

Isolation and Identification of Xanthone from the Root of *Mesua ferrea* L. (Gangaw)

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Abstract

*In this research work, Gangaw, *Mesua ferrea* Linn, was collected from Shwe Kyet Yet Village, Amarapura Township in Mandalay Region. The preliminary phytochemical tests for the roots of this plant were performed. Moreover, the antimicrobial activities of the crude extract in various solvent systems were tested by Agar well diffusion method on six organisms. A pure known compound, 1, 3-dimethoxy- 5, 6-dihydroxy xanthone (21.9mg, 0.73 %) could be isolated from the roots of Gangaw applying solvent extraction and column chromatography. According to the FT-IR spectrum, the isolated compound contains OH functional group, sp² and sp³ hydrocarbon, carbonyl group, aromatic benzene ring, alcohol group, ether group, trans or E and cis or Z alkenic groups respectively. The melting point of this known compound was found to be (275-276°C). The structure of this compound could be identified by FT-IR spectrum and melting point determination.*

Keywords- Agar well diffusion method, column chromatography, FT-IR spectrum, *Mesua ferrea* Linn

1. Introduction

Ceylon Ironwood trees are found throughout the tropical regions of Myanmar and are known as "Gangaw". Botanical name is *Mesua ferrea* Linn. under the family Guttiferae [1].

All parts of the tree (leaves, flowers, barks, fruits, seeds and roots) are used for many different medicinal preparations. *Mesua ferrea* (Ceylon Ironwood) is bitter. It is one of the most effective medicinal plants with antimicrobial, antibacterial, antifungal, antihelminthic, antidiarrhoeic, antidiarrhoeic and anti-inflammatory properties. Flowers are said to be astringent, stomach-ache, expectorant and useful in bleeding piles, the flower buds are used in dysentery. Fresh flowers are also prescribed for excessive perspiration cough and for indigestion. Unripe fruits are used for their aromatic and sudorific effect. Leaves are applied to the head in severe cold. The seeds oil is used for sores, scabies, wounds and rheumatism. The bark is used as an astringent in combination with ginger. Bark and root have sudorific and tonic properties. The root of this herb is often used as an antidote for snake poison [2].

From the plants, xanthones, a number of 4-phenylcoumarin derivatives, friedelin and triterpenes have been isolated. Xanthones are isolated from the heartwood, the coumarin derivatives from the seeds. Recently, a tetraoxxygenated xanthones was isolated from the heartwood and the bark of the plant.

Xanthones are indeed rare antioxidants. Many antioxidants are plant based and play an important role in protecting plants that are exposed to strong sunlight and live under severe oxygen stress. Antioxidants also play an important role in human health because the biologic defense mechanisms cannot operate under severe oxygen stress [7]. According to recent research, activated oxygen is thought to be a major factor in ageing, hardening of the arteries, diabetes, cancer and tissue injury of skin [5]. Indeed approximately 90 % of age-related diseases are linked to activated oxygen. When human skin is exposed to ultraviolet rays active oxygen (free radical) is generated, which is scavenged by excess melanin. Pigmentation from excess melanin can cause the appearance of spots and freckles on the skin. Plants are rich source of antioxidants. Although all parts of the 'Gangaw' trees are very useful, only the roots were selected to study in this research work.

The aim of this research is to study and isolate the xanthone from the roots of Gangaw. It can be identified by FT-IR spectroscopic method and melting point determination respectively.

1.1. Botanical Description

Botanical name	-	<i>Mesua ferrea</i> Linn.
Family name	-	Guttiferae
Myanmar name	-	Gangaw
English name	-	Ceylon Ironwood Tree
Parts used	-	Root
Flowering period	-	March to July



Figure 1. The Plant of Gangaw and the Flowers of Gangaw

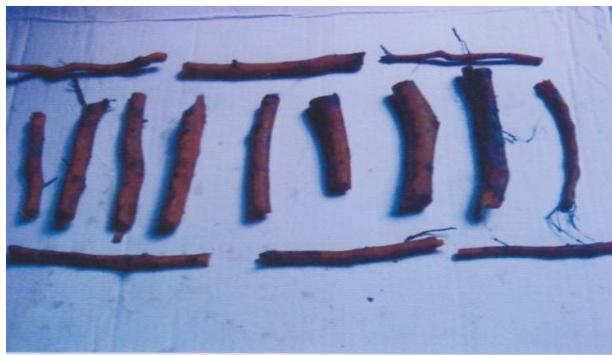


Figure 2. The Roots of Gangaw

1.2. Description

Mesua ferrea is a medium-sized or fairly large evergreen tree up to 100 feet tall, often buttressed at the base with a trunk up to 2 meters in diameter. The plant bears simple, narrow, oblong, dark green leaves with a whitish underside. The leaves possess a length of 7-15 cm. Its flowers are 4-7.5 cm diameter with four petals and a center of numerous yellow stamens. The heartwood is reddish-brown with a purple tinge when fresh, becoming dark red-brown upon exposure. The wood is very heavy, hard and strong.

1.2.1. Distribution. The plant is distributed in Upper Myanmar and Taninthayi. It is native to tropical region from Srilanka but also cultivated in Southern Nepal, Indo China and the Malay Peninsula. According to botanist, this is the only ironwood forest in the dry zone with wet zone vegetation. The plant has commonly grown along roadsides and in the parks [1].

1.2.2. Medicinal Uses. Gangaw has been used as cough, asthma, fever, dysentery, antimicrobial, antidiarrhoeal, antihelminthic, antidiarrhoeal, bleeding piles, burning feet, astringent, stomach-ache, antidote for snake poison and skin diseases [2].

2. Materials and Methods

2.1. Instrumentation and Materials

2.1.1. Instrumentation. The IR spectrum was measured by using Matlson FT-IR (Fourier Transform Infrared Spectrophotometer) at Department of Chemistry, University of Mandalay. The occurrence of UV absorption on TLC plate was checked by UV detector and iodine vapour. The apparatus for extraction and chromatography were used with common laboratory equipments.

2.1.2. Materials. Commercial available reagents and solvents were used by further purification. Iodine vapour was used for location of the spots. Analytical and preparative thin-layer chromatography was performed by using precoated silica gel plates. Silica gel (Merk Inc; Kiesel gel 60, 70-230 mesh ACTM) was used for column chromatography.

2.2. Sample Collection

The roots of Gangaw were collected from Shwe Kyet Yet Village, Amarapura Township in Mandalay Region. The roots were washed and cut into small pieces and allowed to air dry well. It was stored in a well stoppered bottle and used throughout the experiments.

2.3. Preliminary Phytochemical Screening of Root of Gangaw

Phytochemical tests were done on the extracted sample according to the procedures by test tube methods [3].

2.4. Antimicrobial Activities of Root of Gangaw

The plant extract with five solvent systems were sent to DCPT, Insein, Yangon for the investigation of antimicrobial activities. Table (1) described the results of the antimicrobial activities of root of Gangaw determined by Agar well diffusion method towards on six organisms.

2.5. Isolation of Pure Organic Compound by Column Chromatography

2.5.1. Preparation of Crude Sample by Solvent Extraction Method. Gangaw, the air-dried powder (350 g) was percolated with ethanol (1.5 L) for two

months. Percolated solution was filtered and evaporated. Then ethanol crude extract was concentrated and the residue was extracted with ethyl acetate (200 ml). The crude extract was evaporated to dryness and separated by column chromatography.

2.5.2. Determination of Ethyl acetate Crude Extract by Thin Layer Chromatography Method. Thin layer chromatography was conducted on the crude extract in a solvent system of n-hexane and ethyl acetate. The crude extract was spotted on TLC plate. After spotting, the plate was dried and run in the chosen solvent system and then it was dried. Then TLC was inspected in short wave UV detector and iodine vapour.

2.6. Flow Sheet for the Isolation of Xanthone

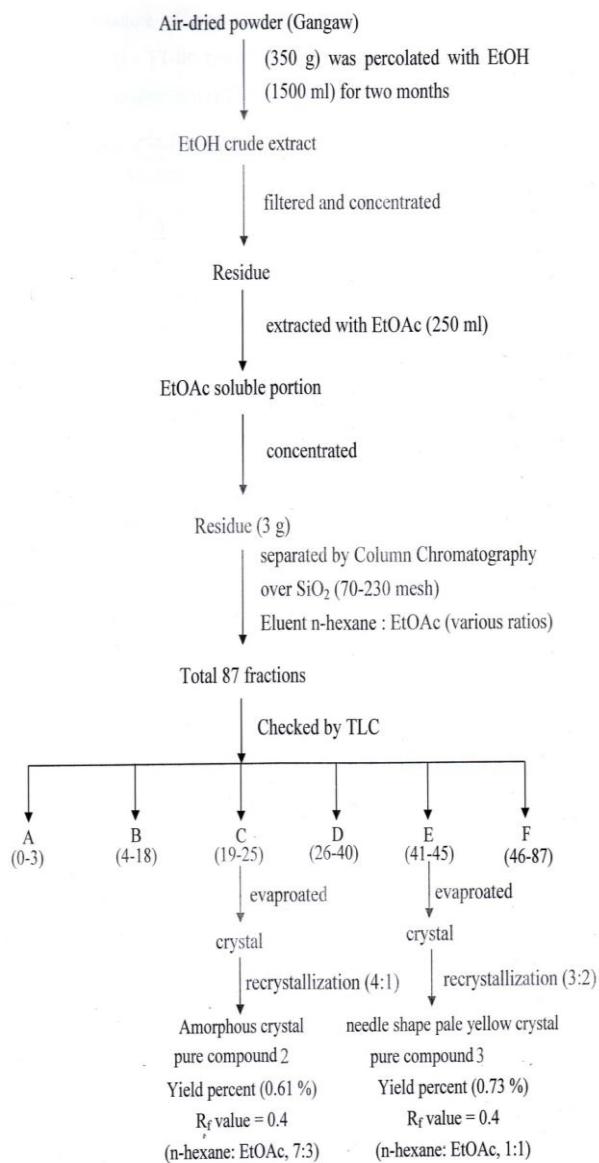


Figure 3. Flow Sheet for the Isolation of Xanthone

2.6.1. Procedure. Two pure compounds were separated from ethyl acetate crude extract by column chromatography as shown in Figure (3) and (4).



Figure 4. Separation of Ethylacetate Extract by Column Chromatography

2.7. Study of the FT-IR Spectrum of Pure Organic Compound (3)

The structure of pure organic compound (3) was studied by FT-IR spectroscopy [4]. The FT-IR spectrum was illustrated in Figure (8) and its assignment were described in Table (3).

2.8. Polyphenol Test for Pure Compound (3)

A small amount of pure crystal was boiled with ethanol in a water-bath for a few minutes. The mixture of 1% FeCl_3 and 1% $\text{K}_3\text{Fe}(\text{CN})_6$ solution was added to it.

2.9. Determination of Melting Point of Pure Organic Compound (3)

A small amount of crystal was inserted into a capillary tube and the tube was attached to the thermometer. It was then inserted into the test tube containing liquid paraffin. After the tube was gently heated, the crystal in the capillary tube was melted at (275-276°C).

3. Results and Discussion

3.1. The Results of Preliminary Phytochemical Tests of Root of Gangaw

The phytochemical screening of the root extracts of Gangaw has been done as shown in Figure (5) and the results are shown in Table (1).



Figure 5. Preliminary Phytochemical Tests for Root of Gangaw

Table 1. The Results of Preliminary Phytochemical Tests for Root of Gangaw

No.	Test	Reagent Used	Observation	Inference
1.	Alkaloids	(i) Dragendorff's reagent (ii) Mayer's reagent	Orange ppt Cream color	+
2.	Flavonoids	Conc: HCl + Mg	Red	+
3.	Glycosides	10% lead acetate	White ppt	+
4.	Polyphenols	1% FeCl ₃ + 1 % K ₃ Fe(CN) ₆	Bluish green	+
5.	Steroids	(CH ₃ CO) ₂ O, con: H ₂ SO ₄	Reddish brown	+
6.	Terpenes	CHCl ₃ , Con:H ₂ SO ₄ , (CH ₃ CO) ₂ O	Red	+
7.	Saponins	NaHCO ₃	Frothing	+
8.	Sugars	Benedict's solution	Red ppt	+
9.	Lipophilic acids	0.5 M KOH	-	-
10.	Tannins	2% NaCl, 1 % FeCl ₃	-	-

(+) = presence of constituent, (-) = absence of constituent

According to Table (1), the root of Gangaw contains alkaloids, flavonoids, glycosides, polyphenols, steroids, terpenes, saponins and sugars respectively.

3.2. The Results of Antimicrobial Activities of Root of Gangaw

Table 2. The Results of Antimicrobial Activities for Root of Gangaw

Sample	Solvent	Organisms					
		<i>B. sub</i>	<i>S. aureus</i>	<i>Pseudo monas</i>	<i>B. pumalis</i>	<i>Candida</i>	<i>E. coli</i>
Gangaw (root)	n-hexane	-	20 mm (+++)	-	20 mm (+++)	20 mm (+++)	-
	CHCl ₃	-	20 mm (+++)	-	20 mm (+++)	20 mm (+++)	-
	EtOH	-	25 mm (+++)	-	25 mm (+++)	25 mm (+++)	-
	EtOAc	-	28 mm (+++)	-	30 mm (+++)	30 mm (+++)	-
	Acetone	-	26 mm (+++)	-	25 mm (+++)	25 mm (+++)	-

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 pumalis*

(5) *Candida albican*

(6) *E. coli*

According to the results in Table (2), all five solvents extracts give high activity on three tested organisms (such as *S. aureus*, *B. pumalis* and *Candida albican*) as shown in Figure (6).

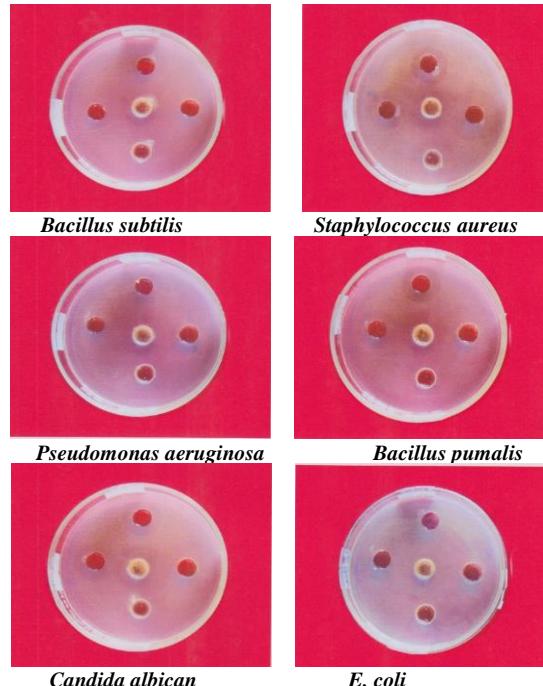


Figure 6. Antimicrobial Activities of Root of Gangaw

3.3. The Results of Isolation of Pure Organic Compound from the Root of Gangaw

Pure organic compound (3) could be isolated from ethyl acetate extract of root of Gangaw using thin layer and column chromatographic methods. The physical state of compound (3) is pale yellow crystal. The yield percent of compound (3) is (0.73 %) based upon the ethyl acetate extract (3g). R_f value of pure compound (3) was observed as (0.4) by TLC method as shown in Figure (7).



Figure 7. TLC of Pure Organic Compound (3)

3.4 .The Results of FT-IR Spectrum of Pure Compound (3)

According to the FT-IR spectrum, in Figure(8), the isolated compound (3) contains OH functional group, sp^2 and sp^3 hydrocarbon, carbonyl group, aromatic benzene ring, alcohol group, ether group, trans or E and cis or Z alkenic groups respectively as shown in Table (3) [8].

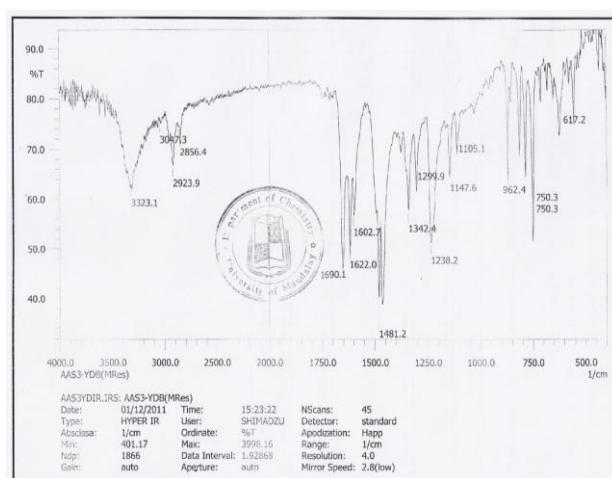


Figure 8. FT-IR Spectrum of Pure Organic Compound (3)

Table 3. The Absorption Peaks and their Assignments of Compound (3)

No.	Absorption peak	Assignments
1	3323.1 cm^{-1}	OH stretching vibration
2	3047.3 cm^{-1}	=C – H stretching vibration of sp^2 hydrocarbon
3	2923.9 cm^{-1} , 2856.4 cm^{-1}	Unsymmetrical and symmetrical C–H stretching vibration of sp^3 hydrocarbon
4	1690.1 cm^{-1}	C=O stretching vibration of carbonyl group
5	1602.7 cm^{-1} , 1622.0 cm^{-1} , 1481.2 cm^{-1}	C=C stretching vibration of aromatic benzene ring
6	1238.2 cm^{-1}	C–C–O stretching vibration of alcohol group
7	1147.6 cm^{-1} , 1105.1 cm^{-1}	C–O–C stretching vibration of ether group
8	962.4 cm^{-1}	C–H out of plane bending vibration of trans or E alkenic groups
9	750.3 cm^{-1}	C–H out of plane bending vibration of cis or Z alkenic groups

3.5. The Results of Polyphenol Test for Pure Compound (3)

Polyphenol was observed as the blue green color solution. This colour reaction test indicates the presence of polyphenol as shown in Figure (9).

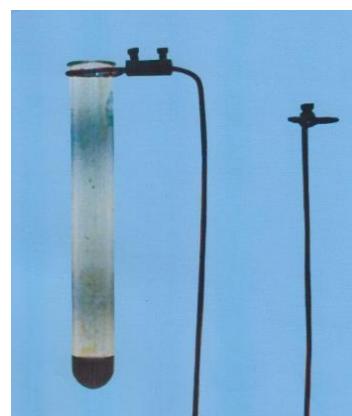


Figure 9. Polyphenol Test for Pure Organic Compound (3)

3.6. The Results of Determination of Melting Point of Pure Compound (3)

Melting point of this compound (M.P 275-276°C) was nearly identical with that of literature value (M.P 275-277°C). Thus, this crystal is estimated as 1, 3-dimethoxy-5, 6-dihydroxy xanthone as shown in Figure (10).

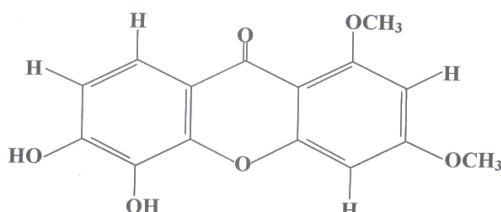


Figure 10. The Structure of 1, 3-dimethoxy-5, 6- dihydroxy xanthone

4. Conclusion

In this research work, the roots of Gangaw were collected from Shwe Kyet Yet Village, Amarapura Township in Mandalay Region.

The phytochemical screening of root of Gangaw gives rise to the positive tests for alkaloids, flavonoids, glycosides, polyphenols, steroids, terpenes, saponins and sugars respectively.

In addition, the antimicrobial activities of the crude extracts in various solvent systems were tested by agar well diffusion method on six selected organisms, namely *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonous aeruginosa*, *Bacillus purnalis*, *Candida albican* and *E-coil*. Among them, all five solvent extracts give high activity on three tested organisms (such as *S. aureus*, *B. pumalis* and *Candida albican*).

Isolation of pure compound (3) was done by solvent extraction and column chromatography. The yield percent of this compound was found to be (0.73 %) based upon the ethyl acetate crude extract. Identification of structure of pure compound (3) was determined by FT-IR spectroscopic method, colour reaction test, determination of melting point and R_f value respectively. From the study of the various results, isolated pure compound (3) may be 1, 3-dimethoxy-5, 6-dihydroxy xanthone [6].

5. Acknowledgement

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6. References

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Analysis of Physical and Chemical Properties of Soil Samples from Nyungouk Village in Ma-Hlaing Township (Mandalay Region)

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Abstract

In this research paper, the soil samples were taken from Nyung-ouk Village in Ma-Hlaing Township. Firstly, these samples were collected from the depth of 6cm on the surface in which three different places for analysis of physical and chemical properties. Physical properties of these samples such as moisture, pH, colour, electrical conductivity and soil texture were determined. The values of pH for all samples were found to be 10.09, 10.04 and 10.21. The texture type of samples 1, 2 and 3 were determined as sandy clay, sandy clay loam and loamy sand respectively. In addition, the determination of chemical components, such as chloride, water soluble sulphate, exchangeable calcium, exchangeable magnesium, exchangeable sodium, carbonate, bicarbonate and CaCO₃ were done. The elemental compositions of soil samples were determined by EDXRF method.

Keywords : chemical properties, EDXRF, physical properties.

1. Introduction

Soil science is the study of soil as a natural resource on the surface of the earth including soil formation, classification and mapping; physical, chemical, biological, and fertility properties of soil; and these properties in relation to the use and management of soil [1]. Soil is a composed of organic and inorganic matter, minerals, gases, liquids, and organisms that together support life. It varies from place to place due to its structure and composition. There are many kinds of soil, such as alkali soil, natural soil, acetic soil and so on. The alkaline soils are the soil with high pH value (> 9). It has a poor soil structure and low infiltration capacity. These types of soil have dominated presence of minerals such as sodium carbonate which causes the soil to swell. They are generally noticed in arid and semi arid regions where there is low rainfall and high temperature causing intense evaporation. There are several causes that result in soil alkalinity. They comprise the accumulation of minerals forming sodium carbonate and sodium bicarbonate due to weathering process and accumulation of sodium that are used in the industrial and domestic application.

There are two principal aspects of the salt problem in irrigation agriculture. One is the improvement of soils are salt affected under natural conditions or have

become salt affected because of mismanagement. The other aspect is the management of productive or slightly salt-affected soils as to prevent increases in the soluble salt and adsorbed sodium contents and this prevent reduction in crop yield [2].

In this research work, soil samples were collected from Nyung-ouk Village in Ma-Hlaing Township, Mandalay Region. The soil samples are called natural soda. The main aim of this research is analysis of physical and chemical properties of selected soil samples and the objectives of this research are to determine some physical, chemical constituent and elemental compositions for the purpose of agricultural used and raw material for soap industry.

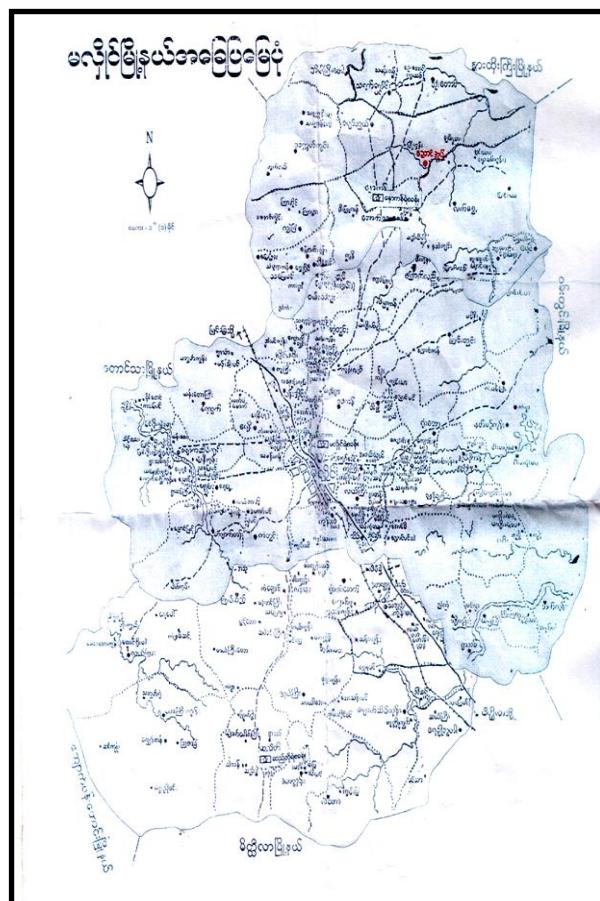


Figure 1. Map of Ma-Hlaing Township Showing the Location of Soil Samples Collected Area

2. Materials and Methods

2.1. Sample Collection

Three types of soil samples were collected from 6 cm depth on the surface of the selected area.



Sample 1 (Lower the Stream)



Sample 2 (Upper the Stream)



Sample 3 (Near the Village)

Figure 2. Collection of Soil Samples

2.2. Preparation of Soil Samples

The soil samples should be drawn towards the end of the dry season, when they contain the maximum concentration of soluble salts. The sample may be kept in drill bags. When the samples are received at the laboratory, it should be broken up into large lumps and spread them on stout sheets of brown paper for air dried. When the samples are air dried, the samples are ground

in a mortar under such conditions that the aggregate particles are crushed but no real grinding or breakdown of the ultimate particles of the soil occurs. The soil should be sifted through a sieve with round holes 2 mm in diameter and returned the coarse material to the mortar for further crushing. The sieving and crushing of this residue are repeated until all aggregate particles are fine enough to pass through the sieve and only stone and organic residue remain on it. Then the fine earth passing through 2 mm sieve should be mixed and stored it in suitable bottles and labelled then properly.

2.3. Determination of Moisture

Constant weight of the weighing bottle was first determined. Then about 5 g of soil sample was transferred into weighing bottle and weighed accurately. It was allowed to dry in electric oven at 105°C. Then it was dried to constant weight. From the loss in weight, the percentage of moisture content was calculated.

Calculation

$$\begin{aligned} \text{Mass of basin} &= W_b \\ \text{Mass of basin} + \text{soil} &= W_1 \\ \text{Mass of oven dry soil} + \text{basin} &= W_2 \\ \text{Mass of soil before heating} &= W_1 - W_b = b \\ \text{Mass of soil after heating} &= W_2 - W_b = a \\ \therefore \text{Moisture \% (M)} &= \frac{(b-a) \times 100}{b} \\ \text{Moisture coefficient, } K &= \frac{100 - M}{100} \end{aligned}$$

2.4. Determination of Soil pH

10.0g of sample was weighed accurately and placed into a conical flask. Then 25 mL of distilled water was added and shaken for 15 min. The pH was measured by pH meter. The pH meter was calibrated with pH 4.0 and pH 10.0 buffer solution before use.

2.5. Determination of Texture

About 10 g of air-dry soil was weighed accurately and placed in a 500 mL conical flask and some amount of distilled water was added. The flask was heated till boiling. 10 mL of 10 % sodium pyrophosphate solution was added to disperse the soil colloids and heating was continued for about fifteen minutes after which it was cooled. After cooling, the contents were transferred to a 1000 mL graduated cylinder and the solution was made up to the mark with distilled water and then kept overnight to allow the soil colloids to settle.

The next day, the contents were stirred for about four minutes, the solution from 9 cm depth was pipetted with 25 mL pipette and then it was transferred to a porcelain basin and evaporated on a water bath. From the weighed amount of residue, the percentage of clay and silt were calculated.

After four hours of the stirring, the solution was pipetted with 25 mL pipette from 4 cm depth and evaporated to dryness. From this residue, the percentage of clay was calculated. Then, the percentage of silt was obtained by difference.

For the determination of the amount of sand, the remaining solution was poured into 53 μm sieve and the clay and silt were washed with water. The percentage of sand was calculated.

Calculation

$$\begin{aligned}\text{Clay \%} &= \frac{c \times 4000}{s \times k} \\ \text{silt \%} &= \frac{(b - c) \times 4000}{s \times k} \\ \text{sand \%} &= \frac{a \times 100}{s \times k}\end{aligned}$$

c = Weight of clay

b = Weight of silt and clay

a = Weight of sand

s = Weight of sample

k = Moisture coefficient

V_1 = Volume of water extract taken for titration
 V_2 = Total volume of water extract
k = Moisture coefficient
s = Weight of sample

2.6.2. Determination of Water Soluble Sulfate. 25 mL of water extract was pipetted into conical flask and the flask was gently warmed to expel carbondioxide till its content began boiling and 10 mL of 0.02 M barium chloride solution was added. It was cooled to room temperature. Then 5 mL of ammonium buffer solution ($\text{pH} = 10$) was added. Eriochrome Black T was used as an indicator. It was titrated with 0.2 M EDTA solution. The end point is reached to change blue color.

Calculation

$$\% \text{ of } \text{SO}_4^{2-} = \frac{V \times M \times 4.803 \times V_2}{V_1 \times k \times s}$$

V = Titrant for blank

M = Molarity of EDTA solution

V_1 = Volume of water extract taken for titration

V_2 = Total volume of water extract

k = Moisture coefficient

s = Weight of sample

2.6. Preparation of 1:5 Soil Water Extract for Determination of Chloride and Water Soluble Sulphate

About 120 g of soil was transferred into a suitable shaking bottle and 600 mL of distilled water and shook the contents in a shaker for 30 minutes. At the end of the period, the bottle was set aside for 3 hours to allow the coarser particles to settle down leaving a clear supernatant liquid. It was decanted carefully into a dry beaker.

Then the extract was passed through a filter rejecting the first portion of 20 to 25 mL. Alternatively the extract was passed through a small Buchner funnel fitted with a dry filter paper (Whatman No. 50) receiving the filtrate into a dry flask rejecting the first portion of 20 to 25 mL. Thus it was collected 500 mL of the clear extract for analysis.

2.6.1. Determination of Chloride. 10 mL of 1:5 soil water extract was taken in a porcelain basin. 0.5 g of sodium carbonate was added to make it slightly alkaline. 1 mL of the potassium chromate solution was added. Then it was titrated against 0.02 M silver nitrate, stirring the contents in chocolate brown precipitate of silver chromate persisted even after stirring. The white milky colour of the basin serves as the background to observe the colour of the precipitate and the end point is marked precisely. The volume of the silver nitrate used was noted.

$$\% \text{ of chloride (as } \text{Cl}^-) = \frac{V \times M \times V_2 \times 100}{V_1 \times k \times s}$$

V = Titrant

M = Molarity of silver nitrate solution

2.7. Determination of Alkali Carbonate and Bicarbonate

Exactly 10 mL of the sample was pipetted into a conical flask and then 2 drops of phenolphthalein indicator solution was added to it. It was titrated with standard HCl solution up till end point where colour changed to colourless. If the solution was colourless, no carbonate was presented but if pink colour was seen titration must be continued slowly and carefully with 0.1 M HCl till pink colour just disappears. This indicated the bicarbonate end point. The volume of the acid required was noted as "A" mL of 0.1 M HCl. 2 drops of methyl orange indicator was added to the flask and continued the titration with 0.1 M HCl till the indicator just changed to light rose red. Let the second portion of the time value be "B" mL of 0.1 M HCl. Hence $2A = \text{carbonate}$ and $B - 2A = \text{bicarbonate}$.

2.8. Determination of Calcium Carbonate

1 g of air-dry soil was weighed into a 250 mL Erlenmeyer flask. 10 mL of 1 M hydrochloric acid solution was added to the flask with a volumetric pipette. This flask was stirred and leaved at overnight or this flask was heated to 50-60°C and the flask was cooled. After cooling, 50 mL of distilled water was added in 2-3 drop of phenolphthalein indicator was added. It was titrated with 1 N sodium hydroxide solution while stirring the flask. The colour of end point is changed from colourless to faint pink.

Calculation

$$\% \text{ CaCO}_3 = [(10 \times N_{\text{HCl}}) - (R \times N_{\text{NaOH}})] \times 0.05 \times \frac{100}{\text{Wt}}$$

N_{HCl} = Normality of HCl solution

R = Volume of NaOH solution used

N_{NaOH} = Normality of NaOH solution

Wt = Weight of air dry soil

2.9. Determination of Electrical Conductivity

A 10 g of sample was weighed and 250 mL of distilled water was added to make a 1 : 2.5 H₂O soil water suspension solution. Then it was allowed to stand for an hour. Conductivity meter was calibrated using 0.01 M KCl solution. Soil solution was filtered and electrical conductivity of filtrate was determined by conductivity meter.

2.10. Determination of Exchangeable Sodium by Flame Photometer

About 5 g of sample was weighed accurately and placed in a 100 mL shaking bottle containing 50 mL of 1 M ammonium acetate solution. The bottle was shaken for one hour and the solution was filtered. The amounts of sodium in the filtrate were measured by using the flame photometer.

2.11. Determination of Exchangeable Calcium and Magnesium

About 2.50 g of sample was weighed accurately and placed in a 500 mL shaking bottle containing 250 mL of 1 M sodium chloride solution. The bottle was shaken for three minutes and kept overnight and the filtered.

To determine calcium and magnesium, 25 mL of filtrate was pipetted into conical flask and then 5mL of ammonium buffer solution (pH = 10) was added. Eriochrome Black T was used as an indicator. It was titrated with 0.02 M EDTA solution until the color changed to blue.

To determine calcium, 25mL of filtrate was pipetted into conical flask and then 2 mL of 10 % sodium hydroxide solution was added. Murexide was used as an indicator. It was titrated with 0.02 M EDTA solution and the end point color was violet.

Calculation

$$\text{Ca(meq/100g)} = \frac{V \times M \times 1000}{s \times k}$$

V = Titrant for Ca

M = Molarity of EDTA solution

s = Weight of sample

k = Moisture coefficient

$$\text{Mg(meq/100g)} = \frac{V \times M \times 1000}{s \times k}$$

V = titrant for (Ca + Mg) – titrant for Ca

3. Results and Discussion

3.1. Physical and Chemical Analysis of Soil Samples

The soil samples were collected from Nyung-ouk Village in Ma-Hlaing Township. They were taken from three places as sample 1, 2 and 3 in that region. These samples were analysed for physical and chemical properties. The results of all samples were described in table 1, 2, 3, 4 and 5.

Table 1. Some Physical Properties of Soil Samples

Sample	pH	Moisture (%)	Electrical Conductivity(m mhos/cm)	Colour
S ₁	10.09	11	62.1	White
S ₂	10.04	8	42.7	White
S ₃	10.21	3	13.2	Gray

According to Table (1), the pH value of all these samples are greater than 10. So these soil samples are strongly alkaline. Moisture content of sample 3 is less than the other two. Soil moisture content may be prevented excess irrigation and leaching of nutrients. The electrical conductivities of soil samples 1 and 2 are greater than sample 3. Therefore, these two samples were contained the higher amount of dissolve materials. According to soil color, all these samples may be presented as silicate and salt.

Table 2. Textural Analysis of Soil Samples

Sample	Composition			Texture class
	Sand (%)	Silt (%)	Clay (%)	
S ₁	54.5	3.3	42.2	Sandy clay
S ₂	72.2	1.3	26.5	Sandy clay loam
S ₃	85.0	7.7	7.3	Loamy sand

In Table (2) texture type of these samples were not the same each other but sand percent were determined very high in all samples. This condition do not maintained the water for a long time.

Table 3. Some Chemical Properties of Soil Samples

Sample	Chloride (%)	Carbonate (%)	Bicarbonate (%)	Calcium carbonate (%)	Water soluble sulphate (%)
S ₁	18	15	25	23	13
S ₂	8	9	15	13.5	11
S ₃	2	2	3	1.5	3.9

In Table (3), the percent of chloride (Cl^-) and water soluble sulphate in soil samples 1 and 2 are higher than sample 3. In addition, the values of carbonate, bicarbonate and calciumcarbonate of sample 1 and 2 are higher than the sample 3. Therefore, sample 1 and 2 may be used as raw material for soap industrial.

Table 4. Exchangeable Cation Analysis of Soil Samples

Sample	Exchangeable Na (cmol/kg)	Exchangeable Ca (cmol/kg)	Exchangeable Mg (cmol/kg)
S ₁	15	0.02	0.1
S ₂	9	0.23	0.11
S ₃	2	0.25	0.16

In table 4, the exchangeable Na^+ values of samples 1 and 2 are very high. Due to the effect of excess exchangeable sodium, these two samples were found to be an adverse effect on the physical and nutritional properties of the soil, with consequent reduction in crop growth significantly or entirely.

3.1. Elemental Analysis of Soil Samples

The elemental analysis of soil samples were determined by EDXRF at Physics Department, University of Mandalay. The results were shown in Table (5).

Table 5. Elemental Analysis of Soil Samples by EDXRF Method

No .	Element	Sample 1 (%)	Sample 2 (%)	Sample 3 (%)
1	Aluminum	0.435	0.6809	1.48
2	Silicon	4.617	8.877	15.95
3	Phosphorus	0.0065	< 0.0003	0.0086
4	Sulphur	0.2532	0.2356	0.073
5	Chlorine	3.407	2.103	0.8245
6	Potassium	0.2696	0.4850	1.034
7	Calcium	0.8569	0.8791	1.086
8	Manganese	0.01	0.0133	0.1973
9	Iron	0.2540	0.3207	0.4682

In Table (5), higher amount of silicon and the lowest amount of phosphorus were contained in all samples. This effect was founded by which the highest amount of sand percent. The amount of Al, K, Ca and Mn in sample 3 were greater than the other two samples. It is good for ion exchange capacity in soil.

According to all these results, these samples were presented an impure efflorescent deposit containing salt of sodium including the carbonate with a large amount of intermixed sand. It may be used for cleaning and washing.

4. Conclusion

The soil samples were collected from Nyung-ouk Village, Ma-Hlaing Township in Mandalay Region. They were taken from three places as sample 1, 2 and 3 in that region. These samples were analysed for physical and chemical properties. From the results, the pH values were greater than 10. So, the soil samples are strongly alkaline. The values of electrical conductivity were very high (62.1 dS/m, 42.7 dS/m and 13.2 dS/m). So, the soil samples are saline alkali soil and more dissolved materials.

The higher amount of chloride and water soluble sulphate were presented in samples 1 and 2. The percent of carbonate, bicarbonate and CaCO_3 of samples 1 and 2 were greater than sample 3. The exchangeable sodium contents were very high in samples 1 and 2. The elemental compositions of the soil samples were found to be higher amount of silicon. According to all these results, the soil samples may be regarded as a class of problem soils that requires special remedial measures and management practices for agricultural purpose. These are suitable for used as raw material in soap industrial.

5. Acknowledgements

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Effect of Synthetic Naphthalene Acetic Acid (NAA) on Rooting Growth and Stem Growth of Cow pea (*Vigna catjang walp*)

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Abstract

*The aim of this research was to study the effect of different media and concentrations of synthesized naphthalene acetic acid (NAA) on rooting growth and stem growth of cow pea (*Vigna catjang walp*). In this work, NAA was successfully synthesized from naphthalene which was extracted from coal tar and naphthalene ball. In each step, isolation, purification and characterization were carried out by Ultraviolet (UV) and Fourier Transform Infrared (FT-IR) spectroscopy. The final product NAA were checked by thin layer chromatography and confirmed by determination of their melting points. The yield percent of naphthalene from coal tar and naphthalene ball are found to be 4.09 % and 39.17%. The yield percent of NAA based on naphthalene is found to be 21.0 %. The effect of NAA on root development was also studied in different concentrations of NAA on cow pea hypocotyl cutting in aqueous and soil media.*

Keywords - coal tar, cow pea hypocotyl cutting, naphthalene ball, naphthalene acetic acid

1. Introduction

Cow pea seeds provide a rich source of proteins and calories, as well as minerals and vitamins. This complements the mainly cereal diet in countries that grow cow peas as a major food crop. A seed can consist of 25% protein and has very low fat content. Cow pea starch is digested more slowly than the starch from cereals, which is more beneficial to human health. The grain is a rich source of folic acid, an important vitamin that helps prevent neural tube defects in unborn babies [1]. In Myanmar, all sorts of legumes are cultivated and exported every year. Actually Myanmar is the second largest legumes exporting country in the world since 2002. At least 18 varieties of legume was cultivated and among them, exported soy bean was 4% and that of cow pea was also nearly 4% of the total export. Varieties of legumes were exported to neighbouring countries such as India, Pakistan, Indonesia, Singapore, Malaysia and Japan [2]. As the market demand is increasing, Myanmar should also increase its legume cultivation. In the hope to fulfill this novel ambition, this research

work was performed for the synthesis of NAA and application of NAA with cow pea in different media.

Growth medium which plays an important role in growth and production of flowers as it provides plants with all the necessary materials and nutrients essential for growth and flowering. The growth in the balanced media diet provides plants with nutrients good to improve the growth of plants and flowers [3]. The hormone NAA does not occur naturally, and, like all auxins, is toxic to plants at high concentrations. NAA is widely used in agriculture shown to greatly increase cellulose fiber formation in plants paired with another phytohormone[4]. Naphthalene acetic acid (NAA) is an organic compound with the formula $C_{12}H_{10}O_2$. This colorless solid is soluble in organic solvents. It features a carboxyl methyl group (CH_2CO_2H) linked to the "1-position" of naphthalene. NAA is a synthetic plant hormone in the auxin family and is an ingredient in many commercial plant rooting horticultural products; it a rooting agent and used for the vegetative propagation of plants from stem and leaf cuttings.[5]

Growth regulators play a key role for developing a specific mode of growth in the cultured cells or tissues, which may be due to accumulation of specific biochemical contents in them. The single or combination of different hormones in the medium causes maintenance of specific and balanced inorganic and organic contents in the growing tissue. This leads the cells or tissues to develop either into shoots/or roots or even death [6]. In tissue culture, plant growth regulators are important media components in determining the development and developmental pathway of the plant cells. Growth regulators are used in different proportions to break dormancy and enhance shoot formation since it is well demonstrated that the apical dormancy is under control of these growth regulators [7]. The cytokinins and auxins are of importance *in vitro* culture as the later are concerned with root formation, the former is mainly required in the media for shoot formation and growth of buds[8]. These growth regulators are required in combination in the media as it is always the manipulation and variation of auxins and cytokinins levels that can successfully change the growth behavior of plant cultures Auxins and other growth regulators such as gibberellins play important roles in the growth and differentiation of cultured cells and tissues[9,10]. Auxins such as

naphthalene acetic acid (NAA) have been reported to promote plant rooting *in vitro* [11, 12].

The aim of this research is to study the effect of naphthalene acetic acid application on rooting, stem growth of cow pea hypocotyl cutting in soil and aqueous medium.

2. Experiment

In my research work, naphthalene acetic acid was successfully synthesized. In this work, coal tar and naphthalene ball were used as naphthalene source.

2.1. Materials and Methods

2.1.1. Chemicals, Apparatus and Equipments. All chemicals used, were of reagent grade from British Drug House (BDH) Chemical Co. Ltd, England and Merck Co. Ltd. Methanol and ethanol 95% were purchased locally and used after double distillation. TLC plates (Kieselgel60 F₂₅₄, Merck),UV lamp (Model UV GL-58,UVP) and Model Genesis II, Fourier Transform Infrared Spectrometer(FT-IR)(Australia),UV Visible Spectrometer (Lambda 40,Perkin-Elmer Co. England) were used. The glassware such as separating funnels, reflux condenser and round bottomed flasks were made of Pyrex glass.

2.1.2. Methodology. In this research work, methods and working procedures were employed according to the methods and procedures given in the standard text books and articles such as Vogel (1968) and internet web sites.

2.1.3. Preparation of naphthalene acetic acid from coal tar and naphthalene ball. The synthesis of naphthalene acetic acid comprised the following four reaction steps. There are

First step: Extraction of naphthalene from coal tar as well as naphthalene ball

Second step: Preparation of α -chloromethyl naphthalene from naphthalene

Third step: Preparation of α -naphthyl acetonitrile from α -chloromethyl naphthalene

Fourth step :Preparation of naphthalene acetic acid (NAA) from α - naphthyl acetonitrile

Step (1)

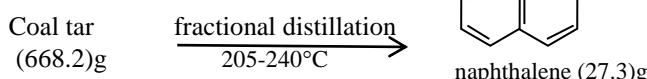


Figure 1. The Reaction Step for the Extraction of Naphthalene from Coal tar

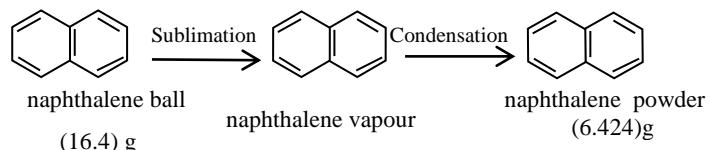


Figure 2. The Reaction Step for the Extraction of Naphthalene from Naphthalene ball

Step (2).

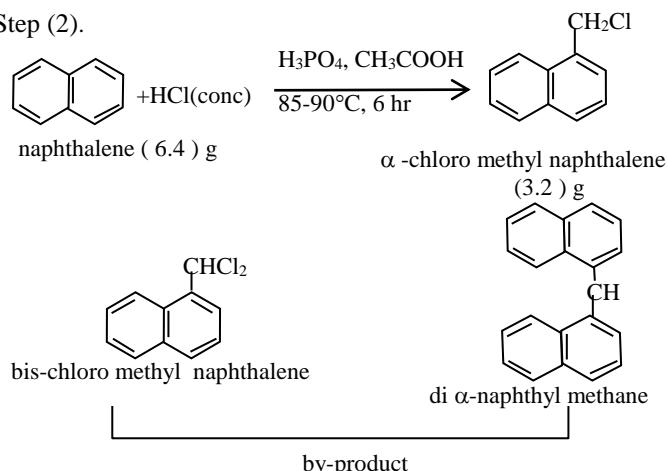


Figure 3. The Reaction Step for the Preparation of α - Chloromethyl naphthalene from Naphthalene

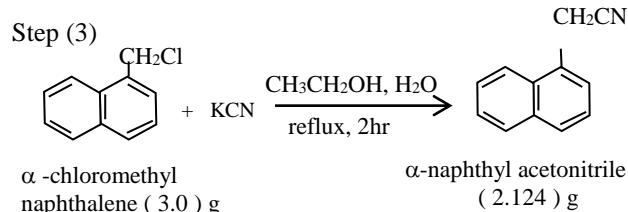


Figure 4. The Reaction Step for the Preparation of α - Naphthyl Acetonitrile from α - Chloro methyl naphthalene

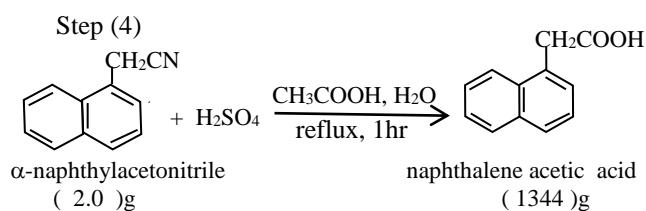


Figure 5. The Reaction Step for the Preparation of Naphthalene acetic acid from α - Naphthyl acetonitrile

In each step, isolation, purification and characterization by molecular spectroscopy such as UV and FT-IR were carried out. The precursor naphthalene was characterized by melting point, color, odor and solubility and also checked by thin layer chromatography. The solvent system used for TLC was carbon tetrachloride or n-hexane for naphthalene. All results for naphthalene, α -chloro methyl naphthalene and α - naphthyl acetonitrile were shown in previous

journals. In this paper, the result data of the final synthetic naphthalene acetic acid were performed. The final product NAA was also checked by thin layer chromatography and confirmed by determination of their melting points. The solvent system used for TLC was Benzene, Acetone, Acetic acid (15:6:1) v/v for NAA. The FT-IR spectrum and Ultra-Violet data of the isolated NAA were also recorded. And, then the effect of NAA on root development was also studied in different concentrations of NAA on cow pea (*Vigna catjang walp*)

2.2. Application of Naphthalene Acetic Acid

Cow pea (*Vigna catjang Walp*) were collected and washed in tap water followed by one rinse in sterile distilled water. Cow pea seeds were sterilized for (1 min) in 70% ethanol, soaked in 1% sodium hypochlorite plus a drop of a liquid detergent and then stirred gently (150 rpm) for 20 minutes, followed by three rinses in sterile distilled water.

Ordinary sand was put into tin tray (21cm×28cm×4cm) and sufficient water was applied until the sand was thoroughly wet. The soil medium was allowed to stand for at least ten hours to get saturated and stable condition. Thoroughly washed and sterilized seeds were grown in the prepared soil. Then the seed-containing tin trays were put in darkness and at 27°C. The sprouts appeared within 3 days. They were watered each day and after 7 days, they became about 5cm tall.

2.2.1. Procedure for Experiment in Aqueous NAA. Cow pea hypocotyl segments (2.5 cm) in length were cut from the connection of two cotyledons of cow pea plants after 7 days of growth in aqueous medium. The segments were placed in each test tubes containing the NAA solution of various concentrations (0.25 ppm, 0.5 ppm and 1ppm).For comparison, distilled water alone (ie.0 ppm NAA) was tested. The test tubes were placed on the racks. The segments were incubated for 7 days at a room temperature, also in darkness.

After incubation period, the segments were then removed from the test tubes and the length of roots were accurately measured and recorded. The number of roots were also counted and recorded.

The resultant data of number of roots, hypocotyl length, in stem length (without root) and root length was described in Table 1.

2.2.2. Procedure for Experiment in Prepared Soil Media. Cow pea seeds (*Vigna catjang walp*) were grown until they were about 5cm tall, as described in 2.2.

Hypocotyl segments (2.5 cm) in length were cut from the connection of two cotyledons of cow pea plants after 7 days of growth in soil medium. The segments were put into beakers containing various concentrations of NAA solution (0.25, 0.5 and 1.0 ppm) for 5 minutes .Then the segments were placed on the

soil medium, the cotyledons being on top. The segments without NAA treatment were also planted for comparison. The segments are cultivated in soil medium for 7 days at room temperature, also in darkness.

After cultivation periods, the segments were then removed from the sand and the length of root, the number of roots, the length of hypocotyl length and the stem length were accurately measured and recorded. The data were described in Table 2. The effect of NAA on root development was determined in different concentrations of cow pea (*Vigna catjang walp*) by bar graph and line graph.

3. Results and Discussion

Naphthalene acetic acid ($C_{12}H_{10}O_2$), melting point (128°C) and the melting points of this naphthalene acetic acid was agreeable with the literature values(129-135°C) [13] . Figure 6. shows Thin Layer Chromatograph of synthesized naphthalene acetic acid comparing with standard sample. According to UV data, the absorption peaks at 223 and 280.25 nm which are almost consisted with those of the literature value 218 and 275 nm. The yield percent of naphthalene acetic acid based on naphthalene is 21%. Figure 8. show FT-IR spectrum of naphthalene acetic acid. It revealed the presence of COOH and intense O-H and three adjacent hydrogen C-H out of plane bending vibration of a substituted naphthalene indicate at 1217.69, and at 931.34,792.36 cm^{-1} , which are almost consisted with those of the authentic NAA sample Figure 9.[13][14]

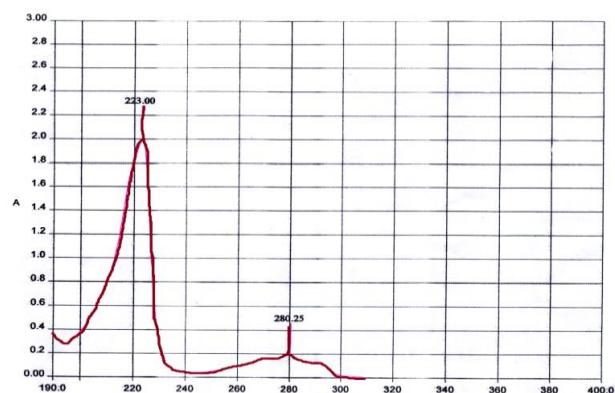


Figure 6.UV Spectrum of Naphthalene Acetic Acid

- 1) Stationary phase : Silica gel
- 2) Mobile phase: Benzene: Acetone:

Acetic acid (15:6:1)
3) Visualization :UV 254 nm
4) R_f value : 0.85

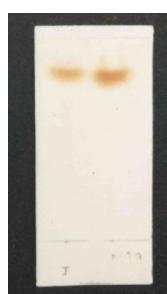


Figure 7. Thin Layer Chromatograph of Naphthalene Acetic Acid (Authentic sample, Synthesized Sample)

Peak Report
File: C:\FIRST\T\MPKMT-6.RAS
Title: Sample for Pure NAA
Filter: Three Point Center of Gravity
cm-1 %T cm-1 %T cm-1 %T cm-1 %T
539.04 29.33 623.72 33.27 778.20 4.90 792.95 16.96
931.52 20.61 1159.41 27.77 1184.36 17.68 1217.69 5.61
1261.53 14.41 1268.06 22.79 1292.55 24.64 1327.80 22.23
1410.47 11.00 1294.88 24.18 1692.14 1.91 2535.75 22.63
2644.63 19.28 2728.03 19.06 2913.53 12.68 2947.35 13.78
3012.60 12.68 3057.65 11.66

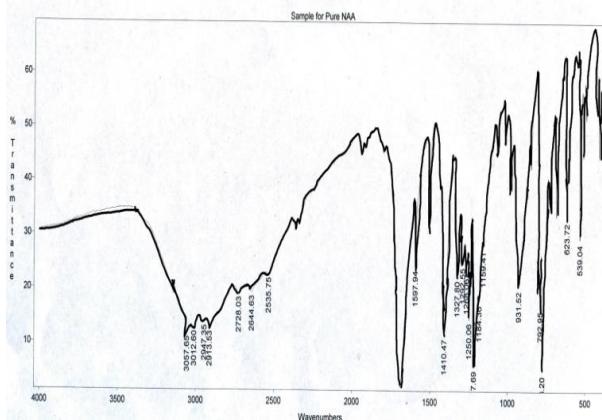


Figure 8. FT-IR Spectrum of Naphthalene Acetic Acid

Peak Report
File: C:\FIRST\T\MPICON-284
Title: 1-naphthaleneacetic acid
Filter: Three Point Center of Gravity
cm-1 %T cm-1 %T cm-1 %T cm-1 %T
541.11 69.05 625.61 72.90 780.68 20.22 937.69 60.85
1187.35 68.43 1221.02 27.49 1252.36 51.26 1270.19 61.96
1294.15 66.60 1330.23 65.00 1413.61 43.18 1513.22 78.36
1600.76 73.56 1695.03 10.00 2654.66 70.95 2739.76 70.31
2917.26 52.67 2967.43 54.60 3017.59 49.89 3061.32 46.83

WinFIRST Report
Name of owner: Ma Khin Mooh Thient
Sample name: (Lban)
Comments: 1%KBr
Operator: Daw Khin Aye Than&Myint Myint Khine

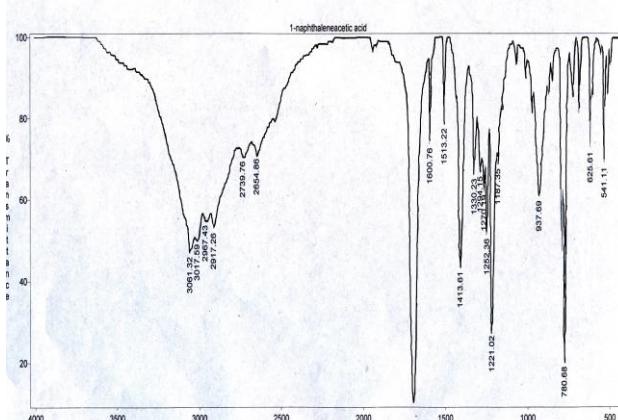


Figure 9. FT-IR Spectrum of Naphthalene Acetic Acid (Authentic Sample)

NAA is a rooting hormone. The natural auxin in plant is indole acetic acid (IAA) but it is less stable than the synthetic auxins as NAA. Consequently NAA is usually used to speed up the root formation in shoot, stem or leaf-cuttings and often allow rooting of cuttings that would not root without auxin application [15]. In this research, cow pea seeds were used in NAA application. Figure 10. The effect of NAA concentration on root formation of cow pea hypocotyl cuttings was examined.

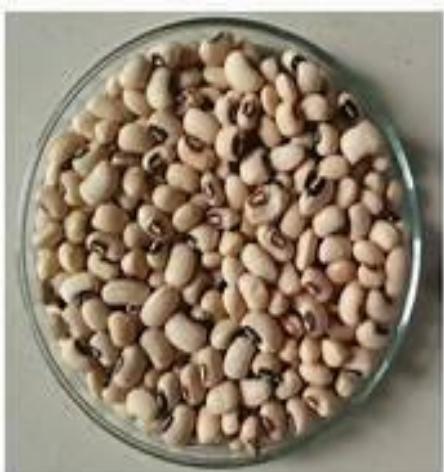


Figure 10. Cow Pea (*Vigna catjang walp*)

NAA was applied to cow pea hypocotyl cuttings in aqueous medium and in soil medium. In aqueous medium, hypocotyl length did not increase but the number of roots, root length and epicotyl length were all highest after applying 0.50 ppm NAA. Table 1. Also in soil medium, the hypocotyl length did not increase but other parameters (number of roots, root length, epicotyl length and hence stem length, i.e; total lengths of hypocotyl and epicotyl lengths) were highest for cuttings treated after 0.50 ppm NAA. Table 2. For this particular plant, the cutting dipped in 0.5ppm NAA gave the promising results.

Table 1. The Effect of NAA on Root Development of Cow pea Hypocotyl Cuttings in Aqueous Media

Sr. No	Root Development	Different Concentration of NAA			
		Control H ₂ O	0.25 ppm NAA	0.50 ppm NAA	1.0 ppm NAA
1	Number of root	3	12	14	12
2	Root length (cm)	0.10	0.10	0.13	0.11
3	Hypocotyl length (cm)	2.5	2.5	2.5	2.5
4	Epicotyl length (cm)	11.1	17.9	19.9	15.6
5	The stem length (cm)	13.6	20.4	22.4	18.1

Table 2. The Effect of NAA on Root Development of Cow pea Hypocotyl Cuttings in Soil Media

Sr. No	Root Development	Different Concentration of NAA			
		Control H ₂ O	0.25 ppm NAA	0.50 ppm NAA	1.0 ppm NAA
1	Number of root	13	18	23	20
2	Root length (cm)	0.12	0.10	0.15	0.11
3	Hypocotyl length (cm)	2.5	2.5	2.5	2.5
4	Epicotyl length (cm)	11.4	17.9	20.9	15.6
5	The stem length (cm)	13.9	20.4	23.4	18.1

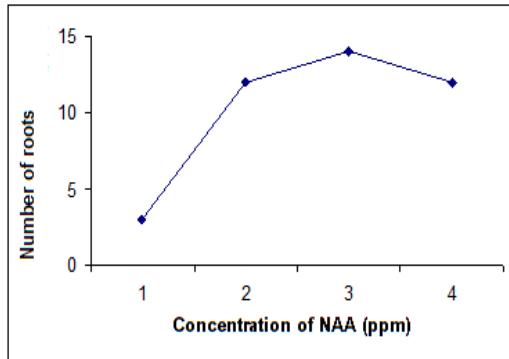


Figure 13. The Graph of the Root Number of Cow pea Cutting vs Concentration of NAA (ppm) in Aqueous Medium

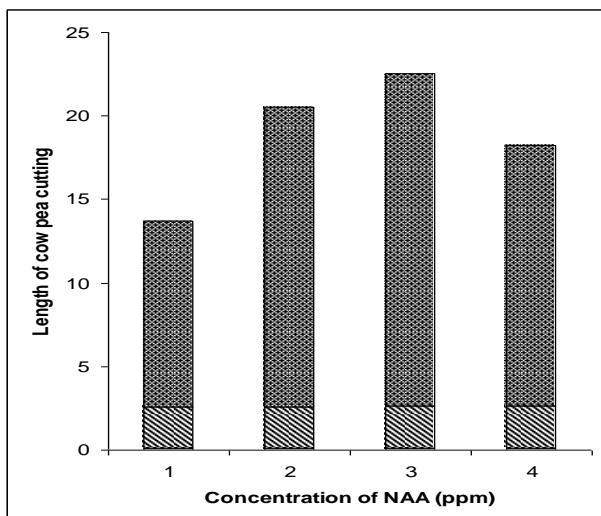


Figure 11. The Graph of the Length of Cow pea Cutting vs Concentration of NAA (ppm) in Aqueous Medium

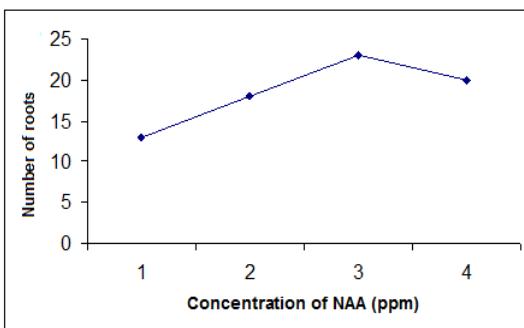


Figure 14. The Graph of Number of Roots of Cow pea Concentration Cutting vs of NAA (ppm) in Soil Medium

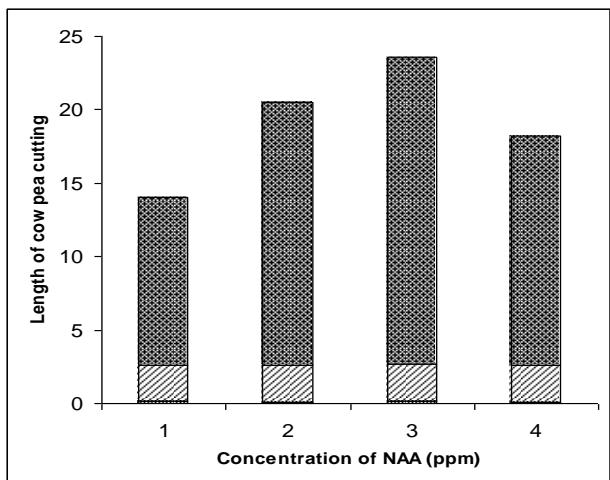


Figure 12. The Graph of the Length of Cow pea Cutting vs Concentration of NAA (ppm) in Soil Medium

- ☒ Epicotyl length (cm)
- ▨ Hypocotyl length (cm)
- Root length (cm)
- 1. Control
- 2. 0.25ppm
- 3. 0.50ppm
- 4. 1.0ppm

It can be clearly seen that NAA does not affect on the root development of cow pea hypocotyl cutting in test tubes. Only the number of root is much more in NAA than that of control. It is concluded that cow pea does not like the root development plant hormone, NAA. The data of different concentration of NAA on the root development of cow pea hypocotyl cutting are illustrated by bar graphs and line graphs, Figure 11 to 14. In conclusion, for cow pea, hypocotyl cuttings should grow after treating with 0.50 ppm NAA.

4. Conclusion

Naphthalene acetic acid is a rooting hormone. It is often used to encourage root development in cuttings to know the effect development in cuttings. In this work, naphthalene acetic acid was successfully synthesized from coal tar and naphthalene ball. NAA was checked

by TLC and identified by UV and FT-IR absorption spectroscopy. The yield percent of naphthalene from coal tar and naphthalene ball is found to be 4.09% and 39.17%.The yield percent of NAA based on the precursor, naphthalene was found to be 21%.

To know the effect of Naphthalene acetic acid (NAA) on root development, cow pea hypocotyls cuttings were used under different concentration of NAA (i.e 0.25 ppm, 0.5 ppm and 1.0 ppm).Control experiment was also done using distilled water. The most remarkable promotion of root formation occurred at 0.50 ppm NAA. In conclusion, for cow pea, hypocotyl cuttings should grow after treating with 0.50 ppm NAA. Actually,Myanmar is the second largest legumes exporting country in the world since 2002. As the market demand is increasing, Myanmar should also increase its legume cultivation. In the hope to fulfill this novel ambition, this research work was performed for the synthesis of NAA and application of NAA with cow pea in different media. Thus, these results would be useful for mass-scale propagation of cow pea.

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Investigation of Water Quality from Selected Areas in Mandalay Region

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Abstract

The study carries out investigation of the quality of ground water in Pyaygyidakhon Township, Industrial Zone (I), and Mandalay.. The water quality was determined by means of physicochemical parameters such as color, pH, total dissolved solid, turbidity, total hardness, total alkalinity, calcium, magnesium, sulphate, chloride and iron. The physicochemical parameters of collected water samples were acceptable at the range of standard value. The bacteriological examination of water samples was also studied at Public Health laboratory. In addition, heavy toxic metals such as arsenic, lead, cadmium, chromium and mercury in water samples were determined by applying Atomic Absorption Spectroscopy. Furthermore, some organic pollutant parameters such as dissolved oxygen, bacterial oxygen demand and chemical oxygen demand of water samples were also analyzed. The results obtained from the analysis were compared with WHO (World Health Organization) standard value for drinking and public consumption.

Keywords - physicochemical parameters, bacteriological, organic pollutants, toxic metals

1. Introduction

Water is a basic nutrient of human body and is critical to human life. It supports the digestion of food, absorption, transportation and the elimination of toxins and wastes from the body. [7] Water is very important in life. It is needed for many purposes in domestic life. [2]

Using water to drink and to prepare food is the most important aspect when considering the quality of water for domestic use as it directly affects the health of the consumer. Then, water to be consumed must be pleasing, in appearance, taste and odor. And water for personal hygiene must be safe and pleasing. However, it is not necessary to have high quality of water to consume. For gardening, the consumer needs only a lower quality of water than for other domestic uses. However, for irrigation of crops such as lettuce, water must not be contaminated since diseases could be transmitted in this way.[3] Domestic water supplies are one of the fundamental requirements for human life. [7]

Good quality of drinking water is very necessary to improve living standards of people and to prevent from diseases. Safe drinking water should be free of pathogens, low in concentrations of toxic chemicals,

clear, tasteless and colorless. [4] Water may contain dissolved substances or micro-organisms which may not necessarily affect the appearance or taste of the water but which may have serious health or other effects, making the water unfit for domestic use. The fitness for domestic use of particular water can only be assessed if water which includes substances of concern is available to be analyzed. [3]

Water has very important functions in the world. It plays a central role for climate and for co-evaluation of lives on earth. Water is an essential food resource for man, since humans must drink at least 2 liters of water daily. People's health also depends on the quality of the water. [5]

When water looks clean and tastes good, many people will regard it as drinking water of good quality. This may be dangerous because water may contain excessive amounts of harmful substances such as mercury or micro-organisms which may have both short-term and long-term health effects on consumers. These substances are not apparent by looking at, or tasting the water. [3]

Water quality can be determined by analysis of physicochemical properties, bacteriological examination, heavy toxic metals and some organic pollutants parameters of ground water samples. Therefore, in this research, water samples collected from Pyaygyidakhon Township, Industrial Zone (I), and Mandalay were analyzed in order to realize whether it is safe to use for drinking and domestic consumption. The present research aims to study the quality of water in those areas and to help the ordinary working class people realize that the water in their areas is enough safe to drink.

2. Materials and Methods

2.1. Sample Collection

Water samples were collected from Pyaygyidakhon Township, Industrial Zone (I), and Mandalay. The samples were collected from four sites such as site (1) in the 62nd street, site (2) in the 60th street, site (3) in the 58th street and site (4) in the 56th street in February, July and November in 2019. All sites of water samples are located between Phoeayarzar Street and, Yawmingyi Street. The depths of the sites range from 200 fts to 380 fts). Before collecting the samples, the glass bottles of 10 liters were rinsed with nitric acid and washed with water which was to be collected.

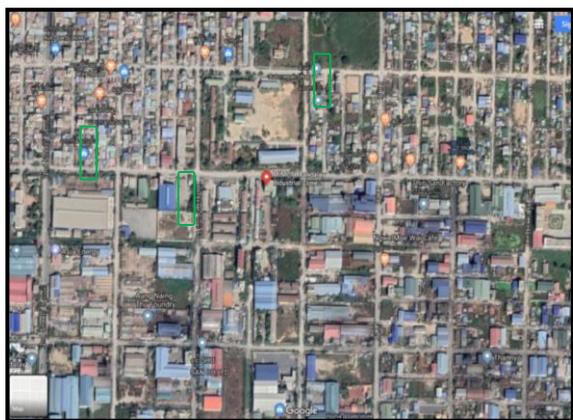


Figure 1. The Map of Industrial Zone I

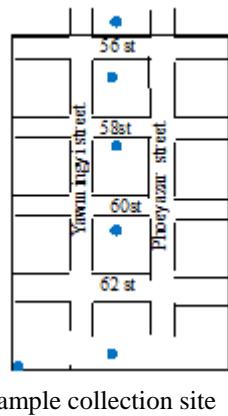


Figure 2. Sample Collection Sites Mandalay Region

2.2. Analysis of Physicochemical Properties of Water Samples

2.2.1. Determination of color. The color of water samples was determined by platinum Cobalt standard method, spectrophotometer. Color can be measured by the comparison of water samples with a series of dilutions of potassium chloroplatinate and crystalline cobaltous chloride. The units are called platinum-cobalt units based on 1mgL^{-1} Pt. [1]

2.2.2. Determination of pH. The water samples were determined by electrometric method direct measurement with pH meter. The pH measures the acid balance of a solution and is defined as the negative of the logarithm to the base 10 of the hydrogen ion concentration. [1]

2.2.3. Determination total dissolved solid. The total dissolved solid of water samples was determined by evaporation method. This parameter comprises the total dissolved solids present in the whole sample which is analyzed directly without filtration. There are two commonly used drying temperatures 105°C and 180°C . [1]

2.2.4. Determination of Turbidity. The turbidity of water samples was determined by turbidimetric method. [1]

2.2.5. Determination of total hardness. The total hardness of water samples was determined by EDTA titrimetric method. Hardness may vary over a wide range. Hardness may also determine from the sum of the divalent ions analyzed individually. [1]

2.2.6. Determination of total Alkalinity. The total alkalinity of water samples was determined by (acid-base titration) titrimetric method. The alkalinity of water is controlled by the sum of titration bases. It is mostly taken as an indication of concentration of carbonate, bicarbonate and hydroxide. [1]

2.2.7. Determination of Calcium. The calcium of water samples was determined by EDTA titrimetric method. Calcium is present in all waters as Ca^{2+} ion and is readily dissolved from rocks rich in calcium minerals, particularly, as carbonates and sulphates, especially limestone and gypsum. [1]

2.2.8. Determination of Magnesium. Magnesium is common in natural water as Mg^{2+} ion, along with calcium, is a main contributor to water hardness.

Method: Calculation Method

Magnesium can be calculated by the following formula
 $\text{Mg mg/L} = (\text{Total hardness as } \text{CaCO}_3/\text{L} - \text{Ca hardness as } \text{mg/L}) \times 0.244 \times 1000$

$$\text{CaCO}_3/\text{L} \times 0.244 \times 1000$$

2.2.9. Determination of Sulphate. The sulphate of water samples was determined by turbidimetric method. Sulphate concentrations in natural waters are usually between 2 and 80 mgL^{-1} although levels may exceed 1000 mgL^{-1} near industrial discharges or in arid regions where sulphate minerals are present. [1]

2.2.10. Determination of Chloride. The chloride of water samples was determined by argentometric method. Chloride is frequently associated with sewage; it is often incorporated into assessments as an indication of possible feces contamination or as a measure of the extent of the dispersion of sewage discharges in water bodies. [1]

2.2.11. Determination of Iron. The iron of water samples was determined by phenanthroline method. Iron is one of the essential minerals for humans and animals. Degree of absorption depends upon solubility and stability of compound. [1]

2.3. Bacteriological Examination of Water Samples

The samples were sent to the Public Health Laboratory, Mandalay to determine the bacteriological examination of water. Monitoring for the presence of pathogenic bacteria is an essential component of any water quality assessment where water use, directly or indirectly, leads to human ingestion.

2.4. Analysis of Heavy Toxic Metals of Water Samples

The content of heavy toxic metals, such as arsenic (As), lead (Pb), cadmium (Cd), chromium (Cr) and mercury (Hg) of water samples were examined by Atomic Absorption Spectrophotometer at Water Laboratory, Chemical Technology Department, Taunggyi University.

2.5. Analysis of Organic Pollutants of Water Samples

The dissolved oxygen of water samples was determined by Winkler's method. [1]

DO, BOD and COD of the water samples were measured from Ministry of Agriculture, Livestock and Irrigation, Department of Fisheries, Aquaculture Division, Fresh water Aquaculture Research, Water and Soil Examination Laboratory, Yangon City.

3. Results and Discussion

Table 1. Results of Physicochemical Properties of Water Samples on February, July and November 2019

Date	Sample	Parameters									
		Site	1	2	3	4	5	6	7	8	9
Feb 2019	1	5	7.7	1005	400	562	110	64	80	220	
	2	5	7.7	1165	420	568	112	64	80	232	
	3	5	7.8	1107	410	600	118	64	100	235	
	4	5	7.8	1288	400	658	120	64	80	238	
July 2019	1	5	7.7	1275	430	630	112	65	90	220	
	2	5	7.8	1155	420	635	110	65	100	220	
	3	6	7.8	1121	400	620	120	65	100	218	
	4	6	7.9	1188	420	638	120	65	100	228	
Nov 2019	1	5	7.6	1158	400	586	112	68	80	220	
	2	5	7.7	1140	410	620	123	60	100	228	
	3	5	7.7	1412	412	563	125	62	100	220	
	4	5	7.7	1255	422	580	120	62	80	210	
WHO	HDL	5	7.0-8.5	500	100	600	75	30	200	200	
	MPL	50	6.5-9.2	1500	500	950	200	150	600	400	

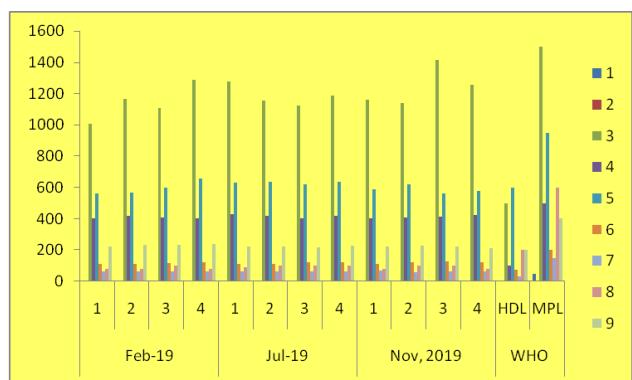


Figure 3. Physicochemical Properties of Water Samples on February, July and November 2019

- 1 = Color (platinum Cobalt Scale)
- 2 = pH
- 3 = Total Dissolved Solid (mg/ L)
- 4 = Total Hardness (mg/L)
- 5 = Total Alkalinity (mg/L)
- 6 = Calcium (mg/L)
- 7 = Magnesium (mg/L)
- 8 = Chloride (mg/L)
- 9 = Sulphate (mg/L)
- WHO = World Health Organization, 2018
- HDL = Highest desirable level
- MPL = Maximum permissible level

According to the physicochemical properties of water samples in February 2019, the color of water samples is compatible with the highest desirable level. The pH value of all water samples is between 7 to 8 and hence they are said to be slightly alkaline. The total dissolved solid of all water samples was lower than the highest desirable level. The amount of total hardness in sites 1 and 4 is the same. Magnesium amount from all the sites is the same but sulphate of water sample in site 4 was greater than other sites of water samples. The contents of total alkalinity of water sample in sites 1, 2, 3 and 4 were found to be 562, 568, 600 and 658. Thus, they were within highest desirable level. The composition of calcium of water sample in sites 4 was higher than other sites 1, 2 and 3. The chloride content in site 3 was higher than other sites.

The physical properties of water samples in July, 2019 the color of water samples goes with the highest desirable level. All the water samples have in the range of pH 7 to pH 8 and so they are supposed to be slightly alkaline. The total dissolved solid was not as high as the most desirable level. Sites 2 and 4 have the same the amount of total hardness and their compositions of the chemical properties are alike, too. Although the magnesium composition is the same in all sites, the portion of sulphate in site 3 was lower than other sampling sites. Total alkalinity of water sample in sites 1, 2, 3 and 4 showed as 630, 635, 620 and 638 which were within highly desirable level. It was also found that chloride was equally composed in sites 1, 2 and 3.

However, the content of calcium of water sample in sites 3 and 4 was higher than other sites 1 and 2.

Examining the physical properties of water samples collected in November, 2019, it showed that the color of water samples was acceptable according to the highest desirable level. The water samples were slightly alkaline because the pH values of all water samples range between 7 to 8. Moreover, the total dissolved solid from all samples were not greater than the highest desirable level. In addition to that, the amount of total hardness in all water samples is not the same. Sites 3 and 4 contain the same amount of magnesium. The sulphate of water samples in site 2 was greater than other sites of water samples. The content of total alkalinity in sites 1, 2, 3 and 4 were found to be 568, 620, 563 and 580 and they were within highest desirable level. But, the content of calcium in water samples is not the same. The chloride content in sites 2 and 3 has the same portion.

The turbidity of water samples was studied the depth to which light is transmitted. All tested water samples were found to be not turbid. The iron content was not found in all sites of water samples. The physicochemical properties of all water sites did not exceed than highest desirable level and maximum permissible level.

Table 2. Results of Bacteriological Examination of Water Samples February, July and November 2019

Date	Site	Probable Coliform Count	<i>Escherichia coli</i>
February 2019	1	0/5	ND
	2	0/5	ND
	3	0/5	ND
	4	0/5	ND
July 2019	1	0/5	ND
	2	0/5	ND
	3	0/5	ND
	4	0/5	ND
November, 2019	1	0/5	ND
	2	0/5	ND
	3	0/5	ND
	4	0/5	ND

ND = Not Detected

When the samples were studied by bacteriological examination, water samples did not show probable Coliform Count. *E.coli* was not found in all water samples. From the point of view of the bacterial, the sampling water is enough clean and clear to use as a drinking water.

Table 3. Results of Heavy Toxic Metals Contents of Water Samples February, July and November 2019

Date	Site	As	Pb	Cd	Cr	Hg
February 2019	1	ND	ND	ND	ND	ND
	2	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND
	4	ND	ND	ND	ND	ND
July 2019	1	ND	ND	ND	ND	ND
	2	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND
	4	ND	ND	ND	ND	ND
November, 2019	1	ND	ND	ND	ND	ND
	2	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND
	4	ND	ND	ND	ND	ND
WHO	HDL	0.05	0.01	0.01	0.01	0.01
	MPL	0.01	0.43	0.45	0.45	0.01

WHO = World Health Organization, 2018

HDL = Highest desirable level

MPL = Maximum permissible level

ND = Not Detected

Units – mg/L

As = Arsenic

Pb = Lead

Cd = Cadmium

Cr = Chromium

Hg = Mercury

According to this table, arsenic, lead, cadmium, chromium and mercury contents were not found in all water samples. All sites of water samples did not have toxic effect on surrounding environment. The water samples could not bring harms to the users.

Table 4. Results of some organic pollutants in water samples February, July and November 2019

Date	Site	DO	BOD	COD
February 2019	1	3.0	2.0	1.472
	2	3.5	1.0	1.104
	3	2.0	1.0	1.472
	4	2.5	0.5	0.784
July 2019	1	3.0	2.0	1.470
	2	3.5	1.0	1.624
	3	2.0	1.25	1.471
	4	2.5	2.0	0.784
November, 2019	1	3.0	1.5	1.572
	2	3.5	1.5	1.624
	3	2.0	2.25	1.672
	4	2.0	2.0	1.684
WHO	HDL	-	6	10
	MPL	4-6	5	5

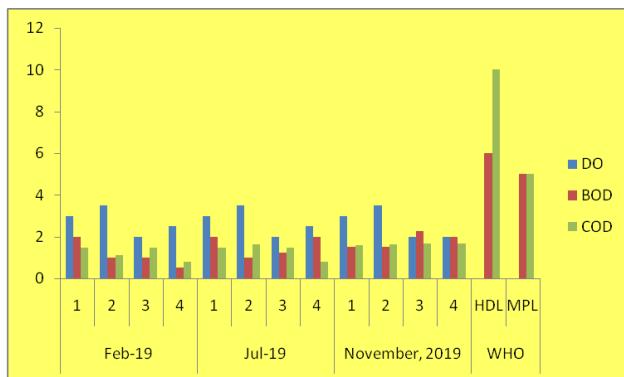


Figure 4. Organic Pollutants in Water Samples February, July and November 2019

WHO = World Health Organization, 2018

EPA = Environmental Protection Agency

Units – mg/L

DO = Dissolve Oxygen

BOD = Biochemical Oxygen Demand

COD = Chemical Oxygen Demand

Here, this table shows that dissolved oxygen (DO), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) values of all sites of water sample were not higher than the standard value. Besides, it was also found that those sampling waters could not bring any side effect on the consumers.

The water is drinkable because the components such as dissolved oxygen (DO), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) of water sample were acceptable according to the standard value.

4. Conclusion

Looking over the findings of the study, the physicochemical properties of water samples collected in three seasons are compatible with the standard value.

In February, 2019 and July 2019 and November, 2019 the physicochemical data of all water samples were fallen within WHO standard value, color, pH, turbidity, chloride and iron amounts of water samples were nearly equal. In February, 2019 the total dissolved solid, total alkalinity, calcium, sulphate amounts of water samples were higher than water samples in July, 2019. In July, 2019, total hardness and magnesium amounts of water samples were higher than in February, 2019. Iron was not observed on three seasons.

Besides, not only coliform count but also E.coli did not appear in all seasons. Therefore, the bacteriological examination results indicate that the quality of water sample is satisfactory for drinking purpose.

The dissolved oxygen (DO) measures the current oxygen level in water. The DO level varies with temperature. DO decreases by an increase in temperature. BOD was the amount of oxygen consumed

by bacterial as they oxidized organic matter in water. The value of DO, BOD and COD of water samples were within WHO standard value. The heavy toxic metals such as arsenic, lead, cadmium, chromium and mercury were not found in all water samples on February, June and November 2019.

According to analysis, data indicated that all sites of water samples were suitable for drinking purposes. The water quality is suitable for public consumption and domestic use. However, the way the consumers store matters to decide the quality of water they drink.

5. Acknowledgment

I would like to express my deepest gratitude to Dr Thida Win, Rector, Dr TinTun Aung, Dr Myint Zu Min, and Dr. Min Min Yee, pro-rectors, University of Mandalay for their interest and encouragement on my research paper. I also wish to express my thanks to Dr Yi Yi Myint, Professor and Head, Department of Chemistry, University of Mandalay for her suggestion and invaluable guidance for this research work.

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Study on the Total Flavonoid and Tannin Contents of Stem Bark of *Morus alba L.* (Po-sa) and Its Radical Scavenging Activity

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Abstract

*In this research, the stem bark of *Morus alba L.* which is flavonoid and tannin rich plant was selected to qualify and quantify the total flavonoid and tannin contents present in it. The barks of *M. alba L.* were collected from Pyin Oo Lwin Township, Mandalay Region, Myanmar. Firstly, the barks were extracted by methanol. The total flavonoid and tannin contents of this extract were evaluated by the spectrophotometric method. The total flavonoid and tannin contents of *M. alba L.* were determined as 86.02 ± 0.28 mg quercetin equivalent per g and 153.7 ± 0.65 mg/g crude extract using the standard curves respectively. Finally, the antioxidant capacity of methanolic extract of *M. alba L.* was measured by DPPH Assay method. IC₅₀ value of methanolic extract was 24.6 µg/mL. *M. alba L.* was found to exhibit significant radical scavenging activity which is comparable to standard ascorbic acid (4.92 µg/mL).*

Keywords- *Morus. alba L.*, flavonoid, tannin, spectrophotometric method, antioxidant.

1. Introduction

Plants have received a lot of attention as a source of biologically active substances, including antioxidants, antimutagens and anticarcinogens [1]. Many herbs, cereals, fruits, vegetables, oils, spices and other plant materials possess antioxidant properties, mainly because of the content of phenolic compounds. The compounds from these materials are indeed useful as alternative therapy agents, or as models for new synthetic substances. Phenolic compounds: flavonoids, phenolic acids, anthocyanins, stilbenes, tannins, lignans, and lignins, are important for normal plant growth and development as well as a defense against infection and injury. These compounds are commonly found in both edible and non-edible plants, and they have been reported to have multiple biological effects, including antioxidant activity [2].

M. alba L. commonly known as white mulberry belongs to family Moraceae is also known as Tut in India. *M. alba L.* is a moderately sized tree, three to six meters high. White mulberry is cultivated throughout the world, wherever silkworms are raised. The leaves of

white mulberry are the main food source for the silkworms. In European countries it is grown for fruit production and it is also used as vegetable in different parts of the World, while in Japan mulberry leaves are used as tea and powder juice [3-5]. Because of its good therapeutic activity and low toxicity, *M. alba* has been extensively used in conventional Chinese medicine [6].

The human body composed of a structure system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the adverse effects of free radicals and other oxidants [7].

Phenol compounds are active as antioxidants in different ways, such as direct reaction with free radicals, scavenging of free radicals, increasing dismutation of free radicals to the compounds with much lower reactivity, chelation of pro-oxidant metals (mainly iron), delaying or strengthening activities of many enzymes. Fresh fruit extracts are an excellent source of polyphenolic compounds with antioxidant activity [8].

Tannins are poly-phenols present in plants, foods and beverages, and are of great economic and ecological interest [9, 10]. They are water soluble. They also form complexes with water-insoluble proteins, alkaloids and gelatin. Many plant species producing tannins are used in folk medicine for different purposes. The tannin's drug applications are mainly related to their astringent properties. They exert internal anti-diarrheal and antiseptic effects by waterproofing the outer layers of more exposed mucous membranes [11]. Many tannins act as radical scavengers, intercepting active free radicals, various degenerative diseases such cancer, multiple sclerosis, atherosclerosis and aging process itself are associated with high concentrations of intercellular free radicals [12].

In the present research, the stem bark of *M. alba L.* was selected for the determination of total flavonoid content, total tannin content and evaluation of antioxidant activity because it is one of the rich sources of flavonoid and tannin compounds which possess antioxidant properties.

1.1. Botanical Description

Family Name	- Moraceae
Botanical Name	- <i>Morus alba</i> Linn.
Myanmar Name	- Po-sa
English Name	- Mulberry
Genus	- <i>Morus</i> Linn.
Species	- <i>Morus alba</i>

Medicinal uses - Cough, wheezing, edema, fever, headache and sore eye.



Figure 1. Plant, Fruits and Stem Barks of *M. alba* L.

2. Material and Methods

2.1. Sample Collection

The stem barks of *M. alba* L. were collected from Pyin Oo Lwin Township, Mandalay Region, Myanmar (Figure 1). The collected samples were cut into small pieces and dried in air. It was stored in a well-stoppered bottle and used throughout the experiment.

2.2. Extraction of Sample

The air dried stem barks of *M. alba* L. (300 g) was extracted with methanol (1 L) at room temperature for three weeks to yield methanol extract. Methanol extract was evaporated to give 5.1 g of dried methanolic extract.

2.3. Qualitative Test for Flavonoids

2.3.1. Ferric Chloride Test. A few drops of neutral ferric chloride solution were added to 1 mL of extract solution. Formation of blackish red color indicates the presence of flavonoids.

2.3.2. Shinoda's Test. To 1 mL of extract solution, a small piece of magnesium ribbon or magnesium foil was added and a few drops of concentrated HCl were added. Change in pink red colour shows the presence of flavonoids.

2.3.3. Lead-acetate Test. To 1 mL of extract solution, a few drops of aqueous basic lead acetate solution were added. Formation of precipitate indicates the presence of flavonoids [13].

2.4. Quantitative Determination of Total Flavonoid Content

2.4.1. Principle. The basic principle of Aluminium chloride colorimetric method is that aluminium chloride forms acid stable complexes with the C-4 keto group

and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B- ring of flavonoids. Quercetin is reported to be suitable for building the calibration curve. Therefore standard quercetin solutions of various concentrations were used to build up the calibration curve [13, 14].

2.4.2. Preparation and Determination of Standard Quercetin. 10 mg of the standard quercetin was taken in a test tube. 100 mL of distilled water was added to the standard compound. The stock solution was obtained. It was diluted with distilled water in various ratios to obtain five ranges of concentration, such as 25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL respectively. Then, 5.0 mL of solution was prepared for each concentration. A 0.5 mL of quercetin at different concentrations was put in test tubes. At the onset of the experiment, 150 µL of 5 % NaNO₂ was added to the test tube. After 5 min, 150 µL of 10 % AlCl₃ was added. After further 6 min, 1 mL of 1 M NaOH was added to the mixture. The solution was diluted to a final volume of 5 mL with water and mixed. After each standard solution was mixed with distilled water, the absorbance values of the solutions were measured with a UV-Visible spectrophotometer at 510 nm with respect to the blank solution [13-14].

2.4.3. Determination of Total Flavonoid Content of *M. alba* L. The total flavonoid content of methanolic extract of *M. alba* L. was measured by aluminium chloride (AlCl₃) according to a colorimetric assay using quercetin as a standard. Firstly, 10 mg of methanolic crude extract was dissolved in 10 mL of methanol. The same procedure and the assay were carried out in triplicate [13, 14].

2.5. Qualitative Tests for Tannins

2.5.1. Test with 2 % Gelatin solution. 2 % gelatin solution (2 mL) was added to 2 mL of extract solution. A curdy white precipitate was formed. This precipitate indicated the presence of tannins.

2.5.2. Test with saturated solution of Potassium iodate. A few drops of a saturated solution of potassium iodate solution was added to a little of the extract solution. The pink-red colour was formed, which changed into brown colour solution on standing for about 20 min. It indicated the presence of tannins like gallic acid and ellagic acid.

2.5.3. Test with Nitrous acid Solution. The freshly prepared nitrous acid (2 mL) was added to the extract solution. Carmine-red colour was developed. Then, it changed to indigo blue colour solution on standing for about 20 min. It indicated the presence of tannins like ellagitannins [15].

2.6. Quantitative Determination of Total Tannin Content

2.6.1. Principle. Tannin-like compounds reduce phosphotungstomolybdic acid in alkaline solution to produce a highly colored blue solution, the intensity of which is proportional to the amount of tannins. The intensity is measured in a spectrophotometer at 700 nm [16].

2.6.2. Preparation of standard Tannic acid stock Solution. Standard tannic acid (0.01 g) was taken into a 100 mL volumetric flask and made up the volume with distilled water. The obtained solution was used as a stock solution.

2.6.3. Estimation of λ_{max} for Tannic acid. To determine the maximum absorption, standard solution of tannic acid in concentration (6 $\mu\text{g/mL}$) was prepared. The volume was made up to 1.6 mL with distilled water. And then, 100 μL of Folin-Denis reagent and 300 μL of sodium carbonate solution were added. The mixture was heated in a water bath at 40 °C for 30 min and then cooled at room temperature. The spectrum of this solution was measured in the wavelength interval 660-740 nm.

2.6.4. Determination of standard Tannic acid. The standard tannic acid stock solution was taken by micropipette into a series of test tubes 20 μL , 40 μL , 60 μL , 80 μL and 100 μL respectively. The volume was made up to 1.6 mL with distilled water in each test tube. And then, 100 μL of Folin-Denis reagent and 300 μL of saturated Na_2CO_3 solution were added. After the each standard solution was heated in the water bath at 40 °C for 30 min and then cooled at room temperature. The values of absorbance of prepared standard tannic acid solutions were measured by PD-303, UV-Visible spectrophotometer at 700 nm with respect to the blank solution.

2.6.5. Determination of total Tannin Content of *M. alba* L. The total tannin content of the methanolic extract solution of *M. alba* L. was measured with the Folin-Denis reagent. Firstly, 1 mg of methanolic crude extract was dissolved in 10 mL of methanol. 30 μL of this extract solution was taken in a test tube. The same procedure and the assay were carried out in triplicate.

2.7. Determination of Antioxidant Activity by Spectrophotometric Method

2.7.1. Chemicals. DPPH, 95 % Ethanol, Ascorbic acid

2.7.2. Preparation of 60 μM DPPH solution. 0.0024 g (2.4 mg) of DPPH powder was weighed and it was thoroughly and gently dissolved in 100 mL of 95 %

ethanol and stored in brown coloured reagent bottle. It must be kept in the fridge for no longer than 24 hours before use.

2.7.3. Preparation of standard Ascorbic acid solution. 0.01 g (10 mg) of ascorbic acid was weighed and was dissolved in 100 mL of 95 % ethanol. It was diluted with 50 % ethanol in various ratios to obtain five ranges of concentration, such as 1 $\mu\text{g/mL}$, 2 $\mu\text{g/mL}$, 4 $\mu\text{g/mL}$, 8 $\mu\text{g/mL}$ and 16 $\mu\text{g/mL}$ respectively and the same volume 5.0 mL of standard ascorbic acid solution was prepared for each concentration.

2.7.4. Preparation of Methanolic extract solution of *M. alba* L. The methanolic extract of *M. alba* L. was diluted with methanol in various ratios to obtain five ranges of concentration, such as 6.25 $\mu\text{g/mL}$, 12.5 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ respectively. Then, 5.0 mL of methanol solution was prepared for each concentration.

2.7.5. Measurement of DPPH Radical Scavenging activity by Spectrophotometric method. The control solution was prepared by mixing 2 mL of 60 μM DPPH solution and 2.0 mL of 95 % ethanol using vortex mixer. Moreover, the blank solution could be prepared by mixing 2.0 mL of methanolic extract solution of *M. alba* L. and 2.0 mL of 95 % ethanol thoroughly in the vortex mixer. Furthermore, the prepared standard ascorbic acid solutions and the test sample solutions were also prepared by mixing gently each of 2.0 mL of 60 μM DPPH solution and 2.0 mL of test sample solution with various concentrations by applying vortex mixer. After that, the solutions were allowed to stand for 30 minutes at room temperature. Then, the absorbance value of each solution at 517 nm was measured by UV-Visible spectrophotometer [17].

3. Results and Discussion

In accordance with the results of extract obtained from the stem bark of *M. alba* L. by using the special qualitative tests of flavonoid, it was observed that sample consists of flavonoid compounds. The calibration curve was plotted against by using the resulting data of standard quercetin solution as shown in Figure 2.

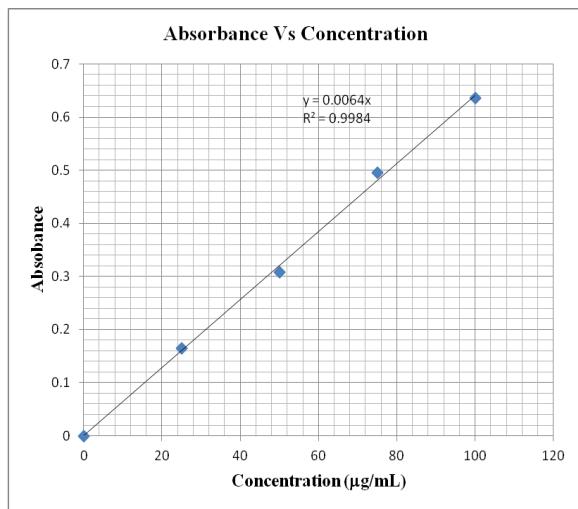


Figure 2. Absorbance Concentration Calibration Curve for Standard Quercetin at 510 nm

From the results of the total flavonoid content of the stem bark of *M. alba* L., the amount of total flavonoid content was obtained by using the standard graph. The results were described in Table 1.

Table 1. The Results of Total Flavonoid Content of Methanolic Extract of *M. alba* L.

Name of Sample	Flavonoid (mg/g)	Flavonoid (mg/g) Mean \pm Standard Deviation
Methanolic extract of stem bark of <i>M. alba</i> L.	86.10	86.02 \pm 0.28
	85.70	
	86.25	

The total flavonoid content present in the methanolic extract of *M. alba* L. was found to be 86.02 ± 0.28 mg quercetin equivalent (QE) per gram crude extract. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

In addition, according to the results of methanolic crude extract by using the special qualitative test of tannins, it was also revealed that the methanolic extract of *M. alba* L. consists of tannin compound.

Determination of total tannin content in methanolic crude extract was carried out by Folin- Denis reagent method using UV-Visible spectrophotometer. From the determination of the maximum absorption of standard tannic acid, it showed a maximum absorbance (λ_{max}) at 700 nm as depicted in Figure 3.

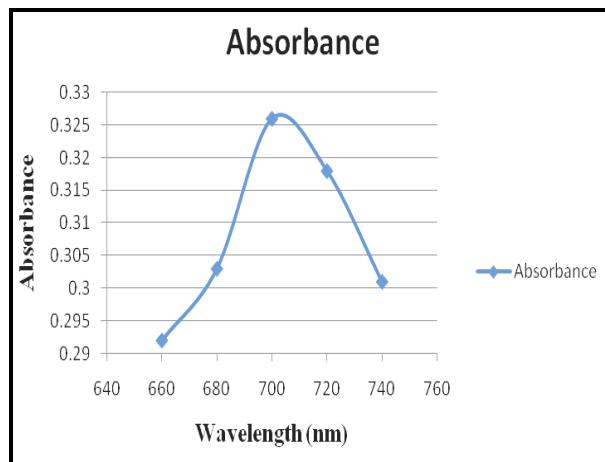


Figure 3. Maximum Wavelength of Standard Tannic Acid

By using the resulted maximum wavelength ($\lambda_{\text{max}} = 700$ nm), the total tannin content present in the stem bark of *M. alba* L. could be measured quantitatively.

The standard tannic acid solutions at concentration 2 to 10 µg/mL in distilled water were measured to know their absorbance values by PD-303 UV-Visible spectrophotometer. The calibration curve was plotted by using the resulting data of standard tannic acid solution as shown in Figure 4.

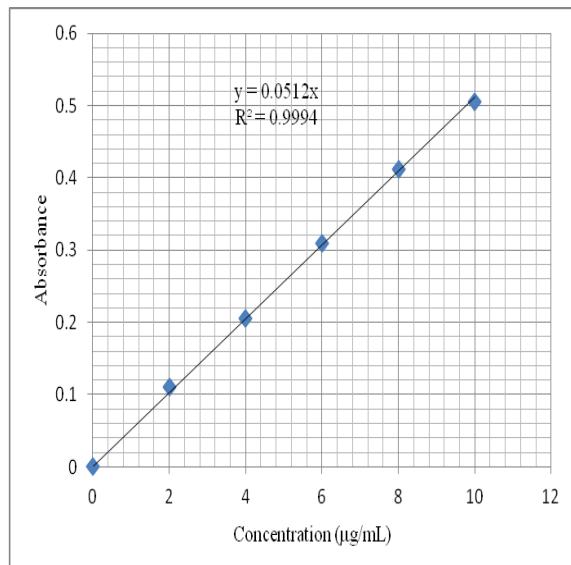


Figure 4. Absorbance Concentration Calibration Curve for Standard Tannic Acid at 700 nm

Moreover, the obtained results from the evaluation of total tannin content are described in Table 2. From these results, the amount of total tannin content of methanolic extract of *M. alba* L. was obtained by using the standard curve.

Table 2. Results of the Absorbance and Concentration of Methanolic Extract of *M. alba* L.

No.	Test sample	Absorbance	Concentration ($\mu\text{g/mL}$)
1	Test 1	0.0237	0.463
2	Test 2	0.0235	0.459
3	Test 3	0.0236	0.461

The total tannin contents of methanolic extract of *M. alba* L. were calculated from different concentrations of tannin in which the same spectrophotometric procedure was followed for the working standard. The calculated results are tabulated in Table 3.

Table 3. Amount of Tannin Content in Methanolic Extract of *M. alba* L.

Name of sample	Tannin (mg/g)	Tannin (mg/g) Mean \pm Standard Deviation
Methanolic extract of stem bark of <i>M. alba</i> L.	154.3	153.7 ± 0.65
	153.7	
	153.0	

In accordance with these results, the total tannin content of the methanolic extract of *M. alba* L. was found to be 153.7 ± 0.65 mg tannic acid equivalent per 1 g of crude extract. The present investigations revealed that the stem bark of *M. alba* L. is rich in tannin compounds. Plant tannins have both positive and negative effects on the body. Therefore, consuming the tannin containing foods moderately is beneficial for health.

Phenolic compounds, tannin and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals.

Among chemical methods applied to determine the antioxidant activity of a compound, DPPH, (2,2-diphenyl-1-picrylhydrazyl) is one of the most used methods because it is practical, fast and stable. Antioxidant activity of methanolic extract of *M. alba* L. was expressed as percentage of DPPH radical inhibition and IC₅₀ values ($\mu\text{g/mL}$). The results of antioxidant activity using DPPH method in methanolic extract of *M. alba* L. using ascorbic acid as a positive control are shown in Figure 5, 6, and Table 4.

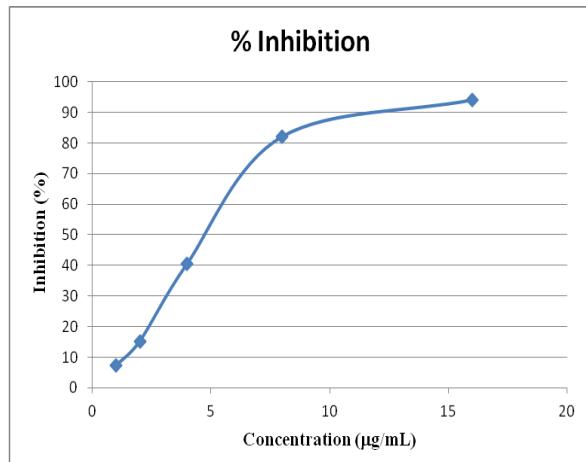


Figure 5. Plot of % Inhibition vs Concentration of Standard Ascorbic Acid

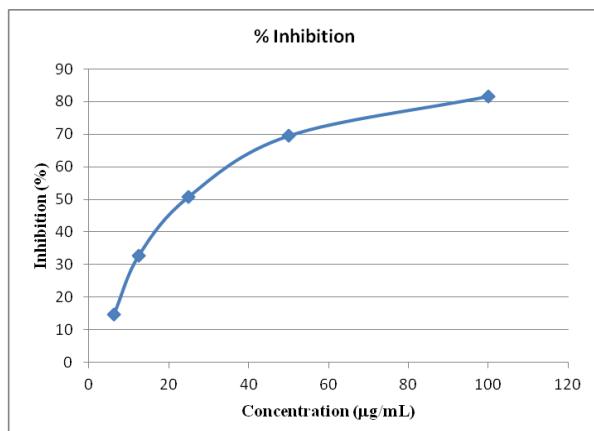


Figure 6. Plot of % Inhibition vs Concentration of Methanolic Extract of *M. alba* L.

Table 4. IC₅₀ Values of Standard Ascorbic Acid and Methanolic Extract of *M. alba* L.

No.	Test Samples	IC ₅₀ Values ($\mu\text{g/mL}$)
1	Ascorbic acid	4.92
2	Methanolic extract of stem bark of <i>M. alba</i> L.	24.6

The IC₅₀ value is a parameter used to measure antioxidative activity and it is defined as the concentration required for 50 % scavenging of DPPH radicals under experimental condition employed. A smaller IC₅₀ value corresponds to a higher antioxidant activity.

In this study, the antioxidant activity of methanolic extract of *M. alba* L. was shown to be influenced by the total flavonoid and tannin contents. The stem bark of *M. alba* L. containing high flavonoid and tannin contents have been found to exert high antioxidant potential. The study of present research has shown a

direct relation between antioxidant activity of methanolic extract of *M. alba* L. and flavonoid and tannin contents. Therefore, the study suggests that the selected *M. alba* L. might be a potential source of natural antioxidants.

4. Conclusion

In this research work, the stem bark of *M. alba* L. was determined the total flavonoid and tannin contents. It was found that the total flavonoid and tannin contents were 86.02 ± 0.28 mg/g and 153.7 ± 0.65 mg/g of crude extract. Moreover, the result of the investigation showed that the methanolic extract of *M. alba* L. gave the significant antioxidant activity in accordance with IC₅₀ value of 24.6 µg /mL, but still lower than ascorbic acid with IC₅₀ value of 4.92 µg /mL which is a positive control. Based on the obtained results, it can be concluded that flavonoid and tannin-enriched *M. alba* L. was effective on DPPH radicals significantly. It has been suggested that the antioxidant capacity of *M. alba* L. is strongly correlated to the type of flavonoid and tannin compounds which are present in it. The *M. alba* L. can be regarded as promising candidates for natural plant sources of radical scavengers with high value.

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Extraction, Characterizations and Application of Pectin from Fruit Peels of *Holocereus undatus* (Haworth) Britton & Rose

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Abstract

In this research work, the fruit peels of *Holocereus undatus* (Haworth) Britton & Rose., Myanmar named Red Dragon fruit were selected for extraction of pectin. The preliminary phytochemical tests and mineral content of Dragon fruit peels were determined. Furthermore, pectin from peels of Dragon fruit was extracted by various extraction times and extracted solvents. The best yield % of pectin from selected sample was found that the extraction time 60 minutes with only distilled water. Extracted pectin was confirmed by FT IR spectrum. The some physicochemical properties, moisture, ash, pH, and characterizations of extracted pectin such as solubility, viscosity, and biodegradable properties were determined. The extracted pectin was prepared the films and which applied in fruit coating. The extending shelf life of coated fruits was investigated. After 10 days period, packaging fruits with prepared pectin films could be observed the no changes and uncoated fruit was changed to the colour of outer surface.

Keywords- *Holocereus undatus* (Haworth) Britton & Rose., biodegradable, pectin, FT IR, physicochemical

1. Introduction

Pectin is one of the main structural components of cell wall polysaccharides that present naturally in higher plants, composed mainly of D-galacturonic acid (Gal A) units linked through by α -1,4-glycoside linkage [1]. The behavior of pectin in food applications is determined with the ratio of esterified to non-esterified galacturonic acid. Pectin is classified according to the degree of methoxylation (DM) as high methoxyl pectin and low methoxylation influences the properties of pectin, especially the solubility and the gel forming characteristics [2].

Red dragon fruit, (*Hylocereusundatus* (Haworth) Britton & Rose) is the fruit of Cactaceae family, known as pitaya or pitahaya, which is native to Mexico, central and South America [2] and has been widely cultivated as fruit crops in south east Asian countries such as Vietnam, Taiwan, Philippines, Myanmar and Malaysia [3]. The peel of dragon fruit approximately represents about a quarter of the fruit mass. It is usually discard as a waste material in fruit juice manufacturing companies

and resulting in pollution of the environment. The utilization of these wastes for commercial pectin production can be considered as an alternative to waste treatment [4].

Fruit pectin is associated with cellulose in plant tissues, where it plays a fundamental role in determining their mechanical properties. Pectin as a food ingredient can be used as a gelling agent, thickening agent, stabilizer, and emulsifier in food applications. The wide spread application of biodegradable packaging materials in order to reduce the volume of inert material currently being disposed of in landfills, occupying scarce available space. Since the peels of dragon fruit are often discarded as waste, it would be an advantage to convert it into a value-added product such as pectin [4]. Dragon fruit is a potential alternative source of pectin that is locally abundant [5].

The aim of present study is to extract the pectin from fruit waste (Dragon fruit peels) and to investigate the characterizations and application of the extracted pectin.

1.1 Botanical Description of *Holocereus undatus* (Haworth) Britton & Rose.



Figure 1. Plant, Flower and Fruits of *Holocereus undatus* (Haworth) Britton & Rose.

Botanical name	- <i>Hylocereus undatus</i> (Haworth) Britton & Rose
Family name	- Cactaceae
Genus	- <i>Hylocereus</i>
English name	- Red Dragon Fruit
Myanmar name	- Nagar-mauk
Part used	- Fruit peels
Medical uses	- Diabetes, digestion, cancer, arthritis, gout, cardiovascular, weight lose, inflammatory, pre-biotic and antioxidant properties [6]

2. Materials and Methods

2.1. Sample Collection and Preparation

The Red Dragon fruits were collected from Kyaukpan-taung Township, Mandalay Region, Myanmar in December, 2018. It was cut into small pieces and dried in air at room temperature for used through out the experiment.

2.2. Preliminary Phytochemical Screenings of Peels of Red Dragon Fruit

Phytochemical Screenings of Peels of Red Dragon Fruit were done on the various extracts of sample by test Tube method [7] and these results were described in Table 1.

2.3. Determination of Mineral Content in Peels of Red Dragon Fruit

Mineral contents of Red Dragon fruit peels were measured at Department of Chemistry, Monywa University by applying EDXRF (Energy Dispersive X-Ray Fluorescence) Spectroscopy method. The results of mineral contents were tabulated in Table 2 and Figure 2.

2.4. Extraction of Pectin with Water Extraction Method by Various Time Intervals

8g of sample powder was placed into a conical flask 250 ml of distilled water was added and heated with various time intervals such as 20, 40, 60 ,80 and 100 minutes. Then the hot extract was filtered through cheese cloth. The filtrate was cooled and then coagulated with 90% ethanol and stirred regularly for 15min, and then the pectin was washed with ethanol. The pectin was dried in oven at 50 °C for few hours until constant weight. The final weights were recorded and pectin yield were calculated by the following equation [8].

$$\text{Yield percent of pectin} = \frac{\text{Weight of dried pectin}}{\text{Weight of crude sample}} \times 100 \%$$

The results of yield % in various time intervals with water extraction method were tabulated in Table 3.

2.5. Extraction of Pectin with D/W:HCl Acid at pH-2 by Different Time Intervals

8g of each sample powder was placed into a conical flask. 150 mL of D/W: 0.1M HCl (25:1) was added and heated for 20, 40, 60 and 80 minutes. Then the hot extract was filtered through muslin cloth. The filtrate was cooled and 95 % ethanol was added on equal

volume basis of extract and kept for overnight. Then the coagulated pectin was removed by filtration and dried in sunlight. The yield percent of pectin was also calculated by the above equation. The results of yield % in various time intervals with D/W: HCl extraction method were tabulated in Table 4.

2.6. Confirmation of Pectin by FT IR Spectroscopic Method

FT IR spectrum of extracted pectin from Red Dragon fruit peels was measured at the Department of Chemistry, Monywa University. The prominent functional groups containing in extracted pectin was identified with the FT IR spectrum of commercial pectin in literature [9]. The FT IR spectra of extracted pectin and commercial pectin were described in Figure 3 and 4.

2.7. Determination of Some Physicochemical Properties of Extracted Pectin from Red Dragon Fruit Peels

2.7.1. Determination of Moisture Content. The moisture content of extracted pectin powder from red dragon fruit peels was determined by oven drying method [10].

2.7.2. Determination of Ash Content. The ash content of extracted pectin powder from red dragon fruit peels was determined by muffle furnace method [10].

2.7.3. Determination of pH. The pH of extracted pectin powder from red dragon fruit peels was dissolved in distilled water and measured by pH meter.

2.8. Solubility Tests of Extracted Pectin

The solubility of extracted pectin powder were tested by cold and hot water, cold and hot alkali and cold and hot acid.

2.9. Determination of Viscosity of Extracted Pectin

The viscosity of extracted pectin powder was measured by Ostwald viscometer. The results of Viscosities of various concentrations of extracted pectin were described in Table 6 and Figure 7.

2.10. Preparation of Bio-films with Extracted Pectin

The bio-films with red dragon fruit peel pectin and commercial pectin were prepared by Casting method [11]. The prepared pectin films with extracted pectin and commercial pectin were shown in Figure 7.

2.11. Biodegradable Properties of Prepared Pectin Films

The biodegradation of red dragon fruit pectin film and commercial pectin film in a soil environment were studied by Soil Burial test [12].

2.12. Investigation of Food Packaging Properties of Prepared Pectin Films

Three fresh apple fruits were taken and two fruits were coated with red dragon fruit pectin film and commercial pectin film. Remaining one was uncoated. These fruits were placed at room temperature and

watched with three days to ten days period. The changes of fruits conditions were investigated.

3. Results and Discussion

3.1. Results of Preliminary Phytochemical Screenings for the Red Dragon Fruit Peels

The phytochemical screenings of selected sample were determined by test tube method and results are shown in Table 1.

Table 1. The Results Phytochemical Tests for Red Dragon Fruit Peels

No	Test	Extracts	Regents	Observation	Remarks
1	Alkaloids	1% HCl	Dargendroff's reagent	Reddish brown ppt	+
2	Carbohydrate	D/W	Fehling solution	Yellow ppt	+
3	Flavonoids	95%EtOH	Mg coil, conc: HCl	Pink color solution	+
4	Glycosides	D/W	10% lead acetate	White ppt	+
5	Terpenes	95%EtOH	(CH ₃ CO) ₂ O, conc:H ₂ SO ₄	Pink color solution	-
6	Saponins	D/W	Shaken	Froth	+
7	Reducing Sugars	D/W	Benedict's reagent	Green ppt	+
8	Phenolic Compounds	D/W	10% FeCl ₃	Brown color solution	+
9	Polyphenols	95%EtOH	10% FeCl ₃ , 1% K ₃ [Fe(CN) ₆]	Blue black color solution	+
10	Steroids	95%EtOH	(CH ₃ CO) ₂ O, conc:H ₂ SO ₄	Green color solution	-
11	Tannins	D/W	10% FeCl ₃ , dilute H ₂ SO ₄	Yellowish brown color solution	+
12	Lipophenols	95%EtOH	0.5N KOH, 0.5N NaOH	Deep color solution	+

(+) = presence of constituents

(-) = absence of constituent

According to this table, the peels of red dragon fruit consists of alkaloids, carbohydrates, flavonoid, glycosides, saponins, reducing sugars, phenolic compounds, polyphenols, tannins and lipophenols respectively.

3.2. Mineral Contents from Red Dragon Fruit Peels

The content of element in red dragon fruit peels powders were determined by EDXRF analysis. The results are shown in Table 2 and Figure 2.

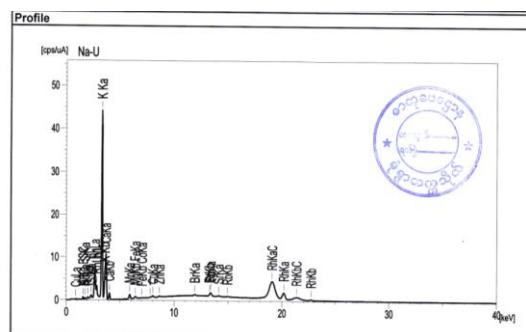


Figure 2. EDXRF Spectrum of Red Dragon Fruit Peel

According to these results, Red dragon fruit peels powder contains potassium, calcium, silicon, phosphorous, sulfur, manganese, iron, copper, rubidium,

zinc, and bromine respectively. Any toxic mineral does not observed in selected sample.

Table 2. Mineral Contents in Red Dragon Fruit Peels

Dragon Fruit Peels	
Element	Relative Abundance (%)
Potassium(K)	2.763
Calcium(A)	0.520
Silicon (Si)	0.142
Phosphorus (P)	0.083
Sulfur (S)	0.076
Manganese(Mn)	0.011
Iron (Fe)	0.004
Copper (Cu)	0.002
Rubidium(Rb)	0.002
Zinc (Zn)	0.001
Bromine(Br)	0.001
CH	93-395

3.3. Extraction of Pectin with Water Extraction Method by Various Time Intervals

Pectin from red dragon fruit peels powders were extracted by using distilled water with different time intervals. The yield percents of pectin are described in Table 3 and Figure 3.

Table 3. The Yield (%) of Red Dragon Fruit Pectin by Using D/W with Different Time Intervals

Sample	Extraction Time (min)	Extracted Pectin Weight (g)	Yield % of Pectin (%)
Red Dragon Fruit Peels (8g)	20	1.42	17.75
	40	1.51	18.88
	60	1.77	22.13
	80	1.68	21.00
	100	1.56	19.5

According to these results, the best yield % of pectin from red Dragon Fruit peels was found that the extraction time 60 minutes with only distilled water.

3.4. Extraction of Pectin with D/W: HCl at pH-2 by Various Time Intervals

Pectin from red dragon fruit peels powders were extracted by using distilled water: HCl (25:1) at pH-2 with various time intervals. The yield percents of pectin are described in Table 4.

Table 4. The Yield (%) of Red Dragon Fruit Pectin with D/W: HCl (25:1) at pH-2 with Different Time Intervals

Sample	Extraction Times (mins)	Extracted Pectin Weight (g)	Yield % of Pectin (%)
Red Dragon Fruit Peels (8g)	20	0.69	8.63
	40	0.87	10.88
	60	0.79	9.87
	80	0.49	6.13

According to the above experimental data, if the extracted solvent used as the distilled water: HCl (25:1 v/v) mixture at pH-2, the best yield % was found that the extraction time 40 minutes in red Dragon fruits peels. But only distilled water extraction is more better yield % than distilled water: HCl (25:1 v/v) mixture.

In accordance with these results, the yield % of extracted pectin depends on the extracted solvent and pH used in extraction procedure. The changes in extraction conditions both of the extracted pectin yield % and the better extraction time interval were changed.

3.5. Confirmation of Pectin by FT IR Spectroscopic Method

FT IR spectrum of extracted pectin from red dragon fruit peels was measured at the Department of Chemistry, Monywa University. The FT IR spectrum was confirmed the prominent functional groups containing in extracted pectin and identified with the FT IR spectrum of commercial pectin in literature. FT IR spectrum of extracted pectin and commercial pectin were shown in Figure-3 and 4.



Figure 5. FT IR Spectrum of Extracted Pectin from Red Dragon Fruit Peels

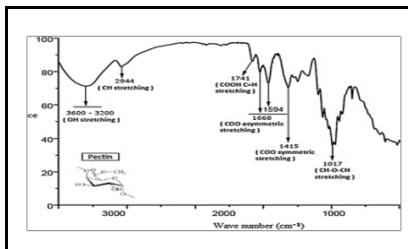


Figure 4. FT IR Spectrum of Commercial Pectin from Literature

According to FT IR assignments, extracted pectin contain O-H group, sp^3 hydrocarbon, ester (C=O) carbonyl group, ether functional group respectively [11].

3.6. Determination of Some Physicochemical Properties of Extracted Pectin from Red Dragon Fruit Peels

Some physicochemical properties of extracted pectin were determined by AOAC method [9] and pH was determined by pH meter. These results are shown in Table 5.

Table 5. Some Physicochemical Properties of Extracted Pectin from Red Dragon Fruit Peels

Extracted Pectin from Red Dragon Fruit Peels	
Moisture (%)	16.67
Ash (%)	9.41
pH	5.50

Pectin from red Dragon fruit peels contains 16.67%, ash 9.41% and pH 5.5 respectively.

3.7. Solubility Tests of Extracted Pectin

The solubility of extracted pectin were tested by cold and hot H_2O , cold and hot 0.1N NaOH, and cold and hot HCl solution. The pectin was slightly soluble in cold water, cold alkali and cold acid but it was completely soluble in hot water, hot alkali and hot acid.

3.8. Determination of Viscosity of Extracted Pectin

The viscosity of Red Dragon fruit pectin was determined by using Ostwald Viscometer. The results were shown in Table 6.

Table 6. The Results of Viscosity Extracted Pectin from Red Dragon Fruit Peels in Various Concentrations

Sample	Concentration of Pectin (M)	Flow Rate Time (s)	Viscosity (cP)
Red Dragon Fruit Peels	0.040	190	0.20
	0.020	127	0.10
	0.010	95	0.09
	0.005	90	0.09
	0.001	72	0.08

The viscosity increases the concentration increase.

3.9. Preparation of Bio-films with Extracted Pectin

The bio-films of red dragon fruit peels and commercial pectin were prepared by Casting method [12]. Figure 5 shows the different types of prepared bio-films.



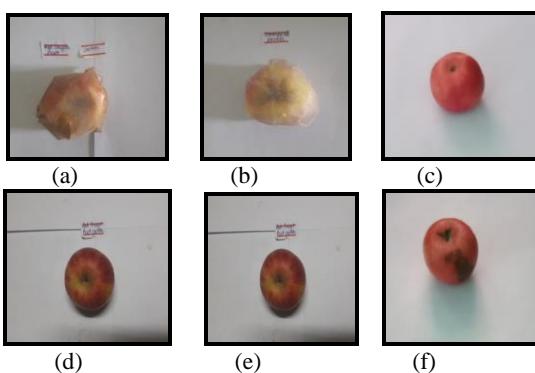
Figure 5. (a)Extracted Pectin Film (b) Commercial Pectin Film

3.10. Investigation of Biodegradable Properties of Prepared Pectin Films

The biodegradation of red dragon fruit pectin film and commercial pectin film in a soil environment were studied by Soil Burial test [13]. After three days, all of prepared pectin films were completely biodegradable in soil.

3.11. Investigation of Food Packaging Properties of Prepared Pectin Films

Two prepared pectin films were used for packaging of fresh fruits and their extended shelf life of coated and uncoated fruits were investigated within 10 days periods. After 10 days period, packaging fruits with prepared pectin films could be observed the no changes. Uncoated fruit was over ripen and changed to the colour of outer surface. These results were described in Figure 6.



**Figure 6. (a) Packaged with Red Dragon Fruit Pectin Film
(b) Packaged with Commercial Pectin Film (c)Unpackaged
Fruit (d) After 10 Days, Extracted Pectin Film Coated
Fruit (e) After 10 Days, Commercial Pectin Film Coated
Fruit (f) Uncoated Fruit**

4. Conclusion

In the present studies, the fruits waste of red dragon fruit peels was selected for extraction of pectin sources. As a result of present studies, pectins from various fruit wastes were easily extracted and it was very useful edible coating biodegradable films.

The uses of renewable resources that can produce biodegradable materials to reduce waste disposal problems continue to be explored as consumer concern rise on limited natural resources and environmental issues.

If the biodegradable pectin based films is replaced in the uses of synthetic non-biodegradable plastic, people will decrease the environmental pollutions and increase in cleaning of our environments.

5. Acknowledgements

We are deeply thankful to Dr Thida Win, Rector, University of Mandalay and Dr Yi Yi Myint, Professor, Head of Department of Chemistry, University for their kind permission and for providing all research facilities.

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Decolorizing Activity of Corncobs Powder for Aqueous Solution of Dyes

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Abstract

The present research work is the Corncobs powder to be used as adsorbent for the decolorization of dyes. The Corncobs were collected from the local market, Mandalay, Mandalay Region. Physicochemical properties (moisture, pH, bulk density) were determined by AOAC (Association of Official Analytical Chemists) and European Pharmacopoeia methods. The sample was heated at 100°C for 1 hour. The color removal of the dyes such as Congo red and Methylene blue solutions on the sample was carried out spectrophotometrically. The effects of sorption parameters (sorbent dose, concentration of dye solution and contact time) of adsorbent were investigated for the removal of specified colored dyes. Langmuir and Freundlich isotherms were used to study Sorption isotherm. So, the sample could be applied in waste water treatment because it gave the significant percent removal on Congo red and Methylene blue.

Keywords- adsorbent, decolorization, Corncobs Congo red, Methylene blue

1. Introduction

The waste product in the environment is a worldwide problem that has been highlighted by various environmentalist groups. Colored organic effluent is produced in industries such as textiles, rubber, paper, plastic, cosmetics, etc. [3]. Discharging of dyes into water resources even in a small amount can affect the aquatic life and food. Dyes can also cause allergic dermatitis and skin irritation [7]. Water pollution due to discharge of colored effluents from textile dye manufacturing and textile dyeing mills are one of the major environmental concerns in the world today. Because of their complex molecular structures and large sizes, most of the dyes are considered non-oxidizable by conventional, physical and biological treatments. Thus their decolorization is one of the indispensable processes in wastewater treatment [9]. Adsorption technology is currently used extensively for the removal of organic pollutants from the drinking water and wastewater, including dyes, pesticides and food contaminants [8]. The corncob has a good flow ability of adsorbent material and many pores which is very advantageous for dyes adsorption [6]. In this research, the decolorizing properties of dyes by using corncobs

were performed. The adsorptions of Methylene blue and Congo red by using corncobs were determined by using UV-visible spectrophotometer.

Botanical Description [4]

Botanical name	- <i>Zea mays</i> L.
Family name	- Poaceae
English name	- Maize
Myanmar name	- Pu-sa-pyaung
Part used	- Corncobs



Figure 1. Plants and Corncobs of Corns

2. Experimental

2.1. Sample Collection

The corncobs were collected from the Local Market, Mandalay region. The collected corncobs were washed with water and then dried in sun light. After that, the dried corncobs were crushed and grounded by using a blender. Then the corncobs powdered sample was sieved with 100-mesh size. Thermal activation of corncobs powder was carried out at 100°C for 1 hour.



Figure 2. Powder of Corncobs

2.2. Physicochemical Properties of Corncobs Sample

2.2.1. Determination of Moisture. 1 gram of sample was placed in an electric oven, and dried at 100°C for one hour. After the heating, the dish was removed from the oven and placed in desiccator. Cooling and weighing were repeated until a constant weight was obtained. The moisture was calculated by the loss in weight [2].

2.2.2. Determination of pH. The sample (1gram) was placed into a beaker and then 100 mL of distilled water was added in a beaker. That beaker was gently shaken and the sample was filtered. The filtrate was cooled at room temperature and pH of the sample was determined by a pH meter [1].

2.2.3. Determination of Bulk Density. A clean dry graduated cylinder (10 mL) was weighed. It was then filled with the dry samples to the 10 mL mark and reweighed. The graduated cylinder was tapped gently until there was no more reduced in volume. The minimum volume was recorded and the bulk density was calculated [5].

2.3. Sorption Studies of Corncobs Sample for the Color Removal of Dye Solutions

To select the maximum absorption wavelength for a particular dye, a spectral curve was first plotted by determining the color index (absorbance) of the dye solution as a function of concentration. The concentrations were chosen for 25 mgL⁻¹ of congo red and 25 mgL⁻¹ of methylene blue. Half dilution of the stock dye solution was made by using distilled water as diluents. In the measurement of the color of the diluted solution, 1 cm cell was used with distilled water as reference. From the spectral curves, maximum absorption wavelengths for Congo red and methylene blue were measured.

2.3.1. Effect of Dosage. Different amount of samples such as 0.01 g, 0.02 g, 0.03 g, 0.04 g and 0.05 g were added into the 25 mL of 50 mgL⁻¹ dye solutions (Congo red and Methylene blue) in each conical flask. The solutions were shaken with a shaker at 150 rpm for 30 minutes at room temperature. Then, each mixture was centrifuged for 15 minutes. The absorbances were determined by using UV-visible spectrophotometer.

2.3.2. Effect of Initial Concentration. 25 mL of different concentrations such as 25 mgL⁻¹, 50 mgL⁻¹, 75 mgL⁻¹, 100 mgL⁻¹, 125 mgL⁻¹ and 150 mgL⁻¹ of dye solutions (Congo red and Methylene blue) were added into the 0.03 g of sample in each conical flask. The solutions were shaken with a shaker at 150 rpm for 30 minutes at room temperature. Then, each mixture was

centrifuged for 15 minutes. The mixture of absorbance was determined by using UV-visible spectrophotometer.

2.3.3. Effect of Contact Time. 0.03 gram of sample was added into 25 ml of 50 mgL⁻¹ dye solutions (Congo red and Methylene blue) in each conical flask. The solutions were shaken with a shaker at 150 rpm for different minutes such as 15minutes, 30minutes, 45 minutes, 60 minutes, 75minutes and 90minutes at room temperature. Then, each mixture was centrifuged for 15 minutes. The mixture of absorbance was determined by using UV-visible spectrophotometer.

2.4. Studies of Adsorption Isotherm

The amount of dyes adsorbed onto the activated sample (corncobs), q_e (mg g⁻¹) was calculated by the following equation.

$$q_e = (C_0 - C_e) \frac{V}{w}$$

where C_0 and C_e the initial and equilibrium concentrations of dye solution, (mg L⁻¹), V used the volume of the solution(L), and w is the weight of the activated sample(g).

$$q_e = \frac{X_m b C_e}{1 + b C_e}$$

Where, q_e = the amount of sorbate adsorbed per unit mass of sorbent

C_e = equilibrium concentration of the sorbate 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 Langmuir adsorption isotherm is mathematically expressed as;

$$\begin{aligned} \frac{x}{m} &= \frac{x_m b C_e}{1 + b C_e} \\ q_e &= \frac{X_m b C_e}{1 + b C_e} \\ \frac{1}{q_e} &= \frac{1}{X_m} + \frac{1}{X_m b C_e} \\ \frac{C_e}{q_e} &= \frac{C_e}{X_m} + \frac{1}{X_m b} \end{aligned}$$

Where, X_m = Langmuir monolayer capacity parameter

b = constant for a given adsorbate and adsorbent at a particular temperature.

The Freundlich adsorption isotherm is mathematically expressed as; $\frac{x}{m} = K C_e^{1/n}$

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

$$\log q_e = \log K + \frac{1}{n} \log C_e$$

where, K and $\frac{1}{n}$ are constants for a given adsorbate and adsorbent at a particular temperature.

3. Results and Discussion

3.1. Physicochemical Properties of Corncobs Powder

The physicochemical properties (moisture content, pH, Bulk density) of Corncobs powder were shown.

Table 1. Physicochemical Properties of Corncobs Powder

Parameter	value
Moisture content (%)	6.1
pH	5.93
Bulk density (gcm^{-3})	0.36

3.2. Maximum wavelength of Congo red and Methylene blue

The maximum wavelength of Congo red was determined by using UV-visible absorption spectroscopy. The wavelength of maximum absorption of Congo red was recorded between 480-520 nm. The maximum wavelength from the experiment was found to be 500 nm. The wavelength of maximum absorption of Methylene blue was measured between 650-690 nm. The maximum wavelength of Methylene blue was found to be 666 nm.

Table 2. Maximum Wavelength of Congo Red by Using UV-Visible Spectrophotometer

Wavelength (nm)	Absorbance
480	0.519
484	0.534
488	0.547
492	0.557
496	0.562
500	0.564
504	0.561
508	0.551
512	0.541
516	0.527
520	0.519

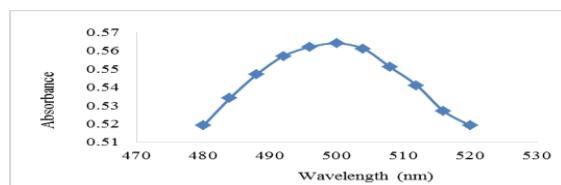


Figure 3. Maximum Wavelength of Congo red
From these Results, the Maximum Wavelength of Congo red was Recorded at 500 nm.

Table 3. Maximum wavelength of Methylene Blue by Using UV-Visible Spectrophotometer

Wavelength(nm)	Absorbance
650	1.214
654	1.542
658	1.856
662	2.372
666	2.488
670	2.474
674	2.312
678	1.918
682	1.471
686	1.139
690	1.057

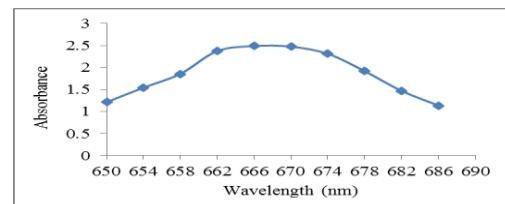


Figure 4. Maximum Wavelength of Methylene Blue

From these results, the maximum wavelength of methylene blue was recorded at 666 nm.

3.3. Calibration Curves for Dye Solutions

Table 4. Determination of Congo Red by Using UV-Visible Spectrophotometer at 500 nm

Concentration(mgL^{-1})	Absorbance
12.5	0.292
6.25	0.158
3.13	0.085
1.57	0.046
0.79	0.029

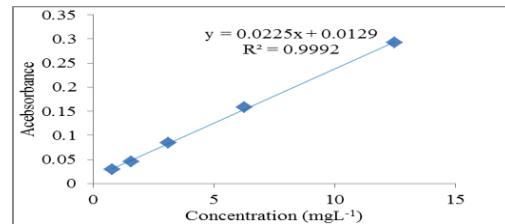


Figure 5. Standard Curve of Congo Red

Table 5. Determination of Methylene Blue by using UV-Visible Spectrophotometer at 666 nm

Concentration(mgL ⁻¹)	Absorbance
12.5	1.502
6.25	0.733
3.13	0.385
1.57	0.163
0.79	0.075

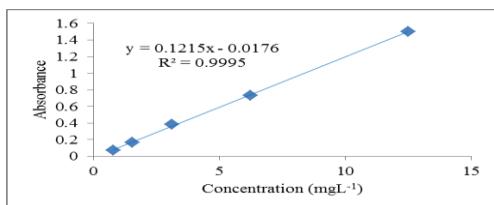


Figure 6. Standard Curve of Methylene Blue

3.4. Adsorption Study

The phenomenon of adsorption is generally applied for the removal of dye from solution. The sorption studies of the sample can be carried out by the sorbent-sorbate interaction with the dye solutions (Congo red and Methylene blue). For the sorption studies, dosage method, concentration method and contact time methods were used.

3.4.1. Effect of Dosage

Table 6. Effect of Dosage on the Sorption of Congo Red

Dosage(g)	C _e (mgL ⁻¹)	Percent removal (%)
0.01	44.75	10.50
0.02	40.13	19.74
0.03	36.00	28.00
0.04	32.79	34.42
0.05	29.25	41.50
0.06	26.71	46.58

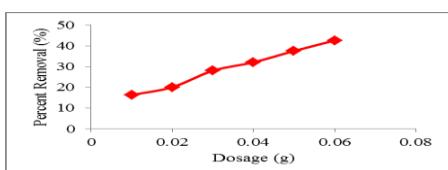


Figure 7. Effect of Dosage on the Sorption of Congo Red

Table 7. Effect of Dosage on the Sorption of Methylene Blue

Dosage (g)	C _e (mgL ⁻¹)	Percent Removal(%)
0.01	26.00	48.00
0.02	16.21	67.58
0.03	11.86	76.28
0.04	8.80	82.04
0.05	6.51	86.98
0.06	4.76	90.48

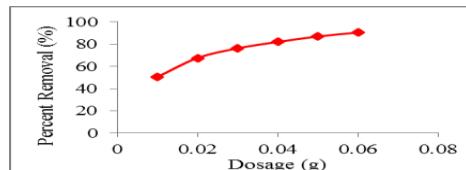


Figure 8. Effect of Dosage on the Sorption of Methylene Blue

From these results, the percent adsorption increases with increase in sorbent dosage. The dosage of sample was more removal on methylene blue than congo red.

3.4.2. Effect of Initial Concentration of Dye Solution

Table 8. Effect of Initial Concentration on the Sorption of Congo Red by Sample

C ₀ (mgL ⁻¹)	C _e (mgL ⁻¹)	Percent removal(%)
25	15.08	39.68
50	37.00	26.00
75	53.88	28.16
100	75.25	24.75
125	99.79	20.17
150	123.79	17.47

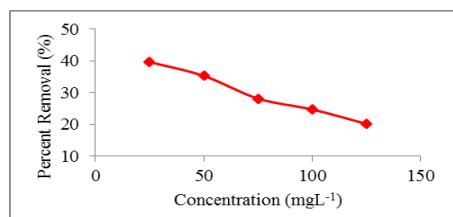


Figure 9. Effect of Initial Concentration on the Sorption of Congo Red

Table 9. Effect of Initial Concentration on the Sorption of Methylene Blue

C ₀ (mgL ⁻¹)	C _e (mgL ⁻¹)	Percent removal(%)
25	3.23	87.08
50	10.99	78.02
75	18.89	74.81
100	28.11	71.89
125	37.91	69.67
150	58.32	61.12

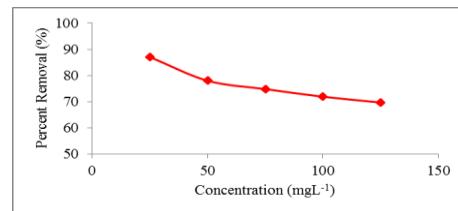


Figure 10. Effect of Initial Concentration on the Sorption of Methylene Blue

From these results, the percent adsorption increases with decrease in concentrations of congo red and

methylene blue. The concentration of methylene blue on sample was more removal than congo red.

3.4.3 Effect of Contact Time

Table 10. Effect of Contact Time on the Sorption of Congo Red

Contact Time (min)	C _e (mgL ⁻¹)	Percent removal(%)
15	42.00	16.00
30	38.17	23.66
45	37.00	26.00
60	34.21	31.58
75	32.83	34.34
90	31.46	37.08

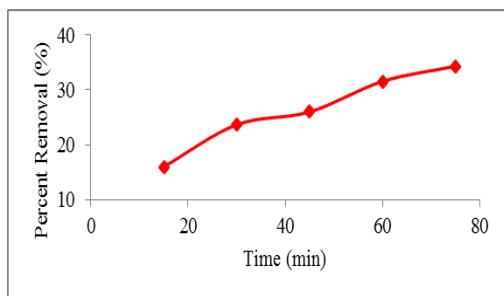


Figure 11. Effect of Contact Time on the Sorption of Congo Red by Sample

From these results, the percent adsorption increases with increase in contact time.

Table11. Effect of Contact Time on the Sorption of Methylene Blue by Sample

Contact Time(min)	C _e (mgL ⁻¹)	Percent removal(%)
15	17.48	65.04
30	12.22	75.56
45	10.21	79.58
60	8.92	82.16
75	7.12	85.76
90	6.98	86.04

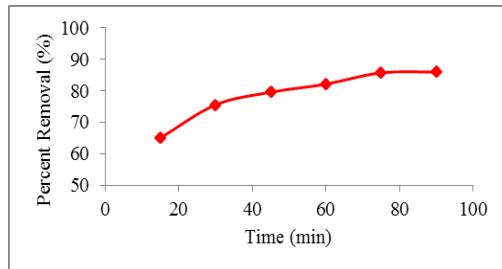


Figure 12. Effect of Contact Time on the Sorption of Methylene Blue By Sample

From these results, the percent adsorption increases with increase in contact time. The contact time of methylene blue on sample was more removal than congo red.

So, the adsorption of corncobs (sorbent dose, concentration of dye solution and contact time) was better in methylene blue than congo red.

In Table 12, the Langmuir and Freundlich parameters were able to differentiate and classify the two types of sorbents were as follows:

Table 12. Adsorption of Different Mass of Sample by Using Congo Red

Mass (g)	C _e (mgL ⁻¹)	q _e (mg g ⁻¹)	C _e /q _e (gL ⁻¹)	log C _e	log q _e
0.01	44.75	13.125	3.410	1.651	1.118
0.02	40.13	12.338	3.253	1.603	1.091
0.03	36.00	11.667	3.086	1.556	1.067
0.04	32.79	10.756	3.049	1.516	1.032
0.05	29.25	10.375	2.819	1.466	1.016
0.06	26.71	9.740	2.742	1.427	0.989

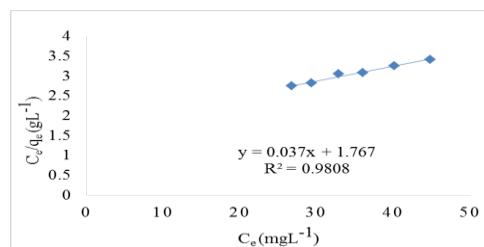


Figure 13. Langmuir Isotherm for Sorption of Congo Red on Sample

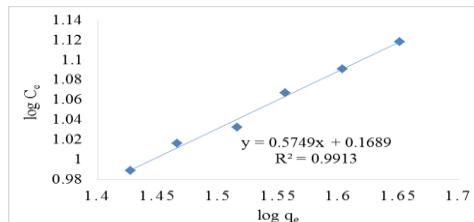


Figure 14. Freundlich Isotherm for Sorption of Congo Red on Sample

Table 13. Adsorption of Different Mass of Sample by Using Methylene Blue

Mass (g)	C _e (mgL ⁻¹)	q _e (mg g ⁻¹)	C _e /q _e (gL ⁻¹)	log C _e	log q _e
0.01	26.00	60.000	0.433	1.415	1.778
0.02	16.21	42.238	0.384	1.210	1.626
0.03	11.86	31.783	0.373	1.074	1.502
0.04	8.80	25.750	0.342	0.944	1.411
0.05	6.51	21.745	0.299	0.814	1.337
0.06	4.76	18.850	0.253	0.678	1.275

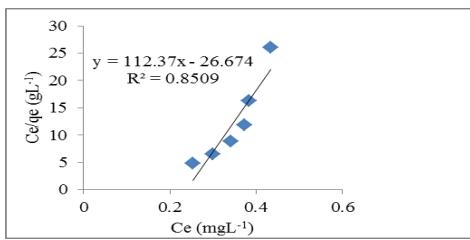


Figure 15. Langmuir Isotherm for Sorption of Methylene Blue on Sample

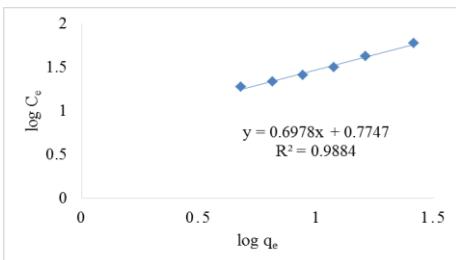


Figure 16. Freundlich Isotherm for Sorption of Methylene Blue on Sample

Table 14. Langmuir and Freundlich Parameters for the Adsorption of Dyes

	Parameter	Methylene blue	Congo red
Langmuir parameter	R ²	0.8509	0.981
	X _m (mg g ⁻¹)	0.0089	27.027
	b (L mg ⁻¹)	4.2127	0.021
Freundlich parameter	R ²	0.988	0.991
	n (gL ⁻¹)	1.433	1.739
	K (mg g ⁻¹)	5.9525	1.0006

From these results, the Freundlich is more effective than the Langmuir for lene blue and congo red on sample. Therefore, both multilayer adsorption (Freundlich) and monolayer adsorption (Langmuir) are possible.

4. Conclusion

In this research work, physicochemical properties of corncob sample such as moisture (6.1%), pH (5.93), and bulk density (0.36 gcm⁻³) were determined. Sample was heated at 100°C for 1hr for adsorption of dyes. The effects of sorption parameters (sorbent dose, concentration of dye solution and contact time) of each sorbent upon the removal of Congo red and methylene blue were investigated. The adsorption of corncobs (sorbent dose, concentration of dye solution and contact time) was better in methylene blue than congo red. Isotherm equations of Langmuir and Freundlich were applied and significant sorption parameters were evaluated. Based on R² (correlation coefficient values),

the isotherm in both Freundlich and Langmuir were known. The Freundlich is more effective than the methylene blue and congo red on sample. Therefore, both multilayer adsorption (Freundlich) and monolayer adsorption (Langmuir) are possible. Moreover, the sample could be applied in waste water treatment because it gave the significant percent removal on Congo red and Methylene blue.

5. Acknowledgement

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Production and Characterization of Activated Carbon from Banana Empty Fruit Bunch and *Delonix regia* Fruit Pod

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Abstract

In the research paper, the sample of *Delonix regia* fruit pods (DRFP) was collected from Shwebo University Campus and also Banana Empty Fruit Bunch (BEFB) was collected from local market in Shwebo. The air dried samples were carbonized by using in a hot oven. These carbons were pulverized to fine powder and sieved to mesh size 40. Then the activated carbon of DRFP and BEFB were prepared by pyrolysis method with H_3PO_4 at 400°C and 450°C. The physicochemical properties such as pH, electrical conductivity, bulk density and hardness of different activated carbon with H_3PO_4 were investigated. Moreover, the morphology of surface area of different activated carbons were examined by SEM. And then the liquid phase adsorption of iodine removal by different activated carbon and untreated carbon were determined.

Keywords- DRFP, BEFB, activated carbon, SEM, liquid phase adsorption.

1. Introduction

Activated carbon (AC), a tasteless, solid, microcrystalline, non-graphitic form of black carbonaceous material with a porous structure has been regarded as a unique and versatile adsorbent because of its extended surface area, micro porous structure, high adsorption capacity, and high degree of surface reactivity. AC is applied widely in a variety of fields such as food and chemical industries, wastewater treatment, solvent recovery, air pollution control and hydrometallurgy for the recovery of gold and silver. AC obtained from agricultural by-products has the advantage of offering an effective, low cost replacement for non-renewable coal-based granular activated carbons (GACs) provided that they have similar or better adsorption efficiency. The abundance and availability of agricultural by-products makes them good sources of raw materials for activated carbon production. In recent years, this has promoted a growing research interest in the use of alternative waste materials from industry and agriculture for activated carbon production [3]. Chemical activation of AC has been reported as more advantageous over physical activation

due to higher yields, more surface area and better development of porous structures in carbon. In this project paper, we report the production of AC from *Delonix regia* fruit pods (DRFP) and banana empty fruit bunch (BEFB) by pyrolysis and chemical activation with H_3PO_4 and its characterization for surface chemistry and liquid adsorption capacity [2].

1.1. Aim and Objectives

Aim

- To characterize and measure the adsorptivity of the prepared activated carbons from agricultural waste.

Objectives

- To prepare the activated carbons from selected samples treated with phosphoric acid
- To determine the physico-chemical properties such as pH, electrical conductivity, bulk density of activated carbon and untreated carbon
- To analyze the surface area of untreated carbon and activated carbon by using SEM spectrogram
- To determine the liquid phase adsorption by removal of iodine

1.2. Botanical Description

Family Name	- Fabaceae
Botanical Name	- <i>Delonix regia</i>
Myanmar Name	- Seinban
Part of uses	- Fruit bunch



Figure 1(a). Fruit Pod of *Delonix regia*

Family Name	- Musaceae
Botanical Name	- <i>Musa acuminata</i>
Myanmar Name	- Hnget-pyaw
Part of uses	- Fruit pod



Figure 1(b). Banana Empty Fruit Bunch

1.2.1. Description. Palmyra palm tree is a tall and swaying tree well known as '*Borassus flabellifer*'. The word '*Borassus*' was derived from a Greek word and it means the leathery covering of the fruit and '*Flabellifer*' means fan-bearer. Palmyra palm tree belongs to the 'Palme' family. This tree is found in the drier areas of India, Sri Lanka, and Burma and also in most of the tropical countries. In its ideal condition the Palmyra palm tree can grow up to a height from 12 metres to 18 metres when it becomes matured. However, in some special occasions, it can achieve the height of 30 metres and a diameter of 60 metres. The tree can be very easily recognized amongst a gathering of trees by its large and fan-shaped leaves. The stem of the tree is black in colour and looks like cylinders. The leaves of Palmyra palm tree are palm-shaped and have the maximum size of 5 metres. The heaving segments are normally 60 to 80 in number. They remain linked for a part of their length.

2. Materials and Methods

2.1. Sample Collection and Preparation

DRFP was collected from Shwebo University Campus. BEFB was collected from local market in Shwebo. Each sample was dried in air and they were pulverized to fine powder, sieved to mesh size 40 and used for characterization and production of activated carbon.

2.2. Activated Carbon Production

Activated carbon of DRFP and BEFB were prepared by following the one step pyrolysis method. For this, the test samples were divided into two parts; the first part mixed with 10% phosphoric acid (H_3PO_4) (100 g sample + 100 mL of H_3PO_4 wt/v) and the second part without any addition. The treated and untreated sample was pyrolyzed at 400°C (DRFP) and 450°C (BEFB) for 1 h in an electric muffle furnace. After activation, the mixture was removed from the furnace and allowed to cool to room temperature. The pyrolysed carbons were leached with 2% HCl (v/v) for 2 h and washed several times with de-ionized hot water until a neutral pH was achieved. Later the carbon paste was dried in an electric oven at 110°C for 24 h. The

activated carbon yield was calculated by applying the formula.

$$X (\%) = \frac{m}{m_0} \times 100$$

Where X is activated carbon yield (%), m is the activated carbon mass (g) and m_0 is the raw sample mass (g).

The carbon preparation experiments were carried out several times to obtain enough activated carbon samples for further analysis and characterization. Thus, X is an average value of all the effective experiment [1].



Figure 2. Lignocellulose Substrates and Activated Carbon

2.3. Determination of the Physical and Chemical Characteristics of Activated Carbon (AC)

The pH, electrical conductivity (EC) and bulk density of the AC samples DRFP AND BEFB were determined by following standard methods. For pH determination, 1% (w/w) suspension of activated carbon in de-ionized water was prepared and the suspension was heated at 90°C with stirring for 20 minutes. The suspension was then allowed to cool at room temperature and the pH was measured using pH meter. For EC determination, 1% (wt/wt) solution of activated carbon in water was stirred at room temperature for 20 min and the electrical conductivity was measured using conductivity meter and values were presented in micro Siemens (μS).

2.4. Determination of the Bulk Density of Activated Carbon

For bulk density, a glass cylinder (25 ml) was filled to a specified volume with 40 mesh activated carbon powder and dried in an oven at 80°C overnight. The cylinder was tapped for 1-2 minutes to compact the carbon and the bulk density calculated and presented as g ml^{-1} following the formula:

$$\frac{\text{Weight of dry material (g)}}{\text{Volume of Packed dry materials (ml)}} \times 100$$

All the experiments were carried out in triplicate and the averages were presented. The bulk density of activated carbon, BEFB, was determined by follow the above procedure.

2.5. Determination of the Hardness of Activated Carbon

The hardness of activated carbon samples was determined using a wet attrition test. One gram of activated carbon sample DRFP (40 mesh size) was added to 100 ml of acetate buffer (0.07 M sodium acetate and 0.03 M acetic acid, pH 4.8) in a 250 ml Erlenmeyer flask. The solution was stirred for 24 h at 25°C on magnetic stirrer (model. REMI, 2MLH) at 200 rpm using ½ inch stir bars. The samples were then poured onto a 40 mesh screen, and the retained carbon was washed with 250 ml of de-ionized water. After washing, the carbon was transferred to a pre-weighed silica crucible and dried at 90°C in a vacuum oven for 4 hrs. The samples were removed and allowed to cool in desiccators and weighed. The percentage of attrition was calculated using the formula:

$$\frac{\text{Weight of carbon retained by sieve (g)}}{\text{Initial sample weight (g)}} \times 100$$

The hardness of activated carbon, BEFB, was determined by using the same procedure.

2.6. Analysis of Surface Area of Untreated and Treated Carbon Sample by Using Scanning Electron Microscope (SEM)

The surface morphological changes of each activated carbon samples (DRFP, BEFB) were investigated by using a Scanning Electron Microscope (SEM, Make: JEOL, Japan) operated at 25kV.

2.7. Determination of Iodine Removal from Treated and Untreated Carbon Samples

Treated and untreated carbon samples (0.2-1.0 g) DRFP were taken in a 250 mL flask and 10 mL of 5% HCl was added. The flask was swirled until the carbon became wet. Then 100 mL of stock iodine solution (2.7 g of Iodine and 4.1 g of potassium iodide in 1 L of de-ionized water) was added to it and the mixture was shaken for 5 minutes in an orbital shaker. Then the samples were filtered through Whatman No.1 filter paper. 50 mL of filtrate was titrated with 0.1 M sodium thiosulphate until the solution becomes pale yellow. 1 mL of starch indicator solution (1%) was added and titration was continued with sodium thiosulphate until the solution becomes colorless. A blank was prepared without adding carbon. The percent iodine removed by each carbon was calculated by applying the following formula:

$$\frac{(A - B) \times 100}{B}$$

Where, A= mL of sodium thiosulphate used for blank
B = mL of sodium thiosulphate used for sample

The iodine values of treated and untreated carbon samples, BEFB, were determined by using the above procedure and formula [4].

3. Results and Discussion

3.1. Prepared Activated Carbon

The activated carbon of each selected sample was produced by chemical activation with H_3PO_4 and the yield percent were calculated. These results were shown in Table 1.

Table 1. Yield of Activated Carbon from DREF and BEFB with or without H_3PO_4 Treatment

Sample	Untreated (%)	Treated with H_3PO_4 (%)
DRFP(400°C)	37.65	40.46
BEFB(450°C)	34.63	36.09

A higher yield of 40.46% was recorded in DRFP treated with H_3PO_4 and untreated DRFP was found to be 37.65% yield. While the untreated BEFB registered a yield of 34.63% and the H_3PO_4 treatment registered 36.09% yield. Several activating agents have been reported for use in the chemical activation process. Among them H_3PO_4 and KOH are widely used in the production of activated carbon because of low energy costs and high carbon yields as well as easy recovery of the activating agents.

3.2. Physicochemical Properties of Activated Carbon

The pH, EC, bulk density and hardness of the activated carbon samples were recorded and presented in Table 2.

Table 2. Physical and Chemical Properties of Activated Carbon Samples

Measurement	BEFB		DRFP	
	H_3PO_4	Untreated	H_3PO_4	Untreated
pH	9.53	10.33	3.73	8.53
EC ($\mu S/cm$)	1225	3038	352.56	320.23
Bulk density (g/mL)	0.34	0.39	0.44	0.39
hardness (%)	10.18	10.28	1.45	1.86

All the carbon samples recorded alkaline pH values except H_3PO_4 treated DRFP 3.73. A comparatively low EC value 352.56 μS for H_3PO_4 treated DRFP was obtained followed by untreated DRFP 320.23 μS respectively. In this study, H_3PO_4 treated DRFP showed a higher bulk density 0.44 g/ml. The hardness or attrition is a measure of the mechanical strength of the carbons and it is an important parameter for understanding its relative loss during the transportation, handling and regeneration. The hardness was high in untreated 10.28% and H_3PO_4 treated 10.18% DRFP. The percentage of hardness observed in carbon, as the results indicate, depends upon

the carbon density or starting materials of the hardness percentage is varied.

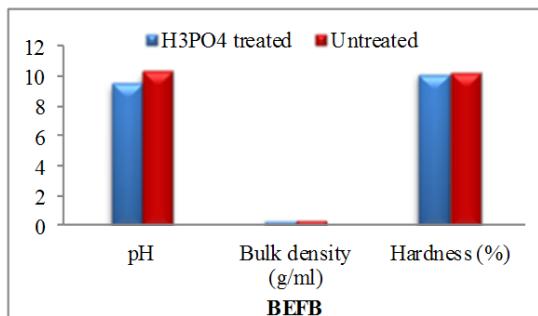


Figure 3. Physicochemical Properties of Treated and Untreated Carbon for BEFB

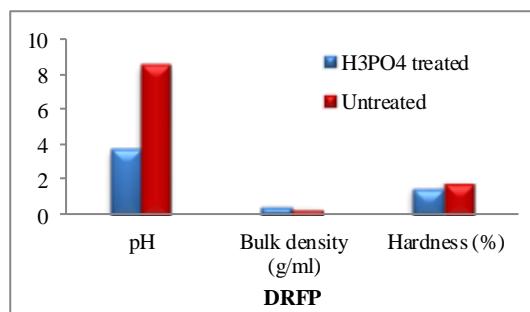


Figure 4. Physicochemical Properties of Treated and Untreated Carbon for DRFP

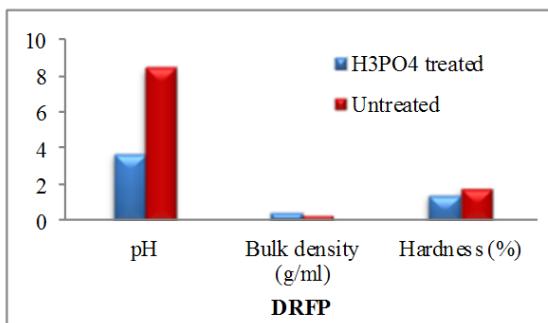


Figure 5. Physical Properties of Treated and Untreated Carbon for BEFB

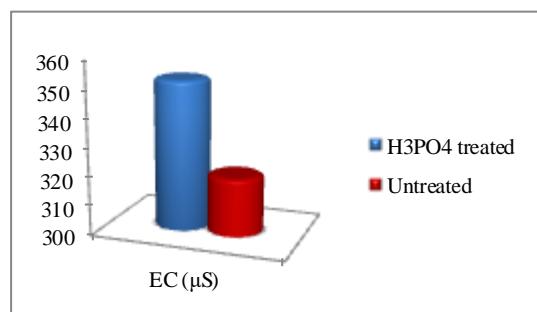


Figure 6. Physical Properties of Treated and Untreated Carbon for DRFP

3.3. Liquid Phase Adsorption

The results of iodine removal by different activated carbon samples at doses between 0.2-1 g were presented in Figure (7).

Table 3. Results of Iodine Removal by Treated and Untreated Samples

Sample	Dose (g)	Untreated (%)	Treated H ₃ PO ₄ (%)
DRFP	0.2	35	20
	0.4	55	40
	0.6	60	45
	0.8	70	50
	1.0	80	60
BEFB	0.2	30	35
	0.4	40	50
	0.6	45	60
	0.8	60	70
	1.0	70	80

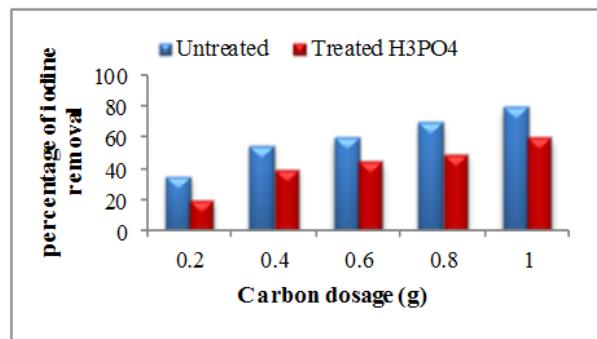


Figure 7. Iodine Removal from Untreated and Treated Carbon of DRFP

The H₃PO₄ treated carbon samples showed lower iodine removal than the untreated samples. While high iodine removal was registered in untreated DRFP samples (80%) and it was low in H₃PO₄ treated DRFP (60%).

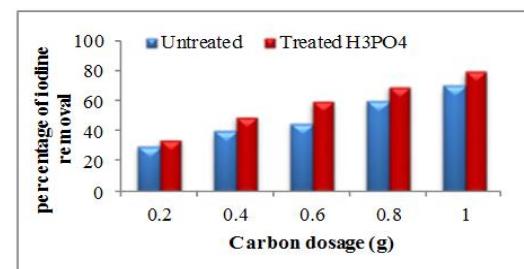


Figure 8. Iodine Removal from Untreated and Treated Carbon of BEFB

The H₃PO₄ treated carbon samples showed higher iodine removal than the untreated samples. While higher iodine removal was registered in H₃PO₄ treated BEFB samples 80% and it was low in untreated BEFB (70%). Higher degrees of iodine adsorption have been reported to indicate a higher surface area and the presence of largely micro and mesoporous structures. Therefore, there exist differences among the different carbons in

iodine adsorption which could be attributed to the differences in their surface areas, porosity and activation methods.

3.4. Surface Area Analysis

The SEM photographs of the untreated and treated BEFB samples showed rod like structures but while the untreated sample was bundled together the treated samples showed more spaced out structures. In the case of DRFP, H₃PO₄ treated samples showed open porous structures. The untreated sample showed holes that were spaced out on the surface with smooth edges.

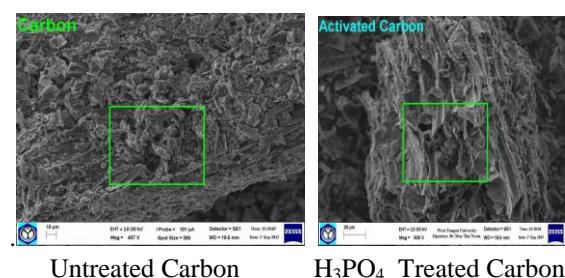


Figure 9. SEM Micrographs of Carbon Samples from Banana Empty Fruit Bunch (BEFB)

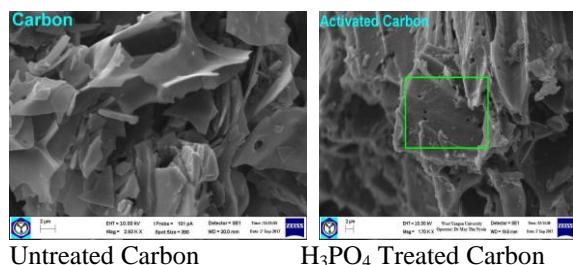


Figure 10. SEM Micrographs of Carbon Samples from *Delonix regia* fruit pods (DRFP)

4. Conclusion

In this research paper, the activated carbons were prepared from the pyrolysis of fruit pods of *Delonix regia* at 400°C and banana empty fruit bunch at 450°C followed by untreated and treated with H₃PO₄. The yields of the cultivated carbons produced by chemical activation with H₃PO₄ were found to be higher than untreated carbon. The prepared activated carbon were characterized by physicochemical properties, surface morphology and adsorptivity. From the results of physicochemical properties, all the activated carbon were recorded alkaline pH values except H₃PO₄ treated DRFP (3.73).

The activated carbons of BEFB have higher electrical conductivity values than activated carbons of DRFP. The bulk density for untreated carbons of DRFP and BEFB showed the same values. But the higher bulk density of H₃PO₄ treated DRFP was found to be 0.44

g/mL. So bulk density decreases and electrical conductivity increases indicate that the porosity of activated carbon increase. The hardness was high in untreated (10.28 %) and H₃PO₄ activated (10.18 %) of DRFP. The hardness is a measure of the mechanical strength of the carbon and it is an important parameter for understanding its relative loss during the transportation, handling and regeneration.

The surface morphological changes of activated carbon samples were investigated using a Scanning Electron Microscope (SEM). Iodine removal from treated and untreated carbon samples were determined by using titration method. According to the results, the H₃PO₄ treated DRFP carbon samples showed lower iodine removal than that of the untreated samples. But good iodine removal of H₃PO₄ treated BEFB carbon samples than that of the untreated samples. The ability to remove iodine reveals that it had an improved adsorption behavior of high performance adsorbents. Consequently, the activated carbons produced from lingo-cellulosic waste biomass can be used as adsorbents for various environmental applications including treatment of drinking water, removing colour from industrial effluents and removal of heavy metals.

5. Acknowledgements

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Removal of Toxic Metal, Cadmium from the Waste Water Discarded from Battery Factory by using Rice Husk, Rice Husk Charcoal and Rice Husk Ash

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Abstract

In this work, the waste water from Naung Yoe Battery Factory, Chanmyathazi Township, Mandalay Region, was collected. The rice husk, rice husk charcoal and rice husk ash were used as adsorbents in removal toxic metal cadmium (Cd^{2+}). The optimum pH was selected by volumetric analysis using standard EDTA and xylenol orange as indicator. In addition, the amount of cadmium removal capacities were investigated by using various mesh size and various amount of rice husk, rice husk charcoal and rice husk ash. In this research work, removal capacities of rice husk, rice husk charcoal and rice husk ash were determined by filtration and complexometric titration method. Rice husk charcoal can adsorb cadmium greater than that of others.

Keywords - cadmium, rice husk, rice husk charcoal, rice husk ash, EDTA.

1. Introduction

Environmental pollution is one of the most serious problem facing humanity and other life forms on our planet today. Environmental pollution is defined as the contamination on the physical and biological components of the earth/atmosphere system to such an extent that normal environmental processes are adversely affected [4].

Some of the main causes of pollution include industrial emissions, poor disposal of wastes, mining, deforestation, use of fossil fuels and agricultural activities. Pollution can affect the air, the land and water bodies throughout the world [10].

Domestic household, industrial and agricultural practices produce wastewater that can cause pollution of many lakes and rivers [11].

An important source of water pollutants is industrial discharge water. Most industrial waste waters are discharged directly into natural water systems without proper management process. Unfortunately, the existence of large dosage of heavy metals in these effluents represents the greatest challenge in water purification, where exceeding the allowed concentration limit of these metals in the human body can end up the acute or chronic death [6].

Like other heavy metals, cadmium is one of major pollutants in waste-water. It is used as a major raw material in battery manufacturing and wastewater from this industry can contain high concentration of cadmium. Cadmium may contaminate surface and drinking water from industrial effluents, waste incinerators and utilities. Cadmium affects the bone, cardiovascular, nervous, kidney and blood diseases.

Several approaches have been employed to remove heavy metal ions from waste water. However, generally, the adsorption process has been proved convenient in terms of cost, simplicity and flexibility [6].

Rice husk is one of the major agricultural wastes. It is a fibrous materials containing cellulose as the major constituent. Agricultural by product materials appear as effective and cheap sorbents for removal of heavy metals from wastewater. In this research work, rice husk, rice husk charcoal and rice husk ash were used as adsorbents for the removal of cadmium from battery factory wastewater.

2. Material and Methods

2.1. Sample Collection

The waste water sample was collected from Naung Yoe battery factory, Chanmyathazi Township, Mandalay Region.

2.2. Determination of the pH Value of Waste Water Sample

The pH of the waste water was determined by using pH meter. The results were shown in Table (1).

2.3. Determination of Optimum pH for the Cadmium Solution

The waste water sample was prepared to obtain the variable pH values by using ammonia solution. Then, each waste water having pH value 3-6 was titrated with 0.001 M EDTA solution using Xylenol orange as indicator. The effect of pH on the waste water was determined. The results were shown in Table (2). From the results, the optimum pH was found to be 5.5. Therefore, the waste water sample was treated to get pH

5.5 by using ammonia solution and this prepared solution was used throughout the research work.

2.4. Preparation of Adsorbent

Among the many kinds of adsorbents, rice husk was selected as adsorbent for the study.

Rice husk sample was collected from Bomgwin village, Kyaukse township, Mandalay Region.

500 g of rice husk was grinded by using motar and pastel. And then these rice husk were passed served through 40, 60, 80 and 100 mesh size sieves.

Three different mesh sizes rice husk sample such as 40> mesh >60, 60> mesh >80, 80> mesh >100 were obtained (RH_1 , RH_2 , RH_3).

500g of rice husk was heated in electric furnace at 400°C about 30 minutes till to obtain charcoal. After burning, the charcoal was cooled at room temperature. The rice husk charcoal was grinded and served to set three different mesh sizes denoted as (RHC_1 , RHC_2 , RHC_3)

1000 g of rice husk was heated in electric furnace at 800°C about 30 minutes to obtain white colour of ash. After burning, the ash was cooled at room temperature and sieved to obtain three different mesh sizes denoted as (RHA_1 , RHA_2 , RHA_3).

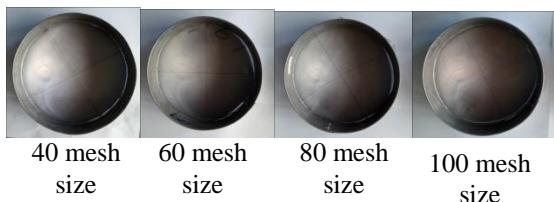


Figure 1. Three Different Adsorbent (Rice husk, Rice husk Charcoal and Rice husk ash)

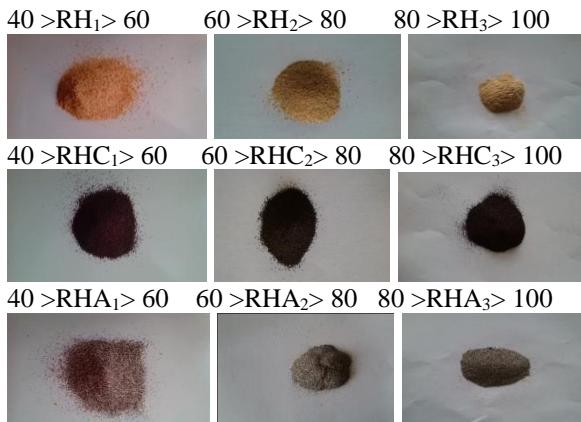


Figure 2. Three Different Adsorbent (Rice husk, Rice husk charcoal and Rice husk ash)

2.5. Determination of Cadmium Removal Capacity of Rice Husk for the Waste Water Discarded from Battery Factory

0.215 g cotton was put in the coloum 2 cm diameter and 70 cm length. Rice husk sample, RH_1 (40> RH_1 > 60

mesh) 1 g was added over the cotton. Then, 25 mL of prepared waste water was added into the coloum. The waste water sample was passed through the adsorbent slowly and steadily along the coloum at room temperature. The flow rate of the sample was 10 drops per minute. The filtrate was collected until no drop comes out from the coloum. One drop of xylanol orange indicator was added into 5 cm³ of filtrate in a conical flask. A few drop of dilute sulphuric acid was added in this solution to obtain yellow colour solution. A small amount of hexamine powder was added in this solution to obtain deeply red colour solution. This prepared solution was titrated with 0.001 M EDTA solution to reach the end point of yellow colour solution. To know the effect of amount of rice husk on removal of cadmium, the various amounts of adsorbent, rice husk, were used in the range of (1-10) g. The results were shown in Table (3). For rice husk samples, RH_2 (60 > RH_2 > 80 mesh), and RH_3 (80 > RH_3 > 100 mesh), similar procedures were performed and the results were described in Table (4) and (5).

2.6. Determination of Cadmium Removal Capacity of Rice Husk Charcoal

0.215 g cotton was put in the coloum 2 cm diameter and 70 cm length. Rice husk charcoal sample, RHC_1 (40> RHC_1 > 60 mesh) 1 g was added over the cotton. Then, 25 mL of prepared waste water was added into the coloum. The waste water sample was passed through the adsorbent slowly and steadily along the coloum at room temperature. The flow rate of the sample was 10 drops per minute. The filtrate was collected until no drop comes out from the coloum. One drop of xylanol orange indicator was added into 5 cm³ of filtrate in a conical flask. A few drop of dilute sulphuric acid was added in this solution to obtain yellow colour solution. A small amount of hexamine powder was added in this solution to obtain deeply red colour solution. This prepared solution was titrated with 0.001 M EDTA solution to reach the end point of yellow colour solution. To know the effect of amount of rice husk charcoal on removal of cadmium, the various amounts of adsorbent, rice husk charcoal, were used in the range of (1-10) g. The results were shown in Table (6). For rice husk charcoal samples, RHC_2 (60 > RHC_2 > 80 mesh), and RH_3 (80 > RHC_3 > 100 mesh), similar procedures were performed and the results were described in Table (7) and (8).

2.7. Determination of Cadmium Removal Capacity of Rice Husk Ash

0.215 g cotton was put in the coloum 2 cm diameter and 70 cm length. Rice husk ash sample, RHA_1 (40> RHA_1 > 60 mesh) 1 g was added over the cotton. Then, 25 mL of prepared waste water was added into the

coloum. The waste water sample was passed through the adsorbent slowly and steadily along the coloum at room temperature. The flow rate of the sample was 10 drops per minute. The filtrate was collected until no drop comes out from the coloum. One drop of xylenol orange indicator was added into 5 cm³ of filtrate in a conical flask. A few drop of dilute sulphuric acid was added in this solution to obtain yellow colour solution. A small amount of hexamine powder was added in this solution to obtain deeply red colour solution. This prepared solution was titrated with 0.001 M EDTA solution to reach the end point of yellow colour solution. To know the effect of amount of rice husk ash on removal of cadmium, the various amounts of adsorbent, rice husk ash, were used in the range of (1-10) g. The results were shown in Table (9). For rice husk ash samples, RHA₂ (60 > RHA₂ > 80 mesh), and RHA₃ (80 > RHA₃ > 100 mesh), similar procedures were performed and the results were described in Table (10) and (11).

3. Results and Discussion

3.1. pH Value of the Waste Water Sample

pH value of the waste water discarded from Battery factory was determined and the results were shown in Table (1).

Table 1. pH Value of Waste Water Sample

No	pH
1	0.5
2	0.5
3	0.5

According to this table, pH of the waste water discarded from Battery factory was found to be 0.5. The discarded waste water is too acidic.

3.2. Determination of Optimum pH for the Solution Containing Cadmium

The waste water sample (pH 0.5) was prepared to obtain various pH values. The observation was made for the titration of waste water solution that contains cadmium with standard EDTA solution. The results were shown in Table (2).

Table 2. Titration of 5 ml Waste Water Solution with Standard 0.001 M EDTA Solution

No	pH	The colour after the addition of indicator xylenol orange	The colour after the addition of dilute sulphuric acid	The colour after the addition of hexamine	Remark
1	3	Yellow	Yellow	-	-
2	3.5	Yellow	Yellow	-	-
3	4	Yellow	Yellow	-	-

4	4.5	Yellow	Yellow	-	-
5	5	Yellow	Yellow	-	-
6	5.5	Yellow	Yellow	Red	Yellow (true colour change)
7	6	Yellow	Yellow	-	-
8	6.5	Yellow	Yellow	-	-

From Table (2), pH 5.5 solution gave the true colour change during the titration. The optimum pH for the solution that contains cadmium found to be 5.5. This pH agrees with the literature value.

Therefore, the waste water sample was prepared to get pH 5.5 by using ammonia solution. The prepared solution was used through the research work.

3.3. Results for Cadmium Removal Capacity of Rice Husk of Three Different Mesh Sizes for the Waste Water Discarded from Battery Factory

To determine the cadmium removal capacity, rice husk, rice husk charcoal and rice husk ash were used as adsorbents and filtration method was used.

Cadmium removal capacity of various amount of three different mesh sizes rice husk samples of were determined for the waste water sample from Battery Factory and the results were described in Table (3), Table (4) and Table (5).

Table 3. Removal of Cadmium from Waste Water Sample by Using Rice husk (40 > RH₁ > 60 mesh)

No	Amount of rice husk used (g)	Amount of removal of Cadmium (mg)	Removal percentage (%)
1	1	0.44	26.25
2	2	0.48	28.75
3	3	0.52	31.25
4	4	0.60	36.25
5	5	0.64	38.75
6	6	0.68	41.25
7	7	0.73	43.75
8	8	0.81	48.75
9	9	0.83	49.99
10	10	0.93	56.25

Table 4. Removal of Cadmium From Waste Water Sample by Using Rice husk (60 > RH₂ > 80 mesh)

No	Amount of rice husk used (g)	Amount of removal of Cadmium (mg)	Removal percentage (%)
1	1	0.52	31.25
2	2	0.56	33.75
3	3	0.62	37.49
4	4	0.66	39.99
5	5	0.70	42.49
6	6	0.77	46.25
7	7	0.81	48.75

8	8	0.87	52.49
9	9	0.91	54.99
10	10	1.04	62.49

Table 5. Removal of Cadmium from Waste Water Sample by Using Rice husk(80 > RH₃> 100 mesh)

No	Amount of rice husk used (g)	Amount of removal of Cadmium (mg)	Removal percentage (%)
1	1	0.56	33.75
2	2	0.62	37.49
3	3	0.66	39.99
4	4	0.73	43.75
5	5	0.77	46.25
6	6	0.81	48.75
7	7	0.85	51.25
8	8	0.91	54.99
9	9	0.95	57.50
10	10	1.06	63.75

From Table (3), (4) and (5), it can be seen that the increase in amount of rice husk used increases the amount of removal of Cadmium from the waste water.

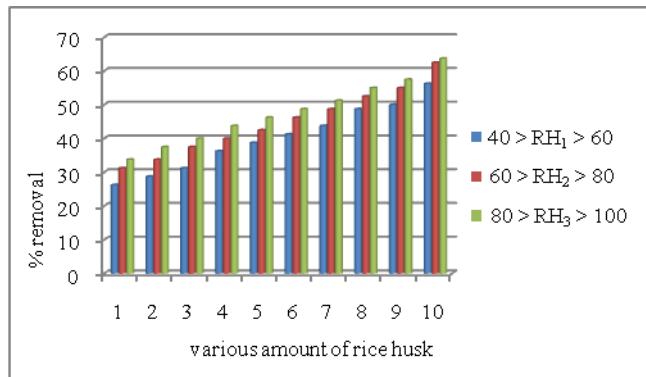


Figure 3. The Percent Removal of Cadmium by Various Amount and Various Size of Rice husk

According to Table (3), (4), (5) and Figure (3), it was found that the smaller the mesh size of rice husk, the more adsorption can occur and the more amount of Cadmium can be removed.

3.4. Results for Cadmium Removal Capacity of Rice Husk Charcoal of Three Different Mesh Sizes

Cadmium removal capacity of various amount of three different mesh sizes rice husk charcoal samples were determined for the waste water sample from Battery Factory and the results were described in Table (6), Table (7) and Table (8).

Table 6. Removal of Cadmium from Waste Water Sample by using Rice husk Charcoal (40 >RHC₁> 60 mesh)

No	Amount of rice husk charcoal used (g)	Amount of removal of Cadmium (mg)	Removal percentage (%)
1	1	0.62	37.47
2	2	0.66	37.49
3	3	0.81	48.75
4	4	0.82	49.99
5	5	0.85	51.25
6	6	0.91	54.99
7	7	1.02	61.25
8	8	1.06	63.75
9	9	1.16	70.00
10	10	1.24	74.99

Table 7. Removal of Cadmium from Waste Water Sample by Using Rice husk Charcoal (60>RHC₂>80 mesh)

No	Amount of rice husk charcoal used (g)	Amount of removal of Cadmium (mg)	Removal percentage (%)
1	1	0.66	40.00
2	2	0.70	42.50
3	3	0.87	52.51
4	4	0.95	57.50
5	5	1.04	62.50
6	6	1.09	66.24
7	7	1.14	68.75
8	8	1.24	75.00
9	9	1.31	78.75
10	10	1.41	85.00

Table 8. Removal of Cadmium from Waste Water Sample by using Rice husk Charcoal (80 > RHC₃> 100 mesh)

No	Amount of rice husk charcoal used (g)	Amount of removal of Cadmium (mg)	Removal percentage (%)
1	1	0.70	42.45
2	2	0.75	45.00
3	3	0.91	54.99
4	4	1.02	61.25
5	5	1.08	64.99
6	6	1.16	70.00
7	7	1.22	73.75
8	8	1.31	78.75
9	9	1.37	82.50
10	10	1.43	86.25

From Table (6), (7) and (8), it can be seen that the increase in amount of rice husk charcoal used increases the amount of removal of Cadmium from the waste water.

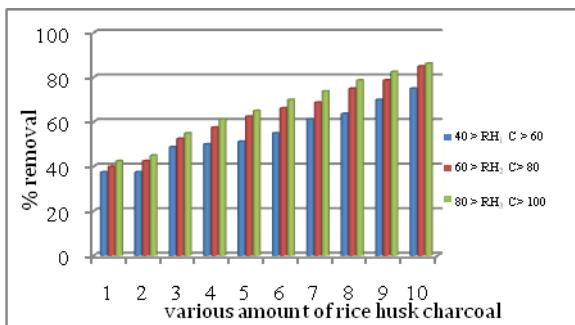


Figure 4. The Percent Removal of Cadmium by Various Amount and Various Size of Rice husk Charcoal

According to Table (6), (7), (8) and Figure (4), it was found that the smaller the mesh size of rice husk charcoal, the more adsorption can occur and the more amount of Cadmium can be removed.

3.5. Results for Cadmium Removal Capacity of Rice Husk Ash of Three Different Mesh Sizes

Cadmium removal capacity of various amount of three different mesh sizes rice husk ash samples of were determined for the waste water sample from Battery factory and the results were described in Table (9), Table (10) and Table (11).

Table 9. Removal of Cadmium from Waste Water Sample by using Rice Husk Ash (40 >RHA₁> 60 mesh)

No	Amount of rice husk ash used (g)	Amount of removal of Cadmium (mg)	Removal percentage (%)
1	1	0.48	28.75
2	2	0.52	31.25
3	3	0.62	37.49
4	4	0.66	39.99
5	5	0.70	42.49
6	6	0.77	46.25
7	7	0.85	51.25
8	8	0.91	54.78
9	9	1.06	63.75
10	10	1.09	66.25

Table 10. Removal of Cadmium from Waste Water Sample by using Rice husk Ash (60 > RHA₂> 80 mesh)

No	Amount of rice husk ash used (g)	Amount of removal of Cadmium (mg)	Removal percentage (%)
1	1	0.56	33.75
2	2	0.60	36.25
3	3	0.64	38.75
4	4	0.70	42.49
5	5	0.81	48.75
6	6	0.85	51.25
7	7	0.95	57.50

8	8	0.99	60.00
9	9	1.09	66.25
10	10	1.22	73.75

Table 11. Removal of Cadmium from Waste Water Sample by using Rice husk Ash (80 >RHA₃> 100 mesh)

No	Amount of rice husk ash used (g)	Amount of removal of Cadmium (mg)	Removal percentage (%)
1	1	0.60	36.25
2	2	0.64	38.75
3	3	0.68	41.25
4	4	0.77	46.25
5	5	0.83	49.99
6	6	0.91	54.99
7	7	1.02	61.25
8	8	1.08	64.99
9	9	1.20	74.41
10	10	1.24	74.99

From Table (9), (10) and (11), it can be seen that the increase in amount of rice husk ash used increases the amount of removal of Cadmium from the waste water.

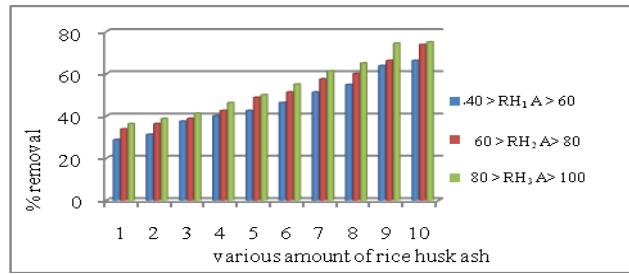


Figure 5. The Percent Removal of Cadmium by Various Amount and Various Size of Rice Husk Ash

According to Table (9) (10), (11) and Figure (5), it was found that the smaller the mesh size of rice husk ash, the more adsorption can occur and the more amount of Cadmium can be removed.

The amount of removal of cadmium from the waste water sample was compared by using same mesh size of rice husk, rice husk charcoal and rice husk ash.

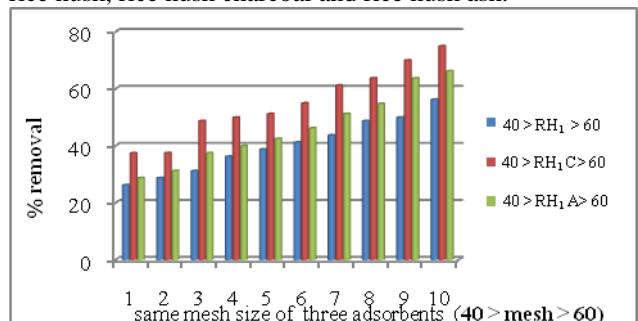


Figure 6. The Effect of Same Mesh Size of Three Adsorbents (40 > mesh > 60)

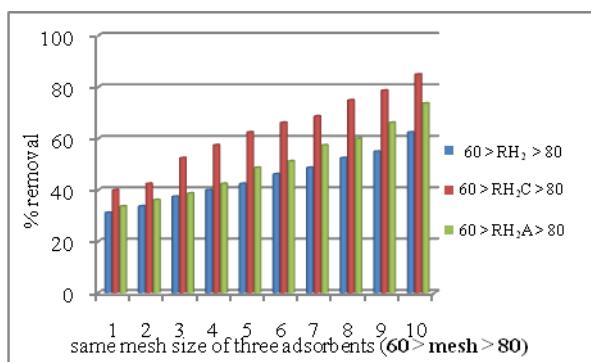


Figure 7. The Effect of Same Mesh Size of Three Adsorbents (60 > mesh > 80)

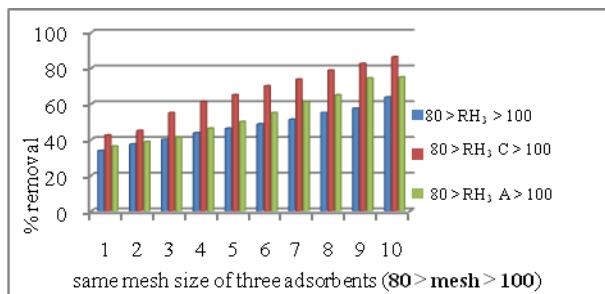


Figure 8. The Effect of Same Mesh Size of Three Adsorbents (80 > mesh > 100)

From Figure (6), (7) and (8) it was found that rice husk charcoal can adsorb more cadmium and the amount of removal of cadmium was greater than that of others.

4. Conclusion

The waste water discarded from battery factory contains some extent of toxic cadmium metal that can pollute the environment (soil, water, etc.). In this research work, the removal of toxic metal cadmium was studied by using rice husk, rice husk charcoal and rice husk ash as adsorbents by applying filtration method. The waste water sample was collected from Naung Yoe Battery Factory, Chanmyathazi, Township, Mandalay Region on August, 2018. Rice husk sample was also collected from Bomgwin village, Kyaukse Township, Mandalay Region.

Rice husk charcoal and rice husk ash were prepared by using muffle furnace. Three different mesh sizes such as 40 > mesh > 60, 60 > mesh > 80, 80 > mesh > 100 were also prepared for each kind of adsorbent. The pH of the sample solution was measured by pH meter and pH of the original waste water sample was found to be 0.5 (very acidic). The amount of cadmium removal was selected by volumetric analysis using standard EDTA and xylenol orange as indicator. The optimum pH was determined and found that it was 5.5. Therefore, the waste water sample was prepared to obtain pH 5.5. The experiments were carried out at room temperature and

flow rate of the sample was 10 drops per minute. To know the effect and amount of rice husk on removal of cadmium, the various amounts of adsorbent, rice husk, were used in the range of (1-10)g. The increase in amount of rice husk used increases the amount of removal of cadmium from the waste water. The smaller the mesh size of rice husk, the more adsorption can occur and the more amount of cadmium can be removed. Rice husk charcoal can adsorb cadmium greater than that of others. Therefore, this sample rice husk can be used for the removal of metal ion (Cd^{2+}) from polluted water.

5. Acknowledgments

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Transformation of Wood Wastes to Usable Product

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Abstract

This research is intended to transform the wood wastes to potentially useful product. This study mainly concerned with the production of particleboards by using “Kyun” sawdust and “In” sawdust with the help of adhesive, urea formaldehyde. The raw materials were purchased from local sawmill, Banmaw township, Kachin State. Some physicochemical properties such as moisture content, hot water solubility, cellulose and lignin content of each species were investigated by Technical Association of Pulp and Paper Industry (TAPPI) standard method. Two types of particleboards were prepared for each species by using different amount of urea formaldehyde resin on the same weight of sawdust. Some of physicomechanical properties of the prepared particleboards such as moisture, density, hardness, water sorption, swelling thickness, Modulus of Rupture (MOR) or bending strength and impact energy were determined. This study indicates that the more content of binding resin, the better quality of particleboards.

Keywords; In, MOR, Kyun, Physicomechanical, TAPPI

1. Introduction

Particleboard is a product in the form of chips or particles of wood or other fibrous ligno-cellulosic matter bonded together with an organic binder by means of pressure and heat. Particleboard can be made from a great number of raw materials such as sawmill residue and forest waste. The particleboards are mostly used in the furniture and the building industries there is an infinite range of application in building such as partitions, doors, flooring [3].

Teak, known as “Kyun” in Myanmar, belongs to Lamiaceae family and its botanical name is *Tectona grandis* L.f. “In” botanically called *Dipterocarpus tuberculatus* Roxb belongs to Dipterocarpaceae family [2]. “In” and Kyun sawdust were introduced in the present work. These plants are grown in Myanmar, Thailand, Bangladesh, Vietnam and Cambodia.

Adhesives are used to manufacture building materials. Urea-formaldehyde (UF) is chemically known as urea-methanal, non-transparent thermosetting resin. It

is a great for bonding particleboard. UF is the commercial resin popularly used for wood-based panel product (Lee, et al., 2011).The empirical formula of UF resin is $C_5H_{10}N_4O_3$.The colour of UF is white. It is also synthetic thermosetting resin, made from urea and formaldehyde. It is used in adhesive, molded objects, binders, bottle caps, electrical plugs and terminals, buttons, limited to interior grade bonds in polywood and furniture industrials. Urea-formaldehyde reacts quickly when composite are not pressed. It is water resistant but not water-proof, inexpensive and has high tensile strength [5].

The main aim of this research is to investigate the physicochemical properties of the wood wastes and to promote the wood waste into usable product as particleboard. In the current study, investigation of physicochemical properties of raw materials, preparation of particleboards and assessment of their physicomechanical properties were conducted.

2. Materials and Methods

2.1. Sample Collection and Preparation

“In” sawdust and “Kyun” sawdust were collected from local sawmill, Banmaw Township, Kachin State. Firstly the sample was made to powder and sieved with 60 mesh sieved. The samples were washed with water and dried in the sun.

2.2. Determination of Physicochemical Properties of Raw Sample Powder

To investigate the quality of raw material, tests on some physicochemical properties such as moisture content, hot water solubility, 1% NaOH solubility, lignin and cellulose contents were carried out according to TAPPI (Technical Association of Pulp and Paper Industry) standard method.

2.2.1. Determination of moisture content. Moisture content of sawdust was determined by oven drying. 1.0 g of sample was placed in pre-weighed porcelain crucible. Then it was dried in an oven at 105°C for two hours. It was cooled in desiccator and weighed again. The process of heating, cooling and weighing was repeated until a constant weight was achieved. The

moisture content was calculated by the following equation.

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

W_1 = Initial weight (g)

W_2 = Oven dried weight (g)

2.2.2. Determination of hot water solubility. 2.0 g of dried sample was placed in a flask and 100 mL of distilled water was added. A reflux condenser was attached to the flask and the apparatus was placed in a gently boiling water bath at 100°C for three hours. Special attention was given to ensure that the level of the solution in the flask remain below that of the boiling water. The residues in the flask were washed with hot distilled water until the flask has no residues. It was kept in the oven at 105°C for constant weight. After drying, it was cooled in the desiccator and weighed. The percentage of dissolved materials in the hot water solubility was calculated as the following.

$$\text{Hot water solubility \%} = \frac{C - B}{A} \times 100$$

A = oven dried sample weight in gram (before extraction)

B = oven dried weight of crucible in gram

C = oven dried weight of cotton bag with sample in gram (after extraction)

2.2.3. Determination of 1% NaOH solubility. 2.0 g of dried sample was placed in a beaker, and 100 mL of 1% NaOH solution was added into it and stirred. After stirring well, the covered beaker was placed in the water bath, which will be boiling steadily for exactly 1 hour, stirring the contents three times, at periods of 10, 15, 25 minutes after the beaker was placed in the boiling bath. After 1 hour, the contents of the beaker were filtered by the cotton bag and washed by 100 mL of hot water, then with 50 mL of 10% acetic acid, and then thoroughly with hot water. The cotton bag with the content was dried to constant weight at 105°C, cooled in desiccator and weighed. 1% solubility of the sample was calculated by the following equation.

$$1\% \text{ NaOH solubility} = \frac{C - B}{A} \times 100$$

A = oven dried sample weight in gram (before extraction) B = oven dried weight of cotton bag in gram

C = oven dried weight of cotton bag with sample in gram (after extraction)

2.2.4. Determination of Lignin content .Oven dried sample (1.0g) was placed in a pestle and motor. Then 15 mL of 72% sulphuric acid was added. The mixture was stirred at 20°C for two hours. After that, the mixture

was transferred into a conical flask and 665 mL of distilled water was added to obtain 3% acid concentration. And then the flask was connected with condenser to attain constant solution level and heated at 100°C for four hours. After being heated, the flask was cooled at the room temperature. The samples were filtered into the crucible. The content in the crucible was washed with hot water until it was free from acid. Then it was dried in the oven for two hours at 105°C. After that, it was cooled in desiccator and weighed and lignin content was calculated [4].

$$\text{Lignin content \%} = \frac{C - B}{A} \times 100$$

A = oven dried sample weight in gram (before testing)

B = oven dried weight of crucible in gram

C = oven dried weight of crucible with sample in gram (after testing)

2.2.5. Determination of Cellulose content. The cellulose content in each raw sample was determined by AOAC method. About 2.0 g of the sample was boiled in ethanol for 15 minutes (four times) and washed thoroughly with distilled water and kept in oven for dry weight. Then it was divided into two parts in which one part is considered as fraction "A". Second part of residue was treated with 24%(w/v) potassium hydroxide for 4 hour at 25°C, followed by washed thoroughly with distilled water and dried at 80°C in oven and the dried weight was taken as fraction "B". The same sample 2 g was again treated with 72% (v/v) sulphuric acid for 3 hours to hydrolyze the cellulose and refluxed with 5% (v/v) sulphuric acid for 2 hours. Sulphuric acid was completely removed by washing it distilled water and it was allowed to dry at 80°C in oven. The dried weight was taken as fraction "C". The cellulose content was calculated by the following equation [4],[6].

$$\text{Cellulose content \%} = \frac{B - C}{A} \times 100$$

A = original weight

B = oven dried weight after treated with 24% potassium hydroxide

C = oven dried weight after treated with 72% sulphuric acid

2.3. Processing of Particleboards Production

In the present work, the particleboards labeled as PB-1 and PB-2 for "Kyun" sawdust however PB-3 and PB-4 for "In" were prepared at Department of Research and Innovation at Yankin Township, Yangon. The composition of binding adhesive, urea-formaldehyde was **30g** for **PB-1 and PB-3**, **60g** for **PB-4**

2 and **PB-4** on constant weight 150g of each raw sample. 150 g of sawdust and different weights of urea-formaldehyde (30g, 60g) were mixed in the Henschel mixer and blended for 5 minutes at ambient temperature. After the whole mass was blended, the blended mixture was then poured into 6" x 6" (15.2 cm x 15.2 cm) configuration molds so as to produce a single layer board and wedged between two steel plates. The single layer wet board was pressed in a hydraulic hot press at 2200 psi at temperature 150 °C for 15 minutes. During pressing the board, moisture content was removed from the board by reducing the valve in each 5 minutes. An original pressure is attained in each time. The parameters employed to produce single layer particleboards are shown in table 1 and the single layer prepared particleboards are shown in figure 1(a) and 1(b).

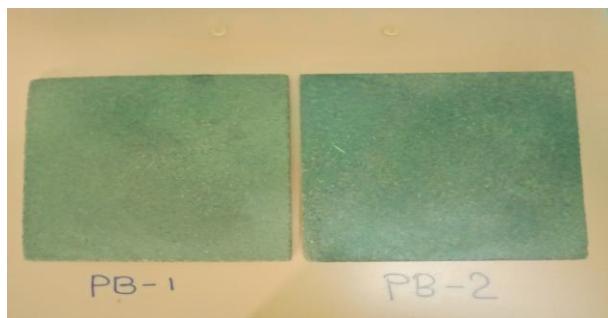


Figure 1(a). Prepared Particleboards PB-1 and PB-2

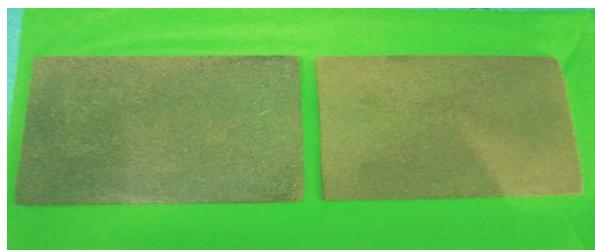


Figure 1(b). Prepared Particleboards PB-3 and PB-4

Table 1. Parameters of Prepared Particleboards

No	parameters	Values
1	Length (cm)	15.24
2	Width (cm)	15.24
3	Thickness (cm)	0.69 – 0.85
4	Pressing temperature(°C)	150
5	Pressing time (min)	15
6	Pressing pressure (psi)	2200
7	Number of boards for each type	3

2.4. Determination of Some Physicomechanical Properties of Prepared Particleboards

The physical properties of the prepared boards such as moisture, density, hardness, thickness swelling, water absorption and heat resistance of the boards were carried out. Moreover, the most important mechanical property, bending strength or modulus of rupture (MOR) of the prepared boards was conducted in this study. All tests were conducted at the department of Research and Innovation at Yankin Township, Yangon. Sample preparation and testing procedure were carried out according to ASTM (American Society for Testing and Material) standard [1]. These parameters were compared.

3. Results and Discussion

Some physicochemical properties of "Kyun" and "In" sawdust are described in table 2.

Table 2 .Some Physicochemical Properties of Samples

No	Parameters	Kyun sawdust	In sawdust
1	Moisture (%)	7.95	8.55
2	Hot water solubility (%)	22.15	20.45
3	1% NaOH solubility(%)	33.65	30.65
4	Cellulose (%)	30.36	28.36
5	Lignin (%)	38.95	35.95

The moisture content of the tested sample was 7.95 for "Kyun" sawdust meanwhile 8.55 for "In" sawdust. The extractive contents of hot water soluble are important in the predetermination of water soluble extractives such as tannin, starch, sugar, pectin and phenolic compounds within the woody materials. If water solubility percent of raw material exceeds 10%, it is not suitable for pulp production. However, these extractives are binding materials and they improve the adherent quality in particleboard making [7]. The results showed that the two raw materials had high solubility of hot water and 1%NaOH. The extractives soluble in this solubility are also binding materials and used to improve the adhesive quality [8].

Chemical analysis proves that the raw sawdust powder had high cellulose content. Cellulose is the natural polymer with high strength and stiffness per weight and it is the building material of long fibrous cells. High cellulose content is highly desirable properties of a fiber to be used as reinforcement in polymer composite [10]. From this investigation, it was found that cellulose content of "Kyun" sample was

30.36% but 28.36% for “In”. Hence, it is a major interesting fact for particleboard making.

A chemical property of lignin content in raw material needs to be found out for its pulping characteristics. Lignin is the natural glue between fibers in wood and annual plants. High amount of lignin content in raw material causes difficulty in pulping bleaching because lignin possesses resin-like property. Therefore high amount of lignin content can cause the adherent quality in particleboard making [9].

Table 3. Physicomechanical Properties of Prepared Boards

Modulus of Rupture =MOR

No	Physico-chemical properties	PB-1	PB-2	PB-3	PB-4	Ref
1	Moisture (%)	6.16	5.98	6.89	6.19	5-8
2	Density (gcm^{-3})	1.13	1.85	0.82	0.87	0.4 - 0.9
3	Water absorption (%)	23.9	14.34	27.90	18.34	20 - 75
4	Swelling thickness (%)	15.8	7.50	17.55	9.5	5 - 15
5	Thickness (cm)	0.70	0.69	0.67	0.69	0.5 0.9
6	Hardness	98	99.00	97.00	99.0	-
7	Impact energy (kJm^{-2})	61.7	49.85	25.55	21.9	
8	MOR (psi)	4431	4599	3675	3705	-
9	MOR (kgcm^{-2})	311.9	223.4	258.4	260.5	100 - 500
10	Tensile strength (lb)	7	7	5.8	6.2	

Ref = Reference Reported

As shown in table 3, it was observed that the moisture contents, density, and water absorption of all of the tested boards were different from each other but they were in the range of the reported reference limit (Anon, 1996).

In technical terms, the modulus of rupture (MOR) is computed as maximum fibre stress in the extreme upper and lower surface fibres of the specimen under test. In simple terms, this value is regarded as the breaking strength of the product under test. It was found that the MORs of each tested board was different significantly from each other. The MOR of standard particleboard is in the range of 100 to 500 kg cm^{-2} . Therefore tested particleboards were in the range of MOR standard.

According to the overall results of physicomechanical properties of the prepared boards, the quality of prepared PB-1 and PB-2 (made from “Kyun” sawdust) are better than that of PB-3 and PB-4 (made from “In” sawdust). Among them PB-2 is better quality than PB-1 as well as PB-4 is better than PB-3. The tested physicomechanical properties of the prepared PB-2 and PB-4 are compared in table 4. Moreover, PB-2 and PB-4 were selected for heat resistant testing. The weight loss percentages due to these testing were calculated based upon the weight of particleboards, 100g before each testing. The results are described in table 5.

Table 4. Comparison of Tested Physicomechanical Properties of PB- 2 and PB-4

No	Physicomechanical properties	PB- 2	PB-4
1	Moisture(%)	5.98	6.19
2	Density (gcm^{-3})	1.85	0.87
3	Water absorption (%)	14.34	18.34
4	Swelling thickness (%)	7.50	9.50
5	Thickness (cm)	0.69	0.69
6	Hardness	99.00	99
7	Impact energy (kJm^{-2})	49.85	21.9
8	MOR (kgcm^{-2})	223.4	260.5
9	Tensile strength (lb)	7	6.2

**Table 5. Heat Resistant Testing of Selected Boards,
PB- 2 and PB-4**

Temperature	Weight loss % PB- 2	Weight loss % PB-4	Colour observation
100°C	4.55 %	5.55%	No change (brown)
150°C	3.45%	5.25%	No change (brown)
200°C	3.02%	4.55%	Pale black
250°C	5.05%	5.80%	Dark black
300°C	7.96%	7.95%	Deep dark black

As described in the above table, it could be seen that there was no change in colour of all of tested particleboards until they were heated at 150°C. Over 150°C, however, the colour of all tested were deeper as the temperature increased. Therefore the heat resistance of prepared particleboards remained until 150 °C.

4. Conclusion

From the experimental results, the raw material, “Kyun” and “In” sawmill residues are good sources of cellulose and lignin. The high content of cellulose and lignin ensure better mechanical strength. The physical properties such as moisture content, density, water sorption and swelling thickness of the prepared particleboards lie in the limit of reference report. In addition, it can be known that one of mechanical properties, modulus of ruptures (MORs) of the prepared boards is significantly different from each other. However, they are conformable with the standard. It can be said that the quality of PB-2 is better than that of PB-1 as well as PB-4 is better than PB-3 according to the results of all tested physicomechanical properties. Moreover, it was observed that PB-2 and PB-4 can resist the temperature until 150° C without colour change. The present study indicates that the more content of the binding resin for any tested raw material, the better quality of the particleboard. Based on the results obtained from the present study, further investigations on different ratios of adhesives to the raw material, preparation of multilayers particleboards and testing on further mechanical properties of prepared particleboards should be carried out.

5. Acknowledgement

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Preparation and Characterization of Cellulose from *Saccharum officinarum* L. Sugarcane Bagasse

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Abstract

Waste recycling has been the main importance of various scientific researches due to environmental management. Sugarcane bagasse fiber residues have been extensively investigated and employed as a source of reinforcement of polymer. For this research, sugarcane bagasse was collected from Mya-kan-thar village, Madayar Township, Mandalay Region, Myanmar. Cellulose pulp was prepared from sugarcane bagasse by using concentrations of nitric acid (3 %, 5%, 7%, 9% HNO₃) and concentrations of sodium hydroxide (1.0 M, 1.5 M, 2.0 M, 2.5 M NaOH) with reaction times (1 hr, 2 hr, 3 hr, 4 hr) respectively. In order to know the effect on the yields of cellulose pulp, various reflexing periods, acid concentration and base concentration were used. Among them, the cellulose pulp from sugarcane bagasse with (5% HNO₃, 2 M NaOH), and the reaction time 4 hr was found to be optimum condition. Morphological structure of cellulose pulp (5% HNO₃, 2 M NaOH, 4 hr) was determined by Scanning Electron Microscope (SEM). Sugarcane bagasse cellulose pulp obtained was confirmed by Fourier Transformed Infrared Spectroscopy (FT-IR). Crystallinity of the resulting cellulose pulp was also determined by X-ray Diffraction (XRD) method.

Keywords- Sugarcane Bagasse, Cellulose, Scanning Electron Microscope (SEM), Fourier Transformed Infrared Spectroscopy (FT-IR) and X-ray Diffraction (XRD).

1. Introduction

As a renewable material, sugarcane-bagasse fiber waste has a huge potential as raw material for production of the cellulose. The photosynthesis process which converts CO₂ to organic compound is the most important step in the growth of biomass. Cellulose, carbohydrate and fatty oil are the main three components in biomass produced by photosynthesis process, so the plantation cellulose is one of the renewable chemical performed in CO₂ photosynthesis conversion. The cellulose in plantation fibre generally is the most dominant organic components in most biomass. Renewable agricultural sources such as

pineapple leaf, sisal, jute, piassava, coir, and sugarcane bagasse are among agro waste, normally known as biomass [5].

Sugarcane bagasse is fibrous material obtained as a residue from the sugarcane after crushing to extract the juice. Its stalk is composed of two components viz, outer rind and inner pith. The rind consists of strong fibrous structure protecting the inner soft spongy structured material (pith). It contain long finer fibers arranged randomly throughout the stem bound together by lignin and hemicellulose, while the inner component contains small fibers with major part being sucrose. Chemically, sugarcane bagasse is composed of cellulose hemicellulose and lignin. The content of these constituents may vary depending on the growth region and condition [4].

Sugarcane bagasse consists of cellulose (41-55 wt %), hemicellulose (20-27.5 wt %), lignin (18-26.3 wt %) and other (~ 7 wt %) attributed to inorganic materials. Sugarcane bagasse can be employed for other applications [3].

The solid waste bagasse from sugar production may be counted as a potential raw material for cellulose production, which can be converted further to some end product, cellulose acetate, carboxyl methyl cellulose, viscose cellulose and other cellulose derivatives.

The aim of this research is to study the optimum conditions of cellulose from sugarcane bagasse with various concentration of acid, alkali and reaction time. Characterization techniques use for this research included SEM, FT-IR and XRD methods.

2. Materials and Methods

2.1. Materials

In this research, sugarcane bagasse was collected from Mya-kan-thar village, Madayar Township, Mandalay Region. The three steps of sugarcane bagasse (SCB) delignification are a combination of acid process, alkaline process and oxidation process. Sugarcane bagasse was used as a raw material in variation of nitric acid and sodium hydroxide concentration were used to delignification process. Hydrogen peroxide was used as an impurity removal of sugarcane bagasse cellulose.



Figure 1. Sugarcane Bagasse Powder

2.2. Preparation of Cellulose Pulp from Sugarcane Bagasse

Sugarcane bagasse was cleaned and dried in shaded area. The dried sugarcane bagasse was cut into small pieces and grinded with grinder. The sugarcane bagasse powder was sieved by using 40×10^3 mm mesh size sieves.

Cellulose pulp was prepared employing four different concentrations of nitric acid (3 %, 5 %, 7 %, 9 %). 10 g of sifted raw sugarcane bagasse powder was mixed with 150 mL of 3 % HNO_3 (3 mL HNO_3 and 97 mL H_2O), heated on oven and stirring with magnetic stirrer at 80°C for two hours and then filtered and wash until neutral. The residue was mixed with 100 mL of 2 M NaOH and stirred with magnetic stirrer at 80°C for two hours and then filtered and wash until neutral. 100 mL of 10 % H_2O_2 was added to the resulting residues and stirred with magnetic stirrer at 80°C for two hours. Then it was filtered and washed with distilled water until neutral. Then, cellulose pulp was oven dried and weighed. Similarly, cellulose pulp was also prepared by different concentrations of nitric acid, sodium hydroxide and reaction times.



Mixed with H_2O_2 ,
and Stirring with Magnetic
Stirrer at 80°C Filter Cellulose
Pulp

Figure 2. Cellulose Pulp Processing

2.3. Scanning Electron Microscope (SEM) Analysis

2.3.1. Analysis of Cellulose from Sugarcane Bagasse.

Scanning electron microscopy was used to study the morphological structures of cellulose by detecting the presence of connected microporosity.

The optimum conditions, such as cellulose (5 % HNO_3), cellulose (2M NaOH), cellulose (4 hr) reaction time were qualitatively analysed by SEM method. The morphological structure of cellulose was measured at University Research Center (URC), Yangon.

2.4. FT-IR Analysis

The Fourier Transform Infrared (FT-IR) Spectroscopy was used to identify the functional group of the prepared active compounds based on the peak value in the region of infrared radiation. The FT-IR spectra of the cellulose from 5 % HNO_3 , cellulose from 2 M NaOH, cellulose from 4 hr reaction time were recorded by FT-IR spectrophotometer (Perkin Elmer, UK, L 1600400) in the wave number range 400-4000 cm^{-1} [7].

2.5. Crystallization Analyzed by XRD

The crystallization of cellulose from 5 % HNO_3 , 2 M NaOH, 4 hr, reaction time were examined by XRD measurement. Performed on a multiplex 2kW (Rigaku, Japan) using Cu/K-alpha ($\lambda = 1.54058 \text{ \AA}$) at 40 kV and 50 mA. XRD analysis was measured at University Research Center (URC), Yangon.

3. Results and Discussion

3.1. Effect of Acid Concentration on Yield of Cellulose Pulp from Sugarcane Bagasse

Four different concentrations of acid were used for the experiment, the results are shown in the following Table 1.

Table 1. Effect of Acid Concentration on Yield of Cellulose Pulp from Sugarcane Bagasse

experiment	sugarcane bagasse (g)	concentration of HNO ₃ (%)	yield (g)	yield (%)
1	10	3 %	0.60 g	6.0 %
2	10	5 %	1.04 g	10.4 %
3	10	7 %	1.27 g	12.7 %
4	10	9%	1.12 g	11.2 %

According to the results, when 3% concentration of HNO₃ was used, the resulting pulp was rigid and coloured. When 7 % and 9 % concentration of HNO₃ were used yield of pulp was higher but the pulp was found to be faint colour 5% concentration of HNO₃ was the most suitable for yields and quality but for consumption of acid. Dried sugarcane bagasse cellulose powders are shown in Figure. 3.

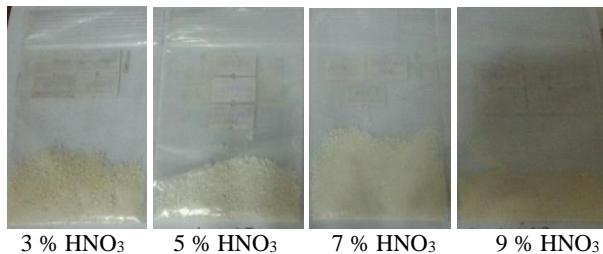


Figure 3. Dried Sugarcane Bagasse Cellulose Powder

3.2. Effect of Alkali Concentration on Yield of Sugarcane Bagasse Pulp

Four different concentrations of alkali were used for the experiment, the results are shown in the following Table 2.

Table 2. Effect of Alkali Concentration on Yield of Sugarcane Bagasse Pulp

experiment	sugarcane bagasse (g)	concentration of NaOH (%)	yield (g)	yield (%)
1	10	1.0 M	1.56 g	15.6 %
2	10	1.5 M	3.10 g	21.0 %
3	10	2.0 M	1.96 g	19.6 %
4	10	2.5 M	1.43 g	14.3 %

According to results, when 1.0 M NaOH was used, the pulp obtained was very hard and coloured. When 1.5 M NaOH was used the resulting pulp obtained high yield but it was faint colour. When 2.5 M NaOH was

used the pulp obtained low yield and faint colour. 2.0 M concentration of alkali was the best condition because it was soft and bright colour. Dried sugarcane bagasse cellulose powders are shown in Figure 4.



Figure 4. Dried Sugarcane Bagasse Cellulose Powder

3.3. Effect of Reaction Time on Yield of Sugarcane Bagasse Pulp

Four different reaction times were used for the experiment, the results are shown in the following Table 3.

Table 3. Effect of Reaction Time on Yield of Sugarcane Bagasse Pulp

Experiment	Sugarcane bagasse (g)	Reaction time (s)	Yield (g)	Yield (%)
1	10	1 hr	2.82 g	28.2
2	10	2 hr	2.01 g	20.1
3	10	3 hr	2.07 g	20.7
4	10	4 hr	1.97 g	19.7

According to results, reaction time 1 hr was used, the pulp obtained was very hard and coloured. When reaction time 2 hr and 3 hr were used the pulp was soft but faint coloured. The reaction time 4 hr was found to be efficient because the pulp obtained was soft and bright colour. Dried sugarcane bagasse cellulose powder are shown in Figure 5.



Figure 5. Dried Sugarcane Bagasse Cellulose Powder

3.4. Investigation of Sugarcane Bagasse Cellulose Pulp (5 % HNO₃, 2MNaOH, 4 hr) by FT-IR

The FT-IR spectrum of sugarcane bagasse cellulose powder obtained using optimum acid concentration (5 % HNO₃), alkali concentration (2MNaOH), optimum reaction time (4 hr) were measured at Delta Science.

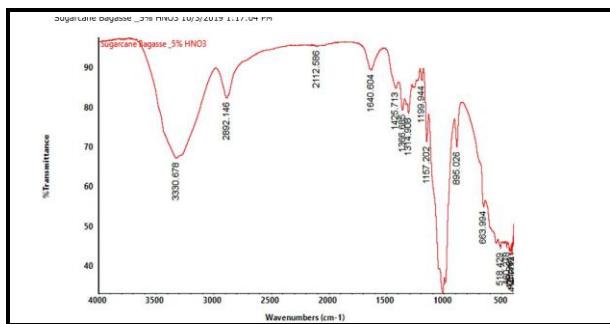


Figure 6. FT-IR Spectrum of Sugarcane Bagasse Cellulose Obtained by Using Optimum Acid Concentration, 5 % HNO₃

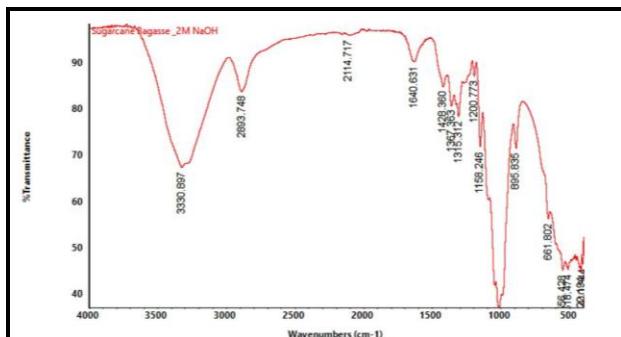


Figure 7. FT-IR spectrum of Sugarcane Bagasse Cellulose Obtained by Using Optimum Alkali Concentration, 2 M NaOH

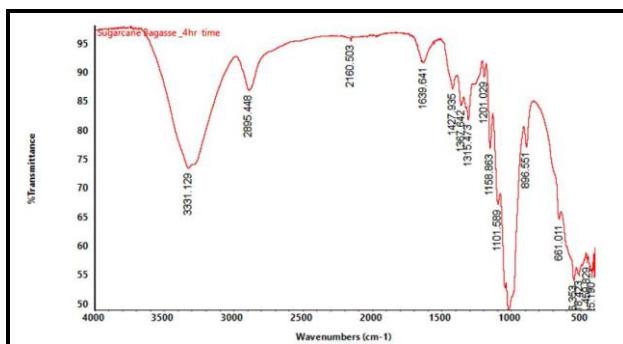


Figure 8. FT-IR Spectrum of Sugarcane Bagasse Cellulose Obtained by Using Optimum Reaction Time, 4 hr

According to FT-IR spectra the absorption bands at 3330.678 cm⁻¹, 3330.897 cm⁻¹ and 3331.129 cm⁻¹ were assigned to the O–H stretching vibration. The spectra show the bands at 2892.146 cm⁻¹, 2893.748 cm⁻¹ and 2895.448 cm⁻¹ were assigned to C–H stretching

vibration of sp² hydrocarbon, which is also characteristic of cellulosic materials.

The peak of 1000 cm⁻¹ indicates the C – O – C pyranose ring stretching vibration. The band at 895.635 cm⁻¹ was assigned to associate with β-glycosidic linkage between glucose. [7]

3.5. Scanning Electron Microscopy of Sugarcane Bagasse Cellulose Pulp

The morphological structure of fibres in sugarcane bagasse cellulosic pulp was determined using Scanning Electron Microscope (SEM).

The SEM image of the cellulose pulp obtained using optimum conditions 5 % HNO₃, 2 M NaOH, 4 hr reaction time are shown in Figure 9.

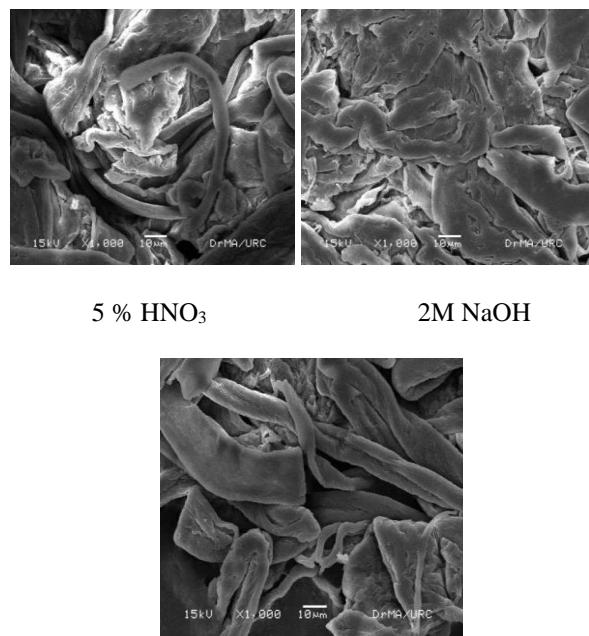


Figure 9. SEM Images of Cellulose from 5 % HNO₃, 2M NaOH, 4 hr Reaction Time

According to the SEM analysis, the flakes of fibres were found in cellulose pulps.

3.6. X-ray Diffraction Pattern Analysis of Sugarcane Bagasse Cellulose Pulp (5 % HNO₃, 2MNaOH, 4hr)

The XRD analysis of the cellulose pulp obtained by using optimum conditions (5 % HNO₃, 2MNaOH, 4 hr reaction time) are shown in Figures (10, 11 and 12).

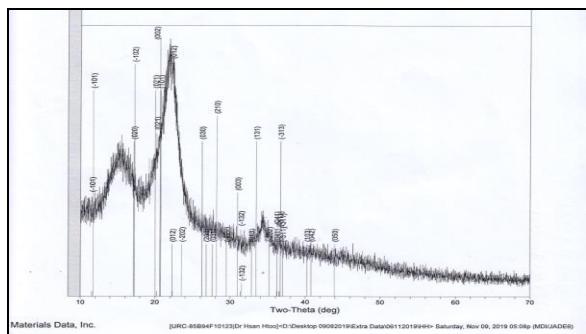


Figure 10. XRD Diffractogram of Sugarcane Bagasse Cellulose Obtained by Using Optimum Acid Concentration, 5 % HNO₃

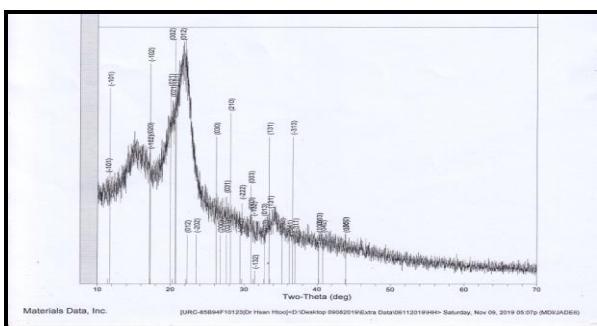


Figure 11. XRD Diffractogram of Sugarcane Bagasse Cellulose Obtained by Using Optimum Alkali Concentration, 2 M NaOH

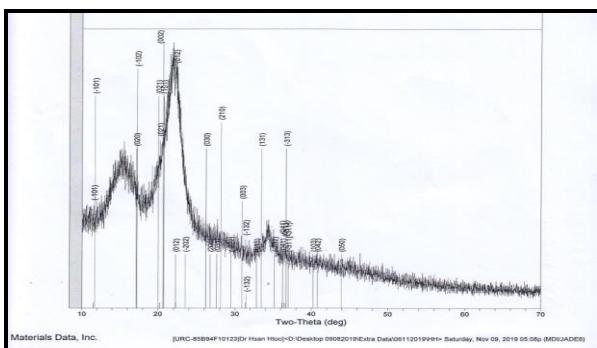


Figure 12. XRD Diffractogram of Sugarcane Bagasse Cellulose Obtained by Using Optimum Reaction Time, 4 hr

According to XRD results, it can be seen that the peak of cellulose are observed at about 22.11° , 22.31° , 22.30° with sharp peak. These peaks are called intensity of crystalline. The crystal lattice system of cellulose using optimum conditions of acid, alkali and reaction time was found to be orthorhombic.

4. Conclusion

In this research, an investigation has been made of the effects of HNO_3 , NaOH and H_2O_2 in three steps delignification process of sugarcane bagasse (SCB) on percent cellulose quality. The delignification process consisted of three steps, using various concentration of

HNO_3 , NaOH , and various reaction time respectively. The result showed that the optimal process condition of the sugarcane bagasse fiber conversion to the percent cellulose content of 19.7 were found to be at 5 % HNO_3 , 2M NaOH and 4 hr reaction time. From FT-IR result, the absorption band at 3331.12 cm^{-1} assigned to the O–H stretching vibration. The peak at 1000 cm^{-1} indicates the C–O–C pyranose ring stretching vibration. The band at 895.63 cm^{-1} implies associated with β -glycosidic linkage between glucose. The spectrum showed at 2893.74 cm^{-1} was assigned to C–H stretching vibration of sp^2 hydrocarbon, which is also characteristic of cellulosic materials. According to SEM analysis, the flakes of fibres were found in cellulose pulps. According to XRD result, crystal lattice system of cellulose using optimum condition of acid, alkali and reaction time was found to be orthorhombic. Cellulose from sugarcane bagasse may be converted to some end product, such as cellulose acetate, carboxyl methyl cellulose and other cellulose chemical derivatives form of useful products. *The use of sugarcane bagasse, sugarcane bagasse ash and its cellulose as reinforcing fillers for polymers should be extended by combining with advanced technology.*

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Determination of Caffeine, Vitamin C and Sugar Contents in Energy Drinks

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Abstract

Globally, energy drinks are typically attractive to young people. Approximately 50 percent of consumers are between the ages of 12 and 30 years. Although energy drinks are popular with 30 to 50 percent of young adults and teens consuming them. There can be the disadvantages to drinking these beverages. This is especially true in the case of children and teenagers, because they cannot safely consume as much caffeine as adults. Therefore, in this research work, the energy drinks were selected for chemical analysis. The five energy drink samples were purchased from Local Market, Mahar Aung Myay Township, Mandalay. Caffeine from energy drinks was extracted by using dichloromethane. The extracted caffeine was confirmed by measuring the melting point with the melting point determination apparatus. The functional group identification was also performed by Fourier Transform Infrared spectrum. The contents of vitamin C and sugar in energy drink samples were also determined by titrimetric method.

Keywords : dichloromethane, caffeine, vitamin C, energy drink, titrimetric.

1. Introduction

Energy drinks are beverages that have a stimulant, usually caffeine. They may have ingredients like sugar, artificial sweeteners, vitamins, minerals, amino acids and herbs. Natural caffeinated beverages including coffee, cocoa, tea and cola drinks are not regarded as energy drinks.^[1]

The main constituent of energy drinks is caffeine. Energy drinks have the effects of caffeine and sugar provide, but there is little or no evidence.^[2] Most of the effects of energy drinks on cognitive performance, such as increased attention and reaction speed, are primarily due to the presence of caffeine.^[3] Energy drinks have been associated with health risks, such as an excessive or repeated consumption can lead to cardiac and psychiatric conditions.^[4]

The sugar in non-diet energy drinks is food energy that can be utilized by the human body.^[5] Consuming a lot of added sugars increases risk for obesity, because added sugars provide extra calories.^[6]

Most energy drinks contain large amounts of caffeine, which can provide a temporary energy boost.

Energy dinks that contain sugar may contribute to weight gain and too much caffeine or caffeine-like substances can lead to nervousness, irritability, insomnia, rapid heartbeat and increased blood pressure.^[7]

In this research work, some commercial energy drinks were selected for the determination of contents of caffeine, vitamin C and sugar.

The aim of this research work is to give the knowledge whether young people should have the energy drinks or not. The objectives are to collect the energy drink samples from Local Market, Mahar Aung Myay Township, Mandalay, to extract the caffeine from energy drinks, to identify the functional groups of extracted caffeine, to check the melting point of the extracted caffeine, to determine the vitamin C content and to measure the sugar content in energy drinks sample.

2. Materials and Method

2.1. Sample Collection

The energy drink samples were purchased from Local Market, Mahar Aung Myay Township, Mandalay.



Figure 1. Energy Drink Samples

2.2. Determination of Caffeine Contents from Energy Drinks^[8]

100 mL of energy drink sample was placed in a conical flask. About 2 g of sodium carbonate was added into the sample and swirled to dissolve. The mixture was transferred to a separating funnel. The 20 mL of dichloromethane was added to the mixture in the separating funnel and shaken gently. The separating funnel was allowed to stand until two layers separate. Once the organic layer was separated into the 50 mL beaker.

About 1 g of anhydrous sodium sulphate was added to the dichloromethane solution to absorb any residual

water in the solution. The dried extract was transferred into the 50 mL boiling flask and evaporated to dryness. The flask was cooled and the caffeine content was calculated.



Figure 2. Extraction of Caffeine from Energy Drinks by Dichloromethane



Figure 3. Extracted Caffeine Crystals

2.3. Measuring the Melting Point of Extracted Caffeine^[8]

The melting point of the extracted caffeine was confirmed by measuring with the melting point determination apparatus (SMP-30).

2.4. Identification of the Functional Groups in the Extracted Caffeine^[9]

The functional groups in the extracted caffeine were identified by FTIR spectrum.

2.5. Determination of Vitamin C Contents in Energy Drinks^[10]

10 mL of 0.0057 M standard ascorbic acid solution was pipetted into a 125 mL conical flask. 10 drops of 1% starch solution were added and then titrated against iodine solution until blue-black color was observed. From the experimental data, the concentration of iodine solution was calculated.



Figure 4. Titrimetric Result of 10 mL of 0.0057 M Standard Ascorbic Acid with Iodine Solution

10 mL of energy drink sample was pipetted into a 125 mL conical flask. 10 drops of 1% starch solution were added and then titrated against iodine solution until blue-black color was observed. From the experimental data, the vitamin C content was calculated.



Figure 5. Titrimetric Result of 10 mL of Energy Drink Sample with 0.0051 M of Iodine Solution

2.6.Determination of Sugar Contents in Energy Drinks^[11]

10 mL of 0.075 M glucose solution was placed in the conical flask. 20 ml of 0.0465 M iodine solution and 45 ml of 0.1 M sodium hydroxide solution were added into the conical flask and closed the flask and left the flask in the dark place for 15 minutes. 6 mL of 1 M hydrochloric acid solution was added to mixture in the flask. The mixture solution was titrated with 0.05 M sodium thiosulphate solution in the burette. When the liquid become straw colour, 1 mL of starch indicator solution was added. The solution become dark again and 0.05 M sodium thiosulphate solution was added until the colourless solution, end point was obtained. From the experimental data, the concentration of iodine solution was calculated.



Figure 6. Titrimetric Result of 10 mL of 0.075 M Standard Glucose Solution with Iodine Solution

10 mL of energy drink sample was mixed with 90 mL of distilled water to obtain the 100 mL of energy drink sample solution.

10 mL of energy drink sample solution was placed in the conical flask. 20 mL of 0.0465 M iodine solution and 45 mL of 0.1 M sodium hydroxide solution were added into the conical flask and closed the flask and left the flask in the dark place for 15 minutes. 6 mL of 1 M hydrochloric acid solution was added to mixture in the flask. The mixture solution was titrated with 0.05 M sodium thiosulphate solution in the burette. When the liquid become straw colour, 1 mL of starch indicator solution was added. The solution become dark again and 0.05 M sodium thiosulphate solution was added until the colourless solution, end point was obtained. From the experimental data, the sugar content was calculated.



Figure 7. Titrimetric Result of 10 ml of Energy Drink Sample Solution with 0.0465 M of Iodine Solution

3. Results and Discussion

Caffeine was extracted from energy drink samples by using dichloromethane. The results were shown in Table (1).

Table 1. The Contents of Caffeine Extracted from Energy Drink Samples

No.	Sample	Caffeine Content (mg/mL)
1.	Sample-1	0.1921
2.	Sample-2	0.1902
3.	Sample-3	0.1838
4.	Sample-4	0.2312
5.	Sample-5	0.1771

From the Table (1,) it was found that the Energy Drink sample-4 contains the highest caffeine amount. It was known that the caffeine contents in all samples were suitable quantities if one drinks one can a day.

The melting point of the extracted caffeine was confirmed by measuring with the melting point determination apparatus. The result was shown in Table (2).

Table 2. The Melting Point of Extracted Caffeine

Sample	Melting Point (°C)
Extracted Caffeine	237-239

From the results, it could be seen that the extracted caffeine was agreed with the melting point, 238 °C, the reference caffeine.

The functional groups in the extracted caffeine were identified by FTIR spectrum. The results were shown in Figure (8) and Table (3).

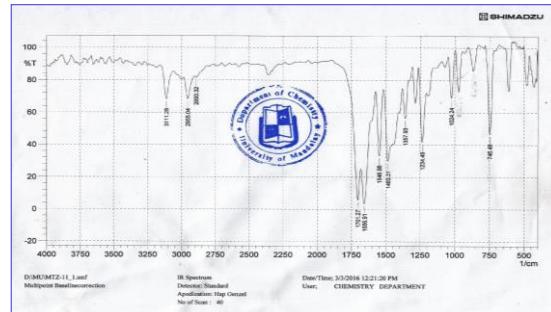


Figure 8. FTIR Spectrum of Extracted Caffeine

Table 3. Characteristic Absorption of FTIR and their Assignments of Extracted Caffeine from Energy Drink Samples

No.	$\lambda_{\text{max}} (\text{cm}^{-1})$	Assignments (Functional group)
1.	3114	-CH stretching vibration of sp^2 hydrocarbon
2.	2956, 2878	-CH stretching vibration of sp^3 hydrocarbon
3.	1706, 1662	C=O stretching vibration of carbonyl group
4.	1550	C=N stretching vibration C=C stretching vibration
5.	1485, 1238, 1025	C-N stretching vibration
6.	1358	C-H bending vibration of methyl group
7.	744	C-H Out of plane bending vibration

According to the results of FTIR spectrum, the functional groups in the extracted caffeine were found to be C=O, C=N, C=C, C-N group.

The vitamin C contents in Energy Drink samples were determined by titration method. The results were shown in Table (4).

Table 4. Vitamin C Contents of Energy Drink Samples

No.	Sample	Vitamin C Content (mg/mL)
1.	Sample-1	0.359
2.	Sample-2	0.448
3.	Sample-3	0.539
4.	Sample-4	0.718
5.	Sample-5	0.898

According to this Table (4), it can be seen that the vitamin C content in sample-5 is the highest. It was considered that the vitamin C contents in all samples were fair quantities.

The sugar contents in Energy Drink samples were determined by titration method. The results were shown in Table (5).

Table 5. Sugar Contents of Energy Drink Samples

No.	Sample	Sugar Content (g/mL)
1.	Sample-1	0.1058
2.	Sample-2	0.1355
3.	Sample-3	0.1499
4.	Sample-4	0.1161
5.	Sample-5	0.1350

According to this Table (5), it was known that the sample-3 has the highest glucose content. It was found that the glucose contents in all samples were fair quantities. This result shows that one should not drink more than one can a day.

4. Conclusion

In this research work, the caffeine was extracted from energy drink samples by using dichloromethane. The caffeine contents of the energy drink samples were 0.1921, 0.1902, 0.1838, 0.2312 and 0.1771 mg/mL. In 2015, the European Food Safety Authority published their Scientific Opinion on Safety of Caffeine. They concluded that it is safe for an adult to consume up to 200 mg of caffeine in a single serving and up to 400 mg of caffeine per day on a regular basis. Therefore, the amounts of caffeine in the samples were suitable if one drinks one can a day. The vitamin C contents in energy drink samples were determined by redox titration. The vitamin C contents of the energy drink samples were 0.359, 0.448, 0.539, 0.718 and 0.898 mg/mL. For adults, the recommended daily amount for vitamin C is 65 to 90 milligrams (mg) a day and the upper limit is 2,000 mg a day. Thus, the vitamin C contents in all samples were good quantities. Furthermore, the sugar contents of the energy drink samples were also determined by redox titration. The sugar contents of energy drink samples were 0.1058, 0.1355, 0.1499, 0.1161 and 0.1350 g/mL. It was found that the sugar contents in all samples were fair quantities. This result shows that one should not drink more than one can a day. Therefore, a tin of energy drink can stimulate the drinker but drinker should not more than one can a day.

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Optimization of Extraction Condition of Yellow dye from Turmeric and Apply on Cotton Fabric

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Abstract

This research work on natural dyes was focused on determining optimal process parameters dye extraction from targeted plant species Curcuma longa L. Firstly, dye solutions were extracted under various conditions such as solvent ratio, pH, and extraction time. The dye percent's were found to be 30 %, 28% and 25% in extraction solvent watery, hydroethanol (1:1) and ethanol. The optical density of dye solutions were determined by UV spectrophotometer. The yellow dye was extracted with solid/liquid ratio (1:10). These dye solutions were applied on the textile dyeing process with pre-mordanting method. Three types of mordant, K₂Cr₂O₇, CuSO₄ and alum were used. The reaction between natural dye, mordant and textile is unique and optimum combinations have to be determined in each case. The physical properties of dyeing fabrics were determined. Turmeric is suitable for used as the precursor material for good quality and high yield yellow dye extraction.

Keywords- yellow dye, turmeric, cotton fabric, mordant, pre-mordanting

1. Introduction

Dyes can be defined as organic chemical compounds which have property of producing the phenomenon of colour by light absorption. These are intensely coloured materials that retained in substances by physical absorption, mechanical retention and by formation of covalent chemical bonds or complexes with salts of metals [1]. There are two types of dye, synthetic dye and natural dye. Synthetic dyes obtained from chemical substance or derived through chemical process and natural dyes obtained from natural source [2]. All the dyes came from the natural sources like plants, animal and minerals. Natural dyes are mostly nonsubstantive and must be applied on textiles by using mordant which are metallic salts, having an affinity for both the colouring matter and the fibre.

Transition metal ions usually have strong coordinating bonding and capable of forming weak to medium interaction forces and thus can act as bridging material to create substantively of natural dyes when a textile material being impregnated with such metallic salt (i.e. mordant) is subjected to dyeing with different

natural dyes, usually having some mordantable groups facilitating fixation of such dye. These metallic mordants after combining with dye in the fibre, it forms an insoluble precipitate or lake and thus both the dye and mordant get fixed to become wash fast to a reasonable level [3]. Traditionally, plants were used for coloring silk, wool and cotton fibres but gradually were replaced by cheaper synthetic dyes. However, there has been a growing interest in the re-introduction of natural dyes and dye-yielding plants for textile application [2].

Natural dyes derived from plants have recently gained economic advantage over synthetic dyes because of their non-toxic, non-carcinogenic and biodegradable nature [4, 5]. The reasons for this new scientific interest in natural dyes are based on the growing awareness to find sustainable and non-toxic alternatives to synthetic dyes. In this research paper, it reports the results on characterization of the colour produced from selected dye-yielding plants for textile and food colouration [6].

Dye-yielding plants, unlike synthetic dyes, may contain more than one chemical constituent, each exhibiting a different colour and properties, operating singly or in combination with the different groups, depending on their chemical structure and composition. Natural dyes are considered eco-friendly as these are renewable and biodegradable; are skin friendly and may also provide health benefits to the wearer. Natural dye can be used for dyeing almost all types of natural fibers. Natural dyes are also used in the coloration of food, medicine, handicraft items and toys, and in leather processing, and many of the dye-yielding plants are used as medicines in various traditional medicinal systems [7].

In this research work the natural yellow dyes were extracted from rhizome of turmeric and applied on textile dyeing process.

2. Material and Methods

The chemicals used were procured from British Drug House (BDH), England and Wako Chemical Co. Inc. Tokyo, Japan. Analar grade reagents and solvents were used thought the experiment. In all the investigations, the recommended standard methods and techniques involved both conventional and modern methods. The apparatus consists of conventional lab ware, glass ware and other supporting facilities. The

instruments used in this experiment were Balance Sartorius AG Gottingen BL 2105 L, Switzerland pH meter Jenway 4330, Lab quip, England, Centrifuge KUBOTA- 5200, Japan, UV spectrometer UV-1800, Shimazu, Made in Japan and Rotary Evaporator EYELA, Japan.

2.1. Preparation of Plant Materials

In this research work, rhizome of turmeric, colorant natural resource was collected from the Kyaukse Township, Mandalay Region. The collected plant materials were properly cleaned and washed with tap water. After cleaning, these samples were placed in good ventilation place for one week. Air dried samples were cut into small pieces and then stored in brown glass bottles in dried and dark place till further used.

2.2. Determination of Phytochemical Constituents

Phytochemical tests were examined according to the standard methods as described by Harbone (1973)[8]. The different classes of potential bioactive compounds such as alkaloid, flavonoid, saponin, glycoside, tannin, phenolic, steroid, terpene, polyphenol and reducing sugar tests were determined.

2.3. Determination of Mineral Contents

The sample powder was sent to Department of Physics, University of Mandalay to determine the mineral content contains in turmeric's rhizome.

2.4. Determination of Optimal Conditions for Dye Extraction

Dye solutions were extracted from the natural sources by using three solvent systems such as EtOH only, hydroethanol (1:1 v/v) and water only. The effects of extraction time, effect of solvents and effect of pH on the color intensities were also determined. The visible color changing intensities were measured by using UV spectrophotometer.

2.5.1. Determination the Effect of Solvent. The dried sample (1 g) was put into the conical flask and added the respective solvent (10 mL). This flask was heated in the water bath at 70 °C for 60 min. The solution was filtered and the filtrate was centrifuged with 4000 rpm for 15 min.



Figure 1. Dye Solution with Different Solvent System

2.5.2. Determination the Effect of Extraction Time

The samples (1 g) was soaked with the solvent (10 mL) such as ethanol only, ethanol water (1:1) and water only in each conical flask and heated the flask at 70 °C for 10 to 70 min in water bath. After extraction, it was centrifuged. The apparent colours were taken a photo and the absorbance of filtrates were checked and recorded.

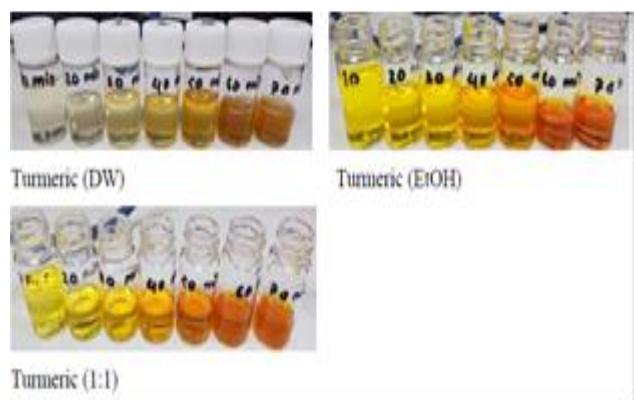
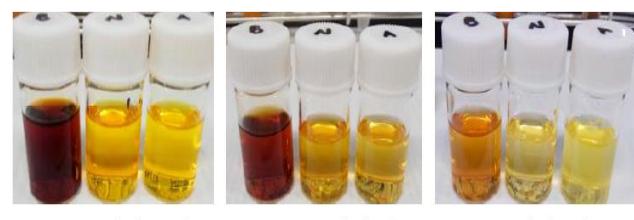


Figure 2. Yellow Dyes with Different Extraction Time

2.5.3. Determination the Effect of pH. Firstly, the pH of extraction solvents was prepared to the three different conditions pH 5, 7 and 9 using 0.1 M NaOH and 0.1 M HCl. And then the samples (1 g) was soaked with the solvent (10 mL) such as ethanol only, hydroethanol (1:1 v/v) and water only in each conical flask and heated the flask at 70 °C for one hour in water bath. After extraction, it was centrifuged. The absorbance of filtrates was measured. When different pH solvent used for extraction, it can clearly be seen wide ranges of colour.



B= alkaline, A = acidic, N= neutral

Figure 3. Yellow Dyes with Different pH Conditions

2.6. Extraction of Dye Solution

Each 10 g dried samples, turmeric was placed into the 2.5 L bottle and it was filled with 100 mL of solvents EtOH only, EtOH: H₂O (1:1) and H₂O only. The bottle was placed into a water bath and the temperature was maintained at 70°C for 60 min. After one hour, the dye solution was cooled and filtered and it was used for dyeing process.

2.7. Application on the Fabric Dyeing Process

2.7.1. Scouring of Fabrics for Dyeing Process. Scouring is the process by which oils, fats, waxes and other nitrogenous matters are removed. For dyeing process, cotton fabrics were collected. Firstly, these were washed with water. 20 mL of vinegar was added into a bowl and 2L of water was added and stirred thoroughly. The cotton fabric was cut into 11 x 11 inch. These fabrics were soaked into 0.1 % vinegar solution for 1 hour to remove unwanted material from the surface of the fabrics. These fabrics were dried in the good ventilation placed and these were used for dyeing process.

2.7.2. Preparation of Mordant Solutions. Three mordant, alum, copper II sulphate, potassium dichromate were used for dyeing process. 0.1 % mordant solutions were prepared.

2.7.3. Treatment of Fabrics before Dyeing. Only pre mordanting method was used in dyeing process. Treated fabric was dipped in 0.1% mordant solution and kept on water bath at 60°C for one hour. It is squeezed and dried.

2.7.4. Dyeing of Fabrics. The sample fabrics were immersed in hot dye solution to facilitate uniform penetration of the dye molecules. Solid/liquid is maintained 1:40 g/ml. The dyeing temperature for cotton was maintained at (90-95°C) for 1 hour. After dyeing, all dyed fabrics were washed with 5g/L non-ionic detergent and then rinsed with water and dried in air at room temperature.

2.8 Determination of Physical Properties of Dyeing Fabrics

2.8.1. Determination of Colour Fastness. Fastness of dyed or printed textile fabrics denotes the resistance which the fabrics opposes to varying or losing its shade when subjected to the action of various agent such as washing, rubbing, etc. which can give rise to loss/change of shade and to staining of the other textiles. Usually a textile fabric faces many external conditions which can affect the colour of the textiles are washing and rubbing. Therefore, the dyed fabrics may be tested for their color fastness properties as per the use of the textile fabrics viz. colorfastness to washing, colorfastness to rubbing and colorfastness to perspiration. The rating of different fastness properties was measured by grey scales. Colour fastness studies of dyed fabrics were carried out according prescribed norms of BIS. Colour fastness to crocking and perspiration was carried out following IS-766: 1988 and IS-971: 1983 respectively.

2.8.2. Washing Test. The washing test was carried out following IS 687: 1979. This method is applied to

determine the effect of washing only on the color fastness of the textiles. It is not intended to reflect the result of the comprehensive laundering procedure. The dyed textile fabrics viz., silk, wool and cotton were tested for their colour fastness to washing. The specimen of each fabric (10 cm × 4 cm) were placed between the two adjacent fabrics and sewed along four sides to form a composite specimen. The adjacent fabrics for silk, wool and cotton were silk and cotton, wool and cotton and cotton and wool respectively. One composite specimen was placed in a container and necessary amount of soap solution (5 g/L) previously heated to 40 ± 2°C to give a liquor ratio of 1:50 was added. Composite specimens were treated for 30 min at 40 ± 2°C in the mechanical washing device. Then composite specimens were removed and rinsed, twice in cold water and then in cold running tap for 10 min, then squeezed. Stitching along the two long sides and one short side were removed. Composite specimens were opened and dried in air. Treated test specimen and the two pieces of the adjacent fabrics were allowed to cool after drying and to regain their normal moisture contents before evaluation. The change in color of the treated test specimen and the degree of staining of the two pieces of the adjacent fabrics were evaluated with the help of grey scale.

2.8.3. Crocking/Rubbing Test. The rubbing test was carried out following IS 766:1988. The rubbing fastness determines the color fastness of textile materials to rubbing off and staining to other materials. The test specimen was fix to the rubbing device by means of clamps such that the long direction of the specimens the track of the device. The rubbing fastness was carried out in dry as well as wet conditions. In the dry rubbing cloth flattened in place over the end of the finger of the testing device, it was rubbed to and fro in a straight line along a track 10 cm long on a dry specimen, 10 times to and fro in 10 seconds, with a downward force 22 N or 9 N on finger. The fresh dry specimen was rubbed with a rubbing cloth that was wetted with water. After rubbing, the cloth was dried at room temperature. Assessment of test fabrics was carried out for the staining of the rubbing cotton cloths with grey scale for evaluating dry and wet staining.

3. Results and Discussion

3.1. Phytochemical Screening

The phytochemical composition of the rhizome of turmeric is presented in Table 1. It reveals the presence of several groups of secondary metabolites. All tested phytochemical constituents are present in all extracts while steroids, terpene and tannin were only absent in the waterry extract. Tannin was found only in ethanol extract. Phytochemical screening of the extracts of turmeric has revealed in various ways the presence of

bioactive compounds such as alkaloid, glycoside, flavonoid, saponin, terpene, phenolic, polyphenol and reducing sugar.

Table 1. Phytochemical Composition of Turmeric

Phytochemical Metabolites	Extracts		
	Ethanol	50% Ethanol	Aqueous
Alkaloid	+	+	+
Flavonoid	+	+	+
Saponin	+	+	+
Glycoside	+	+	+
Tannin	+	-	-
Phenolic	+	+	+
Steroid	+	+	-
Terpene	+	+	-
Polyphenol	+	+	+
Reducing Sugar	+	+	+

+ present - absent

3.2. Mineral Contents

The mineral content of turmeric powder was sent to Physics Department, Mandalay University to determine mineral content of the sample. According to the EDXRF data, the turmeric contains lower mineral contents. Among containing minerals, silica is the highest amount (5.1380%), and calcium is the second higher composition (3.2340 %) in turmeric. Toxic heavy metals are not found in this sample.

Table 2. Metal Content in Turmeric Powder

Metal	Results (%)	Metal	Results (%)
Arsenic	N.D	Silica	5.1380
Cadmium	N.D	Calcium	3.2340
Lead	N.D	Sulphur	0.0117
Mercury	N.D	Zinc	0.0010
Tin	N.D	Iron	0.0050
Copper	N.D	Manganese	0.0020
		Chromium	0.0010

N.D. = Not Detected (Detection limit ≥ 0.050 mg/kg)

3.3. Color Density with Different Conditions

3.3.1. Effect of Solvent. In the experiment three solvents such as EtOH only, hydroethanol (1:1 v/v) and water only were used. Ethanol and hydroethanol solvent give wide range of color between (350-550) nm. But ethanol solvent gives high color density at (400-450) nm.

3.3.2. Effect of Extraction Time. In the experiment, the extraction times for 10 - 70 min in water bath were done for all selected samples. The optical color intensity of the dye samples were shown in following Figure (5, 6 and 7). From these spectral data increasing the

extraction time, increasing the visible absorbance till 60 min. Beyond 60 min, the absorbance dose not appreciably increase. So the extraction time 60 min was selected for the applied dyeing process.

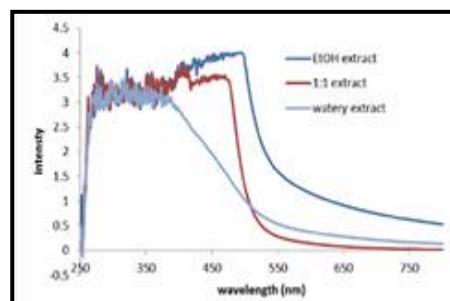


Figure 4. Effect of Solvent for Dye Extraction

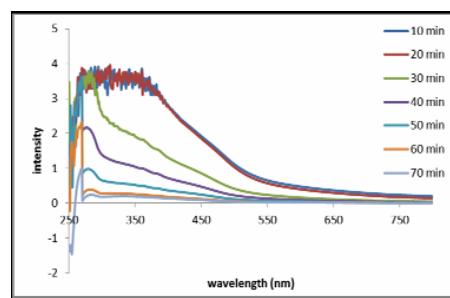


Figure 5. Effect of Extraction Time for EtOH Extract

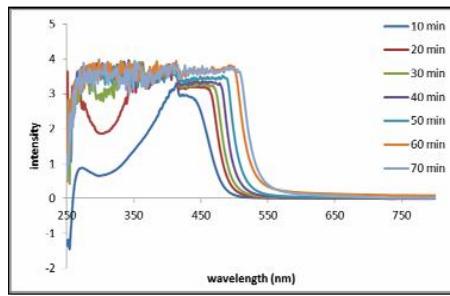


Figure 6. Effect of Extraction Time for 50% EtOH Extract

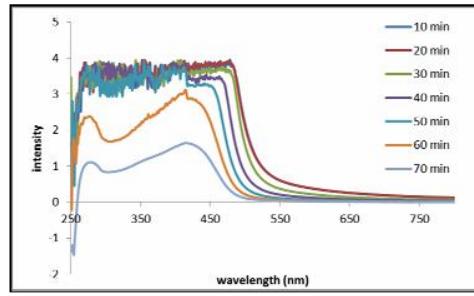


Figure 7. Effect of Extraction Time for Watery Extract

3.3.3. Effect of pH. The optical intensity of the dye samples with different pH conditions were shown in following Figure (8, 9 and 10). When EtOH was used as solvent, color intensities of all pH were found to lower. The additional peak at (550-650 nm) was observed

within in acidic condition. When 1:1 v/v (hydroethanol) was used as solvent, color intensities of all pH were found to higher. The sharp peak at (350-450 nm) was observed within in acidic condition. When H₂O was used as solvent, color intensities of acidic and alkaline condition were found to higher.

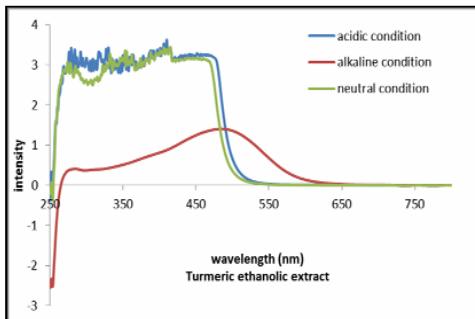


Figure 8. Effect of pH for EtOH Extract

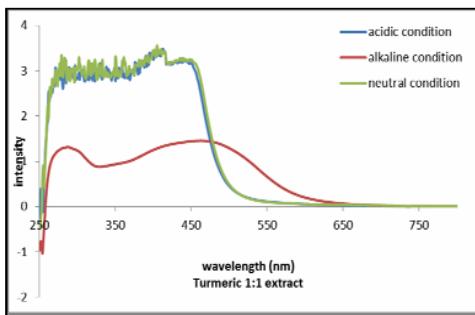


Figure 9. Effect of pH for 50% EtOH Extract

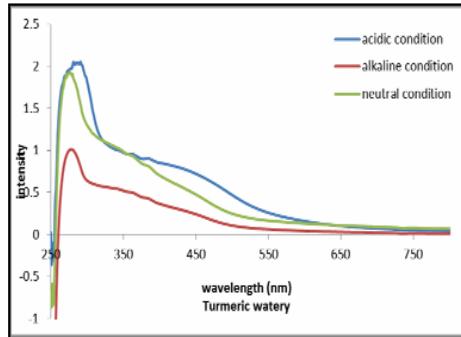


Figure 10. Effect of pH for Watery Extract

3.4. Application on the Fabric Dyeing Process

3.4.1. Dyeing of Fabrics. After dyeing process, the dyeing fabric with different solvent system, and different mordant were taken a photo and shown in figure (11, 12 and 13). The color of dyeing fabrics varies depending of the mordant and pH conditions. When K₂Cr₂O₇ was used as mordant, the fabric color was found to be a few brown. The fabric color was a few reddish yellow when CuSO₄ was used as mordant. The pure yellow color was obtained when alum was used as mordant. When the fabric was dyeing without

mordant, the color of fabric was found to be pale yellow.

Mordant	pH 5	pH 7	pH 9
K ₂ Cr ₂ O ₇			
CuSO ₄			
Alum			
without mordant			

Figure 11. EtOH Extract Dyeing Fabric Colors

Mordant	pH 5	pH 7	pH 9
K ₂ Cr ₂ O ₇			
CuSO ₄			
Alum			
without mordant			

Figure 12. 50% Ethanol Extract Dyeing Fabric Colors

Mordant	pH 5	pH 7	pH 9
K ₂ Cr ₂ O ₇			
CuSO ₄			
Alum			
without mordant			

Figure 13. Watery Extract Dyeing Fabric Colors

3.4.2. Rubbing Fastness Properties. The sample has to be tested in the delivered condition; don't wash and/or tumble it before testing the fabric/sample has to be air conditioned at least 8 hours by standard climate (20°C / 65% relative humidity). The air conditioned specimen has to be rubbed with the dry and wet cotton rubbing cloth in warp, weft or diagonal direction on face side. The change in color of the sample was compared with standard scale. The results for cotton fabric dyed with EtOH and hydroethanol extracts are good condition for both dry and wet tests and the rating is 4. When dyeing without mordent, the rating of all extract fall into 3. The result for cotton fabric dyed with watery extract is acceptable value for dry and wet test.

Table 3. Rubbing Fastness of Samples

Type of Mordent	pH range	Rubbing Fastness 100 Times					
		EtOH		(1:1)		Watery	
		Dry	Wet	Dry	Wet	Dry	Wet
K ₂ Cr ₂ O ₇	pH 5	4	4	4	4	3	3
	pH 7	4	4	4	4	3	3
	pH 9	4	4	4	4	3	3
CuSO ₄	pH 5	4	4	4	4	3	3
	pH 7	4	4	4	4	3	3
	pH 9	4	4	4	4	3	3
Alum	pH 5	4	4	4	4	3	3
	pH 7	4	4	4	4	3	3
	pH 9	4	4	4	4	3	3
without mordent	pH 5	3	3	3	3	2	2
	pH 7	3	3	3	3	2	2
	pH 9	3	3	3	3	2	2

3.4.2. Washing Fastness Properties. Color fastness to washing is the common quality parameter, which is considered very important from the point of view of consumers. This test determines the loss & change of colour in the washing process by a consumer and the possible staining of other garments or lighter portion that may be washed with it. This test is used to predict the performance of any dyed or printed textile product to the common washing process using a detergent and additives. Colour fastness to washing is assessed in three ways are 1) change in shade (loss of colour or tone as compared to the original unwashed sample), 2) Extent of staining on the Multifibre sample and 3) Self staining if any. This Grey Scale is for assessing changes in colour of fastness tests. The scale consists of nine pairs of grey colour chips each representing a visual difference and contrast. The grey scale has the 9 possible values: 5, 4-5, 4, 3-4, 3, 2-3, 2, 1-2, 1. The fastness rating goes step-wise from: Note 5 = no visual change (best rating) to Note 1 = a large visual change (worst rating). The data in Table (3) reveals that the wash fastness grade for colour change was 4-5 for ethanol extract, 4 for hydroethanol extract and 3-4 for watery extract. It mean that the the dye extract with ethanol solvent the best for color stay. The color stay of all extracts fall rating when dyeing without mordent. These result points out the colour fastness rating are depending upon the mordent using.

Table 4. Colour Fastness Grades of Dye on Cotton

Type of Mordent	pH	Washing Fastness 50°C, 45 min				
		EtOH	(1:1)	H ₂ O	Cotton	P/C
K ₂ Cr ₂ O ₇	5	4-5	4	3-4	4	4
	7	4-5	4	3-4	4	4
	9	4-5	4	3-4	4	4
CuSO ₄	5	4-5	4	3-4	4	4
	7	4-5	4	3-4	4	4
	9	4-5	4	3-4	4	4
Alum	5	4-5	4	3-4	4	4
	7	4-5	4	3-4	4	4
	9	4-5	4	3-4	4	4
without mordent	5	3	3	2	4	4
	7	3	3	2	4	4
	9	3	3	2	4	4

4. Conclusion

In this research, the natural dyes were extracted from rhizome of turmeric. According to phytochemical screening, turmeric contains all types of tested secondary metabolites except steroids and terpene. Quantitative elemental analysis by using EDXRF revealed that turmeric has little amount of mineral and does not contain toxic heavy metal. In this research work, only pre-modanting method was used. Different shades of colors are obtained by using different mordants viz. K₂Cr₂O₇, CuSO₄, and alum. The results from washing and rubbing test have shown the yellow dye extract from turmeric is a strong color and is washing resistant. Home Economics and as suitable organic replacements for the chemical colourants used in the textile industry.

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