous Extract ". *Journal of Nanoscienc and Technology* ,**2**(5), 221–223

Effendy,N.,Wahab, Z.A., Abdul Aziz, S.H., Matori,K.A., Mohd Zaid,M.H.andAbdul Rashid, S.S. (2017). " Characterization and Optical Properties of Erbium Oxide Doped ZnO–SLS Glass for Potential Optical and Optoelectronic Materials ". *Mater. Express*, **7** (1), 59-65

Gao, Y. P. , Sisk, C. N. and Hope-Weeks, L. J.(2007). " A Sol-gel Route to Synthesize Monolithic Zinc Oxide Aerogels ". *Chemistry of Materials*, **19** (24), 6007–6011

Ghane, M., Sadeghi, B. , Jafari, A. R. and Paknejhad, A. R. (2010). " Synthesis and Characterization of a Bi-oxide Nanoparticle ZnO/CuO by Thermal Decomposition of Oxalate Precursor Method ". *International Journal of Nano Dimensions*, **1**(1),33-40

Karthik, K., Jaya, N. V., Kanagaraj ,M. and Arumugam, S. (2011). " Temperature- dependent Magnetic Anomalies of CuO Nanoparticles". *Solid State Commun.*, **151** , 564-568

Kumar, H. and Rani, R.(2013). " Structural and Optical Characterization of ZnO Nanoparticles Synthesized by Microemulsion Route ". *International Letters of Chemistry, Physics and Astronomy* , **14** , 26-36

Owens ,G J., Singh, R. K., Foroutan, F., Alqaysi ,M., Han, C. M., Mahapatra, C., Kim, H. W. and Knowles, J. K.(2016). " Sol-gel Based Materials for Biomedical Applications ". *Prog. Mater. Sci.*,**77**, 1

Roy,R.,Roy,R.A.and Roy,D.M.(1986)."Alternative Perspectives on Quasi-crystallinity:Non uniformity andNanocomposites ". *Materials Letters.*,**4**(8-9),323-328

Widiarti, N., Sae, J. K. and Wahyuni, S. (2017). "Synthesis CuO-ZnO Nanocomposite and its Application as an Antibacterial Agent". *IOP Conf. Series: Materials Science and Engineering* ,**172**, 1-11

Study on Nutritional Values, Total Phenolic Content and Antioxidant Activity of *Melastoma Malabathricum* L.

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Abstract

Melastoma malabathricum L. a medium to large size tree as medicinal plant was chosen to investigate nutritional values, total phenolic content and antioxidant activities .The nutritional values were determine by AOAC method , such as moisture , ash, protein , crude fiber , fats and carbohydrate in sample . The content of these value are 8.33% , 4.55%, 9.88%, 25.25% , 0.49%, 9.39% respectively . The total phenol content of ethanol , ethyl acetate and watery extracts were determined by Folim – Ciocalteu method observing that TPC value of ethanol extract (17.2746 ± 0.00023µg GAE/mg) , ethyl acetate (31.39 ± 0.00023µg GAE/mg) and watery extract (23.94 ± 0.00023µg GAE/mg) , respectively . The antioxidant properties of ethanol , ethyl acetate and watery extracts of leaves were investigated by DPPH free radical scavenging assay . The IC_{50} value of these extracts were observed to be 2.127µg/mL , 0.63 µg/mL and 0.750 µg/mL respectively .

Keywords : *Melastoma malabathricum* L. , nutritional values , total phenolic content , antioxidant activities

Introduction

Medicinal plants may be defined as a group of plants that possess some special properties or virtues that quality them as articles of drugs and therapeutic agents, and are used for medicinal purposes. Plants have formed the basis of sophisticated traditional medicine (TM) practices that have been used for thousands of years by people in China. India and many other countries. Plants products still remain the principlesource of pharmaceutical agents used in traditional medicine.

Melastoma malabathricum L. belongs to family Melastomataceae. Melastoma commonly known as the Butter nut tree. Melastoma is a medium to large sized deciduous tree distributed in Nepal, India and Sri Lanka. *Melastoma* is a large, shady, deciduous tree doting much of the central India landscape, both wild and cultivated. Leaves clustered at end of the branches, young branches, leaves and petiole pubescent or tornentoes. Leaves coriaceous, elliptic, shortly acuminate, base cuneate. There are a lot of uses for *M malabathricum* reported in folk medicine, but not supported by clinical data.

Leaves are chewed up, pounded, and applied as paste on cuts or wounds or finely chopped up and squeezed to apply the juice onto the wound to stop bleeding. Leaves are used to prevent scarring from smallpox, to treat dysentery, diarrhoea, piles, and as a tonic. Young leaves are eaten to treat diarrhea. Leaves are also useful to treat ulcers, gastric ulcers, scar, pimple, and black spot at skin. Powdered leaves alone is used as astringent for dysentery.

Materials And Methods

Plant Material

The leaves of *Melastoma malabathricum* L were collected from the Yangon Township. The collected plant was identified as *Melastoma malabathricum* L by the authorized botanist at Department of Botany, Yangon University. The dried samples were cut into pieces and pulverised into powder using a grinding mill. The dried powdered samples were then stored in the air tight container.

Preparation of Plant Extract

The dried powder(100g) of *Melastoma malabathricum* L was extracted with 70% EtOH at R.T(3 x 300 ml) for one week and filtered. The filtrate was evaporated by rotatory evaporator to get 70% EtOH extract. The dried powdered of the plants was also boiled with water to get watery extract. The dried powdered samples (300g) were extracted with methanol (500 ml) for one week by maceration followed by filtration. This procedure was repeated three times. The total combined filtrate was concentrated by rotatory evaporator the one-third volume and further partitioned between pet-ether and water to get the particular soluble extract. The aqueous layer was further dry over water bath to get dry methanol extract. The resulting extract was partitioned with ethyl acetate and ethyl-acetate soluble portion was concentrated by rotatory evaporator and dried over water-bath to get ethylacetate extract. The dried extracts obtained were then stored and kept in the refrigeraior for the isolation of phytochemical organic constituents and for screening of bioactivity.

Nutrient Values and Physicochemical Characterization of Leaves Sample

(a) Determination of Moisture Content

The moisture content of the dried powder was determined by using Air Oven Method.

Procedure

Amount of 2-5 g well prepared test portion was placed in dish. The sample was dried in an air oven at 130°C for 1 hour. After drying was completed, lids were replaced on dishes and transferred to desiccators and then cooled for 30 minutes. It was weighed accurately until constant weight is achieve.

(b) Determination of Total Ash Content

The determination of percentage of the total ash and acid-insoluble ash values of powdered sample is carried out according to the Ashing method (AOAC 2000, 930.05)

Procedure

The crucible appearance was checked. The empty crucible and cover were heated in Muffle furnace at 574 ± 25 °C for 15 minutes and placed in a desiccators and then cooled for 45 minutes when a porcelain or silica crucible was used or for 15 minutes in when using platinum. Nearest 0.1 mg of the prepared test sample was weighed. A suitable test specimen may be about 3 to 5 mg. At that time, test specimens were weighed for moisture determination. The test specimen was transferred to the prepared crucible and placed it, with the cover removed. The test specimen could also be carbonized on a gas burner over a low flame or other technique. The crucible with sample was placed in the Muffle furnace without cover. The temperature was raised gradually to 575 ± 25 °C. Heating should be done after placing the crucible with sample into the Muffle furnace. If Muffle furnace has been up before hand sample is burned immediately and serenely. Muffle furnace should be cooled down before putting sample into it. The material was allowed to ignite at 575 ± 25 °C for a period of 4 hours or longer, if needed to turn away all the carbon. Complete ignition was indicated by the absence of black particles. After ignition was finished completely, the crucible was kept in the Muffle furnace for 2 hours for cooling down to 300 °C. The lids of Muffle furnace were not opened during the cooling down. After cooling down, the crucible was replaced into desiccators. The crucible was covered and cooled for 30 minutes. The calculation of ash results are shown in Table 1.

(c) Determination of Protein Content

Procedure

The protein content was determined by AOAC method (AOAC, 1990). The procedure was as follows: The representative sample (1 g), 50 mL of distilled water, 5 g of digestion mixture (4 g of potassium sulphate and 1 g of copper sulphate) and 15 ml of concentrated sulphuric acid were added into a preheated flask and gently shaken to 'wet' the sample with acid. The exhaust system was attached to the digestion tubes in the rack and wet the water aspirator to full effect. The loaded rack was exhausted into a preheated digestion block (420 °C). After about 5 minutes turn down the water aspirator until the acid fumes were just contained within the exhaust head. Digestion was continued until all samples were clear with a blue or green solution. The rack of tubes with exhaust still in place in the stand was removed to cool for 10-20 minutes. Using a commercial air blower can increase cooling. 80 mL of deionized water was added to the tubes. 25-30 mL of receiver was added to a conical flask and placed it into a distillation unit and then raised the platform so that the distillate outlet was submerged in the receiver solution. 50 mL of 40 % NaOH was dispersed into the tube. The steam valve on the Kjeldahl 1002 was opened and distilled for approximately 4 minutes. At the end of the distillation cycle when using Kjeldahl 1002 was closed the steam valve. The receiver solution in the distillate flask will now be green indicating the presence of an alkali-ammonia. The distillate was titrated with standardized HC 1 (usually 0.100 N or 0.200 N) until the blue or grey end point is achieved. The amount of the protein content was given in Table 1.

(d) Determination of Fiber Content

Procedure

The powdered samples (Ca. 2 g) were weighed and. extracted with petroleum ether (30 mL) by stirring, settling and decanting for three times. The extracted sample was air dried and transferred to a round-bottom flask. Sulphuric acid (0.1275 M) was added to disperse the sample followed by hot sulphuric acid (0.1275 M, 170 mL). The solution was then refluxed for about 30 minutes and filtered through a Buchner funnel. The insoluble matter was washed with boiling water until the final wash liquid was free from acid. The residue was rinsed back into the flask with sodium hydroxide (0.313 M, 30 mL) and 170 mL of boiling sodium hydroxide was added. The mixture was again refluxed for 30 minutes and filtered using a Gooch crucible with an asbestos pad. The residue was washed with boiling water, 1 % hydrochloric acid and again with boiling water until wash liquor was free from acid. It was then washed twice with ethanol and three times with ether. The crucible was dried at 100 °C and weighed. The fiber content was determined by the loss in weight on ignition at 100 °C.

(e) Determination of Fat Content

Accurately weighed 3 g of powdered sample (in a fine form) were weighed accurately and placed into a thimble lined with a circle of filter paper. Thimble and content were then placed in a Soxhlet extractor. Petroleum ether (60-80 °C) was poured into the extractor until some of it over flowed into the flask. The petroleum ether was heated by means of a mantle. The extraction was assumed to be completed when a small amount of extract placed on a watch glass did not leave any residue on evaporation of solvent. A duration of about 6-8 hours was required for the complete extraction during which time the petroleum ether was recycled for about 27 times.

(f) Determination of Carbohydrate

Carbohydrate content of sample can be obtained as the difference between 100 and the sum of the percentage of moisture, ash, protein, fiber and fat contents (Vogel, 1956).

(g) Determination of Energy Value

Energy value of the sample was determined via calculation.

Screening The Antioxidatant Activity of Crude Extracts of *M.malabathricum* Leaves by DPPH Assay.

Preparation of Solutions

(i) Preparation of 60 µM DPPH solution

2.364 mg of DPPH was thoroughly dissolved in 100 mL of 95% ethanol. This solution was freshly prepared in the brown coloured flask. Then it must be stored in the fridge for no longer than 24 hours.

(ii) Preparation of Test Sample Solution

Accurately weighed 2 mg of each test sample and 10 mL of 95% EtOH was thoroughly mixed by shaker. The mixture solution was filtered and the stock solution was obtained. Desired concentrations 20µg/mL, l0µgmL4, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL and 0.625 µg/mL of each solution was prepared from this stock solution by dilution with appropriate amount of 95% ethanol.

(iii) Preparation of Blank Solution

Blank solution was prepared by mixing 1.5 mL of test sample solution with 1.5 mL of 95% ethanol.

Procedure

DPPH radical scavenging activity was determined by UV spectrophtometric method. The control solution was prepared by mixing 1.5 mL of 60 µM DPPH solution and 1.5 mL of 95 % ethanol using shaker. The sample Solution was also prepared by mixing thoroughly 1.5 mL of 60 µM DPPH solutions and 1.5 mL of test sample solution. The solutions were allowed to stand at room temperature for 30 minutes. After 30 minutes, the absorbance of these solutions was measured at 517 nm by using UV spectrophotometer. Absorbance measurements were done in triplicate for each solution and then mean values so obtained were used to calculate percent inhibition of oxidation by the equation and then IC_{50} (50 % inhibitory concentration) value were also calculated by linear regressive excel program. The percent inhibition of oxidation and 50 % inhibitory concentration of *M-malathricum* leaves were also presented in Figure 1 and Table 2.

Determination of Total Phenol Contents of The Samples by FCR Method

The total phenol contents of the samples (TPC) were determined UV-spectrometrically according to the Folin-Ciocalteu Method (Basma *et al*., 2011 and and Poonam, 2014).)

Determination of Total Penolic Contents as Gallc Acid Equivalent in Samples

The total phenolic content in each sample was estimated by Folin-Ciocaitcu method. Each extract (10 mg) was mixed with 10 mL of distilled water. Each extract solution (1 mL) was mixed with 3 mL of FCR solution and incubated for 5 minutes. To each tube, 6 mL of I M sodium carbonate was added and the tubes were kept in room temperature for 2 hours. The absorbance was measured at 765 rim spectrophotomerticially (KWF UV-7504). The experiment was done in triplicate. Results were expressed as microgram of gallic acid equivalents (GAE) per milligram of extract (GAE: gmL dry extract). The concentrations of gallic acid equivalent (GAE) of each of the plant extracts were calculated by using the linear regression equation from the standard curve of gallic acid. Total phenol content (TPC) in the plant samples were expressed as mg gallic acid equivalent per 10 mg dry plant extracts (GAE/mg) (Basma *et al*., 2011 and Kaur and Poonam, 2014) and recorded.

Results

Nutritional Values of *Melastoma malabathricum* Leaves

The quantitative analyses for the determination of total ash and moisture contents have been done according to methods described in the British Pharmacopoeia (1980), Myamnar medicine formulary (1989) and Ayuvedic formulation (1976). The total ash in the sample is the inorganic residue remaining after the organic matter has been burnt away.

The nutritional values such as protein, fiber, fat and carbohydrates were also determined by appropriate methods. The fat content determined by the soxelt extraction method was 0.49 %. In addition, the sample was also studied for fiber content by acid alkali treatment, protein content by AOAC method and ash content by using Muffle furnace. The protein, fiber, carbohydrates, moisture and ash contents for *Melastoma malabathricum* leaves were found to be 9.88 %, 25.25 %, 9.39 %, 8.33 % and 4.55 %, respectively. The results are shown in Table 1.

Table 1 Nutritional Values of the Leaves of *Melastoma malabathricum* L.

No.	Test Parameter	Content (%)
1.	Moisture	8.33
2.	Ash	4.55
3.	Protein	9.88
4.	Fiber	25.25
5.	Fat	0.49
6.	Carbohydrate	9.39
7.	Energy	81.49 kcal/100g

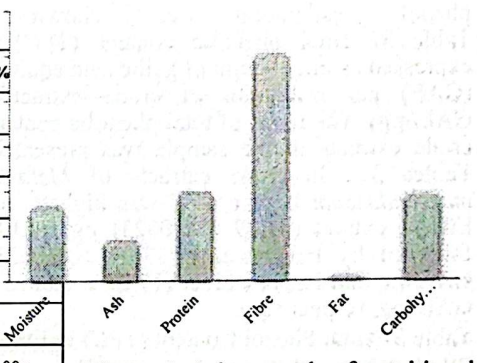

Figure 1 A bar graph of nutritional values of the leaves of *Melastoma malabathricum* L.

Antioxidant Activity of Crude Extracts from *Melastoma malabathricum* Leaves

The present study was carried out to investigate the radical scavenging activity of the leaf samples using two crude extracts such as ethanol, ethyl acetate and water extracts, by using DPPH assay according to the spectrophotometric method. In this experiment, six different concentrations (0.625 µg/mL, 1.25 µg/mL, 2.5 µg/mL, 5 µg/mL, 10 µg/mL and 20 µg/mL) of each crude extract were prepared in ethanol solvent. Ascorbic acid was used as standard and ethanol without crude extract was employed as control. Determination of absorbance was carried out at wave length 517 nm using UV visible spectrophotomeer. Each experiment was done triplicate. The results are shown in table 2 and figure 1 and 2.

The IC_{50} values were found to be 2.127 µg/mL for ethanol extract, 0.631 µg/mL for ethyl acetate extract and 0.750 µg/mL for aqueous extract. Among these extracts, since the lower the $1C_{50}$ showed the higher the free radical scavenging activity ethanol extracts were found to be more effective than aqueous extracts in free radical scavenging activity. However, it was observed that all of these extracts have the higher antioxidant activity than standard ascorbic acid ($1C_{50}$-2.25µg/mL)

Table 2 Radical Scavenging Activity (%RSA) and IC_{50} values of Ethanol, Ethyl acetate and Watery Extracts from Leaves and Standard Ascrobic Acid

Samples	% Inhibition (Mean ± SD) In Different Concentration (µg/ml)						IC_{50} (µg/ml)
	0.625	1.25	2.5	5	10	20	
Ethanol	35.481 ± 0.033	45.747 ± 0.002	51.804 ± 0.010	68.556 ± 0.003	88.597 ± 0.004	90.335 ± 0.001	2.127
Ethyl acetate	39.911 ± 0.004	58.591 ± 0.021	67.010 ± 0.010	71.993 ± 0.011	92.388 ± 0.028	94.450 ± 0.011	0.631
Watery	48.195 ± 0.009	57.216 ± 0.001	61.941 ± 0.003	65.102 ± 0.012	63.487 ± 0.004	72.336 ± 0.003	0.750
Ascrobic acid	34.361 ± 0.00	53.859 ± 0.00	62.379 ± 0.00	66.870 ± 0.00	72.751 ± 0.00	82.509 ± 0.00	2.25

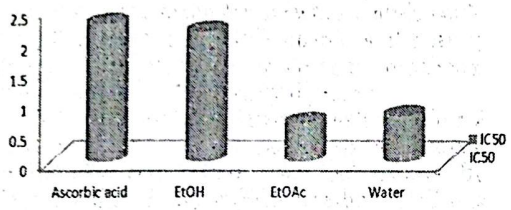

Figure 3 IC_{50} values of ethanol , ethyl acetate and watery extracts of leaves and standard ascorbic acid

In this study, the total phenolic content of the leaves was estimated by FolinCiocalteu method. Phenols react with an oxidizing agent phosphomolybdate in F-C reagent under alkaline conditions and result in the formation of blue coloured complex. The molybdenum blue which is measured at 765 nm colorimetrically. Gallic acid (3,4,5-trihydroxybenzoic acid) was used to construct standard calibration curve for total phenol estimation as shown in Table 3. Total phenolic content (TPC) was expressed as micro gram of gallic acid equivalent (GAE) per milligram of crude extract (µg GAE/mg). The result of total phenolic content of crude extracts of the sample was presented in Tables 3 . In crude extracts of *Melastoma malahathricum* L., the TPC was highest in the EtOAc extract (31.39 ± 0.0023) µg GAE/mg, followed by H_2O extract (23.94 ± 0.0023)µg GAE/mg and EtOH extract (17.27 ± 0.0023) µg GAE/mg, respectively.

Table 3. Total Phenol Content (TPC) in Ethanol, Ethyl acetate and Watery Extracts of Leaves

No	Extracts	TPC (µg GAE/mg ± SD)
1	Ethanol	17.27 ± 0.0023
2	Ethyl acetate	31.39 ± 0.0023
3	Watery	23.94 ± 0.0023

Conclusion

From the overall assessment of some chemical and biological investigation of banana flower, the following inferences could be deduced. The nutritional values of *Melastoma malahathricum* L contains moisture (8.33%), ash (4.55%), fat (0.49 %), fibre (25.25), protein (9.88), carbohydrate (9.33%), and energy value is (81.49%) kilocalories in 100 g of sample. According to the results, the presence of the important nutrients like fat, fibre, protein,

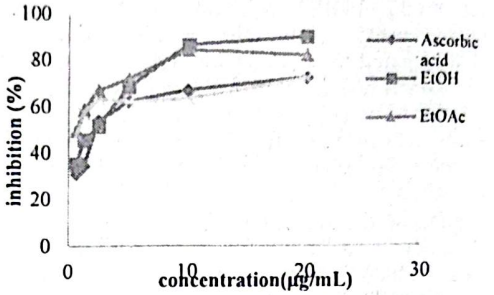

Figure 2 % Radical scavenging activity of ethanol, ethyl acetate and watery extracts of leaves and standard ascorbic acid

carbohydrate and the physical properties like moisture and ash mean the selected sample could be used as a nutritionally valuable and healthy ingredient to improve traditional medicinal formulation and to treat many diseases.

Total phenol content of watery, ethanol and ethyl acetate crude extract of *Melastoma malahathricum* L leaves was determined by Folin-Ciocalteu Reagent (FCR) method. TPC was expressed as milligram of gallic acid equivalent per gram of crude extract (mg GAE/g). Total phenol content of watery extracts (23.94 ± 0.0023 mg GAE/g) , ethanol extract 17.27 ± 0.0023 mg GAE/g and ethyl acetate extract 31.39 ±0.0023 mg GAE/g were obtained. In the antioxidant activity, the IC_{50} values were found to be 2.127µg/mL for 95 % ethanol extract and 0.750 µg/mL for watery extract and 0.631 µg/mL for ethyl acetate and 2.25 µg/mL for standard ascorbic acid. Among these extracts, since the lower the IC_{50} showed the higher free radical scavenging activity, watery extract and ethyl acetate was found to be slightly more effective than the 95 % ethanol extract and standard. From these observation, *Melastoma malahathricum* L. leaves has a lot of nutritional values and medicinal qualities.

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Reference

Adeshina, G. O., Jibo S., and Ehinmidu. J. O. (2011). "Antibacterial Activity of Fresh Juices of A ilium cepa and Zingiber officinale Against Multidrug Resistant Bacteria". Journal of International Pharma and Bio Sciences, 2(2), 289 – 294

Ashokkumar, R. and Ramaswamy. M. (2013). "Determination of DPPH Free Radical Scavenging Oxidation Effects of Methanolic Leaf Extracts of some Indian Medicinal Plant Species". Journal of Chemical, Bological and Physical Sciences, 3 (2), 1273 - 278

Basma, A. A., Zakaria. Z., Latha. L. Y., and Sasidharan. S. (2011). "Antioxidant Activity and Phytochemical

Screening of the Method Extracts of Euphorbia hirta L.". Asia Pacific Journal of Tropical Medicine., 386 – 390

Chen, J. C., Yeh T. Y., Chen P. C., and Hsu. C. K. (2007)."Phenolic Content and DPPH Radical Scavenging Activity of Yam-containing Surimi Antiarthritjc Activities of Centellaasiatica Extracts". Journal of Chem. Rio. Phy. Sd. 1 (2), 260 - 269

Gels Influenced by Salt and Heating".Asian Journal of Health and Information Science,2, 1-8

Chippada S. C. and Vangalapati. M. (2011). "Antioxidant, An Anti-inflammatory and Dockery, M. and Tomkins. S. (2000) Brine Shrimp Ecology. London: Ist Ed., The British Ecology Society, 92 – 93

Feingold, S., Martin. W., and Scott. E. (1978). Diagnostic Microbiology. London: the C.V Mosby Co., 124 – 126

Justin, S. and Shenbagaraman. S. (2011). "Evaluation of Antioxidant and Antimicrobial Activities of the Selected Green Leafs' Vegetables". International Journal of Pharm Tech Research, 3 (1), 148 – 152

Kamiya, K., Tanaka. Y., Endang. H., Umar. M. and Satake. T. (2004). "Chemical onstituents of Morinda citrfolia Fruits Inhibit Copper-Induced Low-Density Lipoprotein Oxidation". Journal ofAgriculture and Food Chemistry, 52, 5843

Kaur, S. and Poonam. M. (2014). "Study of Total Phenolic and Flavonoid Content, Antioxidant Activity and Antimicrobial Properties of Medicinal Plants". Journal of Microbiology and Experimentation, 1(1), 1 – 6

Marinova, G. and Batchvarov. V. (2011). "Evaluation of the Methods for Determination of the Free Radical Scavenging Activity By DPPH". Bulgarian Journal of Agricultural Science, 17 (1), 11-24

Pouorad, F., Hosseinimehr. S. S., and Shahabimajd. N. (2006). "Antioxidant Activity, Phenol and Flavonoid Content of some Selected Iranian Medicinal Plants". Africa Journal of Biotechnology, 5(11), 1142 – 1145

"Saha, M. R., Hasan. S. M. R., Akter,. R. and Mazumder. M. E. H. (2008). "In Vitro Free Radical Scavenging Activity of Menthnol Extract of the Leaves of Mimusops elengi L.". Bangl. J. Vet. Med, 6 (2), 197-202

Sahgal, G., Ramanathan. S., Sasidharan/ S., Mordi. M. S., Ismail. S., and Mansor. S. M. (2010). "Brine Shrimp Lethality and Acute Oral Toxicity Studies on Swietenia mahagoni (Linn.)Jacq. Seed Methanolic Extract". Pharmacognosy Res, 2, 215 - 220

Comparative Study of Cellulose Hydrogel Films Prepared from Rice Straw and Sugarcane Bagass

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Takaomi Kobayashi, Professor, Department of Materials Science, Nagaoka University of Technology, Japan

Abstract

Rice straw (RS) and sugarcane bagasse (SB) are biomass waste products mainly deriving from rice production and sugar production. In this study, conversion of these waste products to valuable products like cellulose hydrogel films is important issues to be concerned. They were used as cellulose resources which were chemically treated using acid (H_2SO_4) and alkali (NaOH). When these pre-treated samples were bleached by sodium hypochlorite (NaOCl), treated cellulose fiber was obtained. Yield percent of treated cellulose fiber depend on the nature of biomass materials. Percent cellulose fibers were found to be 27.63% in rice straw and 32.31% in sugarcane bagasse. SEM and FTIR analyses showed that the amount of liginin and hemicellulose from these samples were successfully reduced by chemical treatment and also proved that the final products were cellulose fibers. XRD measurements were carried out to evaluate the effect of treatment conditions on the crystalline structure of treated samples. The crystalinity index of rice straw cellulose fiber is greater than that of sugarcane bagasse cellulose fiber. Cellulose fibers using with dimethylacetamide/lithium chloride (DMAc/ LiCl) system were used to obtain cellulose hydrogel solutions. Following this, the hydrogel films were prepared by phase inversion method without cross-linker; these films were transparent and flexible. Rice straw cellulose hydrogel films (RSCF) has less water retainable property than sugarcane bagasse cellulose hydrogel films (SBCF). Based on the viscoelasticity measurements, it was observed that the RSCF showed soft and elastic nature in the deformation than the SBCF. Antimicrobial activities of cellulose solutions were tested for the purpose of using hydrogel films to biomedical applications.

Keywords - cellulose hydrogel film, phase inversion method, rice straw, sugarcane bagasse antimicrobial activities

Introduction

In recent years, there has been an increase in the level of research on the development of new biodegradable materials for use in packaging, agriculture, medicine and other areas. Generally, biodegradable polymer materials are increasingly important as environmental contamination and waste disposal problems associated with plastics and related products from synthetic polymers become more severe. Natural polymers have various advantages over synthetic polymers due to their low-cost, great availability and biodegradability [10]. Cellulose hydrogel has become especially attractive to "tissuse engineering" as matrices for repairing and regenerating a wide variety of tissue and organs. Hydrogels consisted of hydrophilic polymer networks which can absorb from 10-20 % up to thousands of times of their dry weight in water. Various hydrogels from natural polymers were fabricated by using hyaluronate, chitosan and its derivatives, and cellulose in which there is a potential application in the biomaterials field. Commonly, all plant biomass consists of cellulose, hemicellulose, lignin, pectin and protein. Most of the plant biomass consists of about 33 % of cellulose as the major component of the rigid cell walls. Cellulose is the most abundant renewable resource on earth and may become a main resource in the future. Therefore, this sustainable material in plants has numerous functional possibilities and can be expected with the demand for environmentally and biocompatible products [8]. A wide range of lingocellulosic agricultural by-products has successfully been converted into cellulose hydrogel film including agave tequilana Weber bagasse, bamboo, sugar cane bagasse, thanaka and rice straw [4-7].

Myanmar is the agricultural country and usually grows paddy crops. Agricultural residues are generated in the flied after being harvested. Also sugarcane bagasse, as the fibrous by-product remaining after sugar extraction from sugarcane, is one of the most important byproducts. And thus these biomass wastes are usually proliferation in many areas. As a result, as sustainable cellulose, rice straw and sugarcane bagasse are agricultural wastes which are applicable to a good product, they will be more valuable items which will lead to the development of new value added materials. For this purpose, this study was carried out to prepare the rice straw cellulose solutions and sugarcane bagasse cellulose solutions using DMAc/LiCl systems to prepare hydrogel films. Antimicrobial activities test of cellulose solutions has been carried out to be utilized to hydrogel films for cell regeneration.

Materials and Methods

Preparation of Treated Fiber

The collected raw materials, rice straws were washed with clean water. Then, they were dried at 40 °C in the drying cabinet. For the preparation of acid-treated sample, 10 g of rice straw were immersed in 500 ml, 4 vol% H_2SO_4 solution and stirred at 90 °C for 2 h by using the oil bath and overhead stirrer. After cooling down the sample to the room temperature, then they are washed with distilled water so many times until neutral in order to remove the acid residues. Then, samples are filtrated and collected acid treated samples (dark brown color). Similarly, for the base-treated sample, the acid-treated samples were again immersed in 500 ml, 10 vol% NaOH solution and stirred for 6 h at the same temperature (90 °C). They were washed, filtrated, done as previous steps and collected the base-treated samples (light brownish yellow color). Again, they were immersed in 200 ml, 10 vol% NaOCl and stirred at 40 °C for 1 h for the color bleaching. The color of the samples changes from light yellow to milky color. Then, they are dried at 50 °C inside the drying cabinet until completely dry and the products of treated rice straw fibers are obtained[4]. Figure 1 shows the schematic diagram for preparation of rice straw cellulose fiber (RSC).

For the bagasse treatment, firstly the bagasse was washed with warm water to remove remaining sugar components and then dried in oven at 40 °C. Preparation of acid-treated sample, base treated sample and bagasse cellulose fiber were carried out above procedures same as rice straw sample. Figure 2 shows the sugarcane bagasse raw sample, acid treated, base treated

sample and bagasse cellulose fiber (SBC). The obtained cellulose fibers (RSC and SBC) were used for preparation of cellulose solutions in DMAc/LiCl system.

Preparation of Cellulose Solution

100 mL of deionized water were added into 1 g of previous treated samples and stirred at room temperature overnight. Then they are filtered and washed with ethanol. After that, 100 mL of ethanol was added to the swelled fibers and the mixture was stirred for 24 h. Then, ethanol was removed and added to 50 mL of DMAc and stirred overnight. Finally, 8 wt% of LiCl and DMAc were added to the swelled cellulose fibers and adjusted the solution concentration. The mixture was stirred at room temperature until to obtain a hydrogel solution. Figure 3 shows the preparation of cellulose hydrogel solution.[4,9]

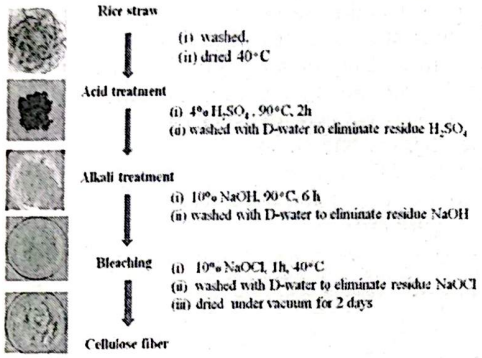


Figure 1. Schematic diagram for preparation of rice straw cellulose fiber (RSC)

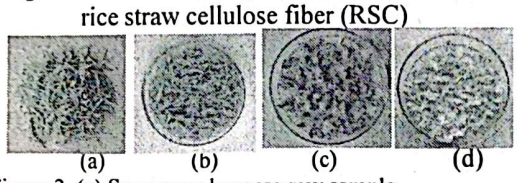


Figure 2. (a) Sugarcane bagasse raw sample (b) acid treated sample (c) base treated sample and (d) cellulose fiber (SBC)

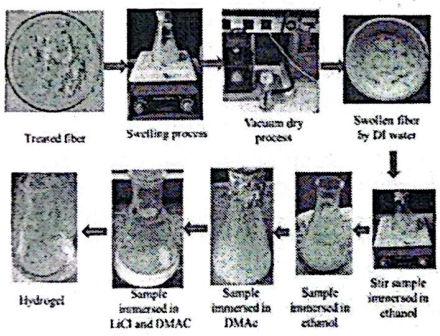


Figure 3. Photo showing the preparation of cellulose hydrogel solution

Preparation of Cellulose Hydrogel Films

For hydrogel films preparation, 10 g of cellulose solution was poured into a Petri dish (10 cm diameter), and kept in a container filled with ethanol for 24 h. After this, a transparent hydrogel films were obtained. Then, these films were washed with distilled water many times and immersed in distilled water and kept at the room temperature. Figure 4 illustrated the rice straw cellulose hydrogel film (RSCF) and sugarcane bagasse cellulose hydrogel film (SBCF) [4].

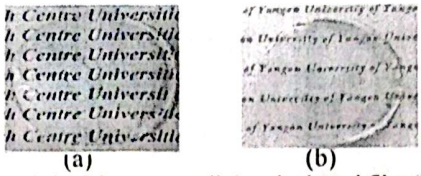


Figure 4. (a) Rice straw cellulose hydrogel film (b) sugarcane bagasse cellulose hydrogel film

Characterization Techniques

The formation of prepared samples was monitored by ultraviolet-visible (UV-Vis) spectroscopy (Shimadzu UV 1800 spectrophotometer). The structural changes of samples were analyzed by FTIR 8400 Shimadzu spectrophotometer by using a KBr pallet method in the MIR radiation with the wavelength from 4000 cm^{-1} to 400 cm^{-1} range with a resolution of 4.0 cm^{-1}. Surface morphology of the samples was investigated by SEM (JEOL 15 kV). The X-ray diffraction (XRD) patterns of raw sample and pretreated cellulose fibers were recorded with CuKα radiation ($\lambda = 1.5418$) at 40 kV and 30 mA in the range of $2\theta = 10°$- 40° by X-ray diffractometer (Smart Lab, Rigaku, Japan). The samples were dried in vacuum at room temperature before the measurement. The crystalinity index (CI) was calculated according to reported method using following equation: $CI\ (\%) = (I_{002}-I_{am})/I_{002}) \times 100$, where I_{002} is the maximum intensity of the peak (002) lattice diffraction and I_{am} is the intensity of diffraction attributed to amorphous cellulose [1]. Viscoelasticity of the hydrogel film with 2 cm in diameter with 5 mm in thickness was determined by Auto Paar-Reoplus equipment (AutonPaar Japan, Tokyo) in wet conditions at 37 °C.

Study on Antimicrobial Activity

The study on antimicrobial activity was performed by agar well diffusion method. Nutrients agar was prepared according to the method of Cruickshank 1975 [3]. Firstly, nutrient agar (medium) was boiled and 20-30 ml of the medium was poured into the test tubes which were plugged with cotton wool. Secondly, the test tubes with medium were autoclaved at 121 °C for 15 minutes and they were cooled down to 30-35 °C. Thirdly, the medium was poured into the sterilized petri-dishes and 0.1-0.2 mL of test organisms were also added into the dishes. The agar was allowed to set for 2-3 hours. Fourthly, 10 mm agar-well was made with the help of sterilized agar-well cutter. Finally, about 0.2 mL of samples were introduced into the agar-well and incubated at 37 °C for 24 to 36 hours. The inhibition zone appeared around the agar-well was measured for the indication the presence of antimicrobial activity.

Results and Discussion

UV spectra showing absorbance and transmittance values of cellulose solution were shown in Figure 5. The transmittance values were observed about 90 % which indicates the prepared cellulose film was transparent (Figure 5).

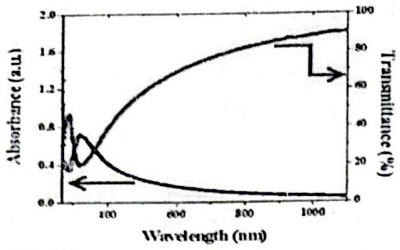


Figure 5. UV-vis spectrum of cellulose solution

The structural changes of each prepared (a) cellulose fiber (b) base-treated (c) rice straw and (d) acid-treated samples are shown in Figure 6. The broad peaks at 3400 cm^{-1} in all samples were due to the stretching vibrations O-H groups of water contents. However, the cellulose fiber exhibited higher O-H stretching band because of higher contents of O-H group resulted from cellulose. In other words, this OH group in the fibers might interact with the surrounded water, supporting the rice straw solutions for hydrogel films. The peak at 2900cm^{-1} was due to C-H stretching. The peaks around 1520 cm^{-1} showed the presence of lignin, hemicellulose in the initial rice straw and acid-treated samples. But, they were absent after base-treated and in the cellulose fiber sample. 1320 and 1430 cm^{-1} peaks were due to symmetric CH_2 bending and wagging. The weak band at 1080 cm^{-1} could be assigned to polysaccharides. The glycosidic bond of C–O–C stretching assigned at 1160 and 901 cm^{-1} [2]. Comparison of the FTIR spectra suggested that treated fiber was cellulose fiber due to lignin and hemicellulose have already been removed.

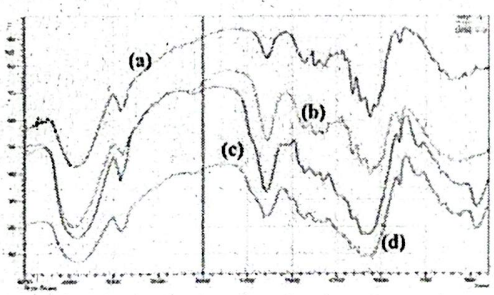


Figure 6 FTIR spectra of (a) cellulose fiber (b) base-treated (c) rice straw and (d) acid-treated samples

Figure 7 shows FTIR spectra of (a) the sugarcane bagasse, (b) acid treated fiber (c) base treated fiber and (d) cellulose fibers at different treatment conditions. From the comparison of the FTIR spectra, the appeared peaks around 1510 cm^{-1} showed the presence of lignin and lignocellulose in the initial raw sample. Moreover, the band around 1200 cm^{-1} disappeared in treated fiber which is assigned the removal of hemicelluloses. This clearly indicated that the amount of lignin from the sugar cane bagasse raw sample was successfully reduced by the chemical and temperature treatments.

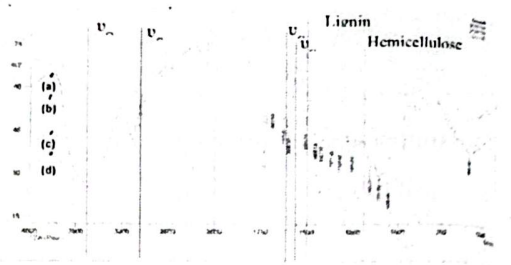
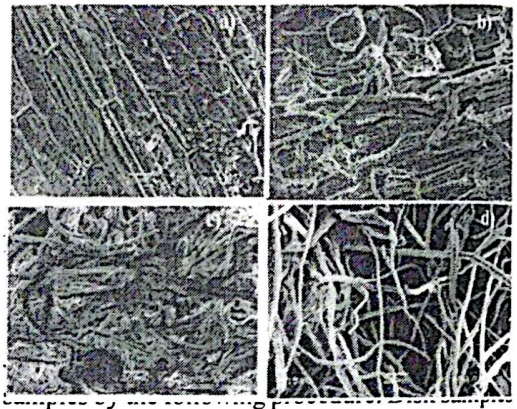


Figure 7 FTIR spectra of (a) sugar cane bagasse raw sample (b) acid treated sample (c) base treated sample and (d) cellulose fiber

The surface morphologies of the prepared samples were shown in SEM images (Figure 8). Morphological changes in the raw, acid and base pretreated samples were clearly seen in these SEM images. The fibrous patterns of plant cell wall appeared in the raw samples shown in Figure 8(a). Figure 8(b) and (c) represented the distorted structure of lignin and hemicellulose of plant cell wall structure due to pretreatment of acid and base. Pretreatment of NaOH tended to decrease the lignin and hemicellulose which in turn increased the content of cellulose. It also resulted into increase in cellulose accessibility like shown in Figure 8(d).



with 5 mm diameter were cut from cast films and dried in vacuum oven for 24 h and weighed (W_0). Then, samples were immersed in distilled water for 36 h. After that, films were removed from water and wrapped with filtered paper in order to remove excess water, and weighed it again (W_1). Finally, the equilibrium water content was calculated from the wet (W_1) and dried (W_0) hydrogel films. For each sample, four independent measurements were done and averaged by the following formula:

$$\text{Water content (\%)} = (W_1 - W_0)/ W_0 \times 100.$$

According to the calculation, water content was 83.63% for RSCF film and 128.47% for SBCF film. Thus, the film has water retainable property which will be formation of interaction of hydrogen bonding networks of the resultant cellulose in the hydrogel films.

XRD measurement was carried out to evaluate the effect of treatment condition on the crystalline structure of raw samples, treated fibers and cellulose fibers. Figure 9 shows the XRD patterns of rice straw and the purified fibers. The patterns of (a) to (d) exhibited typical crystalline lattice of cellulose with peaks at 22.3° and 16.4°. The crystalinity indexes of rice straw raw sample, acid treated sample, base treated sample and

cellulose fiber were 71.43, 75.7, 76.25 and 78.57 %, respectively. Figure 10 shows the XRD patterns of the bagasse and the purified fibers. The crystalinity indexes of sugar cane bagasse raw sample, acid treated sample, base treated sample and cellulose fiber were 44.1%, 58.8%, 59.1% and 60.2% respectively. The increment of crystalinity in cellulose fiber was due to the removal of hemicelluloses and lignin by NaOH and NaOCl treatment. The crystalinity index (CI) was calculated by using following equation:

$$CI\ (\%) = (I_{002} - I_{am}) / I_{002} \times 100$$

I_{002} = the maximum intensity of the peak (002) lattice diffraction

I_{am} = the intensity of diffraction attributed to amorphous cellulose

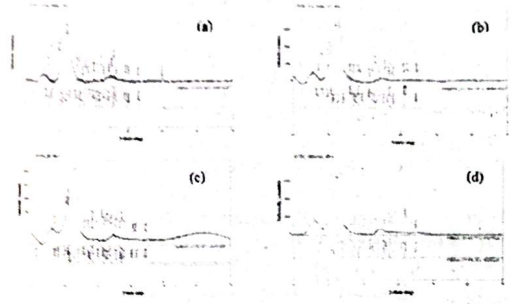


Figure 9 XRD patterns of (a) rice straw raw (b) acid treated fiber (c) base treated (c) fiber and (d) cellulose fiber

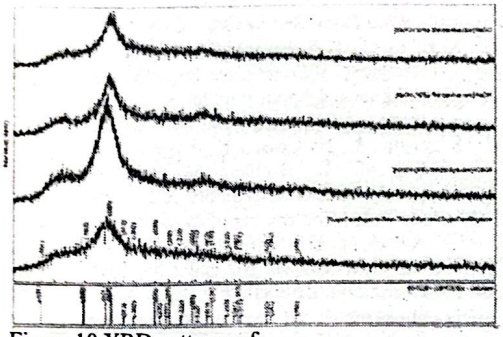


Figure 10 XRD patterns of
(a) sugar cane bagasse raw sample
(b) acid treated fiber
(c) base treated fiber and
(d) cellulose fiber

Viscoelasticity measurements indicates the relationship between G' and G" for the viscoelesticity of the hydrogel films. Figure 11(a) shows the viscoelastic data for rice straw hydrogel film (RSCF) and figure 11(b) shows the viscoelastic data for SBCF films of different LiCl contents. The crossover point of G' and G" meant fracture of materials or inability to follow deformation, since rigid polymer network may not flow. Therefore, crossoverpoint at larger strain indicated flexible structure of the hydrogel films. For RSCF, the crossover point of RSCF was found w% LiCl), the G' and G" values were overlapped at 35.71% strain. It seemed that the RSCF showed very soft and less elastic nature than SBCF.

Figure 12 exhibited the photos showing the antimicrobial activities of RS cellulose solution (left) and SB cellulose solution (right). Each petri dish represented to test results of antimicrobial activities against six organisms including (1) *Bacillus subtilis,* (2) *Staphylococcus aureus,* (3) *Pseudomonas aeruginosa, (4) Bacillus pumilus,* (5) *Candida albicans*and (6) *Escherichia coli* respectively. According to results, rice straw cellulose solution showed inhibition zone more than SB cellulose solution. Table 1 showed the measurements of inhibition zones of cellulose solutions,

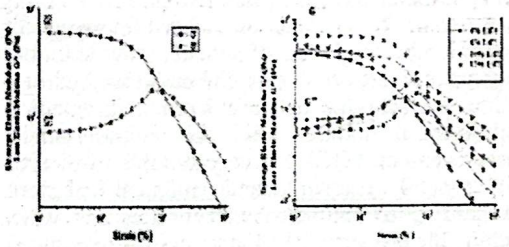


Figure 11 (a) Viscoelasticity of rice straw cellulose hydrogel film (RSCF) and

Table 1 Antimicrobial activity results for test samples

Samples	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus pumilus*	*Candida albicans*	*Escherichia coli*
RS cellulose solution	17 mm (++)	15 mm (+)	26 mm (+++)	17 mm (++)	16 mm (++)	15 mm (++)
SB cellulose solution	15 mm (++)	14 mm (+)	16 mm (++)	17 mm (++)	13 mm (+)	14 mm (+)

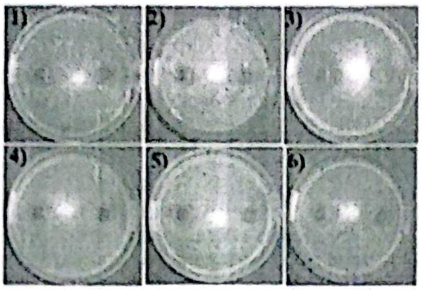


Figure 12 Photo showing the antimicrobial activities of RS cellulose solution (left) and SB cellulose solution (right)

Conclusion

By using LiCl/ DMAc system, rice straw cellulose hydrogel films (RSCF) and sugarcane bagasse cellulose hydrogel films (SBCF) were successfully prepared. H_2SO_4 and NaOH were good reagents to remove the lignin and hemicellulose from the initial raw materials according to the SEM images. FTIR spectra and the increment of their crystalinity in the pre-treated fibers exhibited that final products were cellulose fibers. Phase inversion method led to cellulose solution to hydrogel films. According to UV-vis result, the films were transparent and showed the high transmittance (90 %). The film has water retainable property based on the water content (%). Water retainable property of SBCF was more than that of RSCF. The RSCF showed very soft and less elastic nature than SBCF during the strain sweep measurements. Showing the antimicrobial activities of both cellulose solutions against six test organisms, the cellulose hydrogel films are possible to be utilized in biomedical applications. Base on the viscoelasticity measurement, SBCF are very suitable to be used in wound dressing and medical plasters. According to antimicrobial activities and water retainable property, RSCF are possible to apply as facial masks in biomedical applications.

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References

[1] Azubuike C.P., Rodriguez H. and Okhamafe A. O (2012). "Physicochemical, spectroscopic and thermal properties of microcrystalline cellulose derived from corn cobs", *International journal of recycling of Organic Waste in Agriculture*, 19, 425-433

[2] Binod P., Satyanagalakshmi K., Sindhu R., Janu K. U., Sukumaran R. K., and A. Pandey. (2012). "Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse," *Renewable Energy*., 37, 109-116

[3] Cruickshank R., Duguid J.P., Marmion B.P., Swain R.H.A. (1975). *Medical Microbiology*. NewYork: Churchill Livingstone; 2.

[4] Cho Cho., Cho Cho Thet, Nan Sanda Myint and Wut Yee Aye (2017). "Cellulose Hydrogel Films Prepared from Biomass Waste Rice Straw", *Universities' Research Journal*, vol. 10,2,pp.55-66

[5] Cho Cho and Kobayashi T. (2018) " Preparation and Characterization of Cellulose Hydrogel Films from Myanmar Thanaka Heartwood" Transaction on GIGAKU 5(1)05004/1-8

[6] Karla, L.T., Satoshi S.S., Tagaya M. and Kobayashi T. (2013). " Fibroblast Compatibility on Scaffold Hydrogels Prepared from Agave Tequilana Weber Bagasse for Tissue Regeneration "*J.Ind.Eng.Chem.Res.*,vol.52,pp.11607-11613

[7] Kazuki, N. and Kobayashi T. (2016). " Effect of Pre-treatment of Sugar Cane Bagasse on the Cellulose Solution and Application for the Cellulose Hydrogel Films". *J. Polym. Adv. Technol.*,vol. 27, pp. 973-980

[8] Svensson, A., Nicklasson E., Harrah T., Panilaitis B., KaplanD. L., Brittberg M. and Gatenholom P. (2005). "Bacterial Cellulose as a Potential Scaffold for Tissue Engineering of Cartilage". *Biomaterials*, vol. 26, pp. 419

[9] Thinzar Aye, Thinzar Nu and Cho Cho (2018). " Preparation of Cellulose Hydrogel Films from Sugarcane Bagasse". *J. Myanmar Acad.Arts Sci.*Vol. XVI, No.1B,371-378

[10] Zhou, R., Mo Y., Li Y., Zhao Y., Zhang G. and Hu Y. (2008). "Quality and Internal Characteristics of Huanghua Pears (Pyrus pyrifolia Nakai, cv. Huanghua) Treated with Different Kinds of Coating during Storage". *Postharvest Biology and Technology*, vol 49, pp. 171-179

Investigation of Some Phytoconstituents and Screening of Some Bioactivities of Heartwood of *Biancaea Sappan* L. (Teinnyet)

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Ni Ni Than, Dr, Professor and Head, Department of Chemistry, University of Yangon

Abstract

In this research heartwood of *Biancaea sappan* L. (Heartwood of Teinnyet, TNH) was chosen to be studied. Some phytoconstituents, nutrition value, EDXRF value, and bioactivity studies such as antimicrobial, antioxidant, total phenolic content and acute toxicity of heartwood sample of *B.sappan* were investigated. Antimicrobial activities of pet ether, ethyl acetate, 95 % ethanol, methanol and watery extracts from TNH were investigated against six species of microorganisms such as *Bacillus subtilis, Bacillus pumilus, Candida albicans, Escherichia coli, Staphylococcus aureus, and Pseudomonas areuginosa* by agar well diffusion method. According to DPPH free radical scavenging assay, ethanol extract of heartwood (IC_{50} = 29.40μg / mL) of *B. sappan* was found to have higher antioxidant activity than that of watery extract. Moreover, the total phenol contents in pet-ether and ethyl acetate, ethanol, and watery extracts of heartwood of *B. sappan* was determined by Folin-Ciocalteu Method. From this study, EtOH extract of TNH showed the highest total phenolic content 83.82 ± 0.015 μg GAE/mg. Acute toxicity of 95% ethanol extract of heartwood of *B. sappan* was also investigated by OECD guide line. Nowadays, there has been increasing interest in certain compounds having enormous health effects which include antioxidant and phytochemicals.

Keywords: *Biancaea sappan* L., phytoconstituents, antimicrobial activity, antioxidant activity, acute toxicity

1. Introduction

Biancaea sappan L. (Family: Fabaceae) is a species of flowering tree in the legume family. It is native to tropical Asia. *B. sappan* is widely distributed in Myanmar, Southwest China, Indonesia, Thailand, Vietnam, Sri Lanka, and India and the best used for blood purification. *B. sappan* is used for quenching thirst, treatment of jaundice, cough, respiratory ailments and wounds, curing blood pressure, heart diseases, amenorrhea, dysmenorrhea, blood stasis after delivery [3]. The heartwood is bitter, astringent, acrid refrigerant, vulnerary, depurative, constipating, sedative and hemostatic. It is useful in vitiated condition of pitta, burning sensation, wound, ulcers, leprosy, skin diseases, diarrhea, dysentery, epilepsy, convulsions, menorrhagia, diabetes and leucorrhoea. The leaves can be used to hasten ripening of fruits such as bananas and mangoes. The leaves contain a pleasant smelling volatile oil. The plant has many uses such as to produce a valuable reddish dye used for dyeing fabric and making red paints and inks. The red dye is obtained from its heartwood. The wood is applied a famous tooth paste and tooth powder in India. The heartwood contains (5-hydroxy-1,4-naphthoquinone) juglone that has antimicrobial activity. During the 17th century sappanwood was a major trade good, when it was exported from Southeast Asian nations abroad red seal ship to Japan. *B. sappan* is a thorny and shrubby small to medium-sized tree up to 4-10 m tall. The leaflets are sub-sessile, oblong, 10-20 mm long and 6-10 mm broad, oblique at the base and rounded to emarginated at the apex. The yellow flowers are fragrant and aggregated in terminal and auxiliary panicles. The fruits are woody beaked pods; each contains 3-4 yellowish-brown seeds [2]. *B. sappan* grows wildly in mountainous regions and as an ornamental tree it is cultivated in yard and gardens because of its yellow colored flowers.

Botanical Aspects of *Biancaea sappan* L. (Teinnyet)

Scientific classification

Botanical name : *Biancaea sappan* L.
Myanmar name : Teinnyet
English name :Sappanwood, Indian redwood
Part used : Heartwood

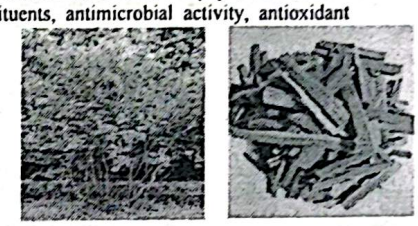

Figure 1 Images of *Biancaea sappan* L. (Teinnyet)

2. Materials and Methods

Sampling of Plant Material and Identification

The plant of *Biancaea sappan* L. (Teinnyet) belonging to the family Fabaceae was collected from Tamwe Township, Yangon region during January and February 2019. After collection, the scientific name of the plant was identified at the Botany Department, University of Yangon. The collected fresh samples of Teinnyet branches were peeled off and obtaining heartwood (TNH) was air-dried at room temperature for two weeks and then they were ground into powder by grinder. The dried powdered samples were stored in air-tight containers.

(a) Preliminary Phytochemical Investigation of the Heartwood of Teinnyet

In order to classify the types of organic constituents present in samples, preliminary phytochemical tests such as alkaloids, α- amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, phenolic compounds, reducing sugars, saponin, starch, steroids, tannins and terpenoids on heartwood samples were carried out according to the appropriate reported methods [5, 9, 17, 18, 21, 22].

(b) Determination of Some Nutrient Values of the Samples

Determination of some nutritional values present in heartwood samples such as moisture, ash, fiber, protein, fat and carbohydrate contents were carried out by Association of Official Analytical Chemists (AOAC) method [1, 7, 15, 16, 20, 23].

(c) Elemental Analysis of the Heartwood of Teinnyet by Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometry

For this measurement, pellets of the sample were first made. X-ray spectrometer permits simultaneous analysis of light element to heavy element. Energy dispersive X-ray fluorescence spectrometer (Shimadzu EDX - 700) can analyze the

elements from Na to U under vaccum condition. X-ray fluorescence uses X-rays to excite and unknown sample. The individual elements in the sample are detected by using semiconductor detector [Si - Li] that permits multi-elements, simultaneous analysis. Qualitative analyses of some elements in Teinnyet were measured by EDXRF method using EDX - 700 instrument at University of Monywa.

Preparation of Petroleum Ether, Ethanol, Methanol, Ethyl-acetate and Watery Soluble Extracts

Procedure

Accurately weighed 50 g of each of dried powdered samples was placed in five conical flasks and 200 mL each of PE (60 – 80 °C), ethyl acetate, 95 % ethanol, methanol and water solvent was added to each flask. These flasks were closed with aluminum foil, shaken for 6 h using shaker machine and then allowed to stand for 18 h. Each one was then separately filtered taking precautions against loss of solution. The volume of each extract solution was measured and 20 mL of each of the extract was separately evaporated in previously weighed evaporating basin. Then each basin was transferred in the oven and dried at 105 °C to achieve constant weight. On the basic of the dry weight of each 20 mL extract, the dry weight of the whole volume of each extract containing selected plant constituents was calculated. The percentages of each solvent soluble extracts were calculated with reference to the dried sample used [4].

Screening of some Biological Activities of the Sample

Screening of antimicrobial activity, antioxidant activity, total phenol content and acute toxicity on the heartwood of Teinnyet were studied.

(d) Screening of antimicrobial activity of the heartwood of Teinnyet

The antimicrobial activity screening of different crude extracts *viz.*, pet-ether, ethyl acetate, 95 % ethanol, methanol and watery extracts of TNH were carried out against six species of microorganism *viz., Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans, Escherichia coli* by employing agar well diffusion method at Myanmar Pharmaceutical Industrial Enterprise Research Department (MPIERD), Yangon[12, 13, 14, 24].

Procedure

The antimicrobial activity of the crude extracts was performed by the agar well diffusion assay. The pathogenic test organisms were incubated in trypticase soy broth at appropriate temperature for 24 h. Nutrient agar medium containing meat extract (0.5 g), peptone (0.5 g), sodium chloride (0.25 g), agar (1.5 g) and 100 mL of distilled water were placed in a beaker and the contents were heated for 30 minutes. The nutrient agar medium was put into sterilized conical flask and plugged with cotton wool and then autoclave at 121 °C for 15 minutes. After cooled down to 40 °C, one drop of suspended strain was inoculated to the nutrient agar with the help of a sterilized disposable pipette near the burner. About 20 mL of medium was poured into the sterilized petri-dishes and allowed to set the medium. Once solidified the dishes were stored for 2 h in a refrigerator. Five wells of 10 mm diameter each were cut out in the inoculated agar to place extract samples to be tested. The volume of each extract sampled placed in each well was 0.2 mL. Five samples extracts, namely, PE extract, EtOAc extract,95 % EtOH extract, MeOH extract and H_2O extract were tested. The petri-dishes were then incubated at 37 °C for 24 h, and the diameters of clear inhibition zone around the well, if appeared were measured with calipers in millimeter. The antimicrobial activity was determined by measuring the clear zones around the wells.

(e) *In vitro* screening of antioxidant activity of some crude extracts from the heartwood of Teinnyet by DPPH assay

DPPH (1, 1-diphenyl-2-picrylydrazyl) free radical scavenging assay was chosen to assess the antioxidant activity of 95 % EtOH and H_2O extracts of the heartwood sample[6, 8].

Preparation of Solutions

(i) Preparation of test sample solution

Accurately weighed 20 mg of each test sample was separately dissolved in 20 mL of 95 % EtOH under vigorous shaking. The mixture solution was filtered and the stock solution was obtained. Desired concentrations (250 µg / mL, 125 µg / mL, 62.5 µg / mL, 31.25 µg / mL and 15.62 µg / mL) of sample solutions were prepared from this stock solution by serial dilution with appropriate amount of 95 % EtOH.

(ii) Preparation of 120 µM DPPH solution

DPPH (4.73 mg) was thoroughly dissolved in 100 mL of 95 % EtOH. This solution was freshly prepared in the brown flask and kept in refrigerator for no longer than 24 h.

(iii) Preparation of standard solution

Accurately weighed 20 mg of ascorbic acid was dissolved in 10 mL of 95 % EtOH and used as a standard solution.

(iv) Preparation of blank solution

Blank solution was prepared by mixing 1.5 mL of the test sample solution with 1.5 mL of 95 % EtOH.

Procedure

DPPH radical scavenging activity was determined by UV spectrophotometric method. The control solution was prepared by mixing 1.5 mL of 120 µM DPPH solution and 1.5 mL of 95 %EtOH. The sample solution was also prepared by mixing thoroughly 1.5 mL of 120 µM DPPH solution and 1.5 mL of test sample solution. The solutions were allowed to stand at room temperature for 30 minutes. After 30 minutes, the absorbance of these solutions was measured at 517 nm by using UV spectrophotometer. Absorbance measurements were done in triplicate for each solution and then mean values so obtained were used to calculate percent inhibition of oxidation by the following equation.

$$\%\ \text{Oxidative Inhibition} = \frac{A_{DPPH} - \left(A_{\text{test sample}} - A_{\text{Blank}}\right)}{A_{DPPH}} \times 100$$

A_{DPPH} = absorbance of DPPH in EtOH solution

$A_{\text{test sample}}$ = absorbance of (sample+DPPH) solution

A_{Blank} = absorbance of (sample + EtOH) solution

$$\text{Average}, \bar{X} = \frac{X_1 + X_2 + X_3 + \cdots + X_n}{n}$$

Standard deviation (SD) =

$$\sqrt{\frac{(\bar{X} - X_1)^2 + (\bar{X} - X_2)^2 + (\bar{X} - X_3)^2 + \cdots + (\bar{X} - X_n)^2}{n-1}}$$

where, $\bar{X}$ = average % inhibition oxidation

$X_1, X_2, X_3, \ldots, X_n$ = % inhibition of test sample solution

n = number of times

Then, IC_{50} (50 % oxidative inhibitory concentration values) was calculated by linear regressive excel programme.

(f) Determination of total phenol content by FCR method

One of the antioxidant factors, total phenol content of pet-ether, ethyl acetate, ethanol and watery extracts from heartwood of *Biancaea sappan* L. was measured spectrophotometrically according to the Folin-Ciocalteu reagent method [19].

Procedure

(i) Construction of gallic acid standard curve

First, 0.5 mL of different concentration of gallic acid solution (31.25, 62.50, 125, 250, 500 and 1000 µg/mL) was added with 0.5 mL methanol and mixed with 5 mL of diluted Folin-Ciocalteu reagent (FCR: H_2O, 1:10 v/v) and incubated for 5 minutes. 4 mL of 1 M sodium carbonate was added to each tube and the tubes was kept at room temperature for 2 h and the UV absorbance of reaction mixture was read at λ_{max} 765 nm. A standard curve was prepared by plotting the absorbance against concentration of gallic acid.

(ii) Determination of total phenol content as gallic acid equivalent in sample

The total phenol content (TPC) in each crude extract was estimated by Folin-Ciocalteu reagent method. First 1 mL of prepared extract solution was mixed with 5 mL of FCR reagent (1:10) v/v and incubated for 5 minutes. 4 mL of 1 M sodium carbonate solution was added to each tube and the tubes were kept at room temperature for 2 h and the UV absorbance of reaction mixture was read at 765 nm. The blank solution was prepared as the above procedure by using distilled water instead of sample solution. Total phenolic content was estimated as microgram gallic acid equivalents per milligram of different extract (µg GAE/ mg).

(g) Acute Toxicity of the Heartwood of *Biancaea sappan* L.

In this study, acute toxicity effect of ethanol extract of *Biancaea sappan* L. (TNH) (two doses) were determined on albino mice at Laboratory Animal Services Division, Department of Medical Research (DMR), Yangon[10, 11].

Procedure

Acute toxicity of different doses of ethanol extracts of *Biancaea sappan* L. (TNH) was evaluated by the methods of OECD Guidelines for the Testing of Chemicals 423. According to the test description, total number of 18 adult female albino mice, weighting (25-30 g) were selected and divided into three groups. Each group contained three animals. They were fasted for 18 h before giving the extracts. Group (A) mice were orally administrated with 95 % EtOH extract of TNH 2000 mg/kg dose. Group (B) mice were given orally with 95 % EtOH extract of TNH 5000 mg/kg dose. Group (C) mice performed as a control group and they were treated with clean water and normal animal food. All groups of mice were kept in the three mouse cages in the separated room at the room temperature of 26 ± 1° C. After administration of extract on each group of animals were observed first 6 h continuously for mortality and behavior changes. Then check the animals each 24 h for fourteen days. The mortality during this period was noted (Nil or percent death). The results obtained from acute toxicity are described in Table 4.

3. Results and Discussion

In this research, the heartwood of *Biancaea sappan* L. (Local name: Teinnyet) collected from Tamwe Township, Yangon region were used in dried powdered form.

Phytochemicals Present in the Heartwood of Teinnyet

From preliminary phytochemical investigation, TNH contained alkaloids, α-amino acids, carbohydrates, glycosides, phenolic compounds, saponins, reducing sugars, terpenoids and tannins but flavonoids, steroids, starch, and cyanogenic glycosides were absent in sample.

Nutrient Values of the Heartwood of Teinnyet

The nutrient values of TNH were estimated by AOAC method. From this observation, moisture content (8.82 %), ash content (5.48 %), the fat content (3.11 %), the crude fiber content (27.12 %), the protein content (1.01 %), the carbohydrate (54.46 %) and the energy values of TNH are 233 kcal / 100 g, respectively.

Elemental Analysis of the Heartwood of Teinnyet

In this study relative abundance of elements present in TNH was determined by EDXRF spectrometer. From the experiment, qualitative EDXRF elemental analysis revealed that Teinnyet (heartwood) contained Si, Ca as major constituent elements, S and K as trace elements and Fe, Cu, and Hg as very trace elements. According to the results TNH is benefit human health due to the presence of some elements necessary for human health.

Various crude extracts from the heartwood of Teinnyet

To prepare some crude extracts from heartwood of Teinnyet, crude extracts were directly extracted by employing shaker. In Teinnyet heartwood, (3.45 g, 6.9 %) in yield of 95 % EtOH extract, (1.15 g, 2.3 %) in yield of H_2O extract, (2.2 g, 4.4 %) in yield of EtOAc extract and (1 g, 2 %) in yield of PE extract, and (3.5 g, 7 %) in yield of MeOH were obtained and these extracts were kept for bioactivity studies.

Biological Activity of Heartwood of Teinnyet

Some biological activities such as antimicrobial activity, antioxidant activity total phenol content and acute toxicity of various extracts of TNH were investigated.

***In vitro* antimicrobial activity of various crude extracts from the heartwood of Teinnyet**

Screening of antimicrobial activity of various crude extracts of TNH has been done by agar well diffusion method. In this investigation, the different crude extracts were tested on six species of microorganisms *viz., Bacillus subtilis, Bacillus pumilus, Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli.* Among the antimicrobial activity of all tested five crude extracts, EtOH extracts of TNH exhibited the highest antimicrobial activity against six species of microorganisms inhibition zone diameters in the range of 27-30 mm whereas, PE extract showed the lowest activity (Inhibition zone diameter 12-18 mm). The results obtained are shown in Table 1 and Figure 2

Table 1 Microbial Inhibition Zone Diameters of Different Crude Extracts from the Heartwood of Teinnyet

Microorganisms	Types of Microorganisms	Inhibition Zone Diameters (mm)				
		PE	EtOH	EtOAc	H_2O	MeOH
Bacillus subtilis	Gram (+) ve	15	30	15	22	27
Staphylococcus aureus	Gram (+) ve	12	29	15	25	25
Pseudomonas aeruginosa	Fungus	-	27	20	20	25
Bacillus pumilus	Gram (-) ve	20	30	17	22	25
Candida albicans	Gram (-) ve	17	30	17	22	26
Escherichia coli	Gram (+) ve	18	30	20	25	30

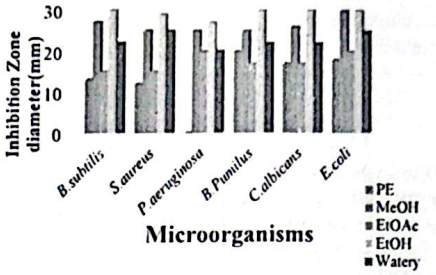

Figure 2 Microbial inhibition zone diameters (mm) of different crude extracts from the heartwood of Teinnyet from the heartwood of Teinnyet was expressed in terms of percent inhibition. The percent oxidative inhibition and IC_{50} values of both crude extracts are summarized in Table 2. EtOH extract of Teinnyet Heartwood (IC_{50}= 29.40 µg mL^{-1}) was found to have higher antioxidant activity than that of water extract. From this observation, due to the presence of the antioxidant activity the extract of TNH may be used in prevention of diseases related to coronary heart disease, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and even in various types of cancer.

In vitro antioxidant activity of some crude extracts from the heartwood of Teinnyet

The antioxidant activities of 95 % EtOH, and H_2O extracts of the heartwood of Teinnyet were studied by DPPH (1, 1-diphenyl-2-pricryhydrazyl) free radical scavenging UV spectrophotometric assay method. This method is based on the reduction of coloured free radical DPPH in ethanolic solution by different concentrations of each sample. The antioxidant activity was expressed as 50 % oxidative inhibitory concentration (IC_{50}). The lower the IC_{50} value, the higher the antioxidant activity of the sample. Antioxidant activity of both crude extracts

Table 2 Percent Oxidative Inhibition and IC_{50} Values of Crude Extracts from the Heartwood of Teinnyet and Standard Ascorbic Acid

Extracts	Percent Oxidative Inhibition (%) (mean ±SD) in different concentrations (µg/mL)					IC_{50} (µg/mL)
	15.62	31.25	62.5	125	250	
EtOH	35.41 ±4.18	67.33 ±3.82	77.41 ±0.06	93.21 ±0.85	98.51 ±0.49	29.40
H_2O	18.9± 0.32	30.18 ±0.06	52.66 ±0.10	75.89 ±0.19	99.02 ±0.25	58.79
Ascorbic acid	14.04 ±2.09	54.83 ±2.48	72.44 ±3.83	77.13 ±1.47	87.4± 2.37	16.17

Total phenol content of crude extract from the heartwood of Teinnyet

The total phenol contents of watery, ethanol, pet-ether and ethyl acetate crude extracts of heartwood of Teinnyet were evaluated with spectrophotometric method using Folin-Ciocalteu reagent. The principle of this method is reduction ability of phenol functional group. Phenols react with an oxidizing agent phosphomolybdate in Folin-Ciocalteu reagent under alkaline condition and resulted in the formation of blue colored complex. The absorbance can be measured at UV 765 nm. Gallic acid (3, 4, 5 – trihydroxybenzoic acid) was used to construct standard calibration curve. Gallic acid standard curve is shown in Figure 3. Total phenol content was expressed as microgram gallic acid equivalent per milligram (µg GAE/mg) was detected in ethanol extract than watery extract. This means that phenolic compounds were more soluble in ethanol. Comparison of TPC in ethanol, watery, petroleum ether, and ethyl-acetate extracts from the heartwood of *Biancaea sappan* L. is represented in Table 3.

Table 3 Result of Total Phenolic Content in Various Crude Extracts from Heartwood of Teinnyet by Folin- Ciocalteu Method

No.	Samples	TPC (µg GAE /mg ± SD) TNH
1	EtOH	83.82 ± 0.015
2	H_2O	60.12 ± 0.017
3	PE	23.68 ± 0.002
4	EtOAc	39.98 ± 0.001

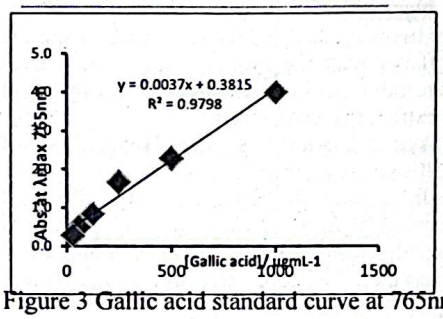

Figure 3 Gallic acid standard curve at 765nm

Acute Toxicity of the Heartwood of Teinnyet

Acute toxicity screening of EtOH extracts of heartwood of Teinnyet was done with the dosage of 2000 mg/kg and 5000 mg/kg body weight in each group of albino mice. The results show no lethality of the mice was observed up to fourteen days administration. Each group of animals were also observed still alive and did not show any visible symptoms of toxicity like restlessness, respiratory disorders, convulsion, aggressive activities, coma and death. The results obtained are shown in Table 4.

Table 4 Acute Toxicity Effect of Ethanol Extract of Heartwood of Teinnyet on Albino Mice Model after Two Weeks Administration

No	Groups	Extract Administration TNH	Dosage (mg/kg)	No. of death	% of death after 14 days
1	Group A	EtOH Extract	2000	Nil	0
2	Group B	EtOH Extract	5000	Nil	0
3	Group C	No administration	Nil	Nil	0

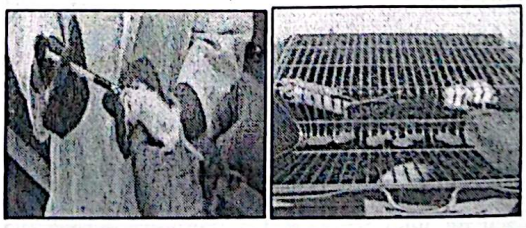

Figure 4 Acute toxicity test of ethanol extract of heartwood of Teinnyet on albino mice model.

4. Conclusion

The present study on *Biancaea sappan* L. (Heardwood of Teinnyet, TNH) provides the following information:

- The preliminary phytochemical investigation revealed that alkaloids, α-amino acids, carbohydrates, glycosides, phenolic compounds, saponins, reducing sugars, terpenoids and tannins were present and flavonoids, steroids, starch, and cyanogenic glycosides were absent in TNH.
- From the results of nutritional value in TNH it was found that sample contained 8.82 % of moisture, 5.48 % of ash, 3.11 % of fat, 27.12 % of crude fiber, 1.01 % of protein and 54.46 % of carbohydrates.
- Elemental analysis by EDXRF method revealed that TNH contained Si, Ca, K, S, Fe, Cu and Hg as consitutent elements.
- According to the antimicrobial activity tests on five crude extracts if TNH, EtOH extracts of TNH exhibited the highest antimicrobial activity against six species of microorganism inhibition zone diameters in the range of 27-30 mm whereas, PE extract showed the lowest activity.
- In the *in vitro* antioxidant activity screening by using DPPH free radical scavenging assay method on two crude extracts of TNH, EtOH extract of TNH was found to have higher antioxidant activity than that of watery extract.
- From the study of total phenolic contents on four crude extracts of TNH, EtOH extract of TNH was found to be the highest content (83.82 μg GAE / mg) than that of other extracts.
- From the screening of acute toxicity test on the 95 % EtOH extract of TNH, the results showed no lethality of the mice was observed up to fourteen days after administration and extract may be free from acute toxic.
- According to this study, the presence of phytoconstituents, antimicrobial and antioxidant activities, high total phenol content and absence of acute toxicity activity confirm that *B .sappan*, TNH has valuable medicinal of properties. Moreover, the presence of phytochemicals such as tannins, and phenolic compounds act as antioxidants. These phytochemicals are reported to have anticancer, antimicrobial, anti-inflammatory and antiallergic properties. The results of this study revealed that the extracts of TNH may be used as a natural source of antioxidant to prevent progression of many diseases.

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References

[1] AOAC. (1990). *Official methods of analysis of the Association of Official Analytical Chemist. Association* of Official Analytical Chemists (AOAC). Washington, D.C: 15th Ed., 1298

[2] Badami S, Moorkoth S, Suresh B. (2004). *B. sappan* L. Medicinal dye yielding plant. Natural Product Radiance; 3: 75-82.

[3] Badami S, Rai SR, Moorkoth S, Rajan S, Suresh B. (2003). Pharmacognostical Evaluation of *B. sappan* heartwood. Ancient Science of Life; 2: 1 – 7

[4] British Pharmacopoeia. (1973). *Determination of Soluble Matter Contents.* London: Pharmaceutical Press, 858-862

[5] Finar, I. L. (1969). *Organic Chemistry, Stereochemistry and Chemistry of Natural Product.* London: 4th Ed., Longman Green and Co.Ltd, 308-338

[6] Hu J, Xiaoling Y, Wei W, Hao WU, Lei H, Lijun D. (2008). Antioxidant Activity In- Vitro of Three Constituents from *B. sappan* L. *Tsinghua Science and Technology*, 13: 474–479.

[7] Lee, R. (1975). *Food Analysis: Analytical and Quality Control Methods.* London: Leonard Hill Books Co. Ltd, 431

[8] Lobo, V., A. Patil and N. Chandra. (2010). "Free Radicals Antioxidant and Functional Foods: Impact on Human health". *Pharmacogn. Rev.*4 (8), 118-126

[9] Marini-Bettole, G.B., M. Nicolettic and M. Patamia. (1981). "Plant Screening by chemical Chromatographic Procedure Under Field Condition". *J. Chromato.* 121, 21-214

[10] OECD. (1998). Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances as Endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Part 2, p.11

[11] OECD. (2000). Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No 24

[12] Panda, S. K. (2012). "Screening Method in the Study of Antimicrobial Properties of medicinal Plants." *IJBTR.* 2 (1), 1-35

[13] Panda, S. K and S. K. Dutta. (2011). "Antibacterial Activity from Bark Extracts of Pterospermumacerifolim (L.) Willd". IJPSR. 2 (2), 584-595

[14] Parekh J, Jadega D, Chanda S. (2005). Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turkish Journal of Biology; 29: 203 – 210.

[15] Pearson, D. (1962). *The Chemical Analysis of Food.*London: J and A Churchill Ltd, 347

[16] Raghuramula, N., K. M. Nair and S. Kalyanosandaram (1983). *Manual of Laboratory Techniques.* India: National Institute of Naturation, Indian Council of Medical Research, 31-33

[17] Robinson, R.L. (1983). *The Organic Constituents of Higher Plants.* North Armberst: 5th Ed., Cordus Press, 285-286

[18] Shriner, R.L., R.C. Fuson, V. Curtin and T.C. Morrill. (1980). *The systematic Identification of Organic Compounds A Laboratory Manual.* New York: John Willey and Sons, 385-425

[19] Song, F. L.,R.Y. Zhang, Q. Xiao, L.Kuang and H.B.Li .(2010). "Total Phenolic Contents and Antioxidant Capacities of Selected Chinese Medicinal Plants". *Int.J.MolSci.*, 11, 2367-2372.

[20] Steyermark, A.I. (1962). *Quatitative Organic Macro Analysis. London*: 2nd Ed., Academic Press, 188

[21] Tin, W.M. (1972)."Phytochemical Screening Methods and Procedures. "*Phytochemical Bulletin of Botanical Society of America,* 5 (3), 4-10

[22] Trease, G. E and W.C. Evans. (1980). *Pharmaconosy.* London: 1st Ed.,
Spottiswoode Ballantyne Ltd, 1089-529

[23] Triebold, H.O. and L.W. Aurand. (1984). *Food Composition and Analysis.* London: 2nd Ed., Van Nostrand Reinhold Co. Ltd, 132

[24] Valgas, C., S. M. D. Souza, E.F.A. Smanial and A. Smania.(2007). "Screening Methods to Determine Antibacterial Activity of Natural Products".*Braz.J.Microbia.* 38, 369-380

Phytochemical Screening and Evaluation of some Biological Activities of the Flower Buds of *Syzygium aromaticum*

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Abstract

The present study investigates the phytochemicals, mineral contents, antioxidant activity and antimicrobial activity of flower buds of *Syzygium aromaticum*. The preliminary phytochemical screening was carried out by reported methods indicating the presence of alkaloids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, starch, saponins, steroids, tannins and terpenoids. The qualitative elemental analysis was detected by EDXRF spectrometry. The nutritional values were determined by AOAC method. In the study of the *vitro* antioxidant activity, ethanol and watery extracts were screened by DPPH radical scavenging assay, with ascorbic acid as positive control. The ethanolic extract possessed excellent antioxidant property than watery extract. In addition, The total phenol content (TPC) of ethanol and watery extracts of the flower buds of *Syzygium aromaticum* were determined by FCR method. The antimicrobial activities of the various crude extracts (PE, EtOAC, EtOH and watery extracts) of the flower buds of *Syzygium aromaticum* were determined against six strains of microorganisms by agar well diffusion method. Among them ethyl acetate and ethanol extracts showed higher antimicrobial activities than other crude extracts.

Keywords: *Syzygium aromaticum*, antioxidant activity, antimicrobial activities, total phenol

Introduction

In Myanmar, traditional medicine has been an important health care service throughout history, and can be regarded as a priceless national asset unique to the Myanmar people. Nowadays, the government has encouraged in traditional medicine products with the genuine quality, safety and efficacy. Nowadays, many reports confirm the antibacterial, antifungal, antiviral and anticarcinogenic properties of spice plants. Clove in particular has attracted the attention due to the potent antioxidant and antimicrobial activities standing out among the other spices (Shan *et al,* 2005). *Syzygium aromaticum* (*S. aromaticum*) (synonym: *Eugenia cariophylata*) commonly known as clove, is an median size tree (8-12 m) from the Mirtaceae family native from the Maluku islands in east Indonesia. For centuries the trade of clove and the search of this valuable spice stimulated the economic development of this Asiatic region (Kamatou *et al*,2011). Cloves may be used to give aromatic and flavor qualities to hot beverages, often combined with other ingredients such as lemon and sugar. They are a common element in spice blends such as pumpkin pie spice and speculoos spices. A major component of clove taste is imparted by the chemical eugenol, Though long-used in traditional medicine, there is little evidence that clove oil containing eugenol is effective for toothache pain or other types of pain, although one review reported efficacy of eugenol combined with zinc oxide as an analgesic for alveolar osteitis (Taberner-Vallverdú, 2015). Studies to determine its effectiveness for fever reduction, as a mosquito repellent, and to prevent premature ejaculation have been inconclusive *(Wonjung, 2014)* It remains unproven whether blood sugar levels are reduced by cloves or clove oil. Use of clove for any medicinal purpose has not been approved by the US Food and Drug Administration, and its use may cause adverse effects if taken orally by people with liver disease, blood clotting and immune system disorders, or food allergies (MedlinePlus, 2014). This study provides antioxidant capacity and total phenolics contents and antimicrobial activity of the *Syzygium aromaticum.*

Materials and Methods

Sample collection of preparation

The flower buds of *Syzygium aromaticum* used in this study were collected from Gangaw Township, Magway Region. The collected sample was washed with water, chopped into small pieces using a stainless knife and air dried to a constant weight at room temperature for one month. The dried sample was subsequently milled into coarse powder using a grinding mill and then stored in an airtight plastic container for the experimental works.

Reagents

1,1-diphenyl-2-picryl-hydrazyl (DPPH), linoleic acid, gallic acid, ferrous chloride, sodium carbonate, dimethyl sulfoxide (DMSO), chloroform, Folin-Ciocalteu's phenol reagent, butylated hydroxytoluene (BHT) and ascorbic acid (Wei Hai Eurasia hihgtech Co.Ltd. from China) were purchased from the British Drug House (BDH) Chemical Ltd, England. All other chemicals used were of analytical reagent grade. In all investigations, the recommended standard methods and techniques involving both conventional and modern methods were provided.

Preparation of extracts

The dried powdered root (300 g) was percolated with 95% ethanol (1000 mL) for one week and filtered. This procedure was repeated for three times. The combined filtrates were evaporated under reduced pressure by means of a rotatory evaporator. Consequently, 95% ethanol soluble extract was obtained. Water extract of three samples was prepared by boiling 300 g of sample with 1000 mL of distilled water for 6 h and filtered. It was repeated three times and the filtrates were combined followed by heating on water bath and sand bath to give water extract. Each extract was stored in refrigerator for screening of biological activities.

Preliminary phytochemical investigation

The preliminary phytochemical tests were carried out according to the appropriate methods to know which types of phyto-organic constituents are present (Marini-Bettolo, 1982). Pet ether, ethyl acetate, ethanol crude extracts of flower buds of *S. aromaticum* were prepared for TLC investigation, which were loaded on the precoated TLC silica gel plate and the chromatography was carried out by using an appropriate standard solvent system. The developed chromatograms were first inspected under UV-254 nm and 365 nm light and then sprayed with detecting reagents to classify the compounds present and their functional groups (M-TinWa, 1972).

Qualitative elemental analysis by energy dispersive X-ray fluorescence (EDXRF) spectrometry

The energy dispersive X-ray fluorescence method was used to determine the elements present in flower buds of *S. aromaticum.*

Determination of nutritional values

Nutritional values such as moisture content, ash content, fiber content, protein content, and carbohydrate content an energy value of flower buds

of *S. aromaticum* were determined by AOAC method. (AOAC, 2002). Screening of antioxidant activity of the the flower buds of *S. aromaticum* by DPPH radical scavenging assay

Preparation of DPPH solution

The 60 µM DPPH solution was freshly prepared in the pure reagent bottle. The stock solutions of ethanol and watery extracts were prepared by adding 20 mg of each tested sample and 100 mL of ethanol thoroughly by using shaker. The sample solutions of each extract with different concentrations of 20, 10, 5, 2.5, 1.25 and 0.625 µg/mL were prepared by serial dilution with ethanol from the stock solution.

Determination of antioxidant activity

The DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical scavenging activity was determined by UV-visible spectrophotometric method according to the procedure described by (Marinova and Batchvarov) (Marinova and Batchvarov, 2011). The control solution was prepared by adding ethanol (1.5 mL) and 60 µM DPPH (1.5 mL). The test sample solutions were also prepared by mixing 1.5 mL of 60 µM DPPH solution and 1.5 mL of each sample solution. These mixture solutions were incubated at room temperature and were shaken on shaker for 30 minutes. Then, the absorbance of these solutions was measured at 517 nm by using UV-7504 spectrophotometer. The IC_{50} values and positive control were calculated by linear regressive excel program from plot of % radical scavenging activity against concentrations.

$$\% \text{ Inhibition} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

Where, $A_{Control}$ = absorbance of control solution

A_{Sample} = absorbance of tested sample solution.

Determination of total phenolic contents

The Folin–Ciocalteu method [11] was used to determine the total phenolic content with some modification. The different concentrations depending on solubility of extracts or fractions (500 µL) in water were mixed with 2.5 mL of Folin- Ciocalteu reagent (0.2 N). After 5 minutes 2 mL of Na2CO3 solution (75 g/ L) was added. The mixture solution were stand for 120 minutes in dark. The optical density was measured at 760 nm against a blank. The total phenolic contents were calculated on the basis of the calibration curve of gallic acid and expressed as gallic acid equivalents (GAE) in milligrams per gram of the sample.

Determination of Antimicrobial Activity of Various Crude Extracts of the flower buds of *S. aromaticum*

Antimicrobial activities of various crude extracts such as PE, EtOAc, EtOH, and H_2O were investigated by agar well diffusion method at the Pharmaceutical Research Department (PRD), Ministry of Industry, Yangon.

The agar well diffusion method was used to test the antimicrobial action of the extracts on 24 hours broth culture of the organisms used. Nutrient agar (4.6 g) and agar (1 g) were dissolved in 20 mL distilled water. The resulting nutrient agar medium was autoclaved at 121°C for 15 minutes and cooled in water bath (40 °C). After cooling, bacteria suspension of each bacteria strain (0.02 mL) was added and poured into Petri dishes. The seeded plates were allowed to dry in room temperature for 10 minutes. A standard cork borer of 10 mm diameter was used to cut uniform wells on the surface of the solid medium. The extracts of PE, EtOAc, EtOH, and H_2O (1 g) of each were dissolved in 1 mL of their respective solvent. Each of the different crude extracts (0.15 mL) were filled into the wells and incubated at 37 °C for 18-24 hours. Antimicrobial activity in terms of zones of inhibition (mm) was recorded after 24 hours of incubation (Lawrence *et al.*, 2009).

Results and Discussions

Phytochemical constituents of the flower buds of *S. aromaticum*

The flower buds of *S. aromaticum* contain alkaloids, α-amino acids, carbohydrate, flavonoids, glycosides, phenolic compound, saponins, starch, steroids, tannins, terpenoids, and organic acids, however, cyanogenic glycosides and reducing sugars were absent.

Nutritional values of the flower buds of *S. aromaticum*

The nutritional values determined by AOAC method indicated that the percentage of moisture, ash, protein, fiber, fat and carbohydrate were 3.98, 6.08, 5.81, 7.24, 12.07, 24.82 and the energy contents were determined to be 245 kcal/100g.

Elements present in the flower buds of *S. aromaticum* by EDXRF method

X-ray spectroscopy permits simultaneous analysis of light elements to heavy elements. In this work, relative abundance of elements present in the flower buds of *S. aromaticum* was determined by EDXRF spectrometer. Figure 1 and Table1 show that Ca and K are the major elements present in the flower buds of *S. aromaticum*. In addition trace element such as Fe, Mn, Cu, and Sr were also found in flower buds of *S. aromaticum*. Among them, the calcium content was the highest (48.34%) in this flower buds of *S. aromaticum.*

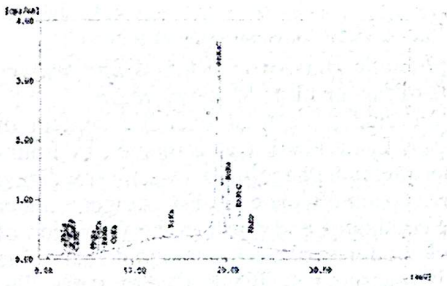

Figure 1. EDXRF spectrum of flower buds of *S. aromaticum*

Table 1. Relative Percent Abundance of Element in the flower buds of *S.*

Element	Relative Percent Abundance (%)
Ca	48.34
K	37.44
Fe	8.86
Mn	3.01
Cu	1.21
Sr	1.14

Antioxidant Activity of the flower buds of *S. aromaticum* by DPPH Method

Antioxidants are essential and important for plants and animals' sustenance that protect cells from the damage caused by unstable molecules known as free radicals. The antioxidant activity of watery and ethanol extracts of the flower buds of *S. aromaticum* were evaluated by DPPH (1, 1-

diphenyl-2-picryl-1hydrazyl) radical scavenging assay (Marinova and Batchvarov, 2011). The resultant % inhibition for different concentrations of the crude extracts are shown in Table 2 and Figure 2. The ascorbic acid was used as standards. It shows that % inhibition increased with increasing the concentrations of crude extracts. The IC_{50} values of ethanol and watery extracts are 42.71 µg/mL and 52.62 µg/mL respectively. Therefore watery extract has more potent antioxidant activity than ethanol extract. Hower antioxidant potency of these extract were weaker than that of standard ascorbic acid (IC_{50} = 9.71 µg/mL).

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Table 2. Oxidative Percent Inhibitions and IC_{50} Values of Crude Extracts of the flower buds of *S. aromaticum*

Test sample	% RSA ± SD of different concentrations (µg/mL)						IC_{50} (µg/mL)
	6.25	12.5	25	50	100	200	
H_2O	20.34±3.61	26.62±3.24	39.25±3.12	48.14±0.75	83.63±2.34	98.37±0.61	52.62
EtOH	21.01±3.35	24.01±2.02	41.39±1.96	53.54±0.79	83.19±0.86	92.16±0.83	42.71
Ascorbic acid	34.95±2.69	62.12±2.48	81.02±3.67	84.87±2.25	93.31± 0.00	95.99±1.42	9.71

Data are expressed as mean ± SD values (n=3).

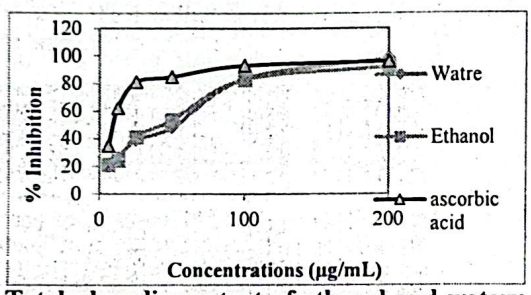

Figure 2 DPPH radical scavenging activities of the flower buds of *S. aromaticum*

Total phenolic content of ethanol and watery extracts of flower buds *S. aromaticum*

In this study, the total phenolic content of (Nann-paw kyawt) bark was estimated by Folin-Ciocalteu method. Phenols react with an oxidizing agent phosphomolybdate in F-C reagent under alkaline conditions and result in the formation of blue coloured complex, the molybdenum blue which is measured at 765 nm colorimetrically. The total phenolic content (TPC) (µg GAE/mg) of ethanol extract (19.43± 0.02) was higher than that of watery extract (16.08± 0.03). The greater the total phenolic content, the higher the antioxidant activity. Therefore, ethanol extract of *S. aromaticum* has more antioxidant activity than watery extract.

Antimicrobial activity of crude extracts of the flower buds of *S. aromaticum*

For the screening of antimicrobial activity of crude extracts such as pet-ether, chloroform, ethyl acetate, ethanol and water extracts from *S. aromaticum* Linn. (Clove) buds against six microorganisms which are *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus , Candida albicans* and *Escherichia coli* by agar well diffusion method, the results are described in Table 3 and

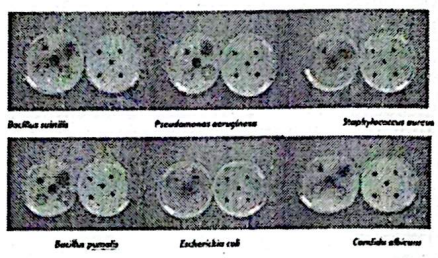

Figure 3and 4. The inhibition zone diameter showed the degree of antimicrobial activity. The larger the inhibition zone diameter, the greater the antimicrobial activity. Among the five extracts, ethyl acetate and ethanol extracts are the most active than others.

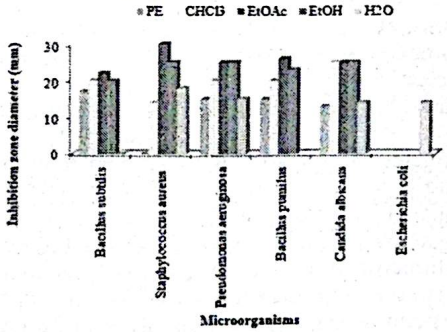

Figure 3. Antimicrobial screening of various extracts of flower buds of the flower buds of *S. aromaticum*

1 .PE extrct 2. EtOH extract 3. EtOAc extract 4. $CHCl_3$ extract 5. H_2O extract

Table 3. Inhibition Zone Diameters of Various Extracts from Flower Buds of the flower buds of *S. aromaticum*

Organisms used	Diameter of inhibition zone (mm)				
	PE extract	$CHCl_3$ extract	EtOAc extract	EtOH extract	H_2O extract
Bacillus subtilis	17	20	22	20	-
Staphylococcus aureus	-	14	30	25	18
Pseudomonas aeruginosa	15	20	25	25	15
Bacillus pumilus	15	20	26	23	-
Candida albicans	13	25	25	25	14
Escherichia coli	-	-	-	-	14

Agar Well – 10 mm 10 mm ~ 14 mm (+) low activity 15 mm ~ 19 mm (++) higher activity 20 mm ~ above (+++) highest activity

Figure 4. Inhibition zone diameters of various extract from the flower buds of *S. aromaticum*

Conclusion

From the overall assessment of present work, the following inferences may be deduced.

Preliminary phytochemical investigation on the *S. aromaticum* buds indicated the presence of alkaloids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, starch, saponins, steroids, tannins and terpenoids by test tube method. According to EDXRF spectrometer , Ca and K are the major elements present in the flower buds of *S. aromaticum*.

In the determination of nutritional values, moisture, fat, ash, fiber, protein contents were found to be 13.98%, 12.07%, 6.08%, 7.24% and 5.81%, respectively. 54.82% of carbohydrate content and 352 kcal/100 g of energy values were observed in buds of *S.aromaticum*. Antioxidant activity of various extracts from buds of *S.aromaticum* was determined by DPPH assay. IC_{50} value of ethanol extract was 42.71 µg/mL and watery extract was 52.62 µg/mL. Thus, the radical scavenging power of plant extracts were observed that 95% EtOH extract had higher anti-oxidative effect than watery extract. Their antioxidant potency were conducted to be very weak if compared with the potency of standard ascorbic acid (IC_{50} = 9.71 µg/mL). Antimicrobial activities of some crude extracts such as pet-ether, $CHCl_3$, EtOAc, 90% EtOH and water obtained from *S. aromaticum* were investigated on six strains of bacteria. It was found that among the five extracts, ethyl acetate and ethanol extracts are the most active than others. This research revealed some biological properties, antioxidant and antimicrobial effects, of the *S. aromaticum* .Therefore it is hoped that the research finding will contribute to some extent to the search for the antioxidative and antimicrobial agent of plant origin and also to the development of the role of Myanmar traditional medicinal formulation, especially in the treatment of oxidative related diseases and diabetes.

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References

AOAC. (2002). "Official Method of Analysis". *International Food Research Journal*, 17, 423-432

Griken, R. E. and Markowictz, A. A. (1993). "Quantification in XRF Analysis of Intermediate Thickness Samples", 1st Ed., Marcel Dekker Inc., New York: 339 – 358

Kamatou GP, Vermaak I, Viljoen AM. (2012). "Eugenol–from the Remote Maluku Islands to the International Market Place: a review of a remarkable and versatile molecule. Molecules, 17(6), 6953–6981

Lawrence, R., Priyanka, T. and Ebenezer, J. (2009). "Isolation, Purification and Evaluation of Antibacterial Agents from *Tamarindus indica* L. (Magyi) Pulp". *J. Microbial*, 40, 906-915

Marini-Bettolo, G.B., Nicoletti, M., Patamia, M. and Patamia, M. (1981). "Plant Screening by Chemical and Chromatography Procedure Under Field Condition, *J. Chromatography*, 213, 113 – 127

Marinova, G. and V. Batchvarov, V.(2011)." Evaluation of the Methods for Determination of the Free Radical Scavenging Activity by DPPH, *Bulgarian Journal of Agricultural Science*, 17, 11 – 24

MedlinePlus. (2014). "U.S. National Library of Medicine and National Institutes of Health". *Retrieved* , 45-98

M-TinWa,(1972)." Phytochemical Screening Methods and Procedures", *Phytochemical Bulletin of Botanical Society of America*, Inc., 5(3), 4 – 10

Shan B., Cai, YZ., Sun, M., Corke, H.(2005). "Antioxidant Capacity of 26 Spice Extracts and Characterization of their Phenolic Constituents. J. Agric. Food Chem., 53(20),7749–7759

Taberner-Vallverdú, M., Nazir, M.; Sanchez-Garces, M. Á.;and Gay-Escoda, C. (2015). "Efficacy of Different Methods used for Dry Socket Management: A systematic review". *Medicina Oral Patología Oral y Cirugia Bucal*.,20(5),633–639

Wonjung, Y. (2018). " Tight Stocks of Quality Cloves Lead to a Price Surge". *Tridge. Retrieved*, 230-267

Evaluation of Antifungal and Chemical Investigation for Conservation of Old Palm-Leaf Manuscripts by Citronella Grass

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Abstract

The conservation of old palm-leaf manuscripts is very important in Myanmar because many of these are destroyed due to harsh climate and insect destruction in Myanmar. The palm-leaf manuscripts which are important documents of historical, national and cultural heritage can be sustained in good condition for longer time. Citronella grass (*Cymbopogon winterianus* jowitt.) possesses many useful biological activities which has been the reason for the selection of this plant for the present paper. Scientific investigation on the inhibition effect of citronella oil on the fungus species infesting the palm-leaf manuscripts stored in University of Yangon Library is still lacking. The aim of the present paper is the detection of fungus from old palm-leaf manuscripts stored in University of Yangon Library and conservation of old palm-leaf manuscripts by citronella oil. Citronella oil of citronella grass was prepared by steam distillation method. Essential oil content of citronella grass was found to be 0.6 % by steam distillation. Fungi were detected from old palm-leaf manuscripts by agar plate streak method. *Penicillin, Aspergillus, Mycorrhiza* fungi were detected from old palm-leaf manuscripts by agar plate diffusion method. Antifungals activity of citronella oil was tested by agar well diffusion method. Citronella oil showed significant antifungal activity (20 mm to 25 mm) against all tested fungi. Antibacterial activity of essential oil was also determined by agar disc diffusion method. According to GC-MS spectrum, citronellal was the major component of essential oil of citronella grass. Therefore, the present research intended to contribute the feasibility of removing the fungal infection of the palm-leaf manuscripts of Yangon University Library by using the citronella oil.

Keywords: Fungi, citronella oil, palm-leaf manuscript, antifungal

Introduction

Like other countries of Asia, Myanmar has thousands of manuscripts written on paper (parabaik) and palm leaf all over the country. Palm leaf, one of the medium of written text, is the most popular material in Southeast Asia countries in 11th century A.D. Palm leaf manuscripts are in an ancient document form that comprises a significant documentary heritage. In Myanmar, these manuscripts were kept in royal palaces, monasteries, libraries, archives and personal collections.

In India and Southeast Asia because of the harsh climate and insect destruction, the earliest examples of palm-leaf manuscripts have not survived. Recent research on conservation of palm- leaf manuscripts with camphor, castor, cedar wood, neem, clove and sesame indicate that cedar wood oil has slower absorption rate than some of the other oils (Agrawal, 1984).

At present, to preserve palm-leaf manuscripts, citronella oil will apply to each leaf. The department of Chemistry the University of Yangon has made much progress in the research on citronella oil "Ei Ei Soe (2006) studied on the mosquito repellent activity of citronella oil" and "Khin San Nwe (2011) on citronella oils from local and China *Cymbopogon winterianus* Jowitt. leaves and their bioactivities". In their study, citronella oil has been found to be active against microorganisms such as some bacteria and fungus species and to be nontoxic to human. It is urgently needed to conserve palm-leaf manuscripts of University of Yangon Library which are infected with fungus because palm-leaf manuscripts constitute precious national and cultural heritage. Conserving of these manuscripts by naturally available substances from Myanmar medicinal plant is an interesting project. On the other hand, scientific investigation on the inhibition effect of citronella oil, which is abundantly available in Myanmar, on the species of fungus infesting the palm-leaf manuscripts stored in University of Yangon Library is still lacking.

Materials and Methods

Plant material: The leaves of citronella grass (*Cymbopogon winterianus* jowitt.) were collected from Paunggyi, Hlegu Township, and Yangon Region. Plant sample was washed, followed by cutting, air-drying grinding into powder, and finally stored separately in air-tight containers for further works.

Extraction of essential oil: Extraction of essential oil from citronella grass was carried by steam distillation method. The dried powder (100 g) and distilled water (500 mL) were placed in 1L round bottomed flask. The flask was fitted for steam distillation and heated. The steam was passed into the flask. The condensed oil and water were collected in a flask and the oil was extracted with PE in a separating funnel. The PE extract was dried over anhydrous sodium sulphate. After filtration, the filtrate was evaporated to get the essential oil which was weighed and kept in air tight bottle for further analysis (Hesham *et al.*, 2016).

Screening of fungus: Detection of fungus from old palm-leaf manuscripts stored in the University of Yangon Library. This study was carried out by agar plate diffusion method.

Antifungal activity

Screening of antifungal activity of citronella oil was carried out by agar well diffusion method. *Penicillin, Aspergillus and Mycorrhiza* fungi were used in the investigation of antifungal activity.

Determination of Organic Compounds in Essential Oil

Gas chromatography-mass spectrometry (GC-MS) is a method of gas chromatography and mass spectrometry to identify different substance with a test sample (Robert and Webster, 1998). Application of GC-MS includes identification and quantitation of volatile and semi-volatile organic compounds in complex mixtures. Organic constituents in essential oil from the leaves of *Cymbopogon winterianus* were detected by GC-MS spectroscopic method.

Results and Discussion

In this paper *Cymbopogon winterianus* Jowit. leaves originated from Myanmar was chosen. According to preliminary phytochemical investigation, it was found that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, steroids, tannins and terpenoids were present. However, there are no cyanogenic glycosides and saponins observed in tested sample, indicating that no toxic effect due to these compounds in tested sample. In this work, relative abundance of elements present in citronella grass was determined by ED-XRF spectrometer. It can be seen that; potassium and chloride peaks are the most predominant peaks. Other elements such as Rb, Mn, and Br are also present in tested sample (Table 1).

Screening of fungus

Three fungi namely *Penicillin, Aspergillus* and *Mycorrhiza* fungus were detected from old palm-leaf manuscripts stored in University of Yangon Library (Figure 1).

Antifungal activity of citronella oil

In vitro antifungal activity of citronella oil has been screened on three fungi: *Penicillin, Aspergillus* and *Mycorrhiza* by agar well diffusion method (Figure 2 and Table 2). According to this experiment, the citronella oil may be used for the treatment of fungal infections (20 mm-25 mm).

Conservation of old palm-leaf manuscripts

The citronella oil can be lightly applied by laying the leaf onto a piece of glass and laid onto the leaf with a soft brush or a piece of cotton (Figure 3). Therefore, the use of natural herbs like citronella grass oil with the manuscripts to keep fungus away. Moreover, application of citronella oil on the surface of the palm-leaf manuscript to keep it flexible. This prevents physical damage due to brittleness.

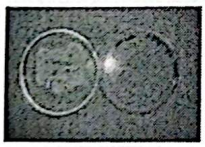
GC-MS spectroscopic study on essential oil of citronella grass

According to GC-MS spectroscopic study, it was found that citronella grass oil contained citronellal (tetrahydro 4-methyl 2H-pyran-2-one), the major constituent of it. It was identified by matching its MS spectrum with the library data (Figure 4).

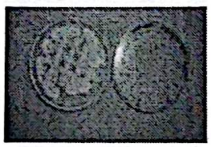
Table 1. Relative Abundance of some Elements in Citronella Grass Leaves by EDXRF Method

Elements present	Relative Abundance (%)
	Citronella grass
K	81.108
Cl	15.347
Rb	1.538
Mn	1.496
Br	0.501
Fe	ND

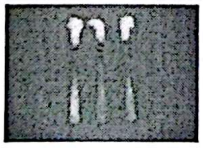
ND = Not detected



Penicillin



Aspergillus



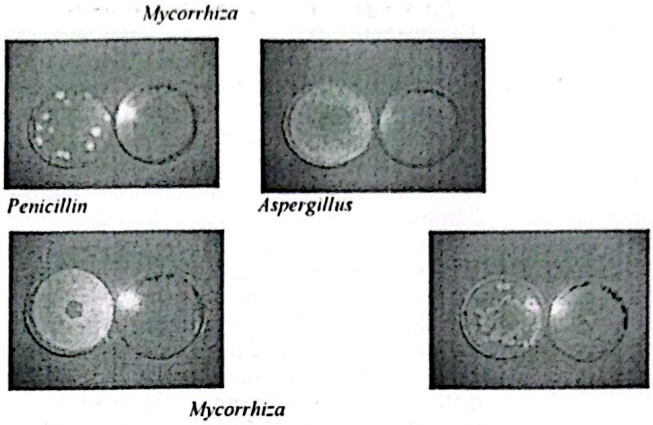


Figure 1. Colony morphology of fungus from old palm-leaf manuscripts

Figure 2. Antifungal screening of citronella oil from citronella grass

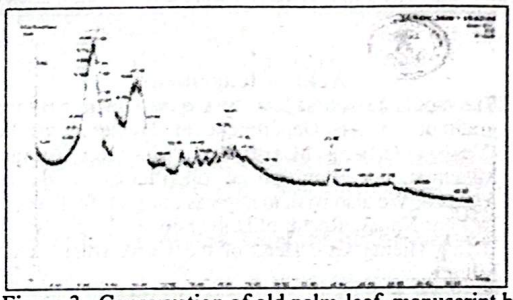


Figure 3. Conservation of old palm-leaf manuscript by citronella oil

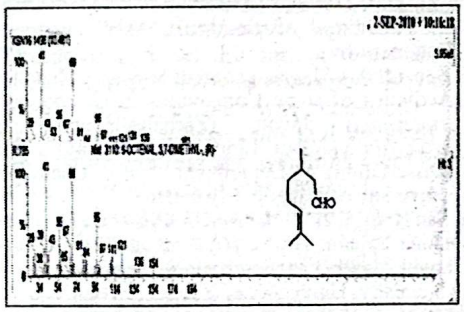


Figure (4 a) GC chromatogram of essential oil from citronella grass

Figure (4 b) Mass spectrum of isolated citronella from citronella grass

Table 2. Results of Antifungal Activity of Citronella Oil from Citronella Grass

Extract	Inhibition Zone Diameter (mm)		
	Penicillin	*Aspergillus*	*Mycorrhiza*
Citronella oil	25 (+++)	25 (+++)	20 (+++)

Agar well - 10 mm, 10 mm - 14 mm (+), 15 mm -19 mm (++), 20 mm above (+++)

Conclusion

From the overall assessments of the present work, the following inferences could be deduced. According to the phytochemical investigation, chemical constituents such as (alkaloids, carbohydrates, flavonoids, glycoside, phenolic compound, reducing sugar steroids and tannins)

were found in leaves of citronella grass. Cyanogenic glycosides and saponins were absent. From ED-XRF spectrum, K, Cl, Rb, Mn, Br and Fe were found in leaves of citronella grass. From atomic absorption spectroscopic determination of some potentially toxic metals such as lead and arsenic were absent in citronella grass and the metals constituents such as K, Ca, Fe, Zn, Cu and Mn were found to be present.

The essential oil content (0.6 %) was obtained from citronella grass by steam distillation method. *Penicillin, Aspergillus, Mycorrhiza* fungi were detected from old palm-leaf manuscripts stored in the University of Yangon Library. Citronella oil can be inhibited the detected fungi by agar well diffusion method (20 mm to 25 mm). From the GC-MS spectrum, the major component, citronellal was detected from essential oil of citronella grass.

Consequently, it can be inferred that citronella oil extracted from citronella grass may be used in the treatment of fungus infection. According to the research finding, currently, citronella oil has already been used for the conservation of palm-leaf manuscripts in University of Yangon Library. Application of citronella oil on the surface of the palm-leaf manuscripts to keep it flexible and prevents fungus away.

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References

Agrawal, O.P. (1984), "Conservation of Manuscripts and Paintings of Southeast Asia", Londaon: Butterworths

Ei Ei Soe. (2006). "Investigation of Mosquito Repellent Activities of some Compounds in *Cymbopogon winterianus* Jowitt. (Zarbalin-Hmwe) and *Artemisia vulgaris* Linn. (Mel-Di-Dote)". PhD (Dissertation), Department of Chemistry, University of Yangon, Myanmar

Khin San Nwe. (2011). "Citronella Oils from Local and China Sabalin Hmwe (*Cymbopogon winterianus* Jawitt.", PhD (Dissertation), Department of Chemistry, University of Yangon, Myanmar

Hesham, H. A. R. A. H. Nour and R. M. Yunus (2016). "Techniques for Extraction of Essential oil from Plants: A Review". *Australian Journal of Basic and Applied Sciences*, 10 (16), 117-127

Robert, M. S and F. X. Webster. (1998). *Spectrometric Identification of Organic Compounds*. New York: 6th Ed., John Wiley & Sons, 367-370

Phytochemical Investigation, Elemental Analysis and Removal of Lead by Using Ash of *Azadirachtaindica* A. Juss.

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Abstract

In this research, leave of AzadirachtaindicaA.Juss (Neem Tree) were collected from University Campus, University of Mandalay, Myanmar. The sample was tested by phytochemical screening which gave rise to positive results for saponin, phenolic compound, polyphenol, alkaloid, and lipophenol. In addition, the mineral contents of leave and ash from AzadirachtaindicaA.Juss(Neem Tree) were also determined by Energy Dispersive X-Ray Fluorescence (EDXRF) method. K, Ca, Cl, Si, P, S, Al, Fe and Sr were present in the dried leave. Among them, K was found to be the highest percent in this sample. Furthermore, K, Ca, Cl, P, Si, Al, Fe and Sr were present in ash. Among them, K was also found to be the highest percent in the ash sample. Moreover, ash of AzadirachtaindicaA.Juss(Neem Tree) leave was used as an adsorbent. The sample was sieved to obtain two different mesh sizes (60 and 80). The percent removal of lead was determined by using complexometric titration with ethylene diaminetetraacetic acid (EDTA) and xylenol orange was used as an indicator. The effect of various sample weights on the adsorption of lead by using shaking method was determined. Finally, the adsorption effect of fixed sample weight with different shaking times was also determined. *According to the results of adsorption data, the ash of AzadirachtaindicaA.Juss(Neem Tree) could be applied in the waste water treatment for removal of lead.*

Keywords: Azadirachtaindica A.Juss , complexometric titration, EDTA, EDXRF, xylenol orange

Introduction

Nowadays lead contamination in an environment is a very important problem of worldwide concerning due to its highly toxic and non-biodegradable in nature. The primary sources of heavy metal in the environment are anthropogenic such as industrial effluent, electroplating metallurgical industries, etc. The industrial activity is the major sources of lead containing in water. Industrial waste water containing lead, copper, cadmium, and chromium can contaminate ground water resources and thus lead to serious ground water pollution. Lead ion has an affinity for ligands containing thiols and phosphate groups and they inhibit the synthesis of heme, causing damage both to kidney and liver. Many processes exist for the removal of heavy metals from aqueous solution such as precipitation, ion-exchange, solvent extraction, reverse osmosis and adsorption [4].Among all of these methods, adsorption process is better than other methods because of conveniences, easy

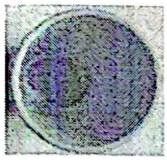


Figure 3 Ash content of the leave of Neem

operating and lower operating cost for the removal of heavy metals. In the waste treatment processes, the adsorption processes are widely used especially for solutions at low metal concentrations [3].In this paper,ash of *Azadirachtaindica*A.Juss. (Neem tree) leave was used as an adsorbent for the removal of lead from aqueous solution.

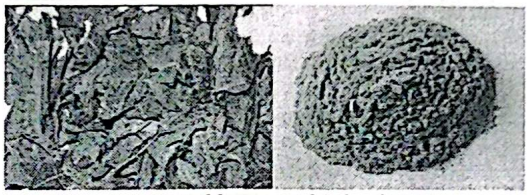
Botanical Description[1]

Family name : Meliaceae
Botanical name : *Azadirachtaindica*A.Juss.
Myanmar name : Tama
English name : Neem
Part used : Leave

Experimental

A. Sample Collection

The fresh leave of Neem was collected from University campus, Mandalay University, Myanmar. The leavesample was dried at room temperature. And then, the air dried leave were burnt in the Muffle Furnace to obtain ash. The ash samples (60 and 80 mesh sizes) were stored in a plastic bottle and used the experiment.



F. *Figure 2 Dried leave and ash of Neem*

G. Phytochemical Investigation of Leave of Neem[2]

The leave sample of Neem was tested by phytochemical screening.

Determination of Ash Content of the Leave of Neem

The air dried sample (2 g) was weighed, and placed in a preheated, cooled, and weighed the crucible. The crucible was heated carefully in the furnace at 550°C for 2 hours, burned off without flaming or until all the carbon was eliminated. When the materials were converted to white ash powder, the crucible was cooled at room temperature, in a desiccator and weighed again. The heating, cooling and weighing were repeated until a constant weight was obtained.

$$\text{Ash \%} = \frac{\text{weight of residue (g)}}{\text{weight of sample (g)}} \times 100$$

H. Determination of Mineral Content from the Dried Leave and Ash of Neem

Mineral contents of the dried leave and ash of Neem were measured at the Department of Physics, University of Mandalay, by applying EDXRF (Energy Dispersive X-Ray Fluorescence

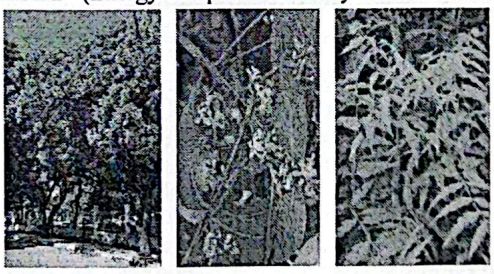


Figure1 Plant, Flowers and Leave of Neem Tree

Spectroscopy) method.

Removal of Lead by Using Ash of Neem Leave(60 mesh size and 80 mesh size for Different Dosages)

0.1g of ash sample (60 mesh size) was mixed with 50 mL of 100 mgL^{-1} lead II nitrate solution and shaken for 30 minutes at room temperature with 180 rpm. After shaking, it was filtered by using filter paper. And then, 10 mL of filtrate was used for titration to determine the remaining content of lead in the solution. 1 drop of xylenol orange indicator was added into 10 mL of filtrate in the conical flask. The color was changed from colorless to wine red and1 drop of 1M HNO_3 was added. The color was changed to yellow. In this solution, a few amount of hexamine powder was added, the color was changed from yellow to orange red. Finally, this solution was titrated with 0.0027M EDTA and the color of the solution waschanged to yellow. This yellow color was checked by using colorimeter at wavelength 570 nm. When absorbance is 0.04, the end point is reached. The same procedure for various dosages of 0.2g, 0.3g, 0.4g and 0.5g for 60 mesh size of ash sample were carried out.

Similar procedure was used for the determination of various dosages of 0.1g, 0.2g, 0.3g, 0.4g and 0.5g for 80 mesh size of ash sample.

I. **Removal of Lead by Using Ash of Neem Leave(60 mesh size and 80 mesh size for Different Shaking Times)**

0.1 g of ash sample (60 mesh size) was mixed with 50 mL of 100 mgL^{-1} lead II nitrate solution and shaken for 30 minutes at room temperature with 180 rpm. After shaking, it was filtered by using filter paper. And then, 10 mL of filtrate was used for titration to determine the remaining content of lead in the solution. 1 drop of xylenol orange indicator was added into 10 mL of filtrate in the conical flask. The color was changed from colorless to wine red and 1 drop of 1M HNO_3 was added. The color was changed to yellow. In this solution, a few amount of hexamine powder was added, the color was changed from yellow to orange red. Finally, this solution was titrated with 0.0027M EDTA, the color of the solution was changed to yellow. This yellow color was checked by using colorimeter at wavelength 570 nm. When absorbance is 0.04, the end point is reached. The same procedure for 0.1g of ash sample (60 mesh size) was carried out at different times (60min, 90min, 120min, 150min, 180min).

Similarly, 80 mesh size of ash for different shaking times was measured by the same procedure.

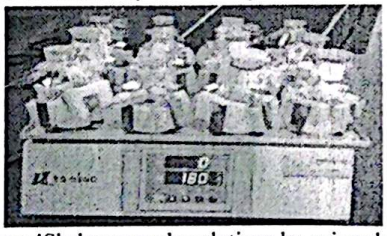


Figure 4Shaken sample solutions by using shaker

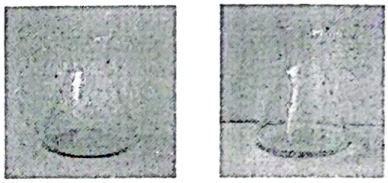


Figure 5 Before titration and after titration

Table 1 Results of Phytochemical Constituents of NeemLeave

Weight of sample (g)	Weight of residue (g)	Ash (%)
2.00	0.16	8
2.00	0.18	9
2.00	0.18	9

Results and Discussion

J. Phytochemical Testsof NeemLeave

Constituents	Reagent used	Observation	Result
Tannin	H_2O, 10%$FeCl_3$, dil: H_2SO_4	No yellowish brown ppt	-
Saponin	H_2O	Froth	+
Flavonoid	Conc:HCl, piecies of Mg	No pink color solution	-
Terpene	EtOH,$CHCl_3$, conc:H_2SO_4	No pink color solution	-
Glycoside	10%lead acetate	No white ppt	-
Steroid	EtOH, acetic anhydride,conc: H_2SO_4, $CHCl_3$	No green color solution	-
Phenolic Compound	H_2O, 10% $FeCl_3$	Brown color solution	+
Polyphenol	EtOH,1 %$FeCl_3$, $K_3[Fe(CN)_6]$	Greenish blue color solution	+
Reducing sugar	H_2O, Benedict's solution	No ppt	-
Alkaloid	Wagner's reagent	Reddish brown ppt	+
Lipophenol	H_2O,0.5M KOH+ NaOH	Deep color solution	+
Carbohydrate	Fehling(A) + (B) + EtOH	No yellow color solution	-

(+) = presence (–) = absence

According to this table, leave of Neemconsist of alkaloid, polyphenol, phenolic compound, saponin and lipophenol.

Determination of Ash Content of NeemLeave

The ash content of Neemleave was determined and the results were presented in Table 2.

According to this table, the ash content of Neem leavewas 8-9 %.

Elemental Composition of Dried Leave and Ash of Neem by EDXRF Spectroscopy

Table 2Results of Ash Contents ofNeem Leave

Dosage (g/50mL)	Shaking Time (min)	Initial conc: C_o (mg/L)	Equilibrium conc: C_e (mg/L)		Percent Removal(%)	
			60 mesh size	80 mesh size	60 mesh size	80 mesh size
0.1	30	100	56.79	47.59	43.21	52.41
0.1	60	100	39.89	32.23	60.11	67.77
0.1	90	100	32.68	25.98	67.32	74.02
0.1	120	100	27.89	18.14	72.11	81.86
0.1	150	100	19.11	7.87	80.89	92.13
0.1	180	100	10.22	4.79	89.78	95.21

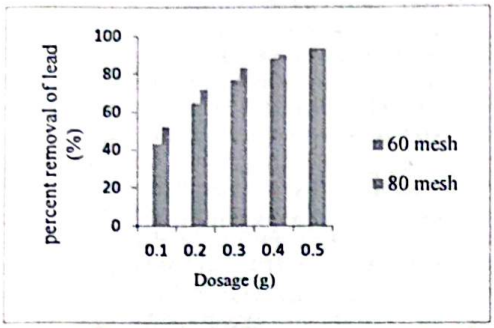


Figure 6 Comparison of percent removal of lead by different dosages

Removal of Lead by Using Ash of NeemLeave (60 mesh size and 80 mesh size for Different Dosages)

The effect of sample weight on the adsorption of lead by shaking method was investigated and the results were shown in Table 4.

Table 4 Removal of Lead by Using Ash of NeemLeavewith different dosages (for 60 and 80 mesh sizes) Shaking Time = 30 minutes, Shaking Rate = 180 rpm

According to Table 4, it was found that the precents removal of lead was increased with increasing the dosage of sample weight (g).

From these results, the percent removal of lead for 80 mesh size of ash sample was better than 60 mesh size. It was found that the smaller the size of the selected sample, the more adsorption of lead on the surface occurred.

K. **Removal of Lead by Using Ash of NeemLeave**

L. **(60 mesh size and 80 mesh size for Different Shaking Times)**

The effect of shaking time on the adsorption of lead was determined and the results were shown in Table 5.

Table 5 Removal of Lead by Using Ash of NeemLeavewith Different Shaking Times (for 60 and 80 mesh sizes) Shaking Rate = 180 rpm

Dosage (g/50mL)	Initial conc: C_o (mg/L)	Equilibrium conc: C (mg/L)		Percent Removal (%)	
		60 mesh size	80 mesh size	60 mesh size	80 mesh size
0.1	100	56.79	47.59	43.21	52.41
0.2	100	35.28	28.12	64.72	71.88
0.3	100	22.87	16.53	77.13	83.47
0.4	100	11.66	9.98	88.34	90.02
0.5	100	6.63	6.47	93.37	93.53

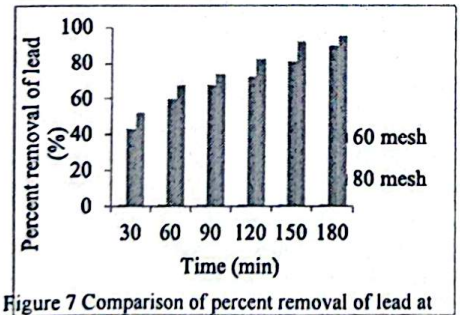


Figure 7 Comparison of percent removal of lead at different shaking times

Table 3 Results of Elemental Composition of Dried Leave and ash of Neem by EDXRF Spectroscopy

Elements	Relative abundance (%) (Leave)	Relative Abundance (%) (Ash)
Potassium	2.1990	21.470
Calcium	1.5040	16.230
Chlorine	1.0510	9.6370
Silicon	0.1342	0.7875
Phosphorus	0.1322	1.4440
Sulfur	0.0979	0.3124
Aluminium	0.0747	0.4181
Iron	0.0396	0.3057
Strontium	0.0139	0.1675

K. Table 3 shows that some of elements (K, Ca, Cl, Si, P, S, Al, Fe, Sr) were found in the dried leave and ash of Neem.Among them, potassium was the highest amount in these samples.

According to Table 5, it was found that the percent removal of lead was increased with increasing the shaking time (min). From these results, the percent removal of lead for 60 and 80 mesh sizes of ash samplewasincreased with increasing the shaking time.

Study on Adsorption Isotherm

The amount of lead adsorbed onto the ash of Neem leave, q_e(mg g^{-1}) was calculated by the following equation. $q_e = (C_o - C_e)\frac{v}{w}$ where C_o and $C_e$were the initial and equilibrium concentrations of lead solution,(mgL^{-1}), V used the volume of the solution(L), and w is the weight of the ash sample(g).

There are two well-known types of adsorption isotherm; the Langmuir isotherm and, Freundlich isotherm.

The Langmuir adsorption isotherm was mathematically expressed as;

$$\frac{x}{m} = \frac{X_m b C}{1+bC}$$

$$q_e = \frac{X_m b C_e}{1+bC_e}$$

$$\frac{1}{q_e} = \frac{1}{X_m} + \frac{1}{X_m b C_e}$$

$$\frac{C_e}{q_e} = \frac{C_e}{X_m} + \frac{1}{X_m b}$$

where X_m= Langmuir monolayer capacity parameter

b = constant for a given adsorbate and adsorbent at a particular temperature.

The Freundlich adsorption isotherm is mathematically expressed as;

$$q_e = K C_e^{1/n}$$

$$\log q_e = \log K + \frac{1}{n} \log C_e$$

where K and $\frac{1}{n}$ were constants for a given adsorbate and adsorbent at a particular temperature.

Table (7 and 9)and Figure (8, 9, 10 and 11) showed the data of lead for Langmuir and Freundlich isotherms.

From the isotherm model plots, the respective model constants were calculated and compared to know which isotherm model fits to the adsorption of lead on ash of Neem leave.

Table 6Adsorption of Different Masses of ash of Neem Leave (60 mesh size)

Mass of Sample(g)	C_e (mg/L)	q_e (mg/g)	C_e/q_e (g/L)	Log C_e (mg/L)	Log q_e (mg/g)
0.1	56.79	21.61	2.63	1.7543	1.3347
0.2	35.28	16.18	2.18	1.5475	1.2089
0.3	22.87	12.86	1.78	1.3593	1.1092
0.4	11.66	11.04	1.05	1.0667	1.0429
0.5	6.63	9.97	0.66	0.8215	0.9987

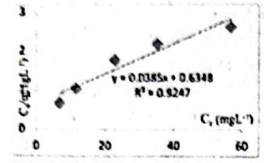


Figure 8 The Langmuir isotherm for lead onto ash of Neem leave (60 mesh size)

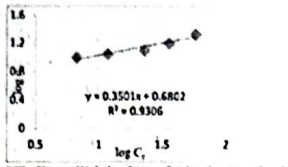


Figure 9TheFreundlich isotherm for lead onto ash of Neem leave (60 mesh size)

Table 7 Langmuir and Freundlich parameters for the adsorption of Lead onto ash of Neem Leave in Dosage method(60 mesh size)

Langmuir parameters			Freundlich parameters		
R^2	X_m (mg g^{-1})	b(L mg^{-1})	R^2	n (gL^{-1})	K(mg g^{-1})
0.9247	25.97	0.0606	0.9306	2.8563	4.7885

Table 8 Adsorption of Different Masses of ash of Neem Leave (80 mesh size)

Mass of Sample(g)	C_e (mg/L)	q_e (mg/g)	C_e/q_e (g/L)	Log C_e (mg/L)	Log q_e (mg/g)
0.1	47.59	26.21	1.82	1.6775	1.4185
0.2	28.12	17.97	1.56	1.4490	1.2545
0.3	16.53	13.91	1.19	1.2183	1.1433
0.4	9.98	11.25	0.88	0.9991	1.0512
0.5	6.47	9.35	0.69	0.8109	0.9708

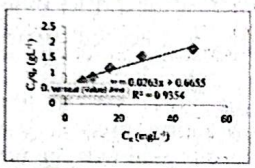


Figure 10The Langmuir isotherm for lead onto ash of Neem leave (80 mesh size)

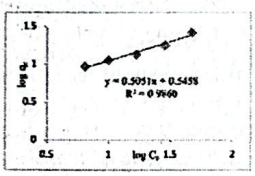


Figure 11TheFreundlich isotherm for lead onto ash of Neem leaves (80 mesh size)

Table 9 Langmuir and Freundlich parameters for the adsorption of Lead onto ash of Neem Leave in Dosage method (80 mesh size)

Langmuir parameters			Freundlich parameters		
R^2	X_m (mg g^{-1})	b(L mg^{-1})	R^2	n (gL^{-1})	K(mg g^{-1})
0.9354	38.02	0.0395	0.9860	1.9798	3.5140

From these results, the correlation coefficient for ash was found to be the best fit with the Freundlich model. R^2 value 0.9306 for lead adsorption onto ash of Neem Leave is the better in Freundlich model thanLangmuir model.

From these results, the correlation coefficient for ash was found to be the best fit with the Freundlich model. R^2 value (0.9860) for lead adsorption onto ash of Neem Leave is the better in Freundlich model thanLangmuir model.

Conclusion

From the phytochemical investigation, the Neem leave consisted of alkaloid, polyphenol, phenolic compound, saponnin, and lipophenol. The elemental contents of dried leave and ash were determined by EDXRF method.***K, Ca, Cl, Si, P, S, Al, Fe and Sr were present in the dried leave and ash.*** The amount of potassium was the highest percent in these samples. In addition 8-9 % of ash was contained in the Neem Leave. The removal properties of lead on ash sample was carried out by using dosage method, and different shaking times methods for two different mesh sizes (60 and 80 mesh sizes). According to experimental data, the percent removal of lead was increased with increasing the dosage of sample weight and shaking time. The percent removal of lead for 80 mesh size of ash sample is better than 60 mesh size. The Langmuir and Freundlich isotherms were examined by using the experimental data of ash of Neem leave. In addition, the value of correlation coefficient (R^2) in Freundlich isotherms for 80 mesh size was better than 60 mesh size. Both methods fit the experimental data but the Freundlich is the best one for 60 and 80 mesh sizes. According to the results of adsorption data, the ash of Neem could be applied in the waste water treatment for removal of lead.

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References

[1]DrKhinMaungLwin&DrMyat Kay ThweLwin (2015), "Medicinal Plant List of Myanmar", 2nd Edition, 272.

[2]Harbone .J. B (1973)."Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis". NewYork, Chapman and Hall.

[3]RashmiVerma and PratimaDwived, (2013). "Heavy Metal water pollution A case study" , Recent Research in Science and Technology, 98-99.

[4]Subapriya, S. Nagini, (2005)." Medicinal properties of Neem leaves", A Review Source, Current Medicinal Chemistry-Anti-Cancer Agents, 149-156.

Study on the Antioxidant Activity of the Root of *Quisqualis Indica* (L.) and Isolation of a Bioactive Pure Compound

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Abstract

In most of the countries of the world, many plants, largely based on the use of roots, leaves, barks, seeds, fruits and flowers are employed in traditional medicine. Also in Myanmar, the study of traditional indigenous medicinal plants and their usage in therapy play is a very important role. In this research work, one Myanmar indigenous medicinal plant, namely *Quisqualis indica* L. (Htar-wei-hmaing) was selected for chemical investigation. The roots of this plant were collected from Maha-aung-myae Township, Mandalay. The preliminary phytochemical screening of the roots of *Quisqualis indica* L. was carried out by phytochemical tests. The roots could respond positive for alkaloid, flavonoid, glycoside, phenolic compound, steroid, reducing sugar and sponin tests. Moreover, antimicrobial activities of the crude extract in various solvent systems of the roots of this plant were examined by Agar- well diffusion method on six tested organisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albican* and *Mycobacterium* species. The crude extracts in various solvent systems gave the antimicrobial activities against all tested organisms. Then, the antioxidant activity of ethanol extract of roots of the plant sample was determined by DPPH (1, 1 diphenyl-2-picryl hydrazyl) assay. In addition, a bioactive pure compound could be isolated as a pale yellow oily form from the roots of *Quisqualis indica* (L.) by using modern separation techniques, such as Thin Layer and Column Chromatographic methods. The yield percent was found to be 1.5% based upon ethyl acetate crude extract. The antimicrobial activities of this pure compound were examined by applying Agar-well diffusion method. It gave activities on all tested organisms, such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albican* and *Mycobacterium* species.

Keywords : phytochemical, roots, antimicrobial, bioactive, organisms.

1. Introduction

In many developing countries, it is estimated that about two third of the population relies heavily on traditional medicinal plants to meet the primary healthcare need. Many plants are have been revealed by researchers based on variation in plant species and their therapeutic chemical principles. (Farnsworth NR *et al.*,1991).

Medicinal plant is an integral part of human life to combat the sufferings from the dawn of civilization. It is estimated that more than 80,000 of total plant species have been identified and used as medicinal plants around the world. (Joy P. *et al*, 1998)

The indigenous medicinal plants and plant-derived drugs are the potential source of alternative medicine and are extensively used to treat various health aliments. (Kavishankar G. *et al*, 2011)

Use of the medicinal plants is a core component at primary health care level due to availability, acceptability, compatibility, and affordability. It is estimated that about 75–80% of people of developing countries and about 25% of people of developed countries depend on medicinal plants for the first line of treatment.(Gupta P .D., 2012)

Therefore, people are encouraging indigenous production and processing of these medicinal plants to use for the treatment of various diseases. Moreover, importance and uses of medicinal plants are also stated in different religious books.(Urbi Z. *et al*, 2014)

Quisquails Indica L. is an important medicinal plant and widely used around the world. It belongs to the family, *Combretaceae*. *Quisquails Indica* L. plant is **used** for traditional medicine in certain regions. **Leaves** can be **used** to relieve pain caused by fever while the roots to treat rheumatism. (Gardino Nursery, Corp Top Tropicals, Medicinal use of Rangoon creeper - bimbima – 2013)

The leaves and flowers are used for treating the weakness, the leucorrhoea, diabetes, digestive disorders, cold, cough. The seeds are taken to expel parasite worm. Decoctions of the root, seed or fruit can be used as anthelmintic to expel parasitic worms or for alleviating diarrhea. Fruit decoction can also be used for gargling. The fruits are also used to combat nephritis. (*Wetwitayaklung, P.etal, 2008)*

Htar-wei-hmaing is one of Myanmar traditional indigenous medicinal plant. In Myanmar, *Quisquails Indica* L. roots are applied for digestive disorders and diarrhea. The roots of Htar-wei-hmaing are also used as an agent promoting the healing of ulcers. In this research, the roots of *Quisquails Indica* L. (Htar-wei-hmaing) were studied.

The aim and objectives of the research are to study the pharmaceutical activities and to upgrade the research level of natural products in organic chemistry, to search biologically active organic compounds from Myanmar indigenous medicinal plants, to isolate by using chromatographic and spectroscopic methods and to provide the effective chemical constituents as traditional remedy in public and provide health service.

2. Materials and Method

2.1 Sampling

In this research work, the roots of *Quisquails Indica* L., **Family name,** *Combretaceae*, Common name, Rangoon creeper, Myanmar name, Hta-wei-hmaing, were collected from Maha-aung-myae Township, Mandalay. They were chopped into pieces, allowed to dry in air and ground to powder. They were stored in well-stopped bottle and used throughout the research work.

Figure 1. The Plant of *Quisquails Indica* L.

2.2 Preliminary Phytochemical Screening of the Roots of Plant Sample (Harbone.J.B ,1984)

Preliminary phytochemical screening of the roots of *Quisquails Indica* L. was carried out by phytochemical tests.

2.3 Antimicrobial Activities of the Roots of Plant Sample

The antimicrobial activities of the crude extracts of the roots of *Quisquails Indica* L. were performed by agar well diffusion method in various solvents, n-hexane, ethanol, chloroform and ethyl acetate systems on six tested organisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albican* and *Mycobacterium* species.

2.4 Antioxidant Activity of the Roots of Plant Sample

The antioxidant activity of ethanol extract of the roots of *Quisquails Indica* L. was determined by DPPH assay using ascorbic acid as standard.

2.5 Extraction and Isolation of Pure Compound from the Roots of Plant Sample

The 500 g of the air dried pieces of the roots of *Quisquails Indica* L. were percolated with 2 liters of ethanol for two months. The extract was filtered off and the resulting ethanol soluble solution was evaporated to yield the concentrated residue. Then, the residue was extracted with 500 ml of ethyl acetate. The ethyl acetate soluble portion was concentrated to obtain the crude extract. It was checked by thin layer chromatogram. The 4.01 g of the crude extract was separated by column chromatographic method using 70-230 mesh of silica gel as an adsorbent and various ratios of n-hexane and ethyl acetate ranging from non-polar to polar were used as eluent.

2.6 Antimicrobial Activities of Pure Compound Isolated from the Roots of Plant Sample

The antimicrobial activities of isolated pure compound were rechecked by using agar well diffusion method with six tested organisms.

2.7 Identification of Functional Groups of Pure Compound Isolated from the Roots of Plant Sample (Silverstein R.M., et al, 2005)

The functional groups of isolated pure compound were identified by FT-IR spectroscopy.

3. Results and Discussion

The results of preliminary phytochemical screening of the roots of *Quisquails Indica* L. were tabulated in Table1.

Table 1.The Results of Phytochemical Screening of the Roots of Plant Sample

Constituents	Reagent Used	Observation	Sample
Alkaloid	Dragendroff's Reagent	Orange ppt	present
Flavonoid	EtOH, Mg turning, HCl	Pink color	present
Terpene	AceticAnhydride, Conc: H_2SO_4	Pink color	present
Steriod	AceticAnhydride,Conc: H_2SO_4	Green color	present
Saponin	Water	Forth like comb	present
Phenolic Compound	10% $FeCl_3$ solution	Green color	present
Glycoside	10% lead acetate	White ppt	present
Tannin	$FeCl_3$, Conc: H_2SO_4	Yellowish brown	absent

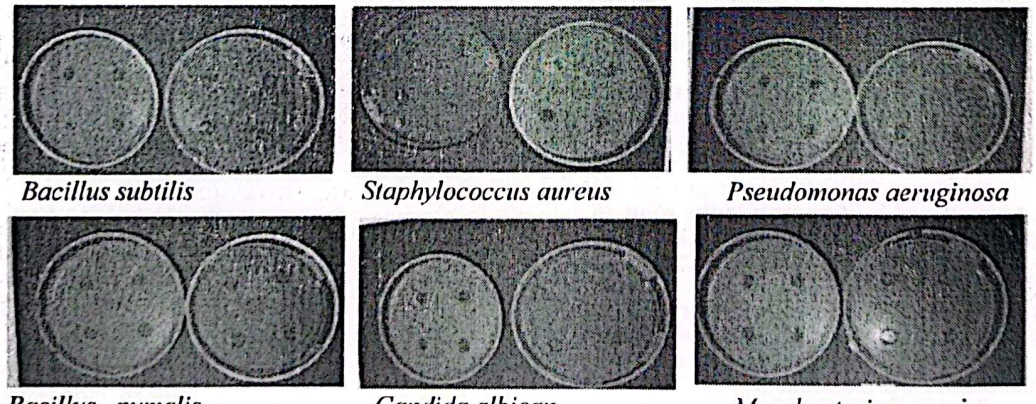
This Table indicated that the crude sample of the roots of *Quisquails Indica* L. contained the alkaloid, flavonoid, glycoside, phenolic compound, steroid, terpene and saponin respectively.

The antimicrobial activities of the roots of *Quisquails Indica* L. were recorded in Table 2. and Figure 2.

Table 2. Antimicrobial Activities of Crude Extracts of the Roots of Plant Sample

	Inhibition Zone			
Organisms	n-hexane	ethyl acetate	ethanol	chloroform
Bacillus subtilis	+	+	+	+
Staphylococcus aureus	+	+	++	++
Pseudomonas aeruginosa	+	+++	+++	++
Bacillus pumalis	+	++	++	++
Candida albican	+	++	++	++
Mycobacterium species	+	++	+	+

Agar Well – 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)



Bacillus subtilis *Staphylococcus aureus* *Pseudomonas aeruginosa*

Bacillus pumalis *Candida albican* *Mycobacterium* species

Figure 2. Antimicrobial Activities of Crude Extracts of Roots of Plant Sample

According to the Table 2., the crude extracts of the roots of the plant sample could respond activities against all tested organisms.

The % inhibition and IC_{50} value of standard ascorbic acid were shown in Table 3 and Figure 3.

Table 3. The % Inhibition and IC_{50} Values of Standard Ascorbic Acid

No.	Concentration (µg/ml)	Mean Absorbance	Mean % Inhibition	IC_{50} Value (µg/mL)
1	50	0.0628	89.936	11.98
2	25	0.224	64.103	
3	12.5	0.288	53.840	
4	6.25	0.341	45.350	
5	3.125	0.397	36.378	

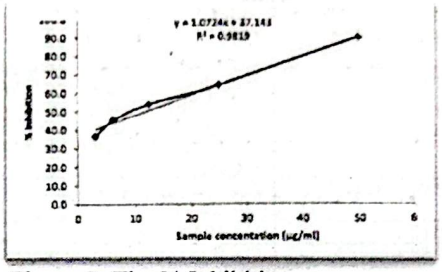


Figure 3. The % Inhibition vs. Concentration of Standard Ascorbic Acid

The % inhibition and IC_{50} value of the ethanol extract of the roots of *Quisquails Indica* L. were shown in Table 4. and Figure 4.

Table 4. % Inhibition and IC_{50} Values of Ethanol Extract of Roots of Plant Sample

No	Concentration (µg/mL)	Mean Absorbance	Mean % Inhibition	IC_{50} Value (µg/mL)
1	100	0.075	50.20	80.12
2	50	0.277	49.02	
3	25	0.296	45.89	
4	12.5	0.38	41.98	
5	6.25	0.472	39.50	

From the results, the IC_{50} value of ethanol extract of the roots of *Quisquails Indica* L. was 80.12 µg/mL and that of standard ascorbic acid was 11.98 µg/mL. It was seen that the ethanol extract of the roots of *Quisquails Indica* L. could have the high antioxidant activity.

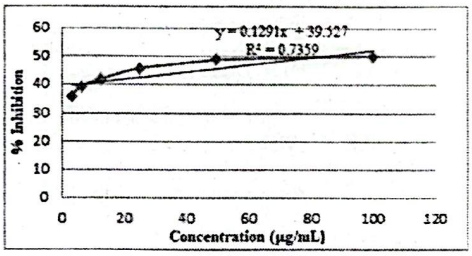


Figure 4. The % Inhibition vs. Concentration of Ethanol Extract of Roots of Plant Sample

After the column separation, totally 142 fractions were obtained. Each fraction was checked by TLC. The same R_f value of fractions were combined. The eight combined fractions were obtained. Among them, the combined fraction (V) gave pale yellow oily form and only one spot on TLC. The 0.0603 g of yellow oily form was obtained. The yield percent was found to be 1.5% based upon the ethyl acetate extract.

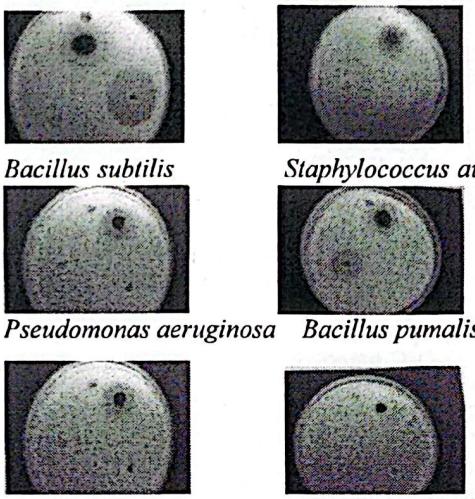
The antimicrobial activities of isolated pure compound were shown in Table 5.

Table 5. Antimicrobial Activities of Isolated Pure Compound

Sample	I	II	III	IV	V	VI
Pure Compound	++	++	++	++	+	+

Agar Well – 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

I=*Bacillus subtilis* II=*Staphylococcus aureus*
III=*Pseudomonas aeruginosa*
IV=*Bacillus pumalis*
V= *Candida albican*
VI=*Mycobacterium* species



Bacillus subtilis *Staphylococcus aureus*

Pseudomonas aeruginosa *Bacillus pumalis*

Candida albican *Mycobacterium* species

Figure 5. Antimicrobial Activities of Isolated Pure Compound

The isolated pure compound could respond medium activities on *Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis* and low activity on *Candida albican* and *Mycobacterium* species. The results gave the information that the pure compound isolated from the roots of *Quisquails Indica* L. was a bioactive compound.

The characteristic absorption frequencies in the FT-IR spectrum of isolated bioactive compound of the roots of *Quisquails Indica* L. was shown in Figure 6. and Table 6.

Table 6. Characteristic Absorption requencies of Isolated Pure Compound

Absorption frequencies cm^{-1}	Assignment of functional group
3340.5	N-H stretching vibration of amino group O –H stretching vibration of alcohol group
3101.3,3070.5	C – H stretching vibration of alkenic hydrocarbon
2962.5, 2862.2	Asymmetric and symmetric C – H stretching vibration of sp^3 hydrocarbon
1705.0	C = O stretching vibration of carbonyl group
1604.7, 1566.1	C —C ring skeletal stretching vibration of aromatic benzene ring.
1512.1	N – H bending vibration
1458.1	C – H in plane bending vibration of allylic hydrocarbon
1434.9	C – N stretching vibration
1265.2	C – C – O stretching vibration of carbonyl group
1095.5,1072.3,	C – O – C stretching vibration of ether group
925.8	C – H out of plane bending vibration of trans or E alkene
802.3	C – H out of plane bending vibration of cis or Z alkene

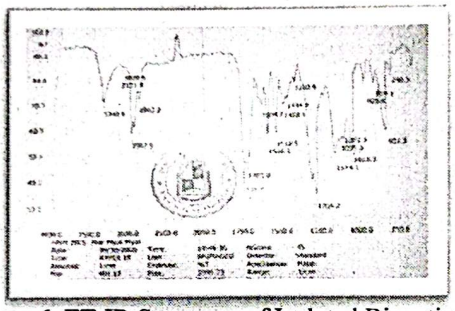


Figure 6. FT-IR Spectrum of Isolated Bioactive Pure Compound

The Table 6. showed that the bioactive pure compound isolated from the roots of *Quisquails Indica* L. may be present O-H,N-H, C=O, C-O-C functional groups.

4. Conclusions

In this research, one Myanmar indigenous medicinal plant, namely *Quisquails Indica* L. (Htar-wei-hmaing) was chosen for chemical analysis. As described, preliminary phytochemical screening of the roots of this plant could respond positive for flavonoid, alkaloid, sponin, phenolic compound, steroid and glycoside, respectively. The results showed that the valuable phytochemicals were present in the roots of the plant sample.

Furthermore, as mentioned in Table 2. the crude extracts of the roots of *Quisquails Indica* L. gave rise to activities against all selected organisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albican* and *Mycobacterium* species.

From the determination of antioxidant activity, the IC_{50} value of ethanol extract of the the roots of *Quisquails Indica* L. was 80.12 µg/mL and that of standard ascorbic acid was 11.98 µg/mL. It was seen that the ethanol extract of the roots of *Quisquails Indica* L. could have high antioxidant activity.

In addition, the separation of ethyl acetate extract of the roots of *Quisquails Indica* L. by modern separation technique such as Thin Layer and Column Chromatography applying silica gel (70-230 mesh) as an adsorbent and n-hexane and ethyl acetate in various ratios as eluent produces a pure pale yellow oily compound. The yield percent was found to be (60.3 mg, 1.5%) based upon the ethyl acetate extract.

Moreover, the isolated pure compound could respond medium activities on four tested organisms and low activity on two tested organisms. The results gave the information that the isolated compound was a bioactive pure compound.

From FT-IR spectrum, it was found that isolated bioactive pure compound may be present N-H, O-H, C=C, C-O-C functional groups, respectively.

Acknowledment

We would like to express our heartfelt gratitude to Dr Thida Win, Rector, University of Mandalay and Dr Myint Myint Sein, Professor and Head (Rtd), Department of Chemistry, University of Mandalay for their interest and encouragement on our research work. We also convey special gratitude to Dr Yi Yi Myint, Professor and Head, Department of Chemistry, University of Mandalay for her invaluable advice.

References

Farnsworth NR, Soejar DD (1991) Global important of medicinal plants. Cambridge University Press .

Gardino Nursery, Corp Top Tropicals, Medicinal use of Rangoon creeper-bimbima (2013) https://www.bimbima.com/ayurveda/medicinal-**use-of-rangooncreeper**/*1298*

Gupta P.D, De A. (2012) "*Diabetes Mellitus and its herbal treatment.*" Int J. Res Pharmaceut Biomed Sci 3: 706-721.

Harbone J.B (1984), Phytochemical Method , A Guid to modern techniques of plant analysis, Chapman and Hall Ltd; U.S.A

Joy P., Thomas J. ,Mathew S., and Skaria B.P.,(1998) "*Medicinal plants,*" Tropical Horticulture, Vol. 2, pp. 449– 632. View at Google Scholar.

Kavishankar G., Lakshmidevi N., Murthy S, M., Prakash H., and Niranjana S., (2011) "*Diabetes and medicinal plants – a review,*" Journal of Pharmaceutical and Biomedical Sciences, vol. 2, no. 3, pp. 65–80. View at Google Scholar.

Silverstein R.M., Webster F.X, Kiemle D.J. (2005)"*Spectroscopic Identification of organic compound*", 7th Edition, John Willey and Sons, Inc. Singapore.

Urbi Z., Hossain S., Hafizur Rahman K.M., and Zayed T.M.,(2014) "*Grape: a medicinal fruit species inthe holy Qur'an and its ethnomedinical importance,*" World Applied Sciences Journal,Vol. 30, No. 3, PP. 253–265.

Wetwitayaklung, P., Phaechamud, T., Linmatvapirat, C. and Keokitichai, S. (2008). Acta Hortic. 786, 185- 192 DOI: 10.17660/ActaHortic.2008.786.20

Characterization and Application of Micro Fibrillated Cellulose Prepared from Bamboo Sawdust

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Abstract

Bamboo sample was collected from Taung-dwin-gyi Township, Magway region, Myanmar. Bamboo sawdust was prepared by cutting the sample with saw and dust was sieved (60 – 100 mesh). The sawdust was treated with 5% HCl followed by 5% NaOH solution. The resulting sample was bleached using 50% H_2O_2 solution. Micro fibrilla cellulose (*MFC*) was prepared by hydrolysing the bleached cellulose sample with (4M H_2SO_4) at a boiling temperature for two hours. Surface morphology of *MFC* was studied through scanning electron microscope (SEM) photography and structural feature was characterized by fourier transform infrared (FT IR) spectrometry. Application of prepared *MFC* was justified by adsorption experiments including the removal of metallic ions and coloring organic substances as well as by water absorbing tendency. Experimental results showed that *MFC* can be used not only as a support for iron for removal of As^{3+} ion but also as a desiccant and water absorbing material because it has high affinity for water.

Keywords: MFC, adsorption, As^{3+} ion, colour substance, desiccant

Introduction

Botanically, bamboos are tall giant perennial grasses belonging to the family Poaceae. Twelve hundred and fifty species of 47 genera of bamboo are known to exist in the world. Bamboo are widely distributed in the tropical and sub-tropical regions of all continents except Europe (Ramadora and *et al.*, 1991).

The quick growth, easy availability and cheapness of the bamboos have always made it an important natural product used extensively in the rural areas. The long lengths of the culms, their straightness, smoothness and lightness, combined with strength and hardness and the easy manner in which they can split, make them ideal for all types of constructions and implements. They can be used to furniture decorations, baskets and implements (Lakkad and *et al.*, 1981).

In addition, it was observed that bamboo was used to manufacture various types of processed products like bamboo insulation-boards, veneer, mats and boards, and for manufacture of pulp and paper products. Bamboo is considered a composite material because it consists of cellulose fibers embedded in a lignin matrix. Cellulose fibers are aligned along the length of bamboo providing maximum tensile flexural strength (Lakkad and *et al.*, 1981).

The main constituents of bamboo culms are cellulose, hemicellulose and lignin (web – 1). The minor constituents of bamboo are resins, tannins, waxes and inorganic salt (Tomalang and et al., 1980). Cellulose fibers are built up fibril bundles, which consist of smaller elements called microfibrils. Through a fibrillation process, the cellulose fibers are converted into a three-dimensional network of microfibrils with an ultra-high surface area. These microfibrils are called micro fibrillated cellulose (web – 2).

As a key forest product, cellulose is widely used in the form of microfibers (Turbak and *et al.*, 1983). The use of natural fibers instead of traditional reinforcement materials such as glass fibers, carbon and talc provide several advantages including low density, low cost, good specific mechanical properties, reduced tool wear and biodegradability (Visakh and *et al.*, 2010).

In this work, we explored the preparation of micro fibrillated cellulose bamboo sawdust from the waste product of bamboo as well as the justification for application of it in adsorption of some metallic ions, organic dye compounds and determination of water absorbing tendency.

Botanical Description

Family name - Poaceae
Botanical name - *Bambu ori*
Common name - Guadua bamboo
English name - Bamboo
Myanmar name - Hmyin-war

The stem is the most commonly part of the plant in use.

Figure 1. *Bambu ori* plant

Materials and Method

Preparation of *MFC*

Bamboo sawdust was obtained from the waste materials of bamboo. They were collected from Taung-dwin-gyi Township, Magway region. Bamboo sawdust sample was sieved to get the range of 60-100 mesh size. The sample was treated with 5% HCl solution at room temperature for 24 hr. After washing with distilled water, it was again treated with 5% NaOH solution for further 24 hr. Then the sample was washed and bleached by soaking with 50% H_2O_2 solution for 24 hr. Finally, the sample was partially hydrolysed with 4M H_2SO_4 solution at the boiling temperature for 4 hours in order to get the *MFC*. It was washed several times with distilled water to become neutral. The prepared *MFC* was dried in an oven at 100°C for three hours and stored it for further work.

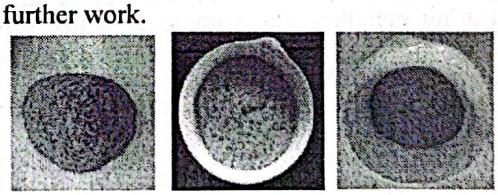


Figure 2. Bamboo sawdust (left), Bleached sawdust (middle), and *MFC* sample (right)

Characterization

MFC sample was characterized by the surface morphology through *SEM* images and the presence of characteristic absorption bands by *FTIR* spectrometric identification method. *SEM* information gave a good agreement for the surface

feature of prepared *MFC* with literature while *FTIR* information supported the confirmation for the structural feature of the prepared *MFC*.

Application

(1) Removal of colored metallic ion from solution

These adsorption experiments were conducted colourimetrically by using various dosage level of *MFC* sample (0.50 g, 1.00 g, 1.50 g and 2.00 g) with each 25 mL solution of 10g/L $NiSO_4$, 10 g/L $CuSO_4$, and 5 g/L K_2CrO_4 respectively. Calibration curve for each of the metallic solutions was also constructed. Wavelength 680 nm was used for absorbance measurement of pale green $NiSO_4$ solution, 620 nm for blue $CuSO_4$, and 450 nm for yellow K_2CrO_4 solution respectively. The effectiveness of *MFC* in these experiments was justified in accordance with the results obtained.

(2) Removal of arsenic ion

The experiment was conducted by using powdered Fe_2O_3 – *MFC* mixture. Optimum for respective mass of Fe^{3+} and *MFC* was determined spectrometrically by using standard arsenic (III) solution. The mixture of 2 mL of (0.01%) Methylorange, 4 mL of 2 M HCl, 4 mL of 2% KI and 4 mL of 2% KIO_3 mixture which was finally made up to 100 mL was used as chromogenic reagent. The presence of As^{3+} ion was found to be linearly proportional to the absorbance at λ_{max} 430 nm. The tendency of percent removal of arsenic was calculated as follow.

% removal of $As^{3+} = (A_0 - A / A_0) \times 100$

where, A_0 and A were amount of As^{3+} before and after adsorption in the solution.

(3) Removal of colored organic dye

The experiment described in application (1) was repeated for the adsorption of methylene blue and rhodamine B dye solutions. Only the difference was concentration of the solutions used in experiment. The concentration for both methylene blue and rhodamine B solution were 0.1 g/L. Wavelength 620 nm was used for methylene blue and 450 nm for rhodamine B solution.

(4) Determination of water absorbing tendency

10.0 mL of water was measured with (10.0 mL) measuring cylinder. Then it was poured into beaker containing 0.10 g of *MFC*. The solution was stirred with glass rod occasionally. After 5 minutes, the solution was filtered through gauze. Filtrate was collected with (10.0 mL) measuring cylinder. The volume of water was noted. Water absorbing tendency of *MFC* was calculated by following formulation.

$$\% \text{ water absorbing tendency} = \frac{10.0 - x}{10.0} \times 100$$

where, x = volume of filtrate (water)

A number of experiment were repeated by increasing mass of *MFC* up to 2.00 g.

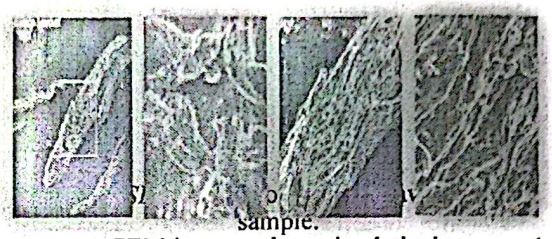
Application of *MFC*

(1) Adsorption tendency to coloured metallic ions

When absorbance was plotted against concentration, straight line was obtained.

Results and Discussion

Characterization of bamboo sawdust *MFC*



sample.

SEM images showed relatively a good surface morphology of *MFC* sample. Both pores and fine lines are observed. Porosity is not very apparent. But parallel fine lines are apparent. The segments are also apparent in the figures. This appearance supports the structure of micro fibrilla cellulose. Characterization is further confirmed by *FTIR* spectral measurement.

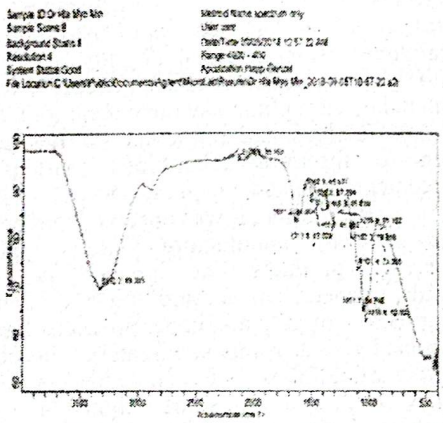


Figure 4. *FTIR* spectrum of bamboo sawdust *MFC*

The strong and broad symmetric band at 3332 cm^{-1} is assigned as stretching vibration of O-H functional group. The medium band appeared at 1661 cm^{-1} is characteristic of acetal group. Strong band observed at 1030 cm^{-1} accounts for the presence of ether groups. This ether link is considered to mix with acetal and polymer chain linkage C-O-C. The combination of these observation gives a strong evidence for the structural requirements of *MFC*.

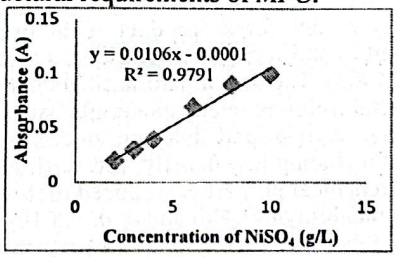


Figure 5. Calibration curve of $NiSO_4$ solution

Removal of Ni^{2+} ion by *MFC* was carried out by using the highest concentration of this solution, 10 g/L. When absorbance of Ni^{2+} (and other coloured metal ions) solution after absorption by *MFC* was

plotted against the dosage, following graph was obtained.

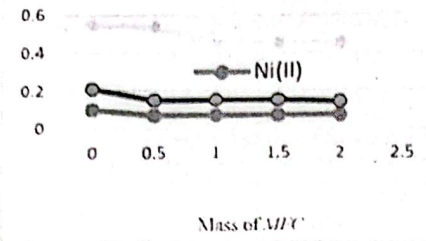


Figure 6. Absorbance of coloured metal solution after adsorption by *MFC*

Adsorption behavior of remaining solutions by *MFC* sample was shown by the graph resulting from the plot of absorbance of corresponding solution against dosage of *MFC*. In each of the experiments, construction of calibration curve was done prior to application of *MFC*. But here, representation for the calibration curve was omitted. When the adsorption tendency of *MFC* sample to metal ions under investigation was compared, a smooth trend was observed.

Table 1. Comparison of adsorption behavior to metal ions

Coloured metal ion	Effective dosage *MFC* (g)	% Adsorption
Ni^{2+}	0.50	30
Cu^{2+}	0.50	28.57
CrO_4^{2-}	1.00	18.18

It was observed that *MFC* material possesses higher affinity for cation over anion. The interaction between *MFC* and cationic species might be higher than anionic species. Moreover, the smaller the size of metal ion, the greater the adsorption tendency of *MFC* sample to these ions. Here, nickel and copper are considered as single ion but chromium as compound ion.

However, the adsorption tendency of all metal ion was found to be lower than 50 % even the dosage of *MFC* has increased to 2.00 g. In addition, the adsorption tendency of *MFC* alone to metallic ions was found to be low though the high concentration of metal solutions were used.

On the basis of this observation, as a supporting material, one metal ion (As^{3+}) removal tendency of *MFC* was further determined by mixing with iron (III) oxide powder. This is because iron is very well known in removal of toxic arsenic metal ion from aqueous solution.

(2) Removal of arsenic ion

Optimum mass for Fe^{3+} (Fe_2O_3) and *MFC* were 0.2 g and 0.1 g respectively. The mixture of 0.2 g Fe_2O_3 and 0.1 g *MFC* sample was observed to have 82.29% removal tendency to As^{3+} from its solution. Std As^{3+} means standard arsenic solution before adsorption experiment, *MFC* means absorbance of As^{3+} solution after adsorption by 0.1 g *MFC* sample, Fe^{3+}(0.1g) means absorbance of As^{3+} solution after adsorption by 0.1 g Fe_2O_3 sample and so on.

For the 100 ppm As^{3+} solution, the adsorption tendency of Fe – *MFC* mixture was:

% removal of As^{3+} (for 100 ppm) = $[(0.480 - 0.085) / 0.480] \times 100 = 82.29\%$

It was observed that the Fe – *MFC* mixture possesses high tendency for the removal of arsenic ion. This point was controversy to adsorption tendency of *MFC* to former metallic ions. Although As^{3+} ion has higher charge density than Ni^{2+} and Cu^{2+} ions, it had been removed higher level by the mixture of Fe – *MFC*. This was probably due to complex formation between Fe hosted by *MFC* sample and arsenic ions in the solution.

(3) Adsorption tendency to organic dyes

It was found that the effective dosage of *MFC* for 25 mL of 0.01 g/L methylene blue and rhodamine R solutions was found to be 0.50 g and its adsorption tendency was 89.06% and 91.35% respectively. From the graph, ED_{50} (effective dosage 50%) of bamboo *MFC* sample for both dyes was assigned as 0.30 g.

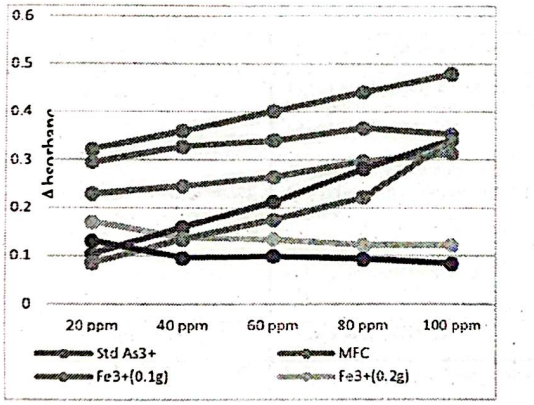


Fig. 7. Absorbance versus concentration of As3+ solutions

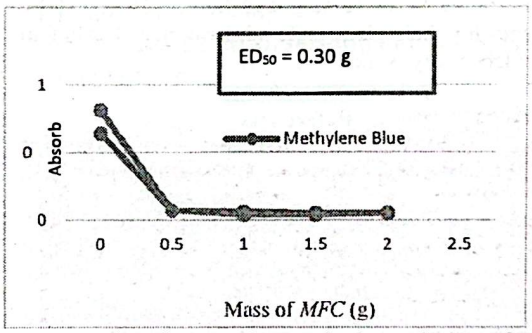


Figure 8. Absorbance of organic dye solution after adsorption by *MFC*

(4) Water absorbing tendency

This experiment was conducted with initial volume 10 mL of water. After being absorbed by *MFC* sample, the volume of remaining water was measured.

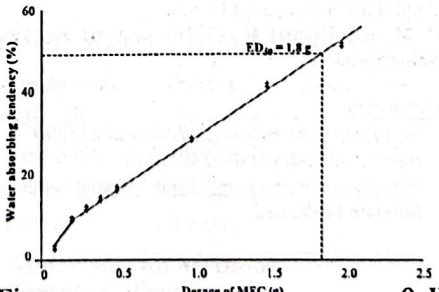


Figure 9. Water absorbing tendency versus dosage of *MFC*

When % water absorbing tendency of *MFC* was plotted against the dosage, relatively smooth line graph was obtained. The relationship between dosage of *MFC* and tendency to absorb water is found to be linear. From the graph, ED_{50} value was taken as 1.8 g. Bamboo sawdust *MFC* 1.8 g can absorb 5.00 mL of water if 10 mL of water is in contact with it.

Research outcome

The waste bamboo sawdust can be transformed into useful material called *MFC*. The preparation of *MFC* is easy and the whole process is very cheap. *MFC* can be used effectively for the removal of dyes and some metallic ions having color from their solutions.

Because of the relatively low number of pores on surface of *MFC*, it showed lesser tendency to adsorption for metallic ions. But this material can be used as a support for iron (III) ion for adsorption of arsenic ion form solution. Due to having the high surface area, *MFC* revealed high tendency to adsorption of organic dyes.

Conclusion

For the purpose of application, it is suggested that *MFC* can be used in removal of coloring inorganic metallic ions (Ni^{2+}, Cu^{2+}, Cr^{6+}) and preferentially organic dyes (methylene blue and rhodamine B) from solutions. Very small amount 0.3 g of *MFC* showed high tendency to adsorb organic synthetic coloring materials. Experimental results pointed out that *MFC* can be used not only as a support for iron for removal of As^{3+} ion but also as an effective desiccant and water absorbing material because it has high affinity for water.

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References

Lakkad H. C., and Patel J. M., (1981), Mechanical properties of bamboo, natural composite. Fiber Sci. and Tech. 14 (4), 319-322.

Ramadora T.S, Trikannad P. M and Ravindranathan R., (1991), Bamboo a Potential raw material for paper making in tropical countries. Non-wood Plant Fiber Pulping. Progress Reports No 12, 57-65.

Tomalang F. N., (1980), Properties and utilization of Philippine erect bamboo.

Turbak A. F., (1983), Micro fibrillated cellulose, a new cellulose product, properties, uses and commercial potential., J. Appl. Polym. Sci., 37, 815-827.

Visakh P. M. and Thomas H., (2010), // waste and biomass valourization 1.

Online Websites

1. http://en. wikipedia.org/w/index.php? title= Hemicellulose& oldid=824841216
2. http://www.exilva.com/About-Exilva/What is micro fibrillated cellulose

Adsorption Capacity of Leaves of *Terminalia catappa* Linn and Utilization on Treatment of Waste Water

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Abstract

The leaves contain many chemical compounds. Due to this chemical richness, the leaves are used in different herbal medicines for various purposes. Furthermore, these leaves are also considered as adsorbents to remove toxic materials in water. This research is mainly concerned with the dried leaves of Badan which is botanically called *Terminalia catappa* Linn. The dried leaves were used as adsorbent. The elemental composition of Badan was determined by Energy Dispersive X-ray Fluorescence (EDXRF) and the surface morphology was examined by Scanning Electron Microscopy (SEM) at different magnification. The adsorptive property of the leaves on heavy metal, lead was determined by complexometric titration. The removal of lead ions from aqueous solution was carried out by using the leaves at various concentrations of lead II nitrate solution (0.006M, 0.008M, 0.01M, 0.012M, 0.014M), pH values ranging from 3 to 6, different contact time (2hr, 4hr, 6hr, 8hr, 10hr) and different adsorbent dosage (0.5g, 1g, 1.5g, 2g, 2.5g). In order to characterize the adsorbent, the experimental results obtained by dosage method were analyzed by using Langmuir and Freundlich isotherms. Based upon the optimum conditions from experiments, the waste water from agricultural sites was treated using dried Badan leaves.

Keywords: adsorption, complexometric titration, Terminalia catappa, EDXRF, SEM

I. Introduction

The adsorption operations exploit the ability of certain solids preferentially to concentrate specific substances from solution onto their surfaces. The components of either gaseous or liquid solutions may be separated in this manner from each other. The substance that is removed from the liquid/gas phase is called the *adsorbate* and the solid surface that is used to remove adsorbate is called *adsorbent.* The phenomenon is called *adsorption.* This process creates a film of the adsorbate (the molecules or atoms being accumulated) on the surface of the adsorbent. The phenomenon of adsorption is essentially an attraction of adsorbate molecules to an adsorbent surface. Adsorption processes can be classified as either physical adsorption (van der Waals adsorption) or chemisorption (activated adsorption) depending on the type forces between the adsorbate and the adsorbent (Gawande, *et al.*,2012).

Table-1 Comparative Summary of Physical and Chemical Adsorption

Physical adsorption	Chemical adsorption
Based on inter-molecular attractive force	Based on chemical interaction between the adsorbent and adsorbate
A number of layers of molecules can build up on the surface and not confined to a single layer	Results in the formation of a single layer of molecules on the surface
A small amount of heat is liberated	A large amount of heat is liberated
Rapid and reversible	Slow and irreversible
It forms multi-molecule layers	It forms mono-molecular layers

In recent years, lead contamination in an environment is a very important problem of worldwide concerning due to its highly toxic and nonbiodegradable in nature. Lead is released into aquatic system by various ways such as natural phenomenon, urban, agricultural and industrial activity. The industrial activity is the major source of lead contamination in water (Akpor *et al.*,2012).

Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver and other vital organs. Long-term exposure may result in slowly progressing physical, muscular and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis. (Islam, *et al.*, 2016).

Therefore, elimination of toxic heavy metals from environment should do by researchers. The main aim of this research is in order to use biological resources as adsorbent to remove toxic element such as lead from waste water.

There are various methods to treat the metal contaminated effluent such as precipitation, reverse osmosis, ion exchange, coagulation and adsorption (Webb, *et al.*, 2003). In the present research work, adsorption process was used to treat waste water collected from agriculture sites near Gwaygyigone village, Patheingyi township Mandalay Region by the use of Badan leaves. Botanical description of Badan is as follow. (Bartram *et al.*, 1996).

1.1 Botanical Description of *Terminalia catappa* Linn.

Figure 1. Badan Tree and dried leaves

Botanical name : *Terminalia catappa Linn.*
Family nam : Combretaceae
English name : Wild or Indian Almond
Myanmar name : Badan
Part used : Leaves

II. Materials and Methods

A. Sample Collection

The dried leaves of *Terminalia catappa Linn* were collected from Mandalay University

sample was reduced by dried Badan leaves based upon previous experimental results.

III. Results and Discussion

A. SEM Image Studies

The surface morphologies of the Badan leaves were examined by scanning electron microscope because it is necessary to characterize the surface morphologies of the species which will be used as adsorbent. In the SEM image of dried Badan leaves, it can be seen clearly that the particles have different size of holes and the size in the range of 3.15 μm to 6.31 μm. This is the most interesting factor to use as an adsorbent. The dried Badan leaves powder is an effective adsorbent due to its surface morphologies. Therefore the leaves powder was considered to remove toxic lead metal by adsorption. The SEM image of the dried leaves powder is shown in figure 2.

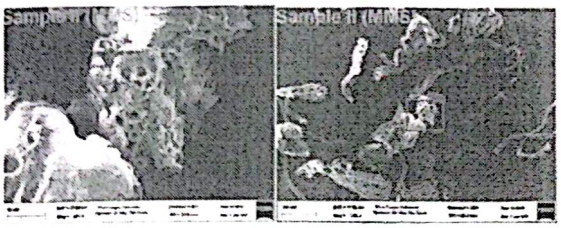

Figure 2. Scanning Electron Micrographic Image of Dried Badan Leaves

B. Elemental Composition of the Dried Badan Leaves Powder by EDXRF

The elemental composition of the dried Badan leaves powder was described in table 2.

Table 2. Elemental Composition of Dried Selected Leaves Powder

No	Element	Badan (%)
1	Potassium (K)	0.970
2	Calcium (Ca)	3.197
3	Chlorine (Cl)	4.325
4	Silicon (Si)	1.059
5	Aluminium (Al)	0.052
6	Phosphorous(P)	0.229
7	Sulphur (S)	0.050
8	Iron (Fe)	0.035
9	Strontium (Sr)	0.016
10	Manganese (Mn)	0.009
11	Titanium (Ti)	0.009
12	Barium (Ba)	0.004
13	Vanadium (V)	0.004
14	Zinc (Zn)	0.005

From the result of EDXRF information, it can be seen that many metals are present in Badan leaves but toxic heavy metals are not in it.

C. Effect of pH on the Removal of Lead II Ions Using the Dried Leaves Powder

The value of pH is greatly affected on the adsorption of heavy metals especially on lead based upon the adsorbent (Islam, 2016).

As shown in table 3, the adsorption of lead ion by dried leaves was studied over a pH range of 3 to 6. According to experimental results, it was observed that the optimum pH for removal of lead in aqueous solution was found to be at pH 5 but the removal of lead is the lowest at pH 3 for the Badan leaves.

Table 3. Effect of pH on the Removal of Lead II Ions by Dried Badan Leaves

No	pH	Initial conc: mg/L	Final conc: mg/L	Reduce mg/L	Percentage
1	3	2072.1	1450.47	621.63	30%
2	4	2072.1	1409.03	663.07	32%
3	5	2072.1	1346.87	725.23	35%
4	6	2072.1	1471.19	600.91	29%

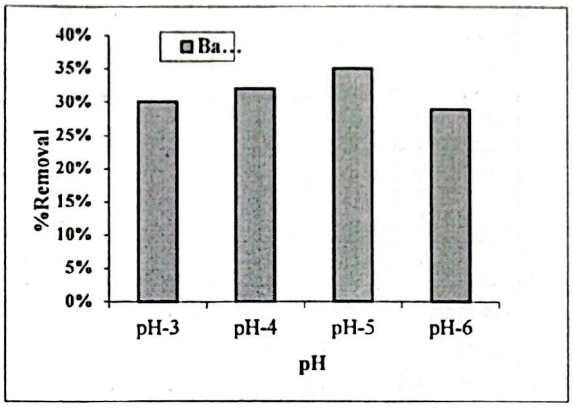

Figure 3. Effect of pH on the Removal of Lead II Ions by Dried Badan Leaves

D. Effect of Concentration on the Removal of Lead II Ions by Dried Badan Leaves

From the observation of concentration effect, it was noticed that the greater the concentration of lead, the lesser the removal percentage. These results are described in table 4.

Table 4. Effect of Concentration of Removal of Lead by Badan Leaves at pH5

No	Lead II nitrate concentration	Initial conc: mg/L	Final conc: mg/L	Reduce mg/L	Percentage
1	0.006 M	2486.52	1554.08	932.44	37.49%
2	0.008 M	2279.31	1450.47	828.84	36.36%
3	0.01 M	2072.1	1346.86	725.24	35.00%
4	0.012M	1864.89	1243.26	621.63	33.33%
5	0.014 M	1657.68	1139.65	518.03	31.25%

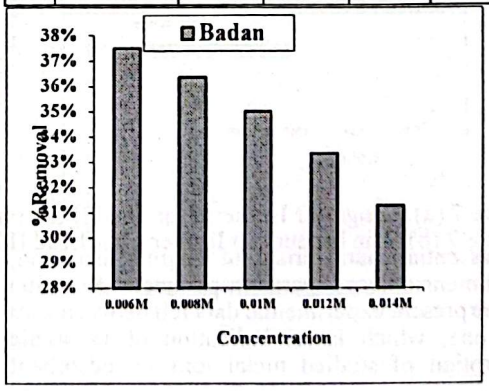

Figure 4. Effect of Concentration on the Removal of Lead by Dried Badan Leaves

E. Effect of Shaking Time on the Removal of Lead at pH 5

Table 5. Effect of Shaking Time on the Removal of Lead by Badan Leaves

No	Time	Initial conc: mg/L	Final conc: mg/L	Reduce mg/L	Percentage
1	2 hr	2072.1	1243.26	828.84	40%
2	4 hr	2072.1	1201.82	870.28	42%
3	6 hr	2072.1	1139.65	932.45	45%
4	8 hr	2072.1	1077.49	994.61	48%
5	10 hr	2072.1	1015.33	1056.77	51%

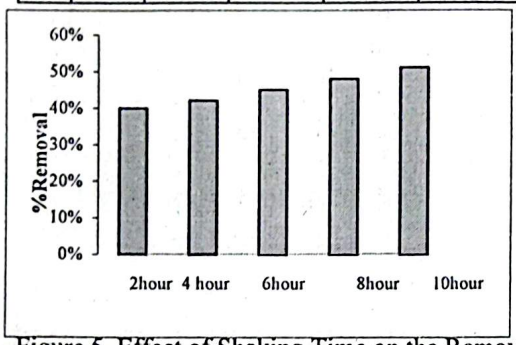

Figure 5. Effect of Shaking Time on the Removal of Lead by Badan Leaves

According to the results of contact time effect, it was found that the greater the contact time, the higher the percentage removal of lead but the optimum contact time for removal of lead is 8 hours.

F. Effect of Dosage of Badan Leaves on the Removal of Lead at pH-5

Table 6. Effect of Dosage of Badan Leaves on the Removal of Lead at pH-5

No	Dosage of adsorbent (g)	Initial conc mg/L	Final conc mg/L	Reduce mg/L	Percentage
1	0.5	2072.1	1699.12	372.98	18%
2	1.0	2072.1	1346.86	725.24	35%
3	1.5	2072.1	1139.66	932.44	45%
4	2.0	2072.1	932.45	1139.65	55%
5	2.5	2072.1	725.24	1346.86	65%

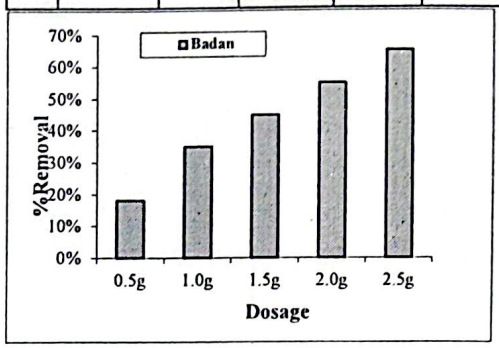

Figure 6. Effect of Dosage of Badan Leaves on the Removal of Lead II Ion

By observation of experimental results, it can be seen that there is the greater the adsorbent weights, the greater the removal of lead ion.

G. Study Adsorption Isotherm

The experimental results obtained from dosage method were analyzed by Langmuir and Freundlich isotherms.

Table 7. Langmuir and Freundlich Isotherms for Removal of Lead II Ion

No	Mass of sample (g)	Co (mgL^{-1})	Ce (mgL^{-1})	q_e (mgg^{-1})	Ce/q_e (gL^{-1})	log Ce ($mg\ L^{-1}$)	log q_e (mgg^{-1})
1	0.5	2072.1	1699.12	79.59	21.35	3.23	1.89
2	1.0	2072.1	1346.86	72.52	18.57	3.13	1.86
3	1.5	2072.1	1139.66	62.16	18.33	3.06	1.79
4	2.0	2072.1	932.45	56.98	16.36	2.97	1.75
5	2.5	2072.1	725.24	53.87	13.46	2.86	1.73

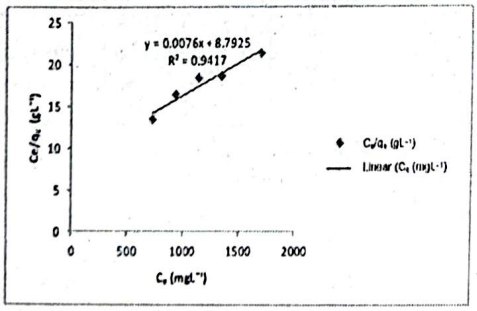

Figure 7 (a). Langmuir Isotherm for Lead II ion onto the Badan Leaves Powder

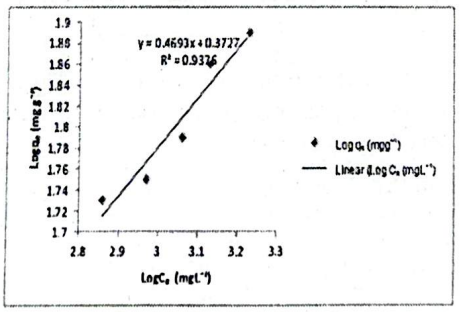

Figure 7 (b). The Freundlish Isotherm for Lead II Ion onto the Badan Leaves

The essential characteristic of langmuir isotherm, the dimensionless separation parameter R_L value for the present experimental data fell between zero and one, which is an indication of favorable adsorption of studied metal ions on adsorbent materials. Based on the Langmuir parameters X_m ($mg\ g^{-1}$) for monolayers formation, the sorption of lead II ion on Badan was 131.58 $mg\ g^{-1}$.

The Langmuir constant 'b' for lead II ion was 0.00086 in Badan. The square of correlation

Campus, Mahar Aung Myae Township, Mandalay Region on September, 2017. The collected leaves were ground by using grinder and then they were sieved by using 100 and 150 mesh size sieves. The resulting powder (between 100 and 150 mesh size sieves) were stored in plastic bottle and was used throughout the research.

B. Decolourization of the Leaves powder by Using Sodium Hypochlorite

The dried leaves powder was dissolved in 2% sodium hypochlorite solution for twenty-four hours and was filtered. The residue was washed with distilled water until it was decolorized. The decolourized sample prevents the disturbance of colour in detecting the end point of titration using this sample.

C. Analysis of the Collected Sample by EDXRF

The elemental composition of the collected leaves was analyzed by using Energy Dispersive X-ray Fluorescence (EDXRF) spectrophotometer.

D. Characterization of Dried Leaves Powder by Scanning Electron Microscope (SEM)

The leaves powder was examined by scanning electron microscope (SEM) for a visual inspection of external porosity and morphology.

E. Determination of Adsorptive Properties of Badan Leaves by Shaking Process

The adsorption capacity of dried leaves of Badan was determined by shaking process based upon various pH and various concentrations of aqueous lead solution, various dosage of the Badan leaves powder (Singh, *et al.*, 2017).

F. Determination of Removal of Lead by Dried Badan Leaves at Various pH of Lead II Nitrate Solution

Each 1 g of dried Badan leaves powder sample was mixed 100 mL of 0.01 M lead (II) nitrate solution at pH 3, 4, 5 and 6. The mixture was shaken for two hours by using shaker at 250 rpm. After shaking, it was filtered and the filtrate was used to determine the adsorptive properties of sample.

The resulting filtrate (10 mL) was placed in a conical flask. 0.2 g of tartaric acid was added. And then 5 mL of buffer solution and 35 mL of distilled water was added into the flask. When 0.2 g of EBT indicator was added, the colour of the solution was changed from colourless to purple. Finally, this solution was titrated with 0.01 M EDTA solution. At the end point, the colour of solution changed to blue.

G. Determination of Removal of Lead by Dried Badan Leaves Powder at Various Concentration of Lead II Nitrate Solution

Each 1 g of dried Badan leaves powder sample was mixed with 100 mL of each lead (II) nitrate solution in the range of 0.006 M, 0.008 M, 0.01 M, 0.012 M and 0.014 M at pH 5. The mixture was shaken for two hours by using shaker at 250 rpm. After shaking, it was filtered and the filtrate was used to determine the adsorptive properties.

The filtrate (10 mL) was placed in a conical flask and 0.2 g of tartaric acid was added. Then 5 mL of buffer solution and 35 mL of distilled water was also added into the flask. After adding 0.2 g of EBT indicator, the colour of the solution changed from colourless to purple. The filtrate (10 mL) was placed in a conical flask and 0.2 g of tartaric acid was added. Then 5 mL of buffer solution and 35 mL of distilled water were also added into the flask. After adding 0.2 g of EBT indicator, the colour of the solution changed from colourless to purple. The purple coloured solution was titrated with 0.01 M EDTA solution. When the colour of solution changed to blue, the titration end point was achieved.

H. Determination of Removal of Lead by Various Dosage of Badan Leaves

In the dosage method, a constant concentration of metal ion (initial concentration of metal ion solution 0.01 M) was used. The dosage of the adsorbent was varied in the range of 0.5 g, 1.0 g, 1.5 g, 2.0 g and 2.5 g. The pH was adjusted to the optimal pH 5 of metal ion. The mixture was shaken for two hours by using shaker at 250 rpm. After shaking, it was filtered and the filtrate was used to determine the adsorptive properties of sample.

The filtrate (10 mL) was placed in a conical flask and 0.2 g of tartaric acid was added. And then 5 mL of buffer solution and 53 mL of distilled water were added into the flask. When 0.2 g of EBT indicator was added, the colour of the solution changed from colourless to purple. When this purple coloured solution was titrated with 0.01 M EDTA solution, the colour of solution changed to blue at the end point.

I. Determination of Removal of Lead by Dried Badan Leaves at Various Shaking Time

Each 1 g of sample powder was mixed with 100 mL of 0.01 M lead (II) nitrate solution at pH 5. The mixture was shaken for 2 hours, 4 hours, 6 hours, 8 hours and 10 hours by shaker at 250 rpm. After shaking, it was filtered and the filtrate was used to determine the adsorptive properties of sample.

The filtrate (10 mL) was placed in a conical flask. 0.2 g of tartaric acid was added. And then 5 mL of buffer solution 35 mL of distilled water were added into the flask. When 0.2 g of EBT indicator was added, the colour of the solution changed from colourless to purple. Finally, this colour solution was titrated with 0.01 M EDTA solution. At the end point the colour of the solution changed to blue.

J. Study the Adsorption Isotherms

The experimental results obtained by dosage method were analyzed by using the Langmuir and Freundlich isotherms (Rashmi *et al.*,2012).

K. Assessment of Water from Agricultural site and Treatment

In the present study, the water samples from agricultural sites near Gwaygyigone village, Pathneingyi Township, Mandalay Region were assessed (Smeth *et al.*,2013). The water sample was collected from the common drainage of paddy growing fields on May 2017.

Some of physicochemical properties and the content of toxic heavy metal especially lead of the collected water sample were investigated (Rai *et al.*,1998). Then the pollution of the water

coefficient (R^2) for lead II ion was within the range of 0.9417 for Badan. The essential characteristic of Freundlich parameters, sorption capacity for Freundlich isotherm k(mg g^{-1}) on Badan was 2.359. The square of correlation coefficient (R^2) for lead II ion was within the range of 0.9376 for Badan. These isotherm data indicate that the experimental data obtained from dosage method are mathematically fit into the Langmuir and Freundlich adsorption isotherms.

According to the overall experimental results, the dried Badan leaves powder was used as adsorbent to reduce water pollution from agricultural sites in Patheingyi Township, Mandalay Region. The effect of adsorbent (Badan leaves powder) on treatment of waste water are shown in following table.

H. Effect of Adsorbent on Treatment of Water from Agricultural Site

Table 8. Effect of Adsorbent on Treatment of Water from Agricultural Site

Parameters	Before treatment	After treatment	WHO standard
pH	7.9	7.10	7-8
Turbidity (NTU)	6.92	0.92	5
TDS(mg/L)	1275	90	500
Alkalinity (mg/L)	575	87	200
Hardness (mg/L)	389	48	100
Chloride (mg/L)	228	30	200
Sulphate (mg/L)	286	155	200
Calcium (mg/L)	152	15	75
Lead (mg/L)	1.5	0.00	-

By observing the results of measured parameters before and after treatment of the parameters of waste water, it can be said that the adsorbent is the effective species to reduce water pollution.

IV. Conclusion

From the whole experimental data, it can be concluded that the dried Badan leaves powder is an effective adsorbent for removal of toxic lead metal ion in aqueous solution. The extent of removal of lead II ion from aqueous solution depends on pH, the mass of adsorbent used, the concentration of lead II ion in aqueous solution and the contact time between adsorbent and adsorbate. The optimum pH for the removal of lead II ion was observed at pH 5. And then it could be known that the greater the dosage of adsorbent, the greater the removal of lead II ion. Similarly, the longer the contact time between adsorbent and adsorbate, the stronger the adsorption capacity. Therefore, the present research shows that biological resources can be used as adsorbents to remove toxic element such as lead from waste water.

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References

Akpor, O.B., Okolomike, U.F., Olaoul, T.D. and Aderive, B.I., (2014), "Trends in Applied Sciences Research", *Remediation of Polluted Wastewater Effluents: Hydrocarbon Removal*, **Vol. 9(4)**, PP-160-173.

Assi, M.A., Hezmee, M.N., Haron, A.W., Sabri, M.Y.M. and Rajion, M.A., (2016), "The Detrimental Effects of Lead on Human and Animal Health", doi: 10.14202/vetworld, Vol. (6), PP. 660-671.

Bartram, J. and Balance, R., (1996), "Water Quality Monitering", *United Nations Environment Programme/ World Health Organization*, ANSI/NISO Z39.48-1992 and ANSI/NISO Z39.48-1984.

Gawande, N.J., Chaudhari, A.R. and Khope, R.U., (2012), "Influence of Surface Characteristics of Adsorbent and Adsorbate on Competitive Adsorption Equilibrium", *Advances in Applies Science Research*, Peligia Research Library, ISSN: 0796-8610, 3(3): 1836-1841

Islam, M.R., Sarkar, M.K.I., Afrin, T., Rahman, S.S.R., Talukder, R.I., Howlader, B.K. and Khaleque, M.A., (2016), "A Study on Total Dissolved Solids and Hardness Level of Drinking Mineral Water in Bangladesh", *AmericanJournal of Applied Chemistry*, doi: 10.11648/ j.ajac.20160404.11, ISSN: 2330-8753 (print); ISSN: 2330-8745, 4(5): 164-169.

Singh, A.K., Srivastava, M., Rajneesh, N.K. and Shukla, S., (2017), "Corncobs as Low Cost Bio-Adsorbent for Water and Waste Water Treatment", *Internal Journal of Innovative Research in Science, Engineering and Technology*, ISSn: 2347-6710, DOI:10.15680.

Webb, P.A., (2003), "Introduction to Chemical Adsorption Analytical Techniques and their Applications to Catalysis", *MIC Technical Publications*, Micromeritics Instrument Crop, Norcross, Georgia 30093.

Rai A-K-S.N. Upadhay, S. Kumer, and Y.D. Upadhay. (1998), "Heavy metal pollution and it control through a cheapter method : A Review, J. IAEM, 25, 220-251.Ju; Okoli, Ibe Ezuma, (2014), "Adsorption Studies of Heavy Metals by Low-Cost Adsorbents; Vol. 18(3) 443-448.

Rashmi Verma and Pratima Dulivedi, (2013), "Heavy metal water pollution. A Case Study", Recent Research in Science and Technology, 5(5): 98-99.

Smeth Khaoya and Ura Pancharoen, (2012), "Removal of lead II from Battery Industry Wastewater by HFSLM," International Journal of Chemical Engineering and Applications, Vol.3, No.2.

Structural Elucidation of (+)-Pinoresinol from the Stem of *Alyxia fascicularis* Benth.

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Abstract

In this study, one Myanmar indigenous medicinal plant, *Alyxia fascicularis* Benth., locally known as Taw Noe Yee, belonging to the family Appocynaceae was selected for the isolation of chemical constituents. The pure compound (KTDS-3) was isolated from *Alyxia fascicularis* Benth..by advanced separation techniques. The structural elucidation of the pure compound (KTDS-3) was done using advanced spectroscopic methods such as FT IR, ^{1}HNMR(600 MHz), ^{13}CNMR (150MHz), DEPT, DQF-COSY, HSQC, HMBC and FAB-mass spectral data respectively. The pure compound (KTDS-3) was identified as (+)-Pinoresinol.

Keywords: spectroscopic, indigenous, structural elucidation, chemical constituents

Introduction

Plants produce an enormous variety of natural products with highly diverse structures. These products are commonly termed 'secondary metabolites' which are essential for plants growth and development. [Anne E. Osbourn. (2009)] In this study, *Alyxia fascicularis* Benth. was selected for isolation of some chemical constituents.

This plant is locally used in Kachin State for several diseases such as paralyses, archorrhagia, arthragrosis, sore throat, hemias and backaches. It can also be used to treat for several diseases. For instance, people suffering stroke can consume the sink in alcohol to get the good health. In this research work, the structural elucidation of pure compound (KTDS-3) was isolated by advanced separation techniques such as Column and High Performance Liquid Chromatography (HPLC). In addition, the structure of isolated compound (KTDS-3) was elucidated by using advanced spectroscopic methods such as FT IR, ^{1}H NMR (600 MHz), ^{13}CNMR(150MHz), DEPT, DQF-COSY, HSQC, HMBC and FAB-mass spectral data respectively.

Materials and Method

General Experimental Procedure

FT IR spectrum (KBr pellets) were recorded on a Perkin Elmer Paragon 100 PC spectrometer. FAB-Mass spectrum was obtained with a JEOL JMS MS-700, respectively. The ^{1}D and ^{2}D NMR spectra were recorded with a Bruker Avance III HD 600 spectrometer equipped with Prodigy liq. Nitrogen cryoprobe. Chemical shifts for ^{1}H-and ^{13}C NMR are given in ppm (δ) relative to solvent signal (MeOH-d_4: δ_H3.30 and δ_C49.0; $CDCl_3$: δ_H7.26 and δ_C77.0) as internal standard. HPLC reversed phase column (C-8-UG-5) was used for purification of compounds. The specific optical rotations were measured on a JASCO P-2200 Polarimeter. Analytical and preparative TLC separations were performed on 0.25 mm silica gel 60 (GF254, Merck) aluminium supported foils.

Plant Material

The stem of *Alyxia fascicularis* was collected from a forest of Inkhaing Bum Hill, Myitkyina, Kachin State in March 2016. The plant was identified by Associate Professor, Dr Sandar Tun and Professor, Dr Moat War Dai Naw, Department of Botany, University of Mandalay, Myanmar.

Extraction and Isolation of Bioactive Compounds

The stem of *Alyxia fascicularis*(800 g) were percolated with 3 L of methanol. The methanolic extract was concentrated in *vacuo*, and dried extract (106.40 g) was obtained. The extract was then partitioned with ethyl acetate and 1-butanol against water successively to give ethyl acetate (25.34 g), 1-butanol (24.70g) and water-soluble fractions (51.25g). The ethyl acetate fraction was partitioned again with acetonitrile and hexane to afford acetonitrile (15.45 g) and hexane (4.40 g). The acetonitrile fraction (5.43 g) was separated on RP-18 and eluted with MeOH: water (decreasing polarity from 3:7 to 9:1, MeOH only) to give 20 main fractions (A-T). The fraction H (110 mg of 810 mg) was purified by HPLC with reversed phase column(C-30, UG-5) with a gradient system of (A) methanol–water (2:98) : (B) MeOH (60 : 40 to 30:70) as a mobile phase to give three subfractions (H-1 to H-3). The subfraction (H-3) was purified by HPLC reversed phase column(C-8, UG-5) with a gradient system of (A) methanol–water (2:98):(B) MeOH (95:5 to 60:40) as a mobile phase to give pure compound (KTDS-3).

Results and Discussion

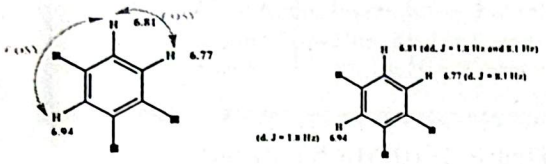
Structural Elucidation of Pure Compound, KTDS-3

The structural elucidation of the pure compound (KTDS-3) was done by some spectroscopic techniques such as ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz), DEPT, DQF-COSY, HSQC, HMBC and FAB-mass spectral data respectively.

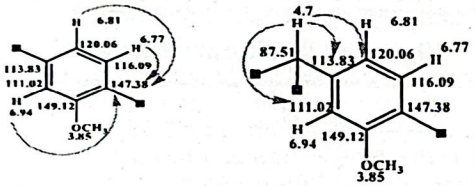
In DQF-COSY spectrum, Figure (7) there was observed the medium graphic area between two aromatic protons δ 6.81 ppm and δ 6.77 ppm, and small graphic area between two aromatic protons δ 6.81 ppm and δ 6.94 ppm producing the following tri-substituted benzene ring. The above fragment could be proved by the splitting patterns and coupling constants of these three aromatic protons δ 6.81 ppm (dd, J = 1.8 Hz and 8.1 Hz), δ 6.77 ppm (d, J ^{1}H NMR spectrum, Figure (2) as shown below.

In the HSQC spectrum supported ^{1}H-^{13}C direct correlation of the above aromatic protons with their respective aromatic carbons as indicated below. According to HMBC spectrum, Figure (6) the existence of α- and β- ^{1}H-^{13}C long range signals of the aromatic protons δ 6.94 ppm and δ 6.77 ppm with sp^2 quaternary carbon at δ 133.83 ppm giving rise the following fragment. In

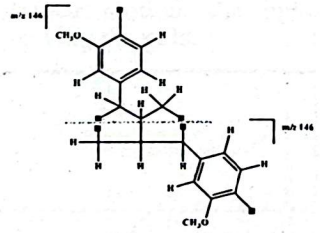
addition, the observation of α- and β- ^{1}H-^{13}C long range signals of the aromatic protons δ 6.94 ppm and δ 6.77 ppm with sp^2quaternarycarbonat δ 149.12 ppm lead to the following fragment. Moreover, in HMBC spectrum, Figure (6) the



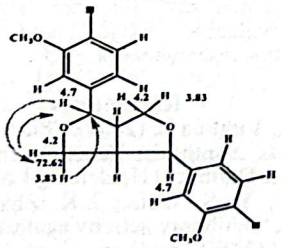
Moreover, the occurrence of α- and β- ^{1}H-^{13}C long range signals of the aromatic protons δ 6.77 ppm, δ 6.81 ppm and δ 6.94 ppm with sp^2 quaternary carbon at δ 147.38 ppm gave the following fragment. Furthermore, the sp^3 methine proton δ 4.7 ppm had α- and β- ^{1}H-^{13}C long range coupling with aromatic quaternary carbons at δ 133.83ppm, δ 111.02 ppm and δ 120.06 ppm in HMBC spectrum, Figure (6).



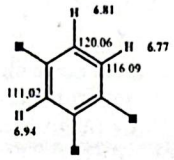
In this stage the partial molecular formula of (KTDS-3) is $C_{10}H_{10}O$ and the partial molecular mass is m/z 146. According to mass spectrum, Figure (8), the molecular mass of (KTDS-3) is 358 suggesting that the pure compound (KTDS-3) possessed the symmetry structure as show below. In addition, the elucidated hydrogen deficiency index is (8).



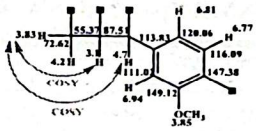
The above fragment could be confirmed by β ^{1}H-^{13}C long range coupling of sp^3 methine proton δ 4.7 ppm with sp^3 methylene carbon at δ 72.62 ppm and sp^3 methylene protons δ 4.2 ppm



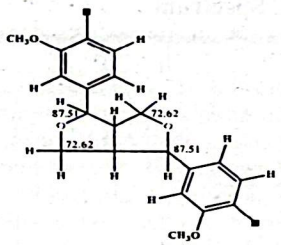
existence of β-^{1}H-^{13}C long range signal of the singlet methyl methoxy group at δ 3.85 ppm with down field chemical shift aromatic quaternary carbon at δ 149.12 ppm producing the following fragment.



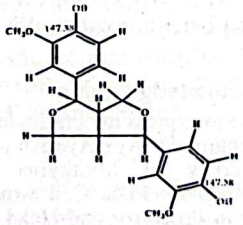
In DQF-COSY spectrum, Figure (7), the sp^3 methine proton δ 4.7 ppm and another sp^3 methine proton δ 3.1 ppm were connected each other with medium graphic area resulting the following fragment. In addition, in DQF-COSY spectrum, Figure (7) the observation of medium graphic area between the sp^3 methine proton δ 3.1 ppm and sp^3 methylene protons δ 4.2 ppm and δ 3.83 ppm resulted the following fragment.



HDI calculated from molecular formula is (10). The remaining HDI (2) must be two cyclic rings. Therefore, the reliable two sets of down field chemical shift carbons at δ 87.51 ppm and δ 72.62 ppm were assumed to be oxygen bearing carbons and gave the following extended fragment.



and δ 3.83 ppm with sp^3 methine carbon at δ 87.51 ppm in HMBC spectrum, Fig (6).Finally, the elucidated partial molecular formula of (KTDS-3) is $C_{20}H_{20}O_4$. The molecular formula of pure compound (KTDS-3) is $C_{20}H_{22}O_6$. The remaining partial molecular formula is H_2O_2. There must be two hydroxyl groups. These two hydroxyl groups could be connected to the two equivalent down field chemical shift aromatic carbons at δ 147.38 ppm to accomplish the complete structure of pure compound (KTDS-3).



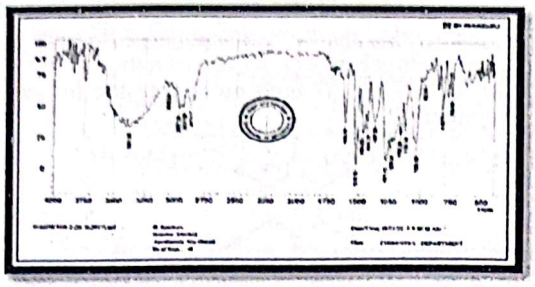


Figure (1) FT IR Spectrum

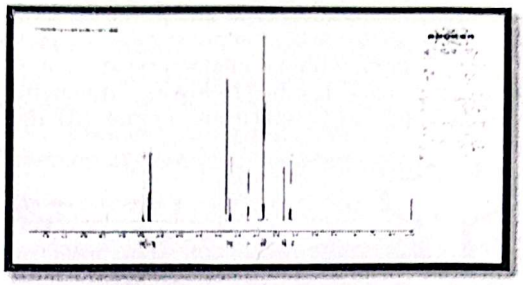


Figure (2)^{1}HNMR Spectrum

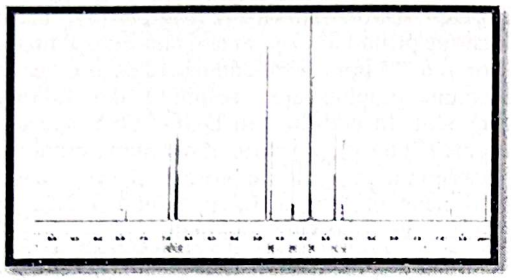


Figure (3)^{13}CNMR Spectrum

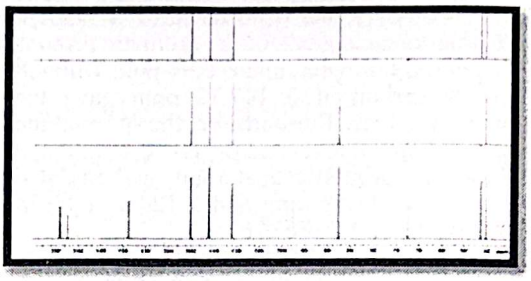


Figure (4) DEPT Spectrum

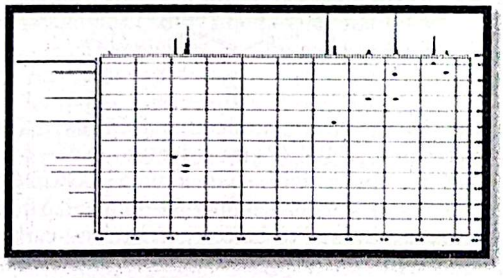


Figure (5) HSQC Spectrum

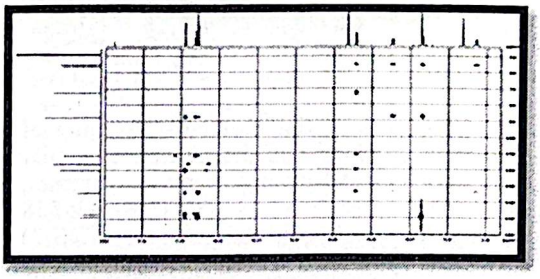


Figure (6) HMBC Spectrum

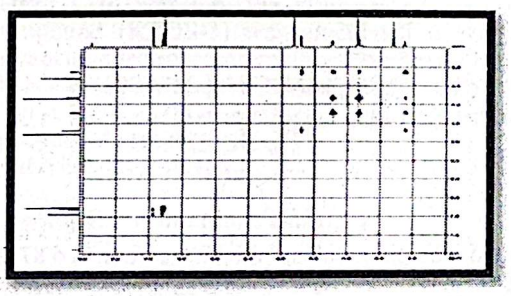


Figure (7) DQF COSY Spectrum

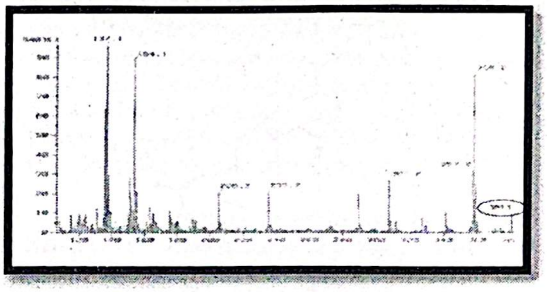


Figure (8) FAB – Mass Spectrum

Conclusion

In this study, pure compound (KTDS-3) was isolated and purified from the stem of *Alyxia fascicularis* Benth. by various chromatographic techniques. The structure of (KTDS-3) was assigned by using FAB-mass, ^{1}D and ^{2}D NMR spectral data and identified as (+)-Pinoresinol. Its IUPAC name is 4,4'– (hexahydrofuro [3,4-c] furan-1,4- diyl) bis (2-methoxyphenol).

Acknowledgements

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References

Anne, E. O., Virginia L. (2009). "Plant-derived Natural Products, Synthesis, Function and Application". Springer Dordrecht Heidelberg London New York.

Ming, A.O.; Y. S. Wein.; Z.K. Zhang.; Y.H. Kuo. (2007), "Inhibitory activity against tobacco mosaic virus (TMW) replication of pinoresinol and syringaresinol lignans and their glycosides from the root of *Rhus javanica* var. *roxburghiana*." *J.Agric. Food Chem*, Vol. 55, pp. 6460-6465.Silverstein, R.M., et al. Spectrometric Identification of Organic Compound, 6 Edition; John Wiley & Sons, Inc. U.S.A, 1998.

Silverstein, R.M., et al. Spectrometric Identification of Organic Compound, 7 Edition; John Wiley & Sons. Inc. U.S.A, 2005.

Chemical Analysis of Soil from Gold Bearing Layer in Tanaing Township, Kachin State

Phyu Phyu Thwe, Lecturer, Dr, Department of Chemistry, Mohnyin Degree College

Abstract

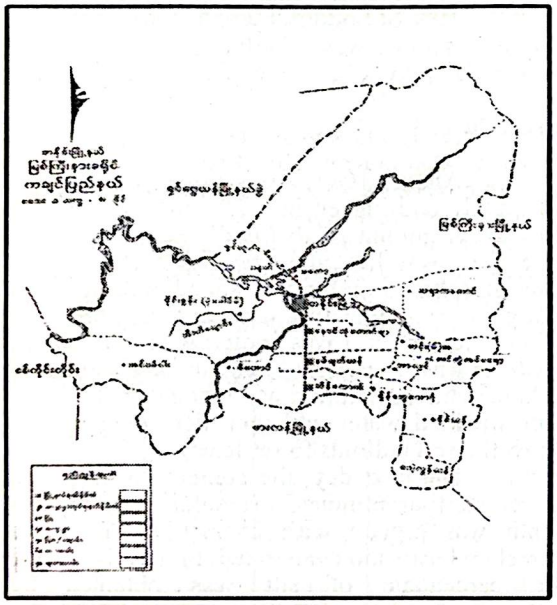
In this investigation, soil samples from four sites of gold producing areas in Tanaing Township, Kachin State were chosen for some chemical analysis. They were collected from Tanaing, Koung-ra, Nan-phyat and Tain-kout village. All these samples were picked up from 5 feets depth.The moisture, soil textures and pH values of four samples were examined by the standard method. Moreover, from the elemental analysis, such as AAS (Atomic Absorption Spectroscopy) and EDXRF (Energy Dispersive X Ray Fluoresence), gold content in Tanaing samples(3.45%) are much greater than that of Koung – ra (1.24%), Nan-phyat (1.59%) and Tain-kout(0.98%), respectively. By calculating the economic purification method, Tanaing gold sample has the highest purity (94%) than Myitkyina (89%) , Hopin (75%) and Indawgyi (88%) respectively.

INTRODUCTION

In the early days, gold could be found loose in riverbeds. Miners would divert streams, sending smaller streams off to each side, leaving stream beds exposed. The dry days of summer and early fall were best as low water levels exposed stone areas where gold may have been hiding.The people land, water, air and sunlight are essential for the existence of living things. People can obtain food from the earth as well as gold, silver, gemstones and many other useful metals and materials. In the present work four samples collected from the depth of 5feets were studied. The gold mine contains different layers of sand, they are soot-sand layer, yellow sand layer, red sand layer, green sand layer and blue sand layer.

Placer mining

Using shallow metal pans, miners mixed water with soil from the riverbed. By gently swirling in circular motion, the lighter soils washed away, leaving the gold.



Map of Tanaing Towship

Quartz or hardrock mining

Shafts were sunk and large machinery was needed to remove the veins of gold from the quartz rock. Drilling could be done by hand or by compressed air-drill. Dynamite was placed into the holes created and detonated.

Hydraulic mining

This method was developed to explore the soil of older, dried riverbeds and later, any available soil.

a. Riveted pipes guided water down from higher elevations. As the water travelled down, its pressure increased.

b. The water was channeled into iron nozzles called monitors.

c. By this time, the pressure was sufficient to blast away hillsides.

d. The monitors were systematically waved back and forth, boring into the land.

e. The soil would run down into a vally and a series of sluices where the silt and water would be separated through traditional mining techniques.

Preventing exposure to arsenic in mine tailings

Arsenic is a substance found in the environment. It occurs naturally in crushed rock. It is often found near gold deposits and is extracted as part of gold mining activities. The extracted material is called mine tailing. Arsenic has also been used as a pesticide to protect animals, timber, fruit and vegetables from insects. Children and adults who live near mine tailings are at risk of exposure to arsenic. The risk can be reduced;

Exposure to mine tailing soil and dust are reduced.

Do not allow young children to play in or eat mine tailings

Washed children's hands and their toys frequently.

Bring in clean soil for vegetable garden beds and ensure all fruit and vegetables are washed before eating.

Do not swim in or eat fish from dams with walls made from mine tailings.

Therefore in the present research paper included the comparision study of gold content from four sites of gold producing areas in Tanaing townships, Kachin State.

Soil

The term soil can be defined simply as the products of weathering, above weathered parent rocks. Thus, soil horizons can be defined as the development of the soils into layers differing from each other in their properties and composition such as, differences in colour, texture, pH, organic matter content, clay mineral type and assembladge and the amount of sesquioxides. The individual layers are referred to as soil horizons. The

thickness may range from a few inches to several feet.

.Gold ores is found associated with Silver, Copper, Iron, Zinc, Lead, Arsenic, Calcium, Titanium, Phosphorus, Manganese, Zirconium, Strontium, Chromium, Rubidium and Vanadium. Gold is the most important metal on the world.

Factors of soil formation

The formation of soil from the parent rock is the resultant of the action of different forces and the nature of the soil thus formed is influenced by the following factors. They are nature or parent rock, climate, vegetation, and time. All of these factors individually and collectively control the nature and properties of the soil formed and also the rate of its formation and leave an imprint in the soil profile that is developed in consequence.

A study of the soil profile can help to characterize the physical nature and properties of the soil. Soil develops from parent material by process of soil formation that differs from process of rock, weathering which produces material.

As a soil is developed from the parent materials certain changes take place which differentiate the various horizons to give soils their distinctive layering. These changes are brought about largely by shifting of materials either mechanically or in solution to various layers with the horizons or completely out of the soil.

Soil texture

The relative size of the soil particles is expressed by the team texture. Texture is the relative proportion of the different size groups or separates. The texture is due to different proportions of sand, silt and clay in the soil.The rate and extent of many important physical and chemical reactions in soils are governed by texture because of determines the amount of surface on which the reactions can occur.

Soil Moisture

The moisture which is contained in a soil is a very important part of the soil as far as plant growth is concerned. Water is formed in soils in various forms.They are hygroscopic moisture and capillary moisture gravitational or free water.

Soil pH

The pH is defined as the negative log of the hydrogen ion activity. The pH range normally found in soils varies from 3 to 9. Various categories of soil pH may be arbitrarily described as follows; strongly acid (pH<5.0), moderately to slightly acid (6.5 – 7.5), moderately alkaline (7.5 – 8.5), and strongly alkaline (>8.5). Sigificance of pH lies in its influence on availability of soil nutrients, solubility of toxic nutrient elements in the soil, physical breakdown of root cells, cation exchange capacity in soils whose colloids (clay/humus) are pH dependent, and most micronutrients, expect boron (B) and molybdenum (Mo), tends to decrease.

Experimental

Preparation of soil samples

Four samples were taken from four sites. Sample I, II, III and IV were taken depth to 5 feets from Tanaing Township, Nan-phyat , Koung- ra and Tain- kout villages. These four samples were broken up into small lamps and spread out in the shade for air-dry. Gravel, root, etc were discarded. After drying, the samples were ground and shifted. The samples passing the sieve were mixed together very thoroughly and used for analysis.

Determination of moisture

Principle

The sample is allowed to dry in an electric oven at 105°C, cooled in a desicator and weighed. From the loss in weight, the percentage of moisture of the sample is calculated.

Procedure

Constant weight of container was first determined. Then about 2g of sample was placed in container and weighed accurately. It was allowed to dry in an electric oven at105°C. Then it was dried to constant weight. From the loss in weight, the percentage of moisture of the sample under analysis was calculated.

Calculation

$$\text{moisture \%} = \frac{b-a}{b} \times 100$$

b = weight of a sample before heating (g)

a = weight of sample after heating (g)

$$\text{moisture coefficient} = \frac{100 - M}{100}$$

M = percentage of Moisture

Determination of texture by pipette method

Reagent

10% of Sodium pyrophosphate solution 10g of a sample was dissolved in distilled water and made up to 100cm^3.

Procedure

About 100g of sample was weight accurately and placed in a 500cm^3 conical flask and some amount of distilled water was added. The flask was heated to boil, 10cm^{3}of sodium pyrophosphate solution was added to disperse the soil colloids and heating was contained for about 15 minutes. Then it was cooled.After cooling the contents were transferred to a 1000cm^3 graduated cylinder and the solution was made up to the mark with distilled water and then kept overnight to allow the soil colloids to settle.

The next day, the contents were stirred for about four minutes; the solution from 9cm depth was pipette with 25cm^3 pipette into a porcelain basin and evaporated. From this residue the percentage of silt was obtained by difference.To determine the amount of sand, the remaining solution was poured into 50cm^3 sieve and the clay and silt were washed with water. The percentage of sand was calculated.

Calculation

$$\text{clay\%} = \frac{c \times 100}{s \times k}$$

$$(b - c) \times 100$$

$$\text{silt\%} = \frac{b - c}{s \times k} \times \frac{100}{a}$$

$$\text{sand\%} = \frac{c}{s \times k}$$

c = weight of clay in 25cm³ (g)
b = weight of silt and clay (g)
a = weight of sand (g)
s = weight of sample (g)
k = weight of coefficient

Determination of pH

Procedure

About 20g of sample was weight accurately and placed into a shaking bottle and 50cm³ of distilled water (pH = 7) was added (the ratio of sample to water was 1;2.5) and shaken for half an hour. Then pH was measured by pH meter using glass electrode. The pH meter was standardized with buffer solution before use.

Results and Discussion

Determination of Moisture, Texture and pH value of Four Soil Samples

The examination of moisture content, soil texture and pH values are assigned in table (1).

According to the data, moisture percent of Tain-kout sample was higher than others. Tanaing Township sample has lowest amount of sand and highest amount of clay than the other samples. This is the good results for soil sample because gold become bear in that soil. Tanaing and Nan-phyat samples have slightly acidic than the two other samples.

Table (1) Moisture, Texture and pH value of four Soil Samples

Sample	Moisture(%)	Texture			pH
		Sand(%)	Silt(%)	Clay(%)	
Tanaing Township	1.16	6.36	28.68	65.96	5.7
Koung-ra	11.83	20.81	14.08	65.11	6.2
Nan-phyat	1.14	28.97	20.20	50.83	5.4
Tain-kout	21.62	31.03	19.68	49.29	6.5

Elemental Analysis of Soil Samples by AAS

Four samples were measured at the Applied Geology Department, Yangon University Campus, and University of Yangon.The results were shown in table (2).

Table (2) Results of Elemental Analysis in four soil samples

Location	Au ppm	As ppm	Ag ppm	Cu ppm	Pb ppm	Sn ppm	Fe ppm
Tanaing	3.45	0.58	1.54	95	**195**	89	11500
Koung-ra	1.24	**0.21**	1.29	**85**	170	70	8700
Nan-phyat	1.59	0.37	1.28	90	**185**	85	9850
Tain-kout	0.98	0.18	1.64	**75**	175	75	8900

According to the data, the amount of gold and other elements fromTanaing sample are the best of others. On the other hands, the amounts of silver are more present in Tain-kout sample.

Interpretation of the EDXRF spectrum of samples

The EDXRF spectra of samples were measured at the Universities Research Centre, Yangon University Campus, and University of Yangon. These individual results were described in table (3). As a result of these data, the present Fe and other elements except Ti and Si was more contain in Tanaing compared to that of Koung-ra, Nan-phyat and Tain-kout. On the other hand, the amount of Si found in Nan-phyat sample was higher than others.

Table (3) Results from the EDXRF spectrum of samples

Sample name	Si (%)	Fe (%)	K (%)	Ca (%)	Ti (%)	S (%)	Mn (%)	Zr (%)	Sr (%)
Tanaing	69.39	20.60	3.65	2.70	1.83	1.22	0.26	0.10	0.07
Koung-ra	75.64	18.84	2.78	ND	2.17	ND	0.297	0.14	0.05
Nan-phyat	83.26	13.9	1.65	ND	1.06	ND	ND	0.10	ND
Tain-kout	75.3	19.77	2.42	ND	1.99	ND	ND	0.16	ND

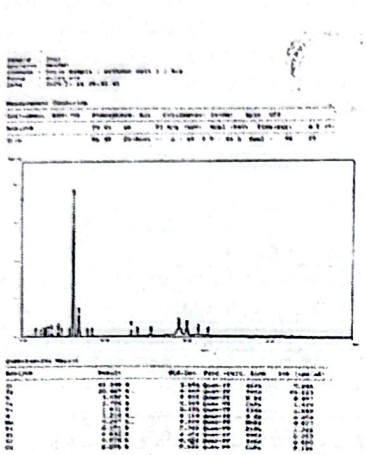
ND means not detected

Comparison Study of gold quality from four sites in Kachin State

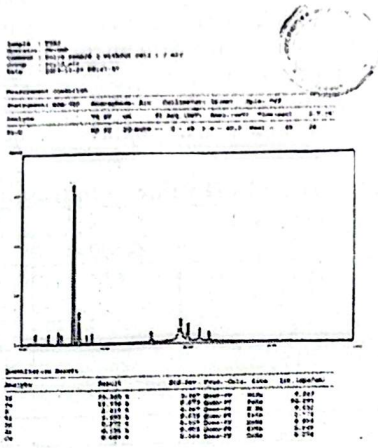
In the Kachin State, there are four gold producing areas. Their location site, producing area, gold color and purity percent were described in the table (4).By calculation of economic purification method (from Kyaw Myanmar gold shopping center), Tanaing gold sample has the highest purity (94%) than that of Myitkyina (89%), Hopin (75%) and Indawgyi (88%) respectively.

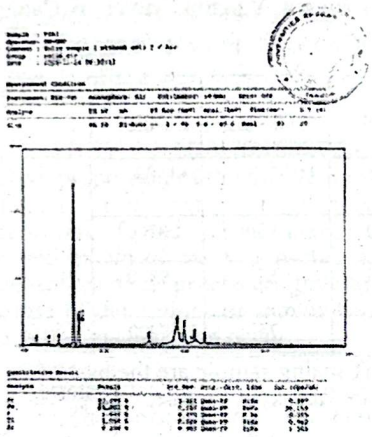
Table(4) Comparison of Gold quality from four producing sites

Location site	Producing area	Gold color	Purity (%)
Myitkyina	Myitsone	Pale yellow	89
Tanaing	Nan phyat	Brick	94
Hopin	Tetlet	Pale greenish yellow	75
Indawgyi	Nanpadae	Pale greenish yellow	88

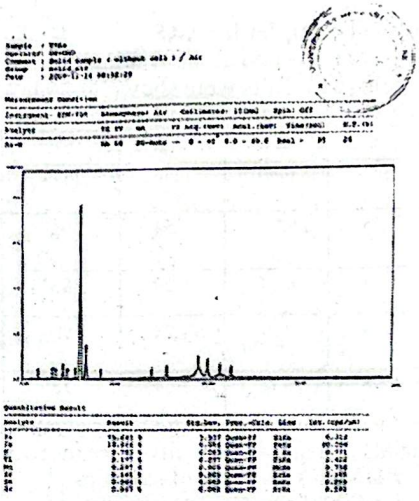


EDXRF Spectrum of Tanaing Soil Sample





EDXRF Spectrum of Nan-phyat Soil Sample



EDXRF Spectrum of Tain-kwat Soil Sample

Conclusion

In this investigation four soil samples from gold producing areas in Tanaing Township, Kachin State were chosen for comparison study on gold content. Firstly soil texture, moisture, and pH contents of all samples were determined by the standard method.

The micro and macro elements were measured by AAS and EDXRF. By the interpretation of AAS and EDXRF spectrum of all samples, gold percent of Tanaing (3.45%) is better than that of Koung – ra (1.24%), Nan-phyat (1.59%) and Tain – kout (0.98%) respectively. Among them, another essential element (Ag) are good values found in Tain – kout sample. By calculation of economic purification method (from Kyaw Myanmar gold shopping center), Tanaing gold sample has the highest purity (94%) than that of Myitkyina (89%), Hopin (75%) and Indawgyi (88%) respectively. Therefore gold may be produced in Tanaing Township are the best of other areas, inversely silver may be produced in Tain- kout village is more preferable than others.

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References

Chhibber, Macmillam, London, (1934).Mineral Resources of Myanmar by H.L.

Govinda Rajan S.V and HG., Gopala Rao.,(1971)."Soil and Group productivity ". Asia publishing House, London.

Johm wiley and sons, Inc. London, (1951).C Millar, C.E;L M Truck; " Fundamental of soil science ", 2nd edition, New York.

Roy, L.D;R.W.Miller; J.C. shick luna; Raymond W miller, John C. Shick Luna,.An introduction to soil and plant growth, Prentice Englewood Cliffs, New Jersey.

Thomspor, L.M and F.R Troch, FRECERICK R. TROEH(1973). Soil and soil fertility 3rd Edition, Mc. Graw Hill, New York .

Websites on Internet

www.cdpr.ca.gov/docs/emon/pubs/ehapreps/eh9404.pdf

www.icarda.org/Publications/lab-Manual/PDF/part5.pdf

www.soils.wisc.edu/soils/forms/Jackson_Order_form1.pdf

http://agronomy.agr.state.nc.us

Natural Treatment of Water from Mayangyan Jetty, Ayeyarwady River

Dr. Thida Kyaw, Associate Professor, Department of Chemistry, Yadanabon University

Abstract

The Ayeyarwady River is the life-blood of Myanmar people and fresh water aquatic organisms. So, Ayeyarwady River water was chosen to treat for my research paper. Firstly, the physical properties, chemical properties and bacteriological properties of water sample were carried out in this research. The samples were collected from Mayangyan Jetty, Chan Aye Thar San Township, Mandalay Region. In this water treatment process, the aquatic plants of water chestnut were used. Furthermore, white marble powder, peanut shell charcoal, coconut shell charcoal, rice husk charcoal and sponge were used in water treatment process. Before and after treatment of water sample, the physical and chemical properties were determined. And then heavy metals such as lead and cadmium were also determined.

Keywords: Water, Treatment, Chestnut Plants, Charcoal, Sponge

Introduction

Water is the most important substance for all living organisms. People cannot exist without water. Although water is colorless, tasteless and odorless, it is essential to human life. Drinking water should always be clean and free from contaminants to ensure proper health and wellness. If any toxic metal is present in water, there would be great danger for human beings. Natural water is never completely pure. Also Ayeyarwady River water is never completely pure for drinking and cooking.

Ayeyarwady River originates from the confluence of two rivers namely Maykha and Malikha. The Ayeyarwady River is the life-blood of Myanmar and is the most important commercial waterway of the country. Therefore, the Ayeyarwady River should always be clean and sustainable for long time. Fresh water is becoming polluted. Water pollution is serious problems globally involving the discharge of dissolved or suspended substances into river, oceans, ground water and streams. So, in this research work, the water sample of Ayeyarwady River at Mayangyan Jetty, Chan Aye Thar San Township, Mandalay Region was selected for water treatment.

Aim and Objectives

Aim

- To assess the quality of water from Mayangyan Jetty, Ayeyarwady river, Chan Aye Thar San Township, Mandalay Region.
- To treat the water sample from Mayangyan Jetty, Ayeyarwady River, Chan Aye Thar San Township, Mandalay Region by phytoremediation and natural materials.

Objectives

- To analyze the physicochemical properties of water sample
- To determine the bacteriological examination of water sample
- To treat the water sample by using water chestnut, geochemical sorbent, bio sorbent and synthetic sorbent.
- To examine the physiochemical properties of water sample after treatment
- To perform the bacteriological examination of water sample after treatment

Botanical Description

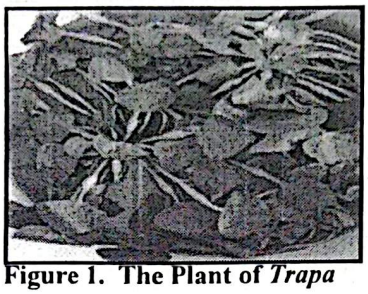

Figure 1. The Plant of *Trapa*

Family name : Trapaceae
Scientific name : *Trapa bispinosa* Roxb.
English name : Water Chestnut
Myanmar name : Kywe-gaung
Part used : The whole plant

Materials and Methods

Sample collection

The water samples were collected from Mayangyan Jetty, Ayeyarwady River, Chan Aye Thar San Township, Mandalay Region on August, 2014 (rainy season) and December, 2014 (cold season). For the sample collection, four plastic bottles of capacity 5 gallons which has been washed with 1 : 1 nitric acid solution and distilled water. And then two or three times with the water of Ayeyarwady River which is to be collected. Water samples were collected from middle of Ayeyarwady Rive r at M ayangyan Jett y and 2 feet depth.

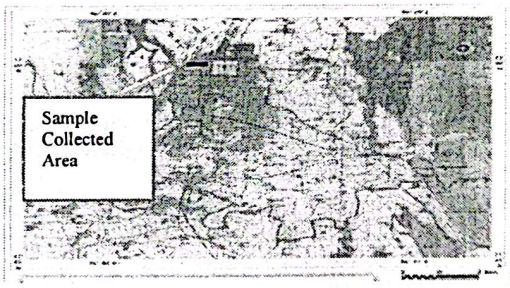

Figure 2. Location Map of Sample Collected Area, Mayangyan Jetty, Ayeyarwady River, Chan Aye Thar San Township, Mandalay Region

Ten plants of water chestnut (*Trapa bispinosa* Roxb.) and marble sample were collected from Sagin Village, Patheingyi Township, Mandalay Region. The collected marble was ground and sieved by using 100 mesh size sieves. After sieving the sample under 100 mesh size was stored in plastic bag and it was used to treat the water sample.

Determination of pH

pH of water sample was determined by pH meter.

Determination of Color

Color of water sample was measured by platinum-cobalt standard method.

Determination of Turbidity

Turbidity of water sample was examined by standard method.

Determination of Conductivity

Conductivity of water sample was directly measured by the conductivity meter.

Determination of Total Dissolved Solid (TDS)

Total dissolved solid value of water sample was determined by gravimetric method.

Determination of Chemical properties

Chemical properties of water sample such as total hardness, calcium, magnesium, total alkalinity, sulphate, chloride and total iron were examined by using titrimetric method.

Bacteriological examination

Probable coliform count and *E. coli* of collected water sample were examined at Mandalay Public Health Association.

Determination of Organic Constituents

Dissolved oxygen (DO), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of collected water sample were measured at Water and Sanitation Department, Mandalay City Development Committee, Water Laboratory, Mandalay.

Planting of water chestnut in water tank

The ten plants of water chestnut were collected in August, 2014 and planted in plastic tank width 3 feet and height 2 feet containing (70L) collected water sample. After one week all of water chestnut plants were taken out from the tank.

Activation of Peanut-Shell Charcoal

250 g of peanut-shell charcoal were placed into a plastic container. 500 ml of 5% NaOH solution were added to the peanut-shell charcoal and stirred. The mixture was stood for 3 hours. After 3hours, the mixture was filtered and washed with distilled water until neutral. Then, 500 mL of 5% NaOCl solution were added into neutral peanut-shell charcoal for decolorization and stirred. The mixture was stood for 24 hours. And then it was filtered and washed with distilled water to obtain neutral, peanut-shell charcoal was dried in sunlight for 24 hours. The result of charcoal was grounded and sieved by using 40-60 mesh size sieves. The activated, decolorized peanut-shell charcoal was obtained.

Determination of Toxic metals

The amount of heavy toxic metals such as lead and cadmium in untreated water sample and treated water were determined by EDTA titrimetric method.

Results and Discussion

The experimental results for physical and chemical parameters of water samples collected from Mayangyan Jetty, Ayeyarwatty River on August, 2014 and December, 2014 were given in Table 1and 2.

Table 1. Comparison of Physical Properties for Water Sample from Mayangyan Jetty, Ayeyarwady River on August, 2014 and December, 2014

No	Test	August, 2014	December, 2014	WHO standard	
				Highest desirable level	Maximum permissible level
1	Color	7	7	7	50
2	Total Dissolved Solid (mg/L)	450	490	500	1500
3	pH	8.1	8.4	7.0-8.5	6.5-9.2
4	Conductivity	70	80	400	800

From this table, the colors of water samples were found to be the same as highest desirable level. The conductivities and total dissolved solid were lower than that of highest desirable level and maximum permissible level. pH of the water samples was found to be alkaline.

Table 2. Comparison of Chemical Properties for Water Sample from Mayangyan Jetty, Ayeyarwady River on August, 2014 and December, 2014

No	Test	August, 2014	December, 2014	WHO standard	
				Highest desirable level	Maximum permissible level
1	Total Hardness (mg/L)	80	100	100	500
2	Calcium(mg/L)	8	16	75	200
3	Magnesium(mg/L)	2.4	12	30	150
4	Total Alkalinity (mg/L)	230	295	600	950
5	Sulphate (mg/L)	78	96	200	400
6	Chloride (mg/L)	25	32	200	600
7	Total Iron	Nil	Nil		

According to above table, the amount of calcium, magnesium, total alkalinity, sulphate and chloride of the water samples were lower than the highest desirable level. The amounts of total hardness, calcium, magnesium, total alkalinity, sulphate and chloride of the water sample on December, 2014 were found to be larger than that of August, 2014. From the point of view of chemical properties of water samples on December, 2014 was found to be more polluted than that of August, 2014. Bacteriological examination of water samples was done and the results were shown in Table 3.

Table 3. Comparison of Bacteriological Examination for Water Sample from Mayangyan Jetty, Ayeyarwady River on August, 2014 and December, 2014

No	Test	August, 2014	December, 2014
1	Probable Coliform Count	5/5	5/5
2	*Escherichia Coli* Count	Isolated	Isolated

According to this table, *Escherichia coli* counts were isolated and probable coliform counts were observed in all water sample. Therefore, from the bacteriological point of view, all water samples were found to be unsatisfactory for drinking purpose.

The Organic constituents such as dissolved oxygen (DO), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of collected water sample were also determined and the results were shown in Table 4.

Table 4. Comparison of DO, BOD and COD Parameters of Ayeyarwady River water sample

No	Test	August, 2014	December, 2014	Effluent standard	Remarks
1	DO (mg/L)	4.98 mg/L	4.72 mg/L	-	-
2	BOD(mg/L)	7 mg/L	8 mg/L	≤ 50 mg/L ≤ 3 mg/L (Drinking std)	Above drinking water limit
3	COD(mg/L)	<30 mg/L	<30 mg/L	≤ 250 mg/L	Normal

The collected water sample on December, 2014 was treated by using phytoremediation, geochemical adsorbent, biosorbents and synthetic sorbent. After treatment, physical and chemical parameters of collected water sample were determined and the results were presented in Table 5 and 6.

Table 5. Comparison of Physical Properties of Treatment for Water Sample from Mayangyan Jetty, Ayeyarwady River on December, 2014

No	Test	Untreated water sample	Treated water by adsorption using						WHO standard	
			Phytoremediation	Marble	Peanut Shell Charcoal	Coconut Shell Charcoal	Rice Husk Charcoal	Sponge	Highest desirable level	Maximum permissible level
1	Color (Platinum Cobalt Scale)	7	6	6	6	6	6	6	7	50
2	Total Dissolved Solid (mg/L)	490	450	430	400	380	300	270	500	1500
3	pH	8.4	8.2	8.0	8.1	7.8	7.4	7.2	7.0 to 8.5	6.5 to 9.2

From above data, the colors of untreated water sample and treated water were found to be nearly the highest desirable level. After treating, the amount of total dissolved solid was found to be decreased. pH of the untreated water sample and treated water was found to be alkaline medium.

Table 6. Comparison of Chemical Properties for Water Sample from Mayangyan Jetty, Ayeyarwady River on December, 2014

No	Test	Untreated water sample	Treated water by adsorption using						WHO standard	
			Phytoremediation	Marble	Peanut Shell Charcoal	Coconut Shell Charcoal	Rice Husk Charcoal	Sponge	Highest desirable level	Maximum permissible level
1	Total Hardness (mg/L)	100	90	80	70	70	60	60	100	500
2	Calcium (mg/L)	16	16	16	14	14	14	14	75	200
3	Magnesium (mg/L)	12	9	9	8	7	7	7	30	150
4	Total Alkalinity	295	260	250	230	200	200	190	600	950
5	Sulphate (mg/L)	96	88	80	75	70	50	50	200	400
6	Chloride (mg/L)	32	30	27	27	25	20	20	200	600

From these data, phytoremediated water, white marble powder, peanut shell charcoal, coconut shell charcoal, rice husk charcoal and sponge were found to reduce the amount of chemical properties of untreated water sample. Marble, rice husk charcoal and sponge were found to decrease that of untreated water sample. Therefore, white marble powder, rice husk charcoal and sponge were selected for the

treatment of phytoremediated water sample due to their better removing capacity of some parameters than others.

Comparison of physico-chemical properties of untreated and treated water sample were presented in table 7 and 8.

Table 7. Comparison of Physical Properties of Untreated Water Sample and Treated Water

No	Test	Untreated water sample	Treated water by adsorption using				WHO standard	
			phytoremediation	Marble	Rice husk charcoal	Sponge	Highest desirable level	Maximum permissible level
1	Color (Platinum, Cobalt Scale)	7	6	6	6	6	7	50
2	Total Dissolved Solid (mg/L)	490	450	400	350	300	500	1500
3	pH	8.4	8.2	8.0	7.5	7.2	7.0-8.5	6.5-9.2

According to above data, the color of untreated water sample and selected treatment of water were found to be the nearly desirable level. pH of the untreated and treated water were found to be alkaline condition. White marble powder, rice husk charcoal and sponge were found to reduce the color, total dissolved solid and pH.

Table 8. Comparison of Chemical Properties of Untreated Water Sample and Treated Water

No	Test	Untreated water sample	Treated water by adsorption using				WHO standard	
			phytoremediation	Marble	Rice husk charcoal	Sponge	Highest desirable level	Maximum permissible level
1	Total Hardness (mg/L)	100	90	80	70	70	100	500
2	Calcium (mg/L)	16	16	14	14	12	75	200
3	Magnesium (mg/L)	12	9	9	8	8	30	150
4	Total Alkalinity	295	260	250	230	220	600	950
5	Sulphate (mg/L)	96	88	80	75	70	200	400
6	Chloride (mg/L)	32	30	25	25	25	200	600

After treating with water chestnut plant, marble powder, rice husk charcoal and sponge, the amount of total hardness, calcium, total alkalinity and sulphate were found to be lower than that of untreated water sample.

Comparison of lead and cadmium contents of untreated and treated water sample were tabulated in table 7 and 8.

Table 9. Comparison of lead and cadmium contents of Untreated Water Sample and Treated Water

No	Test	Untreated water sample	Treated water by adsorption using					
			phytoremediation	Marble	Peanut shell charcoal	Coconut shell charcoal	Rice husk charcoal	Sponge
1	Lead (mg/L)	8.4	7.8	6.6	5.8	4.7	3.9	2.6
2	Cadmium (mg/L)	6.4	5.5	4.3	3.7	2.6	2.2	1.6

According to the treatment of water samples, the amount of lead and cadmium were found to be decreased each filtration step using different types of adsorbent.

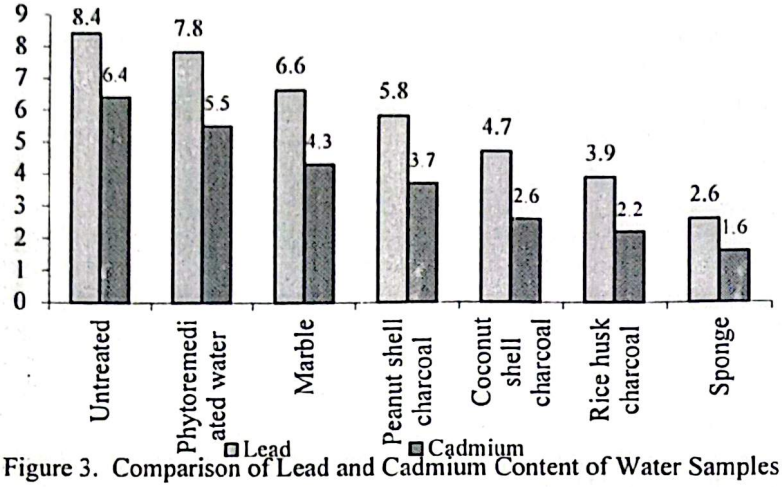

Figure 3. Comparison of Lead and Cadmium Content of Water Samples using different adsorbents

Table 10. Comparison of lead and cadmium contents of Untreated Water Sample and Treated Water (using effective adsorbents)

No	Test	Untreated water sample	Treated water by adsorption using phytoremediation	Marble	Rice husk charcoal	Sponge
1	Lead (mg/L)	8.4	7.8	6.6	5.6	3.2
2	Cadmium (mg/L)	6.4	5.5	4.3	2.3	1.5

From these data, the amount of lead and cadmium were found to be decreased with each filtration step using different types of adsorbent.

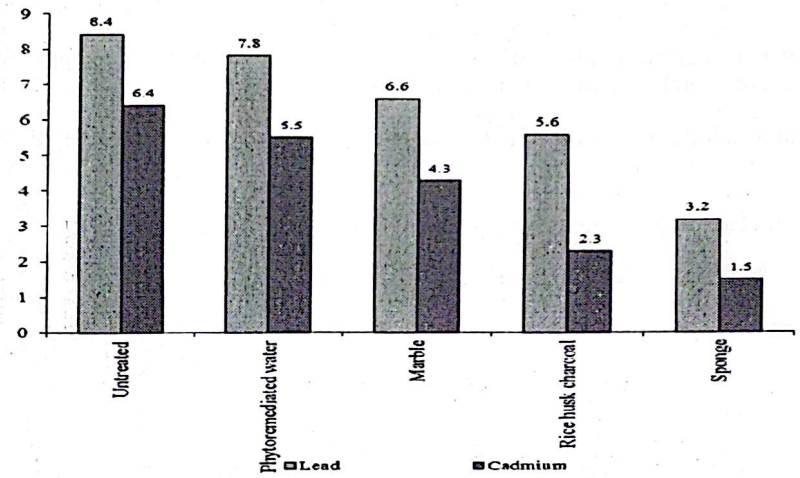

Figure 4. Comparison of Lead and Cadmium Content of Water Samples using Effective Adsorbent

Conclusion

The water samples were collected from Ayeyarwady River of Mayangyan Jetty on August and December, 2014. The water qualities of Ayeyarwady River were compared. From the physico-chemical point of view, the colors of water sample were found to be the nearly highest desirable level. The amount of total dissolved solid of water samples was lower than the highest desirable level and maximum permissible level. pH of the water samples were found to be as the alkaline condition. The chemical properties such as total hardness, total alkalinity, calcium, magnesium, sulphate and chloride of water samples were less than the highest desirable level. Iron was not detected in both samples. The amount of total hardness, calcium, magnesium, sulphate and chloride of the water sample on December, 2014 was found to be larger than that of August, 2014. The water sample of Ayeyarwady River on December, 2014 was found to be more pollutant than that of August, 2014. *E. coli* counts were isolated in all water samples. From the bacteriological point of views, all water samples were found to be unsatisfactory for drinking purpose.

The water samples of Ayeyarwady River collected from Mayangyan Jetty on December, 2014 was treated naturally by phytoremediation

and step by step filtration. Water chestnut plants were used for phytoremediation and three kinds of biosorbent such as peanut shell charcoal, coconut shell charcoal and rice husk charcoal were used for filtration. White marble powder was used as geochemical sorbent. Sponge was used as synthetic sorbent.

After treating with water chestnut plants, white marble powder, peanut shell charcoal, coconut shell charcoal, rice husk charcoal and sponge, the amount of physical properties such as pH, colour and total dissolve solid were found to be lower than that of untreated water sample on December, 2014.

Then, white marble powder, rice husk charcoal and sponge were selected for the treatment of phytoremediated water sample due to their better removing capacity of some parameters than others. After treating with above materials, the amount of total dissolved solid, total hardness, calcium, magnesium, total alkalinity, chloride and sulphate were found to be less than that of untreated water sample.

According to the treatment of water samples, the amount of lead and cadmium were found to be decreased with each step filtration using different types of adsorbent. It was found that, the greater the number of adsorbents has been used, the more amounts of lead and cadmium has been removed.

To reduce the physical and chemical parameters of the water samples, phytoremediation should be carried out with any aquatic plants and then phytoremediated water should be passed through the marble, charcoal and sponge. From the study, it can be concluded that local people should not drink directly Ayeyarwady River water and it should be boiled for drinking. According to the experimental data, Ayeyarwady River water should be only used for domestic and agriculture.

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References

HACH, (1985),"Water Analysis Handbook", Hach Company.

Mantell, C.L. (1951), Adsorption (Chemical Engineering Series). PhD.2nd Edition.

Mitra, L. (1958) "A Text-Book of Inorganic Chemistry", 2nd Ed, Ghosh and Co, Calcutta.

W.H.O, (1984), "Guideline for Drinking Water Quality", Vol. I. Geneva.

Win Win Than (1983), Feasibility Study for Supply of Water to Mandalay, Department of Chemistry, University of Mandalay. MSc. (Thesis)

Appendix

Calculation

Calculation of cadmium present in sample

1 mL of 0.05 M EDTA (titrant) ≡ 5.621 mg of cadmium

(V) cm^3× 0.001M EDTA $= \frac{(10^{-4} \times V) mmol \times 5.621\ mg}{0.05\ mmol}$ = (a) mg

10 mL sample ≡ (a) mg of cadmium

100 mL (1L) = ?

$= \frac{10^3 a}{10}$

V = Volume of EDTA titrant (mL)

a = Weight of cadmium (mg)

Calculation of lead present in sample

1 mL of 0.05 M EDTA (titrant) ≡ 10.3605 mg of lead

(A) cm^3 × 0.0001M EDTA $= \frac{(10^{-4} \times A)\ mmol \times 10.3605\ mg}{0.05 mmol}$

= (b) mg

10 mL sample ≡ (b) mg of lead

1000 mL (1 L) = ?

$= \frac{10^3 b}{10}$

$= (10^2 \times b)\ mgL^{-1}$

A = Volume of EDTA titrant (mL)

b = Weight of lead (mg)

Using of Rice Straw (*Oryza Sativa* L.) for Better Purposes Fabricating and Evaluating of Physical and Mechanical Properties of Fiberboard

Su Su Aung, Dr, Associate Professor, Department of Chemistry, Banmaw University

Abstract

This study investigated the preparation and characterization of rice straw (*Oryza Sativa* L.) fiberboard. The fiberboards were made by treated rice straw and different amounts adhesives urea-formaldehyde. These were manufactured using urea-formaldehyde adhesive. Fabrications of fiberboards were made by compressing molding method. The physicochemical properties of pre-treated rice straw were investigated. The physicomechanical properties of prepared fiberboards such as bending strength , impact , hardness, water absorption, thickness and density were determined. The prepared rice straw fiberboards (FB_r1,FB_r2,$FB_r3$and FB_r4)will also be characterized by modern techniques. Surface morphologies of pre-treated rice straw sample and prepared fiberboards will be analyzed by Microscope.

Keywords : Rice straw , Urea-formaldehyde , Fiberboards, Physicochemical , Physicomechanical

Introduction

1.1 Straw

Straw is the stem of a small grain cereal plant, such as wheat or rice, or the shaft left after the growing of grass for grass seed (Petruzzi, 2001). Rice straw has a relatively low lignin content and high silica content (Cetin and Ozmen 2002) . Using straw as the fiber for fiberboards provides an alternative to burning straw in fields. Straw into fiberboard and particleboard is similar to processing wood residues, breaking straw into fibers requires less processing and less drying, therefore less energy use. In addition, the properties of strawboard, such as internal bond strength, resistance to rupture, moisture resistance, and crew holding strength (as measured by tests developed by the American National Standards institute or the American Society for Testing and Materials (ASTM C208-8a), are equal to or better than wood-based fiberboard and particleboard.

1.2 Rice Straw

The rice straw, which is the main residue in rice production consist mainly of **culm** (Another name for the stem of the rice plant). The culm consists of several **nodes** spaced apart by **internodes** 1 cm apart near the base, and further apart towards the top of the rice plant. The intermodal length can reach 10-30 cm at the formation of the panicle from the last internode (Figure 1).

1.3 Botanical Description

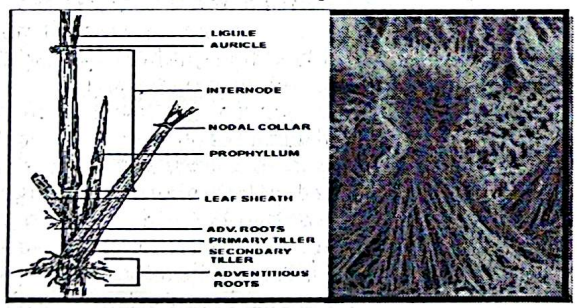

Figure 1 The rice straw

Common name = Rice straw or paddy straw
Botanical name = *Oryza sativa* L.
Family name = Poaceae
Myanmar name = Kaukyoe
Plant part used = Stem
Fiber type = Grass

1.4 Properties of Rice Straw as Material for Board Making

The Rice straw can be easily crushed into chips or particles, which are very similar to wood particles or fibers. The rice straw is mainly consisting of carbohydrate components such as hemicelluloses, cellulose, and lignin (Lee, *et al* 2004). These are the basic requirements in a material to be used for the production of fiberboard (lignocellulosic) as stated by the standard (BS EN 622-1: 2003).The carbohydrates content is variable, as well as other components, such as hand silica. The hydrophilic character of the rice straw is one of the reasons for relatively high moisture content, approximately 60-90% on a wet base, or 10-12% on a dry base (Buzarovska *et a,l* 2008). The ash content of up to 22% and low protein content result in a material decomposing not as readily as other straws (Pan *et al*, 1999).

Materials and methods

2.1 Collection of the Samples

Rice straw (*Oryza sativa L.*) were collected from Namphar Quarter, Banmaw Township , Kachin State. Urea –formaldehyde (UF) was from Wartara glue factory, Shwe Pyi Tha Township Yangon, Myanmar.

Preparation of Rice Straw Fibers

Rice straw was kept under water for several days (about 7 days). The Rice straw (RSF) The materials used for this research were procured from the same source.

Chipping, Washing and Soaking

The process of the manufacture of the board starts with the cutting of the rice straw into chips. This was done manually with the aid of cutlass to reduce the size of the chips to 1cm to 4cm length, so as to ease the handling of material (Ape,1999). The hipped straw is then soaked in clean water for about 20 minutes to remove suspended materials, impurities and debris that settles at the bottom of the container. Then it was later washed. After which the straw was soaked for 48 hours in clean water to soften the material so as to enhance ease and reduce time of cooking.

Cooking, Felting and Drying

After the 48 hours soaking process, the soaked material was then drained of the water. The materials were then cooked for 1 – 2 hours. The

cooking was done in a 'half cut' drum containing some quantity of water using firewood. Fresh water was poured into the drum and allowed to reach boiling point (between 80°C - 100°C) before the material was introduced into it. The essence of the cooking process is to further soften the material, and to enhance complete mechanical disintegration of the material (Zubairu, 1989)

2.2 Effect of Urea-Formaldehyde (UF) on the Preparation of Rice Straw Fiberboards

In this research work, all of the rice straw fiberboards were prepared by Hot press (Compressing molding) method Blending, mat formation , pressing trimming and conditioning processes were carried out in this step.

Procedure

Four types of rice straw fiberboards labeled as FBr1, FBr2, FBr3 and FBr4 were fabricated by using 120g of pre-treated rice straw fiber and 10g, 20g,30g and 40g of urea-formaldehyde as bonding adhesive. The batch process to produce a rice straw fiberboard, 120 g of pre-treated rice straw fiber length (0.5 cm) in the Henschel mixer and blended for 5 min at ambient temperature. After the whole mass was blended, the blended mixture was then poured into 6" x 6"(15.2 cm x 15.2 cm) configuration molds so as to produce a single layer board and wedged between two steel plates. The single layer wet board was pressed in a hydraulic hot press with 2200 psi at temperature 120°C for 10 min. During pressing the board, moisture content is removed from the board by reducing the valve in each 5 minutes. And original pressure is attained in each time by using valve. The parameters employed to produce single layer fiberboard are shown in (Table 3). The process diagram (overall procedure) for the preparation of fiberboards was shown in (Figure 3).

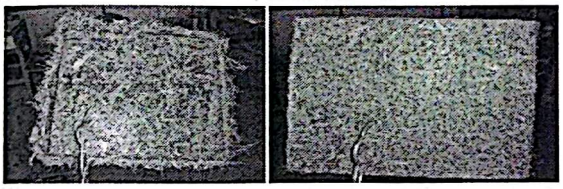

Figure 2 (a),(b) The preparation of fiberboards (a) before and (b) after trimming

2.3 Determination of the Physicochemical and Physicomechanical Properties of Prepared Fiberboards

Density -Density of test pieces was measured according to the British Standard Method (B.S : 1811:1961) (ASTM (1972)

Procedure

The boards to be tested were cut into (2.54 cm x 2.54 cm) in size. The length, width and thickness of each test piece were measured to an accuracy of 0.01 cm. The weight in grams of each test piece was determined to an accuracy of 0.01 mg.

Hardness - Hardness of test pieces was measured according to Durometer hardness tester (Shore Instrument and MFG Co., Freeport, New York)

Procedure

Hardness is the resistance of material to deformation, particularly permanently deformation, indentation or scratching. The Durometer hardness test is used for measuring the relative hardness of soft material. The test method is based on the penetration of a specified indenter forced into the material, under specified condition. Durometer hardness readings were performed according to ASTM D 2240. The boards to be tested were cut into (1.7 cm x 1.7 cm) pieces. The surface of the boards were trimmed and sanded to get smooth surface. The test was carried out by first placing a specimen on a hard, flat surface. The pressure foot of the instrument was pressed onto the specimen, making sure that it was parallel to the surface of the specimen. The durometer hardness was read within one second after the pressure foot was in form contact with specimen. Each specimen was subjected to durometer hardness readings, at designated positions on the sample bases .

Thickness - Thickness of test pieces was measured according to Micrometer or Screw gauze (Veneer clipper).

Procedure

Each type of particleboard was cut into (14 cm x 2.54 cm) size of pieces and thickness of individual fiberboard was measured by veneer clipper at least four points of each bundle. All of samples were measured about five references.

Impact -An impact test measured how much energy is absorbed when an object fractures or breaks under a high speed collision. An impactresistance of prepared rice straw fiberboards were determined according to IZOD Impact Tester.

Procedure

The fiberboards cut into (14 cm x 2.54 cm) pieces and impact resistance of individual fiberboards was measured by IZOD Impact Tester according to the procedure. All of the samples were measured at least five references. Two references were taken from one and three references were taken from another of the same ingredient of boards.

Bending strength-Bending strength has become a common measurement of fiberboards bending strength. Bending strength of prepared rice straw fiberboards were determined according to electro-hydraulic tensile tester (Philadelphia U.S.A)

Procedure

The boards cut into (14 cm x 2.54 cm) pieces and bending strength of individual prepared rice straw fiberboards were measured by Electro-hydraulic tensile tester according to the procedure. All of the samples were measured at least five references. Two references were taken from one and three references were taken from another of the same ingredient of boards.

Water absorption -Water absorption percentage of rice straw fiberboards was determined according to IS: 3087-1965.

Procedure

The fiberboards to be tested were cut into (2.54 cm x 2.54 cm) in size, weighed and placed in dedicator. Then the test specimens were immersed in distilled water for 24 h. After soaking in water, the swollen pieces were wiped with the filter paper to remove water on the surface and then reweighed. Five replicated specimens of individual fiberboards were tested and the results were presented as average of the tested specimens.

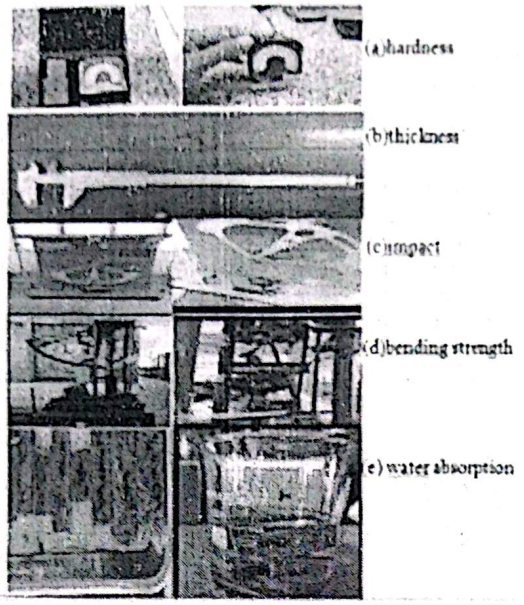

Figure 3The apparatus used for mechanical properties of prepared fiberboards

2.4 Characterization of Prepared Fiberboards

Microscopic and surface chemical analysis Procedure

Specimens were also prepared for microscopic and surface chemical analyses according to the specified by each apparatus. Microscopic images were obtained with Vividia Borescopes & Microscopes for fiberboards surfaces and interior structure along fiberboards thickness for distribution on fiberboards surfaces. A typical spectrum is a plot of electrons detected per unit time versus the bending energy of the electrons detected . The distance between layers ranged from 0.2 to 0.5 mm. The microscopic images of fiberboards were show in (Figure 5) .

2.5 Determination of Heat Resistance for Prepared Fiberboards

Procedure

The fiberboard was cut into (2.54 cm x 2.54 cm) pieces and weighed. Then these pieces of fiberboards were heated at different temperatures (100, 150, 200°C) respectively. The weight loss percentages were determined after heated at different temperatures. The results are presented in (Table 4).

2.6 Determination of Soil Degradation of Prepared Fiberboards

The soil degradation of fiberboards was tested by soil burial test.

Procedure

The soil degradation of fiberboards was tested by Soil Burial Test. Cellulose possesses the tendency to be degraded when it is buried in the soil (having at least 25 % moisture). The fiberboards were cut into (2.54 cm x 2.54 cm) pieces weighed and individually. These pieces were buried in the soil (60 % moisture) for 1 to 8 weeks. Thereafter, samples fiberboards pieces were carefully withdraw and washed with distilled water followed by dried at 105 °C for 1 h and kept for 24 h in desiccator. The fiberboards pieces were reweighed and weights are 51 recorded.

Results and Discussion

On the Aspect of Preparation and Properties of Rice Straw Fiber

The results presented in this chapter are based on the tests carried out on experimental board samples produced from rice straw (*Oryza Sativa* L.) . The fiberboards were made by treated rice straw fiber and different amounts adhesives urea-formaldehyde as binder material. These were manufactured using urea-formaldehyde adhesive. Fabrications of fiberboards were made by compressing molding method.Characterization of rice straw fiber was done according to physicochemical parameters such as moisture content, pH, bulk density and elemental composition of rice straw by ED XRF analysis (Table 1,2) and (Figure 4) .The experimental value of moisture content of rice straw fiber was 5.79 %. The value of bulk density of rice straw fiber was 0.88 g cm^{-3} and pH was 4.68.

Table 1 Physicochemical Properties of Rice Straw Fiber

Physicochemical Proper	Observed value
Moisture content % (w/w	5.79
pH	4.68
Bulk density (g cm^{-3})	0.88

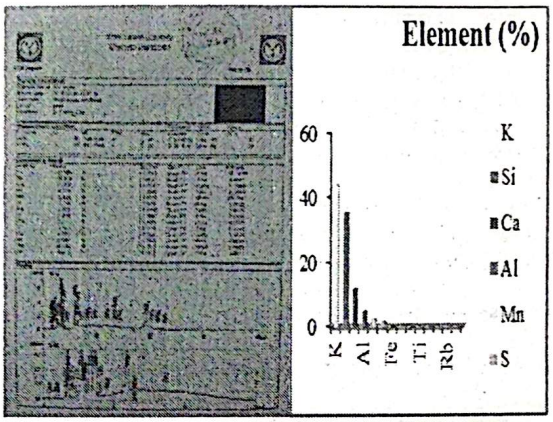

Figure 4 Elemental composition of rice straw fiber by ED XRF

Table 2 Study on Elemental Composition of Rice Straw Fiber by ED XRF Analysis

Element	Element (%)
K	43.566
Si	35.014
Ca	11.606
Al	4.755
Mn	2.424
S	1.704
Fe	0.330
P	0.245
Zn	0.110
Ti	0.103
Cu	0.051
Br	0.044
Rb	0.034
Sr	0.015

Effect of Urea- Formaldehyde on The Preparation of Rice Straw Fiberboards

Rice straw Fiberboards were prepared with various amounts of urea-formaldehyde (10, 20, 30 and 40 g) as adhesive (FBr1, FBr2 , FBr3 and FBr4 shown in (Table 4) at 120 °C of temperature and 2200 psi pressure. The results of physicochemical and physicomechanical

properties including density, hardness, thickness ,impact, bending strength and water absorption of reared fiberboards FBr1, FBr2, FBr3 and FBr4 are presented in (Table 3) and (Figure 5) .It was found that fiberboard (FBr4) prepared using rice straw fiber 120g and 40 g UF has the highest hardness and the lowest water absorption among them. From the comparison of physicochemical and physicomechanical properties of FBr4 had higher than other , less water absorption than other due to using 40g of UF. It was found that the adhesive type is also an important factor for high strength and durable fiberboard characteristics.

Table 3 Physicochemical and Physicomechanical Properties of Prepared Fiberboards with Different Amount of Urea- formaldehyde

Parameters	FBr1	FBr2	FBr3	FBr4
Density (g cm^{-3})	1.01	1.02	1.036	1.149
Hardness (Pa)	81	92	92	97.50
Thickness (cm)	1.74	1.71	1.64	1.54
Impact(kJm^{-2})	48.25	76.65	90.40	99.80
Bending Strength(lb ft)	512	665	890	1222
Water absorption (%)	64.55	43.07	43.04	39.19

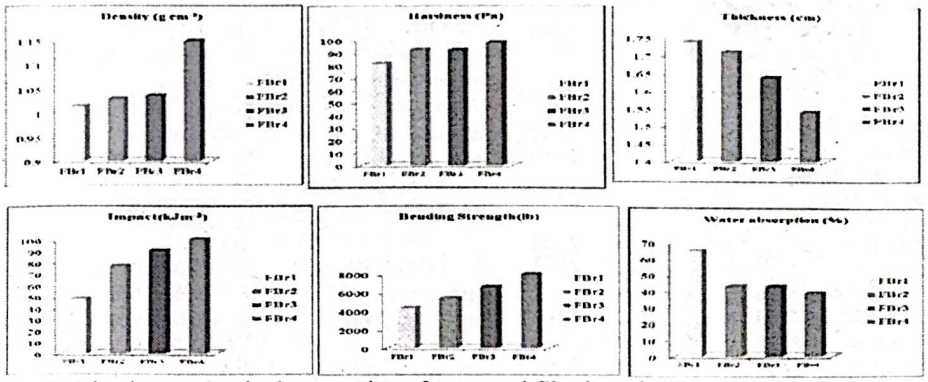

Figure 5 Physicomechanical properties of prepared fiberboards

Study on Surface Morphology of Prepared Rice Straw Fiberboards

Electron microscopic analysis examined the surface morphology of prepare fiberboards. The removal of surface impurities on plant fibers was advantageous for fiber and binder as it facilitates both mechanical interlocking and the bonding reaction due to the exposure of the hydroxyl groups to the chemicals used in treatment.

Microscopic images of rice straw fiberboards are shown in Figure 6(a),(b),(c) and (d). It was observed that the surface fracture of FBr4 fiberboard is more uniform than that of other fiberboard. It was also found that FBr4 fiberboard is better than other fiberboard in compatibility interfacial adhesion between the fibers and binders. When manufacturing the fiberboards, compatibility of the fiber and binders are important. More compatibility between the fibers and binders cause more enhanced the modulus of rupture and less water absorption.

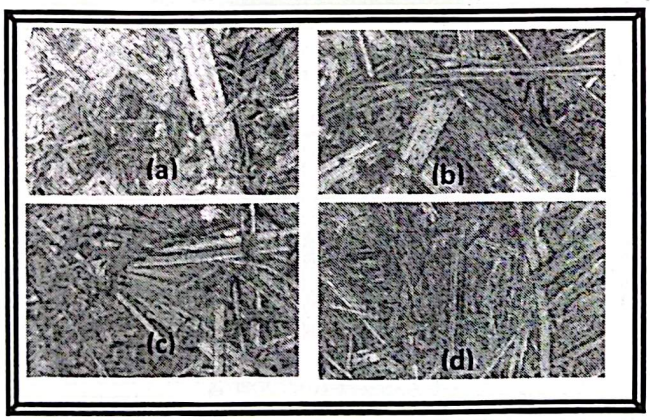

Figure6 (a) Microscopic image of prepared fibreboard (FBr1)
(b) Microscopic image of prepared fibreboard (FBr2)
(c) Microscopic image of prepared fibreboard (FBr3)
(d) Microscopic image of prepared fibreboard (FBr4)

Heat Resistance of Prepared Rice Straw Fiberboards at Different Temperatures

The heat resistance properties of the prepared rice straw fiberboards were shown in (Table 4). The characteristics of the heat resistance usually show the insulating property of fiberboards towards heat and fire. Out of the prepared rice straw fiberboards (FBr4) had the higher hardness value (92Pa) and lower water absorption 39.19% than other. So, (FBr4) was selected to observe heat resistance properties. FBr4 was heated in the electric oven at different temperatures (100, 150 and 200°C) and changes of surface color of rice straw fiberboards were studied (Figure 7). It could be seen that the colour of rice straw fiberboard is deeper as the temperature increases. It was found that the heat resistance of FBr4 rice straw fiberboard was remained until 200 °C.

Soil Degradation of Prepared Rice Straw Fiberboards

Rice straw fiberboards made from rice straw fiber with urea- formaldehyde were buried in soil (60 % water) for a period of 8 weeks in order to study the effect of environmental condition on the degradability of the samples (Figure 8). The weight loss of the rice straw fiberboards sample was periodically measured and the results are given in (Table 5). The weight loss due to degradability is increased as increases in buried time interval. The weight loss of FBr4 (13.71%) is at the maximum period of observation. The less weight loss of FBr4 is due to the presence of large proportion of urea-formaldehyde.

Table 4 Heat Resistance of Selected Prepared Fiberboards at Different Temperatures

Sample	Temperature (°C)	Before heating (g)	After heating (g)	Weight loss (%)	Observation
FBr1	100	26.71	25.64	4.00	No color change (Yellowish brown)
	150	26.71	24.46	8.42	No color change (Yellowish brown)
	200	26.71	24.17	9.50	Brown
FBr2	100	25.55	24.60	3.71	No color change (Yellowish brown)
	150	25.55	23.46	8.18	No color change (Yellowish brown)
	200	25.55	23.27	8.92	Brown
FBr3	100	23.51	22.72	3.36	No color change (Yellowish brown)
	150	23.51	21.67	7.83	No color change (Yellowish brown)
	200	23.51	21.44	8.80	Brown
FBr4	100	22.00	21.27	3.31	No color change (Yellowish brown)
	150	22.00	20.18	8.27	No color change (Yellowish brown)
	200	22.00	20.18	8.27	Brown

Table 5 Comparison for Weight Loss of Prepared Fiberboards by Soil Burial Test

Sample	Soil Buried Period (week)	Weight Loss (%)
FBr1	1	5.96
	2	7.57
	4	9.17
	8	48.44
FBr2	1	2.92
	2	21.95
	4	38.25
	8	59.66
FBr3	1	0.95
	2	4.01
	4	6.22
	8	27.36
FBr4	1	2.87
	2	3.79
	4	6.70
	8	13.71

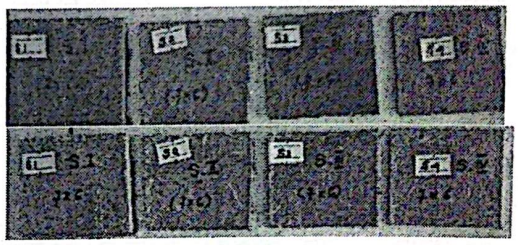

Figure 7 Heat resistance of prepared rice straw fiberboard at 100°C-200°C

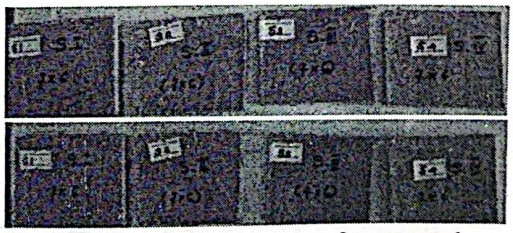

Figure 8 Soil burial test for prepared fiberboards

Conclusion

From the overall assessments of the present work concerning with the results of investigation on four types of prepared fiberboards (FB_r1, FB_r2, FB_r3 and FB_r4) were made by rice straw fiber and different amount of urea – formaldehyde. The following inferences could be deduced. Firstly, the some physicochemical properties of rice straw fiber; moisture content (5.79 %), pH (4.68) and bulk density (0.88 g cm^{-3}) were determined. From the comparison of physicochemical and physicomechanical properties of fiberboards , FB_r 4 prepared from 120g of rice straw fiber and 40g of urea- formaldehyde at 120 °C and 2200 psi was found to possess the highest density value (1.028 gcm^{-3}) together with the hardness (97.5Pa), bending strength(1222$lbft^{-3}$), impact (99.8 kJm^{-2}) and the lowest water absorption value (39.190 %) , thickness (1.54 cm), and the lowest soil degradation value (13.71 %). Therefore, FBr4 indicated the highest bending strength value and surface of FBr4 was more uniform than others which tend to increase bending strength from heat resistance determination, the weight loss of FBr4 at 200 °C was 8.27 % and surface appeared brown due to combustion of fiberboard. The less weight loss of FBr4 (8.27 %) was found due to thermal degradation up to 200 °C. According to the microscopic images of prepared fiberboards, fewer micropores, microcracks and more uniform were observed in FBr 4 .Therefore FBr4 was chosen the most suitable for making fiberboard. The lowest water absorption value (39.190%) and the lowest heat resistance (8.27 %) are good properties for fiberboard. The high content of water absorption caused to grow fungi on the surface and could be broken down. The uniform surface tends to be less water absorption content. It was found that estimated cost of fiberboard (2′ x 2′) sq ft size was 600 kyat, omitting the cost of machine usage and that of commercial fiberboard CPB1 (China- grade1) (2′ x 2′) sq ft size was 1200 kyat. Therefore, prepared fiberboard was cost effective and quality grade fiberboard for making valued-added products. In addition to these facts, the prepared fiberboards from waste products are not only helpful in protecting our environment but also in manufacturing cost effective fiberboards.

Acknowledgements

I would like to express my deepest gratitude to Dr Aung Kyaw Thin, Rector, Dr Aye Aye Han, Pro-rector, Banmaw University, for their interest and encouragement on my research work. I also wish to express my thanks to Dr Myint Myint Sein, Professor and Head, Banmaw University for her kind provision and the research facilities in the Department and numerous invaluable suggestions.

References

ASTM. (1972). *Annual Book of American Society for Testing and Materials, Standard Structural Sandawich Construction, Wood, Adhesives.* U.S.A : Printed in Md, Easton

Apeh A.J. (1999). "*The use of Rice Straw in the production of Compressed Straw Slabs*"(unpublished) An MSc thesis submitted to the Department of Building,

Buzarovska A, Bogoeva-Gaceva G, Grozdanov A, Avella M, Gentile G, Errico M (2008).'*Potential use of rice straw as filler in eco-composite materials*' Australian Journal of Crop Science 1(2):37-42 (2008). ISSN: 1835-2707 www.cropsciencejournal.org

Cetin, N. S. and N. Ozmen. (2002). "Use of Organosoly Lignin in Phenol-Formaldehyde resins for Particleboard Production and Properties". *International Journal of Adhesion and Adhesives,* 22 (6), 481-486

Lee E., Sun X., Karr G. S. (2004). *Adhesive properties of modified soybean flour inwheat straw particleboard.* Composites: Part A. 35:297-302

Petruzzi M (Ed) (2001). '*Particleboard and Medium-Density Fibreboard*'.The Choose www.greenseal.org

Zubair I.K. (1989). "*Suitability of Maize Cob as material for Particle BoardProduction*" (unpublished) An MSc thesis submitted to the Departmentof Building, Ahmadu Bello University Zaria. Nigeria .

Determination for the Crude Protein Content of Poppy Seed Meal Cake *Papaver Somniferum* L. (Bein)

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Abstract

Opium poppy known as "Bein" in Myanmar is a useful medicinal plant. In this paper, the chemical composition of meal cake extracted from poppy seeds purchased from Zegyo market, Chan Aye Thar San Township, Mandalay Region was investigated. When oil from the seeds was removed by solvent extraction with petroleum ether, meal cake for analysis was obtained. Ash moisture and crude fibre content of poppy seed cake were determined. Soluble carbohydrate was determined by phenol sulphuric acid colourimetric method. Micro Kjeldahl's method was used for the determination of protein content.

Keywords: Micro Kjeldahl's method, solvent extraction

Introduction

Opium poppy, *Papaver somniferum*, is a member of family Papaveraceae. Opium poppy, also called Bein in Myanmar is native range of Eastern Mediterranean, it now occurs in most countries as a weed.

Poppy is cultivated as an annual crop in all countries of the world with suitable, temperate climates.

Poppy is grown mainly for its content of opium and oil seed. The seed are used almost exclusively for their oil. The seed of the fruit is the spice as well as a popular food. But the plant is better known as a narcotic plant. The gun from the fruit is known as "Opium" that is ill famed for addition. In fact, the growers grow the plant for opium that fetches them greater value than its seed.

Poppy seeds have high protein content, the major component being a globulin, which accounts for 55% of the total nitrogen. The amino acid make up of the globulin is similar to that of the whole seed protein and is as follows: Arginine, Histidine, Lysine, Tyrosine, Tryptophan, Phenylalinine, Cystine, Methionine, Threonine and Valine. The proteins are deficient in lysine and methionine.

Poppy seeds are rich in linoleic acid and oleic acid, unsaturated fatty acids that useful in human nutrition. Among the volatile components of poppy seeds, aliphatic hydrocarbons and aldehydes have been reported. 2-Pentylfurane is a key aroma compound.

The white seeds are very small. It yields thiamine, riboflavin and nicotinic acid considerably, but carotene is absent. There are also small quantities of minerals such as iodine, manganese, copper magnesium and zinc. The seeds also contain lecithin, oxalic acid, pentosans, traces of narcotine and an amorphous alkaloid, and the enzymes diastase, emulsin, lipase and nuclease.

Opium contains two families of alkaloids: (i) Phenanthrene-type alkaloids include morphine, codeine, thebaine and the synthetic heroin (ii) Benzylisochinoline-type alkaloids, are more common in the plant kingdom; in opium, they are represented by narcotine, papaverine and narceine. Most of these have their special field of application in modern medicine.

The alkaloid content of poppy seed is low (50 ppm) and cannot have any pharmaceutical effect. It is, however, high enough that morphine can be detected in the urine after heavy poppy seed consume, which might make for an unpleasant surprise in drug tests.

Opium poppies yield valuable alkaloids, used as medicines. Medicines produced from opium poppies include morphine and codeine cultivation and production is strictly controlled because opium poppies are also used to make illegal and highly addictive drugs such as heroin.

Opium apart from its addition values is also considered as medicine. Many of the traditional herbal preparations are manufactured with opium as one of the important ingredient.

Poppy seeds are devoid of narcotic properties and are utilized as food and as a source of fatty oil. They are considered nutritive and are used in breads, curries, sweets and confectionary. Seed have a number of medicinal virtues. It was reported that the seeds of poppy were useful for the relief of dysentery, constipation, cough and asthma.

Poppy seed oil appears to be of good quality for human, consumption since it is generally rich in polyunsaturated fatty acids. Seed oil contained an appreciable amount of β-tocopherol (309.5 ppm-567.3 ppm). Stearic, palmlitic, oleic, linoleic and linolenic were determined as the main fatty acids. Poppy seed and oil having nutritive value recommended for processing as healthy food products.

Poppy seed oil is widely used for culinary purposes. It is free from narcotic properties and is used mixed with olive oil or as salad. On hydrogenation, it yields a product similar to hydrogenated groundnut oil, which may also be useful for industrial purposes. Its seeds oil is an edible oil and useful for manufacture of paints and soaps.

In India, the oil is generally extracted by cold pressing the seed in small presses in homes or small establishments (oil yield about 20%). Raw cold pressed oil is pale to golden yellow in colour.

The cake or the meal left extraction of the oil from the seeds is sweet and nutritious and eaten by the poor people. It is readily consumed by cattle and sheep, and may be fed alone or preferably fed with other feeds. However, excessive feeding of the cake (over 1kg/day) to diary cattle may result in decrease of the content of milk and in soft fat.

Aim of this research work is to study the chemical constituents of poppy seed and to determine the crude protein content in the meal left after extraction of the oil from the seeds.

Botanical Description

Family name	: Papaveraceae
Botanical name	: *Papaver somniferum* Linn.
Myanmar name	: Bein
English name	: Opium poppy

Distribution : Poppy is cultivated as an annual crop in countries such as China, India and Czechoslovakia.

Parts Used : Seed

Medicinal value : The seeds have been effective in treating insomnia, inflammation, fever, dysentery and irritation among other disorders. An infusion of seeds is said to help ear and toothache.

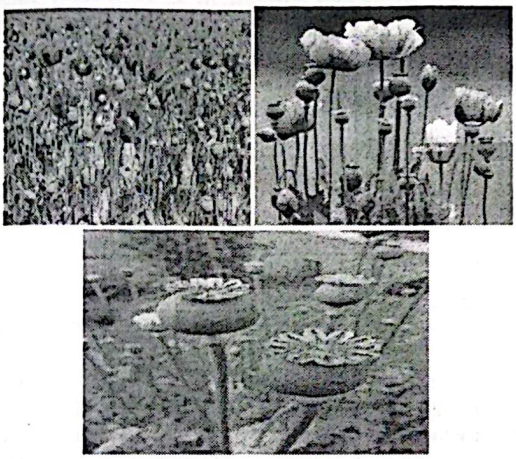

Fig 1. Poppy plants, poppy flowers and poppy fruits

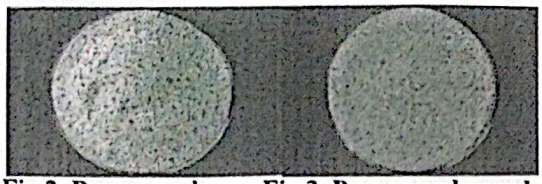

Fig 2. Poppy seeds

Fig 3. Poppy seeds meal cake

Experimental Work

Sample Collection

The sample, poppy seeds were purchased from Zegyo market, Chan Aye Thar San Township, Mandalay Region. It was stored in a well stopped bottle and used throughout the experiment.

Extraction by Soxhlet Apparatus

Accurately weighed poppy seed powder 30g was introduced into the weighed extraction thimble. The open end of the thimble was closed with a plug of cotton wool. The thimble was placed into the extractor and a round bottle flask 250mL which had been weighed was attached. Sufficient petroleum ether (b.p 60°C-80°C) was poured into extractor so as to start the siphon. Then the extractor was attached to the condenser and the flask was hated on a water bath for 10 to 12 hours. When the extraction was completed, the petroleum ether was removed by vacuum distillation. The last trace of the solvent was then removed by placing the content in an oven at about 100°C until constant weight was obtained. After extraction, the thimble containing the meal cake was placed in an oven until no odour of ether remains. The meal cake and oil contents of poppy seeds are tabulated in Table (1)

Table 1. Yield of Meal Cake and Oil from Poppy Seeds

No.of expt.	Wt.of sample (g)	Wt.of oil (g)	Wt.of meal cake (g)	Oil yield (%)	Meal cake yield (%)
1	30.3515	14.3414	16.0101	43.2510	56.7489
2	30.4110	14.3727	16.0383	43.2615	56.7384
3	30.3547	14.3436	16.0113	43.2533	56.7466

Mean = 43.25, 56.75

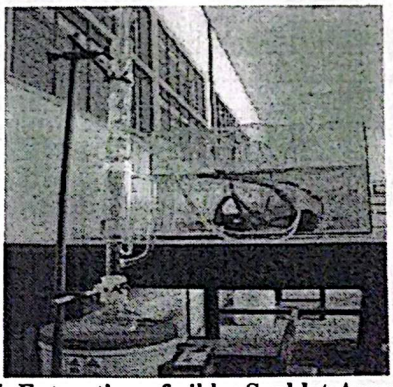

Fig 4. Extraction of oil by Soxhlet Apparatus

Determination of Ash

About 1g to 2 g of defatted poppy seed powder was accurately weighed in a preheated, cooled and weighed porcelain crucible. The crucible and sample were heated carefully over an open flame until the sample was thoroughly carbonized. After heating, the crucible was placed in a muffle furnace at 600°C until the residue was free from carbon. The crucible and contents were then cooled in a desiccator and weighed. Heating in the furnace, cooling and weighting were repeated until a constant weight was obtained.

Calculation

$$\text{Ash (\%)} = \frac{\text{Residue Wt, (g)} \times 100}{\text{sample Wt, (g)}}$$

Table 2. Yield of Ash from Defatted Poppy Seed

No. of expt.	Wt. of sample (g)	Wt. of Ash (g)	Ash (%)
1	1.1025	0.0262	2.3764
2	1.3220	0.0313	2.3676
3	1.2915	0.0306	2.3693
			Mean = 2.37

Determination of Moisture and Volatile matter

Accurately weighed 2g of defatted poppy seed powder was added into a glass dish previously dried and cooled in a desiccator. The dish containing the sample was placed in an oven and dried for 30 minutes at 101± 1°C. The dish then removed from the oven and cooled in a desiccator to room temperature and weighed. The procedure was repeated until the loss in weight did not exceed 0.05% per minute during the drying period.

Calculation

$$\text{Moisture and Volatile Matter (\%)} = \frac{\text{Loss in Wt.(gm)} \times 100}{\text{Wt.of sample}}$$

The results from several determinations of moisture content were tabulated in Table (3).

Table 3. Yield of Moisture and Volatile Matter from Poppy Seed Meal Cake

No. of expt.	Wt. of sample (g)	Loss in Wt. (g)	Moisture (%)
1	2.0012	0.2151	10.7485
2	2.0011	0.2151	10.7491
3	2.0015	0.2153	10.7569
			Mean = 10.75

Determination of Crude Fibre

About 2g of the defatted poppy seed powder was accurately weighed and introduced into a 500mL flask. Then 200mL of 1.25% sulphuric acid solution was added, the flask was connected with a reflux condenser and refluxed for about 30 minutes. The flask was rotated with hand at every 5 minutes in order to mix thoroughly. After 30 minutes the insoluble material was filtered through linen in a funnel. The residue was washed with hot water and the washing was continued until the washing were no longer acidic. The residue was then washed down into the flask with 200mL of sodium hydroxide solution. The flask was boiled gently for exactly 30 minutes. At the end of the period, it was filtered through the same linen. The residue was washed thoroughly with hot water till free from alkali and then with about 15mL of 95% ethanol. The residue was introduced into a crucible and dried to a constant weight. The weight was recorded and residue was incinerated at a dull red heat for about 20 minutes until the carbonaceous matter had been removed. The contents of the crucible were cooled and weighed. Heating, cooling and weighing were repeated until a constant weight was obtained. The loss in weight was taken as crude fibre. The results were tabulated in Table (4).

Calculation

$$\text{Fibre (\%)} = \frac{\text{Wt.of Fibre} \times 100}{\text{Wt. of sample}}$$

Table 4. Yield of Crude Fibre from Poppy Seed Meal Cake

No. of expt.	Wt. of sample (g)	Loss in Wt. (g)	Fibre (%)
1	2.1071	0.2419	11.4843
2	2.1052	0.2415	11.4726
3	2.2065	0.2418	11.4831
			Mean = 11.48

Determination of Water Soluble Carbohydrate

Preparation of sample solution

0.1g of sample powder was dissolved in 100mL of hot water and shaken for ten minutes. 1mL of this solution was then diluted to 10mL with water and this solution was taken as the sample extract.

Preparation of standard sugar solutions

100 mg (0.1g) of hydrated glucose was exactly weighed and dissolved in 100mL of distilled water.1,2,4,6,8 and 10mL of these solutions were drawn out and put in each 100mL volumetric flask and diluted to the mark with distilled water. These solution contained 10, 20,40,60,80 and 100 µg of glucose per ml respectively.

Procedure

1mL of sample solution and six standard sugar solution containing 10, 20,40,60,80 and 100 µg of glucose per ml were put in each test tube. 1mL of 5% phenol solution was also added to each test tube and mixed. A blank also prepared with 1mL of distilled water instead of sugar solution. 5mL of 96% sulphuric acid, was again added to each tube so that the stream hit the liquid surface directly to produce good mixing. Each test tube was agitated during the addition of acid. After ten minutes, the tube were reshaken and placed in water bath at 25-30°C for twenty minutes. The yellow orange colour was stable for several hours. Absorbance were measured at 490 nm using UV-VIS Spectrophotometer (Model-1601).

A standard curve was plotted by the absorbance of the standard solution against the

concentration in μg per mL. Using this standard curve, the concentration of glucose in the sample was calculated.

The absorbance of the standard glucose solutions were listed in Table (5) and the calibration curve for standard glucose solution was shown in figure (5). The results were shown in Table (6).

Table 5. Absorbance of Standard Glucose Solutions

No.	Concentartion of glucose (μg/mL)	Absorbance at 490nm
1	10	0.1052
2	20	0.2070
3	40	0.4100
4	60	0.6100
5	80	0.8593
6	100	1.0870

Table 6. Soluble Carbohydrate in Meal Cake of Poppy Seed

No. of expt.	% of soluble carbohydrate
1	6.84
2	6.83
3	6.82
Mean = 6.83	

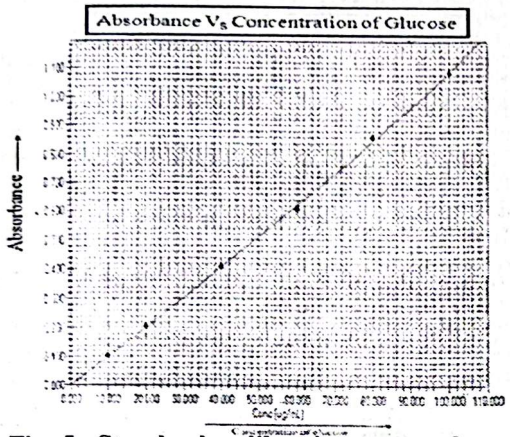

Fig 5. Standard calibration curve for water soluble carbohydrate (as glucose)

Determination of Protein Content by Kjeldahl's method

Accurately weighed defatted powder 2g was placed in the Kjeldahl's digesting flask, a round bottomed flask with a long narrow neck. Anlar potassium sulphate 10g, anhydrous copper sulphate 0.5g and pure sulphuric acid 10 mL were added to the Kjeldahl's digesting flak in such a way as to the wash down any solid adhering to the neck. Then it was shaken until the content were well mixed and heated gently and cautiously in small flame. When foaming ceased, heating was increased gently until mixture boiled brickly and became colourless. The digestion was continued for half an hour to make sure that all the nitrogen in the sample was converted into ammonium sulphate. It was allowed to cool and distilled water 100mL was carefully added into the flask with frequent shaking. The Kjeldahl's distillation apparatus was set up, taking care that the tip of condenser extended well below the surface of the standard sulphuric acid solution 50 mL in the receiver. The digested solution was poured into the distillation flask and then 100 mL of 40% sodium hydroxide solution was added into it through the dropping funnel to make the mixture strongly alkaline. The evolved ammonia was distillate with respect to excess sulphuric acid was titrated with standard N. sodium hydroxide solution using methyl orange as an indicator. A blank determination was carried out exactly as above, but instead of poppy seed powder sample, 20 mL of distilled water was used.

Calculation

$$\text{Protein (\%)} \quad \frac{0.014\times100\times(X-V)N_A}{W}\times6.25$$

where,

X = Volume (mL) of sulphuric acid solution used in blank

V = Volume (mL) of sulphuric acid solution used in test

N_A = Normality of sulphuric acid solution

W = Weight (g) of sample

Table 7. Protein Content in the Meal Cake of Poppy Seed

No. of expt.	% of Nitrogen	% of Protein
1	3.9859	24.9121
2	3.9848	24.9051
3	3.9891	24.9123
		Mean = 24.91

Results and Discussion

For the present investigation, poppy seeds were obtained from Zegyo market, Chan Aye Thar San Township, Mandalay Region. The yield of the meal cake obtained from the poppy seeds by Soxhlet apparatus using petroleum ether (b.pt.60-80) was 56.75%.

The chemical compositions of poppy seed meal cake such as ash, moisture, crude fibre, water soluble carbohydrate and crude protein are shown in Table (8). Oil, ash and moisture contents of the sample are nearly the same as the literature values. Crude fibre and water soluble carbohydrate contents are not described in literature. However, crude protein content of the meal is less than that of literature value. According to these results, poppy seeds are rich in protein and oil. So, poppy seeds can be utilized as food and as a source of fatty oil.

Table 8. Chemical Constituents of Meal Cake of Poppy Seeds

Chemical constituents	Literature (%)	Result (%)
Meal Cake	-	56.75
Ash	2.4	2.37
Moisture	10.8	10.75
Crude fibre	-	11.48
Water soluble carbohydrate	-	6.83
Crude Protein	36.6	24.91

Conclusion

In this paper, investigation of meal cake obtained from poppy seeds by Soxhlet apparatus using petroleum ether (b.pt 60-80) was described. The yield percent of meal cake was observed at 56.75%. The chemical composition of poppy seed meal cake such as ash (2.37%), moisture (10.75%) and crude fibre (11.48%) were also determined. The soluble carbohydrates (6.83%) were estimated by phenol-sulpuric acid colorimetric method. Micro Kjeldahl's method was used for the determination of crude protein content (24.91%). These results reveal that poppy seeds and defatted poppy seeds are nutritionally valuable for human and animals. Hence opium poppy should be produced only for food and medicinal purposes.

Acknowledgements

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References

https:// www.britannica.com>plant

https://en.m.wikipedia.org>wiki

Diamond, P.S. and Denman, R.F. (1966). "Laboratory Technique in Chemistry and Biochemistry", Butter Worth &Co.Ltd., London.

Harold Hart and Robert D. Schuetz, (1968). "Organic Chemistry", 3rd ed., Printed in U.S.A.

I.L. Finar, (1967). "Organic Chemistry", 3rd ed., Printed U.S.A.

J. A. Barnard and R. Chayen, (1965). "Modern Method of Chemical Analysis", McGraw-Hill Publishign Company Ltd., London.

J.E. Hodge and B.THofreller, (1962). "Method in Carbohydrates Chemistry", Academic Press Inc., N.Y, 1962.

KhinThanKyi, (1975). "Analysis of Amino Acids and Carbohydrates in the Defatted Groundnut oil cake", M.Sc.Thesis, University of Mandalay.

M.L. Anson, Kenneth Bailey and John T. Edsall., (1952). "Advances in Protein Chemistry, Vol VII, N. Y.

MyintAung, (1975). "Investigation on the Chemical Constituents of Poppy Seed Oil", M.Sc. Thesis, University of Mandalay.

R.L. Whistler, (1965). "Method in Carbohydrate Chemistry," Vol. V., Academic Press, New York and London, Page 296.

Sharman, (1962). "Chemistry of Food and Nutrition", 8th Ed, The Macmillan Company, New York.

Vogel, A.I., (1961). "Quantitative Inorganic Analysis", 3rd ed., p. 256, Longmans and Green Co. Ltd. London.

Antibacterial and Antioxidant Activities of the Stem Bark from *Bauhinia racemosa* (Lam.) and Isolation of a Pure Bioactive Organic Compound

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Abstract

A pure unknown compound was isolated from the stem bark of *Bauhinia racemosa* (Lam.) by using Thin Layer and Column Chromatographic methods. The plants are grown in Sagaing and Mandalay Regions, Upper Myanmar. According to the phytochemical screening, some of the secondary metabolites were found in the stem bark of this sample. The antibacterial activity of the crude extract sample was examined using various solvents by agar well diffusion method on six organisms. Chloroform extract was the highest activity on three organisms, such as *Enterococcus faecalis*, *Salmonella typhi* and *Shigella*. IC_{50} values of standard ascorbic acid and ethanol crude extract sample were found to be 0.3975µg/mL and 8.257µg/mL. Functional groups of isolated pure compound were determined by applying FT-IR spectrometric method.

Keywords: *Bauhinia racemosa*, Antibacterial Activity, Antioxidant Activity,Isolation

1. Introduction

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed. In developing countries, herbal remedies have become more popular in the treatment of minor ailments and also on account of the increasing costs of personal health maintenance (UNESCO, 1996).

Medicinal plants are distributed worldwide, but they are most abundant in tropical countries. According to the World Health Organization (WHO), because of poverty and lack of access to modern medicine, about 65–80% of the world's population which lives in developing countries depends essentially on plants for primary health care. Secondary metabolites are found in all parts of plant. Some contains high concentration and some of them are present at very low concentrations (Calixto, J.B., 2000).

Most commonly used herbs are extremely safe to use. Some plants, however, can produce side effects, and, like all medicines, herbal remedies must be treated with respect. The ability of an herbal medicine to affect body systems depends on the chemical constituents that it contains. Scientists first started extracting and isolating chemicals from plants in the 18th century. Research into isolated plant constituents is of great importance, for it has given rise to many of the world's most useful drugs (Chevallier, 1996).

Developed countries, in recent times, are turning to the use of traditional medicinal systems that involve the use of herbal drugs and remedies. Medicinal plants are an integral component of research developments in the pharmaceutical industry. Such research focuses on the isolation and direct use of active medicinal constituents, or on the development of semi-synthetic drugs, or still again on the active screening of natural products to yield synthetic pharmacologically active compounds (Hoareau, 1999).

For a long period time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies devoted to natural therapies. About 80% of individuals from developed countries use traditional medicine, which involves compounds drives from medicinal plants. Therefore, such plant should be investigated to better understand their properties, safety, and efficiency (Kirtikar, K. R. & Basu, B. D., 1975).

Most of used in Myanmar drugs are obtained from wild plants and only a few drug plants are cultivated. Myanmar people had own ancient empirical knowledge on the use of plants for medicine. The plants, namely *Bauhinia racemosa* are grown in Myanmar, India, Ceylon, China and Timor. It is named Phalan in Myanmar. It belongs to the Caesalpiniaceae Family. Flowering period in Myanmar is from August to November. It is used in the treatment of headache, fever, skin diseases, tumors, blood diseases, dysentery and diarrhoea. (Yin Yin Aye & Maung Thinn, 2005)

In this research, the preliminary phytochemical screening, antimicrobial and antioxidant activities of the stem bark were carried out. An unknown pure bioactive organic compound had also been isolated from the stem bark of that plant applying Thin Layer and Column Chromatography. The functional groups of this compound were determined by using FT-IR spectrometry.

1.1 Aim and Objectives

The aim of this research work is to investigate phytochemical compounds, elemental contents, antibacterial and antioxidant activities of crude extract, and to determine the functional groups of the pure isolated compound from the stem bark of *Bauhinia racemosa* (Lam.).

Objectives are to collect the stem bark of *B.racemosa,* to examine the phytochemical compounds present or absent in the sample, to analyze the elemental constituents by EDXRF method, to determine the antibacterial activity by Agar well diffusion method and antioxidant activity by DPPH assay, to isolate the pure organic compound from the ethyl acetate crude extract by thin layer and column chromatography and to examine the functional groups in the isolated pure compound by FT-IR spectrometric method.

1.2 Botanical Description of *Bauhinia racemosa* (Lam.)

B. racemosa is a small crooked tree with dark scabrous bark, containing numerous drooping branches. The tree typically reaches a height of 6-12 m and their branches spread 3-6 m outwards. The bark of *B. racemosa* is bluish black, rough, pinkish red inside, which turns brown on exposure. The leaves are broader than long, having size 2-5 cm by 2.5-6.3 cm, divided a little less than half way down into two rounded lobes. The upper surface of leaf being green and glabrous, rigidly

coriaceous, slightly cordate, clothed more or less densely beneath with grey pubescence and base is usually cordate. (Pritipadma, *et al.*, 2015).

2. Experiments

2.1 Instrumentation

In this research work, common laboratory apparatus and equipments were used. UV lamp (lambda-40, Pekin Elmer Co., England) and FT–IR spectrometer (Shimadu, Japan) which are used for functional groups characterization of the isolated pure organic compound.

2.2 Materials and Methods

Commercial grade reagents and solvents were used without further purification. An iodine vapour was used for the location of the sample spots. Analytical preparative thin layer chromatography was performed by using pre-coated silica gel (Mark. Co. Inc. Kieselgel 60 F_{254}). Silica gel (70 to 230 Mesh ASTM) was used for column chromatography.

The stem barks of *B. racemosa* were collected from Shwebo Township, Sagaing Region, Upper Myanmar. They were clean and chopped into small pieces. Then these samples were dried in air and stored in a well stoppered bottle.

Determination of Phytochemical contents in the sample was carried out by standard method (Harborne, 1998). Antibacterial activity of the crude extract of the stem bark was tested on six species by agar well diffusion method. Free radical scavenging activity of ethanol crude extract sample was measured by DPPH (2,2-diphenyl 1-picrylhydrazyl) assay method (Philips, 2010).

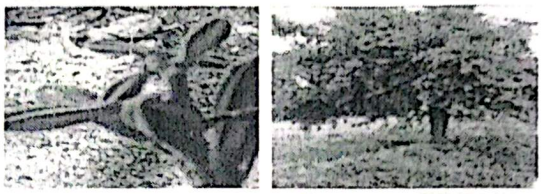


Figure 1. Flower and Plant of *B. racemosa* (Lam.)

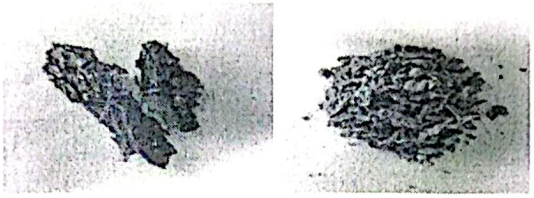


Figure 2 .The Stem Bark of *Bauhinia racemosa* (Lam.)

3. Results and discussion

3.1 Phytochemicals in the Stem Bark of *B. racemosa*

The preliminary phytochemical Screening informed that flavonoid, terpene, phenolic, saponin, glycoside and reducing sugar were found in the stem bark of *B. racem*

Table 1 Phytochemical Contents of the Stem Bark of *B. racemosa*

No.	Phytochemical Tests	Reagent	Observation	Result
1	Alkaloid	Dragendroff's	Yellow ppt	+
2	Saponin	Distilled water	frothing	+
3	Phenolic	10% $FeCl_3$	Brown solution	+
4	Glycoside	10% lead acetate	White ppt	+
5	Reducing Sugar	Benedict's solution	Reddish brown solution	+
6	Tannin	1% $FeCl_3$, 1% Gelatin	White gelly	+
7	Polyphenol	$FeCl_3$, K_4 [Fe(CN)$_6$]	greenish brown solution	+
8	Steroid	Pet-ether, acetic anhydride, Conc.	Violet solution	+
9	Terpene	$CHCl_3$,acetic anhydride,	Reddish brown layer	+
10	Flavonoid	EtOH, Conc.HCl,	Reddish Pink solution	+

+ presence, - absence

The stem bark of *B racemosa* contained alkaloid, saponin, phenolic, glycoside, reducing sugar, tannin, polyphenol, steroid, terpene and flavonoid.

3.2 Elemental Composition in the Stem Bark of *B. racemosa*

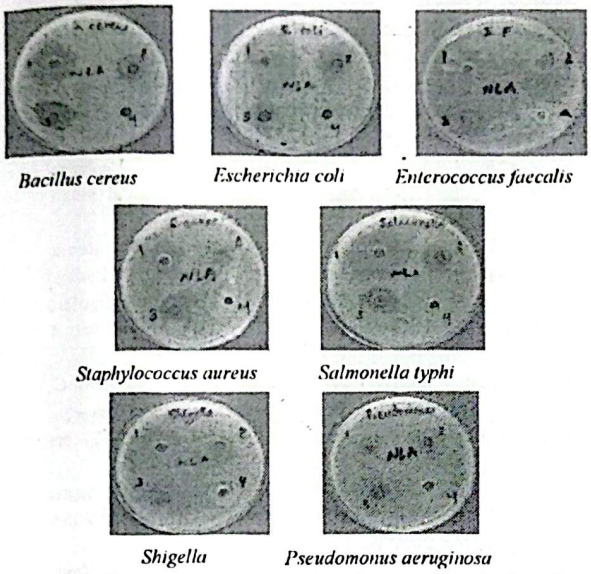
Elemental composition of the stem bark of *B. recemosa* was measured by using EDXRF spectroscopic method at Department of Chemistry, University Research Center, Monywa University.

As tabulated in Table 2, Calcium (Ca) was found to be the highest constituent in the stem bark of *B. recemosa*. Moreover, Silicon (Si), Potassium (K), Sulfur (S), Stronium (Sr) and Iron (Fe) were observed as 0.144%, 0.129%, 0.067%, 0.010% and 0.010% respectively.

Table 2.Elemental Composition in the Stem Bark of *B. racemosa*

No.	Elements	Relative Abundance (%)
1.	Ca	1.972
2.	Si	0.144
3.	K	0.129
4.	S	0.067
5.	Sr	0.010
6.	Fe	0.010
7.	Ti	0.002
8.	Cu	0.002
9.	Mn	0.001

3.3.Antibacterial Activity of the Crude Extract of the Stem Bark of *B. racemosa*



Bacillus cereus *Escherichia coli* *Enterococcus faecalis*

Staphylococcus aureus *Salmonella typhi*

Shigella *Pseudomonas aeruginosa*

Figure 3 Antibacterial Activity of the Stem Bark of *B. racemosa*

Chloroform extract was highly responded on *Enterococcus faecalis*, *Salmonella typhi*, *Shigella* and *Bacillus cereus*. The inhibition of ethanol and ethyl acetate extracts were significantly found on *Enterococcus faecalis*. Antibacterial ctivity of acetone extract of the plant sample was also observed on the remaining organisms, *Bacillus cereus*, *E.coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Shigella*. As ethanol extract did not effect on *Staphylococcus aureus* and also ethyl acetate extract did not inhibit on *pseudomonas aeruginosa*.

Table 3.Antimicrobial Activities of Crude Extract of the Stem Bark of *Bauhinia racemosa*

No.	Organism	Inhibition of various solvent extracts(mm)		
		CHCl3	EtOH	EtOAc
1	*Bacillus cereus*	30	18	22
2	*Escherichia coli*	10	22	20
3	*Enterococcus faecalis*	36	36	35
4	*Staphylococcus aureus*	20	-	22
5	*Salmonella typhi*	35	20	25
6	*shigella*	35	17	22
7	*Pseudomonas aeruginosa*	12	12	-

Agar well = 0.8 mm

1 = $CHCl_3$ extract
2 = EtOH extract
3 = EtOAc extract
4 = control

3.4. Free Radical Scavenging Activity of the Stem Bark of *B. racemose*

According to DPPH assay method, IC_{50} value of standard ascorbic acid and ethanol crude extract were found to be 0.3975 µg/mL and 8.257 µg/mL. The IC_{50} value is inversely proportional to the free radical scavenging activity.The IC_{50} value of sample extract indicates the plant sample has antioxidant activity.

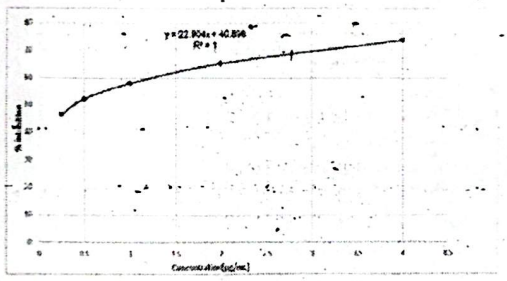


Figure 4 Free Radical Scavenging Activity of Ethanol Crude Extract

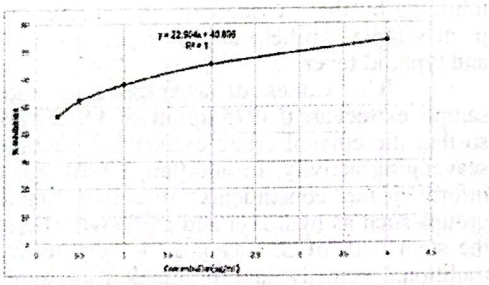


Figure 5 Free Radical Scavenging Activity of Std: Ascorbic Acid

Table 4 Absorbance, % Inhibition, IC_{50} Values of Ethanol Crude Extract and Standard Ascorbic Acid

No.	Extract	Concentration (µg/ mL)	absorbance	% Inhibition	IC_{50} (µg/ mL)
1	Ethanol Extract (Sample)	1.95	0.2674	42.655	8.257
		3.9	0.2517	46.022	
		7.8	0.236	49.389	
		15.62	0.1872	59.854	
		31.25	0.1514	67.532	
2	Ascorbic Acid	0.25	0.2489	46.622	0.3975
		0.5	0.2222	52.348	
		1.0	0.1957	58.031	
		2.0	0.1615	65.365	
		4.0	0.1219	73.858	

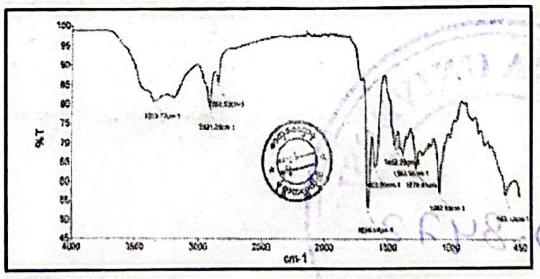


Figure 6 FT-IR Spectrum of Pure Isolated

3.5. Extraction and Isolation of Compound

Air dried sample (850g) was percolated with 95% ethanol (2L) for about two months. The extracted solution was filtered and evaporated. The residue was dissolved in (250 mL) of ethyl acetate and ethyl acetate extract solution was evaporated at room temperature.

The ethyl acetate extract of the stem bark (2.5g) was chromatographed by silica gel column, using n-hexane and ethyl acetate with various ratios from non-polar to polar.

3.6 Functional Groups Analysis of Pure Isolated Compound

In FT-IR spectrum of pure isolated compound, the broad absorption peak at 3353.77 cm^{-1} showed O-H stretching vibration of alcohol groups. The bands which appear at 2921.25 cm^{-1} and 2850.53 cm^{-1} indicate that asymmetrical and symmetrical C-H stretching vibration of sp^3 hydrocarbons. The intense peak at 1658.54 cm^{-1} gave C=O stretching vibration of carbonyl group. The peaks at 1605.20 cm^{-1} and 1452.29 cm^{-1} represented C=C stretching vibration of aromatic ring skeleton. The peak appears at 1383.98 cm^{-1} signified C-H bending vibration of gem-dimethyl group. C-O stretching vibration of alcohol groups was observed at1278.61cm^{-1}. The other peaks at 1092.65 cm^{-1} and 565.12 cm^{-1} indicated C-H in plane and out of plane bending vibration of trans or E and cis or Z alkenic groups.

4. Conclusion

According to the preliminary phytochemical screening, stem bark of *B. racemosa* consists of all tested phytochemical compounds. The presences of secondary metabolites such as flavonoid, terpene, phenolic, saponin, and glycoside indicated that plant sample have some medical activities. Moreover, due to the presence of Calcium, the sample is good for teeth and bone health and other elemental composition can supply the requirements of minerals for human health.

Investigation of antibacterial activity informed that the crude extracts have high activity on *Enterococcus faecalis*, *Shigella*, and *Salmonella typhi*. Especially *Enterococcus faecalis* was highly inhibited by these crude extracts. Therefore, the plant sample can help to cure infectious diseases and typhoid fever.

IC_{50} values of ascorbic acid and the sample extract are 0.3975 μg/mL and 8.257 μg/mL so that the ethanol crude extract has free radical scavenging activity. In addition, FT-IR spectrum informed the constituents of active functional groups such as hydroxyl and carbonyl. Therefore, the stem bark of *B. racemosa* is very useful for traditional remedy and production of synthetic drugs.

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References

Calixto, J.B., (2000). "Brazilian Journal of Medicinal and Biological Research", Vol. 33(2), pp.179-189

Chevallier,A., (2000). "Encyclopaedia of Herbal Medicine", 2nd Edition, A Dorling Kindersley Book

Harborne, J.B., (1998). "Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis", Chapman and Hall Ltd, U.S.A.

Hoareau, L. and Da Silva, E.J., (1999). "*Medicinal Plants : a re-emerging health aid*", Division of Life Sciences, UNESCO Rue Miollis, Paris, France

Kirtikar, K.R. and Basu, B.D., (1975). "*Indian Medicinal Plants*", 2nd Edition. Bishen Singh Mahendra Pal Singh, Dehradum, India. Vol.2, pp. 894- 895,

Philips, A., Philip, S., Arull, V., Padmakeerthiga, B ., Renju, V., Santha, S ., and Sethupathy, S., (2010). " Free Radical Scavenging Activity of Leaf Extracts of Indigofera Aspalathoides – An *in vitro* Analysis", *J. Pharm. Sci. & Res.* Vol.2(6), pp. 322-328

Pritipadma P., Debajyoti D., Priyanka D. and Goutam G. (2015). "Therapeutic Potential of Bauhinia Racemosa – A Mini Review", Int, J. Pharm. Sci. Rev. Res., Vol.32(2), pp. 169-179

Silverstein, R. M. and Webster, F. X . (1998). "Spectrometric Identification of Organic Compounds", 6th Edition, John Wiley and Sons Inc., New York

UNESCO. (1996). "Culture and Health " Orientation Text-World Decade for Cultural Development, Paris, France, pp-129

Yin Yin Aye and Maung Thinn, (2005). "Source plants used in traditional medicine to cure hypertension with various trademarks found in Mandalay City", PhD Dissertation, Department of Botany, University of Mandalay, Myanmar