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Chemistry

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Chemistry

Preparation and Application of Starch Based Wound Dressing Film

Htun Minn Latt[*]

Abstract

Polymer film of polyvinyl alcohol (PVA) - corn starch was prepared by casting method. Firstly, corn starch was extracted from corn grains. Yield % of the corn starch was found to be 74%. Then, PVA – corn starch blended film was prepared. This film showed mild antimicrobial activity on all tested microorganisms. Biomedical application of the prepared film was studied by animal test using Albino Wistar rats and its wound healing activity was observed.

Keywords: PVA, corn starch, citric acid, glycerol, wound healing activity

Introduction

Synthetic polymer materials have been widely used in every field of human activity (Vert *et al.*, 2002) during last decades. These artificial macromolecular substances are usually originating from petroleum and most of the conventional ones are regarded as non-degradable. However, the petroleum resources are limited and the blooming use of non-biodegradable polymers has caused serious environmental problems. In addition, the non-biodegradable polymers are not suitable for temporary use such as sutures. Thus, the polymer materials which are degradable and biodegradable have been paid more and more attention since 1970s. Both synthetic polymers and natural polymers that contain hydrolytically or enzymatically labile bonds or groups are degradable.

The advantages of synthetic polymers are obvious, including predictable properties, batch-to-batch uniformity and can be tailored easily (Nair and Laurencin, 2007). In spite of this, they are quite expensive. Thus it is needed to focus on natural polymers, which are inherently biodegradable (Chiellin and Solaro, 1996).

Recently the term “biomaterial” has been defined as a non-living material used in medical device applications for interaction with a biological system. It is important that the term “biocompatibility” was also formulated; it determines how a tissue responds to foreign material.

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Starch and chitosan are abundant naturally occurring polysaccharide. Both of them are cheap, renewable, non-toxic, and biodegradable (Zhai *etal.*, 2004). Starch is totally biodegradable in wide

variety environments. Starch itself is poor in processability, also poor in the dimensional stability and mechanical properties for its end products (Choi *et al.*, 1999). Therefore, native starch is not used directly.

Aim and Objectives

This research was aimed to prepare PVA - corn starch - glycerol - citric acid film and to study antimicrobial properties and wound healing property.

The objectives of this research include

- To isolate corn starch from corn grain
- To prepare PVA - corn starch blended films
- To characterize the prepared films by modern techniques namely, Scanning Electron Microscopy (SEM), X-Ray Diffraction Analysis (XRD) and Fourier Transform Infrared Spectroscopy (FT IR)
- To study antimicrobial properties of the prepared PVA- corn starch film
- To study the wound healing activity of the prepared PVA-corn starch film on Wistar rats.

Materials and Methods

Extraction of Starch from Corn Grains

Corn starch was extracted from corn grains. Corn grains were ground and the corn powders were passed through 250 mesh screen. Corn powder sample (200g) was placed in a 500 mL beaker. Distilled water (250 mL) was added to the corn powder, stirred manually for 30 min and allowed to settle overnight. The supernatant was decanted off and this starch was subjected to a second washing and settled overnight. The supernatant was then decanted off and filtered through double -layered cotton cloth. Then the starch obtained was dried in air for 48 h at room temperature. The starch lumps were made into powder before it was stored in polyethene bag prior before use. Yield percentage of corn starch was 74 %.

Preparation of PVA - Corn Starch - Glycerol - Citric Acid Films

PVA films with corn starch, glycerol and citric acid with the ratios of 2:0.5:0.3:0.3 were prepared. PVA (2g) was added into a 250 mL beaker. Next, 100 mL of distilled water was added to the beakers. The solution mixture was stirred for 10 min. The corn starch (0.5 g) was added to PVA solution and the solution mixture was stirred for10 min. After that 0.3 g of glycerol and 0.3 g of citric acid were added. The solution mixture was stirred at 150 ^oC by using a magnetic stirrer with temperature control for 45 min at a rate of 1000 rpm and then autoclaved at 0.1 MPa, 121˚C for 20min. The polymeric solution was casted on the melamine plate and oven dried for 2.5 h and air dried for 5.5 days to obtain the PVA-corn starch-glycerol -citric acid film with the ratio of 2:0.5:0.3:0.3.

Determination of Physico-mechanical Properties of PVA - Corn Starch - Glycerol - Citric Acid Films

Fourier Transform Infrared (FT IR) Analysis

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra of the samples was recorded on a Perkin Elmer FTIR spectrometer at Universities' Research Center, Yangon. The samples were taken at random from the flat films and data were collected over 16 scans with a resolution of 4 cm^{-1} at room temperature. FTIR analysis was in a range of wavenumber from 4000 to 550 cm^{-1}.

X-Ray Diffraction Analysis

X-ray diffraction patterns of the samples were recorded on X-ray diffraction instrument (Rigaku, Tokyo, Japan) at Universities' Research Center, Yangon, using CuK_α radiation (λ= 1.54^0A) at 40 kV and 40° mA. The diffraction angle ranged from 10° to 70° of 2θ.

Investigation of Antimicrobial Activities of PVA and Blended Films

Tests for antimicrobial activities of the PVA and its blended films were carried out at Fermentation Department, Central Research and Development Center (CRDC), Ministry of Industry, Yangon.

Wound Healing Activity of Films by Animal Experiment

Wound healing activity of films by animal experiment was studied at Department of Medical Research (Lower Myanmar). Microscopic appearances of animal wounds were checked in days 1, 3, 6, 9, 12, 15 and 18. The results are shown in Figure 4 to Figure 10.

Histological Study (Histopathological Finding)

Specimens of skin from healed wounds from each Wistar rats were taken and fixed in 10% buffered formalin solution for histopathological studies. Sections of the healed skin were made at a thickness of 5 μm with paraffin and, stained with hematoxylin and eosin (H & E), and assessed for histopathological changes in day 18th of wounded skin. The enclosements of wounded surface area (WSA) were conducted by tracing of wounds. The progress of wound healing in macroscopic was taken by photo at 1, 3, 6, 9, 12, 15 and 18 days post wounded. The microscopic slides were photographed.

Results and Discussion

Extraction of Corn Starch

Corn starch was extracted from 200g of corn grain powder and yield percent was found to be 74 %.

Effect of Citric Acid on PVA - Corn Starch Films

Effect of citric acid on the plasticized PVA-corn starch film was studied by the addition of 0.3 g into the mixture of PVA ,corn starch and glycerol (2:0.5:0.3).

Citric acid was added as an additive. In this research work, citric acid was chosen as the additive for the following reasons. First of all, as a result of its multi-carboxylic structure, esterification could take place between the carboxyl groups on citric acid and the hydroxyl groups on the PVA or starch. Such an esterification would improve the water resistibility

(Borredon *et al.*, 1997). Furthermore, because of the multicarboxyl structure, citric acid may serve as a cross-linking agent. Crosslinking of a blend reinforces the intermolecular binding by introducing covalent bonds that supplement natural intermolecular hydrogen bonds (Krumova *et al.*, 2000) so as to improve the mechanical properties and water resistibility. Second, the residual - free citric acid in the PVA - starch blends may act as a plasticizer. As compared to the hydroxyl groups on glycerol, the carboxyl groups on citric acid can thus form stronger hydrogen bonds with the hydroxyl groups on PVA - starch blends, thus improving the interactions between the molecules (Shi *et al.*, 2007; Yu *et al.*, 2005). And as a third point, citric acid is rated as nutritionally harmless since it is a nontoxic metabolic product of the body (Krebs or citric acid cycle). Consequently, it has already been approved by FDA for use in humans (Yang *et al.*, 2004). These nontoxic properties will benefit the incorporation of citric acid in PVA- starch blends films and such films would have potential applications in the food packing industry and in the biomedical field. Citric acid has two functions, i.e., as a cross linker and as a plasticizer. The mechanical properties are not only related to the cross linker but also related to the plasticizer. The cross- linker and the plasticizer always have the contrary effects on the tensile properties.

Characterization of PVA and Blended Films by FTIR Analysis

PVA - corn starch film containing glycerol and citric acid was characterized by Fourier Transform Infrared Analysis.

In the FTIR spectrum of PVA, the stretching and bending vibration of –OH absorption peaks occurred at 3293 and 1261 cm^{-1}, respectively. The peak at 2942 cm^{-1} assigned as C–H stretching vibration was observed.

The peak at around 760 cm^{-1} was the characteristic absorption of starch, and the peak at 1734 cm^{-1} was the characteristic absorption of PVA, which were not overlapped by other absorption peaks. The two peaks existed together in the FT IR spectrum indicating the success of blending of PVA with corn starch. In FT IR spectrum, the peak at 1716 cm^{-1} indicated the esterification (cross-linking) occurred between PVA and citric acid and starch and citric acid.

X-Ray Diffraction Studies

XRD pattern had peaks at 19.3° , 21.4° and 23.8° of 2θ.Some crystalline peaks of starch at 20.1^{o} and 23 ^{o} of 2θ were also observed together with those of PVA peaks. Moreover, the peak at 29.6^{o} was caused by the residual citric acid in the blends.

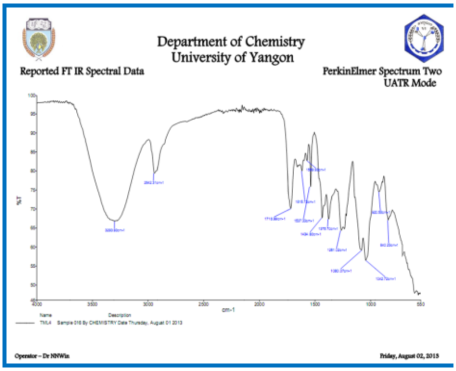


Figure 1. FT IR spectrum of PVA- corn starch - glycerol -citric acid film

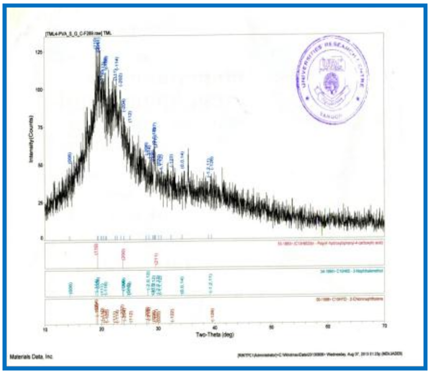
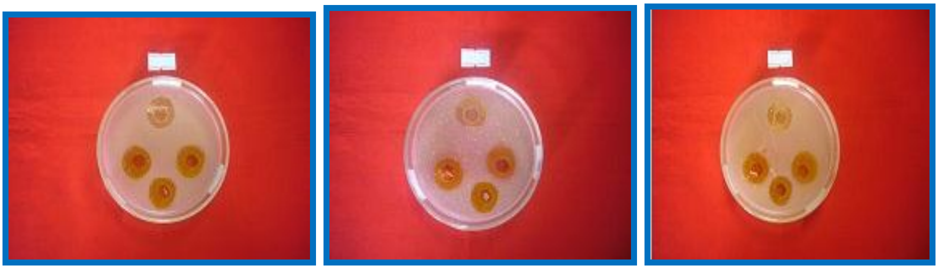


Figure 2. XRD pattern of PVA - corn starch film containing glycerol and citric acid

Antimicrobial Activity

Figure 3 shows antimicrobial test results of PVA-corn starch-glycerol-citric acid (2:0.5:0.3:0.3) films against six different micro-organisms and the results are shown in Table 1. It was found that PVA did not show antimicrobial property against all six microorganisms, *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli*. However, PVA film incorporated with corn starch as well as corn starch, glycerol and citric acid were found to show mild inhibition effect on all tested microorganisms.



Bacillus subtilis aureus *Staphylococcus* *Pseudomonas aeruginosa*

Bacillus pumilus *Candida albicans* *Escherichia coli*

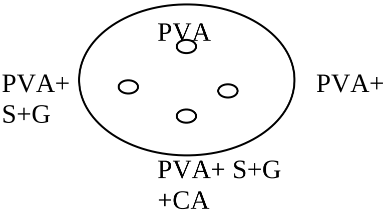


Figure 3. Antimicrobial tests of prepared films

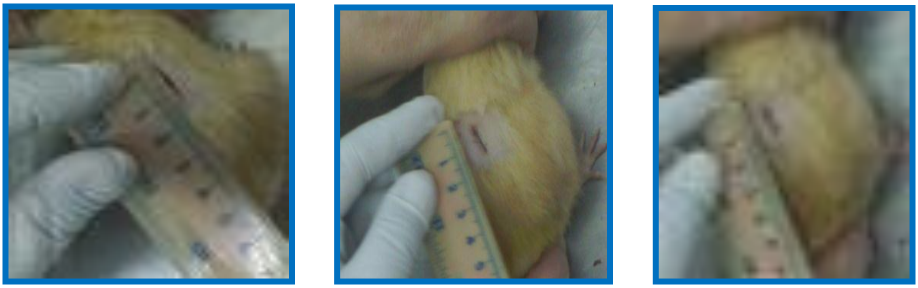
Table 1. Antimicrobial Tests on PVA Film, PVA - Corn Starch Film PVA - Corn Starch - Glycerol Film and PVA - Corn Starch - Glycerol - Citric Acid Film

No	**Organism**	**PVA (2%)**	**PVA – corn starch (2:0.5)**	**PVA – corn starch - glycerol (2:0.5:0.3)**	**PVA – corn starch - glycerol- citric acid (2:0.5:0.3:0.3)**
1	*Bacillus subtilis*	-	+	+	+
2	*Staphylococcus aureus*	-	+	+	+
3	*Pseudomonas aeruginosa*	-	+	+	+
4	*Bacillus pumilus*	-	+	+	+
5	*Candida albicans*	-	+	+	+
6	*Escherichia coli*	-	+	+	+

Agar well – 10 mm
10 mm - 14 mm (+)
15mm - 19 mm (++)
20 mm and above (+++)

Wound Healing Activity of Prepared Films

The biomedical application of cross-linked PVA film was studied by animal experiment. The operation skins of Wistar rats were treated with septidine and PVA - corn starch - glycerol film cross-linked with citric acid. The progress of these operation skins was recorded by photo at specified time interval. The photographs are shown in Figure 4 to Figure 10.



Without Treatment (1 day)

Control (Septidine) (1 day)

PVA-corn starch-glycerol-citric acid film (1 day)

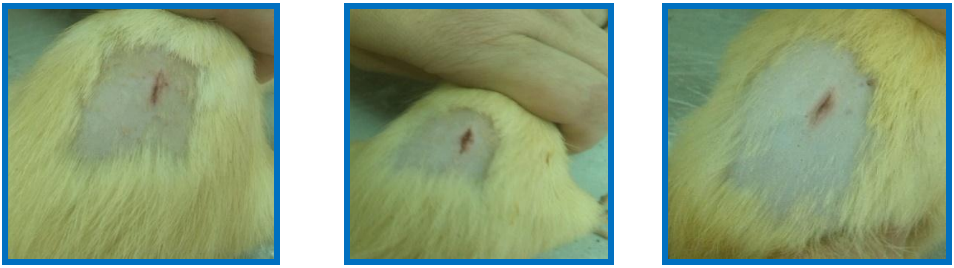
Figure 4. Wound-healing progresses in 1 day

Without Treatment (3 days)

Control (Septidine) (3 days)

PVA-corn starch-glycerol-citric acid film (3 days)

Figure 5. Wound-healing progresses in 3 days

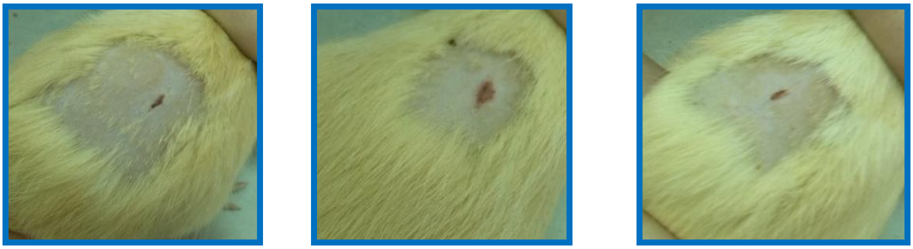


Without Treatment (6 days)

Control (Septidine) (6 days)

PVA-corn starch-glycerol-citric acid film (6 days)

Figure 6. Wound-healing progresses in 6days

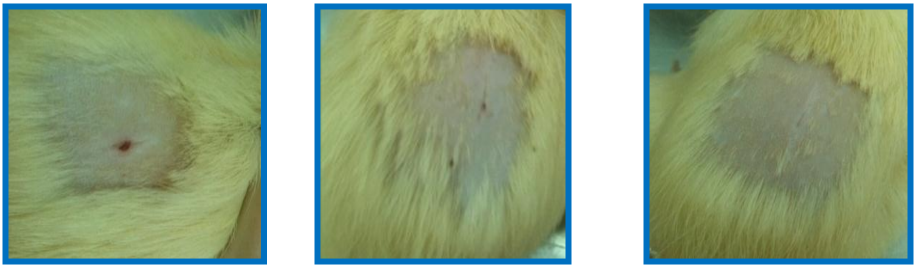


Without Treatment (9 days)

Control (Septidine) (9 days)

PVA-corn starch-glycerol-citric acid film (9 days)

Figure 7. Wound-healing progresses in 9 days



Without Treatment (12 days)

Control (Septidine) (12 days)

PVA-corn starch-glycerol-citric acid film (12 days)

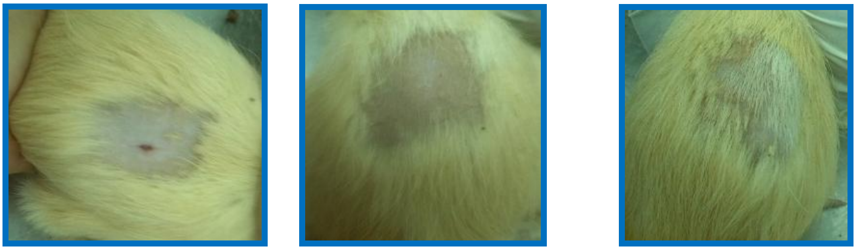
Figure 8. Wound-healing progresses in 12 days

Without Treatment (15 days)

Control (Septidine) (15 days)

PVA-corn starch-glycerol-citric acid film (15 days)

Figure 9. Wound-healing progresses in 15days



Without Treatment (18 days)

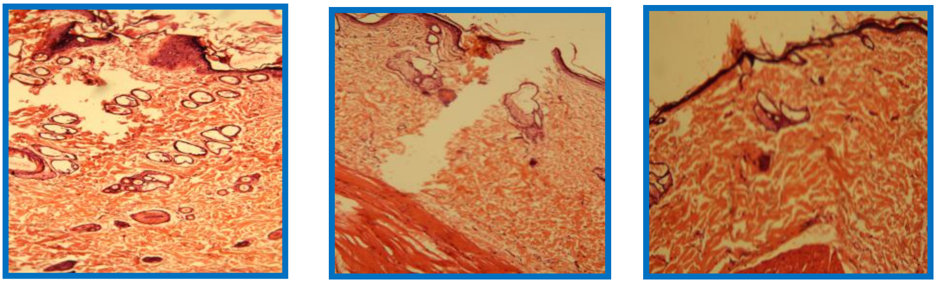
Control (Septidine) (18 days)

PVA-corn starch-glycerol-citric acid film (18 days)

Figure 10. Wound-healing progresses in 18 days

Histopathological Finding of Skin Lesions Samples

Histopathological finding of skin lesions after 18 days was done by (H & E) method and recorded by using light microscopic with specific images. These images are presented in Figure 11.



(a)Without Treatment (after 18 days)

(b) Control (spetidine) after 18days

(c) PVA-corn starch-glycerol-citric acid film (after 18days)

Figure 11. Hematoxyline and eosin-stained section of biopsies for the microscopic lesions of three different wound skins (18 days treatment) of Wistar rats

Conclusion

This research was conducted to prepare Polyvinyl Alcohol (PVA) and corn starch blend films (cross-linked) and to study its antimicrobial activity and wound healing activity.

Firstly, corn starch was isolated from corn grain powder and yield percent was obtained as 74%.

Then, Poly Vinyl Alcohol (PVA) and corn starch blend film was prepared. Finally, these films concerned with the antimicrobial activity and biomedical application, the following inferences could be deduced.

PVA - corn starch film showed mild antimicrobial activity on all tested microorganisms *viz., Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli.*

The last part of this work is biomedical application of cross-linked PVA - corn starch film. Histopathological studies for 18 days showed that the wounded skin of cross-linked PVA - corn starch film dressing area became complete epithelialization in epidermis and dermis and absence of inflammatory cells (polymorph and macrophages) in dermis. The prepared cross-linked PVA - corn starch film has better and faster wound healing activity than septidine on Wistar rats.

In conclusion, biodegradable PVA - corn starch blend films were successfully prepared using corn starch as low cost filler since corn is widely grown in Myanmar and is readily available. Furthermore, cross-linked PVA - corn starch films were found to have wound healing activity and so these films can be used as wound healing agent.

Acknowledgements

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References

Borredon, E., Bikiaris, D., Prinos, J. and Panayiotou, C. (1997). *Properties of Fatty-acid Esters of Starch and their Blends with LDPE, Journal of Applied Polymer Science,* Vol. (*65***)**, pp. 705–721

Chiellini, E. and Solaro, R. (1996). *Biodegradable Polymeric Materials, Advanced Materials*, Vol. (4**)**, pp. 305–313

Choi, E. J., Kim, C. H. and Park, J.K. (1999). *Synthesis and Characterizationof Starch-g-Polycaprolactone Copolymer, Macromolecules,* Vol. (32)**,** pp.7402–7408

Krumova, M., López, D., Benavente, R., Mijangos, C. and Pereña, J. M. (2000). *Effect of*

Crosslinking on the Mechanical and Thermal Properties of Poly (Vinyl Alcohol), Polymer, Vol.(41**)**, p.9265

Nair, L. S. and Laurencin, C. T. (2007). *Biodegradable Polymers as Biomaterials, Progress in Polymer Science*, Vol. (32**)**, pp. 762–798

Shi, R., Zhang, Z. Z., Liu, Q. Y., Han, Y. M., Zhang, L. Q. and Chen, D. F.(2007). *Characterization of Citric Acid / Glycerol Co-plasticized Thermoplastic Starch Prepared by Melt Blending, Carbohydrate Polymers*, Vol**.** (69)**,** pp. 748–755

Vert, M., Santos, I. D., Ponsart, S., Alauzet, N., Morgat, J. L., Coudance, J. and Garreau, H. (2002). *Degradable Polymers in a Living Environment: Where Do You End Up?, Polymer International*, Vol. (51), pp. 840–844

Yang, J., Webb, A. and Ameer, G. (2004*). Novel Citric Acid-Based Biodegradable lastomers for Tissue Engineering*, *Advanced Materials*, Vol. (16), pp.511–516

Yu, J. G., Wang, N. and Ma, X. F. (2005). *The Effects of Citric Acid on the Properties of Thermoplastic Starch Plasticized by Glycerol, Starch/ Stärke*, Vol. (57), pp. 494–504

Zhai, M., Yoshii, F. and Kume, T. (2004). *Radiation Modification of Starchbased Plastic Sheet, Carbohydrate Polymers* Vol. (52)**,** pp. 311-317

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Molecular Formula Determination and Structure Elucidation of Active Compound Isolated from the Stem Bark of *Protium serratum* (Wall.ex Colebr.) Engl. (Gati)

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Abstract

In this research wrok, one of the Myanmar indigenous medicinal plant, *Protium serratum* (Wall. ex Colebr.) Engl., Myanmar name Gati was chosen for chemical analysis and pharmcological investigation. Firstly preliminary phytochemical tests were performed by standard qualitative methods. A pure organic compound, colorless crystal was isolated from the stem bark of Gati by applying advanced separation methods such as Thin-layer and Column Chromatography. This pure compound was checked by phytochemical tests which gave positive for steroid and glycoside test. The yield percent of this compound was found to be (29.2 mg) 1.19% based upon ethyl acetate crude extract. Antimicrobial activity of isolated compound was determined by Agar well diffusion methods tested on six microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Bacillus pumilus, Pseudomona aeruginosa, Candida albicans* and *Escheria coli*. The molecular formula of compound was determined as $C_{46}H_{80}O_7$ by using spectroscopic techniques, such as FT-IR, ^{1}HNMR (500 MHz), ^{13}C NMR (125 MHZ), DEPT, HSQC and DART-mass spectral data respectively. Hydrogen deficiency index of this compound is 7. Finally the complete structure of compound (steriodal glycosidic fatty acid ester) was elucidated by applying DQF-COSY, ^{1}H NMR splitting patterns, coupling constant (*J*-values) and HMBC spectroscopic studies.

Keywords: *Protium serratum* (Wall. ex Colebr.) Engl., Thin-layer and Column Chromatography, Spectroscopic techniques

Introduction

In Myanmar there are many traditional medicinal plants which have been reputed for their various kinds of activities and usefulness in pharmacology. Most of the people in Myanmar depend on traditional medicinal plants and herbal medicines for the treatment of various diseases. The study of traditional indigenous medicinal plants and their usage in therapy plays a very important role in health care system of Myanmar.

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[3] Associate Professor, Dr, Department of Chemistry, Kyaukse University.

Protium serratum (Wall. ex Colebr.) Engl. belong to the family Burseraceae, Myanmar name Gati is an evergreen or briefly deciduous tree with a large, spreading crown, it can grow up to 10 meters tall. The bole is short. The tree is harvested from the wild for local use as a food, medicine and source of wood (Website -1). It is mainly distributed in Bhutan, Cambodia, India, Laos, Myanmar, Thailand and Vietnam (website-2).

In this research work, *Protium serratum* (Wall. ex Colebr.) Engl., Gati in Myanmar was selected for chemical analysis. Local people in Pyin Oo Lwin Township use the stem bark of Gati for the treatment of hypertension, diabetic, dysentry and diarrhea. In Myanmar, medicinal uses of Gati are less known. Thus, the promotion of its medicinal uses such as antiviral, antifungal, antidiabetic, antioxidant activities and the bioactive compounds from the stem bark of Gati are intended to be investigated.

Material and Method

Analar grade reagent and solvents were used throughout the experiment. Analytical preparative Thin-layer Chromatography was performed by using aluminium coated sheet silica gel (Merck, Co. Inc., Kiesel gel 60 F_{254}) and slica gel (70-230 mesh ASTM) was used for Column Chromatography.

The advanced instruments were used in the characterization of sample and structural elucidation of organic compound. These are shown below.

1. UV-lamp (Lambda 40, Perkin Elmer Co. England)
2. FT-IR spectrometer (Shimadzu, Japan)
3. ^{1}H NMR spectometer (500 MHz, Japan)
4. ^{13}C NMR spectrometer (125 MHz, Japan)
5. DART-Mass spectrometer (Japan)

Sample Collection and Preparation

The stem barks of the Gati were collected from Pyin Oo Lwin Township, Mandalay Region. They were cut into small pieces and air dried at room temperature for about two months. Then, the air dried sample was stored in a well stoppered bottle, and used throughout the experiment.

Figure 1. Leave, Fruit and Stem Bark of *Protium serratum* (Wall. ex Colebr.) Engl.

Extraction and Isolation of a Pure Compound

The air dried stem bark sample 1 kg was percolated with 5 L of 95% ethanol for two months. The precolated solution was evaporated and then extracted with (350 mL) of ethyl acetate. When ethyl acetate extract was concentrated, the crude extract (2.5 g) was obtained. This extract (2.5 g) was fractionated by Column Chromatography over silica gel with various volume ratios of *n*-hexane and ethyl acetate from non-polar to polar. Totally (280) fractions were collected. Then each fraction was checked on TLC using iodine as visualizing agent. The fraction with same R_f values was combined. Major combined fractions (V) gave only one spot on TLC and UV inactive. The yield percent of this colorless crystal compound was found to be 1.19% (29.2 mg) based upon the crude ethyl acetate extract.

Flow Sheet for the Isolation of Pure Compound

Ethyl acetate extract

- Chromatographed by Column
  Adsorbent- SiO_2 (70-23 mesh)
  Eluent-*n*-hexane:EtOAc (non-polar to polar)

Total (280) fractions

- Checked by TLC, I_2 vapor and UV lamp
- Combined

(15) Combined fractions

Other fractions

Combine fractions (V) colorless solid compound

(only one spot on TLC and UV inactive)
(29.2 mg) (1.19%) based on EtOAc extract
$R_f = 0.30$
n-hexane: EtOAC
1:4 v/v

Determination of Antimicrobial Activity of Isolated Compound

Antimicrobial activity of isolated compound was tested by Agar well diffusion method tested on six microogranisms.

Phytochemical Tests of Isolated Compound

Glycoside Test

A small amount of compound was placed in a test tube. And then, small amount of 10% lead acetate solution was added. White precipitate was observed and it indicated the presence of glycoside.

Steroid Test

A small amount of compound was placed in a test tube. Then, 2 mL of acetic anhydride solution, 1 mL of concentrated sulphuric acid and a few drops of chloroform were added to the test tube. The presence of blue color solution indicated the presence of steroid.

Spectroscopic Studies of Compounds

Isolated bioactive organic compound was subjected to analyze by FT-IR, ^{1}H NMR, ^{13}C NMR, DEPT, HSQC, DQF-COSY, HMBC, DART-Mass and NOESY spectroscopic techniques.

Results and Discussion

Antimicrobial Activity of Isolated Compound

Antimicrobial activity of isolated compound was tested on six selected organisms by applying Agar well diffusion method. Isolated compound responded medium activity on all tested organisms.

Table 1. Antimicrobial activity of isolated compound

Solvent extract	**Inhibition zone/diameter (mm)**					
	B. subtilis	***S. aureus***	***B. pumilis***	***P. aeruginosa***	***C. albicans***	***E.Coli***
EtOAc	16 (++)	18 (++)	17 (++)	18 (++)	19 (++)	18 (++

Confirmation of Phytochemical Test

Phytochemical test for isolated compound gave positive test for glycoside and steroid test.

Molecular Formula Determination of the Isolated Compound

The isolated compound was obtained as colorless solid crystal. The molecular formula was determined to be $C_{46}H_{80}O_{7}$ from the observation of DART-MS spectrometry. The FT-IR spectrum exhibit absorption bands at 3422.80, 3065.95, 2929.00, 2853.78, 1733.10, 1646.30, 1379.15, 1281.50, 1172.76, 1077.28 and 737.80 cm^{-1} ascribable to hydroxyl, sp^{2} H/C, sp^{3} H/C, ester carbonyl group, allylic H/C, gem-dimethyl group and ether functional group respectively. The ^1H NMR spectrum (Table 4) revealed seven s*p*^{3} methyl protons, twenty s*p*^{3} methylene protons, fifteen s*p*^{3} methine protons and one s*p*^{2} methine proton. The DEPT and FT-IR spectral data display the presence of seven s*p*^{3} methyl carbons, twenty s*p*^{3} methylene carbons, one s*p*^{3} carbinol methylene carbons, ten s*p*^{3} methine carbons, three s*p*^{3} carbinol methine carbons, three s*p*^{3} quaternary carbons, one s*p*^{2} quaternary carbon, one either oxygen and one carbonyl group.

According to ^1H NMR and ^{13}C NMR spectral data, the partial molecular formula is $C_{46}H_{77}$ and the partial molecular mass is 629.

In accordance with FT-IR assignments, this compound should consists of at least one O–H group, one carbonyl group and one ether functional group. Hence, the partial molecular formula could be extended to $C_{46}H_{78}O_3$ and its partial molecular mass is 678.

In addition, the DART-MS spectrum is described in Figure (9).

In this spectrum, M + H (M + 1) peak was observed as *m/z* 745.5959 which indicates that the molecular mass of compound is 744.

Therefore the remaining partial molecular mass is (744 – 678) = 66.

It must be two O–H groups and two ether oxygen atoms.

Consequently, the real molecular formula of this isolated compound could be assigned as $C_{46}H_{80}O_7$.

$$\text{Hydrogen Deficiency Index (HDI)} = C - \frac{H}{2} + 1 = 46 - \frac{80}{2} + 1 = 7$$

^{1}H NMR Spectral Data of Compound

^1H NMR spectrum is shown in Figure (3). According to this spectrum, compound contains (77) protons. The chemical shift values of protons and their assignments are shown in Table (2).

Table 2. ^{1}H NMR Spectral Data of Compound

No.	Chemical shift (δ/ppm)	No. of protons	Splitting pattern	Coupling constant (*J* values Hz)	Proton assignment
1	0.68	3H	s	-	sp^3 methyl proton
2	0.82	3H	d	6.8	sp^3 methyl proton
3	0.82	3H	d	6.8	sp^3 methyl proton
4	0.84	3H	d	7.5	sp^3 methyl proton
5	0.88	3H	t	7.1	sp^3 methyl proton
6	0.91	1H	m	-	sp^3 methine proton
7	0.92	3H	d	6.6	sp^3 methyl proton
8	0.94	1H	m	-	sp^3 methine proton
9	0.99	3H	s	-	sp^3 methyl proton
10	1.01	1H	m	-	sp^3 methine proton
11	1.02, 1.35	2H	m	-	sp^3 methylene proton
12	1.05, 1.84	2H	m	-	sp^3 methylene proton
13	1.1	1H	m	-	sp^3 methine proton
14	1.14, 1.16	2H	m	-	sp^3 methylene proton
15	1.22, 1.26	2H	m	-	sp^3 methylene proton
16	1.15, 1.17	2H	m	-	sp^3 methylene proton
17	1.26	2H	m	-	sp^3 methylene proton
18	1.26	2H	m	-	sp^3 methylene proton

No.	Chemical shift (δ/ppm)	No. of protons	Splitting pattern	Coupling constant (J values Hz)	Proton assignment
19	1.29	2H	m	-	sp^3 methylene proton
20	1.29	2H	m	-	sp^3 methylene proton
21	1.29	2H	m	-	sp^3 methylene proton
22	1.29	2H	m	-	sp^3 methylene proton
23	1.29	2H	m	-	sp^3 methylene proton
24	1.31, 1.62	2H	m	-	sp^3 methylene proton
25	1.32	2H	m	-	sp^3 methylene proton
26	1.36	1H	m	-	sp^3 methine proton
27	1.45, 1.47	2H	m	-	sp^3 methylene proton
28	1.48	1H	m	-	sp^3 methine proton
29	1.52, 1.99	2H	m	-	sp^3 methine proton
30	1.61, 1.30	2H	m	-	sp^3 methine proton
31	1.63	2H	m	-	sp^3 methylene proton
32	1.68	1H	m	-	sp^3 methine proton
33	2.26 2.33	2H	dd dd	12.12, 7.44 12.12, 7.44	sp^3 methylene proton
34	2.32	2H	m	-	sp^3 methylene proton

No.	Chemical shift (δ/ppm)	No. of protons	Splitting pattern	Coupling constant (*J* values Hz)	Proton assignment
35	3.35	1H	t	8.4	sp^3 methine proton
36	3.38	1H	t	8.4	sp^3 methine proton
37	3.46	1H	m	-	sp^3 methine proton
38	3.52	1H	tt	9.8, 4.5	sp^3 methine proton
39	3.54	1H	t	8.4	sp^3 methine proton
40	4.36	2H	m	-	sp^3 methylene proton
41	4.37	1H	d	8.4	sp^3 methine proton
42	5.35	1H	br.dd	-	sp^2 methine proton
Total number of protons = 77					

HSQC Spectral Data of Compound

HSQC spectrum, Figure (5), represents the proton carbon direct correlation. The chemical shift values of carbons and their relative protons are tabulated in Table (3).

Table 3. ^1H-^{13}C Correlation in HSQC Spectrum of Compound

No.	^{13}C NMR chemical shift (δ/ppm)	^1H NMR chemical shift (δ/ppm)	Type of carbon
1	11.88	0.68	sp^3 methyl carbon
2	11.99	0.84	sp^3 methyl carbon
3	14.02	0.88	sp^3 methyl carbon
4	18.82	0.92	sp^3 methyl carbon
5	19.10	0.82	sp^3 methyl carbon
6	19.35	0.99	sp^3 methyl carbon

No.	^{13}C NMR chemical shift (δ/ppm)	^{1}H NMR chemical shift (δ/ppm)	Type of carbon
7	19.77	0.82	sp^3 methyl carbon
8	21.07	1.45, 1.47	sp^3 methylene carbon
9	22.65	1.32, 1.32	sp^3 methylene carbon
10	23.31	1.22, 1.26	sp^3 methylene carbon
11	24.29	1.63	sp^3 methylene carbon
12	24.97	1.31, 1.62	sp^3 methylene carbon
13	26.16	1.15, 1.17	sp^3 methylene carbon
14	29.35	1.30, 1.61	sp^3 methylene carbon
15	29.66	1.68	sp^3 methine carbon
16	29.67	1.29	sp^3 methylene carbon
17	29.67	1.29	sp^3 methylene carbon
18	29.72	1.29	sp^3 methylene carbon
19	29.72	1.29	sp^3 methylene carbon
20	29.74	1.26	sp^3 methylene carbon
21	29.74	1.26	sp^3 methylene carbon
22	31.91	1.29	sp^3 methylene carbon
23	31.92	1.48	sp^3 methine carbon
24	32.00	1.52, 1.99	sp^3 methylene carbon
25	34.09	2.32	sp^3 methylene carbon
26	34.10	1.02, 1.35	sp^3 methylene carbon
27	36.18	1.36	sp^3 methine carbon
28	36.80	-	sp^3 quaternary carbon
29	37.37	1.05, 1.84	sp^3 methylene carbon
30	38.98	2.26, 2.33	sp^3 methylene carbon

No.	^{13}C NMR chemical shift (δ/ppm)	^{1}H NMR chemical shift (δ/ppm)	Type of carbon
31	39.88	1.14, 1.16	sp^3 methylene carbon
32	42.43	-	sp^3 quaternary carbon
33	46.01	0.94	sp^3 methine carbon
34	50.33	0.91	sp^3 methine carbon
35	56.27	1.1	sp^3 methine carbon
36	56.88	1.01	sp^3 methine carbon
37	63.46	4.36	sp^3 methylene carbon
38	70.41	3.38	sp^3 methine carbon
39	73.63	3.35	sp^3 methine carbon
40	73.94	3.46	sp^3 methine carbon
29	37.37	1.05, 1.84	sp^3 methylene carbon
30	38.98	2.26, 2.33	sp^3 methylene carbon
31	39.88	1.14, 1.16	sp^3 methylene carbon
32	42.43	-	sp^3 quaternary carbon
33	46.01	0.94	sp^3 methine carbon
34	50.33	0.91	sp^3 methine carbon
35	56.27	1.1	sp^3 methine carbon
36	56.88	1.01	sp^3 methine carbon
37	63.46	4.36	sp^3 methylene carbon
38	70.41	3.38	sp^3 methine carbon
39	73.63	3.35	sp^3 methine carbon
40	73.94	3.46	sp^3 methine carbon
41	76.22	3.54	sp^3 methine carbon
42	79.64	3.52	sp^3 methine carbon

No.	^{13}C NMR chemical shift (δ/ppm)	^{1}H NMR chemical shift (δ/ppm)	Type of carbon
43	101.27	4.37	sp^3 methine carbon
44	122.07	5.35	sp^2 methine carbon
45	140.09	-	sp^2 quaternary carbon
46	174.30	-	carbonyl carbon
33	46.01	0.94	sp^3 methine carbon
34	50.33	0.91	sp^3 methine carbon
35	56.27	1.1	sp^3 methine carbon
36	56.88	1.01	sp^3 methine carbon
37	63.46	4.36	sp^3 methylene carbon
38	70.41	3.38	sp^3 methine carbon
39	73.63	3.35	sp^3 methine carbon
40	73.94	3.46	sp^3 methine carbon
41	76.22	3.54	sp^3 methine carbon
42	79.64	3.52	sp^3 methine carbon
43	101.27	4.37	sp^3 methine carbon
44	122.07	5.35	sp^2 methine carbon
45	140.09	-	sp^2 quaternary carbon
46	174.30	-	carbonyl carbon

Confirmation of Molecular Formula of Isolated Compound

FT-IR spectrum and DEPT spectrum show the number and kinds of carbon, proton and oxygen. These results are tabulated in Table (4).

Table 4. The Results Given by DEPT Spectrum and FT-IR Spectrum

Assignment	no. of carbon	no. of proton	no. of oxygen
DEPT Spectrum			
Seven sp^3 methyl carbons	7	21	-
Twenty sp^3 methylene carbons	20	40	-
One sp^3 carbinol methylene carbon (δ 63.46 ppm)	1	3	1
Ten sp^3 methine carbons	10	10	-
Three sp^3 carbinol methine carbons (δ 73.63, 76.22, 70.41 ppm)	3	6	3
Three sp^3 quaternary carbons	3	-	-
One sp^2 quaternary carbon	1	-	-
FT-IR Spectrum			
One ether oxygen	-	-	1
One carbonyl group	1	-	1
Partial molecular formula	C_{46}	H_{80}	O_6

∴ The partial molecular formula = $C_{46}H_{80}O_6$

The partial molecular mass = 728

∴ The remaining molecular mass = 744 – 728 = 16

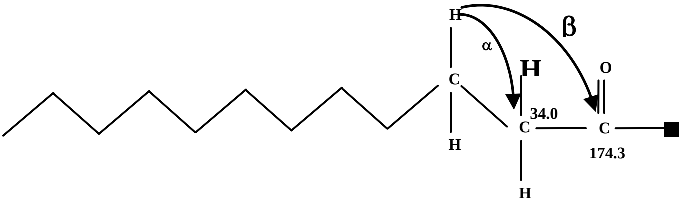
It must be one ether oxygen atom.

∴ Real molecular formula = $C_{46}H_{80}O_7$

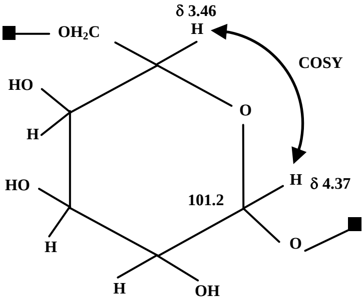
Structure Elucidation of Compound

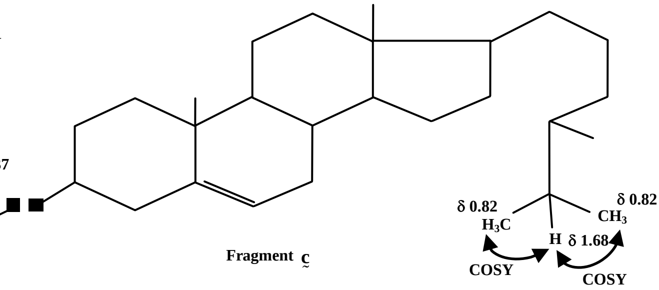
The structure elucidation of pure compound could be determined by ^{1}H NMR, DQF-COSY, HSQC and HMBC spectral data, respectively.

In this structure elucidation, fragment a, b and c could be assigned by DQF-COSY, ^{1}H NMR splitting, coupling constant (*J*-values), HSQC and HMBC spectra.



Fragment a

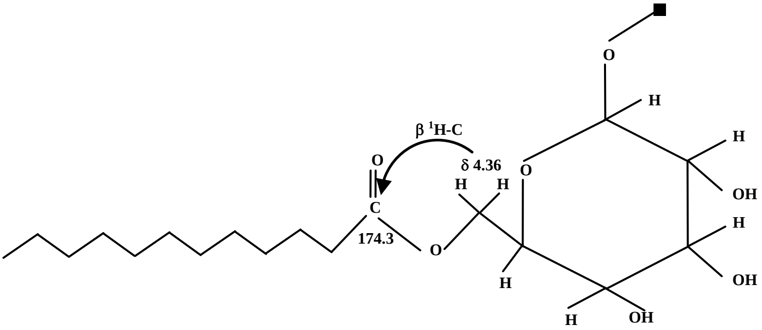




Fragment c

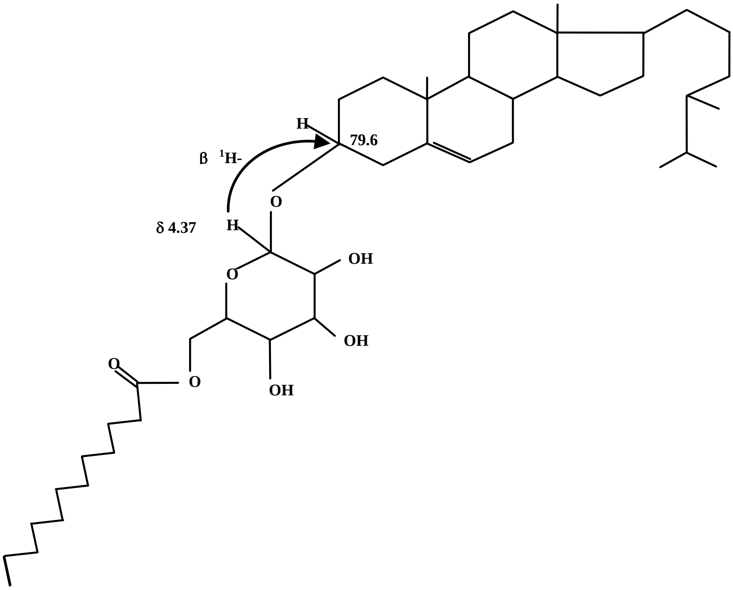
Fragment b

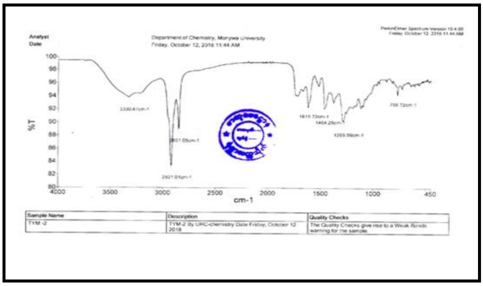
Fragment a and b could be connected by HMBC spectrum gave rise to the fragment d.



Fragment d

Finally, the elucidated partial fragment c and d could be connected by HMBC spectrum in which acetal methine proton (δ 4.37 ppm) has β ^{1}H-C long range coupling with ether bearing carbon (δ 79.64 ppm) which accomplished the complete planar structure of compound.




Analyst
Date

Department of Chemistry, Monywa University
Friday, October 12, 2018 11:44 AM

PerkinElmer Spectrum Version 10.4.00
Friday, October 12, 2018 11:44 AM

Sample Name	Description	Quality Checks
TYM -2	TYM-2 By URC-chemistry Date Friday, October 12 2018	The Quality Checks give rise to a Weak Bands warning for the sample.

Figure 2. FT-IR Spectrum of Isolated Compound

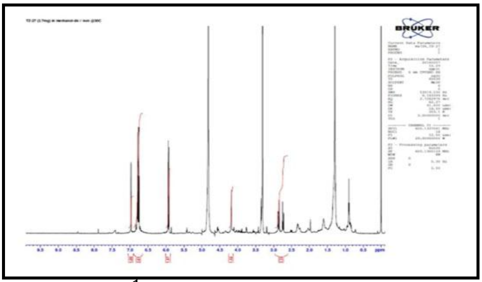


Figure 3. ¹H NMR Spectrum of Isolated Compound

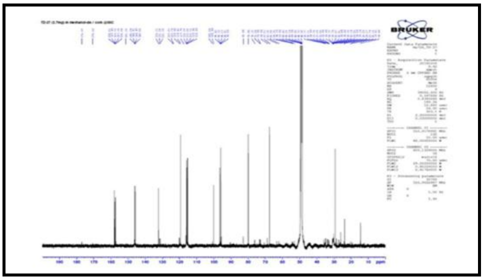


Figure 4. ¹³C NMR Spectrum of Isolated Compound

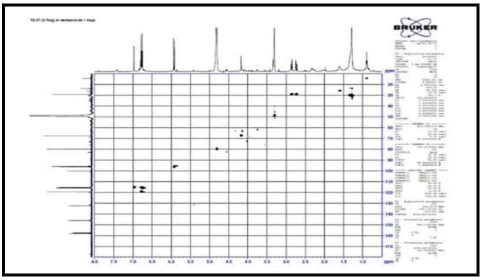


Figure 5. HSQC Spectrum of Isolated Compound

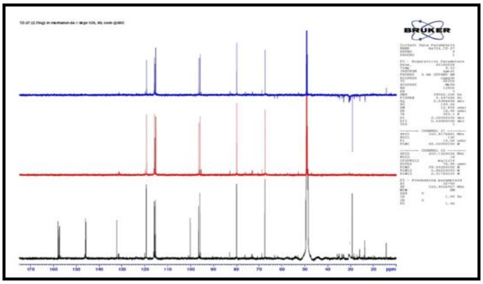


Figure 6. DEPT Spectrum of Isolated Compound

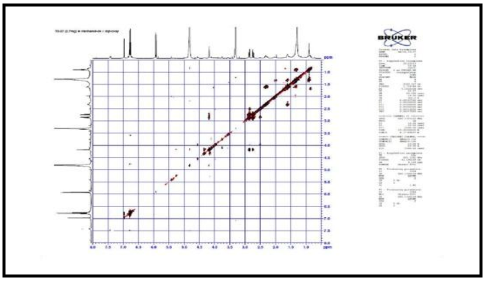


Figure 7. DQF-COSY Spectrum of Isolated Compound

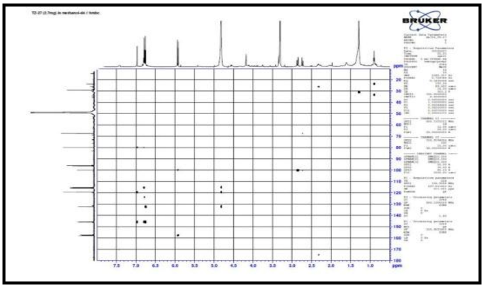


Figure 8. HMBC Spectrum of Isolated Compound

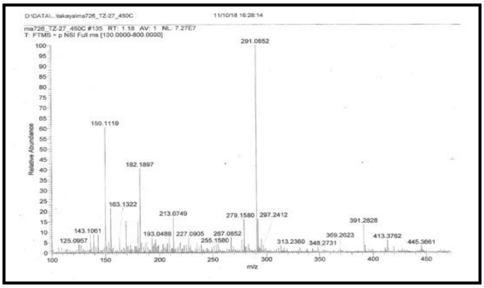
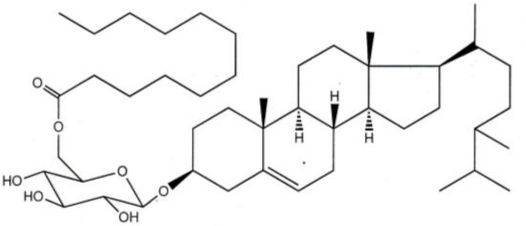


Figure 9. DART-Mass Spectrum of Isolated Compound



The IUPAC name of isolated pure compound is ((2'*R*,3'*S*,4'*S*,5'*R*,6'*R*)-6-(((3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-17-((2*R*)-5,6-dimethylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yl)oxy)-3',4',5'-trihydroxytetrahydro-2*H*-pyran-2-yl)methyl dodecanoate.

Conclusion

In the present investigation, antimicrobial activity was determined from isolated compound which has significant biological activities. Moreover, the fractionated colorless solid crystal compound could be elucidated by using sohicated spectroscopic methods and confirmed by DART-MS spectroscopy. This study supports that basic concepts, uses of different types of spectroscopy and the stem bark of Gati should be used for the treatment of antimicrobial, antifungal, allergy, diarrhea and dysentry. Further studies are required and are in progress here.

Acknowledgements

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References

Bretmaier, E. (2002). *Structure Elucidation by NMR in Organic Chemistry.* A practical Guide, 3rd reverse ed. University of Manchester, West Sussex PO 198s Q, England.

Fransworth, R. N. (2005). *Review on Biological and Phytochemical Screening of Plants*.

Harbonne, J. B. (1984). *Phytochemical methods*: *A Guide to Modern Techniques of Plant Analysis*, 2nd ed, Chapman and Hill Ltd, U.S.A.

Sliverstein, R. M., Webster, F. X. and Kiemle, D. J. (2005), *Spectrometric Identification of Organic Compound*, 7th ed, Singapore, John Wiley & Sons, Inc.

Van Wyk, B. E. and Wink, M., (2004). "*Medicinal Plants of the world*". Selangor Darul Ehsan Alam, Times ed, Singapore, 1-22

Online Materials

1. http://tropical.theferns.info/viewtropical.php
2. http://www.efloras.org/florataxon.aspx?flora

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Isolation, Structure Identification and Activity against Downy Mildew Pathogen *Plasmopara viticola* of Staurosporine Derivatives from Marine *Streptomyces* sp. B 7919

Hnin Yu Win[1] & Hartmut Laatsch[2]

Abstract

In the course of screening for bioactive metabolites from microorganism origin, three staurosporine derivatives, namely, K-252d (1), staurosporine (2), and 7-oxostaurosporine (3) were isolated from the culture broth of marine *Streptomyces* sp. B 7919. Among them, 7-oxostaurosporine (3) exhibited high activity against downy mildew pathogen *Plasmopara viticola*, which had not been reported before. The structures of these metabolites were assigned on the basis of their spectroscopic data.

Keyword: staurosporine, *Streptomyces* sp., *Plasmopara viticola*

Introduction

Streptomycetes are known to produce diverse groups of interesting bioactive secondary metabolites (Blunt, 2009) (Win, 2014). In the course of screening for secondary metabolites from *Streptomyces* sp., three staurosporine derivatives, namely, K-252d (1), staurosporine (2), and 7-oxostaurosporine (3) were isolated from the culture broth of marine *Streptomyces* sp. B 7919. Among them, 7-oxostaurosporine (3) exhibited high activity against downy mildew pathogen *Plasmopara viticola*. This study describes the isolation, structure identification and biological activity of staurosporine derivatives.

Peronosporomycetes cause many devastating diseases in plants, animals, fishes and humans (Islam, 2001 & Dick, 2001). One of the most notorious members of peronosporomycetes is *Plasmopara viticola* which is a serious pathogen of grapevine worldwide. Many fungicides are ineffective against this phytopathogen, and hence, bioactive compounds with new modes of action are needed to combat this economically important pest. Under favorable environmental conditions, the fungus-like stramenopile, *P.*

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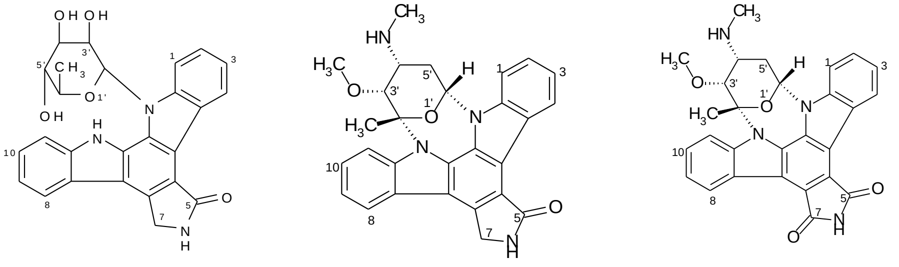
viticola infects grapevine leaves through characteristic biflagellate motile zoospores released from airborne sporangia coming from other infected plants. The zoospores aggregate to stomata of the grapevine leaf by swimming through water film and then rapidly encyst to become round cystospores by shedding their flagella (Kiefer, 2002 & Islam, 2008). The cystospores then rapidly germinate to form germ tubes and penetrate host tissue through the stomata. Disruption of any of these asexual stages eliminates the potential for pathogenesis (Islam, 2005). The success of any zoosporic pathogen can be attributed in part to the speed of asexual differentiation to generate bi-flagellated motile zoospores and their ability to find a host through chemotaxis (Judelson, 2005). Therefore, compounds that can interfere with normal swimming behavior and early development of *P. viticola* are supposed to be important as lead compounds in the management of this notorious phytopathogen (Islam, 2008 & Abdalla, 2011).



1 **2** **3**

Figure 1. Isolated compounds (1-3) from marine Streptomyces sp. B 7919

Materials and Methods

General Experimental Procedures

NMR spectra were measured on a Varian Inova 600 (599.740 MHz) and a Varian Unity 300 (300.145 MHz) spectrometer. ESIMS were measured on a Quattro Triple Quadrupol mass spectrometer with a Finnigan TSQ 7000 with nano-ESI API ion source. EIMS at 70 eV with Varian MAT 731, Varian 311A, AMD-402, high resolution with perflurokerosene as standard. HRESIMS were measured on a Micromass LCT mass spectrometer coupled with a HP 1100 HPLC and a diode array detector. Column chromatography was carried out on MN silica gel 60, 0.05-0.2 mm;

TLC was performed on Polygram SIL G/UV$_{254}$. All silica gel materials were purchased from Macherey-Nagel, Düren, Germany. Size exclusion chromatography was done on Sephadex LH-20 (Lipophilic Sephadex; Amersham Biosciences, Freiburg, Germany, purchased from Sigma-Aldrich Chemie, Steinheim, Germany). XAD-16 adsorber resin was obtained from Rohm and Haas (Frankfurt, Germany) (Win, 2014).

Spray reagents

Anisaldehyde/sulphuric acid: 1 mL anisaldehyde was added to 100 mL of a stock solution containing 85 mL methanol, 14 mL acetic acid and 1 mL sulphuric acid. After spraying, the TLC cards were heated with hot air until colour development.

Biological screening

The crude extract was dissolved in $CHCl_3$/10% MeOH (400 μg/paper disk), in which the paper disks were dipped, dried under sterile conditions (flow box) and put on an agar plates inoculated with *Bacillus subtilis* (ATCC6051), *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57), *Escherichia coli*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus subspicatus*, *Candida albicans* and *Mucor miehei* (Tü 284). The plates were incubated at 37 °C for bacteria (12 hours), 27 °C for fungi (24 hours), and 24-26 °C under day-light for micro-algae (96 hours). The diameter of the inhibition zones was measured by ruler (Win, 2014).

Taxonomy

The marine *Streptomyces* sp. B 7919 was isolated and taxonomically identified by E. Helmke from the Alfred-Wegener Institute for Polar and Marine Research in Bremerhaven, Germany. The strain was deposited in the culture collection at the Institute of Organic and Biomolecular Chemistry, Göttingen, Germany (Win, 2014).

Fermentation and working up

The marine *Streptomyces* sp. B 7919 subculture was used to inoculate a 25 L shaker culture using M_2^+ medium (with sea water) (pH 7.8 before sterilisation). The fermentor broth was harvested after 7 days and the resulting brown culture broth was filtered over Celite using a filter press. The filtrate and mycelia were separately extracted by ethyl acetate (water phase) and acetone (biomass). The combined extracts were evaporated to dryness under vacuum, to give a brown crude extract (2.3 g). Chemical screening by TLC showed blue and yellow fluorescent UV absorbing bands, which changed to brown by spraying with anisaldehyde/sulphuric acid solution.

Isolation

Separation was performed by a silica gel column chromatography (CH_2Cl_2/MeOH gradient, 1.0 L CH_2Cl_2, 0.5 L CH_2Cl_2/2% MeOH, 1.0 L CH_2Cl_2/3% MeOH, 1.0 L CH_2Cl_2/5% MeOH, 0.5 L CH_2Cl_2/10% MeOH, 0.5 L CH_2Cl_2/20% MeOH, 0.5 L CH_2Cl_2/50% MeOH, 0.5 L MeOH). Three fractions were selected based on the spot pattern on TLC.

Fraction II was subjected to a Sephadex LH-20 column with CH_2Cl_2/MeOH (6:4) to afford K-252d (1) (4.5 mg) and two subfractions II-1, II-2. Subfraction II-1 showed blue and yellow fluorescent zones under UV at 254 nm and was subjected to preparative thin layer chromatography with CH_2Cl_2/MeOH (95:5) for 3 times. Staurosporine (2) (33.6 mg) and 7-oxystaurosporine (3) (1.8 mg) were isolated from this fraction.

Results and Discussion

The marine strain *Streptomyces* sp. B 7919 formed white mycelial colonies after incubation on M_2^+ agar medium for 3 days at 28 °C. The antimicrobial assay of this strain showed high activity against the microalgae *Chlorella vulgaris, Chlorella sorokiniana* and *Scenedesmus subspicatus*. It was also highly activity against the fungus *Mucor miehei* (Tü 57) and showed moderate activity against *Staphylococcus aureus* and *Streptomyces Viridochromogenes* (Tü 57).

Table 1. Antimicrobial activity of the crude extract from strain B 7919 on M_2^+ medium [40 μL/paper disk (100 mg/mL)], diameter of inhibition zones in mm.

Test microorganisms	**Inhibition zone Ø [mm]**
Staphylococcus aureus	12
Streptomyces viridochromogenes (Tü 57)	11
Mucor miehei (Tü 284)	23

Test microorganisms	**Inhibition zone Ø [mm]**
Chlorella vulgaris	40
Chlorella sorokiniana	40
Scenedesmus subspicatus	40

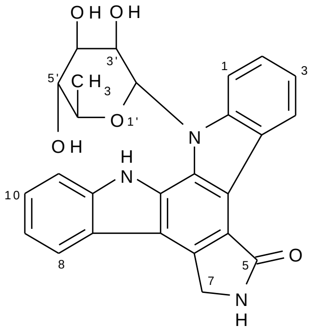
Structure Elucidation of Isolated Compounds (1-3)

K-252d (1)

The Sephadex LH-20 purification of fraction II delivered K-252d (1), which showed a strong blue UV fluorescence and gave brown colour with anisaldehyde/sulphuric acid. Amongst others, this is characteristic for staurosporine derivatives.

The ESI mass spectrum revealed a *pseudo*molecular ion (base peak) at *m/z* 456 $[M - H]^-$ and the high-resolution ESI mass spectrum suggested a molecular formula of $C_{26}H_{23}N_3O_5$. In the aliphatic region, the ^1H NMR spectrum showed a methyl doublet at δ□1.80 and four oxygenated methine signals between δ 4.15 and δ 4.70. This suggested that it contained a sugar moiety. This was confirmed by a doublet at δ 6.50 for the anomeric proton. The spectrum showed the doublet of a methylene group at δ 4.98, which could be connected both with a heteroatom and an sp^2 carbon because of the downfield shift. In the aromatic region, the ^1H NMR spectrum showed four 1H doublets at δ□9.35, 7.98, 7.70, 7.60 and two overlapped triplets at δ□7.40 and 7.25 which revealed the presence of two 1,2-disubstituted benzene rings linked to heteroatoms. (O, N or S).

A search in AntiBase (Laatsch, 2008) with these data gave K-252d (1) as a result. It was further confirmed by comparing with the authentic spectra as well as the literature data (Nakanishi, 1986).



(1)

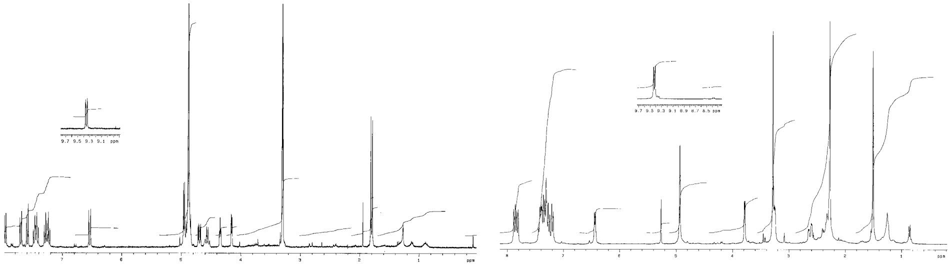


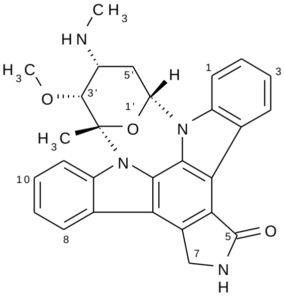
Figure 2. ^{1}H NMR spectrum (CD$_3$OD, 300 MHz) of K-252d (1) and staurosporine (2)

Staurosporine (2)

The PTLC purification of the subfraction II-1 gave staurosporine (2) and 7-oxostaurosporine (3). The former one showed strong blue fluorescence and UV absorption at 254 nm and stained to blue by spraying with anisaldehyde/sulphuric acid. In the aromatic region, the ^{1}H NMR spectrum of staurosporine (2) was similar to that of K-252d (1), however, there was no sugar moiety as in the latter. The spectrum indicated the presence of two indole nuclei with eight proton signals in the aromatic region; doublets at δ 9.44 for H-4, δ 7.88 for H-11 and δ 7.81 for H-8 protons and multiplets between δ 7.20 and 7.50 with the integration of 5 protons as for H-1, H-2, H-10, H-9 and H-3, respectively. Additionally, the spectrum revealed one doublet at δ 6.45 for the anomeric proton H-6', and one methylene singlet at δ 4.98 as in K-252d. In the aliphatic region, two heteroatoms bearing methine proton signals at δ 3.79 (d, H-3'), δ 3.32 (dd, H-4'), an ABX system of methylene (CH$_2$-5') at δ 2.71 (dd) and 2.40 (m), two methyl singlets at δ 1.54 and δ 2.33 and one methoxy group at δ 3.26 were observed.

The ESI mass spectrum showed a *pseudo*molecular ion peak (base peak) at *m/z* 467 $[M + H]^+$ and the high-resolution ESI mass spectrum gave the molecular formula as $C_{28}H_{26}N_4O_3$.

With a search in AntiBase using the above spectroscopic data as well as the molecular weight gave staurosporine (2). It was further confirmed by comparing with authentic spectra as well as the literature data.

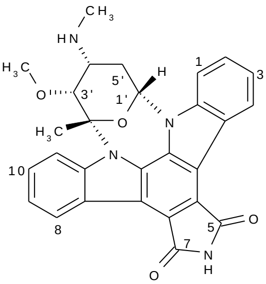


(2)

7-Oxostaurosporine

Comparison of the spectroscopic data of compound (3) with (2) showed similarity in both the aliphatic and aromatic regions except that there was no singlet at δ 4.98 for CH_2-7 in compound (3). The ESI mass spectrum showed a *pseudo*molecular ion peak (base peak) at *m/z* 481 $[M + H]^+$ and the high-resolution mass spectrum gave $C_{28}H_{24}N_4O_4$.

A search in AntiBase using the above spectroscopic data as well as the molecular weight gave 7-oxostaurosporine (3). It was further confirmed by comparing with the authentic spectra as well as literature data (Koshino, 1992).



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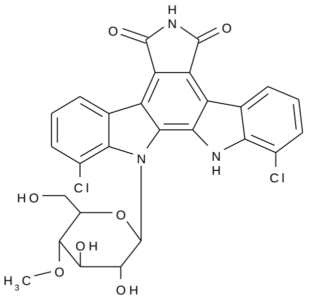

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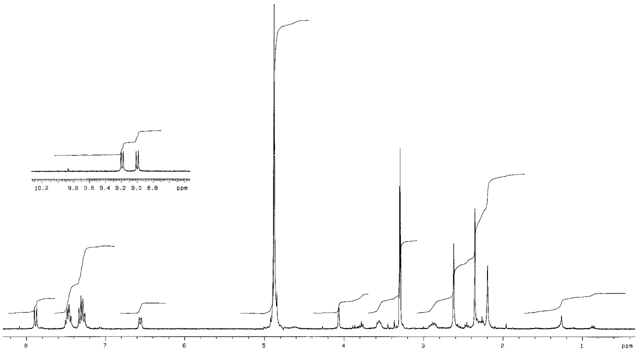


Figure 3. ^{1}H NMR spectrum (CD_3OD, 300 MHz) of 7-xostaurosporine (3)

Staurosporine is an indolo[2,3,α]carbazole alkaloid and was originally isolated in 1977 from bacterium *Streptomyces staurosporeus* Awaya (AM-2282) (Omura, 1977) and subsequently from many other actinomycetes (Oka, 1986). It was the first of over 50 alkaloids with a bis-indole structure. Staurosporine has been reported to have biological activities ranging from anit-fungal to anti-hypertensive (Rüegg, 1989). Staurosporine and its derivatives inhibited mainly PKC (protein kinase C) *in vitro* at an extremely low concentration (Koshino, 1992). The activity is achieved through the prevention of ATP binding to the kinase. Moreover, staurosporine inhibited the platelet aggregation and showed activity against a number of experimental tumors (Tamaoki, 1986) *in vitro* but no effects on bacteria. The aglycone moiety plays an important role for biological activity.

The indolocarbazole chromophore of 7-oxostaurosporine (3) is similar to the aglycone of the antitumor antibiotic rebeccamycin (4) (Nettleton, 1985). According to Meksuriyen *et al.*, staurosporine is most probably constructed from a tryptophan-derived aromatic unit and an amino sugar moiety, which are connected stereospecifically by an unusual double N-glycosidic linkage (Meksuriyen, 1988).

Staurosporine derivatives showed high activity against downy mildew pathogen *Plasmopara viticola*, which had not been reported before. Motility halting and zoosporicidal activity of 7-oxostaurosporine (3) against downy mildew pathogen *Plasmopara viticola* was shown in Figure 4.

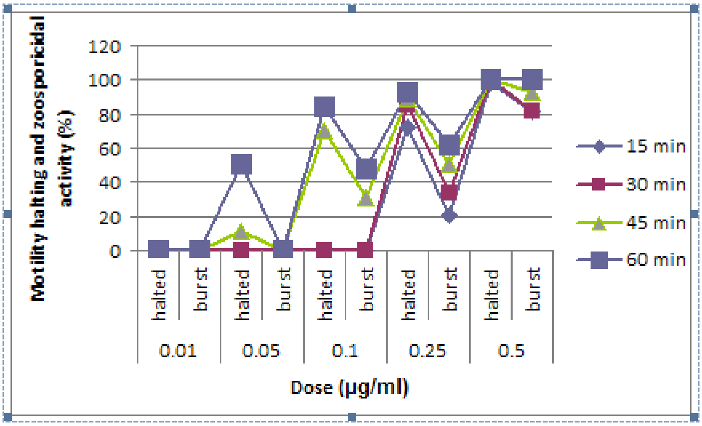
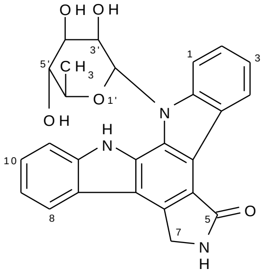
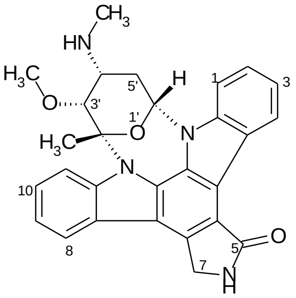


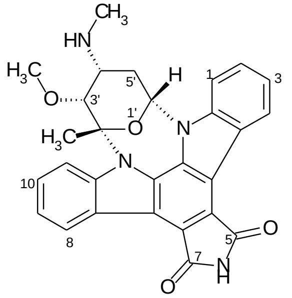
Figure 4. Motility halting and zoosporicidal activity of 7-oxostaurosporine (3) against downy mildew pathogen *Plasmopara viticola*



K-252d (1): brownish solid, 4.5 mg, R_f = 0.4 (CH_2Cl_2/5% MeOH), strong blue UV fluorescent, brown by anisaldehyde/sulphuric acid. – **^{1}H NMR** (CD_3OD, 300 MHz) δ 9.36 (d, ^3J = 7.7 Hz, 1H, H-4), 7.97 (d, ^3J = 7.8 Hz, 1H, H-8), 7.71 (d, ^3J = 8.3 Hz, 1H, H-1), 7.59 (d, ^3J = 7.7 Hz, 1H, H-11), 7.46 (m, 2H, H-2, 10), 7.27 (m, 2H, H-3, 9), 6.54 (d, ^3J = 9.5 Hz, 1H, H-1'), 4.96 (d, ^3J = 5.4 Hz, 2H, CH_2-7), 4.70 (dd, ^3J = 9.6, 3.8 Hz, 1H, H-3'), 4.56 (br q, ^3J = 7.6 Hz, 1H, H-5'), 4.35 (m, 1H, H-2'), 4.15 (br t, ^3J = 3.2 Hz, 1H, H-4'), 1.79 (d, ^3J = 7.3 Hz, 3H, CH_3-6'). – **(+)-ESIMS** *m/z* 937 ([2 M + Na]$^+$, 100), 480 ([M + Na]$^+$, 30). – **(-)-ESIMS** *m/z* 913 ([2 M - H]$^-$, 20), 456 ([M - H]$^-$, 100). – **(+)-HRESIMS** *m/z* 458.17105 [M + H]$^+$, (calcd. 458.17104 for $C_{26}H_{24}N_3O_5$).



Staurosporine (2): brownish solid, 33.6 mg, R_f = 0.23 (CH_2Cl_2/5% MeOH), strong blue fluorescence UV absorbing band at 254 nm, brown colour by anisaldehyde/sulphuric acid. – **^{1}H NMR** ($CDCl_3$, 300 MHz) δ 9.44 (d, ^3J = 7.7 Hz, 1H, H-4), 7.88 (d, ^3J = 8.4 Hz, 1H, H-11), 7.81 (d, ^3J = 7.7 Hz, 1H, H-8), 7.50-7.20 (m, 5H), 6.45 (d, ^3J = 5.1 Hz, 1H, H-6'), 4.94 (d, ^3J = 15.5 Hz, 2H, CH_2-7), 3.79 (d, ^3J = 3.3 Hz, 1H, H-3'), 3.32 (ddd, ^3J = 3.3 Hz, 3.3 Hz, 3.1 Hz, 1H, H-4'), 3.26 (s, 3H, OCH_3-3'), 2.74 (ddd, ^3J = 14.7, 5.3, 3.6 Hz, 1H, H_A-5’), 2.40 (m, 1H, H_B-5'), 2.33 (s, 3H, CH_3-2'), 1.54 (s, 3H, NCH_3-4'). – **(+)-ESIMS** *m/z* 933 ($[2 M + H]^+$, 100), 467 ($[M + H]^+$, 67). – **(+)-HRESIMS** *m/z* 467.20779 $[M + H]^+$, (calcd. 467.20777 for $C_{28}H_{27}N_4O_3$).



7-Oxystaurosporine (3): yellow solid, 1.8 mg, R_f = 0.33 (CH_2Cl_2/5% MeOH), strong blue UV fluorescent (366 nm) or absorbing (254 nm) band, brown colour by anisaldehyde/sulphuric acid. – **^{1}H NMR** (CD_3OD, 300 MHz) δ 9.20 (d, ^3J = 7.3 Hz, 1H, H-8), 9.00 (d, ^3J = 7.9 Hz, 1H, H-4), 7.89 (d, ^3J = 8.5 Hz, 1H, H-11), 7.47 (m, 2H, H-2,10), 7.31 (m, 3H, H-1,3,9), 6.53 (d, ^3J = 5.5 Hz, 1H, H-6'), 4.06 (d, ^3J = 3.7 Hz, 1H, H-3'), 3.51 (ddd, ^3J = 3.7 Hz, 3.7 Hz, 2.5 Hz, 1H, H-4'), 3.26 (s, 3H, OCH_3-3'), 2.91 (br dd, ^3J = 14.7 Hz, 2.5 Hz, 1H, H_A-5'), 2.60 (s, 3H, CH_3-2'), 2.32 (s, 3H, NCH_3-4'), 2.30 (m, 1H, H_B-5'). – **(+)-ESIMS** *m/z* 983 ($[2 M + Na]^+$, 100), 961 ($[2 M + H]^+$, 83), 481 ($[M + H]^+$, 60). – **(+)-HRESIMS** *m/z* 481.18699 $[M + H]^+$, (calcd. 481.18703 for $C_{28}H_{25}N_4O_4$).

Acknowledgements

The authors thank the Deutsche Akademische Austauschdienst (DAAD), Bonn, for a research fellowship of re-invitation programme for former scholarship holders. The authors thank Md. Tofazzal Islam, Department of Crop Sciences, Division of Plant Pathology and Plant Protection, Georg-August-University Göttingen, Göttingen, Germany for biological activity test against *P. viticola*. HYW thanks Dr Yi Yi Myint, Head of Department of Chemistry, and University of Mandalay for her kind permission to submit this paper.

References

Abdalla, M. A., Win, H. Y., Islam, M. T., Tiedemann, A. v., Schüffler, A. and Laatsch, H. (2011) "Khatmiamycin, a motility inhibitor and zoosporicide against the grapevine downy mildew pathogen *Plasmopara viticola* from *Streptomyces* sp. ANK313" *J. Antibiot*. **vol. 64**, pp. 655-659.

Blunt, J. W., Copp, B. R., Hu, W. P., Munro, M. H., Northcote, P. T., and Prinsep, M. R. (2009) "Marine natural products." *Nat. Prod. Rep*. **vol. 26**, pp. 170-244.

Dick, M. W. (2001) "The Peronosporomycetes." In *The Mycota VII, Part A, Systematics and Evolution* (eds McLaughlin, D. J., McLaughlin, E. G. & Lemke, P. A.) 39–72 (Springer-Verlag, Berlin, Heidelberg).

Islam, Md. T., and Tahara, S. (2001) "Chemotaxis of fungal zoospores, with special reference to Aphanomyces cochlioides." *Biosci. Biotechnol. Biochem*. **vol. 65**, pp. 1933-1948.

Islam, M. T. and Tiedemann, A. V. (2008) "Zoosporogenesis and differentiation of grapevine downy mildew pathogen Plasmopara viticola in host-free system." *Phytopathology*. **vol. 98**, S72.

Islam, M. T., Hashidoko, Y., Deora, A., Ito, T. and Tahara, S. (2005) "Suppression of damping-off disease in host plants by the rhizoplane bacterium Lysobacter sp. strain SB-K88 is linked to plant colonization and antibiosis against soilborne Peronosporomycetes." *Appl. Environ. Microb.* **vol. 71**, pp. 3786–3796.

Judelson, H. S. and Blanco, F. A. (2005) "The spores of Phytophthora: weapons of plant destroyer." *Nat. Rev. Microbiol*. **vol. 3**, pp. 47–58.

Kiefer, B., Riemann, M., Kassemeyer, C. B. H. and Nick, P. (2002) "The host guides morphogenesis and stomatal targeting in the grapevine pathogen *Plasmopara viticola*." *Planta Med.* **vol. 215**, pp. 387–393.

Koshino, H., Osada, H. and Isono, K. (1992) "A new inhibitor of protein kinase C, RK-1409 (7-oxostaurosporine)." *J. Antibiot*. **vol. 45**, pp. 195-198.

H. Laatsch, *AntiBase 2008*, A Data Base for Rapid Structural Determination of Microbial Natural Products, **2009** and annual updates, Chemical Concepts, Weinheim, Germany; see http://wwwuser. gwdg. de/~ucoc/laatsch/AntiBase. htm.

Meksuriyen, D. and Cordell, G. A. (1988) "Biosynthesis of Staurosporine, 2. Incorporation of tryptophan." *J. Nat. Prod*. **vol. 51**, pp. 893-899.

Nakanishi, S., Matsuda,Y., Iwahashi, K. and Kase, H. (1986) "K-252b, c and d, potent inhibitors of protein kinsase C from microbial orgin." *J. Antibiot*. **vol. 39**, pp. 1066-1071.

Nettleton, D. E., Doyle, T. W. and Krishnan, B. (1985) "Isolation and structure of rebeccamycin-a new antitumor antibiotic from nocardia aerocoligenes." *Tetrahedron Lett.* **vol. 26**, pp. 4011-4014.

Omura, S., Iwai, Y., Hirano, A., Nakagawa, A., Awaya, J., Tsuchiya, H., Takahashi, Y. and Masuma, R. (1977) *J. Antibiot*. **vol. 30**, pp. 275.

Oka, S., Kodama, M., Takeda, H., Tomizuka, N. and Suzuki, H. (1986) "Staurosporine, a potent platelet aggregation inhibitor from a *Streptomyces* species." *Agric*. *Biol*. *Chem*. **vol. 50**, pp. 2723-2727.

Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. and Tomita, F. (1986) "Staurosporine, a potent inhibitor of phospholipid/Ca^{++}depedent protein kinase." *Biochem*. *Biophys*. *Res*. *Commun*. **vol. 397**, p. 135.

Rüegg, U. T. and Burgess, G. M. (1989) "Staurosporine, K-252 and UCN-01: potent but nonspecific inhibitors of protein kinase." *Trends Pharmacol. Sci.* **vol. 29**, pp. 253-257.

Win, H. Y. (2014) "Metabolites produced by marine and terrestrial bacteria Streptomycetes." *Jour. Myan. Acad. Arts & Sc.* **vol. XII**. No. 1. pp. 385-405.

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Structure Elucidation of an Unknown Bioactive Sterol Compound Isolated from Root Bark of *Vallaris solanacea* (Roth) O.Ktze

Tin Tin Moe[1] , Thinn Myat Nwe[2] & Myint Myint Sein[3]

Abstract

In this research work, the roots bark of *Vallaris solanacea* (Roth) O.Ktze was collected from Taung-thar Township, Mandalay Region, for chemical analysis.The phytochemical constituent of sample was determined by usual method. The antibacterial activities of six extracts such as n-hexane, chloroform, benzene, acetone, ethyl acetate and ethanol of crude sample were tested by Agar-well diffusion method on three selected organisms. In addition, bioactive compound (TTM-1) could be isolated from the root bark of sample by using Thin layer and Column chromatographic methods. The antimicrobial activity of pure compound was determined by Agar well diffusion on six selected microorganisms. The molecular formula (C_{25} H_{38} O_4) of unknown compound (TTM-1) could be determined by FT-IR, ^{1}H NMR ,^{13}C NMR, HSQC, DEPT and EI mass spectral data respectively. The planar structure of this compound could be elucidated by using COSY and HMBC spectrum. Finally, the conformational analysis of rings (A, B, C and D) could be assigned by ^{1}H NMR splitting pattern, coupling constant and NOESY spectral data, respectively.

Keywords *Vallaris solanacea,* phytochemical, antimicrobial, chromatographic

Introduction

In every country, many plants are used in traditional medicine. Plants continue to be a major source of medicines, as they have been throughout human history. The study of traditional indigenous medicinal plants and their usage in therapy play a very important role in Myanmar. The growing interest in natural products from plants in relation to the search for new

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active principle and precursors in biosynthesis has promoted considerable work in the chemical screening of plants in the last decade.

Myanmar traditional practitioners use a variety of medicine mostly containing potent medicinal plants available in Myanmar, for the cure of various diseases. For example, the roots bark of Khin-bok usually used for the treatment of constipation, and carbuncle. Infusion of the herb is used for the treatment of malaria. Seeds are also used as anathematic and conjunctivitis.(Wbsite 1 and 2)

In this research work, the root bark of Khin-bok was selected for phytochemical tests, antibacterial activities and as a new source of compound in this field.

Botanical Description

Family name: Apocyanceae

Scientific name: *Vallaris solanacea* (Roth) O.Ktze

Myanmar name: Khin-bok

Figure 1. The Plant of *Vallaris solanacea* (Roth) O.Ktze

Metarial and Method

Instrumentation and Materials

The occurrence of UV absorption on TLC plate was checked by UV detector and iodine vapor. Analytical and preparative thin-layer chromatography was performed by using precoated silica gel plates. Silica gel (Merck-Co, Inc., Kiesel gel 60, 70-230 Mesh ACTM) was used for column chromatography.

Sampling

The sample of *Vallaris solanacea* (Roth) O.Ktze for chemical analysis was collected from Taung-thar Township, Mandalay Region.

Preliminary Phytochemical Test

Preliminary phytochemical test of sample was carried out by usual method. The results are shown in Table (1)

Determination of pure compound (TTM-1)

The FT-IR spectrum of the isolated compound (TTM-1) was measured at the Department of Chemistry, University of Mandalay. The ^{1}HNMR,^{13}CNMR, HSQC, COSY, HMBC, DEPT and NOESY spectrum of this compound were measured at Faculty of pharmacy, Meijo University, Nagoya, Japan.

Antibacterial Activities

Antibacterial activities of six solvents extract of the root bark of Khin-bok were tested in various solvent systems by using Agar well diffusion method on six selected organisms in CRDT (Central Research Development and Technology), Insein, and Yangon as shown in Figure (2). The results are shown in Table (2).

Extraction and isolation

The sample 300 g was percolated with 95 % ethanol 800 ml for about two months and then filtered and the filtrate was concentrated. The residue was re-extracted with 250 ml of ethyl acetate. The EtOAc extract (3.27g) was separated by column chromatography using silica gel and eluent as n-hexane and ethyl acetate. The obtained crystal was recrystallized by using n- hexane and ethyl acetate (4:1,v/v). The pure colorless needle shape crystal (52.3 mg) was obtained.

Antimicrobial Activities of compound (TTM-1)

The antimicrobial activities of pure compound (TTM-1) were tested by agar well diffusion method with six microorganisms. The results are shown in Table (3).

Determination of Melting Point

The melting point of isolated pure compound (TTM-1) was measured by using liquid paraffin and thermometer.

Results and Discussion

The results of phytochemical constituent of the root bark of *Vallaris solanacea* (Roth) O.Ktze are shown in Table (1).

According to this table, *Vallaris solanacea* (Roth) O.Ktze extract consist of alkaloids, steroids, reducing sugars, saponins, polyphenols and glycosides respectively.

Table 1. Results of Preliminary Phytochemical Test of Sample

No.	**Constituents**	**Reagent used**	**Observation**	**Result**
1.	Alkaloid	(1)Mayer's reagent	Cream ppt	+
		(2)Dragendorff's reagent	Orange ppt	+
2.	Flavonoid	conc: HCl, Mg turning, Δ	No color change	–
3.	Terpene	Pet-ether,acetic anhydride,$CHCl_3$, conc: H_2SO_4	No Red color solution	–
4.	Steroids	Aceticanhydride,$CHCl_3$, conc: H_2SO_4	Green color solution	+
5.	Reducing sugar	Benedict solution	Brick-red ppt	+
6.	Saponin	Distilled water	Frothing	+
7.	Polyphenol	1 % $FeCl_3$, 1 % $K_3[Fe(CN)_6]$	Greenish blue color solution	+
8.	Glycoside	10 % lead acetate	White ppt	+

(+) = presence of constituents (–) = absence of constituents

The results of antibacterial activities of the roots bark of *Vallaris solanacea* (Roth) O.Ktze are shown in Table 2.

Table 2. Antibacterial Activities of *Vallaris solanacea* (Roth) O.Ktze

Sample organisms		**Inhibition Zone**					
		n-hexane	$CHCl_3$	benzene	acetone	EtOAc	EtOH
Khin-bok	I	–	15mm (++)	13mm (+)	16mm (++)	37mm (+++)	15mm (++)
	II	13mm (+)	15mm (++)	12mm (+)	18mm (++)	36mm (+++)	20 mm (+++)
	III	–	15mm (++)	13mm (+)	16mm (++)	33mm (+++)	17 mm (++)

Agar well – 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

Organisms
I= *Bacillus subtilis*
II= *Staphylococcus aureus*
III=*Pseudomonas aeruginosa*

According to this table, n-hexane extract of sample responds low activities on *Staphylococcus aureus.* Chloroform and acetone extract of the sample respond medium activities on all tested organisms. Benzene extrac of sample gives low activities on all tested organisms. Ethyl acetate extract of sample responds high activities on all tested organisms. Ethanol extract of sample gives high activities on *Staphylococcus aureus* and medium activities on *Bacillus subtilis, Pseudomonas aeruginosa*.

Table 3. Antimicrobial Activities of Compound (TTM-1)

Compound (TTM-1)	**Inhibition zone**					
	I	**II**	**III**	**IV**	**V**	**VI**
	15mm (++)	15mm (++)	14mm (+)	14mm (+)	17mm (++)	15mm (++)

Agar well – 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

Organisms
I= *Bacillus subtilis*
II= *Staphylococcus aureus*
III=*Pseudomonas aeruginosa*
IV=*Bacillus pumilus*
V=*Candida albican*

VI=*Mycobacterium* species

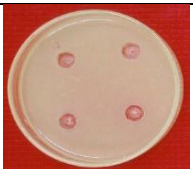
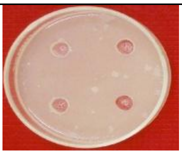
		
Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
		
Bacillus pumilus	*Candida albicans*	*E. coli*

Figure 2. Antimicrobial activities of compound (TTM-1)

The result of melting point of pure compound (TTM-1) was found to be 205-206 °C.

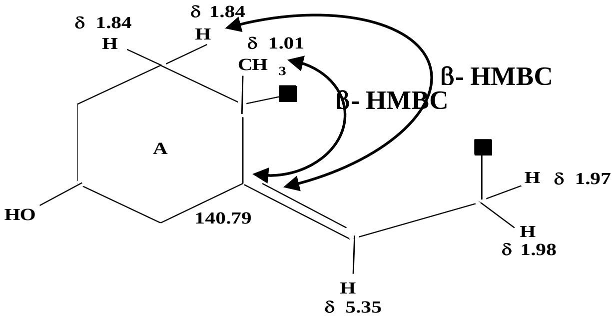
Molecular formula determination of compound (TTM-1)

According to ^{1}HNMR(Figure 4) and ^{13}CNMR(Figure 5) spectral data, the number of protons 35 and the number of carbons 25 are present in this compound. From FT-IR spectrum(Figure 3) , the appears of the band at 3425 cm^{-1} is an evidence for the existence of –OH group and ether group could be observed at 1049 cm^{-1}.Hence, this compound should consist of at least one –OH group and one ether group. Therefore, the partial molecular formula could be assigned as $C_{25}H_{36}O_2$. Therefore, partial molecular mass is 368.From EI mass spectrum (Figure 11), the molecular ion peak; m/z 402 indicates its molecular mass. The remaining partial molecular mass is 34. Hence, the remaining molecular mass 34 must be two-OH groups. Therefore, the real molecular formula of this compound could be assigned as $C_{25}H_{38}O_4$. Hydrogen deficiency index is 7.

Structure Elucidation of Unknown Compound (TTM-1)

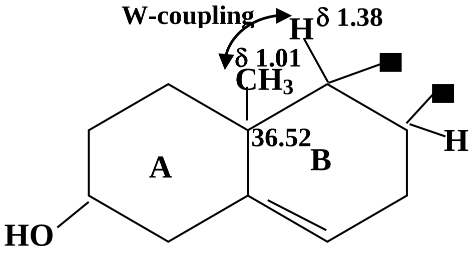
The structure elucidation of unknown compound (TTM-1) could be determined by using HSQC (Figure 7), COSY (Figure 8) and HMBC (Figure 9) spectral data.

In HMBC spectrum, both of these methyl protons (δ 1.01ppm) and methylene protons (δ 1.84ppm) have β proton carbon long range coupling with the sp^2 quaternary carbon (δ 140.79ppm). This evidence gives rise to the following fragment (a) containing ring (A).



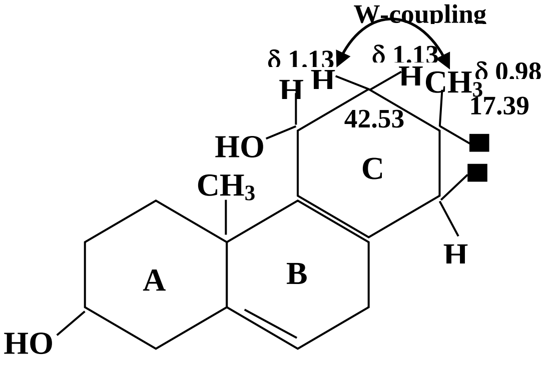
Fragment a

In COSY spectrum, the observation of W-coupling between the methane proton (δ 1.38ppm) and the methyl protons (δ 1.01ppm) with small graphic area produces the following fragement (b) containing ring (B).



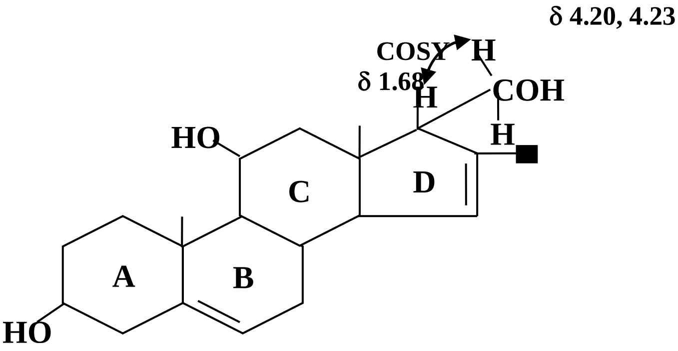
Fragment b

In COSY spectrum, the existence of W-coupling between the methylene protons(δ 1.13ppm) and the methyl protons(δ 0.98ppm) with small graphic area, produces the following fragment(c) containing ring(C).



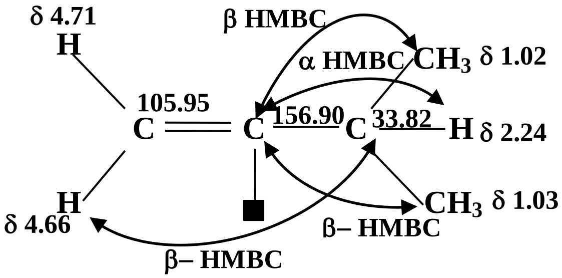
Fragment c

In COSY spectrum, there is observed medium graphic area of the methane proton(δ 1.68ppm) with carbinol methylene protons (δ 4.20,4.23ppm), leading to the following fragment(d).



Fragment d

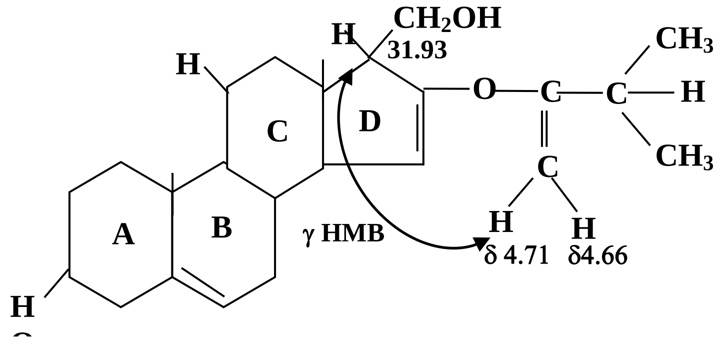
The occurrence of β-proton carbon long range coupling between the exomethylene protons(δ 4.71 and 4.66 ppm) and sp^3 methine carbon (δ 33.82 ppm), reveals the following fragment (e).



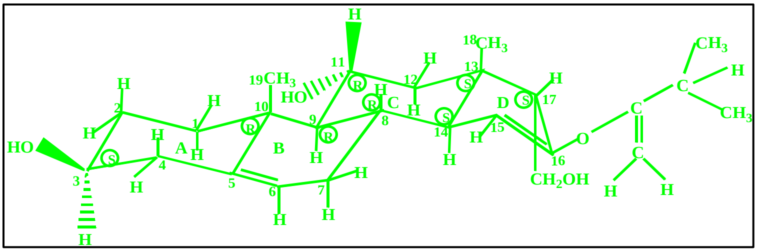
Fragment e

The downfield chemical shift of sp^2 carbon δ145.87 ppm and another sp^2 carbon δ 156.90 ppm should be attached to the one oxygen atom. Hence, the -O- must be a junction group between the fragement (d) and the fragement(e).Thus,the planar structure of an unknown compound was assigned,

The existence of the planar structure of compound (TTM-1) was confirmed by HMBC spectrum. In HMBC spectrum, the two exomethylene protons (δ 4.71 and 4.66 ppm) have γ-proton carbon long range coupling with sp^3 methine carbon (δ 31.93 ppm), confirming the following planar structure of compound (TTM-1).



The planar structure could be confirmed by conformational analysis.



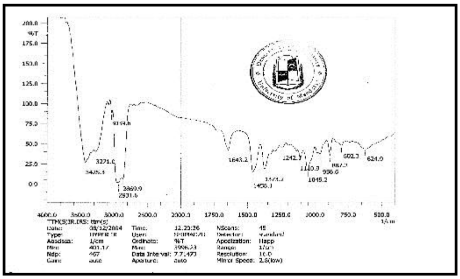


Figure 3. FT-IR spectrum of compound (TTM-1)

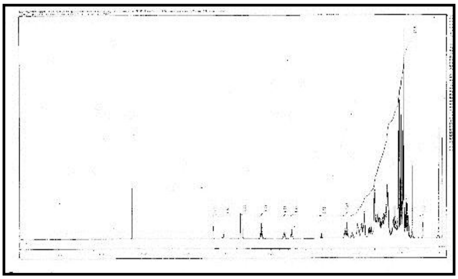


Figure 4. ^{1}HNMR spectrum of compound (TTM-1)

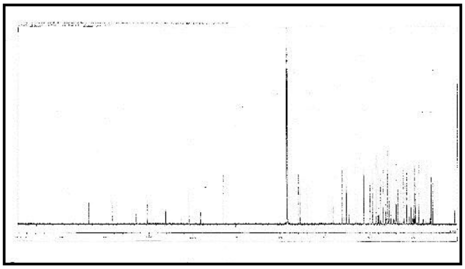


Figure 5. ^{13}CNMR spectrum of compound (TTM-1)

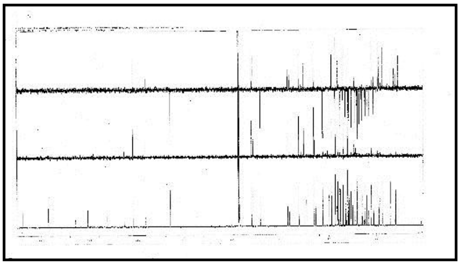


Figure 6. DEPT spectrum of compound (TTM-1)

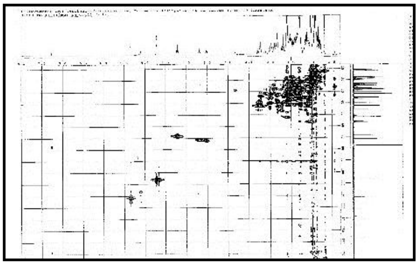


Figure 7. HSQC spectrum of compound (TTM-1)

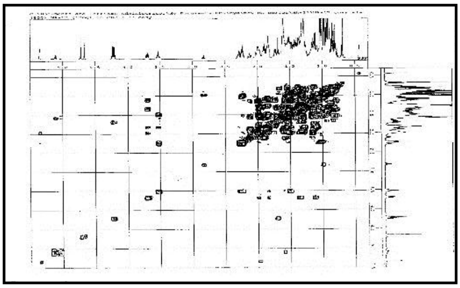


Figure 8. COSY spectrum of compound (TTM-1)

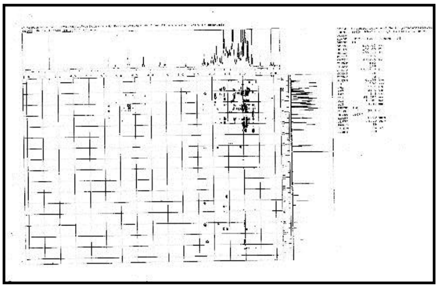


Figure 9. HMBC spectrum of compound (TTM-1)

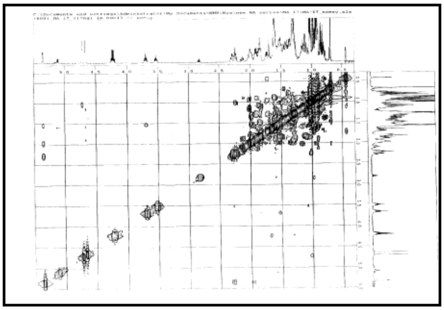


Figure 10. NOESY spectrum of compound (TTM-1)

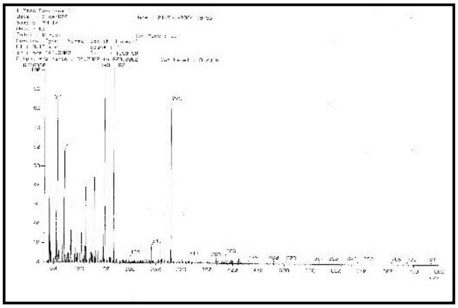


Figure 11. EI mass spectrum of compound (TTM-1)

Conclusion

In this research work, an unknown colorless sterol compound (TTM-1) was isolated by using Thin layer and Column chromatograms with n-hexane: ethyl acetate in various ratios from non-polar to polar. The yield percent is 1.4% based upon ethyl acetate crude extract. The melting point of this compound was determined as 205-206°C. Moreover, the molecular formula ($C_{25} H_{38}O_4$) and the planar structure of this compound could be determined by FT-IR,^{1}HNMR(600MHz),^{13}CNMR (150MHz), DEPT (Figure 6), HSQC, DEPT, COSY, HMBC and EI mass spectral data, respectively.

Finally, the conformational analysis of rings (A, B, C and D) could be determined by ^{1}HNMR splitting pattern, coupling constant and NOESY spectral data (Figure 10), respectively. The absolute configurations of eight chiral centers could be determined.

Acknowledgements

We would like to express our sincere gratitude to Dr Thida Win, Rector, University of Mandalay, for her permission and suggestion upon the research work. We would like to express our sincere thanks to Dr Yi Yi Myint, Professor and Head, Department chemistry, University of Mandalay, for her permission and suggestion upon this research paper.

References

Harbone, J. B., (1973), "Phytochemical Method, A Guide to Modern Techniques of Plant Analysis", Chapman and Hall in association with Methuen, Inc., 733 Third Avenue, New York NY

Merck Index˝, 1983. 10th Edition, Merck & Co, Inc.USA.

Nelson, J. H.,(2003), "Nuclear Magnetic Resonance Spectrocsopic" Pearson Education,Inc.Upper Saddle River, NJ 07458.

Silverstein, R.M and Webster, F.X, (1998), “Spectrometric Identification of Organic Compound”, 6th edition, John Wiley and Sons, Lnc., New York

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An Investigation on Disinfection Byproduct Precursors Production from *Skeletonema costatum* Removal by Chlorination

Thae Su Lwin[1] & Suchat Leungprasert[2]

Abstract

To supply the safe water to public, disinfection process is essential in the water treatment process. One of the interesting problems found at the disinfection process is carcinogenic disinfection byproducts formation at the chlorination process. In the present work, the production and fate of disinfection byproduct precursors from *Skeletonema costatum* removal by chlorination were investigated. Chlorine consumption, chlorophyll a content, dissolved organic carbon and algae organic matter were measured under different revolution per minute, contact time and chlorine dosage. As the results, apparently decreasing of residual chlorine, chlorophyll a with high production of disolved organic carbon under longer contact time while they were not changed apparently on different revolution per minutes and chlorine dosage. Tyrosine, tryptophan and soluble microbial by-product-like were detected by spectrofluorometer.

Keywords: algae organic matter, chlorination, disinfection by-products, *Skeletonema costatum*, spectrofluometer

Introduction

Algae, which are abundant and easy to grow in nature, are oxygenic, phototrophic and eukaryotic microorganisms with nuclei and organelles (Bellinger and Sigee, 2010). The presence of algae poses many problems in water treatment process such as consumption of high coagulant and flocculant dose (Tang X *et al*, 2015), producing of disinfection by-product (DBP) precursors (Qi J *et al*, 2016) and fouling in the filter system (Her N *et al*, 2004) because of their algogenic organic compounds. The algogenic organic matter (AOM) that produced from algae includes intracellular organic matter (IOM) excreted from algae and extracellular organic matter (EOM) from cell lysis. Recent study shows that IOM is mainly comprised

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of aromatic and aliphatic protein-like products and EOM exhibits humic and fulvic-like products with high dissolved organic carbon/dissolved organic nitrogen ratio(DOC/DON) and aromaticity (Hua L *et al*, 2017). The composition and functional groups of AOM differ by depending on the origin (marine and freshwater), species and growth phase (Tang. X *et al*, 2017) and (Villacorte L *et al*, 2015).

Rook (1974) and Bellar *et al* (1974) reported that trihalomethane (THM) and haloacetic acid (HAA) are primary halogenated compounds. The maximum allowable concentration in drinking water is 80 μgL^{-1} for total THM and 60 μgL^{-1} for HAA are regulated by United States Environmental Protection Agency due to their carcinogenic and dangerous potent to human. The DBP formation potential of trichloromethane, trichloronitromethane, dichloroacetonitrile, 1,1 dichloropropanone and 1,1,1 trichloropropanone from two algae were monitored by Liao (Liao X *et al*, 2015).

Borges *et al* (2008) observed that the higher the contact time, pH, temperature, chlorine dose, amount of organic matter, the higher the formation level of DBPs. Hua L's study shows that the intracellular organic matter have higher carbonaceous DBP formation potential than extracellular organic matter because of their high chlorine substitution potential (Hua L *et al*, 2017). The biochemical compositions, vitamins and mineral content of marine diatom *Skeletonema costatum* (*S.costatum*) was analyzed by Kumar *et al*. (2015). Due to the presence of nutritional values such as trace minerals, unsaturated fatty acid, various kind of vitamins and protein, it could be food for human.

The aim of this research is to investigate the DBP precursors from *S.costatum* removal by pre-chlorination by using fluorescence excitation emission matrix (FEEM) and high performance liquid chromatography (HPLC). Chlorophyll a content, algae cell counting, residual chlorine and dissolve organic carbon were also detected under different chlorine dosage, contact time and revolution per minute.

Material and Methods

Cultivation algae

The dominant species at the study area *S.costatum* was received from Department of Fishery Biology, Kasetsart University. The sterilized nutrient F2 medium (Guillard and Ryther, 1962 and Guillard, 1975) was used for cultivation (Anderson, 2005). The cultivation was conducted at room temperature with 16:8 Light/Dark cycle under fluorescent light 18.0714 µmole photon/m^{-1}s^{-1}. Algae stock solution was prepared by centrifuging algae cell at 6000 rpm, 15 min and transfer to distilled water.

Determination of algae density

The chlorophyll a was measured by UV/Visible spectrophotometer before and after chlorination to know the density of the algae according to the procedure of standard methods for the Examination of Water and Wastewater. The optical density measured at wavelength 750 nm, 664 nm, 647 nm and 630 nm. Wavelength 664, 647 and 630 nm are for chlorophyll a,b and c and wavelength 750 nm is for turbidity in the sample. Algae cells were counted by using Segwick rafter counting chamber under the microscope (Lobban C.S *et al*, 1988)

Chlorination of *Skeletonema costatum*

The chlorination process was carried out by using Jar Test (JLT6, VELP Scientifica). Algae solutions were adjusted to get around pH 7 by using 1 N NaOH and N HCl and distilled water was used as a blank. Chlorination processes were done under three different conditions (1) revolution per minute 100, 150 and 200 rpm (3mg/L, 10 min), chlorine concentration 3, 4 and 5 mg/L (200 rpm , 10 min) and (3) contact time 10, 30 and 60 min (3 mg/L, 200 rpm) respectively. 3 mg/L of chlorine dose, 200 rpm or 10 min contact time were chosen base on the pre-chlorination conditions of study area (Bangkhen water treatment plant) for each condition.

Determination of residual chlorine and dissolved organic carbon

The concentration of residual chlorine were measured by Iodometric Titration method after Jar Test. The sample was acidified with 5ml of acetic acid and added 1g of potassium iodide. It was titrated with sodium thiosulphate (0.01N) by using starch indicator. All tests were done in duplicate.

For DOC measurement, 50mL of samples was filtered with Whatman GF/C filter paper and were kept in 4^{o}C refrigerator until analysis by a TOC analyzer (Analytikjena Multi N/C 2000). DOC was obtained by subtracting inorganic carbon from total organic carbon.

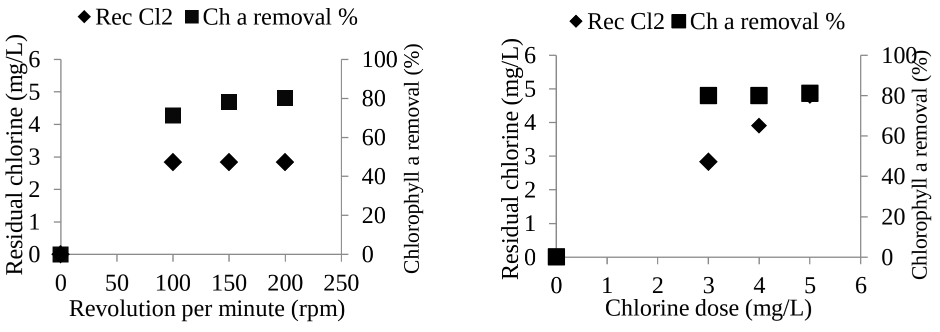
Fluorescence Spectroscopy

Three dimension fluorescence excitation emission matrix is widely used to distinguish natural organic matter by many researcher (Her N *et al*, 2004, Chen J *et al*, 2002 and Henderson R.K *et al*, 2008) because it is easy and give imformation in a short time. FEEM spectra were analyzed by using Jasco FP-8200 (PC) in a range of 210-650 nm. The results were analyzed according to the literture figure (Chen J *et al.,* 2002)

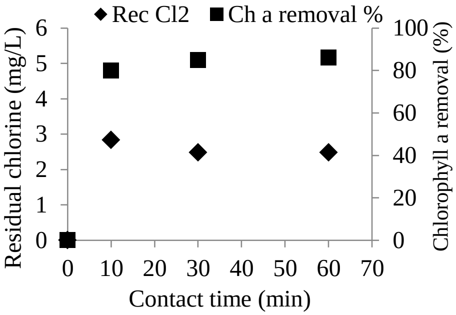
Result and Discussion

Residual chlorine and Chlorophyll a removal

According to the data (Fig 1), residual chlorine remained similarly 2.836, 2.836 and 2.836 mg/L under different revolution, 2.836, 3.8995 and 4.7857 mg/L under different chlorine dose, 2.836, 2.4815, 2.4815 mg/L under different contact time. The residual chlorine was nearly the same on different revolution and dose but the significant reduction of it happened under different contact time (2.836, 2.4815 and 2.4815 mg/L). The highest chlorophyll a reduction 80, 85 and 86% were found under long contact time with highest chlorine consumption but nearly the same cell reduction 71, 78, 80% and 80, 80 and 81% were happened under different revolution and chlorine dose. It was concluded that *S.costatum* is the least resistant algae (Liao X *et al.,* 2015) because 3 mg/L of chlorine, 200 rpm and 30 min can give satisfied cell reduction. It was supposed that this condition was the suitable choice to kill *S.costatum* from economic point of view.



(A) (B)

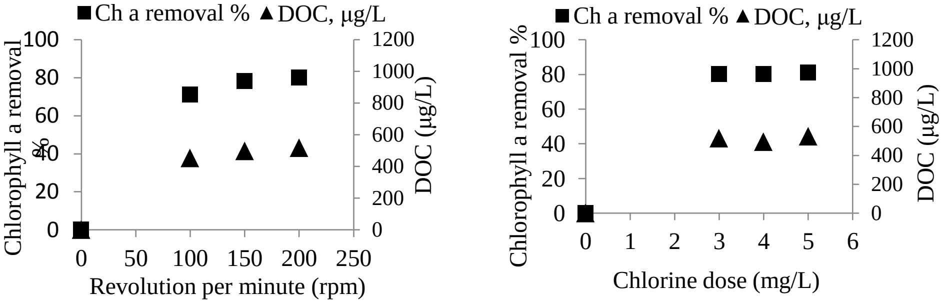


(C)

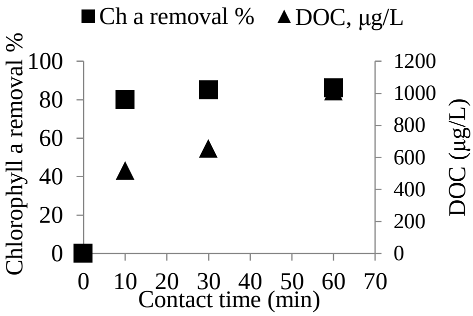
Figure 1. Correlation of residual chlorine (Rec Cl_2) and chlorophyll a removal % (Ch a removal %) of *S.costatum* chlorination under (A) 100, 150 and 200 rpm (B) 3, 4 and 5 mg/L (C) 10, 30 and 60 min

Chlorophyll a reduction and Dissolved Organic Carbon

The correlation of DOC and chlorophyll a removal % are shown in following figures (Fig 2). After chlorination of algae, DOC and particle come out from cell lysis. The DOC concentrations were obtained 451.3, 496.7 and 516.9 µg/L under different rpm, 516.9, 492.9 and 531.3 µg/L under different chlorine, 516.9, 654.8 and 1011.8 µg/L under long contact time respectively. The results showed DOC production increased with chlorophyll a removal under all conditions. DBP formation potential do not correlate with DOC value but the nature of it (Li *et al.*, 2014). The production of DOC correlated to neither chlorophyll a nor cell counting because the high production of DOC from low chlorophyll a of diatom was higher than green and blue green algae (Nguyen *et al.*, 2005).



(A) (B)



(C)

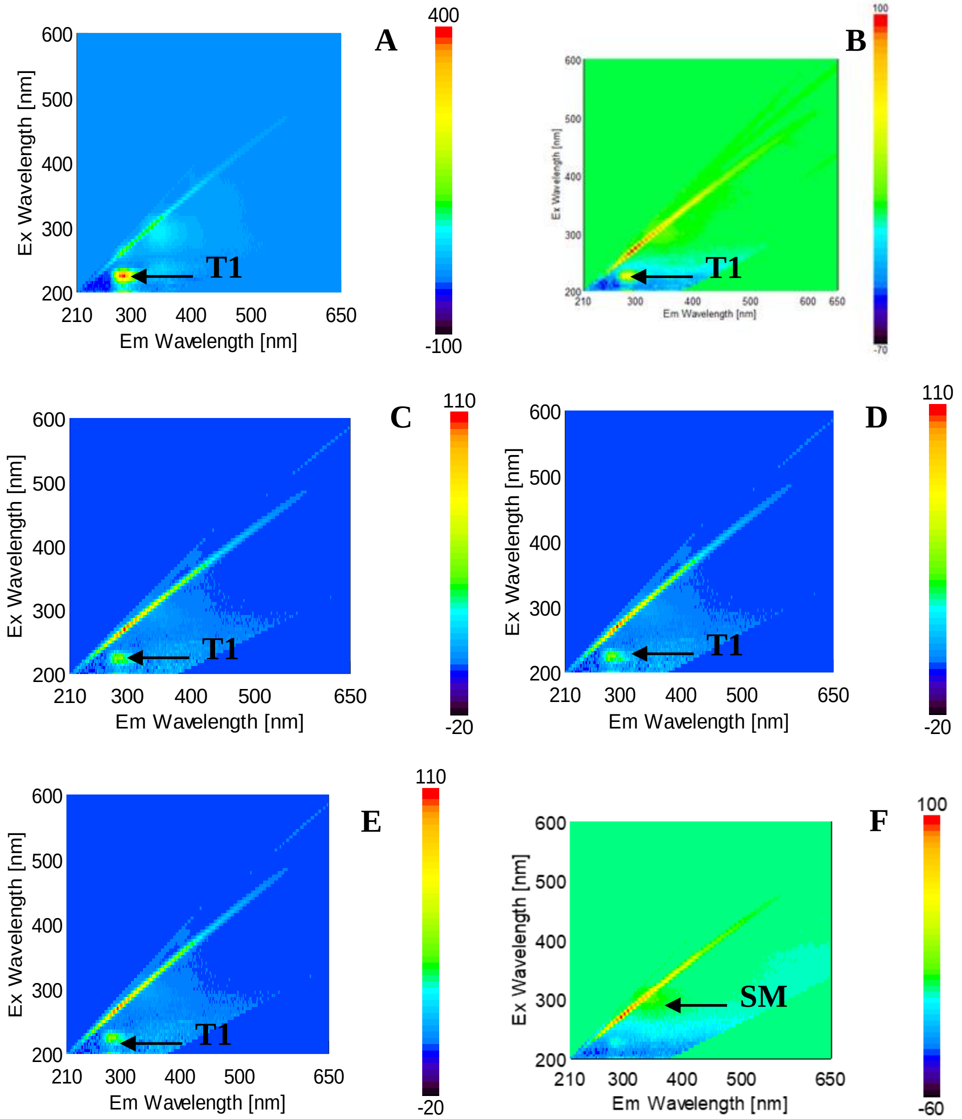
Figure 2. Correlation of chlorophyll a removal % and dissolved organic carbon of *S.costatum* chlorination under (A)100, 150 and 200 rpm (B) 3, 4 and 5 mg/L (C) 10, 30 and 60 min

Disinfection by-product precursors by fluorescence spectroscopy

The spectra before chlorination showed the strong intensity of tyrosine region (T_1). Xie concluded that the appearance of aromatic substances (T_1) can be the bounded dissolved organic matter on the cell membrane (Xie *et al*, 2013). Another study of chlorination of *Microcystic sp* and *Cyclotella sp* also found the originated AOM (before chlorination) was on T_1 and SM regions (Liao X *et al.*, 2015).

The strongly removal of tyrosine was found after chlorination on different revolution and chlorine dose but new region soluble microbial by-product-like (SM) evolved under 4 and 5 mg/L reaction from (~ 80%) chlorophyll decay. ~85% of cell decay gave the presence of tyrosine, tryptophan and soluble microbial by-product like under long contact time reaction with high production of dissolved organic carbon. The protein like substances removed but soluble microbial product-like were difficult to treat by coagulation (Tang X *et al*, 2017).

From the findings of present work, the main compounds that involved in the productionof DBP are T_1, T_2 and SM product compounds. The aromatic protein compounds are primarily responsible for the fomation of N-DBPs. This finding is similar to the studies of Tang *et al.* (2017). The enhance of nitrogen organic compounds leads to the formation of nitrogen DBPs such as dichloronitromethane and trichloronitromethane (Fang *et a.*, 2010).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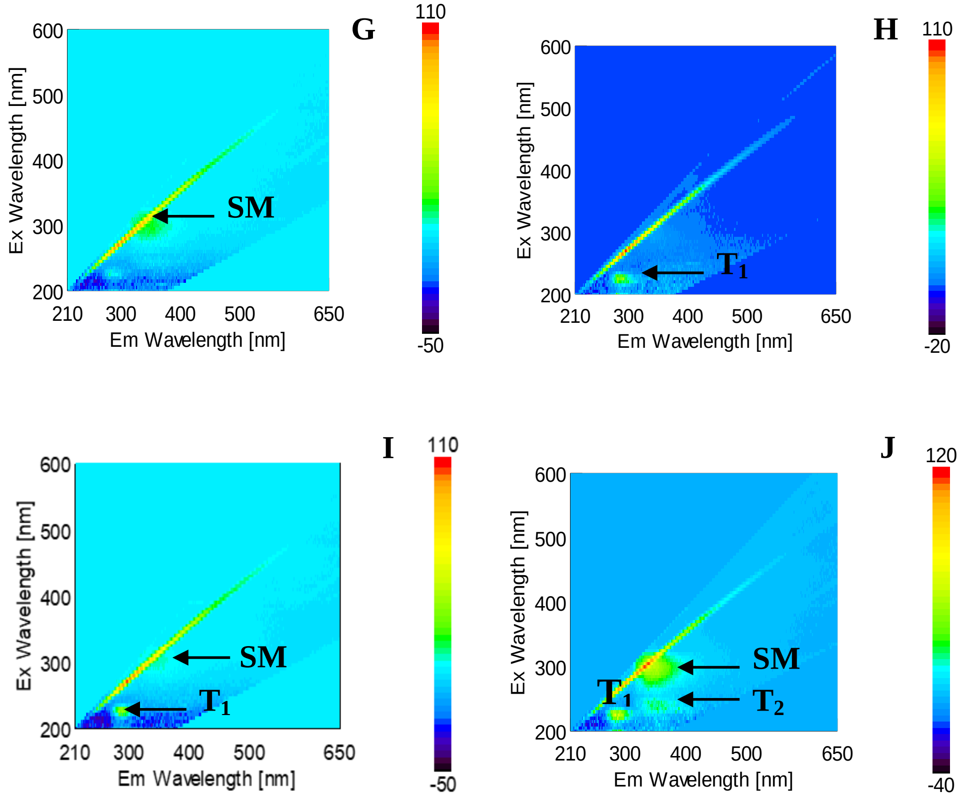

Figure 3. FEEM spectra of *S.costatum* after chlorination under (A) before chlorination (B)100 rpm (C) 150 rpm (D) 200 rpm (E) 3 mg/L Cl (F) 4 mg/L Cl (G) 5 mg/L Cl (H) 10 min (I) 30 min and (J) 60 min

Conclusion

It can be concluded that the major DBP precursors from the removal of *S.costatum* products are aromatic proteins and soluble microbial by-product like. These nitrogenous DBP precursors can lead to the formation of potential nitrogenous DBP such as dichloronitromethane, trichloroacetonitrile, 1,1 dichloropropanone and 1,1,1 trichloropropanone, trichloronitromethane and diethylacetonitrile. The optimum conditions to decay *S.costatum* are 3 mg/L Cl, 200 rpm and 30 min according to the results. To reduce DBP production, the recommended control strategies by

EPA such as changing the point of chlorine addition to a location after sedimentation or clarification, using alternative disinfectants, removing NOM before disinfection process can be desired.

Acknowledgement

The author would like to appreciate to Advisor Assist.Prof. Suchat Leungprasert for his guideline and supporting for my work. The author also would like to thank to Thailand International Cooperation Agency (TICA) for their financial supporting.

References

Ali, R.K., El-Aty, A.M., Badawy, M.I., Abdel-Karim, A., Gad-Allah, T., Ali, M.E. (2015) Impacts of algal cells and humic acid on the formation of disinfection by-products during chlorination of drinking water. J Applied Environmental and Biological Sciences. 5(8) 298-303

Andersen, R.A. (2005). Algal Culturing Techniques Burlington. ISBN

Bellar, T.A., Lichtenberg, J.J., Kroner, R.C. (1974). The occurrence of organohalides in chlorinated drinking waters. J. Am. Water Works Assoc. 66:12: 703-706

Bellinger, E.G and Sigee, D.C. (2010). Freshwater algae: identification and use as bioindicators, 1st. New Delhi, India.

Borges, J.T., Sparrapan, R., Guimaraes, J., Eberlin, M., Augusti, R. 2008. Chloroform formation by chlorination of aqueous algae suspension online monitoring via membrane introduction mass spectrometry. J.Brazilian Chemical Society. 19: 950-955

Chen, J., LeBoeuf, E.J., Dai, S., Gu, B. 2002. Fluorescence spectroscopic studies of natural organic matter fractions. Chemosphere. 50: 639-637

Fang, J., J. Ma., X. Yang and C. Shang. (2010) Formation of carbonaceous and nitrogenous disinfection by-products from the chlorination of Microcystis aeruginosa. Water Research 44: 1934-1940

Henderson, K.R., Baker, A., Parsons, S.A., Jefferson, B. 2008. Characterization of algogenic organic matter extracted from cyanobacteria, green algae and diatoms. Water Research. 42: 3435-3445

Her, N., Amy, G., Park, H., Song, M. (2003) Characterizing algogenic organic matter (AOM) and evaluating associated NF membrane fouling. Water Research. 38: 1427-1438

Kumar, C.S and V.A. Prabu. (2015). Nutritional value of Skeletonema costatum (Cleve,1873) from Parangipettai, Southeast Coast of India. International Journal of Phamaceutical Sciences and Research 6(8): 3463-3466

Liao, X., Liu, J., Yang, M., Ma, H., Yuan, B., Huang, C. (2015). Evaluation of disinfection by-product formation potential (DBPFP) during chlorination of two algae species Blue-green *Microcystis aeruginosa* and diatom *Cyclotella meneghiniana.* Sci. Total Environment. 532: 540-547

Lobban, C.S, Chapman, D.J., Kremer, B.P. (1988) Experimental Phycology: A laboratory Manual Phycological Society of America

Nguyen, M., Westerhoff, P., Baker, L., Hu, Q., Esparza-Soto, M and Sommerfeld, M. (2018). Characteristics and Reactivity of Algae-Produced Dissolved Organic Carbon. J. Environ. Eng., 2005 131(11): 1574-1582

Qi, J., Lan, H., Liu, R., Miao, S., Liu, G., Qu, J. (2016). Prechlorination of algae-laden water: The effects of transportation time on cell integrity, algae organic matter release, and chlorinated disinfection byproduct formation. Water Research. 102: 221-228

Tang, X., Zheng, H., Gao, B., Zhao, C., Liu, B., Chen, W., Guo, J. (2017). Interactions of specific extracellular organic matter and polyaluminium chloride and their roles in the algae-polluted water treatment. Journal of Hazardous Materials. 332: 1-9

Rook, J.J. (1974). Formation of haloforms during chlorination of natural waters. J Water Treatment Examination Journal Water Treat 23: 234-243

Villacorte, L.O., Ekowati, Y., Neu, T.R., Kleijn, J.M., Winters, H., Amy, G., Schippers, J.C., Kennedy, M.D. (2015). Characterisation of algal organic matter produced by bloom-forming marine and freshwater algae. Water Research. 73: 216-230

Xie, P., J. Ma., J. Fang., Y. Guan., S.Yue., X. Li and L. Chen. (2013) Comparison of Permanganate Preoxidation and Preozonation on algae containing water: Cell integrity, characteristics, and chlorinated disinfection byproduct formation. Environmental Science and Technology 47: 14051-14061

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Chemical Investigations of Pomegranate Seeds (*Punica granatum* Linn.) and Its Acute Toxicity

Kyi Nwe Aye[*]

Abstract

In this research, chemical investigations and acute toxicity test of pomegranate *(Punica granatum* Linn.) (Tha-Lae) seeds were carried out. Three crude extracts of pomegranate seeds were prepared by successive extraction with petroleum ether, follow by ethyl acetate and finally with ethanol. By using column chromatographic separation with silica gel in the solvent system of chloroform-methanol-acetic acid, needle shape crystals compound (gallic acid) (0.07% yield, m.pt=234-236) was isolated from ethyl acetate extract of pomegranate seeds. This compound has been identified by spectroscopic measurement of ultra-violet (UV), infra-red (IR) and nuclear magnetic resonance spectroscopic (^{1}HNMR) methods. After that, the acute toxicity testing on mice were done with ethanol and aqueous extract of pomegranate seeds according to the method of Litchfied and Wilcoxon. Both extracts of pomegranate seeds were administrated orally to mice at various dose levels to determine the acute toxic effect in mice. The result showed that pomegranate seeds extracts were free from acute toxic effect.

Keywords*:* Pomegranate, antioxidant, gallic acid, acute toxicity

Introduction

The imbalance between free radicals and the body’s defense against oxidative stress cause chronic diseases. With regards to beneficial properties of antioxidants to neutralize oxidative reactive products, the identification of more effective and less toxic antioxidant compounds is in progress. Antioxidants react with free radicals, protecting human bodies against disease and degeneration. Many antioxidant compounds can be found in fruits and vegetables including phenolics, flavonoids and anthocyanin. Pomegranates contain powerful antioxidants that appear to inhibit the onset of atherosclerosis, reduce the risk of heart disease, and low high blood pressure, antiviral and antibacterial effect. Pomegranate extract also has demonstrated anticarcinogenic properties that effectiveness in

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suppressing a variety of cancers, including skin, breast and colon cancers. Pomegranate is composed of a rich variety of flavonoids, which comprise approximately 0.2% to 1.0% of the fruit. Many polyphenols are found in the whole fruit (peel and seeds). Pomegranate is also a rich source of ellagic acid, a phenolic normally found as a polymer with gallic acid, known as ellagitannin. There is a large body of evidence suggesting that ellagic acid is an inhibitor of chemically-induce cancers. Gallic acid and its derivatives have exhibited for the treatment of diabetes, ischemic heart diseases and ulcer. Freshly prepared pomegranate juices were contained many phenolic compounds such as gallic acid, caffeic acid, ferulic acid, o-and p-cumaric acid, catechin, quercetin and organic acid such as citric acid, malic acid, tartaric acid, oxalic acid (Maria, 2000).

Botanical Descriptions of Pomegranate (*Punica granatum* Linn.)

Family	-	Punicaceae
Botanical Name	-	*Punica granatum* Linn.
Myanmar Name	-	Tha-lae
English Name	-	Pomegranate
Part of Used	-	Seeds

Figure 1. Photograph of fruit and seeds of pomegranate

Sampling of Plant Materials

Pomegranate fruits were collected from Magway Division. Fruits were identified in Department of Botany, University of Yangon. The pomegranate seeds were taken off from these fruits and air-dried at room temperature. Then it was ground into powder and stored in air-tight container to prevent moisture changes and contamination.

Preparation of Seeds Extracts

The dried seed powdered sample (300g) was defatted with 1000mL of petroleum ether 60-80°C for one week at room temperature by percolation method and follow by filtration. The filtrate was evaporated under reduced pressure by means of rotary evaporator. The defatted pet ether extracted samples was then percolated with 95% ethanol (1000mL) for three weeks and the extract was then filtered. This procedure was repeated other three times. The combined filtrates were then concentrated by removal of the solvent under reduced pressure using vacuum rotary evaporator. This concentrated 95% ethanol soluble matter was then partitioned between ethyl acetate and water. After removal of the solvent from organic layer, ethyl acetate soluble extract was obtained. In this way, ethyl acetate extract and ethanol extract of both samples were prepared. After removal of the solvents all of the above different crude extracts were weight and the weights obtained were recorded.

Separation, Purification and Isolation of Compounds from Ethyl Acetate Extract of Pomegranate Seeds

Five gram of pomegranate seeds defatted ethyl acetate (EtOAc) extracts were subjected to silica gel (100 g) column chromatographic separation with gradient elution of PE:EtOAc (9:1, 7:1, 5:1, 2:1, 1:1, 1:2 and ethyl acetate only) solvent systems which was chosen depending on TLC behavior. In this separation, a total of 95 fractions were collected and the fractions which showed similar TLC behaviors were combined to give successively five main fractions F I (f_1-f_{20}), F II (f_{21}-f_{30}), F III (f_{31}-f_{43}), F IV (f_{44}-f_{59}), F V (f_{60}-f_{95}). After concentration of the above fractions by removal of the solvents with air dried, from the fraction F III (f_{31}-f_{43}) pale yellow solid crystals of compound 1 was obtained, but contain a little impurity. Further purification of fraction F III (f_{31}-f_{43}) was performed by column rechromatography method as follows. This column rechromatography was performed by using column (1.2cm x 29cm), silica gel (15 g) and eluting with PE:EtOAc of 4:1, 2:1, 1:1, solvent systems followed by EtOAc and to collect a total 50 fractions. Collected fraction numbers f_{25} to f_{43} showed same thin layer chromatographic feature were combined together, and concentrated to give white solid crystals compound 1 in 0.07% (35 mg) of yield. It was observed as a single spot of TLC chromatogram.

The isolated compound 1 was examined and characterized by thin layer chromatography, using GF_{254} precoated silica gel aluminium plate (Merck) as adsorbent and suitable solvent systems for isolated compounds. Localization of spot was made by viewing directly under UV (254nm-365 nm wavelength) light. The compound on the TLC plate were also localized by spraying with Libermann Burchard reagent, 5% H_2SO_4, anisaldehyde, and vanillin reagents followed by heating at 110°C if necessary and exposure to iodine vapor and 1% $FeCl_3$ were carried out. The R_f values and behavior on TLC of the respective spots were measured and shown in Table 1.

Acute Toxicity Test of Pomegranate Seeds on Albino Mice

To determine the symptomatology consequent to ingestion of the extracts and to determine the nature and degree of toxicity produced by pomegranate seed extracts and to find out the medium lethal doses (LD_{50}) of the extracts, the acute toxicity test was done. Usually the acute lethality of a compound is determined on the basis of deaths occurring in 24 hours but the survivors should be observed for at least seven days in order to detect delayed effects (Loomis, 1968). The acute toxicity effect of the 95% ethanol and aqueous extracts of pomegranate seeds were studied on albino mice after 7 days treatment. The acute toxicity test was done according to the method of Litchfield and Wilcoxon (1949). A total of 70 adult mice, weighting (25-35 g) were used for administration of ethanol and aqueous extracts of samples. They were divided into 7 groups, each containing of 10 animals. Group I to III mice were treated with ethanol extracts and group IV to VI mice were treated with aqueous extracts by orally. The doses employed were (4, 8, 16 g / kg) of extracts in 10 mL of water respectively. The last group VII mice were treated with 0.1 mL/10g of distilled water and served as a control. After administration of various concentrations of extract, the mice were separately house in standard aluminium cages and allowed to access to food and water. The ambient temperature was maintained at 26±1°C. The mice were observed for first six hours continuously for mortality and behavioral changes if any and then every 24 hours for 14 days. The mortality during this period was noted as nil or percent death. Each study was done separately. The mice were also closely examined for sings of intoxication, lethargy, behavioral modification and co-morbid state.

Results and Discussion

Characterization and Classification of Compound 1

Compound 1 was isolated as pale yellow solid crystals shaped from column chromatographic separation of EtOAc crude extract of pomegranate seeds using silica gel GF_{254} as adsorbent and PE: EtOAc (1:2 v/v) as eluent. Its R_f value was found to be 0.45 with $CHCl_3$:MeOH:Acetic acid (7:1:1 v/v) solvent system. The pure crystal of compound 1 was purified by column rechromatography eluting with PE:EtOAc (1:1 v/v) obtained white needle shape crystals, melt between 232-235^{o}C. Compound 1 gave deep blue coloration with 1%$FeCl_3$ showing that it contains phenolic –OH groups. It did not give pink colour with concentrated HCl and Mg turning but it gave brown coloration with KI. Therefore compound 1 may be hydrolysable tannin of phenolic acid. The melting point was found to be similar to that of gallic acid (235-240^{o}C) (Merck Index, 2001). Some physico-chemical properties of compound 1 were listed in table 1. Compound 1 may be assigned as gallic acid and photograph of thin layer chromatogram and isolated compound (gallic acid) was described in figure 5. and figure 6.

Table 1. Some Physico-chemical Properties of Isolated Compound 1

Experiment	**Observation of Compound 1**	**Remark**
Melting point °C	234-235°C	from ethyl acetate extract
R_f value	0.45	chloroform:methanol:acetic acid (7:1:1 v/v)
UV (254nm-365nm)	active	conjugated double bond
1 % $FeCl_3$ solution	deep blue colour	phenolic –OH present
Mg/HCl	no pink colour	flavonoid absent
Liebermann-Burchard reagent, Δ	no coloration	not steroid /terpenoid
KI	brown	tannin

Spectroscopic Identification of Isolated Compound 1

Study on UV Spectrum

In the UV spectrum of compound 1 showed in figure 2 and the data was tabulated in table 2 was consistent with reported λ_{max} value of gallic acid. The observed UV spectrum of compound 1 measured in MeOH solvent showed λ_{max} at 221 nm ($\pi \rightarrow \pi^*$ transition) and 271 nm ($n \rightarrow \pi^*$ transition), indicating the presence of unsaturation and non-bonding electron pairs. In the presence of NaOH, the absorption band at shorter wavelength of 209 nm occurred present of carbonyl group of acid but longer wavelength of 271 nm was found to be shifted to longer wavelength of 287 nm that is bathochromic shift of red shift occurred. This is indicated the presence of phenolic –OH groups.

Table 2. UV Spectral Data of Isolated Compound 1

Shift Reagent	**Observed $\lambda_{\mu ax}$ (nm)**		**Assignment**
	Compound 1	***Gallic acid**	
MeOH	221	-	$\pi \rightarrow \pi^*$ transition
(methanol)	271	272	$n \rightarrow \pi^*$ transition
MeOH+NaOH	209	-	Bathochromic Shift (Red shift)
(methanol +sodium hydroxide)	287	-	

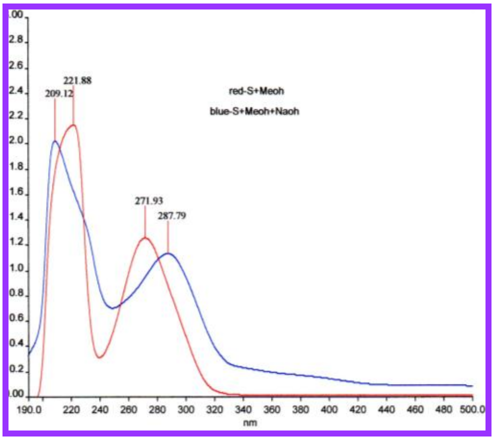


Figure 2. UV spectrum of isolated compound 1 (in MeOH)

Study on FT-IR Spectrum

The functional groups present in the compound 1 could be assigned from FT-IR spectrum described in figure 3 and table 3. The present of phenolic O-H group was confirmed by absorption bands appeared at 3371 and 3282 cm^{-1} due to –OH stretching vibration. The broad band ranging between 3500cm^{-1}~2630 cm^{-1} indicated that compound 1 contained a carboxylic acid –COOH group and it was attributed to –OH stretching vibration. The absorption band at 1707 cm^{-1} referred to C=O stretching of α, β–unsaturated –COOH group and that 1620 cm^{-1} indicated the presence of C=C groups. In addition, the absorption band occurred in the range 1450 cm^{-1}, 1338 cm^{-1} and 1307 cm^{-1} represented the presence of in-plane-bending vibration of aromatic benzene ring C-H group.

Table 3. FT-IR Spectral Data of Isolated Compound 1

Wave number (cm^{-1})	**Band Assignment**
3500 ~ 2630	O-H stretching vibration of –COOH group
3371, 3282	O-H stretching vibration of phenolic –OH group
1701	C=O stretching of α,β–unsaturated –COOH
1620	C=C stretching vibration of aromatic ring
1450, 1338, 1307	C-H in-plane-bending vibration

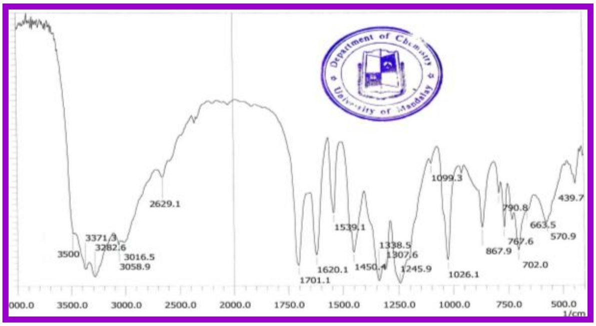


Figure 3. FT-IR spectrum of isolated compound 1(in MeOH)

Study on ^{1}HNMR spectrum

The ^{1}HNMR spectrum of compound 1 was measured in CD_3OD to appear at the chemical shift of δ 7.07 ppm related to two protons of aromatic benzene ring -CH groups by comparing the ^{1}HNMR spectrum with computer assistant ACD Labs software of gallic acid which is described in figure 4 and table 4. It was observed that the chemical shift was identical to that corresponding to H-2 and H-6 protons of gallic acid.

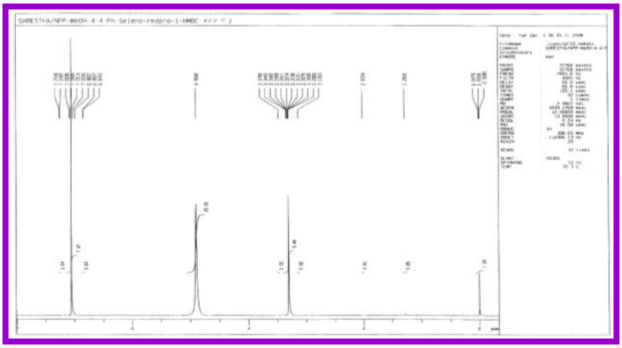
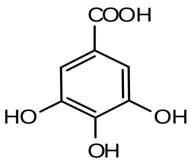


Figure 4. ^{1}HNMR spectrum of isolated compound 1(in CD_3OD)

Table 4. ^{1}HNMR Spectral Data (400 MHz, CD_3OD) of Isolated Compound 1 and Predicted by ACD Labs

δ (ppm)		no. of Protons	Multiplicity	Assignment
Compound	**Predicted by ACD Labs**			
7.07	7.07	2H	s	H-2, H-6 Two protons of aromatic CH groups

Therefore, colour reaction, melting point, UV, FT-IR, ^{1}HNMR spectral data, the literature survey and computer assistant ACD Labs software the isolated compound 1 could be assigned as gallic acid (molecular weight, $C_7H_6O_5$ and molecular mass, 170) with the following molecular structure.



gallic acid

Compound **1** : Gallic acid
* : Authentic gallic acid
Solvent system : Chloroform:methanol:acetic acid (7:1:1v/v)
R_f value : 0.45
Adsorbent : silica gel GF_{254}
Spraying agent : 1% $FeCl_3$

Figure 5. Thin layer chromatogram of isolated compounds

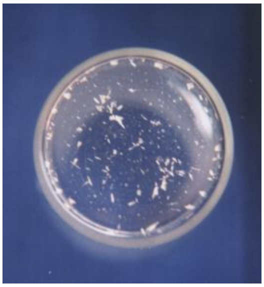


Figure 6. Isolated compound 1 (gallic acid)

Pharmacological Activities of Pomegranate Seeds

Acute Toxicity Test

The acute toxicity testing on mice were done with 95% ethanol and aqueous extracts of pomegranate seeds according to the method of Litchfield and Wilcoxon. Pomegranate seeds extracts were administrated orally to mice at various dose levels to determine the acute toxic effects and the medium lethal dose (LD_{50}) in mice. The acute toxicity screening for both ethanol and aqueous extracts of pomegranate seeds was tested with the dosages of 16, 8, and 4g/kg body weight. The maximum permissible dose (16g/kg body weight) of 95% ethanol and aqueous extracts of both seed on albino mice were found to be alive and healthy during the observation period of two weeks. All animals remained alive and did not show any visible symptoms of toxicity like respiratory disorders, convulsions and death etc. at the dosage tested. Therefore, it was observed that both 95% ethanol and aqueous extracts of pomegranate seeds were clearly free from acute toxic harmful effect. The results of acute toxicity study of pomegranate seeds on albino mice were shown in table 5 and figure 7 indicated the administration of pomegranate extracts in albino mice by oral route.

Table 5. Result of Acute Toxicity Test of 95% Ethanol and Aqueous Extracts of Pomegranate Seeds

Groups	**No. of animals**	**Drug Administration**	**Dosage**	**No.of death per tested**	**% of Death**
I	10	Ethanol extract	4g/kg	1/10	0%
II	10	Ethanol extract	8g/kg	1/10	0%
III	10	Ethanol extract	16g/kg	1/10	0%
IV	10	Aqueous extract	4g/kg	1/10	0%
V	10	Aqueous extract	8g/kg	1/10	0%
VI	10	Aqueous extract	16g/kg	1/10	0%
VII	10	Distilled water	10ml/kg	1/10	0%

All groups of mice were fed normal stock diet and distilled water.

Figure 7. Administration of pomegranate extracts in albino mice by oral route for acute toxicity test

Conclusion

From the overall assessment concerning with chemical investigation on pomegranate seeds, the bioactive compound was isolated which were identified by spectroscopic measurement as compound 1 (gallic acid) (0.07% yield, m.pt=234-235°C). From acute toxicity test of pomegranate seeds, 95% ethanol and aqueous extracts were found to be free from acute toxic or harmful effect at maximum permissible dose of 16g/kg body weight. According to *in vivo* toxicity test, it may be inferred that the edible fruit of pomegranate seeds can be used safely.

Acknowledgements

I would like to thank the former Professor Dr Saw Hla Myint, Professor and Head of Department of Chemistry, University of Yangon for allowing me to do this research program and provision of the research facilities. Next, I would like to express sincere gratitude and thanks to Dr Than Htut Lwin (Principal) and Dr Win Naing (Professor and Head of Department of Chemistry) from Taunggoke Degree College for their kind permission to submit this research paper.

References

Halliwell, B., and Gutteridge, J. M. C., (2000), "*Free radicals In Biology and Medicine*", Department of Biochemistry, Oxford University

Litchfield, J, T and Wilcoxon, F., (1974), "A Exemplified Methods Evaluating Dose Effect Experiments", J. *Pharmacol* Exp., **96**, 110-113

Loomis, T.A., (1968), "Essential of Toxicology", 144-146

Maria. J.G., Francisco. A., Tomas.B., Betty.H.P., Deirdre. M.H., and Adel. A.K., (2000), "Antioxidant Activity of Pomegranate Juice and Its Relationship with Phenolic Composition And Processing", *J. Agric. Food Chem*, **48**, (10), 4581-4589.

Mark Index, (2001), "An Encyclopedia of Chemicals, Drugs and Biological", **13**

Rice Evans, C.a., Miller, N.J and Paganga, G., (1996), "Structure-Antioxidant Activity Relationships of Flavonoids and Phenolic Acids", **20**, 993-956

Silverstein, R.M. and Webster, F.X., (1998), "Spectroscopic Identification of Organic Compounds", 12-28, 81-98, 200-216, 222-249, 250-274

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Investigation of Fungal Secondary Metabolites in the Mushroom *Cortinarius nemorensis* (Fr.) J.E. Lange

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Abstract

In an effort to identify secondary metabolites from edible and medicinal mushrooms, (*R*)-(8*E*,10*E*)-12-hydroxy-7-oxo-8,10-octadecadienoic acid (**1**) and its methyl ester (**2**) together with 3-hydroxybenzaldehyde and 4-hydroxybenzoic acid, were isolated from the acetonitrile fraction of the ethyl acetate extract of the fruiting bodies of *Cortinarius nemorensis*. The structures were elucidated by 1D and 2D NMR spectroscopy, high-resolution mass spectrometry and, comparison with the reported data. Compound **1** and **2** have not previously been reported as secondary metabolites from this genus.

Keywords: *Cortinarius nemorensis*, mushroom, secondary metabolites

Introduction

Mushrooms are used by the local people as food as well as medicines, particularly in the Asian countries such as, Myanmar, China, Japan, and Korea as well as in some part of Africa, and nowadays their popularity as both is growing in many countries. A large number of scientific publications have shown that natural products from medicinal/edible mushrooms play a dominant role in the discovery of leads for the development of drugs for prevention and treatment of many disease (Chen *et al*., 2018). Phytochemical studies on the contents of mushrooms have been increasing steadily in the last decades (Clericuzio *et al*., 2018). Over the years, lectins, lanostanoids and other terpenoids, sterols, phenolic compounds, and alkaloids have been identified as key compound classes responsible for the variety of medicinal properties associated with mushrooms, including antitumor, anti-inflammatory, antifungal, antimicrobial, and antiviral activity (Öztürk *et al*., 2015).

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Cortinarius nemorensis (Fr.) J.E. Lange. belongs to the family Cortinariaceae according to *Index fungorum*. The genus *Cortinarius* is the most widely distributed genus in Agaricales and comprises about 4200 epithets (Teichert *et al*., 2007). Many species in the *Cortinarius* genus are known to produce biologically active natural products, including pigments and toxins (Nicholas *et al*., 2001). In our investigations on the secondary metabolites of fruiting bodies of *Cortinarius nemorensis* we focused on the constituents of the acetonitrile fraction of ethyl acetate extract of *C. nemorensis* which resulted in the isolation and structural elucidation of (*R*)-(8*E*,10*E*)-12-hydroxy-7-oxo-8,10-octadecadienoic acid (**1**), (*R*)-(8*E*,10*E*)-12-hydroxy-7-oxo-8,10-octadecadienoic acid methyl ester (**2**), 3-hydroxybenzaldehyde and 4-hydroxybenzoic acid. Chemical investigations of *Cortinarius* genus are few. This is the first report for the isolation of conjugated ketonic fatty acid and its methyl ester from the mushroom *Cortinarius nemorensis* so far.

Materials and Methods

Optical rotation was measured with a Perkin-Elmer 241 polarimeter. Infrared spectra were recorded on a Shimadzu FTIR-8400 spectrometer; ν in cm^{-1}. NMR experiments were performed on a Bruker AV 300 spectrometer, at 300 MHz (^1H) and 75.47 MHz (^{13}C) without TMS. NMR chemical shifts are reported in ppm and solvent peak was used as internal standard. The abbreviations *s* = singlet, *d* = doublet, *t* = triplet, *dd* = double doublet, and *m* = multiplet are used throughout; coupling constants (*J*) are reported in Hz. The multiplicity of each carbon atom was determined by APT experiments. COSY, DEPT, HSQC and HMBC spectra were recorded using standard pulse sequences. NMR spectra were recorded in $CDCl_3$, purchased from Sigma-Aldrich. ESI-MS data were recorded on a Thermo TSQ mass spectrometer, by flow injection analysis (FIA), with electron spray ionization source (ESI). High resolution mass spectra were measured on a FT-ICR Bruker Daltonics Apex II mass spectrometer. Column chromatography was performed on Merck Kieselgel 60 (40-63 µm). TLC was carried out on precoated aluminium TLC plates with silica gel 0.25 mm silica gel 60 (GF_{254}, Merck). Compounds were visualized under UV light (254 and 366 nm) and, additionally, they were stained by exposure to a 0.5% solution of vanillin in H_2SO_4-EtOH (4:1) or dipping in $KMnO_4$ in acetone, followed by gentle heating at 100 °C. Reagent grade solvents, purchased from Carlo Erba (Milan, Italy) or from Aldrich, were used for extraction and chromatographic separations.

Fungal Materials

The fruiting bodies of *C. nemorensis* were collected from Brallo, Oltrepò hills - Lombardy region, Italy in October 2012 and was identified by the expert mycologist Alfredo Gatti. Collected fruiting bodies were frozen at -20°C in B2 Laboratory, Organic Section, Department of Chemistry, University of Pavia.

Extraction and Isolation

Frozen fruiting bodies of *C. nemorensis* (3.1 kg) were rapidly minced and extracted with ethyl acetate and 2-propanol at -10°C. Each extraction was carried out three consecutive times, for one hour each. The residue (2.5 g) from the EtOAc extract was redissolved in acetonitrile and partitioned with hexane to remove the largest part of triglycerides and free fatty acids.

Acetonitrile soluble fraction (1.3 g) was chromatographed on silica gel and eluted with a gradient mixture of hexane-EtOAc (10-80% EtOAc in hexane) to give subfractions J1-J8. Fraction J2 (12.7 mg) was further chromatographed on silica gel, eluting with 10-50% EtOAc in hexane to give 3-hydroxybenzaldehyde (3.3 mg) (R_f (hexane-EtOAc, 4:1) 0.44). Fraction J7 (21.2 mg) was purified by column chromatography on silica gel and eluted with 100% DCM to give 4-hydroxybenzoic acid (0.5 mg) (R_f (DCM 100%) 0.54). Desired fraction J8 (303.2 mg) was further subjected to chromatography on silica gel, eluting with a gradient mixture of hexane-EtOAc (10-100% EtOAc in hexane) to give compound **2** (3.32 mg) (R_f (hexane-EtOAc, 7:3) 0.28) and compound **1** (8.07 mg) (R_f (hexane-EtOAc, 7:3) 0.09).

Compound **1**: Yellow amorphous; $[\square]_{\mathrm{D}}^{20}$ = + 3.79 (*c* 0.0040, DCM); ESI-MS $[M-H]^-$ *m/z* 309.30 for $C_{18}H_{30}O_4$; ^{1}H NMR Spectral Data (300 MHz, $CDCl_3$): see Table 1; ^{13}C NMR Spectral Data (75 MHz, $CDCl_3$): see Table 1.

Compound **2**: Yellow amorphous; $[\square]_{\mathrm{D}}^{20}$ = + 4.06 (*c* 0.0016, DCM); ESI-MS $[M+H]^+$ *m/z* 325.231 (Yao *et al*., 2015) for $C_{19}H_{33}O_4$; ^{1}H NMR Spectral Data (300 MHz, $CDCl_3$): see Table 1; ^{13}C NMR Spectral Data (75 MHz, $CDCl_3$): see Table 1.

Results and Discussions

Structural Elucidation of Isolated Compound 1-2

Compound **1** was obtained as yellow solid, and its molecular formula, $C_{18}H_{30}O_4$, was established from ESI-MS, which gave a peudomolecular ion peak at *m/z* 309.30 $[M-H]^-$. The ^1H NMR spectrum (Fig 1) showed signals due to two conjugated *E,E*-form enone systems [δ_H 7.27 (1H, *dd*, *J* = 15.6 and 10.6 Hz, H-9), 6.41 (1H, *dd*, *J* = 15.3 and 10.6 Hz, H-10), 6.25 (1H, *dd*, *J* = 15.3 and 5.7 Hz, H-11), 6.20 (1H, *d*, *J* = 15.6 Hz, H-8)], one methine [δ_H 4.17 (1H, *m*, H-12)], ten methylene [δ_H 1.25-1.48 (10H, *m*, H-4, 14, 15, 16, 17), 1.50-1.55 (2H, *m*, H-13), 1.55-1.70 (4H, *m*, H-3 and 5), 2.62 (2H, *t*, J = 7.3 Hz, H-6), and 2.28 (2H, *t*, J = 7.3 Hz, H-2)], and one methyl [δ_H 0.91 (3H, *t*, *J* = 6.5 Hz, H-18)] hydrogens.

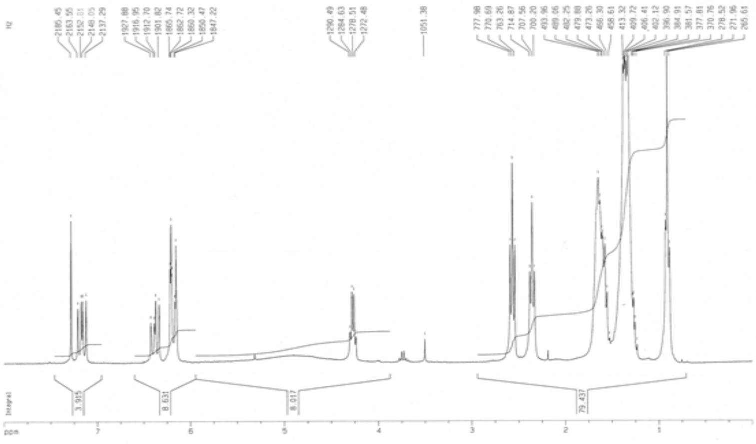

Figure 1. The ^1H NMR spectrum of compound **1** in $CDCl_3$

The ^{13}C NMR and APT spectra exhibited a total of 18 carbon signals, including characteristic signals due to a carboxylic acid at δ_C 177.8 (C-1), a ketone at δ_C 203.6, and four olefinic carbon [δ_C 148.5 (C-11), 144.3 (C-9), 130.3 (C-8), and 128.8 (C-10)], along with a secondary carbinol carbon at δ_C 72.6 (C-12).

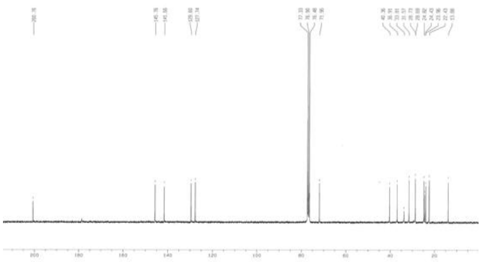

Figure 2. ^{13}C NMR spectrum of compound **1** in $CDCl_3$

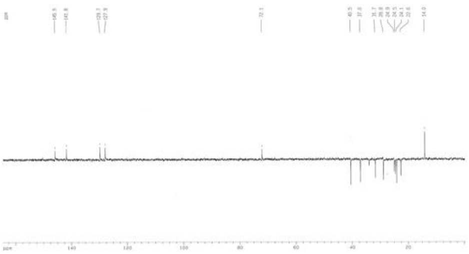

Figure 3. APT spectrum of compound **1** in $CDCl_3$

The ^{1}H-^{1}H COSY (Fig. 6) showed connectivity among H-2, H-3, H-4, H-5 and H-6, and among H-8, H-9, H-10, H-11, H-12, H-13 and H-14, and between H-17 and H-18. The HMBC spectrum (Fig. 4) gave the following long-range correlations: H-2 and C-1, 3, 4; H-6 and C-4, 5, 7; H-8 and C-7, 9; H-9 and C-7, 8, 10, 11; H-11 and C-12, 13; H-12 and C-10, 11, 13; H-18 and C-16, 17, indicating the locations of a carbonyl carbon at C-7 and a tertiary carbon bearing a hydroxyl group at C-12. Moreover, TOCSY spectrum (Fig. 5) in methanol-*d_4* showed correlations between protons H2-6 and H2-2, and between H-12 and H3-18, confirming the position of the carbonyl and the carbinol groups at C-7 and C-12, respectively.

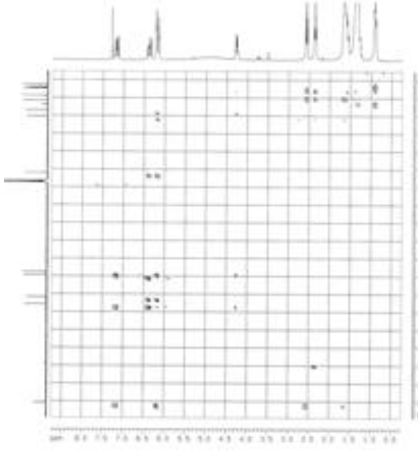

Figure 4. HMBC spectrum of compound **1** in

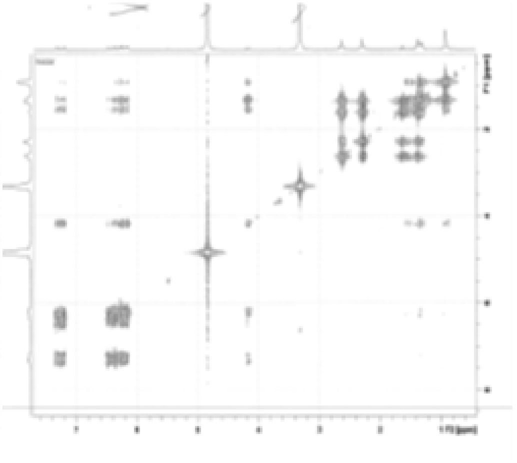

Figure 5. TOSCY spectrum of compound **1** in methanol-*d_4*

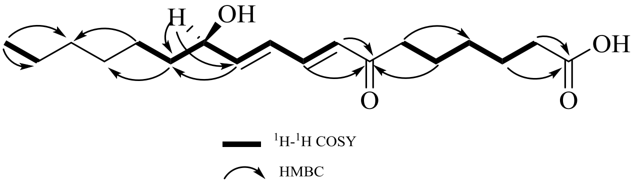

Figure 6. COSY (bold bond) and Key HMBC Correlations of compound **1**

In the ESI-MS of compound **1**, in negative mode, the ion at *m/z* 195 may correspond to the loss of the fragment C(1)-C(6) from the molecular ion at *m/z* 309 $[M-H]^-$.

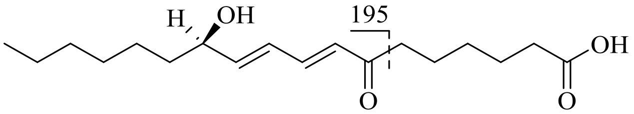

Figure 7. ESI-MS Analysis of Compound **1**

Compound **1** was optically active and the optical rotation $[\square]^{20}_{D}$+3.79 (*c* = 0.0040, DCM) was detected. Thus compound **1** was determined to be (*R*)-(8*E*,10*E*)-12-hydroxy-7-oxo-8,10-octadecadienoic acid.

Compound **2** had the molecular formula $C_{19}H_{32}O_4$ as determined by the ESI-MS, ^{13}C NMR, and APT spectral data. The ^{1}H and ^{13}C NMR data (Table 1) were very similar to those of compound **1**, except for one singlet at 3.67, indicating that this compound was a C18 fatty acid derivative having an α, β, γ, δ-dienone group and the methyl ester of compound **1**.

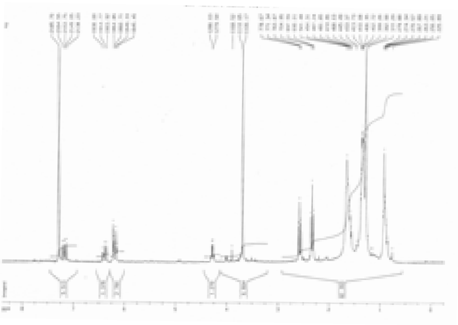

Figure 8. ^{1}H NMR spectrum of compound **2** in $CDCl_3$

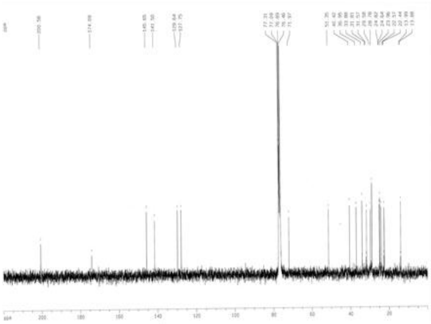

Figure 9. ^{13}C NMR spectrum of compound **2** in $CDCl_3$

Compound **2** contained a straight-chain methyl ester with a conjugated dienone system, indicated by the sequence of COSY correlations and the FT-IR absorption bands. When the optical rotation [□]$^{20}_{D}$+4.06 (*c* = 0.0016, DCM) was measured, the structure of compound **2** was determined to be (*R*)-(8*E*,10*E*)-12-hydroxy-7-oxo-8,10-octadecadienoic acid methyl ester. Chemical assignment for compound **1-2** are in good agreement with those reported data (Yao *et al*., 2015).

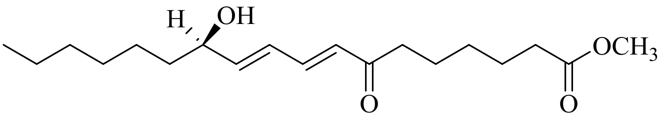

Figure 10. Structure of Compound ZMT.CN-2

Table 1. The ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR Spectral Data of Compound **1-2** in $CDCl_3$

Position	**Compound 1**		**Compound 2**	
	^{1}H NMR (*J* in Hz)	**^{13}C NMR(δ)**	**^{1}H NMR (*J* in Hz)**	**^{13}C NMR (δ)**
1	-	177.8	-	174.5
2	2.28 (2H, *t*, 7.3)	35.0	2.30 (2H, *t*, 7.4)	34.3
3	1.55-1.70 (2H, *m*)	25.9	1.61 (2H, *m*)	25.1
4	1.25-1.48 (2H, *m*)	30.0*	1.33 (2H, *m*)	29.2

Position	**Compound 1**		**Compound 2**	
	^{1}H NMR (*J* in Hz)	**^{13}C NMR(δ)**	**^{1}H NMR (*J* in Hz)**	**^{13}C NMR (δ)**
5	1.55-1.70 (2H, *m*)	26.2	1.61 (2H, *m*)	24.4
6	2.62 (2H, *t*, 7.3)	41.0	2.55 (2H, *t*, 7.4)	40.8
7	-	203.6	-	200.8
8	6.20 (1H, *d*, 15.6)	130.3	6.19 (1H, *d*, 15.5)	130.1
9	7.27 (1H, *dd*, 15.6 and 10.6)	144.3	7.15 (1H, *dd*, 5.5 and 10.8)	141.9
10	6.41 (1H, *dd*, 15.3 and 10.6)	128.8	6.37 (1H, *dd*, 15.3 and 10.8)	128.2
11	6.25 (1H, *dd*, 15.3 and 5.7)	148.5	6.18 (1H, *dd*, 15.3 and 3.9)	146.1
12	4.17 (1H, *m*)	72.6	4.26 (1H, *m*)	72.4
13	1.50-1.55 (2H, *m*)	38.0	1.61 (2H, *m*)	37.4
14	1.25-1.48 (2H, *m*)	25.5	1.33 (2H, *m*)	25.2
15	1.25-1.48 (2H, *m*)	30.0*	1.33 (2H, *m*)	31.9
16	1.25-1.48 (2H, *m*)	32.9	1.33 (2H, *m*)	30.0
17	1.25-1.48 (2H, *m*)	23.7	1.33 (2H, *m*)	22.9
18	0.91 (3H, *t*, 6.5)	14.4	0.87 (3H, *m*)	14.3
1-OCH_3	-	-	3.69 (3H, *s*)	51.8

*Assignments can be interchanged.

Conclusion

Isolation of biologically active compounds from various mushroom species has been increasing due to the wide range of medical activities of mushrooms. Chemical analysis of the fruiting bodies of the mushroom *Cortinarius nemorensis* yielded one conjugated ketonic fatty acid, its methyl ester, 3-hydroxybenzaldehyde and 4-hydroxybenzoic acid. This report describes the isolation and structure elucidation of compound **1-2** which were characterized as (*R*)-(8*E*,10*E*)-12-hydroxy-7-oxo-8,10-octadecadienoic acid and (*R*)-(8*E*,10*E*)-12-hydroxy-7-oxo-8,10-octadecadienoic acid methyl ester. The structures of compounds 1-2 were determined by analysis of NMR and MS data.

Acknowledgements

The authors are indebted to Dr Thar Htun Maung, Rector of Kalay University for his kind permission to submit this paper, Professor Giovanni Vidari for the laboratory facilities, Dr Andrea Gandini for ESIMS, and Professor Mariella Mella for recording NMR spectra on a 300 MHz spectrometer.

References

Chen, H.-P., Z.-Z. Zhao, Z.-H. Li, Y. Huang, S.-B. Zhang, Y. Tang, J.-N. Yao, L. Chen, M. Isaka, T. Feng and J.-K. Liu, (2018). "Anti-proliferative and anti-inflammatory lanostane triterpenoids from the polish edible mushroom *Macrolepiota procera*", *Journal of Agricultural and Food Chemistry*, **66**, 3146-3154.

Clericuzio, M., G.G. Mellerio, P.V. Finzi and G. Vidari, (2018). "Secondary Metabolites Isolated from *Tricholoma* Species (Basidiomycota, Tricholomatacee): A Review", *Natural Product Communications*, **13** (9), 1213 – 1224.

Nicholas, G.M., J.W. Blunt and M.H.G. Munro, (2001). "Cortamidine Oxide, a Novel Disulfide Metabolite from the New Zealand Basidiomycete (Mushroom) *Cortinarius* Species", *Journal of Natural Products*, **64** (3), 341-344.

Öztürk, M., G. Tel-Cayan, A. Muhammad, P. Terzioglu, M.E. Duru, (2015) "Chapter 10 - Mushrooms: A Source of Exciting Bioactive Compounds", *Studies in Natural Products Chemistry*, **45**, 363-456.

Teichert, A., J. Schmidt, A. Porzel, N. Arnold and L. Wessjohann, (2007). "Brunneins A–C, β-Carboline Alkaloids from *Cortinarius brunneus*", *Journal of Natural Products*, **70** (9), 1529–1531.

Yao, D.-L., C.-H. Zhang, R. Li, J. Luo, M. Jin, J.-H. Piao, M.-S. Zheng, J.-M. Cui, J.K. Song and G. Li, (2015). "Two new conjugated ketonic fatty acids from the stem bark of *Juglans mandshurica*", Chinense Journal of Natural Medicines, **13** (4), 0299-0302.

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In Vitro α-Amylase and α-Glucosidase Inhibitory Activity of *Dioscorea bulbifera* Tubers (Hpwut-Sa-U)

Kalya Cho[1], Myat Thazin Mon[2] & Ohnmar Tun[3]

Abstract

One of the antidiabetic therapeutic approachs is to reduce gastrointestional glucose production and absorption through the inhibition of carbohydrate digesting enzymes such as α-amylase and α-glucosidase. The aim of the current study was to screen the phytoconstituents present in the PE, EtOAc and EtOH extracts of *D. bulbifera* tubers and their efficiency to inhibit α-amylase and α-glucosidase. The phytochemicals tests of crude extracts of sample revealed that the presence of alkaloids, saponins, phenolic compounds, steroids and terpenoids. *In vitro* α-amylase inhibitory activity was determined by starch iodine method and the resultant activities were compared with antidiabetic drugs; acarbose and metformin. The order of α-amylase inhibitory activities were found as acarbose > metformin > EtOAc extract > EtOH extract > PE extract. Furthermore, α-glucosidase inhibitory effect of these crude extracts was evaluated by using p-nitrophenyl-α-D-glucopyranoside as substrate. The IC_{50} values of acarbose, metformin, PE, EtOAc and EtOH extracts were found to be 28.00, 40.50, 49.50, 35.0 and 37.50 μg/mL, respectively.

Keywords: *D. bulbifera*, antidiabetic, α-amylase, α-glucosidase

Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia in postprandial and fasting state (Quaisar *et al.*, 2014). One therapeutic approach which may prove to be benefical for treatment of diabetes is to decrease the postprandial hyperglycemia. This can be achieved by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes in the digestive tract. The α-amylase and α-glycosidase enzymes are responsible for the breakdown of polysaccharide or disaccharide to monosacharide. Inhibitors of these enzymes delay carbohydrate digestion and causing a marked decrease in the rate of glucose absorption thereby blunting the post prandial plasma glucose

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rise (Bhutkar and Bhise, 2012). Thus, discovery of a suitable inhibitor of α-amylase and α-glycosidase with minimum side effects poses a challenge in the search for a potent therapeutic agent (Ghosh *et al*., 2012).

Several enzyme inhibitors have been isolated from medicinal plants for the development of new drugs with increased potency and lower adverse effects than existing drug (Quaisar *et al*., 2014). *Dioscorea bulbifera* L. is a glabrous nonspiny climber of 10-20 feet high with bulbils 1-8 cm in size. Tubers are renewed annually and possess profound therapeutic potential due to the presence of bioactive secondary metabolites such as steriodal saponin, phenolic compounds and terpenoids (Mbiantcha *et al*., 2011). The present study was undertaken to evaluate the inhibitory effect of the extracts of *D. bulbifera* tubers on α-amylase and α-glycosidase enzymes.

Materials and Methods

Reagents

α-amylase (Jiangsu Boli Bioproducts Co., Ltd), starch, iodine, disodium hydrogen phosphate, sodium dihydrogen orthophosphate, acarbose (Bayer Pharma AG, Kaiser-Whilheim-Allee, Leverkusen, Germany), Metformin (Denk Pharma Gmbtt Co., Ltd Munchen, Germany), α-glucosidase (Cool Chemical Science and Technology Ltd. Beijing), p-nitrophenyl-α-D-glucopyronoside (Wako Chemical. Ltd), sodium carbonate (BDH) and deionized water

Plant Material

Tubers of *D. bulbifera* were collected from the Mawlamyine University Campus. The plant material was identified and authenticated at the Department of Botany, Mawlamyine University. The collected tubers were chopped into pieces and shade dried at room temperature. Dried plant material was subjected to size reduction to a coarse powder by using grinder.

Preparation of Plant Extracts

About 100 g of powdered plant material was extracted successively with petroleum ether, ethyl acetate and ethanol by hot percolation method using Soxhlet apparatus. PE, EtOAc and EtOH extracts were evaporated to dryness under reduced pressure at 40 °C in rotary evaporator and stored in air-tight container in refrigerator at 4 °C.

Phytochemical Screening from Crude Extracts

Phytochemical examinations of all the extracts were carried out by using the following tested and reagents; alkaloids with Mayer's and Dragendroff's tests, glycosides with Keller Killani test, saponin with foam test, phytosterols with Libermann Burchard's test, phenols with Ferric chloride test, tannins with Gelatin test, and flavonoids with alkaline reagent test (Tiwari *et al.,* 2011).

α-Amylase Inhibition Assay

The α-amylase inhibitory activity of PE, EtOAc and EtOH extracts of *D. bulbifera* tubers was assayed using a modified Bernfeld method using starch as substrate (Genapaty *et al*., 2013).10 μL of α-amylase solution (0.3 U/mL) was mixed with 390 μL of each different concentrations of sample and standard solution. After incubation at 37 °C for 10 min, 10 μL of 1 % starch solution was added to the mixture and re-incubated at 37 °C for 30 min. Then 0.1 mL of 1 % iodine solution was added to the reaction mixture. After adding 5 mL of distilled water, the absorbance of the reaction mixture was measured at 565 nm by using UV-spectrometer. Control determination without test sample and blank without starch were carried out under the same reaction conditions. Inhibition of enzyme activity was calculated by the following equation

$$\text{\% inhibition of enzyme activity} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

where, A_{control} = absorbance of the control

A_{test} = absorbance of the sample

The IC_{50} value was calculated by linear regressive excel program.

α-Glucosidase Inhibition Assay

The α-glucosidase inhibitory activity was assessed by standard method with slight modification (Quisar *et al*., 2014). 50 μL of α-glucosidase solution (0.1 U/mL) was mixed with 60 μL of each different concentration of test samples and standard drugs and incubated at 37 °C for 20 min. After pre-incubation, 50 μL of 2 μM p-nitrophenyl-α-D-glucopyranoside solution in 80 mM phosphate buffer (pH-6.8) was added to each tube and incubated at 37 °C for another 20 min. Then the reaction was stopped by adding 160 μL of 100 mM Na_2CO_3 and the absorbance readings were recorded at 405 nm. Control determination (without test samples) and blank determination (without α-glucosidase) were done under the same reaction conditions. All experiments were done in triplicate for each sample solution. The α-glucosidase inhibitory activity was expressed as inhibition % and was calculated as follows.

$$\% \text{ Inhibition } = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

where, A_{control} = absorbance of the control

A_{test} = absorbance of the test sample

The concentration of inhibitors required for inhibiting 50 % of the α-glucosidase activity under the assay conditions was defined as the IC_{50} value. Then IC_{50} values was calculated by linear regressive excel program.

Statistical Analysis

All the analyses were carried out in triplicate and the results were expressed as mean ± standard deviation.

Results and Discussion

Phytochemicals present in crude extracts of tubers of *D. bulbifera*

In order to know types of phytochemical constituents that are present in crude extracts of Tubers of *D. bulbifera,* the phytochemical tests were carried out and the results obtained from these experiments are summarized in Table 1.

According to these experiments, the secondary metabolites such as glycosides, saponins and flavonoids were found to be present in all extracts. However, some phytoconstituents were absent in some extracts. The variation in the result could be due to the difference in the polarity of solvents used for extraction. Phenolic compounds, tannins and terpenoids were found to be present in EtOAc and EtOH extracts but absent in PE extract. Alkaloids were found to be present in EtOH extract. The results of these experiments showing ethylacetate and ethanol could be the ideal solvent system for the extraction of phytochemicals due to the presence of bioactive secondary metabolites such as saponins, flavonoids, steroids, phenolic compounds, tannins and terpenoids.

Table 1. Phytochemical Analysis of Various Extracts of Tubers of *D. bulbifera*

Phytoconstituents	**Tubers of *D. bulbifera***		
	PE Extract	**EtOAc Extract**	**EtOH Extract**
Alkaloids	–	–	+
Glycosides	++	+	+
Saponins	+	+	+
Phenolic compounds	–	+	++
Flavonoids	+	++	++
Tannins	–	+	+
Steroids	+	++	++
Terpenoids	–	++	++

(–) = Not present + = Present (in lower amount)
++ = Present (in higher amount)

In vitro Antidiabetic Effect of Crude Extracts from Tubers of *D. bulbifera*

The treatment goal of diabetic is to maintain near normal level of glycemic control in both fasting and post-prandial conditions. Many natural sources have been investigated with respect to suppression of glucose production from the carbohydrates in the gut or glucose absorption from the intestine. α-Amylase catalyses the hydrolysis of α-1,4-glucosidic linkages of starch, glycogen and various oligosaccharides to disaccharide and α-glucosidase further breaks down these disaccharides to simple sugars for intestinal absorption. The inhibition of their activities in the digestive tract is considered to be effective tool to control diabetes (Genapaty *et al.*, 2013).

α-Amylase inhibitory activity

α-Amylase inhibitory activity was measured *in vitro* by hydrolysis of starch in the presence of α-amylase enzyme. The amount of starch remained was determined by using iodine which gave blue colour with starch. The reduced intensity of blue colour indicated the enzyme-induced hydrolysis of starch. In the other words, the more the intensity of blue colour, the higher the inhibitory activity of the test sample on α-amylase enzyme. The extracts exhibited IC_{50} less than 100 μg/mL will be considered active in comparison with antidiabetic drug metformin and acarbose (Ganapaty *et al.*, 2013). Table 2 and Figure 1 show the inhibitory of α-amylase by the extracts of tubers of *D. bulbifera*. All test extracts showed dose dependent inhibitory of enzyme. The IC_{50} values of pet-ether, ethyl acetate, ethanol as well as metformin and acarbose were found to be 172.50, 165.00, 62.50, 60.00 and 42.50 μg/mL, respectively. Among these extracts, ethyl acetate extracts showed the significant α-amylase inhibitory activity (Figure 2). Its IC_{50} value (62.50 μg/mL) was higher than acarbose (IC_{50} 42.50) but nearly the same as metformin (IC_{50} 60.00 μg/mL).

Table 2. α-Amylase Inhibition % and IC_{50} Values of Various Crude Extracts of Tubers of *D. bulbifera* Compared with Metformin and Acarbose

Sample	**% Inhibition (mean ± SD) in different concentrations (μg/mL)**					**IC_{50} (μg/mL)**
	25	**50**	**100**	**200**	**400**	
PE (extract)	14.75 ± 0.69	24.35 ± 1.84	38.44 ± 1.06	55.82 ± 1.53	69.26 ± 1.76	172.50
EtOAc (extract)	34.10 ± 1.38	44.71 ± 0.89	72.59 ± 1.95	78.11 ± 0.91	84.26 ± 0.61	62.50
EtOH (extract)	15.65 ± 0.73	28.37 ± 0.89	43.02± 0.65	55.34 ± 1.17	61.81 ± 1.00	165.00
*Metformin	21.85 ± 0.73	45.30 ± 1.01	66.76± 1.07	75.09 ± 0.85	86.61 ± 0.90	60.00
*Acarbose	25.98 ± 0.66	60.32 ± 0.21	79.86± 0.73	85.96 ± 1.11	95.06 ± 0.89	42.50

Data are expressed as means of triplicate determination ± standard deviation.
* = Antidiabetic drug

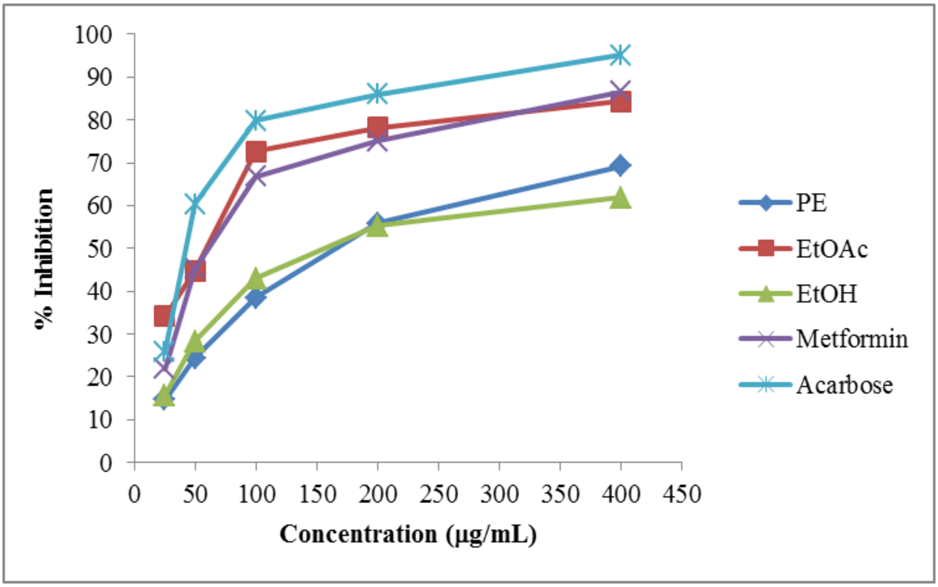

Figure 1. α-Amylase inhibition activity of crude extracts of tubers of *D.bulbifera*, metformin and acarbose at different concentrations

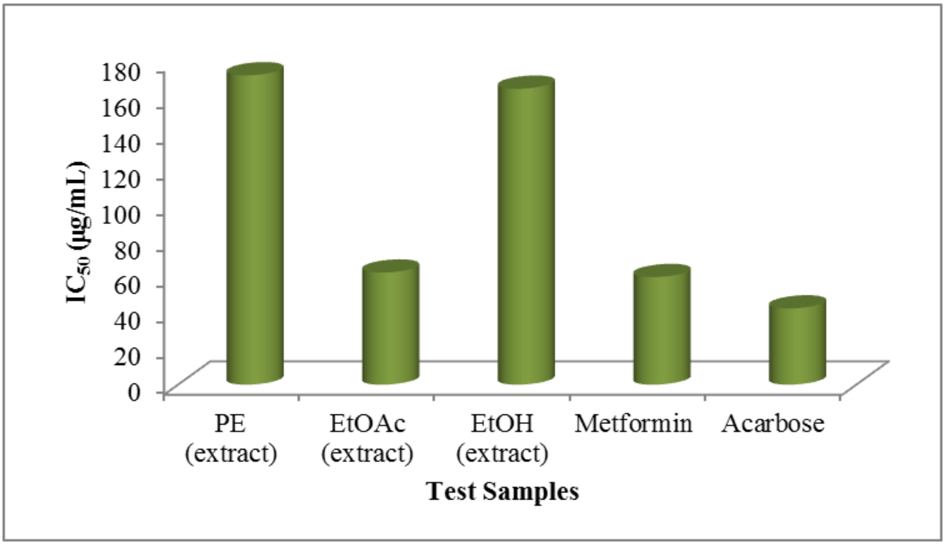

Figure 2. 50% α-Amylase inhibitory concentration (IC_{50}) values of crude extracts of tubers of *D. bulbifera* compared with metformin and acarbose

α-Glucosidase inhibitory activity

α-Glucosidase inhibitory activity was assayed by using the substrate p-nitrophenyl-α-D-glucopyranoside which was hydrolyzed by α-glucosidase to release the product p-nitrophenol, a colour agent that could be monitored at 405 nm. Table 3 and Figure 3 show the α-glucosidase inhibitory activity of each extract of tubers of *D. bulbifera*. The order of α-glucosidase inhibitory activity was found to be acarbose (IC_{50} 28.00 μg/mL) > ethyl acetate (IC_{50} 35.00 μg/mL) > ethanol (IC_{50} 37.50 μg/mL) > metformin (IC_{50} 40.50 μg/mL) > pet ether (IC_{50} 49.50 μg/mL). The ethyl acetate and watery extracts exhibited significant α-glucosidase inhibitory activity in comparison with the metformin and acarbose (Figure 4). From the result, it was evident that the ethyl acetate extract showed the highest α-glucosidase inhibitory activity due to its IC_{50} value was smaller than that of ethanol and pet-ether extracts.

Table 3. α-Glycosidase Inhibition % and IC_{50} Values of Various Crude Extracts of Tubers of *D. bulbifera* Compare with Metformin and Acarbose

Sample	**% Inhibition (mean ± SD) in different concentrations (μg/mL)**					**IC_{50} (μg/mL)**
	25	**50**	**100**	**200**	**400**	
PE (extract)	28.31± 0.45	51.81± 0.53	70.05± 0.45	76.36± 0.39	80.22± 0.81	49.50
EtOAc (extract)	39.78± 0.47	65.85± 0.85	75.20± 0.88	85.21± 0.95	86.14± 0.64	35.00
Watery (extract)	31.35± 0.69	68.18± 0.35	76.34± 0.59	82.11± 0.58	88.73± 0.73	37.50
*Metformin	34.04± 1.09	58.33± 1.08	68.44± 0.84	75.18± 1.07	81.41± 1.30	40.50
*Acarbose	44.30± 1.40	72.87± 0.98	85.33± 1.13	89.22± 0.59	93.32± 0.78	28.00

Data are expressed as means of triplicate determination ± standard deviation.

* = Antidiabetic drug

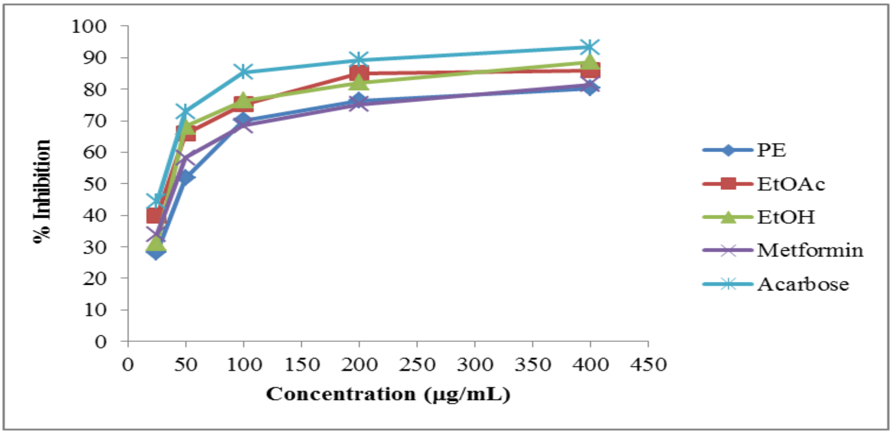

Figure 3. α-Glucosidase inhibition activity of crude extracts from tubers of *D. bulbifera*, metformin and acarbose at different concentrations

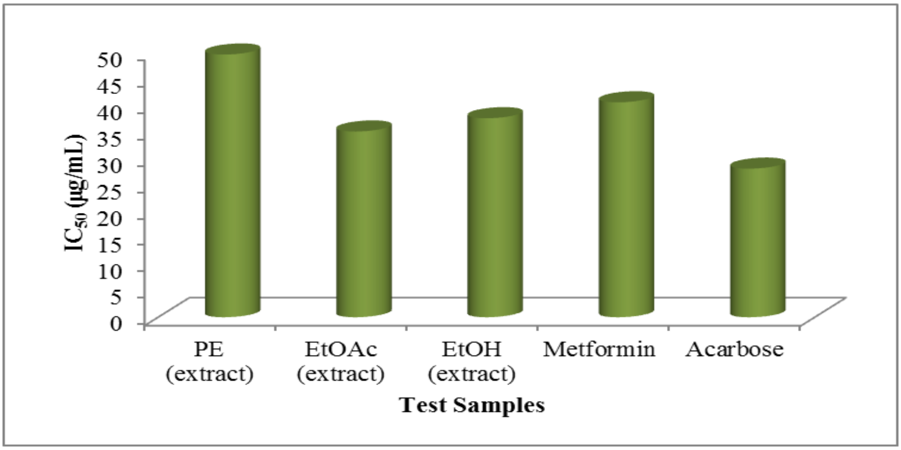

Figure 4. 50% α-Glucosidase inhibitory concentration (IC_{50}) values of crude extracts of tubers of *D. bulbifera* compared with metformin and acarbose

Conclusion

From the overall assessment of the present research work, the following inferences could be drawn. The phytochemical screening of PE, EtOAc and EtOH extracts observed that the secondary metabolites such as alkaloids, saponins, phenolic compounds, steroid and teprenoids were found to be present in each extract.

In vitro α-amylase inhibition activity was measured by starch iodine method and it was found that IC_{50} value of PE, EtOAc and EtOH extract were 172.50, 62.50 and 165.00 μg/mL, respectively. Among these extracts, EtOAc extract showed significant α-amylase inhibition activity due to its IC_{50} value was nearly the same as antidiabetic drug metformin (IC_{50} = 60 μg/mL).

In vitro α-glucosidase inhibition activity was determined by using p-nitrophenyl-α-D-glucopyranoside as substrate and 50 % α-glycosidase inhibitory concentration (IC_{50}) of PE, EtOAc and EtOH were found to be 49.50 μg/mL, 35.00 μg/mL and 37.50 μg/mL, respectively. These IC_{50} values were compared with IC_{50} values of metformin (40.50μg/mL) and acarbose (28.00μg/mL). The *in vitro* α-glucosidase inhibition activity of EtOAc extract was found to be higher than that of PE and EtOH extracts but lower than antidiabetic drugs.

From the result, it can be concluded that the EtOAc extract of *D. bulbifera* tubers has adequate antidiabetic potential by its ability to inhibit α-amylase and α-glucosidase.

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References

Bhutkar, M. A. and Bhise, S. B. (2012). "*In Vitro* Assay of Alpha Aymlase Inhibitory Activity of some Indigenous Plant". *Int.J.Chem.Sei*, "**10**",.437-462

Ghosh, S., Ahire, M., Patil, S., Jabgunde, A., Dusane, M. B., Joshi, B. N., Pardesi, B. N., Jachak, S., Dhavale, D. and Chopade, B. A. (2012). "Antidiabetic

Activity of *Gnidia glauca* and *Dioscorea bulbifera* : Potent Amylase and Glucosidase Inhibitors". *Handawi*, **10**”,1-10

Ganapaty, S., Nandeesh, R., Veerapur, V. P., Thippeswamy, B. S. and Shivasharan, D. (2013). "*In Vivo* and *In Vitro* Anti-Diabetic Effects of *Madhuca indica* Roxb., in Alloxan-Induced Diabetic Rats". *IJAPBC,* **2**”,282-290

Mbiantcha, M., Kamanyi, A. and Watcho, P. (2011). "Analgesic and Anti-Inflammatory Properties of Extracts from Bulbils of *Dioscorea bulbifera* L. var sativa (*Dioscoreaceae*) in Mice and Rats", Hindawi Publishing Corporation, “**10**”, 1-9

Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and Kaur, G. (2011). "Phytochemical Screening and Extraction: A Review". *IPS,* **1**”, 98-106

Qaisar, M. N., Chaudhary, B. A., Sajid, M. U. and Hussain, N. (2014). "Evaluation of α-Glucosidase Inhibitory Activity of Dichloromethane and Methanol Extracts of *Corton bonplandianum Biall*". *Trop.J.Pharm.Res.*, **13**”,1833-1836

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Study of the Physicomechanical Properties and Biodegradability of Banana Peel—Starch Based Bioplastics

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Abstract

Plastic causes environmental pollution because of having non-biodegradable characteristics and they are full of harmful by-products and chemicals which are released during their break down process. Thus, the biodegradable plastic becomes a promising solution to solve all this problems. This research was based on the preparation of bioplastics from banana peel starch. Bioplastics of banana peel and Polyvinyl alcohol (PVA) blend were prepared by casting method. Different blends were made by varying the concentration of PVA and banana peel starch. The physico-mechanical properties of thickness, tensile strength (TS), elongation at break (EB) and tear strength of the prepared bioplastics wereinvestigated. The solubility of the prepared bioplastics were determined in different solvents. The biodegradable nature of prepared blended bioplastics were studied according to the soil burial test.

Keywords: biodegradable plastics, banana peel starch, polyvinyl alcohol

Introduction

The major sources of land pollution include plastics, metal and glass containers, food wrapping, worn-out machinery, old furniture, garbage, etc (Modebelu M. N., Edward I,2014). The plastics in excess produced will be deposited as a landfill and are degraded very slowly, which can cause the original products to remain in the landfills for hundreds or even thousands of years (Raaz., M, and et.al, 2013). The prominence of plastic pollution is correlated with plastics being economical and durable, which lends to high levels of plastics used by humans (Hester and et.al,2011).Since plastics are vital to people's everyday lives, production of biodegradable plastics to make plastics more compatible with the environment is necessary.

Biodegradable plastics are made from biomass which is a completely biodegradable and renewable resource. The use of starch based films for flexible packaging has been limited because of their weak mechanical

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properties and high sensitivity to moisture. (Azeredo, H.M.C.,de., 2009). Due to the solubility and biodegradability of PVA, PVA films are increasingly used in packaging applications(Pavol, and et.al., 2002).

Starch is one of the major sources in the development of bioplastics. Many previous studies have been conducted by using starch as a natural biopolymer. Due to its large availability, low cost, renewability and biodegradability, starches are commonly used in the production of bioplastics.

Plasticizer is a material incorporated into a plastic material, and increases the flexibility and applicability of the plastic material. Plasticizer molecules penetrate starch granules, and destroy the inner hydrogen bonds of starch in high temperature, pressure, and shearing.

One of the most common waste form of starch is the banana peels. Banana (*Musa paradisiaca)* belongs to family Musaceae is a very common plant in tropical and subtropical countries. The present research aims at preparation of biodegradable plastics from fruit waste, banana peels, using plasticizers.

Botanical Description

Family Name	-Musaceae
Englisg Name	-Banana
Botanical Name	-*Musa acuminate*
Part Used	-Peels

Figure 1. Photograph of banana fruits

Aim and Objectives

Aim

The aim of this research is to study the physicomechanical properties and biodegradabilityof banana peel starch-based bioplastics.

Objectives

- To collect banana peels, polyvinyl alcohol (PVA) and plasticizers
- To prepare banana peel starch
- To determine the qualitative test for starch

- To prepare the blended bioplastic
- To measure the physico-mechanical properties of prepared blended bioplastic
- To investigate the biodegradability of blended bioplastic

Materials and Methods

Sample Collection

Banana peels were collected from Amarapura Township, Mandalay Region.

Preparation of Treated Banana Peel Paste

Banana peels were removed using stainless steel knife and converted into small pieces. Approximately 100 g of banana peels were dipped in sodium metabisulphite (0.2M) solution for 45 minutes. It is used as antioxidant and preservative. The peels were placed in the beaker containing 800 mL distilled water and boiled for 30 minutes. After the boiling process, the peels were left to dry on gauze pad for 30 minutes at room temperature. Using the blender, the peels were pureed until a fluid paste was formed. The fluid paste was filtered to produce banana peel starch.

Qualitative lodine Test for Starch

Iodine (3.0g) and potassium iodide (3.0 g) were ground and the mixture was dissolved in 60 ml of distilled water. Then the solution was diluted to 100 ml with distilled water in a volumetric flask. The prepared banana peel paste was qualitatively examined by iodine staining method. Banana peel paste (1g) was added into a beaker containing 10 ml of boiled distilled water and then boiled for 10 min. The solution was cooled down to room temperature. Two drops of iodine solution was added in the solution. The colour changes was examined.(BeMiller.1986, and Fruton, Simonds, 1958).

Preparation of Bioplastics from Banana Peel Starch

A starch based film was prepared using casting method.

Banana peel starch (6 g) was placed in a 250 mL beaker. After that, 3 mL of (0.5 M) HCl was added to the beaker and the mixture was stirred using a glass rod. And then 3 mL (0.5 M) NaOH was added to the mixture and stirred. Polyvinyl alcohol was added and the mixture was stirred once more. The solution was mixed by gentle stirring for about 15 min while slow heating. Stirring and heating were ended when the solution reaches temperature of 70-75°. The film forming solution was taken out and spread evenly into a Petri dish. The sheet was removed from Petri dish after cooling at room temperature for 3 days. In this way the bioplastics with starch and PVA (2.5 g) was obtained. Similarly, bioplastics with starch and PVA (2 g and 3 g) were prepared by adding different amounts of PVA .

Preparation of Bioplastics from Banana Peel Starch with Different Plasticizers

From the bioplastics from banana peel starch and PVA (2 g, 2.5 g, and 3 g), banana peel starch and PVA blended bioplastics (6 g: 2.5 g) was chosen to find the plasticizers such glycerol and sorbitol. Glycerol (0.13g) was added to the banana peel starch and PVA blended solution (6 g: 2.5 g). The solution was mixed by gentle stirring for about 15 min while slow heating. Stirring and heating were ended when the solution reaches temperature of 60-65°C. Then, 50 mL of the film forming solution was take out and spread evenly into a petri dish and kept at room temperature for 3 days. Thus, the ratios of bioplastics were found to be (2 g: 2.5 g: 3 g).

Determination of Solubility of Blended Bioplastics with and without Plasticizers

All the samples were cut into small pieces and weighed. Then these samples were inserted into the test tubes containing different solvents. The suspension were mixed and allowed to dehydrate for an hour.

Determination of Swelling Test of Blended Bioplastics with and without Plasticizers

A preweighed piece of samples were taken in the test tube and were carried out on the medium containing various solvents such as water, chloroform, and pet-ether medium. The deliberated samples were kept in the medium for about 2 hours and the results were recorded accordingly.

Determination of Mechanical Properties of Prepared Blended Bioplastics with and without Plasticizers

Determination of Thickness

Thickness of the prepared banana peel starch- PVA blended bioplastics was measured by using NSK micrometer. The thickness of the blended bioplastics was measured at five locations (center and 4 corners) using micrometer (Chandra, 1995 and website 1).

Determination of Tensile Strength and Percent Elongation at Break

Dog bone shaped test specimens were cut from the blended bioplastics. Both ends of the test pieces were firmly clamped in the jaw of the test machine. One jaw was fixed and the other was moveable. The rate of moveable jaw was hold 100 mm/min. The recorder of the machine showed the tensile strength in Mpa. This procedure for tensile strength was repeated for three times.$Tensile\ Strength(MPa) = \frac{LoadedWeight(N)}{crosssectionalarea\ (mm^2)}$

$$Elongationat\ Break(\%) = \frac{\Delta L \times 100}{L}$$

Where, ΔL = Different Length, L= Original Length

Determination of Tear Strength

Test specimen was cut off by a die from the above blended bioplastics. Specimen was cut with a single nick (0.04 mm) at the entire of the inner concave edge by a special cutting device using a razor blade. The clamping of the specimen in the jaw of test machine was aligned with travel direction of the grip in 100 mm/min. The recorder of the machine was shown the highest force to tear from a specimen nicked. The procedure was done in triplicate. Tear strength can be calculated according to the formula.

$$Tear\ Strength\left(\frac{kN}{m}\right) = \frac{ForceatBreak}{Thickness}$$

Biodegradability of Blended Bioplastics with and without Plasticizers

In Dry Soil

Biodegradability test was done by using the soil burial method (Naryan, R., et.al., 1999). The samples were cut into 30 × 50 mm pieces and buried in the soil at a depth of 10 cm. The sample was dried in desiccators until their weights became constant (W1). These samples were then buried in dry soil for 1 week. After that, the samples were dried in a desiccators until their weights became constant (W2). The results are shown in Table (5). The percentage of weight loss, (%W) can be calculated from the following equation: $\% W = \frac{W1-W2}{W1} \times 100$

In Wet Soil

The samples were cut into 30 × 50 mm pieces and buried in the soil at a depth of 10 cm. The soil was placed in the laboratory, and the moisture of the soil was maintained by sprinkling water at regular time intervals. The excess water was drained through a hole at the bottom of the pot. The degradation of the samples was determined at regular time intervals (7 days) by carefully removing the sample from the soil and washing it gently with distilled water to remove soil from the film. The sample was dried under vacuum until a constant weight was obtained. Weight loss of the sample over time was used to indicate the degradation rate of the soil burial test. The results are shown in Table (6).The percentage of weight loss, (%W) can be calculated from the following equation:

$$Weightloss\ (\%) = \frac{Wi - Wd}{Wi} \times 100$$

where *Wd* is the dry weight of the film after being washed with distilled water, and *Wi* is the initial dry weight of the specimen.

Results and Discussion

Qualitative Examination of Starch

Iodine was used test for starch. The colour of prepared starch solution and after addition of iodine solution was shown in Figure (2) . Blue colour appeared by the reaction of iodine with starch. The iodine amylose complex gives the characteristic blue colour. Blue colour appeared due to the fact that the iodine (I_3^- and I_5^-) fits inside the coils of amylose, the charge transfers between I_2^- and starch, and the energy level spacing in the resulting complex compound to the absorption in the visible light region.

Examination of Solubility for Blended Bioplastics with and without Plasticizers

In order to characterize blended bioplastics, solubility tests were carried in different solvents and solutions. The results were shown in Table (1). It was found that blended bioplastics are soluble in water but insoluble in dilute hydrochloric acid, dilute sulphuric acid, chloroform, pet-ether, acetone, ethanol and methanol. Thus blended bioplastics is a hydrophilic polymer.

Table 1. Solubility Tests of Blended Bioplastics with and without Plasticizers

No	Solvent /Solution	Observation
1	Distilled water	+
2	Ethanol	-
3	Methanol	-
4	Dilute hydrochloric acid	-
5	Dilute sulphuric acid	-
6	chloroform	-
7	acetone	-
8	Pet-ether	-
9	Dilute potassium hydroxide	+

(+ = soluble, - = insoluble)

Determination of Swelling Tests of Blended Bioplastics

Swelling study is generally conducted to check whether developed material retains the original properties when it was formed during the preparations.

Table 2. Swelling Test Results of Blended Bioplastics

	Solvent medium	Quality (mL)	Initial weight of the sample (g)	Final weight of the sample (g)	Difference of weight (g)
Bioplastic derived from banana peel	Distilled water	20	0.20	0.32	0.12
	Chloroform	5	0.13	0.14	0.01
	Dilute hydrochloric acid	20	0.13	0.16	0.03

According to the data, the highest solubility was found in distilled water because the amylopectin branch structure of the starch is present in analyzed sample. The water absorption and water solubility of the bioplastics are directly proportionate. This finding is a normal behavior as an increase in water absorption will make the bioplastics easier to dissolve in water. When water is absorbed onto the hydrophilic group, mainly through hydrogen bonding or weak electrostatic interactions, the bioplastics can be easily dissipated with water molecules. In this data, the weight loss of water solubility in distilled water is higher than that in acidic solution. This is because the bioplastics is capable of dissolving in distilled water rather than in acidic solution.

Physico-mechanical properties of Prepared Blended Bioplastics with and without Plasticizers

Banana peel starch based bioplastics with various ratios of polyvinyl alcohol blended bioplastics were prepared. Then, starch- polyvinyl alcohol-glycerol bioplastics, starch-polyvinyl alcohol-sorbitol bioplastics and starch-polyvinyl alcohol-glycerol and sorbitol bioplastics were prepared by using banana peel starch with various ratios of polyvinyl alcohol. Physico-mechanical properties, such as, thickness, tensile strength, elongation at break and tear strength of prepared blended bioplastics were investigated. These results were described in Table(3), Table(4) and Figure (2), Figure(3).

Starch: PVA 6 g:2 g Starch: PVA 6 g:2.5 g Starch: PVA 6 g:3 g

Figure 2. Starch and various ratios of polyvinyl alcohol blended bioplastics

Table 3. Physico-mechanical Properties of Starch-PVA Blended Bioplastics

Properties	**Starch-PVA Blended Bioplastics**		
	6 g : 2 g	**6 g : 2.5 g**	**6 g : 3 g**
Thickness (mm)	0.15	0.18	0.20
Tensile Strength (MPa)	12.20	15.80	27.80
Elongation at break (%)	285.00	301.00	328.00
Tear Strength (kN/m)	62.70	57.30	53.40

According to these table, banana peel starch-polyvinyl alcohol (6 g : 3 g) gave the highest values of tensile strength (27.80 MPa) and elongation at break (328.00%). Starch-polyvinyl alcohol (6 g : 2 g) blended bioplastics shows the highest tear strength (62.70 kN/m).

Starch:PVA:Sor+Gly Starch:PVA:Sorbitol Starch:PVA:Glycerol

Figure 3. Starch and various ratios of polyvinyl alcohol blended bioplastics with different plasticizers

Table 4. Physico-mechanical Properties of Starch-PVA Blended Bioplastics with Different Plasticizers

Properties	**Starch-PVA Blended Bioplastics with Plasticizers**		
	Sorbitol+ Glycerol	**Sorbitol**	**Glycerol**
Thickness (mm)	0.18	0.20	0.30
Tensile Strength (MPa)	13.90	26.60	10.80
Elongation at break (%)	365.00	342.00	402.00
Tear Strength (kN/m)	34.00	63.00	22.30

According to these table, banana peel starch-polyvinyl alcohol-glycerol gave the lowest values of tensile strength (10.80 MPa) and highest elongation at break (402.00%). Starch-polyvinyl alcohol-sorbitol blended bioplastic shows the highest tensile strength (26.60 MPa) and tear strength (63.00 kN/m).

Biodegradability of Blended Bioplastics

In this work, biodegradation of banana peel starch-PVA (2 g, 2.5 g, and 3 g) with and without plasticizers were tested by soil burial method. Soil burial is a traditional way to test sample for degradation because of this similarity of actual condition of waste disposal. The results are shown in following Tables (5), (6). These bioplastics clearly showed that some deformation of these bioplastics started after 3 days and significant degradation was found after one week for blended bioplastics.

Table 5. Characteristics of Biodegradable Blended Bioplastics

Test		**Biodegradable blended bioplastics**		
		Starch (6 g)+ PVA (2g)	**Starch (6 g)+ PVA (2.5g)**	**Starch (6 g)+ PVA (3g)**
Biodegradability (%)	In Dry Soil	51.82	40.59	40.00
	In Wet Soil	93.75	81.82	73.08

Table 6. Characteristics of Biodegradable Blended Bioplastics with Different Plasticizers

Test		**Biodegradable plastics**		
		Sorbitol	**Glycerol**	**Sor + Gly**
Biodegradability (%)	In Dry Soil	69.63	76.54	61.43
	In Wet Soil	94.09	95.41	72.14

Conclusion

Starch PVA blend is a biodegradable blend made from sustainable resources. This blend may be used for various applications which include food packaging, biodegradable packaging etc. as a single material or in combination with other materials. For these above mentioned applications, the studies of mechanical and thermal properties are important.

In this research work, blended bioplastic consisting of banana peel starch and various amounts of polyvinyl alcohol with different plasticizer (Glycerol and Sorbitol) were prepared. Among the various amount of PVA solution, 2.5 g PVA solution was selected for the blended bioplastic with different plasticizers. To investigate the effect of plasticizers, two different plasticizers such as glycerol and sorbitol were chosen. The blended films were also prepared by using banana peel starch, PVA and different plasticizers such as glycerol and sorbitol.

The solubility of blended bioplastic was found to be increased with increasing temperature. Blended bioplastic is soluble in water and dilute potassium hydroxide but insoluble in ethanol, methanol, dilute hydrochloric acid, dilute sulphuric acid, dilute acetic acid, chloroform, pet-ether and acetone. Thus blended bioplastic is a hydrophilic polymer.

From the prepared blended bioplastics, the amount of PVA increases with the highest tensile strength and elongation at break and the lowest the tear strength. Banana peel starch-PVA-glycerol blended bioplastics gave the highest elongation at break and lowest in tear strength. Therefore, it is suitable to use food packaging material because it can dissolve in water and it is also swelled by testing with water.

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References

Azeredo, H.M.C.de., (2009), “Nano-composites for Food Packaging Applications” Food Research International, 42, 1240-1253.

BeMiller, J.M., (1986), “Chemistry and Function of Pectins”, American Chemical Society, Washington , DC. Blackwell, J. Walton, A.G., (1973), "Biopolymers", New York, 474-89.

Chandra, R., (1995), "Rubber and Plastic Technology", CBS Publishers and Distributors, New Delhi, India.

Fruton, J. S., Simonds, S., (1958), "General Biochemistry", John Wiley & Sons, New York, 417.

Hester, Ronald E.; Harrison, R. M. (2011), “Marine Pollution and Human Health”, Royal Society of Chemistry, 84-85.

Modebelu M. N., Edward I., (2014), “Environmental Health Hazards and Rural Community Development in Abia State of Nigeria,” International Letters of Natural Science. Vol. 20: 129-138.

Naryan, R., et.al., (1999), “Testing for Biodegradable Plastics”, US Patent 5.

Pavol, A., Darina, K., Miroslav, K., Dusan, B. &Barbora, S., (2002), “Poly(vinyl alcohol) Stabilisation in Thermoplastic Processing*”, Polym.Degrad. Stab.*, 78(3), 413–421.

Raaz, M., Bina, R., Sangeeta, P.,and Anju, S., (2013), “Eco-friendly Bioplastic for Uncontaminated Environment”, Research Journal of Chemical and Environmental Sciences, Vol 1 (1): 44-49.

Online Material

1. http://pharosproject.net/ uploads/ sources/ lyondell-dispersants. pdf

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Structural Identification of Organic Compound Isolated from the Bark of *Acrocarpus fraxinifolius* Wight et. Arn.

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Abstract

The bark of *Acrocarpus fraxinifolius* Wight et. Arn., (Mayanin) was selected for chemical analysis. The phytochemical constituents in the sample were tested by using standard method. The antimicrobial activities of the crude extracts and the pure organic compound were tested by agar well diffusion method using six micro-organisms. The pure organic compound (I) was isolated from the bark of Mayanin by thin layer and column chromatographic separation techniques. The pure compound was obtained as pale yellow feather shape crystal. Phytochemical test for pure compound (I) was carried out and it gave positive test for flavonoid. The complete structure of flavonoid compound (I) was identified by applying 1D and 2D NMR spectroscopy as well as EI-Mass spectrometry. The name of the coumpound (I) could be assigned as 3-(benzo [d] [1,3]dioxol -5-yl)-4,5,7-trimethoxy-2H-chromen-2-one.

Keywords: *Acrocarpus fraxinifolius*, Antimicrobial activity, FT-IR, NMR spectroscopy, MS spectrometry

Introduction

Humans have long used naturally occurring substances for medical purposes. Plants have played a leading medical role in most cultures. With the development of the science of chemistry at the beginning of the 19th century, plants began to be examined more closely to understand why they were medically useful. (Beutler, 2009) Natural products, including plants, animals and minerals have been the basis of treatment of human diseases (Lahlou, 2007, Patwardhan, 2004 and Lahlou 2013).

History of medicine dates back practically to the existence of human civilization. Historically, the majority of new drugs have been generated from plants and animals (secondary metabolites) (Lahlou, 2007) Before 20th century, crude and semi-pure extracts of plants, animals, microbes and

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minerals represented the only medications available to treat human and domestic animal illnesses. The 20th century revolutionized the thinking in the use of drugs, as the receptor theory of drug action. The idea that effect of drug in human body are mediated by specific interactions of the drug molecule with biological macromolecules (proteins or nucleic acids in most cases) led scientist to the conclusion that individual chemical compounds in extracts, rather than some mystical “power of life” are the factors required for the biological activity of the drug. This lead to the beginning of a totally new era in pharmacology, as pure, isolated chemicals, instead of extracts, became the standard treatments for diseases. Indeed, many bioactive compounds, responsible for the effects of crude extract drugs, and their chemical structure was elucidated. (Lahlou, 2013)

Plant research continues to explore a variety of lead structures, which may be used as templates for the development of new drugs by the pharmaceutical industry. There is no doubt that plants have been, and will be, important sources of new pharmaceutical compounds. (Lahlou, 2007)

Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin. Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine. Many food crops have medicinal effects, for example garlic. The medicinal effects of plants are due to metabolites especially secondary compounds produced by plant species.

At present, a lot of research concerning with antibacterial activity, antioxidant activity of Myanmar medicinal plants have been investigated in *vitro* and *vivo* methods. This research has been made on the interesting upgrade level of Myanmar medicine isolated from the Myanmar indigenous medicinal plants, and has been studied chemically. Medicinal plants were and still continue to be an important therapeutic aid and for alleviating ailments of human kind.

In this research work, *Acrocarpus fraxinifolius* was selected for the investigation of phytochemical constituents and antimicrobial activities of the various crude extracts and pure isolated compound because this plant has the anti-proliferative, anti-inflammatory, anti-oxidant and anti-diabetic as well as hepato-protective activities (Abd El-Ghffar, 2016, Abou Zeid, 2012, Abou Zeid, 2011, El-Kashak, 2016). And then the pure organic compound (I) was isolated from the bark of Mayanin by using chromatographic separation methods. In addition, the functional groups of organic compound (I) were assigned by IR spectral data.The complete structure of isolated compound (I) could be elucidated by NMR spectroscopy and Mass spectrometry.

Botanical Description

Family name	: Caesalpiniaceae
Scientific name	: *Acrocarpus fraxinifolius*
Local Name	: Mayanin
English name	: Pink cedar
Medicinal properties	: anti-proliferative, anti-inflammatory, anti-oxidant, anti-diabetic and hepato-protective activities

Figure 1. The plant of Mayanin

Materials and Methods

General

Column chromatography was performed using silica gel (SiO_2; Merck, 0.063–0.200 mm) and TLC: silica gel 60 F_{254} plates (Merck). UV-Lamp (lambda – 40, Perkin – Elmer Co, England) and iodine vapour were used as visualizing agents Gallenkamp melting point apparatus (England) was used to measure the melting point of the compound. The functional groups of the compound were identified by FT-IR spectrometer (Shimadsu, Japan). ^{1}H-and ^{13}C NMR spectroscopy was carried out on JEOL at 500 MHz and 125 MHz respectively. Chemical shifts values are given in δ-value (ppm) with tetramethylsilane (TMS) as internal standard. EI-mass spectrometer (JEOL, JMS-600 MHz) was employed to assign the molecular formula of the compound.

Plant Material

The bark of *Acrocarpus fraxinifolius* Wight et. Arn. was collected at Singaung-gyaung forest, Kani Township, Sagaing Regionand identified by Dr Soe Myint Aye at Department of Botany, Mandalay University.

Preliminary Phytochemical Screening

A few grams of each sample were subjected to the tests of alkaloids, flavonoids, terpenoids, steroids, glycosides, reducing sugars, polyphenols, saponins and lipophilic compounds as the preliminary phytochemical test according to the reported methods (Harborne, 1973, Thamaraiselvi, 2012, Geetha, 2014 and Tiwaki, 2011).

Antimicrobial Activity Tests of the Crude Extracts and the Isolated Compound

The antimicrobial activities of the crude extracts from the bark of *Acrocarpus fraxinifolius* Wight et. Arn. (Mayanin) and the isolated compound (I) were tested by Agar well diffusion method on six selected microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E. coli* at PRD (Pharmaceutical Research Department), Yangon.

Extraction and Isolation

The air-dried powdered bark (500 g) was extracted with 95% ethanol (2000 mL) at room temperature for 3 months. It was concentrated and was successively extracted with ethyl acetate. The ethyl acetate crude extract (2.41 g) was packed with 50g of silica gel in a column (75cm x 2.0cm) and eluted with n-hexane; n-hexane: ethyl acetate mixtures (19: 1-1: 19) and ethyl acetate. The progress of separation was monitored by thin layer chromatography using n- hexane: ethyl acetate mixtures. Twelve combined fractions with same R_f values were obtained.

The combined fraction VII (54.3 mg) was subjected to repeated column chromatography over silica gel eluted with n- hexane/ EtOAc (9:1, 4:1, 3: 2, 1: 1, 2: 3 and 1: 9) to afford a pale yellow feather shape crystal 25.2 mg (1.046 % based upon EtOAc extract). TLC using solvent system (n-hexane: EtOAc- 3:2) gave a single spot with R_f (0.51). The molecular formula determination and complete structure assignment of the compound (I) were accomplished by ^{1}H-NMR, and ^{13}C-NMR, DEPT, DQF-COSY, HMQC and HMBC spectroscopies and MS spectrometry. (Silverstein, 1981 and Silverstein, 1998)

Results and Discussion

Preliminary Phytochemical Tests for the Bark of Mayanin

The results of phytochemical tests of crude extract of *Acrocarpus fraxinifolius* (Mayanin) are tabulated in Table (1). According to the preliminary phytochemical screening, the bark of Mayanin contains alkaloids, flavonoids, terpenes, glycosides, reducing sugars, polyphenols, saponins and lipophilic compounds.

Table 1. Phytochemical Constituents of the Bark of Mayanin

No.	Tests	Reagents	Observation	Results
1.	Alkaloids	Dragendorff's reagent Wagner's reagent	Orange ppt Reddish brown ppt	+ +
2.	Flavonoids	conc: HCl, Mg tunning	Pink color solution	+
3.	Terpenes	Acetic anhydride, conc: H_2SO_4, $CHCl_3$	Pink color solution	+
4.	Steroids	Acetic anhydride, conc: H_2SO_4	No green color solution	-
5.	Glycosides	10 % lead acetate	Yellow ppt	+
6.	Reducing sugars	Benedict solution	Red ppt	+
7.	Polyphenols	10 % $FeCl_3$	Green color solution	+
8.	Saponins	vigorously shaken	Frothing	+
9.	Lipophilic compounds	0.5N KOH	Deep color solution	+

(+) = Presence of constituents (–) = Absence of constituents

Antimicrobial Activities of Crude Extracts from the Bark of Mayanin

The antimicrobial activities of the crude extracts of the bark of *Acrocarpus fraxinifolius* (Mayanin) shown in the Table (2).

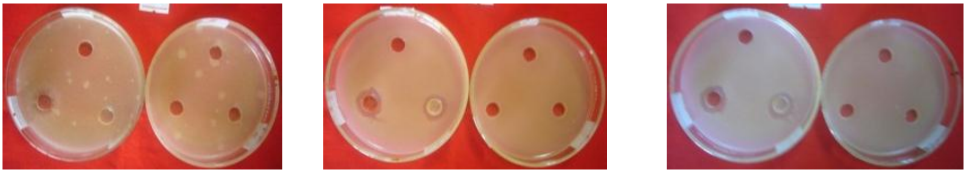
Table 2. Antimicrobial Activities of Crude Extracts

Crude Extract	**Diameter of Inhibition Zone (mm)**					
Solvent	I	II	III	IV	V	VI
n- hexane	-	-	-	-	-	-
EtOAc	13(+)	19 (++)	18 (++)	15 (++)	-	22 (+++)
EtOH	-	18 (++)	18 (++)	-	-	-

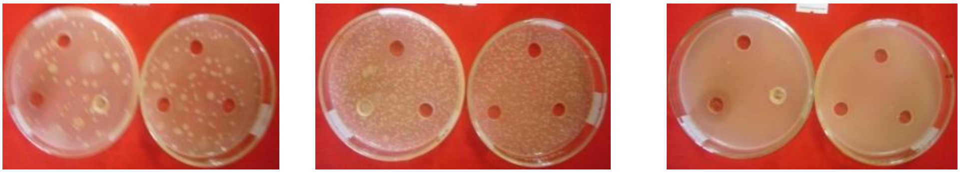
Microorganisms

I = *Bacillus subtilis*
II = *Staphylococcus aureus*
III = *Pseudomonas aeruginosa*
IV = *Bacillus pumilus*
V = *Candida albicans*
VI = *E. coli*

Agar well-10mm, 10mm~14mm(+); 15mm~19mm(++); 20mm above (+++)



Bacillus subtilis *Staphylococcus aureus* *Pseudomonas aeruginosa*



Bacillus pumilus *Candida albicans* *E. coli*

Figure 2. Antimicrobial activities of the crude extracts

The ethyl acetate extract of the bark responds high activity on *E. coli* and median activity on all microorganisms except *Candida albicans.* The ethanol extract shows median activity on *Staphylococcus aureus* and *Pseudomonas aeruginosa* and no activity on other organisms. But n-hexane extract of Mayanin has no activity on all microorganisms. Hence, the ethyl acetate crude extract was valuable for the isolation of pure organic compound.

Antimicrobial Activities of Pure Compound (I)

The results of the antimicrobial test relevant to different types of organisms are tabulated in Table (3). From this table, the pure compound (I) responds median activity on all microorganisms except *Bacillus subtilis* and *Candida albicans* (Figure 3).

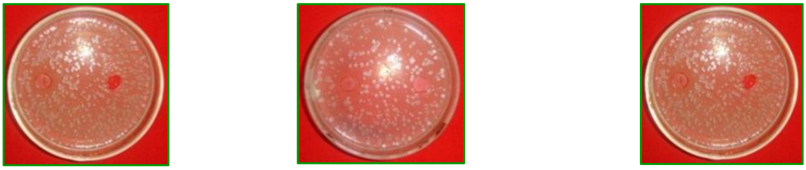
Table 3. Antimicrobial Activities of Pure Compound (I)

Sample	**Solvent**	**Diameter of Inhibition Zone (mm)**					
		I	II	III	IV	V	VI
Compound (I)	EtOAc	-	19 (++)	18 (++)	17 (++)	-	19 (++)

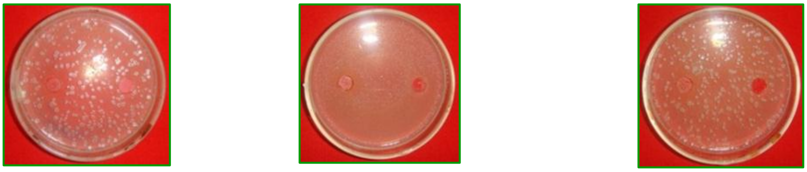
Microorganisms

I = *Bacillus subtilis*
II = *Staphylococcus aureus*
III = *Pseudomonas aeruginosa*
IV = *Bacillus pumilus*
V = *Candida albicans*
VI = *E. coli*

Agar well-10mm, 10mm~14mm(+); 15mm~19mm(++); 20mm above (+++)



Bacillus subtilis *Staphylococcus aureus* *Pseudomonas aeruginosa*



Bacillus pumilus *Candida albicans* *E. coli*

Figure 3. Antimicrobial activities of the pure compound (I)

Structure Elucidation of Isolated Compound

The concentrated ethyl acetate extract of the bark of *Acrocarpus fraxinifolius* was separated by column chromatography on silica gel resulted in the isolation of the flavonoid compound (I) with yield of 1.046 % based upon EtOAc extract. It was isolated as pale yellow feather shape crystal compound with melting point of 146-148°C.

The FT- IR spectrum (Figure 5) showed the characteristic signal bands of flavonoid compound [(3095.85 cm^{-1}, sp^2 hydrocarbons), (1716.7 cm^{-1}, C=O stretch of aromatic carbonyl), (1606.69 cm^{-1}, C=C stretch of aromatic hydrocarbons), (991.44 cm^{-1} and 815.92 cm^{-1}, C-H bending of trans or E and cis or Z)]. (Silverstein, 1981)

The ^{1}H NMR spectrum (Figure 6) revealed nine proton signals (three methyl, one methylene, and five methine) representing 16 protons in the compound. The ^{13}C-NMR and DEPT spectra (Figures 7 and 8) showed 19 carbon signals (ten sp^2 quaternary carbons, five sp^2 methine carbons, one sp^3 methylene carbon and three sp^3 methyoxyl carbons). Four signals between δ 165.13 and 157.86 ppm represented the aromatic carbons directly connected with oxygen atoms. The singlet pattern of the methylene protons at δ 6.03 ppm in ^{1}HNMR spectrum shows the presence of one acetal methylene group in the compound. (Silverstein, 1998)

The ^{1}H-NMR spectrum reflected three methoxyl groups (δ_H 3.48, 3.87 and 3.89 ppm) corresponding to δ_C 60.77, 55.90 and 56.48 ppm (Figure 9). The existence of flavonoid type compound could be confirmed by the ^{13}C- NMR spectral data at δ_C 165.13, 162.95, 161.76, 157.86, 155.68, 112.12, 100.82, 95.88 and 93.39 ppm. From the FT- IR, ^{1}H NMR, ^{13}C NMR, DEPT and HMQC spectral data, the partial molecular formula of compound (I) could be assigned as $C_{19}H_{16}O_3$ and its partial mass is 292.

But the molecular mass of pure organic compound (I) is 356 Da according to EI-MS spectrum (Figure 10). So the remaining molecular mass 64 should be one carbonyl oxygen and three ether oxygen atoms according to FT- IR spectral data. Thus, the molecular formula of the pure compound (I) should be $C_{19}H_{16}O_7$. The calculated molecular mass is in agreement with the measured molecular mass (m/z = 356 Da). (Porter, 1971)

Careful interpretation of the cross signals in the DQF- COSY and HMBC spectra (Figures 11 and 12) combined with the coupling constants of the signals in the ^{1}H NMR spectrum resulted in the complete structure of the compound (I).

Table 4. ^{1}HNMR, ^{13}CNMR, DEPT and HMBC Data of the Compound (I) (at 500 and 125 MHz, resp. in DMSO; 296 K; *δ* in ppm, *J* in Hz)

Position C/H	**DEPT**	**δ_H**	**δ_C**	***J* correlations (HMBC)**
2	C		161.76	H-8,
3	C		112.12	H-2', H-6'
4	C		165.13	H-6, -OCH_3,
5	C		157.86	H-6, -OCH_3,
6	CH	6.53 (d, 2.3)	95.88	H-8,
7	C		162.95	H-6, H-8, -OCH_3,
8	CH	6.63(d, 2.3)	93.93	H-6,
9	C		155.68	H-8,
10	C		100.82	H-6, H-8,
1/	C		125.38	H-2', H-5',
2/	CH	6.92 (d, 1.7Hz)	110.90	H-6',
3/	C		146.69	H-2', H-5', -O-CH_2-O-,
4/	C		146.52	H-6', -O-CH_2-O-,
5/	CH	6.94 (d, 8.0Hz)	107.73	H-6'
6/	CH	6.84 (dd, 8.0Hz, 1.7 Hz)	115.81	H-5'
-OCH_2O-	CH_2	6.03 (s)	100.94	
OMe at 4	CH_3	3.48 (s)	60.77	
OMe at 5	CH_3	3.89 (s)	56.48	
OMe at 7	CH_3	3.87 (s)	55.90	

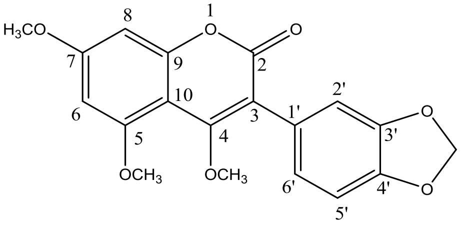


Figure 4. Plannar structure of compound (I)

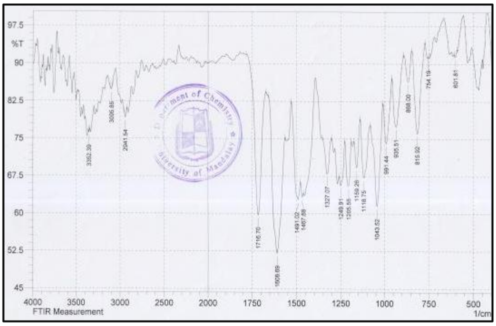


Figure 5. FT- IR spectrum of the compound (I)

Figure 6. ^{1}H NMR spectrum of the compound (I)

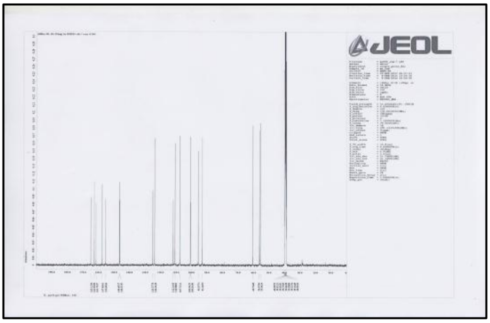


Figure 7. ^{13}C NMR spectrum of the compound (I)

Figure 8. DEPT spectrum of the compound (I)

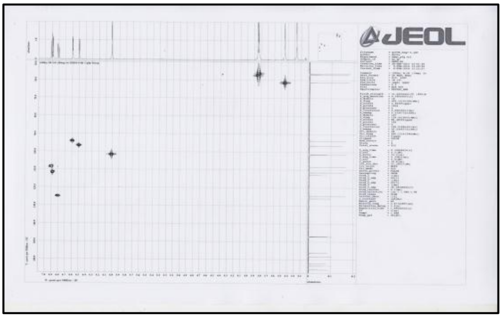


Figure 9. HMQC spectrum of the compound (I)

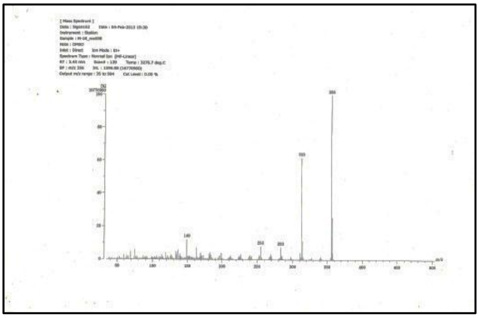


Figure 10. EI- MS spectrum of the compound (I)

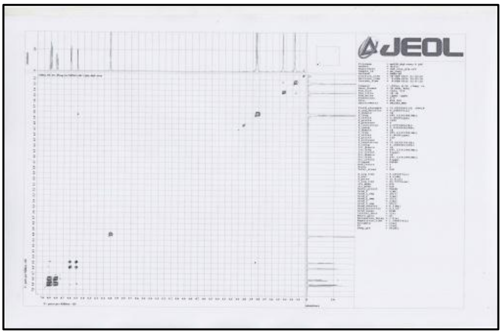


Figure 11. COSY spectrum of the compound (I)

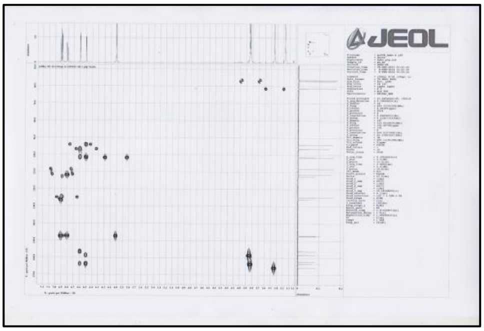


Figure 12. HMBC spectrum of the compound (I)

Conclusion

The bark of *Acrocarpus fraxinifolius* Wight et. Arn., (local named-Mayanin) was used for this research. The bark sample contains alkaloid, glycoside, flavonoid, polyphenol, sugar, lipophilic, terpene, saponin and phenolic compounds. The pure organic compound (I) could be isolated by chromatographic separation techniques. Phytochemical test for pure compound (I) was done and it showed the positive test for flavonoid. The melting point of this compound was found to be 146-148 ºC and its yield percentage is 1.046% (25.2 mg) based upon the EtOAc crude extract. This compound (I) possess median activity on all selected organisms except *Bacillus subtilis* and *Candida albicans* by Ager well diffusion method.

The structure assignment of the compound (I) was accomplished by NMR and MS spectral data. The MS displayed (M^+) at m/z 356 (corresponding to $C_{19}H_{16}O_7$) and significant peaks at m/z 313, 283, 255 and 149 characteristic fragmentation of chromen skeleton. There was found to be a carbonyl group at C-2 of chromen type flavonoid compound in the compound (I). The name of the compound (I) could be assigned as 3-(benzo[d] [1,3] dioxol- 5- yl) -4, 5, 7- trimethoxy- 2H- chromen- 2-one. According to the references, this coumarin compound from the bark of *Acrocarpus fraxinifolius* was isolated for the first time. (Rosales-Castro, 2015)

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References

Abd El-Ghffar, E.A., et al., (2016), "GC-MS Analysis and Hepatoprotective Activity of the n- Hexane Extract of *Acrocarpus fraxinifolius* Leaves against Paracetamol- Induced Hepatotoxicity in Male Albino Rats", *Pharmaceut Biol*, **55**, 441- 449

Abou Zeid, A. H., Mohammed R.S., Soliman, F.M., Sleem A. A. and El-Dokrory, Y. M., (2012), "Galloyl Derivatives and Biological Activities of *Acrocarpus fraxinifolius* Weight & Arn. Leaves", *Planta Med*, **78**, 1192- 1192

Abou Zeid, A. H., Soliman, F.M., Mohammed R.S., Sleem A. A. and El-Dokrory, Y. M., (2011), "Anti-inflammatory Effect and Lipoidal Content of *Acrocarpus fraxinifolius* Weight & Arn. Leaves", *Planta Med*, **77**, DOI. 10.1055/s-0031-1282657

Beutler, J. A, (2009), "Natural Products as a Foundation for Drug Discovery", *Curr Protoc Pharmacol*, **1 (**46), 1-30

El-Kashak, W.A., Hamed, A.R., El-Raey, M., Elshamy, A.I. and Abd-Ellatef, G.E.F., (2016), "Antiproliferative and Antioxidant and Antimicrobial Activities of Phenolic Compounds from *Acrocarpus fraxinifolius*", *J Chem Pharmaceut Res*, **8**, 520- 528

Geetha, T. S. and N. Geetha, (2014), "Phytochemical Screening, Quantitative Analysis of Primary and Secondary Metabolites of *Cymbopogan citratus* (DC) stapf.

Leaves from Kodaikanal hills, Tamilnadu", *International Journal of Pharm Tech Research*, **6** (2), 521- 529

Harbone, J. B., (1973), "Phytochemical Method, A Guide to Modern Techniques of Plant Analysis", Chapman and Hall in association with Methuen, Inc., 733 Third Avenue, New York NY

Lahlou, M., (2007), "Screening of Natural Products for Drug Discovery", *Expert Opinion on Drug Discovery*, **2** (5), 697-705

Lahlou, M., (2013), "The Success of Natural Products in Drug Discovery", *Pharmacology & Pharmacy*, **4**, 17-31

Patwardhan, B., A. D. B. Vaidya and M. Chorghade, (2004), "Ayurveda and Natural Products Drug Discovery", *Current Science*, **86** (6), 789-799

Porter, QN, et al., 1971, “Mass Spectrometry of Heterocyclic Compounds”, University of Melbourne, John Wiley and Sons, Inc. New York, London, Sydney, Toronto

Rosales-Castro, M et al., 2015 May, “Antioxidant Phenolic Compounds of Ethanolic and Aqueous Extracts from Pink Cedar (*Acrocarpus fraxinifolius* Whight & Arn.) Bark at Two Tree Ages”, Journal of Wood Chemistry and Technology, 35 (4): 270- 279

Silverstein, R.M and Webster, F.X, (1981), “Spectrometric Identification of Organic Compound”, 4th edition, John Wiley and Sons, Inc., New York

Silverstein, R.M and Webster, F.X, (1998), “Spectrometric Identification of Organic Compound”, 6th edition, John Wiley and Sons, Lnc., New York

Thamaralselvi, P. L. and P. Jayanthi, (2012), "Preliminary Studies on Phytochemical and Antimicrobial Activity of Solvent Extracts of *Eichhornia crassipes* (Mart) Solms", *Asian Journal of Plant Science and Research*, **2** (2), 115-122

Tiwari, P., B. Kumar, M. Kaur, G. Kaur and H. Kaur, (2011), "Phytochemical Screening and Extraction: A review", *Internationale Pharmaceutica Sciencia*, **1 (**1), 98- 106

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Phytochemical Screening, Total Phenolic Content, Total Flavonoid Concentration, Antioxidant and Antimicrobial Activities of Two Medicinal Plants Collected from Sagaing Region

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Myint Myint Khine[5]

Abstract

In this study, two medicinal plants collected from Sagaing Region were analysed for phytochemical screening, total phenolic content, total flavonoid concentration, antimicrobial and antioxidant activities. Preliminary phytochemical screening was performed with standard procedures. Antimicrobial activities of the methanolic crude extracts of the two medicinal plants on five microorganisms were determined by agar dilution-streak method. The determination of total phenolic and flavonoid contents of the methanolic extracts of two medicinal plants were determined by spectrophotometric techniques. Then, the antioxidant activities of methanolic extracts of two medicinal plants were evaluated using 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay.

Keywords: phytochemical, spectroscopic, DPPH, antioxidant

Introduction

Plants and herbs have been used in the indigenous systems of medicine in Myanmar and also in other countries. Many medicinal plants contain a number of phenolic and flavonoid compounds, having antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic activities. Some studies have been reported that phenolic compounds in herbs significantly contributed to their antioxidant (Siripongvutikorn *et al*., 2008; Saeh *et al*., 2010) and pharmaceutical properties (Srinivasan *et al*., 2005). The present study is an attempt to perform phytochemical screening, total phenolic content, total flavonoid concentration, antioxidant and antimicrobial activities of two medicinal plants (*Neolamarckia cadamba* and *Salacia*

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smaliana) collected from Sagaing Region. *Neolamarckia cadamba* (Myanmar name – Ma-U) of distribution is Bangladesh, Borneo, Cambodia, China South-Central, China Southeast, East Himalaya, Guatemala, India, Jawa, Laos, Malaya, Myanmar, New Guinea, Sri Lanka, Sulawesi, Sumatera, Thailand and Vietnam. In folk medicine, various parts of *Neolamarckia cadamba* are used in the treatment of various ailments such as fever, uterine complaints, blood diseases, skin diseases, tumour, anaemia, eye inflammation and diarrhoea. *Salacia smaliana* (Myanmar name – Nwe Ngan) are widely distributed in South-West India, Penincular region of India, Sri Lanka, Vietnam, China, Indonesia, Brazil, South Africa, Malaysia, Thailand, Philippines and Myanmar. The aerial parts and roots of *Salacia smaliana* are used in Myanmar for treatment of diabetes, gonorrhoea, rheumatism, itching, asthma, ear diseases, leukaemia and inflammations.

Materials and Methods

Plant Material

The medicinal plants of *Neolamarckia cadamba* and *Salacia smaliana* were collected from Mahar Myaing forest, Kalay-wa Township, Sagaing Region (Myanmar) and voucher specimens were deposited in the Department of Chemistry, Kalay University, Myanmar. The collected samples were allowed to dry for one week in well ventilated shade. Then, the air dried samples were grounded into powder by grinding machine.

Phytochemical Analysis of the Samples

The preliminary phytochemical analysis of alkaloids, flavonoids, phenolics, glycosides, tannins, saponins, steroids and terpenoids in the extracts were carried out using standard methods (Tiwari *et al*., 2011).

Antimicrobial Assay by Agar Dilution-streak method

The agar-streak method was used to assess the antimicrobial activities of the methanol extracts of two medicinal plants against *Bacillius subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus* and *Escherichia coli*. Cultures of bacteria and fungus were grown on nutrient broth (Basal Media) at 37°C for 24 h and were maintained on respective agar slants at 4°C. The test organisms maintained on agar slants were recovered for testing by inoculating into nutrient broth and incubated at 37°C in a shaker at 180 rpm. Antibacterial and antifungal activity was carried out by agar dilution-streak method (Mitscher *et al*., 1972). Plant extract was incorporated into the media and poured into the different petriplates and allowed to solidify. Bacteria or fungi inocula were then streaked at different areas on the respective agar plates. Plates were incubated at 37°C and observed after 24 h. Blank plates each containing only NA were prepared. Growth of bacteria and fungus were observed after one day. The results were compared with neomycin as a standard.

Determination of Total Phenolic Contents in Plant Extracts

Total phenolic content of methanolic extracts of two medicinal plants were determined with Folin-Ciocalteu's method (Singleton*et al*., 1999). The Folin–Ciocalteu (F–C) reagent is sensitive to reducing compounds, polyphenols and thus produces a blue colour complex. The F-C assay relies on the transfer of reducing equivalents (electrons), in the alkaline medium, from phenolic compounds to phosphomolybdic/ phosphotungstic acid complexes, manifested in the formation of blue colour complexes [possibly (PMoW11O40)4−] that were determined on a UV-visible spectrophotometer by monitoring the absorbance at 765 nm (Singleton *et al*., 1999). Ethanolic solution of the extract in the concentration of 1 mg/mL was used in the analysis. The reaction mixture was prepared by mixing 0.5 mL of ethanolic solution of extract, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 mL of ethanol, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45°C for 45 mins. The absorbance was determined using spectrophotometer at λ_{max} = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. To obtain a calibration curve, various concentrations of gallic acid solutions (100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL) were prepared. Based on the measured absorbance, the concentration of phenolics was read (mg/mL) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

$$\text{Total Phenolic Content} = c\,\frac{V}{m}$$

c = concentration from calibration curve

m = mass of the extract used
v = volume of the extract

Determination of Total Flavonoid Contents in Plant Extracts

The content of flavonoids in the examined plant extracts was determined using spectrophotometric method (Quettier *et al.*, 2000). The sample contained 1 mL of ethanol solution of the extract in the concentration of 1 mg/mL and 1 mL of 2% $AlCl_3$ solution dissolved in ethanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λ_{max} = 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of quercetin and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg/mL) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of quercetin equivalent (mg of Q/g of extract).

$$\text{Total Flavonoid Content} = c\,\frac{V}{m}$$

c = concentration from calibration curve
m = mass of the extract used
v = volume of the extract

Determination of Antioxidant Activity by DPPH Radical Scavenging Assay

The antioxidant activities of the extracts of two medicinal plants were determined by DPPH scavenging activity assay (Yamaguchi *et al.*, 1998). Its reaction principle was based on mechanism of free radicals inhibition by hydrogen transfer, the antioxidant activity of sample was expressed in EC_{50}. 500 μL of test solutions in various concentrations (100 μg/mL, 50 μg/mL and 10 μg/mL) and 500 μL of 0.2 M acetate buffer pH 5.5 solutions are mixed in a test tube. 250 μL of 5×10^{-4} M DPPH solution was added to the mixture in dark. The mixture was homogenized using a vortex mixer in a dark room (resistant to UV light) and stand for 30 minutes at room temperature. After that, the mixture was measured by a spectrophotometer UV absorbance at λ_{max} 517 nm. Vitamin C was used as a reference compound in the same concentration range as the test compounds. A control solution was prepared by mixing 500 μL of buffer (pH 5.5) solution, 500 μL of ethanol and 250 μL of 5×10^{-4} M DPPH solution in the test tube. Blank solution was prepared by mixing 500 μL of buffer (pH 5.5) solution with 750 μL of ethanol in the test tube. The mean values were obtained from triplicate experiments. The capability of scavenging DPPH radicals as a percentage of DPPH remaining in the resulting solution was determined using the following equation:

$$\text{DPPH}\ (\%) = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}$$

where Abs control is absorbance of control and Abs sample is absorbance of sample. The antioxidant power (EC_{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. EC_{50} (50% effective concentration) values were calculated by linear regressive excel program.

Results and Discussions

The air dried samples were extracted with Methanol. After solvent removal by evaporation, the residue was used for the determination of total phenolic, total flavonoids, antimicrobial and antioxidant activities.

Figure 1. The plant of *Neolamarckia cadamba* (Roxb.) Bosser

Figure 2. The plant of *Salacia smaliana* Brandis

Preliminary Phytochemical Test of the Plant Extracts

The results for the phytochemical screening of the stem bark of *Neolamarckia cadamba* and *Salacia smaliana* by the standard procedures were shown in Table (1).

Table 1. The Results of Phytochemical Screening of the Extracts of Two Medicinal Plants

No	**Constituents**	**Observation**	**Results**	
			I	**II**
1	Alkaloids	Orange ppt	+	-
2	Flavonoids	Reddish pink color solution	+	+
3	Glycosides	White ppt	+	+
4	Phenolic compounds	Dark green color solution	+	+
5	Steroids	Blue color solution	+	+
6	Saponins	Formation of froth	+	+
7	Terpenoids	Red or Pink color solution	+	+
8	Tannins	White ppt	+	+

(+) sign indicates the presence of the constituent.

(-) sign indicates the absence of the constituent.

I = *Neolamarckia cadamba* II = *Salacia smaliana*

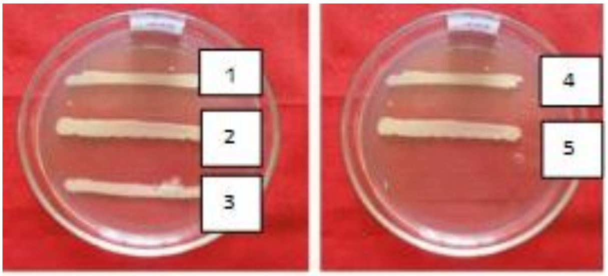
Phytochemical anlaysis revealed that the extracts of *Neolamarckia cadamba* contained all tested phytochemical constituents. The extracts of *Salacia smaliana* showed the presence of all tested phytochemical constituents except alkaloids.

Antimicrobial Activities of Selected Medicinal Plants

The results of antimicrobial activities of selected medicinal plants were shown in Table (2).

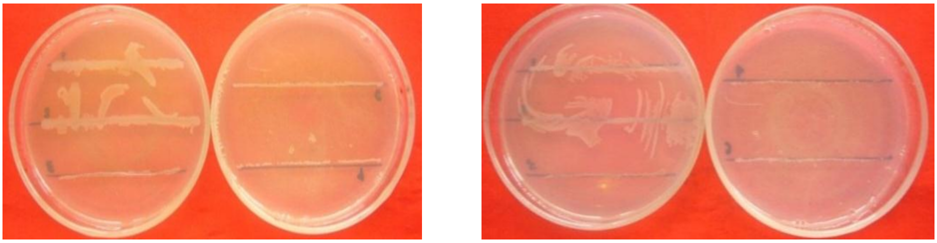
Table 2. Antimicrobial Activities of Selected Medicinal Plants

Plants	***B.subtilis***	***S.aureus***	***P.aeruginosa***	***B.pumilus***	***E. coli***
N.cadamba	-	-	-	+	+
S. smaliana	-	-	-	+	+



Test Organisms in NA medium (Control)

1. *Bacillus subtilis,* 2. *Staphylococcus aureus,* 3. *Pseudomonas aeruginosa,* 4. *Bacillus pumilus* 5. *E. coli*



Neolamarcka cadamba *Salacia smaliana*

Figure 3. Antimicrobial activities of selected medicinal plants

According to the antimicrobial experiments, the methanolic extract of *Neolamarcka cadamba* and *Salacia smaliana* revealed low sensitivity on *Bacillus pumilus* and *E. coli* and no activity on *Bacillus subtilis, Staphylococcs aureus* and *Pseudomonas aeruginosa.*

Determination of Total Phenolic Contents in Plant Extracts

The total phenolic contents of the extracts of two medicinal plants were determined with Folin-Ciocalteu reagent according to the (Singleton *et al*., 1999) method using gallic acid as a standard. The calibration curve is shown in Figure (4).

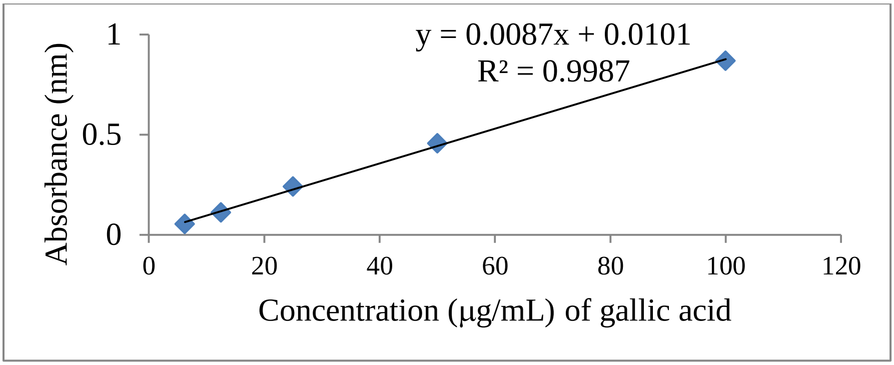


Figure 4. Plot of standard calibration curve of gallic acid (GA) for the determination of total phenolic content

The results of total phenolic content were found to be 163 mg GAE/g (*Neolamarckia cadamba*) and 126 mg GAE/g (*Salacia smaliana*). These results are shown in figure (5)

.

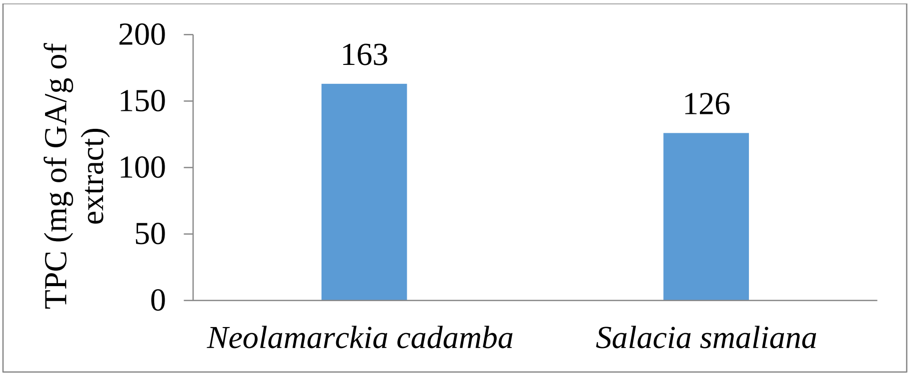


Figure 5. Total phenolic contents in the plant extracts expressed in terms of gallic acid equivalent (GAE) (mg of GA/g of extract)

Determination of Total Flavonoid Contents in Plant Extracts

The total flavonoid contents in the extracts of two medicinal plants were determined using quercetin as a standard.

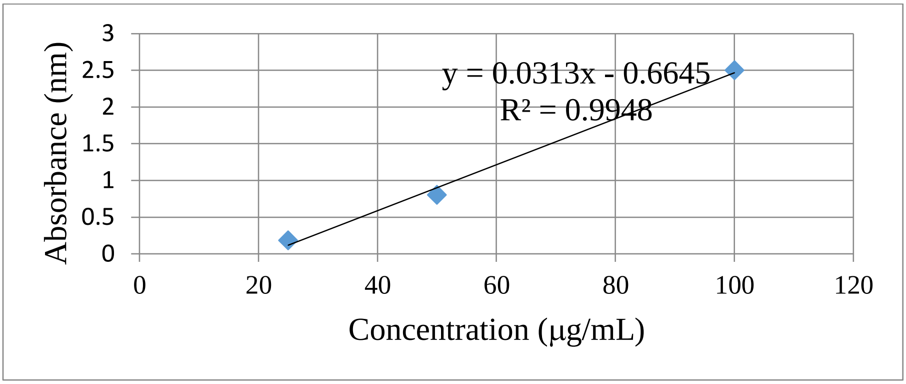


Figure 6. Plot of standard calibration curve of quercetin (Q) for the determination of total flavonoid content

The result of total flavonoid contents were found to be 30.75 mg QE/ g (*Neolamarckia cadamba*) and 88.93 mg QE/g (*Salacia smaliana*).

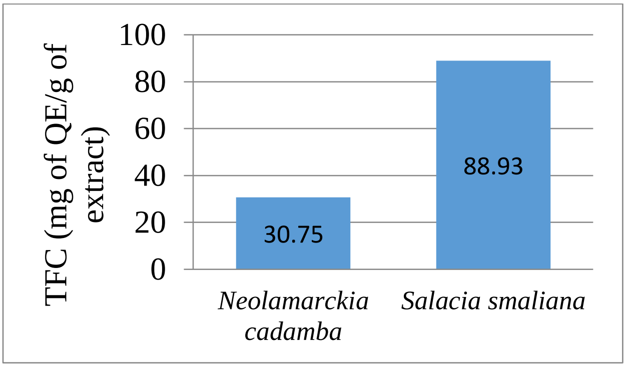


Figure 7. Total flavonoid contents in the plant extracts expressed in terms of quercetin equivalent (QE) (mg of Q/g of extract)

Antioxidant Activity by DPPH Scavenging Activity Assay

The result of antioxidant activity using DPPH assay in standard ascorbic acid was shown in Figure (8).

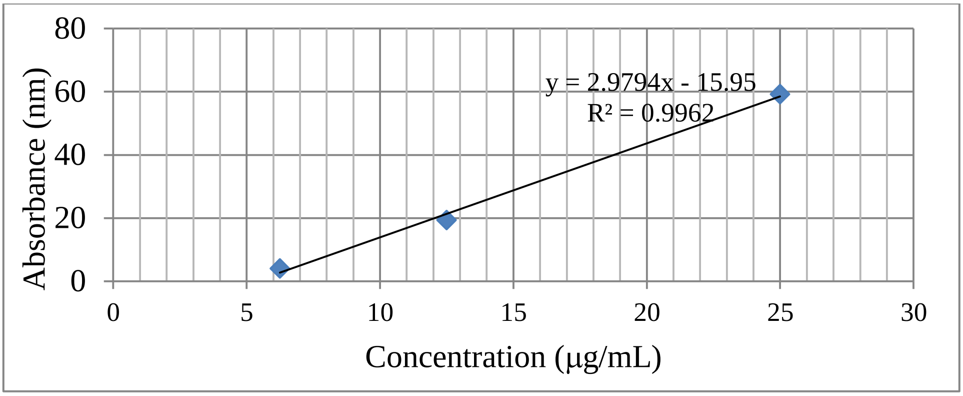


Figure 8. Standard calibration curve of ascorbic acid for the determination of DPPH scavenging activity

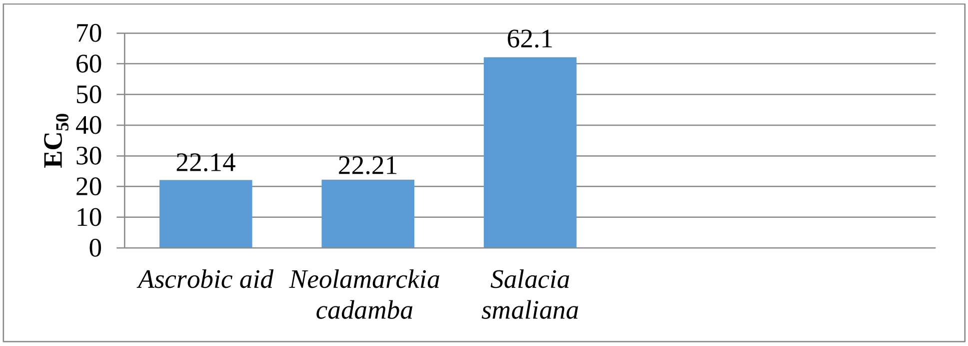


Figure 9. DPPHradical scavenging activity of ascrobic acid and methanolic extracts of ascorbic acid and two medicinal plants

As shown in figure (9), DPPH radical scavenging activities of the methanolic extracts of *Neolamarckia cadamba* showed potent free radical scavenging activity with EC_{50} 22.21 µg/mL whereas that of *Salacia smaliana* exhibited the medium free radical scavenging activity with 62.1 µg/mL.

Conclusion

In this study, two medicinal plants (*Neolamarcka cadamba* and *Salacia smaliana*) were selected for phytochemical investigation, determination of total phenolic content, total flavonoid concentration, antimicrobial and antioxidant activities. The results of phytochemical analysis revealed that the extract of *Neolamarckia cadamba* contained all phytochemical constituents. The extracts of *Salacia smaliana* showed the presence of all chemical constituents except alkaloids. According to the antimicrobial experiments, the methanolic extracts of *Neolamarcka cadamba* and *Salacia smaliana* revealed low sensitivity on *Bacillus pumilus* and *E. coli* and no activity on *Bacillus subtilis*, *Staphylococcs aureus* and *Pseudomonas aeruginosa.* The results of total phenolic content were found to be 163 mg GAE/ g (*Neolamarckia cadamba*) and 126 mg GAE/g (*Salacia smaliana*). The flavonoid concentrations in two medicinal plants were 30.75 mg QE/ g (*Neolamarckia cadamba*) and 88.93 mg QE/g (*Salacia smaliana*). According to antioxidant experimental data, the methanolic extract of *Neolamarcka cadamba* was found to be potent antioxidant activity with EC_{50} = 22.21 μg/mL. The methanolic extract of *Salacia smaliana* (EC_{50} = 62.1μg/mL) was found to be medium antioxidant activity. There was a direct relation between antioxidant activity and the content of phenols in some extracts in this study. For example, *Neolamarckia cadamba* showed high phenolic contents and showed good antioxidant activity. These results suggest that the higher levels of antioxidant activity were due to the presence of phenolic components. The result of the present study suggests that selected plants can be used as a source of antioxidants for pharmacological preparations which is very well evidenced by the present work.

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References

Mitscher, L.A., Leu, R., Bathala, M.S., Ivu, I.V. and Beal, J.L., (1972), "Antimicrobial agents from higher plants. Introduction, rational and methodology", *L10ydia*, vol. **35(2)**, pp. 157-166.

Quettier, D.C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M.C., Cayin, J.C., Bailieul, F. and Trotin, F., (2000), “Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour”, *J. Ethnopharmacol*, vol. **72**, pp. 35-42.

Seah, R., Siripongvutikorn, S. and Usawakesmanee, W., (2010). “Antioxidant and antibacterial properties in Keang-hleung paste and its ingredients”, *Asian Journal Food Agro-Industry*, vol. **3**, pp. 213-220.

Siripongvutikorn, S., Pengseng, N., Ayusak, S. and Usawakesmanee, W., (2008), “Development of green curry paste marinade for white shrimp (Litopenaeus vannamei)”, *Songklanakarin Journal Science and Technology*, vol. **30**, pp. 35-40.

Srinivasan, K., (2005), “Role of spices beyond food flavouring: Nutraceuticals with multiple health effects”, *Food Review International*, vol. **21**, pp. 167-188.

Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M., (1999), "Analysis of Total Phenols and Ot her Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent Methods", *Enzymol*, vol. **299**, pp. 152-178.

Tiwari, P., Kumar, B., Kaur,M., Kaur, G. and Kaur, H.,(2011), "Phytochemical Screening and Extraction: A Review", *Internationale Pharmaceutica Sciencia*, vol. **1(1)**, pp. 98-106.

Yamaguchi, T., H. Takamura, T. Matoba and J. Terao, (1998). “HPLC method for evaluation of the free radical-scavenging activity of foods by using 1, 1-diphenyl-2-picrylhydrazyl”, *Bioscience, Biotechnology and Biochemistry*, vol. **62**, pp. 1201-1204.

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Sorption of Free Fatty Acids in Peanut Oil and Palm oil Using Calcinated Rice Husk Ash

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Abstract

This research focuses on understanding sorption process and developing a cost effective technology for treatment of industrial foodstuff. Majandaw, rice husk sample obtained from Mahlaing township was treated with hydrochloric acid and calcinated at the temperature of 600° C. Edible oil is a major dietary compound and plays an important nutritional role. Edible oil is mixture of triglycerides of saturated and unsaturated fatty acids. The quality of edible oil may deteriorate due to oxidation, hydrolysis, cross contamination and contamination with undesirable substances. The present study seeks to investigate the adsorption of free fatty acids in palm oil and peanut oil with rice husk ash. Physicochemical properties (free fatty acid, acid values, iodine values, peroxide values, saponification values, unsaponifiable matter,and colour) of edible oils were determined by using appropriate methods.

Keywords: Palm oil, peanut oil, rice husk ash, adsorption

Introduction

Adsorption is a process in which a soluble chemical (adsorbate) is removed from a fluid by contact with a solid surface (adsorbent). It is used in industry for product separation and wastewater treatment. Adsorption is actually a mechanism in which the force of interaction between surface atoms and the adsorbate molecules (La Grega*et al.*, 1994). There are both attractive forces and repulsive forces with the net force depending on the distance between the surface of the adsorbent and the adsorbate molecule (Cooney, 1999). In general, adsorption is a process by which a component moves from one face to another while crossing some boundary.

There are several factors that impact physisorption. The major factors which affect physisorption include the surface area, pore structure,

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surface Chemistry of the adsorbent, nature of the adsorbate, pH of the solution and the presence of compaction adsorbates. So physisorption is considered to be a complex phenomenon by these factors.

Surface area of the adsorbent is one of the most important factors which consist of external surface area and internal surface area (pore walls). The external surface of the adsorbent contributes to the external superficial surface area. The pores of the adsorbent largely contribute to the internal surface area. The pore diameter of most media ranges from less than 10-100,000. If the adsorbate molecules were larger than the pore diameter, lesser adsorption would take place because of stearic hindrances (Hassler, 1963).

Adsorption occurs when an adsorbent comes in contact with a liquid containing the adsorbate and adsorption sites on the adsorbent become filled. Equilibrium occurs when the adsorption sites are filled. Equilibrium is a phenomenon when the rates of adsorption and rate of desorption are equal. The relationship between the amount of adsorbate and the surface of adsorbent may be estimated by various adsorption isotherms at a constant temperature.

Palm oil

Palm oil is a major dietary component and plays an important nutritional role (Liew, 1993). Palm oil is a mixture of triglycerides of saturated and unsaturated fatty acids. Fatty acids contribute about 95% of the total weight of triglyceride molecules. It contains pigment (carotenes) and impurities. It is used in cooking, cosmetics and in the manufacture of soap.

Composition of Palm Oil

Palm oil is made up of triglycerides and partial glycerides with one percent of minor components (Liew, 1993).The fatty acid components of palm oil consist of 50% saturated fatty acid and 50% unsaturated fatty acid. Thus it has a balance composition. The major components of the saturated fatty acids are palmatic acid (44%) and stearic acid (5%). The unsaturated fatty acids are made of oleic acid (39%) and linoleic acid (10%).

Uses of Palm Oil

Palm oil is part of our world in many more ways than we imagine (Abdullah, 1994). It is present at homes, work, restaurants, and even cars rely on the countless uses of palm oil. Palm oil is rich in carotene from which it derives its bright, tropical, red colour. In fact, the carotene content of palm oil is 16 times higher than levels found in a carrot with the same mass and weight. This makes palm oil one of the main and richest sources of carotene and as such is important in combating vitamin A deficiency common in many developing countries.

Peanut Oil

Peanut oil is a mild - tasting vegetable oil derived from peanuts. It is also known as groundnut oil. The oil is available with a strong peanut flavor and aroma. It is analogous to sesame oil. The peanut oil is often used in China, South Asian nations and South -east Asian nations, both for cooking and in the case of roasted oil, for added flavour. Peanut oil has a high smoke point relative to many other cooking oils, so is commonly used for frying foods. Its major component fatty acids are oleic acid (46.8% as olein), linoleic acid (33.4% as linolein) and palmaticacid(10.0% as palmitin). The oil also contains some stearic acid, arachidic acid, behenic acid and other fatty acids.

Health Benefits of Peanut Oil

Some of the health benefits of peanut oil include its ability to lower cholesterol levels, benefit heart health, prevent cancer, boost cognitive function, improve the nervous system, strengthen the immune system, lower blood pressure and protect from the skin. Peanut oil comes in a number of varieties, including refined, unrefined, roasted and cold-pressure, which have slight differences in their nutritional value and health benefits.

Most of the health benefits of peanut oil come from its diverse types of fatty acids such as oleic acid, palmatic acid, and linoleic acid among others. While unbalanced levels of fatty acids can be dangerous for health, peanut oil has a very safe balance that can boost health in a variety of ways in addition to the health benefits from the other vitamins, minerals and organic compounds found in peanuts.

Experimental

Preparation of the Oil Samples by Using Rice Husk Ash

Rice husk ash (50 g) was treated with 0.4M hydrochloric acid solution and kept for 24 hours. This mixture was washed with distilled water, dried at room temperature and calcinated at 600˚C to obtain acid activated rice husk ash. Peanut oil sample and palm oil sample (1.0 g), 30 ml of ethanol and 1.0 g of acid activated rice husk ash were mixed in a 250 ml conical flask. The mixture was shaken and placed for 3 hours. Then the solution was filtered to obtain treated oil samples. The whole of the above procedure was carried out by using various weight of acid activated rice husk ash sample 1g, 2g, and 3g respectively.

Determination of Free Fatty Acid

The oil sample (0.2 g) was dissolved with 25 ml of isopropanol. And then three drops of phenolphthalein indicator was added and the solution was titrated with 0.1M potassium hydroxide solution until the pink color developed.

Determination of Acid Value

The procedure was the same as the determination of free fatty acid.

Determination of Iodine Value

The oil sample (0.2g) was added into a 250 ml conical flask. It was dissolved in 10 ml of chloroform and added 25 ml of Wjis solution. The flask was kept in a dark room for one hour at 20˚ C. And then 20ml of 15% potassium iodide solution and 100 ml of distilled water were added to the pale yellow color solution. The color of the solution changes from pale yellow to blue color. This solution was titrated with 0.1 M sodium thiosulphate solution using starch as an indicator. The titration was continued until color has disappeared. A blank solution determination was carried out under similar conditions at the same time in the absence of selected oil sample (AOAC, 1998).

Determination of Peroxide Value

The oil sample (5.0g), 15 ml of glacial acetic acid and 10 ml of chloroform solution were added into a 250 ml conical flask. The flask was shaken to dissolve the treated oil sample. And then 1 ml of saturated potassium iodide solution was added into the pale yellow color solution.

The color of solution changes from pale yellow to blue color. The flask was placed in the dark room for 5 minutes and 75 ml of distilled water was added into the blue color solution. This solution was titrated with 0.01M sodium thiosulphate solution using starch solution as an indicator. The titration was continued until blue color has disappeared (Chen, 2003).

Determination of Saponification Value

The oil sample (2.0 g) and 25ml of alcoholic potassium hydroxide solution were added into a 250 ml round bottom flask. The flask was connected with the air condenser and the solution was refluxed to complete saponification. After heating, the condenser was disconnected and the flask was cooled to room temperature for 10 minutes. 3 drops of phenolphthalein indicator was added into the flask and titrated with 0.1M hydrochloric acid solution until pink color had just disappeared. A blank determination was carried out under similar conditions at the same time in the absence of the oil sample.

Determination of Unsaponifiable Matter

The oil sample 2.5 g was accurately weighed into a 250 ml flask, and 25 ml of the ethanolic potassium hydroxide solution was added. The mixture was boiled under reflux for one hour on a steam-bath, with occasional shaking to affect saponification. The solution was transferred to a 250 ml separating funnel. Equal volumes of 50 ml each of water and ether were added to the contents of the separating funnel. The mixture was shaken well while it was still warm and then the layers were allowed to separate. The lower layer was 250 ml separating funnel containing 20 ml of water. In this way, further extracts were carried out each time with 50 ml ether and the extracts were combined.

The combined ether extract was washed successively three times with 20 ml, 0.5 M potassium hydroxide solution. Finally the mixture in the separating funnel was washed several times with 20 ml of water until the washing was neutral with phenolphthalein. Then the ether extract solution was placed in a weighed 50 ml beaker and 3 ml of acetone was added into it. The beaker was placed on a water-bath to evaporate and it was removed when any residue is left. The beaker was placed in an oven until a constant weight was gained.

Determination of Color using Lovibond Tintometer

The viewing tube was inserted in the square viewing tube sleeve in the molded plastic case, with the viewing tube are checked to be free from dust. Both apertures of the cabinet are covered with the magnesium carbonate blocks, using the magnet and spring to hold them as position. The lid was replaced before tank color measurements. The transparent sample is poured into one of the glass cells. The cell was placed with one cell face against the lower window of the black molded plastic case. The lamps are checked that the correct voltage for the supply and then controlled by the switches. The instrument light was switched on for reading the values if glasses used, after a match has been obtained. The fixed yellow value was set and the red rocks light was switched on and the color values are to be read.

Results and Discussion

Physicochemical Properties of Peanut Oil and Palm oil

The chemical analysis of food enables people to know the composition of such material and with the aid of nutritional and biochemical knowledge, to know what should be eaten and should not be eaten. In this work, the physiochemical properties of the edible oil samples such as free fatty acid, acid value, iodine value, peroxide value, saponification value, unsaponifiable matter and color were determined by appropriate methods. The results are shown in Table 1 and Table 2. Crude oils contains the largest amount of unsaponifiable matter and completely refined oil the least.

Free Fatty Acid of the Selected Oil Samples

Fatty acid is an important industrial material in the oleochemical industry, for the production of various important oleochemical such as fatty alcohols, soaps, drugs, plastics, lubricants and other detergents which are widely used in the pharmaceutical and food industry. Free fatty acids in vegetable oil resulted from the breaking of the triglyceride ester bonds, are normally removed during the refining process in the industry. In this research, the sample was treated with various amounts (1.0 g, 2.0 g and 3.0 g) of acid activated rice husk ash. After treatment with 3.0 g of acid activated rice husk ash, free fatty acid was decreased. It can be seen that percentage of free fatty acid present in the oil sample was decreased with increase in weight of rice husk ash.

Table 1. Physicochemical Properties of Crude Peanut Oil Sample and Treated Peanut Oil Sample

Properties	**Crude Peanut Oil Sample**	**Treated Peanut Oil with 1 g Acid Activated Rice Husk Ash**	**Treated Peanut Oil with 2 g Acid Activated Rice Husk Ash**	**Treated Peanut Oil with 3 g Acid Activated Rice Husk Ash**	**FAO Limit**
Free Fatty Acid (%)	2.306	0.768	0.717	0.602	-
Acid value (%)	5.050	1.70	1.56	1.33	< 6.0
Iodine value	94.355	96.855	99.755	104.255	84-105
Peroxide value (meqO_2/kg)	0.185	0.177	0.169	0.161	< 10
Saponification value	192.782	194.582	197.382	198.580	190-200
Unsaponifiable matter (%)	0.183	0.20	0.23	0.24	< 1.0
Color	11.2R/12Y	5.6R/13Y	5.4R/13Y	5.2R/13Y	-

Acid Value of the Selected Oil Samples

Most fat and oil consists small amounts of free acid. The acid value, also known as the acid number or acidity index, is the number of milligrams of potassium hydroxide required to neutralize the free acids percent in one gram of oil, fat, wax or similar organic substances of complex composition. In the determinations of the acid value, dilute alkali must be used since many of the esters normally present in essential oils are capable of saponification even in the cold in the presence of strong alkalis. The value 5.61 is the number of milligrams of potassium hydroxide equivalent to 1ml of 0.1 M sodium hydroxide solution. The acid value of an oil often increase as the oil ages, especially if the oil is improperly stored processes such as oxidation of aldehyde and hydrolysis of esters increases the acid value.

In this research, the oil sample was treated with various amounts (1.0 g, 2.0 g and 3.0 g) of activated rice husk ash. After treated with 3.0 g of acid activated rice husk ash, acid value in the oil sample decreased. It can be seen that percentage of acid value present in the oil sample decreased with increased in weight of acid activated rice husk ash.

Iodine Value of the Selected Oil Samples

The iodine number is expressed as the grams of iodine absorbed by one hundred grams of fat or oil. Under certain conditions, iodine is absorbed quantitatively by unsaturated acids or triglycerides at the point of unsaturation. There are various reagents of determining the iodine value. One of the earliest reagents for this purpose was Hubl reagent, which contained an alcoholic solution of iodine in the presence of mercuric chloride. After modification, the two most widespread reagents are those of Wijs and House employing iodine monochloride and iodine monobromide in glacial acetic acid respectively. The Committee on Analysis of Commercial Fats and Oils of the Division of Industrial Chemicals and Chemical Engineers of the American Chemical Society has accepted the Wijs procedure as the standard.

The oil sample was treated with various amounts (1.0 g, 2.0 g and 3.0 g) of acid activated rice husk ash. After treatment with 3.0 g of acid activated rice husk ash, iodine value increase. It was found that iodine value present in the oil sample increased with increased in weight of acid activated rice husk ash. The higher iodine value indicates the present of more unsaturated fatty acid in the oil sample.

Peroxide Value of the Selected Oil Samples

The peroxide value is a measure of the active oxygen present in the oil or fat. It is defined as the weight in mil equivalent of peroxide per 1000 grams of the sample, which oxidize potassium hydroxide under the conditions of the test. These are generally assumed to be peroxides or other similar products of fat oxidation. Due to the oxidation effect of lipase enzyme, heat and light on oil, peroxide formed in the oil. The oil sample was treated with various amounts (1.0 g, 2.0 g and 3.0 g) of acid activated rice husk ash. After treatment with 3.0 g of acid activated rice husk ash, peroxide value decreased. It can be seen that peroxide value in the oil sample decreased with increased in weight of rice husk ash. Low peroxide value indicates the oil may be fresh oil.

Saponification Value of the Selected Oil Samples

Saponification is the chemical reaction in which an ester is heated with aqueous alkali to form an alcohol (usually glycerol), and the salt of the acid corresponding to ester. Saponification value or number includes the free fatty acids, and thus is a measure of both free and combined fatty acids. It may be defined as the milligrams of potassium hydroxide necessary to neutralize the free fatty acids, and to saponify the esters in one gram of the fixed oil or wax. The saponification number is inversely proportional to the molecular weight of the fat. The determination of saponification value is great importance in the evaluation of many lipids. In the case of natural fats and oils which are largely mixed triglycerides containing several varieties of acids, the saponification values usually lie from 190 to 200 (Anderson, 1962). The oil sample was treated with various amounts (1.0 g, 2.0 g and 3.0 g) of acid activated rice husk ash, saponification value increases in all selected samples. It was found that saponification value in the oil sample increases with weight of rice husk ash. High saponification value indicates presence of lower molecular weight of fatty acid. Saponification value is inversely proportional to molecular weights of fatty acid present in the oil sample.

Table 2. Physicochemical Properties of Crude Palm Oil Sample and Treated Palm Oil Sample

Properties	**Crude Palm Oil Sample**	**Treated Palm Oil with 1 g Acid Activated Rice Husk Ash**	**Treated Palm Oil with 2 g Acid Activated Rice Husk Ash**	**Treated Palm Oil with 3 g Acid Activated Rice Husk Ash**	**FAO Limit**
Free Fatty Acid (%)	6.54	0.768	0.717	0.602	-
Acid value (%)	14.58	1.70	1.56	1.33	< 6.0
Iodine value	48.42	96.855	99.755	104.255	84-105

Properties	Crude Palm Oil Sample	Treated Palm Oil with 1 g Acid Activated Rice Husk Ash	Treated Palm Oil with 2 g Acid Activated Rice Husk Ash	Treated Palm Oil with 3 g Acid Activated Rice Husk Ash	FAO Limit
Peroxide value (meqO_2/kg)	5.8	0.177	0.169	0.161	< 10
Saponification value	186.72	194.582	197.382	198.580	190-200
Unsaponifiable matter (%)	1.72	0.20	0.23	0.24	< 1.0
Color	11.2R/10Y	5.6R/13Y	5.4R/13Y	5.2R/13Y	-

Unsaponifiable Matter of the Selected Oil Samples

Crude fats and oils obtained from various vegetable and animal sources contain variable amounts of unsaponifiable matter. In the refined oil approximately (2) or (3) percent of unsaponifiable matter is still present. Sterols are characteristics components of all fats and oils, comprising in most cases the greater part of the unsaponifiable matter. They may be separated from the unsaponifiable constituents of fats through their solubility in hot alcohol and may be concentrated from oils without saponification to latter, by means of molecular distillation, or extraction of the oil with alcohol or other suitable solvents. Sterol occurs in fats and oil both in the free from and as esters of fatty acids.

The amount of unsaponifiable matter present in edible oil depends on the degree of refining to which it has been subjected. Crude oils contains the largest amount of unsaponifiable matter and completely refined oil the least.

Conclusion

In this research, the selected oil samples were calcinated by using prepared rice husk ash sample (1g, 2g and 3g). The rice husk sample was obtained from Mahlaing Township, Mandalay Region and calcinated at

600˚C and treated with hydrochloric acid to obtain the acid activated rice husk ash. Some physicochemical properties of the selected oil samples of peanut and palm oil free fatty acid (2.306 %, 6.54%), acid value (5.050 %, 14.58%), iodine value (94.355, 48.42), peroxide value (0.185, 5.8 meq O_2/kg), saponification value (192.782, 186.72), unsaponifiable matter (0.183 %, 1.72%), and color (R11.2/12Y, R11.2/10Y).

Peanut oil samples and palm oil samples were treated with various amounts (1.0 g, 2.0 g and 3.0 g) of acid activated rice husk ash. After treatment with 3.0 g of acid activated rice husk ash, some crucial values of free fatty acid ,acid value, peroxide value decreased from (2.306 % to 0.602 % and 6.54% to 0.60%), (5.050 % to 1.33 % and 14.58 to 1.63%) (0.185meq O_2/kg to 0.161 meq O_2/kg and 5.8 meq O_2/kg to 3.8 meq O_2/kg). Whereas iodine value increased from (94.355 to 104.255 and 48.42 to 59.23) , saponification value increased from (192.782 to 198.582 and 186.72 to 192.45), unsaponifiable matter also increased from (0.183 % to 0.24%)for peanut oil but for palm oil sample it decreased from (1.72% to 0.265%).

According to the FAO standards, the range of acid value for edible oils is not more than 6.0 %, iodine value is 84-105, peroxide value is < 10 meq O_2/kg, saponification value is 190-200 and unsaponifiable matter is not more than 1.0 %. According to these ranges, the physicochemical properties of the all edible oil samples were in the range of acceptable.

After treated with acid activated rice husk ash, free fatty acid, acid value and peroxide value of the oil decreased with increased the weight of acid activated rice husk ash and iodine value, saponification value, unsaponifiable matter also increased with increased in weight of acid activated rice husk ash. Therefore rice husk ash can be used as an adsorbent material for the purification of edible oil and treated peanut oil samples and palm samples should be consumed without side effects. And this adsorption technique is a cost effective technology for the treatment of industrial foodstuff. Because of decreasing activity in free fatty acid, acid value, peroxide value, and increasing behavior in the case of iodine value, saponification value, unsaponifiable matter of the calcinated rice husk ash, sorption process plays a key role in purification of edible oil industry.

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References

A.O.A.C., (1998), " *Official Methods of Analysis* '' ,Association of Official Analytical Chemists . 18th Ed., Washington, DC, pp. 265-270.

Anderosn, A.J.C., (1962), "*Refining of Oils and Fats For Edible Purposes* '',Pergamon Press ,Co., London, pp. 196-203

Atkins,P.W.,(1994),"*Physical Chemistry*", 5th Ed., oxford University Press, New York, pp. 986-989

Bugge, C., (1993), "*Techniques for Enhancing Quality in Edible Oil Proceeding of the Word Conference on Oil Seed*", Technology and Utilization, AOCS Press, Champaign, pp. 198-206

Chen, C.Y., C.I. Lin and H.K. Chen, (2003), "Kinetics and Adsorption of β-Carotene from Soy Oil with Activated Rice Hull Ash'',*J.Chem.Eng.Jpn*., vol. 36, pp.265-270

Cooney, D.O., (1999), "Adsorption Design for Wastewater Treatment", Lewis Publishers, CRC Press LIC, Baca Raton, Florida

(http:// www. agroecology. org/cases/ rice hull-compost. Htm)(Accessed 17 July 2016)

FAO,(1997),"Rice By-products in Asia", RAP publication, The UN.RAP. Bangkok, Thailand, pp. 42-60

Gutcho, M.,(1979), "*Edible Oils and Fats Recent Develovpments*'',Nayes Data Corporation, New Jersey, pp. 350-355

Hassler,J.W.,(1963),"Activated Carbon", Chemical Publishing Co., Inc., New York, pp. 224-239

Lin, S.P., C.I. Lin and H.K. Chen, (2001), "Sesame Oil Bleaching with Activated Rice Hull Ash," *J. Chem. Eng. Jpn.*, vol. 34, pp. 737-747

LaGrega, M., P. Buckingham, J. Evans and the Environmental Resources Management Group,(1994). Hazardous Waste Management. McGraw- Hill Inc., New York, N (http://passpsarala.com/alio0.4thm/rice husk ash) (Accessed 2 August 2016)

Liew, K.Y., A.H. Yee and M.R. Nordin, (1993), "Adsorption of Carotene from Palm Oil by Acid Treated Rice Hull Ash", (http:// www.organicfacts.net >oils >oils)(Accessed 2 August 2016)

Norris, F.A., (1982), “*Extraction of Fats and Oils in Baileys*’’, John Wiley and Sons, New York, pp. 175-201

Pearson, D., (1970), “*The Chemical Analysis of Foods*’’, J. and A. Churchill Ltd , London, pp. 11-28

Proctor,A., and S. Palaniappan, (1989), “Soy Oil Lutein Adsorption by Rice Hull Ash’’, *J.Am.OilChem.Soc.*, vol. 66, pp. 1618-1621

Pryor, J.N., (1998), Bogdanor, J.M., Welsh, W.A.US pat. 4781864

(http:// en.m – wikipedia. org> wiki> Peanut….) (Accessed 17 July 2016)

Routh, J.I., (1955), “*Fundamental of Inorganic*’’, Organic and Biological Chemistry, W.B, Saunders Co., London, pp. 224-239

White, E.H., (1964),"*Chemical Background for the Biological Science*", Prentice-Hall, Inc., New Jersey, pp. 145-152

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Assignment of the Structure and Determination of Antimicrobial Activity of an Organic Compound Isolated from One Myanmar Indigenous Medicinal Plant, *Schisandra chinensis* (Turcz.) Baillon (Wu-Wei-Zi)

Thandar Soe[1], Yin Kay Khaing[2], Khin Mar Yee[3] & Myint Myint Sein [4]

Abstract

In this research work, one Myanmar indigenous medicinal plant, namely *Schisandra chinensis* (Turcz.) Baillon (Wu Wei Zi), which collected from Nan-khan Township, Northern Shan State, Myanmar was selected for the determination of phytochemical constituents and antimicrobial activities. This test gave rise to positive for alkaloid, glycoside, flavonoid, phenolic, polyphenol and sugar respectively. Moreover antimicrobial activity of crude extract of the stem of this plant was performed by agar well diffusion method in various solvent systems on six microorganisms. Among them ethyl acetate extract responded medium activity on four tested microorganisms. Furthermore, a bioactive compound could be isolated from ethyl acetate extract by using advanced separation techniques, such as Thin Layer and Column Chromatograms. The physical state of this compound (TDS-1) is yellow oily form and the yield percent is found to be (93.6 mg, 1.007 %) based upon the crude extract. In addition, the antimicrobial activity of this compound gave medium activities on three organisms, such as *Bacillus subtilis*, *Bacillus pumalis* and *E. coli* by applying agar well diffusion method. The molecular formula ($C_{29}H_{43}N_3O_8$) and the structure of this compound were assigned by using some sophisticated spectroscopic methods.

Keywords: *Schisandra chinensis* (Turcz.) Baillon (Wu Wei Zi), antimicrobial activities, agar well diffusion method, spectroscopic methods

(1Z, 2Z, 5E, 7E, 9Z, 11Z, 14E)2, 14 diamino-7-((E)-2 amino-3-hydroxybuta-1, 3-dienyl)-3-(2-hydroxypropan –2-yl)-12 isopropyl 5, 9, 10-trimethoxy cyclohexadeca-2, 5, 7, 9, 11, 14, 16-heptaene- 1, 4, 8-triol

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Introduction

A natural product is a chemical compound or substance produced by a living organism found in nature that usually has a pharmacological or biological activity for use in pharmaceutical drug discovery and drug design. A natural product can be considered as such even if it can be prepared by total synthesis. Many today's medicines are obtained directly from a natural source. Many of herbs and species used by humans to season food yield useful medicinal compounds.

A medicinal plant is (1) any plant used in order to relieve, prevent or cure a disease or to alter physiological and pathological process or (2) any plant employed as a source of drugs or their precursors. The developing countries including Myanmar have been investigated in the research of medicinal plants.

The present research may be of value for the development of new drugs and may stimulate further investigations. In this research, one Myanmar indigenous medicinal plant, namely, *Schisandra chinensis* (Turcz.) Baillon. (Anonymous, 2010) belongs to the Schisandraceae Family. In Myanmar, Northern Shan State, Nan-khan Township, there are plenty of this plant in the forests. Local people employ this plant in various diseases such as irritating and allergic skin conditions for external uses and dry cough, asthma, poor memory, hepatitis, palpitation, gastric ulcer for internal uses. So, this plant was selected. As a preliminary examination, the phytochemical screening and antimicrobial activities of crude extract of the stem of *Schisandra chinensis* (Turcz.) Baillon was carried out. A pure bioactive compound (TDS-1) could be isolated from the stem of *Schisandra chinensis* (Turcz.) Baillon.

For the detail study of the compound, its bioactivity and the structure should be determined. The planar structure of compound (TDS-1) was assigned by using modern spectroscopic methods, such as FT-IR, ^{1}H NMR, ^{13}C NMR, DEPT, DQF-COSY, HMQC, HMBC and EI-Mass spectra respectively.

Botanical Description of Selected Plant

Botanical name	-	*Schisandra chinensis* (Turcz.) Baillon
Family	-	Schisandraceae
Japanese name	-	Chosen gomishi
Chinese name	-	Wu Wei Zi (five taste fruit)
English name	-	five flavour fruit, magnoliavine, schisandra
Myanmar name	-	Unknown
Medicinal uses	-	gastric ulcer, asthma, hepatitis and cancer

Materials and Methods

Commercial grade reagents and solvents were used with further purification. Analytical preparative thin layer chromatography was performed by using precoated silica gel (Merk. Co. Inc, Kiselgel 60 F_{254}) and silica gel 70 to 230 mesh ASTM was used for column chromatography.

Common laboratory tools were used in the isolation and purification of compound (TDS-1). The advanced instruments which are used in the characterization of samples and elucidation of pure compound are shown below.

1. UV lamp (Lambada-40, Perkin-Elmes Co. England)
2. FT-IR spectrometer (Shimadzu, Japan)
3. ^{1}H NMR spectrometer (500 MHz)
4. ^{13}C NMR spectrometer (125 MHz)
5. EI- Mass spectrometer
6. UV spectrometer (PD-303 UV)

Sample Collection

The medicinal plant, *Schisandra chinensis* (Turcz.) Baillon, was collected from Nan-khan Township, Northern Shan State, Myanmar. The stem of the collected sample was cut into small pieces and was allowed to dry in air. Then, the raw material was kept in the glass bottle with stopper and used throughout the experiment. Air dried sample (1025 g) was percolated with ethanol (3400 mL) for two months.

Phytochemical Constituents of the Stem of *Schisandra chinensis* (Turcz.) Baillon Extract

In order to know the type of chemical constituents consisting in the stem of the selected plant, phytochemical tests (Harbonne, 1993) were carried out. The crude extract gave rise to positive for alkaloid, glycoside, flavonoid, phenolic, sugar and polyphenol respectively.

Antimicrobial Activities of the Stem of *Schisandra chinensis* (Turcz.) Baillon

The antimicrobial activities of the stem of *Schisandra chinensis* (Turcz.) Baillon were tested in various solvent systems on six organisms by using Agar well diffusion method. This plant extract was sent to Development centre for Pharmaceutical Technology (DCPT), Insein, Yangon. The applying organisms are *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus, pumalis, Candida albicans* and *E. coil* species. Ethyl acetate extract responded medium activity on four tested organisms. Hence, the ethyl acetate crude extract was used for further column separation.

Extraction and Isolation of Compound (TDS-1) from the Stem of *Schisandra chinensis* (Turcz.) Baillon

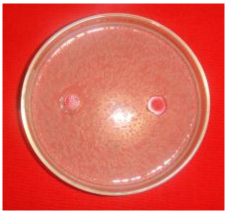
The air dried pieces of the stem of *Schisandra chinensis* (Turcz.) Baillon (1025 g) was percolated with 95 % ethanol for about two months. Then, the percolated solution was filtered and evaporated to concentrate. The residue was dissolved in EtOAc, and the extract of EtOAc was evaporated at room temperature.

The EtOAc crude extract (9.3 g) was chromatographed by Silica gel column by using n-hexane and EtOAc solvent system, with various solvent ratios from non-polar to polar. Totally 170 fractions were obtained. Each fraction was checked by TLC.

The same R_f value fractions were combined. Entirely, 20 combined fractions were obtained. Among them, the fraction XVII (129-132) has been found as main fraction that showed only one spot on TLC, and UV active. The yellow oily form of pure unknown compound (TDS-1), 93.6 mg, was obtained. The yield percent was found to be (1.007 %) based upon EtOAc crude extract. The R_f value of pure compound was 0.395 (n-hexane:EtOAc) (3:2).

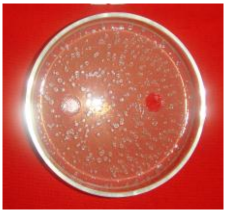
Antimicrobial Activities of an Unknown Compound (TDS-1)

The antimicrobial activities of an unknown compound (TDS-1) were tested by agar well diffusion method on six microorganisms. According to results, this compound responded medium activities on three organisms such as *Bacillus subtilis*, *Bacillus pumalis* and *E. coli*.

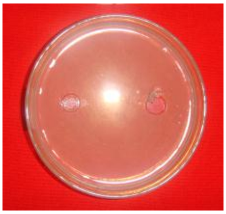


Bacillus subtilis

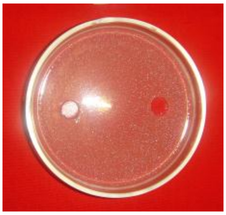
Staphylococcus aureus



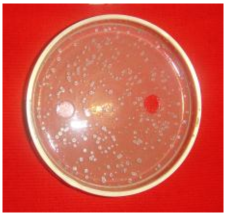
Pseudomonas aeruginosa



Bacillus pumalis



Candida albicans



E. coli

Spectroscopic Studies of an Unknown Compound (TDS-1)

This unknown compound (TDS-1) was subjected to analysis by Infrared Spectrophotometer (Hyper-IR, SHIMADZU) at the Department of Chemistry, University of Mandalay. EI-mass, ^{13}C Nuclear Magnetic Resonance (^{13}C NMR, 125 MHz), Distortionless Enhancement by Polarization Transfer (DEPT), Double Quantum Filtered Correlation Spectroscopy (DQF-COSY), Proton Nuclear Magnetic Resonance (^{1}H NMR, 500 MHz), Heteronuclear Multiple Quantum Coherence (HMQC), Heteronuclear Multiple Bond Coherence (HMBC), spectral data were measured at Department of Natural Resource Chemistry, Faculty of Pharmacy, Meijo University, Japan.

Results and Discussion

Molecular Formula Determination of An Unknown Compound

According to FT-IR spectrum, alcohol group, amine group, sp^{2} hydrocarbons, sp^{3} hydrocarbons, ether group and trans or E and cis or Z alkene could be assigned (Silverstein, 2005).

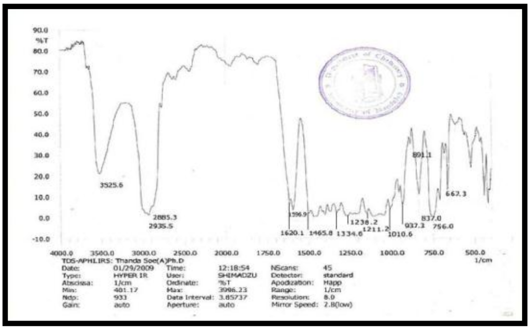


Figure 1. FT-IR spectrum of compound (TDS-1)

The ^{1}H NMR (500 MHz) spectrum, responded the total number of protons present in compound. In this spectrum, total 32 protons could be observed (Timothy, D.W, 1999).

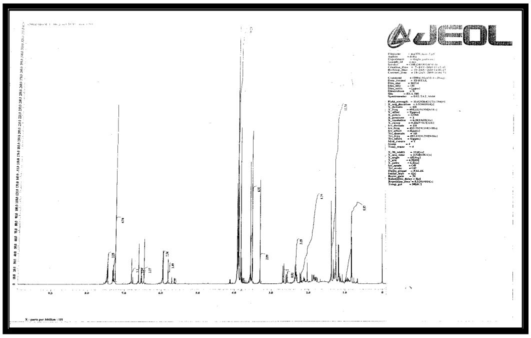


Figure 2. ^{1}H NMR spectrum of compound (TDS-1)

^{13}C NMR spectrum indicated the total number of carbons to be 29 (Johnson, L.F and Jankowaki, W.C, 1972).

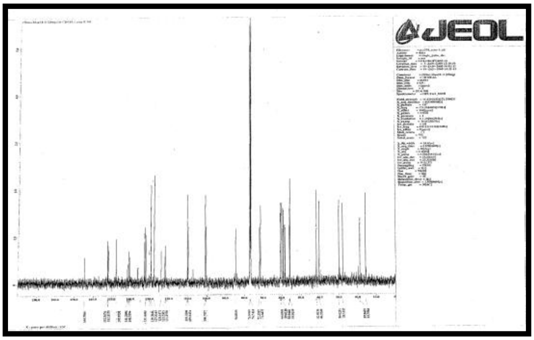


Figure 3. ^{13}C NMR spectrum of compound (TDS-1)

DEPT spectrum gave rise to the number and kinds of carbons as well as protons (Silverstein, 2005).

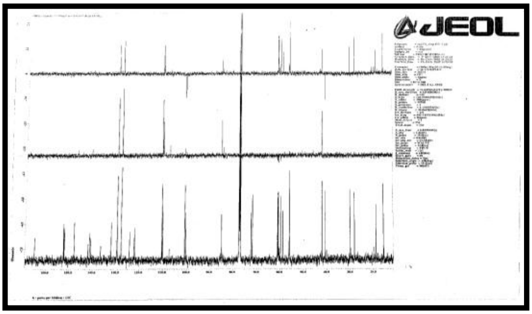


Figure 4. DEPT spectrum of compound (TDS-1)

HMQC spectrum indicated the direct proton carbon correlation (Timothy, D.W, 1999).

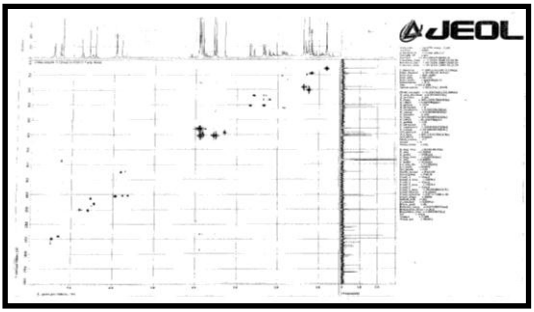


Figure 5. HMQC spectrum of compound (TDS-1)

In EI-MS spectrum of this compound, the molecular ion peak was (m/z 561) which represented its molecular mass (Porter, Q.N and Baldas, J., 1971).

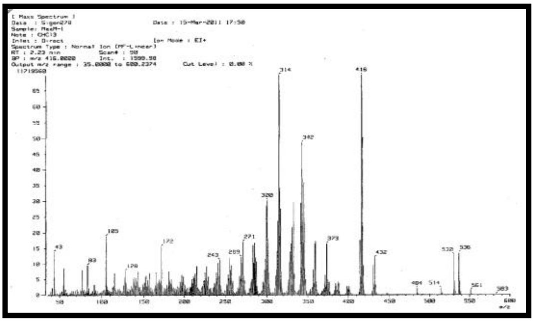


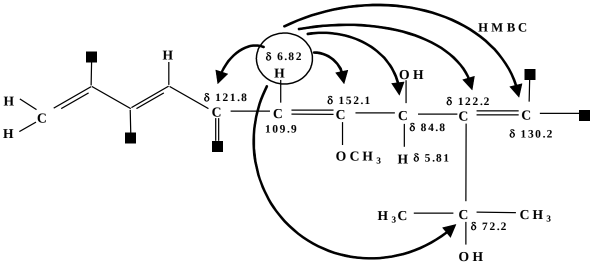
Figure 6. EI-Mass spectrum of compound (TDS-1)

According to above spectra, the molecular formula of the pure organic compound could be assigned as $C_{16}H_{16}O_4$. Hydrogen Deficiency Index of compound = 10.

Structure Elucidation of an Organic Compound

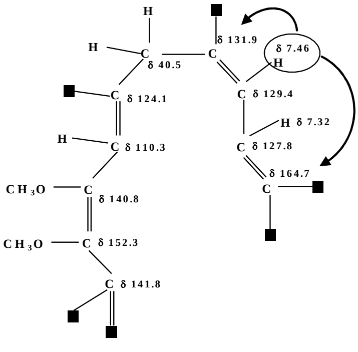
Structure elucidation of an organic compound was done by applying FT-IR, ^{1}H NMR, ^{13}C NMR, DEPT, DQF-COSY, HMBC, HMQC and EI-MS respectively. The following fragments could be assigned by DQF-COSY, ^{1}H NMR, HMQC and HMBC spectra.

HMBC correlation of methine proton (δ 6.82 ppm) with respect to the carbon atoms at δ 84.8, δ 121.8, δ 152.1, δ 122.2, δ 130.2 and δ 72.2 ppm supported the presence of fragment A.



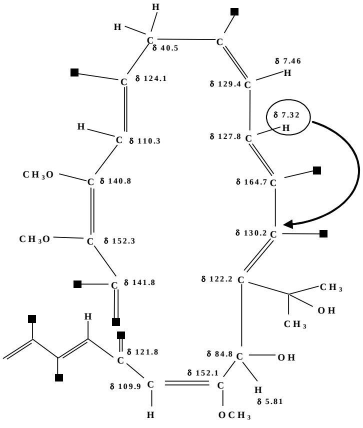
fragment A

The alkenic proton (δ 7.46 ppm) represented proton-carbon correlation to the sp^2 quaternary carbons at δ 131.9 and d 164.7 ppm. These HMBC correlation gives the partial structure of fragment B.



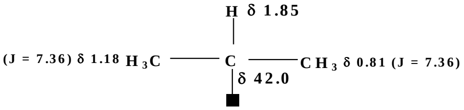
Fragment B

On the basis of ^{1}H-C long range correlation cross peak between δ 7.32 ppm proton from fragment B and δ 130.2 ppm quaternary carbon from fragment A, the combined fragment can be assigned.

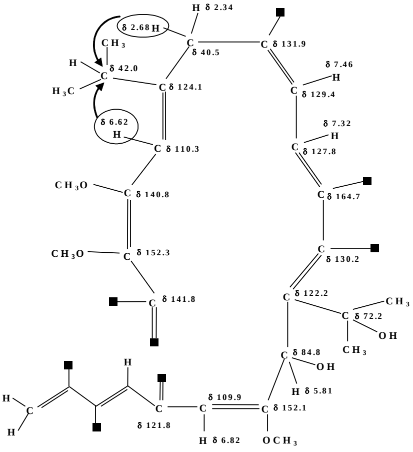


Combined Fragment

In DQF-COSY spectrum, the two doublet methyl groups were coupled to adjacent methine proton with medium graphic area leading to this isopropyl fragment.

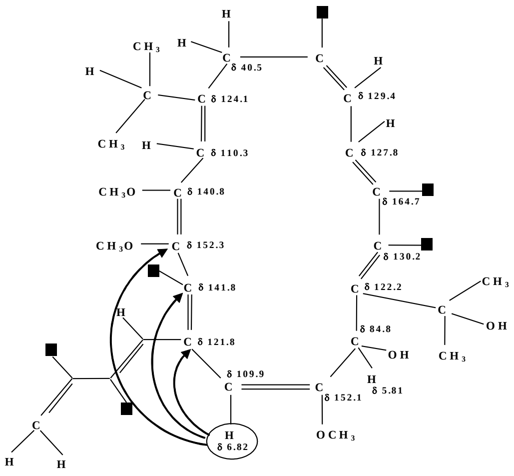


This iso-propyl group can be attached to combined fragment according to the HMBC correlation between both alkene proton at δ 6.62 ppm, methylene proton at δ 2.68 ppm from the combined fragment and methine carbon δ 42.0 ppm. So, fragment C can also be assigned.



Fragment C

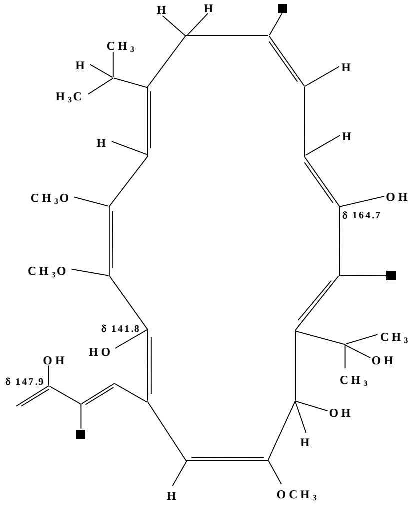
The observation of α, β and γ H-C long range coupling between an alkenic proton (d 6.82 ppm) and sp^2 quaternary carbons (δ 121.8 ppm, δ 141.8 ppm and δ 152.3 ppm) produced the 16 membered ring skeleton fragment D.



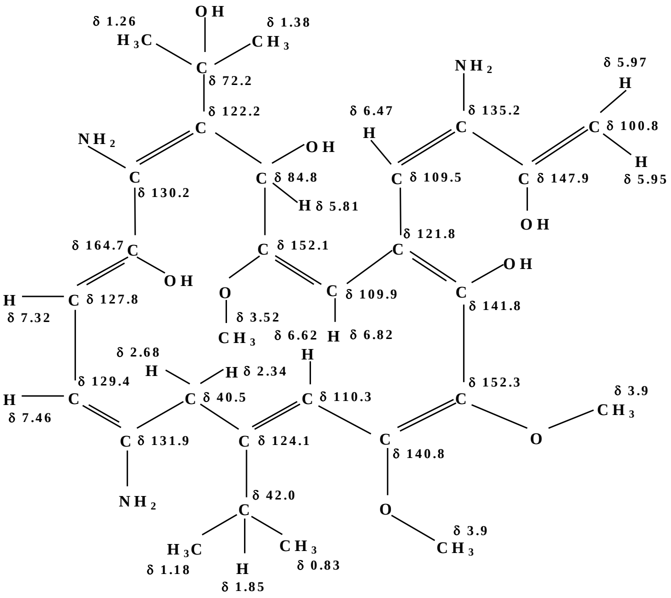
Fragment D

The molecular formula of isolated compound was $C_{29}H_{43}N_3O_8$. The partial molecular formula of this isolated compound was calculated as $C_{29}H_{34}O_5$. Hence, the remaining unassigned partial fragment was $H_9N_3O_3$.

Reliable attachment of three – OH groups to the downfield chemical shift alkenic carbons (δ 164.7, δ 147.9 ppm and δ 141.8 ppm) gave rise to the following fragment.



Finally, the logical correlation of the remaining – NH_2 groups to their respective alkenic carbons (δ 131.9 ppm, δ 130.2 ppm and δ 135.2 ppm) accomplished the complete structure assignment of this potent organic compound.



Conclusion

In this Ph.D research work, one Myanmar indigenous medicinal plant, *Schisandra chinensis* (Turcz.) Baillon (Wu wei zi) was chosen for phytochemical screening and antimicrobial activity on crude extract. A yellow oily form compound was isolated from *Schisandra chinensis* (Turcz.) Baillon (Wu wei zi) in pure state. This compound is found to have the medium antimicrobial activity on *Bacillus subtilis, Bacillus pumalis* and *E. coli*. The molecular formula ($C_{29}H_{43}N_3O_8$) and the structure of this compound could be assigned by spectral analysis consisting of FT-IR, ^{1}H NMR, 13C NMR, DEPT, HMQC and EI-MS respectively.

Acknowledgement

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References

Anonymous, (2010). Flora of China, Schisandraceae. People's Republic of China.

Eller K.I., Doctor of chemical science, (RAMS Nutrition Research Institute) Vlasov A.M. (Sechenov MMA).

Harbonne, J.B. (1993). "Phytochemical methods: A Guide to modern Techniques of Plant Analysis," Chapman and Hill Ltd, U.S.A.

John. H. Nelson, (2003). "Nuclear Magnetic Resonance Spectroscopy," Pearson Education, Inc. Upper Saddle River. NJ07458.

Komarova, E.L., Doctor of pharmacological science (ZAO "Natural Ingredients", Moscow).

LeRoy F. Johnson & Willian C-jankowski, (1972). Carbon-13 NMR. Spectra, A collective of Assigned, Coded, and Indeed Spectra", A Wiley-Inter Science Publication.

"Merk Index", (1983), 10th Edition, merk & Co. Inc. USA.

Nakanishi. (1969). "Infrared Absorption Spectroscopy," 3rd-Edition, ELBS with macmillan.

Phillip Crew, Jaime Rodriguez, Marcel. Jaspar, (1998)". Organic Structure Analysis", University of California, Santa Cruz, Oxford University, New York.

Raoelison Emmanuel, (2002)," Phytochemical Survey of Plants from Madagascar," Faculte des. Sciences, Institute de pharmacognosic *et al*, phytochimie, University de Lausanne.

Silverstein, R.M. *et al* (1989). "Spectrometric Identification of Organic Compound 6th Edition, John Willy & Sons, Inc, Singapore.

Silverstein, R.M. *et al* (1989). "Spectrometric Identification of Organic Compound 7th Edition, John Willy & Sons, Inc, Singapore.

Timothy, D.W. Claridge, (1999). "High resolution NMR Techniques in Organic Chemistry", Pergamon, An imprint of Elsevier Science, Ansterdam-Lausanne, New Yoke, Oxford-Shannon, Singapore, Tokyo.

Kemp, W., (1994). "Organic Spectroscopy", 3rd Edition, ELBS with Macmillan.

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Study on the Production of Ecofriendly Liquid Biofertilizer from Organic Wastes

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Abstract

The present research work deals with the study on the production and characterization of liquid biofertilizer from organic wastes. In the present study, liquid biofertilizer was made from the mixture of fresh cow manure, rice husk, corn cob and kokko leaves at 26 ˚C by using the anaerobic fermentation. Fermentation was taken for the period of one and half months. pH of fermented sample was determined by pH meter reading on the time interval basis. Physical parameters like electrical conductivity, organic carbon and humus and chemical parameters, total P_2O_5, total K_2O, total nitrogen and semiquantitative elemental analysis were determined using standard procedure. From this study, it has been revealed that the pH values of liquid biofertilizer was neutral in nature i.e., pH = 7.2 after fermentation period of 42 days. The electrical conductivity, organic carbon and C/N ratio of prepared liquid biofertilizer were found in acceptable range. The amounts of macronutrient, total nitrogen, total phosphorus (P_2O_5) and total potassium (K_2O) were 1.57 %, 0.22 % and 0.13 % were contained in prepared liquid biofertilizer. The high content of calcium was found to be 0.21 % followed by 0.15 % of magnesium and 0.09 % sulphur in prepared liquid biofertilizer. White Mustard leaves, Corn and Okra were cultivated on the soil of Demawso, Ngwedaung Township, Kayah State to study the effect of prepared liquid biofertilizer on plants, compared to that of control. The growth rate of plant heights and leaves sizes were measured seven days regularly. From these results it was found that the growth activity of plants enhanced by using prepared liquid biofertilizer. Therefore, the production of liquid biofertilizer from organic waste can save time, money and to increase crop output and help the environment.

Keywords: Liquid biofertilizer, anaerobic fermentation, macro-nutrients, organic waste, physicochemical parameter

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Introduction

Composting is generally defined as the biological oxidative decomposition of organic constituents in wastes under controlled conditions which allow development of anaerobic micro-organisms that convert biodegradable of organic matter into a final product sufficiently stable for storage and application without adverse environmental effects. The main products of anaerobic composting are CO_2, H_2O, mineral ions and humus. Also the process destroys pathogens, converts nitrogen from unstable ammonia to stable organic forms and reduces the volume of waste (Frey, 1999).

Moreover, biofertilizer contain microorganisms that help plants to grow by increasing the quantity of nutrients. Since these fertilizers contain living organisms, it increases or promotes the supply of important nutrients crucial for the overall productivity of the soil. An increasing number of farmers and agriculturists are turning to the use of biofertilizers as these are gentler on the soil as against chemical fertilizers (Ngampimol, 2008).

One of the major reasons of carrying out the experiment is that there is high rate of maize cobs from agricultural waste, cow dung from cattle farm and rice husk from paddy field that have become nuisance to that environment. One of the ways of getting rid of these is to convert them to a useful material that can be of help the plant. Also, biofertilizer improves the quality of soil for more productivity because they contain natural components which improve plant growth and do not cause any damage to plant. In case of chemical fertilizers the soil loses its natural balance and that in turn leaves a harmful effect on the environment (Ajiboye, 2013).

Materials and Methods

This part deals with all experimental procedures. The chemicals used were from the chemical suppliers; 'British Drug House Chemicals Ltd., Poole, England' and 'Kanto Chemical Co., Ltd., Tokyo, Japan', unless otherwise stated. Various conventional and modern techniques and instruments were used throughout the experimental procedures. All analytical works were according to recommended standard texts (Volgel, 1964). All experimental data were computed on the statistical basis. The apparatus consists of both conventional labware and glassware and modern equipment.

Sample Collection and Handling

The raw materials used for prepared liquid biofertilizer such as fresh cow manure, rice husk, corn cob, kokko leaves and molasses were collected from surrounding areas of Loikaw Township, Kayah State. The fresh cow manure was dried in the shade and was sieved with (80) mesh. The collected samples was stored in polyethylene bag and clearly labeled.

Sample Preparation for Liquid Biofertlizer

Liquid biofertlizer was prepared by the following steps.

Step 1

Fresh cow manure (400 g), corn cob (400 g), rice husk (200 g) and kokko leaves (200 g) were mixed in 35 L of plastic container. The 10 L of water was added to the solution and mixed with thoroughly.

Step 2

Molasses (200 mL) was added in 20 L of plastic bucket. Then, 1 L of water was added into the solution.

Step 3

The solution from step 1 and 2 were mixed in the 35 L of plastic container and stirred thoroughly with a wooden rod for 10 minutes.

Step 4

One end of a half inches plastic pipe was inserted in a 35 L plastic container. The other end of plastic pipe was inserted into plastic bottle filled up with water. The container was air tight leavened and placed in the shade for at least one month and half hours. After that every seven days, condition of prepared liquid biofertilizer was checked.

Determination of Physicochemical Properties

During fermentation, pH variation of prepared liquid biofertilizer was measured in water (1:1.25 w/v) using pH meter. The electrical conductivity was measured by conductivity meter. The chemical properties of prepared liquid biofertilizer were monitored including carbon to nitrogen ratio. Organic carbon content in the sample was determined by Walkley - Black method (Nathan, 1998). Total nitrogen analysis was performed by the Micro-Kjeldhal method, available phosphorus by spectrophotometer (Robotham, 2004). Calcium (Ca), magnesium (Mg) and sulphur (S) were determined by atomic absorption spectroscopy (Evans, 1968) and for potassium (K) and sodium (Na), by flame photometer. The texture for the cultivated soil sample was determined by pipette method (AOAC, 1984).

Results and Discussion

Composting is generally defined as the biological decomposition of organic constituents in wastes under controlled conditions which allow development of anaerobic micro- organisms that convert biodegradable organic matter into a final product sufficiently stable for storage and application without adverse environmental effects. In this study, purpose to build a platform that liquid biofertilizer obtained from organic waste are better than the conventional fertilizer.

Physicochemiacal Properties of Prepared Liquid Biofertilizer

Determination of pH variation during fermentation

Table 1. pH of Liquid Biofertilizer at Different Fermentation Period

No	Time (days)	pH values
1	1	6.8
2	7	7.2
3	14	7.8
4	21	8.1
5	28	8.5
6	35	8.2
7	42	7.2

The pH values of the prepared liquid biofertilizer are shown in Table 1. In this research, pH values of prepared liquid biofertilizer exhibit a slight declined in the beginning of anaerobic fermentation process. These were later inclined in the next 14 and 21 days. The pH initially decreased due to the degradation of the organic matter leading to the production of organic and inorganic acids. Further increasing of pH was caused by (a) decomposition of organic matter containing nitrogen (organic nitrogen mineralization), leading to formation of NH_3 which reacts with water and forms NH_4OH that neutralized the existent acids. (b) reaction of amino acids which was released from proteins forming ammonia (Sarkiyayi, 2011).

Total organic carbon (TOC) and organic matter

The contents of total organic matter and organic carbon of the prepared biofertilizer are shown in Table 2. In this research, the organic matter and organic carbon contents from prepared biofertilizer were 56.19 % and 25.6 %, respectively. High total organic carbon caused increase in the nutrient. These results revealed that the prepared biofertilizer was saturated with organic matter. Thus, it had the ability to affect plant growth as both source of energy and trigger for nutrients availability through mineralization. An increase in compost organic microorganism and total carbon, leads to biological diversity in the compost, thus increasing biological control of plant diseases and pests.

Electrical conductivity and carbon-to-nitrogen ratio (C/N ratio)

The electrical conductivity and C/N ratio for prepared liquid biofertilizer are shown in Table 2. In this research, the electrical conductivity and C/N ratio was 3.25 dSm^{-1} and 20.76. For agricultural purposes, fertilizer should have an initial C/N ratio of 20-30:1. To improve the C/N ratio of the fertilizers, it is necessary to mix substantial amount of other organic substances with high carbon and low amount of cow dung.

Table 2. Organic Matter, Organic Carbon, Electrical Conductivity and Carbon to Nitrogen Ratio of the Prepared Liquid Biofertilizer

Parameter	**Unit**	**Result**
Organic matter	**(%)**	56.19
Organic carbon	**(%)**	25.6
Electrical conductivity	(dSm^{-1})	3.25
Carbon to nitrogen ratio		20.76

Macronutrients (N, P, K) contents, total Ca, total Mg and total S of the prepared liquid biofertilizer

Total nitrogen (N), total phosphorus (P_2O_5) and total potassium (K_2O) are known to be macronutrients. Therefore, it is a vital work to analyze these elements not only in qualitative but also in quantitative. Macronutrients content in prepared liquid biofertilizer are shown in Table 3 and Figure 1. In this result, the content of total nitrogen, total P_2O_5 and total K_2O in liquid biofertilizer were found to be 1.57 %, 0.22 % and 0.13 %, respectively. Therefore, quantity of total nitrogen in prepared liquid biofertilizer was higher than the other nutrients total P_2O_5 and K_2O.

Table 3. Macronutrients (N, P, K) Contents, Total Ca, Total Mg and Total S of the Prepared Liquid Biofertilizer

Parameter	**Result (%)**
Total Nitrogen	1.57
Total Phosphorus	0.22
Total Potassium	0.13
Total Calcium	0.21
Total Magnesium	0.15
Total Sulphur	0.09

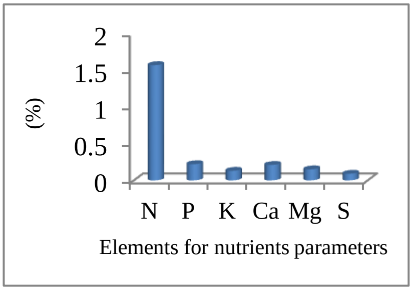

Figure 1. Histogram for NPK, Ca,Mg and S contents in liquid biofertilizer

The content of total calcium and total magnesium were found to be 0.21 % and 0.15 %. The predominant cation content is calcium which is necessary not only preserve desirable physical, chemical and biological properties in liquid biofertilizer but also to serve as a source of plant nutrient. The content of total sulphur in prepared liquid biofertilizer was found to 0.09 %. The result showed that the prepared liquid biofertilizer contained good amount of NPK content as well as Ca and Mg. Therefore, these nutrients may be used as for all parts of the plants.

Physicochemical Properties of Cultivated Soil Sample

In this research, the soil type of cultivated soil sample is shown in Table 4. According to data, the soil texture for cultivated soil sample is sandy clay loam. Generally, the best agricultural soils are those contained 10-20 % clay. Clay particles tend to retain or fix nutrient in soil.

The pH value of the cultivated soil sample is shown in Table 4. The data indicated that the soil sample was neutral nature (pH 6.94). The value of electrical conductivity of cultivated soil sample was observed to be 0.05 mScm^{-1} which was not very high.

The moisture contents, organic carbon and humus of the cultivated soil sample are shown in Table 4. In this research, the moisture content were found to be 1.12 %. The values of organic carbon and humus were 1.37 % and 2.35 % in cultivated soil sample. From this result, it was found that the contents of humus and organic carbon of the cultivated soil sample was found to be low which was suggested that the soils can be classified as low which was suggested that the soils can be classified as low level organic soil (3 to 19 % humus) (Magdoff, 2000).

Table 4. Physicochemical Properties of Cultivated Soil Sample

Parameter		**Results**
Moisture	%	1.12
pH		6.94
Electrical conductivity	mScm^{-1}	0.05
Texture (%)	Sand	69.76
	Silt	10.00
	Clay	20.24
		Sandy clay loam
Organic Carbon	%	1.37
Humus	%	2.35
Total Nitrogen	%	0.18
Available Phosphorus	ppm	1.25
Available Potassium	ppm	8.69

Macronutrients contents in cultivated soil samples are shown in Table 4. Total nitrogen (N) content of the cultivated soil sample was found to be 0.18 %. The contents of available phosphorus (P_2O_5) and available potassium (K_2O) were 1.25 ppm and 8.69 ppm. From these results, it was found that the cultivated soil sample contained a good amount of potassium contents but insufficient nitrogen and phosphorus. Hence, suitable fertilizers should be added to the soils for ensuring the maximum crop production.

Application for Liquid Biofertilizer

The obtained liquid biofertilizer can be applied in green areas of the homes which attached to this study. All these are fundamental for growth and development of all types of plants (fruits and vegetables included). It has an appropriate pH to improve conditions in the crop of vegetables and fruits. The effect of growth activity of White Mustard, Corn and Okra are shown in Table 5, 6 and 7 and Figure 2, 3 and 4. The growth rates of plant heights and leaves sizes were measured seven days interval. Plants can ascend by using liquid biofertilizer.

Table 5. Effect of Prepared Liquid Biofertilizer on White Mustard

No	**Time (days)**	**Plant height (in)**		**Leaf length (in)**		**Leaf Width (in)**	
		Control	**Spraying**	**Control**	**Spraying**	**Control**	**Spraying**
1	0	2.5	2.5	1.5	1.5	1.0	1.0
2	7	6.0	6.5	3.2	3.5	2.5	2.8
3	14	7.0	8.5	4.8	5.0	3.9	4.2
4	21	9.4	11	6.0	5.2	4.8	5.2
5	28	14.0	15.5	6.0	8	5.0	5.5
6	35	22.0	24.0	6.0	9.8	5.0	5.5

Figure 2. The growth rate of White Mustard using the liquid biofertilizer

Table 6. Effect of Prepared Liquid Biofertilizer on Corn (Maize)

No	Time (days)	Plant height (in)		Leaf length (in)		Leaf Width (in)	
		Control	Spraying	Control	Spraying	Control	Spraying
1	0	6	6	6	6	0.7	0.7
2	7	12.7	13.0	13.5	14.0	2.0	2.2
3	14	23.0	25.0	21.0	23.0	2.5	2.8
4	21	32.0	40.0	26.0	32.0	2.8	3.5
5	28	54.0	58.0	32.0	38.0	3.5	3.8
6	35	62.0	65.0	32.0	38.0	3.5	3.8
7	42	75.0	78.0	32.0	38.0	3.5	3.8

Figure 3. The growth rate of Corn using the liquid biofertilizer

Table 7. Effect of Prepared Liquid Biofertilizer on Okra

No	Time (days)	Plant height (in)		Leaf length (in)		Leaf Width (in)	
		Control	Spraying	Control	Spraying	Control	Spraying
1	0	2.2	2.2	1.4	1.4	1.2	1.4
2	7	3	3.5	2.5	2.8	2.2	2.5
3	14	5	6	3	3.4	3.3	3.8
4	21	10.4	11	4.8	5.2	6	6.5
5	28	14.7	15.5	7.4	8	9	10
6	35	22	25	9	9.8	10	12
7	42	28	30	10	11	10.5	12

Figure 4. The growth rate of Okra using the liquid biofertilizer

Conclusion

From the present work on the study of production and characterization of liquid biofertilizer from organic waste, the following inferences could be deduced. The electrical conductivity and pH value of prepared liquid biofertilizer was found to be 3.25 dSm^{-1} and 7.2. The results showed that the liquid biofertilizer is neutral in nature. The percent of organic matter, organic carbon and C/N ratio of prepared liquid biofertilizer were contained 56.19 %, 25.6 % and 20.76, respectively. The macronutrients, total nitrogen, total P_2O_5 and K_2O were found to be 1.57 %, 0.22 % and 0.13 % and the contents of total Ca (0.21 %), Mg (0.15 %) and S (0.09 %) were contained in prepared liquid biofertilizer. Prepared liquid biofertilizer was applied on the cultivated plants, White Mustard leaves, Corn and Okra. The growth rates of plant height and leaves sizes were measured for seven days interval. The results showed that the plants treatment with biofertilizer had significant differences from the control. The positive effects produced by prepared liquid biofertilizer can for giving a great amount of available nutrients to plants. Therefore, the results in the study showed the benefits of biofertilizer used in the cultivation of vegetables. This information will help to obtain biofertilizer with high nutritive value for plants and good amendments of soil physical, chemical and microbiological properties.

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References

Ajiboye, B., O. Akinremi and G. Razz. (2013). "Effects of Manure and Fertilizer on Soil Fertility and Soil Quality", *J. Environ Qual*, **33**, 1-61

AOAC. (1984). "Official Methods of Analysis", 14th Ed., Association of Official Analytical Chemists, Washington, DC

Evans, E.H. (1968). “Introduction to Analytical Atomic Absorption Spectroscopy”, John Wiley & Sons, New York

Frey, S.D. (1999). “Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients”. *J Biochem*. 31 (**4**), 573-585

Magdoff, F and H. Van Es, (2000), “Building Soils for Better Crops”, 2nd Ed. Jarboe Printing. Washington, D. C., pp. 240.

Nathan, M. V and K. Beegle. (1998). “ *Recommended Chemical Test Procedures*”. North Central Regional Research Publication, **21** 5-35

Ngampimol, H. and V. Kunathigan. (2008). “The Study of Shelf Life for Liquid Biofertilizer from Vegetable Waste”, *AUJI*, **11**(4), 204-208

Robotham, M. and C. Smith. (2004). “*Testing for Soil Nitrogen and Phosphorus for Environmental Pollution Monitoring*” Soil and Crop Management, **6** 1-4

Sarkiyayi, S. and M. Shehu. (2011). “Effects of Boiling and Fermentation on the Nutrient Composition of Cow Milk in Kaduna Metropolis”, *Research Journal of Chemical Science*, **1**(7), 81-84

Vogel, A.I. (1968). “*A Text Book of Quantitative Inorganic Analysis Including Elementary Instrumental Analysis”.* London: Longmang Green Co., Ltd, 884

Zheng, L., N. Hui and B. Junguo. (2014). “Effect of Nitrogen Fertilization and Genotype on Rice Grain Macronutrients and Micronutrients”. *Journal of Rice Science*, 21(4), 223-242

In vitro Screening of Antiasthmatic Activity in *Tamarindus indica* L. (Ma-gyi) on Isolated Guinea Pig Trachea

Thin Thin Win[1], Myint Myint Khine[2] & Saw Hla Myint[3]

Abstract

In Myanmar Traditional Medicine, there are many plants origin used to treat bronchial asthma. *Tamarindus indica* L. (Ma-gyi) stem barks were selected for this study because they are widely grown in our country and used as folk medicine for the treatment of asthma and chronic bronchitis. The direct relaxant effect of the aqueous extract and 70% ethanol extract of *T. indica* stem barks was studied on the *in vitro* model of tracheal chain preparation using guinea pig. The extracts inhibited the contraction of guinea pig tracheal is muscle induced by histamine stimulation. The aqueous and 70% ethanolic extracts of *T. indica* stem barks showed 59.6% and 74.2% relaxation response respectively. So, it can be inferred that *T.indica* stem barks can be used as a remedy for the treatment of bronchial asthma.

Keywords: *Tamarindus india* L. (Ma-gyi), antiasthmatic activity, direct relaxant effect

Introduction

Tamarindus genus belongs to subfamily Caesalpiniaceae in Leguminosae. Members of this subfamily Caesalpinoideae are trees or shrubs; leaves pinnate or bipinnate; flowers zygomorphic; typical floral formula K5, C5, A5+5, Gl. Drugs derived from this subfamily are senna leaves and pods, cassia pods and tamarinds (Evans, 2002)□. The family Caesalpiniaceae consists of about 150 genera and 2200 species, widespreaded in tropical and subtropical regions, only a few species grow in distinctly temperate climates. 26 genera and 124 species including *T. indica* L. grow in Myanmar (Kress & Yin Yin Kyi, 2003).

The bark is astringent and is given in diarrhoea; in lotions and poultices, it is also applied to sores boils. In some countries, the bark is reported to be prescribed in asthma, amenorrhoea, and as a tonic and

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febrifuge. The bark contains about seven percent tannin and is reported to be used in tanning (Website).

Tamarind Tamarin Tamarind Close up to tamarind

Figure 1. Images of the different parts of *Tamarindus indica* L.

Bronchial asthma is a common disease affecting both men and women equally. It has also become a major health problem especially in industrialized countries. In some countries approximately 4 % of population have signs and symptoms consistent with a diagnosis of asthma (John, 1981). Asthma is a lung disorder in which spasms and inflammation of the bronchial passages leads to restriction of air in and out of the lungs. The number of people with asthma and the death rate from this have been increasing since the late 1980s (Website).

Asthma is characterized by difficulty in breathing, wheezing and constriction in the chest. Asthma attacks are the partial obstruction of the air way from an increase of mucous secretion and muscle wall constriction around the bronchioles. There is no cure for asthma but there are treatment to help to relieve the asthmatic attack. Some asthmatic attacks will subside on their own while others will need to be treated (Website).

There are many traditional medicine and medicinal plants used for the treatment of Asthma. Commonly used medicinal plants include *Ageratum conyzoides* L. (Khway-thay-pan). *Piper betle* L. (Kun-ywet), *Ocimum sactum* L. (Pin-zein), *Piper longum* L. (Peik-chin), *Coelus aromaticus* Benth (Ziyar-ywet-htu), *Adhatoda vasica* Nees (Mu-ya-gyee) and *Aegle marmelos* Correa (Okshit), *Tamarindus indica* L. (Ma-gyi), *Acacia concinna* DC. (Kin-mun-gyin). They are employed for treating conditions such as bronchitis, sore throat, tightness of chest, cough and expectorant. The bronchodilating activities of some medicinal plants were also reported by Indian Council of Medical research Alcoholic extract of

Tylophora indica (Burn. F) Merr. (Oo-pa-tha-ka) showed non specific smooth muscle relaxant action on isolated guinea pig tracheal smooth muscle.

Materials and Methods

Sample Collection

The stem barks of *Tamarindus indica* L. (Ma-gyi) were collected from Kamayut Township, Yangon Region. After collection, the sample was identified by authorized botanist at the Department of Botany, University of Yangon. The collected samples were washed with water and dried at room temperature for two weeks. It was then powdered in a grinding machine. The dried powdered samples were then stored in air-tight containers so as to prevent from fungus attack.

Preparation of Crude Extracts

The dried powdered samples (100 g) were extracted with (250 cm^3) of 70 % ethanol and water in separate conical flask, respectively for at least 7 days and then filtered. The filtrates were evaporated by using rotatory evaporator and desiccated. Then the dried extracts were weighed. Each extract was stored in refrigerator for screening of antiasthmatic activity.

in vitro Screening of Anti-asthmatic Activity of Various Crude Extracts from *T. indica* Stem Barks by Using Isolated Tracheal Chain from Guinea Pig

(a) Preparation of Isolated Tracheal Chain from Guinea Pig

The tracheal chain from guinea pig was isolated according to the method described by Castillo and De Beer (Castillo, 1947).

A guinea pig was killed by a blow on a head and cutting the throat. The neck and upper thorax were opened and the muscles surrounding the trachea were cleared. A length of trachea about 4-6 cm was dissected out from guinea pig and placed in a oxygenated Krebs solution in a petridish. By transverse cuts, each tracheal ring containing two cartilage bands were made. Tracheal rings were tied with a fine thread in a series so that smooth muscle was in a longitudinal plane and consecutive rings have muscle on opposite sides. About 8-10 tracheal rings were tied to obtain a chain as shown in (Figure 2).

The chain was placed in an organ bath, a thread from one end of the chain was fixed to the tissue holder and a thread from the other end connected to a simple lever which was attached with a writing point. The organ bath was filled with Kreb's solution and aerated with oxygen and temperature of the bath kept at 37 ℃. The magnification of the lever for recording was 10-12 folds and a constant tension on tissue was kept between 0.4-0.6 g. An appropriate passive tension was applied and the strip was allowed to equilibrate for half an hour (Figure 2).

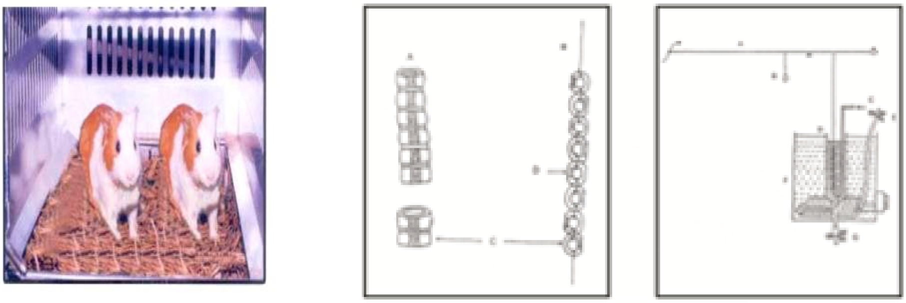
(b) Action of Extracts on Tracheal Chain Isolated from Guinea Pig

Following the equilibrating period, the effect of extracts on isolated tracheal chain was studied at different doses i.e., 0.5 mg, 1 mg, 2 mg, 3 mg, 4 mg, 5 mg per cm3 bath concentration. Each dose of extract was left in contact with tissue for exactly 5 mins and the effect was recorded. Then organ bath was washed 3 times and allowed to rest for 15 mins or more until the tissue recovered to the normal base line.

The same procedure was carried out on the strip which was applied with different concentration of different extracts. Each concentration of different extracts was tested for 3 times.

(c) Action of Extracts on Histamine Induced Tracheal Chain

Following the equilibrating period, the strip was allowed to contract by adding histamine (1 mg/cm3 bath conc.) to the organ bath, left for 5 min, washed 3 times and allowed to rest for 15 min or more until the tissue recovered to the normal base line. After that, histmine (1 mg/cm3 bath conc.) was added to the organ bath, left 3 min and followed by extract (1 mg/cm3 bath conc.) without washing the bath and left for another 2 min and the effect was recorded. Then the organ bath was washed 3 times and allowed to rest for 15 min or more until the tissue recovered to the normal base line. The same procedure for each extract was carried out 3 times.



(a) (b) (c)

Figure 2. Picture of (a) guinea pig (b) guinea pig tracheal chain (c) set up of apparatus for isolated guinea pig tracheal chain preparation

Results and Discussion

in vitro Screening of Anti-asthmatic Activity of Different Crude Extracts from *T. indica* Stem Barks

Effect of Plant Extracts on Normal Contraction of Isolated Tracheal Chain from Guinea Pig

The relaxation response (%) of aqueous and alcoholic extracts from *T. indica* stem barks with different doses (0.5mg, 1mg, 2mg, 3mg, 4mg and 5mg per cm3 bath conc) are given in (Table 1).

This Table shows the relaxing effect of the *T. indica* stem barks extracts with different doses on normal tracheal chain. The maximum dose for both aqueous and alcoholic extracts of Ma-gyi was found to be 2mg/cm3.From the data of these two extracts of Ma-gyi, it was observed that the alcoholic extract (77.8%) was slightly more potent than the aqueous extract (72.0%).

From the result of the experimental carried out on the normal contraction of tracheal chain isolated from guinea pig, it had been observed that the 70% ethanol extract of Ma-gyi had more bronchodilating activity than that of the watery extracts of Ma-gyi. According to literature review, both of these extracts of Ma-gyi have relaxation activities and those are popularly used as an anti-asthmatic.

Effect of Plant Extracts on Histamine Induced Tracheal Chain from Guinea Pig

When different extracts were added to the organ bath after stimulating the trachealis muscle with histamine (1mg/cm3), the extracts inhibited the contraction. The relaxation effect of aqueous extract and alcoholic extract from stem barks of Ma-gyi on isolated tracheal chain are shown in (Table 2). The results of present study have demonstrated that all these extracts possessed anti-histaminic activity. Ma-gyi aqueous extract and 70% EtOH extract were found to have and relaxation response, respectively, in histamine induced tracheal chain. Ma-gyi 70% EtOH extract was found to possess the most pronounced activity in trachealis muscle relaxation than the watery extracts of Ma-gyi (Figure 4).

Thus the beneficial effect of Ma-gyi stem barks in bronchial asthma and cough expectorant appears to be due to its anti-histaminic activity. So, it could be concluded that both aqueous and ethanolic extracts of Ma-gyi stem barks studied may some possess active principles with bronchodilating activity on the trachealis muscle.

For Ma-gyi stem barks, direct relaxant activity of ethanolic extract was observed to be higher than that of aqueous extract. Therefore the constituents present in ethanolic extract may be more active than that in aqueous extract.

Table 1. Relaxation Effect of Different Extracts from *T. indica* Stem Barks on Tracheal Chain Isolated from Guinea Pig

Bath conc. (mg/cm^3)	**Relaxation response (%)**	
	MG (aqueous)	**MG (70 % EtOH)**
0.5	40.3 ±1.2	38.7 ±3.5
1	62.7 ±3.7	40.3 ±1.2
2	72.0 ±6.9	77.8 ±1.3
3	68.0 ±2.4	75.3 ±2.4
4	65.3 ±2.3	74.7 ±2.9
5	70.0 ±5.8	74.0 ±2.3

Table 2. Relaxation Effect of Aqueous Extracts and Ethanolic Extracts of *T. indica* Stem Barks on Histamine Induced Tracheal Chain Isolated from Guinea Pig

Extracts (1 mg/cm^3 bath conc.)	**Height of contraction after addition of histamine (1 mg/cm^3 bath conc.) (mm)**	**Height of contraction after addition of extract (mm)**	**Relaxation response (%)**
MG (watery)	17.0±1.0	7.0±1.6	59.6±7.1
MG (70 % EtOH)	19.0±1.4	5.0±0.59	74.2±1.8

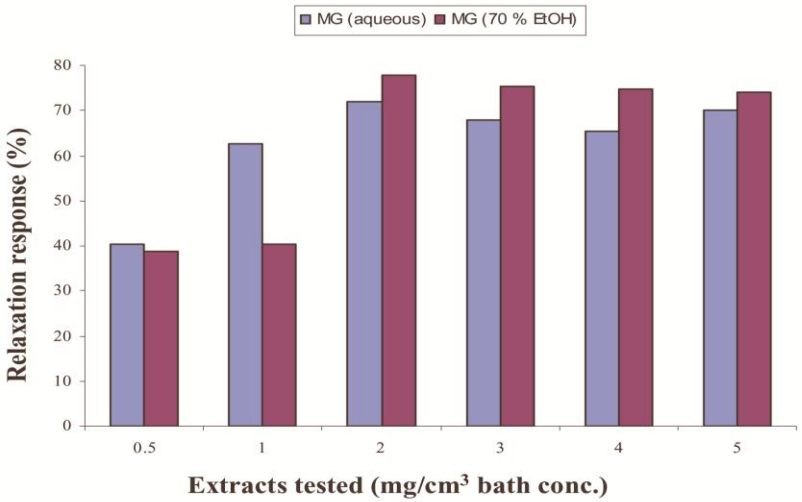


Figure 3. Effect of extracts on tracheal chain isolated from guinea pig

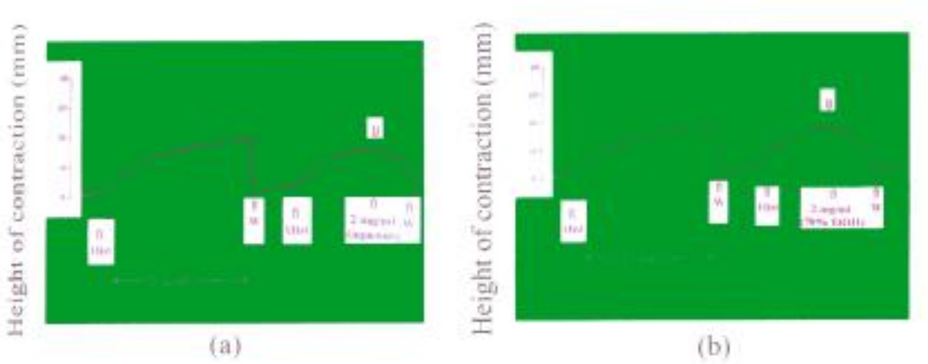


Figure 4. Effect of (a) Ma-gyi stem barks (water), (b) Ma-gyi stem barks (70% EtOH) extracts on histamine induced tracheal chain isolated from guinea pig

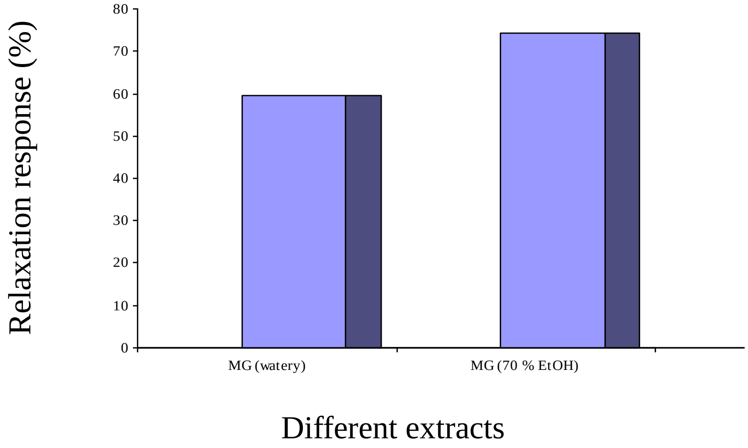


Figure 5. Effect of different extracts on histamine induced tracheal chain

Conclusion

From the overall assessment of the present work on the selected Myanmar Indigenous Medicinal Plants: Ma-gyi used to treat bronchial asthma, the following inferences can be deduced.

Crude extracts of 70% EtOH and water have been prepared from *T. indica* stem barks.

Direct relaxant effect of the extracts (water and 70% EtOH) of the *T. indica* stem barks were studied by in vitro model of tracheal chain preparation using guinea pig. The maximum doses of *T. indica* stem barks extracts were found to be 2 mg/cm3.

The effect of extracts on histamine induced contraction was screened on isolated tracheal chain. From this study, the relaxation response of histamine induced tracheal chain was observed to be 59.6% and 74.2% after treating with aqueous extract and 70% EtOH extract from *T. indica* stem barks respectively.

Therefore, the in vivo relaxation response of histamine induced tracheal chain of guinea pig for different extracts of the *T. indica* stem barks could be found in the order of 70% EtOH extract of the *T. indica* stem barks is greater than aqueous extract of *T. indica* stem barks.

So, from this experimental results, *T. indica* stem barks can be suggested as a remedy (or) folk medicine for the treatment of bronchial asthma.

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References

Castillo, J.C, and De Beer, E.J., (1947), *Journal of Pharmacologyand Experimental Therapeutics*, **90**, 104-109

Evans, W.C, (2002), Trease and Evans Pharmacognosy, 15th Ed., Toronto, 26

John Oroften, Sir., "Andrew Douglas, (1981), A Test Book of Respiratory disease", 3rd Edn.

Kress, K. J, R. A. De Fillipps, E. Farr and Daw Yin Yin Kyi, (2003), "A Checklist of the Trees, Shrubs, Herbs, and Climbers of Myanmar". Washington D. C: Department of Systematic Biology-Botany National Museum of National History, 243

Websites

http://www-bacterial/Escherichia coli-wikipendia, the Free encyclopeda. htm

http://www-What is asthma. htm

http://www-Asthma Facts, Disease Prevention and treatment strategies. htm

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Determination of Antimicrobial Activities and Structure Elucidation of Organic Compound Isolated from the Root of Michelia Champaca L. (Saga War)

Thinn Myat Nwe[1] & Ohnmar Myint[2]

Abstract

In this research work, the Myanmar Indigenous Medicinal Plant, *Michelia champaca L.* (Saga war) was chosen for chemical analysis. It gave rise to positive for the alkaloid, flavonoid, glycoside, polyphenol, terpene, steroid, saponin and reducing sugar respectively. Furthermore, antimicrobial activities of the ethyl acetate extract of the sample responded high activities on four tested organisms such as *Bacillus subtilis, Staphylococcus aureus, Bacillus pumilus* and *E. coli,* the ethanol extract of the sample responded high activities on three tested organisms such as *Bacillus subtilis, Staphylococcus aureus, Bacillus pumilus* and n-hexane extract gave high activities on three tested organisms such as *Staphylococcus aureus, Bacillus pumilus* and *E. coli*. In addition, a pure unknown compound was isolated from the root of Saga war by using thin layer and column chromatography. The pale yellow oily form compound 60.8 mg was obtained and the yield percent was found to be (2.432 %) based upon the ethyl acetate crude extract. Furthermore, the antimicrobial activities of isolated compound were rechecked in ethyl acetate solvent system by using Agar well diffusion method. This pure compound gave high activities on all selected organisms. Finally, the molecular formula $C_{16}H_{24}O_6$ and the complete structure of isolated compound were elucidated by spectroscopic methods such as, FT-IR, ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC, DQF-COSY and EI-Mass spectral data. The IUPAC name of isolated compound was 5-hydroxy-6-(3-hydroxy-3-methyl-6-oxotetrahydro-2H-pyran-2-yl)methyl)-2-methyl cyclo- hexen-2-en-1-yl acetate.

Keywords: *Michelia champaca L.,* phytochemical analysis, antimicrobial activities, chromatography

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Introduction

Nearly all culture, from ancient times, has used plants as a source of medicine. In many developing countries, traditional medicines are still the mainstay of health care and most of the drugs and the resultant remedy come from plants. In developed countries, many people are turning to herbal remedies, especially for minor ailments, and modern scientific medicine still depends on plants, and the knowledge for some essential drugs. People in India and China are known to have used plant in organized health care regimes for over 5,000 years (Mukherjee, 2004).

During the past decade, traditional system of medicine had become topics of global interest. World Health Organization (WHO) has also urged countries to promote the role of traditional practitioners in the health care system of developing countries and to allocate financial support for the development of traditional medical system (Damintoti Karou, *et al*, 2005).

The people in the countries mostly rely on traditional medicines. This traditional medicine involves the use of different plant extracts. Folkloric uses of some parts of Saga war are also described. Flowers of Saga war are used to produce an essential oil for perfume. Externally flower oil is used as an application in gout and rheumatism. An infusion or decoction of the Saga war flowers are used for dyspepsia, (indigestion), nausea and fevers. The flowers buds of Saga war are used for diabetes and kidney diseases. The bark is bitter, tonic, astringent. The Saga-wa root is used as purgative. Powdered bark of Saga war is also used for fevers, rheumatism. The crushed leaves of Saga war is mixed with oil and is applied on affected joints. Seeds of Saga war are used for healing cracks in feet.

Materials and Methods

Instrumentation and Materials

Instrumentation

(i) The FT-IR spectrum was measured at the Department of Chemistry, University of Mandalay.

(ii) Analytical and preparative Thin Layer Chromatography was performed by using precoated silica gel plates (Merck Co. Inc, Kiesel gel $60F_{254}$).

(iii) Common laboratory apparatus and equipments were used throughout the research work.

Materials

(i) Commercial grade reagents and solvents were used.
(ii) Iodine-vapour and UV lamp were used for location of the spots.
(iii) Silica gel G-60, (70-230) mesh was used for column chromatography.

Results and Discussion

Botanical Description

Family name - Magnoliaceae
Botanical name - *Michelia champaca* L.
English name - Golden or yellow champa
Myanmar name - Saga war
Part used - Root
Therapeutic uses - Purgative, carbuncles, haematemesis, stimulant and diuretic

Figure 1. Plant, Flower and Root of *Michelia champaca* L. (Saga war)

Sample Collection

The root of the *Michelia champaca* L. was collected from Kyauk Pantaung Township, Mandalay Region.

Extraction and Isolation of Organic Compound

Air dried sample (1000 g) was percolated with ethanol (2.5 L) for about two months. Percolated solution was filtered and concentrated to yield residue. It was extracted with ethyl acetate (300 mL) and evaporated. The ethyl acetate crude sample (2.5 g) was obtained. It was fractionated by column chromatography over silica gel (70-230 mesh) eluted by various solvent ratio of n-hexane and ethyl acetate from non- polar to polar. Totally, (225) fractions were obtained. These fractions were combined according to same R_f values under UV lamp and iodine detector. Seven combined fractions were obtained. The combined fraction (III) was checked by TLC for purity amount. It gave one spot on TLC in (R_f = 0.4) with n-hexane and EtOAc (3 : 2 v/v) and UV active. The pure compound, pale yellow oily form (60.8 mg) was obtained. The yield percent was found to be (2.432%) based upon the EtOAc crude extract.

The Results of Antimicrobial Activities of the Root of Saga war

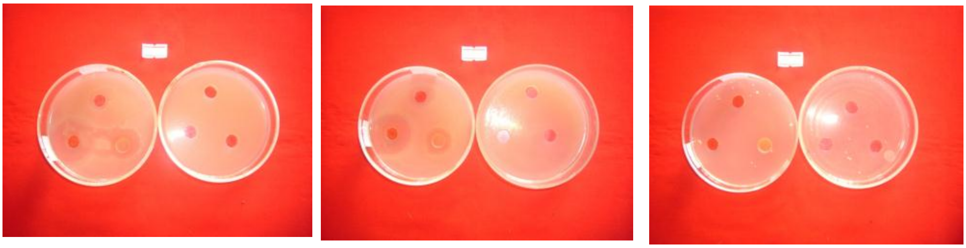
Table 1. Antimicrobial Activities of the Root of Saga war

Sample	**Solvents**	**Organisms**					
		(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**
Sga war	n-hexane	17mm (++)	26mm (+++)	-	27mm (+++)	-	26mm (+++)
	EtOAc	27mm (+++)	28mm (+++)	-	28mm (+++)	-	29mm (+++)
	EtOH	25mm (+++)	25mm (+++)	-	24mm (+++)	-	-

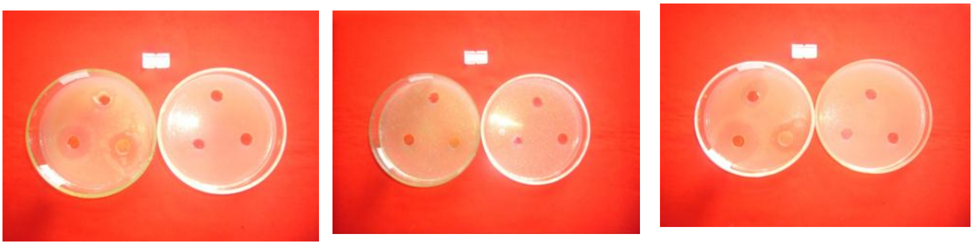
Agar well – 10 mm	
10 mm ~ 14 mm	(+)
15 mm ~ 19 mm	(++)
20 mm above	(+++)

Organisms
(1) *Bacillus subtilis*
(2) *Staphylococcus aureus*
(3) *Pseudomonas aeruginosa*
(4) *Bacillus pumilus*
(5) *Candida albican*
(6) *E. coli*

According to this table, ethyl acetate extract responded high activity on *Bacillus subtilis, Staphylococcus aureus, Bacillus pumilus* and *E. coli*. Furthermore, ethanol extract responds high activity on *Bacillus subtilis, Staphylococcus aureus* and *Bacillus pumilus*. In addition, hexane extract responded high activity on *Staphylococcus aureus, Bacillus pumilus* and *E. coli*. respectively.



Bacillus subtilis *Staphylococcus aureus* *Pseudomonas aeruginosa*



Bacillus pumilus *Candida albican* *E. coli*

Figure 2. Antimicrobial Activities of Root of *Michelia champaca* L.

Preliminary Phytochemical Screening of the Root of *Michelia champaca* L. (Dos, A.K., *et al*, 1970, Harbone, J.B, 1984)

The root of *Michelia champaca* L. was tested by phytochemical screening and these results are shown in Table (2).

Table 2. The Result of Phytochemical Test for *Michelia champaca* L.

No.	Constituents	Reagent used	Observation	Result
1.	Alkaloid	Dragendorff's solution Mayer's reagent	Yellow ppt White ppt	+ +
2.	Glycoside	10 % lead acetate	White ppt	+
3.	Flavonoid	Conc: HCl, Mg turning	Brown red color solution	+
4.	Steroide	$CHCl_3$, conc. H_2SO_4, Acetic anhydride	Greenish blue color	+

No.	Constituents	Reagent used	Observation	Result
5.	Saponin	Distilled Water	Frothing	+
6.	Terpene	Petether, conc. H_2SO_4, Acetic anhydride	Red purple color solution	+
7.	Reducing Sugar	Benedict solution	Brick red ppt	+
8.	Polyphenol	1 % $FeCl_3$ + 1 % $K_3[Fe(CN_6)]$	Greenish blue color solution	+

(+) = presence of constituents (–) = absence of constituents

According to these results, the root sample consisted of alkaloid, glycoside, flavonoid, steroid, saponin, terpene, reducing sugar and polyphenol respectively.

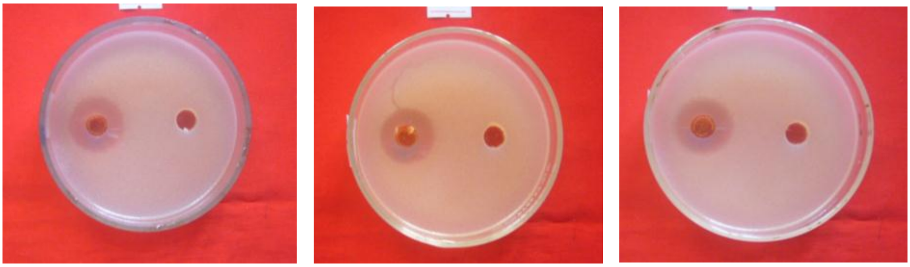
The Results of Antimicrobial Activities of the Isolated compound

Table 3. Antimicrobial Activities of the Isolated Compound

Sample	Solvents	Organisms					
		(1)	(2)	(3)	(4)	(5)	(6)
Isolated Compound	EtOAc	27mm (+++)	28mm (+++)	-	28mm (+++)	-	29mm (+++)

According to this table, ethyl acetate extract of isolated pure compound responded high activity on all selected organisms.

Bacillus subtilis *Staphylococcus aureus* *Pseudomonous aeruginosa*



Bacillus pumilus *Candida albican* *E. coli*

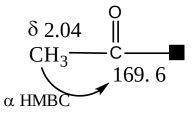
Figure 3. Antimicrobial Activities of Isolated Pure Compound

Molecular Formula Determination of Isolated Compound

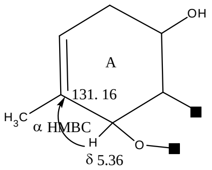
From ^{1}H NMR and ^{13}C NMR spectral data, the partial molecular formula was $C_{16}H_{22}$. From FT-IR spectral data, there were at least one-OH group and one ester group. Therefore, the extended partial molecular formula was $C_{16}H_{23}O_3$. Information from the DEPT spectrum, there was observed that two carbonyl carbons, one carbinol carbon and oxygen bearing methine carbon. Thus, the partial molecular formula was $C_{16}H_{23}O_4$ and the partial molecular mass was m/z 279. In EI-Mass spectrum, the molecular ion peak is m/z 312. Hence, the remaining partial molecular mass was m/z 33. The remaining partial molecular mass 33 may be one $–NH_2$ and one –OH groups or one oxygen atom and one –OH group. According to nitrogen rule, the remaining partial molecular mass 33 must be one oxygen atom and one OH group. Therefore, the complete molecular formula was $C_{16}H_{24}O_6$ and the hydrogen deficiency index of isolated compound is 5.

Structure Elucidation of Isolated Compound from the Root of *Michelia champaca* L.

According to the DEPT, HMQC and DQF-COSY spectral data, the following fragments **a** and **b** could be assigned.

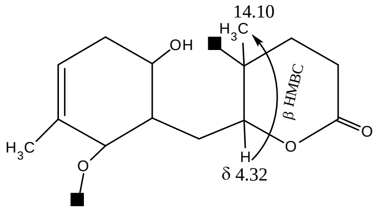


fragment **a**



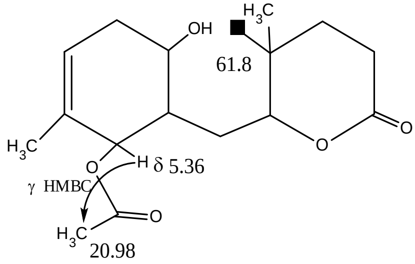
fragment **b**

In addition, the observation of α and β ^{1}H-C long range signal in HMBC spectrum produced the following fragments **c**.

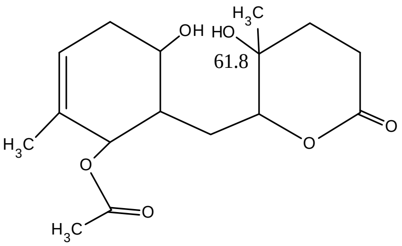


fragment **c**

The fragment **a** and **c** could be connected by the occurrence of γ ^{1}H–^{13}C long range signal between sp^3 oxygen bearing methine proton (δ 5.63 ppm) and the other methyl carbon (δ 20.98 ppm) in HMBC spectrum. It was described the following fragment.

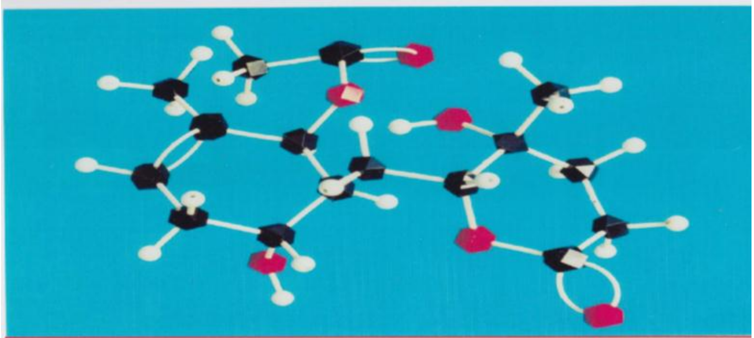


Consequently, the partial molecular formula of the above fragment could be assigned as $C_{16}H_{23}O_5$. The real molecular formula was $C_{16}H_{24}O_6$. Therefore, the remaining partial molecular formula must be OH group. This OH group must be attached to more down filed chemical shift (δ 61.8 ppm) of quaternary carbon. Therefore, the complete structure of isolated compound could be elucidated as follows.

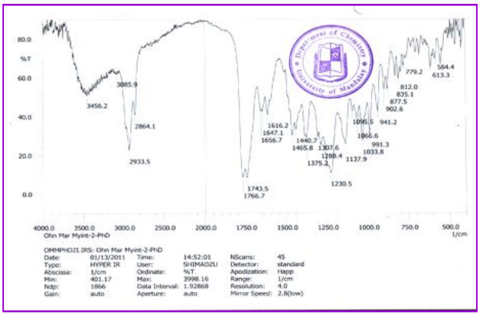


Conclusion

In the root extract of *Michelia champaca* L., alkaloid, flavonoid, glycoside, polyphenol, terpene, steroid, saponin and reducing sugar were observed when this sample was tested by phytochemical screening. Furthermore, the antimicrobial activities of root extract in various solvent systems were tested by agar well diffusion method on six selected organisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albican* and *E. coli*. According to these results, the ethyl acetate extract of isolated compound was rechecked the antimicrobial activity by agar well diffusion method on six selected organisms. It gave high activities on all selected organisms. On the other hand, the pale yellow oily compound was isolated from the root extract of *Michelia champaca* L. by using Thin Layer and Column Chromatography. The planar structure of isolated organic compound is as follows.



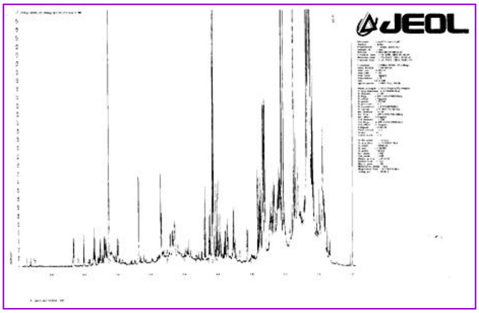
5-hydroxy-6-(3-hydroxy-3-methyl-6-oxotetrahydro-2H-pyran-2-yl)methyl)-2-methyl cyclohexen-2-en-1-yl acetate



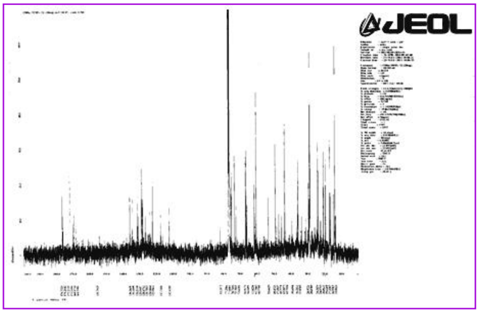
Ohn Mar Myint-2-PhD

OMMPHD2I.IRS: Ohn Mar Myint-2-PhD
Date: 01/13/2011 Time: 14:52:01 NScans: 45
Type: HYPER IR User: SHIMADZU Detector: standard
Abscissa: 1/cm Ordinate: %T. Apodization: Happ
Min: 401.17 Max: 3998.16 Range: 1/cm
Ndp: 1866 Data Interval: 1.92868 Resolution: 4.0
Gain: auto Aperture: auto Mirror Speed: 2.8(low)

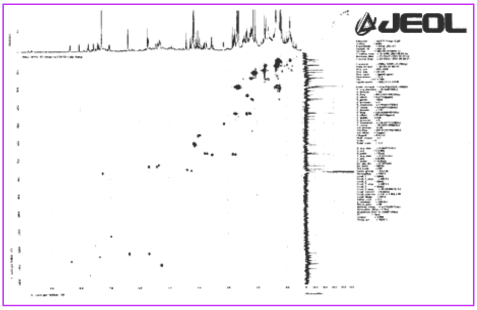
FT-IR Spectrum of Isolated Compound



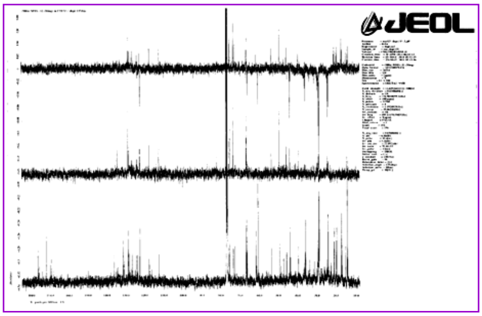
^{1}H NMR Spectrum of Isolated Compound



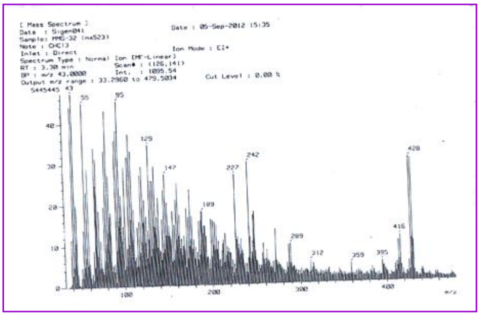
^{13}C NMR Spectrum of Isolated Compound



HMQC Spectrum of Isolated Compound

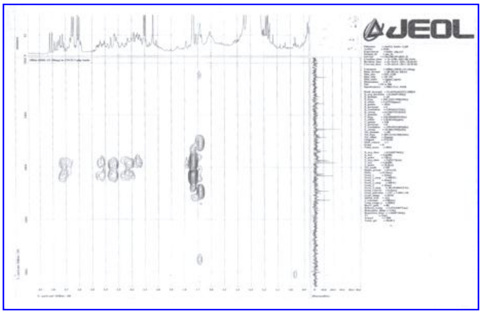


DEPT Spectrum of Isolated Compound

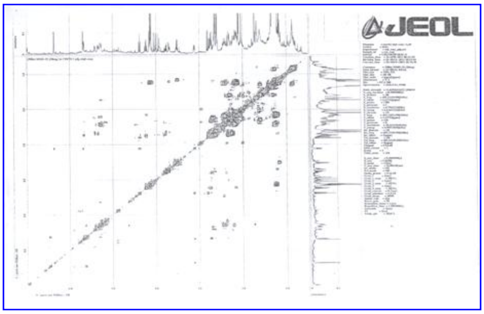

[Mass Spectrum]
Data : Sigen041
Sample: MMG-32 (ma523)
Note : CHCl3
Inlet : Direct
Spectrum Type : Normal Ion [MF-Linear]
RT : 3.30 min
BP : m/z 43.0000
Output m/z range : 33.2960 to 479.5034

Date : 05-Sep-2012 15:35
Ion Mode : EI+
Scan# : (126,141)
Int. : 1095.54
Cut Level : 0.00 %

EI-Mass Spectrum of Isolated Compound



HMBC Spectrum of Isolated Compound



DQF-COSY Spectrum of Isolated Compound

Acknowledgements

The authors would like to thank Professor and Head, Dr Khin Mar Yee and Professor Dr Aye Aye Myint, Department of Chemistry, Mandalay University of Distance Education, Myanmar, for their valuable advice and permission. The authors are also thankful to Dr Myo Myint, Deputy Director, Fermentation Department, Development Centre of Pharmaceutical and Food Technology for identification of antimicrobial activity of the root extract of *Michelia champaca* L.

References

Bruneton, J. (1995) *Pharmacognosy, Phytochemistry, Medicinal Plants* pp. 434-436. England, Intercept , Hampshire

Dos, A.K., *et al*, (1970). A Systematic Approach to Phytochemical Screening. Vol. 12 (1). Botanical Survey of India, Edinburgh.

Damintoti Karou, *et al*, (2005) Antibacterial Activities of Polyphenols from Ethanomedicinal Plants Burkina Faso, African Journal of Biotechnology. Vol. 4(8), pp 823 – 828, Academic Journals.

Harbone, J.B, (1984). Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 2nd Edition. Chapman and Hall Ltd. USA.

Mukherjee T. (2004), *Medicinal Plant Utilization and Conservation*. pp. 391–404.

Silverstein, R.M, *et al*. 1989. "Spectrometric Identification of Organic Compound" 6th Edition, John Willy & Sons, Inc. Singapore. p-126.

Online References

1. http://www.Springerreference.com/docs/html/chapterdbid/ 68946. html.
2. http://toptropica/s.com/pics/garden/c17/9856.J.pg

Universities Research Journal 2019, Vol. 12, No. 1

Study on the Effectiveness of *Moringa Oleifera* Seed for the Treatment of Water from Pakokku Industrial Zone

Than Than Aye[*]

Abstract

Safe drinking water is essential to the health and welfare of a community, and water from all sources must have some form of purification before consumption. Various methods are used to make water safe and attractive to the consumer. The natural coagulants/flocculants have shown advantages over chemicals, particularly in relation to biodegradability, low toxicity and low production of waste sludge. The water tested for this research was collected from the Pakokku Industrial Zone, Pakokku Township, Magway Region in cold season, January 2019. Coagulation/flocculation tests were carried out by using *Moringa oleifera* Lam. natural coagulant. The treatments included the control culture (no *Moring*a seed) and 4 g of *Moringa oleifera*. Before and after treatment of seed powder with water the parameters such as total alkalinity (TA), total hardness (TH), chloride, pH, total dissolved solids (TDS), and electrical conductivity (EC) were determined. Total alkalinity values (498.8 mg/L to 453.6 mg/L), total hardness values (336.2 mg/L to 314.2 mg/L), chloride values (492.2 mg/L to 239.2 mg/L), the pH values (9.0 to 8.9), total dissolved solids (534.0 mg/L to 480.0 mg/L), and electrical conductivity values (1060.0 µS/cm to 982 µS/cm) obtained from the treatment were in the recommended range set by the World Health Organization (WHO). Analysis of the trace metals (cadmium, copper, chromium, lead, zinc, iron, calcium, and magnesium) was performed before and after treatment of water with the *Moringa oleifera* seed powder by using Atomic Absorption Spectrophotometer (AAS). The results showed that *Moringa* seeds were capable of absorbing the trace metals tested. Thus the application of this low cost *Moringa oleifera* seeds is recommended for eco-friendly, nontoxic and simplified water treatment for rural people living in extreme poverty.

Keywords : Pakokku Industrial Zone, Coagulation/flocculation tests, *Moringa oleifera* Lam., natural coagulant

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Introduction

Specifically, water supply is a basic requirement for living creatures and human being. In this world the amount of resources available to living creatures are limited. About 75% of the present world population lives in the developing countries of the world. About 1.2 billion people still lack safe drinking water and more than 6 million children die from diarrhea in developing countries every year (Eman, *et al*., 2009).

Safe drinking water is essential to the health and welfare of a community, and water from all sources must have some forms of purification before consumption. Various methods are used to make water safe and attractive to the consumer. The method employed depends on the character of the raw water. One of the problems with treatment of surface water is the large seasonal variation in turbidity (McConnachie, *et al*., 1999).

Current operational procedures at many treatment works in developing countries are based on arbitrary guidelines, particularly in relation to the dosage of chemicals. Besides that, there is also the problem of inadequate number of skilled workers and inadequate laboratory facilities to monitor process performances required to operate the plants (Muyibi, 1998).

Conventional treatment is mostly used for water treatment. Coagulation-flocculation followed by sedimentation, filtration and disinfection, often by chlorine, is used worldwide in the water treatment industry before distribution of treated water to consumers.

Many coagulants are widely used in conventional water treatment processes for potable water production. These coagulants can be classified into inorganic coagulant, synthetic organic polymer, and naturally occurring coagulant. Synthetic polyelectrolytes are used as primary coagulant as well as coagulant aid to improve the strength of particle aggregates, enhance coagulation and deposition.

Naturally, occurring coagulants is usually presumed to be safe for human health while there is fear by using aluminum salts that may induce Alzheimer’s disease. Some studies on natural coagulants have been carried out and various natural coagulants were produced or extracted from microorganisms, animals or plants (Ganjidoust, *et al*., 1997)

Materials and Methods

Study Area and Sample Collection

Pakokku Industrial Zone is located in Pakokku Township, Magway Region. In this study, Water from Pakokku Industrial Zone was used for treatment by *Moringa* seeds in powdered form (Figure 1). The water sample was collected in cold season (January 2019). It was collected in plastic containers. Before collecting, plastic containers were cleaned with diluted hydrochloric acid (1:1) and washed with distilled water. However, plastic containers were rinsed again 3 to 4 times with sampling water before collection.

Figure 1. Collection of water sample from Pakokku Industrial Zone

Coagulants Used in the Study

Coagulation/flocculation tests were carried out by using *Moringa oleifera* Lam. natural coagulant. Fresh seeds of *Moringa oleifera* Lam. were used for water treatment in order to prevent degradation of the coagulant protein. Dry *Moringa oleifera* seeds used in this study were collected from Kyun Gyi Village, Taung Tha Township, Mandalay Region. Figures 2 and 3 showed the tree and pods with seeds.

The seeds were harvested when they were fully matured. This is determined by observing if there are any cracked pods on the plants. The pods that were plucked were cracked to obtain the seeds which were air-dried at 40°C for two days. The shells surrounding the seed kernels were removed by using knife and the kernels were pounded, using laboratory mortar and pestle into a fine powder. This method is a slight modification of the one proposed by (Ghebremichael, 2004). This was the coagulant prepared from *Moringa*.

Sample Preparation

The concentration of the stock solution for the loading dose was prepared by weighing 4.0 g of *Moringa* powder into a beaker containing 1000 mL of distilled water. The mixture in the beaker was stirred using a glass rod to obtain a clear solution. A 1000 mL of distilled water with no *Moringa* powder was kept as the control treatment.

A 3 mL of the 4.0 g/L concentration of *Moringa oleifera* and the control were added separately into a beaker containing 1000 mL of the water from Pakokku Industrial zone. The solutions were mixed rapidly for 2 min; followed by 10 min of gentle mixing, using glass rod to aid in coagulant formation. The suspensions were left to stand without disturbance for 2 h. The supernatants formed were decanted and subjected to total dissolved solids (TDS), pH, electrical conductivity (EC), total alkalinity (TA), total hardness (TH), and chloride measurements.

Figure 2. *Moringa oleifera* tree

Figure 3. *Moringa oleifera* pods and seeds

Determination of Water Parameters

Table 1. Applied Methods for Determination of Water Parameters

No.	Parameters	Methods / Apparatus
1.	pH	pH meter (Pocket pH-Meter ad 110 pH)
2.	Electrical Conductivity (μScm^{-1})	Conductivity Meter (SensoDirect 150)
3.	Total Dissolved Solids (mg/L)	TDS meter
4.	Total Hardness(mg/L)	EDTA Titrimetric Analysis
5.	Total Alkalinity(mg/L)	Titrimetric Analysis
6.	Chloride(mg/L)	Mohr's Method
7.	Trace Elements(mg/L) (Cd, Cu, Cr, Pb, Zn, Fe, Ca, and Mg,)	Atomic Absorption Sepctrophotometer

Results and Discussion

Sample Collection

In this research work, water sample was collected from Pakokku Industrial Zone, Pakokku Township, Magway Region and *Moringa oleifera* seeds were obtained from Kyun Gyi Village, Taung Tha Township, Mandalay Region.

Effect of *Moringa oleifera* on the Physicochemical Parameters of Treated Water

Physicochemical properties of water from Pakokku Industrial Zone were investigated before and after treatment. Total alkalinity was reduced from 498.8 mg/L to 453.6 mg/L for the treatment. Water hardness was reduced from 336.2 mg/L to 314.2 mg/L, chloride value was reduced from 492.2 mg/L to 239.2 mg/L, the pH value was reduced from 9.0 to 8.9, total dissolved solids were reduced from 534.0 mg/L to 480.0 mg/L, and electrical conductivity was reduced from 1060.0 µS/cm to 982 µS/cm for the treatment, respectively (Table 2 and Figures 4, 5, and 6). The results of the parameters were found within the range of WHO standard values for the water treated with *Moringa oleifera*.

Removal of Trace Metals

In this study, the concentrations of trace metals were determined before and after treatment of water with *Moringa oleifera* seed powder by using AAS. The Pb, Cr, Cu, and Fe were not detected in the water of Pakokku Industrial Zone. Table 3 and Figure 7 showed that *Moringa* seeds removed the trace metals tested (Mg, Zn, and Ca) but *Moringa* seeds were found not to be very effective in the removal of Cd.

The observed values of Mg, Zn, and Ca of before and after treatment of water with *Moringa oleifera* seed powder were within permissible limit of WHO but Cd values were above the permissible limit of WHO.

Table 2. Investigation of Physicochemical Properties of Water from Pakokku Industrial Zone Before And After Treatment with ***Moringa oleifera* (MO)**

Physicochemical Parameter	**Results**		**WHO Standard Value**
	Control (without MO)	**Treatment (with MO)**	
Total Alkalinity (mg/L)	498.8	453.6	200 - 500
Total Hardness (mg/L)	336.2	314.2	500
Chloride (mg/L)	492.2	239.2	250
pH	9.0	8.4	6.5 - 8.5
Total Dissolved Solids (mg/L)	534.0	480.0	500
Electrical Conductivity (μS/cm)	1060.0	982.0	1400

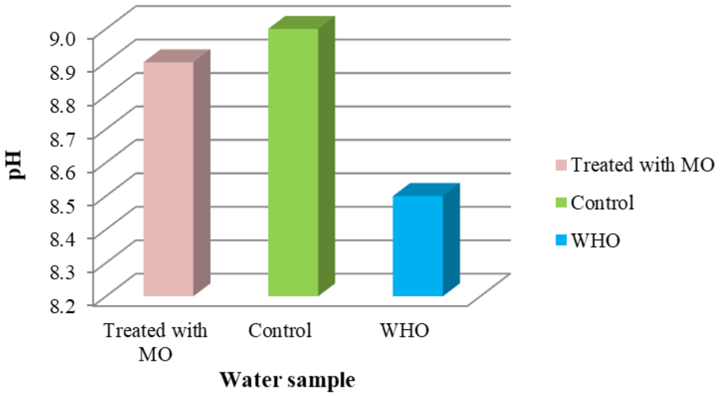

Figure 4. Influence of *Moringa oleifera* (MO) on pH of water

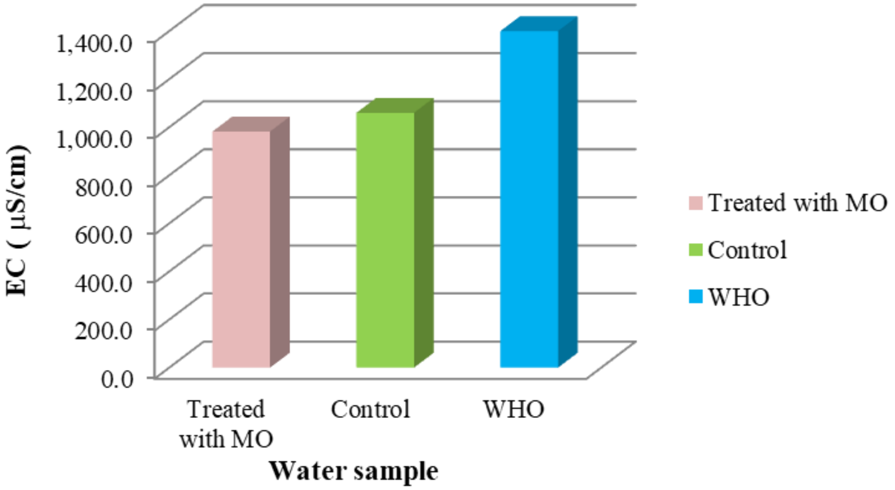

Figure 5. Influence of *Moringa oleifera* (MO) on electrical conductivity (EC) of water

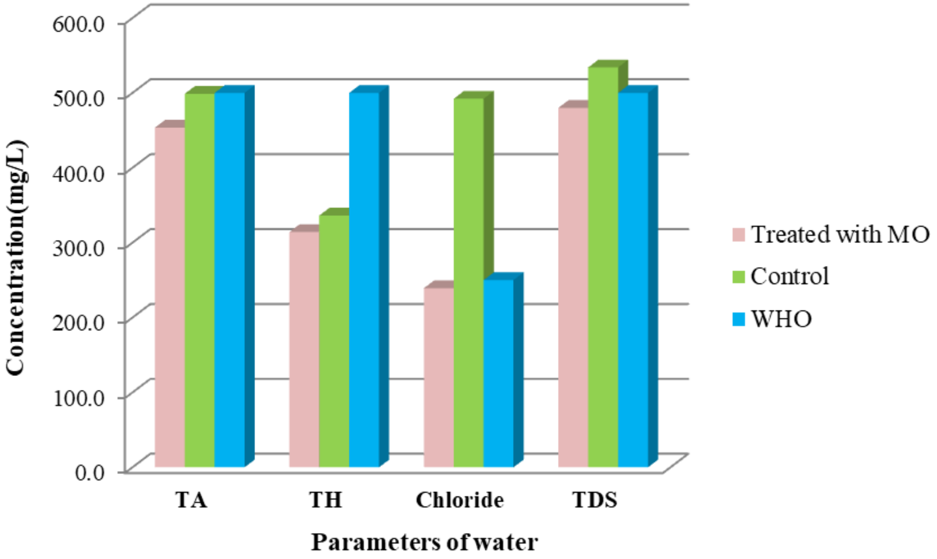

Figure 6. Influence of *Moringa oleifera* (MO) on total alkalinity (TA), total hardness (TH), chloride, and total dissolved solids (TDS) of water

Table 3. Concentrations of Trace metals before and after treatment of water with *Moringa oleifera* (MO) seed powder

Trace Metals	**Concentrations (mg/L)**		**Permissible Limit of WHO**
	Before Treatment with MO	**After Treatment with MO**	
Pb	ND	ND	0.01
Cr	ND	ND	0.05
Mg	5.215	4.213	50
Zn	0.030	0.015	5.0
Cd	0.079	0.078	0.05
Cu	ND	ND	2.0
Fe	ND	ND	0.3
Ca	17.58	16.31	75

ND = Not Detected, WHO = World Health Organization

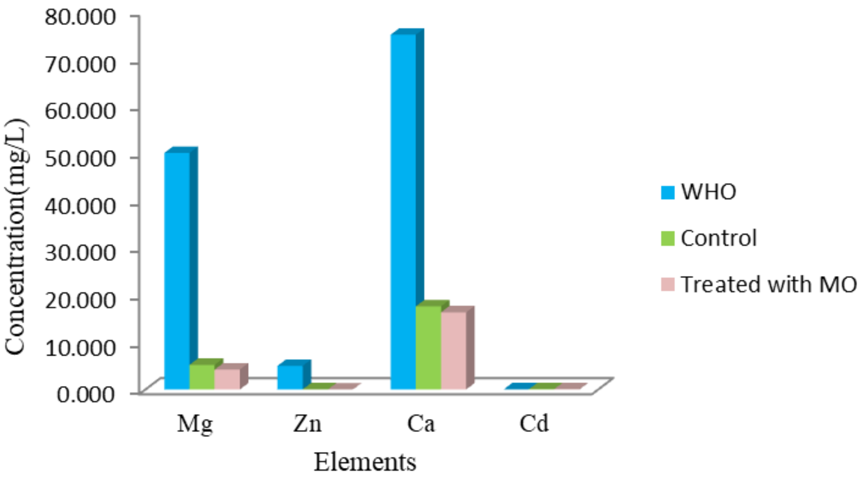

Figure 7. Influence of *Moringa oleifera* on trace metals (Mg, Zn, Ca, and Cd) of water

Conclusion

This research was carried out to confirm the effectiveness of powder extracted from mature-dried *Moringa oleifera* seeds which is commonly available in most rural communities. The treatment of *Moringa* gave values that are acceptable according to the World Health Organisation (WHO) guidelines for safe drinking water. The control sample gave the higher values which are unacceptable. *Moringa* coagulum can be locally produced, its use in water purification should be encouraged. This is likely to reduce the high cost of the current water treatment systems.

Until now, no evidence was found that these seeds can cause some side effects in humans, especially with the doses required to water treatment (Schwarz, 2000). Thus, it can be stated that treatment with *Moringa oleifera* Lam. seeds does not have health risks.

Acknowledgement

I would like to express my thanks to Professor Dr. Khin Maw Maw Soe, Head of the Department of Chemistry, Pakokku University, for her kind permission, encouragement, for providing the departmental facilities during my research work. I am also very thankful to Professor Dr. Mi Mi Hlaing, Department of Chemistry for her kind encouragement during my research.

References

Eman, N. Ali1, A. M. Suleyman , M. S. Hamzah, M. S. Mohd Ramlan and M. A. Zahangir. (2009). "Moringa oleifera seeds as natural coagulant for water treatment". Thirteenth International Water Technology Conference, IWTC 13. **Vol. 7**(3), pp. 163-168.

Ganjidoust, H., K. Tatsumi, T. Yamagishi, and R.N. Gholian. (1997). "Effect of Synthetic and natural coagulant on lignin removal from pulp and paper wastewater". *Water Science Technol*. **Vol. 35**, pp. 286-291.

Ghebremichael, Ka. (2004). "Moringa seed and pumice as alternative natural materials for drinking water treatment". PhD dissertation, Royal institute technology (kth), Stockholm, Sweden.

McConnachie, G.L., G.K. Folkard, M.A. Mtawali, and J.P. Sutherland. (1999). "Field Trials of Appropriate Hydraulic Flocculation Processes". *Wat. Res*. **Vol. 33**(6), pp. 1425-1434.

Muyibi, S. A. (1998). "*Moringa oleifera* seed extract in water treatment". *Journal Institution of Engineering*, Malaysia. **Vol. 59**(3), pp. 37-50.

Schwarz, D. (2000). "Water clarification using Moringa oleifera". *Technical Information*. **Vol. W1e** pp. 1-7.

Universities Research Journal 2019, Vol. 12, No. 1

Kinetic and Isotherm Studies of Heavy Metal Removals from Model Solutions and Wastewater Using Steam Activated Sulphurised Carbon

Ma San Myint*

Abstract

Liquid waste discharged from process industries, when not correctly handled and disposed, poses a great threat to both man and the environment. In this work, removal of some heavy toxic metals (Pb^{2+}, Cu^{2+} and Cd^{2+}) from aqueous solutions were studied by using steam activated sulphurised carbon(SASC) prepared from sugarcane bagasse pith. Batch sorption experiments were performed as a function of solution pH, contact time and adsorbent dose. The results obtained indicated that 1 g of adsorbent per 100 mL of water was enough to remove 79 % for Cu, 95 % for Pb 73% for Cd, respectively after 1 hour contact time. The maximum removal took place at pH 5 for Cu^{2+} and pH 6 for both Pb^{2+} and Cd^{2+}.The dosage method of sorption of the metal cations studied under the designated conditions, revealed a normal sorption pattern was followed. It fits the Freundlich and Langmuir adsorption isotherm. It showed on the basis of each speciation run, the sorption or removal capacity of SASC for the metal ions were in the following decreasing order: $Pb^{2+} > Cu^{2+} > Cd^{2+}$. The possible mechanism can be attributed as surface sorption accompanied by co-precipitation. The sorption study was applied by using a mixture of the metal ions ($P_b{}^{2+}$, Cu^{2+} & Cd^{2+}). Selective sorption data was achieved following the same order of selective sorption capacity: $Pb^{2+} > Cu^{2+} > Cd^{2+}$.Adsorption kinetic data further more showed that rather than pseudo-first order, pseudo-second order rate equation was followed. It can be inferred that sorption of metal ions (Pb^{2+}, Cu^{2+} & $Cd^{2+)}$ on modified bagasse pith carbons is probably a two-step process. The resulting sorption study when applied to a drained out battery wastewater body showed the selective removal capacity followed the same order: $Pb^{2+} > Cu^{2+} > Cd^{2+}$.

Keywords: Heavy metals, steam activated sulphurised carbon, kinetic studies, isotherm studies

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Introduction

Today, activated carbon adsorption is one of the low cost commercial technologies which is generally employed in the liquid phase adsorption, separation and purification processes (Allen, 2005). Activated carbons have been produced from every conceivable form of carbonaceous substances. The most common materials are wood, coal, peat, lignin, nutshell, sawdust, bone and petroleum coke (Hassler, 1974). Alkaline activated carbon which can be used for removing metals such as gold and silver from their solution has been reviewed by Mantell (1945). The preparation of low cost adsorbent for wastewater treatment has been reported by Pollard et al. (1992).

A wide range of lingocellulosic agricultural by-products has successfully been converted into activated carbon including coconut shell (Hichcocket al., 1983), jute stick (Banarjee and Mathew, 1985), sarkada grass (Chugtaiet al., 1987), palm-tree cobs (Renouprez&Avom, 1988), rice hull (Teker, 1999), spent activated clay (Weng et al., 2007).

Materials and Methods

Carbonization of Sugarcane bagasse pith

Carbonization (i.e., pyrolysis) of raw sugarcane bagasse was done by using Muffle furnace. The prior carbonizations of raw sample were first done on a microscale (TG-DTA analysis). The samples were carbonized at different temperatures (100,150, 200, 250, 300, 350, 400 ºC) and various times (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 hr). The content was then removed from the muffle furnace after the set period and cooled in an open air for one hr. This process was repeated until a substantial amount of carbonized sample was obtained. Carbonaceous material produced were then characterized by SEM, FT-IR and EDXRF analysis.

Chemical modification of Carbonized Materials

Activation of the carbonaceous material produced using superheated steam was carried out at 300 300 ºC for 2 hr in accordance to the description reported by Yalc, et al., 2000. The steam activated carbon was subjected to sulphurisation by SO_2 and H_2S for 4 hr gasification time to obtain sulphurised carbon designated as SASC (Kobya, et al., 2005).

Determination and Identification of Sulphur Content

The percentage content of sulphur found out by using Eschka's method. It was about 5.484 %. The EDXRF analysis inferred the presence of slphur in the steam activated sulphurised carbon.

Results and Discussion

Heavy Metal Analysis

Industrial effluent discharge is recognized as one of the major sources of toxic chemicals in the environment. In the present study, metal ions contained in model solution and industrial wastewater were removed using activated sulphurised carbon. Table 1 shows the results of the analysis as well as the allowable limits of the heavy metals in wastewater. From the table, it can be seen that heavy metal analysis of the effluent sample has revealed the presence of Cu, Pb and Cd ions at various concentrations even beyond the permissible limits set by the regulatory authorities.

Table 1. Measured heavy metals ion concentration of the wastewater

Metal	**Wastewater sample (mg L^{-1})**	**Safe limit (mg L^{-1})**		
		PEPA (2001)	**USEPA (1979)**	**FAO (1992)**
Cu^{2+}	5.457	0.01	1.00	0.2
Pb^{2+}	3.744	0.01	0.015	0.05
Cd^{2+}	1.285	0.05	0.005	0.01

Sorption Isotherms

The sorption of metal (II) ions (Cu^{2+}, Pb^{2+} and Cd^{2+}) by activated sulphurised carbon (SASC) was based on dosage method. The dosage method is the practical method of assessing the sorption capacity of a sorbent towards an adsorbate. The dosage method involves the use of different masses of sorbent, but a fixed initial concentration of the sorbate at an ambient temperature. Here, to reveal the significance of the removal of metal (II) ions, two well-known isotherm were applied (*i.e.*, Langmuir isotherm and Freundlich isotherm). The Ferundlich isotherm is an empirical relation. It was used on a large practical scale to estimate what amount of sorbent will be able to remove unwanted or decolourised substances from a certain volume of solution.

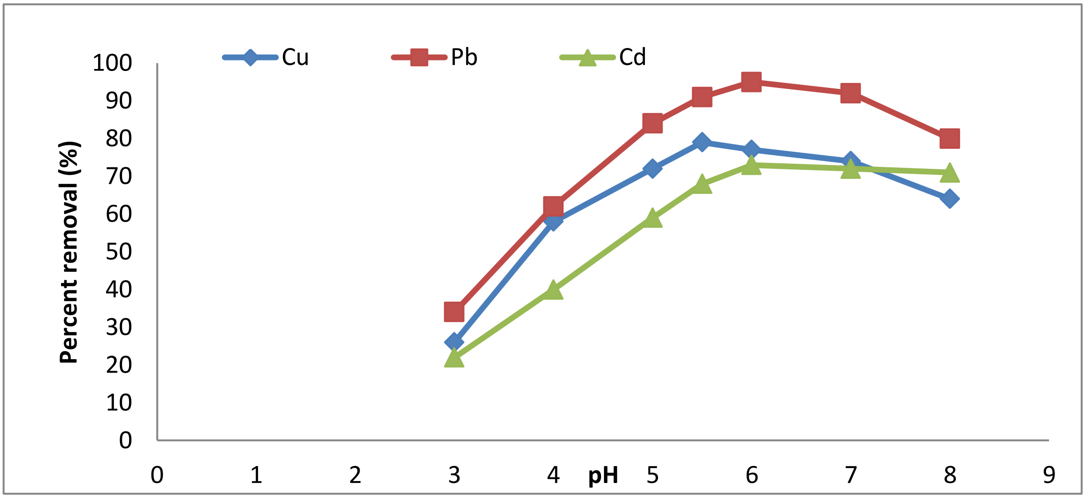


Figure 1. Effect of pH (3-8) on the removal of metal-II ions (Pb^{2+}, Cu^{2+} and Cd^{2+}) by 1 g of SASC in 100 mL sample solution; time 1 hr at ambient temperature

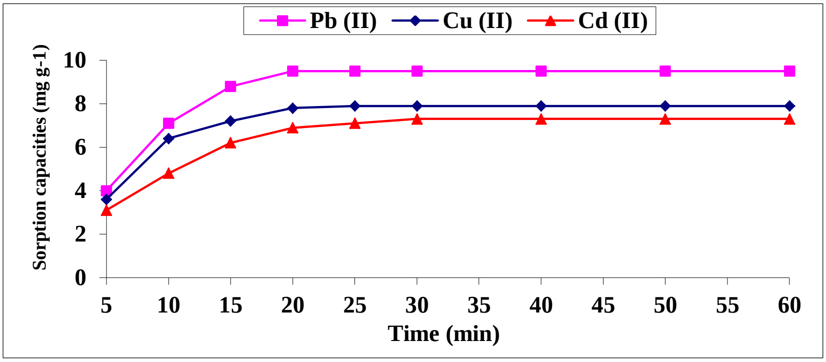


Figure 2. Effect of contact time (5-60 min) on the removal of metal-II ions (Pb^{2+}, Cu^{2+} and Cd^{2+}) by 1 g of SASC in 100 mL sample solution; pH 5 for Cu^{2+} and 6 for Pb^{2+} & Cd^{2+}

The Langmuir isotherm is more meaningful and a potential plot. It is based on theoretical concepts. The parameter constants reflect the significance of sorption phenomena. The Langmuir sorption equation (1) was used to fit the experimental sorption data:-

$$X = \frac{X_m b C_e}{1 + b C_e} \tag{1}$$

where, X = x/m, the amount of sorbate (x) adsorbed per unit mass of sorbent

C_e = equilibrium concentration of the srobate in solution

X_m = the maximum monolayer amount of sorbate per unit mass of sorbent

b = Langmuir constant related to the affinity between the sorbent and sorbate

The intercept equation is

$$\frac{1}{x/m} = \frac{1}{b X_m} \cdot \frac{1}{C_e} + \frac{1}{X_m} \tag{2}$$

where, slope is $1/bX_m$ and intercept is $1/X_m$.

The Freundlich equation is:-

$$\frac{x}{m} = K C^{1/n} \tag{3}$$

where, ‘x’ is the amount of adsorbate held by the sorbent, and ‘m’ is the mass of sorbent with which it is affected. ‘C’ is the concentration of substance remaining unadsorbed at equilibrium. The two constants ‘K’ and ‘1/n’ are particular to the system, *i.e.*, to both the sorbent used and nature of the sorbent. The equation, thus, correlates sorption capacity per unit mass of sorbent, x/m, with concentration of sorbate remaining solution at equilibrium. The equation in logarithmic form is:-

$$\log \frac{x}{m} = \frac{1}{n} \log C_e + \log K \qquad (4)$$

The calculated results of the Langmuir and Freundlich isotherm constants are presented in Tables 1.The essential feature of Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter, R_L which is used to predict if an adsorption system is “favourable” or “unfavourable”.

The separation factor, R_L is defined by:-

$$R_L = \frac{1}{1 + b\,C_o} \qquad (5)$$

where, ‘C_o’ is the initial concentration (ppm) and ‘b’ is the Langmuir adsorption equilibrium constant. Based on the effect of separation factor on isotherm shape, the R_L values are in the range of $0 < R_L < 1$, which indicates that the adsorption of metal ions (Cu^{2+}, Pb^{2+} and Cd^{2+}) on both activated carbons were favourable.

Adsorption Kinetic Study

Kinetic models have been used to investigate the mechanism of sorption and potential rate controlling steps, which is helpful for selecting optimum operating conditions for the full-scale batch process (Kalavathy*et al.*, 2005). Two kinetic models were applied mainly pseudo-first order and pseudo-second order rate equations.

Table 2. Values of Langmuir and Freundlich isotherm constants for sorption of metal (II) ions by SASC

Metal ions	Langmuir constants			Freundlich constants	
	X_m	**b**	**R_L**	**K**	**n**
Pb^{2+}	90.98	0.0040	0.7143	0.7858	1.2673
Cu^{2+}	83.33	0.0087	0.5348	0.6462	1.1579
Cd^{2+}	69.19	0.0102	0.4950	0.1975	1.0120

The First Order Adsorption

The pseudo-first order rate equation (Ho & McKay, 1998) is expressed as $dq/dt = k_1\ (q_e - q_t)$, where q_e and q_t are the amount of metal adsorbed (mg/g) at equilibrium and at any time ‘t’, respectively and k_1 is the pseudo-first order rate constant (min^{-1}). The integrated rate law, after applying the initial condition of $q_t = 0$ at $t = 0$, is $\log (q_e - q_t) = \log q_e - (\frac{k_1}{2.303}\ t)$. In this equation, the rate of adsorption is assumed to be proportional to the difference between the adsorption capacity (q_e) at equilibrium and the capacity at any time. The plots of $\log (q_e - q_t)$ *vs.* t were found as curves and inferred that the reaction did not follow the pseudo-first order as in Figure (3).

The Second Order Adsorption

The pseudo-second order rate equation (Ho &Mckay, 2000) is expressed as $\frac{t}{q_t} = \frac{1}{k_2\, q_e^2} + \frac{t}{q_e}$, where $h = k_2\, q_e^2$ (mg/g min) is the initial adsorption rate can be regarded as the initial adsorption rate as $t \rightarrow 0$ and k_2 (g/mg min) is the rate constant of the pseudo-second order adsorption.

The correlation coefficient R^2 for the pseudo-second order adsorption model has the highest value of 0.992. This suggests a strong relationship between the parameters and also explains that the process of adsorption follows the pseudo-second order kinetics in Figure (4).

Table 3. Kinetic model rate constants for the adsorption of Pb^{2+}, Cu^{2+} and Cd^{2+} on SASC-4

Concentration (mg L^{-1})	**Pb^{2+}**		**Cu^{2+}**		**Cd^{2+}**	
	$k_2 \times 10^3$ (g/mg min)	**h (mg/g min)**	**$k_2 \times 10^3$ (g/mg min)**	**h (mg/g min)**	**$k_2 \times 10^3$ (g/mg min)**	**h (mg/g min)**
100	33.68	3.608	25.06	1.828	21.10	1.225
200	12.19	4.526	11.80	3.495	8.06	1.813
500	3.88	7.786	4.25	6.631	2.45	2.428

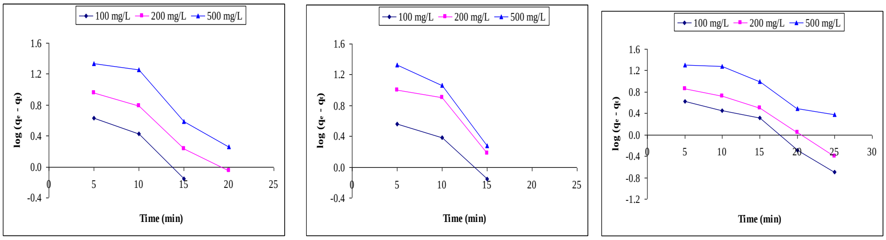


Figure 3. Natural logarithm of metal-II ions (Cu^{2+} ion, Pb^{2+} ion and Cd^{2+} ion) adsorbed with contact time by SASC

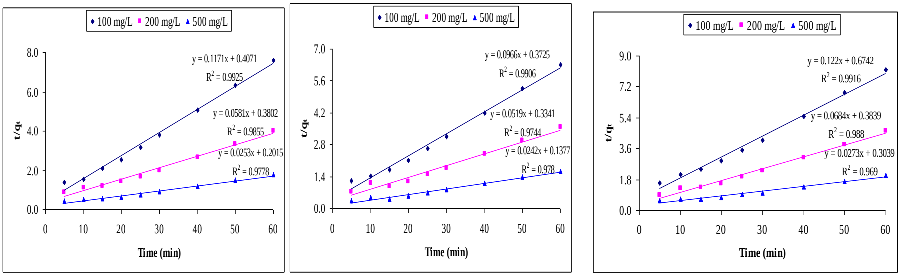


Figure 4. Reciprocal of metal-II ions (Cu^{2+} ion, Pb^{2+} ion and Cd^{2+} ion)adsorbed with contact time by SASC

Metal Sorption Selectivity of Steam Activated Sulphurised Carbon in the Mixture of Metal (II) Ions

Figure 5 shows the EDXRF spectrum of the presence of Pb^{2+}, Cu^{2+} and Cd^{2+}sorbed on SASC. from the EDXRF spectrum it can be inferred that Pb^{2+}, Cu^{2+} and Cd^{2+} were sorbed. The results are semiquantitative only. They indicate the relative quantitative amount in the SASC.Table 4 shows the metal sorption selectivity of SASC toward the metal ions Pb^{2+}, Cu^{2+} and Cd^{2+} from a solution mixture of the metal ions at pH 5. It can be observed that the removal of Pb^{2+} was more pronounced than that of Cu^{2+} and Cd^{2+}. For instance, the percent removal of metal ions is 49 % for Pb^{2+}, 35 % for Cu^{2+} and 16 % for Cd^{2+}. The decreasing order of metal ions removal selectivity, *i.e.*, $Pb^{2+}> Cu^{2+}> Cd^{2+}$, at pH 5 was found to be consistent with those of the single-species metal removal capacity. However, the percent removals for the single species metals were higher than that of the mixture of metal ion removal selectivity. This same order of preference indicates that the adsorption mechanism-taking place is the same. The pronounced selective uptake for Pb^{2+} and Cu^{2+} may be attributed to the intermolecular chelating power of SASC, chelating and complexing power of metal ions, charge density of the ions, sizes of the ions and also due to the solubility product of the metal ions at certain pH of the solution. The low percent values of Cd^{2+} reflect that hydrated ions do take part in the sorption mechanism. It is obvious (Table 4) that the percent removal of multi-species metal ions was lower than that of single species metal ions which may be due to the increased mass transfer resistance that was caused by the presence of more metal ions in the solution.

Table 4. Percent removal of mixture of metal (II) ions by SASC

Metal ions	**Percent removal (%)**	
	Removal capacity Metal ion only (Single species)	**Removal selectivity Metal ions mixture (Multiple species)**
Pb^{2+}	95	49
Cu^{2+}	79	35
Cd^{2+}	73	16

Initial concentration=100 mg L^{-1}; Dosage=1.0 g in 100 mL of sample solution; Time=1 hr; pH=5; removal efficiency= Pb> Cu > Cd

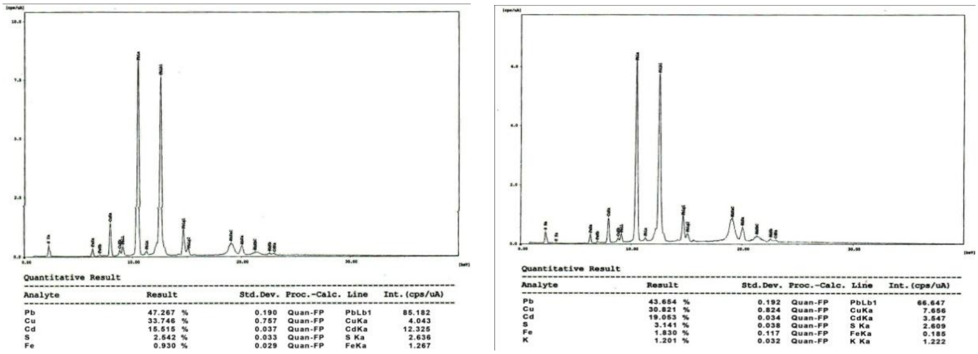
AAS Analysis of Copper, Lead and Cadmium from Wastewater Treatment

Industrial wastewater sample from industry of GS Battery (Hlaing Tharyar Industrial Zone) was collected. The metal ion (copper, lead and cadmium) concentrations in industrial wastewater were determined by atomic absorption spectrophotometer.

Table 5. Metal removal efficiency in industrial wastewater

Metal	**Concentration of metal ions in industrial wastewater (ppm)**		**Removal efficiency (%)**
	Before treatment	**After treatment**	
Lead	3.744	1.984	47
Copper	5.457	3.656	33
Cadmium	1.285	1.028	20

Volume of wastewater = 10 mL, Dosage = 0.1 g, pH = 5, removal efficiency= Pb> Cu > Cd



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Quantitative Result
-----------------------------------------------------------------------
Analyte          Result          Std.Dev. Proc.-Calc. Line   Int.(cps/uA)
-----------------------------------------------------------------------
Pb               47.267  %          0.190  Quan-FP    PbLb1       85.182
Cu               33.746  %          0.757  Quan-FP    CuKa         4.043
Cd               15.515  %          0.037  Quan-FP    CdKa        12.325
S                 2.542  %          0.033  Quan-FP    S Ka         2.636
Fe                0.930  %          0.029  Quan-FP    FeKa         1.267
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Quantitative Result
-----------------------------------------------------------------------
Analyte          Result          Std.Dev. Proc.-Calc. Line   Int.(cps/uA)
-----------------------------------------------------------------------
Pb               43.654  %          0.192  Quan-FP    PbLb1       66.647
Cu               30.821  %          0.824  Quan-FP    CuKa         7.656
Cd               19.053  %          0.034  Quan-FP    CdKa         3.547
S                 3.141  %          0.038  Quan-FP    S Ka         2.609
Fe                1.830  %          0.117  Quan-FP    FeKa         0.185
K                 1.201  %          0.032  Quan-FP    K Ka         1.222
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Figure 5. EDXRF spectrum of mixture of metal sorbed & real wastewater of metal sorbed on SASC

On the average of copper concentration 5.457 ppm, lead concentration 3.744 ppm and cadmium concentration 1.285 ppm were found in industrial wastewater. The contents of these metals in the wastewater samples were found to be reduced significantly by sorbent material such as SASC. On examination of the resulting data, removal efficiencies of steam activated sulphurised carbon were 33 % copper, 47 % lead and 20 % cadmium, respectively. The resulting data are consistent with that found out by working with model solutions consisting of either single ionic species or with multiple ionic species.

Thus, the process of removing the three metal ions (copper, lead and cadmium) from wastewater sample by steam activated sulphurised carbon can be used as an effective sorbent material in the treatment of industrial effluents. Figure 9 shows the EDXRF relative semi-quantitative data regarding the removal of the metals (Pb, Cu and Cd)consisting in the GS Battery wastewater drainage systems. The significance of the data indicates that the removal efficiency of the sorbent SASC followed the same selective trend in the removal efficiency. The order becomes Pb^{2+}> Cu^{2+}> Cd^{2+}.

Conclusion

From the adsorption studies, the resulting data were found to fit the Langmuir and Freundlich isotherms. The maximum adsorption capacity determined from theLangmuir isotherm was found to be 90.98 mg g^{-1} Pb^{2+}, 83.33 mg g^{-1} Cu^{2+} and 69.19 mg $g^{-1}$$Cd^{2+}$ for SASC. The kinetic process of metal (II) adsorption onto SASC was tested by applying the pseudo-first order and pseudo-second order rate equations. Adsorption was best fitted by the pseudo-second order model. This means the possible sorption behaviour follows the two-step wise process of surface and co-precipitation process. Selective sorption study of metal ions Pb^{2+}, Cu^{2+} and Cd^{2+} from a mixture of the three cations show that SASC selectively remove in the same order as tested with single cation model solutions. Selective sorption capacity follows the decreasing order, *i.e.*, Pb^{2+}> Cu^{2+}> Cd^{2+}.

The removal of lead, copper and cadmium from wastewater located at GS Battery Factory, Hlaing Tharyar Insustrial Zone, was analyzed. It was found that the removal efficiency of SASC were 47 % lead, 33 % copper and 20 % cadmium, respectively. The resulting data confirmed the pre-experimental data, *i.e.*, Pb^{2+}> Cu^{2+}> Cd^{2+}. The content of lead, copper and cadmium was assayed by AAS analysis. The results suggested the applicability of using the adsorbent (SASC) for metal (II) removal from real wastewater effluent. Since the steam activated sulphurised carbon, the raw material used in the investigation, is freely and locally available, the adsorbent is expected to be economically viable for wastewater treatment.

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References

Allen, S.J. and B. Koumanova. (2005), “Decolourization of Water/Wastewater Using Adsorption”, *J. Metallurgy*, Bulgaria, **40**, 175-192

Chugtai, F.A., Fakhar-un-Nisa, A. Illahi,Ejaz, W-Haque and N. Parveen. (1987), “Sarkada Grass”, *J. Pure Appl. Sci.*, **6**, 60-67

Hassler, J.W., (1974), “Active Carbon”, Chemical Publishing Co. Inc., New York

Hitchcock, S.J., B. McEnaney, and S.J. Watling.(1983), “Coconut Shell”, *J. Chem. Tech. Biotechnol.*, John Wiley & Sons, Inc., New York, **33** (A), 157-163

Ho, Y.S, and G. McKay. (1998), “A Comparison of Chemisorption Kinetic Models Applied to Pollutant Removal on Various Sorbents”, *Process Safe Environ. Protec.*, **76**, 332-340

Ho, Y.S, and G. McKay. (2000), “The Kinetics of Sorption of Divalent Metal Ions onto Sphagnum Moss Peat”, *Water Res.*, **24**, 735-741

Kalavathy, M.H., T. Karthikeyan,S. Rajgopal, and L.R. Miranda. (2005), “Kinetics and Isotherm Studies of Cu (II) Adsorption onto H_3PO_4 – Activated Rubber Wood Sawdust”, *J. Colloid & Interface Sci.*, **292**, 354-362

Kobya, M., E. Demirbas, E. Senturk, and M. Ince.(2005). “Adsorption of heavy metal ions from aqueous solutions by activated carbon prepared from apricot stone”.Bioresource Technology, **96**, 1518-1521.

Mantell, C.L., (1945), “Adsorption”, Chemical Engineering Series, 2nd Ed., McGraw-Hill Book Co., Inc., New York

Pollard, S.J.T., G.F. Flower, C.J. Sollar, and R. Perry. (1992), “Environmental”, *Sci. Total Envi.*,**116**, 31-52

Renouprez, A. and J. Avom. (1988), "Characterization of Porous Solids", *Elservier*, Amsterdam, 49-54

Teker, M, and M. Imanoglu. (1999), "Adsorption of Copper and Cadmium Ions by Activated Carbon from Rice Hulls", *Turk J. Chem.*, **23**, 185-191

Weng, C.H., C.Z. Tsai, C.H. Chu, and Y.C. Sharma.(2007), "Adsorption Characteristics of Copper (II) onto Spent Activated Clay", *Sep. Purifn. Technol.*, **54**, 187-197

Yalc, N., and V. Sevinc.(2000). "Studies of the surface area and porosity of activated carbons prepared from rice husks, Carbon" **38**, 1943-1945

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Screening on Phytochemical Constituents, Nutritional Composition and Some Pharmacological Properties of Seeds of *Parkia speciosa* Hassk.

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Abstract

In this research work, seeds of *Parkia speciosa* Hassk. was collected for chemical investigation such as phytochemical constituents, nutritional composition, mineral contents, antimicrobial activities, toxicity and antioxidant activity. The main aim of this research work was to study on nutritional value and some pharmacological properties of seed sample. The phytochemical tests gave rise to positive tests for alkaloids, flavonoids, glycosides, phenolic compounds, polyphenols, saponins, steroids, tannins and terpenes, respectively. In nutritional composition of this sample, protein content was found to be the highest value (28.75%). The mineral contents were analyzed by EDXRF(Energy Dispersive X-ray Fluorescence) spectroscopy. In this study, potassium was found to be highest. in this sample. The antimicrobial activities of three solvents extracts of sample were determined by agar-well diffusion method on six microorganisms such as *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi* and *Shigella boydii*. The toxicity of water extract of sample were also investigated. The antioxidant activity of ethanol extract of sample was determined by DPPH (1,1-diphenyl-2-picryl hydrazyl) assay.

Keywords: *Parkia Speciosa* Hassk., Phytochemicals, Nutritional composition, Antimicrobial , Toxicity, Antioxidant

Introduction

Parkia speciosa Hassk. is a tropical leguminous tree in the family of fabaceae found in most Southeast Asian countries such as Malaysia, Indonesia, Thailand, Philippines and Myanmar. It grows up to 40 meter high. It bears green long and flat edible beans which are called pods in stalks. The seeds are in pods, approximately 35-45 They are also a good source for minerals. They have a considerable amount of vitamin C and α-tocophenol(Nattausut Ngogedee,2005).

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The seeds are known to have important chemical and medicinal compounds, such as several cyclic polysulphide, which are used for the treatment of antibacterial activity in kidney, ureter and urinary bladder infections and thiazolidine-4-carboxylic acid, which is used for its anticancer activity. The seeds also have a hypoglycemia effect due to the synergistic action of β-sitosterol and stigmasterol. High concentration of tannins was detected in its seeds coats and pods compared to other fruit vegetables. Tannins has been reported to decrease protein and amino acid digestibility.

Almost all major chemical compounds are present in the seeds. Phenolic compounds are also present in almost all parts of the plant. The seeds have a high content of total polyphenol, phytosterol and flavonoids. It demonstrates a good antioxidant activity. It displays many beneficial properties (Yus Kamisha,2013).

This research was undertaken to investigate the phytochemical constituents, nutritional composition, antimicrobial activities, toxicity and antioxidant activity of seeds of *Parkia speciosa* Hassk.

Botanical Description

Scientific Name -- *Parkia speciosa* Hassk.
Family Name -- Fabaceae
English Name -- Stink bean
Myanmar Name -- Myauk ngo

Figure 1. Stink bean fruits

Figure 2. Stink bean seeds

Objectives

- To collect the seed sample from Kalay Township, Sagaing Region.
- To carry out the phytochemical screening of the seed sample.
- To determine the nutritional composition of the seed sample
- To analyze the elemental contents of the seed sample by EDXRF Spectroscopy
- To examine the antimicrobial activities of the seed sample by agar- well diffusion method
- To determine the toxicity of the water extract of seed sample
- To investigate the antioxidant activity of the ethanol extract of the seed sample by DPPH scavenging assay

Materials and Methods

Sample Collection and Preparation

The fruits of *Parkia speciosa* Hassk. were collected from Kalay Township, Sagaing Region, Myanmar. The seeds were separated from the pods and dried in air. Then dried seeds were ground into powder by electric grinder. The powdered sample was stored in an air tight container and used throughout the experiment.

Phytochemical Screening

The various solvents of seed sample were prepared to analyse the presence of certain phytochemicals. Analysis was done for alkaloids, flavonoids, phenolic compounds, polyphenols, saponins , steroids, tannins and terpenes.

Determination of Nutritional Composition of Seed Sample

The study of nutritional composition of seed sample such as moisture, ash, mineral, oil, crude fibre, nitrogen and protein, and soluble carbohydrate was performed.

Table 1. Determination of Nutritional Composition of Seed Sample

No.	Nutritional Content	Methods / Instruments
1.	Moisture	Oven drying method
2.	Ash	Muffle furnace
3.	Mineral	EDXRF Spectroscopy
4.	Oil	Soxhlet Apparatus
5.	Crude fibre	Muffle furnace
6.	Nitrogen and protein	Micro Kjeldahl’s method
7.	Soluble carbohydrate	UV-visible spectroscopy

Determination of Some Pharmacological Activities of Extracts of Seed Sample

The three solvents such as n-hexane, ethyl acetate and ethanol were used to analyze the antimicrobial activities of seed sample. The antimicrobial activities of seed sample were analyzed by agar-well diffusion method on six microorganisms such as *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi* and *Shigella boydii*.

The water extract of seed sample were used to determine the toxicity of seed sample. The animal models (Mus. Musculus) were used in this study. The mice with 30 g of body weight and age of 4-6 weeks were selected to use and kept separately.

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of seed sample. In this study, the ethanol extract of seed sample was used. The DPPH radical-scavenging assay measures the ability of the compound to donate hydrogen to DPPH radical. The DPPH radical-scavenging activity of seed sample was also determined based on median inhibition concentration (IC_{50}). In this experiment, ascorbic acid was used as standard antioxidant.

Table 2. Determination of Some Pharmacological Activities of Crude Extract of Seed Sample

No.	Activities	Method
1.	Antibacterial Activities	Agar-well diffusion method
2.	Toxicity	Litchfield and Wilcoxon's method
3.	Antioxidant Activity	DPPH Radical Scavenging Assay

Results and Discussion

Phytochemical Constituents of Seed Sample

Preliminary phytochemical analysis was performed in order to know different types of organic compounds present in seed sample. Analysis of the extracts of the seed sample revealed the presence of phytochemicals such as alkaloids, flavonoids, glycosides, phenolic compounds, polyphenols, saponins, steroids, tannins and terpenes, respectively. These phytochemicals are known to exhibit medicinal as well as physiological activities. The results are shown in Table 3.

Table 3. Results of Phytochemical Constituents of Seed Sample

No	Constituents	Extract	Reagents	Observation	Result
1.	Alkaloids	1% HCl	Dragendorff's reagent	Orange ppt.	+
2.	Flavonoids	95% ethanol	Conc: HCl, Mg	Pink colour	+
3.	Glycosides	Water	10% lead acetate	White ppt.	+
4.	Phenolic compounds	Water	1% $FeCl_3$	Purplish colour	+
5.	Polyphenols	95% ethanol	1% $FeCl_3$ + 1% $K_3[Fe(CN)_6]$	Green blue colour	+
6.	Saponins	Water	-	Froth	+

No	Constituents	Extract	Reagents	Observation	Result
7.	Steroids	95% ethanol	$CHCl_3$,acetic anhydride,H_2SO_4	Green color	+
8.	Tannins	95% ethanol	10%$FeCl_3$,H_2SO_4	Brown ppt.	+
9.	Terpenes	Pet-ether	$CHCl_3$,acetic anhydride,H_2SO_4	Pink colour	+

(+) = Presence of Constituents

Nutritional Composition of Seed Sample

The determination of nutritional composition of dried seed powder was carried out to know the nutritional values of seed sample. From the experimental data, it can be observed that the seed sample contain many nutritional values which have benefits to humans. Among them, protein content was found to be the highest value (28.75%) in the seed sample. The results are shown in Table 4.

Table 4. Results of Nutritional Composition of Seed Sample

No.	Nutritional Composition	Content (%)
1.	Moisture	7.06
2.	Ash	4.50
3.	Oil	23.30
4.	Fibre	1.20
5.	Nitrogen	4.62
6.	Protein	28.75
7.	Soluble carbohydrate	14.80

Mineral Content of Seed Sample by EDXRF Spectroscopy

The seed sample contains minerals .It displays many beneficial properties. Minerals are important for our body to stay healthy. The elemental composition in seed sample was examined by EDXRF spectroscopy. The results are shown in Table 5.

Table 5. The Results of Mineral Contents in Seed sample

No.	**Elements**	**Symbols**	**Results (mass %)**
1	Potassium	K	1.091
2	Calcium	Ca	0.5237
3	Sulfur	S	0.4044
4	Chlorine	Cl	0.3314
5	Phosphorus	P	0.2137
6	Aluminium	Al	0.1264
7	Silicon	Si	0.0675
8	Iron	Fe	0.0210
9	Manganese	Mn	0.0114

Table (5) shows that potassium was the highest amount in the sample. Therefore, seeds of *Parkia speciosa* Hassk. was found to contain the essential minerals for human health

Antimicrobial Activities of Seed Sample

The study of antimicrobial activities of seed sample was performed by agar-well diffusion method on six microorganisms. The results are tabulated in Table 6 and Figure 4.

Table 6 . Results of Antibacterial activities of Seed Sample

Sample	**Solvents**	**Organisms**					
		I	**II**	**III**	**IV**	**V**	**VI**
Stink bean Seed	EtOH	7mm (++)	7mm (+)	8mm (++)	9mm (++)	8mm (++)	8mm (++)
	EtOAc	17mm (++)	13mm (++)	14mm (++)	15mm (++)	15mm (++)	9mm (++)
	n-hexane	0mm (-)	0mm (-)	0mm (–)	0mm (-)	0mm (-)	0mm (-)

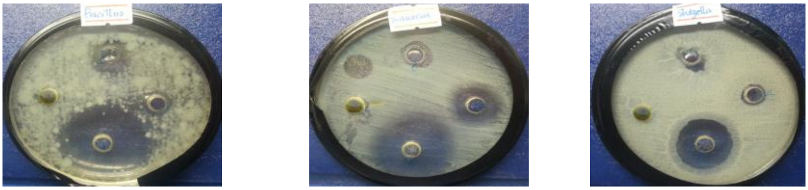
Clear Zone diameter

Well diameter=5mm,“0”mm = (-),5mm-7mm=(+),above 7mm =(++)

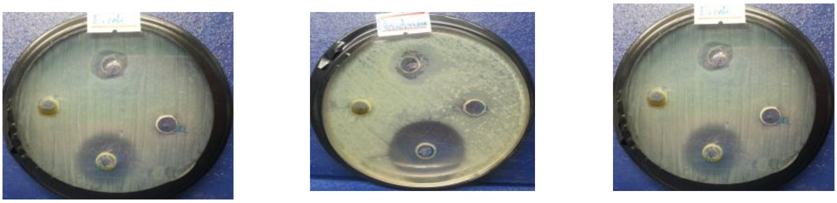
Organisms

I = *Bacillus cereus*
II = *Escherichia coli*
III= *Pseudomonas aeruginosa*
IV = *Staphylococcus aureus*
V = *Salmonella typhi*
VI = *Shigella boydii*

According to the experimental data, ethyl acetate extract of sample gave rise to high activities on all tested organisms.



(a) *Bacillus cereus* **(b) *S.aureus*** **(c) *Pseudomonus aeruginosa***



(d) *Escherichia coli* **(e) *Shigella*** **(f)** *S. typh*

Figure 3. Antimicrobial activities of crude extracts of seed

Toxicity Test for Seed Sample

0.5g/kg/day,1g/kg/day, 1.5g/kg/day, 2.0g/kg/day and 2.5g/kg/day does were used to examine the toxicity test for water extract of seeds of *Parkia speciosa* Hassk. 30 mice were administrat orally. The water extract of seed gave the normal condition of their health. They were survived 10 days after administration. No mortalities were observed among the mice tested with water extract.

Therefore, it was found that 2.5g/kg/day of water extract of seed sample showed confidence dose and considered as safe. The following Table 7 showed the results for toxicity test.

Table 7. Results of Toxicity Test for Seed Sample

No.	Dose	Tested Mice	Dead	Alive
1	0.5 g/kg/day	5	-	5
2	1.0 g/kg/day	5	-	5
3	1.5 g/kg/day	5	-	5
4	2.0 g/kg/day	5	-	5
5	2.5 g/kg/day	5	-	5

Antioxidant Activity of Ethanol Extract of Seed Sample

The antioxidant activity was studied on the ethanol extract of sample by DPPH assay. In this experiment, ascorbic acid was used as a standard antioxidant. On the basis of absorbance values, percent inhibition of ethanol extract in different concentrations was calculated. According to results, it can be seen that as the concentration was increased, the percent of inhibition of oxidation was also increased. The IC_{50} values were determined by using linear regressive excel program. IC_{50} value for extracted sample (IC_{50} = 0.91 µg/ml) was higher than that of standard ascorbic acid (IC_{50} = 0.53 µg/ml). Therefore, the extracted sample has lower antioxidant activity than standard ascorbic acid.

Table 8. % Inhibition and IC_{50} Values of Ethanol Extract of Seed sample

Concentration (µg/mL)	**0.1568**	**0.3125**	**0.625**	**1.25**	**2.5**	**5**	**10**
Blank solution	0.001	0.001	0	0.002	0	0	0
Absorbance	0.162	0.164	0.143	0.049	0.05	0.016	0.007
	0.163	0.161	0.134	0.069	0.045	0.015	0.008
Net Absorbance	0.161	0.163	0.143	0.047	0.05	0.016	0.007
	0.162	0.16	0.134	0.067	0.045	0.015	0.008
Control	0.203	0.203	0.203	0.203	0.203	0.203	0.203
% Inhibition	20.69	19.70	29.56	76.85	75.37	92.12	96.55
	20.20	21.18	33.99	67.0	77.83	92.61	96.06
Average	20.44	20.44	31.77	71.92	76.60	92.36	96.31
SD	0.35	1.04	3.13	6.97	1.74	0.35	0.35
IC_{50}	0.91						

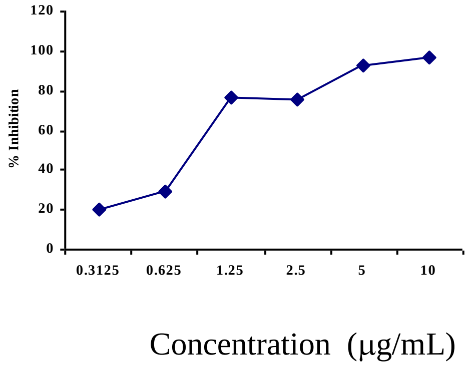


Figure 4.% Inhibition Vs Concentration of ethanol extract of sample

Table 9. % Inhibition and IC_{50} Value of Standard Ascorbic Acid

Concentration (µg/mL)	**0.1568**	**0.3125**	**0.625**	**1.25**	**2.5**	**5**	**10**
Blank solution	0	0	0	0	0	0	0
Absorbance	0.158	0.188	0.072	0.053	0.048	0.063	0.031
	0.165	0.155	0.074	0.051	0.048	0.039	0.034
Net Absorbance	0.158	0.188	0.072	0.053	0.048	0.063	0.035
	0.165	0.155	0.074	0.051	0.048	0.039	0.034
Control	0.203	0.203	0.203	0.203	0.203	0.203	0.203
% Inhibition	22.17	37.39	64.53	73.89	76.35	78.97	84.73
	18.72	23.65	63.55	74.88	76.35	80.79	83.25
Average	20.44	15.52	64.04	74.38	76.35	74.88	83.99
SD	2.44	11.49	0.70	0.70	0.00	8.36	1.04
IC_{50}		0.53					

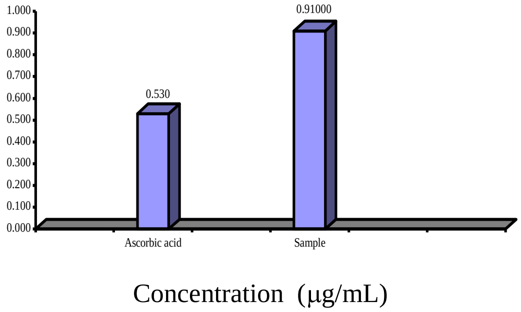


Figure 5.% Inhibition Vs Concentration of Standard Ascorbic Acid

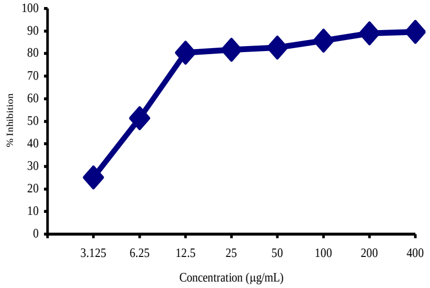


Figure 6. Bar-graph of IC_{50} Values for Standard Ascorbic Acid

Conclusion

In this study, seeds of *Parkia speciosa* Hassk were collected from Kalay Township, Sagaing Region for chemical investigation. The phytochemical screening revealed that the presence of medicinally important constituents in the seeds of *Parkia speciosa* Hassk. It was found that *Parkia speciosa* Hassk seeds contain many nutritional values such as moisture, ash, oil, nitrogen, protein, crude fiber and soluble carbohydrate. According to experimental data, the protein content was found to be the highest value (28.75%) in the seeds of *Parkia speciosa* Hassk. The elemental analysis indicated that potassium is the highest value (1.091%) in this sample.

In the study of antimicrobial activities of three solvents extracts of this sample, ethyl acetate extracts gave rise to high activities on all tested organisms. Therefore, ethyl acetate extracts seeds of had the greatest potentiract of sample was studied by DPPH assay and IC_{50} value (IC_{50}=0.91µg/ml) of sample extract was compared to that of standard antioxidant, ascorbic acid (IC_{50}= 0.53µg/ml). It can be seen that antioxidant activity of seed sample have lower activity compared to ascorbic acid.

According to experimental data, it can be seen that seeds of *Parkia speciosa* Hassk. contains valuable phytochemical constituents and nutrients for human. This seed sample shows antimicrobial activities and antioxidant activity. The water extract of sample has no toxic effect for 2.5g/kg/day dose and it showed confidence dose and considered as safe. Therefore, the seeds of *Parkia speciosa* Hassk. should be consumed for nutrients and health of human.

Acknowledgement

I would like to express my heartfelt gratitude to Acting Rector, Dr. Aung Win , Myitkyina University for his interest and encouragement on my research work. I deeply thank to Dr. Ni Ni Aung, Professor and Head ,Department of Chemistry ,Myitkyina University for her valuable suggestion and encouragement.

References

Association of Official Analytical Chemists (AOAC),(1990). Official Methods of Analysis, 14 th Ed; virginia.

Bacinac, D, (2001). “Evaluation of Antioxidative Activity of some Antioxidant by means of a combined optothermal window and DPPH free Radical colorimetry” . Chit Ko Ko, (1980). "Myanmar Medicinal Plants".

Harbone, J., B and Baxter, H., (1993). “Phytochemical Dictionary”, A Handbook of Bioactive compounds from plants, Taylor and franis Ltd. Washington DC.

Nattausut Ngoendee, (2005). "study on seed, seed storage and some physiological characteristics of *Parkia speciosa* Hassk. Seedling.

Yus Kamisha, Faizah Othman, Hj Mohd Saad Qodriyah, and Kamsiah Jarrin , (2013). “*Parkia speciosa* Hass: A potential phytomedicine.”

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A Study on Chemical Constituents, Antimicrobial and Antioxidant Activities of *Desmodium triquetrum* Linn. (Lauk-Thay) Leaves

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Abstract

Plants have been very important sources of drugs for human beings. The phytocomponents in *Desmodium triquetrum* L. are used in folk remedies for the treatment of inflammation, dysentery and high blood pressure. This project paper is concerned with the determination of minerals, investigation of phytoconstituents, antimicrobial activity and antioxidant activity in the leaves of *Desmodium triquetrum* Linn. (Lauk-thay). From the phytochemical investigation, alkaloids, phenolic compounds, flavonoids, α-amino acids, tannins, saponin, glycosides, and carbohydrates, reducing sugar, steroids, terpenoids, starch were present in the sample but cyanogenic glycoside was not present in the sample. From heavy metal determination by AAS, it was found that the value of As was 1.046 ppm but Pb and Cd were not found in the sample. Relative composition of elements in *Desmodium triquetrum* was determined by EDXRF spectrometer. It was found that there were contained K, Ca, Cl, S, P, Mn, Rb, Br, Zn, Ti, Sr, and Cu in the sample. Antimicrobial activity was studied on crude extracts such as PE and 95% EtOH extracts. According to these results, 95% EtOH extract had more potent activity than the PE extract of *Desmodium triquetrum* leaves. According to the antioxidant activity screening of EtOH and EtOAC extracts were determined by using DPPH assay. The EtOH leaves extract (IC_{50}=14.77 µg/mL) was more potent than EtOAC leaves extract.

Keywords: Phytoconstituents, mineral, antimicrobial activity, antioxidant activity, *Desmodium triquetrum*

Introduction

Medicinal plants played an important role in the treatment of diseases and health disorders for thousands of years and are still important in traditional medicine systems around the world. Many herbal remedies individually or in combination have been recommended in various medical

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treatments for the cure of different diseases. The plant kingdoms serve as invaluable source of new chemical products which may be important due to their biological properties and their potential use in medicine. Numerous medicinal plants are useful for treatment of various diseases. The study of medicinal plants and their therapeutics play a very important role in health care system of Myanmar. It has a long history of health care system by herbal and medicinal plants and it has been accepted as a national heritage. Herbal medicine is a major remedy in traditional medicine system, which is largely based on the use of roots, leaves, barks, seeds and flowers of the plants.

Botanical description of D*esmodium triquetrum* Linn.

Species - *D. triquetrum*
Genus - *triquetrum*
Family - Fabaceae (Papilionaceae)
Botanical name - *Desmodium triquetrum* Linn.
Common name - Country mallow
Myanmar name - Lauk-thay, Shwe-gu-than-hlet

Figure 1. *Desmodium triquetrum* Linn. (Lauk-thay)

Desmodium triquetrum is one of the popular medicinal plants in Myanmar. It was distributed in Chin, Kachin, Kayin, Mandalay, Sagaing, Shan, through lower Myanmar, central and eastern Himalayas, South India and Sri Lanka. It is widespread in all South Asian, East Asian, and Southeast Asian countries.

Medicinal uses

This herb is efficacious because it prevents fainting from the heat (heat stroke), fever, colds; inflammation of the tonsils, acute inflammation of the kidney, swollen, inflammation of the intestine, dysentery, tapeworm infection in the liver; vomiting in pregnancy, malnutrition in children; jaundice. The fresh leaves are applied to wounds. 2 g of leaves powder is mixed with honey and a little amount of salt. This mixture is given two times a day for helminthic infection.

Antimicrobial Activity

An antimicrobial activity is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungal are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called biostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis. With the development of antimicrobials, microorganisms have adapted and become resistant to previous antimicrobial agents.

Antioxidant Activity

Antioxidants are substances which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Antioxidants can be naturally found in vegetables, fruits and plants in general. Antioxidants are also synthesized in our body. Antioxidants are essential for proper function of the immune system. This is partly because immune cell produce free radicals for normal defense functions. If the level of free radicals in the immune cells, surpasses beyond the normal level, they negatively affect the immune system, on the other hand, antioxidants act as scavengers of the free radicals in cells and therefore promote our immunity. Imbalance between free radicals and antioxidants in cells, due to deficiency in single or multiple antioxidants has been reported to result in weakness of immunity function. Antioxidants boost immunity system also by playing other important roles such as in cellular metabolism, signal transduction, gene activation and transcription.

Materials and Methods

Sample Collection and Preparation

Desmodium triquetrum Linn. (Lauk-thay) leaves were collected from Dawei University campus, Launglone Township, Dawei district, Tanintharyi Region, Myanmar. The leaves were washed and air dried at room temperature for two weeks and then grounded into powder by using grinding machine. The dried powdered samples were stored in air-tight container.

Determination of Some Heavy Metals by Atomic Absorption Spectrophotometry

Some heavy metals (As, Pb and Cd) in leaves of *Desmodium triquetrum* were measured by AAS method using Perkin Elma A Analyst 800 Spectrophotometer instrument at the Universities' Research Center (URC), Yangon.

Determination of Relative Composition of Some Elements by Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometry

Relative compositions of some elements in leaves of *Desmodium triquetrum* were measured by EDXRF method at the West Yangon University, Yangon.

Phytochemical Investigation by Test Tube Method

Phytochemical test was carried out to investigate the presence of chemical constituents of *Desmodium triquetrum* Linn. (Lauk-thay) by reported methods.

Screening of Antimicrobial Activity of *Desmodium triquetrum* Linn.

In this Section, the screening of antimicrobial activity of various crude extracts such as PE, EtOAc, 70 % EtOH, MeOH extracts of flower and rhizome of *Desmodium triquetrum* were carried out by agar disc diffusion method at Fermentation Department, Development Center of

Pharmaceutial and Food Technology (DCPFT), Ministry of Industry (1), Yangon, Myanmar. Six microorganisms namely *Bacillus subtilis* (JAP-022/215), *Staphylococcus aureus* (ATCC-12277), *Pseudomonas aeruginosa* (IFO-3080), *Bicilluspumilus* (IFO-12102), *Candida albicans* (IFO-1060) *and Escherichia coli* (ACCT-25922) were used for this test.

Screening of Antioxidant Activity of Crude Extracts from *Desmodium triquetrum* Linn.

DPPH (2, 2-diphenyl-1-picry-hydrazyl) radical scavenging assay was chosen the antioxidant activity of plant materials. Then, the absorbance of sample solutions was measured at 517 nm by using UV-7504 spectrophotometer.

Results and Discussion

Determination of Some Heavy Metals by Atomic Absorption Spectrophotometry

In the present work, content of As, Pb and Cd in *Desmodium triquetrum* were determined by AAS. It was found that the value of As was 1.046 ppm but Pb and Cd were not found in the sample.

Table 1. Some Heavy Metals in *Desmodium triquetrum* Linn.

Part used	**Content (ppm)**		
	As	**Pb**	**Cd**
leaves	1.046	ND	ND

Determination of Relative Composition of Some Elements by EDXRF Spectrometry

In this work, relative composition of elements in *Desmodium triquetrum* was determined by EDXRF spectrometer applying fundamental parameter method. In this result, K, Ca, and Cl were found as major constituents and S, P, Mn, Zn, Cu, Br, Ti, Rb and Sr were found as minor constituents. Among them, it was found that potassium was the highest content in the sample.

Table 2. Relative Composition of Some Elements in *Desmodium triquetrum* Linn.

Sr. No.	Elements	Relative Composition (%)	Sr. No.	Elements	Relative Composition (%)
1	K	13.799	8	Br	0.029
2	Ca	3.859	9	Zn	0.010
3	Cl	2.847	10	Ti	0.008
4	S	0.643	11	Sr	0.006
5	P	0.565	12	Cu	0.005
6	Mn	0.037	13	Br	0.029
7	Rb	0.033			

Investigation of Phytochemical Constituents of Plant Samples by Test Tube Method

From the phytochemical screening of the extracts of *Desmodium triquetrum* Linn. (Lauk-thay), leaves contain the presence of organic compounds such as alkaloids, α –amino acids, carbohydrates, flavonoids, glycosides, saponins, tannins, steroids, terpenoids, reducing sugars, organic acids, phenolic compounds and starch. Cyanogenic glycoside is absent.

Table 3. Phytochemical Screening of *Desmodium triquetrum* Linn.

No.	Tests	Extracts	Reagents	Observation	Remark
1	Alkaloids	1% HCl	Mayer's reagent Wagner's reagent Dragendorff's reagent	White ppt. Brown ppt. Orange ppt.	+ + +
2	α-amino acids	H_2O	Ninhydrin reagent	Purple colour	+
3	Carbohydrates	H_2O	α-naphthol, conc. H_2SO_4	Red ring	+
4	Flavonoids	EtOH	conc. HCl, Mg turning	Pink colouration	+
5	Glycosides	H_2O	10% lead acetate	White ppt.	+
6	Saponins	H_2O	Distilled water	Frothing	+
7	Steroid and Terpenoids	P.E	Acetic anhydride, conc. H_2SO_4	Color change	+
8	Tannins	EtOH	$K_3Fe(CN)_6$, NaCl solution	Color change	+
9	Reducing sugars	5N H_2SO_4	5N NaOH solution Benedict's solution	Brick red ppt.	+
10	Phenolic compounds	H_2O	1% $K_3Fe(CN)_6$, 1% $FeCl_3$ solution	Deep blue color	+
11	Starch	H_2O	KI solution, I_2 solution	Blue ppt.	+
12	Cyanogenic glycoside	H_2O	2M H_2SO_4, Sodium picrate	No red ppt.	-

P.E = Petroleum Ether EtOH = Ethanol ppt. = precipitate
(+) = present (-) = absent

Antimicrobial Activity of *Desmodium triquetrum* Linn.

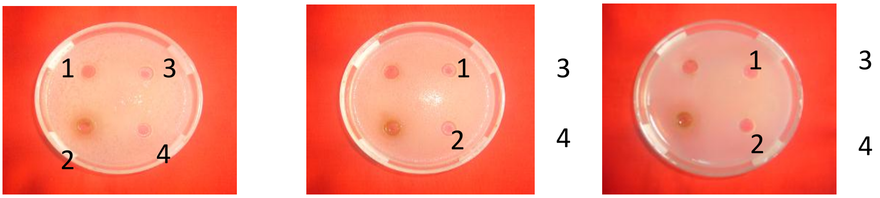
Invitro antimicrobial activities of *Desmodium triquetrum* was studied on crude extracts such as PE and 95% EtOH extracts by agar well diffusion method on six species of microorganisms such as *Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas aureginosa and Staphylococcus.* The inhibition zone diameter for the sample against the tested microorganisms is shown in Figure 3. According to these results, 95% EtOH extract had more potent activity than the PE extract of *Desmodium triquetrum* leaves.

Table 4. Antimicrobial Activities of *Desmodium triquetrum* Linn.

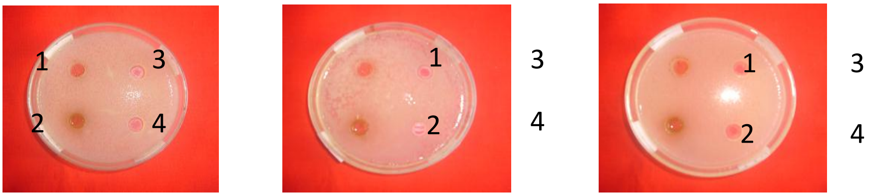
Sample	**Solvent**	**Organisms**					
		B-sub	***S-aureus***	***Pseudo-monas***	***B-pumilus***	***Candida***	***E-coli***
plant	P.E	13 mm (+)	12 mm (+)	11 mm (+)	12 mm (+)	12 mm (+)	12 mm (+)
	EtOH	18 mm (++)	17 mm (++)	20 mm (+++)	17 mm (++)	15 mm (++)	15 mm (++)
Control	P.E	-	-	-	-	-	-
	EtOH	-	-	-	-	-	-

Agar well – 10mm
10mm ~ 14 mm (+)
15mm ~19 mm (++)
20mm above (+++)

Organisms
(1) *Bacillus sublilis* (NCTC-8236)
(2) *Staphylococcus aureus* (NCPC-6371)
(3) *Pseudomonas aeruginosa* (6749)
(4) *Bacillus pumilus* (NCIB-8982)
(5) *Candida albican*
(6) *E-coli* (NCIB-8134)



Bacillus sublilis *Staphylococcus aureus* *Pseudomonas aeruginos*



Bacillus pumilus *Candidaalbican* *E-coli*

(1) PE extract (2) EtOH extract (3) PE control (4) EtOH control

Figure 3. Inhibition well diameter of *Desmodium triquetrum* Linn.

Antioxidant Activity of Crude Extracts of *Desmodium triquetrum* Linn.

The antioxidant activity of 70 % EtOH and water extracts of two samples, one isolated compound (p-hydroxy benzoic acid) and standard vitamin C was studied by DPPH (2, 2 - diphenyl - 1 - picryl - hydrazyl) free radical scavenging assay method. This method is the most widely reported method for screening of antioxidant activity on many plant drugs. It is based on the reduction of color of free radical DPPH in ethanolic solution by different concentrations (6.25, 12.5, 25, 50, 100, 200 μg/mL) of each crude extracts, one isolated compound and standard vitamin C in ethanol solvent were used. Determination of the absorbance of each solution (control, sample) was measured at λ_{max} 517 nm using UV-visible spectrophotometer. The results are shown in Table 5. It was found that as the concentrations increased, the absorbance values decreased. The radical scavenging activity of crude extracts was expressed in term of % inhibition and IC_{50} (50 % inhibition concentration) values were calculated regressive excel program. The results are shown in Table 6. The IC_{50} values were 38.84 μg/ml for EtOAC extract and 14.77 μg/ml for EtOH extract.

Table 5. Percent Free Radical Scavenging Activity of Crude Extracts and Standard Ascorbic Acid of *Desmodium triquetrum* Linn.

Sample	**% Inhibition (mean ± SD) in different concentrations (µg/mL)**						
	6.25	**12.5**	**25**	**50**	**100**	**200**	**IC_{50} (µg/mL)**
Lauk-thay leaves (EtoAc)	33.13 ±44.53	38.65 ±45.48	45.22 ±41.89	53.88 ±38.09	74.038 ±27.65	85.07 ±26.59	38.84
Lauk-thay leaves (EtOH)	21.64 ±0.21	43.73 ±1.26	79.47 ±7.49	96.71 ±0.21	97.91 ±0.63	100.22 ±0.73	14.77
Standard (Ascorbic Acid)	62.46 ±7.94	73.50 ±42.95	94.40 ±13.61	100.22 ±5.80	100.89 ±6.12	101.19 ±5.91	14.56

Table 6. IC_{50} Values of Crude Extracts from *Desmodium triquetrum* Linn.

Sample	**IC_{50} (µg/mL)**
Lauk-thay leaves (EtOAC)	38.84
Lauk-thay leaves (EtOH)	14.77
Standard (Ascorbic Acid)	14.56

Conclusion

This project revealed that the study on investigation of phytochemical investigation, determination of minerals contents, anitbacterial activity and antioxidant activity of *Desmodium triquetrum* Linn. (Lauk-thay) leaves. Lauk-thay is one of the popular and traditional medicinal plants in Myanmar.

From heavy metal determination, it was found that the value of As was 1.046 ppm but Pb and Cd were not found in the sample. Relative composition of elements in *Desmodium triquetrum* was determined by EDXRF spectrometer applying fundamental parameter method. It was found that there were contained K, Ca, Cl, S, P, Mn, Rb, Br, Zn, Ti, Sr, and Cu in the sample. Among these elements, the potassium content was 13.799 %, calcium content was 3.859 % and Cl content was 2.847 % as major constituents in the sample. Potassium works together with calcium. These maintain water balance and acid balance, normal excitability of muscle and especially heartbeat muscle.

In preliminary phytochemical investigation of *Desmodium triquetrum*, alkaloids, phenolic compounds, flavonoids, α-amino acids tannins, saponin, glycosides, and carbohydrates, reducing sugar, steroids, terpenoids, starch were present in the sample but cyanogenic glycoside was found to be absent in the sample. Phytochemical are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for human. Therefore, Lauk-thay that contains many effective phytoconstituents may be an important source of many essential nutrients and used as traditional medicine.

Antimicrobial activities of *Desmodium triquetrum* was studied on crude extracts such as PE and 95% EtOH extracts. According to these results, 95% EtOH extract had more potent activity than the PE extract of *Desmodium triquetrum* leaves.

According to the antioxidant activity screening of EtOH and EtOAC extracts were determined by using DPPH assay. The EtOH leaves extract (IC_{50}=14.77 µg/mL) was more potent than EtOAC leaves extract but less that standard vitamin C (IC_{50}=14.56 µg/mL). Medicinal plant plays a vital role in preventing various diseases. Based on above scientific findings, lauk-thay leaves can be used as antioxidant agents for traditional medicine**.**

The plant kingdoms serve as invaluable source of new chemical products which may be important due to their biological properties and their potential use in medicine. The study of medicinal plants and their therapeutics play a very important role in health care system of Myanmar. It has a long history of health care system by herbal and medicinal plants and it has been accepted as a national heritage. According to the World Health Organization, trading and marketing of medicinal plants and herbal drugs are increasing throughout the world, and it indicates increasing applications of natural plant materials due to various reasons.

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References

Harbone, J.B. (1984). *Phytochemical Methods.* A Guide to Modern Techniques of Plant Analysis, 2nd Ed. Chapman and Hall. New York

Halliwell, B. (1999). "Antioxidant Defense Mechanism from the Begin to the End", *Freem Radical Research*, **31**, 261-272.

Kingston, W. (2008). "Irish contributions to the origins of antibiotics". *Irish Journal of Medical Science*. **177**(2):87-9.

Linsted, R.P. (1995). "A Course of Modern Technique of Organic Chemistry". *Scientific Publications*, 2nd Ed., London

M-Tin Wa. (1972). "Phytochemical Screening Methods and Procedures". *Phytochemical Bulletin of Botanical Society of America, Inc.*

Medicinal Plants of Myanmar, (2007). Department of Traditional Medicine, Ministry of Health, Myanmar

Priestnan, J., and F.C.G. Edwards, (1953). Aid to Quantitative Pharmaceutical Analysis. Page and Bros. (Norwish) Ltd., London

Robinson, T., (1985). The Organic Constituents of Higher Plants, 5th Ed., Cordus Press, North Amberst

San Thandar and Ohn Mar Tun (2015). "In vitro micropropagation of *Desmodium triquetrum* DC., Myanmar medicinal plant", *Internal Journal of Technical Research and Applications*

Vedpal, Dhanabal, S.P., et al., (2016). "Ethnopharmacological and phytochemical profile of three *Desmodium* species: *Desmodium gangeticum* L., *Desmodium triflorum* L. *and Desmodium triquetrum* L.", *Journal of Chemical and Pharmaceutical Research*, India

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Phytochemicals, Investigation of Isolation of Endophytic Fungus and Toxic Effect of the Leaves of *Justicia gendarussa* Burm.f. (Nat-pan)

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Abstract

The research work deals with the isolation of endophytic fungus that are relatively unexplored producers of metabolites useful to pharmaceutical and agricultural industries. The fungus was isolated from the leaf of Myanmar medicinal plant: *Justicia gendarussa* Burm. F. (Nat-pan) which are widely distributed in the whole country. The fungus was firstly isolated from *Justicia gendarussa* Burm. F. (Nat-pan) and identified its morphology and growth parameters on different solid media. The isolated fungus (*Colletotrichum gloeosporioides)* is well cultured on PDA medium (pH = 6 ± 1) at 26 ± 1 °C between 6-7 days. The determination of maximum radial growth on five different carbon sources (glycerol, mannose, dextrose, sucrose and fructose), the maximum growth of *Colletotrichum gloeosporioides* was observed at the best on PDA (9.0 cm) after 5 days. The estimation of fungal biomass on potato dextrose broth, the minimum dry weight of isolated *Colletotrichum gloeosporioides* was 62.60 mg after 2 days incubation and the maximum was 310.60 mg on 12th day. The toxicity of leaves sample was revealed within 10.75 g/kg to 12.52 g/kg by using Litchfield and Wilcoxon method. That information indicated for not only safe to use as drug but also isolate the endophytic fungus, potential sources of bioactive agent.

Keywords: endophytic fungius, *Justicia gendarussa* Burm. F., *Colletotrichum gloeosporioides*

Introduction

According to chemical and pharmacological point of view, it was found out a plenty of medicinal as well as aromatic plants in Myanmar. Most of people in Myanmar depend on traditional medicinal plants and herbal medicine rather than modern medicine for the treatment of diseases. Plants are natural resources for all living things including human and

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animals as a food or drugs to cure their injuries and aliments. Moreover, plants are all main sources of bioactive natural compounds as well as drug-producing endophytic microbes. Endophytic fungi are relatively unexplored producers of metabolites useful to pharmaceutical and agricultural industries (Gangadevi, *et al*., 2007). The endophytic fungi are expected to be a potential source for new natural bioactive agents. In recent years, the quest for the isolation of new compounds from medicinal plants has become a fascinating area of research (Kumaran, *et al*., 2008). Endophytic fungi have been examined in conifers, including *Pinus* spp., *Toxus* spp., and *Juniperus* spp., and they are presumed to be ubiquitous (Wang, *et al*., 2008). The plant species are important interested due to their production of taxol (paclitaxol) and it has been used alone or in combination with other chemotherapeutic agents for the treatment of a variety of cancers, as well as AIDS-related kaposi-sarcoma. It was first isolated from the bark of the pacific yew tree, *Taxus brevifalia* since 1971, but obtaining taxol from this source requires destruction of trees. The limited supply of the drug has prompted efforts to find alternative sources of taxol. The first report on the isolation of taxol producing fungi from *Taxus brevifolia* appeared in 1993. Alternative methods for taxol production, such as chemical synthesis, tissue and cell cultures of the *Taxus* species are expensive and give low yields. Cost-effective taxol production is possible by industrial fermentation. (Chakravarthi, *et al.,* 2008).

Several taxol producing fungi have been identified such as, *Taxomyces andrenae* (Stierle, *et al.*, 1993), *Pestatotiopsis microspore, Fusarium solani* (Chakravarthi, *et al*., 2008) and *Colletotrichum gloeosporioides* (Gangadevi and Muthumary, 2008) *etc*.

There have been a few reports on the isolation of taxol-producing endophytic fungi (*Colletotrichum gloeosporioides*), demonstrating the mentioned organisms other than *Taxus* spp. could produce taxol. *Colletotrichum gloeosporioides* were isolated from *Taxus* species : *T.baccata, Taxus brevifolia* and *Taxus chinensis*. *Collectotrichum* isolates were obtained from lesions on leaf, petiole and fruit banana (*Musa acuminate*), longan (*lunguas galangal*), mango (*Mangifera indica*), and ornamental plant (*Draceana sanderiana*) and soybean (*Glycine max*) from Chiany Mai, Thailand (Photita, *et al*., 2004). In 2008, *Colletotrichum gloeosporioides* was newly recorded as an endophyte of *Taxus mairei* tree in Taiwan (Wang, *et al*., 2008). In this year, there was firstly reported the isolation of taxol producing fungi *Colletotrichum gloeosporioides* from a medicinal plant *Justicia gendarussa* Burm.F. As the above information, the current research was performed and revealed the chemical constituents, toxic effect and isolation of taxol-producing endophytic fungi from the leaves of *Justicia gendarussa* Burm.F.

Botanical name - *Justicia gendarussa* Burm.F
Genus - *Justicia*
Species - *Gendarussa*
Family - Acanthaceae
English name - Daun Rusa, Gandarusa, Warer willow
Chinese name - Chin Jiao
Myanmar name - Nat-Pan
Synonyms name - *Gendarussa vulgaris* Nees
Common name - Bawanet, Htigra-hpraw, Pha-wa-net
Part used - Leaves

Figure 1. Leaves and plants of *Justicia gendarussa* Burm.F

The plant is traditionally used for the treatment of inflammation, bronchitis, eye diseases and the decoction of leaves are given in chronic rheumatism. It is exhibits antioxidant, heptoprotective, anthelmintic, anti-inflammatory, antiarthritic, antiangiogenic, antimicrobial, analgesic and antianxiety activities (Kavitha, et al., 2014).

Materials and Methods

Preliminary Phytochemical Tests of *Justicia gendarussa* Burm. F. (Nat-pan)

The medicinal plant *Justicia gendarussa* Burm. F. (Nat-pan) was selected to be extract the fungus. The leaves were collected from Dagon Myothit (East) Township, Yangon Region, Myanmar at December 2010. The collected plant sample was washed with water to remove impurities. They were dried at room temperature to prevent some reaction of the organic constituents present in sample with sun light. Qualitative chemical analyses were conducted in order to identify the various phytochemicals present in the leaves powdered of *Justicaia gendarussa* Burm. F. Phytochemical investigations on powder sample were carried out according to the standard procedures (Marini, *et al*., 1981). The results of preliminary phytochemical investigation were presented at Table 1.

Isolation of Fungus from the Leaf of *Justicia gendarussa* Burm. F.

The leaf samples were thoroughly washed in running tap water and cut into small fragment (approximately 0.5 cm long segment) with the aid of flame sterilized blade. Then the leaf segments were surface sterilized by immersion in 70 % EtOH for a few seconds (3-5 sec) and rinsed thrice in sterile distilled water for 10 second each. The excess moisture was blotted on a sterile filter paper. The surface sterile leaf segments were evenly placed in Petri dishes (9 cm diameter) containing potato dextrose agar (PDA) medium (amended with chloramphenicol 150 mg/L). The Petri dishes were sealed using paraffin and incubated at 26 ± 1°C in day light with 12 hours followed by 12 hours of dark. The Petri dishes were monitored every day to check the growth of endophytic fungal colonies from the leaf segments.

The hyphal tips, which grew out from leaf segments were isolated and sub-cultured on PDA media and allowed to grow at 26 ± 1°C for another 10 days brought into pure culture.

Identification of Isolated Fungi

The morphology of fungal spore were checked under high power objective using Nikon Japan Microscope and compared with reported literature data (Nithya and Muthumary, 2009). The radial growth of mycelium of the fungus was measured by growing it on different sugar media such as glycerol, mannose, dextrose, sucrose and fructose. The Petri dishes were incubated at 26±1°C for 7 days and the diameter of the mycelium was measured at daily interval.

In addition, the estimation of dry mycelial weight was carried out on potato dextrose broth method. The isolated fungus was grown in 250 mL Elenmeyer flasks containing 50 mL of potato dextrose broth. The culture were incubated on different days; i.e. 2, 4, 6, 8, 10, 12 and 14. The dry mycelial weight was recorded at regular intervals.

Determination of Acute Toxicity of Leaf of *Justicia gendarussa*

The acute toxicity test on mice was performed with 70 % ethanol extract and water extract from the leaves of *Justicia gendarussa* according to Litchfield and Wilcoxon method at the Pharmacological Research Division, Department of Medical Research (Lower), Yangon, Myanmar.

Results and Discussion

The phytochemicals of, plant derived products were not only nutrients for human but also valuable chemicals for all living things. Therefore, the preliminary phytochemicals studies of the plant was essentially carried out before the extraction of phytoconstituents from various parts of plant samples. In this research, the phytochemical investigation of *Justicia gendarussa* Burm. F. showed that the leaves contained valuable metabolites such as alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, terpenoids and tannins (see Table 1).

Table 1. Phytochemical constituents from the leaves of *Justicia gendarussa* Burm. F.

Test	**Extracts**	**Test reagent**	**Observation**	**Remark**
		(i) Dragendroff reagent (ii) Sodium picrate solution iii) Wagner		+

Test	Extracts	Test reagent	Observation	Remark
Alkaloids	1%HCl	reagent iv) Dragendroff reagent (v) Sodium picrate solution vi) Wagner reagent	Orange ppt. White ppt. Brown ppt	
α- amino acids	H_2O	Ninhydrin reagent	Violet	+
Carbohydrates	H_2O	10% α-naphthol , conc. H_2SO_4	Red ring	+
Flavonoids	MeOH	Mg turning, conc. HCl	Light pink	+
Glycosides	H_2O	10% Lead acetate solution	White ppt	+
Phenolic Compounds	H_2O	1%$FeCl_3$ solution , $K_3Fe(CN)_6$	Deep blue	+
Reducing Sugars	H_2O	Benedict solution	Brick-red ppt.	+
Saponins	H_2O	Distilled Water	Frothing	+
Steroids	Pet-ether	Acetic anhydride and conc. H_2SO_4 solution	Green	+
Terpenoids	EtOAc	Acetic anhydride and conc. H_2SO_4 solution	Yellow	+
Tannins	H_2O	1%gelatin , 10%NaCl solution	White ppt.	+

+ = presence

Isolation and Identification of Fungus

According to background information, the taxol-producing endophytic fungus, *Colletotrichum gloeosporioides* can be explored in the leaves of *Justicia gendarussa*. The isolated fungi were identified by morphological studies and cultural studies. Based on the morphology of the mycelial colony and the characteristics of the conidia (fungal sporulation), the endophytic fungus was identified as *Colletotrichum gloeosporioides*. Colonies are pale brown or grayish white, consisting of hyaline, septate, branched mycelium during 7 days (figure 2 (a)). The fungus which grew out from leaves were further cultured on PDA media and allowed at the same temperature with above procedure to get pure culture (figure 2 (b)).

Figure 2. (a) 1st culture of isolated fungus from a piece of leaf
(*Justicia gendarussa*),
(i) leaf tip on PDA medium
(ii) after 1 weeks
(iii) after 2 weeks
(b) 2nd culture, (i) after 2 days
(ii) after 1 week
(iii) after 1 week under microscope

The cultural studies was carried on different sugar media such as potato glycerol agar, potato mannose agar, potato dextrose agar, potato sucrose agar and potato fructose agar and it was checked within 14 days. The results of cultural studies on different solid media, indicated that there was no broader diameter after one day. After 2 day, there was wider than original radius of fungus at 5 media with not similar growth rate. Five days later, the radial growth of *Colletotrichum gloeosporioides* was maximum (9.0cm) on potato dextrose agar, potato sucrose agar and potato fructose agar. During these 5th day, the growth rate on potato dextrose agar (PDA) medium was faster than the other two media. On potato mannose agar, the growth of fungus was maximum at 6th day and the minimum radial growth (7.5cm) was found on potato glycerol agar until 7 days duration. After 6 days, there was no more changes on potato glycerol agar medium. The resultant data were shown in table 2 and described as bar graph in figure 3.

Table 2. Estimation of Radial Growth for *Colletotrichum gloeosporioides* on Different Carbon Sources

Medium	**Day 0 (cm)**	**Day 1 (cm)**	**Day 2 (cm)**	**Day 3 (cm)**	**Day 4 (cm)**	**Day 5 (cm)**	**Day 6 (cm)**	**Day 7 (cm)**
PGA	0.7	0.7	1.5	2.0	4.5	6.5	7.5	7.5
PMA	0.7	0.7	1.7	4.0	7.5	8.5	9.0	9.0
PDA	0.7	0.7	2.3	4.8	8.5	9.0	9.0	9.0
PSA	0.7	0.7	2.2	4.7	8.3	9.0	9.0	9.0
PFA	0.7	0.7	2.1	4.5	8.0	9.0	9.0	9.0

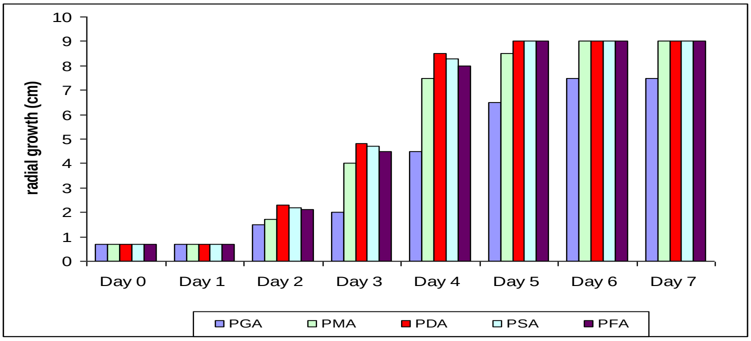

Figure 3. Growth rate of *Colletotrichum gloeosporioides* on different carbon sources

PGA = potato glycerol agar
PMA = potato mannose agar   Disc Diameter = 9.0 cm
PDA = potato dextrose agar
PSA = potato sucrose agar
PFA = potato fructose agar

Table 3. Estimation of Dry Mycelial Weight for *Colletotrichum gloeosporioides* in Potato Dextrose Broth

Sr. no.	**Day after inoculation**	**Mycelial weight in mg**	
		Observed	**Literature ***
1	0	1.25	-
2	2	62.60	65.56
3	4	118.00	127.66
4	6	178.25	199.33
5	8	230.25	245.33
6	10	285.55	292.33
7	12	310.60	330.33
8	14	300.20	307.00

(* Ashoka, 2005)

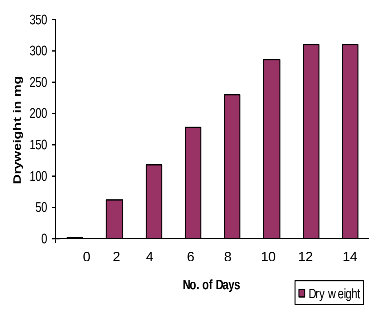

Figure 4. Dry mycelial weight (mg) different day after inoculation

Acute Toxicity Test

By viewing of the results from table 4, aqueous extract of *Justicia gendarussa* leaves has found to be free toxic effect or harmful effect at 12 g/kg and low dose. In other words, all animals alive and did not showed any visible symptom of toxicity like respiratory disorder, convulsions and death, etc. The ethanol extract of leaves, by contrast, has been found to be toxic at 8 g/kg body weight dose of *Justicia gendarussa* leaves, 20 % of mice were dead. From the calculation, LD_{50} of ethanol extract was shown as 11.6 g/kg and 95 % confidence limits were between 10.75 g/kg – 12.52 g/kg. By the study of scales of the Hodge and Sterner, any compound with an oral LD_{50} of between 500 – 5000 mg/kg should be considered slightly toxic (Ahmed, M., 2015). Therefore, the current study can be approved that ethanol and water extracts of *Justicia gendarussa* leaves were safe as orally drugs as well as production of natural bioactive agent from endophytic fungi.

Table 4. Result of Acute Toxicity on Aqueous and 70 % Ethanol Extract of *Justicia gendarussa* Burm. F. (leaves)

Test extract	**Group No.**	**Number of tested animal**	**Dose (g/kg)**	**Ratio of dead to tested animal**	**% of dead**	**LD_{50} (g/kg)**
Aqueous extract	I	10	12	0/10	0	Nil
	II	10	10	0/10	0	
	III	10	8	0/10	0	
	IV	10	6	0/10	0	
70 % EtOH extract	I	10	12	5/10	50	11.6
	II	10	10	3/10	30	
	III	10	8	2/10	20	
	IV	10	6	0/10	0	

Conclusion

In this study, screening of phytoconstituents and taxol-producing endophytic fungus: *Colletotrichum gloeosporioides* from *Justicia gendarussa* Burm. F. (Nat-pan) is reported. In addition, there can be a supporting evidence that the leaves of *Justicia gendarussa* may be use at the production of taxol from fungus which presented in the leaves due to the relatively less toxic by comparing the reported acceptable level.

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References

Ahmed, M., (2015), "Acute Toxicity (Lethal Dose 50 Calculation) of Herbal Drugs Somina in Rats and Mice", *Pharmacology and Pharmacy*, **6**, 185 – 189

Chakrararthi, B.V.S.K., Das, P., Surendranath, K., Karande, A. and Jayabaskaran, C., (2008), "Production of Paclitaxel; by *Fusarium solani* isolated from *Taxus celebica*", *J. Biosci*. Indian Academy of Sciences, **33** (2): 259-267, http://www.ias.ac.in/biosic Academy (accessed date 2.6.2010)

Gangadevi, V. and Muthumary, J., (2007), "Preliminary Studies on Cytotoxic Effect of Fungal Taxol on Cancer Cell lines", *African Journal of Biotechnology*, **6** (12) : 1382-1386

Gangadevi, V. and Muthumary, J., (2008), "Isolation of *Colletotrichum gloeosporioides*, a Novel Endophytic Taxol-Producing Fungus from the leaves of a Medicinal Plant, *Justicia gendarussa*", *Mycologia Balcanica*, **5** : 1-4, http://www.mycobalcan.com (accessed date 16.6.2010)

Kumaran, R.S., Muthumaryl, J. and Hur, B.K., (2008), "Isolation and Identification of Taxol, an Anticancer Drug from *Phyllosticta melochiae* Yates, an Endophytic Fungus of *Melochia corchorifolia* L.", *Food Sci. Biotechnol*., **17** (6) : 1246-1253

Marini, G. B., Bettolo - Nicoletti, M., Patamia, M., Galeti, C. and Messana, I., (1981), "Plant Screening by Chemical and Chromatographic Procedure Under Field Conditions". *J. Chromato*. **46**(2), 359-363

Nithya, K. and Muthumary, J., (2009), "Growth Studies of *Colletotrichum gloeosporioides* (Penz.) Sacc. - a Taxol Producing Endophytic Fungus from *Plumeria acutifolia*", *Indian Journal of Science and Technology*, **2** (11) : 14-18, http://www.injst.org (accessed date 16.1.2011)

Photita, W., Taylor, P.W.J., Ford, R., Hyde, K.D. and Lumyong, S., (2004), "Morphological and Molecular Characterizatic of *Colletotrichum* Species from Herbaceous Plants in Thailand", *Fungal Diversity*, 117-133

Stierle, A., Strobel, G.A. and Stierle, D., (1993), "Taxol and Taxan Production by *Taxomyces andreanae*, and Endophytic fungus of Pacific Yew", *Science*, **260** : 214-216

Wang, Y.T., Lo, H.S. and Wang, P.H., (2008), "Endophytic Fungi from Taxus mairei in Taiwan : First Report of *Colletotrichum gloeosporioides* as an Endophyte of *Taxus mairei* ", *Botanical Studies*, **49** : 39-43

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Study on Preparation and Characterization of Pectin–ZnO Nanocomposite from Fruit Peel of *Durio zibethinus* L. (Durian)

Than Than Nu[*]

Abstract

Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields. The hybrid inorganic-organic nanocomposite materials are also great power of attracting because of their multifunctionality owing to a combination of different compounds incorporated. The research work focused on preparation and characterization of pectin-ZnO nanocomposite from fruit peel of *Durio zibethinus* L. (Durian). The pectin was extracted from fruit peel of Durian by acid hydrolysis followed by precipitation with ethanol. The extracted pectin was characterized by some chemical tests as well as FT IR spectroscopic method. In addition, Pectin-ZnO nanocomposite using extracted pectin was prepared by precipitation method. The prepared pectin-ZnO nanocomposite was studied by FT IR, XRD and SEM methods. These measurements confirmed that the prepared pectin-ZnO composite from Durian pectin was nanoscale composite.

Keywords: *Durio zibethinus* L. (Durian), Pectin, Acid hydrolysis, Precipitation, Pectin-ZnO nanocomposite

Introduction

Plant and their products have always been a source of various drugs and excipients used in pharmaceutical formulations. A valuable by-product that can be obtained from fruit peels is pectin. It is a naturally occurring polysaccharide, has in recent years gained increased importance. Pectin is the methylated ester of polygalacturonic acid. The pectin is employed in pharmaceutical industry as a carrier for drug delivery to the gastrointestinal tract, such as matrix tables, gel beads, film-coated dosage form (Srivastava and Malviya, 2011). Therefore, pectin is an excellent candidate for eco-friendly biodegradable application. In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport

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and properties. Particles are further classified according to diameter. Coarse particles cover a range between 2,500 and 10,000 nanometers. Fine particles are sized between 100 and 2,500 nanometers. Ultrafine particles, or nanoparticles, are between 1 and 100 nanometers in size. Nanoclusters have at least one dimension between 1 and 10 nanometers and a narrow size distribution. Nanoparticles often possess unexpected optical properties as they are small enough to confine their electrons and produce quantum effects. Zinc oxide particles have been found to have superior UV blocking properties compared to its bulk substitute. This is one of the reasons why it is often used in the preparation of sunscreen lotions, and is completely photo-stable. Nanocomposite of ZnO wrapped in pectin will survive the gastric environment and become available in the intestine and readily absorbed due to their nanoscale size. The incorporation of nanocrystalline ZnO into pectin to form nanocomposite may impart unique functionalities to the nanocomposite prepared (Buzea, *et al*., 2007).

Durio zibethinus L. (Durian) belongs to family Bombacaceae. The fruits are ovoid or ovoid-oblong to nearly round, 6 to 12 in long, 5 to 6 in wide. The yellow or yellowish-green rind is thick, tough, semi-woody, and densely set with stout, sharply pointed spines, 3 to 7 sided at the base. Inside there are 5 compartments containing the creamy-white, yellowish, pinkish or orange-colored flesh. The durian is believed to be native to Borneo and Sumatra. It is found in South Tenasserim, Lower Myanmar, Malaysia and is commonly cultivated India. Durian is rich in dietary fiber and source of antioxidant vitamin-C. Further, it also contains a good amount of minerals like manganese, copper, iron and magnesium. Fresh durian fruit is a very rich source of potassium and high levels of essential amino acid (Amid, 2012). Decoctions of the leaves and fruits are applied to swellings and skin diseases. The odor of the flesh is believed to be linked to indole compounds which are bacteriostatic. The toothpaste flavored with durian is currently marketed for durian fanciers (Yumoto Takakazu, 2000).

The present work deals with the preparation and characterization of pectin-ZnO nanocomposites from fruit peels of Durian. The images of fruits and peels of Durian are shown in Figure 1.


(a) (b)

Figure 1. Images of (a) fruits and (b) peels of Durian

Materials and Methods

All chemicals were obtained from British Drug House. The following instruments were used for the determination of physical data: IR spectra; Perkin Elmer GX FT IR spectrophotometer, XRD diffractograms; X-ray diffractometer and Scanning Electron Microscopy; SEM micrograph at the Universities' Research Centre, University of Yangon.

The Durian fruits were collected from Theingyi Zay, Pabedan Township. The green parts of fruit shell were cut with a knife. The white peels (mesocrap) of the fruits were cut into thin slices of about 2.5 mm thick. They were oven-dried at 50 °C for two days and then made into powder. The dried powdered sample was stored in the air tight containers.

Extraction of Pectin by Alcohol Precipitation Method

About 100 grams of prepared sample was washed thrice with water and the holding time for 10 minutes. Sugar and other soluble solids were thus removed. The sample was then filtered and then squeezed by pressing through a cotton bag for further elimination of sugar and soluble solids. The pressed pulps were then boiled with 2 times its own volume of 0.01 N hydrochloric acid at 100 °C for 1h, in which the protopectin was loosen from its binding with cellulose and is hydrolyzed into pectin, which was soluble in water. The extract was separated by squeezing hot suspension through a bag of cloth. It was cooled immediately, allowed to settle and clarified by centrifuging. Decolourization, if required, was done by activated carbon. The pulp left over from the first extraction was extracted second time by boiling for 1 hour with equal volume of 0.01 N hydrochloric acid. The experiments were repeated until four successive extractions had been done and the residue was finally discarded. The clarified extracts were concentrated at 50 °C under reduced pressure. The extraneous materials which separated out during concentration were removed, if necessary, by centrifuging. The solution was finally concentrated to syrup by low pressure evaporating method. The syrup was poured in a thin stream with a vigorous stirring into alcohol to give a final concentration of 70 %. The precipitated pectin was allowed to stand overnight. The gelatinous pectin precipitate was separated by squeezing the material through a mull cloth. It was washed twice with alcohol and finally dehydrated with acetone. Pectin obtained was dried at oven (37 °C) for 5 h. The dried pectin was then powdered and the yield percent of extracted pectin was calculated.

Determination of Physicochemical Properties of the Extracted Pectin

The ash content, equivalent weight, molecular weight, methoxyl content, anhydrouronic acid, degree of esterification and setting time of extracted pectin were examined by appropriate analytical methods.

Characterization of the Extracted Pectin

Extracted pectin was characterized by some chemical tests such as alcohol test, sugar and citric acid test, Fehling’s solution test, lead acetate test and iodine solution test. The extracted pectin and commercial pectin were also characterized by Perkin Elmer GX FT IR Spectrophotometer measured with KBr pellets at the Universities’ Research Centre (URC), University of Yangon.

Preparation of Pectin-ZnO Nanocomposite from Extracted Pectin

A 0.4 g of extracted pectin, 2.4 g of $Zn(NO_3)_2.6H_2O$ and 80 mL distilled water were added into a 200 mL beaker. After complete dissolution, 80 mL of 0.125 M NaOH solution was added dropwise under constant stirring. The reaction was allowed to proceed at 28 ± 0.5°C for 2 h stirring and kept for 24 h. The white precipitate obtained was centrifuged at 10000 rpm for 10 min and collected and washed with distilled water for several time to remove the by-products. After drying at 37 ºC for 5 h, the final product was obtained as white powder.

Characterization of Prepared Pectin-ZnO Nanocomposite

The prepared pectin-ZnO nanocomposite was charactrized by FT IR, XRD and SEM methods. The FT IR spectrum of prepared pectin-ZnO was measured with KBr pellet by Perkin Elmer GX FT IR spectrophotometer. The overall crystalinity of the product was examined by X-ray diffractometer (XRD) with the solid specimens mounted on a low background quartz holder. Powder X-ray diffraction was performed on powder X-ray diffractometer (Rigaku, Print 2000/ PC software, Japan). The overall surface morphology of prepared pectin-ZnO composites was examined by scanning electron microscopy (SEM) analysis.

Results and Discussion

Extraction of Pectin from Durian Peel

In this research work, the pectin was extracted from Durian peel by alcohol precipitation method. The yield based on dry peel of extracted pectin, light brown powder was found to be 13.46 %. A source with a yield of above 10 % coupled with good jelly grade is usually considered commercially variable (Mohamed and Hasan, 1995). Therefore, Durian pectin was found to contain in good jelly grade.

Some Physicochemical Properties of the Extracted Pectin

(a) Ash Content

Ash content can affect the gelling of pectin. The low ash content is more favorable for gel formation. The ash content in extracted pectin was observed to be 1.63 %. Commercial high methoxyl and low methoxyl pectin has been reported to contain about 1.9~5.2 % ash content (Mohamed and Hasan, 1995).

(b) Equivalent weight, Methoxyl content, Anhydrouronic acid and Degree of Esterification

The equivalent weight of extracted pectin was observed to be 806.45 g eq^{-1}. The molecular weight is one of the important criteria to characterize the extracted pectin. In this research work, the relative molecular weight was determined by viscosity method by applying the Mark-Houwink equation. The molecular weight for Durian, pectin was 12.06 × 10^4 Dalton. The methoxyl content of the extracted pectin was

10.91 %, indicating the high methoxyl (HM) pectin. The anhydrouronic acid (AUA) of Durian was observed to be 83.77 %. The % AUA was greater than 70 % showed the good pectin potential. The degree of esterification (DE) is an important means to classify pectin. Consequently low methoxyl (LM) pectins have a DE < 50 %. The degree of esterification of extracted pectin from Durian peels was 73.94 %. The extracted pectin is HM pectin (> 50 % DE). In general HM pectin is hot water soluble and often contains a dispersion agent such as dextrose to prevent lumping (Ismail *et al*., 2012).

(c) Setting time

The setting time of Durian pectin was found to be 12 min. If the jelly set in 10 min to 25 min, the pectin was considered rapid setting (Mohamed and Hasan, 1995). Rapid set pectin is traditionally used for jams and marmalades and also important for higher sugar products like bakery and biscuit jams, sugar confectionary. Therefore, the pectin from Durian has rapid gel-setting time, including in commercially marketable pectin product.

Characterization of the Extracted Pectin

(a) Some Chemical Tests

The extracted pectin (Figure 2) was characterized by some chemical tests. The pectin was found to give a white flocculent precipitate with ethanol and a firm jelly with sugar and citric acid solution. So, they showed the jelly properties of pectin (Ismail *et al*., 2012). Fehling's solution test of extracted pectin exhibited the green-blue gel precipitates due to non-reducing sugar. Basic lead acetate test of pectin gave yellow-white gelatinous precipitate so it was glycoside. The yellow gel with iodine solution showed that it was non-starch polysaccharide (Figure 2).

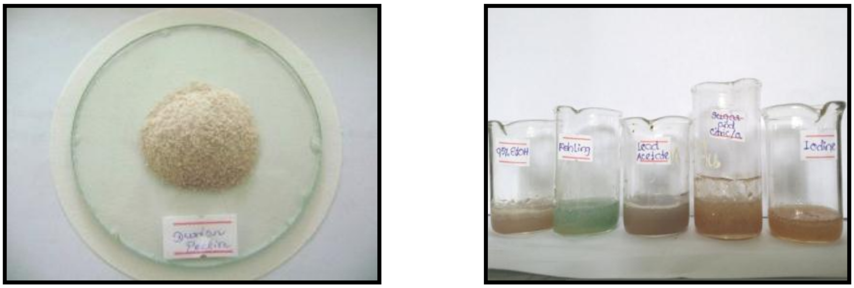


Figure 2. Images of extracted pectin and its chemical tests

(b) Extracted Pectin Compared with Commercial Pectin by FT IR Method

Extracted pectin : Light brown powder; FT IR υ (cm^{-1}), 3444 (υ_{O-H} in alcohol and acid groups), 2956, 2862 ($\upsilon_{asym-C-H}$ and $\upsilon_{sym-C-H}$), 1743, 1688 ($\upsilon_{C=O}$ of ester and acid carbonyl group), 1444 (δ_{C-H} of CH_2 and CH_3), 1383 (δ_{O-H} of OH in plane bending), 1265 and 1147 (υ_{C-O-C} and υ_{C-O}), 914 (δ_{oop} of C-H) and 621 (δ_{oop} of O-H).

Commercial Pectin: Light brown powder; FT IR υ (cm^{-1}), 3396 (υ_{O-H} in alcohol and acid groups), 2943, 2857 ($\upsilon_{asym-C-H}$ and $\upsilon_{sym-C-H}$), 1739, 1665 ($\upsilon_{C=O}$ of ester and acid carbonyl group), 1433 (δ_{C-H} of CH_2 and CH_3), 1380 (δ_{O-H} of OH in plane bending), 1269 and 1147 (υ_{C-O-C} and υ_{C-O}), 916 (δ_{oop} of C-H) and 623 (δ_{oop} of O-H).

Preparation of Pectin-ZnO Composite from Extracted Pectin

In the present work, preparation of pectin-ZnO composite was performed by using extracted pectin in aqueous solution at 28 ± 0.5 °C. In the hydrothermal alkaline solution, the two oxygen atoms of zinc hydroxide are highly repulsive since they all have lone pair of electron and quick dehydration of zinc hydroxide yielded the products of ZnO. Pectin binds with ZnO molecules with COO^{-} and CH_2 groups in a hydrogen bond system to restrain the formed ZnO nanoparticles from further agglomeration by the action of steric hindrance. After a long time of reaction, the pectin-wrapped ZnO nanocomposite is formed. The yield percent of pectin-ZnO nanocomposite was found to be 68.46 %.

Characterization of Pectin-ZnO Composite

(a) FT IR Spectroscopic Method

Pectin- ZnO : White powder; FT IR υ (cm^{-1}), 3465 (υ_{O-H} in alcohol and acid groups), 2926, 2854 ($\upsilon_{asym-C-H}$ and $\upsilon_{sym-C-H}$), 1743, 1685 ($\upsilon_{C=O}$ of ester and acid carbonyl group), 1510 and 1448 (δ_{C-H} of CH_2 and CH_3), 1384 (δ_{O-H} of OH in plane bending), 1143 and 1016 (υ_{C-O-C} and υ_{C-O}), 920 (δ_{oop} of C-H), 642 (δ_{oop} of O-H) and 472 (υ_{Zn-O}) (Figure 3, a).

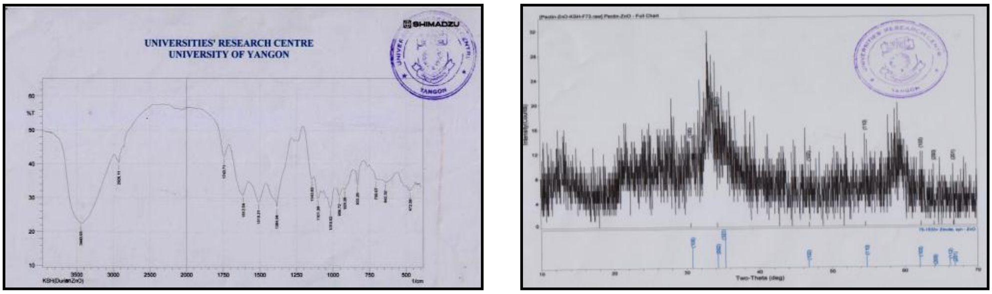
(b) XRD Analysis

The particle sizes of prepared pectin-ZnO composites were calculated by following Sherrer equation.

$$D = \frac{K\ \lambda}{\beta\ \cos\ \theta} = \frac{0.9\ \lambda}{\beta\ \cos\ \theta}$$

D = Crystallite size
β = Full width half maximum (FWHM) of the observed peak
θ = angle of diffraction
K = Scherrer constant

The XRD pattern of pectin- ZnO composite is shown in Figure 3(b). The diffractive peaks indexed to those of hexagonal ZnO. The lattice constants obtained from the XRD data were a=3.3558 Å, c=5.2016 Å which were consistent with the reported values of ZnO (a=3.353 Å, c=5.209 Å) (Shi and Gunasekaran, 2008). The average crystallite particles sizes were found to be 78.22 nm based on the Scherrer equation (Table 1). According to the result, the prepared pectin-ZnO composite from Durian was observed to be nanoscale size.



(a) (b)

Figure 3. (a) FT IR spectrum and (b) XRD diffractogram of pectin-ZnO composite

Table 1. Crystallite Size Distribution of ZnO from Pectin-Zn Composite

No.	**FWHM**	**β**	**λ**	**2θ**	**Cos θ**	**D**
1	0.595	0.010	1.54056	30.591	0.9645	14.3
2	0.119	2.077_10^{-3}	1.54056	34.260	0.9556	69.8
3	0.085	1.488_10^{-3}	1.54056	46.866	0.9175	101.9
4	0.128	2.234_10^{-3}	1.54056	54.640	0.8884	69.8
5	0.066	1.152_10^{-3}	1.54056	62.300	0.8558	140.5
6	0.141	2.461_10^{-3}	1.54056	64.060	0.8477	66.4
7	0.112	1.955_10^{-3}	1.54056	66.708	0.3352	84.88
			Average		78.22 nm	

(c) SEM Analysis

A Scanning Electron Microscope (SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the sample’s surface topography and composition. Scanning electron microscopes can magnify objects 100,000 times or more. They are particularly useful and powerful optical microscopes, They can produce detailed three-dimensional images of the surface of objects. The SEM microgram of Durian pectin-ZnO composite is shown in Figure 4. According to SEM image, the composite granules were found to be irregular. The ZnO particles were wrapped with pectin. In addition, it was found that the composite grain size of pection-ZnO was observed to be 194 nm, indicating the nanocomposite grain size (Table 2).

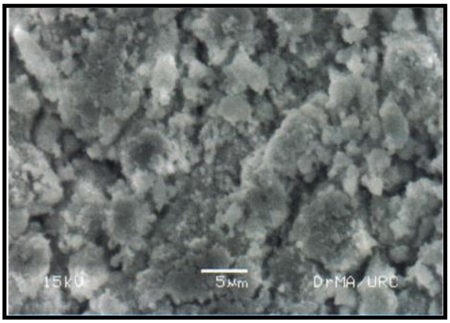


Figure 4. SEM micrograph of pectin-ZnO composite with magnification 5 µm

Table 2. SEM Grain Size Distributions of Pectin-ZnO Composite

Sr. No.	**Counting NB Total (100)**	**Grain size µm**
1	35	4.55
2	54	11.88
3	11	2.97
	Average	0.194 (194 nm)

Conclusion

From the research work, it could be concluded that pectin was extracted from Durian peel by alcohol precipitation method. The yield percents based on dry peels of extracted pectin was found to be 13.46 %. The extracted pectin was characterized by some chemical tests and FT IR spectroscopic method. In addition, Pectin-ZnO nanocomposite using extracted pectin was prepared by precipitation method. The yield percent of pectin-ZnO nanocomposite was found to be 68.46 %. The average crystallite sizes obtained from XRD analysis were found to be 78.22 nm. The lattice constants obtained from the XRD data were a=3.3558 Å, c=5.2016. Thus the broadening of the XRD peak suggested that the prepared pectin-ZnO composite was nanoscale size and the structure was found to be hexagonal. In the SEM observation, the composite granule was irregular and their average composite grain size was about to be 194 nm. It confirmed that the Pectin-ZnO nanocomposite from Durian peels was nanoscale grain size composite. Consequently, the prepared pectin-ZnO nanocomposite may contribute to many new developments in the fields of biomedicine, biosensors and bionanotechnology.

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References

Amid, B.T. (2012). "Chemical Composition and Molecular Structure of Polysaccharide Biopolymer from *Durio Zibethinus* Seed Extraction and Purification Process". *Chem. Cent. J.*, **6**(1), 117-120.

Buzea, C., T. L. Pacheco, and K. Robbie. (2007). "Nanomaterials and Nanoparticles: Sources and Toxicity". *Biointerphases*, **2**(4), 17-71.

Ismil, N. S. M., N. Raml, N. M. Hani, and Z. Meon. (2012). "Extraction and Charaction of Pectin from Dragon Fruit (*Hylocereus polyrhizus*) using Various Extraction Conditions". *Sains Malaysiana*, **41** (1), 41- 45.

Mohmed, S., and Z. Hasan. (1995). "Extraction and Characterisation of Pectin from Various Tropical Agrowastes". *ASIAN Food Journal*, **10** (2), 43- 49.

Shi, L., and S. Gunasekaran. (2008). "Preparation of Pectin ZnO Nanocomposite". *Journal Nanoscale .Res. Lett.*, **3** (12), 491- 495.

Srivastava, P., and R. Malviya. (2011). "Source of Pectin, Extraction and its Application in Pharmaceutical Industry-An Overview". *Indian Journal of Natural Products and Resources*, **2** (1), 10 -18.

Yumoto Takakazu. (2000). "Bird-pollination of Three Durio Species in A Tropical Rainforest in Malaysia". *American Journal of Botany*, **87** (8), 87-92.

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In vitro Antioxidant and Antitumor Activities of the Rhizome of *Rhynchanthus longiflorus* Hook.f. (Kyauk-gin)

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Abstract

The rhizomes of the Zingiberaceae family are widely used as vegetables in many Asian countries, and their medicinal functions have been broadly discussed and accepted in many traditional recipes. In the current research, a rare, endangered plant with tuberous rhizome, viz., *Rhychanthus longiflorus* Hook.f. (Myanmar Name: Kyauk-gin) belonging to the family Zingiberaceae collected from Kachin State, Myanmar, was chosen to investigate some phytoorganic constituents, antioxidant activity and antitumor activity. The qualitative phytochemical analysis revealed that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids and terpenoids were present in the rhizome of *R. longiflorus*. In the *in vitro* antioxidant activity screening of 95 % EtOH and watery extracts of the sample by 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging assay, EtOH extracts of the rhizome showed higher antioxidant activity (IC_{50}= 119.42 µg mL^{-1}) than that of watery extract(IC_{50}= 216.36 µg mL^{-1}). The antitumor activity of 95 % EtOH and watery extracts of the rhizome of *R. longiflorus* was screened on *Agrobacterium tumefacien* by Potato Disc Assay method. Both 95 % ethanol and watery extracts exhibited antitumor activity against *Agrobacterium tumefacien* with the minimum tested concentration of 25 mg mL^{-1}at the observation periods. The results revealed the positive demonstration of the utility of *R. longiflorus* for food and medicinal uses in Myanmar.

Keywords: *Rhychanthus longiflorus* Hook.f., phytoorganic constituents, antioxidant activity, antitumor activity

Introduction

Antioxidant is a substance that can fight and destroy excess free radicals and repair oxidative damage. Insufficient levels of antioxidants, cause oxidative stress and may damage cells. Oxidative stress is damage to cell structure and cell function by overly reactive oxygen-containing molecules and chronic excessive inflammation. Oxidative stress seems to

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play a significant role in many human diseases, including cancers (Lobo *et al*., 2010).

Nowadays, the synthetic antioxidants are widely used in food industry and also included in the human diet. The use of natural antioxidant is safer than synthetic ones (Melanathuru et al., 2017).

A tumor is an abnormal growth of body tissue and actually refers to any type of lump or swelling in the body. Also known as a neoplasm (meaning "new formation"), a tumor can be either benign (non-cancerous or not serious) or malignant (cancerous or deadly). In general, tumors appear to occur when there is a problem with the dividing of cells in the body. Cells are the building blocks of living things. Typically, the division of cells in the body is strictly controlled. New cells are created to replace older ones or to perform new functions. Cells that are damaged or no longer needed die to make room for healthy replacements. If the balance of cell division and death is disturbed, a tumor may form (Nordvist, 2017).

Many plants belonging to the Zingiberaceae family have a history of medicinal use in systems of traditional medicine and are famous for their use as spices. Zingiberaceae is one of the major tropical plant families (Chang et al., 2008). Rhizomes of several species of Zingiberaceae are also used as insect repellents and have various arrays of pharmacological activities such as antioxidant, anticancer, antimicrobial, immunomodulatory effects, gasterointestinal effects, analgesic, antipyretic and antitumor. Zingiberaceae species typically have thickened rhizome. Many compounds with novel structures and a large number of biologically active compounds have been identified from these plants (Zahara et al., 2018).

This research is focused on phytochemical analysis and evaluation of antioxidant and antitumor activities of the rhizomes of a rare, endangered Zingiberaceous plants, *Rhychanthus longiflorus* Hook.f.

The Selected Medicinal Plant for the Present Research

Botanical name	: *Rhychanthus longiflorus* Hook.f.
Family	: Zingiberaceae
Genus	: *Rhychanthus*
Species	: *R. longiflorus*
Myanmar name	: Kyauk-gin
Part used	: Rhizome

(a) (b) (c)

Figure 1 (a) Plants of *Rhynchanthus longiflorus* Hook. f. (Kyauk-gin)
(b) Flowers of *Rhynchanthus longiflorus* Hook. f.
(c) Rhizomes of *Rhynchanthus longiflorus* Hook. f.

Distribution and description

Rhynchanthus logiflorus Hook.f. is native to Myanmar, Yunnam and the Assam region of eastern India. The plant is generally grown in mountainous regions. This genus is represented by 6 species distributed in Myanmar, India and South China only and is a rear, endangered species (Prasanthkumar et al., 2004).

Rhynchanthus longiflorus is a low herb with tuberous rhizome. It Inflorescences terminal on the leafy shoot; bracts large and each sub-tending 1 sessile flower. The bracteole is small, tubular and flowers are yellowish to red.

Materials and Methods

Sampling of Plant Material and Identification

The rhizomes of *Rhynchanthus longiflorus* Hook.f.(Local name: Kyauk-gin) belonging to the family Zingeberaceae were collected from the mountain side nearby Sein Lung village, Momauk Township, Kachin State, Myanmar, during November and December, 2018. After collection, the scientific name of the plant was identified at the Botany Department, Myitkyina University. The collected fresh samples of rhizomes of *R. longiflorus* were washed with tap water and air-dried at room temperature for two weeks and then were ground into powder by grinder. The dried powdered sample was stored in air-tight containers.

Qualitative Phytochemical Analysis of the Rhizomes of *R. longiflorus*

In order to classify the types of organic constituents present in the selected plant sample, qualitative phytochemical tests such as tests for alkaloid, α-amino acid, phenolic compounds, carbohydrates, flavonoids, terpenoids, steroids, reducing sugars, glycosides, saponin, starch,, tannins and cyanogenic glycosides were carried out according to the appropriate reported methods.

Preparation of Various Crude Extracts

Dried powered sample of *R. longiflorus* (ca.50 g) was percolated in 95 % EtOH (300 mL) 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.

Watery extract of the dried powdered sample was prepared by boiling (ca.50 g) of sample with 300 ml of distilled water for 6 h and filtered. The filtrates were concentrated by removal of the water to give watery extract. These crude extracts were kept for screening of antioxidant activity and antitumor activity.

Investigation of Some Bioactivities

(a) *In vitro* screening of antioxidant activity of some crude extracts from the rhizome of *R. longiflorus*

The *in vitro* antioxidant activity of 95 % EtOH and H_2O extracts of *R. longiflorus* was determined by DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay.

The sample solutions were prepared by dissolving 10 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 (1000.0, 500.0, 250.0, 125.0, 62.5 µg/mL) of sample solutions were prepared from this stock solution by serial dilution with appropriate amount of 95 % EtOH. 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

(b) *In vitro* Screening of Antitumor Activity of *R. longiflorus*

The *in vitro* antitumor activity of different crude extracts *viz*., 95 % ethanol and watery extracts of the rhizome of *R. longiflorus* was carried out against *Agrobacterium tumefacien* by Potato Crown Gall test or Potato Disc Assay method at the Pharmaceutical Research Department, Ministry of Industry, Yangon, Myanmar.

Fresh, disease free potato tubers were obtained from local markets and were used within 48 hrs of transfer to the laboratory.

Tubers of moderate sizes were surface-sterilized by immersion in 50 % sodium hypochlorite (Clorox) for 20 min. The ends were removed and soaked for 10 min more in Clorox. A core of the tissue was extracted from each tuber by using surface-sterilized (ethanol and flame) 1.5 cm wide cork borer. And, 2 cm pieces were removed from each end and discarded and the remainder of the cylinder is 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.

Extract of 50 mg, 100 mg and 150 mg were separately dissolved in 2 mL of dimethyl sulphoxide (DMSO), 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 3-5×10^9cells/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 hrs 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 must be conducted within 30 min. The plates were sealed with tape to minimize moisture loss and incubated at room temperature for 12 days. After incubation, Lugol's solution (I_2-KI) was added and the tumors were counted with a microscope and compared with control. The antitumor activity was examined by observation of tumor produced or not.

All materials (petri dishes and potato discs used) were sterilized before clean-up or discarding.

(a) (b) (c) (d)

Figure 2. Photographs for screening of antitumor activity by Potato Crown Gall (PCG) test

(a) Surface sterilization of potato in Clorox
(b) Immersion of potato in another Clorox after removing both ends
(c) Extraction of potato core with cork borer
(d) Cutting 0.5 cm diameter of potato disc

Results and Discussion

Phytochemicals Present in the Rhizome of *R. longiflorus*

Qualitative phytochemical analysis was carried out to know the different types of phytoconstituents present in the rhizome of *R. longiflorus*. The results show that *R. longiflorus* contains alkaloids, flavonoids, terpenoids, steroids, starch, saponins, reducing sugars, phenolic compounds, glycosides, tannin, carbohydrates and α-amino acids. But cyanogenic glycosides were found to be absent. The constituents such as phenolic compounds, terpenoids, flavonoids and steroids present in the sample may contribute to possess bioactivities such as antimicrobial, antioxidant, anticancer, antitumor and antipyretic properties in *R. longiflorus*.

Antioxidant Activity

The antioxidant activities of 95 % EtOH and H_2O extracts of *R. longiflorus* was studied by DPPH (1, 1-diphenyl-2-pricryl hydrazyl) free radical scavenging UV spectrophotometric assay method. The antioxidant activity was expressed as 50 % oxidative inhibitory concentration (IC_{50}). From the results, it was found that the antioxidant activity of 95 % EtOH extract of *R. longiflorus* (IC_{50}= 119.42 µg/mL) was higher than that of watery extracts (IC_{50}= 216.36 µg/mL) but both were lower than standard ascorbic acid (IC_{50} = 1.78 µg/mL). The % oxidative inhibition and IC_{50} values of watery and 95 % EtOH extracts of *R. longiflorus* and standard ascorbic acid were summarized in Tables 1 and 2. Plots of percent oxidative inhibition Vs. concentration of the crude extracts of *R. longiflorus* and standard ascorbic acid are illustrated in Figures 3(a) and (b). From the observation, it can be inferred that both the tested crude extracts of *R. longiflorus* showed antioxidant activity due to the presence of important bioactive components such as flavonoids, phenolic compounds in the rhizome *R. longiflorus*.

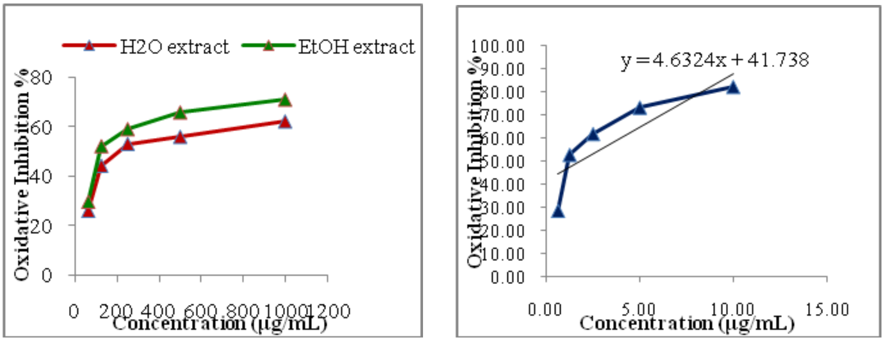
Table 1. Percent Oxidative Inhibition and IC_{50} Values of Crude Extracts from the Rhizome of *R. longiflorus* (Kyauk-gin)

Extracts	**Oxidative Inhibition %(mean ± SD) in different conc: (µg/mL)**					**IC_{50} (µg/mL)**
	62.5	**125**	**250**	**500**	**1000**	
KG (watery)	25.97± 0.57	44.23± 0.87	53.02± 1.63	55.91± 1.48	62.12.± 1.48	216.36
KG (EtOH)	29.45± 0.81	52.01± 1.43	58.91± 0.55	65.68± 1.99	70.88± 1.13	119.42

KG= *R. longiflorus*(Kyauk-gin)

Table 2. Percent Oxidative Inhibition and IC$_{50}$ Values of Standard Ascorbic Acid

Extracts	**Oxidative Inhibition %(mean ± SD) in different conc: (µg/mL)**					**IC $_{50}$ (µg/mL)**
	0.625	**1.25**	**2.50**	**5.00**	**10.00**	
Ascorbic acid	28.54± 0.57	52.78± 0.87	61.78± 1.63	73..19± 1.48	82.15.± 1.48	1.78



(a) (b)

Figure 3. (a) Plot of oxidative inhibition percent vs concentration (µg/mL) of crude extracts of *R. longiflorus*

(b) Plot of oxidative inhibition percent vs concentration (µg/mL) of standard ascorbic acid

Antitumor Activity

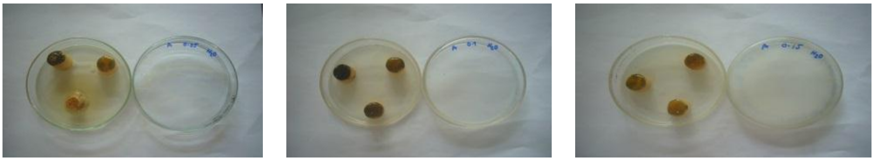
The antitumor activity screening of different crude extracts *viz*., 95 % ethanol and watery extracts of *R. longiflorus* was carried out against *Agrobacterium tumefacien* by Potato Crown Gall test method. The photographs illustrating the different concentration of plant crude extracts against *Agrobacterium tumefacien* are presented in Figures 4 and 5 and the observed antitumor activity of various concentrations of different crude extracts of *R. longiflorus* are summarized in Table 3.

Both 95 % ethanol and watery extracts of *R. longiflorus* exhibited antitumor activity against *Agrobacterium tumefacien* at observation periods of Day-5 and Day-7 with the minimum concentration of 25 mg mL^{-1}in DMSO. The obtained results strongly validate the using of *R. longiflorus* in traditional medicine.

Table 3. Antitumor activity of different crude extracts of *R. longiflorus*

Samples	**Concentration of extracts (mg ml^{-1}) in DMSO**	**observation of tumor at**	
		Day-5	**Day-7**
EtOH extract of KG	25	-	-
	50	-	-
	75	-	-
Watery extract of KG	25	-	-
	50	-	-
	75	-	-

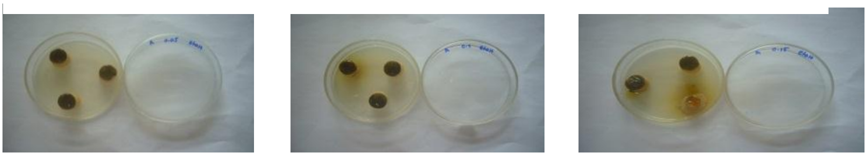
(-) tumor negative KG= *R. longiflorus*(Kyauk-gin)
(+) tumor positive



Watery extract of KG (25 mgmL^{-1}DMSO)

Watery extract of KG (50 mgmL^{-1}DMSO)

Watery extract of KG (75 mgmL^{-1}DMSO)



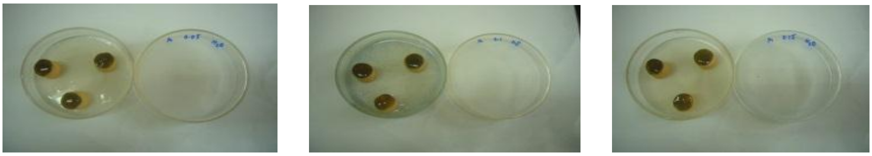
EtOH extract of KG (25 mgmL^{-1}DMSO)

EtOH extract of KG (50 mgmL^{-1}DMSO)

EtOH extract of KG (75 mgmL^{-1}DMSO)

KG= *R. longiflorus* (Kyauk-gin)

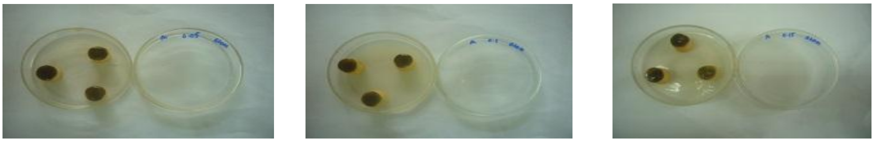
Figure 4. Photographs of screening antitumor activity of different concentrations of watery and EtOH extracts of *R. longiflorus* at day 5



Watery extract of KG (25 mgmL^{-1}DMSO)

Watery extract of KG (50 mgmL^{-1}DMSO)

Watery extract of KG (75 mgmL^{-1}DMSO)



EtOH extract of KG (25 mgmL^{-1}DMSO)

EtOH extract of KG (50 mgmL^{-1}DMSO)

EtOH extract of KG (75 mgmL^{-1}DMSO)

KG= *R. longiflorus* (Kyauk-gin)

Figure 5. Photographs of screening antitumor activity of different concentrations of watery and EtOH extracts of *R. longiflorus* at day 7

Conclusion

In this research work, the phytochemical constituents, antioxidant activity and antitumor activity of the rhizomes of *R. longiflorus* were investigated. According to the phytochemical screening, the rhizomes of *R. longiflorus* consisted of alkaloids, flavonoids, terpenoids, steroids, starch, saponins, reducing sugars, phenolic compounds, glycosides, tannin, carbohydrates and α-amino acids. According to the results of antioxidant activity examination, IC_{50} value of ethanol extract of *R. longiflorus* was found to have 119.42 μg/mL and was higher antioxidant activity than that of watery extract (IC_{50}=163.6 μg/mL). The results revealed that the sample contains phytoconstituents that have antioxidant activity and the potent antioxidant activity of the *R. longiflorus* may be efficient as preventive agents in many diseases.

The antitumor activity of 95 % EtOH and watery extracts of *R. longiflorus* was screened on *Agrobacterium tumefacien* by Potato Disc Assay method. Both the tested crude extracts of *R. longiflorus* exhibited antitumor activity against *Agrobacterium tumefacien* with the minimum concentration of 25 mg mL^{-1} at the observation periods of Day-5 and Day-7.

The present outcome of this research indicated that the rhizome of *R. longiflorus* exerted high antioxidant activity and antitumor potential.

Acknowledgements

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References

Cheng, I. N., C. Chang, C. Ng, C. Wang, Y. Shyu and T. Chang. (2008). "Antioxidant and Antimicrobial Activity of Zingiberaceae Plants in Taiwan", *Plant Food Hum Nutr*. **63**:15-20

Lobo, V., A. Patil, A. Phatak and N. Chandra. (2010). "Free Radicals, Antioxidants and Functional Foods: Impact on Human health", *PHCOG Rev.*

Melanathuru, V., S. Rengarajan, N. Thangavel. (2017). "Comparative Study of Antioxidant and Anticancer Activity of *Alpinia calcarata* and *Alpinia galangal*", International Journal of Pharmacy and Pharmaceutical Sciences, **9**(12):186-193

Nordvist, C. (2017). "What are the Different Types of Tumors?", *Medical News Today*

Prasanthkumar, M. G., J. Skornickova, M. Sabu and S. Jayasree. (2004). "Conservation Priority and Phytogeographical Significance of Rhynchanthus longiflorus Hook. f.(Zingiberaceae): A Rare, Endangered Species from Mizo Hills, NE India", Current Science, **88**(6):977-980

Zahara, M., M. Hasanah and R. Zalianda. (2018). "Identification of Zingiberaceae as Medicinal Plants in Gunung Cut Village, Aceh barat daya, Indonesia", *Journal of Tropical Horticulture*, **1**(1):24-28

Some Bioactivities of the Resin of *Ferula assafoetida* Linn. (Shein –ngo)

April Khin Chaw Chaw Su Kyi[1], Myint Myint Soe[2] & Myint Myint Khine[3]

Abstract

In the present study, investigation of some phytochemical constituents and some biological activities such as acute toxicity, antipyretic, antimicrobial, antioxidant activities has carried out on the resin of *Ferula assafoetida* L. (Shein-ngo). *In vitro*, antimicrobial activities of various crude extracts; PE, 98% ethanol and EtOAc extract were tested on strains of microorganisms such as *Bacillus substilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus pumilus*, *Escherichia coli* and *Candida albicans* by agar well diffusion method. When compared with all crude extracts ethyl acetate extract may posses higher antimicrobial potency than ethanol and petroleum ether extracts. Antioxidant activity of PE, ethanol and defatted EA (sesquiterpene coumarin-rich fraction) extract from Shein-ngo (resin) was determined by DPPH assay method. The IC_{50} value of PE, defatted EA (sesquiterpenecoumarin rich fraction) and ethanol extracts were >286 µg/mL, 561 µg/mL and 869 µg/mL respectively. Thus sesquiterpene coumarin-rich fraction and ethanol extracts of this plant showed the dose dependent antioxidant activity comparable to ascorbic acid, antioxidant might be developed from this plant.

Keywords: *F.assafoetida* L., Antimicrobial activity, Antioxidant activity

Introduction

Plants have been a constant source of drugs and recently, much emphasis has been placed on finding noval therapeutic agents from medicinal plants. Today many people prefer to use medicinal plants rather than chemical drugs. Ferula is a genus of about 170 species of flowering plants in the family Apiaceae, native to the Mediterranean region east to central Asia mostly growing in arid climates (Armand *et al*.,2012). *Ferula assafoetida*, is a species of Ferula grows in Iran, Afghanistan and Kashmir. It is a hard, resinous, oily herbaceous gum, belonging to the plant of the

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family Umbelliferae, also called Apiaceae. It is a perennial shrub that attains a height of 5 to 8 feet. Leaves are parted and dentate. Flowers are small and dirty yellow coloured produced in large compound umbels. It has a strong sulfurous unpleasant smell. The resin of the plant contains various resquiterpere coumarins and polysulfideshave been isolated from this plant. The old traditional phytomedicine asafoetida, an oleo-gum-resin obtained from the roots of different *Ferula assafoetida* L. is used in different countries for various purposes. Asafoetida is not only used as a culinary spice but also traditionally used to treat various diseases, including asthma, bronchitis, whooping cough, gastrointestinal disorders, intestinal parasites, etc. This oleo-gum-resin also has been known to possess antifungal, anti-diabetic, anti-inflammatory, anti-mutagenic and antiviral activities (Iranshahy and Iranshahi, 2010). They have been used for their perceived anthelmintic, anticarcinogenesis, anti-HIV, antimicrobial, antirheumatic, antispasmodic, diuretic and emmenagogue actions in folk medicine Sesquiterpene coumarin compounds of *Ferula assafoetida* L. have been demonstrated *in vitro* against the influenza A virus (H1N1). Asafoetida is a potent antioxidant and ferulic acid, a component of the resin, has shown promise as a chemopreventive agent, suggesting that asafoetida may offer some protection against carcinogenesis. *In vitro* studies have shown some cytotoxicity against lymphoma ascites, tumor cells and human lymphocytes (Kareparamban *et al*., 2012).

Botanical Aspects *of Ferula assafoetida* Linn.

Plant resin

Figure 1. Photographs of the plant and resin of *Ferula assafoetida* Linn.

Material and Methods

Sample Collection

The medicinal plant namely *Ferula assafoetida* L. (Shein-ngo) was chhosen to be studied. The resin of Shein-ngo was collected from the local market, Pabedan Township, Yangon, Myanmar. The resin was grounded into powder by ginding machine. These powder was stored in air tight container until used.

Preliminary phytochemical tests

A few grams of sample powder were subjected to the preliminary phytochemical test according to reported methods.

Preparation of different crude extracts from *Ferula assafoetida* L.

The resin of *Ferula assafoetida* L (1.5) kg was firstly extracted with petroleum ether (60-80) for 48 hrs and removed fat. The mixture was filtered and the petroleum layer was evaporated in rotary evaporator to make complete dryness that provided brown residue (17.34)g.The remaining marc was again extracted with ethyl acetate(2L×3times) for two days and filtered.. Then all filtrate (ethyl acetate layer) was combined and concentrated under reduced pressure by using rotary evaporator yellowish brown residue (ethyl acetate extract) 1.93g was obtained.

Screening of Bioactivities

In this section, screening of bioactivities of the selected plant (the resin of Shein-ngo) was performed including acute antimicrobial activity, *in vitro* antioxidant activity and *in vivo* antipyretic activity.

Materials

Plant materials

Samples used in this study were various crude extracts of *Ferula assafoetida* L. (Shein-ngo).

Antimicrobial activity

Test organisms: *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Escherichia coli* and *Candida albicans*

Test extracts: PE, EtOAc and 98 % ethanolic extracts

Antioxidant activity

Test Extracts: PE, defatted EtOAc, EtOH

Antimicrobial activity of *Ferula assafoetida* L.

Antimicrobial activity was screened by agar well diffusion method in CRDC, Yangon.

For the examination of *in vitro* antimicrobial activity, crude extracts such as PE, EtOAc, and 98 % EtOH of plant sample were prepared by using various solvents.

(a) Preparation of inoculums

The microorganisms used in the investigation of antimicrobial activity were *Bacillius subtilis, Bacillius pumilus, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Candida albicans.*

A few colonies of the organisms to be tested were incubated at 37 °C for 24 hr in an incubator. These organisms were introduced into the trypticase soy broth and incubated for 3 hrs at 37 °C to obtain the bacterial suspension of moderated cloudiness.

(b) Preparation of nutrient agar medium

Three grams of agar powder, 1 g of sodium chloride, 1 g of peptone, 0.4 g of yeast extract and 200 mL of distilled water were mixed in a 500 mL sterile conical flask and the contents were heated on a hot plate for 30 min. The mouth of the flask was covered with a piece of aluminum foil. This medium was adjusted to pH 7.4 with 0.1 M sodium hydroxide solution and was sterilized in an autoclave at 121 °C for 30 min (Nicklin *et al.,* 1999).

After autoclaving, half of this medium (100 mL) was dispensed, 5 mL each, into sterile test tubes inclined at about 15° from the horizontal position for the subculture of bacteria and the remaining half of this medium (100 mL) was used for the culture of bacteria.

(c) Procedure

The agar well disc diffusion method was used to test the antibacterial action of the extracts on 24 hours broth culture of the organisms used.

The extracts of PE, EtOAc, and EtOH (1 mg each) were dissolved in 1cm^3 of their respective solvent. 1mL each of the bacterial suspension of 24 hr of nutrient agar was streaked evenly onto the surface of trypticase soy agar plates with sterile cotton swab. Immediately after hardening of the agar well were made with a 7 mm sterile cork borer from each seeded 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 hr. The diameters of the inhibition zone were measured and recorded in mm.

Radical scavenging activity by DPPH assay method

(a) Extraction of plant

The resin of *Ferula assafoetida* L. was air dried, powered and 100 gm of the powdered plant material was extracted by maceration using petroleum ether, ethylacetate and ethanol. The ethyl acetate extract was then defatted by mixing with petroleum ether and removing the petroleum ether fraction. Finally, petroleum ether, defatted ethylacetate and ethanol portions were evaporated by vacuum evaporator at temperature below 50 °C to obtain the dried powder.

(b) Preparation of DPPH stock solution

The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method (Braca *et al*., 2002). 3.94 mg of DPPH powder and 100 mL of 98 % ethanol (100 μM) were thoroughly mixed by vortex mixer. This solution was freshly prepared in a brown coloured flask.

(c) Procedure

The diluted working solutions of the test extracts were prepared in ethanol. Ascorbic acid was used as standard in 0.3 mg/mL, 0.15 mg/mL, 0.075mg/mL, 0.0375 mg/mL, 0.0187 mg/mL, 0.0093 mg/mL solutions. The stock solutions of PE, defatted EA, and EtOH crude extracts prepared in 98% ethanol were 2.86 mg/mL, 2.13 mg/mL, 5.4 mg/mL repectively. The working solutions of the extracts were prepared from the stock solutions using double dilution. 1.5 ml of DPPH solution (100 μM) was mixed with 1.5 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV/Vis spectrophotometer. Ethanol (1.5 mL) with DPPH solution (100 μM, 1.5 mL) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below (Bors *et al*., 1992).

$$\%\ \text{Inhibition of oxidation} = \frac{\text{DPPH alone - (Sample - Blank) solution}}{\text{DPPH alone}} \times 100$$

Average, $\overline{X} = \dfrac{x_1 + x_2 + ..,... + x_n}{n}$

$\overline{X}$ = average % inhibition of oxidation

$x_1 + x_2 + ..,... + x_n$ = % inhibition of oxidation for test sample

n = number of times

Then IC_{50} (50 % inhibitory concentration) values were also calculated by linear regressive excel program.

Chemical evaluation on bioactive extracts of the resins of Shein-ngo

Most of drugs have definite chemical constituents to which their biological or pharmacological activity is attributed. Qualitative chemical tests are used to identify certain drug or to test their purity. Therefore the bioactive extracts were analysed by phytochemical screening, TLC, spectroscopic determination to know the types of chemical constituents.

Result and Discussion

Phytoconstituents present in *Ferula assafoetida* L. (resin)

According to the phytochemical result, the resin of *F.assafoetida* **(**Shein-ngo) generally contains alkaloids, α- amino acids, carbohydrates, flavonoids, saponins, carbohydrate, steroids, and terpenoids were found in the sample.

Phytochemical screening of plant extracts by TLC method

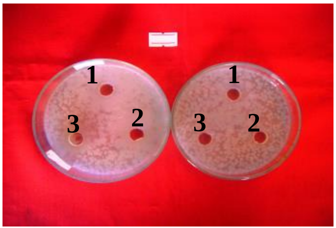
The phytochemical constituents of pet-ether, ethyl acetate and ethanol extracts were analyzed by TLC screening method using detecting reagents. These TLC results also confirmed the presence of steroids, terpenoids, essential oils, phenolic compounds, and coumarins in sample. Sesquiterpenes coumarins and glycerides are major constituents.

Screening of antimicrobial activity of the extracts of resins by agar well diffusion method

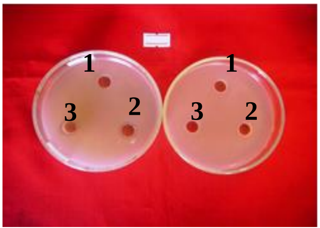
Screening of antimicrobial activity of plant extracts was carried out on different strains of microorganisms by agar well diffusion method. The measurable zone diameter of growth inhibition reflects the degree of antimicrobial activities. The PE, defatted EtOAc and EtOH extracts were investigated on 6 strains microorganisms of which include *Bacillus subtilis, Staphylococcus aureus, Pesudomonas aeruginosa, Bacillus pumilus, Escherichia coli and Candida albicans* by agar well diffusion method.

The results of antimicrobial activities on three crude extracts of sample tested against selected organisms are presented in Table 1.In Figures 2 and 3**,** defatted ethyl acetate and ethanol extracts of *Ferula assafoetida* L. showed antimicrobial activity against all strains with inhibition zone diameter (13-15 mm) except *Bacillus subtilis*, whereas petroleum ether extract showed no antimicrobial activity against all six strains species.

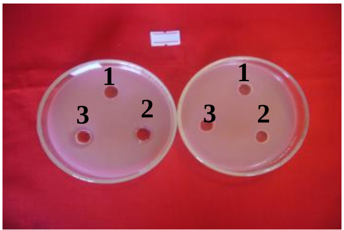
When compared with all crude extracts of resin from *Ferula assafoetida* L. ethyl acetate extract showed more significant zone of inhibition except *Bacillus subtilis*. Therefore, ethyl acetate extract may possess higher antimicrobial potency than ethanol and petroleum ether extracts. According to phytochemical screening and isolation, ethylacetate extract contained umbelliferone coumarin and sesquiterpene coumarins such as galbanic acid, conferol, feselol and ferulsinaic acid. Umbelliferone has antifungal and antibacterial activity (Harborne *et al*., 1993). Sesquiterpene coumarins have weak antibacterial activity.Thus umbelliferone and sesquiterpene coumarins of *Ferula assafoetida* L. can be referred to be responsible for the antibacterial activity of ethylacetate and ethanol extracts.



Sample Control
Bacillus subtilis



Sample Control
Staphylococcus aureus



Sample Control
Pseudomonas aeruginosa

Sample Control
Bacillus pulmalis

Sample Control
Candida albicans

Sample Control
Escherichia coli

Clockwise rotation 1 = PE, 2 = EtOH, 3 = EtOAc

Figure 2. Antimicrobial activity screening of different crude extracts from *F. assafoetida* Linn. (Resin) by agar well diffusion method

Table 1. Results of Antimicrobial Activity Screening on Various Crude Extracts from *F. assafoetida* L. (Resins) by Agar Well Diffusion Method

Type of Microorganisms	**Diameter of Inhibition zone (mm)**		
	PE	**EtOAc**	**EtOH**
Bacillus subtilis	-	-	-
Staphylococcusaureus	-	13	14
Pseudomonas aeruginosa	-	15	13
Bacillus pumilus	-	15	13
Candida albican	-	14	14
Escherichia coli	-	14	13

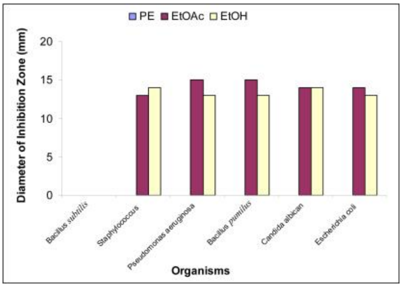


Figure 3. Histogram of inhibition zone diameter of different extracts of *F. assafoetida* L. (Shein-ngo)

Diameter of agar well = 10 mm
10 ~ 14 mm (+) 15 ~ 19 mm (++)
20 mm above (+++)

The antioxidant activity of *Ferula assafoetida* L. (Shein-ngo) by DPPH

The defatted EA (sesquiterpenecoumarin-rich fraction) and ethanol extracts were found to have antioxidant property in dose dependent manner as shown in Table 2. The IC_{50} value of PE, sesquiterpenecoumarins-rich fraction and ethanol extract were found to be > 2.86 mg/mL, 0.561 mg/mL (561 μg/mL) and 0.869 mg/mL (869 μg/mL) respectively and that of ascorbic acid was 0.044 mg/mL (44 µg/mL) from regression equation. The sesquiterpenecoumarin fraction showed more potent antioxidant activity than ethanol extract. The UV/Vis spectra showing antioxidant activity are shown in Figure 4. In UV/Vis spectra for the antioxidant activity of defatted EA and ethanol extracts, the violet colour of DPPH faints into the yellow colour of its reduced congener (DPPH-H), with a high λ-shift in the visible spectra (from 517 nm to 330 nm).

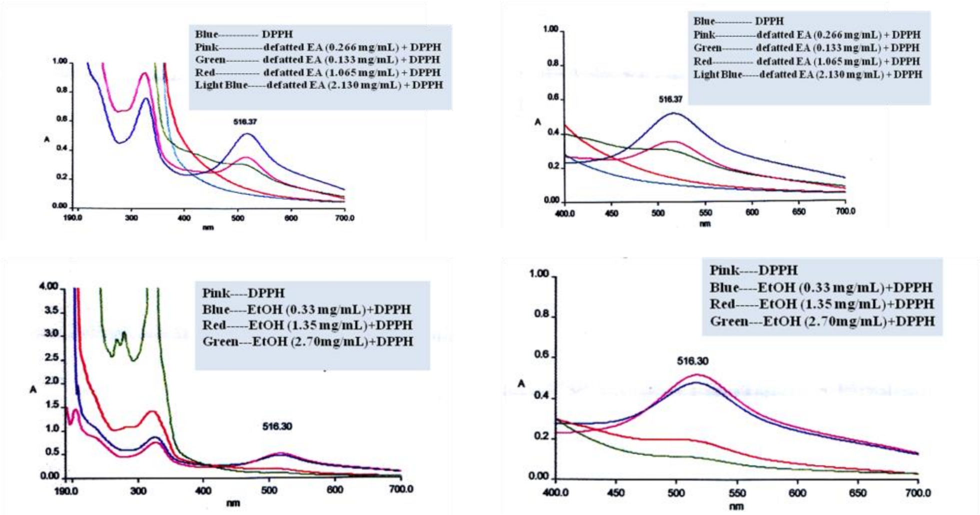


Figure 4. The changing of antioxidant activity by UV/vis

Table 2. DPPH Free Radical Scanvenging Activity of Various Extracts of *F. assafoetida* Linn. (Shein-ngo)

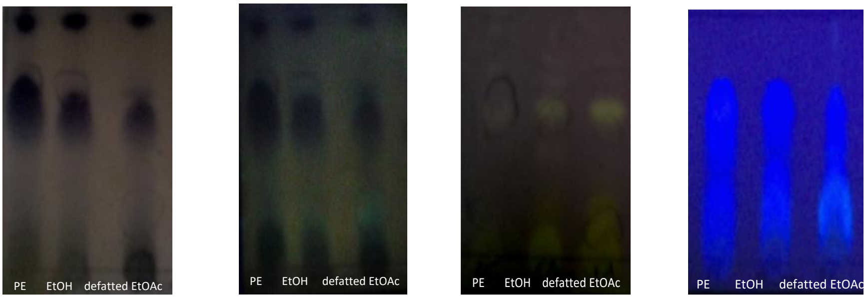
Extract	**Concentration (mg/mL)**	**% Inhibition ± SD**	**IC_{50} (mg/mL)**
EtOH	2.7	99.9863 ± 0.0007	0.869
	1.35	64.8743 ± 0.001	
	0.675	44.6128 ± 0.0007	
	0.337	35.5963 ± 0.0006	
	0.168	29.4338 ± 0.0005	

Extract	Concentration (mg/mL)	% Inhibition ± SD	IC_{50} (mg/mL)
EA (PE wash)	1.065	93.0551 ± 0.0006	0.561
	0.532	48.3677 ± 0.001	
	0.266	24.2136 ± 0.0010	
	0.133	12.278 ± 0.0007	
	0.066	8.00096 ± 0.0006	
PE	2.86	46.421361 ± 0.0003	> 2.86
	1.43	25.623738 ± 0.0004	
	0.715	20.763341 ± 0.0003	
Vitamin C	0.3	95.458 ± 0.0006	0.044
	0.15	69.98861 ± 0.0004	
	0.075	56.55281 ± 0.0009	
	0.0375	48.13964 ± 0.0003	
	0.0187	45.72407 ± 0.0002	
	0.0093	42.77427 ± 0.0002	

Chemical evaluation on bioactive extracts of Shein-ngo

Based on the investigation of bioactivities, defatted ethyl acetate extract and ethanol extract exhibited the antibacterial activity and antioxidant activity. To highlight the type of chemical components in bioactive extracts, the chemical evaluation of defatted ethyl acetate and ethanol extracts was carried out by means of TLC analysis in Figure 5 and UV/vis spectroscopy in Figures 6 and 7. The UV/vis spectral data and R_f values are listed in Tables 3 and 4.

The free radicals are produced in aerobic cells due to consumption of oxygen in cell growth (Barros *et al*., 2007). These free radicals are involved in different disorders like ageing, cancer, cardiovascular disease, diabetes, rheumatoid arthritis, epilepsy and degradation of essential fatty acids. Antioxidant helps in treatment of above disorders. As sesquiterpenecoumarin rich fraction and ethanol extracts of this plant showed the dose dependent antioxidant activity comparable to ascorbic acid, antioxidant agent might be developed from this plant for the treatment of above disorders associated with free radicals. Phenolic compound containing free hydrogen such as umbelliferone and sesquiterpene coumarins are largely responsible for antioxidant activity as well as antipyrectic activity of *Ferula assafoetida* L.



PE : EA = 7 :1 (After dipping in 1% Vanillin + H_2SO_4, Δ)

PE : EA = 7 :1 (After dipping in 1% Vanillin + H_2SO_4, Δ, 10% KOH, under UV_{365})

PE : EA = 7 :1 (After dipping in DPPH)

PE : EA = 7 :1 (under UV_{365})

Figure 5. Thin layer chromatograms for bioactive extracts of *F. assafoetida* Linn. (Shein-ngo)

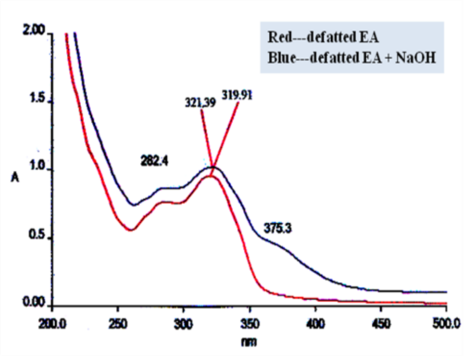


Figure 6. UV-vis spectrum of bioactive defatted EA extract of *Ferula assafoetida.* (Shein-ngo)

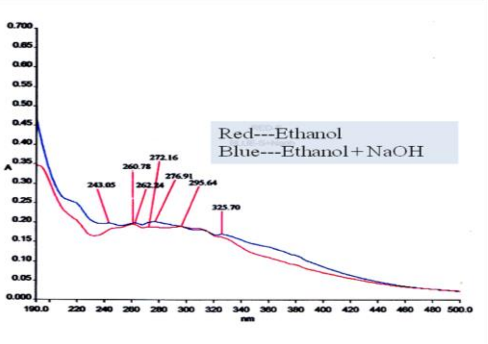


Figure 7. UV-vis spectrum of bioactive EtOH extract of *Ferula assafoetida.*(Shein-ngo)

Table 3. Chemical Evaluation of Bioactive Extracts of *Ferula assafoetida* Linn.by UV/vis

Extract	**Solvent**	**λ_{max}(nm)**		**Transition**	**Remark**
		Observed	**Reference**		
Defatted EA	MeOH	282,319		π-π*transition n-π* transition	Presence of a coumarin nucleus oxygenated at the C-7 position
	MeOH/ NaOH	282,321, 375	321		Presence of phenolic OH group
Ethanol	MeOH	260 272 295 325	289 321	π-π*transition n-π*	Presence of a coumarin nucleus oxygenate

Extract	Solvent	λ_{max}(nm)		Transition	Remark
		Observed	Reference		
				transition	d at the C-7 position
	NaOH/ MeOH	260 272 295 325		π-π*transition n-π* transition	not free 7-hydroxy group in coumarin nucleus

Table 4. Chemical Evaluation of Bioactive Extracts of *Ferula assafoetida.* L. (Shein-ngo)

	R_f value (PE:EA=7:1)					
Extract	**UV light**		**1%Vanillin/ H_2SO_4**	**1%$FeCl_3$**	**1%Vanillin / 10% ethanolic KOH/UV_{365}**	**DPPH**
	254 nm	**366 nm**				
PE	N.D	0.69	0.93, 0.62,	N.D	-	0.56
Defatted EA	N.D	0.69, 0.18	0.93, 0.51	N.D	0.51	0.51 0.18
EtOH	N.D	0.69	0.93, 0.51, 0.075	N.D	0.51	0.51

Conclusion

From the overall assessment for the present work concerning with the chemical and bioactivity investigation on the resin of *Ferula assafoetida* L. the following inference could be deduced. In biological investigation, Shein-ngo (resin) was found to possess a variety of pharmacological activities such as antipyretic, antimicrobial and antioxidant activities. *In* *vitro* screening of antimicrobial activity by agar well diffusion method, EtOAc and 98 % EtOH extracts of *F. assafoetida* L. showed antimicrobial activity on six strains of crude microorganisms (inhibition zone diameter 12-15 mm). According to this results, ethyl acetate extract showed more significant zone of inhibition than ethanol extract. Screening of in *vitro* antioxidant activity of PE, sesquiterpenecoumarin-rich fraction and ethanol extract was done by using DPPH assay method. The IC_{50} value of PE, sesquiterpenecoumarins-rich fraction and ethanol extract were found to be > 2.86 mg/mL, 0.561 mg/mL (561 μg/mL) and 869 μg/mL respectively and that of ascorbic acid was 44 μg/ml from regression equation. The sesquiterpenecoumarin-rich fraction showed more potent antioxidant activity than ethanol extract. A number of pharmacological studies conducted on the species *Ferula foetida* revealed that it poses a number of activities such as antioxidant, antiulcer, hepatoprotective, antimicrobial, antifungal but some of the recent studies has revealed that it poses antiviral activity which is active against influenza A (H1N1) virus.

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References

Armand,N. and Jaahantab,E.,(2012), "Ferula Assafoetida, avaluable medicinal plant to correct and cultivate rangelands in Boyer Ahmad City"," *International Journal of Agriculture and Crop Sciences*",**4**(21),1596-1598

Barros, L., Ferreira, M.J., Queiros, B., Ferreira I.C.F.R. and Baptista, P., (2007), "Total Phenols, Ascorbic acid, β-carotene and Lycopene in Portuguese Wild Edible Mushrooms and Their Antioxidant Activities", *Food Chemistry*, **103**, 413

Bors, W., Saran, M. and Elstner, E.F., (1992), "Screening of Plant Anti-oxidants. In: Linskens H. F., Jackson, J. F., eds. Modern Methods of Plant Analysis-Plant Toxin Analysis-New Series, Springer, Berlin, **13,** 277-295

Braca, A., Sortino, C. and Politi, M., (2002), "Anti-oxidant Activity of Flavonoids from *Licania licaniaeflora*", *J. Ethnopharmacol.,* **79**, 379-381

Harborne, J.B. and Baxtr, H., (1993), "A Handbook of Bioactive Dictionary", Taylor and Francis Ltd, London, 365.

Iranshahy, M., and Iranshahi, M. (2010),"Traditional uses, phytochemistry and pharmacology of asafetida (Ferula assa-foetida oleo-gum-resin)-A review", *Journal of Ethnopharmacol*, **3**, 5-8

Kareparamban, J. A., Nikam, P. H., Jadhav, A. P., and Kadam, V. J. (2012),"*Ferula foetida*"Hing"; A Review", *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **3** (2), 775-786

Nicklin, J., Gracme Cook, K., Paget, T. and Killington, R.A., (1999), "Instant Notes in Microbiology", 1st Edition, Viva Books Private Limited

Universities Research Journal 2019, Vol. 12, No. 1

Study on Biological Activities and Antioxidant Activity of *Citrus sinensis* L. (Orange Peels)

Htay Htay Khaing [1] & May Myat Nwe[2]

Abstract

As the peels of *Citrus sinensis* L. (Orange) have a long history of medicinal use, this research deals with the investigation of some chemical constituents and screening of antimicrobial and antioxidant activities on the peels of *Citrus sinensis* L. (Orange). Preliminary phytochemical tests revealed that the secondary metabolites such as alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, tannins, steroids and terpenoids were found to be present but starch was not detected in the orange peels. The elemental analysis of peels determined by ED-XRF spectrometer indicated that the highest abundance percentage of K (0.858 %) and Ca (0.668 %) for peels. Nutritional values determined by AOAC method indicated that the percentage of moisture, ash, fiber, protein, fat and carbohydrate were 19.73, 2.98, 11.61, 7.26, 1.56 and 56.86 for peels with the energy values of 274 kcal/100 g. Agar well diffusion method was used to determine in the *vitro* antimicrobial activity on the different extracts such as EtOAc, PE, EtOH and water extracts. It was found that the EtOH extract of orange peels exhibited the highest activity (12-21) mm against *Bacillus subtilis, Bacillus pumilus, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and Candida albicans*. Antioxidant activity of orange peels was also investigated by using DPPH radical scavenging assay. The IC_{50} values of EtOH and water extracts from orange peels were observed to be 14.4 and 16.6 $\mu g\ mL^{-1}$, respectively. The EtOH extracts are more effective than water extracts. Besides, terpene (0.7 %) and terpenoidal flavanones (0.8 % and 0.4 %) may be isolated from ethanol extract of peels. These isolated compounds were characterized by TLC, chemical tests, UV and FT IR spectroscopic techniques. The present work reveals that orange peels may be used as food source as well as medicine for the treatment of diseases related to bacterial infections.

Keywords : *Citrus sinensis* Linn peels, antioxidant Activity

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Introduction

The World Health Organization (WHO) estimates that about 80% of the population still depends upon herbal medicines for the treatment of various diseases due to easy availability, economic reasons and less side effects. *Citrus sinensis* peel has many medicinal properties and is widely used against various ailments, such as colic, upset stomach, cancer, diuretic cormunative, immune-enhancing, stomachic, tonic to digestive system, immune system and skin. Periodontal disease is one of the most common dental health problems in the human communities. Several oral microorganisms have been studied as period on to pathogens. It is also used to treat and prevent vitamin deficiencies, colds, flu and scurvy and helping to fight viral and bacterial infections. Antibacterial effects of orange peel have demonstrated in the literature (Lawal, 2013). The peels obtained from *Citrus* fruits constitute between 50 and 65% of the total weight of the fruits. When not processed further, this by-product becomes a very worrisome waste capable of causing serious environmental pollution (Hegazy, 2012).

Materials and Methods

The medicinal plant of orange (*Citrus sinensis* L.) peels were chosen to be studied in the present research because orange peels have antibacterial and antioxidant activities. The sample of orange (*Citrus sinensis* L.) was identified in Department of Botany, University of Yangon, Myanmar. The collected oranges sample was removed peels which cleaned by washing thoroughly with water and air-dried at room temperature. The dried samples were cut into small pieces and ground into powder by a grinding machine. This powder sample was obtained and stored in air-tight container. The dried powdered sample was used to investigate for their chemical and biological activities such as phytochemical constituents were determined by test tube method. Nutritional values such as moisture content, ash content, fat content, fibre content, protein content, carbohydrate content and energy value of the selected sample were determined by AOAC method (AOAC, 2000). Relative abundance of element in peel of orange by ED-XRF spectrometry. The column chromatographic method was used for isolation of compounds from the extract of EtOH. The antimicrobial activity of four crude extracts as petroleum ether, ethyl acetate, ethanol and water from the peels of orange was determined against six strains of microorganisms such as *Bacillus subtilis*, *Bacillus pumilus*, *Staphylococcus aureus*, *Pseydomonas aeruginosa*, *Escherichia coli* and *Candida albicans* by employing agar well diffusion method. The antioxidant activity of EtOH and H_2O crude extracts were by studied by DPPH assay method.

Results and Discussion

Preliminary Phytochemical Investigation of Peels of Orange

The phytochemical investigation of orange peels indicated the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, tannins, steroids and terpenoids were found to be present but starch was not detected, in the orange peels.

Nutritional Value of Peels of Orange

Table 1. Some Nutritional Values of Orange Peels Sample

Sr. No.	Test parameter	FIDSL Result (%)
1	Moisture	19.73
2.	Ash	2.98
3.	Crude Protein	7.26
4.	Crude Fiber	11.61
5.	Crude Fat	1.56
6.	Carbohydrate	56.86
7.	Energy value (kcal/100 g)	274

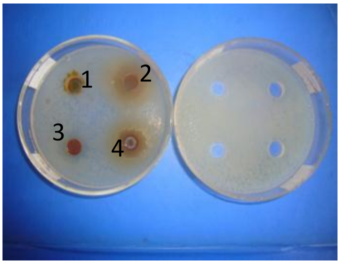
Elemental Analysis in Peels of Orange by Energy Dispersive X-ray Fluorescence ED-XRF Spectrometry

Table 2. Relative Abundance of Elements in *Citrus sinensis* L. (Orange Peels) (ED-XRF)

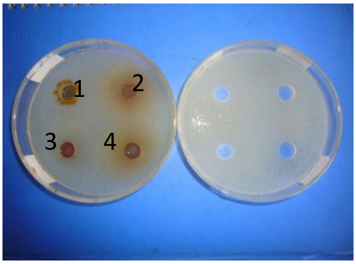
No.	Element	Relative abundance (%)
1	K	0.858
2	Ca	0.668
3	S	0.131
4	Fe	0.004
5	Mn	0.003
6	Sr	0.002
7	Cu	0.001
8	COH	98.332

Antimicrobial Activity of Various Crude Extracts of Peels of Orange

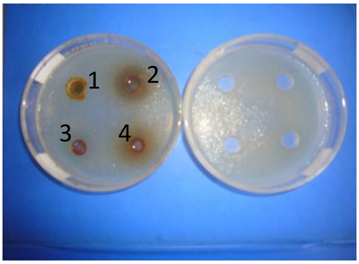
Agar well diffusion method was used to determine in vitro antimicrobial activity on the different extracts such as EtOAc, PE, EtOH and water extracts. It was found that the EtOH extract of orange peels exhibited the highest activity (12-21 mm) against *Bacillus subtilis*, *Bacillus pumilus*, *Staphylococcus aureus*, *Pseydomonas aeruginosa*, *Escherichia coli* and *Candida albicans.* These results are showed in Table3, Fig. 1.



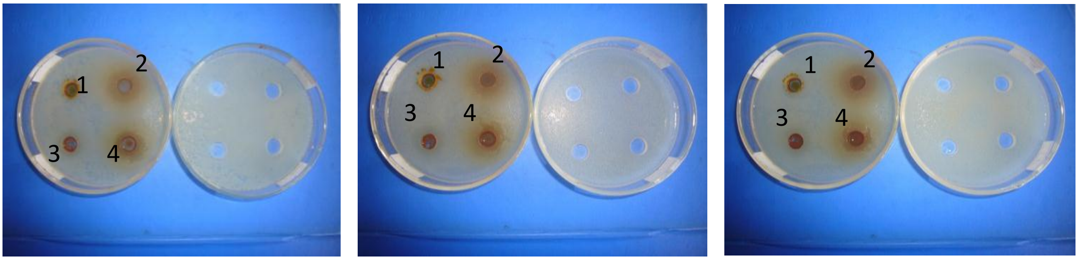
Bacillus subtilis



Staphylococcus aureus



Pseudomonas aeruginosa



Bacillus pumilus *Candida albicans* *Escherichia coli*

1 = PE extract

2 = Water extract

3 = EtOAc extract

4 = EtOH extract

Figure 1. Antimicrobial activity screening of *Citrus sinensis* L. (Orange Peels)

Table 3. Antimicrobial Activity of *Citrus sinensis* L. (Orange Peels) Against Six Microorganisms

Organisms	**Inhibition Zone Diameter of Extracts (mm)**			
	PE	**EtOAc**	**EtOH**	**H_2O**
Bacillus subtilis	13(+)	12(+)	15(++)	11(+)
Bacillus pumilus	11(+)	17(++)	18(++)	11(+)
Staphylococcus aureus	13(+)	16(++)	12(+)	11(+)
Pseudomonas aeruginosa	12(+)	17(++)	21(+++)	20(+++)
Escherichia coli	-	-	18(++)	11(+)
Candida albicans	11(+)	13(+)	18(++)	11(+)

Diameter of agar well = 10 mm

10mm ~ 14 mm = (+) (low)

15mm ~ 19 mm = (++) (medium)

20mm above = (+++) (high)

No activity = (-)

Antioxidant Activity of Crude Extracts of Peels of Orange

The antioxidant activity was studied on 95% ethanol and water extracts by using DPPH assay according to the spectrophotometric method. In this experiment, six different concentrations (200 µgmL^{-1} and 100 6.25 µgmL^{-1}) of each crude extract in 95% ethanol solvent were used. Ascorbic acid was used as standard and ethanol without crude extract as employed as control. The IC$_{50}$ values of EtOH and water extracts from orange peels were observed to be 14.4 and 16.6 µgmL^{-1} , respectively. The EtOH extracts are more effective than water extracts. These results are showed in Fig. 2, 3 and Table 4.

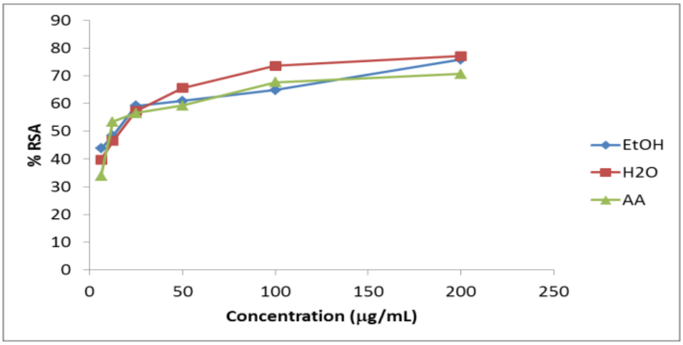


Figure 2. A plot of % RSA Vs concentration of two crude extracts from orange peels and standard ascorbic acid on antioxidant activity

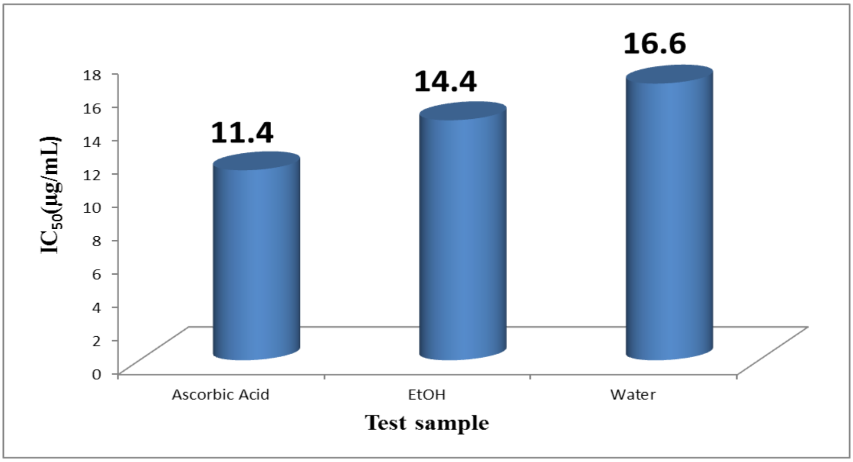


Figure 3. IC$_{50}$ values of crude extracts from orange peels and standard ascorbic acid

Table 4. %RSA of Various Concentrations and IC$_{50}$ Values of Two Crude Extracts from Orange Peels and Standard Ascorbic Acid on Antioxidant Activity

Test samples	**% RSA ± SD in Different Concentration (µg/mL)**						**IC$_{50}$ (µg/mL)**
	6.25	12.5	25	50	100	200	
Water extract	39.75 ± 0.99	46.49 ± 2.98	57.3 ± 1.19	65.59 ± 0.60	73.6 ± 1.38	77.11 ± 0.60	16.6
EtOH extract	43.8 ± 1.58	48.37 ± 0.86	59.15 ± 0.57	60.87 ± 0.14	64.84 ± 0.86	75.91 ± 1.58	14.4
Standard Ascorbic Acid	33.91 ± 1.99	53.44 ± 0.88	56.56 ± 1.33	59.22 ± 1.55	67.66 ± 0.22	70.63 ± 0.44	11.4

Isolation of Compounds from Orange Peels

4 g of ethanol extract was subjected to silicagel column chromatography and solvent system such as (PE:EtOAc, 99:1, 49:1, 29:1, 19:1, 9:1, 7:1, 5:1, 4:1, 3:1, 2:1, 1:1, EtOAc only, EtOAc:MeOH, 19:1, 9:1, 7:1, 5:1, 3:1,2:1, 1:1) and MeOH only. The collected fractions were checked by TLC using Liberman Burchard, 5% Sulphuric acid and 5%FeCl$_3$ as spraying reagents. The fractions that showed similar TLC patterns were combined together and concentrated. In this way, eight major fractions, fractions F$_1$ to F$_8$ were obtained. Fraction F$_2$ (Compound A), fraction F$_5$ (Compound B) and, fraction F$_7$ (Compound C) were obtained after removal of the solvent. The Compound A obtained as a white needle shape crystal was crystallized with CHCl$_3$ to give a white needle shape crystal (0.5 g, 0.7 %). The compound B obtained as a pale yellow needle shape crystal was crystallized with CHCL$_3$ to give a pale yellow needle shape crystal (0.6 g, 0.8%). And then, the Compound C obtained as a white needle shape crystal was crystallized with CHCL$_3$ to give a white needle

shape crystal (0.3g, 0.4%). Isolated compounds A, B, and C were shown in Figure 4.

(a) compound A (b) compound B (c) Compound C

Figure 4. Isolated compounds A, B and C

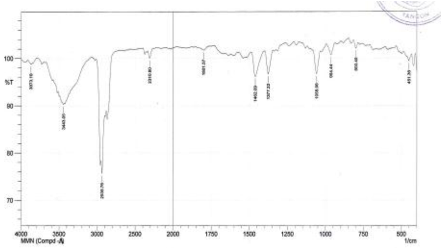


Figure 5. FT IR spectrum of isolated compound A (in KBr)

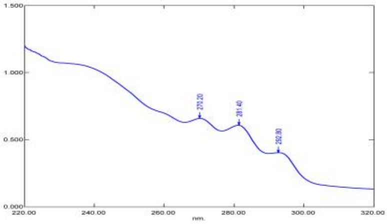


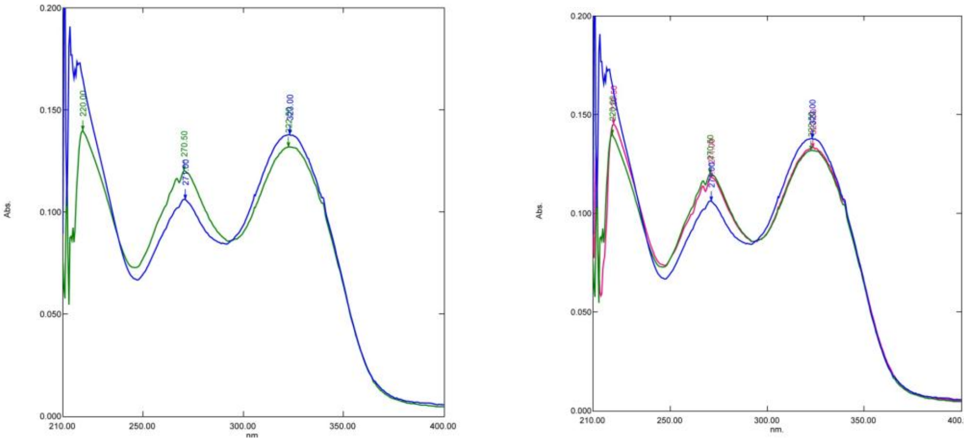
Figure 6. UV visible spectra of isolated compound A + MeOH

Table 5. FT IR Spectrum Data of Isolated Compound A

Wave number/cm^{-1}	**Band assignment**
3443	$\nu_{O\text{-}H}$ of hydroxyl group
2936	$\nu_{C\text{-}H}$ of CH_2 and CH_3
1462	$\delta_{C\text{-}H}$ of CH_2 and CH_3
1058	$\nu_{C\text{-}OH}$ of alcohol

Table 6. UV visible Spectra Data of Observed Isolated Compound A

Type of compound	**Solvent**	**λ_{max} (nm)**				**Interpretation**
		Band I		**Band II**		
		Observed	**Reported***	**Observed**	**Reported***	
Compound A	MeOH	270	270	281	280	Terpenoid



Compound B + MeOH
Compound B + MeOH + NaOH

Compound B + MeOH
Compound B + MeOH + NaOH
Compound B + MeOH + ALCL$_3$

Figure 7. UV visible spectra of isolated compound B

Table 7. UV Visible Spectra Data of Observed Isolated Compound B with Different Shift Reagents

Type of Compound	**Solvent**	**l_{max} (nm)**		**Interpretation**
		Band I	**Band II**	
Compound B	MeOH	323	271	Flavonoid
	MeOH+NaOH	322	270	no free phenolic OH
	MeOH+ALCL$_3$	323	270	no o-diOH or no free 5–OH or 3–OH

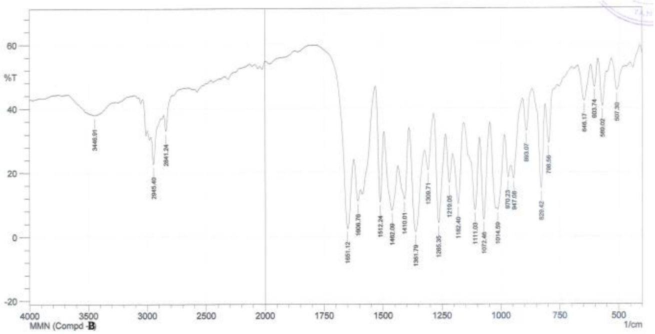
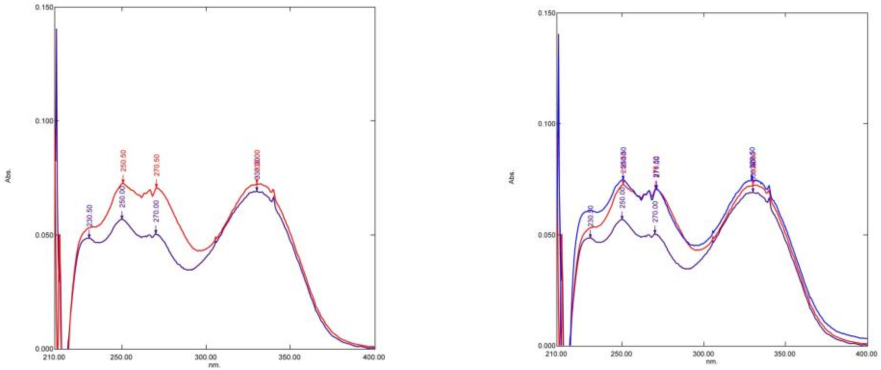


Figure 8. FT IR spectrum of isolated compound B (in KBr)

Table 8. FT IR Spectrum Data of Isolated Compound B

Wave number/cm^{-1}	**Band assignment**
3446	ν_{O-H} of hydroyl group
2945-2841	ν_{C-H} of CH_2 and CH_3
1651	$\nu_{C=C}$ of aromatic ring
1462	δ_{C-H} of CH_2 and CH_3
1072-829	δ_{C-H} of methyl group



Compound C + MeOH
Compound C + MeOH + NaOH

Compound C + MeOH
Compound C + MeOH + NaOH
Compound C + MeOH + $ALCL_3$

Figure 9. UV visible spectra of isolated compound C

Table 9. UV Visible Spectra Data of Observed Isolated Compound C with Different Shift Reagents

Type of Compound	**Solvent**	**λ_{max} (nm)**		**Interpretation**
		Band I	**Band II**	
Compound C	MeOH	330	270	Flavonoid
	MeOH+NaOH	329	270	no free phenolic OH
	MeOH+$ALCL_3$	330	270	no o-diOH or no free 5–OH or 3–OH

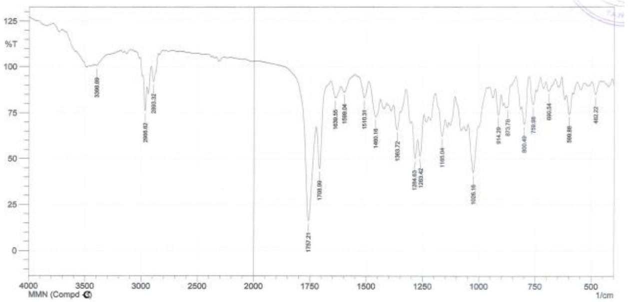


Figure 10. FT IR spectrum of isolated compound C (in KBr)

Table 10. FT IR Spectrum Data of Isolated Compound C

Wave number/cm^{-1}	**Band assignment**
3398	ν_{O-H} of hydroxyl group
2966-2893	ν_{C-H} of CH_2 and CH_3
1751-1708	$\nu_{C=C}$ of aromatic ring
1264-1263	ν_{C-O} of phenolic group
1026-914	δ_{C-H} of methyl group

Conclusion

The phytochemical test revals that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, tannins, steroids and terpenoids are present but starch is absent. Nutritional values determined by AOAC method indicated that the percentage of moisture, ash, fiber, protein, fat and carbohydrates were respectively to be 19.73, 2.98. 11.61, 7.26, 1.56 and 56.86% for peels with the energy values of 274 kcal/100 g. Elemental analysis of plant sample by ED-XRF method revealed the presence of K (0.858%), Ca (0.668%), S (0.131%), Fe (0.004%), Mn (0.003 %), Cu (0.001%) and COH(98.332%).

Agar well diffusion method was used to determine in vitro antimicrobial activity on the different extracts such as EtOAc, PE, EtOH and water extracts. It was found that the EtOH extract of orange peels exhibited the highest activity (12-21 mm) against *Bacillus subtilis*, *Bacillus pumilus*, *Staphylococcus aureus*, *Pseydomonas aeruginosa*, *Escherichia coli* and *Candida albicans.* Antipxidant activity of orange peels was also investigated by using DPPH radical scavenging assay. The IC_{50} values EtOH and water extracts from orange peels were observed to be 14.4 and 26.6 μgmL^{-1}, respectively. The EtOH extracts are more effective than water extracts. The three compounds A,B and C (Yield percent 0.7%,0.8% and 0.4%) were also isolated from ethanol extract of orange peels. These isolated compounds were characterized by chemical tests, TLC, UV and FTIR spectroscopic techniques. Isolated compound A may be terpene and two isolated compounds B and C may be terpenoidal flavanones. The present work reveals that orange peels may be used as food source as well as medicine related to bacterial infections.

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References

Anderson J. (1984). "Effect of Dietary Carbohydrate and Fiber on Tilapia *Oleochromis ninoticus* Linn.". *Aquaculture*, **4**, 303-314

Anthana, A. and C. Paniker. (1983). *Text Book of Microbiology*, New Delhi: Orient Longman Ltd., 584

A.O.A.C. (2000). *Official and Tentative Methods of Analysis, Association of Official Analytical Chemist*, Washington, D.C: 17th Ed., 526-530

Atwater, W.O. and C.D. Woods. (1907). *The Chemical Composition of American Food Material*. Washington D.C: US Office Experiment Stations, Experiment Station Bulletin, **28**

Bylka, W., I. Matlawska and N.A. Pilewska. (2004). "Natural Flavonoids as Anti-microbial Agents", *Journal of American Natraceutical Association*, **7(2)**, 24-31

Dermaxime. (2009). Orange extract in cosmetic use, http://www.dermaxime.com/orange.htm.

Grunnet, J. (2010). "Advantages and Disadvantages of Herbal Medicine". http://herbs.lovetoknow.com/Advantages and Disadvantages of Herbal Medicine

Hanif, R., Z. M. Lqbal, S. Hanif and M. Rasheed. (2006). "Use of Vegetables as Nutritional Food: Role in Human Health". *Journal of Agricultural and Biological Science,* **1(1)**, 18-20

Harbone, J.B. (1984). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis,* New York: 2nd Ed, Chapman and Hall, 120-126

Joslyn, M.A. (1973). *Methods in Food Analysis*. New York: Academic Press Inc., 375-376.

Malika, N., F. Mohamad and E. Chakib. (2004). "Antimicrobial Activity of Natural Honey from Aromatic and Medicinal Plants on Antibio Resistant Strains of Bacteria", *J. Agric. Biol.*, **6**, 289-293

Pearson, D. (1970). *The Chemical Analysis of Foods*. London: J. and A. Constable Ltd., 191

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Investigation of Phytochemical Constituents and Some Bioactivities of Bark of *Dolichandrone spathacea* (Thakhut)

Khin Khin Shwe[*]

Abstract

The present research focuses on the investigation of some phytochemical constituents and some bioactivities such as antimicrobial and antioxidant activities of the bark of *Dolichandrone spathacea* (Thakhut). The selected plant sample (*Dolichandrone spathacea*) were collected from Ayeyawaddy Region. The preliminary phytochemical investigation of Thakhut bark revealed the presence of carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugar, saponins, steroids and terpenoids and the absence of alkaloids, α-amino acids, starch and tannins. The qualitative elemental analysis was conducted by EDXRF; Ca content was found to be the highest. The antimicrobial activity of *D. spathacea* extracts (PE extract, EtOAc extract, EtOH extract and water extract) were evaluated on bacterial and fungal strains like *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia* coli. Various solvent extracts were examined using agar well diffusion method against bacterial and fungal microorganisms. EtOAc and EtOH extracts showed moderate antimicrobial activity. PE and water extract showed mild activity. The investigation of the rapid screening of antioxidant activity of ethyl acetate extract and watery extract of Thakhut bark by Dot-Blot and 2,2-diphenyl-1-picrylhydrazyl (DPPH) staining method was also performed. It was found that both extracts had the radical scavenging activity. Ethylacetate extract showed significant antioxidant property. From these findings, Thakhut bark can also be a potential source of natural antioxidant supplement in order to reduce oxidative stress related diseases.

Keywords: *Dolichandrone spathacea* (Thakhut), Phytochemicals, EDXRF, antimicrobial activity, antioxidant activity

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. In fact that plant produces a diverse range of bioactive molecules,

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making them a rich sources of medicinal compounds, these compounds have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Over 50 % of all modern clinical drugs, one of the natural products origin and natural products play an important role in drug development programs in the pharmaceutical industry (Baker *et al.,* 1995).

Dolichandrone spathacea or commonly known as mangrove trumpet tree is a back-mangrove tree species that has a large creamy white flowers and large seed pods with thick corky wings. This plant could be found in coastal swamps from the southern India and Bengal to Malaysia and New Caledonia. Its lightweight wood was usually used as firewood, fishing net floats and saddles by Javanese Fisher-men and into wooden shoes in the Philippines Island (Azmi *et. al.,* 2011). The stem extract possesses high activity compared with the leaves extract (Ibrahim *et al.*, 2013).

Flower **Trumpet shaded flower**

Fruits **Bark**

Figure 1. Photographs of *Dolichandrone spathacea* (Thakhut)

Materials and Methods

Sample Collection and Preparation

Several samples of Thakhut collected from Mawlamyinegyunn Township, Ayeyarwaddy Region, the bark was removed from the stem. The bark was chipped into very small pieces and allowed to dry well. The dried pieces were made into powder by using grinding machine. The powdered sample was stored in air-tight container to prevent moisture changes and other contaminations. The dried powdered sample was used for chemical and biological investigations.

Preliminary Phytochemical Investigation of Bark of *Dolichandrone spathacea* (Thakhut)

Phytochemical tests for bark of *Dolichandrone spathacea* (Thakhut) was carried out according to the reported methods to investigate the presence and absence of phytochemical constituents such as alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, starch, steroids, terpenoids and tannins.

Qualitative Elemental Analysis of Plant Samples by Energy Dispersive Fluorescence (EDXRF)

The sample was placed in the sample chamber of EDX-8000 spectrometer. The chamber was pumped up to the vacuum. The vacuum pressure was about 38 pa and the detector temperature was about 170°C. Therefore, liquid nitrogen needs to be added at the time of analysis. The measurement condition of the X-ray spectrometer used was Rh target. Each sample was run for a counting time of about 100 seconds and the spectrum obtained as stored and analyzed in PC based multi- channel analyzer using EDX-8000 software.

Screening of Antimicrobial Activity of Crude Extracts from the Bark of *D. spathacea* by Agar Well Diffusion Method

For the examination of *in vitro* antimicrobial activity, crude extracts such as PE, EtOAc, 95% EtOH and water extracts of the plant sample from *Dolichandrone spathacea* (Thakhut) were prepared. The solvent extracts were tested against six pathogenic microorganisms by using agar well diffusion method. The extent of antimicrobial activity was measured from the diameter zone of inhibition.

Basic test organisms used in the research were kindly supplied from the Pharmaceutical Research Department for the determination of the antimicrobial activity. These include *Bacillus subtilis, Staphylococcus aureus, Pseudomonas areuginosa, Bacillus pumalis, Candida albican* and *Escherichia coli.*

Nutrient agar was prepared according to the method described by Cruickshank, (1975). Nutrient agar was boiled and 20-25 mL of the medium was poured into the test tube and plugged with cotton wool and sterilized at 121 °C for 15 minutes in an autoclave. After this, the tubes were cooled down to 30-35 °C and poured into sterilized petridishes and 0.1-0.2 mL of the test organisms were added into the dishes. The agar was allowed to set for 2-3 hours; then 10 mm agar well was made by the help of sterilized agar well and cutter. After that, about 0.2 mL of the sample was introduced into the agar well and incubated at 37 °C for 24 hours. The inhibition zone which appeared around the agar well, indicated the presence of antimicrobial activity.

Rapid Screening of Antioxidant Activity by Dot-Blot and DPPH Staining Preparation of 60 µM DPPH solution

2.364 mg of DPPH powder and 100 mL of 95 % ethanol were thoroughly mixed. This solution was freshly prepared in the brown coloured flask. Then it must be stored in the fridge for no longer than 24 hours.

Preparation of test sample solution

20 mg of test sample (ethyl acetate and water extract) and 10 mL of 95 % ethanol were thoroughly mixed. The mixture solution was diluted with 95 % ethanol to obtain desired concentrations.

Investigation of antioxidant by Dot – Blot and DPPH staining method

A suitable dilution of each extracts were carefully loaded on the 3 × 18 cm TLC Layers (Silica gel GF_{254}) Merck) by using microlitre syringe and dried for 8 min. Test amount were 400, 200, 100, 50, 25, 12.5, 6.25 µg (2, 1, 0.5, 0.25, 0.125 , 0.0625 and 0.03125 mg mL^{-1}). For crude extracts loaded samples gave 1cm diameter (area 78 mm^2). Drop of each sample was loaded in order of increasing concentration along the row. The staining of the silica plate was based on the procedure of Soler- Rivas *et al*., (2000).

The sheet bearing the dry spot was placed upside down for 10 sec in 60 µM DPPH solution. Then the excess solution was removed with a tissue paper, and the layer was dried with a dryer blowing cold air. The stained silica Layer revealed a purple background with white spots at the location of the drops, which showed radical scavenger capacity. The intensity of the white colour depends upon the amount and nature of radical scavenger present in the sample.

Results and Discussion

Phytochemicals in the Bark of *Dolichandrone spathacea* (Thakhut)

The preliminary phytochemical tests were carried out on the stem bark of Thakhut. The results obtained are summarized in Table 1. It was found that carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids and terpenoids were found to be present in the stem bark of Thakhut.

Table 1. Phytochemical Results of the Bark of *Dolichandrone spathacea* (Thakhut)

No	Tests	Extract	Test reagents	Observation	Remark
1	Alkaloids	1% HCl	Dragendorff's reagent	No orange ppt	-
			Mayer's reagent	No white ppt	-
			Wagner's reagent	No brown ppt	-
2	α-Amino acids	H_2O	Ninhydrin reagent	No purple spot	-
3	Carbohydrates	H_2O	10% α- Naphthol, conc : H_2SO_4	Red ring	+
4	Flavonoids	EtOH	Mg ribbon, conc: HCl	Pink colour	+
5	Glycosides	H_2O	10% lead acetate solution	White ppt	+
	Phenolic compounds	EtOH	5%$FeCl_3$ sol:, 1%$K_3Fe(CN)_6$	deep blue solution	+
7	Reducing sugars	H_2O	Benedict's solution	Brick-red ppt	+
8	Saponins	H_2O	Distilled water	Frothing	+
9	Starch	H_2O	1% Iodine	No blackish blue colouration	-
10	Steroids	PE	Acetic anhydride, Conc. H_2SO_4	Green colour	+
11	Tannins	H_2O	1 % Gelatin, 5 % $FeCl_3$ sol:	No white ppt	-
12	Terpenoids	$CHCl_3$	Acetic anhydride, Conc. H_2SO_4	Pink colour	+

(+) = presence, (-) = absence, (ppt.) = precipitate

Elements in the Bark of *Dolichandrone spathacea* (Thakhut) by EDXRF

X- ray spectrometer permits simultaneous analysis of light element to heavy metal. Shimadzu EDX- 8000 spectrometer can analyze the elements from Al to U and C to Sc under vacuum condition.

In this research work relative abundance of elements present in the bark of Thakhut was determined by EDXRF spectrometer. The EDXRF spectrum of the sample was shown in Figure 2. It can be seen that mineral elements such as Ca is predominant in the sample (4. 57 %) followed by smaller amounts of Si, S, K, Fe, Sr, Mn, Ti, Br, Cu, Zn, Rb. From these results it can be observed that, the element calcium is important to normal kidney function and reduces blood cholesterol level. It can be found that these predominant elements can be very effective to human health. The elemental results obtained by EDXRF method are shown in Table 2.

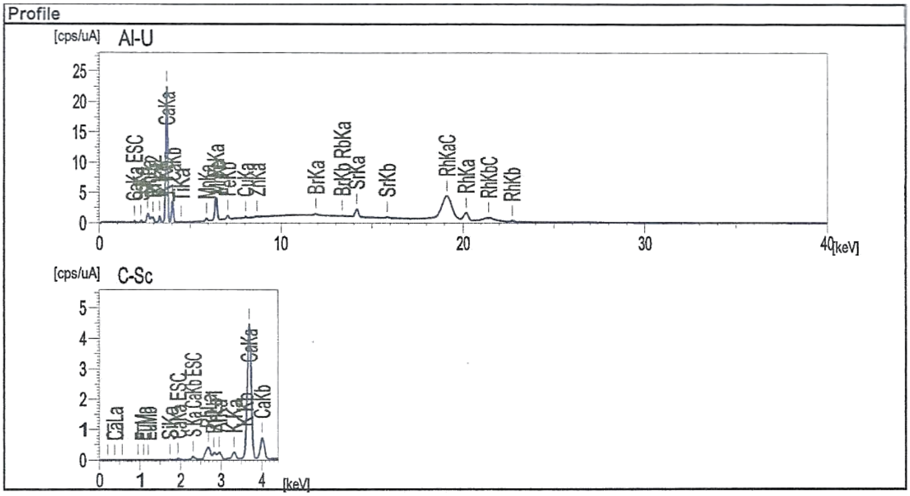


Figure 2. EDXRF spectra of the bark of *Dolichandrone spathacea* (Thakhut)

Table 2. Relative Percent Abundance of Element in the Bark of *Dolichandrone spathacea* (Thakhut) by EDXRF

No.	Element	Relative Abundance (%)
1	Ca	4.571
2	Si	0.743
3	S	0.395
4	K	0.302
5	Fe	0.122
6	Sr	0.019
7	Mn	0.019
8	Ti	0.012
9	Br	0.004
10	Cu	0.003
11	Zn	0.002
12	Rb	0.001

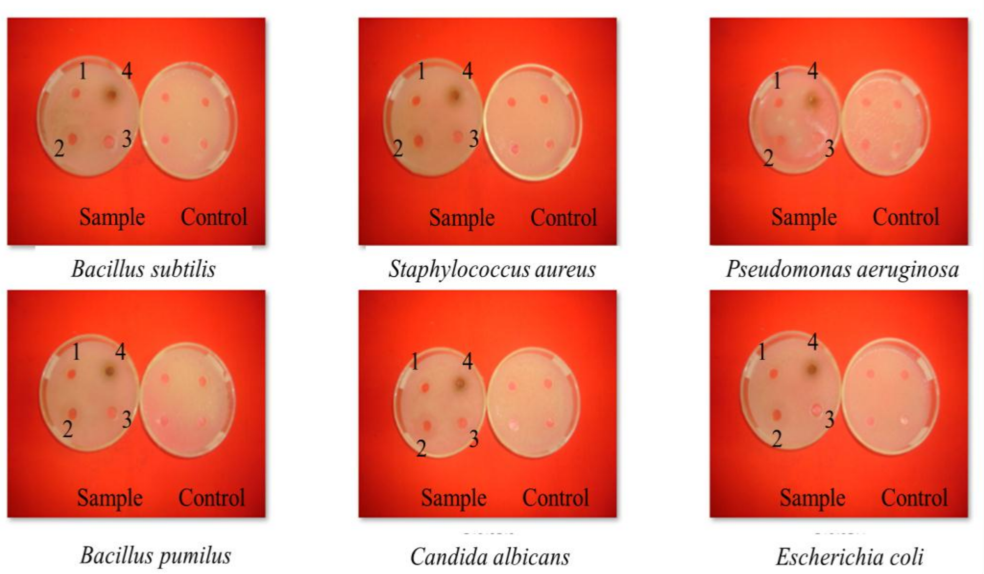
Antimicrobial Activity of Various Crude Extracts from the Bark of *Dolichandrone spathacea* (Thakhut)

Screening of antimicrobial activity of various crude extracts such as pet-ether, ethyl acetate, 95 % ethanol and water were done by agar well diffusion method.

In the present work, the microorganisms used were *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli.* The measurable zone diameter, including the agar well diameter, showed the degree of antimicrobial activity. The larger the diameter of clear zone, the more potent the antimicrobial activity. The antimicrobial activity of crude extracts: petroleum ether, ethyl acetate, ethanol and watery extract of plant sample were screened by using agar well diffusion method on six bacterial strains.

Pet-ether extract has low inhibition zone but no activity on *Bacillus pumilus*. Ethyl acetate extract has moderate activity on five strains expect

Bacillus subtilis. Ethanolic extract showed mild activity on all six strains. Watery extract showed low activity only *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa*. The results are shown in Figures 3 and and Table 3.



1. PE extract **2.** EtOAc extract **3.** EtOH extract **4.** H_2O extract

Figure 3. Antimicrobial activity of various extracts *Dolichandrone spathacea* (Thakhut) bark on six microorganisms

Table 3. Inhibition Zone Diameters of Crude Extracts of *Dolichandrone spathacea* (Thakhut) Bark against Six Microorganisms by Agar Well Diffusion Method

Microorganisms	**Inhibition Zone Diameter (mm)**			
	EP	**EtOAc**	**EtOH**	**H_2O**
Bacillus subtilis	12	16	17	11
Staphylococcus aureus	12	15	16	12
Pseudomonas aeruginosa	11	13	12	11
Bacillus pumilus		-	12	-

Microorganisms	**Inhibition Zone Diameter (mm)**			
	EP	**EtOAc**	**EtOH**	**H_2O**
Candida albicans	12	20	13	-
Escherichia coli	12	11	13	-

Agar well - 10mm

10 mm ~ 14 mm	Mild activity
15 mm ~ 19 mm	Medium activity
20 mm above	Highest activity

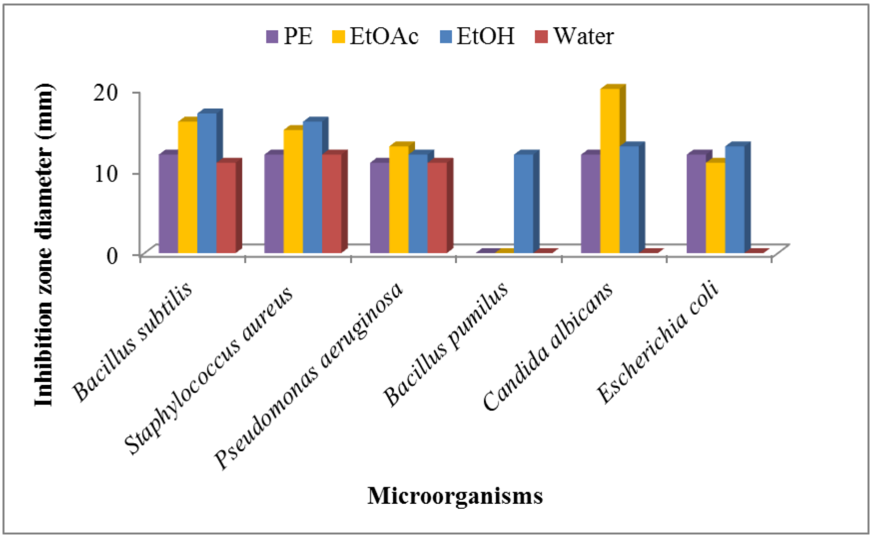


Figure 4. Histogram of inhibition zone diameters of different extracts of *Dolichandrone spathacea* (Thakhut) bark against six microorganisms by agar well diffusion method

Antioxidant Activity by Dot-Blot and DPPH Staining

To make a semi-quantitative visualization possible, ethylaceate and watery extracts of selected plant, *Dolichandrone spathacea* (Thakhut) were detected in the TLC plates by the DPPH staining method. For the rapid screening each prepared sample was applied as a dot on a TLC plate that was then stained with DPPH solution. The appearance of white colour in the spots has a potential value for the indirect evaluation of test samples in the dot blot (Soler-Rivas *et al.*, 2000; Chang *et al.*, 2002). The method is typically based on the inhibition of the accumulation of oxidized products since the generation of free radicals is inhibited by the addition of antioxidants.

When the two extracts (ethyl acetate and watery extracts from the bark of Thakhut) were analysed, white spots with strong intensity appeared at 12.5 μg dried matter for ethylacetate extract, 25 μg dried matter for watery extract. According to these results, the ethylacetate extract has more potent antioxidant activity than that of water extract from the bark of *Dolichandrone spathacea* (Thakhut) (Figure 3.10). ?

The results from DPPH staining experiments demonstrated that the major phytoconstituents in selected plant sample may have antioxidant activity. Hence, *Dolichandrone spathacea* (Thakhut) may be used as an easy accessible source of natural antioxidants as a food supplement, or in the pharmaceutical and medical industries.

Watery Extract

Ethylacetate Extract

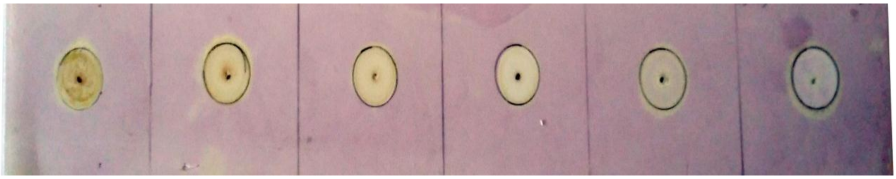


Figure 5. Antioxidant activity of watery and ethylacetate extracts by Dot-Blot and DPPH staining method

Conclusion

In this study, investigation of phytochemical constituents, extractable matter contents of the bark of *Dolichandrone spathacea* (Thakhut) were reported.

- The result of preliminary phytochemical screening of different crude extracts of *Dolichandrone spathacea* (Thakhut) revealed the presence of carbohydrates, flavonoids glycosides, phenolic compounds, reducing sugars, saponins, steroids and terpenoids. Alkaloids, α-amino acids, starch and tannins are absence in the bark of *Dolichandrone spathacea* (Thakhut).

- Elemental analysis by EDXRF revealed Ca (4.571 %) as major elements and Si (0.743 %), S (0.395 %), K (0.302 %), Fe (0.122 %), Sr (0.019%), Mn (0.019 %), Ti (0.012 %), Br (0.004%), Cu (0.003 %), Zn (0.002 %) and Rb (0.001%) as trace elements in the bark.

- The antimicrobial activity of crude extracts : petroleum ether, ethyl acetate, ethanol and watery extract of plant sample were screened by using agar well diffusion method on six microorganisms strains. Finally, it is concluded that EtOAc and EtOH extracts have (15 ~ 17 mm) medium inhibition zone against on *B. subtilis* and *S.aureus.* EtOAc extract has (20 mm) highest inhibition against on *Candida albicans*. Pet-ether extract has (11 ~ 12 mm) low inhibition zone but no activity on *B. pumilus*. The ethanolic extract is effective against all six strains. The watery extract shows inhibitory effect against *B. subtilis, S. aureus* and *P. aeruginosa* only.

- Screening of antioxidant activity by DPPH staining assay revealed that white spots with strong intensity appeared at 12.5 μg dry matter for ethylacetae extract , 25 μg dry matter for watery extract of *Dolichandrone spathacea* (Thakhut). Both extracts showed antioxidant activity.

- Based on above scientific data, *Dolichandrone spathacea* (Thakhut) seems to be promosing as the natural products uses and may be utilized for diseases caused by oxidation and six microorganisms.

Acknowledgement

The authors would like to express profound gratitude to Rector and Pro-Rectors from Dagon University for their kind permission to present this paper. I would also like to express our appreciation to Professor and Head, Dr Cho Cho Win, Department of Chemistry, Dagon University for her kind supervision and encouragement to do this research work.

References

Azmi, J. S., I. M. Mohamed, and N. Hoshim. (2011). “Inhibitory Potential Against Methicillin– Resistant *Staphylococcus aureus* (MRSA) of *Dolichandrone spathacea*, Mangrove Tree Species of Malaysia”. *Am. J. Pham.,* **30** (2), 359- 62

Baker, J. T., R. P. Borris and B. Carte. (1995). “Natural Product Drug Discovery and Development New Perspective an International Collaboration”. *J. Nat Prod.,* **58**, 1325 - 1357

Chang, W.C., S. C. Kim, S.S. Hwang, B.K. Choi, H.J. Ahn, M.Y. Lee, S.H. Park and S. K. Kim. (2002). "Antioxidant Activity And Free Radical Scavenging Capacity between Korean Medicinal Plants and Flavonoids By Assay-Guided Comparison". *Plant. Sci.*, **163**, 1161-1168

Cruickshank, R., J. P. Diguid, G. P. Marmior and R. H. A. Swain. (1975). *Medical Microbiology*. London : Churchill Livingstone Ltd.,

Farombi, E. (2003). “Afrian Indigenous Plants with Chemotherapeutic Potentials and Biotechnological Approach to the Production of Bioactive Propylactic Agents”. *African J. Biotech.,* **2**, 662-671

Harborne, J. B. (1984). *Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis*. London : 2nd Ed., Chapman and Hall, 120-126

Ibrahim.M.S.Eldeen and Mohd. A.W. Effendy. (2013). *Antimicrobial Agents from Mangrove Plants and Their Endophytes.* Malaysia : University of Khartoum, 873-874

Marini-Bettolo, G. B., M. Nicolettic and M. Patamia. (1981). “Plant Screening by Chemical and Chromatographic Procedure Under Field Conditions”. *Journal of Chromatography*, **113** (1), 113-127

M-Tin Wa, (1972).“Screening Methods and Procedures”. *Phytochemical Bulletin of Botanical Society of America*, **5** (3), 4 –10

Soler-Rivas, C., J. C. Espin and H. J. Wichers. (2000). "An Easy and Fast Test to Compare Total Free Radical Scavenger Capacity of Foodstuffs". *Phytochem. Anal.*, **11**, 330-338

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Investigation of Phytochemical Screening, and Antimicrobial Activities and Extraction of Oil from the Leaves of *Coleus aromaticus* Benth. (Ziyar-ywet)

Khin Thandar Shwe[1] & No No Win Ei[2]

Abstract

The research work is focused on investigation of antimicrobial activity and identification of chemical constituents in extracted oil extracted from the leaves of *Coleus aromaticus* Benth. (Ziyar-ywet). Preliminary phytochemical tests were carried out by test tube methods. In addition, the soluble matter of the sample in pet-ether, ethyl acetate, ethanol and water were done by solvent extraction method. The elemental analysis were determined by using EDXRF spectrometry. The crude extracts such aspet-ether, ethyl acetate, ethanol and water of Ziyar-ywet were subjected for screening antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Escherichia coli and Candida albicans* by agar well diffusion method. The oil from Ziyar-ywet was extracted by water distillation method. The chemical constituents in the extracted oil of Ziyar-ywet were detected by GC-MS method.

Keywords: *Coleus aromaticus*Benth. (Ziyar-ywet), EDXRF, antimicrobial activity, *carvacrol*

Introduction

Coleus aromaticus Benth. of the family Lamiaceae is native to East Indies and widely cultivated in Africa and almost all tropical countries. It is also popularly known as "Indian Oregano". Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value. According to the World health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. *Coleus aromaticus* is a tender fleshy perennial plant belonging to the family lamiaceae with an oregano-like flavor and odor. It is a small plant with certain features that resemble a climber. Its stem is hairy, fleshy and attains a height of 1-3 feets. Leaves are 1-2 inch in length, roundish, dentate, hairy, thick and fleshy resembling heart. Flowers are small and blue or purplish in

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color with four petals and it flowers in early summer. The leaves of the plant are thick, succulent and juicy (Pritima, 2007).*Coleus aromaticus* Benth. of the family Lamiaceae is native to East Indies and widely cultivated in Africa and almost all tropical countries. It is also found in Asia, Australia, the Malay Archipelago and Philippines (Nostro*et al.*,2000). Leaves of *Coleus aromaticus* contain flavones salvigenin, quercetin, luteolin, apigenin, flavononeeriodyctol, flavanoltoxifolin, triterpenic acids, oleanolic acid, 2,3-dihydroxyoleanolic acid and 2,3,19,23-tetrahydroxyursolic acid. Volatile constituents of *Coleus aromaticus* contains camphor (39%) along with corvacrol (41.3%). Other constituents reported are (Z)-1,3-hexadiene (0.1%), (Z)-3-hexenol (0.6%), (E, 2) farnesene (0.2%), (E, E) farnesene (0.2%), and murolene (0.2%) (Khare, 2011).

The leaf juice is a powerful aromatic, used in digestive disorders in children. The expressed juice is applied round the orbit to relieve the pain in conjunctivities. In India (South) this herb is traditionally applied externally for burns and insect bites, while internally it is used as a carminative and to control asthma. *Coleus aromaticus* is a medicinal plant having diverse pharmacological properties such as antiepileptic, antimutagenicand antigenotoxic effects anticlastogenicity potency, anti-inflammatory, antitumor, diuretic, radioprotective activities. The essential oil of *Coleus aromaticus* has been showed anti-fedant and antimicrobial activities (Warrier, 1995).

Figure 1. Images of Ziyar-ywet plant and leaves

The present work focused on evaluation of some phytochemical constituents, soluble matter contents, elemental contents and extraction of oil from Ziyar-ywet (*Coleus aromaticus*Benth.).

Materials and Methods

The sample of Ziyar-ywet was collected from Kaymarthiri Quarter, Myitkyina Township, Kachin State in January,2018. It was identified at the Department of Botany, University of Myitkyina. The collected dried sample was ground into power by grinder. The dried powered sample was stored in the air-tight containers to prevent the moisture and other contaminations. Various crude extracts were prepared by using various solvents such as pet-ether, ethyl acetate, ethanol and water.

Screening of Phytochemical constituents on the Ziyar-ywet (*Coleus aromaticus*Benth.)

Qualitative phytochemical tests to determine the presence of alkaloids, α-amino acids, carbohydrates, glycosides, organic acids, reducing sugars, saponins, starch, flavonoids, phenolic compounds, tannins, steroids and terpenoids were carried out on Ziyar-ywet according to the test tube methods.

Determination of Soluble Matter Contents by Direct Extraction Method

25g of dried powdered sample was percolated with 75cm^3 of pet-ether for 6hand filtered. The filtrate was placed in a weighed porcelain basin and then evaporated to dryness on a water-bath until it was completely dried. The residue with the basin was weighed. The different in weight of before and after, the experiment was taken to be the pet-ether soluble matter content. Ethyl acetate, ethanol and watery soluble matter contents were done the same procedure.

Elemental Analysis of Ziyar-ywet 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 vacuum condition. Analysis of some elements in the Ziyar-ywet were measured by EDXRF method using EDX-700 instrument at the Universities' Research Centre (URC), Yangon.

Screening of Antimicrobial Activity of Various Crude Extracts by Agar Well Diffusion Method

The antimicrobial activities of different crude extracts such as pet-ether, ethyl acetate, 95% ethanol and watery extracts from the Ziyar-ywet was determined against six strains of microorganisms such as *Bacillus subtilis, Bacillus pumilus, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*and *Escherichia* coli by employing agar well diffusion method at Fermentation Department, Development Center of Pharmaceutical Technology, Ministry of Industry I, Yangon, Myanmar.

Extraction of Oil from the leaves of *Coleus aromaticus*Benth. (Ziyar-ywet) by Water Distillation Method

The fresh leaves sample of Ziyar-ywet (300g) and distilled water (2L) were placed in the 500 mL round-bottomed flask. The flask was fitted to dean and stark apparatus which has regulated to maintain a rate of condensate of 1-3 hour. The volume of essential oil was collected in the receiver flask. After the water distillation process, the product was collected and separated two layers of immiscible liquids such as water and oil by using separator funnel. The oil was then partitioned with pet-ether in a separating funnel. The pet-ether extract was dried anhydrous sodium sulphate, filtered and evaporated to get the extracted oil which has then weight until to the constant and kept in air tight bottle. Yield percent of extracted oil was calculated according to the following equation.

$$\text{Yield percent } \% = \frac{\text{Volume of extracted oil (g) x } 100\%}{\text{Weight of sample in (g)}}$$

Identification of Chemical Constituents in Oil Extracted from the leaves of *Coleus aromaticus*Benth. (Ziyar-ywet) by GC-MS methods

Gas chromatography mass spectrometry (GC-MS) is that combines features of gas chromatography and mass spectrometry to identify different substances within a test sample (Shriner *et al*., 1981). The GC separates the compounds from each other, while the mass spectrometer helps to identify them based on their fragmentation pattern. For identification of chemical constituents in oil, GC-MS spectrometer was used to examine the molecular weight and formula of organic compound.

Results and Discussions

Phytochemical Constituents on the Ziyar-ywet

Qualitative phytochemical test revealed the presence of alkaloids, α-amino acids, carbohydrates, glycosides, organic acids, reducing sugars, saponins, flavonoids, phenolic compounds, tannins, starch, steroids and terpenoids were present in Ziyar-ywet. However, starch is absent.

Soluble Matter Content of Ziyar-ywet

In this research work, the soluble matter contents of Ziyar-ywet were done by solvent extraction method. The resultant content of these samples is shown in Table (1). From the results, it was observed that the amounts of polar constituents were higher than nonpolar constituents in Ziyar-ywet. In addition, Watery soluble matter content was found to be the highest in this sample.

Table 1. Soluble Matter Content of Ziyar-ywet

Extract	**Yield % of Ziyar-ywet**
Pet-ether	0.8284
Ethylacetate	1.9472
Ethanol	2.0844
Watery	10.32

Elemental Analysis of Ziyar-ywet

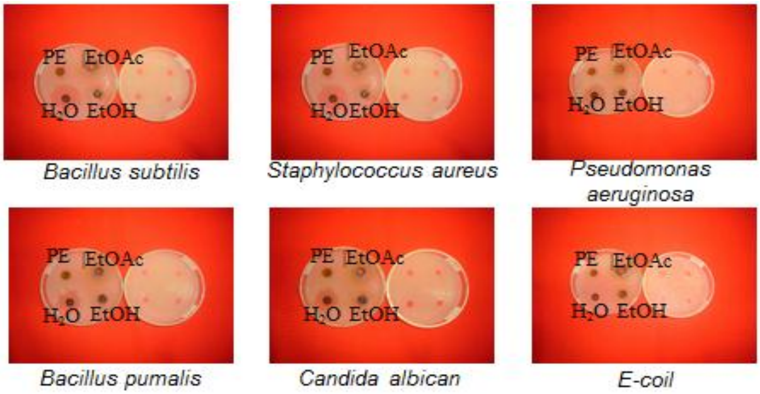
The element contents present in Ziyer-ywet were determined by EDXRF spectrometer (Table 2). According to the results, potassium and calcium were present as macro element in Ziyar-ywet sample. In addition, sulphur, iron, zinc, titanium, strontium, manganese and copper were observed to be micro elements.

Table 2. Relative Abundance of Some Elements in the leaves of Ziyar-ywet

No.	Elements	Relative Abundance (%)
1	Potassium (K)	3.942
2	Calcium (Ca)	2.844
3	Sulphur (S)	0.187
4	Iron (Fe)	0.060
5	Zinc (Zn)	0.010
6	Titanium (Ti)	0.010
7	Strontium (Sr)	0.006
8	Manganese (Mn)	0.006
9	Copper (Cu)	0.002

Screening of Antimicrobial Activity of Various Crude Extracts by Agar Well Diffusion Method

Screening of antimicrobial activity of various crude extracts such as pet-ether, ethyl acetate, ethanol and watery extracts from the Ziyar-ywet investigated by employing agar well diffusion method. In this study, the samples were tested on six species of microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* species. The inhibition zone diameter shows the degree of the antimicrobial activity. The layer inhibition zone diameters, the higher the antimicrobial activity. It was found that ethyl acetate extract and watery extract of Ziyar-ywet showed the most pronounced antimicrobial activity against six microorganisms with inhibition zone diameter ranged in 20mm to 35mm. Moreover, ethanol extract (inhibition zone diameter ranged in 13mm to 22mm), and pet- ether extract inhibition zone diameter ranged in 13mm to 15mm) were also observed to exhibit antimicrobial activity against six species of microorganisms tested.



Bacillus subtilis *Staphylococcus aureus* *Pseudomonas aeruginosa*

Bacillus pumalis *Candida albican* *E-coil*

Figure 2. Images of inhibition zones of various crude extracts against Six microorganism

Table 3. Inhibition zone diameter of various crude extracts of ziyar-ywet against Six microorganisms by Agar Well Diffusion Method

		Organisms					
Sample	**Solvent**	**B-Sub**	**S-aureus**	**Pseudo-monas**	**B-pumilus**	**Candida albicans**	**E-coli**
Ziyar-ywet	Pet-ether	15mm (++)	14mm (+)	13mm (+)	15mm (++)	13mm (+)	14mm (+)
	EtOAc	25mm (+++)	23mm (+++)	35mm (+++)	28mm (+++)	32mm (+++)	25mm (+++)
	EtOH	22mm (+++)	15mm (++)	13mm(+)	13mm(+)	15mm (++)	13mm (+)
	Water	20mm (+++)	20mm (+++)	20mm (+++)	20mm (+++)	20mm (+++)	20mm (+++)

Identification of Chemical Constituents in extracted oil from the leaves of Ziyar-ywet (*Coleus aromaticus*Benth.) by GC-MS Method

The GC-MS chromatogram of oil extracted from Ziyar-ywet is shown in Figure 3. According to GC-MS chromatogram, the peak appears at the retention time 7.38 min with 100% relative abundance. At this retention time 7.38 min, the GC-MS spectrum (Figure 4) shows the molecular ion peak at m/z 150, indicating the molecular weight of compound is carvacrol.

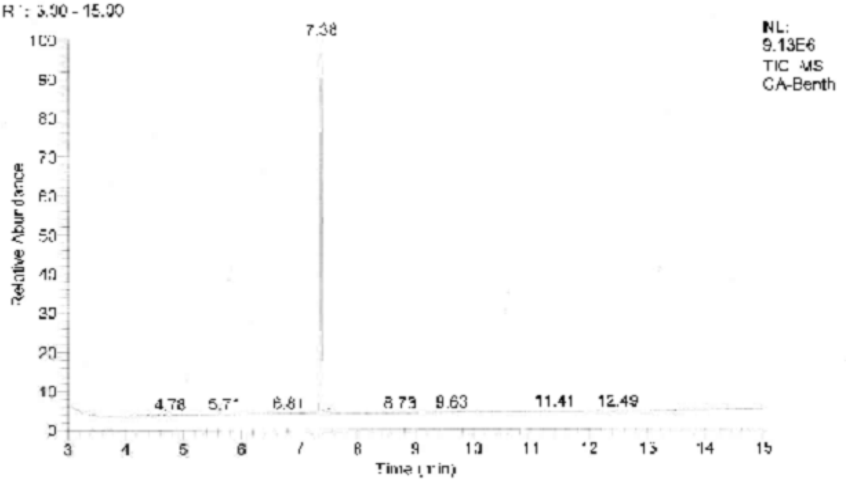


Figure 3. GC-MS Chromatogram of essential oil from the leaves of *Coleusaromaticus*Benth. (Ziyar-ywet)

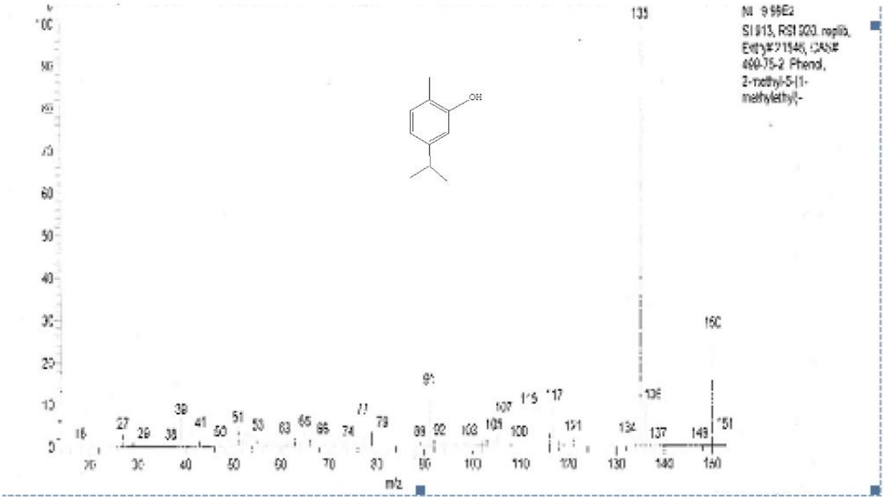


Figure 4. Mass spectrum of compound at retention time 7.38 min and Carvacrol

Conclusion

The research paper concerns with the investigation of phytochemical screening antimicrobial activity and the GC-MS analysis of oil from the leaves of *Coleus aromaticus*Benth.(Ziyar-ywet). The preliminary phytochemical screening of Ziyar-ywet revealed the presence of alkaloids, α-amino acids, carbohydrates, glycosides, organic acids, reducing sugars, saponins, flavonoids, phenolic compounds, tannins, steroids and terpenoids. However, starch is absent. In addition, the crude extracts were prepared in various solvents systems. The water soluble matter was found to be highest (10.32%) and pet-ether soluble matter was the lowest (0.8284%).According to EDXRF elemental analysis, potassium (K) and calcium (Ca) are the highest content. It prevents hypertension and dementia. The sulphur(S), iron(Fe),zinc(Zn), titanium(Ti), strontium(Sr), manganese (Mn) and copper (Cu) were determined to be trace amount. Moreover, from the results of antimicrobial activity by agar well diffusion method, ethylacetate extracts and watery extract of Ziyar-ywet exhibited the highest antimicrobial activity against six selected microorganisms. In addition, ethanol extract and pet-ether extract were also observed medium activity on six selected microorganisms. The yield present of extracted oil was found to be 0.03%. By the GC-MS spectral data, the major component was identified to be carvacrol (m/z 150, R_t 7.38 min, 100% Relative abundance). Consequently, carvacrolposesesbiocidal activity against many artropodes of the importance for veterinary and human medicine. It acts as repellent, larvicide, insecticide and acaricide. Thus, the Ziyar-ywet is very useful for traditional medicine due to the presence of carvacrol compound, the rich of potassium(K) and calcium(Ca) and then it also the highest antimicrobial activity on six selected microorganism.

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References

Khare, R.S., S. Banerjee and K. Kundu. (2011), "A nutritive medicinal plant of Potential therapeutic value of *Coleus aromaticus*Benth". *International Journal of Pharma and Bio Science.***2**, 488-500

Nostro, A., M. Germano, V. D. Angelo and M. Cannatelli. (2000). “Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity.”Lett. Appl, Microbial, **30 (**5**)**: 379-384

Pritima R.A., R.S. Pandian.(2007). “Antimicrobial activity of *Coleus aromaticus* (Benth) against microbes of reproductive tract infections among women”. *Afr. J. Infect.Dis.***1**, 18-24

Shriner, R.L., R.C. Fuson and D.Y. Curtin. (1981). “The Systematic Identification of Organic Compounds; A Laboratory Manual,” John Wiley and Sons, New York, 385-425

Warrier, P.K., V.P. Numbiar and Ramankutty. (1995). *Indian Medicinal Plants*. India: 1st Edn, Orient Langman Limited Madras. **4**, 315-317.

Weli, A.M., J.N. Al-Sabahi and Q. Al-Riyami.(2011). "Phytochemical investigation and Antimicrobial screening of *Coleus aromaticus* grown in oman". *Electronic Journal of Environment, Agricultural and Food Chemistry*. **10**(11), 3083-3090.

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Screening of Phytochemicals, Physicochemical Properties and Antimicrobial Activities of *Calophyllum inophyllum* Linn. (Pong-nyet) Flowers, Leaves and Seeds

Moh Moh Tun[*]

Abstract

Calophyllum inophyllum Linn. (Pong-nyet) has several biological activities such as antimicrobial, antioxidant, anti HIV-I reverse transcripts, antimalarial, antiinflammation, antiviral, anticoagulation activities and cytotoxic effect. In the present work, flowers, seeds and leaves of Pong-nyet, collected from Mayangone Township, Yangon Region were chosen for screening of phytochemicals, physicochemical properties and their antimicrobial activities. The preliminary phytochemical tests revealed the presence of carbohydrates, flavonoids, glucosides, organic acids, phenolic compounds, reducing sugars , starch , steroids, tannin and terpenoids as major constituents and alkaloids, a-amino acids, cyanogenic glycosides and saponins present as minor constituents in all of the three samples. From the results of some physicochemical analyses, flowers of Pong-nyet was found to contain 10.4 % of moisture, 5.17% of ash, 9.55 % of crude fibre, 8.47 % of protein, 8.60 % of fat and 57.81 % of carbohydrates, leaves of Pong-nyet found to contain 9.74 % of moisture, 4.87 % of ash, 11.62 % of crude fibre, 5.88 % of protein, 7.15 % of fat and 60.72 % of carbohydrates, and seeds of Pong-nyet found to contain 23.00 % of moisture, 4.5 % of ash, 5.6 % of crude fibre, 6.90 % of protein, 55.67 % of fat and 4.33% of carbohydrates. Qualitative ED XRF elemental analysis showed the presence of potassium and calcium as major elements and iron and copper as minor elements present in all samples. Sulphur, zinc and yttrium are present in flowers but not in seeds and leaves. The antimicrobial activity of PE, EtOAc, $CHCl_3$, 95% EtOH and H_2O extracts of flowers, leaves and seeds of Pong-nyet was determined by agar well diffusion method against six species of microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albicans* and *Escherichia coli*. It was found that all of these extracts except H_2O extracts have pronounced antimicrobial activity with inhibition zone diameter ranged between 18 mm ~ 29 mm in seeds,12 mm ~ 25 mm in leaves and 12 mm ~ 29 mm in flowers of Pong-nyet. Among three different samples such as flowers, leaves and seeds, flowers extracts show the pronounce antimicrobial activity.

Keywords: *Calophyllum inophyllum* (Pong-nyet), phytochemical, physicochemical analyses, antimicrobial activity

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Introduction

Medicinal plants are one of the important natural wealth of a country. Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinical drugs are of natural product origin, and natural products play an important role in drug development programs in the Pharmaceutical Industry. Of the 250,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal value and around 5,000 species have specific therapeutic value (antimalarial, anticancer, antidiabetic, antibacterial, anti-inflammatory, antidiarrhea, diuretic, cardiotonic, anti-allergic and hepatoprotective, etc.,). The drugs are derived either from the whole plant or from different parts of the plant like root, stem, bark, leaf, flower, fruit and seed etc.,. These medicinal principles are separated by different process; of which the most common is extraction. Extraction is the separation of the required constituents from plant materials using a solvent (Krantz, 1969).

Calophyllum inophyllum Linn. (Pong-nyet) which is also known as “bintangor” by the locals in Malaysia, belong to the Guttiferae family. *Calophyllum inophyllum* Linn. is native to tropical South-East Asia. It is found in Thailand, Myanmar, Malaysia, South India, Srilanka, but is most abundant in exotic Melanesion Islands and Polynesion Island of Tahiti (Allen, 2002). Several species of this genus are known to be used in folk medicine. This species of *Calophyllum* has proved that its virtues not only withstand the scruting of independent traditional use, but also can be proven in modern *in vivo* studies to be as effective as its legend suggested. The chemistry is complex and unusual, perhaps helping to explain some of the impressive physiological actions possessed by this plant (Dharmarate, 1996). *Calophyllum inophyllum* Linn. (Pong-nyet) has several biological activities such as antimicrobial, antioxidant, anti HIV-I reverse transcriptas, antimalarial, antiinflammation, antiviral, anticoagulation activities and cytotoxic effect. Different parts of *Calophyllum inophyllum* Linn. have been widely used in traditional Chinese folk medicines in the treatment of eye-diseases, wounds rheumatism and inflammation for many centuries (Dweck, 2002).

At present, the government of Myanmar has urged to upgrade further and extensive research on traditional methods on potent traditional drugs which can be used against certain diseases that cannot be cured by western medicine and to conduct biomedical research in order to make it safer and more reliable to people. The government is giving impetus to developing Traditional Medicine systematically up to international standards and to manufacturing potent and efficacious Traditional Medicines based on scientific evidences and practices.

Fruits Flowers

Tree

Figure 1. Photographs of *Calophyllum inophyllum* Linn. (Pong-nyet)

Materials and Methods

Sample Collection and Preparation

Flowers, leaves and seeds of *Calophyll inophyllum* Linn. (Local name : Pong-nyet) were collected from Mayangon Township, Yangon Region, Myanmar. The collected fresh samples were washed and air dried at room temperature for one month and then they were ground into powder by grinder. The dried powdered samples were separately stored in the air tight containers.

Preliminary Phytochemical Investigation of *Calophyll inophyllum* Linn. (Pong-nyet)

In order to classify the types of organic constituents present in plant samples, preliminary phytochemical tests on the plant samples were carried out according to the appropriate reported methods.

Determination of Some Physicochemical Properties of the Samples

Determination of physicochemical properties of flowers, leaves and seeds of pong-nyet including the determination of some nutritional values such as moisture, ash, fiber, protein, fat and carbohydrate contents was carried out by appropriate reported methods and qualitative determination of some elements was carried out by ED XRF technique.

Screening of antimicrobial activity of the samples

The antimicrobial activities of different crude extracts such as pet-ether, chloroform, ethyl acetate, 95 % ethanol and watery extracts from flowers, leaves and seeds of Pong-nyet were determined against six strains of microorganisms such as *Bacillus subtilis, Bacillus pumalis, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Escherichia coli* by employing agar well diffusion method at Fermentation Department, Development Centre of Pharmaceutical Technology, Ministry of Industry I, Yangon, Myanmar.

The extract (1g each) was introduced into sterilized petri dishes and dissolved in 1 mL of their respective solvents: pet-ether. chloroform, ethyl acetate, 95 % ethanol and distilled water.

Meat extract (0.5 g), peptone (0.5 g) and sodium chloride (0.25 g) were mixed with distilled water and the solution made up to 100 mL with distilled water. The pH of this solution was adjusted at 7.2 with 0.1 M sodium hydroxide solution and 1.5 g of agar was added. The nutrient agar medium was put into sterilized conical flask and plugged with cotton wool and then autoclaved at 121ºC for 15 minutes. After cool down to 40 ºC, one drop of suspended strain was inoculated to the nutrient agar medium with the help of a sterilized disposable pipette near the burner. About 20 mL of medium was poured into the sterilized petri dishes and left 10-15 mins in order to set the agar. After that the agar wells were made with a 10 mm sterilized cork bare and the wells were filled with 0.1 mL of each extract sample to be tested. And the plates were incubated at 27 ºC for 24 hours. After incubation, the diameters of inhibition zones including 10 mm wells were measured.

Results and Discussion

Phytochemicals of *Calophyll inophyllum* Linn. (Pong-nyet)

In order to know the types of phytoorganic constituents present in flowers, leaves and seeds of Pong-nyet, the phytochemical investigation was preliminarily carried out according to the conventional methods. The results obtained from these experiments were summarized in Table 1. The phytochemical tests revealed the presence of the secondary metabolites such as carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, starch, steroids, tannin and terpenoids in all of the samples. But alkaloids, α-amino acids, cyanogenic glycosides and saponins were found to present as small amounts in all of the samples.

The main constituents such as phenolic compounds, terpenoids, flavonoids and steroids present in Pong-nyet may contribute to possession of bioactivities such as anti-bacterial, analgesic, anti-fungal, anti-inflammatory, antimalarial and cytotoxic properties in Pong-nyet samples

Table 1. Results of Preliminary Phytochemicals Tests on Flowers, Leaves and Seeds of Pong-nyet

No	**Test**	**Extract**	**Test Reagent**	**Observation**	**Remark**		
					Flowers	**Leaves**	**Seeds**
1	Alkaloids	1% HCl	Wagner's Reagents	Reddish brown	+	+	+
			Mayer's Reagent	White ppt.	+	+	+
			Dragendroff's Reagent	Orange ppt.	+	+	+
			Sodium picrate	Yellow ppt.	+	+	+
2	α-Amino acids	H_2O	Ninhydrin Reagent	Pink colouration	+	+	+
3	Carbohydrates	H_2O	10% α-naphthol and conc.H_2SO_4	Red ring	+ +	+ +	+ +
4	Cyanogenic glycosides	H_2O	Sodium picrate solution	Brick-red colouration	+	+	+
5	Flavonoids	EtOH	Mg and conc.HCl	Brick-red colouration	+ + +	+	+ +
6	Glycosides	EtOH	10% lead acetate	White ppt.	+ +	+ +	+ +
7	Organic acids	H_2O	Bromocresol blue	Yellow colouration	+ +	+	+ + +
8	Phenolic compounds	EtOH	1% $FeCl_3$	Brown ppt.	+ + +	+ +	+ + +
9	Reducing sugars	H_2O	Fehling's A and B	Brick-red ppt.	+ +	+	+ +
10	Saponins	H_2O	Distilled water	Frothing	+	+	+
11	Starch	H_2O	Iodine solution	Blue colouration	+ +	+	+ +
12	Steroids	Toluene	Acetic anhydride & conc.H_2SO_4	Green colouration	+ + +	+ +	+ +
13	Tannins	EtOH	2% Gelatin	White ppt.	+ +	+ +	+ +
14	Terpenoids	$CHCl_3$	Acetic anhydride & conc.H_2SO_4	Greenish blue colouration	+ + +	+ +	+ +

(+)	= small amount present	(+ + +)	= large amount present
(+ +)	= medium amount present	(-)	= absent

Some Physicochemical Contents of Flowers, Leaves and Seeds of Pong-nyet

The nutrient values such as moisture, ash, fibre, protein, fat and carbohydrate contents are shown in Table 2. Moisture content of pong-nyet seeds (23%) was found to be higher than flowers (10.47 %) and leaves (9.74 %). It may be due to the fresh seeds and that of flowers (10.47 %) was also higher than leaves (9.74 %) due to the sample collecting period, i.e., flowers were collected in October (rainy season). Ash contents of three samples : flowers, leaves and seeds were 5.17 %, 4.87% and 4.5 % respectively and were observed not to be different to each other. The crude fibre contents of flowers (9.55 %) and leaves (11.62 %) were found to be comparable and seeds (5.6 %) contained lower amount of fibre. The protein contents for flowers, leaves and seeds were observed to be 8.47 %, 5.88 % and 6.9 %, respectively. The fat content of fresh seeds was found to be 55.67 % and those for flowers and leaves were to be 8.60 % and 7.15 %, respectively. The carbohydrate contents in flowers, leaves and seeds were observed to be 57.81 %, 60.72 % and 4.33 %, respectively.

Table 2. Some Physicochemical Contents of Flowers, Leaves and Seeds of Pong-nyet

No.	**Parameters**	**% Contents (w/w)**		
		Flowers	**Leaves**	**Seeds**
1.	Moisture content	10.47	9.74	23.00
2	Ash content	5.17	4.87	4.50
3.	Fibre content	9.55	11.62	5.60
4.	Protein content	8.47	5.88	6.90
5.	Fat content	8.60	7.15	55.67
6.	Carbohydrate content	57.81	60.72	4.33

Some Elements Present in Flowers, Leaves and Seeds of Pong-nyet by ED XRF Method

X-ray spectrometer permits simultaneously analysis of light element to heavy element (Griken, *et.al.*, 1986) Shimadzu EDX-700 spectrometer can analyze the elements from Na to U under vacuum condition. In this work, relative abundance of elements present in flowers, leaves and seeds of pong-nyet were determined by ED XRF spectrometer. The ED XRF spectra of above three samples are shown in Figures 1, 3 and data are listed in Table 3. It can be observed that flowers and leaves of Pong-nyet were found to contain K and Ca as major elements and Pong-nyet seeds contained K, Ca, Fe and Cu as major elements. In addition, Fe, S, Zn and Cu as minor elements and Y and Sr as trace elements were present in flowers of Pong-nyet. Moreover, leaves of Pong-nyet contained Fe, Cu and Sr as minor elements. But S, Zn and Y in Pong-nyet leaves and S, Zn, Y and Sr in Pong-nyet seed were not detected.

Table 3. Relative Abundance of Some Elements in Flowers, Leaves and Seeds of Pong-nyet by ED XRF Method

No.	**Elements**	**Relative Abundance (%)**		
		Flowers	**Leaves**	**Seeds**
1.	Potassium (K)	44.736	26.214	36.952
2	Calcium (Ca)	35.422	61.419	38.304
3.	Iron (Fe)	7.698	9.315	12.861
4.	Sulphur (S)	7.835	-	-
5.	Zinc (Zn)	1.450	-	-
6.	Copper (Cu)	1.339	1.968	11.883
7.	Yttrium (Y)	0.768	-	-
8.	Strontium (Sr)	0.754	1.084	-

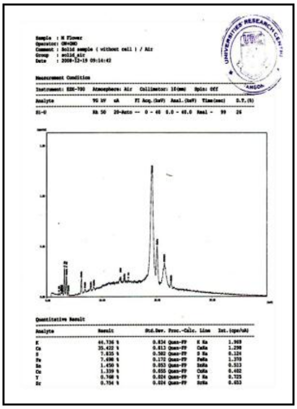

Figure 1. ED XRF spectrum of leaf of Pong-nyet

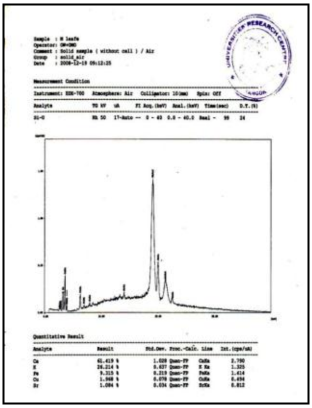

Figure 2. ED XRF spectrum of flower of Pong-nyet

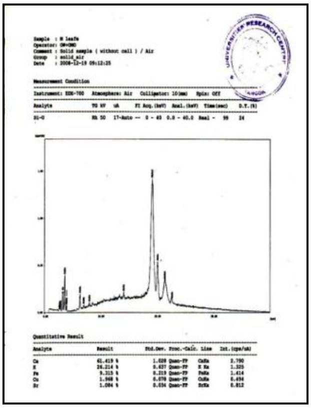

Figure 3. ED XRF spectrum of seed of Pong-nyet

Antimicrobial activity of some crude extracts by agar well diffusion method

Screening of antimicrobial activity of various crude extracts such as PE, $CHCl_3$, EtOAc, 95% EtOH and H_2O extracts from flowers, leaves and seeds of Pong-nyet were investigated by employing agar well diffusion method. In this study, the samples were tested on six species of microorganisms such as *Bacillus substilis*, *Staphylococcus aureus* (ATCC-12277), *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albicans* and *Escherichia coli* species. The inhibition zone diameter shows the degree of the antimicrobial activity. The larger the inhibition zone diameters, the higher the antimicrobial activity. The inhibition zones of crude extracts against six microorganisms tested are shown in Figures 4 to 9 and the observed data are summarized in Table 4.

From these results, it was found that watery extracts of all samples did not exhibit antimicrobial activity against all tested microorganisms. Among the crude extracts tested, EtOAc extracts of flowers, leaves and seeds showed the most pronounced antimicrobial activity against six microorganisms with inhibition zone diameter ranged in 19 mm to 29 mm.

In addition, PE extracts (inhibition zone diameter ranged in 12 mm to 25 mm), $CHCl_3$ extracts (inhibition zone diameter ranged in 14 mm to 20 mm) and 95 % EtOH extracts (inhibition zone diameter ranged in 12 mm to 26 mm) of flowers, leaves and seeds of Pong-nyet were also observed to exhibit antimicrobial activity against six species of microorganisms tested.

According to the results, all crude extracts of flowers, leaves and seeds of Pong-nyet; except watery extracts showed significant antimicrobial properties against both Gram (+)ve and Gram (-) ve microorganisms tested and flowers and seeds extracts were found to possess higher antimicrobial activity than leaves extract .

Table 4. Inhibition Zone Diameters of Various Crude Extracts of Flowers, Leaves and Seeds of Pong-nyet Against Six Microorganisms by Agar Well Diffusion Method

Microorganisms	**Types of Microorgani-sms**	**Sample**	**Inhibition Zone Diameters (mm)**				
			PE	**$CHCl_3$**	**EtOAc**	**EtOH**	**H_2O**
Bacillus substilis	Gram (+)ve	Seeds	20	19	21	22	10
		Leaves	10	14	20	10	10
		Flowers	10	14	24	14	10
Staphylococcus aureus	Gram (+)ve	Seeds	21	20	19	22	10
		Leaves	10	10	20	10	10
		Flowers	15	17	25	12	10
Pseudomonas aeruginosa	Gram (-)ve	Seeds	19	20	20	21	10
		Leaves	12	17	24	16	10
		Flowers	16	17	29	16	10
Bacillus pumalis,	Gram (+)ve	Seeds	20	20	20	21	10
		Leaves	10	20	22	18	10
		Flowers	17	17	25	17	10
Candida albicans	Gram (+)ve	Seeds	20	18	20	21	10
		Leaves	20	25	20	25	10
		Flowers	19	18	27	20	10
Escherichia coli	Gram (-)ve	Seeds	25	18	29	26	10
		Leaves	15	20	25	22	10
		Flowers	20	20	25	19	10

Agar well diameter - 10 mm   15 mm -17 mm (+ +)
10 mm – 14 mm (+)   20 mm -above (+ + +)

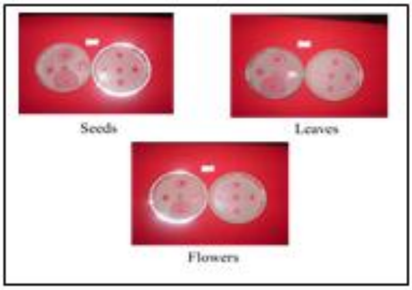

Figure 4. **Inhibition zones of various crude extracts against *Bacillus subtilis***

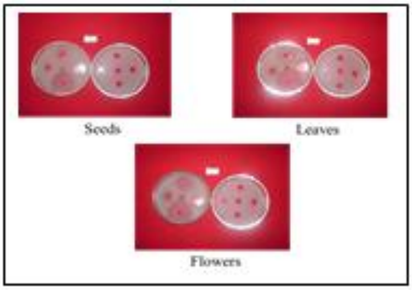

Figure 5. **Inhibition zones of various crude extracts against *Staphylococcus aureus***

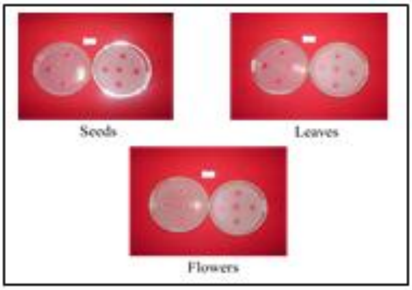

Figure 6. **Inhibition zones of various crude extracts against *Pseudomonas aeruginosa***

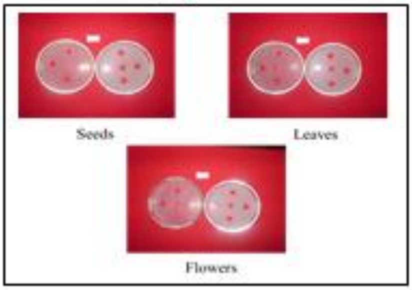

Figure 7. **Inhibition zones of various crude extracts against *Bacillus pumalis***

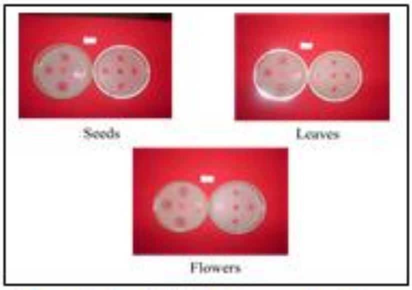

Figure 8. **Inhibition zones of various crude extracts against *Candida albicans***

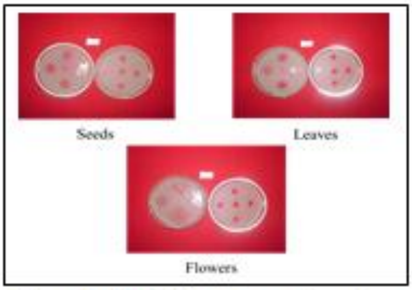

Figure 9. **Inhibition zones of various crude extracts against *Escherichia-coli***

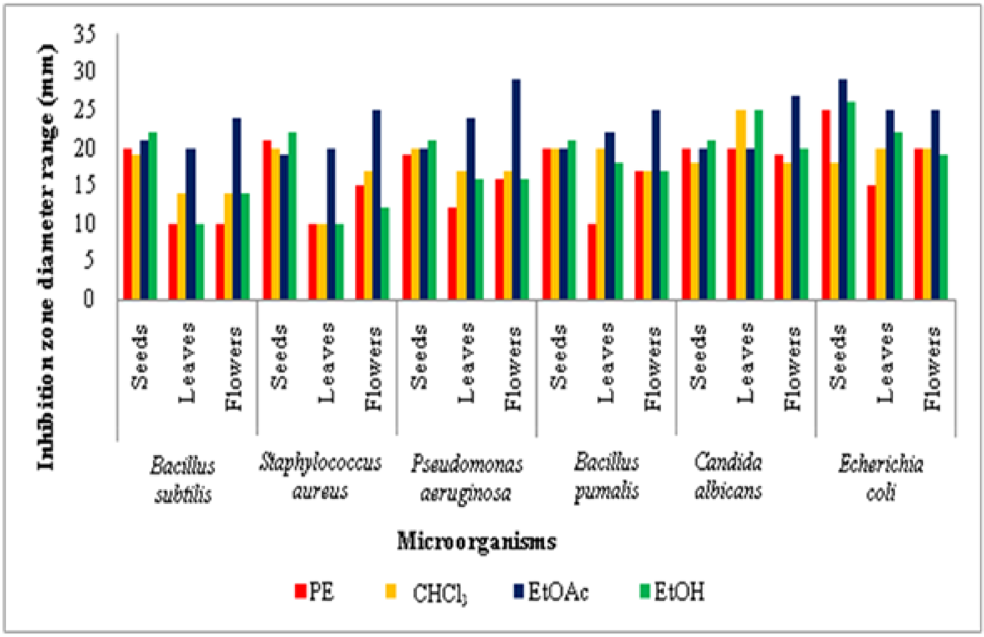

Figure 10. Comparison of inhibition zone diameters for extracts of flower, leaf and seed of Pong-nyet against six microorganisms

Conclusion

From overall assessment of the present work concerning with the investigation of some bioactive phytochemical constituents in *Calophyllum inophyllum* Linn. (Pong-nyet) and evaluation of some biological activities, the following inferences could be deduced.

- The preliminary phytochemical tests revealed the presence of carbohydrates, flavonoids, glucosides, organic acids, phenolic compounds, reducing sugars, starch, steroids, tannin and terpenoids are present as major constituents and alkaloids, α- amino acid, cyanogenic glycosides and saponins present as minor constituents in all of the three samples.
- From the results of some physicochemical analyses, flowers of Pong-nyet was found to contain 10.4 % of moisture, 5.17% of ash, 9.55 % of crude fibre, 8.47 % of protein, 8.60 % of fat and 57.81 % of carbohydrates, leaves of Pong-nyet found to contain 9.74 % of moisture,

4.87 % of ash, 11.62 % of crude fibre, 5.88 % of protein, 7.15 % of fat and 60.72 % of carbohydrates and seeds of Pong-nyet found to contain 23.00 % of moisture, 4.5 % of ash, 5.6 % of crude fibre, 6.90 % of protein,55.67 % of fat and 4.33% of carbohydrates, respectively.

- Qualitative ED XRF elemental analysis revealed that potassium and calcium as major elements and iron and copper as minor elements present in all samples. Sulphur, zinc and yttrium are present in flowers but not in seeds and leaves.
- Screening of antimicrobial activity by agar well diffusion method showed that all of the crude extracts except watery extract have pronounced antimicrobial activity with inhibition zone diameters ranged between 18mm ~ 29 mm in seeds, 12 mm~ 25mm in leaves and 12mm~ 29 mm in flowers of Pong-nyet respectively.

Acknowledgement

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References

Allen, J.A. (2002). "*Calophyllum inophyllum* Linn.", In: Vozzo, J.A., (Edn.), Tropical Tree Seed Manual, Agriculture Handbook 721. U.S. Forest Service, Washington, D.C, 357-358,

Dweck, A.C. and T. Meadows. (2002). "Tamanu *C. inophyllum*- the African, Asian, Polynesian and Pacific Panacea". *Inter, J. of Cosm. Sci*., **24**, 18-23

Dharmarate, H.R.W. and W.M. A. P. Aahigasekera. (1996). "Xanthones from Root Bark of *C.thwaitesi*i". *Phytochemistry,* **42**, 249-250

Harborne, J. B. (1984). *Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis*. London : 2nd Ed., Chapman and Hall, 120-126

Krantz, J.C. and C. J. Carr. (1969). "Pharmacologic Principles of Medical Practice". 7th Edn., Calcutta, Williams and Wilkins, **55**, 952-956

Marini-Bettolo, G. B., M. Nicolettic and M. Patamia. (1981). “Plant Screening by Chemical and Chromatographic Procedure Under Field Conditions”. *Journal of Chromatography*, **113** (1), 113-127

Tin Wa, M. (1972).“Screening Methods and Procedures”. *Phytochemical Bulletin of Botanical Society of America*, **5** (3), 4 –10

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A Comparative Study on Bioactivities of Silver Nanoparticles, Gold Nanoparticles and Gold-Silver Bimetallic Nanoparticles

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Abstract

In this study, the silver nanoparticles (AgNPs), gold nanoparticles (AuNPs) and gold-silver bimetallic nanoparticles (Au-Ag NPs) were synthesized from silver nitrate ($AgNO_3$) and chloroauric acid ($HAuCl_4$) by using chitosan solution as reducing agent. The existence of nanoparticles in colloidal solution was confirmed by Tyndall effect. In the UV-vis spectra, the maximum absorption of AgNPs, AuNPs and Au-Ag NPs were observed at the wavelengths of near 399 nm, 520 nm and 479 nm which confirms the presence of AgNPs, AuNPs and Au-Ag NPs in colloidal solution. These samples were characterized by XRD, FT IR, EDXRF and SEM. XRD spectral data indicated that the prepared NPs were crystalline nature with nanosizes. The corresponding functional groups and the relative abundance of elements in nanoparticles were observed from FT IR and EDXRF analyses. From the EDXRF results, the relative abundance of Ag and Au were found to be 8.28 % of Ag in AgNPs, 10.72 % of Au in AuNPs and 15.09 % Ag, 10.32 % Au in Au-Ag NPs. From the SEM analyses the shape of AgNPs and AuNPs were spherical shape and Au-Ag NPs was irregular shapes which may be due to the formation of alloy. The antimicrobial activities of prepared AgNPs, AuNPs and Au-Ag NPs were carried out. According to the results, bimetallic nanoparticle is the highest activity on *S.aureus and P.aeruginosa* than monometallic nanoparticles such as silver nanoparticles and gold nanoparticles.

Keywords: Chitosan, silver nanoparticles, gold nanoparticles, gold-silver bimetallic nanoparticles, antimicrobial

Introduction

Nanoscience and nanotechnologies are widely seen as having huge potential to bring benefits to many areas of research and application, and are attracting rapidly increasing investments from Governments and from

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businesses in many parts of the world. Nanoparticles are being used in medical field to create different therapies and treatment for different diseases. Nanoparticles are being used in many branches of medicine, one attribute these particles have in common is that they need to be biocompatible. Silver nanoparticles have widely been applied in medical domains due to their antimicrobial and wound healing properties (Balashanmugam *et al.,* 2017). Gold nanoparticles have been a great source of interest due to their novel characteristic such as size-related electronic, optical, chemical, and magnetic properties that are potentially useful in biological applications. Gold nanoparticles become strong antioxidant agent and play an important role in burn healing (Pik *et al.,* 2016). Bimetallic nanoparticles (BMNPs) have excelled monometallic nanocrystals owing to their improved electronic, optical and catalytic performances (Ramos *et al.,* 2010). Gold-silver alloy nanoparticles might have additional biomedical applications because of their distinct optical properties ranging from visible to the near- IR wavelength region (Kim *et al.,* 2008). Chitosan is a modified natural carbohydrate polymer prepared by the partial acetylation of chitin, a natural biopolymer derived from crustacean shells such as crabs, shrimps and lobsters. The biocompatibility and antibacterial properties of chitosan and its being an environmentally friendly polyelectrolyte makes it attractive in academic research (Mansor *et al.,* 2011).

Materials and Methods

Materials

Commercial chitosan sample from shrimp shell was purchased from Asian Technology Groups Co., Ltd., Local Industry, Yangon, Myanmar. 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.

Synthesis of Silver Nanoparticles, Gold Nanoparticles and Gold-Silver Bimetallic Nanoparticles

Silver nanoparticles (AgNPs), gold nanoparticles (AuNPs) and gold –silver bimetallic nanoparticles (Au-Ag NPs) were prepared using chitosan solutions (1 % w/v) as reducing agents as well as stabilizing agents. 50 mL of 1 % (w/v) chitosan solution was heated to 80 ± 3 ºC. 4 mL of silver nitrate (0.01 M) for AgNPs, 4 mL of chloroauric acid (0.001 M) for AuNPs and 25 mL of each silver nitrate and chloroauric acid for Au-Ag NPs were added rapidly to heated chitosan solution at a constant stirring rate. Colour changes from pale yellow to brown for AgNPs, pale yellow to wine red for AuNPs and pale yellow to purple for Au-Ag NPs were observed due to the complex formation between chitosan and Ag^+ ion, Au^{3+} ion. The reaction was carried out under dark conditions.

Characterization of Prepared AgNPs, AuNPs and Au-Ag NPs

The prepared AgNPs, AuNPs and Au-Ag NPs were characterized by Tyndall effect, UV-vis spectroscopy, XRD analysis, FT IR, EDXRF, SEM and determined their antimicrobial properties.

Results and Discussion

Tyndall Effect

The presence of nanoparticles in solutions was detected by using laser beam as Tyndall effect. The scattering of laser beam through solution indicated the presence of nanoparticles. Tyndall effects on silver nanoparticles, gold nanoparticles and gold-silver bimetallic nanoparticles are shown in Figure 1.

AgNPs AuNPs Au-Ag NPs

Figure 1. Tyndall effect on silver nanoparticles, gold nanoparticles and gold-silver bimetallic nanoparticles

Analysis of Gold-silver Bimetallic Nanoparticles by UV-vis Spectroscopy

The prepared AgNps, AuNPs and Au-Ag NPs samples were subjected to UV-visible spectrophotometer and recorded the spectra. Figure 2 showed UV-visible spectra of AgNPs, AuNPs and Au-Ag NPs. The maximum absorption peaks were observed at 399 nm for AgNPs, 520 nm for AuNPs and 479 nm for Au-Ag NPs which correspond to the wavelengths of surface plasmon resonance (SPR). These results are in agreement with the various reports (Zhang *et al.*, 2007). The maximum absorption wavelengths of prepared AgNPs, AuNPs and Au-Ag NPs are summarized in Table 1.

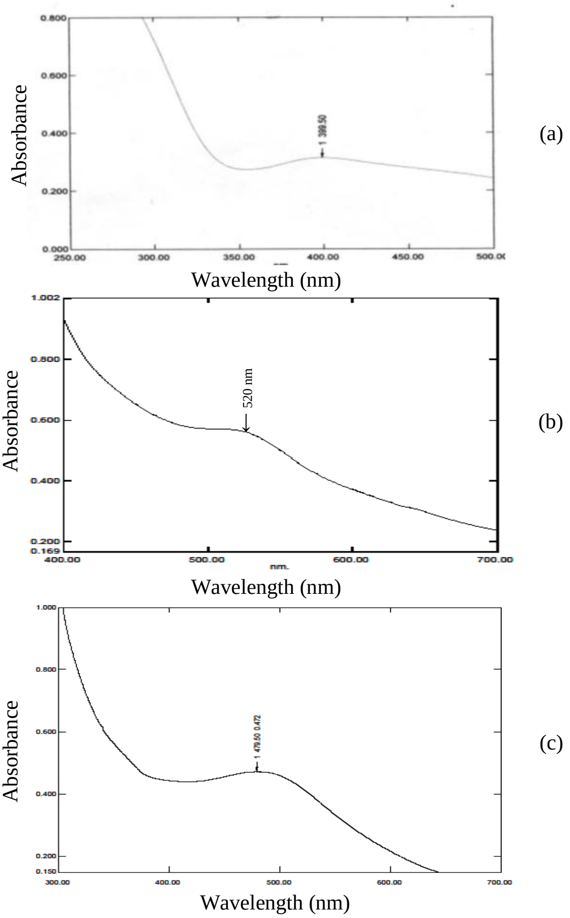


Figure 2. UV-vis spectra of (a) AgNPs, (b) AuNPs and (c) Au-Ag NPs

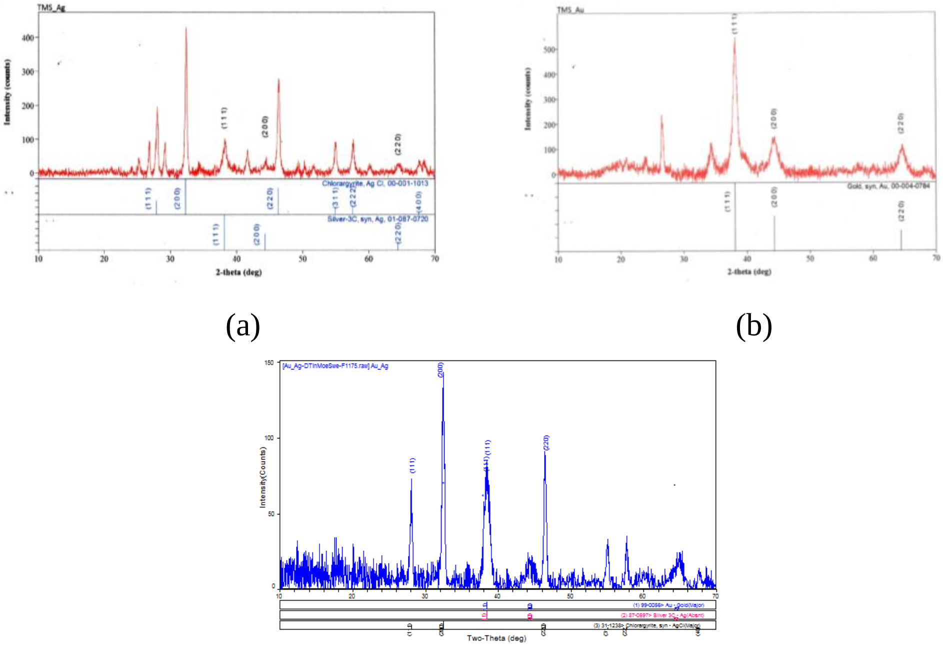
Table 1. The Maximum Absorption Wavelength of Silver Nanoparticles, Gold Nanoparticles and Gold-silver Bimetallic Nanoparticles

Nanoparticles	**Observed wavelengths of maximum absorption (nm)**	**Literature value (nm)**
AgNPs	399	*390-420
AuNPs	520	**514-550
Au-Ag NPs	479	*396-524

* (Zhang *et al.*, 2007) ** (Martinez *et al*., 2012)

X-ray Diffraction Analysis

Structural characterization had been performed using XRD analysis and the XRD diffractogram of AgNPs, AuNPs and Au-Ag NPs are illustrated in Figure 3. The mean crystallite size of AgNPs, AuNPs and Au-Ag NPs 2 were 17.3 nm, 8.56 nm and 12.91 nm respectively.



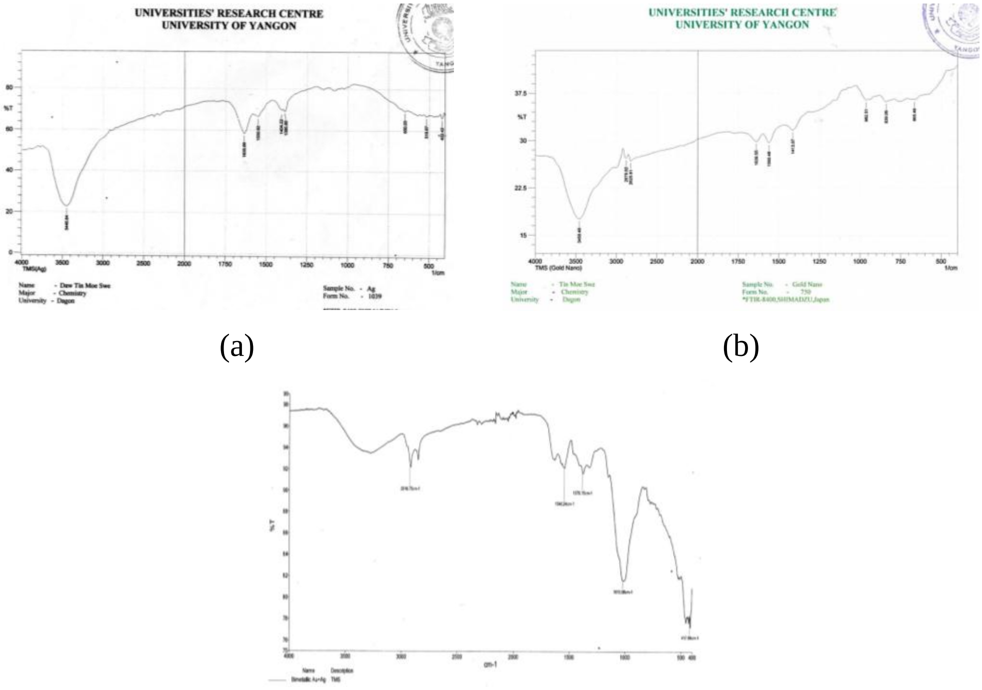
(a) (b)

(c)

Figure 3. X-ray diffractograms of (a) AgNPs, (b) AuNPs and (c) Au-Ag NPs

Fourier Transform Infra Red Spectrpscopy (FT IR)

Fourier transform infra-red spectroscopy of AgNPs, AuNPs and Au-Ag NPs were performed to study the interaction of nanoparticles with that of chitosan. The respective spectra are presented in Figure 4. All spectra exhibited the functional groups of chitosan. The band appearing at 3200 cm^{-1} to 3600 cm^{-1} correspond to stretching vibration of NH. The bands nearly 1560 cm^{-1} to1650 cm^{-1} were due to the stretching of C=O of amide group. This C=O functional group may be due to the presence of chitin left in the chitosan (incomplete the deacetylation step in synthesis of chitosan from chitin). The stretching of C-O group appears at 1550 cm^{-1} to 1600 cm^{-1}. The stretching of C-N bond observed at nearly 1315 cm^{-1} to 1441 cm^{-1}.The results indicated that chitosan may have the ability to perform reducing agent as well as capping agent.



(a) (b)

(c)

Figure 4. FT IR sprectra of (a) AgNPs, (b) AuNPs and (c) Au-Ag NPs

Table 2. FT IR Band Assignment for Silver Nanoparticles and Gold Nanoparticles and Gold-silver Bimetallic Nanoparticles

Observed wave number (cm^{-1})			***Literature wave number (cm^{-1})**	**Band assignment**
AgNPs	**AuNPs**	**Au-Ag NPs**		
3448	3458	3446	3200-3600	-OH stretching and N-H stretching
-	2825	2875	2800-2950	C-H stretching
1635	1639	1633	1560-1650	C=O stretching of chitin
1550	1560	1540	1550-1600	C-O stretching
1404	1413	1384	1315-1441	C-N stretching in amine group

*(Silverstein and Basseler 1967)

Energy Dispersive X-ray Florescence (EDXRF) Analysis

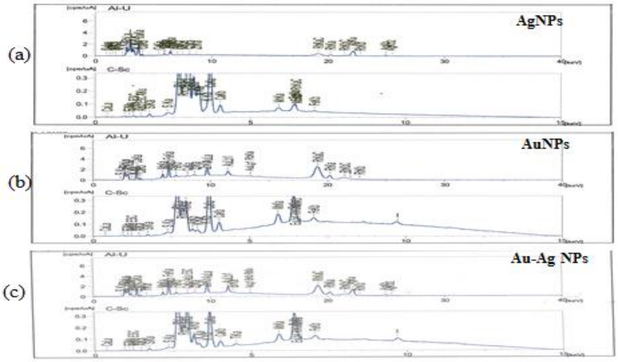


Figure 5. EDXRF spectra of (a) AgNPs, (b) AuNPs and (c) Au-Ag NPs

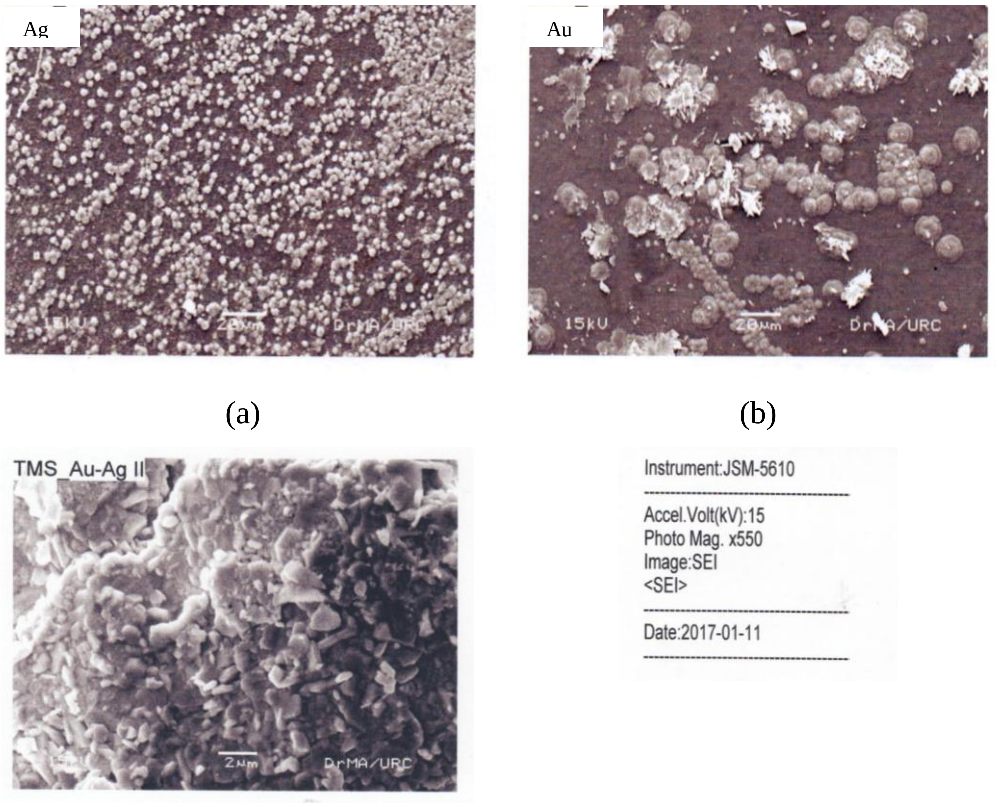
EDXRF technique was used for the determination of relative abundance of elements present in samples. The film form of samples AgNPs, AuNPs and Au-Ag NPs were conducted with EDXRF analyses. 8.28 % of Ag in AgNPs, 10.72 % of Au in AuNPs, 15.09 % Ag and 10.325 % Au in Au-Ag NPs were detected (see Figure 5). Calcium was attributed from commercial chitosan used which may be incomplete demineralization in chitosan production.

Table 3. Relative Abundance of Elements in Silver, Gold and Gold-silver Bimetallic Nanoparticles

Elements	**Relative Abundance of Elements (%)**		
	AgNPs	**AuNPs**	**Au-Ag NPs**
Ca	51.896	49.065	33.421
K	17.774	2.374	1.687
Si	13.306	9.606	12.511
Ag	**8.282**	-	**15.093**
Au	-	**10.720**	**10.325**
Fe	3.066	10.971	9.752
S	2.391	3.540	2.762
Mn	1.955	4.824	3.219
Cr	0.398	-	-
Cu	0.383	0.533	0.434
Zn	0.204	0.966	0.432
Ni	0.174	-	-
Al	0.170	-	8.703
Rb	-	0.091	0.069
Ti	-	-	1.244
Br	-	-	0.350

Scanning Electron Microscopic (SEM) Analysis

Scanning electron microscopy gave the morphological examination with direct visualization. This techniques offer several advantages in morphological and limited information about the size distribution. Structural and surface morphology of silver nanoparticles, gold nanoparticles and gold-silver bimetallic nanoparticles were analyzed by SEM analysis. The micrographs are presented in Figure 6. The various spherical shapes were observed in AgNPs and AuNPs and Au-Ag NPs was irregular shape which may be due to the formation of alloy.



(a) (b)

Instrument:JSM-5610

Accel.Volt(kV):15
Photo Mag. x550
Image:SEI
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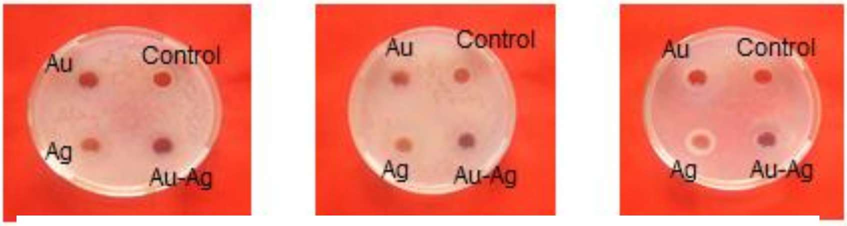
(c)

Figure 6. SEM micrographs of (a) AgNPs, (b) AuNPs and (c) Au-Ag NPs

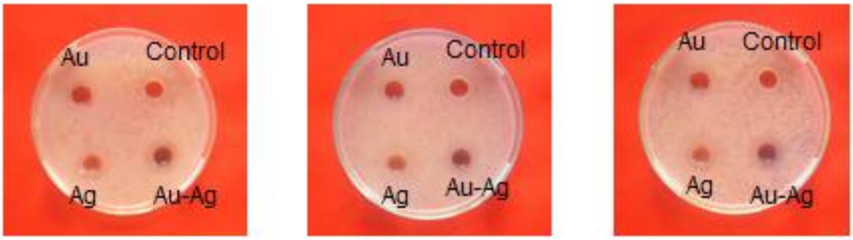
Antimicrobial Activities

Screening of antimicrobial activities of AgNPs, AuNPs and Au-Ag NPs were evaluated against with different strains of microorganisms by agar well diffusion method. The antimicrobial activities of these nanoparticles are shown in Figure 7 and the results are summarized in

Tables 4. According to the results, all of the samples active to all tested microorganisms especially on *S.aureus and P.aeruginosa.*



B. subtiilis ***S. aureus*** ***P. aeruginosa***



B. pumilus ***C. albicans*** ***E.coli***

Figure 7. Antimicrobial Activities of gold, silver and gold-silver Bimetallic nanoparticles

Table 4. Antimicrobial Activities of the Prepared AgNPs, AuNPs and Au-Ag NPs

Sample	**Inhibition Zone Diameters (nm)**					
	B. subtilis	***S. aureus***	***P. aeruginosa***	***B. pumilus***	***C. albicans***	***E. coli***
AuNPs	17mm (++)	13mm (+)	15mm (++)	13mm (+)	16mm (++)	17mm (++)
AgNPs	18mm (++)	13mm (+)	25mm (+++)	15mm (++)	19mm (++)	7mm (++)
Au-Ag NPs	17mm (++)	20mm (+++)	23mm (+++)	17mm (++)	18mm (++)	8mm (++)
Control	-	-	-	-	-	-

Agar well – 10 mm, 10 mm ~ 14 mm (+), 15 mm ~ 19 mm (++), 20 mm above (+++)

Conclusion

From the overall assessment of the present work, yellow, wine red and purple colour of silver, gold and gold-silver bimetallic nanoparticles were successfully synthesized. By the Tyndall effect, the laser light scattering indicates the presence of nanoparticles. From the UV-visible spectrum, the maximum absorption wavelengths of prepared silver, gold and gold-silver bimetallic nanoparticles were found to be 399 nm, 520 nm and 479nm respectively. The X-ray diffractograms of nanoparticles showed the crystalline nature. The FT IR spectra of selected nanoparticles showed the absorption bands of functional groups of chitosan. It also confirmed that the reducing agents have ability to perform the capping agent. From the EDXRF results, the relative abundance of Ag and Au were found to be in AgNPs, AuNPs and Au-AgNPs. From the SEM micrograph, AgNPs and AuNPs have spherical shape but Au-Ag NPs have undefined shape due to the formation of alloy. From the results of antimicrobial investigation, all samples are active to all tested microorganisms. Among them, bimetallic nanoparticles are more active than monometallic nanoparticles.

Acknowledgements

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References

Balashanmugan, P., J.K. Hyung, T.K. Puthupalayam and V. Singh. (2017). " An in Vitro Study on the Burn Wound Healing Activity of Cotton Fabrics incorporated with Phytosynthesized Silver Nanoparticles in Male Wister Albino Rats", *European Journal of Pharmaceuticals Science,* **100**(2017), 187-196

Kim, J. H., W. W. Bryan, T. R. Lee. (2008). "Preparation, Characterization, and Optical Properties of Gold, Silver, and Gold-silver Alloy Nanoshells Having

Silica Cores". http://www. doi.org/10.1021/la 8016497 (Accessed 10th October, 2016)

Mansor, B. A., M. Y. Tay, K. Shameli, M. Z. Hussein and J. J. Lim. (2011). "Green Synthesis and Characterization of Silver/ Chitosan/ Polyethylene Glycol Nanocomposites without Any Reducing Agent". *International Journal of Molecular Science*, **12**, 4872-4884

Martinez, J. C., N. A. Chequer, J. L. Gonalez and T. Cordova. (2012). "Alternative Metodology for Gold Nanoparticles Diameter Characterization Using PCA Technique and UV-VIS Spectroscopy". *Nanoscience and Nanotechnology,* **2**(6), 184-189

Pik, S. L., B. Hazri and N. Wan. (2016). "Influence of Gold Nanoparticles on Wound Healing treatment in Rat Model: Photobiomdulation Therapy", *Lasers in Surgery and Medicine*, **8**(5), 1-7

Ramos, M., D. A. Ferrer, R. R. Chianelli, V. Correa, J. S. Matos and S. Flores. (2010). "Synthesis of Ag-Au Nanoparticles by Galvanic Replacement and Their Morphological Studies by HRTEM and Computational Modeling". *Journal of Nanomaterials*, **20**(11), 1-5

Silverstein, R. M. and G. C. Basseler. (1967). "*Spectrometric Identification of Organic Compounds; Ch 4*". New York: Willey and Sons

Zhang, Q., J. Y. Lee, J. Yang, C. Boothroyd and J. Zhang. (2007). "Size and Composition Tunable Ag-Au Alloy Nanoparticles by Replacement Reaction". *Nanotechnology*, **18,** 1-8

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Bleaching of Palm Oil by Acid Activated Local Bentonite Clay

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Abstract

The bleaching potential of bentonite, from Kyaukpadaung Township, Mandalay Region, for removal of pigments from palm oil was investigated. In this study, raw bentonite clay was treated with 4 N HCl solution to get better adsorption properties. Characterizations of raw bentonite clay and 4 N HCl activated bentonite clay samples were carried out by using FT IR, XRD and chemical analyses. Before and after bleaching processes, some physicochemical properties of crude palm oil were determined by AOAC and AOCS methods. In this research, bleaching efficiencies of the bentonite clay on palm oil were determined spectrophotometrically. In this research, bleaching efficiencies of the raw bentonite clay and 4 N HCl acid activated bentonite clay samples were determined at 65, 80 and 90 ºC for different contact time. The bleaching efficiencies of raw bentonite clay samples and 4 N HCl activated bentonite clay were 14.3 and 42.8%, respectively, for 100 min contact time. Consequently, 4 N HCl activated bentonite clay sample gave the higher bleaching efficiencies than raw sample as well as bleaching efficiency increased with increase in contact times and temperature. Langmuir and Freundlich models were used to study the adsorption mechanism. The equilibrium data was analyzed by using Freundlich and Langmuir adsorption isotherms and it was found that the former isotherm provided a better fit for the data.

Keywords: Bentonite, bleaching, palm oil, physicochemical properties and absorption isotherm

Introduction

Palm oil in its raw form contains impurities such as organic pigments, oxidation metals, trace metals and trace soaps. For palm oil to be edible these impurities which negatively influence the taste and smell of the oil as well as its appearance and shelf life stability, thus reducing consumer acceptance and marketability have to be removed. Thus the refining of palm

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oil through adsorptive bleaching remains inevitable in the oil refining industry. Bleaching remove pigments and other unwanted compounds which negatively influence the taste of the oil by bringing into contact with a surface-active substance that adsorbs the undesired particles. Bleaching of palm oil is done by the use of adsorbents; usually activated carbons and activated bleaching earths. Natural clays are acquiring prominence as low-cost adsorbents over the last few decades due to their local and abundant availability and the capability to undergo modification to enhance the surface area, adsorption capacity, and range of applicability (Salawudeen *et al.*, 2007).

Therefore, the adsorption properties and range of applicability, a number of physical and chemical methods have been investigated to modify the clays which including heat treatment, acid activation, treating the cationic surfactants and polymer modification. In Myanmar, Bentonite occurs in several places around the Kyauktaga area in Kyaukpadaung Township, Mandalay Region (Economic and Social, 1996).

Acid activation of bentonites is one of the most important processes that have been performed to achieve desirable structural condition in edible oil bleaching applications. Activated bentonites are removing chlorophyll, carotenoids, phospholipids, metals and oxidation products from oils (Makhoukhi *et al.*, 2009). Among different bleaching methods, adsorptive bleaching is most commonly practiced because of its nondestructive nature and can simultaneously remove the undesired components (Bijay, 2009).

The aim of this research is to study of efficiency in removal of colouring material and impurities from crude oil by using clay mineral. Local bentonite clay was used for the removal of colouring materials and impurities in palm oil sample. At first physicochemical properties (moisture and impurities, iodine value, peroxide value, free fatty acid content, saponification value, unsaponifiable matter, refractive index and specific gravity) of palm oil was determined by using appropriate methods. Acid activation of bentonite clay samples were carried by using 4 N solution for better adsorption properties and characterized by using FT IR, XRD and chemical analysis. In this study, the bleaching efficiencies of raw and acid activated bentonite clay on palm oil sample was studied by using the various contact times and different temperatures.

Materials and Methods

All chemicals used in the research were from British Drug House Chemical Limited, Poole, England. The chemicals had been used as it was received unless otherwise stated. Apart from the glassware and other supporting equipment are balance (E Mettler, AE 160), Thermostatic shaker (Yamaha), XRD, (Regaku; D/Max 2200 Japan), FT IR (Perkin Elmer 1600) and TG-DTA (Hi-TGA 2950 Thermo Gravimetric, Shimadzu Analyzer).

Sample collection and characterization

The grey coloured bentonite clay sample was obtained locally from Kyaukpadaung Township, Mandalay Region. The crude palm oil used for this study was obtained from Myeik Township, Tanintharyee Region. Petroleum ether and hydrochloric acid used were of analytical grade. The physicochemical properties of the palm oil were determined according to the AOAC and AOCS methods.The clay sample was characterized using FT IR and XRD techniques. Some physicochemical properties of local bentonite clay samples were determined. The chemical properties (loss on ignition, SiO_2, Al_2O_3, CaO, MgO and Na_2O + K_2O percent) were determined according to standard methods of analysis of ceramic materials (Hikichi, 1998).

Preparation of acid activation of bentonite clay

The local bentonite clay sample was ground and sieved to 200 meshes. The sample was rinsed with water several times. The washed bentonite clay was dried at 105-110 °C for four hours. Then the samples were ground and stored for further use. A 20 g of clay sample was weight into a beaker. A 200 mL of 4 N hydrochloric acid was added and stirred. Acid treatment was carried out at 90 °C for 2 hours under reflux and the clay was separated by filtration. The clay residue was rinsed with water several times to remove remaining chloride ion (checked with a 1 % $AgNO_3$ solution). The washed clay material was dried at 55 °C and again crushed and passed to 200 mesh sieve. Then, the 4 N HCl acid activated clay was obtained. Acid activation was done according to the method described by Foletto *et al*., 2006.

Effect of contact time and different temperature on adsorption of palm oil sample

The adsorption treatment of palm oil with bentonite clay samples (raw and 4 N HCl acid activated bentonite clay) were carried out for different contact time (5, 15, 20, 30, 40, 50, 60, 70, 80, 90 and 100 min) at different treatment temperature (65, 80 and 90 °C). A 5.0 g of crude palm oil was placed in a glass stopper conical flask held in a shaking water bath thermostated at a temperature that is 65 °C ± 1. When the temperature reached at 65 °C, 0.2 g of clay sample was added to the hot oil, which was then heated in the shaken for different contact time (5 to 100 min) at 65 °C. After 5 min, the oil was removed from clay sample by filtration. Similarly, the respective conical flasks for different contact time (5 to 100 min) were filtered at 65°C. All kinetic experiments were determined at temperature of 65, 80 and 90 ºC.

Bleaching process and adsorption isotherms

The absorbance of the palm oil was measured using a UV-visible spectrophotometer. The bleaching efficiencies of palm oil sample after treating with bentonite clay samples were determined for different contact time (5, 15, 20, 30, 40, 50, 60, 70, 80, 90 and 100 min) at different treatment temperature (65, 80 and 90 °C). A 0.1 g of palm oil sample after bleaching with bentonite clay for 5 min contact time at 65°C was put into a conical flask and diluted by dissolving in 7.5 mL of petroleum after (analytical grade). The resulted solution for 5 min at 65°C was measured at λ_{max} 445 nm using UV-visible spectrophotometer. The percentage bleaching efficiency (% BE) was determined by the equation;

$$\% \text{ BE} = \frac{A_0 - A_t}{A_0} \times 100 \quad \text{---------} \ (1)$$

Where, A_0 is absorbance of unbleached palm oil and A_t is absorbance of bleached palm oil at time, t. Similarly, the bleaching efficiencies of palm oils after beaching with bentonite clay for different contact time (5, 15, 20, 30, 40, 50, 60, 70, 80, 90 and 100 min) at treatment temperature 80 and 90 °C were carried out in the same way as above procedure.

The models frequently used to represent the adsorption isotherms of pigments from vegetable oils or from solvents are the Langmuir and

Freundlich equations (Kamga *et al*., 2007,).The adsorption equation of Freundlich can be given by the equation;

$$\text{Log}\,(X/m) = \text{Log}\,(\lambda) + (1/n)\,\text{Log}\,(Xe) \qquad \text{------ (2)}$$

Where, λ is the constant reflecting the measurement of the capacity of adsorption. n the constant reflecting the affinity of the pigments for the adsorbent. X/m is the relative amount of pigments adsorbed per gram of adsorbent and Xe the relative amount of pigment at equilibrium. The value of the correlation coefficient obtained show that the Freundlich equation is applicable to the adsorption of palm oil carotenes by clays. Langmuir adsorption equation is given as equation,

$$\frac{X_e}{X/m} = \frac{1}{a} + \left(\frac{b}{a}\right) X_e \qquad \text{---------- (3)}$$

Where, a and b are Langmuir constants and m is the amount of adsorbent. The plot of X_e/X versus X_e was used to evaluate the values of constant a and b.

Results and Discussion

In this research, raw bentonite clay sample was used as adsorbent to remove the colouring materials and impurities in crude oils. Natural occurring bentonites may show very little activity for bleaching oils and fats. For this reason, bentonite clay mineral was treated with 4N HCl for two hours in order to evaluate important structural properties modifications that may affect oil bleaching. Figure 1 showed raw and 4 N HCl acid activated bentonite clay.

FT IR analysis

Figure 2 showed FT IR spectra of raw and 4 N acid activated bentonite clay samples. It can be observed that the hydroxyl, Si-OH, Si-H and Si-O-Si groups were presented in these spectrums of bentonite clay samples. The peak at 3622 cm^{-1} is assigned to stretching vibrations of the OH group of water molecules. The broad band at 3430 cm^{-1} and the band at 1640 cm^{-1} belong to the OH stretching and bending vibration of sorbed water molecules present in the clay, respectively. Furthermore, the strong absorption band at 1033 cm^{-1} and the band at (681-820) cm^{-1} are a result of Si–O stretching vibrations, whereas the bands at 466 and 424 cm^{-1} are assigned to the deformation vibrations of Si–O–Al and Si–O–Si, respectively.

XRD analysis

XRD measurement was carried on raw and acid activated bentonite clay samples. Figure 3 showed XRD measurements of raw and acid activated bentonite clay samples. The presence of smectite (SiO_2), feldspar and quartz compounds in bentonite clay were found in the XRD diffractogram.

Chemical analysis

Chemical constituents (SiO_2, Al_2O_3, Fe_2O_3, CaO, MgO, K_2O + Na_2O and loss on ignition) of raw and acid activated bentonite clay samples were determined according to the standard method of analysis of ceramic materials. Determination of silica percent was carried out by using alkaline fusion. Table 1 shows chemical composition of clays samples. After treating the raw bentonite clay with acid solution was found on increase in SiO_2 concentration. This may be due to the partial destruction (dissolution) of the octahedral layer (Al, Fe, Mg–O layers) and the exposure of the tetrahedral layers (Si–O layers) in acid solution.

Physicochemical properties of palm oil

Crude palm oil was determined the physicochemical properties and then treated with various adsorbent (raw bentonite and 4 N HCl acid activated bentonite clays).The physicochemical properties of bleached palm oils after treating with raw and acid activated bentonite clay were determined and shown in Table 2. The percentage of free fatty acid in palm oil sample which is treated with various adsorbents were 10.90 % (raw) and 7.24 % (4 N) respectively. Thus, it was found that free fatty acid in oil samples treated with 4 N HCl acid activated clay was less than that treated with raw bentonite clay. The free fatty acid is among the undesirable constituent to be removed and thus its low percentage enhances the efficiency of the refining process. It can be seen that the iodine values of palm oil after treating with 4 N HCl acid activated bentonite clay were larger than that after treating with the raw bentonite clay. When iodine value in oil sample is greater, the unsaturated fatty acid in oil sample is greater. The saponification value was more increase after treating with the 4 N HCl acid activated bentonite clay. High saponification value indicates present of low molecular weight fatty acid. Unsaponifiable matter means hydrocarbon, alcohols and sterols. Unsaponifiable matter in palm oil samples were 0.179 %(crude), 0.177 % (raw) and 0.073 % (4 N) respectively. After treating with 4 N HCl acid activated bentonite clay, unsaponifiable matter in palm oil samples was found to decrease significantly.

Bleaching efficiencies (% BE)

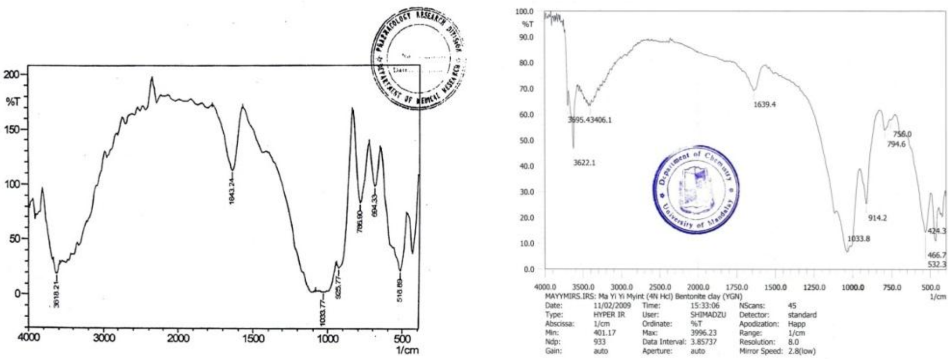
In this research, the bleaching efficiencies (%BE) of raw and 4 N HCl acid activated clay on palm oil sample at three different temperatures (65, 80 and 90 °C) and various contact time were determined. Table 3 mentioned the bleaching efficiencies of raw and 4 N HCl acid activated clay for different contact times at 65, 80 and 90 ºC. As seen in the table, the bleaching efficiency increases as the temperature and contact time increases. It was observed, the percent of bleaching efficiencies for 100 min contact time are 42.8 at 65 °C, 46.0 % at 80 °C and 47.8 at 90 °C for 4 N acid activated bentonite clay. Figure 4 showed the plot of bleaching efficiencies of raw and 4 N activated bentonite clays as a function of contact time at 65, 80 and 90 °C. As the consequence, the bleaching efficiency of 4 N HCl acid activated bentonite clay is better than raw bentonite clay.

Adsorption Isotherms of Carotene in Palm Oil

The kinetic study was shown that the required temperature are 65, 80 and 90°C, the various contact time are 5, 15, 20, 30, 40, 50, 60, 70, 80, 90 and 100 min. It this research, these parameters were selected to build the adsorption isotherms. Adsorption isotherms of carotene in palm oil on 4 N HCl acid activated bentonite clay sample were determined. Figures 5 and 6 showed the Freundlich and Langmuir isotherms for the bleaching of palm oil with 4 N HCl acid activated bentonite clay at 65, 80 and 90°C. In these Figures, Log X against Log Xe is plotted, the constants may be evaluated from slope 1/n and the intercept, λ. In these figures, the constants may be evaluated from slope 1/a and the intercept b/a in the plot Xe/X versus Xe. The parameters of Freundlich and Langmuir isotherm are mentioned in Tables 4. It can be seen that 4 N HCl activated bentonite clay has a higher activity or decolorizing power at higher temperatures compare with raw bentonite clay.

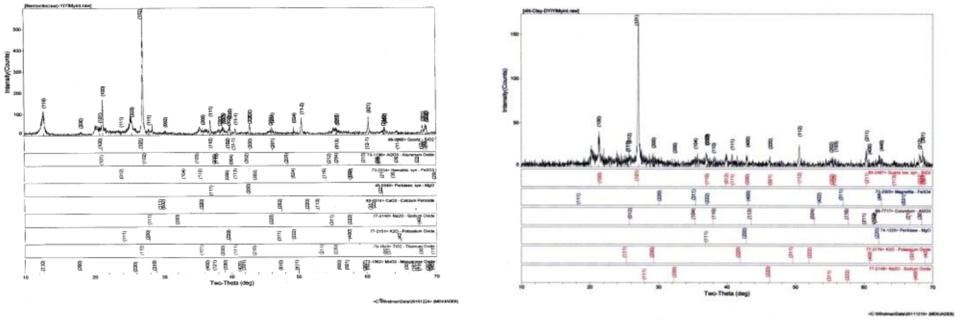
(a) (b)

Figure 1. Photographs of (a) raw and (b) acid activated bentonite clay samples



(a) (b)

Figure 2. FT IR spectrum of (a) raw and (b) acid activated bentonite clay samples



(a) (b)

Figure 3. XRD diffractogram of (a) raw and (b) acid activated bentonite clay samples

Table 1. Chemical Composition of Bentonite Clay Samples

Sr. No.	Compounds	Raw Bentonite clay (%)	4 N acid activated Bentonite clay (%)
1	SiO_2	52.54	54.68
2	Al_2O_3	30.28	29.44
3	Fe_2O_3	3.36	3.52
4	CaO	0.08	0.4
5	MgO	2.50	0.80
6	$K_2O + Na_2O$	1.55	0.30
7	Loss on ignition	10.61	10.80

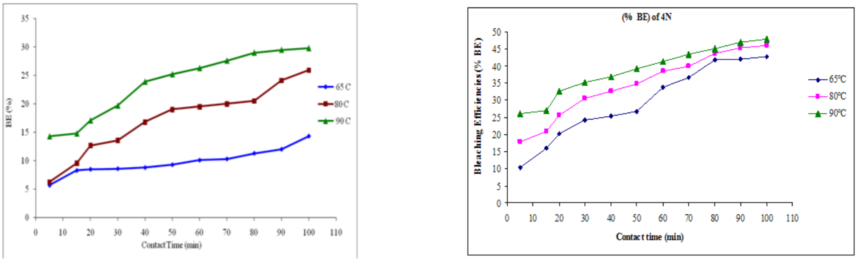
Table 2. Physicochemical Properties of Raw and Acid Activated Palm Oil Samples

Sr. No.	Properties	Palm oil		
		Crude	After bleaching with raw bentonite	After bleaching with 4 N acid activated bentonite
1	Specific gravity (20º C)	0.907	0.914	0.912
2	Iodine value (%)	46.380	48.650	50.700
3	Peroxide value (mEq/kg)	1.420	2.010	1.860
4	Saponification value (mgKOH/g)	201.290	192.720	193.610
5	Unsaponifiable matter (%)	0.179	0.177	0.073
6	Free fatty acid (%) (as oleic)	11.800	10.900	7.240
7	Moisture and Impurities (%)	0.129	0.102	0.078

* obtained from Myeik Township, Tanintharyee Division

Table 3. Bleaching Efficiencies of Raw and Acid Activated Bentonite Clay Sample for Different Contact Time at 65, 80 and 90 ºC

Sr. No.	**Contact time(min)**	**Raw clay (%BE)**			**Acid activated clay (% BE)**		
		65ºC	**80ºC**	**90ºC**	**65°C**	**80°C**	**90°C**
1	5	5.7	6.3	14.3	10.4	17.9	26.0
2	15	8.3	9.6	14.8	16.0	20.8	27.1
3	20	8.5	12.7	17.1	20.3	25.5	32.6
4	30	8.6	13.6	19.7	24.2	30.4	35.1
5	40	8.8	16.8	23.9	25.2	32.7	36.9
6	50	9.3	19.0	25.2	26.3	34.7	39.3
7	60	10.1	19.5	26.3	33.7	38.5	41.3
8	70	10.3	20.0	27.6	36.7	39.9	43.4
9	80	11.3	20.5	29.0	41.8	43.6	45.0
10	90	12.0	24.1	29.5	42.1	45.4	47.0
11	100	14.3	25.9	29.8	42.8	46.0	47.8



(a) (b)

Figure 4. Plot of bleaching efficiencies of (a) raw and (b) acid activated clay samples as a function of contact time at 65, 80 and 90 ºC

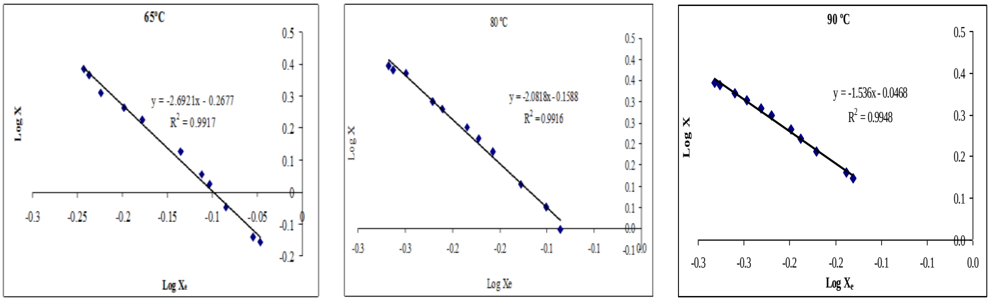


Figure 5. Freundlich isotherm for the bleaching of palm oil with acid activated bentonite clay at 65, 80 and 90 ºC

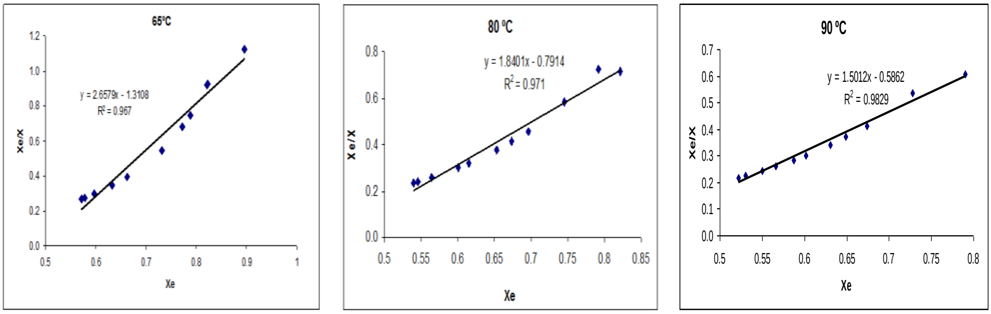


Figure 6. Langmuir isotherm for the bleaching of palm oil with acid activated bentonite clay at 65, 80 and 90º C

Table 4. Parameters of Freundlich and Langmuir Equation for the Adsorption of Carotene in Palm Oil onto Acid Activated Bentonite Clay

Isotherm models	**Parameters**	**Temperature (ºC)**		
		65ºC	**80ºC**	**90ºC**
Freundlich	λ	0.2677	0.1588	0.0468
	n	0.3715	0.4804	0.6510
	R^2	0.0468	0.9916	0.9948
Langmuir	a	0.7629	1.2636	1.7059
	b	2.0277	2.3251	2.5609
	R^2	0.9670	0.9710	0.9829

Conclusion

In this research, palm oil sample was collected to carry out the adsorption by using raw bentonite and 4 N HCl treated bentonite clay. In this study, raw bentonite clay was treated with 4 N HCl solutions to get better adsorption properties. The bleaching efficiencies of raw and 4 N HCl acid activated clays were 14.3% and 42.8%, respectively, for 100 min contact time. Therefore, 4 N HCl activated clay sample gave the highest bleaching efficiencies. This may concern with the structure of 4 N HCl activated clay sample. According to XRD data, 4 N HCl activated clay sample showed the amorphous nature due to the clay structure collapsing. The adsorption isotherms obtained follow Freundlich and Langmuir equation for the adsorption of pigments from palm oil. The Langmuir isotherm plots were present randomly on the line whereas a better fit with a straight line was obtained in the Freundlich isotherm plot. Since the correlation coefficient (R^2) values in Langmuir isotherm are less than in Freundlich isotherm, it can be seen that the Freundlich equation is more applicable than the Langmuir equation to the adsorption isotherms in the case of bleaching of palm oil with bentonite clays. The improvement of the quality of palm oil obtained after discoloration showed that activated local bentonite clay could be used for palm oil bleaching as well as for the adsorption of pigments and free fatty acids.

Acknowledgement

The authors would like to express my sincere gratitude and thanks to Professor Dr Pho Kaung (Rector) and Dr Ni Ni Than (Professor and Head of Department of Chemistry) from University of Yangon for their kind permission to publish this paper.

References

Bijay, K.D. and Jignesh, D. P. (2009). "Bleaching of Mustard Oil with Some Alternative Bleaching Agents and Acid Activated Clay", *J. Oleo. Sci.*, 58, 57- 63

Economic and Social Commission for Asia and the Pacific. (1996). "Atlas of Minerals Resources of the Escape Region", Geology and Minerals Resources of Myanmar, New York, pp. 113-114

Foletto, E.L., Volzone, C. and Porto, L.M., (2006), "Classification of Cottonseed Oil", *Lat.Am.Appl.Res*., Vol. 36, pp. 1-7

Hikichi, Y., (1998), "Chemical and Physical Analysis of Ceramic Materials (Standard Laboratory Manuals)", Minerals Research Laboratory, Nagoya Institute of Technology, Nagoya, Japan, 3-20

Kamga,R., and Nguetnkam, J.P., (2007),"Adsorption Palm Oil Carotene and Free Fatty Acids onto Acid Activated Cameroonian Clays", J. Applied. Sci, 17, 2462-2467

Makhoukhi, B., Didi, M.A. and Azzouz, A., (2009), "Acid Activation of Bentonite for Use as a Vegetable Oil Bleaching Agent", *J. App. Clay, Sci.,* 4, 343 – 349

Salawudeen, T. O, Dada, E. O. and Alagbe, S. O. (2007). “Performance Evaluation of Acid Treated Clays for Palm Oil Bleaching”. *Journal of Engineering and Applied Sciences*, 2(11), 1677-1680

Universities Research Journal 2019, Vol.12, No. 1

Recovery of Silver from Waste X-Ray Film by Using Proteolytic Enzyme in Latex of *Calotropis gigantea* L. (Mayo-gyi)

Yu Yu San[1] & Myint Myint Than[2]

Abstract

In this research, proteolytic enzyme was extracted from latex of Mayo-gyi plant using sodium chloride salt solution. Then, successive ammonium sulphate precipitations (20% and 60% saturation) were used. The optimum pH, temperature, reaction time and enzyme concentration of proteolytic enzyme activities were determined by spectrophotometric method using Folin-Ciocalteau (phenol) reagent. Silver is a precious metal which is used in industry of photographic and X-ray film. The X-ray films contain black metallic silver which is linked to gelatin in the emulsion layer. The extracted proteolytic enzyme hydrolyzed the gelatin from waste X-ray films followed by the release of silver. The recovered crude silver powder (black precipitate) was found to be 0.65g. The recovered silver grain (0.18 g) was obtained by heating crude silver powder in petroleum stove with air blow pipe. The yield percent (0.67%) was calculated from waste X-ray film (27g).The purity of silver was found to be 91.14 % and the metal impurities (Si, Al, K, S, Ca, Fe and Cu) in the recovered silver grain were determined by using EDXRF technique.

Keyword: Silver, waste X-ray film, Latex of Mayo-gyi plants, Proteolytic enzyme , EDXRF

Introduction

Proteolytic enzyme, also called protease, proteinase or peptidase any of a group of enzymes that break the long chain like molecules of proteins into shorter fragments (peptides) and eventually into their components, amino acids. Proteolytic enzymes are present in bacteria, archaea, certain types of algae, some viruses, and plants; they are most abundant, however in animals (Godfrey and West, 1996). There are different types of proteolytic enzymes which are classified according to sites at which they catalyze the cleavage of proteins. The two major groups are the

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exopeptidases, which target the terminal ends of proteins and the endopeptidases which target sites within proteins (Encyclopaedia, 2018). *Calotropis gigantea* L. is a poisonous plant. The active principles are uscharin, calotoxin, calactin, and calotropin (Alikhan and Khanum, 2005).The leaves and stem when incised yield thick milky juice. All parts of the plant are toxic, due to the presence of several cardiac glycosides. It is used as an arrow poison, cattle poison, rarely for suicide and homicide and mostly an accidental poison. The plant is also known to cause allergic contact dermatitis, and the latex causes kerato-conjunctivitis. *Calotropis gigantea* L. is reported to exhibit mosquito controlling properties against *Culex gelidus* and *Culex tritaeniorhynchus* mosquitoes which serve as vectors for Japanese encephalitis.

Botanical Description of *Calotropis gigantea* L. (Mayo-gyi)

Kingdom : Plantae
Botanical name : *Calotropis gigantea* L.
Myanmar name : Mayo-gyi
Genus : *Calotropis*
Species : C. *gigantea*
Family : Apocynaceae
Common name : Sodom apple

Figure 1. The Plant of Mayo-gyi *(Calotropis gigantea* L.)

Materials and Methods

Extraction of Proteolytic Enzyme from Latex of Mayo-gyi Plants

Mayo-gyi latex (25 mL) was prepared by blending with 45mL of cold distilled water and 20 mL of 0.1M sodium chloride solution in a blender for 15 min at room temperature. After homogenization, the resulting slurry was suspended in 20 mL of 0.1M acetate buffer solution (pH 5.5) and stirred for 15 min. This suspension was filtered through cheesecloth. The filtrate was centrifuged at 3000 rpm for 30 min to obtain 95mL extract. After centrifugation, the supernatant liquid was collected and qualitatively assayed for proteolytic enzyme activity.

The supernatant liquid was then brought to 20 % saturation with (10.74 g) of solid ammonium sulphate and stirred overnight at 6°C. After standing overnight at 6°C, the suspension was centrifuged 3000 rpm, 30 min, the precipitate was discarded (no proteolytic enzyme activity), and the supernatant liquid was brought to 60 % saturation with (20.8 g) of solid ammonium sulphate and stirred overnight at 6°C. After standing overnight, the precipitated protein containing proteolytic enzyme was collected by centrifugation for 30min at 3000 rpm. Finally, crude enzyme (2.61 g) was obtained. The prepared enzyme solution was kept in refrigerator at 6ºC.

Determination of Proteolytic Enzyme Activity in Latex of Mayo-gyi Plant

A 0.5 mL enzyme solution from the latex of the Mayo-gyi plant was added to a test tube containing 1mL of 1 % casein solution and 0.5 mL of acetate buffer solution (pH 5.5). The mixture was shaken well and incubated at 30 °C for 30 min. After incubation time, 1mL of 5% trichloroacetic acid (TCA) was added. The mixture was centrifuged and 1 mL of supernatant was taken out. It was added to 5 mL of 0.5M sodium carbonate solution and 1 mL of sodium hydroxide solution and kept for 10 min and 0.5 mL of Folin-Ciocalteau’s reagent was added. The tubes were incubated for 30 min, the blue color development.

A blank solution was prepared by carrying out the procedure as described above except that 0.5 mL of acetate buffer solution (pH 5.5) was used instead of 0.5 mL of enzyme solution. The absorbance of both test and blank solutions were measured at 560 nm by using UV-Visible spectrophotometer.

Treatment of Waste X-ray Film Using Proteolytic Enzyme from Latex of Mayo-gyi Plant

The waste X-ray films were washed with distilled water and wiping ethanol with cotton , and cut into 1 × 1 cm^2 pieces after drying. Each of the film was submerged in 50 ml of enzyme solution and the pH of the solution was adjusted to 5.5. The solution along with submerged film was stirred at room temperature until the gelatin-silver layer is stripped completely. The obtained slurry was dried. The black power was obtained.

Purification of Black Silver Powder from Waste X-ray Films

The silver powder of waste X-ray film (0.65 g) was mixed with borax (0.65 g) placed in a clay crucible and pre-smelted for 5 hour at fire place. The fuse silver was obtained and cooled at room temperature and then heated with air blow pipe furnace. Finally, the pure silver grain (0.16 g) was obtained.

Results and Discussion

Extraction of Proteolytic Enzyme Activity from Mayo-gyi plants

The proteolytic enzyme catalyzes the breakdown of the complex casein into its tyrosine product. In the present research, the proteolytic enzyme was isolated from the latex of the Mayo-gyi plants obtained from Pyay Township, Bago Region and was partially purified by solid ammonium sulphate precipitation method.

Wavelength of Maximum Absorption of Tyrosine

For the determination of wavelength of maximum absorption (λ_{max}), ultraviolet and visible absorption spectroscopy is employed (McDonald and Chen, 1965).In the present research, the absorption spectrum of tyrosine compound was recorded in the range from 400 to 900 nm. The wavelength of maximum absorption was found to be 560 nm.

Effect of Reaction Time on Proteolytic Activity of Enzyme-Catalyzed Reaction

The reaction time is an important criterion in determining the enzyme activity. The action of the enzyme on casein was studied in acetate buffer solution (pH 5.5). The amount of tyrosine liberated during the various reaction times such as 10, 20, 30, 40, 50 and 60 min were determined by UV-Visible Spectroscopic method and listed in Table 1.

Tyrosine concentration increased with an increase reaction time and after 30 min the concentration became nearly constant. The plot of concentration *vs.* reaction time of protease catalyzed reaction, showed that as the time increase the concentration of product increased, as shown in Figure 2. At initial period the reaction is very fast and then velocity of proteolytic enzyme-catalyzed reaction is gradually small. The plot of velocity *vs.* reaction time for sample revealed that the velocity decreased linearly with time up to 60 min so that the reaction time of 30 min was chosen for the enzyme reaction experiment as shown in Figure 3.

Table 1. Relationship between Reaction Time and Velocity of Proteolytic Enzyme- Catalyzed Reaction

Reaction time (min)	**Absorbance at 560 nm**	**Concentration of Enzyme (mM)**	**Velocity (mM min^{-1})**
10	1.08	95.45	9.55
20	1.356	119.87	5.99
30	1.529	135.16	4.51
40	1.571	138.87	3.47
50	1.596	141.08	2.82
60	1.619	143.12	2.39

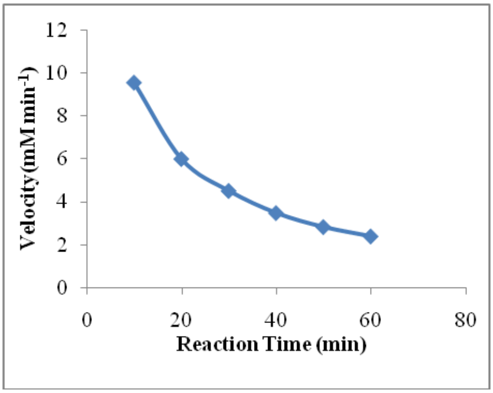


Figure 3. Plot of velocity of enzyme - catalyzed reaction as a function of reaction time

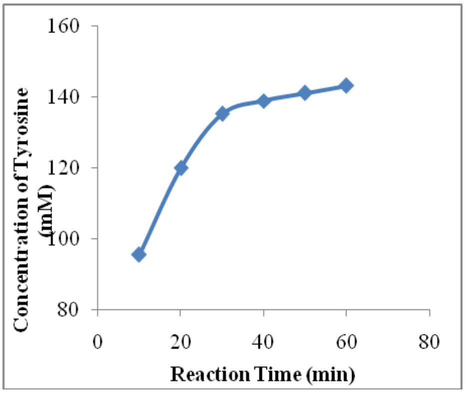


Figure 2. Determination of the enzyme concentration on enzyme-catalyzed

Optimum pH for Enzyme-Catalyzed Reaction

When enzyme activity was determined at several pH values, optimal activity was usually seen between pH values. At optimum pH, enzyme activity is maximum. Above and below this pH, the activity is low. In the present work, acetate buffers at pH values ranging from 3.0 and 7.0 were used to determine the proteolytic activity of the enzyme sample. The nature of the proteolytic enzyme activity *vs*. pH curve of the enzyme as shown in Table 2 and Figure 4 was found to be symmetrical and the optimum pH was attained at pH 5.5 for the sample.

Table 2. Relationship between Proteolytic Enzyme Activity of Enzyme Catalyzed Reaction and pH Buffer Solution

No.	pH	Absorbance at 560 nm	Proteolytic Enzyme Activity (μ mol min^{-1} mL^{-1})
1	3.0	0.953	42.12
2	3.5	0.962	42.52
3	4.0	0.971	42.92
4	4.5	0.975	43.09
5	5.0	0.993	43.89

No.	pH	Absorbance at 560 nm	Proteolytic Enzyme Activity (μ mol min^{-1} mL^{-1})
6	5.5	1.010	44.64
7	6.0	0.973	43.01
8	6.5	0.921	40.71
9	7.0	0.892	39.43

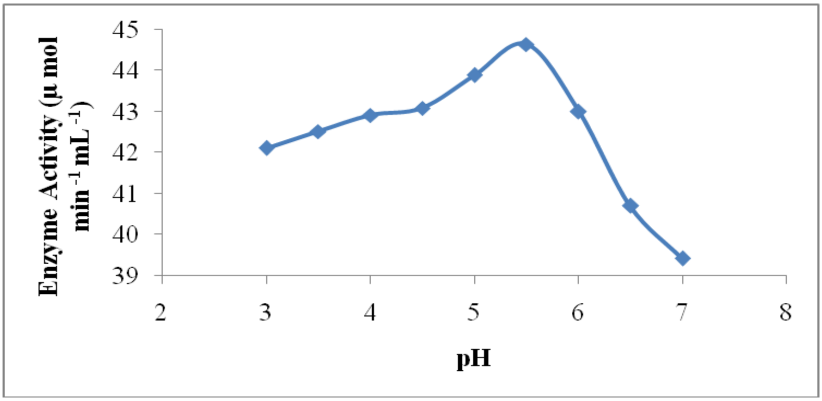


Figure 4. Plot of Proteolytic enzyme activity as a function of pH of the buffer solution

Optimum Temperature for Enzyme- Catalyzed Reaction

Enzyme-catalyzed reactions are influenced by temperature. As temperature increases, enzyme becomes more active until finally rapid thermal denaturing takes place. As the enzyme is inactivated the reactions which it catalyzes slow down and finally stop. In this study, the functional temperature was increased from 10°C to 80°C while the substrate medium (1% casein) was prepared and the optimum pH 5.5 was fixed. The optimum temperature for the enzyme was found to be 40°C in the acetate buffer solution pH 5.5 as shown in Figure 5. The optimum temperature happens to be high since it is a plant enzyme. It was obvious that the activity of enzyme was increased from 20°C to 40°C and then decreased from 40°C to 80°C described in Table 3.

Table. 3 Relationship between Proteolytic Enzyme Activity and Temperature of the Solution at pH 5.5

No.	**Temperature (°C)**	**Absorbance at 560 nm**	**Proteolytic Enzyme Activity (μ mol min^{-1}mL^{-1})**
1	10	0.605	26.74
2	20	0.927	40.97
3	30	1.012	44.73
4	40	1.041	46.57
5	50	1.045	46.19
6	60	0.963	42.56
7	70	0.801	35.40
8	80	0.656	28.99

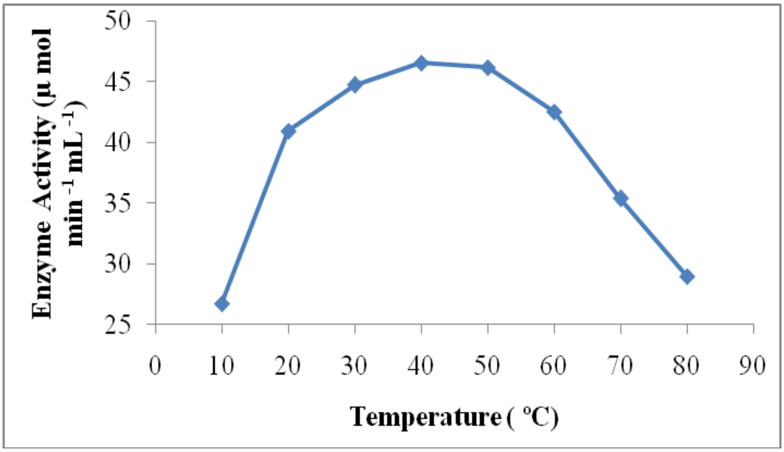


Figure 5. Plot of Proteolytic enzyme activity as a function of temperature of the solution at pH 5.5 acetate buffer solution

Qualitative Test for Proteolytic Enzyme Activity

X-ray film is made by a plastic backing covered with an emulsion of AgCl in gelatin. Gelatin is a protein which can be hydrolyzed by a proteolytic enzyme. The waste X-ray film and crude enzyme solution from the latex of Mayo-gyi plants were placed in the beaker. After 3 hours, the black precipitate was found in the beaker and clear film was obtained due to the reaction of proteolytic enzyme activity in Mayo-gyi latex as shown in Figure 6 and 7.

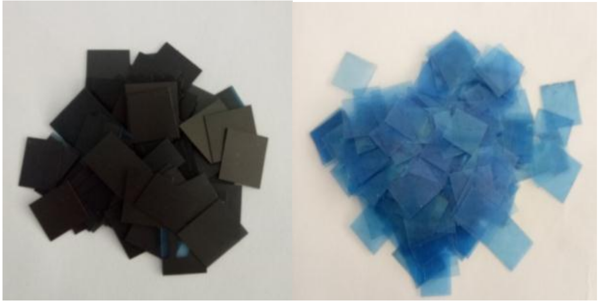
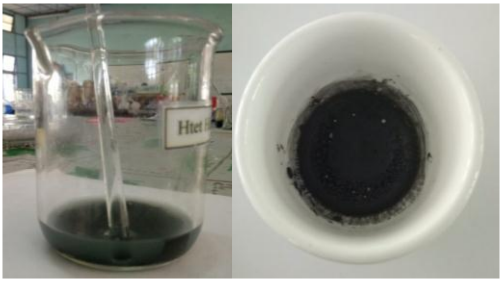


Figure 6. The waste X- ray film before and after treatment of Proteolytic enzyme



(a) (b)

Figure 7. (a) The waste X- ray film submerged in Proteolytic enzyme solution (b) The recovered silver

Treatment of X-ray film using Proteolytic enzyme from the Latex of Mayo-gyi Plants

The waste X-ray films were treated using the crude enzyme solution to strip the silver at 40 ºC and pH 5.5 for 30 min. Under the condition (pH 5.5and 40 ºC), 0.65 g of silver powder was recovered. The silver content of the used waste X-ray films was calculated to be 0.67%.The silver powder purification process is pollution free, efficient and without any harm to the environment .The purity of silver was determined 91.14% by using EDXRF method. Therefore, the recovered silver was almost pure.

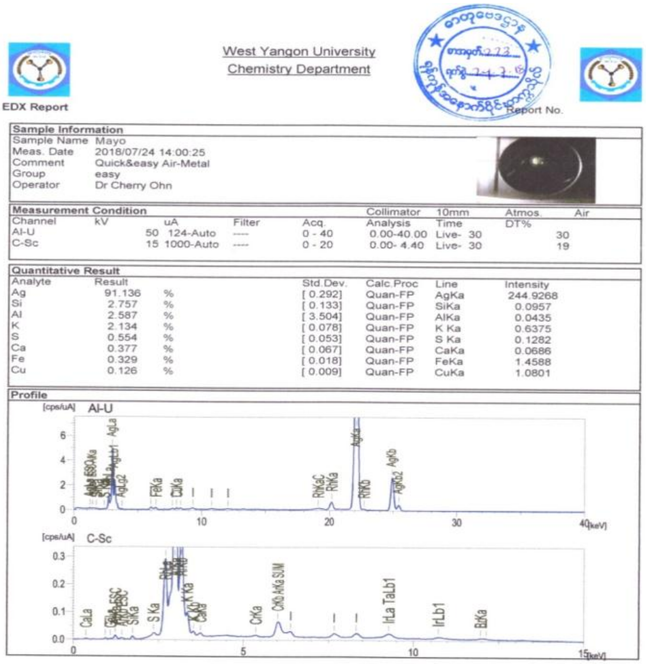
Purification of Black Silver Powder from Waste X-ray Films

The silver powder of waste X-ray film (0.65 g) was mixed with borax (0.65 g) placed in a clay crucible and pre-smelted for 5 hour at fire place. The fuse silver was obtained and cooled at room temperature and then heated with air blow pipe furnace. Finally, the pure silver grain (0.18 g) was obtained as shown in Figure 8.

Figure 8. Purification process of recovered silver grain by heating in petroleum stove with air blow pipe

Qualitative Analysis of Silver from Waste X-ray film by EDXRF Technique

The elements present in the sample were qualitatively determined by EDXRF technique. This technique can give the qualitative result of the silver. The relative abundances of some elements present in the sample were listed in Figure 9. The ED XRF results show that the recovered silver grain has highest purity among the other elements. The purity of recovered silver grain (91.14%) and the metal impurities (2.76 % Si, 2.59 % Al, 2.14% K, 0.55 % S, 0.377% Ca, 0.33 % Fe and 0.126 % Cu) were determined.



West Yangon University
Chemistry Department

EDX Report

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Report No.

Sample Information

Sample Name	Mayo
Meas. Date	2018/07/24 14:00:25
Comment	Quick&easy Air-Metal
Group	easy
Operator	Dr Cherry Ohn

Measurement Condition Collimator 10mm Atmos. Air

Channel	kV	uA	Filter	Acq.	Analysis	Time	DT%
Al-U	50	124-Auto	----	0 - 40	0.00-40.00	Live- 30	30
C-Sc	15	1000-Auto	----	0 - 20	0.00- 4.40	Live- 30	19

Quantitative Result

Analyte	Result		Std.Dev.	Calc.Proc	Line	Intensity
Ag	91.136	%	[0.292]	Quan-FP	AgKa	244.9268
Si	2.757	%	[0.133]	Quan-FP	SiKa	0.0957
Al	2.587	%	[3.504]	Quan-FP	AlKa	0.0435
K	2.134	%	[0.078]	Quan-FP	K Ka	0.6375
S	0.554	%	[0.053]	Quan-FP	S Ka	0.1282
Ca	0.377	%	[0.067]	Quan-FP	CaKa	0.0686
Fe	0.329	%	[0.018]	Quan-FP	FeKa	1.4588
Cu	0.126	%	[0.009]	Quan-FP	CuKa	1.0801

Profile

Figure 9. EDXRF spectrum of silver from waste X-ray film

Conclusion

In this research proteolytic enzyme was extracted from Mayo-gyi latex, using sodium chloride salt solution. Then, successive ammonium sulphate precipitations (20% and 60%) were used. The wavelength of maximum absorption of blue colour compound formed by Folin-Ciocalteu’s phenol reagent was found at 560 nm. The standard curve is linear and passed through the origin. Therefore, Beer- law was obeyed. The optimum pH and temperature of the proteolytic enzyme were found to be 5.5 and 40°C, respectively. Tyrosine concentration increased with an increase reaction time and after 30 min the concentration became nearly constant. At initial period, the reaction is very fast and then velocity of proteolytic enzyme-catalyzed reaction is gradually small. The proteolytic enzyme activity increased with an increase volume of the enzyme solution. Silver is a precious metal used in photographic and X- ray film industry. The waste X-ray films contain black metallic silver which was recovered and it can be reused. Silver is linked to gelatin in the emulsion layer. The proteolytic enzyme from latex of Mayo- gyi plant hydrolyze the gelatin from waste X- ray films, the silver was released. The recovery of silver from waste X-ray film was found to be 0.65 g. The recovered silver grain (0.18 g) was obtained by heating crude silver powder in petroleum stove with air blow pipe. The yield percent (0.67%) was calculated from waste X-ray film (27g). The purity of silver was found to be 91.14% and the metal impurities (Si, Al, K, S, Ca, Fe, Cu) in recovered silver grain were determined by using ED XRF technique. Therefore, this process is easy, cheap, pollution free, efficient, high purity and without any harm to the environment.

Acknowledgements

The author would greatly thankful to Dr Aung Aung Min (Rector-in-charge), Dr Thwe Linn Ko (Pro-rector), Dr Myint Myint Than (Professor and Head) Department of Chemistry, Pyay University for allowing to carry out this research program.

References

Alikhan I, and A Khanum(2005), “Medicinal and Aromatic Plants of India”,Ukaaz Publication; 133–4. Encyclopedia, (2017). “Proteolytic enzyme”, https://www.britannica.com/science/Proteolytic-enzyme. (Accessed,February21, 2018)

Godfrey, T. and West, S. (1996). “Industrial Enzymology, 2nd edn”, NY: Macmillan Publishers, Inc. ISBN 0-33359464 – 9.3

Hedges, L.J. and Lister, C. E. (2008). “Nutritional Attributes of Indian Vegetables” ,*Plant & Food Research Confidential Report*, No. 2291, 19-20

Hooper, N. M. (2002).“Proteases in Biology and Medicine”. London; Portland Press, ISBN 1-85578-147-6Hudson, B. J. F. (1982), “Development in Food Protein”, *Applied Science Pub*, **1**. 53-59

Jabalia N., P.C. Mishra and N. Chaudhary, (2014). “Applications, Challenges and Future Prospects of Proteases: An Overview”, *Journal of Agroecology and Natural Resource Management*, **1**(3), 179-183.

Jayant P .P ., S .P . Patil , I .D . Patil and U .B .Deshannavar ,(2015). “Extraction of Silver from waste x-ray films using protease enzyme”, *International Journal of Advanced Biotechnology and Research (IJBR)*, **6**(2), 220-226.

Kumar G., K. Loganathan and K. V. B. Rao. (2011). “A Review on Pharmacological and Phytochemical Profile of *Calotropis Gigantea* Linn”, Pharmacology online ,**1**,1-8.

McDonald, C. E. and Chen, L. L., (1965), “Lowory Modification of the Folin Reagent for Determination of Proteinase Activity”, *Ann. Biochem*, **10**, 175-179

Mukhtar, H. and Ikarm, U. H., (2008), "Production of Alkaline Proteases by Bacillus Substilies as A Depilating Agent in Leather Processing", *Pak. J, Bot*., **40,**1673-1679

Narendra, N., P.Gaurav, D.Lokesh and K.J.Naveen. (2009). "Wound Healing Activity of Latex of *Calotropis Gigantea*", *International Journal of Pharmacy and Pharmaceutical Sciences*, **1** (1),176-181.

Radha., K .V. and C .Arun ,(2010). "Recycling of exposed photographic X-ray films and recovery of silver using Bromelain",*WIT Transactions on Ecology and the Environment* ,**142**,421-429.

Raghubir, R, Rasik, M. and Gupta, A.J. (1999). "Healing Potential of *Calotropis procera* ondermal wounds in guinea pigs''. *J Ethnopharmacol*, **68**, 261–6.

Rosenberg, I. M. (1996), Protein Analysis and Purification, Birkhauser, Boston,127-132

Singh.,N. (2014),"Calotropis gigantea: A Review on its Phytochemical and Pharmacological profile." *International Journal of Pharmacognosy*, (**1**) 1-8.

Tyagi.S P., (2018). "X-ray film Accessories" www.hillagric.ac.in/edu/covas/vsr/ pdf/ teaching (Accessed March 7, 2018)

Verma, S. K., (2003). "A Text Book of Plant Physiology and Biochemistry",78-99

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Effect of Gamma Irradiation on Shelf Life and Quality of Mature Green Chilli (*Capsicum Annuum* L.)

Mar Mar Khine[1] & Kyaw Naing[2]

Abstract

In this research work, the mature green chillies samples were irradiated with different doses (0.5, 1.0, 1.5, and 2.0 kGy) of the gamma ray by using Co-60 gamma source. The non-irradiated sample was used as comparative study. Induced radioactivity of γ-irradiated mature green chilli samples with different doses were monitored by using NaI (Tl) Scintillation Detector. The shelf life of non-irradiated and γ-irradiated samples were studied by assessing post-harvest changes (color, ripening) at room temperature. And then, nutritional qualities, elemental contents and some important physico-chemical parameters of chilli were studied by using analytical methods, EDXRF analysis, iodometric titration, acid base titration method and pH meter respectively. From these results, γ-irradiated samples were found no distinct activity above background. In studying the shelf life of each sample, it was found that all gamma irradiated chilli samples have more shelf life extension than the non-irradiated samples under same condition. Besides, there is no significantly changes was found in the elemental contents of non-irradiated and γ-irradiated sample with 2.0 kGy dose. The quality of ripening, vitamin C contents, the total acidity and pH values of non-irradiated and γ-irradiated samples were also studied on a dry weight basic. From these overall results, 2.0 kGy dose gamma irradiation prolonged the ripening stage of chillies two times than that of non-irradiated sample. The sample with 2.0 kGy dose does not badly suffer on macronutrients (ashes, fats) although micronutrient (vitamin C) is slightly reduced but do not affect vitamin deficiency. This benefit can be achieved to distribute as fresh product without harmful to eat. Therefore, food irradiation on chilli (with 2.0 kGy dose of gamma ray) provides the extending storage life and is still wholesome enough to eat.

Keywords : chilli, Co-60, shelf life, gamma irradiation

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Introduction

Irradiation extends shelf life of food by slowing the ripening process of fruits and vegetables. The radionuclide cobalt-60 is the most commonly used source of gamma radiation for use in radiotherapy, sterilization of medical products and the irradiation of food (WHO, 1988).

Chilli is a fruit of the plants '***Capsicum annuum'*** and ***'Capsicum frutecens'*** that come from the genus 'Capsicum', belonging to the family of 'Solanaceae', which also includes tomato and potato (Tiwari, 2010).

Irradiation has the same objectives as other food processing methods the reduction of losses due to spoilage and deterioration and control of the microbes and other organisms that cause food-borne diseases. As with other forms of food processing, radiation produces some useful chemical changes in food. It also increases the yield of juice from grapes, and speeds the drying rate of plums. Irradiation extends shelf life of food in two ways. First, it reduces spoilage bacteria and moulds that can grow even under refrigeration. The second way, irradiation extends shelf life is by slowing the ripening process of fruits and vegetables (Charlotte, 1995). Irradiation of food is the use of ionizing radiations from radioactive isotopes of cobalt or cesium or from accelerators that produce controlled amounts of beta rays or x-rays on food (Fan, 2008).

Table 1. Application of Food Irradiation

Type of Food	**Radiation Dose in kGy**	**Effect of Treatment**
Meat, poultry, fish, shellfish, some vegetables, baked goods, prepared foods	20 to 71	Sterilization. Treatment products can be stored at room temperature without spoilage. Treated products are safe for hospital patients who require microbiologically sterile diets.
Spices and other seasonings	Up to a maximum of 30	Reduces number of microorganisms and insects. Replaces chemicals used for this purpose.

Type of Food	Radiation Dose in kGy	Effect of Treatment
Meat, poultry, fish	0.1 to 10	Delays spoilage by reducing the number of microorganisms in the fresh, refrigerated product. Kills some types of food poisoning bacteria and renders harmless disease causing parasites (e.g., trichinae).
Strawberries and some other fruits	1 to 5	Extends shelf life by delaying mold growth.
Grain, fruit, vegetables and other foods subject to insect infection	0.1 to 2	Kills insects or prevents them from reproducing. Could partially replace post-harvest fumigants used for these purpose.
Bananas, mangoes, papayas, guavas, and certain other non-citrus fruits	1.0 maximum	Delays ripening.
Potatoes, onions, garlic, ginger	0.05 to 0.15	Inhibit sprouting.
Grain, dehydrated vegetables, other foods	Various doses	Desirable changes (e.g., reduced rehydration times).

(Snyder and Poland, 1995).

Materials and Methods

Firstly, the mature green chillies were harvested from Lakyinwet Village, Pekon Township, Southern Shan State. And then, the chilies were transported to the Department of Atomic Energy, Ministry of Education for irradiation. The period between harvesting and irradiation was approximately (2) days. The sample was treated with four different doses of gamma radiation (0.5, 1.0, 1.5 and 2.0 kGy) from Co-60 source (Co-60 gamma chamber 5000) which has dose rate of 1.22 kGy/h. For study on safety consumption, the induced activities of four γ-irradiated samples of different doses were monitored by NaI (Tl) Scintillation Detector (LUDLUM MODEL 730) at Nuclear Laboratory, Department of Chemistry, University of Yangon.

The shelf life of non-irradiated and γ-irradiated samples were studied by assessing post-harvest changes (color, ripening) at room temperature. Effects of gamma irradiation on nutritional qualities, elemental contents and some important physico-chemical parameters (such as vitamin C, total acidity and pH) of chilli were studied by using analytical methods, EDXRF analysis, iodometric titration, acid base titration method and pH meter respectively. The non-irradiated sample was used as comparative study in the whole research work.

a b

Figure 1. (a and b) Irradiation of chilli with gamma radiation

Results and Discussions

The induced activities of each irradiated sample (0.5, 1.0, 1.5 and 2.0 kGy) were monitored by NaI (Tl) Scintillation Detector (LUDLUM MODEL 730) at Nuclear Chemistry Laboratory at University of Yangon. From study the results (Table 2), it was found that there are positive values as well as negative values due to fluctuation. This monitoring indicated that there was no distinctive activity from the background.

The shelf life of non-irradiated and γ-irradiated chilli samples of different doses were assessed by seeking post-harvest changes such as (color and ripening) at room temperature under same condition. According to the study of shelf life in chillies, it was observed that 2.0 kGy dose of γ-irradiated sample has the longest shelf life. Therefore, this sample was selected to study the nutritional values compared with non-irradiated sample.

According to the nutritional point of view, 2.0 kGy dose of gamma irradiation provides improving the carbohydrate with higher ash content and lower moisture content of chilli. Thus, the most effective dose for the chilli was 2.0 kGy in the present study. From the observations of the elemental contents in γ-irradiated sample of (2.0 kGy) dose did not differ significantly from that of non-irradiated sample. The results are shown in Table 5. Therefore, it can be inferred that irradiation has no detrimental effect on elemental contents of chilli.

From the study on some important physic-chemical parameter, vitamin C contents of γ-irradiated samples were decreased than that of the non-irradiated sample but do not affect in vitamin deficiency. The total acidity of γ-irradiated samples were found to be increased than that of the non-irradiated sample, thus pH values of these samples decreased than that of the non-irradiated sample. Effect of gamma irradiation can prolong the ripening time.

Table 2. Monitoring of Induced Activity of Different Doses of γ-Irradiated Mature Green Chillies

No.	Samples	Induced activity relative to background (%) (cp 100 s)
1	CG 0.5	+1.55
2	CG 1.0	- 2.81
3	CG 1.5	+1.01
4	CG 2.0	- 3.02

± = due to fluctuation Note: activity no distinct above background

Table 3. Shelf Life of Non-irradiated and Different Doses of γ- Irradiated Mature Green Chillies at Room Temperature

Storage period (days)	**Samples**				
	CG 0	**CG 0.5**	**CG 1.0**	**CG 1.5**	**CG 2.0**
1	0	0	0	0	0
3	4	5	6	0	0
5	50	16	15	14	11
7	100	74	78	70	62
9	Nd	88	86	85	75
11	Nd	100	100	92	85
13	Nd	Nd	Nd	100	91
15	Nd	Nd	Nd	Nd	100

Nd = not detected as samples are spoiled

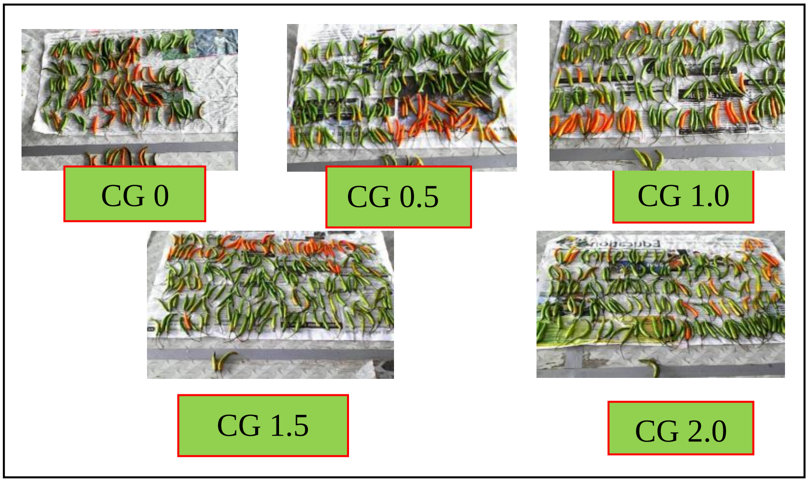


Figure 2. Post-harvest change of non-irradiated and different doses of γ-irradiated mature green chillies during storage 5th days

Table 4. Nutritional Values of Non-irradiated and 2.0 kGy Dose of γ-Irradiated Mature Green Chillies

No.	**Nutritional parameters (%) DW**	**Samples**	
		CG 0	**CG 2.0**
1	Moisture	80.46	72.25
2	Ash	1.27	1.90
3	Protein	3.20	2.80
4	Fiber	6.50	5.80
5	Fat	0.52	0.65
6	Carbohydrate	8.05	16.60
7	Energy value (kcal/100g)	49.68	83.45

DW = dry weight basis

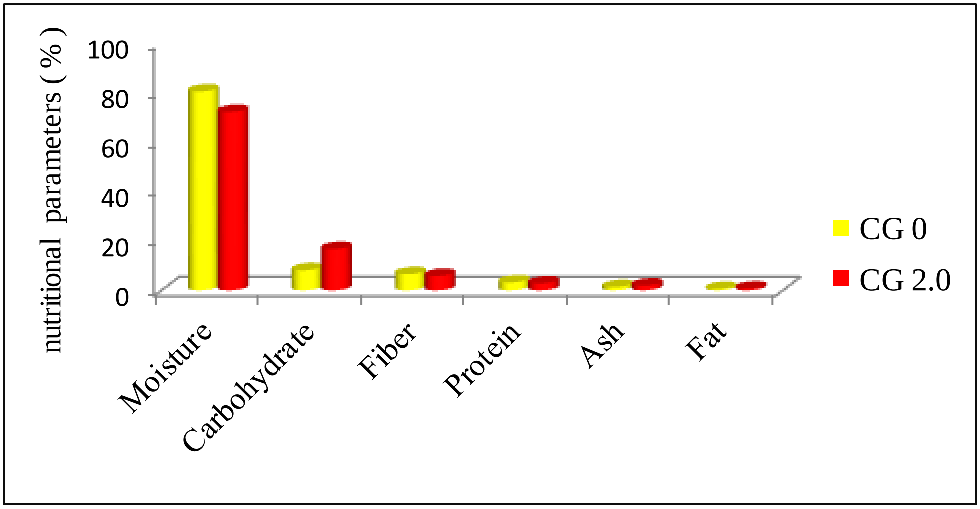
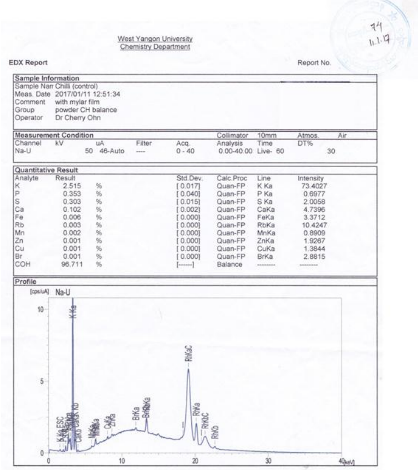


Figure 3. A bar graph of nutritional values of non- irradiated and 2.0 kGy dose of γ-irradiated mature green chillies



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West Yangon University
Chemistry Department

EDX Report Report No.

Sample Information
Sample Nam Chilli (control)
Meas. Date 2017/01/11 12:51:34
Comment with mylar film
Group powder CH balance
Operator Dr Cherry Ohn

Measurement Condition Collimator 10mm Atmos. Air

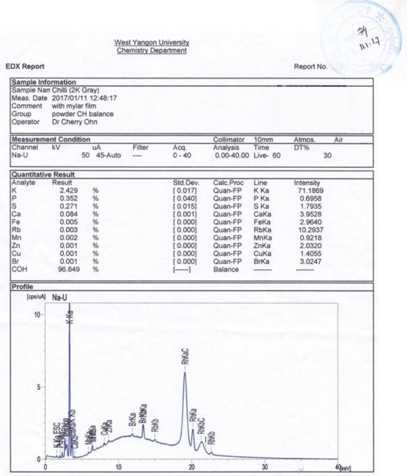
Channel	kV	uA	Filter	Acq.	Analysis	Time	DT%
Na-U	50	46-Auto	----	0 - 40	0.00-40.00	Live- 60	30

Quantitative Result

Analyte	Result		Std.Dev.	Calc.Proc	Line	Intensity
K	2.515	%	[0.017]	Quan-FP	K Ka	73.4027
P	0.353	%	[0.040]	Quan-FP	P Ka	0.6977
S	0.303	%	[0.015]	Quan-FP	S Ka	2.0058
Ca	0.102	%	[0.002]	Quan-FP	CaKa	4.7396
Fe	0.006	%	[0.000]	Quan-FP	FeKa	3.3712
Rb	0.003	%	[0.000]	Quan-FP	RbKa	10.4247
Mn	0.002	%	[0.000]	Quan-FP	MnKa	0.8909
Zn	0.001	%	[0.000]	Quan-FP	ZnKa	1.9267
Cu	0.001	%	[0.000]	Quan-FP	CuKa	1.3844
Br	0.001	%	[0.000]	Quan-FP	BrKa	2.8815
COH	96.711	%	[------]	Balance	--------	--------

Profile

Figure 4. EDXRF spectrum of non-irradiated mature green chilli



74
11.1.17

West Yangon University
Chemistry Department

EDX Report Report No.

Sample Information
Sample Nam Chilli (2K Gray)
Meas. Date 2017/01/11 12:48:17
Comment with mylar film
Group powder CH balance
Operator Dr Cherry Ohn

Measurement Condition Collimator 10mm Atmos. Air

Channel	kV	uA	Filter	Acq.	Analysis	Time	DT%
Na-U	50	45-Auto	----	0 - 40	0.00-40.00	Live- 60	30

Quantitative Result

Analyte	Result		Std.Dev.	Calc.Proc	Line	Intensity
K	2.429	%	[0.017]	Quan-FP	K Ka	71.1869
P	0.352	%	[0.040]	Quan-FP	P Ka	0.6958
S	0.271	%	[0.015]	Quan-FP	S Ka	1.7935
Ca	0.084	%	[0.001]	Quan-FP	CaKa	3.9528
Fe	0.005	%	[0.000]	Quan-FP	FeKa	2.9640
Rb	0.003	%	[0.000]	Quan-FP	RbKa	10.2937
Mn	0.002	%	[0.000]	Quan-FP	MnKa	0.9218
Zn	0.001	%	[0.000]	Quan-FP	ZnKa	2.0320
Cu	0.001	%	[0.000]	Quan-FP	CuKa	1.4055
Br	0.001	%	[0.000]	Quan-FP	BrKa	3.0247
COH	96.849	%	[------]	Balance	--------	--------

Profile

Figure 5. EDXRF spectrum of 2.0 kGy dose of γ-- irradiated mature green chilli

Table 5 Elemental Contents of Non-irradiated and 2.0 kGy Dose of γ-Irradiated Mature Green Chillies by EDXRF Analysis

		Sample	
No.	**Elemental Content (%)**	**CG 0**	**CG 2.0**
1	K	2.515	2.429
2	P	0.353	0.352
3	S	0.303	0.271
4	Ca	0.102	0.084
5	Fe	0.006	0.005
6	Rb	0.003	0.003
7	Mn	0.002	0.002
8	Zn	0.001	0.001
9	Cu	0.001	0.001
10	Br	0.001	0.001

Table 6. Important Physico-chemical Parameters of Non-irradiated and Different Doses of γ-Irradiated Mature Green Chillies

No.	Physico-chemical parameters (DW)	Samples				
		CG 0	**CG 0.5**	**CG 1.0**	**CG 1.5**	**CG 2.0**
1	Vitamin C (mg/100 mL)	48.58	44.00	47.52	45.40	45.40
2	Total acidity (%)	0.58	0.70	0.77	0.64	0.58
3	pH	7.52	7.37	7.28	7.11	6.99

CG 0 = Gamma dose of 0 kGy of chilli
CG 0.5 = Gamma dose of 0.5 kGy of chilli
CG 1.0 = Gamma dose of 1.0 kGy of chilli
CG 1.5 = Gamma dose of 1.5 kGy of chilli
CG 2.0 = Gamma dose of 2.0 kGy of chilli

Conclusion

In this work, effects of different doses of gamma irradiation on shelf life and quality of mature green chilies were studied. On the basis of the present study, it is concluded that 2.0 kGy dose of gamma irradiation do not make food radioactive. This dose is effective in extending the storage life and do not badly suffer on macronutrients within chilli (ashes and fats) although micronutrient (vitamin C) are slightly reduced but this samples do not affect vitamin deficiency. According to the nutritional point of view, 2.0 kGy dose of gamma irradiation is more effective due to higher, carbohydrates and energy value and lower moisture, protein and fiber content of chilli than that of non-irradiated sample. From overall results, all irradiated mature green chillies have no induced activity. In the study of 2.0 kGy dose of gamma irradiated chilli sample, the nutritional qualities are significantly unchanged and this sample can be prolonged about two times than that of the non-irradiated sample at room temperature. This benefit can be achieved to distribute as fresh product without harmful to eat.

Acknowledgements

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References

Charlotte, P. (1995). “Ten Most Commonly Ask Questions about Food Irradiation”. http:// www. epa.gov/ rpdweb00/ sources/ food irrad.html

Fan, X. (2008). “Irradiation, Food Processing and Food Safety”. *Food Technol,* **62** (3), 36-43

Snyder, O. P and D. M. Poland. (1995). “Food Irradiation Today”. Hospitality Institute of Technology and Management, 7-8

Tiwari, R. K. (2010). “Post-Harvest Profile of Chilli”. *Government of India Ministry of Agriculture,* **2,** 6-9

WHO. (1988). “Food Irradiation a Technique for Preserving and Improving the Safety”. *Technical Report Series*, **705**, 8-24