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# Contents

# Natural Science

# Determination of Antimicrobial and Antioxidant Activities of the Fruits of Momordica charantia L. (Bitter Gourd)

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**ABSTRACT :** *Momordica charantia* L. *has long been regarded as a food and medicinal plant. In the present study, Momordica charantia* L., *Myanmar name Kyet-hinn-khar was selected for chemical analysis. The fruits of Momordica charantia* L. *were collected from ShweKyet Yet village, Amarapura Township, Mandalay Region, Myanmar. Firstly, the preliminary phytochemical test of the selected fruits was performed which gave positive for alkaloids, glycosides, flavonoids, saponins, phenolic, polyphenol, lipophilic and terpenoids. The antimicrobial activities of the crude extract in five solvent systems were determined against six tested microorganisms by Agar well diffusion method. In addition, the total phenolic content of the fruits was measured by Folin-Ciocalteau reagent using UV spectrophotometric method as 20.28 mg GAE/g. The antioxidant activity of ethanolic extract of the fruits of Momordica chrantia* L *was investigated by free radical scavenging activity (DPPH assay). The results show that the fruit extract has significant antioxidant capacity. The findings indicate the potential use of fruit extract as biopreservativesas it demonstrated high antimicrobial and antioxidant activities, respectively.*

**Keywords :** Fruit of bitter gourd, antimicrobial activity, antioxidant activity, DPPH assay

## 1. INTRODUCTION

For thousands of years, mankind has been benefitting from plants. Plant extracts were highly regarded by ancient civilizations for the treatment of various ailments [7]. Even today, plant materials remain as an important resource to combat illnesses. Many of these plants have been investigated for novel drugs for the development of new therapeutic agents [12]. Numerous studies has been carried out on various natural products by screening their antimicrobial activity [3].

*Momordica charamtia* L. commonly known as bitter gourd is an important vegetable grown in tropical and subtropical regions of Africa, India, China, Myanmar and several Caribbean countries [1]. Bitter gourd belongs to the cucumber family (Cucurbitaceae) a group comprising about 130 genera and 800 species [15]. Bitter gourd is a herbaceous plant that can grow up to 10 meters tall. The plant bears simple, alternate leaves that are 4-5 cm in width with 3-7 deeply separated lobes. The plant bears oblong fruits with a distinct waxy exterior. The interior of the fruit is somewhat hollow, containing white pith and seeds. When ripe, the fruits become yellow and split into segments that curl back to reveal the seeds. The fruits are eaten while still green and unripe. They have been used for generations by indigenous populations in Africa, India, and Latin America for food and folk medicine [10].

Bitter gourd has been the subject of intensive investigation for biologically active compounds and for its medicinal properties[13]. Fruits and seeds of *M.charantia* L. possess medicinal properties such as anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, antimicrobial and antitumor [19]. Bitter gourd contained minerals and nutrients such as calcium, iron, phosphorus, manganese, sodium, potassium, magnesium, zinc, thiamine, beta-carotene, folate and riboflavin. The immature fruits are eaten as vegetables and are good sources of vitamin A, E, and C [4].

Bitter gourd shows a significant antimicrobial activity and is of great use in medicine for treatment of many diseases such as piles, leprosy, jaundice, diabetes and snake bite [2]. Free radical play an important role in development of tissue role and pathological events in living organisms [8][11]. There are evidences that explain that increased uptake of fruits and vegetables reduce the risk of cancer [6][18].This is attributed by antioxidants presents in fruits and vegetables[17][20]. The aim of this paper was to investigate the antimicrobial and antioxidant activities of *Momordica charantia*L.

### 1.1 Botanical Description

| | | |
|---|---|---|
| Family | : | Cucurbitaceae |
| Genus | : | *Momordica* |
| Species | : | *charantia* |
| Botanical name | : | *Momordica charantia* L. |
| English name | : | Bitter Gourd or Bitter Melon |
| Myanmar name | : | Kyet-hinn-khar |
| Parts Used | : | Fruits |

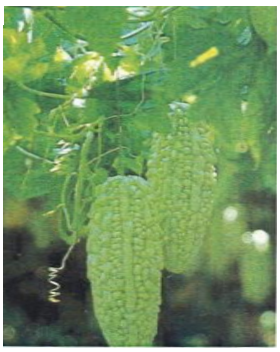

**Figure1. Fruit of *Momordica charantia* L.**

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

The fruits of the sample bitter gourd were collected from ShweKyet Yet village, Amarapura Township, Mandalay Region. The fruits were cut into small pieces and allowed to air-dry well. The dried samples were ground in a mortar. Then they were stored in a well stoppered bottle and used throughout the experiments.

### 2.2 Chemicals and Apparatus

Ethanol, ethyl acetate, n-hexane, pet-ether and chloroform were used to get crude extract for antibacterial activities. Ethanol was used to get crude extract for antioxidant activities. Some chemicals were used to examine the phytochemical constituents by general methods. Common laboratory apparatus was used for phytochemical tests.

### 2.3 Phytochemical Screening on the Fruits of Momordica charantia L.

Phytochemical screening on the fruits of *Momordica charantia* L. was performed in order to know the presence of general classes of phytochemical constituents in the plant sample according to the standard procedures [8].

### 2.4 Determination of Antimicrobial Activities on the Fruits of Momordica charantia L.

Antimicrobial activities of the crude extract of the fruits of *Momordica charantia* L. were tested in various solvent systems by using Agar-well diffusion method on six selected microorganisms at PRD (Pharmaceutical Research Department), Insein, Yangon.

### 2.5 Determination of Total Phenolic Content

Total phenolic content was determined by Folin-Ciocalteau method by using 754-UV spectrophotometer [14, 16].

### 2.6 Preparation of Standard Gallic Acid

The standard gallic acid (10 mg) was dissolved in 10 mL of distilled water and used as standard solution.

### 2.7 Determination of Total Phenolic Content of Momordica charantia L.

The total phenolic content of extract solution of *Momordica charantia* Lwas measured with the Folin-Ciocalteau reagent. Firstly, 10 μL of extract solution were taken in each test tube. Each test tube was made up to 1.6 mL with distilled water. 100μL of Folin-Ciocalteau reagent was mixed, then 300 μL of saturated $Na_2CO_3$ (20 %) was added. These mixtures were heated in a water bath at 40°C for 30 minutes and then cooled in an ice-bath. The absorbances of these prepared sample solutions were measured at 765 nm using a UV-spectrophotometer. The results are shown in table (7). The total phenolic content of the extract solution of *Momordica charantia* L was expressed as mg gallic acid equivalent (GAE)/g DW [14, 16].

### 2.8 Determination of Antioxidant Activity of Fruits of Momordica charantia L. by DPPH Radical Scavenging Assay

DPPH (1,1-Diphenyl-2-picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of plant materials. This assay has been widely used to evaluate the free radical scavenging activity [5] [21].

### 2.9 Preparation of Crude Extracts

Each powdered sample (300 g) was firstly extracted with 95 % ethanol for two months. After filtration, the filtrate was concentrated by the removal of the solvent to give the respective ethanol crude extract [5] [21].

### 2.10 Preparation of Standard Sample Solution

Each crude extract (5 mg) was separately dissolved in 95 % ethanol (10 mL) under vigorous shaking. After filtration, the filtrate was used as a stock solution. Desired concentration (20, 10, 5, 2.5, 1.25 μg/mL) of sample solutions were prepared from this stock solution by dilution with appropriate amount of 95 % ethanol [5] [21].

### 2.11 Preparation of 60 μM DPPH Solution

DPPH (2.364 mg) was thoroughly dissolved in 95 % ethanol (100 mL). This solution was freshly prepared in the brown coloured flask and kept in refrigerator for no longer than 24 hours [5] [21].

### 2.12 Preparation of Standard Solution

Ascorbic acid (5 mg) was dissolved in 10 mL of 95 % ethanol used as a standard solution.

### 2.13 Measurement of DPPH Radical Scavenging Activity by UV Spectrophotometric Method

(i) Sample solution was prepared by thoroughly mixing 1.5 mL of 60 μM DPPH solution and 1.5 mL of test sample solution. The solution was then allowed to stand to room temperature for 30 minutes.

(ii) Control solution was prepared by mixing 1.5 mL of 60 μM DPPH solution and 1.5 mL of 95 % ethanol.

Absorbance of these solutions was measured at 517 nm by using UV spectrophotometer. Experiment was done in triplicate for each sample solution and % inhibition was calculated by using the following equation.

$$\% \text{ inhibition of oxidation} = \frac{A - B}{A} \times 100\%$$

A = Absorbance of DPPH solution
B = Absorbance of sample + DPPH solution

Finally, $IC_{50}$ (50 % inhibition concentration) was determined by using linear regressive excel program [5] [21].

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical Constituents of Fruits of Momordica charantia L.

Phytochemical constituents of fruits of *Momordica charantia* L. was investigated. The results are shown in table 1.

**Table(1) Results of Preliminary Phytochemical Screening on the Fruits of Momordica charantia L.**

| Constituent | Reagent | Observation | Result |
|---|---|---|---|
| Alkaloids | Dragendroff's solution<br>Mayer's reagent | Brown ppt<br>Cream colouredppt | +<br>+ |
| Glycosides | 10 % lead acetate | White ppt | + |
| Flavonoids | Conc: HCl, Mg coil | Pink color solution | + |
| Saponins | Conc: $H_2SO_4$ | Red color solution | + |
| Phenolic | 10 % $FeCl_3$ solution | Purplish color solution | + |
| Polyphenol | 1 % $FeCl_3$ + 1 % $K_3[Fe(CN)_6]$ | Greenish blue color solution | + |
| Lipophilic | 0.5 N KOH | Deep colour solution | + |
| Reducing Sugar | Benedict's solution | No brick red | - |
| Terpenoid | $CHCl_3$, conc: $H_2SO_4$ | Reddish colour solution | + |

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

According to the results,alkaloids, glycosides, flavonoids, saponins, polyphenol, phenol, lipophilic and terpenoids were found to be present in the fruits of Momordica charantia L.

### 3.2 Antimicrobial Activities of Fruits of Momordica charantia L.

The antimicrobial activities on the fruits of Momordica charantia L. in five solvent systems were determined by Agar well diffusion method on six tested microorganisms. The results are shown in Table (2).

**Table (2) Results of Antimicrobial Activities on the Fruits of Momordica charantia L.**

| Sample | Solvents | Microorganisms | | | | | |
|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI |
| *Momordicacharantia*L. (fruit) | n-hexane | - | - | - | - | - | - |
| | Pet-ether | - | - | - | - | 14 mm (+) | - |
| | $CHCl_3$ | 14 mm (+) | 14 mm (+) | 13 mm (+) | 13 mm (+) | 14 mm (+) | 13 mm (+) |
| | EtOAc | 20 mm (+++) | 20 mm (+++) | 20 mm (+++) | 22 mm (+++) | 25 mm (+++) | 20 mm (+++) |
| | EtOH | 15 mm (++) | 15 mm (++) | 15 mm (++) | 15 mm (++) | 15 mm (++) | 16 mm (++) |

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

**Microorganisms**
(I) *Bacillus subtilis*
(II) *Staphylococcus aureus*
(III) *Pseudomonas aeruginosa*
(IV) *Bacillus pumalis*
(V) *Candida albicans*
(VI) *Escherichia coli*

From these results, ethyl acetate crude extract gave high activity on six tested microorganisms. Moreover, ethanol crude extract showed medium activity on six tested microorganisms.

### 3.3 Determination of Total Phenolic Content

The total phenolic contents (TPC) of *Momordica charantia* L. were determined spectrophotometrically according to the Folin-Ciocalteau colorimetric method using gallic acid as the standard. Results of absorbance of standard gallic acid are shown in Table (3). Phenols react with an oxidizing agent phosphomolybdate in Folin-Ciocalteau reagent under alkaline conditions and result in the formation of the

colored complex, the molybdenum blue which is measured at 765 nm colorimetrically.

The absorbances of prepared sample solution of 10 µL of sample was measured with UV-visible spectrophotometer at 765 nm with respect to blank solution. The results are described in Table (4).

**Table (3) Results of Absorbances of Standard Gallic Acid Solution**

| No. | Test sample | Concentration (µg/mL) | Absorbance |
|---|---|---|---|
| 1 | StdGA1 | 2 | 0.153 |
| 2 | StdGA2 | 4 | 0.261 |
| 3 | StdGA3 | 6 | 0.336 |
| 4 | StdGA4 | 8 | 0.459 |
| 5 | StdGA5 | 10 | 0.538 |

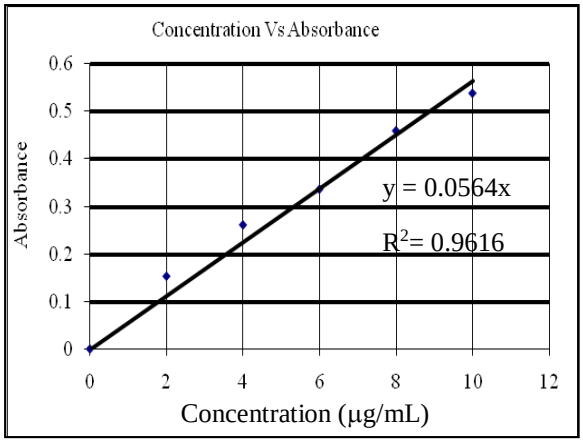


**Figure 2. Concentration absorbance calibration curve for standard gallic acid**

**Table(4) Results of absorbance and concentrations of Momordica charantia L.**

| No. | Test sample | Absorbances | Concentration (µg/mL) |
|---|---|---|---|
| 1 | Fruit of *M.charantia* L. (10 µL) | 0.358 | 6.39 |

From these results, the amount of total phenolic content of analyzed samples was obtained by using the standard graph. Total phenolic of fruits of Momordica charantia L. was expressed as gallic acid equivalent and it was 20.28 mg of gallic acid equivalent (GAE) per g DW.

### 3.4 Antioxidant Activities of Fruits of Momordica charantia L.

Antioxidant activity on the fruits of *Momordica charantia* L. was also determined by DPPH radical scavenging method. On the basis of absorbance, the % inhibition of different concentrations was calculated and $IC_{50}$ values were determined. The results are shown in Table (5) and (6).

**Table (5) The Results of Mean Absorbance and Mean % Inhibition of Fruits of Momordica charantia L. Extract and Standard Ascorbic Acid**

| Test Samples | Concentration (µg/mL) | Mean Absorbance | Mean % Inhibition |
|---|---|---|---|
| *Momordica charantia* L.fruits extract | 1.25 | 0.202 | 29.86 |
| | 2.50 | 0.199 | 30.90 |
| | 5.00 | 0.107 | 62.84 |
| | 10.00 | 0.099 | 65.62 |
| | 20.00 | 0.094 | 67.36 |
| Ascorbic acid | 1.25 | 0.085 | 33.20 |
| | 2.50 | 0.034 | 66.61 |
| | 5.00 | 0.037 | 69.59 |
| | 10.00 | 0.029 | 77.13 |
| | 20.00 | 0.020 | 84.63 |

**Table (6) % Inhibition and $IC_{50}$values of Ethanolic Extract from the Fruits of *Momordica charantia* L. and Standard Ascorbic Acid**

| Test Samples | % inhibition | | | | | $IC_{50}$ |
|---|---|---|---|---|---|---|
| Conc: (µg/mL) | 1.25 | 2.5 | 5.0 | 10 | 20 | |
| *Momordica charantia* L. | 29.861 | 30.903 | 62.847 | 65.625 | 67.361 | 3.38 |
| Ascorbic Acid | 33.20 | 66.61 | 69.59 | 77.13 | 84.63 | 1.88 |

The plot of % inhibition in five kinds of concentration of sample extract and those of standard ascorbic acid give rise to the following Figure (3) and (4).

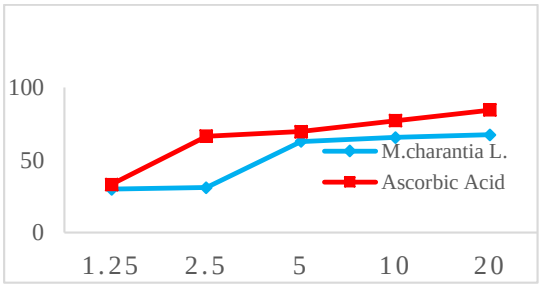


**Figure3. Plot of % Inhibition Vs Concentration (µg/mL) of Crude Ethanol Extract of Momordica charantia L. and Standard Ascorbic Acid**

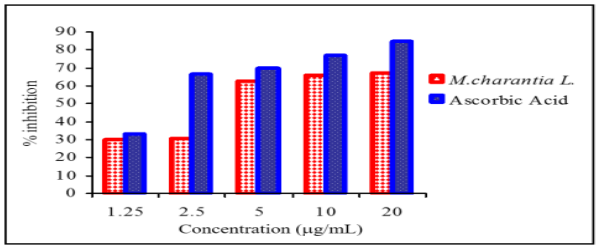


**Figure5. Histogram of % Inhibition Vs Concentration of Ethanolic Extract from *Momordica charantia* L. and Standard Ascorbic Acid**

As described in Figure (3) and (4), percent inhibition of oxidation of standard ascorbic acid increases with the increase in concentration of this standard compound, but in the case of bitter gourd, percent inhibition of oxidation of this sample is nearly equal at lower concentration and increase with the increase in higher concentration.

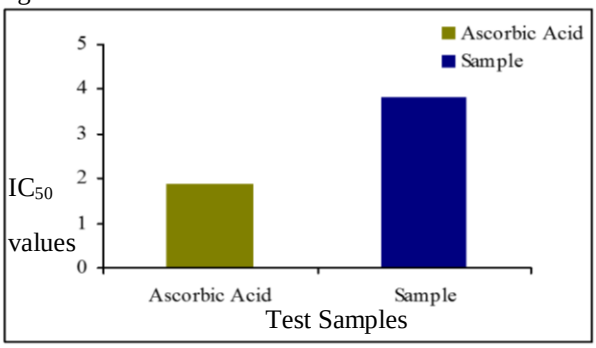


**Figure 5. Bar Graph of $IC_{50}$ Values for Sample and Standard Ascorbic Acid**

The bar graph shows for $IC_{50}$ values of the sample and standard ascorbic acid. The $IC_{50}$ value of ethanolic extract of the sample was found to be 3.38 µg/mL where as standard ascorbic acid was 1.88 µg/mL. So, the sample extract has significant antioxidant activity as standard ascorbic acid.

## 4. CONCLUSIONS

In this research, phytochemical screening on the fruits of Momordica charantia L. was determined. According to the results of phytochemical test, the fruits of Momordica charantia L. contained alkaloids, glycosides, flavonoids, saponins, phenolic,polyphenol, lipophilic, and terpenoids. Moreover, antimicrobial activities on the fruits of sample in five solvents were also determined by agar well diffusion method on six selected microorganisms namely, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albicans* and *E. coli*. The results show that EtOAc extract gave the high activity on six selected microorganisms. Total phenolic content in *Momordica charantia* L. was observed to be 20.28 mg GAE per gram DW. Furthermore, antioxidant activity of ethanolic extract of the sample was determined with five different concentrations (1.25, 2.5, 5.0, 10, 20)µg/ml by DPPH radical scavenging method. The $IC_{50}$ value of ethanolic extract of the sample was found to be 3.38 µg/mL.It showed the significant antioxidant activity. Thus, fruits of bitter gourd contained many phytochemical constituents, these compounds possessed antimicrobial and antioxidant activities. Therefore, these species have great relevance in the prevention and treatment of diseases.

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# Physicochemical Characterization of Water Soluble Chitosan and Its Acute Toxicity and Weight Loss Activity

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**ABSTRACT:** *The main aim of the research is to study the physicochemical characterization of water soluble chitosan and to evaluate scientifically on acute toxicity and weight loss activity. The yield percent (86.10 %) of low molecular weight water soluble chitosan (WS-chitosan) was produced from chitosan by oxidative depolymerization method. By altering chitosan to WS-chitosan can overcome many disadvantages such as many environmental pollution and chemical toxic effect. Some physicochemical properties: moisture content (8.12 %), ash content (0.72 %), molecular weight ($2.1258\times10^4$) and degree of deacetylation (91.65 %) of WS-chitosan was examined. The solubility of WS-chitosan was determined by various solvents including water at room temperature. The acute toxicity study of prepared WS-chitosan was systematically performed by OECD Guideline for the Testing of Chemicals 423. The acute toxicity test on albino mice indicated no toxic effect in both extracts of sample. According to the study on the weight loss activity of WS-chitosan on albino mice carried out by the method of Han et al., 1999, it was found to be evidently effective in weight loss activity. The 600 mg/kg b.wt dose of WS-chitosan is the most eligible dose to reduce the body weight.*

**Keywords:** water soluble chitosan; physicochemical properties; acute toxicity; weight loss activity

## 1. INTRODUCTION

Chitosan is natural linear polysaccharides comprising copolymers of glucosamine and N-acetyl glucosamine and can be obtained by the partial deacetylation of chitin. Nowadays, chitosan is used in versatile applications [9]. Chitosan has been widely used in vastly diverse fields ranging from waste management to food processing, medicine and biotechnology. It becomes an interesting material in pharmaceutical applications due to its biodegradability and biocompatibility, and low toxicity. If chitosan was degraded into low molecular weight water soluble chitosan (WS – chitosan) with the molecular weight less than 25000, its water solubility increased greatly, conductive to human intestinal digestion and absorption, and possess the functions such as promoting to produce splenic antibody, and lowering cholesterol, blood pressure, blood sugar and blood lipid level in serum and liver [8].

Water soluble chitosan (WS-chitosan) was prepared from chitosan that was produced by oxidative depolymerization method. In the present study, some physicochemical properties of WS-chitosan were investigated. Some pharmacological activities such as acute toxicity and weight loss activity of WS- chitosan were also studied.

Acute toxicity study: To know the harmful effects of a new chemical or drug, toxicity test must be done. The potential toxicity of new chemicals or drugs must be evaluated first on the laboratory animals. The principle purpose for conducting toxicity test on animals is to evaluate the nature and the degree of harmful effects or deaths. Animal toxicity test also prevent distinctly harmful agents from become readily available to man [5]. Weight loss activity: Obesity is now a worldwide problem. More than one billion people are currently overweight with 300 million considered clinically obese. If a person body weight is at least 20% higher than it could be, he or she is considered obese. If your Body Mass Index (BMI) is between 25 and 29.9, you are considered overweight. If you BMI is 30 or over you are considered obese [3]. In general, the BMI measurement can be a useful indicator for the average person. Several studies suggest that chitosan may have utility in the treatment of obesity. Low molecular weight water soluble chitosan is more effective than high molecular weight chitosan in pharmaceutical applications because high molecular weight chitosan was very difficult to absorb fat and enter the blood [4]. In addition, studies examining water soluble chitosan as an agent for weight control will also be reviewed. Water soluble chitosan is viewed as potentially useful for increase fecal fat excretion. However, in human studies, the chitosan with low molecular weight can accelerate the weight loss when subjects are on a weight reduction diet, but that when food intake is not restricted, no weight loss should be expected.

## 2. MATERIALS AND METHODS

### 2.1 Preparation Of Low Molecular Weight Water Soluble Chitosan (WS-chitosan)

The water soluble chitosan (WS-chitosan) was prepared from chitosan (purchased from FMI City) by oxidative depolymerization method. The chitosan was completely dissolved in 50 mL of 1 % acetic acid in a water-bath shaker. The conditions were set as $H_2O_2$ (6 %), time (4 hours) and temperature (60 °C). After reaction, a few drops of 10 % NaOH were used to adjust the solution to neutrality. The residue was removed by filtration. The filtrate was concentrated to about 1/5 with a rotary evaporator. After that, the two fold volumes of ethanol were added to the filtrate. The crystal of water soluble chitosan was liberated after incubation at ambient condition overnight, and dried in an oven at 50 ºC [6].

## 2.2 Determination Of Physicochemical Properties Of Prepared WS-Chitosan

Some physicochemical properties of the prepared WS-chitosan such as moisture content, ash content, molecular weight and degree of deacetylation were determined.

The moisture content of the prepared chitosan sample was examined by electronic moisture balance, EB-280 MOC, Shimadzu, Japan). The ash content of the prepared chitosan sample was determined by AOAC method. The prepared chitosan sample was characterized in their average molecular weight by means of viscosity measurements. The degree of deacetylation (DDA) of prepared chitosan sample was determined by hydrogen chloride titrimetric method. The resulting data are summarized in Table 2.

## 2.3 Measurement On Solubility Of Prepared WS-Chitosan

Solubility of prepared WS-chitosan was examined by various solvents. 1.0 g of each sample was weighed and put into a test tube following by adding 100 mL of 1 % (v/v) of acetic acid solution. The suspensions were mixed and allowed to hydrate for 3 hours at room temperature. The physical appearance was observed and recorded in Table 3. Similarly, the solubility of each sample in other solvents was determined as the same procedure.

## 2.4 Screening of Pharmacological Activities of Prepared Water Soluble Chitosan

The water soluble chitosan (WS-chitosan) was converted form chitosan by oxidative depolymerization method. Some pharmacological activities of water soluble chitosan such as acute toxicity and weight loss activity were presently studied.

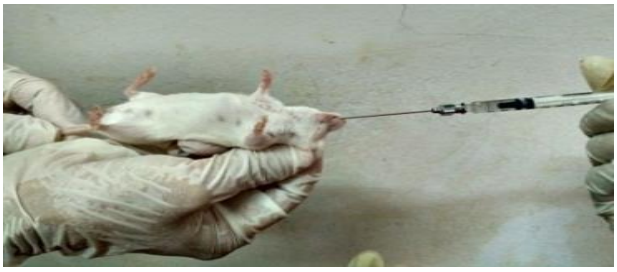
### 2.4.1 Study on Acute Toxicity of Water Soluble Chitosan

To define the consequent of the chitosan sample and to determine the nature and degree of toxicity produce by these products was done. Usually the acute lethality of a compound is determined on the basic of deaths occurring in 24 hours but the survivors should be observed for at least seven days in order to detect delayed effects. In the study, acute toxicity effect of WS-chitosan sample (1g/kg, 2g/kg, 3g/kg and 4g/kg doses) were determined on albino mice at Laboratory Animal Services Division, Department of Medical Research (DMR), Yangon.

Acute toxicity of different doses of WS-chitosan was evaluated by the methods of OECD Guidelines for the Testing of Chemicals 423. According to the test description, total number of 15 adult female albino mice, weighting (25-30 g) were selected and divided into five groups. Each group contained three animals. They were maintained in accordance with the recommendation of the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication N. 85-23, revised 1996) for studies involving experimental animals. They had free access to feed and clean drinking water during the three days' acclimatization period and throughout the experimental period.

They were fasted for 18 hours before giving the WS-chitosan. Group (I) ,(II), (III) and (IV) mice were orally administrated with WS-chitosan (prepared solution dissolved in distilled water) 1 g/kg, 2 g/kg, 3 g/kg and 4 g/kg (b. wt) dose. Group (V) mice performed as a control group. All groups of mice were kept in the mouse cages at the room temperature of 25 ± 1°C and they were treated with clean water and normal animal food which procedure are shown in Figure 1. After administration of WS-chitosan on each group of animals were observed first 6 hours continuously for mortality and behavior changes. The mortality during 14 days was noted (number of death or percent death) and the results obtained from acute toxicity effect are described in Table 4. Then check the animals each 24 hours for 14 days and the general appearance and behavioral observation are presented in Table 5.

(a)

(b)

**Figure 1. Acute toxicity test on albino mice (DDY strain) mice were put in laboratory conditions:**

**(a) standardized boxes, natural light and ambient temperature, allowed free access to both water and animal feed**

**(b) administered the prepared WS-chitosan by oral route using intragastric syringe**

### 2.4.2. Study On Weight Loss Activity Of Water Soluble Chitosan (WS-Chitosan)

Chitosan is viewed as potentially useful for inducing weight loss due to its ability to increase fecal fat excretion. This effect has been demonstrated in animal studies [1].

In this study, three doses of sample such as 400 mg/ kg, 600 mg/kg and 800 mg/kg were prepared. The weight loss activity of WS-chitosan tested on albino mice was carried out according to the method of Han *et al*., (1999). Eighty healthy albino mice (ddy strain) of male and female (32-35 g) were used for this study. They were separated into 4 groups, each group containing of 10 mice. Group I was given distilled water only and served as control group. Group I, II and IV were orally given with three different doses (400, 600 and 800mg/kg) of prepared sample respective and orally administered once a day for 4 weeks shown in Figure 2. The experimental results are summarized in Table 6 and 7.

(a) (b)

**Figure 2. Weight loss test on albino mice (DDY strain)**

**(a) Administered the WS-chitosan in different concentrations by oral route using intragastric syringe**

**(b) Checked the weight of tested mice by weighing using animal balance**

## 3. RESULTS AND DISCUSSION

### 3.1 Preparation Of Low Molecular Weight Water Soluble Chitosan (WS-Chitosan)

Low molecular weight water soluble chitosan (WS-chitosan) was prepared from chitosan by oxidative depolymerization method [6]. Hydrogen peroxide was used to degrade the chitosan into WS-chitosan. The long term aim of this work is to apply the WS-chitosan in pharmacological activity. By altering the chitosan to WS-chitosan can overcome many disadvantages such as environmental pollution and chemical toxic effect. The resultant WS-chitosan showed 91.65 % degree of deacetylation. The yield percent of WS-chitosan was 86.10 % based on chitosan.

### 3.2 Physicochemical Properties of Prepared WS- Chitosan

The physicochemical properties of WS-chitosan such as moisture content, ash content, molecular weight and degree of deacetylation were determined. Some physicochemical properties data of WS-chitosan are summarized in Table 2.

#### 3.2.1. Moisture Content of WS-chitosan

In the research work, determination of moisture content of prepared WS-chitosan was determined by using moisture balance. The moisture content is most widely used analytical measurements for the processing and testing of quality products. The moisture content of WS-chitosan was found to be 8.12 %.

#### 3.2.2 Ash Content of WS-chitosan

The ash content in WS-chitosan is determined by weighing the dry mineral residue of organic material of a food has been destroyed by heating and it is the most convenient assessment of the total mineral matter of sample [7]. It was observed that the ash content of WS-chitosan product is 0.72 %.

#### 3.2.3 Average Molecular Weight of WS-chitosan

In the present work, the relative molecular weight of prepared WS-chitosan was determined by the intrinsic viscosity method. Table 1 shows the relationship among flow time, intrinsic viscosity [$\eta$ / $\eta_0$], specific viscosity [$\eta$ /$\eta_0$-1], and reduced viscosity [ln $\eta$ / $\eta_0$]. By using the data in the table, the graphs of ([$\eta$ / $\eta_0$-1] /c) versus concentration of each chitosan sample and (1/c ln $\eta$ / $\eta_0$) versus concentration of each of the sample were constructed (Figure 3). The intrinsic viscosity was determined by the intercept value of reduced viscosity from the plot of specific viscosity *Vs* concentration plot using Huggin's equation ($\eta_{sp}/c = [\eta] + K_H[\eta]c$) and Karamer's equation ($\ln \eta_r/c = [\eta]c + K_k[\eta]c$). The sum of $K_H$ and $K_K$ in one way gives the Mark-Houwink's equation exponent "a", which is found to be 0.9289 in WS-chitosan. When the polymer molecules are rigid rods, "a" is ~2. If the polymer are hard spheres, a is ~0. The nature of chitosan sample showing a value of a is ~1 can be considered to be more a semi coiled polymer [2]. The Mark-Houwink constant parameter ($K = 1.18 \times 10^{-3}$) for chitosan was used to deduce the viscosity average molecular weight. According to the Mark-Houwink equation $M^a = [\eta] / K$, the measured average molecular weight of WS-chitosan was found to be $2.1258 \times 10^4$ Da.

**Table 1. Relationship among Flow Time, Intrinsic Viscosity, Specific and Reduced Viscosity in WS-chitosan**

| WS-chitosan c ($gcm^{-3}$) | Flow Time (s) | $t/t_0 = \eta/\eta_0$ | $[\eta/\eta_0]$ -1 | $[\eta/\eta_0]$ -1/c | 1/c $\ln\eta/\eta_0$ |
|---|---|---|---|---|---|
| Pure solvent | 45 | 1.000 | 0.000 | 0.000 | 0.000 |
| 0.02 | 64 | 1.429 | 0.429 | 21.450 | 17.849 |
| 0.04 | 91 | 2.022 | 1.022 | 25.550 | 17.602 |
| 0.06 | 128 | 2.833 | 1.833 | 30.555 | 17.355 |
| 0.08 | 169 | 3.750 | 2.750 | 34.375 | 16.522 |
| 0.10 | 216 | 4.812 | 3.812 | 38.123 | 15.711 |

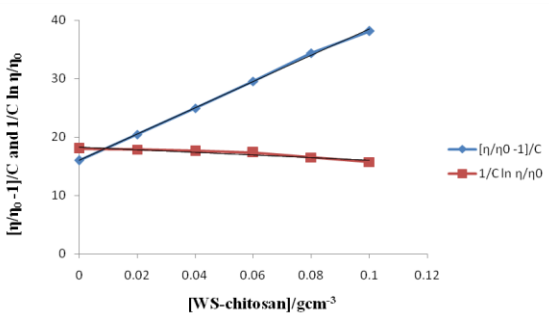



**Figure 3. Huggin's and Karamer's plot of WS-chitosan product for determination of intrinsic viscosity**

### 3.2.4 Degree of Deacetylation of WS-chitosan

The determination of degree of deacetylation (DD %) of chitosan is one of the major analyses in quality control of chitosan. The degree of deacetylation of WS-chitosan was determined by hydrogen chloride titrimetric method. In this work, degree of deacetylation 91.65 % in WS-chitosan was observed. When the more degree of deacetylation, the easier to become the chitosan. According to result, the WS-chitosan is effectively low in molecular weight and high in DD % because the molecular weight of chitosan depends on the deacetylation process. The structure of large molecular weight chitosan containing abundant hydrogen bounds and it only soluble in dilute acid, insoluble in water and tough to be assimilated by human bodies. But, low molecular weight water soluble chitosan (WS-chitosan) overcome above problems. So, WS-chitosan applied in pharmacological applications.

**Table2. Physicochemical properties of water soluble chitosan (WS-chitosan)**

| Sample | Moisture content (%) | Ash content (%) | Molecular weight (Da) | DD (%) |
|---|---|---|---|---|
| WS-chitosan | 8.12 | 0.72 | $2.1258\times10^4$ | 91.65 |

## 3.3 Comparative Study on Solubility of Purchased Chitosan and Prepared WS-chitosan

The chitosan was soluble in dilute organic acid aqueous solution of g/mL at ambient temperature 28 ˚C. The chitosan products are hydrophobic polymer because they are not soluble in water. Chitosan in the form of free amine is insoluble in water, soluble in dilute organic acid but is insoluble in dilute sulphuric acid and alcohol at room temperature. The prepared low molecular weight water soluble chitosan is soluble in dilute organic acid and water at ambient temperature. By introducing carbonyl groups in chitosan structure with either oxidizing process, the water solubility was shown to be enhancing from all the oxidized sample groups. The solubility improvement should be considered for enhancing biological activity such as bile acid-binding capacity. According to the solubility test, the prepared WS-chitosan was found to be soluble in (1 % v/v) of hydrochloric acid, nitric acid, acetic acid, formic acid and especially in water but insoluble in sulphuric acid, ethanol, methanol and (1 % w/v) sodium hydroxide solution**.** The experimental data are shown in Table 3.

**Table 3. Solubility of Purchased Chitosan and Prepared WS-chitosan in Different Solvents at Ambient Temperature**

| Solvents | Solubility of 1 g of sample in 100 mL of solvent | |
|---|---|---|
| | Chitosan | WS-chitosan |
| Hydrochloric acid (1% v/v) | ± | + |
| Sulphuric acid (1% v/v) | – | – |
| Nitric acid (1% v/v) | ± | + |
| Acetic acid (1% v/v) | + | + |
| Formic acid (1% v/v) | + | + |
| Ethanol | – | – |
| Methanol | – | – |
| Water | – | + |
| Sodium Hydroxide (1% w/v) | – | – |

**+ = soluble, - = insoluble, ± = slightly soluble**

## 3.4. Pharmacological Activities of Water Soluble Chitosan (WS-chitosan)

Some pharmacological activities of water soluble chitosan (WS-chitosan) such as acute toxicity and weight loss activity were investigated.

### 3.4.1. Acute Toxicity Study of Prepared WS-chitosan

Acute toxicity screening of WS-chitosan was prepared with the dosage of 1 g/kg, 2 g/kg, 3 g/kg and 4 g/kg body weight in each group of albino mice. The conditions of mice groups were recorded after fourteen days' administration. The results shown no lethality of the mice was observed up to fourteen days. Each group of animals was also observed still alive and did not show any visible symptom of toxicity like restlessness, respiratory disorders, convulsion, aggressive activities, coma and death. Even with the dose up to 4 g/kg body weight administration, there is no lethality after 14 days. Therefore, the prepared WS-chitosan was free from acute toxic effect under condition. The acute

toxicity results and general appearances are distributed in Table 4 and 5.

**Table 4. Results of Acute Toxicity Test of WS - Chitosan on Mice Two Weeks Treatment**

| No. | Groups | Drug Administration | Dosage g/kg (b.wt) | No. of death per tested mice | % of death |
|---|---|---|---|---|---|
| 1. | I | WS-chitosan | 1 | 0/3 | 0 |
| 2. | II | WS-chitosan | 2 | 0/3 | 0 |
| 3. | III | WS-chitosan | 3 | 0/3 | 0 |
| 4. | IV | WS-chitosan | 4 | 0/3 | 0 |
| 5. | V(Control) | Distilled Water | - | 0/3 | 0 |

Note: Each group contains 3 no: of mice
Medium lethal dose $LD_{50}$> 4 g/kg body weight

**Table 5. General Appearance and Behavioral Observations (Checked within 24 hours of 14 days) for Test and Control Group after Administrating WS-chitosan**

| General Behavior | Observations | | | | |
|---|---|---|---|---|---|
| | Group (I) | Group (II) | Group (III) | Group (IV) | Group (V) |
| Skin and fur | Normal | Normal | Normal | Normal | Normal |
| Eyes | Normal | Normal | Normal | Normal | Normal |
| Mucous | Normal | Normal | Normal | Normal | Normal |
| Behavioral | Normal | Normal | Normal | Normal | Normal |
| Salivation | Normal | Normal | Normal | Normal | Normal |
| Lethargy | Normal | Normal | Normal | Normal | Normal |
| Sleep | Normal | Normal | Normal | Normal | Normal |
| Diarrhea | Normal | Normal | Normal | Normal | Normal |
| Coma | No | No | No | No | No |
| Tremors | No | No | No | No | No |

### 3.4.2. Study on Weight Loss Activity of WS-chitosan

In this research, the weight loss activity of WS-chitosan tested on albino mice by Han *et al.*, 1999. In this test, 80 healthy albino mice (32-40 g b.wt) of male and female used. They are subdivided into four main groups. Each group contains 10 male mice and 10 female mice.

The Group I was the control group, it was given distilled water only. Groups II, III, IV were test groups treated with three different doses (400 mg/kg, 600 mg/kg and 800 mg/kg) of water soluble chitosan, respectively. They are orally administered once a day for 4 weeks.

In Group II, water soluble chitosan (400 mg/kg body weight) treated group is slightly decreased in third week in fourth week compared with "0" week. In fourth week, (29.0 g to 28.6 g) decreases in male albino mice and (29.7 g to 26.0 g) in female albino mice.

In the WS-chitosan (600 mg/kg b.wt) treated group III, the weight loss activity is significantly high at fourth week compared with "0" week, 31.4 g reduced to 29.1 g in male albino mice and 31.0 g reduced to 27.3 g in female albino mice.

In the water soluble chitosan (WS-chitosan), 800 mg/kg body weight treated Group IV, the weight loss activity showed high at third week compared with "0" week, 36.0 g lowered to 34.6 g in male and 30.8 g lowered to 28.5 g in female albino mice.

According to the data, (600 mg/kg body weight) dose of WS-chitosan showed the highest activity than others. WS-chitosan possessed more weight loss effect on female albino mice than male albino mice by the resulting data which are presented in Table 6 and 7.

**Table 6. Weight Loss Effect of Different Concentrations of WS-chitosan on Male and Female Albino Mice within 1st and 2nd Weeks**

| Test Groups | Weight (g) at 0 week | | Weight (g) at 1st week | | Weight (g) at 2nd week | |
|---|---|---|---|---|---|---|
| | M | F | M | F | M | F |
| Group I | 30.8 ±1.12 | 31.2 ±0.69 | 30.7 ±1.10 | 31.1 ±0.32 | 31.3 ±0.65 | 30.9 ±1.32 |
| Group II | 29.0 ±0.02 | 29.7 ±0.05 | 29.2 ±0.01 | 27.0 ±0.04 | 29.0 ±0.10 | 26.7 ±0.10 |
| Group III | 31.4 ±0.08 | 31.0 ±0.21 | 30.4 ±0.10 | 30.3 ±0.08 | 31.2 ±0.01 | 29.3 ±0.01 |
| Group IV | 36.0 ±0.04 | 30.8 ±0.02 | 34.8 ±0.03 | 30.5 ±0.01 | 35.8 ±0.10 | 30.8 ±0.07 |

**Table 7. Weight Loss Effect of Different Concentrations of WS-chitosan on Male and Female Albino Mice within 3rd and 4th Weeks**

| Test Groups | Weight (g) at 0 week | | Weight (g) at 3rd week | | Weight (g) at 4th week | |
|---|---|---|---|---|---|---|
| | M | F | M | F | M | F |
| Group I | 30.8± 1.12 | 31.2± 0.69 | 31.1± 0.12 | 31.1± 0.13 | 30.8± 1.11 | 31.1± 0.12 |
| Group II | 29.0± 0.02 | 29.7± 0.05 | 28.8± 0.06 | 26.5± 0.03 | 28.6± 0.01 | 26.0± 0.02 |
| Group III | 31.4± 0.08 | 31.0± 0.21 | 30.6± 0.01 | 27.6± 0.08 | 29.1± 0.10 | 27.3± 0.04 |
| Group IV | 36.0± 0.04 | 30.8± 0.02 | 34.6± 0.10 | 28.5± 0.10 | 34.6± 0.04 | 28.3± 0.06 |

## 4. CONCLUSIONS

From the overall assessments of the research work, the following inferences could be deduced. In this research, water soluble chitosan (WS-chitosan) 86.10% was produced with molecular weight of ($2.125 \times 10^4$ Da) and high degree of deacetylation (91.65 %) could be converted from chitosan by oxidative depolymerization method. The obtained water soluble chitosan (WS-chitosan) is low molecular weight chitosan because its molecular weight was found to be lower than 25,000 Da. It can be inferred that there will be a variety of application by using this prepared WS-chitosan.

WS-chitosan was effectively applied in some pharmacological activities because it is easily soluble in water. In vivo acute toxicity of water soluble chitosan was studied by OECD guidelines using albino mice model, it was observed that the water soluble chitosan (WS-chitosan) was free from toxic effect in the range of 1-4 g/kg body weight of albino mice. Weight loss activity of water soluble chitosan (WS-chitosan) was examined on albino mice model using the method of Han *et al*., 1999. It was found that water soluble chitosan can promote the weight loss activity evidently. The concentration (600 mg/kg) of WS-chitosan showed the highest activity and more weight loss effect on female albino mice than male albino mice.

The water soluble chitosan (WS-chitosan) was successfully converted from chitosan to employ in many pharmacological activities. And also, WS-chitosan has higher solubility in neutral aqueous solutions than chitosan and which broadens their applications as medicinal agents. In addition, WS-chitosan can be attributed and suited for some applications particularly in health care products, nutrition, pharmacology and cosmetic additive process.

## 5. ACKNOWLEDGEMENTS

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# Determination of Dyeing Process and Colour Fastness Properties of Natural Dye Extracted from Mango Bark (*Mangifera indica* L.) on Cotton Cloth

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**ABSTRACT**: *The purpose of this study is to investigate the sorption (adsorption and desorption) and colour fastness properties for the brown natural dye extracted from Mango bark (Mangifera indica L.). The extracted natural dye was characterized by FT IR and UV-visible analysis. The three dye sample solutions ($S_1$, $S_2$ and $S_3$) were prepared by natural dye and natural mordant (banana petaloid and alum). Sorption properties of natural dye solution ($S_1$) on cotton cloth at different temperatures (40, 60 and 80) °C were carried out to select the more effective dyeing temperature. Dyeing of the three dye solutions on cleaned cotton cloth were determined by UV-vis spectrophotometer $\lambda_{max}$ 465 nm. The colour intensities of these dyeing cotton cloth were determined by Reflection Transmission colour Densitometer. $S_1$ showed low colour intensities in the comparison of the other two samples. Depending on the mordant, colour intensity was increased in the order of alum, banana petaloid. Therefore, $S_2$ (alum) and $S_3$ (banana petaloid or natural mordant) can be applied in home- made dyeing process.*

**Keywords**: natural dye, mordants, sorption, colour fastness

## 1. INTRODUCTION

Natural dyes are those obtained from plants, animals and minerals. The majority of natural dyes are acid dyes (anionic) and are attached to the cationic site in keratin (the alpha-protein in wool), mediated by a metal mordant. The use of non-allergic, non-toxic and eco-friendly natural dyes on textile have become a matter of significant importance due to the increased environmental awareness in order to avoid some hazardous synthetic dyes. Natural dyes are considered to be eco-friendly as these are obtained from renewable resources as compared to synthetic dyes which are derived from non-renewable petroleum resources. Most of the natural dyes have no substantivity for the fibre and mordants must be used [1].

Natural dyes are known for their use in colouring of food substrate, leather as well as natural fibres like wool, silk and cotton as major areas of application since pre-historic times. Natural dyeing of different textiles and leather has been continued mainly in the decentralized sector for speciality products besides the use of synthetic dyes in the large scale sector for general textiles [2].

Most of the natural dyes are found to be non-carcinogenic in nature. Moreover, natural dyes have positive effect on antifungal and antibacterial growth. Application of natural dyes has potential to earn carbon credit by reducing consumption of fossil fuel based synthetic dyes. Some of its constituents are anti-allergens, prove safe for skin contact and are mostly non-hazardous to human health. Some of the natural dyes are enhanced with age, while synthetic dye fade with time. Natural dyes bleed but do not stain other fabrics. Natural dyes are usually moth proof and can replace synthetic dyes in kids garments and food-stuff for safety [3].

### 1.1 Mangifera indica L.(Mango)

Mangifera Mangifera is genus of flowering trees in the Anacardiaceae family. There are total 72 species of genus Mangifera today most of them surviving in the rain forests of Malaysia and Indonesia. Mango trees require tropical and warm, subtropical areas with temperatures ranging from 20 to 30 ˚C [4]. Tree is medium to large (10-40 m in height), evergreen with symmetrical, rounded canopy ranging from low and dense to upright and open. Bark is usually dark grey-brown to black, rather smooth, superficially cracked or inconspicuously fissured, rather thick pieces. Mango was concerned mainly with the identification of sugar and phenolic compounds. Amino acid, ambonic acid, indicoside A and B, gallotanning gallic acid and m-digallic acid, ehylgallate, isoquercentin and β-sitosterol are found in various parts of plants [5]. The bark is reported to contain protocatechuic acid, catechin, mangiferin, alanine, glycine, γ-amino-butyric acid, quinic acid, shikimic acid and were separated from the hexane extract of the stem bark of Mangifera indica [6].

## 2. MATERIALS AND METHODS

### 2.1 Extraction of Dye

The bark of Mango Bark (Mangifera indica L.) was collected from Yangon University campus. After collection, the scientific name of Mango was identified by authorized botanist at Botany Department, Yangon University. The sample was washed with tap water to

remove impurities and then air-dried under shade to prevent some reaction of sunlight with organic constituents of sample. The dried sample was separately cut into pieces and ground in a grinding machine. In the procedure of dye extraction, 5 g of powder sample (mango bark) was boiled with 2000 mL of distilled water at 90 °C for 2 hours and then pre dye solution was obtained. This dye solution was heated at 80 °C until dried pasty dye, which was powdered and sieved with 90 μm aperture size. The dye powder sample was separately stored in the air-tight container so that the sample was free from getting molds to prevent moisture, as well as other contaminations and was ready to be used for the experimental works.

### 2.2 Methods

Extracted dye from Mango bark was used as adsorbate. Cotton cloth was used as adsorbent. Extracted natural dye was characterized by FTIR and UV visible spectrometry. Cotton dyeing was carried out by these dyes in the absence and presence of natural mordants (alum and banana petaloid). Sorption properties of three different dye solutions were studied according to the contact time and different temperatures. These data was analysed using Langmuir adsorption isotherm models. The colour intensities of these dyeing cotton cloth were determined by Reflection Transmission Colour Densitometer. The dyed material was tested for light fastness and wash fastness. Light fastness was analyzed by exposing the dyed material to direct sunlight for 24 hours. The wash fastness was carried out by washing the dyed fiber with non-ionic soap solution (1 g $L^{-1}$) for 15 minutes. Colour densities of the tested cotton cloths were measured.

**Figure 1 Mangifera indica L. (Mango)**

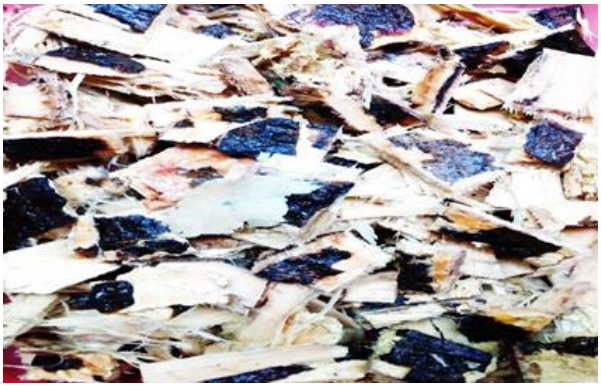

**Figure 2 Bark of Mangifera indica L**

## 3. RESULTS AND DICUSSIONS

### 3.1. FT IR analysis

The Fourier Transformed Infrared spectrum of natural dye extracted from Mangifera indica L. was shown in Figure 3 and the band assignments are represented in Table 1. FT IR investigation is widely used for studies of Natural Dye. In Figure 3, absorption band at about 3373 $cm^{-1}$ indicate O-H stretching of Mango bark dye, while C-H stretching was observed at 1467 $cm^{-1}$. The absorption peaks at 1612 $cm^{-1}$ are characteristic of the C=C stretching vibration. The broad band at 1260 and 1078 $cm^{-1}$ in natural dye are caused by phenolic O-H bending respectively.

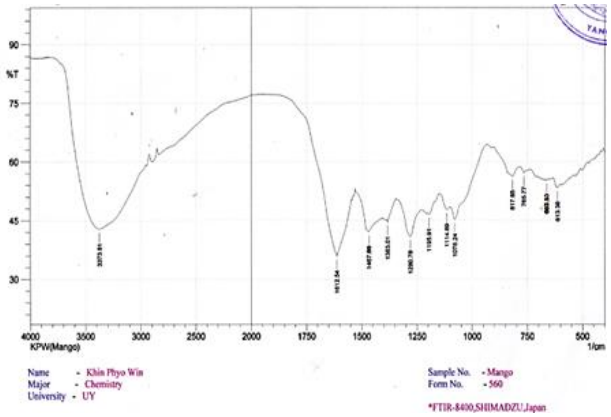


**Figure 3 FT IR spectrum of natural dye extracted from mango bark**

**Table 1 FT IR Spectral Assignments of Natural Dye Extracted from Mango Bark**

| Wave number ($cm^{-1}$) (Mango Bark) | Band Assignment |
|---|---|
| 3373 | $\nu_{O-H}$ of hydroxyl group |
| 1612 | $\nu_{C=C}$ aromatic ring |
| 1467 | $\delta_{C-H}$ of $CH_2$ and $CH_3$ |
| 1260 and 1078 | $\delta_{C-OH}$ phenolic group |

### 3.2 UV Analysis

Ultraviolet spectrum of natural dye extracted from Mango bark was described in Figure 4. The maximum absorbance of the wavelength ($\lambda_{max}$) values and possible transition are summarized in Table 2. The maximum absorbance of the wavelength was found to be 425 nm and 455 nm for Mango bark

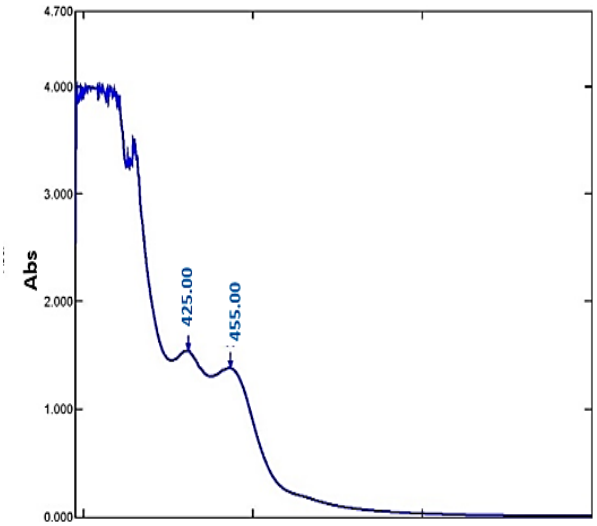


**Figure 4. UV-visible spectrum of atural dye extracted from Magifera indica L (Mango) bark**

**Table 2. Maximum Absorbance of Wavelengths of Natural Dye Extracted from Mango Bark**

| Wavelength (nm) | Transition |
|---|---|
| 425 | $n \rightarrow \pi^*$ |
| 455 | $n \rightarrow \pi^*$ |

### 3.3 Effect Temperature on Brown Natural Dye ($S_1$) Dyeing on Cotton Cloth

If the adsorption properties of natural dye increase, decrease in temperature is said to be exothermic process. Therefore, adsorption properties $S_1$ (natural dye extracted from Mango bark) was examined at 40, 60 and 80 °C, respectively. It was found that $S_1$ undergoes exothermic process, its suitable dyeing temperature was 60 °C. Table 4 and Figure 6 were shown in the adsorption properties of $S_1$ dyeing on cotton cloth at 40, 60 and 80 °C. Similarly, the uptake of $S_2$ and $S_3$ samples increased with decreasing temperature at the same adsorption time.

**Table 3 Effect of Temperature on the Brown Natural Dye ($S_1$) Dyeing on Cotton Cloth**

| Time (min) | $q_t$ (mg/g) | | |
|---|---|---|---|
| | 40 ℃ | 60 ℃ | 80 ℃ |
| 10 | 5.18 | 9.64 | 0.25 |
| 20 | 12.12 | 21.44 | 0.59 |
| 30 | 25.94 | 34.29 | 6.26 |
| 40 | 33.88 | 43.52 | 15.02 |
| 50 | 48.11 | 56.55 | 25.86 |
| 60 | 49.63 | 64.96 | 34.69 |
| 70 | 63.99 | 68.95 | 41.52 |
| 80 | 64.16 | 75.79 | 50.54 |

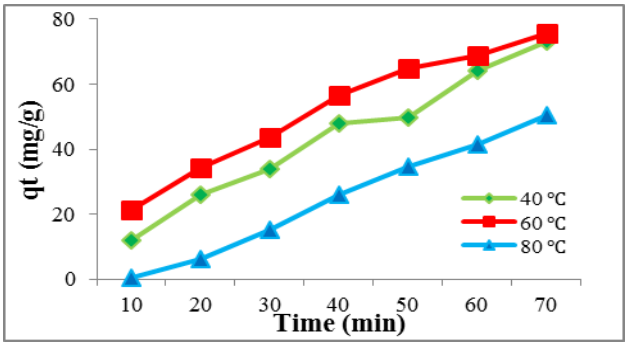


**Figure 5 Effect of dyeing temperature on the brown natural dye ($S_1$) cotton cloth**

### 3.4 Effect of Contact Time for Three Dye Solutions Dyeing on Cotton Cloth at 60 °C

Table 3 and Figure 5 show the amount of dye on cotton cloth with different dye solutions ($S_1$, $S_2$ and $S_3$) with respect to contact time 80 minutes at 60 °C. It was found that maximum sorption capacities were reached at contact time 80 minutes. The resultant data were calculated by following equation;

$$q_e = \frac{C_o - C_e}{1g} \times 1\ L$$

$C_o$ = initial concentration, $C_e$ = equilibrium concentration, $q_e$ = the amount of dye adsorbed per gram of cotton cloth (mg/g cotton) at equilibrium

**Table 4 Comparison of Contact Time for Six DyeSample Solutions Dyeing on Cotton Cloth at 60 ˚C**

| Time (min) | $q_t$(mg/g) | | |
|---|---|---|---|
| | $S_1$ | $S_2$ | $S_3$ |
| 10 | 9.64 | 12.01 | 8.51 |
| 20 | 21.44 | 23.81 | 18.19 |
| 30 | 34.29 | 39.94 | 19.34 |
| 40 | 43.52 | 50.21 | 23.29 |
| 50 | 56.55 | 60.32 | 32.81 |
| 60 | 64.96 | 72.42 | 53.62 |
| 70 | 68.95 | 72.91 | 68.08 |
| 80 | 75.79 | 76.85 | 84.51 |

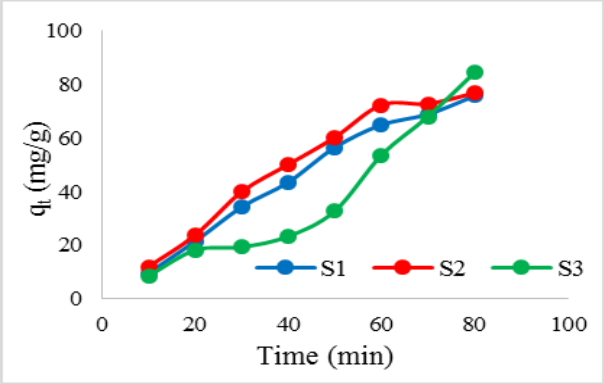


**Figure 6 Comparison of contact time for six dye sample solutions dyeing on cotton cloth at 60 ˚C**

## 3.5 Colour Fastness Properties of Six Dye Solutions Dyeing on Cotton Cloth

Colour density on the cotton cloth was increased significant when a mordant was used. Colour fastness cotton cloth samples were prepared using pre mordanting, simultaneous mordanting and post mordanting, 0.5 % w/v dye concentration and 60 minutes dyeing time because those conditions are resulted in the highest colour strength for cotton cloth. In pre mordanting, simultaneous mordanting and post mordanting of colour fastness results of three dyeing cotton cloth were shown in Table 5 (a) and Figure 7 (a). The sample $S_1$ (natural dye) was seen the lowest colour density and the sample $S_2$ (alum mordant) and $S_3$ (banana petaloid) are higher in colour density. The colour density for three dyeing cotton cloth before and after colour fastness testing were compared in Table 5 (b), (c) and (d) and Figure 7 (b), (c) and (d). Poor substantivity and fastness properties are often found in natural dyes for cotton cloth and can be improved dyes for cotton cloth was first treated with a solution containing mordants such as a salt of alum. . The colour density values of three dyeing cotton cloth after colour fastness testing (lighting fastness and washing) were no significant change before testing the fastness tests.

**Table 5(a) Colour Density of Cotton Cloth Dyed with Water Extract**

| Mordant | Colour densities/ Mordants | | |
|---|---|---|---|
| | Without ($S_1$) | Alum ($S_2$) | Banana petaloid($S_3$) |
| Pre mordanting | 0.26 | 0.32 | 0.29 |
| Simultaneous mordanting | | 0.35 | 0.32 |
| Post mordanting | | 0.37 | 0.35 |


**Figure 7 (a) Colour of cotton cloth dyeing with water extract (i) premordanting (ii) simultaneous mordanting (iii) post mordanting**

**Table 5 (b) Variations in Colour Density of Cotton Cloth after Fastness Test (pre mordanting)**

| Mordant | Colour densities/ Mordants | | |
|---|---|---|---|
| | Without ($S_1$) | Alum ($S_2$) | Banana petaloid($S_3$) |
| Before | 0.26 | 0.37 | 0.35 |
| Lighting | 0.24 | 0.29 | 0.31 |
| Washing | 0.21 | 0.31 | 0.30 |


**Figure 7 (b) Variation in colour of cotton cloth after fastness test (pre mordanting)**

**Table 5 (c) Variations in Colour Density of Cotton Cloth after Fastness Test (simultaneous mordanting)**

| Mordant | Colour densities/ Mordants | | |
|---|---|---|---|
| | Without ($S_1$) | Alum ($S_2$) | Banana petaloid($S_3$) |
| Before | 0.26 | 0.35 | 0.32 |
| Lighting | 0.24 | 0.32 | 0.3 |
| Washing | 0.21 | 0.33 | 0.29 |

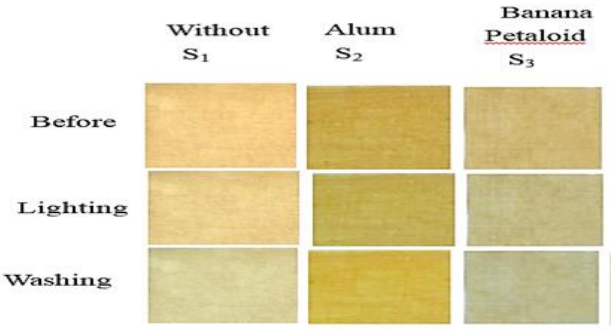


**Figure 7 (c) Variation in colour of cotton cloth after fastness test (simultaneous mordanting)**

**Table 5 (d) Variations in Colour Density of Cotton Cloth after Fastness Test (post mordanting)**

| Mordant | Colour densities/ Mordants | | |
|---|---|---|---|
| | Without ($S_1$) | Alum ($S_2$) | Banana petaloid($S_3$) |
| Before | 0.26 | 0.32 | 0.29 |
| Lighting | 0.24 | 0.3 | 0.26 |
| Washing | 0.21 | 0.31 | 0.25 |

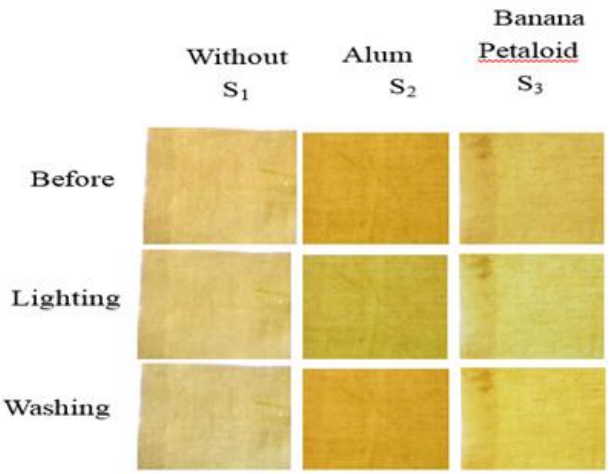


**Figure 7 (d) Variation in colour of cotton cloth after fastness test (post mordanting)**

## 4. CONCLUSION

The natural dyes are clinically more safe than their synthetic analogues in handling and use because of non-carcinogenic and biodegradable nature. Consequently, this study investigated the dyeing process for adsorption capacities (contact time, different temperatures 40, 60 and 80 ˚C) and colour fastness properties (pre-mordanting, simultaneous mordanting and post mordanting). According to the results, the rate of adsorptionof $S_1$, $S_2$ and $S_3$ dyeing on cotton cloth decreased at higher dyeing temperature which indicated the process was exothermic. Banana petaloid was waste of the bud but it can be used as natural mordant for this dyeing process. $S_3$ is found to be the adsorption properties as well as colour desity ability. So, adsorption and colour density are found to be in relation. The present study showed that natural dye extracted from bark of plant was non-toxic and cost effective for the eco-system. Colour fastness of $S_2$ (alum), $S_3$ (banana petaloid or natural mordant) can be applied in home-made dyeing process. The study carried out is significant because organic dyeing helps to presence the traditional art of dyeing and also provides employment and yields economic and ecological benefits.


## ACKNOWLEDGEMENT

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# Isolation and Characterization of Essential Oils from Houttuyania cordata Thunb (Fish Mint)

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**ABSTRACT:** *The whole plant of fish mint is a harbaceous prennial plant belonging to the Saururaceae family. Saururaceae plants constitute one of the main valuable sources of essential oil used in foods and medicinal purpose. This study was designed to examine the some phytochemical constituents, antioxidant activity, antimicrobial activity, total phenol content and to extract the essential oils from fish mint. The total phenolic content was determined by Folin-Ciocalteu reagent method. The total phenolic content of ethanol extract was higher than that of watery extract of the sample. The antioxidant activity was on the watery and 95 % ethanol extracts from fish mint sample by DPPH free radical scavenging assay method. In the screening of the antioxidant activity, ethanol extract of sample was found to be more potent than watery extract. In this study, a significant linear correlation was observed between the values for the total phenol content and antioxidant activity. The high contents of phenolic compounds indicated that these compounds contribute to the antioxidant activity. The essential oils of fish mint were isolated by stream distillation method. The chemical constituents of essential oils from fish mint were identified by Gas Chromatography - Mass Spectrometry method. The six compounds were found in essential oils of fish mint whereas, butylphthalide, hexadecanoic acid methyl ester, heptadecanoic acid methyl ester, methyl sterate, 9-octadecenoid acid methyl ester, and eicosanoic acid methyl ester.*

**Keywords:** fish mint, Saururaceae family, essential oils, Gas Chromatography- Mass Spectrometry method

## 1. INTRODUCTION

The Saururaceae family contains four genera and six species. *Houttuynia cordata* Thunb. (*H. cordata*), a perennial herb, is the sole member of the genus *Houttuynia* [8]. *Houttuynia cordata* Thunb is a flowering plant native to Japan, southern China and Southeast Asia, where it grows in moist shady places. It contains volatile oil, sterols, fatty acids, flavonoid and alkaloids [7]. Moreover, it possesses a variety of pharmacological functions including anti-platelet aggregation, anti-bacterial, anti-microbial, anti-tumor, anti-inflammatory, anti-leukemic and immunomodulatory effects [4]. *Houttuynia cordata* is a well know traditionally used medicinal material in the indigenous medicine systems of Southeast Asia. There is a long history of herbal medicine in far Eastern countries; in particular, Chinese people have utilized herbs and plants to treat various diseases for more than 8000 years. Fish mint is a herbaceous perennial plant with pungently orange-scented, heart-shaped leaves and tiny yellow flowers in spikes with usually 4 prominent white bracts at the base. The leaf has an unusual taste that is often described as fishy, so it is not enjoyed as universally as basil, mint or other more commonly used herbs. It is taken raw as salad and cooked along with fish as fish curry.

Fish mint is important traditional medicine of Southeast Asia, especially China, Japan and Thailand. Due to the various pharmacological activities this herb uses in many types of products, including food supplements, drugs, beverages and cosmetics. It is defective against blood deficiency, cholera and skin diseases and in snake bites. The herbs also contain young shoots and leaves juice is taken in case of cholera, dysentery and curing blood deficiency [8]. In other parts of North-east India this plant is used in stomach disorders and urine problems, to treat wound, in china used to treat earache and pneumonia. In Korea, it has been used for the treatment of cough, pneumonia bronchitis, dysentery, dropsy, leucorrhea, uteri is, eczema, herpes simplex, acne, chronic sinusitis and nasal polyps. In Thailand, it has been used for immune stimulation and as anticancer agent [6].

Essential oils are the fragrant oils that are present in many plants. Hundreds of plants yield essential oils that are used as perfumes, food flavorings, medicines, and as fragrant and antiseptic additives in many common products. Essential oils are natural products that plants produce for their own needs. In general, they are complex mixtures of organic compounds that give characteristic odor and flavor to the plants. They are located in different parts of the plant. They can be found in the root, stems, leaves, flowers, fruit and even seeds. These volatile compounds have diverse ecological functions, acting as defensive substances against microorganisms and herbivores, but can also be important to attract insects for the dispersion of pollens and seeds [2].

**Figure. 1.1 The plant of fish mint**

## 2. MATERIAL AND METHODS

### 2.1 Collection Of Plant Sample

Selected sample used in this work was the whole plants of (fish mint) *Houttuynia cordata* Thun. The sample was collected from Myitkyina Township, Kachin State, on December, 2018. The collected sample was washed with water and dried in air. The dried pieces were made into powder by using grinding machine. The powdered sample was stored in air-tight container to prevent moisture changes and other contaminations. The dried powdered sample was used for chemical and biological investigations.

### 2.2 Preliminary Phytochemical Investigation Of Sample

Preliminary phytochemical screening was done on the aqueous extract and the ethanolic extract of the powdered of the whole plant of fish mint. According to the reported methods ,the sample was investigated the presence and absence of phytochemical constituents such as alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, , saponins, starch, steroids, tannins and terpenoids.

### 2.3 Determination Of Total Phenol Content As Gallic Acid Equivalent In Sample

The total phenol content (TPC) in each crude extract was estimated by Folin-Ciocalteu reagent method. The prepared extract solution (1mL) was mixed with 5 mL of FCR reagent (1:10) and incubated for 5 minutes. Sodium carbonate solution (4 mL of 1 M ) was added to each tube and the tubes were kept at room temperature for 2 hours and the UV absorbance of reaction mixture was read at $\lambda_{max}$ 765 nm. The blank solution was prepared as the above procedure by using distilled water instead of sample solution. Total phenolic content was estimated as microgram Gallic acid equivalents per milligram of different extract (μg GAE/ mg).

### 2.4 Determination Of Antioxidant Activity Of Crude Extract From The Fish Mint By DPPH Free Radical Scavenging Assay

The free radical scavenging activity of crude extracts from the fish mint was measured using DPPH free radical scavenging assay. DPPH radical scavenging activity of watery and ethanol extracts from the fish mint was determined by UV-visible spectrophotometer [3].

### 2.5 Extraction Of Essential Oil from The Fish Mint By Steam Distillation Method

The essential oils from the whole plant of fish mint were extracted by steam distillation set. The sample of the whole plant of fish mint (100 g) was placed in the insert of glass jacket. The glass jacket is filled with distilled water and fitted to set which was joined to water condenser. When the glass jacket was heated, the condensed oils and water coming out from condenser will collected in the receiver flask. The oil was extracted with n-hexane in a separating funnel. The n-hexane was evaporated at 60-70 °C to get the essential oils which was then weighed until to be constant weight and kept in air tight bottle [1].

## 3. RESULT AND DISCUSSION

### 3.1 Preliminary Phytochemical Investigation Of The Whole Plant Of Fish Mint

The phytochemicals constituents present in the fish mint were investigated by test tube method. The phytochemical tests revealed that alkaloids, flavonoids, α-amino acids, carbohydrates, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids were present in sample. However, cyanogenic glycosides and starch were not detected in fish mint.

### 3.2 Total Phenol Content Of Crude Extracts From Fish Mint

The total phenol contents of watery and ethanol crude extracts of fish mint were evaluated with spectrophotometric method using Folin-Ciocalteu reagent. The absorbance can be measured at UV 765 nm. Gallic acid (3, 4, 5 - trihydroxybenzoic acid) was used to construct standard calibration curve. According to the analysis, the higher TPC (μg GAE/mg) was detected in ethanol extract (482.56 μg GAE/mg) than watery extract

(267.95 µg GAE/mg). This means that phenolic compounds were more soluble in ethanol.

### 3.3 Antioxidant Activity Of Crude Extracts From Fish Mint

The antioxidant activity was studied on the watery and 95 % ethanol extracts from fish mint sample by DPPH free radical scavenging assay method. This method is based on the reduction of colored free radical DPPH in ethanol solution by different concentration of the samples. The antioxidant activity was expressed as 50 % oxidative inhibitory concentration ($IC_{50}$). Determination of absorbance was carried out at wavelength 517 nm using UV-visible spectrophotometer. The $IC_{50}$ values were found to be 24.37µg/mL for 95 % ethanol extract and 40.69 µg/mL for water extract. Among these extracts, since the lower the $IC_{50}$ showed the higher the free radical scavenging activity, the 95 % ethanol extract was found to be slightly more effective than watery extract in free radical scavenging activity.

### 3.4 Identification Of Volatile Compounds From Fish Mint By GC-MS Method

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, molecular weight and structure of the components of the test materials were ascertained. The active compounds present in the essential oils of fish mint were identified by GC-MS analysis. The GC-MS analysis shows the presence of compounds in the volatile compounds of fish mint by comparing their retention times and by interpretation of their mass spectra. However, the available literature supports that total six compounds were identified in fish mint. Whereas, butylphthalide (MW= 190, RT= 12...32 mins), hexadecanoic acid methyl ester (MW= 270, RT= 17.79 mins), heptadecanoic acid methyl ester (MW = 284, RT= 19.57 mins), methyl sterate (MW =298, RT= 21.51 mins), 9-octadecenoid acid methyl ester (MW =296, RT = 21.12 mins), and eicosanoic acid methyl ester (MW = 326, RT =24.88 mins). The identified compounds and their retention time, molecular formula and molecular weight (MW) are presented in the in figures.

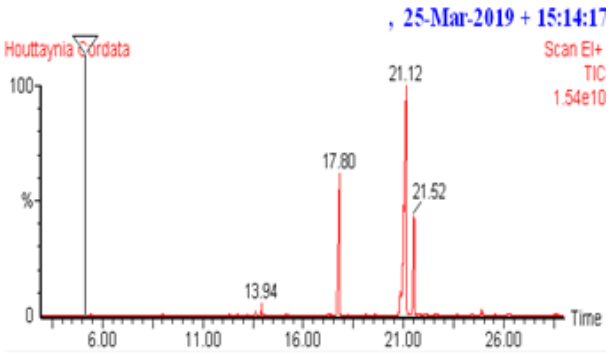


**Figure 3.1 GC Chromatograms of essential oils from fish mint according to retention time**

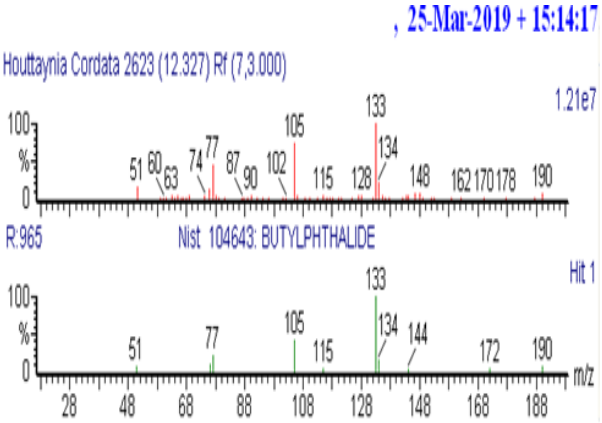


**Figure 3.2 Mass spectra of butylphalide (MW-190) in fish mint essential oils and in library data at retention time (12.32 mins)**

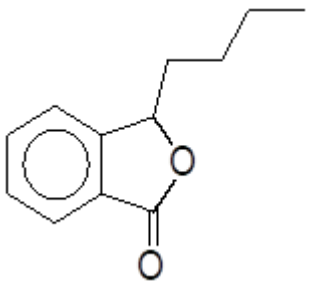

**Figure 3.3 Structure of butylphalide**

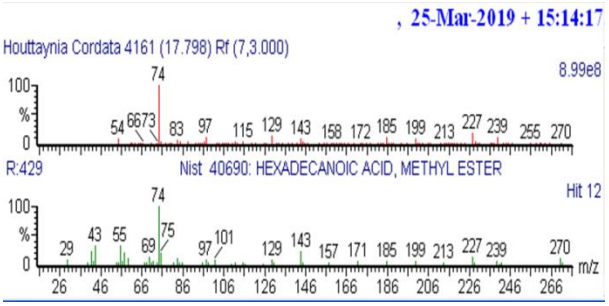


**Figure 3.4 Mass spectra of hexadecanoic acid, methyl ester (MW-270)) in fish mint essential oils and in library data at retention time (17.798 mins)**

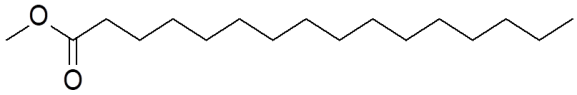

**Figure 3.5 Structure of hexadecanoic acid, methyl ester**

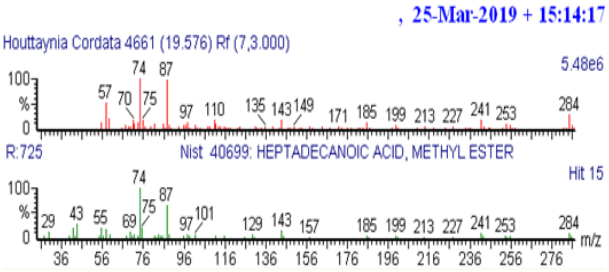


**Figure 3.6 Mass spectra of heptadecanoic acid methyl ester in fish mint essential oils and in library data at retention time (19.576 mins)**

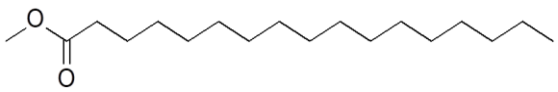

**Figure 3.7 Structure of heptadecanoic acid, methyl ester**

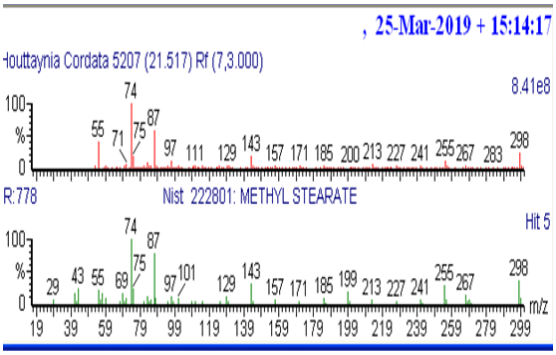


**Figure 3.8 Mass spectra of methyl stearate in fish mint essential oils and in library data at retention time (21.517 mins)**

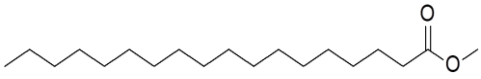

**Figure 3.9 Structure of methyl stearate**

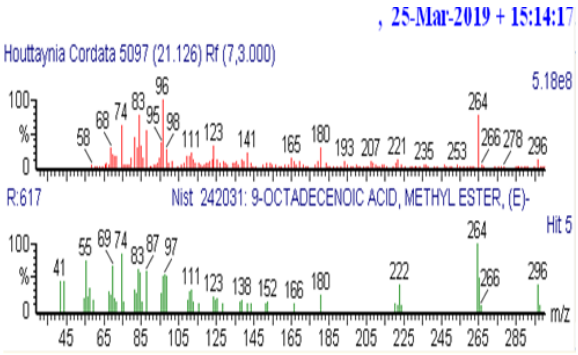


**Figure 3.10 Mass spectra of, 9-octadecanoic Acid, Methyl Ester in fish mint essential oils and in library data at retention time (21.126 mins)**

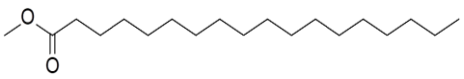

**Figure 3.11 Structure of 9-octadecenoic acid methyl ester**

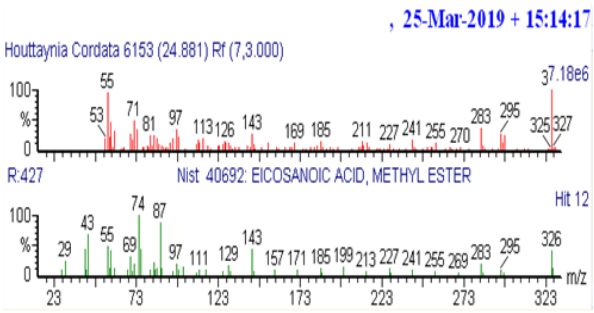


**Figure 3.12 Mass spectra of eicosanoic Acid, Methyl Ester in fish mint essential oils and in library data at retention time (21.126 mins)**

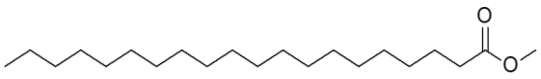

**Figure 3.13 Structure of eicosanoic acid, methyl ester**

## 4. CONCLUSION

The present study was undertaken to isolate and analyze the essential oils of the fish mint by GC-MS. In the present inquiry, 6 bioactive compounds have been identified from the essential oils of fish mint by Gas Chromatography-Mass Spectrometry (GC–MS) analysis. The six compounds were found in essential oils of fish mint whereas, butylphthalide, hexadecanoic acid

methyl ester, heptadecanoic acid methyl ester, methyl sterate, 9-octadecenoid acid methyl ester, and eicosanoic acid methyl ester.

## ACKNOLEDGEMENTS

The author feel indebted to Professor and Head Dr Ni Ni Than, Department of Chemistry, University of Yangon, Yangon, Myanmar, for her stimulating suggestions.

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# Preparation of Biodiesel from Waste Fried Palm Oil Using Zeolite Catalyst

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***Abstract*****:** *As crude oil price reach a new high, the need for developing alternate fuels has become acute. Alternate fuels should be economically attractive in order to complete with currently used fossil fuels. In this research work, biodiesel (methyl ester) was prepared from waste fried palm oil collected from a local restaurant in Hla-dan Market, Kama-yut Township, Yangon Region, Myanmar. The zeolite catalyst sample was collected from Myanmar Pioneer Star Co, Ltd. Methyl alcohol with zeolite as a catalyst was used for the transesterification process. The formation of fatty acid methyl ester (biodiesel) from palm oil was characterized by FTIR spectrosocopy. Moreover, GC-MS spectral data confimed that methtl ester of linoleic acid, oleic acid, stearic acid and palmitic acid were major components of waste fried palm oil methyl ester. The biodiesel was characterized by its physical and fuel properties incluing kinematic viscosity, specific gravity, acid value, flash point, water and sediment content, pour point, cetane index, copper strip corrosion, color and conradson carbon residue according to ASTM standards. To obtain a high quality biodiesel fuel that complies the specification of standard methods ( ASTM D 6751 ), some important variables such as volumetric ratio, types of catalysts, reaction time, temperature and catalyst concentration were selected. The operation variables used were oil : methanol volume ratio (2:1 to 5:1 ), catalyst concentrations ( 0.4 to 1.5 %), temperature ( 40 to 70*ºC *) and reaction times ( 3 to 6 h ). The evolution of the process was followed by determining the yield % and kinematic viscosity of methyl ester at different reaction conditions. The highest approximately 83 % methyl ester yield acquired under optimum conditions of 4 : 1 v/v ratio of oil to methanol, 0.6 % zeolite catalyst for 6 h reaction time. Production of biodiesel from waste fried palm oils for diesel substitute is particularly important because of the decreasing trend of economical oil reserves, environmental problems caused due to fossil fuel use and the high price of petroleum products in the international market.*

**Keywords**: Waste fried palm oil, biodiesel, zeolite catalysts, transesterification.

## 1. INTRODUCTION

Palm oil is a vegetable oil derived from the fleshly part of the oil palm fruit. The oil palm produces fruits having two types of oil, palm oil from the mesocrap and palm kernel oil from the kernel. The two oils differ widely in composition and properties. Palm oil has a wide range of uses due to its unique chemical oil composition. Palm oil containing more short chain triglycerides finds more application in fats in edible uses. [1]

Groundnut oil is very important vegetable oil in Myanmar. Nevertheless, palm oil is also widely used as cooking oil where it is a normal widely used as cooking oil where it is a normal practice of households blending oleic with groundnut and sesame oil. Palm oil is used, alone or with other oils, in an increasing number of foods. It is used around the world in such foods as margarine, shortening, cooking oil, soups, sauces, crackers and other baked goods and confectionary products. [2]

Biochemical research indicates that palm oil, which is high saturated fat and low in polyunsaturated fat, promotes heart disease. Though less harmful than partially hydrogenated vegetable oil, it is far more conductive to heart disease than such heart-protective oils as olive soy and canola.

Huge qualities of waste fried oils and animal fats are available throughout the world, especially in the developed countries. Management of such oils and fats pose a significant challenge because of their disposal problems and possible contamination of the water and land resources. Even though some of this waste fried oil is used for soap production, a major part of it is discharged into the environment.[3] As large amounts of waste fried oils are illegally dumped into rivers and landfills, causing environment pollution, the use of waste fried oil to produce biodiesel as petrodiesel substitute offers significant advantages because of the reduction in environmental pollution. Diesel fuel consumption significantly contributes to the formation of greenhouse gases and other global pollutant emissions. Klass pointed out that petroleum diesel is also the major source for the emission of NOx, SOx , CO, particulate matter and volatile organic compounds. Emission of such pollutants not only has negative impacts to the global environment but also severe impacts in human health due to their persistence in the environment.[4]

The use of waste fried palm oil as biodiesel feedstock reduces the cost of biodiesel production since the feedstock costs constitutes approximately 70-95 % of the overall cost of biodiesel production. Waste fried palm oil, which is otherwise wasted, is one of the most economical choices to produce biodiesel. Since one of

the major concerns on biodiesel production is the price of feedstock, utilization of waste fried oil significantly enhances the economic viability of biodiesel production [5].

## 2. MATERIALS AND METHODS

### 2.1 Collection of Waste Fried Palm Oil

Waste fried palm oil was collected from Hla-dan Market located in Kama-yut Township, Yangon Region, Myanmar on October, 2016. After being received, the sample was kept in the sealed airtight containers and placed in a cold and dark place.

### 2.2 Production of Waste Fried Palm Oil Methyl Ester

Waste fried palm oil methyl ester was prepared by using zeolite catalyst in methanol solvent. In this experiment the catalyst concentration of zeolite, alcohol / oil volume ratio, reaction time, reaction temperature and mixing intensity were changed and their effects on the progress of transesterification reactions were studied to get optimal reaction conditions for production of waste fried palm oil methyl ester. Optimal conditions were evaluated on the basis of methyl ester yield and their respectively viscosity.

### 2.3 Zeolite catalyzed transesterification of waste fried palm oil to methyl easter

The sample waste fried palm oil was filtered to remove the particle ( if any ) by simple filtration using a coffee filter. In order to eliminate the moisture, the sample was heated to 100 ºC. The water was separated out and densed at the bottom of the container. The water was then drained off to avoid steam explosion. The filtered and dewatered oil was used for biodiesel processing.

### 2.4 Optimization of reaction conditions Study of reaction time

Methanolysis of waste fried palm oil was carried out at different reaction times such as 3.0, 4.0, 5.0 and 6.0 h. The Zeolite catalyst concentration of 0.6% ( w/v of oil), MeOH / oil volume ratio of 1:4 ( v/v ), reaction temperature of 60 ºC and stirring speed of 600 rpm were maintained with the reaction time variations.

### 2.5 Study of catalyst concentration

Methanolysis of waste fried palm oil was carried out using zeolite catalyst at 0.3, 0.5, 0.6, and 1.0 % ( w/v of oil ) concentration level were varied at a specific condition set of 1:4 ( v/v ) MeOH to oil volume ratio, 6 h reaction time, reaction temperature at 60 ºC and 600 rpm of mixing intensity.

### 2.6 Study of volume ratio of methanol and oil

Studies were done by varying the MeOH / oil volume ratios in the range of 1:5, 1:4, 1:3 and 1:2 ( v/v ) in the preparation of palm oil methyl ester. The constant zeolite catalyst concentration of 0.6 % ( w / v of oil ), 6 h reaction time, reaction temperature at 60 ºC and mixing intensity of 600 rpm were maintained.

### 2.7 Study of reaction temperature

Studies were undertaken at different temperature of 40, 50, 60 and 70 C. In all experiments, methanol to oil volume ratio of 1:4 ( v/v ), zeolite catalyst concentration at 0.6 % ( w/v of oil ), 6 h reaction time and 600 rpm mixing intensity were maintained.

## 3. RESULTS AND DISCUSSION

Waste fried palm oil as a feed stock for Biodiesel Production Oil palm ( *Elaeis guineensis* Jacq ) a member of the composite family is an important oil seed crop worldwide, yielding approximately 45-50 % oil ( dehulled seed mass basis ). Waste fried palm oil, used in the production of biodiesel, manufacture of soaps, glycerin and candles.

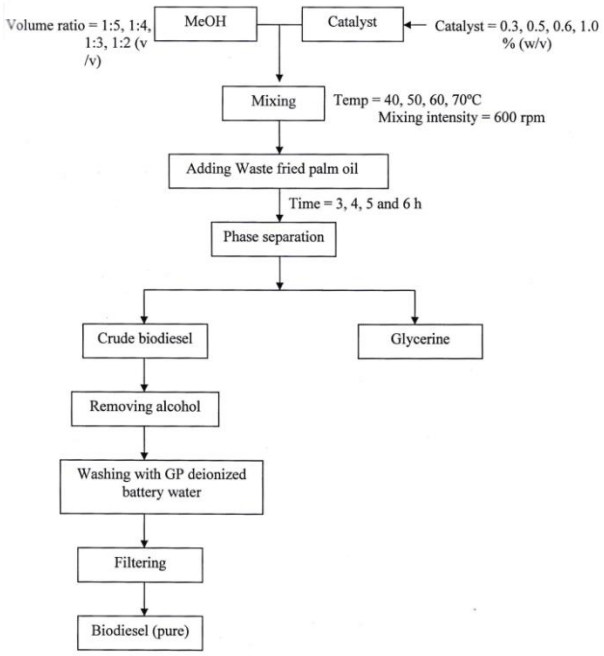


**Figure 1. Process flow for transesterification of waste fried palm oil**

**Table 1. Physico chemical properties of waste fried palm oil**

| No. | Physicochemical properties | Present work | Literature* |
|---|---|---|---|
| 1 | Specific gravity at 60º/60ºF | 0.93 | 0.900~0.926 |
| 2 | Kinematic viscosity at 40ºC(cSt) | 63.40 | 33.9 |
| 3 | Acid value (mg KOH/g) | 0.37 | 2.5~5.2 |
| 4 | Free fatty acid, FFA (%) | 0.18 | 1.5~3.0 |
| 5 | Saponification value (%) | 140.94 | 180~200 |
| 6 | Unsaponifiable value (%) | 4.40 | 1.5 |
| 7 | Moisture (%) | 1.5 | 1.6 |

*ASTM, 2002,*AOCS, 1995

**Table 2. Effect of Methanol on Yield Percent of Biodiesel Prepared from Waste Fried Palm Oil in the Presence of Zeolite Catalyst**

| | | |
|---|---|---|
| Oil | = | 100 mL |
| Zeolite Catalyst | = | 0.6 g |
| Temperature | = | 60 °C |
| Time | = | 6 h |

| Oil | : | Methanol (v/v) | Yield % of Prepared Biodiesel % (v/v) |
|---|---|---|---|
| 5 | : | 1 | 80% |
| 4 | : | 1 | 83% |
| 3 | : | 1 | 60% |
| 2 | : | 1 | 65% |

Note: (1) When oil and methanol ratio (6:1) v/v was used more soap formation occurred.

(2) At the ratio of oil to methanol 4:1, the best yield percent of biodiesel (83%) was obtained.

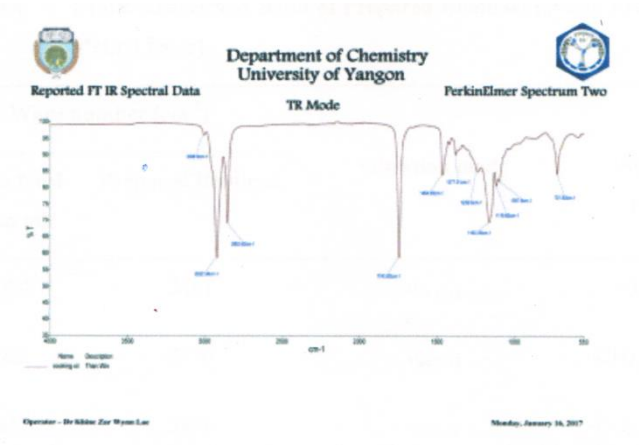


**Figure 2. FT IR spectrum of waste fried palm oil**

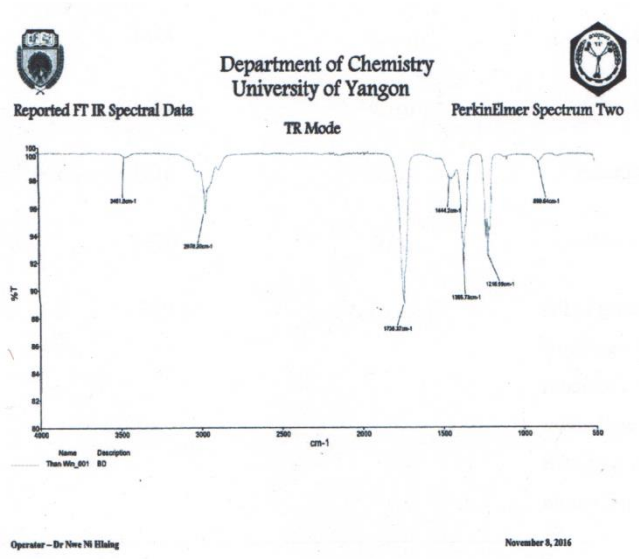


**Figure 3. FT IR spectrum of prepared biodiesel**

**Table 3. FT IR Absorption Band of Prepared Biodiesel (Waste Fried Palm Oil Methyl Ester)**

| Properties | Waste Fried POME | ASTM test method | Limits |
|---|---|---|---|
| Specific Gravity at $60^0$/60 F (Unitless) | 0.08851 | D 1298 | 0.8829~0.8873 |
| Kinematic viscosity at $40^0$C (Cst) | 4.67 | D 445 | 1.9 ~ 6.0 |
| Acid number mg(KOH) /g | 0.67 | D 664 | 0.8 ~ 1.5 max |
| Flash Point $^0$C | 164 | D 93 | 100 min |
| Pour Point $^0$C | 15 | D 97 | -15 to 10 |
| Cetane Index | 48 | D 976 | 48 ~ 65 |
| Copper Strip Corrosion | No.(1)a | D 130 | No.3 max |
| Canradson Carbon residue(%) | 0.0158 | D 189 | 0.05 max |
| Water and Sediment (%) | Trace | D 2709 | 0.05 max |
| Yield % liquid fuel | 83% | - | - |

Waste Fried POME - Using Zeolite Catalyst ASTM, 2002
POME = Palm Oil methyl ester

**Table 4. Fuel properties of prepared biodiesesl (waste fried POME) from acid-catalyzed transesterification compared with ASTM test method reported data**

| Wave number ($cm^{-1}$) | | | |
|---|---|---|---|
| Waste fried Palm oil | Prepared Biodiesel | Vibration mode | Structural Features |
| 3000 | 3481 | $\nu_{=C-H}$ | = CH alkene |
| 2922 | 2970 | $\nu_{as\ C-H}$ | $-CH_2$, $CH_3$ groups |
| 2852 | 2856 | $\nu_{S\ C-H}$ | $-CH_2$, $CH_3$ groups |
| 1743 | 1738 | $\nu_{C=O}$ | Carbonyl group of ester |
| 1464 | 1444 | $\delta_{as\ C-H}$ | $-CH_3$, $CH_2$- |
| 1379 | 1365 | $\delta_{s\ C-H}$ | $-CH_3$, $CH_2$- |
| 1239 | 1216 | $\nu_{C-O-C}$ | Ester group |
| 1097 | 1020 | $\nu_{C-O}$ | C-O- of ester |
| 721 | 899 | $\delta_{CH-CH-CH_2}$ | Alkyl groups Rocking vibration mode of a chain of methylene longer than four carbon atoms (cis alkene) |

*Mohan, Johan, 2000

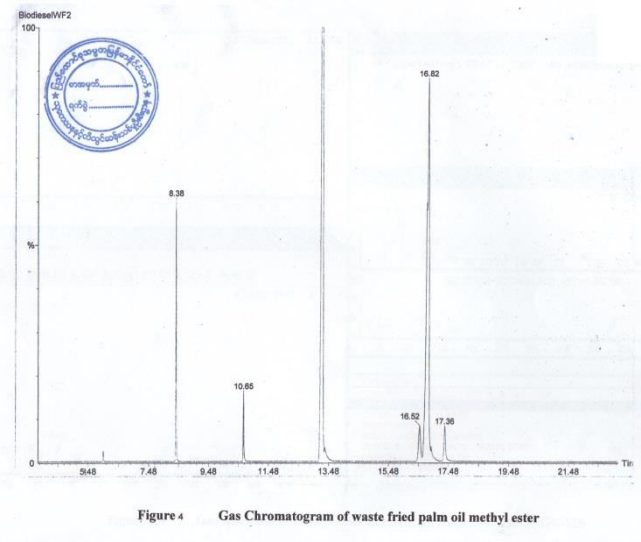


Figure 4 Gas Chromatogram of waste fried palm oil methyl ester

**Figure 4. Gas chromatogram of waste fried palm oil methyl ester**

**Table 4. GC-MS structural Assignment Data for waste fried palm oil methyl ester**

Table 4 GC-MS Structural Assignment Data for Waste fried Palm Oil Methyl Ester

| Retention time (min) | Molecular Weight | Compound |
|---|---|---|
| 3.701 | 158 | Octanoic Acic, methyl ester ($C_9H_{18}O_2$) |
| 5.968 | 186 | Decanoic acid, methyl ester($C_{11}H_{22}O_2$) |
| 13.282 | 270 | Hexadecanoic acid, methyl ester (methyl palmitate) C 16:0 |
| 10.647 | 242 | Methyl Tetradecanoate ($C_{15}H_{30}O_2$) |
| 16.834 | 296 | 9-Octadecanoic acid, methyl ester (methyl oleate) C 18:1 |
| 8.833 | 214 | Dodecanoic acid, methyl ester ($C_{13}H_{26}O_2$) |

From the table, the properties of waste fried oil is nearly similar value to the ASTM test reported data. So, the waste fried oil is used to the biodiesel. It is less toxic than petroleum diesel. Biodiesel is normally considered safe for disel fuel.

## 4. CONCLUSIONS

Some physicochemical characteristics of the prepared oil were determined by standard AOCS methods and these parameters such as specific gravity ( 0.93 ), viscosity value L3,RPM 100, (cP) ( 63.40 cSt ), acid value ( 0.37 mg/g), free fatty acid ( 0.18 ), iodine value ( 55.8 0), unsaponifiable matter ( 4.40 ) and moisture content (0.34 % ) were also observed. The transesterification reaction of waste fried palm oil by means of methanol, using zeolite as a catalyst, was also studied. Results showed that the optimum conditions of waste fried palm oil methyl ester production are 4:1 oil / methanol ratio and 0.6 % catalyst concentration in 6 h reaction time. The highest approximately 83 % methyl ester yield was acquired under the optimum condition. The product of final biodiesel was determined by FTIR and GC-MS analyzed and found that the pure biodiesel contains mainly methyl esters of linoleic acid, oleic acid, stearic acid and palmitic acid in the final products. The biodiesel was characterized for its physical and fuel properties using ASTM standard methods for biodiesel fuel quality assurance. From this tests, the flash point was found to be 164ºC, copper strip corrosion was N0.1 ( a ), color was 1.0, conradson carbon residue was 0.0158 % wt, specific gravity at 60º /60º Fwas 0.8851, water and sediment was trace amount, total acid number was 0.67 mg KOH/g, Kinematic viscosity at 40ºC was 4.67 cSt, cetane index was 48, and pour point was 15 ºC.

Production of biodiesel from waste fried palm oils for diesel substitute is particularly important because of the decreasing trend of economical extracts oil reserve and the environmental problems caused due to the use of fossil fuel.It can be used in most diesel engines and emits less air pollutants and greenhouse gases other than nitrogen oxides. It safer to handle and has the same energy effiency as petroleum diesel. Waste fried palm oil can be an important source for biodiesel production in Myanmar as there is large quantity of waste fried palm oil available. Use of waste fried palm oil helps improve the biodiesel economics.

## ACKNOWLEDGMENT

The authors feel indebted to Professor Dr. Pho Kaung, Rector, University of Yangon, Yangon, Myanmar, for his stimulating suggestions.

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# Phytochemical Investigation and Antioxidant Activities of Three Selected Medicinal Plants from Ayeyarwady Region, Myanmar

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**ABSTRACT-***An antioxidant is a molecule that inhibits the oxidation of other molecules. Antioxidants are widely being used as dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Natural antioxidants can be found in fruits and vegetables. In the present research, the leaves extracts of Calotropisgigantea R.Br. (Mayo), the fruits extracts of Haplophragmaadenophyllum (Wall.) Dop. (Phet-tham), and the leaves and roots extracts of GarciniacowaRoxb. (Taung-ta-le) were chosen for investigation of some phytoconstituents and antioxidant activities. Phytochemicals are variety of non-nutritional biologically active compounds occurring in plant foods. In comparison with all three plants crude extracts, the antioxidant activities were demonstrated in a trend of EtOAc extracts of leaf of Taung-ta-le >EtOAc extract of root of Taung-ta-le > Watery extract of fruits of Phet-tham> PE extract of leaves of Mayo. In Myanmar, no scientific study was carried out to assess antioxidant activities of these three plants. Therefore, present study was conducted to determine the phyytochemical constituents and antioxidant activities of these three plants. In the recent years, there has been increasing interest in certain compounds having enormous health effects which include antioxidant and phytochemicals.*

**Keywords:** CalotropisgiganteaR.Br.,Haplophragmaadenophyllum (Wall.) Dop.,GarciniacowaRoxb. phytoconstituents, antioxidant activity

## I. INTRODUCTION

### (1) *Calotropisgigantea*R.Br.

*Calotropisgigantea*R.Br. (Mayo), a member of the Apocynaceae family, is a well-known plant throughout the tropical world and they are native to the tropical and subtropical parts of Asia and Africa [1]. These plants are commonly known in English as Giant Milk Weeds or Swallow-worts.

They are commonly known as milkweeds because of the latex they produce. Calotropis species are considered common weeds in some parts of the world. Flowers of these plants are fragrant and are often used in making floral tassels in some mainland Southeast Asian cultures. Fibers of these plants are called madar or mader [2]. Calotropis species are usually found in abandoned farmland.

Each flower of *Calotropisgigantea* R.Br. (Mayo) consists of five pointed petals and a small "crown" rising from the center which holds the stamens. In India, Thailand, Philipines and Hawaii the long-lasting flowers of *Calotropisgigantea* R.Br. are used in various floral arrangements in temples and in rosaries. It is also widely planted as an ornamental [3].

In Myanmar, the leaves of *Calotropisgigantea*R.Br. (Mayo) are useful in the treatment of paralysis, arthralegia, swellings, and intermittent fevers.

### 1.1 Botanical Aspect of *Calotropisgigantea* R.Br.

Family :Apocynaceae
Sub-family : Asclepiadaceae
Botanical name :*Calotropisgigantea*R.Br.
Myanmar name : Mayo
English name : Swallow-Wort, Milk weed
Synonyms : *Asclepiasgigantea*L.
Part used : Leaves

**Figure 1.*Calotropisgigantea*R.Br. (Mayo)**

### (2) *Haplophragmaadenophyllum* (Wall.) Dop.

*Haplophragmaadenophyllum* (Wall.)Dop. (Phet-tham), a member of the Bignoniaceae family, is a deciduous tree grown in tropical and subtropical climates of Southeast Asia and Africa[4]. These trees are commonly known in English as Karen wood. This tree is grown as an ornamental plant in parks and gardens due to the unique magical shape of its pods that distinguishes it amongst other trees of horticultural significance. *H adenophyllum* have been used for various ailments treatment which includes cancer, gastrointestinal disorders, cholera, rheumatoid arthritis, hepatic disorders, leucorrhea and diabetes [5].

### 1.2 Botanical Aspect of *Haplophragmaadenophyllum* (Wall.) Dop.

Family : Bignoniaceae

Botanical name :s*Haplophragmaadenophyllum*(Wall.) Dop.
Myanmar name : Phet-tham
English name : Karen wood
Synonyms : Fernandoaadenophyllum
Part used : Fruits

**Figure 2.***Haplophragmaadenophyllum* **(Wall.)Dop. (Phet-tham)**

(3) *Garcinia**cowa***Roxb.**

*Garcinia**cowa***Roxb. (Taung-ta-le) grows widely in tropical rainforest area of Southeast Asia, West and East Africa, and central and South America[6]. *Garcinia* genus plants are well known to be rich in a variety of oxygenated and prenylatedxanthones which were associated to the therapeutic [7]. The plants are small to medium size trees, which grow up to 30 m in height and widely distributed in the tropical regions of the world [8].

This genus has various biological activities such as antioxidant [9] cytotoxic [10] and antimicrobial activities [11]. Due to these properties, *Garcinia* genushas attracted attraction as important sources formedicinal treatment. Moreover, natural products of plants origin are important sources of new chemical compounds leading to the future discovery of new drugs and more effective treatments. Some phytochemical investigation on the root was reported [12]. In addition, the order activities such as antimicrobial and antioxidant activities were evaluated for crude extracts.

### 1.3 Botanical Aspect of *Garciniacowa***Roxb.**

Family :Gluttiferae
Sub-family : Clusiaceae
Botanical name :*Garciniacowa*Roxb.
Myanmar name :Taung-ta-le
English name : Cowa fruit, Cow tree
Synonyms :*Garciniaroxburghii Wight*
Part used : Leaves and Roots

**Figure 3.***Garciniacowa***Roxb. (Taung-ta-le)**

### 1.4 Phytochemicals

Phytochemicals are naturally occurring chemicals produced by plants. They are biological active and may affect human health, however, unlike vitamins and minerals, they are not considered to be essential nutrients. Dietary source of phytochemical include fruits, vegetables, nuts, seeds and legumes. In broad terms, chemicals that plants synthesize during all or part of their normal life cycles are called phytochemical. These processes involve plant metabolism and help explain, for example, how wood created cellulose, sugar cane forms sucrose, and opium poppies produce morphine. Within the context of nutrition and natural health, phytochemical refers only to plant chemical that humans eat and use medicinally. The bioactive phytochemical in these plants have significant positive effects on human metabolism[13].

### 1.5 Antioxidants

Antioxidants are found in vegetables, fruits, grain cereals, eggs, meat, legumes and nuts. Antioxidants such as lycopene and ascorbic acid can be destroyed by long term storage or prolonged cooking. Other antioxidant compounds are more stable, such as the polyphenolic antioxidants in foods such as whole-wheat cereals and tea. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and even altitude sickness. Antioxidant also have many industrial used, such as preservatives in food and cosmetics and to prevent the degradation of rubber and gasoline [14].

## 2. MATERIALS AND METHODS

### 2.1.Sampling of Plant Material and Identification

The leaves of *Calotropisgigantea*R.Br. (Mayo) from Ngawon Kyung Tha Street, Pathein Township, the fruits of *Haplophragmaadenophyllum*(Wall.) Dop.(Phet-tham) from LaputtaTownship, Ayeyarwady Region and the leaves and roots of *Garciniacowa*Roxb.

(Taung-ta-le) from Kyat Paung village, Pathein Township, Ayeyarwady Region, Myanmar, were collected during January to February, 2018. The collected fresh fruit samples were washed and air dried at room temperature for two weeks and dried leaves, fruits and roots were ground into powder and then they were stored in air tight container, separately.

### 2.2 Preliminary Phytochemical Investigation of Three Selected Medicinal Plants

In order to classify the types of organic constituents present in samples, preliminary phytochemical tests such as alkaloids, α- amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, phenolic compounds, reducing sugars, saponin, starch, steroids, tannins and terpenoids on

leaves, roots and fruits samples were carried out according to the appropriate reported methods such as the presence of alkaloid was tested by Dragendorff's, Mayer's, and Hager' reagents. Ninhydrin test was performed for amino acids. Bromine test has been performed for glycosides, and so on [15, 16, 17, 18].

### 2.3 Preparation of Crude Extracts by Direct Extraction Method for Screening of Antioxidant Activities

Dried powdered each sample (50 g) was extracted with 150 mL of pet-ether (60-80 °C) for 6 h by using Soxhlet extractor. The filtrate was concentrated by removal of the solvent under reduced pressure to give the respective PE crude extract. Ethyl acetate, 95 % ethanol and watery extracts were also prepared by similar manner mentioned in above procedure. Each plant extract was dried at normal pressure on a water bath and stored under refrigerator for screening some bioactivities.

### 2.4 Bioactivity

#### 2.4.1 *In Vitro* Screening of Antioxidant Activity of Some Crude Extracts from the Leaves, Fruits and Roots of Three Selected Medicinal Plants by DPPH Assay

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay was chosen to assess the antioxidant activities of the plant samples [19, 20, 211, 22, 23].

PE, EtOAc, 95 % EtOH, and $H_2O$ extracts of the leaves of Mayo, fruits of Phet-tham, and leaves and roots of Taung-ta-le samples were used for experiment..

The Chemicals used in this study are 95 % EtOH, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid.

*Procedure*

DPPH radical scavenging activity was determined by UV spectrophotometric method. The control solution was prepared by mixing 1.5 mL of 60 μM DPPH solution and 1.5 mL of 95 % EtOH. The sample solution was also prepared by mixing thoroughly 1.5 mL of 60 μM DPPH solution and 1.5 mL of test sample solution. The solutions were allowed to stand at room temperature for 30 minutes. After 30 minutes, the absorbance of these solutions was measured at 517 nm by using UV spectrophotometer. Absorbance measurements were done in triplicate for each solution and then mean values so obtained were used to calculate percent inhibition of oxidation by the following equation.

Where,

$$\%\,\text{Oxidative Inhibition} = \frac{A_{DPPH} - (A_{Test\,sample} - A_{Blank})}{A_{DPPH}} \times 100$$

A DPPH = absorbance of DPPH in 95% EtOH solution

A Test sample = absorbance of (sample + DPPH) solution

A Blank = absorbance of (sample + 95% EtOH solution)

$$\text{Average, } \overline{X} = \frac{X_1 + X_2 + X_3 + \cdots + X_n}{n}$$

Standarddeviation(SD)=

$$\sqrt{\frac{(\overline{x} - x_1)^2 + (\overline{x} - x_2)^2 + (\overline{x} - x_3)^2 + \cdots + (\overline{x} - x_n)^2}{n-1}}$$

where, $\overline{X}$ = average % inhibition of oxidation

$x_1, x_2, x_3, \ldots, x_n$ = % inhibition of test sample solution

n = number of times

Then, $IC_{50}$ (50 % oxidative inhibitory concentration values) was calculated by linear regressive excel programme.

## 3. RESULTS AND DISCUSSION

Preliminary phytochemical investigation was carried out to know the different types of phytoconstituents present in the samples. The investigation was carried out according to the test tube methods. The results show that the leaves of Mayo contain alkaloids, α-amino acids, glycosides, steroids, saponins and tannins. But carbohydrates, cyanogenic glycosides, flavonoids, phenolic compounds, reducing sugars, starch and terpenoids were found to be absent in the leaves of Mayo.

In the phytochemical investigation of fruits of Phet-tham, the results showed the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids. But starchs, and cyanogenic glycosides were found to be absent in the fruits of Phet-tham.

From the investigation of phytoconstituents of Taung-ta-le, the results showed that both LTTL and RTTL contain α-amino acids, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins and tannins. Moreover, carbohydrates, starch and terpenoids were found to be present in the RTTL. Alkaloids and steroids were present in LTTL. But cyanogenicglycosides were found to be absent in both of the samples. The constituents such as alkaloids, phenolic compounds, terpenoids, flavonoids and steroids present in the sample may contribute to possess bioactivities such as antimicrobial, antioxidant, anticancer, antitumor, antipyretic, antiulcer and diuretic properties.

### 2.5 Antioxidant Activities of Three Selected Medicinal Plants

#### *(a) In vitro* antioxidant activities of some crude extracts from the leaves of Mayo

The antioxidant activities of 95 % EtOH, EtOAc, PE and $H_2O$ extracts of the leaves of Mayo were studied by DPPH (1, 1-diphenyl-2-pricryhydrazyl) free radical scavenging UV spectrophotometric assay

method. This method is based on the reduction of coloured free radical DPPH in ethanolic solution by different concentrations of each sample.

The antioxidant activity was expressed as 50 % oxidative inhibitory concentration ($IC_{50}$). The lower the $IC_{50}$ value, the higher the antioxidant activity of the sample. In this experiment, ascorbic acid was used as a standardFigure 4, 5, 6, and 7. The antioxidant activities of the crude extracts of all samples were determined for five different concentrations; 0.625 µg/mL, 1.25 µg/mL, 2.5 µg/mL, 5 µg/mL and 10 µg/mL of each samples in 95 % EtOH solvent.

The absorbance of control solution (DPPH in 95 % EtOH solvent), blank solution (sample in 95 % EtOH) and sample solution (sample + DPPH in 95 % EtOH) were measured at wavelength 517 nm using UV spectrophotometer. From the average values of percent inhibition, $IC_{50}$ values (50 % inhibition concentration) were calculated by linear regressive excel programme. The percent oxidative inhibition and $IC_{50}$ values of all crude extracts of the leaves of Mayo are summarized in Table 1.

From these experimental results, it was found that as the concentrations increased, the absorbance values were found to decrease and the antioxidant activity increased.

In the leaves of Mayo, the antioxidant activities were found to be PE extract ($IC_{50}$ = 6.11 µg/mL) >EtOAc extract ($IC_{50}$ = 6.89 µg/mL) > $H_2O$ extract ($IC_{50}$ =9.54 µg/mL) > 95 % EtOH extract ($IC_{50}$= 9.78 µg/mL).

In the present study all crude extracts of the leaves of Mayo were found to have antioxidant activity. Moreover, PE extract of the leaves of Mayo ($IC_{50}$ =6.11 µg/mL) showed the highest antioxidant activity among the tested four crude extracts.

**Table 1. Percent Oxidative Inhibition and $IC_{50}$ Values of Crude Extracts from the Leaves of Mayo and Standard Ascorbic Acid**

| Extracts | Percent Oxidative Inhibition (%) (mean ±SD) in different concentration (µg/mL) | | | | | $IC_{50}$ µg/mL |
|---|---|---|---|---|---|---|
| | 0.625 | 1.25 | 2.5 | 5 | 10 | |
| Mayo (watery) | 18.99±3.57 | 19.79±6.04 | 24.7±0.40 | 25.89±1.05 | 52.46±0.61 | 9.54 |
| Mayo (EtOH) | 9.02±0.86 | 13.82±1.28 | 19.19±1.15 | 26.68±1.16 | 51.06±3.10 | 9.78 |
| Mayo (EtOAc) | 17.41±4.29 | 23.41±0.69 | 30.65±0.87 | 44.59±1.92 | 58.93±2.90 | 6.89 |
| Mayo (PE) | 13.69±0.59 | 12.48±1.16 | 28.73±1.78 | 44.02±1.82 | 71.02±2.16 | 6.11 |
| Ascorbic acid | 27.58±0.04 | 32.91±0.03 | 66.31±0.04 | 70.45±0.04 | 83.32±0.04 | 1.89 |

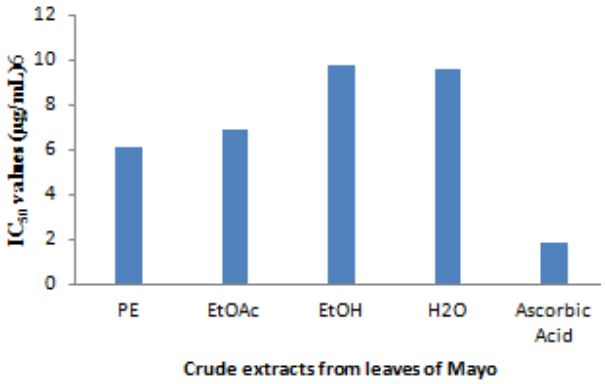


**Figure 4. Bar graph of $IC_{50}$ values (µg/mL) vs various crude extracts from the leaves of Mayo and standard ascorbic acid**

***(b)In vitro* antioxidant activities of some crude extracts from the fruits of Phet-tham**

The antioxidant activities of PE, EtOAc, 95 % EtOH and $H_2O$ extracts of the fruits of Phet-tham were also studied by DPPH (1, 1-diphenyl-2-pricryhydrazyl) free radical scavenging UV spectrophotometric assay method. The percent oxidative inhibition and $IC_{50}$ values of all crude extracts of the fruits of Phet-tham are summarized in Table 2 and Figure 5.

In the fruits of Phet-tham, the antioxidant activity were found to be $H_2O$ extract ($IC_{50}$ = 3.72 µg/mL) >EtOAc extract ($IC_{50}$ = 4.52 µg/mL) > PE extract ($IC_{50}$ = 8.78 µg/mL)> 95 % EtOH extract ($IC_{50}$ = 9.63 µg/mL). All crude extracts of the fruits of Phet-tham were also found to have good activity. Moreover, $H_2O$ extract of the fruits of Phet-tham ($IC_{50}$ = 3.72 µg/mL) showed the highest antioxidant activity among tested four crude extracts.

**Table 2. Percent Oxidative Inhibition and $IC_{50}$ Values of Crude Extracts from the fruits of Phet-tham and Standard Ascorbic Acid**

| Extracts | Percent Oxidative Inhibition (%) (mean ±SD) in different concentration (µg/mL) | | | | | $IC_{50}$ µg/mL |
|---|---|---|---|---|---|---|
| | 0.625 | 1.25 | 2.5 | 5 | 10 | |
| Phet-tham (PE) | 1.34±0.15 | 1.05±3.76 | 18.24±0.68 | 39.58±1.57 | 51.51±0.53 | 8.78 |
| Phet-tham (EtOAc) | 19.39±0.23 | 34.38±0.23 | 45.17±0.23 | 64.21±0.31 | 70.66±0.31 | 4.52 |
| Phet-tham (EtOH) | 2.69±0.15 | 2.24±0.15 | 8.19±2.21 | 22.34±2.48 | 53.32±0.43 | 9.63 |
| Phet-tham ($H_2O$) | 21.43±0.30 | 38.13±0.23 | 46.57±0.23 | 68.51±0.15 | 79.71±0.23 | 3.72 |
| Ascorbic acid | 14.04±2.09 | 54.83±2.48 | 72.44±3.83 | 77.13±1.47 | 87.4±2.37 | 1.87 |

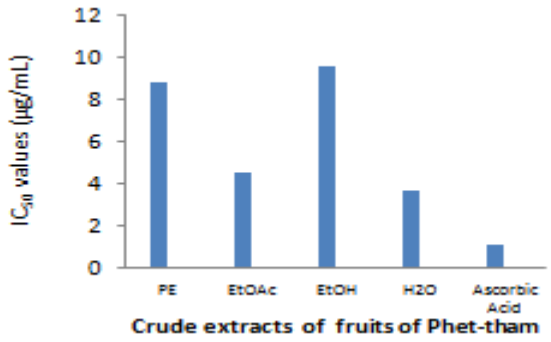


**Figure 5. Bar graph of $IC_{50}$ values (µg/mL) vs various crude extracts from the fruits of Phet-tham and standard ascorbic acid**

***(C)In vitro* antioxidant activities of some crude extracts from the leaves and roots of Taung-ta-le**

The antioxidant activities of 95 % EtOH, EtOAc, PE and $H_2O$ extracts of the LTTL and RTTL were also studied by DPPH (1, 1-diphenyl-2-pricryhydrazyl) free radical scavenging UV spectrophotometric assay method.

The percent oxidative inhibition and $IC_{50}$ values of all crude extracts of the LTTL and RTTL are summarized in Tables 3,4 and Figure 6 and 7 . From these experimental results, it was found that as the concentrations increased, the absorbance values were found to decrease and the antioxidant activity increased.

The antioxidant activity of crude extracts of LTTL was found to be in the order of EtOAc extract ($IC_{50}$ = 1.34 µg/ mL) > $H_2O$ extract ($IC_{50}$ = 1.36 µg/mL) > PE extract ($IC_{50}$ = 1.56 µg/ mL) > 95% EtOH extract ($IC_{50}$ = 5.06 µg/mL). The antioxidant activity of crude extracts of RTTL was found to be in the order of EtOAc extract ($IC_{50}$ = 3.71 µg/ mL) > 95% EtOH extract ($IC_{50}$ = 5.71 µg/mL) > $H_2O$ extract ($IC_{50}$ = 6.27 µg/ mL) > PE extract ($IC_{50}$ = 13.31 µg/mL).

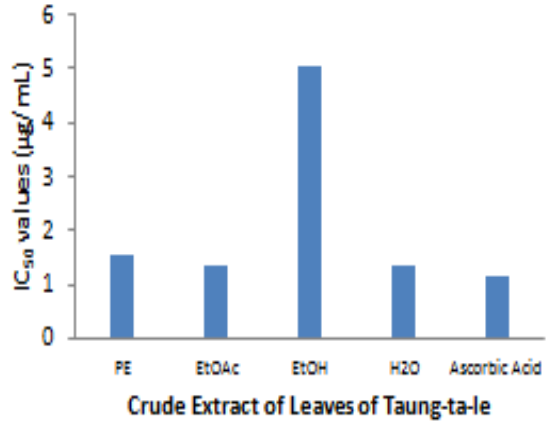


**Figure 6.Bar graph of $IC_{50}$ values (µg/mL) vs various crude extracts from the leaves of Taung-ta-le and standard ascorbic acid**

**Table 4. Percent Oxidative Inhibition and $IC_{50}$ Values of Crude Extracts from the Roots of Taung-ta-le and Standard Ascorbic Acid**

| Extracts | Percent Oxidative Inhibition (%) (mean ±SD) in different concentrations (µg/ mL) | | | | | $IC_{50}$ (µg/ mL) |
|---|---|---|---|---|---|---|
| | 0.625 | 1.25 | 2.5 | 5 | 10 | |
| PE | 1.97±0.56 | 4.26±0.78 | 7.25±2.26 | 21.33±0.66 | 36.00±2.08 | >10 |
| EtOAc | 32.42±2.79 | 40.05±0.40 | 48.97±0.57 | 62.61±0.56 | 74.74±0.40 | 3.71 |
| EtOH | 7.00±0.83 | 21.38±1.20 | 42.00±0.69 | 48.57±0.64 | 73.17±0.60 | 5.71 |
| $H_2O$ | 0.00±1.05 | 18.61±0.78 | 31.41±0.40 | 41.97±0.48 | 73.81±0.88 | 6.27 |
| Ascorbic acid | 14.04±2.09 | 54.83±2.48 | 72.44±3.83 | 77.13±1.47 | 87.40±2.37 | 1.17 |

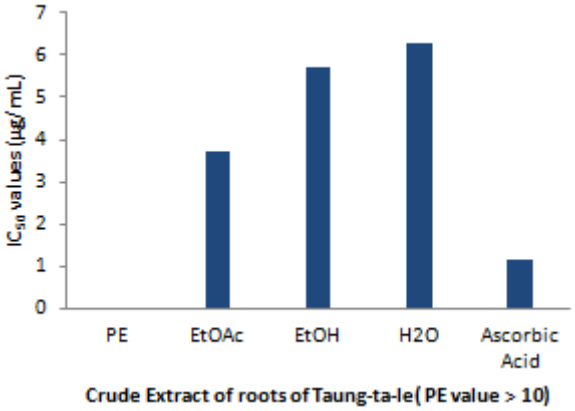


**Figure 7. Bar graph of $IC_{50}$ value (µg/mL) vs various crude extracts from the roots of Taung-ta-le and standard ascorbic acid**

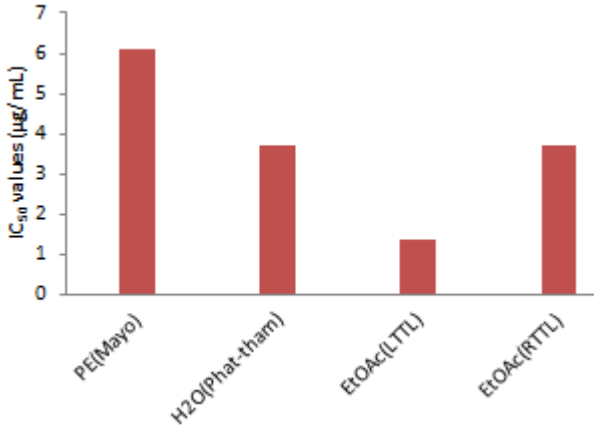


**Figure 8.Bar graph of the lowest $IC_{50}$ value (µg/mL) from crude extracts of three selected medicinal plants**

According to DPPH free radical scavenging method, PE extract from Mayo leaves, $H_2O$ extract from

fruitsofPhat-tham, EtOAc extracts from LTTL and RTTL were found to be the highest antioxidant activity.

Among them EtOAc extract of LTTL showed the most potent antioxidant activity (Figure 8). According to this study, $IC_{50}$ values of three selected medicinal plants were in the low range, so, it could be inferred that all three selected medicinal plants have high antioxidant activity. Among the three selected medicinal plants, EtOAc extracts of leaves of Taung-ta-le are the highest antioxidant activity.

## 4. CONCLUSION

From the overall assessment of the present study, the following inferences could be deduced. Preliminary phytochemical investigation of the leaves of Mayo contained alkaloids, α-amino acids, glycosides, steroids, saponins and tannins.

The fruits of Phet-tham showed the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids. And the phytochemical investigation of the leaves and roots of Taung-ta-le by the test tube method, alkaloids, α-amino acids, glycosides, phenolic compounds, saponins, reducing sugars, flavonoids, steroids and tannins were present. However, all three samples did not show cyanogenic glycoside, natural plant toxin.

In th*e in vitro* antioxidant activity screening by using DPPH free radical scavenging assay method, PE extract of the leaves of Mayo ($IC_{50}$= 6.11 μg/mL) was found to have the highest antioxidant activity than that of other extracts of the leaves of Mayo.

On the other hand, in th*e in vitro* antioxidant activity screening by using DPPH free radical scavenging assay method, $H_2O$ extract of the fruits of Phet-tham ($IC_{50}$= 3.72 μg/mL) was found to have the highest antioxidant activity than that of other extracts.

*In vitro* antioxidant activity of PE, EtOAc, 95 % EtOH and $H_2O$ of both LTTL and RTTL were screened by using DPPH free radical scavenging assay method. Among the tested four crude extracts, EtOAc extract of LTTL was found to have the highest antioxidant activity ($IC_{50}$ = 1.34 μg $mL^{-1}$)and its $IC_{50}$ value was found to be a little higher than that of standard ascorbic acid ($IC_{50}$ = 1.17 μg $mL^{-1}$). On the other hand, EtOAc extract of RTTL ($IC_{50}$ = 3.71μg $mL^{-1}$) was found to have the highest antioxidant activity than that of other extracts.

According to the experimental studies, three plant samples contain some bioactive phytochemicals and extracts may have antioxidant activity. Moreover, the presence of phytochemicals such as tannins, flavonoids and phenolic compounds act as antioxidants. These phytochemicals are reported to have anticancer, antimicrobial, anti-inflammatory and anti-allergic properties.

According to the *in vitro* tests, it could be inferred that leaves, fruits and root samples of three selected medicinal plants have valuable medicinal properties. From the results, due to the presence of the antioxidant activity, the leaves of Mayo, fruits of Phet-tham and leaves and roots of Taung-ta-le may be used in prevention of diseases related to oxidative stress such as coronary heart disease, neurodegenerative diseases and even in various types of cancer.

This DPPH free radical scavenging method provides advantages of being rapid, simple and inexpensive method. Therefore, the results of this study revealed that the extracts may be used as a natural source of antioxidant to prevent progression of many diseases. Finally, due to the complex nature of biological systems, there is no single universal method for measuring antioxidant capacity: for this reason, an examination of various antioxidant assays is required for the development of standard methods.

## 5. SUGGESTION FOR FURTHER WORK

Separate the whole extract (methanol or water) into different fractions by extracting with solvents such as Hexane, Chloroform, etc., then try checking the *in vitro* antioxidant capability of each of the separated fractions. If we still require, we can run a column and separate each of the fractions into sub-fractions for precise results. In this way, we can further extend our work to isolation of active antioxidant component from the fraction (isolation and purification). Moreover other bioactivities such as anti-tumor, anti-inflammatory, anti-cancer etc. should be found out.


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# Assessment of Soil Quality in Ywar Thar Gyi Village, Kyaiklat Township, Ayeyarwady Region

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**ABSTRACT:** *This study concerned with the quality assessment of Paddy Soils in Ywar Thar Gyi Village, Kyaiklat Township, Ayeyarwady Region. Most of the land areas in Ywar Thar Gyi Village are Paddy Lands. Three different types of lands; High Land, Middle Land and Low Land were chosen for this investigation. Soil samples were collected in April (2019) and June (2019).These periods were before cultivation and after harvesting of Summer Paddy. The determination of physicochemical properties, soil textual classes, nutrient values and some heavy metal contents were examined. The pH values (4.82 to 5.12), moisture contents (1.61% to 4.31%), bulk density (0.6762gL$^{-1}$ to 0.8154gL$^{-1}$), particle density (1.0640gL$^{-1}$ to 1.2171gL$^{-1}$) and porosity (33% to 40%) were observed. Soil textural classes are good for growing paddy because of the silt loam and clay lome types. The nutrients (N.P.K) for paddy soils were also examined. Nitrogen contents were in the range of 0.19 % to 0.22% , Phosphorus contents were 0.0001% to 0.0005% and Potassium contents were 0.02% to 0.03%. These values are favourable for cultivation of paddy.Based on some heavy metals (Fe,Cu,Pb) contents, the selected areas were not heavymetal contimination. The soil parameters varied considerably in general but all the values were suitable for plant growth.*

**Keywords:** physicochemacal properties, textualclasses, nutrientvalues, heavymetals

## 1. INTRODUCTION

Soil is the main source of nutrients for crops. Soil also provides support for plant growth in various ways. Essential plant nutrients such as N, P, K, Ca, Mg and S are called macronutrients, while Fe, Zn, Cu, Mo, Mn, B and Cl are called micronutrients. It is necessary to assess the capacity of a soil to supply nutrients in order to supply the remaining amounts of needed plant nutrients. The methods and procedures for obtaining soil samples vary according to the purpose of the sampling. Analysis of soil samples may be needed for engineering and agricultural purposes [1]. Soil makes up the "thin" layer of the earth where we live. The soil supports the plants that provide us with food, fiber, and forest products. Soil is located at the interface where the atmosphere and land meet. Soil may be defined as the naturally deposited unconsolidated material which covers the earth's surface whose chemical, physical, and biological properties are capable of supporting plant growth. Soil is a product of natural decomposition forces acting upon native literally thousands of years. The different components of a soil are referred to as fractions [2]. Paddy soils are soils that are managed in a special way for the wet cultivation of rice [1]. Paddy soils exhibit their own specific properties as a result of variant use of soil and cultivation practices. This makes them significant from other agriculture soils. Paddy soils have variations in pH which results mainly from alternate wetting and drying in soils. This change is mainly caused by water content of soil, amount of neutral salts, and type of cations in soil solution and on exchange complex [3].From the point of view of the soil scientists, as stated by the United State, Department of Agriculture Soil Survey Manual, soil is described as the collection of natural bodies occupying portions of the earth's surface that support plants and that have properties and living matter, acting upon parent material, as conditioned by relief, over periods of time [4].

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Soil samples were collected in April(2019) and June (2019) from High Land, Middle Land and Low Land paddy fields before and after harvesting of Summer Paddy in Ywar Thar Gyi village, Kyaiklat Township, Ayeyarwady Region. The sampling sites and locations are shown in Figure-1 to 4 and Table-1.

### 2.2 Sample Handling

Paddy soil samples were taken about 25 cm depth from the soil surface. The paddy soil samples were mixed and dried in air before grinding. Then, roots, gravel were discarded. The soil samples were passed through the (80) mesh. The soil samples were placed in plastic bags and clearly labeled. In all analytical investigations, the methods and techniques involved both conventional method and modern instrumental techniques by using standard recommended techniques.


. **Figure 1. Satellite image of studied area**

**(A)Before Cultivation (B) After Harvesting**
**Figure 2. Sampling site-1,High Land**

**(A)Before Cultivation (B) After Harvesting**
**Figure 3. Sampling site-2, Middle Land**

**(A)Before Cultivation (B) After Harvesting**
**Figure 4. Sampling site-3, Low Land**

**Table 1. Sampling Sites of Soil Samples From Ywar Thar Gyi Village**

| Sampling Site | Location | Place |
|---|---|---|
| Site-1 | 16º 30’ 57’’ N<br>95º 29’ 23’’ E | High Land<br>(Village Paddy Field) |
| Site-2 | 16º 30’ 52’’ N<br>95º 29’ 26’’ E | Middle Land<br>(South Paddy Field) |
| Site-3 | 16º 30’ 44’’ N<br>95º 29’ 29’’ E | Low Land<br>(U Htun Yi Paddy Field) |

The followings were some of the instruments used in the experimental work.
Oven (Ambient 2 ± 2ºC, Griffin, England), pH meter (MD 100, Photometer, Lovibond), Balance (Balance Sartorius AG Gottingen BL 1015), Atomic Absorption Spectrophotometers (AA-7000 Series), Shimadzu, Japan

## 3. RESULTS AND DISCUSSION

### 3.1 Collection Of Soil Samples

In this research, soil samples were collected from three sampling sites in Ywar Thar Gyi Village, Kyaiklat Township Area. The research was carried out the paddy soils before cultivation and after harvesting of Summer Paddy and Monsoon Paddy. The sampling areas were chosen High Land (Site-1), Middle Land (Site-2) and Low Land (Site-3).

### 3.2 Physicochemical Properties

#### 3.2.1 pH

Soil pH is a measure of acidity and alkalinity in soils. Acid soils have a pH below 7 and alkaline soils have a pH value greater than 7. The pH of soils has influence to concentration of heavy metals. Before cultivation of Summer Paddy, the pH values were 5.12 for Site-1, 4.83 for Site-2 and 4.88 for Site-3. After harvesting, the pH values were 5.04, 4.55 and 4.82. All the soil samples from the selected areas showed the acidic character. The optimal pH range for most plants is between 5.5 and 7.0; however, many plants have adapted to thrive at pH values outside this range [5].

#### 3.2.2 Moisture Contents In Soil

The moisture content of soil is the quantity of water contained in a material, such as soil, rock, ceramics, crops or wood. In paddy field, soil moisture represents water availability for the plants and it is

required for irrigation scheduling and water resource allocation, management and planning. If the moisture content of a soil is optimum for plant growth, plants can readily absorb soil water. In this research work, the moisture contents of soils before cultivation of Summer Paddy were 3.78 %, 3.46 % and 4.31 % respectively. After harvesting, the moisture contents of soils were lower than due to the loss of water. The results were 1.89 % for Site-1, 1.61 % for Site-2 and 2.10 % for Site-3.

### 3.2.3 Bulk density

Bulk density of a soil is inversely related to the porosity of the same soil, the more pore space in a soil the lower the value for bulk density. Bulk density is an indicator of soil compaction. It is calculated as the dry weight of soil divided by its volume. This volume includes the volume of soil particles and the volume of pores among soil particles. Bulk density is typically expressed in $gmL^{-1}$. High bulk density is an indicator of low soil porosity and soil compaction. It may cause restrictions to root growth, and poor movement of air and water through the soil [7]. In the present work, the bulk density values of soils before cultivation of Summer Paddy were found to be 0.7920 $gmL^{-1}$ for Site-1, 0.6809 $gmL^{-1}$ for Site-2 and 0.7494 $gmL^{-1}$ for Site-3. According to the results, the bulk density of the soil is a good indication of the suitability for root growth of paddysoil. After harvesting, the values were slightly different for each site. These values were 0.8154 $gmL^{-1}$, 0.6762 $gmL^{-1}$ and 0.7719 $gmL^{-1}$.

### 3.2.4 Particle density

Soil particle density is an important soil property for calculating soil porosity expressions. If the particle density is high, the soil consists of minerals that have a high. Soil particle density depends on the chemical composition and structure of the minerals in the soil. Particle density focuses on just the soil particles themselves and not the volume they occupy in the soil [6] ions. Before cultivation, particle density of each soil sample was 1.2$gmL^{-1}$for Site-1, 1.064$gmL^{-1}$ for Site-2 and 1.1529 $gmL^{-1}$ for Site-3.It was also found that 1.2171 $gmL^{-1}$,1.1270 $gmL^{-1}$ and 1.2062 $gmL^{-1}$ for after harvesting. Based on these results, the soils from collected area were suitable for plants growth.

### 3.2.5 Porosity

Porosity of surface soil typically decreases as particle size increases. Porosity is related the pore space of soils. The relationship of bulk density and porosity is reciprocal. The good soil for growing most plant contains about 50 % of pore space and 50 % solids [10]. The porosity of soils in collected area were 34 %, 36 % and 35 % for each site in before cultivation and 33 %,40 % and 36 % in after harvesting of summer paddy.

## 3.3 Soil Texture Soil Texture

Soil texture refers to the relative percentage of sand, silt and clay in a soil. It is critical to determine the impact of soil properties on different production systems related to water regime along with rice cultivar [10]. In the present work, the soil texture classes were silt loam for sampling sites -1 and 3 before cultivation and after harvesting of summer paddy. Soil texture class for Site - 2 was found to be clay loam for both periods. Silt loams are the best soil types for plant growth and agriculture. Clay loam contains a good deal of plant nutrients and supports most types of plants and crops. Slit loam and Clay loam are also suitable for paddy field.

## 3.4 Nitrogen, Phosphorus, Potassium (N,P,K)

Soil chemistry is highly complex; most nutrients exist in different chemical forms, and not all forms are equally plant-available. For most nutrients, the commonly used extraction procedures attempt to rank relative nutrient availability, not the total soil content of that nutrient [10] Nitrogen, phosphorus and potassium are the main components of soil fertilizer. Nitrogen is used by plants for lots of leaf growth and good green color. Nitrogen (N) promotes rapid plant growth and improves grain yield and grain quality.In the present work, nitrogen contents of summer soils before cultivation and after harvesting were about 0.20 % for all Sites. These values line for the plant growths.

Phosphorous is used by plants to help form new roots, make seeds, fruit and flowers. It's also used by plants to help fight disease. Potassium helps plants make strong stems and keep growing fast. It is especially deficient in sandy soils with low organic matter contents, in very acid soils and in alkaline soils. Phosphorus contents of Summer Paddy soils for sampling site -1 were found to be 0.0003 % and 0.0005 %. For Site-2, phosphorus contents were 0.0002 % and 0.0005 % and for Site-3, phosphorus contents were 0.0001 % and 0.0002 %.

Potassium can quickly become yield limiting in high yielding rice systems where most of the straw is removed because rice straw is rich in K. Potassium contents of sampling Site - 1 were found to be 0.02 % before cultivation and after harvesting of Summer Paddy. For Site-2 and 3, the potassium contents were slightly different (0.03 %) in before cultivation of summer paddy .In the present research, the order of nutrient values N >K >P were found. All nutrients NPK values of collected soils in after harvesting were slightly lower than before cultivation.

## 3.5 Some heavy metals in soil samples

The concentrations of heavy metals in soil of Ywar Thar Gyi Village were analyzed. Some heavy metals (Fe, Cu, Pb) contents in soil samples collected from three sampling sites were determined by using Atomic Absorption Spectrophotometer (AAS). The

distribution among water-soluble, exchangeable and complex (with the organic matter of the solid phase) ferrous iron is strongly pH-dependent [12]. Iron contents of all sampling sites were in the range of 4223 mg/kg to 6576 mg /kg. It is higher than some metal contents but these values are allowable range of EPA guideline.

Copper contents of soils that are collected before cultivation and after harvesting of summer paddy were found to be 0.0794 mg/kg and 2.4131 mg/kg for Site-1, 0.1373 mg/kg and 0.9477 mg/kg for Site-2 and 0.0762 mg/kg and 0.5135 mg/kg for Site-3. all lie in the range of EPA guideline.

Lead contents of soils in all sampling sites were found to be in the range of 0.0204 mg/kg to 0.1368 mg/kg for before cultivation and after harvesting of Summer Paddy. Lead contents present of some contaminants but all are allowable range of EPA guideline. The results indicate that the Fe content was found to be the highest in all the soil samples. The iron constituent in the study area might be in the form of iron oxides and hydroxides in the soil. The some metals (Cu and Pb) content in soil samples were found to be lower the contamination levels of EPA guideline (2008). The results are shown in Table-2

**Table 2. Comparative Data of Heavy Metal Contents in Collected Soil Samples**

| Summer paddy | Sampling Sites | Heavy Metals (mg/kg) (dried wt) | | |
|---|---|---|---|---|
| | | Fe | Cu | Pb |
| Before Cultivation | Site-1 | 4223 | 0.0794 | 0.0226 |
| | Site-2 | 4226 | 0.1373 | 0.0204 |
| | Site-3 | 4232 | 0.0762 | 0.0215 |
| After Harvesting | Site-1 | 6576 | 2.413 | 0.1368 |
| | Site-2 | 5424 | 0.9477 | 0.1093 |
| | Site-3 | 4252 | 0.5135 | 0.0983 |
| EPA (2008) | | 6800 | 31.6 | 35.8 |

EPA = Environmental Protection Agency Value for Soil (2008)

## 4. CONCLUSION

In this research work, the soil samples were collected from three sampling sites of Ywar Thar Gyi Village.The various modern techniques and instruments were used to determine the physicochemical properties, nutrient and some metal contents of soil samples.

For the before cultivation of summer paddy**,** the pH value of collected soil samples were found to be in the range of 4.83 to 5.12. The moisture content of soil samples were observed in the range of 3.46 % to 4.31 %. The value of bulk density was found in the range of 0.6809 $gmL^{-1}$ to 0.7920 $gmL^{-1}$. The particle density of soil sample was observed in the range of 1.0640 $gmL^{-1}$ to 1.2000 $gmL^{-1}$in all sampling sites. The porosity of soil samples were found in the range of 34 % to 36 %. The soil texture class was found in silt loam for sampling Site-1 and Site-3 and clay loam for sampling Site-2, respectively. In nutrient values of soil samples, the nitrogen content of soil was found to be in the range of 0.20 % to 0.22 %. The phosphorus content of soil was observed to be in the range of 0.0001 % to 0.0003 %. The potassium value of soil was found to be in the range of 0.02 % to 0.03 %. In the metal contents of soil samples, the Fe content of soil was found to be in the range of 4223 mg/kg to 4232 mg/kg. The Cu value of soil was observed in the range of 0.0762 mg/kg to 0.1373 mg/kg and the content of Pb of soil was found to be in the range of 0.0204 mg/kg to 0.0226 mg/kg, respectively. The results indicate that the metal (Fe, Cu and Pb) contents in soil samples were found to be lower than the contamination levels of EPA guideline (2008).

For the after harvesting of summer paddy, the pH value of collected soil samples were found to be 5.04, 4.55 and 4.82 in sampling site-1, site-2 and site-3, respectively. In the moisture content of the soil sample, the lowest moisture content, 1.61 was found in sampling site-2 and the highest moisture value, 2.10 was observed in sampling site-3. The bulk density of the soil samples was found to be in the range of 0.6762 $gmL^{-1}$ to 0.8154 $gmL^{-1}$. The minimum value, 0.6762 $gmL^{-1}$ was found in sampling site-2 and the maximum value, 0.8154 $gmL^{-1}$ was observed in sampling site-1. The particle density of the soil samples was found to be in the range of 1.1270 $gmL^{-1}$ to 1.2171 $gmL^{-1}$ . The lowest porosity value, 33 % was observed in sampling site-1 and the highest value, 40 % was found in sampling site-2. The soil texture class was found silt loam for sampling Site-1 and Site-3, and clay loam for sampling site-2. In nutrient values of soil samples, the nitrogen content of soil was found to be in the range of 0.19 % to 0.21 %. The phosphorus content of soil was observed to be in the range of 0.0002 % to 0.0005 %. The potassium value of soil was found to be 0.02 % for all sampling sites. In the metal contents of soil samples, the Fe content of soil was found to be in the range of 4223 mg/kg to 6576 mg/kg. The Cu value of soil was observed in the range of 0.5135 mg/kg to 2.4131 mg/kg and the content of Pb of soil was found to be in the range of 0.0983 mg/kg to 0.1368 mg/kg, respectively. The observed some metals (Fe,Cu and Pb) content in soil samples were within the range of EPA guideline (2008).

Overall assessments of these observations, it can be concluded that the quality of paddy soil was not greatly affected by using fertilizers.

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# Assessment of Toe River Water Quality around Maubin Township from Ayeyarwady Region in Myanmar

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**ABSTRACT:** *This research is focused on the assessment of Toe river water samples were seasonally collected from five different sites around Maubin Township, Ayeyarwady Region. Some physicochemical parameters were determined by conventional method. The concentration of metals (Fe, Zn, Cu, Pb, Mn and As) contents in the river water samples were measured by AAS. Microbiological activities were measured by most probable number methods. Physicochemical properties of pH, temperature, total alkalinity, total hardness, total suspended solid, total dissolved solid, turbidity, BOD, COD, DO, chloride, nitrate nitrogen, nitrite nitrogen, orthophosphate, sulphate, chloride and bromide were found to be observed acceptable limit of EPA guideline standard (2018). Ammonia-nitrogen values of samples (1, 4 and 5) in cold season, all samples in hot season and sample (1) in rainy season were found to be higher than the EPA standard. Zn, Cu, Pb, and As were not detected in studied area. Fe contents in cold and rainy seasons were found to be observed higher than the EPA standard. According to microbiological characteristics results of three seasons from Toe river water were found E-coli positive.*

***Keywords****: river water, Fe , coliform, E-Coli*

## 1. INTRODUCTION

Water has always been a vital material for man's existence. People cannot exist without water, so there has always been a demand and all the nearest and more obvious sources have already been exploited [2]. It is an important component of the tissues of most other living things [3]. Water makes up more than two thirds of the weight of the human body and without it humans could die in a few days [10]. Natural water is often important parts of wonders of the world. River are important sources of natural water apart from serving as a source of natural water, irrigation and fishing; they are generally of immense important in geology, biology, history and culture. Rivers represent about 0.001 % of the total amount of water in the world. They are vital carries of water and nutrients to area all around the earth. An importance sources of valuable deposits of sands, gravels and even electrical energy [14]. Waste from industrials, domestic sewage and agricultural practices into rives resulted in large scale deterioration of the water quality [4]. The consumption of highly contaminated water can cause injury to the heart and kidneys [1]. Myanmar is primarily an agricultural country and the economy of the nation is also mainly depends on the agricultural products. To increase the agricultural productions for the rising demand of a growing population, double crop cultivation of paddy rice and other crops has been introduced by implementing the large-scale irrigation systems. Maubin District is a district of the Ayeyarwady Region in south western Myanmar. It contains four cities. They are Maubin, Pantanaw, Nyaungdon and Danubyu. Maubin district is plain land and rich in streams. Maubin Township is 1362 feet high above sea level. Among the population of Maubin district is 51542. The majority of people are Myanmar and Karen nationals. The area of Maubin district is 1651.49 square miles and 1056,952 areas. It is a port lying on the west bank of the Ayeyarwady River delta and is protected by flood-control embankments. The southern coastline lies along the Andaman Sea. Rice growing and fishing are the major contributors to the economy. It is developing town with growing transportation and communication services [9]. Toe river is one of the eastern most distributaries of the Ayeyarwady Delta, Myanmar and it diverges from Ayeyarwady River near Maubin Township and it is characterized the extreme flat and low plain with and elevation less than 15.24 m above sea level. The channel patterns, width, sinuosity of the main river channel and sediment distributions along the channel are dramatically changed by annual floods in the study area. The sediment deposition rate are affecting on the shifting of river course and influencing on the river channel [13]. The Ayeyarwady Delta comprises the main arms of Pathein River, Pyapon River, Bogale River and Toe River. In Maubin district, no extensive study on water quality assessment of Toe river water has been made. The scope of the present work is to study the assessment of Toe river water quality from five different sites, around Maubin Township at Ayeyarwady region in Myanmar. In this research work, the Toe river water quality of five different collected water samples were investigate the physicochemical parameters, trace elements and microbiological assay of generic bacteria, total coliform and ***E-coli***.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

In this present work, the water samples were collected in January, April and July (2019) in the Toe water from five different sites as located at Maubin Township, Ayeyarwady Region, Myanmar. Figure 1 shows Google map of sampling sites from Toe River around Maubin Township. The photographs of sampling sites were shown in Figure 2 to 6. Toe river water sample was taken 13 feet away from the bank and 5 feet depth from the surface water level. The water samples were collected by mean of a water sampler. Experiment was continuously made within 24 hours.

**Figure 1. Five different sampling sites from Toe River (Google Map)**

**Figure 2. Maubin Bridge ($S_1$)**

**Figure 3. Maubin Harbour ($S_2$)**

**Figure 4. Near B.E.H.S (2)($S_3$)**

**Figure 5. Paw Taw Mu Pagoda ($S_4$)**

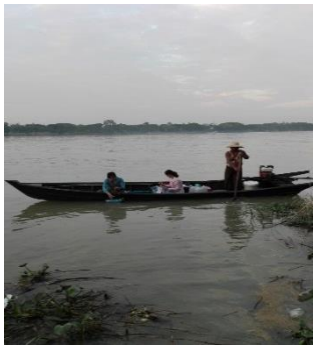

**Figure 6. Nyaung Waing Village ($S_5$)**

**$S_1$ - 16° 44' 54" Longitude and 95° 39' 8" Latitude**

**$S_2$ - 16° 43' 55" Longitude and 95° 39' 22" Latitude**

**$S_3$ - 16° 43' 28" Longitude and 95° 39' 19" Latitude**

**$S_4$ - 16° 43' 16" Longitude and 95° 39' 34" Latitude**

**$S_5$ - 16° 43' 7" Longitude and 95° 39' 47" Latitude**

### 2.2 Physicochemical Examination Of Water Samples

Physicochemical parameters such as pH, temperature, total alkalinity, total hardness, total suspended solids, total dissolved solids, turbidity, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, nitrite nitrogen, nitrate nitrogen, ammonia nitrogen, orthophosphate, sulphate, chloride and bromide were carried out by using conventional method. In this research, modern techniques were used such as Tintometer (MD-100), DO meter, pH meter and COD meter.

### 2.3 Determination Of Trace Elements In Water Samples

Trace elements (Fe, Zn, Cu, Pb, Mn and As) in water samples were determined by Atomic Absorption Spectrophotometer (AA-7000) Series.

### 2.4 Determination Of Some Microbiological Quality In River Water

Some microbiological such as generic bacteria, coliform and ***E-coli*** in water samples were analyzed by using standard plate count, most probable number and traditional agar method.

## 3. RESULTS AND DISCUSSION

### 3.1 Sample Collection

The present study was carried out in the month of January, April and July in 2019. The water samples were collected from the Toe River of five different sites in Maubin Township, Ayeyarwady Region in Myanmar. These water samples are collected from near Maubin bridge (sample 1), near harbor (sample 2), near B.E.H.S (2) (sample 3), near Paw Taw Mu Pagoda (sample 4) and near Nyaung Waing village (sample 5).

### 3.2 Physicochemical Characteristics Of Water Samples

In this work, physicochemical results of all collected water samples for three seasons were shown in Table (1-3).

### 3.2.1 pH In River Water Samples

The Toe River water samples from five different locations were observed in moderately alkaline pH. The values of pH in all water samples were found to be observed within the permissible limit (6.5 – 8.5) of water standards in (EPA 2018). Most fish can be observed at pH values of about (5-9). So the observed pH value is suitable for fish and other aquatic live.

### 3.2.2 Temperature In River Water Samples

The temperature of five different sites of river water sample were found to observe within the range of EPA standard values of 2018 (<40 ˚C).

### 3.2.3 Total Alkalinity In Collected Water Samples

All of the water samples of total alkalinity values were lower than the EPA guideline standard 2018. The EPA guideline standard is (200 ppm). The alkalinity of water is caused mainly due to $OH^-$, $CO_3^{2-}$ and $HCO_3$- ions. Alkalinity is an estimate of the ability of water to resist change in pH upon addition of acid.

### 3.2.4 Total Hardness In Toe River Water Samples

In this study, the highest total hardness value of water sample (1) was found to be observed 50 ppm in January (cold season).

### 3.2.5 Total Suspended Solids In River Water Samples

The highest value of total suspended solids were recorded during rainy season, which indicates the river run off, industrial effluent and municipal sewage [15].

### 3.2.6 Total Dissolved Solids In River Water Samples

In this research, the total dissolved solid values of all water samples were found to be acceptable range of EPA standard (500 ppm).

### 3.2.7 Turbidity In River Water Samples

According to the obtained results, the values of turbidity were below the permissible level of EPA standard (<700 FTU). Turbidity in water cause by suspended matter such as clay, silt, finely divided organic and inorganic matter, soluble organic compound and plankton and other microscopic organism [18].

**Table 1. Physicochemical Parameters Of Collected Water Sampls Of Cold Season**

| Water Parameters | Sampling Sites | | | | | EPA |
|---|---|---|---|---|---|---|
| | Cold Season | | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | |
| pH | 8.17 | 8.11 | 8.11 | 8.11 | 8.09 | 6.5-8.5 |
| Temp: (°C) | 25.80 | 25.82 | 25.79 | 25.99 | 25.85 | 32 |
| TA (ppm) | 87.00 | 92.00 | 95.00 | 87.00 | 91.00 | 200 |
| TH (ppm) | 50.00 | 30.00 | 30.00 | 30.00 | 30.00 | 200 |
| TSS (ppm) | 89.60 | 94.10 | 58.10 | 58.60 | 56.60 | 200 |
| TDS (ppm) | 140.0 | 80.00 | 60.00 | 120.0 | 150.0 | 500 |
| Turbidity (FTU) | 2.15 | 2.50 | 3.50 | 2.15 | 1.45 | <700 |
| DO (ppm) | 5.01 | 5.12 | 3.96 | 4.25 | 3.94 | 5 |
| BOD (ppm) | 2.50 | 1.50 | 2.50 | 2.00 | 2.50 | 5 |
| COD (ppm) | 5.15 | 3.31 | 5.88 | 4.04 | 5.52 | 10 |
| $NH_3$-N (ppm) | 0.06 | 0.05 | 0.04 | 0.08 | 0.09 | 0.05 |
| $NO_2$-N (ppm) | 0.05 | 0.05 | 0.06 | 0.02 | 0.05 | 3 |
| $NO_3$-N (ppm) | 1.05 | 1.04 | 1.06 | 1.07 | 1.08 | 10 |
| $PO_4$-P (ppm) | 0.06 | 0.06 | 0.05 | 0.05 | 0.05 | 1 |
| $SO_4^{2-}$ (ppm) | 0.01 | 0.01 | 0.01 | 0.02 | 0.02 | 250 |
| $Cl^-$ (ppm) | 49.99 | 44.99 | 39.99 | 44.99 | 44.99 | 250 |
| $Br^-$ (ppm) | 0.21 | 0.23 | 0.16 | 0.22 | 0.17 | 65-80 |

**Table 2. Physicochemical Parameters of Collected Water Samples in Hot Seasons**

| Water Parameters | Sampling Sites | | | | | EPA |
|---|---|---|---|---|---|---|
| | Hot Season | | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | |
| pH | 8.03 | 8.04 | 7.98 | 7.99 | 8.09 | 6.5-8.5 |
| Temp: (°C) | 36.00 | 36.00 | 36.00 | 36.00 | 36.00 | 32 |
| TA (ppm) | 69.00 | 73.00 | 72.00 | 75.00 | 73.00 | 200 |
| TH (ppm) | 20.00 | 30.00 | 30.00 | 30.00 | 30.00 | 200 |
| TSS (ppm) | 91.80 | 96.80 | 105.20 | 89.20 | 101.60 | 200 |
| TDS (ppm) | 130.00 | 90.00 | 140.00 | 83.00 | 85.00 | 500 |
| Turbidity (FTU) | 3.21 | 3.25 | 3.23 | 2.98 | 2.23 | <700 |
| DO (ppm) | 4.73 | 5.05 | 4.79 | 4.65 | 4.77 | 5 |
| BOD (ppm) | 3.00 | 3.00 | 3.50 | 3.00 | 3.00 | 5 |
| COD (ppm) | 6.05 | 4.12 | 6.12 | 5.13 | 6.62 | 10 |
| $NH_3$-N (ppm) | 0.12 | 0.15 | 0.14 | 0.15 | 0.13 | 0.05 |
| $NO_2$-N (ppm) | 0.02 | 0.05 | 0.05 | 0.06 | 0.06 | 3 |
| $NO_3$-N (ppm) | 2.00 | 2.00 | 2.40 | 2.50 | 2.00 | 10 |
| $PO_4$-P (ppm) | 0.58 | 0.57 | 0.56 | 0.55 | 0.56 | 1 |
| $SO_4^{2-}$ (ppm) | 0.12 | 0.15 | 0.14 | 0.12 | 0.23 | 250 |
| $Cl^-$ (ppm) | 49.99 | 47.99 | 49.99 | 49.99 | 49.99 | 250 |
| $Br^-$ (ppm) | 0.22 | 0.24 | 0.24 | 0.19 | 0.21 | 65-80 |

**Table 3. Physicochemical Parameters of Collected Water Samples in Rainy Seasons**

| Water Parameters | Sampling Sites | | | | | EPA |
|---|---|---|---|---|---|---|
| | Rainy Season | | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | |
| pH | 7.79 | 7.51 | 7.54 | 7.47 | 7.37 | 6.5-8.5 |
| Temp: (°C) | 28.00 | 28.00 | 28.00 | 28.00 | 28.00 | 32 |
| TA (ppm) | 21 | 29 | 29 | 27 | 23 | 200 |
| TH (ppm) | 10 | 10 | 10 | 10 | 10 | 200 |
| TSS (ppm) | 91.60 | 109.80 | 121.60 | 116.20 | 87.80 | 200 |
| TDS (ppm) | 370 | 180 | 500 | 300 | 120 | 500 |
| Turbidity (FTU) | 17.6 | 16.3 | 16 | 14.8 | 16.7 | <700 |
| DO (ppm) | 5.34 | 5.57 | 5.63 | 5.30 | 5.53 | 5 |
| BOD (ppm) | 2 | 1.5 | 2.5 | 2 | 2 | 5 |
| COD (ppm) | 2.232 | 2.325 | 4.352 | 2.321 | 4.321 | 10 |
| $NH_3$-N (ppm) | 0.06 | 0.05 | 0.04 | 0.05 | 0.03 | 0.05 |
| $NO_2$-N (ppm) | 0.01 | 0.03 | 0.03 | 0.05 | 0.05 | 3 |
| $NO_3$-N (ppm) | 1 | 1 | 1.5 | 2 | 1.5 | 10 |
| $PO_4$-P (ppm) | 0.118 | 0.142 | 0.120 | 0.115 | 0.116 | 1 |
| $SO_4^{2-}$ (ppm) | 0.055 | 0.035 | 0.045 | 0.025 | 0.027 | 250 |
| $Cl^-$ (ppm) | 49.99 | 49.99 | 37.89 | 39.90 | 29.99 | 250 |
| $Br^-$ (ppm) | 0.28 | 0.25 | 0.28 | 0.26 | 0.21 | 65-80 |

## 3.3 Dissolved Oxygen Gases In River Water

### 3.3.1 Dissolved Oxygen

The dissolved oxygen (DO) values of river water samples (1) and (2) (5.01 and 5.12 ppm) in January. Sample (2) 5.05 ppm in April and all collected water samples (1) to (5) (5.34, 5.57, 5.63, 5.30 and 5.53 ppm) in July were found in higher than the EPA standard rainy season were found in higher than the EPA standard values. At higher temperatures, the decreased solubility of oxygen, combined with the increased respiration rate of aquatic organisms (Manahan, 2000).

### 3.3.2 Biochemical Oxygen Demand

In this study, all BOD values of river water samples were below the permissible level of EPA standard (5 ppm) [7].

### 3.3.3 Chemical Oxygen Demand

Chemical oxygen demand, biochemical oxygen demand and dissolved oxygen of five different sites of water samples were shown Table (1-3). All COD values of river water samples were below the permissible level of EPA standard (10 ppm).

## 3.4 Nutrient In Water

Nutrients are essential for plant growth and development. Many nutrients are found in wastewater and fertilizers [16].

### 3.4.1 Ammonia-nitrogen In Water

The $NH_3$-N values of collected water samples 0.06, 0.08, 0.09 ppm in ($S_1$, $S_4$ and $S_5$) were found in higher than the EPA standard range and the values of samples 0.05 and 0.04 ppm ($S_2$ and $S_3$) were found in acceptable level of the EPA standard range in January (cold season). The values of $NH_3$-N in all water samples (0.12, 0.15, 0.14, 0.15 and 0.13 ppm) in April (hot season and $S_1$ (0.06 ppm) in July (rainy season) were found to be observed higher than the EPA standard. Figure (7) showed nutrient value of five different water samples in three seasons. Most of the ammonia in the environment comes from the natural breakdown of manure, dead plants and animals. Higher concentrations of ammonia may cause corrosive injury including skin burns, eye damage or blindness. Ammonia nitrogen can lead to gill damage and a substantial reduction in growth rates [8].

### 3.4.2 Nitrite-nitrogen In Water

The $NO_2$-N values of all collected water samples were found in lower than the EPA standard value (3 ppm).

### 3.4.3 Nitrate-nitrogen in Water

The observed $NO_3$-N values were found in lower than the EPA standard value (10 ppm).

### 3.4.4 Orthophosphate In Water

All orthophosphate values of collected water samples were found to be observed permissible level of EPA standard range (1 ppm). According to the water nutrients values, Toe river water samples are not polluted.

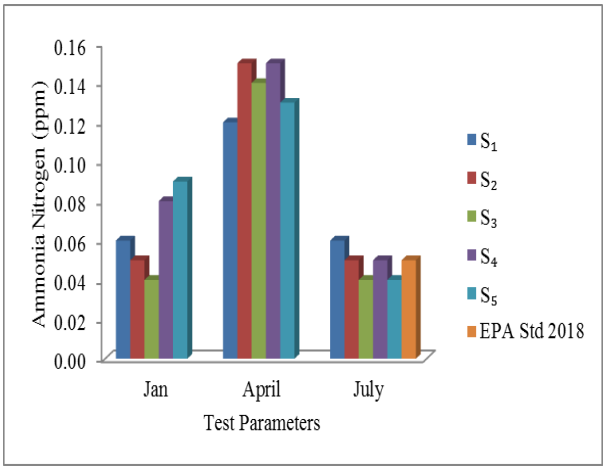


**Fig 7. Histogram of ammonia nitrogen values of five different sampling sites in Toe River**

## 3.5 Inorganic Constituents

### 3.5.1 Sulphate In River Water Samples

The sulphate in Toe river samples were determined by spectrophotometer method. The concentrations of sulphate in all collected water samples in three seasons were found to be very low concentration in EPA standard value (250 ppm).

### 3.5.2 Chloride In River Water Samples

The chloride of the collected water samples from five different sites were shown in Table 1-3. According to the obtained data the chloride concentrations were found in lower than EPA value (250 ppm) [7].

### 3.5.3 Bromide In River Water Samples

The bromide of the collected water samples from five different sites were found in very low level of EPA value (65-80 ppm).

## 3.6 Trace Elements Of River Water Samples

The concentration of trace elements and heavy metals (Fe, Zn, Cu, Pb, Mn and As) of the collected Toe river water samples from five different sites were shown in Table 4. The concentration of Fe values of water samples in cold season and rainy season were found in higher than the EPA standard whereas in hot season was found in lower than the EPA standard (0.3 ppm). The increase in iron content might be due to discharge into the river through industrial, agricultural and other human activities in the area [17]. The concentrations of Zn, Cu, Pb and As were not detected from all collected water samples in three seasons. The contents of Mn in cold season of sample (1, 2, 3) and sample (5) in rainy season were found to observe lower than the EPA standard.

**Table 4. Comparisons of Trace Elements of the Collected Water Samples from Toe River**

| Seasons | Sites No. | Fe (ppm) | Zn (ppm) | Cu (ppm) | Pb (ppm) | Mn (ppm) | As (ppm) |
|---|---|---|---|---|---|---|---|
| Cold Season | $S_1$ | 0.66 | ND | ND | ND | 0.03 | ND |
| | $S_2$ | 0.69 | ND | ND | ND | 0.03 | ND |
| | $S_3$ | 0.73 | ND | ND | ND | 0.03 | ND |
| | $S_4$ | 0.72 | ND | ND | ND | ND | ND |
| | $S_5$ | 0.72 | ND | ND | ND | ND | ND |
| Hot Season | $S_1$ | 0.14 | ND | ND | ND | ND | ND |
| | $S_2$ | 0.16 | ND | ND | ND | 0.01 | ND |
| | $S_3$ | 0.06 | ND | ND | ND | ND | ND |
| | $S_4$ | 0.01 | ND | ND | ND | ND | ND |
| | $S_5$ | 0.049 | ND | ND | ND | ND | ND |
| Rainy Season | $S_1$ | 0.40 | ND | ND | ND | ND | ND |
| | $S_2$ | 0.64 | ND | ND | ND | ND | ND |
| | $S_3$ | 0.61 | ND | ND | ND | ND | ND |
| | $S_4$ | 0.45 | ND | ND | ND | ND | ND |
| | $S_5$ | 0.52 | ND | ND | ND | ND | ND |
| | **EPA (2018)** | 0.3 ppm | 0.5 ppm | 1.0 ppm | 0.05 ppm | 0.05 ppm | 0.01 ppm |

**ND = Not Detected, EPA = Environmental Protection Agency**

## 3.7 Some Microbiological Quality In River Water

### 3.7.1 Generic Bacteria

The generic bacteria of all water samples from five different sites were observed >500 spc. Bacteria live in every climate and location on earth. High concentration of general bacteria can be removed by boiling and chlorination.

### 3.7.2 Coliform

The coliform values in all water samples ($S_1$ – $S_5$) were found to be observed 180 (M.P.N) in all seasons. The observed coliform values in all water samples were not agree with WHO standard 2018. High levels of fecal coliform in the water may cause typhoid fever, hepatitis, gastroenteritis, dysentery and eat infection. Some factors which may affect the concentration of these bacteria are the presence of wastewater and septic system, animal wastes, run-off, high temperature and nutrient-rich water [11]. Coliform can be removed by chlorination, UV irradiation and distillation method.

### 3.7.3 Escherichia Coli

The all samples ($S_1$ – $S_5$) found to be ***E-coli*** positive in all seasons. The presence of ***E-Coli*** in water is a strong indication of recent sewage or animal waste contamination. Some kind of ***E-Coli*** can cause diarrhea, while other cause gastroenteritis, Urinary tract

infections, respiratory illness and neonatal meningitis [6]. The presence of ***E-coli*** can be removed by water to a rolling boil for one minute (WHO 2018).

## 4. CONCLUSION

This research work is focused on the assessment of five different sites of river water samples were seasonally collected from Toe River around Maubin Township, Ayeyarwady Region in Myanmar. The values of dissolved oxygen of sample (1 and 2) in cold season, sample 2 in hot season and all samples in rainy seasons were slightly exceeded than the EPA standard 5 ppm. All collected river water samples are not harmful to human health. According to the observed data, ammonia-nitrogen values of sample (1, 4 and 5) in cold season, all collected river sample in hot season and sample (1) in rainy season were found to be higher than the EPA standard. Some trace metals Zn, Cu, Pb and As were not detected in studied area. The element of Mn was found to lower limit of EPA standard and Fe contents in cold and rainy seasons were found to be observed higher than the EPA standard. According to the physicochemical point of view and elemental analysis the studied area of Toe river water not polluted and it can be used for recreational activities. However, all river water samples showed numbers of coliform counts and generic bacteria were found to observe above permissible levels of WHO standard. ***E-coli*** in all river water samples were positive for seasonally. So microbiological remarked were unsatisfactory for collected water samples. It was unfit for drinking and cooking but other activities can be used. The present results, indicate that the water quality of Toe river in studied area may be polluted from microorganism point of view.

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# Mineral Elements, Essential Nutritive Elements and Toxic Elements in Some Myanmar Indigenous Medicines Containing Kyauk-Thway

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**ABSTRACT**: *In this paper, ten brands of Myanmar indigenous medicines reportedly containing Kyauk-thway (Ferric ammonium citrate) were analysed in terms of ash, moisture, nitrogen, citric acid and trace metals. The ash and moisture contents of samples were determined by ashing method and oven dried method. The mineral elements (Na, K, Mg, Ca), essential nutritive elements (Fe, Zn, Cu), non-nutritive toxic elements (Pb and As) were determined by Atomic Absorption Spectroscopy (AAS). Nitrogen contents were determined by micro-Kjeldahl method. Citric acid contents were determined by gravimetric method. In the ten brands of samples, the ash percents and moisture contents were found in the range of 4.9875 to 20.8959 % and 6.00 to 12.00 %. According to their treatment order, daily dosage for adults are sodium, 0.174 to 9.423 mg; potassium, 0.299 to 7.895 mg; magnesium, 0.207 to 9.486 mg; calcium, 0.256 to 10.955 mg; iron, 0.185 to 7.160 mg respectively. According to the experimental results, the level of mineral contents in these medicines are allowable, the toxic metal, arsenic, was not detected and lead content was found to be very low quantity. By knowing the above facts, it may be of much benefit to produce high quality Myanmar indigenous medicines.*

**Keywords**: Myanmar indigenous medicines, Kyauk-thway, Mineral Elements, AAS, Toxic Elements

## 1. INTRODUCTION

Myanmar indigenous medicines are originally prepared from medicinal plants, chemical origin and animal origins. Kyauk-thway contained in Myanmar indigenous medicines is a good constituent for giving strength according to literature as well as traditional medicine practitioners (Ministry of Industry (1), 1988).'Thway Hsay' as known in Myanmar is the most commonly used indigenous drug from time immemorial. Local indigenous physicians use Thway Hsay in the treatment of various kinds of diseases caused by blood. It is specially suitable for monks, elder people and females in the treatment of dizziness, giddiness, gynaecological disorders and fever by using different vehicles. It can be used by snuffing applying on the skin and taken orally. Myanmar Thway Hsay is well known as blood tonic for anemic patients. It is being used for all anemic patients including children. According to the literature, elements are found to have important role in biological processes and are essential for life and maintenance of plants and animals (Frieden, 1984). Naturally occurring minerals have been used as active principles in pharmacy for centuries (Hidreth, 1977).

## 2. MATERIALS AND METHODS

Ten brands of Myanmar indigenous medicines containing Kyauk-thway were purchased from Bogyoke, Hledan, and Hlegu markets. All the samples were stored at room temperature. The ash contents of samples were determined by using a porcelain crucible and burning initially on a hot plate, then finally in a muffle furnace at 400˚C. In this work, the moisture content of samples was determined by oven-dried method. The mineral elements (Na, K, Mg, Ca), essential nutritive elements (Fe, Zn, Cu), non-nutritive toxic elements (Pb and As) were determined by Atomic Absorption Spectroscopy (AAS). Nitrogen contents were determined by micro-Kjeldahl method (Stansby and Dassow, 1963). Citric acid contents were determined by gravimetric method (Driver, 1955).

## 3. RESULTS AND DISCUSSION

Traditional medicine involves not only the use of herbal medicines but also the use of animal parts and minerals. In the present work, ten brands of Myanmar medicine samples containing Kyauk-thway were collected from shops selling Myanmar indigenous medicines (Parasay Shops) and Ministry of Health, Department of Traditional Medicine.

### 3.1. Determination Of Ash And Moisture Contents

The ash percent in a foodstuff is actually the residual inorganic materials remaining after the organic matter has been burnt (Pearson, 1970). The ash % is a measure of the quality of food. The smaller the ash %, the better will be the food quality. However, in the case of Myanmar medicines, the active principles are the inorganic materials so that the ash % should be reasonable amount. Therefore, for this work, determination of ash content is an important picture or later determinations. Generally, it was found that the elemental contents were directly proportional to the ash content. In the present work, the ash contents of various

Myanmar medicines containing Kyauk-thway were found to be in the range of 4.9875 % to 20.8959 % (Table 1).

Many substances absorb moisture on storage (Beckett and Stenlake, 1975). The amount of heat to which the substance is submitted varies considerably according to the nature of the substance. The temperature must be sufficiently high to produce the required result within a reasonable time, but not so high as to cause decomposition. If the substance is stable that is usually applied by drying to constant weight at 105˚C. Table 2 shows the moisture contents of ten brands of Myanmar medicines containing Kyauk-thway. The moisture contents of various Myanmar medicines containing Kyauk-thway were found to be in the range of 6.00 % to 12.00 % (Table 2).

### 3.2. Determination Of Single Dose And Daily Dose Of Myanmar Indigenous Medicines

In Myanmar traditional medicines, the amount of single dose is specified as the amount of ywei gyi or ywei lay seed, as the amount of tamarind seed, as the amount of betel nut, the amount of tea-spoon full, as the amount of pe-tha (of weight) and as the number of tablets and capsules. The daily dose equals the amount of single dose multiplied by maximum intakes for one day. In the present work, the single dose and daily dose of Myanmar medicine samples were determined according to their instruction pamphlets. The weight of single dose, daily dose and maximum intake of ten brands of Myanmar indigenous medicines containing Kyauk-thway are shown in Table 3.

### 3.3. Determination Of Elemental Contents By Atomic Absorption Spectroscopy

Atomic absorption is a popular technique for the determination of metals in many types of samples. It is commonly used for the analysis of food (Christian and Feldman, 1970).

#### 3.3.1. Determination Of Mineral Element (Na, K, Ca, Mg) Contents

The mineral elements are essential to many vital processes (Chatterjee, 1981). Certain mineral elements, principally sodium and potassium are the major factors in osmotic control of water metabolism. According to literature, mineral elements, sodium, potassium, magnesium and calcium are currently known or thought to be required for normal biological functions in humans (Lippard, 1990).

Although potassium is excreted into the intestine in the digestive fluids, much of this is later reabsorbed. The kidney is the principal organ of excretion for potassium. A prolonged deficiency of potassium may produce severe damage to the kidney(Harper, 1965).

In the present work, mineral elements(Na, K, Mg, Ca)contents were shown in Table 4.

#### 3.3.2. Determination Of Essential Nutritive Elements (Fe, Zn, Cu) Contents And Toxic Elements (Pb, As)

Iron is a component of hemoglobin, myoglobin and cytochrome as well as the enzymes catalase and peroxidase. The role of iron in the body is almost exclusively confined to the processes of cellular respiration (Harper, 1965). The iron content in Myanmar medicines were shown in Table 5.

Zinc is a mineral that is essential for a healthy immune system, production of certain hormones, wound healing, bone formation and clear skin. It is required in small amounts and is thus known as a trace mineral. Zinc is found in nearly every cell of the body and is a key to the proper function of over 300 enzymes. The recommended daily allowance for zinc is 15 mg per day for adults (Sackheim and Lehman, 1977).

Copper seldom gives rise to poisoning, and has been less frequently used in medicine than many of the other heavy metals. Large quantities, however, include corrosion of the walls of the stomach and intestine and give rise to violent vomiting and purging, blood appearing in them later from the corrosion of the mucous membrane. Violent pain in the abdomen is complained of, and the usual symptoms of acute corrosive poisoning may follow: delirium, coma, headache, convulsion, paralysis, nausea, vomiting and gastrointestinal mucosa. The human requirement for copper is 2.5 mg per day (Harper, 1965).

Lead is present in small amounts in many foods and is a normal constituent of the blood and tissues. It is classified as a category 2B carcinogen by the International Agency for Research on Cancer (Manahan, 1989). It is accumulated mostly in gill, liver, kidney and bone (World Health Organization, 1988). The daily requirement level of lead is 20-514 µg per day. In this research, the zinc, copper and lead contents in Myanmar medicines were shown in Table 6.

Arsenic is carcinogenic and chronic toxicity is known to occur as a result of exposure to natural sources or from accidental contamination of foods (Manahan, 1989). Human adult intakes in the range 12-25 µg per day are probably adequate to meet any possible

requirement. The arsenic contents of ten brands of Myanmar indigenous medicines were not detected.

### 3.3.3. Determination Of Iron, Nitrogen And Citric Acid (As Kyauk-thway) Contents

Iron's major function is to combine with protein and copper in making hemoglobin. Hemoglobin transports oxygen in the blood from the lungs to the tissues which need oxygen to maintain basic life functions.

Proteins are complex nitrogenous substances. Nitrogen occurs in many important materials such as proteins, fertilizer, synthetic drugs (Skoog, West and Holler, 1992). The total nitrogen content in certain organic compounds is determined by the Kjeldahl method, which is one of the most widely used in all analytical methods (Stansby and Dassow, 1963).

The citric acid is a white crystalline soluble inorganic tribasic acid. It is a sour taste and occur as the free acid in lemons (6%) and other sour fruits. It is a most important clearing go use of metabolic intermediates, since it deals with the final stages of the oxidation of carbohydrates and fats and is also involved in the synthesis of some amino acid.

The iron content, nitrogen content and citric acid content in Myanmar medicines were shown in Table 5.

### 3.3.4. Elemental Contents In Daily Dose Of Myanmar Indigenous Medicines

A safe and adequate intake of sodium ranges from 1100 to 3000 mg per day for adults. Daily requirements of 5 to as much as 15 g of sodium chloride have been recommended for adults by various authorities.

The normal intake of potassium in food is about 4 g per day.

The daily requirements of calcium for men and women are 800 mg (Grallam, 1974) during later half of pregnancy and lactation are 1.5 to 2 g and for children, 1 to 1.4 g.

Magnesium requirement of adult man has been estimated by balance techniques to lie between 200 and 300 mg per day. The recommended allowances are set at 350 mg per day for adult men and 300 mg per day for adult women (Seeling, 1964).

The daily requirement of an adult for iron is usually taken as being from 10 to 15 mg.

The elemental (Na,K, Ca, Mg, Fe) contents in daily dose of Myanmar indigenous Medicines were shown in Tables 7, 8, 9, 10 and 11.

**Table 1. The ash percentage of Myanmar medicines containing Kyauk-thway**

| No. | Sample | Mean ash (%) |
|---|---|---|
| 1 | Gold Tonic Powder | 8.9702 ± 0.0298 |
| 2 | Shwe Htarwara | 7.6413 ± 0.0254 |
| 3 | Shwe Ohn Thee | 6.7864 ± 0.0134 |
| 4 | Hman Cho | 8.6380 ± 0.0286 |
| 5 | Hnalone Thukha | 19.9008 ± 0.0990 |
| 6 | Ayubayda Hnalone Theejay Hsay | 20.8959 ± 0.1039 |
| 7 | Ayubayda Hnalone Arsay | 6.7332 ± 0.0168 |
| 8 | Shwe Nan Dwin | 6.3123 ± 0.0209 |
| 9 | Ahbinyin (U Nyan Htun) | 6.6442 ± 0.0221 |
| 10 | Mae Myanmar | 4.9875 ± 0.0124 |

**Table 2. Moisture content (g %) in Myanmar medicines containing Kyauk-thway**

| No. | Sample | Mean Moisture (%) |
|---|---|---|
| 1 | Gold Tonic Powder | 9.00 ± 0.10 |
| 2 | Shwe Htarwara | 10.68 ± 0.07 |
| 3 | Shwe Ohn Thee | 12.00 ± 0.11 |
| 4 | Hman Cho | 11.33 ± 0.02 |
| 5 | Hnalone Thukha | 11.00 ± 0.05 |
| 6 | Ayubayda Hnalone Theejay Hsay | 6.00 ± 0.12 |
| 7 | Ayubayda Hnalone Arsay | 6.67 ± 0.03 |
| 8 | Shwe Nan Dwin | 9.66 ± 0.08 |
| 9 | Ahbinyin (U Nyan Htun) | 9.33 ± 0.07 |
| 10 | Mae Myanmar | 6.50 ± 0.04 |

**Table 3. Weight of single dose, daily dose and maximum intake of drug according to drugs manufacturer's specification**

| Sample | Adult | | |
|---|---|---|---|
| | Single dose (g) | Maximum intake (time) | Daily dose (g) |
| Gold Tonic Powder | 0.272 | 3 | 0.816 |
| Shwe Htarwara | 0.870 | 3 | 2.610 |
| Shwe Ohn Thee | 0.830 | 3 | 2.490 |
| Hman Cho | 0.272 | 3 | 0.816 |
| Hnalone Thukha | 1.020 | 3 | 3.060 |
| Ayubayda Hnalone Theejay Hsay | 2.040 | 3 | 6.120 |
| Ayubayda Hnalone Arsay | 2.040 | 3 | 6.120 |
| Shwe Nan Dwin | 0.272 | 2 | 0.544 |
| Ahbinyin (U Nyan Htun) | 0.272 | 2 | 0.544 |
| Mae Myanmar | 2.230 | 3 | 6.690 |

**Table 4. Mineral element contents (%) in Myanmarmedicines containing Kyauk-thway**

| Sample | Mineral Element Contents (%) * | | | |
|---|---|---|---|---|
| | Na | K | Ca | Mg |
| Gold Tonic Powder | 0.056 | 0.061 | 0.038 | 0.065 |
| Shwe Htarwara | 0.080 | 0.038 | 0.058 | 0.064 |
| Shwe Ohn Thee | 0.049 | 0.051 | 0.073 | 0.093 |
| Hman Cho | 0.133 | 0.088 | 0.123 | 0.102 |
| Hnalone Thukha | 0.040 | 0.040 | 0.023 | 0.033 |
| Ayubayda Hnalone Theejay Hsay | 0.042 | 0.027 | 0.044 | 0.027 |
| Ayubayda Hnalone Arsay | 0.154 | 0.129 | 0.179 | 0.155 |
| Shwe Nan Dwin | 0. 064 | 0.055 | 0.097 | 0.086 |
| Ahbinyin (U Nyan Htun) | 0.032 | 0.058 | 0.047 | 0.038 |
| Mae Myanmar | 0.071 | 0.051 | 0.090 | 0.056 |

* based on sample weight

**Table 5. Iron, nitrogen and citric acid (as Kyauk-thway) contents in Myanmar medicines containing Kyauk-thway**

| Sample | Contents (%) * | | |
|---|---|---|---|
| | Fe | N | Citric acid |
| Gold Tonic Powder | 0.049 | 1.54 | 0.34 |
| Shwe Htarwara | 0.015 | 1.54 | 0.70 |
| Shwe Ohn Thee | 0.038 | 2.52 | 0.99 |
| Hman Cho | 0.091 | 1.12 | 1.03 |
| Hnalone Thukha | 0.014 | 0.70 | 0.51 |
| Ayubayda Hnalone Theejay Hsay | 0.020 | 1.40 | 0.70 |
| Ayubayda Hnalone Arsay | 0.117 | 1.26 | 0.67 |
| Shwe Nan Dwin | 0.046 | 1.40 | 1.03 |
| Ahbinyin (U Nyan Htun) | 0.034 | 1.12 | 1.25 |
| Mae Myanmar | 0.088 | 1.26 | 0.66 |

* based on sample weight

**Table 6. The essential nutritive elements contents and toxic elements contents in Myanmar medicines containing Kyauk-thway**

| Sample | Elemental Contents (ppm) | | |
|---|---|---|---|
| | Cu | Zn | Pb |
| Gold Tonic Powder | 0.598 | 0.598 | ND[#] |
| Shwe Htarwara | 0.509 | ND | ND |
| Shwe Ohn Thee | ND | ND | 0.452 |
| Hman Cho | ND | ND | ND |
| Hnalone Thukha | 0.306 | ND | ND |
| Ayubayda Hnalone Theejay Hsay | 0.499 | 0.332 | 0.499 |
| Ayubayda Hnalone Arsay | ND | ND | ND |
| Shwe Nan Dwin | ND | ND | ND |
| Ahbinyin (U Nyan Htun) | 0.664 | 0.443 | 0.443 |
| Mae Myanmar | ND | ND | ND |

[#] not detected

**Table 7. Sodium contents in daily dose of Myanmar indigenous medicines**

| Sample | Based on daily dose Na (mg) | |
|---|---|---|
| | Adult | Child |
| Gold Tonic Powder | 0.457 | 0.457 |
| Shwe Htarwara | 2.088 | 1.392 |
| Shwe Ohn Thee | 1.220 | 0.610 |
| Hman Cho | 1.085 | 1.085 |
| Hnalone Thukha | 1.224 | 0.408 |
| Ayubayda Hnalone Theejay Hsay | 2.570 | 1.285 |
| Ayubayda Hnalone Arsay | 9.423 | 4.712 |
| Shwe Nan Dwin | 0.348 | 0.348 |
| Ahbinyin (U Nyan Htun) | 0.174 | 0.174 |
| Mae Myanmar | 4.750 | 2.375 |

**Table 8. Potassium contents in daily dose of Myanmar indigenous medicines**

| Sample | Based on daily dose K (mg) | |
|---|---|---|
| | Adult | Child |
| Gold Tonic Powder | 0.498 | 0.498 |
| Shwe Htarwara | 0.992 | 0.661 |
| Shwe Ohn Thee | 1.270 | 0.635 |
| Hman Cho | 0.718 | 0.718 |
| Hnalone Thukha | 1.224 | 0.408 |
| Ayubayda Hnalone Theejay Hsay | 1.652 | 0.826 |
| Ayubayda Hnalone Arsay | 7.895 | 3.947 |
| Shwe Nan Dwin | 0.299 | 0.299 |
| Ahbinyin (U Nyan Htun) | 0.316 | 0.316 |
| Mae Myanmar | 3.412 | 1.706 |

**Table 9. Magnesium contents in daily dose of Myanmar indigenous medicines**

| Sample | Based on daily dose Mg (mg) | |
|---|---|---|
| | Adult | Child |
| Gold Tonic Powder | 0.530 | 0.530 |
| Shwe Htarwara | 1.670 | 1.113 |
| Shwe Ohn Thee | 2.315 | 1.158 |
| Hman Cho | 0.832 | 0.832 |
| Hnalone Thukha | 1.010 | 0.336 |
| Ayubayda Hnalone Theejay Hsay | 1.652 | 0.826 |

| | | |
|---|---|---|
| Ayubayda Hnalone Arsay | 9.486 | 4.743 |
| Shwe Nan Dwin | 0.468 | 0.468 |
| Ahbinyin (U Nyan Htun) | 0.207 | 0.207 |
| Mae Myanmar | 3.746 | 1.873 |

**Table 10. Calcium contents in daily dose of Myanmar indigenous medicines**

| Sample | Based on daily dose Ca (mg) | |
|---|---|---|
| | Adult | Child |
| Gold Tonic Powder | 0.310 | 0.310 |
| Shwe Htarwara | 1.514 | 1.009 |
| Shwe Ohn Thee | 1.817 | 0.909 |
| Hman Cho | 1.003 | 1.003 |
| Hnalone Thukha | 0.704 | 0.235 |
| Ayubayda Hnalone Theejay Hsay | 2.693 | 1.346 |
| Ayubayda Hnalone Arsay | 10.955 | 5.477 |
| Shwe Nan Dwin | 0.528 | 0.528 |
| Ahbinyin (U Nyan Htun) | 0.256 | 0.256 |
| Mae Myanmar | 6.021 | 3.010 |

**Table 11. Iron contents in daily dose of Myanmar indigenous medicines**

| Sample | Based on daily dose Fe (mg) | |
|---|---|---|
| | Adult | Child |
| Gold Tonic Powder | 0.400 | 0.400 |
| Shwe Htarwara | 0.392 | 0.261 |
| Shwe Ohn Thee | 0.946 | 0.473 |
| Hman Cho | 0.743 | 0.743 |
| Hnalone Thukha | 0.428 | 0.143 |
| Ayubayda Hnalone Theejay Hsay | 1.224 | 0.612 |
| Ayubayda Hnalone Arsay | 7.160 | 3.580 |
| Shwe Nan Dwin | 0.250 | 0.250 |
| Ahbinyin (U Nyan Htun) | 0.185 | 0.185 |
| Mae Myanmar | 5.887 | 2.943 |

## 4. CONCLUSIONS

Kyauk-thway is ferric ammonium citrate, so the iron, nitrogen and citric acid were contained in Myanmar medicines. The ash and moisture content (%) of ten brands of Myanmar indigenous medicines containing Kyauk-thway were found to be in the range of 4.9875 to 20.8959 % and 6.00 to 12.00 %. The mineral elements (Na, K, Mg, Ca) contents and essential nutritive elements (Fe, Zn, Cu) were found to be allowable limits. Among the samples of the ten brands of Myanmar indigenous medicines, 7 samples were found to be free from lead. Arsenic contents were not detected in all samples. Therefore, the samples of Myanmar indigenous medicines containing Kyauk-thway so far studied are found to have no undesirable side effects to health.

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# Isolation of Lupeol Compound and Pharmacological Actions of *Crataeva nurvala* Ham.Bark (Kadet)

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ABSTRACT: *The present study deals with the screening of antimicrobial activity, antioxidant activity and investigation of some chemical constituents from C. nurvala (kadet bark). Nutritional values such as contents of moisture, ash, protein, fiber, fat and carbohydrate were also determined on the C. nurval bark. The elemental analysis was carried out by Energy Dispersive X-ray Fluorescence (EDXRF) method. In accord with EDXRF spectral results, it was found that potassium was the highest content and heavy toxic elements were not detected. In antimicrobial screening, water, methanol, ethanol, ethyl acetate and petroleum ether extracts for this plant were examined by using agar well diffusion method. Among these extracts, ethyl acetate extracts show more potent antimicrobial activity (zone of inhibition ranged from 25 to 35 mm) than that of other crude extracts. In vitro antioxidant activity of crude extracts from C. nurvala (kadet) bark were investigated by using DPPH free radical scavenging assay method. The chemical nature of isolated compound-1 was classified by some physicochemical properties; such as TLC, UV, FT IR and* $^{1}H$ *NMR.*

*Keywords*— *C. nurvala (kadet bark), Antimicrobial activity, Antioxidant activity*

## 1. INTRODUCTION

Capparidaceae family contains about 45 genera and 700 species of trees. Those are distributed mainly in the tropical parts of the world. *Crataeva nurvala* is belonging to the family Capparidaceae. This plant is a leafy small to medium sized soft wooded tree with fragrant white flowers. The evergreen tree grows widely in all parts of Bangladesh, Pakistan, India, Philippine, Myanmar, South America, China, and Africa. It is distributed throughout India and tropical regions of the world cultivated. *C. nurvala* is also called three leaved caper. It is commonly known as steaved tree in English. Whole of the plants are used for medicinal purpose. The bark of the tree is an important drug for problem affecting the kidneys and bladder. It is especially effective in the urinary complaints, kidney and bladder stones, fever, vomiting and gastric irritation. Root and bark are also laxative and lithotripter. They increase appetite and biliary secretion. The bark of *C. nurvala* is contraceptive and cytotoxic, It is also useful in urinary disorders, kidney bladder stones, fever, vomiting and gastric irritation [4].

This plant is found along streams and also in dry, deep boulder formations in sub Himalayan tract. Stem bark of the plant contains saponins, flavonoids, sterols. Leaves are externally used in rheumatism, febrifuge and tonic. The major component isolated from this plant is lupeol, which is used to treat hypercrystalluria, hyperoxaluria and hypercalciuria. This compound also decreases elevated concentration of oxalate, phosphorus and magnesium in renal tissue [6].

## 2. MATERIALS AND METHODS

### 2.1. Collection and Preparations of Sample

The *C. nurvala* (kadet bark) were collected from Danuphyu Township. The collected sample was identified by authorized botanists, in Department of Botany, Maubin University. After cleaning, the *C. nurvala* was cut and air-dried. The air-dried sample were made powdered by using blender and then stored in air-tight container for preventing moisture changes, other contamination and further research works.

### 2.2. Phytochemical Investigation of Plant Sample

Preliminary phytochemical examination was carried out on dried powdered *C. nurvala* (kadet bark) samples with a view to determine the presence or absence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, starch, steroids and tannins.

### 2.3. Nutritional Values of *C. nurvala* (kadet) bark

The nutritional value of the dried powder sample was determined by AOAC methods. The moisture content of dried powdered samples was determined by using oven drying method. The protein content was determined by micro kjeldahl method. The fat content was determined by soxhlet extraction method. Fiber content detected by acid-base treatment

method. The total carbohydrate content of any food can be obtained as the difference between 100 and the sum of the percentages of moisture, protein, fat, ash and fiber.

### 2.4. Elemental Analysis of Plant Sample by EDXRF

In order to determine the heavy toxic metals and macronutrient elements in the bark of *C. nurvala* were determined by Energy Dispersive X-ray Fluorescence method [5] at the West Yangon University.

### 2.5. Screening of Antimicrobial Activity

A total of 6 different species of microorganisms will be cultured in Pharmaceutical Research Department, Ministry of Health and Sports, Yangon. Crude extracts sample were investigated by six pathogenic strains using agar well diffusion method [2].

### 2.6. Rapid Screening of Antioxidant by Dot-Blot and DPPH Staining

DPPH (1,1-diphenyl-2-picryl–hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of plant materials. Each methanol extracts sample was carefully loaded onto a 6 cm × 6 cm TLC layer (silica gel $GF_{254}$ precoated plates; Merck) and allowed to dry 3 min. Drops of each sample were loaded, in order of decreasing concentration (400 to 12.5µg/mL), along the row [3]. The sheet bearing the dry spots was placed upside down for 10 s in a 0.4 mM DPPH solution. The intensity of the yellow color depends upon the amount and nature of radical scavenger present in the sample.

### 2.7. Isolation of Some Organic Constituents from Ethanol Extract of *Crataeva nurvala* (kadet) bark

The dried powdered sample of selected kadet barks (200g) were firstly extracted by maceration with 75 % EtOH for two days. This ethanol extract was filtered and then concentrated under reduced pressure at 45 ºC using rotatory evaporator. The marc remained was similarly extracted, and filtered again successively more than two times for nearly two weeks. The alcohol soluble matter was partitioned between water and petroleum ether (60-80 ºC) to remove the fat. The

dark brown colour crude extract was obtained. The crude extract was washed with PE: EA (9:1) and then recrystallized with methanol. The purified compound-1(colorless crystal, 1.2%) was obtained.

## 3. RESULTS AND DISCUSSION

### 3.1. Preliminary Phytochemical Investigation of Plant Sample

According to these experiments, alkaloids, α-amino acids, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, steroids, terpenoids and tannins were present in sample. Reducing sugars were not detected in *C. nurvala* (kadet bark).

### 3.2. Determination of Nutritional Values of *C. nurvala* (kadet) bark

The nutritional values such as fats, protein, carbohydrates and fiber were determined. As a result, it was found that carbohydrates were present as major nutrient in samples. The determination of ash, fat, fiber, moisture and protein content were made according to method described in the AOAC method (2000) [1].

**Table 1. Nutritional values (%) in *Cataeva nurvala* (kadet) bark**

| No. | Nutrients | Content |
|---|---|---|
| 1 | Moisture (%) | 12.00 |
| 2 | Ash (%) | 3.20 |
| 3 | Protein (%) | 2.63 |
| 4 | Crude Fiber) (%) | 31.00 |
| 5 | Crude Fat (%) | 3.26 |
| 6 | Carbohydrate (%) | 47.91 |
| 7 | Energy Value (k cal / 100g) | 231.5 |

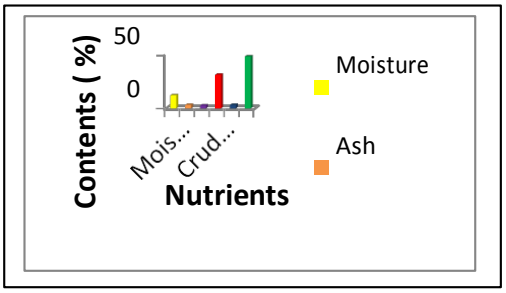


**Figure1. Histogram of nutrition values from *Crataeva nurvala* (kadet) bark**

### 3.3. Elemental Analysis of Plant Sample by EDXRF Method

The elemental analysis was performed by Energy Dispersive X-Ray Fluorescence spectrometer. As a result K (1.42 %), S (1.12 %), Ca (0.84%), Si (0.12 %) and Fe (0.03 %) were found to be present. They are essential elements for various metabolism and activity of enzyme of body. Among them, potassium peak was also the most predominant and so it showed potassium was the highest content. According to EDXRF, toxic elements were found to be absent in the selected plant sample.

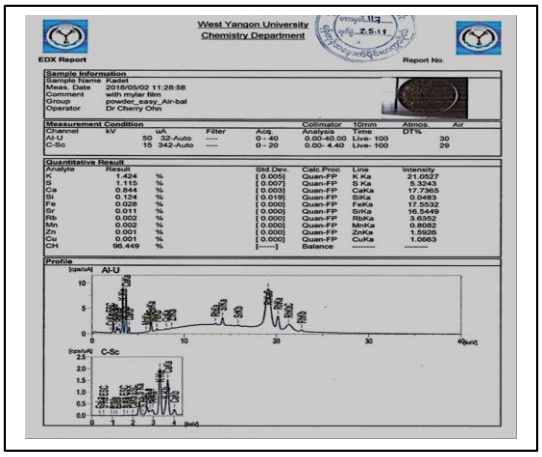


**Figure 2. EDXRF spectrum of *Crataeva nurvala* (kadet) bark**

### 3.4. Screening of Antimicrobial Activity

Water, petroleum ether, ethyl acetate, methanol and ethanol extracts have been tested against two strains of *Bacillus, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Escherichia coli*.

It was found that ethyl acetate extract showed more significant zone of inhibition when compared with other extracts of this plant The activities of ethyl acetate extract showed high activity (zone of inhibition ranged from 25 to 35 mm). The ethanol and methanol extracts exhibited moderate activity (zone of inhibition ranged from 14 to 17mm). The petroleum ether extract showed low activity (zone of inhibition showed less than 14). It can be obviously seen that watery extract of *C. nurvala* bark did not exhibit the potent antimicrobial activity.

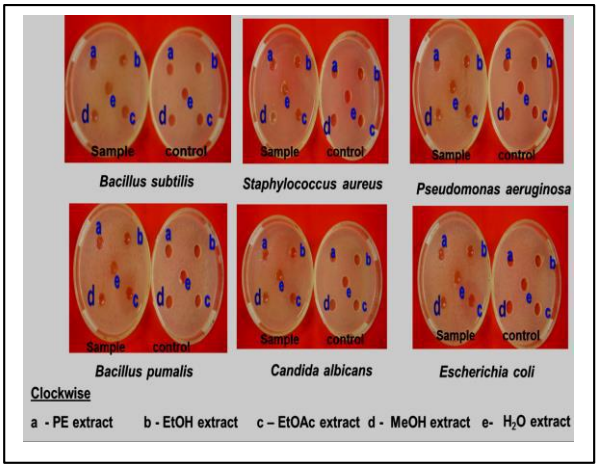


**Figure 3. Antimicrobial screening of crude extracts of *Crataeva nurvala* (kadet) bark**

### 3.5. Rapid Screening of Antioxidant by Dot-Blot and DPPH Staining

The antioxidant activity was studied on the methanol extracts from the selected plant samples by rapid screening of antioxidant by dot-blot and DPPH staining method as mentioned in section 2.6. Methanol extracts fractions were applied as a dot on a TLC layer that was then stained with DPPH solution. Yellow spots with strong intensity appeared quickly at the concentration of 12.5 µ g/ml of *C. nurvala* (kadet bark) per application.

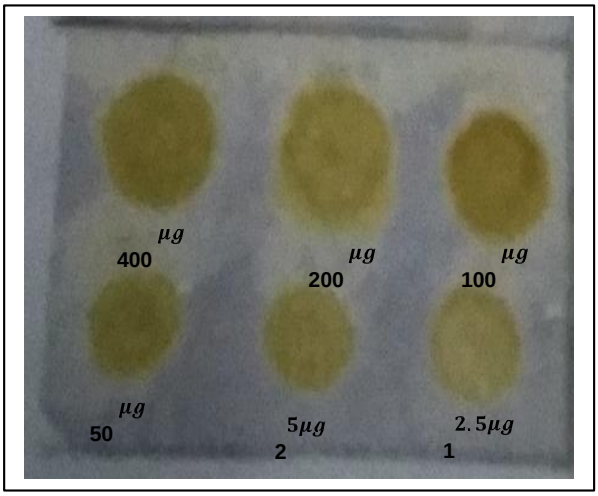


**Figure 4. Screening of antioxidant activity of *Crataeva nurvala* (kadet) bark extracts by dot-blot and DPPH staining**

### 3.6. Identification of Isolated Compound-1 from *Crataeva nurvala* ( kadet ) bark

Compound-1 was isolated as colourless needle shape from EtOH extracts of kadet bark. It was the UV inactive but, it gave a purple on TLC chromatogram by spraying with vanillin-$H_2SO_4$, brown coloured spot with 5% $H_2SO_4$ and purple colouration with Libermann-Burchard reagent. It was classified as a terpenoid. In addition, the $R_f$ 0.45 (PE: EtOAc, 9:1 v/v) was also coincident with that of standard luperol. In the UV spectrum of compound-I in methanol, the maximum absorption at the wavelength 203 nm illustrated the π to π* transition indicating the presence of isolated double bonds (see Table 2 and Figure 5). FT-IR spectrum of compound-I and its corresponding spectra data assignments and that of luperol are described in Table 3. On the basic of observed data of FTIR spectrum(Figure 6), absorption band appeared at 3420 $cm^{-1}$ due to the OH stretching vibration, 3070 $cm^{-1}$ due to the $C=CH_2$ stretching vibration group, 2962 $cm^{-1}$, 2924 $cm^{-1}$ due to C-H stretching vibration of $–CH_3$, $-CH_2$ groups. The band at 1637 $cm^{-1}$, 1580 $cm^{-1}$ indicated the stretching band of C= C group. The band at 1451 $cm^{-1}$, 1378 $cm^{-1}$, 1334 $cm^{-1}$ suggested that the in plane banding of $CH_2$ and $CH_3$ group (gem-dimethyl). The band at1188 $cm^{-1}$, 1042 $cm^{-1}$ were due to the asymmetric and symmetric C-O stretching vibration of CH-OH and out of plane banding vibration of CH group indicated the 827 $cm^{-1}$ respectively. In $^1H$ NMR spectrum (in $CDCl_3$) (Figure 7), the olefinic protons showed as *brods* at δ 4.56 and 4.68 ppm. The six tertiary methyl signals as singlets at δ 1.03, 0.97, 0.93, 0.83, 0.79, 0.75 ppm were coincident with that of reported lupeol (Table 4). The vinyl methyl proton appeared as singlet at δ 1.68 ppm like other methyl protons. The proton signal at δ 3.2 ppm as *dd* indicated that the secondary carbinol proton. The complex multiplets between δ 1.20-2.39 ppm are due to the protons of $CH_2$ and $CH_3$ group. All these $^1H$ NMR data were found to identical with those of lupeol.

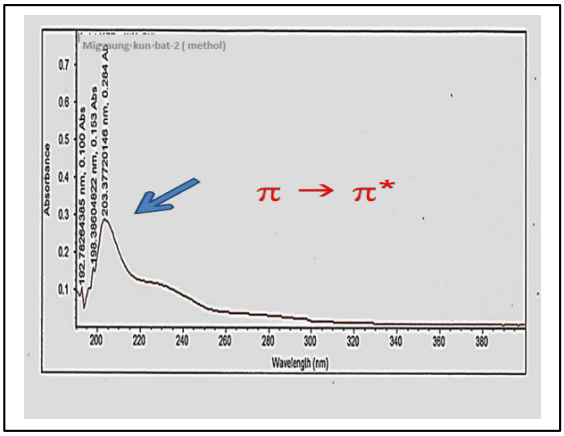


**Figure 5. UV spectrum of isolated compound-1 (lupeol) from *Crataeva nurvala* (kadet) bark**

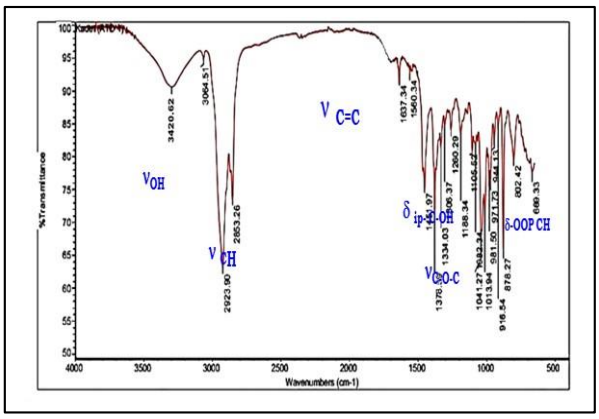


**Figure 6. FTIR spectrum of isolated compound-1(lupeol) from *Crataeva nurvala* (kadet) bark**

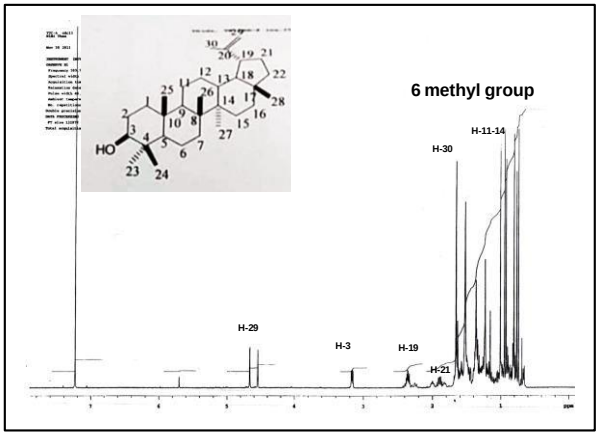


**Figure 7. $^1H$ NMR spectrum of isolated compound-1 (lupeol) from *Crataeva nurvala* (kadet) bark**

**Table 2. UV spectral data of isolated compound-1**

| Observed $\lambda_{max}$ (nm) | Assignment |
|---|---|
| 203 | π → π* of isolated double bond |

**Table 3. FT IR spectral data of isolated compound-1 and reported lupeol**

| Wave number ($cm^{-1}$) | | Band assignments |
|---|---|---|
| Lupeol | Compound-1 | |
| 3463 | 3420 | $\nu_{OH}$ |
| 3007 | 3064 | $\upsilon_{C=CH2}$ |
| 2949 | 2923,2853 | $\upsilon_{asyCH}$ and $\nu_{syCH}$ |
| 1643 | 1637,1580 | $\nu_{C-=C}$ |
| 1457,1380 | 1451,1378 | $\divideontimes$ ip-CH3 |
| 1189, 1076 | 1188, 1042 | $\upsilon_{C-O-C}$ |
| 825 | 827 | $\divideontimes$ oop-CH |

**Table4. $^1$H NMR (600MHz, $CDCl_3$) spectral data of isolated compound-1 and compared with reported lupeol**

| H-atom | δ-H ppm | | Multiplicitv | Assignment |
|---|---|---|---|---|
| | Compound-1 | Reported lupeol | | |
| 3 | 3.20 | 3.18 | *dd* | -CHOH |
| 23 | 0.97 | 0.97 | *s* | $-CH_3$ |
| 24 | 0.75 | 0.74 | *s* | $-CH_3$ |
| 25 | 0.83 | 0.81 | *s* | $-CH_3$ |
| 26 | 1.03 | 1.03 | *s* | $-CH_3$ |
| 27 | 0.93 | 0.94 | *s* | $-CH_3$ |
| 28 | 0.79 | 0.77 | *s* | $-CH_3$ |
| 29 ( a, b) | 4.56. 4.68 | 4.57, 4.67 | *brods* | $C=CH_2$ ( olefinic) |
| 30 | 1.68 | 1.66 | *s* | $-CH_3$ |
| OH | 3.62 | 3.64 | - | O-H |
| Other 'p' | 1.20-2.39 | | *m* | CH and $CH_2$ protons |

Reported data [4]

## 4. CONCLUSION

From this study of chemicals and bioactivity investigation of *Crataeva nurvala* Ham. bark (kadet), the following inferences can be concluded.

As the biological screenings, such as antimicrobial activities and DPPH staining were demonstrated from activity guided plant extracts. Biological active compound-1 (lupeol, 1.2%) from *Crataeva nurvala* was also carried out. The structures of isolated compound-1 was identified and elucidated by Co-TLC method and Joint application of modern spectroscopic techniques. According to phytochemical investigation of selected plant was found to contained α-amino acids, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, steroids, terpenoids, alkaloids and tannins were present in plant samples. Reducing sugars were not detected in kadet bark.

Nutritional values such as moisture content (12.00 %), ash content (3.20%), protein content (2.625 %), fiber content (3.1%), fat content (3.26%) and carbohydrate content (47.91%) were also determined on *C. nurvala* (kadet) bark. In plant samples, carbohydrates were present as major nutrients.The elemental analysis of C. *nurvala* (kadet) bark was carried out by EDXRF method. By EDXRF method, it was found that potassium was the most abundant element and no heavy toxic elements were detected. In the *C. nurvala* bark, K (1.42%), S (1.12 %), Ca (0.84%), Si (0.12 %) and Fe (0.03 %) were found to be present. Water, petroleum ether, ethyl acetate, methanol and ethanol extracts were screened by using agar well diffusion method against by six pathogenic strains. The activities of ethyl acetate extract on the organisms are considerably high (zone of inhibition ranged from 25 to 35 mm). The ethanol and methanol extracts exhibited moderate activity (zone of inhibition ranged from 14 to 17mm). It can be obviously seen that watery extract of *C. nurvala* bark did not exhibit the potent antimicrobial activity. In antioxidant activity, methanol extracts of kadet bark showed potent activity at dry matter amount (12.5 µg to 400µg dry matter/mL). The yellow spots with strong intensity appeared quickly at the concentration of 12.5 µg/ml of kadet bark per application. The appearances of yellow colored spots have a potential value of antioxidant activity. In brief, based on above scientific findings, kadet bark can be used for antimicrobial and antioxidant agents in traditional medicine. Phytochemical constituents such as terpenoids, phenolic compounds and flavonoids present in this plant may be responsible for these activities.

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# Effect of Gamma Radiation on Agronomic Characteristics of Maize (*Zea Mays* L.)

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**ABSTRACT:** *The effect of gamma radiation on maize was investigated in this research. The seeds of maize were irradiated by gamma radiation from $^{60}$Co source with different doses (0.05 kGy, 0.25 kGy, 0.45 kGy and 0.65 kGy) respectively. After irradiation, these irradiated seeds and non-irradiated seeds (GM 0 kGy) were cultivated for first generation at Min Thar Kyun Village, Pantanaw Township, Ayeyarwady Region. The agronomical characteristics, nutritional values and antioxidant activity of mutant maize as a result of gamma irradiation were studied. GM 0.65 kGy was found to be the lethal dose for germination of maize. Moreover, the growth of GM 0.45 kGy very slowed as compare to the others. The agronomic characteristics of maize such as (height of plants, length, weight, number of rows of seeds and yield) of maize were studied. From these studies, it was observed that the length, weight, numbers of rows of seeds of GM 0.25 kGy (17 rows) were greater than the others. However, GM 0.05 kGy was found to be the highest yield of maize (72.7 %). GM 0.45 kGy was a diminutive figure among them. The nutritional values of control (GM 0 kGy) and irradiated maize are not quite different. In the DPPH staining, the methanol extract of GM 0.05 kGy had the highest radical scavenging activity, since it showed from minimum value of 3.125 μg to 400 μg. GM 0 kGy extract also showed from minimum value of 25 μg to 400 μg. Therefore, GM 0.05 kGy may possess higher antioxidant potency than that of GM 0 kGy. Therefore, main constituent of GM 0.05 kGy may contribute significantly to potent antioxidant activity.*

**Keywords:** gamma irradiation, nutritional values, $^{60}$Co, GM

## 1. INTRODUCTION

Maize is mostly consumed in all countries including Myanmar. In Ayeyarwady region, most of people cultivate maize as one of the economies. The present work is part of the study on effect of gamma irradiation on maize. To realize the differences between the non-irradiated and irradiated maize plants, this research was conducted.

### 1.1. Maize

| | | |
|---|---|---|
| Family | : | Poaceae |
| Genus | : | Zea |
| Species | : | *Z. mays* |
| Botanical name | : | *Zea mays* Linn |
| English name | : | maize, corn |
| Myanmar name | : | Pjaun |

### 1.2. Types of Maize

Maize has been classified in different types according to its use and/or starch content viz., Sweet corn (Zea mays var. Saccharata), Dent corn (Zea mays var. Indentata), Flint corn (Zea mays var. Indurata), flour corn (Zea mays var. Amylacea), Pod corn (Zea mays var. Tunicate), Baby corn (Zea mays L.) and Waxy corn (Zea mays var. Ceratina) [1].

### 1.3 Cultivation Regions in Myanmar

The main corn producing areas in Myanmar are primarily found in hilly and dry zones of the country with smaller production taking place in the delta and coastal regions. According to government sources, Shan state which is located in the central part of country, accounts for 52 % of Myanmar's total corn production area while the Ayeyarwady (delta regions), Magwe, and Sagaing regions make up the balance.

### 1.4 Health Benefits of Maize

Maize has various health benefits. The B-complex vitamins in maize are good for skin, hair, brain, and proper digestion. They also prevent the symptoms of rheumatism because they are believed to improve the joint motility [2]. The presence of vitamins A, C and K together with beta-carotene and selenium helps to improve the functioning of thyroid gland and immune system. Potassium is a major nutrient present in maize which has diuretic properties. Maize silk has many benefits associated with it [3]

## 2. SAMPLE COLLECTION AND IRRADIATION

The seeds of maize (pan brand) (Figure 1) were bought from the market, Maubin Township, Ayeyarwady Region, Myanmar. The seeds of maize were irradiated with gamma rays at the Department of Atomic Energy, Ministry of Education, Hmawbi Township (Figure 2). The seeds were irradiated with different doses of gamma radiation (0.05 kGy, 0.25 kGy, 0.45 kGy and 0.65 kGy) from Co-60 gamma source.


(a) (b)

**Figure 1. (a) Pan brand (b) Seeds of maize**

**(a)** **(b)**

**Figure 2. (a) Gamma Source (b) Irradiation of maize with gamma**

## 3. CULTIVATION PROCEDURE OF MAIZE

The irradiated and non-irradiated maize were cultivated at Min Thar Kyun Village, PantanawTownship, Ayeyarwady Region. Firstly, the humus was filled into the each polypropylene bags, this is called the grow bag. The top of each grow bag was poked with fingers, creating a hole from 2 to 4 cm deep. One or two seeds of maize was dropped in each grow bag and then the top of the hole was covered with soil. Watering should be sprayed daily. Sprouts were appeared between 4-9 days. The water should be wetting on the soil before planting. After nine days, the height of stalk of the maize reached to 6 cm these maize plants were ready for cultivation. The holes of soil with the depth of 5-10 cm were made at the small maize plants from the grow bags were put in these holes. Then, the hole was slightly filled with soil. After cultivation, the water was sprayed one time in two days. The cultivated maize plants are shown in Figure 3. After three weeks, the fertilizer was feed if plants are grown at 18-28 cm height. And then, the age of maize plant between 3 to 5 weeks were sprayed insecticide. The weeds were removed at least twice in one month. Because weeds were drain the nutrients of the soil. The fertilizers were feed the maize plant at the age of 6 and 9 weeks. After two months, when the maize plant have fully grown and the ears start to develop. At about three months, when tassels begin to turn brown and cob start to swell and kernels were full, the ears were harvested (Figure 3).

**(a)**

**(b)**

**(c)**

**Figure 3. The cultivated maize (a) Germination (b)Maize plants after cultivation for one month (c) The ears of maize (GM 0.25 kGy)**

## 4. STUDY OF AGRONOMICAL CHARACTERISTICS OF MUTANT MAIZE

The agronomical characteristics (such as height of plant, weight of seeds, row of seeds, length of maize, quality and yield) of mutant maize were investigated. Non-irradiated (GM 0 kGy) was used as comparative study.

### 4.1 Plant Height

The observed plant height are shown in (Table1).

**Table l. Observed Plant Heights of Mutant Maize from 3st to 12rd Week**

| Maize | Plant Height (cm) | | | |
|---|---|---|---|---|
| | 3 w | 6 w | 9 w | 12 w |
| GM 0 kGy | 29.3 | 124.4 | 249.9 | 255.1 |
| GM 0.05 kGy | 26.2 | 135.9 | 256.3 | 261.5 |
| GM 0.25 kGy | 28.0 | 132.9 | 240.5 | 243.8 |
| GM 0.45 kGy | 15.5 | 83.8 | 125.6 | 138.7 |
| GM 0.65 kGy | 5.5 | NG | NG | NG |

NG= not grow, GM= Gamma Irradiated Maize, GM 0 kGy= non-irradiated maize, W=week

### 4.2 Length of Mutant Maize

The measured length of mutant maize and non-irradiated maize are shown in (Figure 4) and (Table 2).

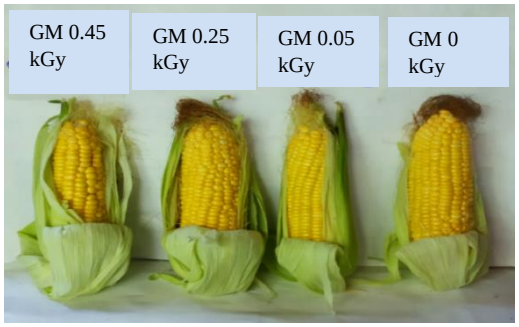



**Figure 4. Maize with different gamma doses**

**Table 2. Measured length of selected mutant maize plants**

| Maize | Length of Maize (cm) | | | | Average |
|---|---|---|---|---|---|
| GM 0 kGy | 19.5 | 19.6 | 19.8 | 20.2 | 19.8 |
| GM 0.05 kGy | 19.1 | 19.5 | 19.8 | 20.4 | 19.2 |
| GM 0.25 kGy | 18.5 | 18.9 | 22.6 | 23.1 | 20.8 |
| GM 0.45 kGy | 14.5 | 15.0 | 15.5 | 16.2 | 15.2 |

### 4.3 Rows of Mutant Maize

The rows of non-irradiated and mutant maize are described in (Table 3) and (Figure 5). GM 0.45 kGy has the least number of rows. Moreover, the highest number of rows (17) was found in GM 0.25 kGy.

**Table 3. Numbers of rows of maize seeds**

| Maize | Rows of Seeds per Maize | | | | Average Rows |
|---|---|---|---|---|---|
| GM 0 kGy | 16 | 16 | 16 | 17 | 16 |
| GM 0.05 kGy | 15 | 16 | 16 | 18 | 16 |
| GM 0.25 kGy | 16 | 16 | 17 | 17 | 17 |
| GM 0.45 kGy | 9 | 12 | 13 | 13 | 12 |

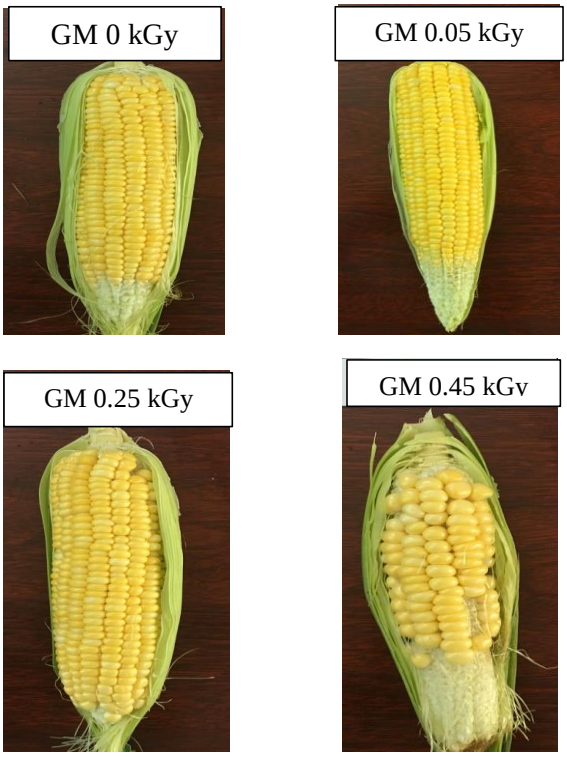



**Figure 5. Rows of mutant maize**

### 4.4 Weight of Mutant and Non-irradiated Maize

The measured weights of maize are presented in (Table 4).

**Table 4. Weight of mutant maize**

| Maize | Weight of Maize (g) | | | Average weight |
|---|---|---|---|---|
| GM 0 kGy | 254.01 | 285.8 | 313.0 | 299.4 |
| GM 0.05 kGy | 172.36 | 199.6 | 172.4 | 199.6 |
| GM 0.25 kGy | 226.79 | 285.8 | 340.2 | 303.9 |
| GM 0.45 kGy | 127.01 | 140.6 | 140.6 | 154.2 |

### 4.5 Yield Percent of Mutant Maize

The yield percent of mutant maize were also studied in this research. From this study, average yield percent of GM 0 kGy, GM 0.05 kGy, GM 0.25 kGy and GM 0.45 kGy were found were to be 66.7 %, 72.7 %, 25.0 % and 20.0 % respectively. It was found that the GM 0.05 kGy has the highest yield percent of maize than the others and GM 0.45 kGy is the lowest yield percent of maize (Table 5). These data were also clearly shown in (Figure 6).

**Table 5. Yield Percent of Mutant Maize**

| Maize | Yield % of maize |
|---|---|
| GM 0 kGy | 66.7 |
| GM 0.05 kGy | 72.7 |
| GM 0.25 kGy | 25.0 |
| GM 0.45 kGy | 20.0 |

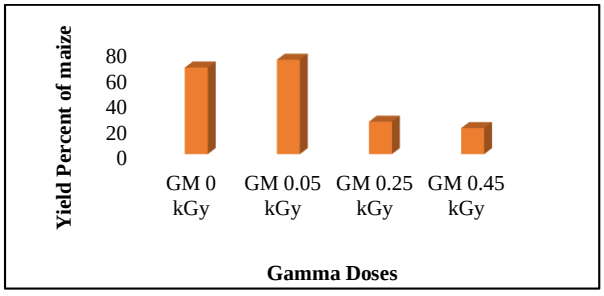
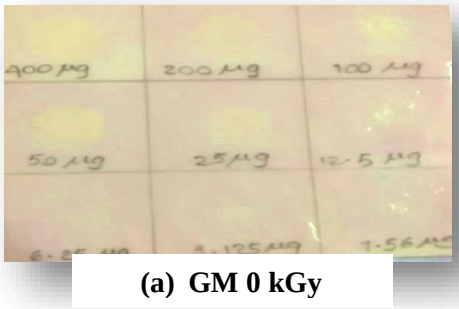


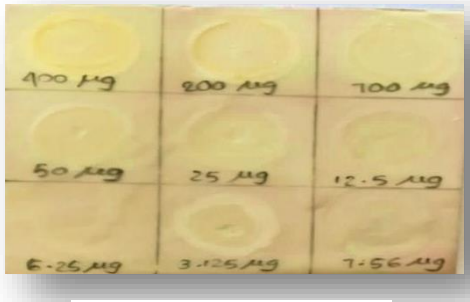

**Figure 6. Comparison of yield percent of maize**

## 5. RAPID SCREENING OF ANTIOXIDANT BY DOT-BLOT AND DPPH STAINING

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of maize. In this experiment, the antioxidant activity was studied on methanol extracts from the sample. Each diluted sample of methanol extracts the maize (GM 0 kGy and GM 0.05 kGy) was carefully loaded onto a 6 cm x 6 cm TLC layer (silica gel 60 $F_{254}$; Merck) and allowed to dry (3 min). Drops of each sample were loaded, in order of decreasing concentration (400, 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/mL), along the row. The sheet bearing the dry spots was placed upside down for 10 s in a 60 µM DPPH solution. Then the excess of solution was removed with a tissue paper and the layer was dried with a hair-dryer blowing cold air. Stained silica layer revealed a purple background with white spots at the location where radical-scavenger capacity presented. The intensity of the yellow color depends upon the amount and nature of radical scavenger present in the sample. The observed results are shown in (Figure 7 (a) and (b)).




**(a) GM 0 kGy**




**(b) GM 0.05 kGy**

**Figure 7. Screening of antioxidant activity of maize**

## 6. NUTRITIONAL VALUES OF MUTANT MAIZE AND NON IRRADIATED MAIZE

Nutritional values of irradiated maize and non-irradiated maize were studied by AOAC methods. The results are shown in (Table 6). It was found that the nutritional values of maize are not significantly changed.

**Table 6. Nutritional values of mutant maize and non-irradiated maize**

| Nutritional Parameter | GM 0 kGy | GM 0.05 kGy | GM 0.25 kGy | GM 0.45 kGy |
|---|---|---|---|---|
| Carbohydrates (%) | 15.7 | 15.7 | 16.4 | 17.0 |
| Protein (%) | 4.2 | 4.0 | 4.2 | 3.2 |
| Fiber (%) | 1.5 | 1.2 | 1.7 | 1.7 |
| Fat (%) | 2.7 | 2.9 | 2.9 | 2.8 |
| Moisture (%) | 73.8 | 74.1 | 72.6 | 73.4 |
| Ash (%) | 2.2 | 2.1 | 2.2 | 2.0 |
| Energy value (kcal/100g) | 103.5 | 104.5 | 108.6 | 105.5 |

## 7. CONCLUSIONS

All irradiated (GM 0.05 kGy, GM 0.25k Gy, GM 0.45 kGy, GM 0.65 kGy) and non-irradiated (GM 0 kGy) maize are cultivated. However, the growing of GM 0.45 kGy and GM 0.65 kGy are inhibited by gamma irradiation. High dose of gamma radiation causes damaging effects on the growing of maize plant. Low doses of radiation actually improve plant growth compared to non-irradiated maize (GM 0 kGy). From this research, gamma radiation can affect the growth of maize. From these studies, it can be seen that plant height of GM 0.05 kGy (261.5 cm) is greater than others. Moreover, It was observed that the length of mutant maize of GM 0.25 kGy (20.8 cm) is longer than others. GM 0.25 kGy has the highest 17 numbers of rows of seeds. The weight of mutant maize is observed the highest in GM 0.25 kGy (303.9 grams). The yield percent of maize of GM 0.05 kGy, GM 0 kGy, GM 0.25 kGy and GM 0.45 kGy are 72.7, 66.7, 25.0 and 20.0 % respectively. The highest yield is given by GM 0.05 kGy. In the DPPH staining, the methanol extract of GM 0.05 kGy had the highest radical scavenging activity, since it showed from minimum value of 3.125 μg to 400 μg. From the observed results, GM 0.25 kGy is greater in length, weight and number of rows of seeds than the others. Although GM 0.25 kGy has such good factors, GM 0.05 kGy is the most saleable in the market. Because GM 0.25 kGy has some defects in row of seeds. The worst effects were observed in GM 0.45 kGy due to high gamma radiation dose. So, it can be suggested that high gamma doses are not suitable for growing maize. Moreover, the yield of maize is the best for GM 0.05 kGy. The nutritional values of GM 0 kGy, GM 0.05 kGy, GM 0.25 kGy and GM 0.45 kGy are not significantly different.

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# Synthesis and Characterization of Silver Nanoparticles Using Three Different Leaves

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**ABSTRACT:** *In this research work, the leaves of Thinbaw, Thinbaw Ma-hnyo and Tha-Khut were collected from Maharaungmyae Township, Mandalay Region, Myanmar. The preliminary detection of phytochemical compounds present in leaves were carried out by phytochemical tests. The elemental composition of these leaves were also determined by using EDXRF (Energy Dispersive X-ray Fluorescence) Spectroscopy. Silver nanoparticles were synthesized by using three kinds of leaf extracts. The size distribution of nanoparticles surface and morphology of silver nanoparticles were determined by Scanning Electron Microscopy (SEM)and the crystallite size of silver nanoparticles were determined by X-ray diffraction (XRD) method.*

**Keywords:** Silver nano particles, green synthesis, XRD, SEM

## 1. INTRODUCTION

In the modern material science, nanotechnology plays a remarkable role with its eminent salient features such as manipulating nanoscale structures, engineering of atoms and designing of materials with improved properties.[1]The term "Nanotechnology" was defined by Tokyo science university Professor Norio Taniguchi in 1974 as "Nanotechnology mainly consists of the processing of separation, consolidation and deformation of materials by one atom or by one molecule".[2] One nanometer (nm) is one billionth of meter. Nanoparticles possess unique electrical, optical as well as biological properties and are thus applied in catalysis, biosensing, imaging, drug delivery, nanodevice fabrication and in medicine.[3] Nano-scale particles with size range of 1-100 nm and different shapes were commonly synthesized either by top-down or bottom-up strategies. At present development of reliable green chemistry route to synthesis nanoparticles is essential for their potential applications in diverse fields, specifically in biology and medicine.[4]

The nanoparticles are synthesized through physical, chemical and biological methods.[5]The physical and chemical methods are extremely pricey (Li *et al.*, 1999).[6] To synthesize the silver nanoparticles several approaches are available. There is an increasing demand for "green nanotechnology".[19] Biologically active compounds present in the plant extracts such as proteins, polysaccharides and vitamins play a major role in the reduction of silver nitrate into silver nanoparticles.[19] Silver nanoparticles have been synthesized using various plant leaf extracts such as *Carica papaya* leaf, *Vincarosea* leaf and *Dolichandronecrispa* leaf. These leaf contains vitamin A, C, and E which are antioxidants. Plant leaf extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles.[21] Bioactive compounds are rich in plant extracts which have recently been used for the synthesis of nanoparticles. Silver nanoparticles have potential in treating a variety of diseases, including retinal neovascularization, immunodeficiency syndrome, infection and cancer.(7)

## 2. BOTANICAL DESCRIPTION

### 2.1 Botanical Description of Three Different Leaves

Family name : Caricaceae
Botanical name : *Carica Papaya* Linn
English name : Papaya
Myanmar name: Thinbaw
Part used : Leaves

Family name : Apocynaceae
Botanical name : *Vincarosea* Linn
English name : periwinkle
Myanmar name: Thinbaw Ma-hnyo
Part used : Leaves

Family name : Bignoniaceae
Botanical name : *Dolichandronecrispa* Seem.
Myanmar name : Tha-Khut
Part used : Leaves

**Figure 1. Three different leaves used for synthesis of silver nanoparticles**

## 3. MATERIAL AND METHOD

### 3.1. Sample Collection

The leaves of Thinbaw, Thinbaw Ma-hnyo and Tha–Khut were collected from Maharaungmyae Township, Mandalay Region, Myanmar. Then, they are cut into small pieces and used for the experiment.

### 3.2. Preparation of Plant Leaf Extract

The collected samples were washed with distilled water and cut into small pieces. The 25g of this samples were boiled in 200 mL of deionized water for 30 min at

room temperature. The solution was filtered by using Whatman filter paper and the obtained clear solution was used as leaves extract.

### 3.3. Determination of Elemental Analysis

The elemental analysis of leaves of Thinbaw, Thinbaw Ma-hnyo, and Tha-Khut were determined by using EDXRF (Energy Dispersive X-ray Fluorescence) Spectroscopy at Department of Physics, University of Mandalay.

### 3.4. Preliminary Phytochemical Test of Leaves of Sample

Phytochemical tests were done on the various extracts of samples.

### 3.5. Synthesis of Silver Nanoparticles

One millimolarity silver nitrate solution was prepared by dissolving 0.0169g of $AgNO_3$ in 100 mL of deionized water. The 100 mL of this solution were mixed with 25 mL of freshly prepared plant extract. This mixture was stirred on a magnetic stirrer with 300 rpm. This reaction mixture was maintained at 50 °C for reaction times intervals of 2, 6, 8 hours for the reduction of silver ions. The mixture was centrifuged with 6000 rpm for 30 mins and the supernatant poured out. The dark paste obtained was redispersed in deionized water to remove excess biological molecules. The process of centrifugation and redispersion in deionized water was repeated three times to completely purify the nanoparticles. The dark paste collected was then dried in petridish. The weight of dark paste silver nanoparticles was shown in Table.


**Figure 2. Procedure for synthesis of silver nanoparticles**

### 3.6. Characterization of silver nanoparticles

Morphology and size distribution of nanoparticles were determined by Scanning Electron Microscopy (SEM) and the size of nano crystallites were measured by X-ray diffraction (XRD) method.

Estimation of crystalline size is carried out by using Debye- Scherrer's equation;

$$L = \frac{K\lambda}{\beta \cos\theta}, \quad d = \frac{K\lambda}{2\sin\theta} \qquad (1)$$

L = average crystallite size
K = constant (shape factor)
λ = wave length of x ray
β = the peak width of the diffraction peak profile at half maximum height (FWHM)
θ = the angle of diffraction
d = spacing

## 4. RESULTS AND DISCUSSION

### 4.1. Phytochemical Test for Three Kinds of Leaves Sample

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

**Table 1. The results of phytochemical test for leaves sample**

| No. | Tests | Reagents | Observation | Results | | |
|---|---|---|---|---|---|---|
| | | | | I | II | III |
| 1 | Alkaloid | Wagner's solution | Reddish brown ppt | + | + | + |
| | | Dragendroff's solution | Pale orange ppt | + | + | + |
| 2 | Flavonoid | 10% Lead acetate | Yellow colour ppt | + | + | + |
| 3 | Glycoside | 10% Lead acetate | Pale yellow ppt | + | + | + |
| 4 | Phenolic | 10 % $FeCl_3$ | Brown colour solution | + | + | + |
| 5 | Saponin | Distilled water | Forth | + | + | + |
| 6 | Steroid | Acetic anhydride, Conc: $H_2SO_4$ | Blue green colour solution | + | + | + |
| 7 | Polyphenol | 1 % $FeCl_3$ + 1 % $K_3[Fe(CN)_6$ | Greenish blue colour solution | + | + | + |

(+) = presence of constituents
I = Thinbaw, II = Thinbaw ma-hnyo, III = Tha-khut

### 4.2. Determination of Mineral Content of Leaves of Thinbaw, Thinbaw Ma-hnyo and Tha-Khut

The mineral content for leaves of Thinbaw, Thinbaw ma-hnyo and Tha-khut were determined by using EDXRF method at Physics Department, University of Mandalay.

**Table 2. Mineral content of leaves of Thinbaw, Thinbaw ma-hnyo, Tha-khut**

| No. | Element | Symbol | Relative Abundance (%) | | |
|---|---|---|---|---|---|
| | | | Thinbaw | Thinbaw ma-hnyo | Tha-khut |
| 1 | Potassium | K | 1.786 | 1.074 | 1.531 |
| 2 | Calcium | Ca | 1.643 | 1.593 | 1.167 |
| 3 | Chlorine | Cl | 0.784 | 1.538 | 0.524 |
| 4 | Phosphorus | P | 0.527 | 0.267 | 0.192 |

| | | | | | |
|---|---|---|---|---|---|
| 5 | Silicon | Si | 0.364 | 0.125 | 0.095 |
| 6 | Sulfur | S | 0.112 | 0.175 | 0.133 |
| 7 | Aluminium | Al | 0.115 | 0.102 | 0.078 |
| 8 | Iron | Fe | 0.033 | 0.083 | 0.029 |

From the experimental results, the higher amount of potassium and calcium were found in the leaves of three samples. Moreover chlorine, phosphorus, silicon sulphur, aluminium and iron were also found in that order. These mineral contents indicate that the leaves of three samples powder were rich source of mineral.

**Table 3. The result of the amount of silver nanoparticles at different time interval**

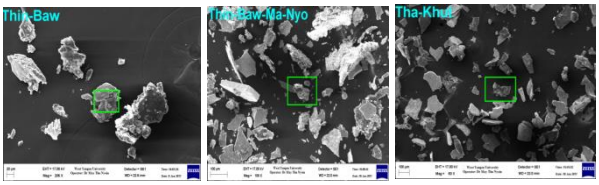
| Sample : $AgNO_3$ | Time (hr) | Weight of silver nanoparticles (g) | | |
|---|---|---|---|---|
| | | Thinbaw | Thinbaw ma-hnyo | Tha-khut |
| 1 : 4 | 2 | 0.0102 | 0.0101 | 0.0093 |
| 1 : 4 | 6 | 0.0115 | 0.0112 | 0.0101 |
| 1 : 4 | 8 | 0.0130 | 0.0128 | 0.0119 |

volume ratio → Leaves extract : $AgNO_3$
1 : 4

According to Table 3 silver nanoparticles were determined by using different time intervals. It was found that, the amount of silver nanoparticles depend on the contact time. When stirring time increase, the amount of silver nanoparticles also increase.

### 4.3. SEM Images of Silver Nanoparticles

Scanning Electron Microscopy was used to determine the size of distribution. The surface morphologies of the sample Thinbaw, Thinbaw Ma-hnyo and Tha-Khut were observed by Scanning Electron Microscopy images as shown in figure (3).




Thin baw Thinbaw ma- hnyo Tha- khut

**Figure 3. SEM micrographs of silver nanoparticles using three different leaves**

According to all SEM micrograph, it was found that AgNPs was aggregate structure. SEM studies provided further insight into the morphology and size details of the silver nanoparticle. The size of the silver nanoparticles synthesized using three different leaves were recorded to be 1 μm.

### 4.4. XRD Analysis of Silver Nanoparticles

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

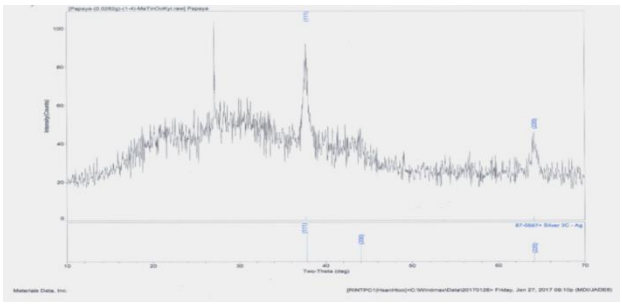


**Figure 4. XRD diffratogram of silver nanoparticles using Thin baw leaves**

**Table 4. XRD results of crystallite size of silver nanoparticles by using Thinbaw leaves extract**

| Sample Name | 2θ | FWHM | β(radian) | Crystallite size(L) nm | d-spacing (nm) |
|---|---|---|---|---|---|
| Thinbaw | 37.740 | 0.465 | $5.254\times10^{-3}$ | 18.05 | 0.2143 |
| | 64.120 | 0.473 | $8.256\times10^{-3}$ | 19.85 | 0.1306 |

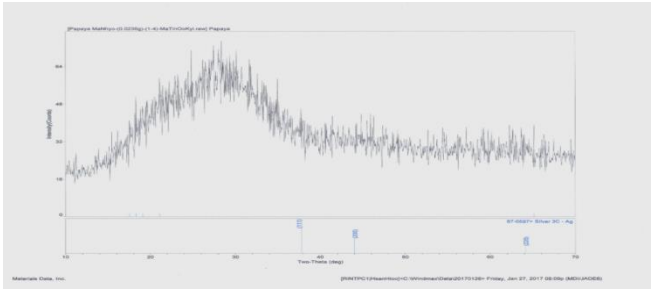


**Figure 5. XRD diffratogram of silver nanoparticles using Thin baw ma- hnyo leaves**

**Table 5. XRD results of crystallite size of silver nanoparticles by using Thinbaw-ma-hnyo leaves extract**

| Sample Name | 2 θ | FWHM | β(radian) | Crystallite size(L) nm | d-spacing (nm) |
|---|---|---|---|---|---|
| Thinbaw ma-hnyo | 17.509 | 0.199 | $3.474\times10^{-3}$ | 40.38 | 0.4555 |
| | 18.304 | 0.111 | $1.937\times10^{-3}$ | 72.50 | 0.4358 |
| | 19.082 | 0.405 | $7.069\times10^{-3}$ | 19.88 | 0.4182 |
| | 21.118 | 0.394 | $6.877\times10^{-3}$ | 20.50 | 0.3783 |
| | 65.153 | 0.269 | $4.695\times10^{-3}$ | 35.04 | 0.1287 |

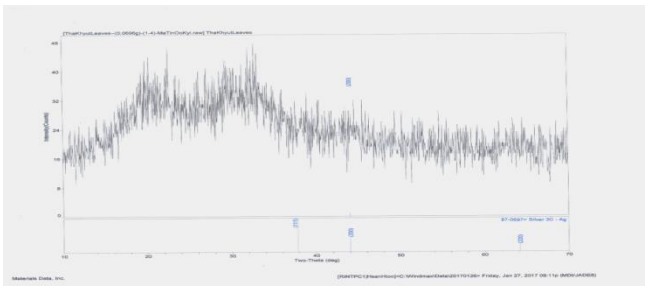


**Figure 6. XRD diffratogram of silver nanoparticles using Tha-khut leaves**

**Table 6. XRD results of crystallite size of silver nanoparticles by using Tha-khut leaves extract**

| Sample Name | 2 θ | FWHM | β (radian) | Crystallite size(L) nm | d-spacing (nm) |
|---|---|---|---|---|---|
| Tha-khut | 44.007 | 0.301 | $5.254\times10^{-3}$ | 28.46 | 0.1850 |

According to XRD results, the crystallite size of silver nanoparticles were found within the range of 18-72 nm. Interplanar spacing between silver nanoparticles were found within the range of 0.1287 to 0.4555. The peaks in XRD pattern using leaves of Thinbaw and Tha-Khut extracts contain only metallic silver peak and leaves of Thinbaw Ma-hnyo extracts contain metallic silver as well as peaks of other impurity crystallite phase were detected.

## 5. CONCLUSIONS

The result of this investigation reveal that synthesis and characterization of silver nanoparticles using leaves extracts. The different times (2hr, 6hr and 8hr) were used in this investigation. Characterization of particle size and morphology were determined by scanning Electron Microscopy(SEM) and the crystallite size of nanoparticles were determined by x-ray diffraction (XRD) method.

In addition, the detection of phytochemical compounds present in leaves were carried out by phytochemical tests. Furthermore, the determination of elemental composition of leaves samples were carried out by using EDXRF (Energy Dispersive X-ray Fluorescence) spectroscopy. According to EDXRF method, it was found that the amount of potassium, calcium and chlorine are higher than the other.

Silver nanoparticles were determined by using different time intervals. It was found that, the amount of silver nanoparticles depend on the contact time. When stirring time increase, the amount of silver nanoparticles also increase. The particle size distribution of nanoparticles surface morphology were characterized with Scanning Electron Microscopy (SEM) that informs nano aggregate structure of silver nanoparticles.

The crystallite size of nanoparticles were determined by using x-ray diffractometer. According to XRD results, the crystallite size of silver nanoparticles were found within the range of 18 to 72 nm. The peaks in XRD pattern of AgNPs prepared by using leaves of Thinbaw and Tha-khut extracts contained only metallic silver peak and that prepared by using leaves of Thinbaw-ma-hnyo extracts contained metallic silver as well as peaks of other impurity crystallite peak. The XRD patterns of silver nanoparticles are in high quality accord with the values of standard card (JCPDS card no: 04-0783). According to XRD peak search report of AgNPs, the crystallite size of the AgNPs was found to be 18.05 nm (Thinbaw), 28.46 nm (Tha- khut) and 19.88 nm (Thinbaw ma-hnyo).

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# Comparative Study on Nutritional Values, Antimicrobial Activities and Antioxidant Activities of Papaya Leaves and Tea Leaves

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**ABSTRACT:** *Foods are complex combinations of nutrients and other compounds. The processing of papaya leaves and tea leaves determine the contents of bioactive ingredients. The analyses included examination of the dried papaya leaves and tea leaves for their chemical composition (moisture, ash, fiber and protein contents). This research deals with the study of phytochemical constituents, nutritional values, antimicrobial activities and antioxidant activities of papaya leaves and tea leaves. Firstly, the preliminary phytochemical test of these selected samples was carried out. The nutritional values of these samples were determined by AOAC method. The mineral contents of these samples could be measured by EDXRF method. The antimicrobial activities of these two samples in three solvent systems (PE, EtOAc, EtOH) were determined by agar well method. It was observed that the ethyl acetate extract of two samples showed high antimicrobial activities against all tested organisms with inhibition zone diameter ranging 20-32 mm. The total phenolic contents of these samples were measured by Folin-Ciocalteau reagent using UV spectrophotometeric method. Total phenolic content of tea leaves and papaya leaves were observed to be 14.50 mg GAE per gram DW and 6.13 mg GAE per gram DW respectively. Antioxidant activity of MeOH extract of two samples were also investigated by using DPPH (1,1-diphenyl-2-picrylhydrazyl radical scavenging assay). The $IC_{50}$ values of MeOH extract of tea leaves and papaya leaves were observed to be 5.3 μg/mL and 18.8 μg/mL respectively. These two samples showed significant activity by comparing with standard antioxidant ascorbic acid ($IC_{50}$ = 2.39 μg/mL).*

**Keywords***: Papaya leaves; Tea leaves; Total phenolic content; Antioxidant activity; DPPH radical scavenging assay*

## 1. INTRODUCTION

Papaya (*Carica papaya* L) belongs to the family Caricaceae. The properties of papaya leaves and other parts of the plant are also well known in traditional system of medicine. Papaya leaves have been used traditionally for the treatment of malaria, dengue, jaundice and antiviral activity [1]. Young leaves are rich in flavonoids, alkaloids and phenolic compounds which possess antioxidant property. Papaya leaf juice helps increase white blood cells and platelets, normalizes clotting, and repairs the liver. Papaya leaf extracts inhibit cancer cell growth. Many benefits of papaya owed due to high content of vitamin A, B, C and E. [2]

Tea leaves (*Camellia sinensis* (L) (Letphet) are a Myanmar traditional meal. In terms of chemical composition teas contain tannin substances, flavonols, proteins and mineral compounds. Tea leaves extracts are powerful antioxidants due to presence of chemical compound catechins. Catechins are natural antioxidants that help prevent cell damage. These substances can reduce the formation of free radical in the body, protecting cells from damage.[10]. The tea leaf is used to treat cancer, asthma, diabetes and coronary heart decrease [5]. The tea leaf is packed with vitamin such as vitamin $B_2$, vitamin C and vitamin E, and minerals like potassium, calcium, phosphorus, magnesium, and manganese. Tea leaf is rich in polyphenols that have effects like reducing inflammation and helping to fight cancer.

### 1.1. Aim and Objectives

The main aim of this research is to determine the nutritional values, antimicrobial activities and antioxidant activities of papaya leaves and tea leaves.

- To carry out the phytochemical screening of sample
- To determine nutritional valves of samples by AOAC method
- To analyze the mineral contents of samples by EDXRF method
- To determine the antimicrobial activities of samples by agar well method
- To investigate total phenolic content and antioxidant activity of samples by Folin-Ciocalteau and DPPH radical scavenging assay

### 1.2. Botanical Description

**Figure1. Tree and Leaves of Papaya**

Botanical name - *Carica papaya* L.
Family - Caricaceae
English name - Papaya
Myanmar name - Thinbaw
Part used - Leaf

**Figure 2. Tree and Leaves of Tea**

Botanical name - *Camellia sinensis* (L)
Family - Theaceae
English name - Tea
Myanmar name - Letphet
Part used - Leaf

## 2. MATERIALS AND METHODS

### 2.1. Sample Collection

In December 2016, papaya leaves were collected from Mahaaungmyae Township, Mandalay region. Tea leaves were collected from Ywa Ngan Township, Southern Shan State, Myanmar. The two samples were cut into small pieces and air dried at room temperature. These samples were ground into powder in an electric blender and stored in air tight container.

### 2.2. Preliminary Phytochemical Test

A few grams of dried sample powder was subjected to the test of alkaloids, glycosides, flavonoids, phenolic compounds, reducing sugar and saponins according to the standard procedures [4, 8]. The results are shown in table (1).

### 2.3. Determination of Nutritional Values by AOAC Method

The moisture content was determined by the oven drying method. The ash content was determined by using muffle furnace. Protein content was determined by Kjeldahl digestion method. Fiber content was investigated by digestion with concentrated sulphuric acid [6, 9].

### 2.4. Determination of Mineral Content

Mineral content of the samples was determined by EDXRF (Energy Dispersive X-Rays Fluorescence) technique at the Physics Department, Taunggyi University.

### 2.5. Determination of Antimicrobial Activity

Antimicrobial activities of the crude extract of the sample were tested in three solvent system (PE, EtOAc, EtOH) by using agar well diffusion method on six selected organisms at PFRD (Pharmaceutical and Food Research Department), Ministry of Industry, Yangon.

### 2.6. Determination of Total Phenolic Content

Total phenolic content was determined by Folin-Ciocalteau method by using 754-UV spectrophotometer [7, 11].

### 2.7. Determination of Total Phenolic Content of Papaya and Tea Leaves

The total phenolic content of extract solution of papaya leaves and tea leaves was measured with the Folin-Ciocalteau reagent. Firstly, 10 µL of extract solution were taken in each test tube. Each test tube was made up to 1.6 mL with distilled water. 100 µL of Folin-Ciocalteau reagent was mixed, then 300 µL of saturated $Na_2CO_3$ (20 %) was added. These mixtures were heated in a water bath at 40°C for 30 minutes and then cooled in an ice-bath. The absorbances of these prepared sample solutions were measured at 765 nm using a UV-spectrophotometer. The results are shown in table (7). The total phenolic content of the extract solution of papaya leaves and tea leaves was expressed as mg gallic acid equivalent (GAE)/g DW [7,11].

### 2.8. Measurement of DPPH Radical Scavenging Activity by UV Spectrophotometric Method

Absorbance of prepared solutions was measured at 517 nm by using UV spectrophotometer. Experiment was done in triplicate for each sample solution and % inhibition was calculated by using the following equation.

$$\% \text{ inhibition of oxidation} = \frac{A-B}{A} \times 100\% \quad (1)$$

A = Absorbance of DPPH solution
B = Absorbance of sample + DPPH solution

Finally, $IC_{50}$ (50 % inhibitory concentration) was determined by using the linear regressive excel programme [3, 12].

## 3. RESULTS AND DISCUSSION

### 3.1. Preliminary Phytochemical Constituent of Papaya and Tea Leaves

The phytochemical constituents of papaya leaves and tea leaves were investigated by test tube method. Alkaloids, phenolic, polyphenols, flavonoids, saponins, glycosides and reducing sugars are found to be present in papaya leaves and tea leaves in Table 1.

**Table 1. Results of Preliminary Phytochemical Screening of Papaya and Tea Leaves**

| No | Constituent | Reagent | Result | |
|---|---|---|---|---|
| | | | I | II |
| 1 | Alkaloids | Dragendroff's sol: | + | + |
| 2 | Glycosides | 10 % lead acetate | + | + |
| 3 | Flavonoids | Conc: HCl, Mg coil | + | + |
| 4 | Saponins | Conc: $H_2SO_4$ | + | + |
| 5 | Phenolic | 10 % $FeCl_3$ | + | + |
| 6 | Polyphenol | 1 % $FeCl_3$ + 1 % $K_3[Fe(CN)_6]$ | + | + |
| 7 | Reducing Sugar | Benedict's solution | – | + |

I = Papaya Leaves, II = Tea Leaves
(+) = Presence, (–) = Absence

### 3.2. Some Nutritional Values of Papaya and Tea Leaves

The ash, fiber and protein contents of the papaya leaves are slightly higher than tea leaves. The fiber can help lower blood cholesterol and glucose levels. Protein is an important building block of bones, muscles, tissues, skin and blood. The results are shown in Table 2.

**Table 2. Results of Nutritional Values of Papaya and Tea leaves**

| No. | Parameters | Observed data (%) | |
|---|---|---|---|
| | | Papaya leaves | Tea leaves |
| 1 | Moisture | 8.44 | 8.88 |
| 2 | Ash content | 16.53 | 5.96 |
| 3 | Fibre content | 12.50 | 11.7 |
| 4 | Protein content | 32.24 | 31.87 |

### 3.3. Determination of Mineral Contents of Papaya Leaves and Tea Leaves

Mineral contents of papaya leaves and tea leaves were measured at the Department of Physics, Taunggyi University, Myanmar by applying EDXRF (Energy Dispersive X-ray Fluorescence Spectroscopy). The results are tabulated in Table (3 and 4).

**Table 3. Results of Mineral Contents of Papaya Leaves**

| No | Minerals | Contents % |
|---|---|---|
| 1 | Ca | 2.94 |
| 2 | K | 2.43 |
| 3 | Si | 1.75 |
| 4 | Mg | 1.51 |
| 5 | S | 0.609 |
| 6 | Al | 0.460 |
| 7 | P | 0.312 |
| 8 | Fe | 0.400 |
| 9 | Cu | 0.258 |
| 10 | Ti | 0.041 |
| 11 | Mn | 0.009 |
| 12 | Zn | 0.006 |

**Table 4. Results of Mineral Contents of Tea Leaves**

| No | Minerals | Contents % |
|---|---|---|
| 1 | K | 2.41 |
| 2 | Ca | 0.768 |
| 3 | Si | 0.404 |
| 4 | Mg | 0.344 |
| 5 | S | 0.351 |
| 6 | P | 0.264 |
| 7 | Al | 0.200 |
| 8 | Fe | 0.084 |
| 9 | Mn | 0.076 |
| 10 | Zn | 0.008 |
| 11 | Ti | 0.007 |
| 12 | Cu | 0.003 |

As described in Table 3 and 4, it was found that calcium (Ca), potassium (K), silicon (Si), magnesium

(Mg), sulphur (S), phosphorus (P) and aluminium (Al) contained the high values in these analyzed samples. Mineral contents of papaya leaves except Mn are higher than tea leaves. Minerals play a key role in various physiological functions of the human body, especially in building and regulation processes. Potassium regulates acidic and alkaline level of human body fluid. Calcium is important to bone growth and formation, blood clotting nerve and muscle function. Silicon gives strength and support to all the tissues of the body. Sulphur is a healing mineral. Phosphorus helps to build strong bones and teeth. It also helps convert food into energy and helps with metabolism. It indicates that papaya leaves and tea leaves contain the essential minerals for our body.

### 3.4. Antimicrobial Activities of Papaya Leaves and Tea Leaves

Antimicrobial activity of papaya leaves and tea leaves in three solvent systems was investigated against six species of microorganisms by employing agar well diffusion method. The samples were tested on six species of organisms including *Bascillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli*. It was found that the ethyl acetate extracts of two samples showed high antimicrobial activity against all tested organisms with inhibition zone diameter ranging 20-32 mm. Moreover, ethanol crude extract gave medium activity on six tested organisms.

### 3.5. Determination of Total Phenolic Content

The total phenolic contents (TPC) of papaya leaves and tea leaves were determined spectrophotometrically according to the Folin-Ciocalteau colorimetric method using gallic acid as the standard. Results of absorbance of standard gallic acid are shown in Table 5. Phenols react with an oxidizing agent phosphomolybdate in Folin-Ciocalteau reagent under alkaline conditions and result in the formation of the colored complex, the molybdenum blue which is measured at 765 nm colorimetrically.

The absorbances of prepared sample solutions of 10 µL of papaya leaves and tea leaves were measured with UV-visible spectrophotometer at 765 nm with respect to blank solution. The results are described in Table 6.

**Table 5. Results of Absorbance of Standard Gallic Acid Solution**

| No. | Test sample | Concentration (µg/mL) | Absorbance |
|---|---|---|---|
| 1 | Std GA1 | 2 | 0.153 |
| 2 | Std GA2 | 4 | 0.261 |
| 3 | Std GA3 | 6 | 0.336 |
| 4 | Std GA4 | 8 | 0.459 |
| 5 | Std GA5 | 10 | 0.538 |

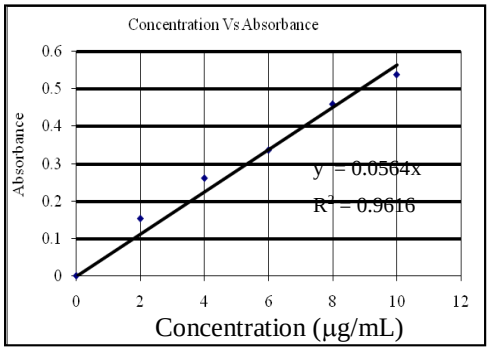


**Figure 3. Concentration absorbance calibration curve for standard gallic acid**

**Table 6. Results of absorbance and concentrations of papaya leaves and tea leaves**

| No. | Test sample | Absorbances | Concentration (µg/mL) |
|---|---|---|---|
| 1 | Papaya leaves (10 µL) | 0.358 | 6.39 |
| 2 | Tea leaves (10 µL) | 0.141 | 2.52 |

From these results, the amount of total phenolic content of analyzed samples was obtained by using the standard graph. Total phenolic of papaya leaves and tea leaves was expressed as gallic acid equivalent and it was 6.13 mg and 14.5 mg of gallic acid equivalent (GAE) per g DW, respectively.

### 3.6. Antioxidant Activities of Papaya Leaves and Tea Leaves

Antioxidant activities of papaya leaves and tea leaves were determined by the DPPH radical scavenging method. In DPPH scavenging assay, the antioxidant activity was measured by the decrease in absorbance as the DPPH radical received an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule. DPPH radical scavenging activity expressed in % inhibition of the samples. In this study, ascorbic acid was used as a standard antioxidant.

Antioxidant activities of samples were expressed as percentage of DPPH radical inhibition and $IC_{50}$ values (µg/mL). $IC_{50}$ values of the samples were calculated from the concentration Vs percent inhibition curve. Values of ascorbic acid, papaya leaves and tea leaves in percentage ranged from 38.21 % to 88.01 %, from 23.01 % to 85.54% and from 30.50 % to 87.08 % respectively. The results of antioxidant activity using DPPH method in papaya leaves and tea leaves using ascorbic acid as a positive control are shown in Figure (4, 5 and 6) and Table (7, 8 and 9).

**Table 7. Absorbance Values and % Inhibition of Standard Ascorbic Acid**

| Ascorbic Acid | Std. 1 | Std. 2 | Std. 3 | Std. 4 | Std. 5 |
|---|---|---|---|---|---|
| Conc: (µg/mL) | 1.25 | 2.5 | 5 | 10 | 20 |
| Absor-bance | 0.21 | 0.14 | 0.13 | 0.10 | 0.04 |
| Inhibition (%) | 38.21 | 55.32 | 60.25 | 69.21 | 88.01 |

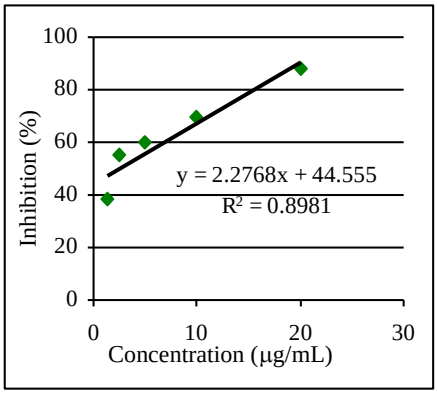


**Figure 4. Plot of % inhibition Vs concentration of standard ascorbic acid**

**Table 8. Absorbance Values and % Inhibition of Papaya leaves**

| Papaya Leaves | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 |
|---|---|---|---|---|---|
| Conc: (µg/mL) | 3.125 | 6.25 | 12.5 | 25 | 50 |
| Absorbance | 0.267 | 0.243 | 0.208 | 0.085 | 0.050 |
| Inhibition (%) | 23.01 | 30.04 | 40.01 | 75.49 | 85.54 |

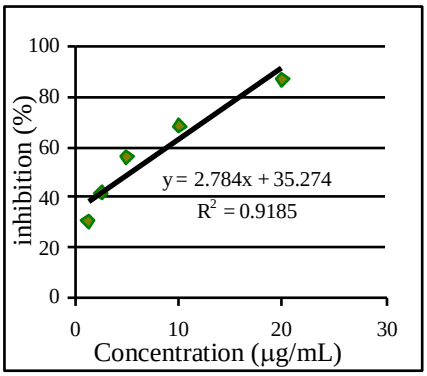


**Figure 5. Plot of % inhibition Vs concentration of papaya leaves**

**Table 9. Absorbance Values and % Inhibition of Tea leaves**

| Tea leaves | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 |
|---|---|---|---|---|---|
| Conc: (µg/mL) | 1.25 | 2.5 | 5 | 10 | 20 |
| Absor-bance | 0.241 | 0.201 | 0.152 | 0.109 | 0.044 |
| Inhibition (%) | 30.50 | 42.08 | 56.12 | 68.47 | 87.08 |

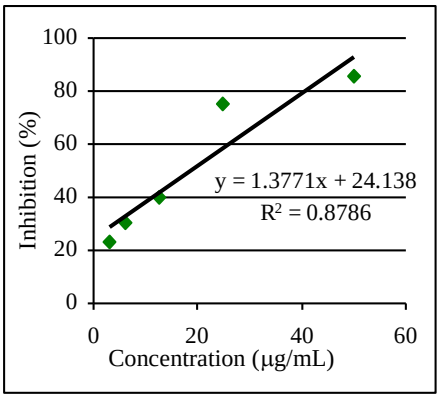


**Figure 6. Plot of % inhibition Vs concentration of tea leaves**

**Table 10. $IC_{50}$ Values of Standard Ascorbic Acid, papaya and tea leaves**

| Test Samples | $IC_{50}$ Values |
|---|---|
| Ascorbic Acid | 2.39 |
| Tea leaves | 5.3 |
| Papaya leaves | 18.8 |

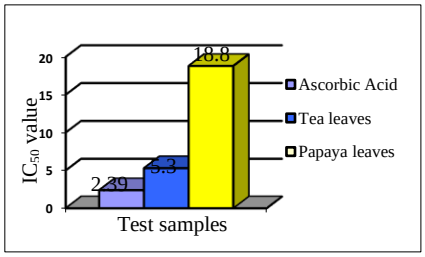


**Figure 7. $IC_{50}$ value of ascorbic acid, papaya and tea leaves**

According to Figure 7, $IC_{50}$ value of papaya leaves is higher than that of tea leaves. The lower the $IC_{50}$ value, the greater the antioxidant activity becomes. The antioxidative potential of samples can be determined by $IC_{50}$ (50 % inhibition concentration). It means that the concentration of the samples can inhibit the oxidation in 50 %.

In the present research, the papaya leaves and tea leaves proved to be scavenging against DPPH radical. The results revealed that antioxidant activity of tea leaves is higher than that of papaya leaves but lower than ascorbic acid as positive control ($IC_{50}$ = 2.39 μg/mL). These two leaves contained a significant amount of antioxidant agents. Therefore, the study suggests that these two leaves might be a potential source of natural antioxidants.

## 4. CONCLUSIONS

In the present research work, antioxidant activities of papaya leaves and tea leaves were analyzed by DPPH radical scavenging assay. The test tube method showed the presence of phenolic compound, polyphenol, flavonoids, glycosides, saponins, reducing sugar and alkaloids in analyzed leaves. Nutritional values of these two leaves obtained by AOAC method were found to be 8.88 % moisture, 5.96 % ash, 31.87 % protein and 11.70 % fiber in tea leaves and 8.44 % moisture, 16.53 % ash, 32.24 % protein and 12.50 % fiber in papaya leaves. The mineral content determined by EDXRF method had the high value of Ca, K, Si, Mg, Al, S, P and Fe. In antimicrobial activity by agar well method, ethyl acetate extract of samples responded the high activity against all six organisms. Total phenolic content in two samples was observed to be 14.50 mg GAE per gram DW in tea leaves and 6.13 mg GAE per gram DW in papaya leaves. In DPPH assay, $IC_{50}$ values by tea leaves and papaya leaves were found to be 5.3 μg/mL and 18.8 μg/mL respectively. Antioxidant activity of tea leaves is more effective than that of papaya leaves. These two kinds of leaves showed significant activity by comparing with the standard ascorbic acid ($IC_{50}$ = 2.39 μg/mL).

The findings of the present study support that papaya leaves and tea leaves are potential sources of natural antioxidant that could have great importance as a therapeutic agent. The fiber, protein and mineral enriched tea leaves and papaya leaves should be eaten daily for health benefits.

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# Analysis of Elemental Concentrations on the Vegetables

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**ABSTRACT:** *Globally, vegetables are highly consumed due to their high nutritional values. To be a healthy person consuming balanced diet, it should be consumed for correct proportion of correct vegetables. The present study was conducted to access the risk to human health by elements potassium (K), phosphorus (P), sulphur (S), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), hafnium (Hf), samarium (Sm), silicon (Si) and zinc (Zn) through the intake of common vegetables (rice, maize, lima bean, mint, cabbage, chayote, and carrot ) grown near Loilem area at Loilem Township by using indexes for vegetables. The elemental concentrations of samples were checked by EDXRF method. The results were described.*

**Keywords**: Vegetables, Index for vegetables, Concentration of elements, Elements, EDXRF Spectrometer

## 1. INTRODUCTION

A balanced diet is important because your organs and tissues need proper nutrition to work effectively. Without good nutrition, your body is more prone to disease, infection, fatigue, and poor performance.

Balanced diet consists of five major food groups are CHO, Fat, Protein, Vitamin and Minerals. Most of vitamins and minerals can be obtained by eating vegetables.

Various vegetables are composed of various elements. Among them rice, maize, lima bean, mint, cabbage, chayote, and carrot vegetables were studied for their compositions (K,P, S, Ca, Mn, Fe, Cu, Hf, Sm, Si and Zn).

Each element has been shown their daily requirements to be healthy diet. If a person eat too much or too low minerals, he will be unhealthy. So, must consume the normal daily requirement of vegetables.

To consume normal daily requirements, a person should know the amount of elements in each vegetable. So, this study was conducted to access the elemental compositions of common vegetables.

Human nutrition deals with the provision of essential nutrients in food that is necessary to support human life and health. Poor nutrition is a chronic problem often linked to poverty, food security or a poor understanding of nutrition and dietary practices.

Malnutrition and its consequences are large contributors to deaths and disabilities worldwide. Good nutrition is necessary for children to grow physically, and for normal human biological development.

In Myanmar, there are three groups of food (Energy yielding group, Body building group and Disease protective group). Figure 1 shows three groups of food.

Among them, rice, maize, lima bean, mint, cabbage, chayote, and carrot vegetables in Loilem Township were studied for the compositions of K, P, S, Ca, Mn, Fe, Cu, Hf, Sm, Si and Zn.

**Figure 1. Three groups of food**

### 1.1 Physical Background of Loilem Township

Loilem is a town in the Shan State of central- easten Myanmar. It is the principal town in Loilem Township in Loilem District. Brodering Kyaukmw District , Hsipaw Township, Lasho District, Tang Yan Township in the north,Taunggyi District Hopong Township, Lawksawk Township in the west, Mong Hsat District, Mongping Township in the east and Langkho District, Mongnai Township, Mawkmai Township in the sourth, it is located between latitudes 20˚25/ and 22˚41/ north between latitudes 97˚10/ and 99˚00/ east. The total area is 7624.87 square miles (2018-2019). Mong Kung, Namsang, Keythi, Laihka, Mong Hsu and Kunhing Townships consist of this District. And then Panglong (26-7-2002), Mong Nawng (3-12-2003), Kho Lam (12-1-2011), Karli (12-1-2011), Mong San ( 12-1-2011) Townships also consist in this region.

Generally, Loilem Township is several of hills Myanmar Naing Ngan. The whole area is composed of wavy land. Several mountains can be found in this region. The mountains are generally found 3000/ to 8000/ height. The whole Township is based on 3000/ above sea level.

The Loilem Township falls within cool and dry zone. It is very cool during cold season (minus temperature). The average four years rainfall is 57.15// (2015-2018). The average maximum temperature is (30.95℃) and the minimum temperature is (-2℃) (2015-2018). She feels cool in the climate.

The main soil types of Loilem Township are designed red brown humid soil. The main types of natural vegetation are rice, maize, avocado, various of bean, carrot, brinjal, cabbage, chayote, chive root, quince, jack fruit, pineapple, strawberry, mulberry, ginger vegetables and so on. They can be grown the whole township.

## 2. INSTRUCTION OF ENERGY DISPERSIVE X-RAY FLUORESENCE SPECTROMETER

Energy Dispersive X-ray Fluorescence spectrometer can be used for a tremendous variety of elemental analysis application. It can be used to measure virtually every element from 11Na to 92U in the periodic table, in concentrations ranging from a few ppm to nearly 100 percent. The sample such as a small amount of milligrams of solid, powder or gas slurry can be analyzed by using EDXRF method. As EDXRF method is recently introduced to a transparent window detector system. It can provide a uniform sensitivity for all element concentration of the materials and can take place energy dispersive X-ray fluorescence (EDXRF).

A detector is a transducer for converting X-ray photon energy into voltage pulses. Each X-ray photon entering the detector a voltage pulse is activated and if the height of the voltage pulse is proportional to the photon energy. The elements and their concentration are identified by counting the pulses at the different energy levels. The purpose of the multichannel pulse-height analyzer is to measure the height of each amplifier output pulse. The number of times a pulse of each height has been detected is accumulated in the analyzer memory to form to spectrum of pulse heights. Subsequently, this information can be displayed as a picture of the analyzed energy spectrum. The Si (Li) detector operating under liquid nitrogen boiling temperature (77 K), is most commonly being used due to their high resolution.

The Energy Dispersive X-ray Fluorescence Spectrometer EDX-7000 measures the energy (keV) and intensity of the generated fluorescent X-rays to determine the type and content of the elements comprising a sample.

It is applied for non-destructive elemental analysis of solid, powder, and liquid samples. It is widely used by electronics and automobile manufacturers around the world. Figure 2 shows Photographs of EDX-7000 spectrometer.


(i) (ii)

**Figure 2. Photographs of EDX-7000 spectrometer**

### 2.1 EDXRF Analysis Method

There are two types of EDXRF analysis. These are Qualitative analysis and Quantitative analysis.

**Qualitative analysis** means determining which elements are contained in a sample. An example of qualitative analysis is given below. The horizontal axis plots the energy of fluorescent X-ray (units: keV) and the vertical axis plots the intensity (unit: counts, cps, cps/μA, etc.)

The energy of fluorescent X-ray is peculiar to each element, and by measuring these energies we can determine which elements are present. Figure 3 shows spectrum of qualitative analysis for EDXRF system.

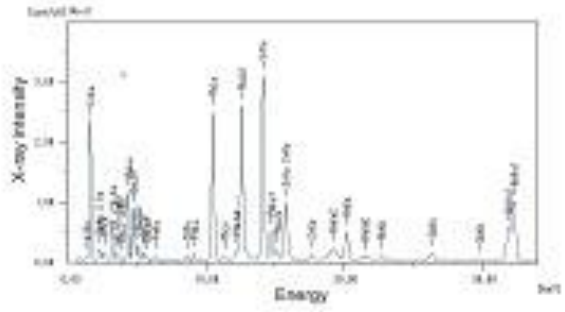


**Figure 3. Spectrum of Qualitative Analysis**

Units for intensity of fluorescent X-rays are

**counts:** This is the number of fluorescent X-rays detected within the measuring time.
**cps:** This stands for "counts per second " and is the number of fluorescent x-ray detected in one second.
**cps/μA**: This is the abbreviation of " cps per micro ampere" and indicates the number of fluorescent x-rays detected per unit of current in one second.

The intensity is proportional to the quantity of an element contained in the sample.
**Quantitative analysis** means determining how of each element is contained in the sample. In qualitative analysis, horizontal axis describes the energy of the spectrum, but in quantitative analysis, the vertical axis describes the intensity.

## 3 . MATERIALS AND METHODS

### 3.1 Collection and Preparation of Vegetables

In the present research work, the vegetable samples had been collected grown at Loilem region. Loilem Township is a Township of Loilem District in South Shan State. The seven vegetables samples collected are rice, maize, lima bean, avocado, cabbage, chayote, Carrot are shown in Figure 4. Samples name and their nature are shown in Table 1. After collecting, the vegetable samples were cut into suitable pieces and each sample was weighted using the digital balance to obtain the needed amount 5g. The size of sample is 10 mm. After preparing, the sample was put in the EDXRF machine. By the nature of EDXRF experiment, the sample must be in the form of solids. After preparing, the sample was put in the EDXRF machine.

(i) Rice

(ii) Maize

(iii) Lima Bean

( iv) Mint

( v) Cabbage

(vi) Chayote

(vii) Carrot

**Figure 4. Photographs of analyzed samples**

**Table 1. Name of vegetable samples and their nature**

| Sr No | Sample | Common Name | Scientific Name | Family |
|---|---|---|---|---|
| 1 | Sample (i) | Rice | Oryza sativa | Gramineae |
| 2 | Sample (ii) | Maize | Zea mays | Poaceae |
| 3 | Sample (iii) | Lima Bean | Phaseoius lunatus | Fabaceae |
| 4 | Sample (iv) | Mint | Mentha | Limiaceae |
| 5 | Sample (v) | Cabbage | Headed cabbage | Brassica oleracea |
| 6 | Sample (vi) | Chayote | Sechium edule | Cucurbitaceae |
| 7 | Sample (vii) | Carrot | Daucus carota | Apiaceae |

## 4. RESULT AND DISCUSSION

A quantitative analysis of the elemental concentration of vegetables was measured by using EDXRF Spectrometer (EDX-7000) at Material Science Lab, Taungoo University. Among these samples, rice is crop or grain, maize and lima bean are seed, mint and cabbage are leaf, chayote is fruit, and carrot is root. The results from EDXRF Spectrometer are shown in Table 2. The concentration of elements in these samples is shown in Figure 5.

According to the results obtained, potassium (K) is high in mint and carrot. Potassium is extremely important to cells and without it, could not survive. Without potassium, the nurse cell could not send the message to brain. Excess of this element in the blood can lead to abnormal heart beat and evenly heart attack. High potassium levels occur mainly due to some underlying health problems (kidney disease). Potassium need to avoid (K) rich food such as mint and carrot vegetables. Low potassium in blood can cause weakness, lethargy and flaccid person. Such potassium get (K) by consuming mint and carrot vegetables. The daily recommended amount of potassium for human is 3800 mg.

Sulphur (S) in vegetables was studied and result shows that it is rich in rice than others vegetables. Sulphur is important for healthy skin, hair and nails. According to the culture, Burmese eat the rice as a major diet group. So, requirement of sulphur can be obtained in Myanmar people. About 0.25% of human body mass is sulphur. In this study, calcium (Ca) is involved in all vegetables except maize and rice. Calcium is important for bone and teeth formation and cardiovascular system. Hypocalcaemia can cause tetany, rickets and draeart problems. Patient with hypocalcaemia can be treated with herbals such as maize and rice. The daily recommended amount of calcium for human is 600 mg.

According to the results obtained, it is found that rice is the concentration of phosphorus (P) is higher than those of other vegetables samples analyzed in this project. Phosphorus element is very important. It works with calcium to help built bones. It is important structural role in nucleic acids and cell membranes. And it is involved in the body's energy production. A person with high phosphorus levels can also experience itching and red eyes. More severe cause of high phosphorus may include severe constipation, nausea, vomiting and diarrhea. The daily recommended amount of phosphorus for human is 700 mg for adults (19 years and older),1250 mg for children (9 to 18 years), 500 mg for children (4 to 8 years), 460 mg for children (1 to 3 years), 275 mg for infants (7 to 12 months), 100 mg for infants (0 to 6 months).

Silicon (Si) is a mineral and it was found in mint. Si supplements are used for weak bones (osteoporosis), heart disease and stroke (cardiovascular disease), Alzheimer's disease, hair loss, and improving hair and nail quality. It is also used for improving skin bealing: and for treating sprains and strains, as well as digestive system disorders. The human body contains approximately 7 grams of silicon, which is present in various tissues and body fluids.

Iron (Fe) was found in maize, lima bean, mint and chayote. Fe was important in blood product and disease protection. Reduced Fe intake can cause anemia and its complications. Fe was restricted in patient with liver disease. Recommended Daily Intakes (RDI) (per day) for iron is 17 mg. By studying these results, human can know the suitable vegetables for each.

Other elements (Cu, Hf, Zn, Sm, Fe, and Mn) were present in trace amount. These amounts cannot affect the human health.

**Table 2. The results from EDXRF Spectrometer**

| **Elements** | **Concentration (%)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | Sample (i) | Sample (ii) | Sample (iii) | Sample (iv) | Sample (v) | Sample (vi) | Sample (vii) |
| K | 0.171 | 0.138 | 0.152 | 0.313 | 0.137 | 0.057 | 0.210 |
| S | 0.131 | 0.038 | 0.045 | 0.048 | 0.048 | 0.033 | - |
| Ca | - | - | 0.017 | 0.086 | 0.025 | 0.013 | 0.034 |
| P | 0.626 | 0.060 | - | 0.021 | 0.049 | - | - |
| Cu | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.002 |
| Fe | - | 0.001 | 0.001 | 0.001 | - | 0.001 | - |
| Zn | 0.001 | 0.001 | - | - | - | - | - |
| Hf | - | - | - | 0.001 | - | - | - |
| Mn | - | 0.001 | - | - | - | - | - |
| Sm | - | - | - | 0.001 | - | - | - |
| Si | - | - | - | 0.108 | - | - | - |

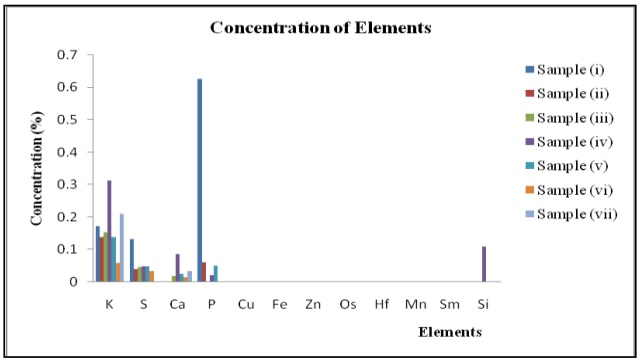


**Figure 5. The concentration of elements in seven samples**

In this research, the elemental concentrations of the vegetables are analyzed for Loilem Township only. And this research can be extended to analyze for the vegetables in other regions.

## 5. CONCLUSIONS

According to result obtained, potassium (K) is high in mint and carrot, phosphorus is high in rice. Calcium (Ca) present in all vegetable except maize and rice. Silicon (Si) is rich in mint and iron (Fe) is present in maize, lima bean, mint and chayote.

As a conclusion, each element plays an important in human health. But some persons need to avoid some of these elements due to their underlying health conditions.

In this paper, the concentrations of each element in common vegetables are studied and results are obtained. By applying this paper, human can get normal healthy life by eating or avoiding the vegetables.

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