Assessment of Heavy Metal Contamination in Sea Water and Sediment Samples Around the Maungmagan Beach Area in Tanintharyi Region

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ABSTRACT*:* Nowadays, Pollution of heavy metals in aquatic environment of ecosystem was growing problem worldwide and currently it has reached with alarm rates. There are many sources of heavy metals; most of them originate from anthropogenic activities like draining of sewerage and recreational activities. As heavy metals could not be degraded, they are being deposited continuously and incorporated in water and sediment, this causes heavy metals were polluted in water bodies. In this research sea water and sediment samples were collected seasonally and annually in (2017-2019). Heavy metal (Cr, Mn, Fe, Cu, Zn, As, Pb, Cd and Hg) contents were determined to identify pollution hot spots in the studied area. Sampling sites were recorded by using GPS detector. The concentrations of metals were determined by atomic absorption spectrometry (AAS) technique and compared with acceptable levels of ASEAN, EPA and CBSQGs standards.

KEYWORDS: *sea water, sediment, heavy metals, Gps detector,AAS, ASEAN, EPA standards*

1. INTRODUCTION

Heavy metals would be added to an aquatic system in natural or anthropogenic sources. Other possible sources of pollution included are domestic effluents, urban storm water runoff, landfill leachate, atmospheric sources and boating activities. Heavy metals were reduced to aquatic systems, generally bound to particulate matter, which are incorporated into sediments. Marine pollution is an environmental problem of ecosystem human activities in the coastal area and marine water were contributed to the discharge of many kinds of pollutants such as heavy metals into the marine ecosystems. Thus, sediments were an efficient mean of accumulation and downstream transport of inorganic contaminants, like heavy metals. It is a prevent fact that heavy metals induce toxic effects on living organisms can pose a high risk when found in high concentrations in sediments [1].

2. MATERIALS AND METHODS

2.1 Sample Collection

The sea water and sediment samples were collected from Maungmagan area in Tanintharyi region. Sea water samples were taken 1.5 miles away from the Sea Shore and 3m depth from the surface water level. Sediment samples were collected about 7 to 14 cm thickness of the surface sediment with a stainless-steel grab sampler during low tide. The concentrations of metals have been determined by Atomic Absorption Spectrometry (AAS) techniques at Yangon University Research Center (URC). The analytical data were tabulated and compared with ASEAN, EPA and CBSQGs Standards.

2.2 Study Area

The studied area Maungmagan is the most beautiful beach in Dawei vicivity, Tanintharyi Region, Myanmar. It is located on the western coast of Maungmagan Village near Dawei where the waves of the Bay of Bengal (BOB) and the Indian Ocean lap the shores.

Table 1. Geolocation of Sampling Sites of Study Area

No.	Samples	GPS position
1	Sea Water	14^0 8′41.12″ N
		98^0 6′27.75″ E
2	Sediment	14° 8′21.62″ N
		98° 6′47.65″ E

Fig 1. Satellite image of Maungmagan in Tanintharyi Region

3. RESULTS AND DISCUSSIONS

3.1 Chromium (Cr) content in sea water and sediment samples

In the present study, the chromium (Cr) content in sea water samples were found to be in the annual range of 0.010 - 0.020 ppm in 2017, 0.003 - 0.050 ppm in 2018 and 0.006 - 0.050 ppm in 2019, respectively. Of these three seasons, the highest Chromium (Cr) content (0.050 ppm) was found in cold season due to the decreasing rate of organic matter decomposition and low water temperature. The lowest value 0.003 ppm was found in rainy season. According to the data, all measured values were within the range of ASEAN standard 0.05 ppm (Table 2 and Figure 2) [3].

The concentration of chromium (Cr) in sediment samples were found to be in the annual range of 0.01 - 0.12 mg/kg in 2017, 0.22 - 0.23 mg/kg in 2018 and 0.24 - 0.26 mg/kg in 2019, respectively. The highest value 0.26 mg/kg was found in cold season and the lowest value 0.12 mg/kg was found in rainy season. Observed values were lower than the CBSQGs (TEC) (2016) value 43 mg/kg for aquatic life protection (Table 2 and Figure 3) [5].

Table 2. Seasonal Variation of Chromium (Cr) Content in Sea Water and Sediment Samples

Year	Season	Sea Water	Sediment
		Cr(ppm)	Cr(mg/Kg)
2017	Hot	ND	0.01
	Rainy	0.010	0.12
	Cold	0.020	ND
2018	Hot	0.020	ND
	Rainy	0.003	0.22
	Cold	0.050	0.23
2019	Hot	0.040	ND
	Rainy	0.006	0.24
	Cold	0.050	0.26
ASEAN (2016)		0.05	-
EPA (2016)		-	-
CBSQGs (2016)		-	43

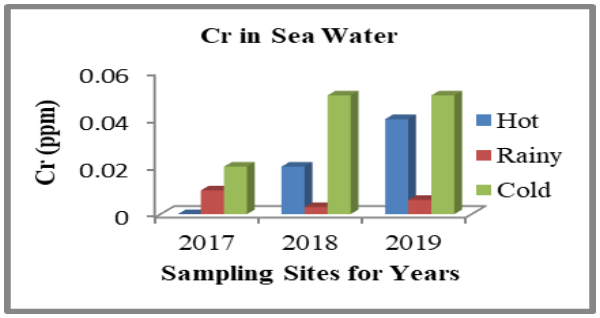

Fig 2. Histogram of Cr in sea water samples Vs study period

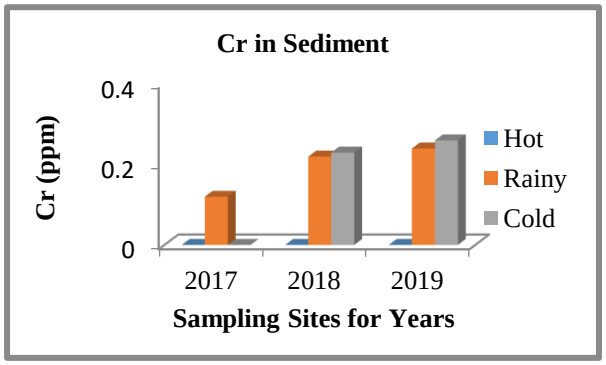

Fig 3. Histogram of Cr in sediment samples Vs study period

3.2 Copper (Cu) content in sea water and sediment samples

The concentration of copper (Cu) in sea water samples were found to be in the annual range of 0.003 - 0.036 ppm in 2017, 0.004 - 0.060 ppm in 2018 and 0.003 - 0.013 ppm in 2019. Of these three seasons, the highest value of 0.036 ppm was found in hot season and the lowest value of 0.003 ppm was found in rainy and cold seasons. The resultants values exceeded the permissible level of ASEAN standard 0.008 ppm because of the sewage and industrial waste are the major sources of copper contamination in aquatic environment (Table 3 and Figure 4) [2].

The concentration of copper (Cu) in sediment samples were found in the annual range of 3.18- 3.85 mg/kg in 2017, 4.25- 4.85 mg/kg in 2018 and 6.20- 6.40 mg/kg in 2019, respectively. The highest copper (Cu) value of 6.40 mg/kg was found in hot season and the lowest value of 3.18 mg/kg was found in rainy season. Observed values were within the CBSQGs (TEC) (2016) value of 32 mg/kg for aquatic life protection (Table 3 and Figure 5) [3].

Table 3. Seasonal Variation of Copper (Cu) Content in Sea Water and Sediment Samples

Year	Season	Sea Water	Sediment
		Cu(ppm)	Cu(mg/Kg)
2017	Hot	0.036	3.85
	Rainy	ND	3.18
	Cold	0.003	3.45
2018	Hot	0.060	4.85
	Rainy	0.004	4.60
	Cold	0.009	4.25
2019	Hot	0.013	6.40
	Rainy	0.003	6.30
	Cold	0.008	6.20
ASEAN (2016)		0.008	-
EPA (2016)		-	-
CBSQGs (2016)		-	32

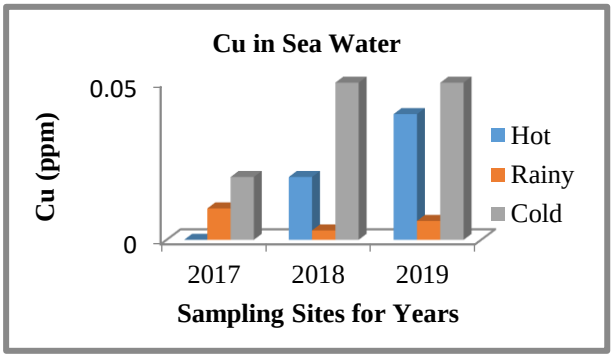

Fig 4. Histogram of Cu in sea water samples Vs stud period

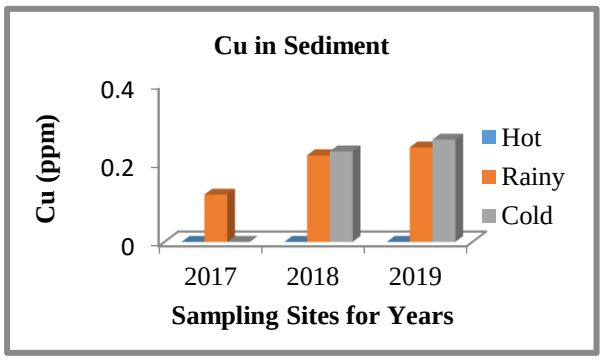

Fig 5. Histogram of Cu in sediment samples Vs study period

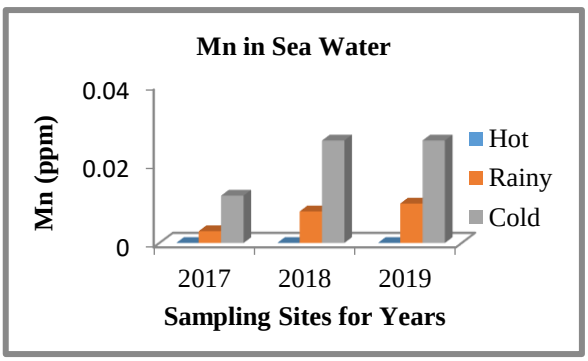

Fig 6. Histogram of Cu in sea water samples Vs study period

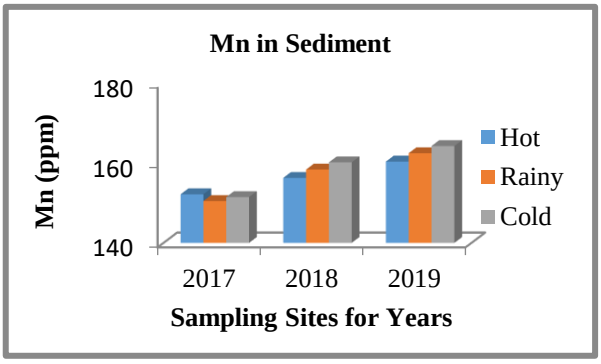

Fig 7. Histogram of Mn in sediment samples Vs study period

3.3 Manganese (Mn) content in sea water and sediment samples

Manganese (Mn) is the naturally occurring metal usually present in many types of rocks. In sea water Mn tends to form particles in the water or settle into the sediment. The manganese (Mn) content in sea water samples were observed in the annual range of 0.003-0.012 ppm in 2017, 0.008-0.026 ppm in 2018 and 0.010-0.026 ppm in 2019, respectively. The highest value of 0.026 ppm was observed in cold season. These values exceeded the EPA standard 0.05 ppm because of the environmental pollution by the waste products of visiting people and villagers (such as, batteries, materials made by iron). The lowest value of 0.003ppm was observed in hot season (Table 4 and Figure 6) [3].

The concentration of manganese (Mn) in sediment samples were in the annual range of 150.5- 152.2 mg/kg in 2017, 156.3-160.2 mg/kg in 2018 and160.4 - 164.3 mg/kg in 2019, respectively. The highest content of 164.3 mg/ kg was observed in cold season and the lowest value of 150.5 mg/kg was found in rainy season. The resultant data were within the CBSQGs (TEC) (2016) value 460 mg/kg for aquatic life protection (Table 4 and Figure 7) [4].

Table 4. Seasonal Variation of Manganese (Mn) Content in Sea Water and Sediment Samples

Year	Season	Sea Water	Sediment
		Mn(ppm)	Mn(mg/Kg)
2017	Hot	ND	152.2
	Rainy	0.003	150.5
	Cold	0.012	151.5
2018	Hot	ND	156.3
	Rainy	0.008	158.4
	Cold	0.026	160.2
2019	Hot	ND	160.4
	Rainy	0.010	162.5
	Cold	0.026	164.3
ASEAN (2016)		NC	-
EPA (2016)		0.05	
CBSQGs (2016)			460

3.4 Iron (Fe) content in sea water and sediment samples

The iron (Fe) concentration in water may be present in varying quantities depending upon the geological area and other chemical component of the water way. The iron (Fe) contents were found to be in the annual range of 0.140-0.143 ppm in 2017, 0.160-0.190 ppm in 2018 and 0.169- 0.210 ppm in 2019, respectively. Fe content is not of concern (NC) in the ASEAN Standard. The highest iron (Fe) content of 0.210 ppm was found in hot season due to the geological nature of the sewage and some industrial waste and the lowest value 0.140 ppm was found in hot season (Table 5 and Figure 8) [2].

The iron (Fe) content in sediment samples were found to be in the annual range of 1200- 1260 mg/kg in 2017, 1300-1500 mg/kg in 2018 and 1400-1700 mg/kg in 2019, respectively. The maximum value1700 mg/kg was found in hot season and the minimum value 1200 mg/kg was found in rainy season. These values were lower than the CBSQGs (TEC) (2016) value of 20000 mg/kg for aquatic life protection (Table 5 and Figure 9). [5].

ASEAN = Standard for human health protection (2016) (for recreational activities); EPA = Standard for aquatic life protection (2016); CBSQGs = Consensus Based Sediment Quality Guide; TEC = Threshold Effect Concentration; ND = Not Detected

Table 5. Seasonal Variation of Iron (Fe) Content in Sea Water and Sediment Samples

Year	Season	Sea Water	Sediment
		Fe(ppm)	Fe(mg/Kg)
2017	Hot	0.140	1260
	Rainy	0.142	1200
	Cold	0.143	1250
2018	Hot	0.190	1500
	Rainy	0.160	1400
	Cold	0.160	1300
2019	Hot	0.210	1700
	Rainy	0.169	1600
	Cold	0.172	1400
ASEAN (2016)		NC	-
EPA (2016)		-	-
CBSQGs (2016)		-	20000

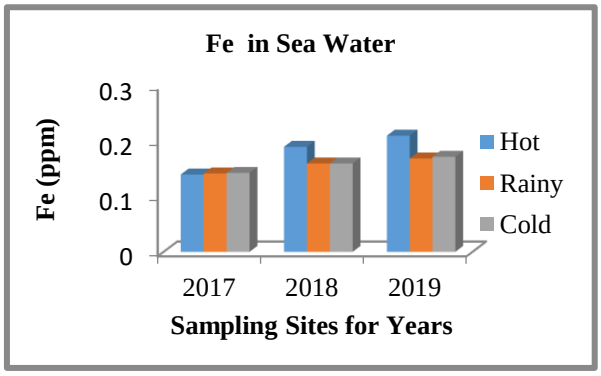

Fig 8. Histogram of Fe in sea water samples Vs study Period

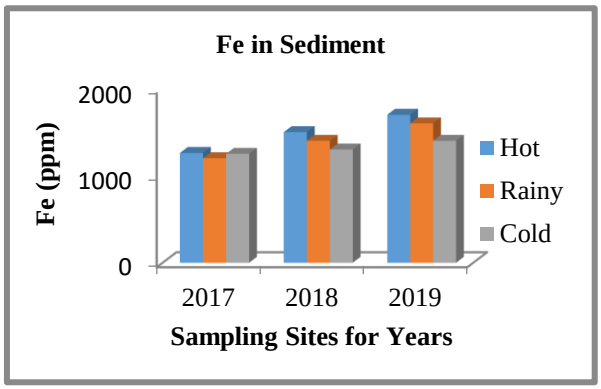

Fig 9. Histogram of Fe in sediment samples Vs study period

3.5 Zinc (Zn) content in sea water and sediment samples

The zinc (Zn) concentration in sea water samples were observed in the annual range of 0.060 - 0.130 ppm in 2017, 0.079 - 0.139 ppm in 2018 and 0.088 - 0.130 ppm in 2019, respectively. The maximum Zn value 0.139 ppm was observed in hot season due to the evaporation of water and temperature effect. The minimum Zn value 0.060 ppm was observed in rainy season (Table 6 and Figure 10). Zn value is not of concern (NC) in the ASEAN standard [3].

The concentration of Zinc (Zn) in sediment samples may be present in the annual range of 50.70- 57.55 mg/kg in 2017, 56.50–58.70 mg/kg in 2018 and 60.20 - 60.80 mg/kg in 2019, respectively. The maximum content of (Zn) 60.80 mg/kg was observed in hot season and the minimum value of (Zn) 50.70 mg/kg was found in rainy season. The resultant data were within the CBSQGs (TEC) (2016) value of 120 mg/kg for aquatic life protection (Table 6 and Figure 11) [5].

Table 6. Seasonal Variation of Zinc (Zn) Content in Sea Water and Sediment Samples

Year	Season	Sea Water	Sediment
		Zn(ppm)	Zn(mg/Kg)
2017	Hot	0.130	57.55
	Rainy	0.060	50.70
	Cold	0.101	55.60
2018	Hot	0.139	58.70
	Rainy	0.079	57.60
	Cold	0.110	56.50
2019	Hot	0.120	60.80
	Rainy	0.088	60.40
	Cold	0.130	60.20
ASEAN (2016)		NC	-
EPA (2016)		-	-
CBSQG (2016)		-	120

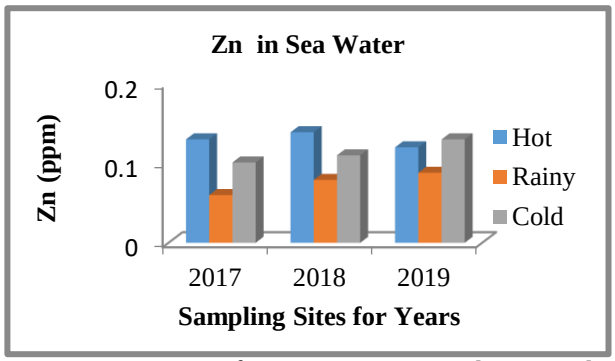

Fig 10. Histogram of Zn in sea water samples Vs study period

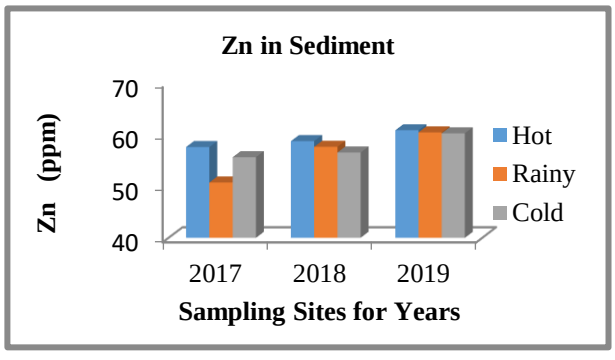

Fig 11. Histogram of Zn in sediment samples Vs study period

3.6 Lead (Pb) content in sea water and sediment samples

The Lead (Pb) concentration in sea water samples were observed in the annual range of 0.001 - 0.003 ppm

in 2017, 0.002 - 0.007 ppm in 2018 and 0.003 - 0.008 ppm in 2019, respectively. The highest Pb value 0.008 ppm was examined in hot season. The lowest Pb value 0.001 ppm was observed in rainy cold seasons (Table 7 and Figure 12). These values were lower than the ASEAN standard 0.009 ppm [2].

The concentration of Lead (Pb) in sediment samples were in the annual range of 0.016 – 0.019 mg/kg in 2017, 0.024 – 0.028 mg/kg in 2018 and 0.024 – 0.045 mg/kg in 2019, respectively. The highest content 0.045 mg/ kg was examined in hot season and the lowest value of 0.016 mg/kg was examined in cold season. Both of sea water and sediment samples the highest values were found in hot season. It would be attributed to the industrial and agricultural discharge as well as from spill of lead petrol from fishing boats and dust (Table 7 and Figure 13) [5].

Table 7. Seasonal Variation of Lead (Pb) Content in Sea Water and Sediment Samples

Year	Season	Sea Water	Sediment
		Pb(ppm)	Pb(mg/Kg)
2017	Hot	0.003	0.018
	Rainy	0.001	0.019
	Cold	0.001	0.016
2018	Hot	0.007	0.028
	Rainy	0.002	0.024
	Cold	0.004	0.027
2019	Hot	0.008	0.045
	Rainy	0.003	0.024
	Cold	0.006	0.025
ASEAN (2016)		0.009*	-
EPA (2016)		-	-
CBSQG (2016)		-	-

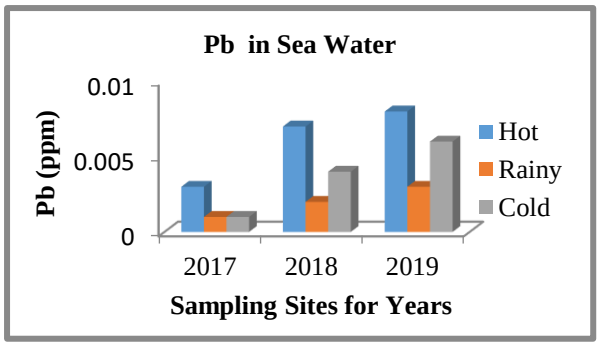

Fig 12. Histogram of Cu in sea water samples Vs study Period

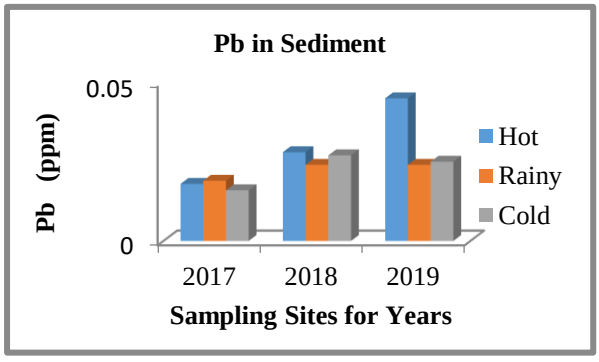

Fig 13. Histogram of Pb in sediment samples Vs study period

3.7 Cadmium (Cd) content in sea water and sediment samples

Cadmium (Cd) is certainly a dangerous water pollutant, causing a major water quality problem. Source of cadmium is industrial discharge, mining waste, metal plating and water pipes. The concentration of cadmium (Cd) in sea water samples were found to be in the annual range of 0.003-0.004 ppm in 2017, 0.004- 0.007 ppm in 2018 and 0.006 - 0.008 ppm in 2019, respectively. Of these three seasons, the highest value 0.008 ppm was found in cold season due to the decreasing rate of organic matter decomposition and low water temperature. The lowest value of 0.003 ppm was found in rainy season (Table 8 and Figure 14). These values were lower than the ASEAN standard of 0.010 ppm [3].

The concentration of cadmium (Cd) in sediment samples were found to be in the annual range of 0.011-0.021 mg/kg in 2017, 0.020-0.024 mg/kg in 2018 and 0.012-0.031 mg/kg in 2019, respectively. The highest cadmium (Cd) value 0.031 mg/kg was found in cold season and the lowest value 0.011mg/kg was found in rainy season. The high level of cadmium contamination may be due to the soil composition and environmental pollution in the study area (Table 8 and Figure 15) [5].

Table 8. Seasonal Variation of Cadmium (Cd) Content in Sea Water and Sediment Samples

Year	Season	Sea Water	Sediment
		Cd(ppm)	Cd(mg/Kg)
2017	Hot	ND	ND
	Rainy	0.003	0.011
	Cold	0.004	0.021
2018	Hot	ND	0.020
	Rainy	0.007	0.024
	Cold	0.004	0.021
2019	Hot	ND	0.012
	Rainy	0.006	0.025
	Cold	0.008	0.031
ASEAN (2016)		0.010	-
EPA (2016)		-	-
CBSQG (2016)		-	-

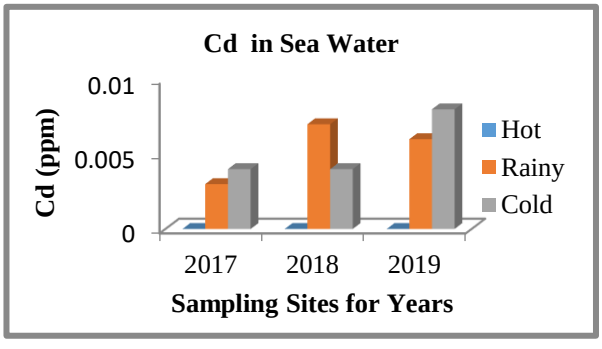

Fig 14. Histogram of Cd in sea water samples Vs study period

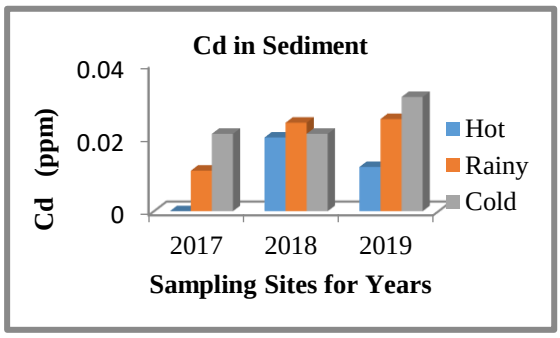

Fig 15. Histogram of Cd in sediment samples Vs study Period

3.8 Mercury (Hg) and Arsenic (As) contents in sea water and sediment samples

The mercury and arsenic contents were not detected in sea water and sediment sample (Tables 9 and 10).

Table 9. Seasonal Variation of Mercury (Hg) Content in Sea Water and Sediment Sample

Year	Season	Sea Water	Sediment
		Hg(ppm)	Hg(mg/Kg)
2017	Hot	ND	ND
	Rainy	ND	ND
	Cold	ND	ND
2018	Hot	ND	ND
	Rainy	ND	ND
	Cold	ND	ND
2019	Hot	ND	ND
	Rainy	ND	ND
	Cold	ND	ND
ASEAN (2016)		0.036	-
EPA (2016)		-	-
CBSQG (2016)		-	-

Table 10. Seasonal Variation of Arsenic (As) Content in Sea Water and Sediment Sample

Year	Season	Sea Water	Sediment
		As (ppm)	As (mg/Kg)
2017	Hot	-	ND
	Rainy	ND	ND
	Cold	ND	ND
2018	Hot	ND	ND
	Rainy	ND	ND
	Cold	ND	ND
2019	Hot	ND	ND
	Rainy	ND	ND
	Cold	ND	ND
ASEAN (2016)		0.16	-
EPA (2016)		-	-
CBSQG (2016)		-	-

4. CONCLUSIONS

The present research work is carried out to give the environmental awareness to the residents. By evaluating the heavy metals accumulation content in sea water and sediments, it can be concluded that heavy metals are highly accumulated in sediments than sea water. Some metal (Cr, Mn, Fe, Zn, Pb and Cd) contents in sea water samples (except Cu) were found within the permissible level of ASEAN and EPA standards. In sediment samples (Cr, Cu, Mn, Zn, Fe, Pb and Cd) values were lower than the Consensus Based Sediment Quality Guide lines (TEC) standards for aquatic life protection showing not toxic on aquatic life especially benthic-dwelling organisms. The mercury and arsenic values were not detected during the study period in sea water and sediment sample. In this comparison, indicate that this sediment samples is deterioration of site quality with the studied heavy metals in studied area. The study revealed that on the basis of computed indexes, the Maungmagan beach area is classified uncontaminated to moderately contaminated. Thus, the studied region in the period from 2017-2019, is free of heavy metals pollution and the marine decoys ohm is still safe to for aquatic life and fisheries [7].

Conversions of Units of Heavy Metals accumulation (sea water and Sediment)

For Liquid, ppm = mg/L, 1ppm = 1 mg/L (sea water)
For Solid, ppm = mg/Kg, 1ppm = 1 mg/Kg (sediment)
*ppm = parts per million

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REFERENCES

[1] ASEAN Environmental Management, Water Quality Criteria and Standards for Freshwater and Marine Aquaculture, vol.9, pp.171-189, June 2016.

[2] Cenci, Pando Mazzola, S "Heavy Metals in Coastal Water System, A Case Study from the North Western Gulf of Thailand". *Chemosphere*, vol.7, pp-67-76, March 2018.

[3] Consensus-Based Sediment Quality Guidelines (CBSQGs), Contaminated Sediment Standing Team Recommendations for Use & Application. *Washington DC: Equal Opportunity Office*, Department of Interior, vol.12, pp1-17, July 2016

[4] EPA, "Nutrient Water Quality Standards". Washington, DC: Office of Water Research, 2016

[5] F. Moore, G. Frogman and A. Qishlaqs, "Assessment of heavy metal contamination in water", vol-8, pp.34-37, March 2018.

Determination of Antimicrobial Activity, Elemental Compositions and Total Tannin Content of *Cassia glauca* Lam. (Pyiban-nyo)

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ABSTRACT*:* In this research work, the leaf of *Cassia glauca* Lam., Myanmar name Pyiban-nyo, which is tannin rich plant, was selected to determine the antimicrobial activity and elemental compositions present in it. Firstly, the antimicrobial activities of the leaf of *C. glauca* Lam. were determined by ager well diffusion method on six tested organisms with various solvent extracts. Ethyl acetate extract responds high activity on all tested organisms except *Candida albicans*. Hence, it was selected for detailed chemical analysis. Moreover, the mineral contents of the leaf of *C. glauca* Lam. were evaluated by EDXRF technique. In addition, leaf of *C. glauca* Lam. was extracted with distilled water to analyze the total tannin content. Furthermore, this extract was checked for qualitative tests of tannin. And then, the total tannin content of this selected sample could be evaluated by the spectrophotometric method using PD 303 - UV Visible spectrophotometer using the Folin-Denis reagent. The amount of tannin compound was calculated from the standard tannic acid curve. The total tannin content in the leaf of *C. glauca* Lam. was found to be 15.032 ± 0.283 mg tannic acid equivalent per g dry weight.

KEYWORDS: *Cassia glauca Lam., tannin, EDXRF technique, Antimicrobial activity, spectrophotometric method, Folin-Denis reagent.*

1. INTRODUCTION

The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security – Joy, et al. (2001). Many reports have shown that some *Cassia* species contain antibacterial, anti-diabetic, antimalarial, anti-carcinogenic and hepato protective substances - Nanasombat & Teckchuen (2009); Sharma, et al. (2000).

The genus *Cassia* is closely, related to Mimosaceae and Papilionaceae, but can be distinguished by few stamens and five free petals. Caesalpinioideae consist of trees, shrubs and a few woody herbs found in the tropics. Economically, woody Caesalpiniaceae is important for its timber. This diverse genus is native throughout the tropics, with a small number of species reaching into temperate regions. The number of species is usually estimated to be about 260 - Marazzi, et al. (2006) but some authors believe that there are as many as 350 – Randell & Barlow (1998). About 50 species of *Senna* are known in cultivation - Huxley, et al. (1992). This genus is distributed all over India, Pakistan, Cylon, Malaysia, China and South Africa. About 26 species of *Cassia* have been reported to contain anthrocene derivatives either in their free form or as glycosides. The anthraquinone molecules are widely distributed in the genus *Cassia* and have remarkable biological properties - Jafri, et al. (1999); Kritikar & Basu (1990).

C. glauca Lam. found throughout India, tropical Asia and Australia. The leaves are long linear, acute, curved in shape. The flower is yellow in color and shorter than the leaves - Kritikar & Basu (1944). Phytochemical study of stem of *C. glauca* Lam. has been indicated the presence of chrysophenol, physcion, stearic acid, β-sitosterol and β-D glucoside – Hemlat & Kalidhar (1994). In folk medicine, bark and leaves of *C. glauca* are used for the treatment of diabetes and gonorrhea. This plant is also a good pollution tolerant and reduces chemical pollutants from atmosphere - Warrier, et al. (1996).

In the present study, the leaf of *C. glauca* Lam. have been investigated for its antibacterial activity, elemental compositions and total tannin content under laboratory conditions.

1.1. Botanical Description

Family : Caesalpiniaceae
Genus : *Cassia*
Botanical name : *Cassia glauca* Lam.
Myanmar name : Pyiban-nyo
Part used : Leaf


Fig 1. Plant, Flower and Leaves of *Cassia glauca* Lam

2. MATERIAL AND METHODS

2.1. Sample Collection

The leaves of *C. glauca* Lam. were collected from Mandalay Technological University Campus, Patheingyi Township, Mandalay Region, in Myanmar. It was cut into small pieces and dried for about one month.

2.2. Antimicrobial Activity Test of Leaf of *C. glauca* Lam.

The antimicrobial activities of Leaf of *C. glauca* Lam. were tested by applying "Agar well diffusion" method on six selected organisms.

2.3. Elemental Compositions in Leaf of *C. glauca* Lam.

The elemental compositions of leaf of *C. glauca* Lam. were determined by EDXRF Spectroscopy Method. The spectrum of selected sample was measured at the Department of Chemistry, Monywa University.

2.4. Qualitative Tests for Tannins

The obtained extract solution was generally tested by three reagents – Buzarbarua (2000).

Preparation of sample: The air dry leaf sample (3 g) was boiled with distilled water. After boiling and cooling, this extract was filtered and the filtrate solution was used for qualitative tests of tannins.

Test with 2 % gelatin solution: 2 % gelatin solution (2 mL) was added to 2 mL of extract solution. A curdy white precipitate was formed. This precipitate indicated the presence of tannins – Buzarbarua (2000).

Test with saturated solution of potassium iodate: A few drops of a saturated solution of potassium iodate solution was added to a little of the extract solution. The pink-red colour was formed, which changed into brown colour solution on standing for about 20 min. It indicated the presence of tannins like gallic acid and ellagic acid – Buzarbarua (2000).

Test with nitrous acid solution: The freshly prepared nitrous acid (2 mL) was added to the extract solution. Carmine-red colour was developed. Then, it changed to indigo blue colour solution on standing for about 20 min. It indicated the presence of tannins like ellagitannins – Buzarbarua (2000).

2.5. Quantitative Determination of Total Tannin Content in Leaf of *C. glauca* Lam.

Principle: Tannin-like compounds reduce phosphortungstomolybdic acid in alkaline solution to produce a highly colored blue solution, the intensity of which is proportional to the amount of tannins. The intensity is measured in a spectrophotometer at 700 nm - Meghashri, et al. (2010).

Preparation of standard tannic acid stock solution: Standard tannic acid (0.01 g) was taken into a 100 mL volumetric flask and made up the volume with distilled water. The obtained solution was used as a stock solution - Meghashri, et al. (2010).

Estimation of λ_{max} for tannic acid: To determine the maximum absorption, standard solution of tannic acid in concentration (6 µg/mL) was prepared. The volume was made up to 1.6 mL with distilled water. And then, 100 µL of Folin-Denis reagent and 300 µL of sodium carbonate solution were added. The mixture was heated in a water bath at 40 °C for 30 min and then cooled at room temperature. The spectrum of this solution was measured in the wavelength interval 660-740 nm - Meghashri, et al. (2010).

Determination of standard tannic acid: The standard tannic acid stock solution was taken by micropipette into a series of test tubes 20 µL, 40 µL, 60 µL, 80 µL and 100 µL respectively. The volume was made up to 1.6 mL with distilled water in each test tube. And then, 100 µL of Folin-Denis reagent and 300 µL of saturated Na_2CO_3 solution were added. After the each standard solution was heated in the water bath at 40 °C for 30 min and then cooled at room temperature. The absorbance values of prepared standard tannic acid solutions were measured by PD-303 UV Visible spectrophotometer at 700 nm with respect to the blank solution - Meghashri, et al. (2010).

Preparation of sample solution: 1 g of dry powdered sample was placed into a beaker with 100 mL of distilled water and boiled for 30 minutes. After boiling, the extract solution was centrifuged at 3000 rpm for about 20 minutes. The supernatant was taken in a 100 mL volumetric flask and made up the volume with distilled water. The obtained solution was used as a stock solution. Then, 1 mL of this solution was taken and made up to 10 mL with distilled water.

Evaluation of total tannin content of leaf of C. glauca Lam.: The total tannin content of the leaf of *C. glauca* Lam. was measured with the Folin-Denis reagent. Firstly, 40 µL of this extract solution was taken in a test tube. It was made up to 1.6 mL with distilled water. 100 µL of Folin- Denis reagent was mixed and then 300 µL of saturated Na_2CO_3 was added. The mixture was heated in a water bath at 40 °C for 30 min and then cooled at room temperature. The absorbance of this prepared sample was measured at 700 nm by using UV Visible spectrophotometer. The assay was carried out in triplicate. The total tannin content of leaf of *C. glauca* Lam. was expressed as mg tannic acid equivalent per gram of dry weight - Meghashri, et al. (2010).

3. RESULTS AND DISCUSSION

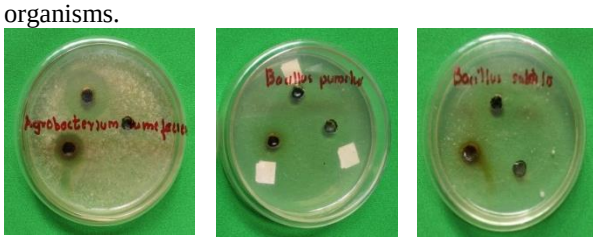
The leaf of *C. glauca* Lam. was investigated the antimicrobial activities, elemental compositions and total

tannin content. The results of antimicrobial activities of the crude sample were shown in table 1 and figure 2.

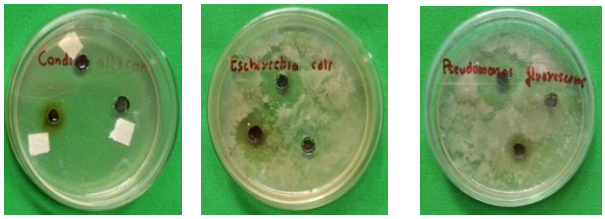
Table 1. The Results of Antimicrobial Activities of the Leaf of *C. glauca* Lam.

No	Test organisms	Ethyl acetate	Methanol	n-Hexane
1	*A. tumefaciens*	31.52 mm	20.43 mm	-
2	*B. pumilus*	27.00 mm	20.09 mm	-
3	*B. subtilis*	31.65 mm	-	-
4	*C. albicans*	-	-	-
5	*E. coli*	33.32 mm	19.55 mm	-
6	*P. fluorescens*	42.13 mm	16.47 mm	-

According to this table, the ethyl acetate extract of the leaf of *C. glauca* Lam. showed high activities on five tested organisms except *Candida albicans.* But, methanol extract gave high activities on *Agrobacterium tumefaciens*, *Bacillus pumilus*, *Escherichia coli* and *Pseudomonas fluorescens.* Furthermore, n-hexane extract did not show antimicrobial activities on all tested organisms.



Agrobacterim tumefaciens *Bacillus pumilus* *Bacillus subtilis*



Candida albicans *Escherichia coli* *Pseudomonas fluorescens*

Fig 2. The antimicrobial activities of leaf of *C. glauca* Lam.

Moreover, the mineral contents in the leaf of *C. glauca* Lam. were also analyzed by EDXRF spectroscopy. The results are shown in Table 2 and Figure 3.

Table 2. Mineral Contents in Leaf of *C. glauca* Lam.

No.	Elements	Relative Abundance (%)
1.	Calcium (Ca)	1.930
2.	Potassium (K)	0.997
3.	Sulfur (S)	0.327
4.	Silicon(Si)	0.166
5.	Phosphorus (P)	0.097
6.	Iron (Fe)	0.017
7.	Strontium(Sr)	0.008
8.	Manganese (Mn)	0.003
9.	Copper (Cu)	0.002
10.	Titanium(Ti)	0.002
11.	Zinc (Zn)	0.001
12.	Bromine(Br)	0.001

Fig 3. EDXRF spectrum of leaf of *C. glauca* Lam.

Table 2 shows that twelve essential elements (Ca, K, S, Si, P, Fe, Sr, Mn, Cu, Ti, Zn and Br) were found in the leaf of *C. glauca* Lam. Among them, calcium is the highest amount in the sample. These results indicate that the leaf of *C. glauca* Lam is a rich source of minerals for health benefit. Minerals play a key role in various physiological functions of the body, especially in building and regulation processes. A number of factors (eg. soil condition, maturity stages, soil type and irrigation regime) may cause variation in the mineral and trace elemental contents in different types of plant as well as within different parts of the same plant. Furthermore, the leaf of *C. glauca* Lam. was examined by using the special qualitative test of tannins. The results are tabulated in Table 3.

Table 3. Results of Qualitative Tests for Tannins

No	Experiment	Observation	Inference
1.	2 mL of 2 % gela- tin was added to 2 mL of extract solution.	A curdy white precipitate forms.	The presence of tannin was indicated.
2.	A few drops of a saturated solution of potassium iodate was added to a little	Pink-red colour forms which changes to brown on standing.	The presence of tannins like gallic and ellagic acid was indicated.
3.	2 mL of freshly prepared nitrous acid was added to a extract solution.	Carmine red colour develops which changes to an indigo blue on standing.	The presence of ellagitannins was indicated.

From these results, it was observed that the aqueous extract of leaf of *C. glauca* Lam. consists of tannin compound. Determination of total tannin content in leaf of *C. glauca* Lam. was carried out by Folin- Denis reagent method using spectrophotometer. The maximum absorption of standard tannic acid in concentration (6 µg/mL) was determined by scanning of the complex in a wavelength range from 660 nm to 740 nm. It showed a maximum absorbance (λ_{max}) at 700 nm as depicted in Table 4 and Figure 4.

Table 4. Wavelength and Corresponding Absorbance of Tannic Acid

No.	Wavelength (nm)	Absorbance
1.	660	0.292
2.	680	0.303
3.	700	0.326
4.	720	0.318
5.	740	0.301

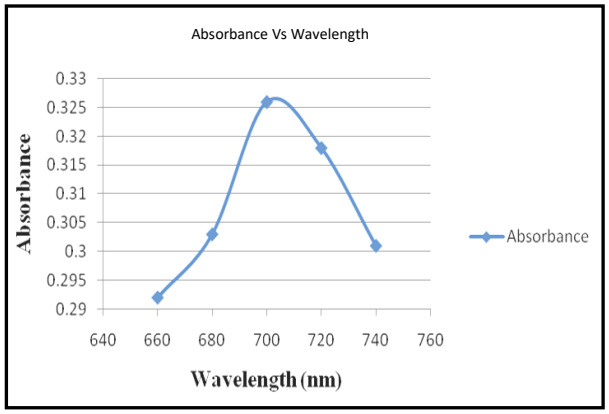


Fig 4. Maximum wavelength of standard tannic acid

By using this maximum absorbance (λ_{max}) at 700 nm, the total tannin content of leaf of *C. glauca* Lam. could be measured. The standard tannic acid solutions at concentration 2 to 10 µg/mL in distilled water were measured to know their absorbance values by PD-303 UV Visible spectrophotometer at 700 nm. The resulted data are described in Table 5. The calibration curve was plotted by using the resulted data of standard tannic acid solution as shown in Figure 5.

Table 5. Results of Concentration and Absorbance of Standard Tannic Acid Solutions

No	Test Sample	Concentration (µg/mL)	Absorbance
1.	Std TA 1	2	0.110
2.	Std TA 2	4	0.20
3.	Std TA 3	6	0.30
4.	Std TA 4	8	0.41
5.	Std TA 5	10	0.50

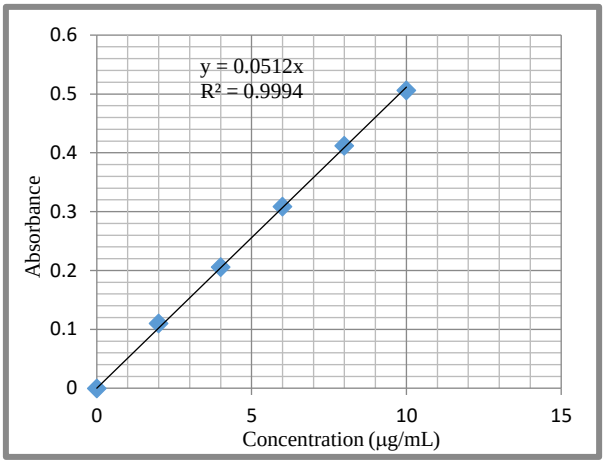


Fig 5. Absorbance concentration calibration curve for standard tannic acid

In addition, the total tannin content of the leaf of *C. glauca* Lam. was quantitatively carried out by spectrophotometric method using the Folin-Denis reagent. The absorbance values of prepared sample solutions were measured with by PD -303 UV Visible spectrophotometer at 700 nm with respect to the blank solution for three times. The results are described in Table 6. From these results, the amount of total tannin content of the leaf of *C. glauca* Lam. was obtained by using the standard curve.

Table 6. Results of the Absorbance and Concentration of The Leaf of *C. glauca* lam.

No	Test sample	Absorbance	Concentration (µg/mL)
1	Test 1	0.031	0.6078
2	Test 2	0.031	0.6078
3	Test 3	0.030	0.5882

The total tannin content of leaf of *C. glauca* Lam. was calculated from different concentrations of tannin in which the same spectrophotometric procedure was followed for the working standard. The calculated results are tabulated in Table 7.

Table 7. Amount of Tannin Content in the Leaf of *C. glauca* Lam.

Name of sample	Tannin (mg/g)	Tannin (mg/g) Mean ± Standard
leaf of *C. glauca* Lam	15.195	15.032 ± 0.283
	15.195	
	14.705	

In accordance with these results, the total tannin content of the leaf of *C. glauca* Lam. was found to be 15.032 ± 0.283 mg tannic acid equivalent per g of dry weight. Thus, the total tannin content in the selected sample was observed to be significant amount. Many plant tannin (phenolic) compounds exhibiting antioxidant properties have been studied and proposed for production against oxidation. Extracts from plants which contribute health benefits to consumers, arising

from protection from free radical mediated deteriorations had stronger antioxidant activity than that of synthetic antioxidants.

5. CONCLUSION

The endeavour of present study pointed out the determination of antimicrobial activity and mineral content of leaf of *C. glauca* Lam. Further study is focused on the evaluation of total tannin content. In accordance with the qualitative test for tannins, it was confirmed that the selected sample contained the tannin compounds. Based on the obtained results, it was found that the total tannin content was 15.032 ± 0.283 mg tannic acid equivalent per g of dry weight. The ethyl acetate extract and methanolic extract of *C. glauca* Lam. exhibited high antimicrobial properties due to it have high tannin content which may be responsible for these activities. The present study revealed the tannin spectrum of medicinally important plants. The good contents of tannin (phenolic) compounds indicated that these compounds contribute to the antioxidant activity. The leaf of *C. glauca* Lam. can be regarded as promising plant species for natural plant sources of free radical scavengers with high potential value for drug preparation.

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REFERENCES

[1] P.P. Joy, J. Thomas, S. Mathew, and B.P. Skaria, "Medicinal Plants. In: Tropical Horticulture", (Ed: T.K. Bose, J. Kabir and P.P. Joy) Naya Prakash, Culcutta, 2001, Vol. 2, pp. 449-632.

[2] S. Nanasombat, and N. Teckchuen, "Antimicrobial, antioxidant and anticancer activities of Thai local vegetables", *J. Med. Plants Res.*, 2009, 3(5), pp. 443- 449.

[3] N. Sharma, P.Trikha, M. Athar, and S. Raisuddin, "*In vitro* inhibition of carcinogen-induced mutagenicity by *Cassia occidentails* and *Emblica officialis*", *Drug Chem. Toxicol.*, 2000, 23: pp. 477-484.

[4] B. Marazzi, K.J. Peter, Endress, Luciano Paganucci de Queiroz and Elena Conti, "Phylogenetic relationship within Seena (Leguminosae, Cassiinae) based on three chloroplast DNA regions: Patterns in the evolution of floral symmetry and extra floral nectarines", *Am. J. Bot.*, 2006, 3(2): pp. 288-303.

[5] B.R. Randell, and B.A. Barlow, Seena, "In: Flora Australia, Alexander S. George (executive editor)", Volume 12. Australian Government Publishing Srevice, Canberra, Australia, 1998, pp. 89-138.

[6] A. Huxley, M. Griffiths, and M. Levy, "The New Royal Horticultural Society Dictionary of Gardening", The Macmillan Press Limited: London. The Stockton Press, New York, 1992.

[7] M.A. Jafri, M.S. Jalis, K. Javed, and S. Singh, "Hepatoprotective activity of leaves of *Cassia occidentalis* against paracetamol and ethyl alcohol intoxication in rats", *J. Ethnopharmacol*., 1999, 66(3): pp. 356-361.

[8] K.R. Kritikar, and B.D. Basu, "Indian medicinal plants", 2nd ed. Periodical experts, New Delhi; 1990, p. 2333.

[9] K.R. Kirtikar, and B.D. Basu, "Indian Medicinal Plants", Vol. IV, Allahabad, India. Lalit Mohan Basu, 1944, p.1139

[10] A. Hemlat, and S.B. Kalidhar, "Chemical components of *Cassia glauca* Lam.", *Indian J. Pharmaceutal. Sci.*, 1994, 1: pp. 33-34.

[11] P.K. Warrier, V.P.K. Nambiar, and C. Ramankutty, "Indian Medicinal Plants: A Compendium of 500 species", Orient Longman Pvt. Ltd., Hyderabad, 1996, p. 423.

[12] A. Buzarbarua, "A Text Book of Practical Plant Chemistry", Schand & Co. Ltd, 2000.

[13] S. Meghashri, V. Kumar and S. Gopal, "Antioxidant properties of a novel flavonoid from leaves of *Leucasaspera". Food Chemistry*, 2010; 122, 105–110.

Study on Some Physicochemical Properties and Antioxidant Activity of Prepared Wine using Fruits of *Elaeagnus latifolia* Linn. (Hman- gu)

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ABSTRACT*:* In this research work, *Elaeagnus latifolia* Linn (Hman- gu) fruit was collected from Mogkok Township, Mandalay Region. The phytochemical investigation of the fruit was tested by standard methods. It consists of alkaloids, flavonoids, glycoside, phenolic, reducing sugar, saponin, polyphenol, steroid, tannin and terpene respectively. Then, two types of Hman-gu wine (X-type) were prepared with sugar, Y- type with sugar and yeast. The physical parameters such as pH, alcohol content and total dissolved solid (TDS) were determined. The pH value in X-type was 3.75 and Y-type was 4.3. Both type of prepared wines are acidic condition. The TDS content in X-type was 995 mg/L and Y-type was 798 mg/L at 30 °C. The sensory evaluation test on prepared wine were also performed. The antibacterial activity of the Hman- gu fruit wine were also examined by using agar well diffusion method. No activities respond on selected microorganisms by prepared wine. The reducing sugar content in Hman- gu wine was determined by using iodometric titration method. The sugar content in X-type was 5.37 g/L and Y-type was 4.91 g/L. The antioxidant activities of four months wine X-type and Y-type were examined by using DPPH assy.

KEYWORDS: *Hman-gu wine, yeast, antioxidant, antimicrobial.*

1. INTRODUCTION

Wine fermentation is one of the most ancient of human's technologies and is now one of the most commercially prosperous biotechnological processes - Arribas MV (2005). The technique of wine making is known since the dawn of civilization and has followed human and agricultural progress - Chambers PJ (2010).

Elaeagnus latifolia belongs to family *Elaeagnaceae* locally known Northern part of Myanmar. The people of Myanmar have found many uses of besides enjoying it as fresh fruit. The fruit is oblong in shape with dark pink color at the time of ripening. The fruit contains a single large seed that has very less viability. The fruits are eaten raw with salt and used for making chutney and fruit pulp is used for making jam, jelly, refreshing drink and wine. The fruit is considered to be a very rich source of vitamins and minerals and other bioactive compounds. It is also a fairly unusual for a fruit. It is being investigated as a food that is capable of reducing the incidence of cancers. The sugar composition of *Elaeagnus latifolia* Linn (Hman- gu) has a key role in wines. *Elaeagnus latifolia* Linn (Hman- gu) sugar composition and concentration change during *Elaeagnus latifolia* Linn (Hman- gu) ripening and can be influenced by many factors, such as environment and agriculture management. Alcohol is the most abundant volatile compound in wine and it can modify both the sensory perception of aromatic attributes and detection of volatile compounds- Goldner (2009).

Therefore, alcohol is important for wine sensory sensations but also by their interaction with other wine components, such as aromas -Goldner (2009) and tannins -Fontoin, H,(2008)& Meilion(2009) also influencing wine viscosity and body -Pickering G.J(1998) and our perceptions of astringency, sourness, sweetness, aroma and flavor-Fischer,U(1994). Antioxidants are the substances which delay, prevent or even inhibit the oxidizable substrate system in which they are present, by inhibiting the propagation or initiation of oxidative chain reaction -M. Oroian and I. Escriche(2005).

Antioxidants possess free radical chain reaction breaking properties thus defend living cells against oxidative damage. They help in reducing and quenching of singlet oxygen formation and function as radical scavengers- V. Lobo, A(2010)&. C. Papuc(2017).

In this research, fruits of *Elaeagnus latifolia* Linn (Hman- gu) was chosen for wine making. Some physicochemical properties of prepared wines were determined by standard method. The antioxidant and antimicrobial activities of both types of Hman-gu wine were also studied.

1.1. Botanical Description of *Elaeagnus latifolia* Linn

Fig 1. *Elaeagnus latifolia* Linn (Hman- gu)

Botanical Name - *Elaeagnus latifolia* Linn

Family Name - *Elaeagnaceae*

Part used - Fruits

2. MATERIALS AND METHODS

2.1. Sample collection

The fruits of *Elaeagnus latifolia* (Hman-gu) were collected from the Mogkok Township, Mandalay region. Yeast and Sugar can be brought in local market.

2.2 Preliminary phytochemical tests for fruits of Hman- gu

The phytochemical composition of furit of *Elaeagnus latifolia* (Hman-gu) was determined by standard phytochemical tests- J. B. Harbone (1984).

2.3. Sample Preparation

The experiments were performed in Mandalay Technological University. The Hman-gu fruits were washed with water to remove the dust particles and other impurities, then washed with distilled water.

1) Wine Making (X-type) : The sugar (960 g) was placed in a wine making pot and 4 L of distilled water was added and stirred until all sugar were dissolved. Then, were put into the pot. The *Elaeagnus latifolia* (Hman-gu) sample 600 g was placed in wine making pot and 960 g of sugar and was also added 4 L of distilled water, respectively. The solution was stirred until all sugar was dissolved. After that the pot was covered and set-up airlocks on. The air pipe was imbedded into lime water. The fermentation process was allowed to four months at room temperature. X-type of wine was obtained.

2) Wine Making with Yeast (Y-type): The *Elaeagnus latifolia* (Hman-gu) sample 600 g was placed in wine making pot and 960 g of sugar was also added. The 1L (0.3 g/L) of yeast solution was then added. Distilled water was added until the prepared solution make up to 3L. The solution was stirred until all sugar was dissolved. After that the pot was covered and set- up airlocks on. The air pipe was imbedded into lime water. The fermentation process was allowed to four months at room temperature. Y-type of wine was obtained.

Fig 2. Wine Making Pots

Table 1. Type of Hman-gu wine and its constituents

Type of wine	Hman-gu fruits (g)	Sugar (g)	Yeast (g/L)	Distilled water (L)
X-type	600	960	-	4
Y-type	600	960	1	3

2.4. Determination of pH, Alcohol Content and TDS

The pH content of the prepared Hman-gu wine was directly measured by using pH meter. 100 mL of prepared wine is placed in beaker and the pH meter was immersed in this beaker. The pH of the wine was recorded. The alcohol content of the prepared Hman-gu wine was directly measured by using alcohol meter. 100 mL of prepared wine was placed into 100 mL of measuring cylinder and the alcohol meter was immersed in this wine and the alcohol percent of wine was read in the marked of alcohol meter. The TDS content of the prepared Hman-gu wine was directly measured by using TDS meter.5o mL of prepared wine was placed into 100 mL of beaker and TDS meter was immersed in wine solution. The TDS amount was read.

2.5. Determination of Sugar Content

1) Iodometric Titration: 1 mL of Hman-gu wine was added 9 mL of distilled water to obtain 10 mL of dilute solution. 10 mL of diluted sample solution was taken into conical flask and 20 mL of 0.05 M iodine solution and 45 mL of 0.1 M sodium hydroxide solution were added into the flask. The flask was closed and left in dark place for 15 minutes. Then 6 mL of 1 M hydrochloric acid was added and titrated with the 0.05 M sodium thiosulphate solution. When the solution become light yellow, 1 mL of starch solution was added. The solution become dark blue again and titrated until the colorless content was obtained. shown in table (3) the experimental data, the decrease amount of sugar can be calculated- J. Memdham(2000), M. Ferguson (1996) & W. F. Gobel(1927).

Fig 3. Color Changing Steps in Iodometric Titration

2.6. Sensory Evaluation

The sensory evaluation of Hman-gu wine was performed by six potential involving of staff and students of Mandalay Technology University. The samples were appraised using a standard "Scoring Difference Test" method (Hodgson, 2008) following the instructions provided in the sensory evaluation surveys. The evaluated the clarity, color, flavor, taste and overall acceptability of the Hman-gu wine. The results are shown in Table (4) and (5).

2.7. Determination of Antioxidant Activity by DPPH Radical Scavenging Method

The antioxidant activity of Hman-gu wine was determined by DPPH (1,1-Diphenyl-2-picryl- hydrazyl) free radical scavenging assay at Medical Biotechnology Laboratory, Biotechnology Research Department (BRD), Kyaukse, Mandalay.

The antioxidant activity of Hman-gu wine was determined by DPPH (1,1-Diphenyl-2-picryl- hydrazyl) free radical scavenging assay. The samples were diluted with 50% EtOH for various concentrations. Briefly, the reaction mixture containing 50 µL of diluted test sample of various concentrations and 50 µL of DPPH (300

µmol) dissolved in method, was taken in a 96. well micro-titer plate and kept standing at 37 ℃ for 30 min. The absorbance was measured at 515 nm by using 96 well microplate reader (Spectrostar Nano, BMG Labtech Microplate reader). Ascorbic acid was used as a standard. 50% EtOH was used as the control and added to the 96-well plate instead of the sample. Percent Radical Scavenging Activity (%RSA) was calculated by using the following formula.

%RSA= [1-(OD test compound/OD control)] × 100

Finally, IC_{50} (50% inhibition concentration) was determined by using linear regressive excel program. Ascorbic acid was used as a standard [15].

2.8. Determination of Antibacterial Activity of Hman-gu Wine by Agar Well Diffusion Method

The Agar Well Diffusion method was used to test the antibacterial activity of the Hman-gu wines, Medical Biotechnology Laboratory, Biotechnology Research Department (BRD), Kyaukse, Mandalay. In this determination, Escherichia coli, Enterococcus faecalis, Staphylococcus aurous and Bacillus cereus are used as tested microorganisms for this experiment.

1) Agar Well Diffusion Method : The antibacterial activity of Hman-gu wine was made by the following procedure. The agar well diffusion method was used for antibacterial activity evaluation by altering the method described by Schlegel. Test microorganisms were protected in Muller Hinton Broth at 37 °C for overnight. On the next day, the overnight broth culture was diluted with Normal saline to obtain the OD_{600} at 0.08 to 0.1 with the approximate cell density of 1.5×10^8 CFU/ mL. Muller Hinton Agar plates were prepared and sterilized by autoclaving at 121°C for 15 mins. The broth inoculums were evenly spread out with sterile cotton swabs on the Muller Hinton Agar plates to obtain the uniform inoculums. After the plate was inoculated, 8 mm diameter wells were made on the agar medium by using a sterile cork borer. Each 50 µL of wine samples were presented into each well labeled. Chloramphenicol 30µg well was used as the positive control. Then, the plates were placed in an incubator at 37 ℃ for 16 to 18 hours. After incubation were measured and recorded to the closest millimeter -H.G Schlegel(199).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Constituents of Hman-gu Fruit Sample

Preliminary phytochemical analysis was performed in order to know different type of organic compound present in Hman-gu fruit. The constituents of phytochemical tests are described in table (2).

Table 2. Phytochemical Constituents of Hman-gu Fruit Sample

No.	Constituent	Extract	Reagent used	Observation	Result
1.	Alkaloids	1% HCl	Dragendroff's reagents	Orange ppt	+
2.	Flavonoid	95 % ethanol	Con: HCl,Mg	Pink color solution	+
3.	Glycoside	Distilled water	10 % lead acetate	White ppt	+
4.	Phenolic	Distilled water	10 % $FeCl_3$	Purplish color solution	+
5.	Reducing sugar	Distilled water	Benedict's solution	Green color	+
6.	Saponin	Distilled water		Forth like comb	+
7.	Polyphenol	95 % ethanol	1 % $FeCl_3$,1% $K_3[Fe(CN)_6]$	Green blue color solution	+
8.	Steroid	95 % ethanol	$CHCl_3$, acetic anhydride, conc:H_2SO_4	Green color solution	+
9.	Tannin	95 % ethanol	10% $FeCl_3$, dil:H_2SO_4	Yellowish brown ppt	+
10.	Terpene	Pet-ether	$CHCl_3$, acetic anhydride, conc:H_2SO_4	Pink color solution	+

(+) = presence of Constituent

(-) = absence of Constituent

According to the Table (2), the Hman-gu fruits contained alkaloids, flavonoid, glycoside, phenolic, reducing sugar, saponin, polyphenol, steroid, tannin and terpene.

3.2 The Result of pH, Alcohol, TDS and Sugar Content of Hman-gu wine

Table 3. Results of pH, Alcohol, TDS and Sugar Content of Hman-gu wine

sample	pH	Alcohol (%)	TDS (mg/L)	Sugar Content (g/L)
X-type	3.75	6	995	5.37
Y-type	4.30	7	798	4.91

Table (3) shows the physicochemical properties of prepared Human-gu wines. According to the result, the pH of wine were found to be 3.75- 4.3. The alcohol content of X- type of wine was found to be 6% and Y-type of wine was found to be 7%. The range of alcohol percent (5 to 10%) stand for good quality of wine. TDS content of Hman-gu wine were contained 995 and 798 mg/L of X-type and Y-type. The sugar content of wine sample was determined by using Iodometric titration method. The sugar content X-type and Y-type of wine remain 5.37 g/L and 4.91 g/L. Therefore, Hman-gu wine is suitable for drink.

3.3The Sensory Grading Evaluation of Hman-gu wine

Table 4. Sensory Grading Evaluation of Female

Type of wine	Clarity	Color	Odour	Taste	Over all acceptance
X-type	1.5	1.6	2	2	2.4
Y-type	1.2	1.5	1.3	1.7	2

Table 5. Sensory Grading Evaluation of Male

Type of wine	Clarity	Color	Odour	Taste	Over all acceptance
X-type	1.3	1.4	2.2	2.4	2.3
Y-type	1.5	1.5	1.3	1.2	1.9

Grade 1= excellent, Grade 2= good, Grade 3= fair,

Grade 4= unfair, Grade 5= bad

According to the sensory evaluation test, female like 80% (X-type) and 66% (Y-type) of Hman-gu wine and male like 77% (X-type) and 63% (Y-type) of Hman-gu wine.

3.4. Antioxidant Activity of Hman-gu Wine

The antioxidant activity of Hman-gu wine was determined by DPPH Assay and the results are shown in Table 6, 7 and Figure 4, 5 respectively.

Table 6. % inhibition of IC_{50} values of X-type wine

Type of sample	% inhibition at Different Concentrations (µg/mL)										IC_{50} (µg/mL)
	10	20	30	40	50	60	70	80	90	100	
X-type				11.11	20.24	34.00	37.60	46.14	52.02	55.56	87.8456

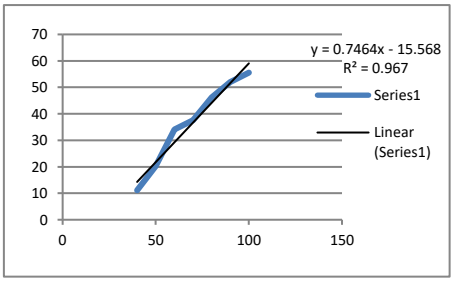

Fig. 4. Plot of % Inhibition Vs Concentration X-type of Wine

Table 7. % inhibition of IC_{50} values of Y-type wine

Type of sample	% inhibition at Different Concentrations (µg/mL)										IC_{50} (µg/mL)
	10	20	30	40	50	60	70	80	90	100	
Y-type	14.94	19.65	30.83	36.72	49.20	58.79	64.68	73.22	83.22	96.17	52.0496

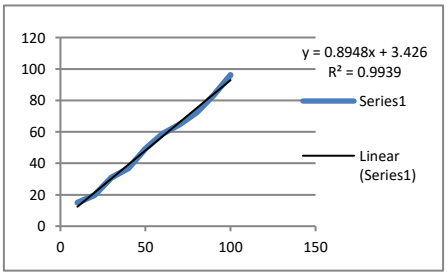

Fig. 5. Plot of % Inhibition Vs Concentration Y-type of Wine

All data were represented as mean standard deviation from three replicate experiments. Ascorbic acid was used as a positive control for DPPH Radical Scavenging Assay. The concentration of DPPH used for this experiment was 0.3 mM. According to the table (6), the higher concentration of samples with the greater amount of % inhibition. % inhibition of ascorbic acid at 500 µg/mL is 94.63. % inhibition of (X-type) wine at 100 µg/mL is 55.56 and (Y-type) wine at 100 µg/mL is 96.17 respectively.

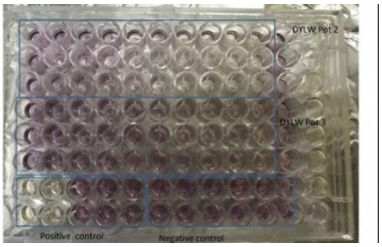

Fig 6. The antioxidant activity of prepared wine against DPPH radical scavenging activity using 96 Well Microplate

The IC_{50} value is a parameter used to measure antioxidant activity and it is defined as the juice concentration required for 50% scavenging of DPPH radicals under experimental condition employed. A smaller IC_{50} value corresponds to a higher antioxidant activity. IC_{50} value was calculated by using linear regressive equation. As a result, IC_{50} value of X-type of wine was 87.85µg/mL and Y-type of wine was 52.05 µg/mL respectively. The both types of Hman-gu wine have significant antioxidant activity. Therefore, the prepared wine should drink because they have health benefits of human body.

3.5. Antibacterial Activities of Hman-gu Wine

The antibacterial activity of Hman-gu wines was determined by Agar Well diffusion method on selected microbes such as *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus* and *Bacillus cereus.*

The results show that both types of prepared wines have no activity on selected microorganism.

4. CONCLUSIONS

In this research paper, the fruits of *Elaeagnus latifolia* (Hman-gu) were selected for wine making and their physicochemical properties, antioxidant activity ad antibacterial activity were determined. The phytochemical tests of *Elaeagnus latifolia* (Hman-gu) fruits give positive for alkaloid, flavonoid, glycoside, phenolic, reducing sugar, saponin, polyphenol, steroid, tannin and terpene respectively. The results of phytochemical analysis showed that the fruits contain valuable phytochemical constituents.

Most wine of pH's fall 3 to 4. The pH of X- type and Y- type of wines were 3.75 and 4.3 respectively. Two types of wine were acidic. Both prepared wines contain suitable contents of alcohol, total dissolve solid and sugar. The antioxidant activity of wines were determined by using 1, 1- diphenyl 1-2 picryl hydrazyl (DPPH) assay. The prepared wines have significant antioxidant

activity. Therefore, Hman-gu wine is suitable for drink because they have health benefits of human body.

The sensory evaluation showed that two type of wine have an acceptable clarity, color, odour and taste but further research is needed to improve the body of the final product. According to the sensory evaluation test, female like 80% (X-type) and 66% (Y-type) of Hman-gu wine. Male like 77% (X-type) and 63% (Y-type) of Hman-gu wine.

This study indicates that Hman-gu wine could be used for fruits wine production and other industrial application.

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REFERENCES

[1] Arribas MV, polo MC. "Wine making biochemistry and microbiology: current knowledge and future trends". Crit Rev Food Sci Nutr 2005;45:265-86.

[2] Chambers PJ, Pretorius IS. "Fermenting knowledge"; The history of winemaking, science and yeast research. EMBO Rep 2010,11:914-20.

[3] Goldner, M.C.; Zamora, M.C.; di Leo Lira, P.; Ginanninoto, H.; Bandoni, A. "Effect of ethanol level in the perception of aroma attributes and the detection of volatile compounds in red wine". *J. Sens. Stud*. 2009, 24, 243-257.

[4] Fontoin, H.; Saucier, C.; Teissedre, P.L; Glories, Y. "Effect of pH. Ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution". *Food Qual. Preference* 2008, 19, 286-291.

[5] Meilion, S.; Urbano, C.; Schlich, P. "Contribution of the temporal dominance of sensations (TDS) method to the sensory description of subtle differences in partially dealcoholized red wines". *Food Qual. Preference* 2009, 20, 490-499.

[6] Pickering G.J.; Heatherbell, D.A.; Vanhanen, L.P.; Barnes, M.F. "The effect of ethanol concentration on the temporal perception of viscosity and density in white wine". *Am. J. Enol. Vitic. 1998, 49,306-318*.

[7] Fischer, U., Noble, A.C. "The effect of ethanol, catechin concentration, and pH on sourness and bitterness of wine". *Am. J. Enol. Vitic. 1994,45, 6-10*.

[8] M. Oroian and I. Escriche, *Food Res. Int*., 74, 10 (2015); http://doi,org/10.1016/j.foodres 2015. 04. 018.

[9] V. Lobo, A. Patil, A. Phatak, and N. Chandra, *Pharmacogn. Rev*.,4,118 (2010);

[10] C. Papuc, G.V. Goran, C.N. Predescu, V. Nicorescu and G. Stefan, *Comp. Rev. Food Sci. Food Saf*,.16,1243 (2017); https:// doi,org/ 10.1111/1541-4337.12298.

[11] J. Memdham, R. C. Denney, J. D. Barnes, M. Thomas, "Vogel's Textbook of Quantitive Chemical Analysis", Pearson Education Ltd, England, 2000.

[12] J. B. Harbone, "Phytochemical methods, *A Guide to modern techniques of plant analysis"*, Chapman and Hall Ltd. U. S. A 1984.

[13] M. Ferguson, "Volumetric Analysis", Chemistry Review , September,1996.

[14] W. F. Gobel, "On the oxidation of Glucose in Alkaline Solution of Iodine", J. Biol Chem. 72, pp. 801-807, 1927.

[15] S.Lee, D. Son, J. Ryu, Y. S. Lee, S. H. Jung, S.Y. Lee, K.H. Shin, "Antioxidant activities of Acanthopananaxsenticosus stems and their lignin components". Archives of Pharmacal Research, 27, 106-110.2004

[16] H.G Schlegel.; C. Zabarosch., "General microbiology". Cambridge university press: 1993.

Identification Of Chemical Constituents, Antimicrobial Activities And Isolation Of Organic Compounds From The Whole Plants Of *Leucas Aspera Spreng* (Pingu-Hteik-Peik)

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A**BSTRACT**: In this research work, one of Myanmar traditional indigenous plants, *Leucas aspera* (Willd) Spreng has been chosen for chemical analysis. The plant sample was collected from the cultivated land beside the east bank of Chindwin River, Monywa Township, Sagaing Region. Preliminary phytochemical analysis was performed in order to know different types of chemical constituents present in the selected plant. Elemental composition of the whole plant of *Leucas aspera* was investigated by applying Energy Dispersive X-ray Fluorescence Spectrophotometric (EDXRF) method. Antimicrobial activities of crude extract in various solvent systems were tested by using agar well diffusion method on six selected microorganisms. Isolation of pure compound of the whole plant of *Leucas aspera* was performed by using column and thin layer chromatographic method. Then the melting point of the resulting pure compound was determined. The functional groups of the pure compound were also assigned by FT IR spectral data. In addition, this compound was reconfirmed by phytochemical tests.

KEYWORDS*: Microorganism, energy dispersive X-ray fluorescence, phytochemical, agar-well Diffusion, antimicrobial activities*

1. INTRODUCTION

The importance of plants is known to us well. The plant kingdom is a treasure house of potential drugs and in the recent years. There has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects-Srinivasn.R.(2011). The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs, antimicrobial drugs and antihepatotoxic compounds-John (2013).

Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances. Secondary Plant Metabolites are biosynthetically derived from the primary metabolites and their distribution in plant Kingdom is restricted. Attempts are being made on the research of producing phytochemicals-Srinivasan, et al (2011). *Leucas aspera* is a medicinal herb that belongs to the family Lamiaceae. It is popular as "Thumbai" throughout the Indian sub-continent. *Leucas aspera* possesses immense medicinal properties- antipyretic, insecticide, anti-inflammatory, antimicrobial and antioxidant. *Leucasaspera* is an edible rainy season weed-Harbon J.B (1982).

1.1Botanical Description

Botanical name	: *Leucasaspera* (Willd) Spreng
Family name	: Lamiaceae
Genus	: Leucas
English name	: Spider wort
Myanmar name	: Pingu- hteik- peik
Part used	: The whole plant
Medicinal Uses	: Skin diseases, chronic malaria, eye diseases, snake bites, poisonous insect bites, rheumatism, jaundice, urinary tract infection, gastrointestinal disorders, paralysis, inflammation, expectorant, ulcers, headache, coughs and colds

Fig 1. The plant of *Leucas aspera* (Willd) Spreng (Pingu-hteik-peik)

1.2 Medicinal uses

The plant is used traditionally as an antipyretic and insecticide. A decoction of the plant is used in the treatment of malarial fever and a domestic medicine for

snake bite. The whole plant was used to treat bronchitis, inflammation, asthma, dyspepsia, paralysis and skin diseases-Ilango.k.s.et al (2008). The whole plant powder is used to cure epileptic convulsions and cerebral function disorders. The juice of the leaves is used as local application for psoriasis, chronic skin eruptions and chronic rheumatism and painful swelling-John.T.A.R.(2013). The juice of the flowers can also be for intestinal worn infections in children. The flowers are administered in the form of syrup or with honey as a domestic remedy for cough and colds. The decoction of the herb as wash liquid is useful in ulcers and is also applied externally to poisonous insect bites. It is good to relieve indigestion, low digestive strength-Yashvanth.s.et al (2013).

2. MATERIALS AND METHODS

2.1 Sample Collection

Plant materials were collected from the cultivated land beside the east bank of Chindwin River, Monywa Township, Sagaing Region. The plant sample was cut into small species and allowed to dry in good ventilation place. The dried sample was stored in stopper bottle and used throughout the experiment.

2.2 Preliminary Phytochemical Analysis on Sample

Preliminary phytochemical analysis was performed in order to know different types of chemical constituents present in the selected plant by using standard methods. Commercial grade reagents and solvents were used-Harbon.J.B.(1982).

2.3 Determination of Elemental Composition on the whole plant of *Leucas aspera*

Elemental composition of the sample was determined by using EDXRF Spectrophotometer at URC, Department of Chemistry, Monywa University.

2.4 Determination of antimicrobial activities of the whole plant of *Leucas aspera*

For the examination of antimicrobial activities of crude extracts on six selected microorganisms, agar well diffusion method was performed in Pharmaceutical and Food Research Department (PFRD), Insein, Yangon.

2.5 Isolation and Purification of the Compounds

Common laboratory tools were used in the isolation and purification of the compounds. The advanced instruments, UV lamp and FTIR spectrometer were used in the characterization of sample and elucidation of pure compound. An iodine vapour was used for the location of the sample spots. Analytical preparative thin layer chromatography was performed by using precoated silica gel. Air dried sample (285g) was percolated with ethanol (2L) for two months and filtered. The filtrate was evaporated. The ethylacetate extract solution was concentrated to produce crude sample (12g). 2g of crude extract was separated by column chromatography and eluenting n-hexane and ethylacetate with various ratios from non-polar to polar. Totally 104 fractions were obtained. Each fraction was checked by TLC under UV detector and the same R_f values were combined. Totally (10) combined fractions were obtained. Among them the fraction IX, pale yellow crystal which gave one spot on TLC. The pure crystals were obtained and named (SS-1). The yield percent was found to be 2.5% based upon the crude EtOAc extract and R_f value 0.449 was observed. The fraction VII is greenish-yellow amorphous form which gives one spot on TLC. This form of unknown compound was obtained and namely as (SS-2). The yield percent was found to be 2.3% based upon the crude EtOAc extract and R_f value 0.523 was observed-Silverstein R.M.et al (1989).

2.6 Determination of the melting point of compound (SS-1)

The pure sample was inserted into a capillary tube was attached to the thermometer and then the thermometer was immersed into the test tube containing liquid paraffin. After the tube was gently heated, the pure sample in the capillary tube was melted at (183.5°C), which was identical with the melting point of reference 5- hydroxy-4', 7- dimethoxy flavone-Aye.M.N.(2014).

2.7 Confirmatory Tests for Compounds (SS-1 and SS-2)

Alkaline reagent test(Flavonoid Test) for compound (SS-1)

A small amount of compound was tested a few piece of magnesium turning in a test tube. Then a few drops of concentrated hydrochloric acid was added to this test tube. Yellow colour was observed which indicates the presence of 5-hydroxy -4', 7-dimethoxy flavone.

Salkowskitest (Steroid Test) for compound (SS-2)

A small amount of compound was dissolved in sufficient volume of chloroform in a test tube. Then 1mL of concentrated H_2SO_4 was added to this test tube. Green colour was observed which indicates the presence of steroid.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening of the Whole Plant of *Leucas aspera*

The results for the phytochemical constituents of the whole plant of *Leucas aspera* are tabulated in Table 1.

Table 1. The Results of Phytochemical Tests of the Whole Plant of *Leucas aspera*

No.	Tests	Reagent used	Observation	Remark
1.	Alkaloids	Dragendorff's reagent	reddish brown ppt	+
2.	Glycosides	10% lead acetate	white ppt	+
3.	Tannins	10% $FeCl_3$, dilute H_2SO_4	deep black ppt	+
4.	Reducing Sugars	Benedict's solution	yellow ppt	+
5.	Saponins	conc: H_2SO_4	frothing	+
6.	Flavonoids	conc: HCl, Mg turning	greenish-yellow ppt	+
7.	Steroids	conc: H_2SO_4, $(CH_3CO)_2O$	reddish brown colour solution	+
8.	Polyphenol	1% $FeCl_3$, 1% $K_3Fe(CN)_6$	deep blue colour solution	+
9.	α-amino acid	ninhydrin	purple colour	+

(+) = presence of constituent

(-) = absence of constituent

According to this table, the whole plant of the Pinguhteik-peik consists of alkaloids, glycosides, tannins, reducing sugars, saponins, flavonoids, steroids, polyphenols and α - amino acids. Therefore, all main phytochemcial constituents are present in this sample and it can be used as natured remedy against many diseases.

3.2 Relative Composition of Elements in the Whole Plant of *Leucas aspera*

Relative composition of elements in the whole plant of *Leucasaspera* were determined by using EDXRF Spectrometer as shown in Figure 2. The data obtained were tabulated in Table 2. The total of twelve elements, K, Ca, Si, P, S, Fe, Ba, Mn, Cu, Zn,Sr and Cr were detected. Among them K, Ca, Si, P, S and Fe were found to be 1.348%, 0.731%, 0.570%, 0.299%, 0.180% and 0.040% respectively.

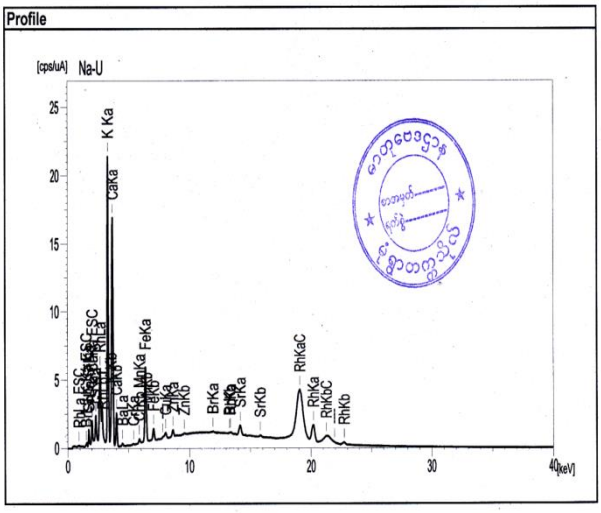

Fig 2. EDXRF spectrum of the whole plant of *Leucasaspera*

Table 2. Elemental Composition of Elements in the Whole Plant of *Leucasaspera*by EDXRF

Qualitative and Quantitative Results		
Analyte		Result
Element	Symbol	
Potassium	K	1.348 %
Calcium	Ca	0.731 %
Silicon	Si	0.570 %
Phosphorus	P	0.299 %
Sulfur	S	0.180 %
Iron	Fe	0.040 %
Barium	Ba	0.013 %
Manganese	Mn	0.003 %
Copper	Cu	0.002 %
Zinc	Zn	0.002 %
Strontium	Sr	0.002 %
Chromium	Cr	0.001 %

From the results of EDXRF, the whole plant of *Leucasaspera* consists of macro minerals such as K, Ca, P, S and micro minerals such as Fe, Mn, Cu, Zn and Cr. The rich of potassium and calcium contents of the plant might supplement the K and Ca to a great extent. Roles of these elements in disease curing are well documented.

3.3 Antimicrobial Activities of the Whole Plant of *Leucasaspera*

Antimicrobial activities of crude extract from the whole plant of Pinguhteikpeik were determined by agar

well diffusion method in various solvent systems on six selected organisms. The results are shown in Table3.

Table 3. Results of Antimicrobial Activities on the Whole Plant of Pingu-hteik-peik

Solvent	Inhibition zone Diameter (mm) of various Crude Extracts Against Different Organisms					
	B-subtilis	***S-aureus***	***P-aeruginosa***	***B-pumilus***	***C-albicans***	***E-coli***
n-hexane	11 mm (+)	13 mm (+)	12 mm (+)	13 mm (+)	13 mm (+)	12 mm (+)
$CHCl_3$	12 mm (+)	12 mm (+)	11 mm (+)	11 mm (+)	11 mm (+)	13 mm (+)
EtOAc	16 mm (++)	15 mm (++)	16 mm (++)	15 mm (++)	18 mm (++)	17 mm (++)
EtOH	15 mm (++)	14 mm (+)	15 mm (++)	14 mm (+)	14 mm (+)	15 mm (++)

Organisms	Agar well – 10 mm
Bacillus subtilis	10 mm – 14 mm (+)
Staphylococcus aureus	15 mm – 19 mm (++)
Pseudomonas aeruginosa	20 mm – above (+++)
Bacillus pumilus	
Candida albicans	
Escherichia coli	

As the result of activity test, ethyl acetate extract of this sample responds medium activity on all six selected microorganisms. Both chloroform and n-hexane extract of this sample shows low activities on all six microorganisms. Ethanol extract of this sample shows medium activities on *Bacillus substilis*, *Pseudomonas aeruginosa* and *E-coli*, low activities on *Staphylococcus aureus*, *Bacillus pumilus* and *Candida albicans*. Therefore all the four solvent extracts exhibited a significant antimicrobial activity against all the screened microorganisms.

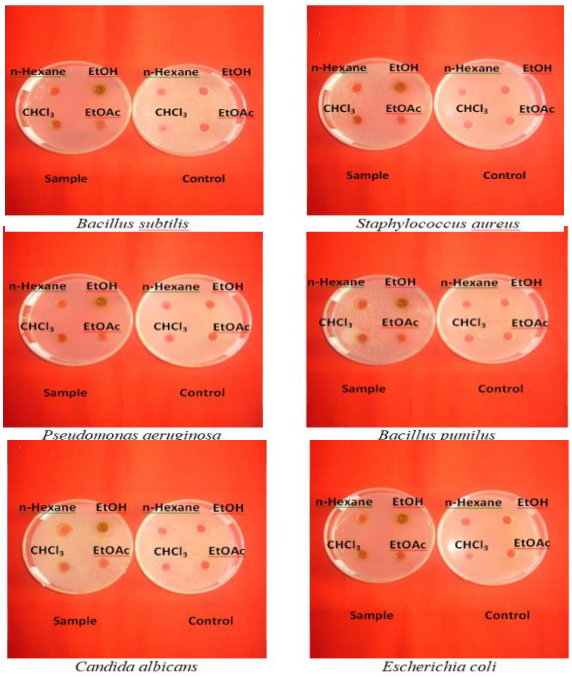

Fig 3. Antimicrobial activities of the whole plant of Pingu hteik peik

3.4 FT IR Assignments of an Isolated compounds

SS-1 and SS-2

The structures oftheisolated compounds were studied by infrared spectra.The series of these spectra were illustrated Figure 4, Figure 5 and FT IR assignments were described in Table 4 and 5.

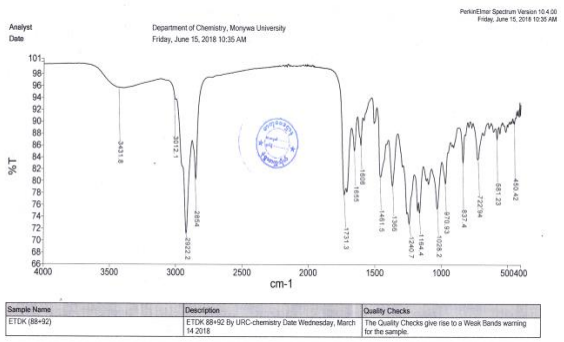

Fig 4. FT IR Spectrum of Isolated Compound SS-1

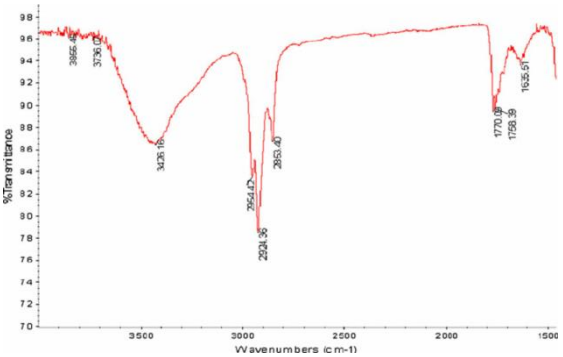

Fig 5 Reference FT IR Spectrum of 5-hydroxy - 4', 7-dimethoxy flavone

Table 4 FTIR Assignments of an IsolatedCompound (SS – 1)

No.	Bands (cm^{-1})	Assignment (Functional group)
1	3431.8	-OH stretching vibration of hydroxyl group
2	3012.1	C-H stretching vibration of sp^2 hydrocarbon
3	2922.2, 2854	Unsymmetrical and symmetrical C-H stretching vibration of sp^3 hydrocarbons
4	1731.3, 1655	C=O stretching vibration of carbonyl group
5	1606	C=C ring skeletal stretching vibration of aromatic benzene ring
6	1461.5, 1366	C-H bending vibration of methylene group
7	1240.7	C-C-O stretching vibration of alcohol group
8	1164.4	C-O-C stretching vibration of ether group
9	1028.2, 970.93	C-H out of plane bending vibration of trans or E alkenic group
10	837.4	C-H out of plane bending vibration of trisubstituted cis or Z alkenic group
11	722.94	C-H out of plane bending vibration of disubstituted cis or Z alkenic group

According to the FT IR spectrum and its assignments, the isolated compound may assume to be 5-hydroxy-4′, 7-dimethoxy flavone. Since authentic this flavone compound is not available, matching of fingerprint region has not yet done. But similar functional groups, same color, same melting point with reference showed the compound (SS-1) should be 5-hydroxy -4′, 7- dimethoxy flavone.

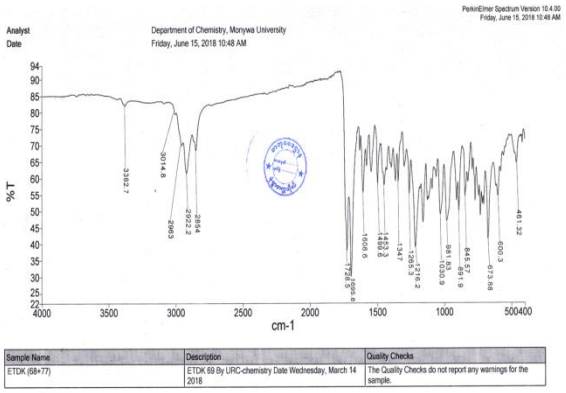

Fig 6 FT IR Spectrum of Unknown Compound SS-2

Table 5 FTIR Assignments of an Unknown Compound (SS-2)

No	Bands (cm^{-1})	Assignment (functional group)
1	3382.7	-OH stretching vibration of alcohol group
2	3014.8	C-H stretching vibration of sp^2 hydrocarbon
3	2922.2, 2854	Unsymmetrical and symmetrical stretching vibration of sp^3 hydrocarbons
4	1728.5, 1695.8	C=O stretching vibration of carbonyl group
5	1608.6	C=C ring skeletal stretching vibration of aromatic benzene ring
6	1453.3	C-H in plane bending vibration of allylic hydrocarbons
7	1347	C-H out of plane bending vibration of gem-dimethyl group
8	1265.3	C-C-O stretching vibration of alcohol group
9	1216.2	C-O-C stretching vibration of ether group
10	1030.9, 981.83	C-H out of plane bending vibration of trans or E alkenic group
11	891.9,845.57	C-H out of plane bending vibration of cis or Z alkenic group

According to FT IR spectrum, -OH stretching vibration, sp^2 hydrocarbon, sp^3 hydrocarbons, C=O carbonyl group, C=C ring skeletal vibration, C-H bending allylic hydrocarbons, gem-dimethyl group, C-C-O alcohol group, C-O-C ether group, trans or E alkenic group and cis or Z alkenic group were found to be present in compound II (SS-2).

4. CONCIUSION

Leucasaspera is well known herb in the Ayurvedic and Modren systems of medicine to cure various disorders. This plant was selected for chemical analysis. Phytochemical tests carried out on the whole plant of *Leucasaspera* show the presence of alkaloids, glycosides, tannins, reducing sugars, saponins, flavonoids, steroids, , polyphenols and α-amino acids.

Moreover, qualitative and quantitative elemental analysis was performed by EDXRF spectrophotometer. The total of twelve elements, K, Ca, Si, P, S, Fe, Ba, Mn, Cu, Zn, Sr and Cr were detected. Among them K, Ca, P, and S are major elements and Si, Fe, Mn, Cu, Zn, Cr and Sr are trace elements. Furthermore antimicrobial activities of the crude extracts of the whole plant of Pingu-hteik-peikwere determined by using agar well diffusion method on six selected organisms. All the solvent extracts exhibit the significant antimicrobial activities against all the screened microorganisms. Among them ethylacetate extract responded the highest activities.

Moreover, the pure organic compounds SS-1 and SS-2 were isolated from ethylacetate extract by modern separation techniques, such as thin layer and column chromatography. The physical state and yield percent of compound (I) were pale yellow crystal and (2.5%) based on ethylacetate crude extract. The crystal of pure compound (I) was confirmed by special test for flavonoids (Alkaline reagent test) and gave positive test. The structure of 5-hydroxy -4′, 7- dimethoxy flavone (SS-1) could be identified by comparing with melting point (183.69ºC) and FTIR spectrum of reference 5-hydroxy -4′, 7-dimethoxy flavone. In addition, amorphous form the compound SS -2 was greenish - yellow and yield percent was 2.3%. The functional group identification of compound II was assigned by FTIR spectroscopic method. In addition this compound II was checked by Salkowski test (steroids test).Therefore, the present result concludes that as this plant sample contains many potent phytochemicals, elemental content and antimicrobial activities, this plant can be used as natural remedy against many diseases.

ACKNOWLEDGEMENTS

We wish to express our deepest thanks to principle, Dr Myint Myint Khaing, Technological University (Pakokku) for her permission to do this research. We are also gratitude to all my teachers, friends and all persons who directly or indirectly contribute towards the successes of this paper.

REFERENCES

[1] Aye.M.N (2014). "Elemental Analysis, Phytochemical Screenings, Antimicrobial Activities and Isolation of Isoquinaline Derivatives and Other Unknown Compound from the Stem of *Cissampelospareira* Linn." Monywa: (M Res Thesis).

[2]Harbon J.B. (1982). "Phytochemical Method, A Guide to Modern Techniques of Plant Analysis, "2nd Edition, Chapman and Hall Ltd. USA.

[3]Ilango.K. S. Ramyu and G. Gopinath.(2008). "Antibacterial Activity of LeucasAsperaSpreng" Department of pharmaceutical chemistry, S.R.M. University, India, 526-530.

[4]John. T.A R. Mata. and J. T. Romeo. (2013). Phytochemistry of Medicinal Plants, Springer Science & Business Media.

[5] Moe.M.S.D.T, (2017)."Investigation of Phytochemical Constituents, Antimicrobial Activities, Isolation and Functional Groups Identification of the Pure and Unknown Compound from the Whole Plants of *PhyllanthusMaderaspatensis* L."Monywa (M Res Thesis).

[6]Silverstein, R. M. *et al*. (1989)."Spectroscopic Identification or Organic Compounds", 6th Edition, John Wiley and Sons, Inc. New York. P.78-126.

[7]Soetan. K. O., C.O.Olaiya, and O.E.Oyewole. (2010). "The Importance of Mineral Elements for Humans, Domestic Animals and Plants: A Review", Nigeria, *African Journal of Food Science*, **4**(5), 200-22.

[8]Srinivasan.R. (2011). "International Journal of pharma and Bio Sciences", *LeucasAspera*-Medicinal plant: A Review", Andhra pradash, India-2. 153-159.

[9]Yashvanth.S. and S.S. Madhavendra.(2013). "Micro Chemical (Elemental) Analysis of *Leucasaspera* (Willd) SprengEmploying SEM-EDAX", *International Journal of Pharmaceutical Sciences and Drug Research*, Andhra pradesh, India.**5**(1): 32-35.

SHELF LIFE EXTENSION OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL) BY GAMMA IRRADIATION

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ABSTRACT: In this work, tomatoes were harvested from Kyar Paing Village, Monywa Township, Sagaing Region. After harvested, these samples were irradiated with different doses (0.25, 0.50, 0.75 and 1.00 kGy) of the gamma ray by using Co-60 gamma source. The non-irradiated sample (control, 0 kGy) was used as comparative study. Induced radioactivity of γ-irradiated tomato samples with different doses were monitored by using NaI (Tl) Scintillation Detector. The shelf life of non-irradiated and irradiated samples were studied by assessing postharvest changes (color, ripening) at room temperature. The nutritional values of non-irradiated and γ-irradiated sample with 0.75 kGy dose were also studied. From these studies, it was observed that 0.75 kGy dose gamma irradiation has the longest shelf life and no significantly change on macronutrients (fat and fiber). Moreover, it was occurred that all gamma irradiated samples have no distinct activity above background. Thus, this work was found that 0.75 kGy dose of γ-irradiation was the most effective dose on the shelf life extension of tomato.

KEYWORDS: *tomato, Co-60, shelf life, γ-irradiated*

1. INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most important vegetables worldwide. As it is a relatively short duration crop and gives a high yield, it is economically attractive and the area under cultivation is increasing daily. Tomato belongs to the Solanaceae family. Tomato is an annual plant, which can reach a height of over two meters (Shankara, *et al.*, 2005).[1]

The postharvest losses in terms of quality and quantity of food are major problem all over the world. There are many processing methods that have been developed to help prevent food spoilage and improve safety. Extending the shelf life of tomatoes is very important for domestic and export marketing (Ullah, 2009).[2]

Irradiation is one of the few food technologies that can maintain food quality and address food safety and security problems without significantly affecting a food's sensory or nutritional attributes (IAEA, 2015).[3]

Cobalt-60 is the most commonly used radioactive source for industrial radiation processing including food irradiation Cobalt-60 is produced in a nuclear reactor via neutron bombardment of highly refined cobalt-59 (^{59}Co) pellets, while cesium-137 is produced as a result of uranium fission. Co-60 is a man-made radionuclide and half-life of 5.26 years. So the activity decays by 12.35% per year (Christopher, *et al*., 2006).[4]

Irradiation treatments do not change the nutritional quality of foods any more than do other methods of food processing such as cooking, freezing or canning. Irradiation does not make food radioactive (Loaharanu, 2003).[5]

The tomato used in this work is classified as follow:

Kingdom - Plantae
Family - Solanaceae
Genus - Lycopersicon
Species - esculentum
Botanical name - *Lycopersicon esculentum* Mill
English name - Tomato

Myanmar name - Kha-yan-gyin
Parts used - Fruits

Fig.1 Cultivation of tomato

2. MATERIALS AND METHODS

Firstly, the tomatoes were harvested from Kyar Paing Village, Monywa Township, Saging Region. And then, the tomatoes were immediately transported to the Department of Atomic Energy, Ministry of Education for irradiation. The period between harvesting and irradiation was approximately (24) hours. The sample was treated with four different doses of gamma radiation (0.25, 0.50, 0.75 and 1.00 kGy) from Co-60 source (Co-60 gamma chamber 5000) which has dose rate 1.22 kGy/h.

Induced radioactivity of γ-irradiated tomato samples with different doses were monitored by using NaI (Tl) Scintillation Detector (LUDLUM MODEL 730) in Nuclear Chemistry Laboratory at University of Yangon. The shelf life of non-irradiated and irradiated samples were studied by assessing postharvest changes (color, ripening) at room temperature. Effects of gamma irradiation on nutritional qualities of tomato (moisture, ash, protein, fat, fiber and carbohydrate) were studied by using analytical methods.

Fig.2 Irradiation of tomato by gamma radiation

3. RESULTS AND DISCUSSIONS

In this study, the tomato (*Lycopersicon esculentum* Mill) samples were collected from Kyar Paing Village, Monywa Township, Sagaing Region. Firstly, these samples were irradiated with different doses (0.25, 0.50, 0.75 and 1.00 kGy) of the gamma ray by using Co-60 gamma source. Non-irradiated tomato sample (control) in a similar way was done on other irradiation cases for check. After γ- irradiated with different doses (0.25, 0.50, 0.75 and 1.00 kGy), the induced activity of each irradiated sample were monitored by NaI (Tl) Scintillation Detector. This monitoring indicated that there was no distinctive activity from the background (Table 1). Therefore,γ-irradiated tomatoes can be handled, stored, cooked and consumed in the same way as non-irradiated fruits. Table 2 shows the shelf life of non-irradiated and γ-irradiated tomatoes of different doses at room temperature. From all of these results, 0.75 kGy dose of γ-irradiated sample has longest shelf life.

The nutritional values of non-irradiated and γ-irradiated tomatoes of 0.75 kGy dose are shown in Table 3 and Figure 4. According to these results, some nutrient (moisture, ash, and protein) contents of 0.75 kGy γ-irradiated samples are slightly increased and carbohydrate content of that sample is slightly decreased than that of non-irradiated sample. Fat and fiber contents of 0.75 kGy γ-irradiated sample was no significantly change from non-irradiated sample. However, these changes were occurred no special nutritional problems in food.

Table 1 Monitoring of Induced Activity in Gamma Irradiated Tomatoes of Different Doses

No	Samples	Induced activity relative to background (%) (cp 300s)
1	TG 0.25	+1.01
2	TG 0.50	+1.45
3	TG 0.75	- 0.32
4	TG 1.00	-0.74

± = due to fluctuation

TG 0.25 = tomatoes with 0.25 kGy gamma dose

TG 0.50 = tomatoes with 0.50 kGy gamma dose

TG 0.75 = tomatoes with 0.75 kGy gamma dose

TG 1.00 = tomatoes with 1.00 kGy gamma dose

Table.2 Shelf Life of Non-irradiated and γ-Irradiated Tomatoes of Different Doses at Room Temperature

Storage period (days)	**Tomato ripening (%)**				
	TG 0	**TG 0.25**	**TG 0.50**	**TG 0.75**	**TG 1.00**
1	0	0	0	0	0
5	0	0	0	0	0
10	9.52	4.76	0	4.76	4.76
15	33.32	9.52	19.04	14.28	14.28
20	66.64	28.56	33.32	23.80	38.08
25	100	61.88	52.36	42.84	66.64
30	Nd	100	80.92	71.40	90.44
35	Nd	Nd	100	85.68	100
40	Nd	Nd	Nd	100	Nd

TG 0 = tomatoes with 0 kGy gamma dose

Nd = Not Detected

TG 0 TG 0.25 TG 0.50 TG 0.75 TG 1.00

Fig.3 Postharvest change of non-irradiated and γ-irradiated tomatoes with different doses during storage at 25 days

Table 3. Nutritional Values of Non-irradiated and γ-Irradiated Tomatoes

No.	Nutritional parameters (%) DW	Samples	
		TG 0	TG 0.75
1	Moisture	93.99	94.55
2	Ash	0.45	0.51
3	Protein	0.81	1.12
4	Fiber	0.84	0.85
5	Fat	0.13	0.10
6	Carbohydrate	3.78	2.87
7	Energy value (kcal/100g)	19.53	16.86

DW = Dry Weight

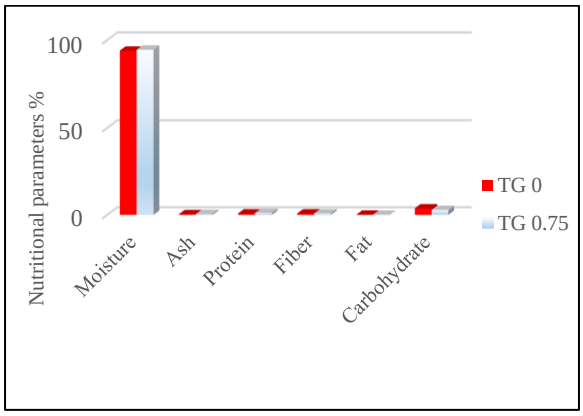

Fig.4 A bar graph of nutritional values of non- irradiated and γ-irradiated tomatoes with 0.75 kGy dose

4. CONCLUSION

In this work, effects of different doses of gamma irradiation on the shelf life extension of tomatoes were studied. The shelf life of γ-irradiated samples with different doses (0.25, 0.50, 0.75 and 1.00 kGy) can be extended than that of non-irradiated sample (5-15 days). Therefore, it was observed that effect of gamma irradiation can prolong the ripening time of tomatoes. Among them, it can be seen that 0.75 kGy dose gave longer shelf life. According to the monitoring of gamma radiation in the irradiated samples, these irradiation processes cannot cause sample to be radioactive. And then, it was also found that some nutrient (moisture, ash, and protein) contents of 0.75 kGy γ-irradiated samples are slightly increased and carbohydrate content of that sample is slightly decreased than that of non-irradiated sample. Besides, fat and fiber contents of 0.75 kGy γ-irradiated sample was no significantly change from non-irradiated sample. However, these changes were occurred no special nutritional problems in food. From these overall results, gamma irradiation process promotes the shelf life of tomato samples. Among them, gamma radiation with 0.75 kGy dose in postharvest stage of tomato is effective in maintaining a fresh product appearance with improving shelf life.

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REFERENCES

[1] N.Shankara, J. L. Jeude, M. Goffau, M. Halmi and B. Dam, "*Cultivation of Tomato*," Netherland:PROTAFoundation,Wageningen/Backhuys Publishers, 2005, 6-8

[2] J. Ullah, "*Storage of Fresh Tomatoes to Determine the Level of ($CaCl_2$) Coating and Optimum Temperature for Extended Shelf Life*,"Department of Food Science and Technology, Pakiston: NWFP Agricultural University, 2009, 4-10

[3] IAEA. "Manual of Good Practice in Food Irradiation," *Technical Reports Series*, 2015, **481**, 1-5

[4] H. S. Christopher, and X. Fan, "*Food Irradiation Research and Technology*," USA: Blackwell Publishing, IFT Press Book, 2006, 21-22

[5] P. M. S. Loaharanu, "*Irradiated Food*," New York: American Council of Science and Health, Inc., 2003,1-4

Studies on the Chemical Constituents of Essential Oil (Linalool and Skatole) From *Jasminum Auriculatum* Vahl. (Zun-Pan)

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ABSTRACT: This research paper includes extraction of essential oil from the *Jasminum auriculatum* Vahl, Myanmar name "Zun-pan", which belongs to Oleaceae family. Zun-pan were collected from Mingun, Sagaing Region, Myanmar. In this research work, the essential oil was extracted from the fresh Zun-pan by using steam distillation method. The yield of essential oil is 0.38 % based on the sample weight. The phytochemical screening has been done and it gave rise to positive for alkaloids, flavonoids, glycosides, phenolics, polyphenols, sugars, terpenes and lipophilic groups. Linalool and skatole were isolated from essential oil by column and thin layer chromatographic techniques. Then their structures were identified by FT-IR method

KEYWORDS *column chromatography, FT-IR method, steam distillation method*

1. INTRODUCTION

Medicinal plants have been used by human beings since ages in traditional medicine due to their potential. The research on medicinal plants has led to the discovery of novel drug used against diverse diseases. According to the World Health Organization's report that nearly 65-80 % of world's population in developing countries depends on the traditional medicine for their primary health care and treatment of ailments. The reason for wide acceptance of herbal medicine are of their being comparatively less expensive, lesser side effects and being natural in origin and hence socially and culturally acceptable (Muniappan Ayyanar et al., 2011).

Jasminum auriculatum Vahl, is a species of jasmine, in the family Oleaceae. It is native to southern and south eastern Asia and distributed and cultivated more or less throughout South India, Shri Lanka, Pakistan, Nepal, Malaysia, Indonesia and Australia. (M.Almeida, 1998).

Jasminum auriculatum is an evergreen shrub and it is used in traditional medicines. It is commercially cultivated for its fragrant flowers mainly in Nepal, E. Asia, Thailand. (Sing Baljinder, et al). Highly used in development of *Jasminum auriculatum* perfumes and jasmine creams. The flowers are worn by women in their hair in Southern and Southeast Asia. It is also used for making cosmetic and string in funerals. So they are also cultivated on a commercial scale in Myanmar.

The chemical constituents of *Jasminum auriculatum* are indole, benzyl alcohol, linalool and skatole. Linalool is one of the constituents of perfumery importance present in bud.

The main purpose of this research is to isolate linalool and skatole from distillation method and column chromatography. The structure of these compounds was determined by FT-IR spectroscopic method.

2. LITERATURE SURVEY

2.1 Botanical description

Family name -Oleaceae
Botanical name -*Jasminum auriculatum* Vahl.
Genus - Jasminum- jasmine
English name - Nil
Myanmar name - Zun-pan
Part used - Flowers (R.P Sing, 1982)

Fig 1. The flowers of *Jasminum auriculatum*

Fig 2. The plant of *Jasminum auriculatum*

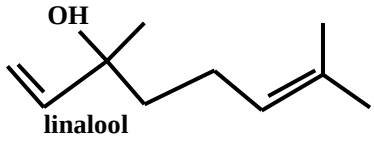
2.2. Medicinal uses

Roots, leaves and flowers of *Jasminum auriculatum* are widely used to cure a number of diseases. The roots are useful in skin diseases especially for ring-worm. Flowers are fragrant but they are useful in burning sensation. Leaves, roots and flowers are also useful in stomatopathy, antiseptic, emollient, anthelmintic, ulcers, leprosy, skin diseases and wounds (Muniappan Ayyanar et al., 2011).

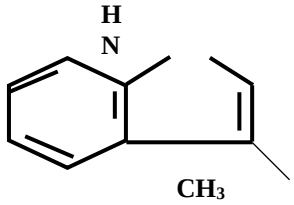
2.3 Chemical constituents of *Jasminum auriculatum*

The chemical constituents of *Jasminum auriculatum* flowers are indole, benzyl benzoate, methyl anthranilate, benzyl alcohol, linalool, skatole.

2.4 Structure of linalool and Skatole



linalool



skatole

2.5 Uses of linalool and skatole

Linalool is used as a scent in 60-80 % of perfumed hygiene products and cleaning four agents including soaps, detergents, shampoos and lotions. It is also used as a chemical intermediate. One common product is linalool is Vitamin E. Additionally, linalool is used by pest professionals as a flea and cockroach insecticide.

Skatole or 3-methylindole is a mildly toxic white crystalline organic compound belonging to the indole family. It is used as a fragrance and fixative in many perfumes and as an aroma compound (E pichersky., RA Raguso,1999)

3. EXPERIMENT

3.1 Material and methods

Commercially available reagents and solvents were used in this research work. Before using the solvent were purified by simple distillation. Analytical preparative thin layer chromatography was performed by using percolated alumina plates (Merck Co. Inc, Kiesilgel 60 F_{256}) visualization was taken via UV and iodine vapor. Silica gel (Merck Co. Inc, Kiesilgel 60F_{256}, 70-230 mesh-ASTM) was used for column chromatography.

3.2 Sample collection

Jasminum auriculatum (Zun-pan) flowers were collected from Mingun, Sagaing Region, Myanmar.

3.3 Preliminary phytochemical Test

Phytochemical tests were carried out to detect the presence of organic constituents in the flowers (Harbone JB, 1973). The result of phytochemical constituents of *Jasminum auriculatum* as shown in Table 2.

3.4 Extraction of essential oil by steam distillation method

Fig 3. Steam distillation apparatus

The essential oil is extracted by steam distillation methods. The above experiment was repeated four times, each time by using (400) g of sample together with 4 dm^3 of distilled water. The percent yield (w/w) of essential oil were shown in Table 1.

Table 1. Yield of oil content

Number of experiment	Weight of sample (g)	Weight of oil (g)	Yield in %
1.	400	1.5	0.38 %
2.	400	1.6	0.40%
3.	400	1.52	0.38%
4.	400	1.53	0.38%

Average percent of essential oil = 0.38 %

3.5 The Study of essential oil by TLC method

The extracted essential oil was subjected to thin layer chromatography using pet-ether (60-80 °C) and ethyl acetate (9:1) solvent systems. The sample was spotted on the plate at the distance 2.1cm from the base line. After spotting, the plate was dried and run in the chosen solvent system. This dried was developed in the iodine vapor. More than one spot was observed. Therefore, it was separated by column chromatography.

3.6 Column separation of extracted essential oil

Fig 4. Separation of crude extract by column chromatography

3.6 Procedure of column chromatography

The crude oil (1.2) g was separated by column chromatography as shown in figure (4). The column was vertically clamped and filled with a small amount of pet-ether. A small pieces of cotton wool was inserted and tamped at the bottom of the column to prevent the packing materials from falling down. The silica gel slurry prepared by mixing 15g of gel and 25 ml of pet-

ether was introduced into the column. The external wall of column was tapped with the small rubber tube to remove air bubbles that was trapped in a column. Care must be taken not to dry the adsorbent while the experimental was carried out.

When the solvent level in the column had just reached to the adsorbent, the tap was closed and the concentrated solution of the crude oil that was prepared by dissolving (1.20) g of crude oil in minimum volume of pet-ether was introduced along the walls of the column by using a micropipette.

When the sample had reached to the adsorbent, about 3g of purified sand was added on the solute to obtain about 1 cm thickness of sand layer. Pet- ether was opened and into the column. Then the tap was opened and adjusted the flow rate. As the layer began to separate, a small poured and dried bottle was placed under the tap of the column to collect the about 3ml per bottle.

After separation, 100 fractions were collected. Each fraction obtained by column chromatography was checked by thin layer chromatography. The fractions of the same R_f value were combined. Solvent systems employed in this chromatography are –

(i) pet-ether: EtOAc (9:1, v/v)

(ii) pet-ether: EtOAc (4:1, v/v)

3.7 Thin layer chromatogram of combined fractions

According to the thin layer chromatogram eight combined fractions were obtained. Among them, fractions V and VIII gave only a single spot on TLC plates.

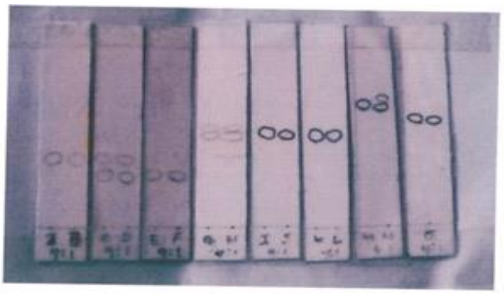


Fig 5. Thin layer chromatography

According to thin layer chromatography, combined fraction (V and VIII) were designates wwk-1 and wwk-2. The physical state of pure wwk-1 is a colorless oily form and wwk -2 is a white crystal.

3.8 Determination of melting point of compound wwk-2

A small quantity of crystal was inserted into a capillary tube and the tube was attached to the thermometer. It was then inserted into the tube containing liquid (paraffin). After the tube was gently heated, the crystal in the capillary tube was melted at 95-97 °C. Its melting point was nearly identical with that at authentic compound (mp 95°C). Thus, the crystal is estimated as skatole.

4. RESULTS AND DISCUSSION

4.1 Preliminary phytochemical screening of *Jasminum auriculatum*

From the experimental results, the flowers of *Jasminum auricualtum* consist of alkaloids, flavonoids, glycosides, lipophilics, phenolics, polyphenols, sugars and terpenes. However, saponins and proteins were not detected.

Table (2) The result of phytochemical tests

No.	Test	Reagent	Observa-tion	Res-ult
1	alkaloid	Dragendroff's solution	orange ppt	+
2.	flavonoid	Conc:HCl and Mg	pink	+
3.	glycoside	10% lead acetate	yellow ppt	+
4.	lipophilic	0.5M KOH	deep orange	+
5.	phenolic	1% $FeCl_3$ and EtOH	Greenish brown	+
6.	polyphenol	10% $FeCl_3$	blue	+
7.	sugar	Benedict' solution	green	+
8.	terpene	Acetic anhydride, Conc:H_2SO_4 and $CHCL_3$	red	+
9.	saponin	Conc: H_2SO_4	-	-
10.	protein	NaOH and $CuSO_4$ solution	-	-

(+) = presence (-) = absence

4.2 FT-IR spectroscopic determination for isolated compound wwk-1

The FT-IR spectrum of isolated compound wwk-1 is shown in fig 6 and results of described in Table 3.

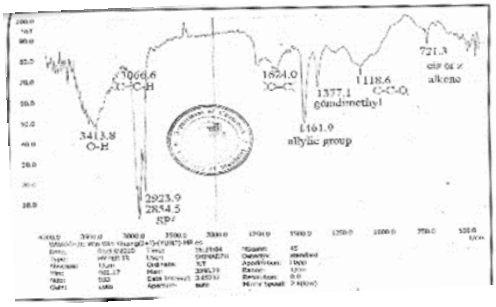


Fig 6. FT-IR spectrum of compound wwk-1

Table 3. The absorption peak and their assignments of compound wwk-1

No.	Wave No. (cm^{-1})	Assignments(functional group)
1	3413	O-H stretching vibration of alcohol group
2	3066	C-H stretching vibration of alkenic group
3	2923,2854	Symmetrical and unsymmetrical stretching vibration of sp^3 hydrocarbon
4	1624	C=C stretching vibration of alkenic group
5	1461	C-H in plane bending of allylic group
6	1377	Gem dimethyl group
7	1118	C-C-O stretching vibration
8	721	C-H out of plane bending vibration of cis or z alkene

According to the above spectrum data the compound wwk-1 may be linalool.

4.3 FT-IR Spectroscopic Determination for Isolated Compound wwk-2

The FT-IR spectrum of isolated compound wwk-2 is shown in fig 7 and results of described in Table 4.

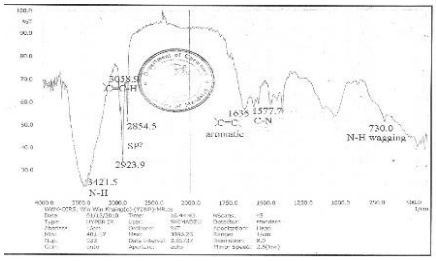


Fig 7. FT-IR spectrum of compound wwk-2

Table 4. The absorption peak and their assignments of compound wwk-2

No.	Wave No.(cm^{-1})	Assignments(functional group)
1.	3421	C-H stretching vibration
2.	3058	C-H stretching vibration of alkenic group
3.	2923,2854	Symmetrical and unsymmetrical stretching vibration of sp3 hydrocarbon
4.	1635	C=C stretching vibration of aromatic ring
5.	1577	C-N stretching vibration
6.	730	N-H wagging vibration

According to this spectrum data, the compound wwk-2 may be skatole.

5. CONCLUSIONS

The main task of this research work is chemical investigation of essential oil from *Jasminum auriculatum* Vahl, locally called "Zun-pan ". In this research work, the Jasmine absolute oil is produced by using steam distillation method. The yield percentage of essential oil is 0.38%. The phytochemical screening of *Jasminum auriculatum* show the presence of alkaloids, flavoids, glycosides, phenolics, polyphenols, sugars and terpenes, and lipophilic groups. From the essential oil of *Jasminum auriculatum*, two compounds were isolated by thin layer and column chromatography. The structures of these two compounds were determined by FT-IR spectra.

From the resulting functional groups of FT-IR spectrum, the isolated compound wwk-1 may be estimated as "Linalool". The R_f value of compound wwk -1 is 0.44. In literature, the R_f value of linalool is 0.39. Therefore, the compound wwk-1 may also be linalool. The yield percent of linalool in this research was found to be 1.5% on the basic of the amount of essential oil.

Furthermore, the other related compound wwk-2 was also assigned by FT-IR spectrum. The melting point of this compound was done by usual capillary tube method giving rise to (95-97 °C) is nearly identical with the melting point of skatole (95 °C). Therefore, the compound may be skatole. The yield percent of skatole was found to be 0.35%.

Finally, Linalool is oil possessing a pleasant oduor. It is one of the most important essential oil, used widely in perfume, cosmetic soap and flavor industries. Therefore, the *Jasminum auriculatum* Vahl supplies the most important and indispensable natural flower oil employed in modern perfumery.

ACKNOWLEDGEMENT

I would like to express my gratitude to Dr Saw Pyone Naing, Rector, Sagaing University of Education, and Dr Myat Myat Thaw, Pro-rector, Sagaing University of Education for their permission, help suggestions, and encouragements. We are also extremely grateful to Dr Kathy Myint Thu, Professor and Head, Department of Chemistry, Sagaing University of Education, for her valuable guidance, advice and kindness on this study.

REFERENCES

[1] E. Pichersky, RA., Raguso A day in the life of a linalool molecule: chemical communication in a plant–pollinator system. Part 1: linalool biosynthesis in flowering plants. Plant Species Biol, 1999, pp.14:95-120.

[2] J.B., Harbone, "*Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*". Chapman and Hall Ltd, U.S.A, 1982.

[3] M. Almeida. Flora of Maharashtra, Orient Press, Mumbai., 1998; pp.1-5.

[4] M., Ayyanara, S., Ignacimuthu. Ethnobotanical survey of medicinal plants commonly used by Kani

tribals in Tirunelveli hills of Western Ghats of India. Journal of Ethnopharmamology 2011; pp.138:851-864.

[5] "Merck Index", 1983. 10th Edition, Merck & Co. Inc USA.

[6] R.P Sing, T.A More The production and perfume potential of jasminum collections. Indian perfume. 26, 1982, pp.156-159.

[7] Sing Baljinder, et al. Antimicrobial potential of poly herbo-mimeral formulation Jatvadi Taila- Taila-A Review Int.J of Research in Ayrveda and Pharmacy''. 2(1),2011, pp151-156.2

Seasonal Variation in Water Quality of Shan Ywar and Nan Kyin Creeks in Myitkyina Township, Kachin State

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ABSTRACT: In this research work, physicochemical properties have been made to assess the quality of water samples obtained from Shan Ywar and Nan Kyin creeks in Myitkyina Township, Kachin State. The experimental works have been done in physical examination, determination of chemical constituents and elemental analysis. Furthermore, seasonal variation of microbiological organisms in creek water was investigated. In addition, the some toxic metals were also determined by using Atomic Absorption Spectrophotometer. The water samples from the studied areas were seasonally detected by using conventional methods and modern instrumental techniques. Most of the experimental data obtained in this investigation are WHO guideline limits for drinking water.

KEYWORDS: Creek water, physicochemical properties, bacteriological parameters

1. INTRODUCTION

Water is essential for plant and animal life. Water is the most abundant and the most important liquid on earth. The uses of water increase with increase in population. Some of water is diverted to irrigation for agriculture. Drinking water is essential to humans and other life forms even through it provide no calories or organic nutrients. This water storage is important, since clean, fresh water is essential to human and other land-based life (Jamie and Richard, 1993).

Water quality and suitability for use are determined by its taste, odor, color, and concentration of organic and inorganic matters. Contaminants in the water can affect the water quality and consequently the human health (Dissmeyer, 2000).

The main purpose of this research is to assess the quality of creek water from two sampling sites. It is located Myitkyina Township in Kachin State. In the present research work, seasonal variation of physical and chemical properties of the creek water was thoroughly investigated. Examination of bacteriological parameters and determination of metals and toxic chemical substances were also being carried out. The results are shown in Tables and compared with the drinking water standards proposed by WHO standard.

2. MATERIALS AND METHODS

In this research, all the investigations, methods, techniques and analyses were carried out by standard recommended procedures (Sadhana & Anand, 2014) and (Vogel, 1968) and specific catalogues. Instruments employed in this work consist of glassware, labware and other supporting facilities mentioned in each experimental section.

2.1 Sample Collection

The creek water samples were seasonally collected during cold season (the period of January), hot season (the period of May) and rainy season (the period of July) in 2019.

The creek water samples were collected from two different study sites (Shan Ywar, and Nan Kyin) in Myitkyina Township (Fig 1 and 2). Shan Ywar creek located in south-west of Myitkyina. It is far about 4 miles from Myitkyina downtown. Nan Kyin creek is situated north-west of Myitkyina, and about 4 miles from Myitkyina downtown.

Fig 1. Location map and sampling site of Shan Ywar creek in Myitkyina Township

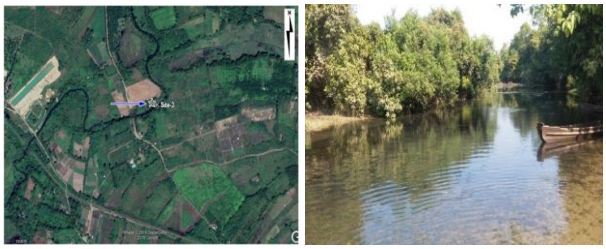

Fig 2. Location map and sampling site of Nan Kyin creek in Myitkyina Township

2.2 Physicochemical Analysis of Creek Water

Some physicochemical parameters were analyzed in this paper including pH by the digital pH meter, electrical conductivity by a conductivity meter, turbidity by absorptometric method, total dissolved solid and total alkalinity by acid-base titrimetric method, total hardness by EDTA titrimetric method, sulphate by iodometric method, dissolved oxygen and biochemical oxygen demand by incubating method and chemical oxygen demand by permanganate titration method.

2.3 Determination of Heavy Metals

The heavy metals of samples were determined by Atomic Absorption Spectrophotometer at Water and Soil Examination Laboratory, Department of Fisheries, Aquaculture Division, Freshwater Aquaculture Research, Ministry of Agriculture, Livestock and Irrigation, Yangon.

2.4 Bacteriological Examination

The *Coliform and Escherichia Coli* (*E.coli*) of water samples were tested at Public Health Laboratory, Mandalay.

3. RESULTS AND DISCUSSION

The physicochemical properties, contents of heavy metals and microbiological indicators in creek water from sampling site-1 (Shan Ywar creek) and sampling site-2 (Nan Kyin creek) were recorded in Tables and corresponding histograms are shown in Figures.

Table 1. Results of pH, temperature and turbidity values in creek water from two sampling sites

Parameters	Site-1			Site-2			WHO Standard (2016)
	Cold Season	Hot Season	Rainy Season	Cold Season	Hot Season	Rainy Season	
pH (scale)	7.7	7.9	6.9	7.49	7.79	7.12	6.5-8.5
Temperature (ºC)	21.73	28.49	28.69	18.94	30.19	28.37	-
Turbidity (NTU)	17.8	16.5	48.1	6.25	7.47	25.01	< 5

According to this table, the pH values of sampling sites 1 and 2 were within the acceptable limit. Among three seasons, the highest value of pH was observed in hot season and the lowest value of pH was found in rainy season. The pH is influenced by acidity of the bottom sediment and biological activities.

The temperature values of two sampling sites were observed the range of 18.94°C and 21.73°C in cold season, 28.49°C and 30.19°C in hot season and 28.37°C and 28.69°C in rainy season. As water temperature increases, the rate of chemical reactions generally increased together with the evaporation and volatilization of substance from the water.

The observed values of turbidity in two sampling sites were above the permissible value. Turbidity in water is caused by the presence of suspended matter such as clay, silts, finely divided organic and inorganic matter, plankton and other microscopic organisms.

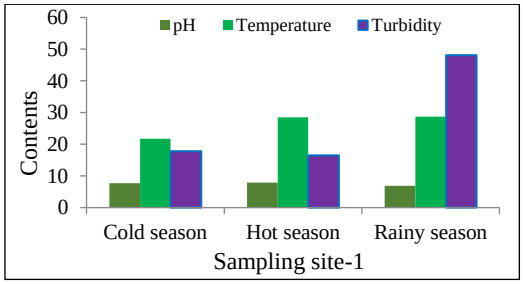

Fig 3. Seasonal variations of pH, temperature and turbidity in the water bodies of sampling site-1 (Shan Ywar creek)

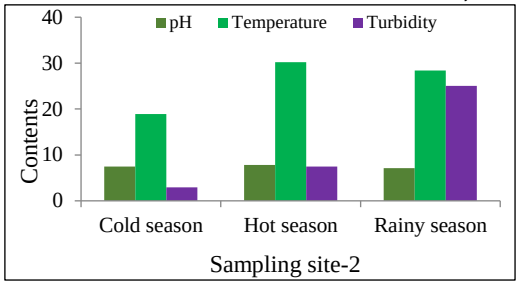

Fig 4. Seasonal variations of pH, temperature and turbidity in the water bodies of sampling site-2 (Nan Kyin creek)

Table 2. Conductivity, sulphate and nitrogen nitrate values in creek water from two sampling sites

Parameters	Site-1			Site-2			WHO Standard (2016)
	Cold Season	Hot Season	Rainy Season	Cold Season	Hot Season	Rainy Season	
Conductivity (µmho/cm)	0.175	0.186	0.069	0.083	0.097	0.022	250
Sulphate (ppm)	0.052	0.162	0.123	0.015	0.155	0.125	400
Nitrogen-nitrate (ppm)	0.250	1.20	1.02	0.188	1.12	1.05	250

According to results, the observed values of conductivity in two sampling sites were lower than permissible value. The conductivity of a solution of water is highly dependent on its concentration of dissolved salts and sometimes other chemical species which tend to ionize in the solution. The values of alkalinity and total dissolved solids can be correlated to conductivity.

Sulphate contents were below than the permissible value in sites 1 and 2. Sulphate is widely distributed in nature and may be present in natural waters in concentrations ranging from a few to several thousand mg/L. Most of the sulphate compounds are readily soluble in water but calcium sulphite is the least soluble.

The observed values of nitrogen nitrate were lower than standard WHO value in two sampling sites. Lower nitrates were due to decrease in degradation of organic matter by microbial activities. Nitrogen is essential for plant and animal growth and nourishment, but the overabundance of certain nutrients in water can cause a number of adverse health and ecological effects.

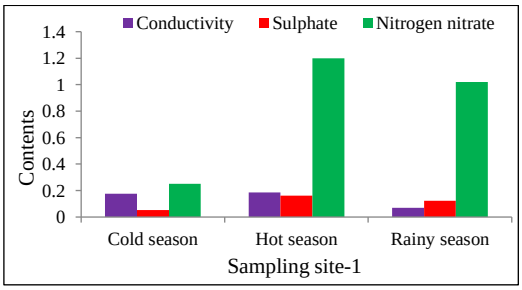

Fig 5. Seasonal variations of conductivity, sulphate and nitrogen nitrate in the water bodies of sampling site-1 (Shan Ywar creek)

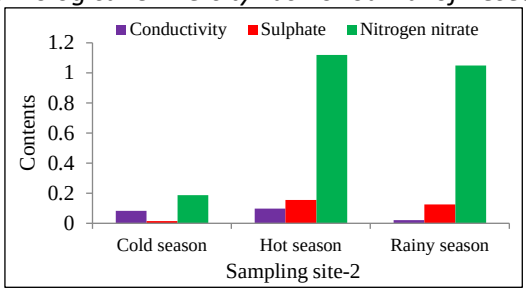

Fig 6. Seasonal variations of conductivity, sulphate and nitrogen nitrate in the water bodies of sampling site-2 (Nan Kyin creek)

Table 3. Total alkalinity, total hardness and TDS values in creek water from two sampling sites

Parameters (ppm)	Site-1			Site-2			WHO Standard (2016)
	Cold Season	Hot Season	Rainy Season	Cold Season	Hot Season	Rainy Season	
Total alkalinity	40	55	48	26	40	42	950
Total hardness	250	280	210	70	110	98	500
TDS	113	121	45	54	63	13	500

From the results, the observed values of total alkalinity were below than standard WHO value in two sampling sites. In the seasonal variation of total alkalinity contents, the low total alkalinity values were found in cold season indicating that they have low buffering capacity. Alkalinity results due to the presence of carbonate, bicarbonate and hydroxides of the alkaline earth metal.

The observed values of total hardness were lower than standard WHO value in two sampling sites. Hardness is a measure of mainly a quality of calcium and magnesium.

The total dissolved solid (TDS) values of two sampling sites were lower than the permissible value. The highest value of TDS was observed in hot season than the other seasons. The sources of TDS are clay minerals and soil, plant fibers, dead vegetation and animals and domestic sewage. TDS indicate the presence of filterable residue in water.

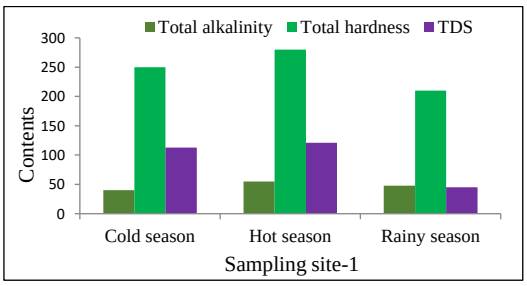

Fig 7. Seasonal variations of total alkalinity, total hardness and TDS in the water bodies of sampling site-1 (Shan Ywar creek)

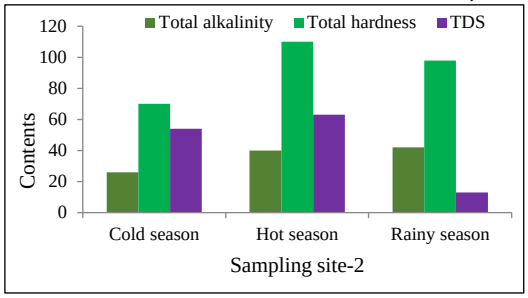

Fig 8. Seasonal variations of total alkalinity, total hardness and TDS in the water bodies of sampling site-2 (Nan Kyin creek)

Table 4. Results of heavy metal concentration in creek water from two sampling sites

Parameters (ppm)	Site-1			Site-2			WHO Standard (2016)
	Cold Season	Hot Season	Rainy Season	Cold Season	Hot Season	Rainy Season	
Arsenic	0.002	0.0002	0.0003	0.003	0.0002	0.0001	0.01
Lead	0.001	0.002	0.002	0.002	0.003	0.001	0.01
Mercury	0.0557	0.0463	0.0132	0.0385	0.0347	0.0098	0.002

According to this table, arsenic contents in water samples from two sampling sites were lower than the permissible value. Arsenic has been used as a human poison since ancient times. The most prominent use of arsenic is in pesticides.

The values of lead were below than the WHO permissible value in water samples from two sampling sites. Lead comes from deposition of exhaust from vehicles in the atmosphere, batteries and waste from lead ore mines, lead smelters and sewage discharge.

The observed values of mercury in two sampling sites were higher than the permissible value. The highest value of mercury was observed in cold season than the other seasons in site 1 and 2. Mercury is the reagent of choice for gold mining in Kachin State. It is relatively inexpensive. Gold mining is carried out on a large scale in Kachin State; much of the mercury used is brought from China. Mercury is a naturally occurring element that is found in air, water and soil. The toxic effects of mercury depend on its chemical form and the route of exposure. Mercury exposure at high levels can harm the brain, heart, kidneys, lungs, and immune system of people of all ages.

Table 5. Results of elemental analysis in creek water from two sampling sites

Parameters (ppm)	Site-1			Site-2			WHO Standard (2016)
	Cold Season	Hot Season	Rainy Season	Cold Season	Hot Season	Rainy Season	
Calcium	42.05	45	25	40.85	45	38	200
Magnesium	207.94	210.23	96.04	29.15	32.35	20.45	150
Potassium	20	22	12	20	34	25	100
Sodium	76	55	25	25	35	20	200
Chloride	29.994	99.99	49.99	29.994	99.98	49.99	600

Cadmium	0.0003	0.0005	0.0002	0.0002	0.0005	0.0001	0.005
Chromium	0.01	0.0001	0.0002	0.02	0.0002	0.0002	0.05
Copper	0.05	0.005	0.002	0.06	0.005	0.002	0.2
Iron	0.27	0.23	0.0002	0.25	0.21	0.0002	1.0
Manganese	0.01	0.003	0.0001	0.03	0.002	0.0001	0.2

According to results, the observed values of calcium were lower than standard WHO value in two sampling sites. Calcium which decreases the toxicity of other ions is a major mineral constituent of the body. The calcium of the diet is prime important to the growth and development of the skeleton. Calcium is widely distributed in foods. Calcium and magnesium are essential elements for all life forms.

The highest values of magnesium were observed at sampling site-1 (Shan Ywar) and the lowest values of magnesium were observed at sampling site-2 (Nan Kyin). Magnesium is needed by all living cells as well it is the second last dominant cation next to potassium. Excessive magnesium intake can be lethal, especially in children and an aesthesia.

The potassium and sodium values in two sampling sites were below than WHO standard value. Potassium is an important in regulating the body fluid volume. It is associated with sodium in promoting relaxation of muscles. It is also essential for the life activities of all cells in the body. The potassium and sodium are generally considered as non-toxic, however, so much present in irrigated water will affect the plants. The sources of sodium are human wastes, water softeners and industrial wastes and water salinity increase.

The chloride ion contents in water samples of two sampling sites were below than the permissible value. High chloride concentration in water body may be harmful to plant life. In natural water, high concentration of chloride is considered to be an indicator of pollution due to organic wastes of animal organ which has high quality of chlorides along with nitrogenous wastes.

The cadmium contents in water samples from two sampling sites were lower than the permissible value. Cadmium is a rare element in the crust of the earth. This metal has gained its rightful place among the most serious contaminants of man's environment, because adsorbed cadmium is retained in the body for decades and is extremely toxic.

The chromium contents were lower than the WHO permissible value in water samples from two sampling sites. Chromium often accumulates in aquatic life, adding to the danger of eating fish that may have been exposed to high levels of chromium.

The copper contents in two sampling sites were below than WHO standard value. Among three seasons variation, the highest value of copper was observed in cold season and the lowest value of copper was found in rainy season. Copper occurs in water as a result of the use of copper salt which is controlling algae growth.

The iron contents in two sampling sites were lower than the permissible value. Iron is wide distribution. Natural waters contain variable but minor amount of iron. Iron is an essential in human nutrition. The precipitation of excess iron gives an objectionable reddish-brown color to the water body.

The manganese contents in two sampling sites were lower than the permissible value. Manganese is a naturally occurring element that can be found ubiquitously in the air, soil and water. Manganese is also an essential nutrient for humans and animals.

Table 6. Results of DO, BOD and COD values in creek water from two sampling sites

Parameters (ppm)	Site-1			Site-2			WHO Standard (2016)
	Cold Season	Hot Season	Rainy Season	Cold Season	Hot Season	Rainy Season	
DO	9.82	5.53	13.69	12.25	7.13	9.52	4 - 7
BOD	2.57	2.0	1.0	2.5	2.7	1.5	<5
COD	4.42	3.62	1.32	3.68	3.27	1.32	<10

According to this table, dissolved oxygen values (DO) in sampling sites 1 and 2 were higher than the permissible value. It was found that the values of DO of hot season were lower when compared with those of other seasons. It is due to the fact that the solubility of oxygen decreases with increasing temperature. Biological decomposition of organic matter uses dissolved oxygen. Dissolved oxygen is necessary for the life of fish and other aquatic organisms.

The observed values of biochemical oxygen demand (BOD) in two sampling sites were below than the permissible value. The highest value of BOD was observed in cold season and the lowest value of BOD was found in rainy season. The BOD depends on the temperature of the environment and on the particular kinds of microorganisms and nutrient presents. It was found that COD values were higher than BOD values because oxidizing agent can oxidize some compounds such as cellulose that are biodegradable under natural conditions.

The observed values of chemical oxygen demand (COD) were lower than WHO standard value in two sampling sites. Among three seasons, the highest value of COD was observed in cold season. The highest value of COD may be due to the influx of forest debris, work disposal and chemical deposited form the respective fertilizer used in agricultural forms. The chemical oxygen demand indicates the quantity of the oxidation materials present in water.

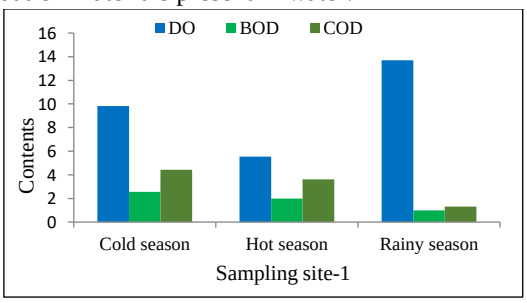

Fig 9. Seasonal variations of DO, BOD and COD in the water bodies of sampling site-1 (Shan Ywar creek)

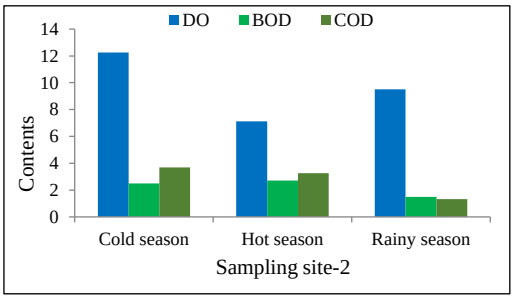

Fig 10. Seasonal variations of DO, BOD and COD in the water bodies of sampling site-2 (Nan Kyin creek)

Table 7. Microbiological indicators in creek water from two sampling sites

Parameters (cfu/mL)	Site-1			Site-2			WHO Standard (2016)
	Cold Season	Hot Season	Rainy Season	Cold Season	Hot Season	Rainy Season	
E.coli	0	0	0	0	0	1	-
Coliform	2	5	2	0	3	1	-

In bacteriological examination of water samples, *E.coli* was detected only in rainy season of sampling site-2. *Coliform* was observed in two sampling sites of water samples. *Coliform* group of bacteria is used as an indicator of bacteria in polluted water. Therefore, the quality of water samples of two sampling sites was not satisfactory for drinking purpose according to bacteriological point of view. The high values of *E-coli* and *Coliform* causes a spread several infectious diseases and harmful effects.

4. CONCLUSIONS

In this research, the seasonal investigation of physicochemical properties, seasonal detection of heavy metals concentration and microbiological organisms of water samples were carried out to assess the quality of water from site-1 (Shan Ywar) and site-2 (Nan Kyin) creeks in Myitkyina Township. During the course of study, pH values and total alkalinity values of two sites were within the acceptable limit. Total hardness values of two sampling sites were below the permissible limit. The values of turbidity in two sampling sites were above the WHO permissible values. Moreover, total dissolved solid (TDS) values were below the permissible level so that these creeks water were good for irrigation purpose and found to have favorable conditions for cultivation of plants. Dissolved oxygen in two sampling sites was higher than the desirable limit so that these creeks water were suitable for irrigation purposes. In the hot season, the decrease of DO value was observed to be lower than the cold and rainy seasons due to the fact that solubility of oxygen decreases with temperature increases. The dissolved oxygen test was measured the current oxygen level in water. The biochemical oxygen demand (BOD) values and chemical oxygen demand (COD) values of two creeks water were within the allowable limits of WHO standard for aquatic life protection.

Furthermore, the contents of heavy toxic metals were detected. Arsenic and lead values were below the permissible limit but the value of mercury was above the toxicity limit. Moreover, the *Coliform* was presented in two sampling sites and *E.coli* was observed in sampling site-2. According to the observed data, it can be suggesting that the water from two sampling sites should not be used for drinking and domestic uses. Therefore, it was concluded that there is a necessary for continuous assessment for the water body of creeks.

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We would like to express our sincere and deepest gratitude to Dr Aung Win, Rector, and Dr Aye Aye Ko, Pro-rector, Myitkyina University for their interest and encouragement on our research work.

REFERENCES

[1] Dissmeyer G. E., (2000). "*Drinking water from Forests and Grasslands"*,South Research Station, USDA Forest Service, Ashville, NC, USA.

[2] Jamie B., and B. Richard., (1993)."*Water Quality Monitoring*", 1st Ed., New York, London.

[3] Sadhana C., & D.G. Anand., (2014). "*Hand Book of Water, Air and Soil Analysis (A Lab Manual)*", Department of Energy & Environment, Faculty of Science and Environment MGGV, Chitrakkot, satna MP, 485331, India.

[4] Vogel, A. I., (1968). "*A Text-Book of Quantitative Inorganic Analysis. London*", 3rd Ed., The English Language Book Society.

[5] WHO., (2016). "*Guideline for Drinking Water Quality Recommendation*", 8th Ed, World Health Organization, Geneva, WHO. p.31.

BIOGENIC SYNTHESIS OF SILVER OXIDE NANOPARTICLES USING *Tamarindus indica* L. LEAVES EXTRACT AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT – In this research work, *Tamarindus indica* leaves were selected, because of its low cost, easily available and medicinal value. The preliminary detection of phytochemical constituents present in the leaves was carried out by phytochemical tests. Biogenic (Green) synthesis of silver oxide nanoparticles – Ag_2O NPs were synthesized by using aqueous silver nitrate with *Tamarindus indica* leaves extract as reducing and stabilizing agent. The synthesized Ag_2O NPs were characterized by applying manifold available techniques. The optical properties was detected by UV-visible spectrometer as well as the characteristic absorption peaks were identified by Fourier Transform Infrared Spectroscopy – FT IR, the relative abundance of elemental analysis of the composition was observed by Energy Dispersive X-ray Fluorescence – EDXRF and X-ray diffraction – XRD was used for analyzing structural property of nanoparticle crystals, the surface morphologies of the Ag_2O NPs were studied by Scanning Electron Microscopy – SEM. Antimicrobial activity of Ag_2O NPs were measured by agar well diffusion method. Ag_2O NPs can be applied as nano medicinal and nano therapeutic drugs due to their anti-cancer, anti-viral properties and other surface charge property which might be beneficial for treating cancer and other viral/bacterial diseases.

***Keywords**:* Biogenic synthesis, silver nanoparticles, antimicrobial activity, XRD, SEM

1. INTRODUCTION

Nanotechnology is rapidly increasing field that making an impact on human life such as pharmaceutical, health, food, electronics, chemical industry, energy science, cosmetics, environmental science and space industries etc. There are many ways to synthesize nanoparticles such as sol gel method, chemical reaction, solid state reaction and co-precipitation (Punnoose *et al.*, 2014). Compared to those methods green synthesis method is one of the best method for the synthesis of nanoparticles in recent years. This method have several advantages namely low cost, simple, use of less toxic materials, most important is eco-friendly. In this method, the plant extract has been used as reducing agent for the synthesis of silver nanoparticles (Dinker *et al*., 2015). Silver nanoparticles have been used extensively as anti-bacterial agents in the health industry, food storage, textile coatings and a number of environmental applications (Kholoud *et al*., 2010). Various plants were used for the synthesis of nanoparticles using green synthesis method. Nanoparticle was synthesized from all the parts of the plant separately like seed, stem, flower and leaf (Dinker *et al*., 2015).

To obtain nanomaterials of desired sizes, shape (Jagpreet *et al*., 2018), and functionalities, two different fundamental principles of synthesis (i.e., top down and bottom up methods) have been investigated in the existing literature (Figure 1). Synthesis of nanoparticles by plants is a green chemistry approach that interconnects nanotechnology and plant biotechnology. Plant extracts are used for the metal ions bio-reduction to form nanoparticles. It has been demonstrated that plant metabolites like sugars, terpenoids, polyphenols, alkaloids, phenolic acids, and proteins play an important role in metal ions reduction into nanoparticles and in supporting their subsequent stability. Nanoparticles gain much popularity due to biological, therapeutic and medical applications in the present era.

Silver oxide nanoparticles have strong antibacterial, antiviral and antifungal effects. Nanoparticles are being rapidly used as nano medicine, nano therapeutic, nano theranostic and cancer treatment (Iqbal *et al.*, 2019).

There are many ways to synthesize nanoparticles such as sol gel method, chemical reaction, solid state reaction and co-precipitation (Punnoose *et. al.*, 2014). Compared to those methods green synthesis method is one of the best method for the synthesis of nanoparticles in recent years. This method have several advantages namely low cost, simple, use of less toxic materials, most important is eco-friendly. In this method, the plant extract has been used as reducing agent for the synthesis of silver nanoparticles (Dinker *et al.*, 2015). Sliver nanoparticles have been used extensively as anti-bacterial agents in the health industry, food storage, textile coatings and a number of environmental applications (Kholoud *et al.*, 2010). Various plants were used for the synthesis of nanoparticles using green synthesis method. Nanoparticles were synthesized from all the parts of the plant separately like seed, stem, flower, and leaf (Dinker *et al.*, 2015). Plant extracts comprise of a wide range of naturally occurring chemical compounds, which are generally recognized as natural products (Lorimer *et al.*, 1996). These natural products possess varieties of biological activities due to their exceptional variety in their chemical structures (Harvey, 2008).

In my research work the synthesis of Ag_2O NPs by using the leaves extract of *Tamarindus indica* L. (Figure 1) as a stabilizing agent and aqueous silver nitrate solution. After synthesis, the resulting Ag_2O NPs were characterized using, EDXRF, UV-visible spectroscopy, FT IR, XRD and SEM analysis. Antimicrobial activity of the silver oxide nanoparticles were studied against human pathogenic bacteria (Raghupati *et al.*, 2011).

Botanical Description

Figure 1. The Habitat of *Tamarindus indica* L.

Family name – Fabaceae (or) Leguminosae
Botanical name – *Tamarindus indica* L.
English name – Tamarind
Myanmar name – Ma-Gyi
Part used – Leaves

2. MATERIALS AND METHODS

2.1 Collection of Plant Material and Extract Preparation

Leaves of the plant were collected from No. (13), Quarter, Pakokku Township, Magway Region. Plant leaves were washed with deionized water and then they were dried under shade condition at room temperature to remove soil and unwanted dust particles. 100g of tamarind leaves were weighed and transferred into a 1000mL beaker containing 500mL deionized water, mixed well and boiled for 45 minutes at 80°C. The extract was filtered through Whatman No.1 filter paper and the filtrate was collected and stored to carry out the further process.

2.2 Qualitative Analysis of Phytochemicals

The aqueous extract was screened for the presence of alkaloids, saponins, tannins, glycosides, flavonoids, reducing sugar, carbohydrates and sterols using the standard methods by the following (Harborne 1998; Veena 2016).

2.3 Preparation of 50mM Silver Nitrate ($AgNO_3$) Solution

Molarity is moles per liter. Since the molar mass of $AgNO_3$ is 169.87 g/mol, a 1 M solution of $AgNO_3$ would be 169.87 g (1 mole $AgNO_3$) in 1 Liter. For the preparation of 77.5 mL, 50 mM solution of $AgNO_3$, 0.658 g of silver nitrate was taken.

2.4 Biogenic Synthesis of Ag_2O nanoparticles

The silver oxide nanoparticles were synthesized by chemical reduction process using the leaves extract of *Tamarindus indica* L. and aqueous (50mM) silver nitrate solution. 75mL of 50mM silver nitrate solution was added to 300mL of *Tamarindus indica* L. leaves extract with continuous stirring using magnetic stirrer. This reaction mixture was maintained at about 35°C for the duration of reaction times 8 hours for the reduction of silver ions. After this process was observed by monitoring the color change from light chocolate color to dark gray color. It means that indicated the formation of silver oxide nanoparticles by leaves extract of *Tamarindus indica* L. (Figure 2.).

And then, the resulting solution was taken from the magnetic stirrer and allowed to settle overnight and the supernatant solution was then discarded cautiously. The precipitates were separated out from the solution by pouring the sample solution into a petridish, washed with deionized water and ethanol for two times to make sure that the residual impurities were removed. Subsequently, the washed precipitates were dried in oven, gradually increased to 200°C for 2 h to form the Ag_2O powders. After drying, the sample was stored in a glass bottle for further analysis. (Markarova, V., *et al*., 2014).

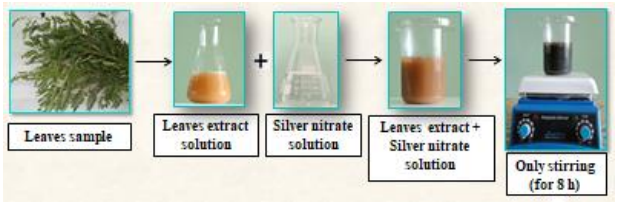


Figure 2. Color Changes for the Synthesis Process of Ag_2O NPs

2.5 Characterization of Silver Oxide Nanoparticles (Ag_2O NPs)

The present study described the process of synthesis of Ag_2O NPs using the leaves extract of *Tamarindus indica* L. and aqueous silver nitrate solution. The synthesized Ag_2O NPs were further characterized by using UV-vis absorption spectroscopy, EDXRF, FT IR, XRD, SEM analysis. The development of Ag_2O NPs was observed by appearance of the solution and UV-vis spectroscopy. The FT IR analysis was used to identify the functional group present in the leaves extract of *Tamarindus indica* L. which were responsible for the reduction of silver nanoparticles. The XRD studies were used to confirm the synthesized Ag_2O NPs were found to be highly stable and nanocrystalline in nature. The elemental analysis of silver oxide nanoparticles was determined by using EDXRF spectroscopy. SEM analysis provided the size and shape of the nanoparticles. The as-synthesized Ag_2O NPs were tested in the sterilized deionized water for their antimicrobial activity by the agar well diffusion method.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Tests on Leaves of *Tamarindus indica* L.

Preliminary phytochemical analysis was performed in order to know different types of organic compounds

present in leaves of *Tamarindus indica* L. The qualitative phytochemical analysis of these samples exhibited the presence of alkaloids, flavonoids, glycosides, phenolic compound, saponin, polyphenol, and lipophilic group.

3.2 EDXRF Analysis of Synthesized Ag_2O NPs

Examination of Ag_2O NPs was carried out by EDXRF spectroscopy. The relative abundance of elemental analysis of the percent compositions were shown in Figure 3. The EDX Spectrometry can analyze the element from Si to U under non vacuum condition. It can be utilized for qualitative identification and quantitative estimation of elements is solids, powders and liquids with appropriate sample preparation techniques.

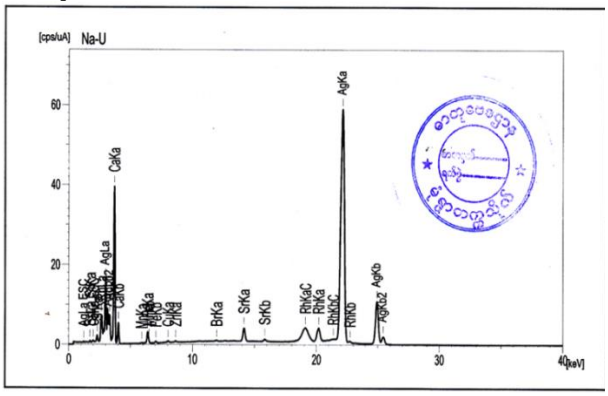


Figure 3. EDXRF pattern of synthesized Ag_2O NPs

According to Table 1, it was found the Ag_2O NPs contained calcium, silver, sulfur, iron, silicon, strontium, copper, manganese, zinc and bromine. Among the present elements, calcium and silver were obtained relative amount in the synthesized Ag_2O NPs with the significant (49.476% and 46.543%).

Table 1. Relative Elemental Composition of Synthesized Ag_2O NPs

No.	Element	Symbol	Result (%)
1	Calcium	Ca	49.476
2	Silver	Ag	46.543
3	Sulfur	S	1.970
4	Iron	Fe	0.922
5	Silicon	Si	0.581
6	Strontium	Sr	0.278
7	Copper	Cu	0.079
8	Manganese	Mn	0.077
9	Zinc oxide	Zn	0.054
10	Bromine	Br	0.019

3.3 Fourier Transform Infrared (FT IR) Measurement

The Fourier Transform Infrared spectrum (FT IR) measurement was done to identify the reducing, capping and stabilizing capacity of biomolecules in synthesized Ag_2O NPs using leaves extract of *Tamarindus indica* L. FT IR analysis of green synthesized nanoparticles via plant extracts confirmed that nascent nanoparticles were repeatedly found to be associated with proteins. Also, amino acids have different ways of reducing the metal ions. Plant extracts are made up of carbohydrates and proteins biomolecules, which act as a reducing agent to promote the formation of metallic nanoparticles. Therefore, the organic compounds involved in the synthesized Ag_2O NPs were identified by FT IR spectroscopy. The FT IR spectrum (Figure 4) reported the characteristic peaks of synthesized Ag_2O from the leaves extract of *Tamarindus indica* L. The synthesized Ag_2O, was observed in the range 406 – 743 cm^{-1} (Yong, N. L., *et al*., 2013). The broad absorption peak at around 3313.67 cm^{-1} was caused by absorbed water molecules since the nano-crystalline materials exhibited a high surface to volume ratio and thus absorbed moisture (Radhakrishnan *et al*., 2014; Silverstein *et al*., 2005).

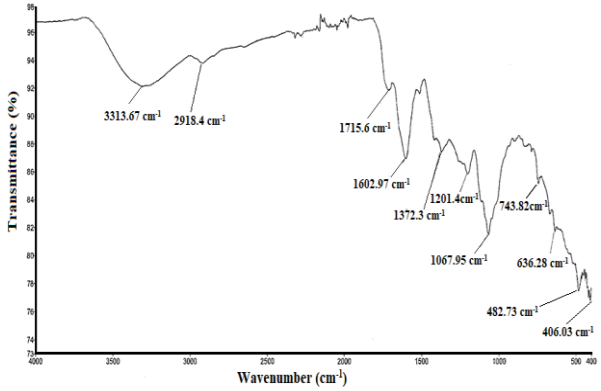


Figure 4. FT IR spectrum of green synthesized Ag_2O NPs

3.4 UV-visible Assignments for Ag_2O NPs

The UV-visible spectroscopic measurements were used to confirm the formation of Ag_2O NPs. The reduction of silver ions to silver nanoparticles by using UV-vis spectrum was shown in Figure 5. It was well known that the Ag_2O NPs showed light brown colour in water. These colors occurred due to the observable fact of surface plasmon excitations in the metal nanoparticles. The absorption spectrum of Ag_2O NPs formed in the reaction mixture was obtained by the UV-visible analysis at the range between 200-600 nm. The sharp absorption peak was observed at 220 nm which indicates the formation of silver oxide (Suman, T., *et al*., 2013).

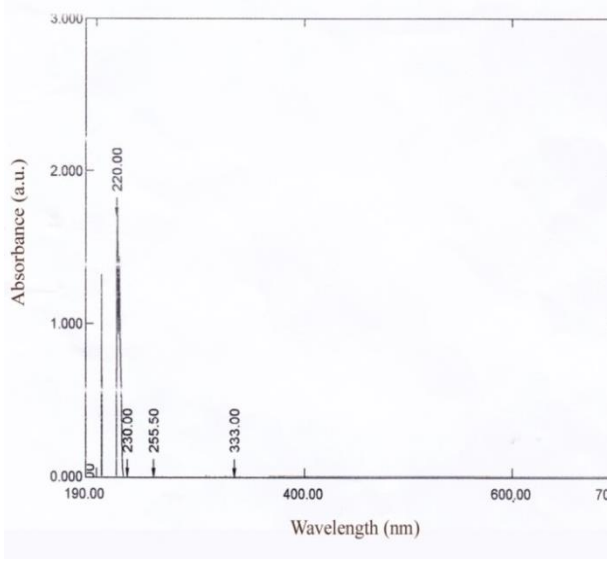


Figure 5. UV-visible spectrum of synthesized Ag_2O NPs

3.5 XRD Assignments for Ag_2O NPs

The X-Ray Diffraction (XRD) spectrum analysis indicated different diffraction peaks at 38.09°, 44.26° and 64.42° and they are shown in figure 6. The XRD analysis revealed that the crystalline structure of silver was face centered cubic in nature. The diffracted intensities were recorded from 10° to 70° at 2 theta angles, which corresponding to the planes (1 1 1), (2 0 0), and (2 2 0) and these planes approximately coincided with the shape formation of SEM analysis in Figure 6. The synthesized crystalline silver oxide nanoparticles were calculated from the width of the XRD peaks, the Debye-Scherrer equation was used to determine the average grain particle size of the nanoparticles (Jayaprakash *et al.*, 2014).

$D = K \lambda / \beta \cos\theta$, where, D is the crystalline size of nanoparticles, λ is the wavelength of the X ray source (0.1.54 nm) used in XRD, β is the full width at half maximum of the diffraction peak, K is the Scherrer constant with a value from 0.9 and θ is the Bragg angle. Using Debye- Scherrer equation the average nanocrystalline size was determined for the Ag_2O NPs formed in the bio reduction process was 27.6 nm.

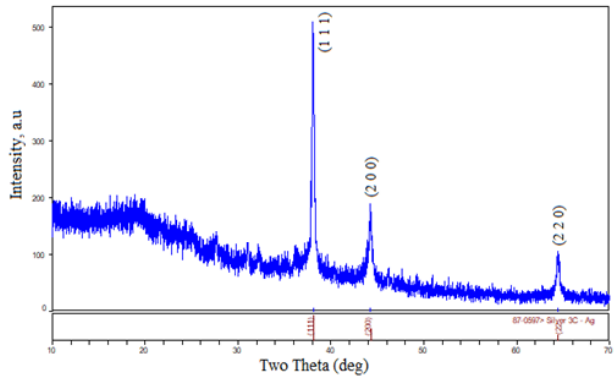


Figure 6. XRD spectrums of synthesized Ag_2O NPs

Table 2. The structure and Geometric Parameters of Ag_2O NPs

No	2θ (deg)	h k l	Cos θ	Nanocrystalline size, *D* (nm)
1	38.087	1 1 1	0.945	32.9
2	44.260	2 0 0	0.926	23.3
3	64.420	2 2 0	0.846	26.8
Average				27.6

According to Table 2, the nanocrystallite sizes of Ag_2O NPs were found to be within the range of 23-33 nm.

3.6 SEM Micrograph of Ag_2O NPs

Scanning Electron Microscopic (SEM) analysis provided the size and shapes of the nanoparticles. The SEM image showed the presence of high-density and compact agglomerates of silver particles. The interactions such as hydrogen bond and electrostatic interactions between the bio-organic capping molecules bond are the reason for synthesis of silver oxide nanoparticles using leaves extract of *Tamarindus indica* L. (Mano, P., *et al.*, 2011). It was shown that the presence of various shapes such as icosahedron, pyramid, plate, nanobar, and cube shapes and their sizes were recorded to be 10μm (Figure 7). The direct contacts even within the aggregates of nanoparticles were not found, indicating the stabilization of the nanoparticles by a capping agent (Awwad, A. M., 2012).

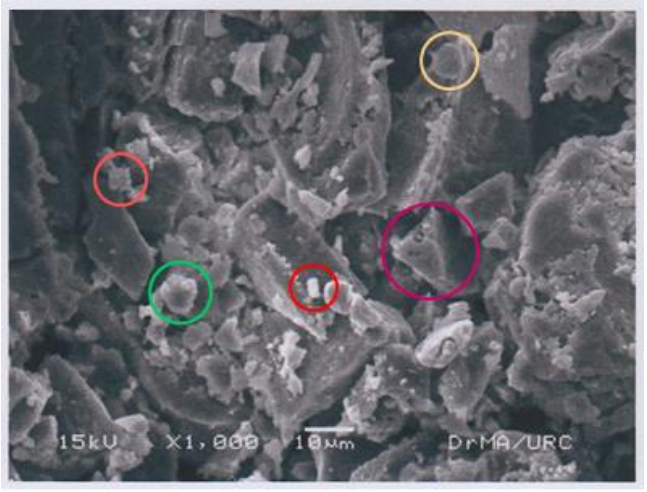
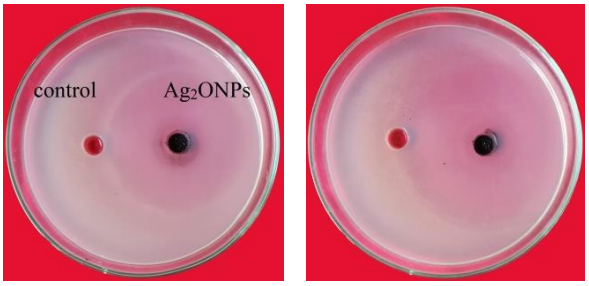


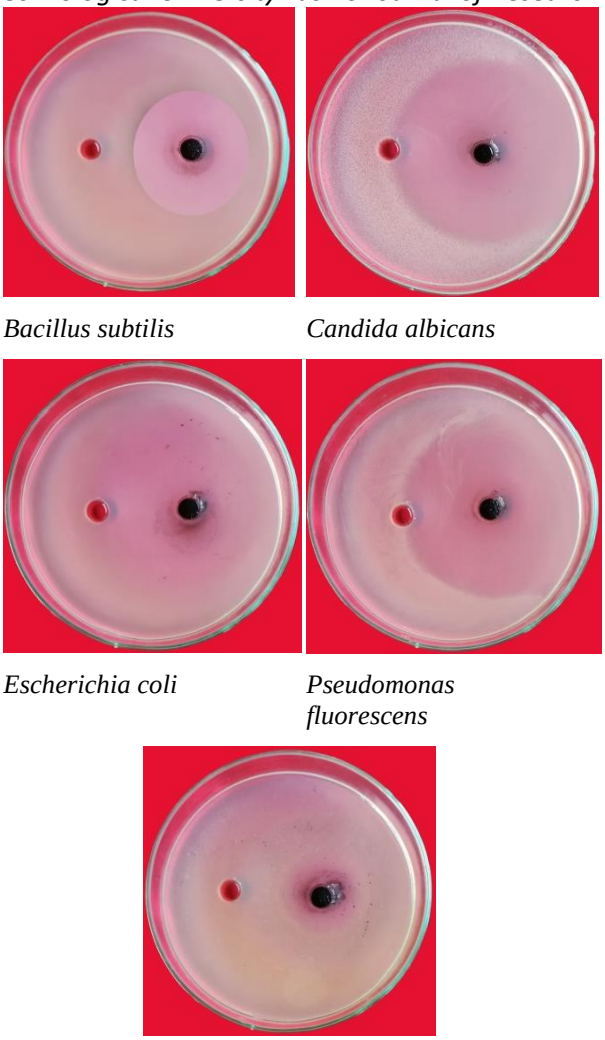
Figure 7. SEM micrograph of synthesized Ag_2O NPs

3.7 Antimicrobial activity studies of Ag_2O NPs

Antimicrobial activities of Ag_2O NPs were tested in the sterilized deionized water for their antimicrobial activity by agar diffusion method on seven selected microorganisms. According to the antimicrobial studies (Table 3), Ag_2ONPs showed high activity against *Agrobacterium tumefaciens*, *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and but it did not inhibit the growth of *Escherichia coli* (Figure 8). Hence, the as-prepared Ag_2ONPs are good to be used as antimicrobial agents for inhibition of some microorganisms.



Agrobacterium tumefaciens *Bacillus pumilus*



Bacillus subtilis *Candida albicans*

Escherichia coli *Pseudomonas fluorescens*

Staphylococcus aureus

Figure 8. **Antimicrobial Activities of synthesized Ag_2O NPs**

Table 3. Results Antimicrobial Activities of synthesized Ag_2O NPs

No.	Microorganisms	Inhibition Zone
1	*Agrobacterium tumefaciens*	52.38 mm (+ + +)
2	*Bacillus pumilus*	59.03 mm (+ + +)
3	*Bacillus subtilis*	33.83 mm (+ + +)
4	*Candida albicans*	62.78 mm (+ + +)
5	*Escherichia coli*	–
6	*Pseudomonas fluorescens*	63.31 mm (+ + +)
7	*Staphylococcus aureus*	30.23 mm (+ + +)

Agar well diameter = 8 mm
Not effective = (–)
8 mm to 12 mm = (+) Low activity
13 mm to 17 mm = (+ +) Medium activity
18 mm above = (+ + +) High activity

4. CONCLUSION

In the present study, Ag_2O NPs were successfully synthesized by using aqueous sliver nitrate solution and leaves extract of *Tamarindus indica* L. as reducing agent as well as stabilizing agent. The synthesized Ag_2O NPs solution exhibited absorbance bands at 220 nm attributed to the excitation of valence electrons of Ag_2O NPs arranged in the nanoparticles. The FT IR analysis was used to identify the functional group present in the leaves extract of *Tamarindus indica* L. which are responsible for the reduction of silver nanoparticles. The FT IR spectrum showed the characteristic absorption peak of Ag_2O NPs was observed within 400-750cm^{-1}. The EDXRF gave strong signal for Ag_2O NPs at energies 992.15 units and its amount was found to be 46.54 %. The XRD studies were used to confirm the synthesized Ag_2O NPs are found to be highly stable and nanocrystalline in nature. According to XRD peak report of Ag_2O NPs, the average nanocrystalline size of Ag_2O NPs was found to be 27.6 nm. The SEM studies revealed the formation of different shaped nanoparticles and their size were recorded to be 10 µm. The antimicrobial activity of synthesized Ag_2O NPs were screened by using Agar well diffusion method against *Agrobacterium tumefaciens*, *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Pseudomonas fluorescens*, *Staphylococcus aureus*. According to the antimicrobial screening, Ag_2O NPs were observed to possess high activity on all the tested microorganisms, except *Escherichia coli*. Thus the progress of green chemistry with the use of plants in the synthesis of NPs has engrossed a great attention.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Punnoose, A.; Dodge, K.; Rasmussen, J.W.; Chess, J.; Wingett, D.; Anders, C. (2014), "Cytotoxicity of ZnO nanoparticles can be tailored by modifying their surface structure: A green chemistry approach for safer nanomaterials". *ACS Sustain. Chem. Eng.* **2**, 1666–1673.

[2] Dinker Pawar, Shahista Shaikh, Deshmukh Shulaksana, RohiniKanawade, (2015). "Green Synthesis of copper nanoparticles using Gloriosa superbal leaf extract". *BGP Sahyadri junior college, Department of Biology, Sangamner.* **2**(3). 422605

[3] Kholoud, M.M., Nour, A.E., and *et.al.*, (2010),. "Synthesis and applications of silver nanoparticles". *Arabian Journal of Chemistry*, **3**, 135-140.

[4] Jagpreet Singh, Tanushree Dutta, and *et al.*, (2018) "Green synthesis of metals and their oxide nanoparticles: applications for environmental remediation". *Journal of Nanobiotechnology*. **Vol. 16 (84)**. 1-24

[5] Iqbal,S., M, Fakhar-e-Alam., F,Akbar., *et al.*, (2019), "Application of Silver oxide Nanoparticles for the Treatment of Cancer". *Journal of Molecular Structure*. **10**, 1016.

[6] Lorimer, S.D., N.B, Perry., L.M, Foster., E.J, Burgess., P.G, Douch., M.C, Hamilton., M.J,

Donaghy., R.A, McGregor. (1996), "A nematode larval motility inhibition assay for screening plant extracts and natural products". *J. Agric. Food Chem.* **44**, 2842–2845.

[7] Harvey, A.L.(2008), "Natural products in drug discovery". *Drug Discov.* **13**, 894–901.

[8] Raghupati K. R., Koodali RT, Manna AC. (2011) "Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles" *Langmuir*. **27**, 4020-4028.

[9] Harborne, J.B. (1998). *Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis*. 5th Edition, London; Chapman and Hall Ltd, 21-72.

[10] Veena S. and Urmila C. (2016). "Pharmacognostic and Phytochemical Screening of Helicteres Isora Roots". *Asian J Pharm Clin Res*, **9**(2), 96-101

[11] Markarova, V., Love, A. J., Sinitsyna, O. V., Markarova, S., Yaminsky. (2014), "Green nanotechnologies: synthesis of metal nanoparticles using plants". *Acta Naturae*. **6**(1), 35-44.

[12] Radhakrishnan, A.A. and Beena, B.B. (2014), "Structural and Optical Absorption Analysis of CuO Nanoparticles". *Indian Journal of Advances in Chemical Science*. **2**, 158-161.

[13] Silverstein, M, Robert., X, Francis., J, David. (2005), *Spectrometric identification of organic compounds*, 6th Edition., P. 450.

[14] Suman, T. Y., R, Rajasree., S. R, Kanchana., S.B, Elizabeth. (2013), "Biosynthesis, characterization and cytotoxic effect of plant mediated silver nanoparticles using Morinda citrifolia root extract". *Colloids and Surfaces B: Biointerfaces.* **106**, 74–78.

[15] Jayaprakash N., Judith Vijaya J., John Kennedy L., Priadharsini K., Palani P. (2014). "One step phytosynthesis of highly stabilized silver nanoparticles using *Piper nigrum* extract and theirantibacterial activity". *Mater. Lett.* **137**, 358.

[16] Mano, P. M., Karunai, S. B., John, P. JA. (2011), "Green Synthesis of Silver Nanoparticles From The Leaf Extracts of Euphorbia Hirta And Nerium Indicum". *J. Nanomat. Biostruct*. **6**, 869–877.

[17] Awwad, A.M., and Nidà, M. (2012), "Green Synthesis of silver nanoparticles by Mulberry leaves Extract". *Nanoscience and Nanotechnology*. **2**, 125-128.

Extraction of Essential Oil and Antimicrobial Activity from Fresh Leaves of *Apium graveolens* Linn. (Tayoke-nan-nan)

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ABSTRACT*:* In this study, leaves of Tayoke-nan-nan (*Apium graveolens* Linn.) were used to analysis. The sample was collected from local market. Essential oils was obtained from fresh leaves of Tayoke-nan-nan by steam distillation method. Phytochemical screening of Tayoke-nan-nan were also done. According to phytochemical screening, leaves of Tayoke-nan-nan sample contain alkaloid, glycoside, reducing sugar, terpene, sterol saponin, phenolic, polyphenol and tannin. Moisture content and ash content of leave of Tayoke-nan-nan were determined by conventional procedures. The yield percent of essential oil from leave of Tayoke-nan-nan were found to be (0.633 %) based upon the raw materials. Moreover, the antimicrobial activities of leaves of Tayoke-nan-nan were also investigated.

KEYWORDS: *Tayoke-nan-nan, essential oil, Steam Distillation Method, antimicrobial activity*

1. INTRODUCTION

Essential oil are volatile, natural, complex compounds characterized by a strong, odour and are formed by aromatic plants as secondary metabolites. They are usually obtained by steam on hydrodistillation first developed in the Middle Ages by Arabs. In nature, essential oils play an important role in the protection of the plants as antibacterials, activirals, antifungals, insecticides and also against herbivores by reducing their appetite for such plants. They also may attract some insects to flavor the depression of pollens and seeds or repel undesirable ethers. Essential oil can be synthesized by all plant organs i.e. buds, flowers, leaves, stems, twigs, seeds, fruits roots, woods, bark and are stored in secretary cells, cavities canals epidermic cells or glandular trichomes [3].

Essential oil have been largely employed for their properties already observed in nature, i.e for their antibacterial, antifungal and insecticidal activities. All present, approximately 3000 essential oils are known, 300 of which are commercially important especially for the pharmaceutical agonomic, food, sanitary, products in dentistry in agriculture as food preserves and additives, and as natural comedies for example, limonene, geranyl acetate and carvone are employed in perfumes arems, saps as flavor additives for food, as fragrances for household cleaning products and as industrial solvents. Moreover, essential oils are used in massage as mixtures with vegetal oil or in baths but most frequently in aromatherapy. Some essential oils appear to exhibit particular medicinal properties that have been claimed to care one or another organ dysfunction or systemic disorder [3].

Since the middle ages, essential oils have been widely used for bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal and cosmetic applications, especially nowadays in pharmaceutical, sanitary, cosmetic, agricultural and food industries. Because of the mode of extraction mostly by distillation from aromatic plants, they contain a variety of volatile molecule such as terpenes and terpenoids, pheonol-derived aromatic components and aliphatic components [8].

The aim and objective of research work is to conduct the antibacterial activity of the extraction of essential oil and its constituents from the leave of Tayoke-nan-nan.

1.1. Terpene

Many terpenes are aromatic hydrocarbon and thus may have had a protective function. The difference between terpenes and terpenoids is that terpenes are hydrocarbons, whereas terpenoids contain additional functional groups. When terpenes are modified chemically, such as by oxidation of rearrangement of the carbon skeleton, the resulting compounds are generally referred to as terpenoids. Some authors will use the term terpene to include all terpenoids. Terpenoids are also known as isoprenoids. Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers. Essential oils are used widely as natural flavor additives for food, as fragrances in perfumery, and in traditional and alternative medicines such as aromatherapy. According to literature, a variety of phytoconstituents are presence in the both leaves and seeds of *Apium graveolens* Linn. [7].

1.2. Medicinal uses of *Apium graveolens* Linn.

The leaves and stalks are used as salad and soup. They are stimulant, nervine sedative and toxic. The dried ripe fruits are used as spice. A domestic remedy for rheumatism is a decoction. The fruits also yield 17 % of a fatty oil (oil of celery). This is used as an antispasmodic and nerve stimulant. It has been employed in rhematoidic and nerve stimulant. It has been employed in rheumatoid arthritis. The root is used as aperitive, diuretic and used for jaundice, nephritic, colic and obstruction in the urinary passage.

Mixture of essential oils of this plant and cuminum cyminum in 1: 1 ratio showed potent fungistatic and insect repellant activities.

1.3. Botanical Description

Family : Umbelliferae

Botanical name : *Apium graveolens* Linn.
Genus : *Apium*
Species : *graveolens*
English name : Celery
Myanmar name : Tayoke-nan-nan

Part used : Leaves

Fig 1. The plant of *Apium graveolens* Linn. (Tayoke-nan-nan)

2. MATERIAL AND METHODS

2.1 Methods of Production of Essential Oils

There are three basic methods of production steam distillation: expression and extraction.

2.2 Steam Distillation Methods

This is simplest and oldest method. A vessel containing water and the crushed plant material is heated by a direct flame, and the water vapour and volatile oil are recovered by a water cooled condenser. The oil is separated from water phase by gravity.

The production of essential oils from powdered almonds, rose petals and orange blasmons must be employed this method. It is because of the moving freely of plant material in boiling water [8].

2.3 Water and Steam distillation Methods

The plant material is support at on a perforated grind or screen inserted some distance above the bottom of the soil. The lower part of the still is filled with water, to a level some what below this grind. The water may be heated by any of the methods previously mentioned saturated, in this case, wet steam of low pressure rises through the plant material. This method can be widely used for production of many oils but compound containing high concentration of very low volatile compounds in oils cannot be employed. This method is well suitable for herb and leaf material [8].

2.4 Extraction with Volatile Solvents

This method is based on the fact that the volatile solvent such as pet-ether penetrate the petals and dissolve all of the natural perfume, also the waxes and coloring matters. The fresh flowers are extracted several times with a fully purified solvent which is subsequently removed, usually by vacuum distillation [8].

The remaining products is a semisolid concentrated flower oil known as "concrete". These oils contain considerable amounts of plant waxes, albuminous material and colour pigments and are only partly soluble in alcohol [8].

2.5 Chemical Constituent of *Apium graveolens* Linn.

According to the literature, a variety of phytoconstituents are presence in the both leaves and seeds of *Apium graveolens* Linn.

Essential oil components such as carvone, eugenol, α-pinene, menthone, limonene and β-selinene will determined in the plant. And also triglycerides, free fatty acid, monoglycerides, diglyceride and sterol ester will determined in the oil of the plant.

2.6 Collection of Tayoke-nan-nan

Leaves of Tayoke-nan-nan were collected from local marked in Mandalay Division. They were washed and cleaned well.

2.7 Phytochemical Examination of Fresh Leaves of *Apium graveolens* Linn. by Test Tube Method

Fresh leaves of Tayoke-nan-nan were dried in shaded area at room temperature. The dried leaves were grind by grinder. The dried leaf powder were carried out by preliminary phytochemical test as shown in Figure.

Fig 2. Phytochemical Screening of Sample of Tayoke-nan-nan

2.8 Extraction of Essential Oil Content by Stem Distillation Method

The apparatus was used shown in Figure. About 6 dm^3 of distilled water was poured into the still body and perforated cone was set over at 200 g of the cut sample was placed on the perforated cone of the still. It was heated carefully without decomposition of oil. The time taken was six hours per day.

After heating 2 hours a mixture of volatile oil and steam was come out and passed into the condenser. The oil collecting on the surface of the water was separated by using separating funnel. The filtrate (cohobation

water) was stored for the use of next extraction. Essential oils were extracted by adding 50 mL of pet ether to the 300 mL of distillate using separating funnel. The solvent was evaporated using evaporator. The dehydrated oil obtained by passing through the sodium sulphate preserves the best quality.

The above experiment was carried out three times, each time using 200 g of sample 6 dm^3 of distilled water at 2 dm^3 of cohobate water.

Fig 3. Extraction of Essential Oil by Steam Distillation Method

2.9 The Study of Essential Oil from Leave of Tayoke-nan-nan by TLC Method

Thin Layer Chromatography was conducted on the essential oil by solvent system, n-hexane and ethylacetate (9:1). The sample (essential oil) was spotted on the base line. And then the plate was dried and run in the chosen solvent system and it was dried. This TLC plate was developed in iodine vapour and more than one spot were obtained.

2.10. Determination of Moisture Content

The moisture content was determined by oven drying method. The sample 5 g was placed in pre-weighed porcelain crucible. Then, it was kept in an oven at 105°C for 4 hours. It was cooled in desiccators and then weight again. The process of cooling and weighing were repeated until a constant weighed again. The process of cooling and weighing were repeated until a constant weights was achieved. Then, the amount of moisture was calculated.

$$\text{Moisture (\%)} = \frac{A-B}{A} \times 100$$

A = before drying, sample weights in gram,

B = after drying, sample weights in gram

2.11. Determination of Ash Content

Sample (1 g) was accurately weighed and placed in a preheated cooled and weighed porcelain crucible. The crucible was carefully heated oven an open flame until the sample was thoroughly carbonized. It was then heated in a heater at (180°C) until the residue was free from carbon. Then, it was slightly cooled, placed in a desiccator to cool down to room temperature and weighed. The process of heating, cooling and weighing was repeated until a constant weighed 0.714 g was obtained.

The ash content was calculated by using the following relation.

$$\text{Percent ash content} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100$$

2.12. Antimicrobial Activities of Leaves of Tayoke-nan-nan

The antimicrobial activities of the solvent extract of leave of Tayoke-nan-nan were performed by Agar well diffusion method on six selected organisms at Yadanabon University, Department of Botany, Mandalay. The resulting data of different type of organisms are shown in Table (5).

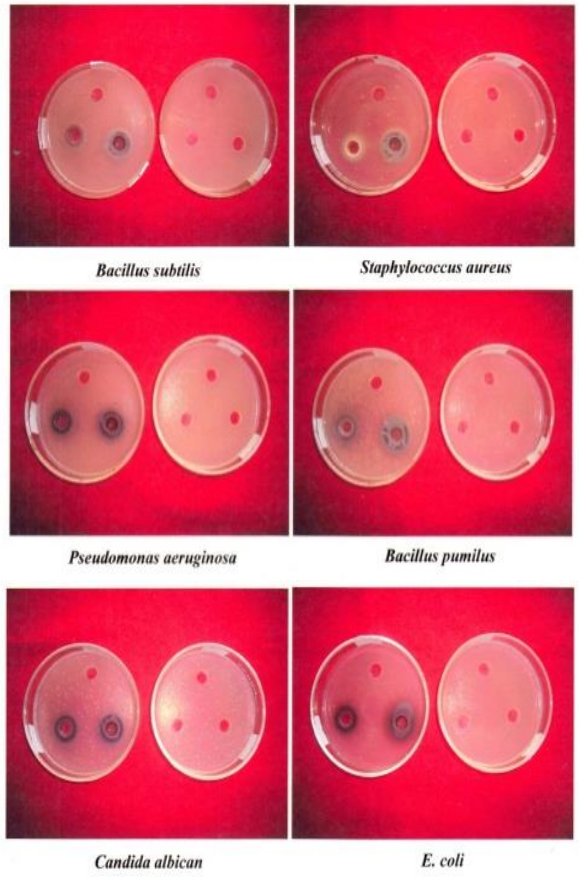

Fig 4. Antimicrobial activities of leaves of Tayoke-nan-nan

3. RESULTS AND DISCUSSION

3.1 Preliminary Phytochemical Test for Leave of Tayoke-nan-nan

Table 1. The Results of Phytochemical Test for *Apium graveolens* Linn. (Tayoke-nan-nan)

No.	Constituents	Reagent used	Observation	Result
1.	Alkaloid	(1) Wagner's reagent (2) Dragendroff's solution	Orange ppt Reddish brown ppt	+ +
2.	Glycoside	H_2O, 10 % lead acetate	White ppt	+
3.	Reducing sugar	H_2O, Benedict solution	Red ppt	+
4.	Sterol	EtOH, Acetic anhydride, $CHCl_3$, conc: H_2SO_4	Greenish colour ppt	+
5.	Saponin	EtOH, conc: H_2SO_4 shake	Formation of frothing	+
6.	Flavonoid	EtOH, conc: HCl, Mg turning	Pink color ppt	–
7.	Polyphenol	EtOH, 1 % $FeCl_3$, 1 % $K_3[Fe(CN)_6]$	Blue green	+
8.	Terpene	$CHCl_3$, conc; H_2SO_4	Pink colour	+
9.	Lipophilic	H_2O, 0.5 M KOH	Deep colour	–
10.	Tannin	10 % $FeCl_3$ + dil (H_2SO_4)	Yellowish brown ppt	+
11.	Phenolic	H_2O, 10 % $FeCl_3$	Greenish blue	+

(+) presence, (–) = absence

According to this table, the leaves of Tayoke-nan-nan consist of alkaloid, glycoside, reducing sugar, sterol, saponin, terpene, polyphenol, tannin and phenolic respectively.

Table 2. Determination of Moisture Content of Leave of Tayoke-nan-nan

No	Sample (g)	Moisture (g)	Moisture (%)
1	5	4.4878	89.756
2	5	4.4826	89.256
3	5	4.4376	89.716

Average percentage of moisture content 89.756 %.

Table 3. Determination of Ash Content of Leave of Tayoke-nan-nan

No	Sample (g)	Ash (g)	Ash (%)
1	5	0.714	14.28
2	5	0.718	14.36
3	5	0.712	14.26

Average percentage of ash content 14.28 %.

Table 4. Yield of Essential Oil Content from Leave of Tayoke-nan-nan

No	Wt of Sample (g)	Wt of oil (g)	Yield in %
1	200	1.29	0.645
2	200	1.28	0.640
3	200	1.23	0.615

Average percentage of oil content is 0.633 %.

3.2 The Study of Essential Oil from Leave of Tayoke-nan-nan by TLC Method

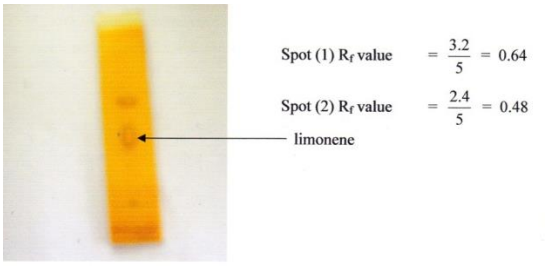

Fig 5. Thin Layer Chromatogram of Essential Oil from Tayoke-nan-nan

From the TLC result, the essential oil from leaves of Tayoke-nan-nan may be contain limonene compound of R_f value compare with its authentic R_f value.

3.3 Antimicrobial Actives of Leave of Tayoke-nan-nan

The antimicrobial activities of leave of Tayoke-nan-nan were performed by Agar well diffusion method on six selected organisms.

Table 5. Antimicrobial Activities of leave of Tayokenan-nan

Sample	Solvent	Organisms					
		Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus pumilus*	*Candida albicans*	*E. coli*
Tayoke nan nan	EtOAc	+++	++	+++	++ +	++ +	+ +
	EtOH	+	++	++	++	++	+

Agar well – 10 mm

10 mm ~ 14 mm (+)

15 mm ~ 19 mm (++)

20 mm above (+++)

According to antimicrobial activity test, ethyl acetate extract showed moderate activities against *Staphylococcus aureus, E.coli* and high activities against tested four organisms. Moreover, ethanol extract showed medium activities against four tested organism, and low activities against two tested organisms, such as *E. coli, Bacillus subtilis*.

5. CONCLUSION

Tayoke-nan-nan is a naturalized tree of Myanmar. There are well cultivated in all part of the country. Extraction of essential oils obtained from leaves of Tayoke-nan-nan by using steam Distillation method. The best quality of oil was dehydrated by using anhydrous Na_2SO_4. In this research work, the moisture content (89.756 %) and ash content (14.28 %) were determined. Moreover, the essential oil percent (0.633%) was found to be based upon the raw materials. And then, antimicrobial activity of two extract (EtOAc ad EtOH) of fresh leave of Tayoke-nan-nan with six tested organisms were determined. According to antimicrobial result, high activity of EtOAc extract with four selected organism, medium activity of EtOH extract with four tested organisms and low activity of two tested organism were observed. Essential oil extracted from leave of Tayoke-nan-nan may contain high amount of limonene compound due to the compare of its authentic R_f value by TLC method. So Tayoke-nan-an is well known for health and should take daily in the diet.

ACKNOWLEDGEMENT

I express my gratitude to Rector Dr Maung Maung Naing and Pro-rectors Dr Si Si Khin and Dr Tint Moe Thu Zar, Yadanabon University, for providing necessary resources. I also wish to express many thanks to Professor Dr Hlaing Hlaing Myat, Head of Chemistry Department, Yadanabon University for her kind permission to carry out this work and his constructive comment and suggestions.

REFERENCES

[1] Aparna, B. (2000). "A Text book of Practical Plant chemistry", First Edition, s.chaudand Company Ltd.

[2] A.A Joslyn, (1970). " Method in Food Analysis 2nd Ed, Academic Press, Inc, New York, 112

[3] Guenther, E. *Essential oil, constituents*, Vol.2., D. Van Nostrand, Co., Inc. Newyork, p-7, 141, 306, 1949

[4] Thiri Lwin, (1998). "Extraction of Essential oil from the peel of Lemon", MSc Thesis, Department of Chemistry, Mandalay University.

[5] Harboen, J. B. (1993). "Phytochemical Methods". A Guide to Modern Techniques of Plant analysis, Champman and Hall Ltd. U.S.A.

[6] Tin Wa, Maung (1992). "Phytochemistry Screening, Methods and Procedure Phytochemical Bulletin of Botanical Society of Marica". Inc. 5 (3) : 4.10.

[7] www.wikipedia.org/wiki/Terpene

[8] www.wikipedia.org/wiki/Essential oil

Preparation of Rice Husk Ash-Alginate and Study on the Removal Properties of Heavy Metals from Aqueous Solution

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ABSTRACT: This experiment investigates the preparation of rice husk ash-alginate beads and study on adsorption properties. Physicochemical properties such as moisture, pH, bulk density of rice husk ash and prepared beads were also determined. The moisture percent of ash (2.46 %) and rice husk ash-alginate beads (93.82 %) were observed. The pH of two samples were nearly neutral. The physicochemical properties were measured by conventional methods and modern technique. The effective biosorbent was successfully prepared by using 1 % of rice husk ash, 2 % $CaCl_2$ in 6 % sodium alginate solution. The prepared beads were used to remove heavy metals, Pb (II) and Cd (II) ions in synthetic solution. The adsorption property were conducted using different parameters such as initial concentration, amount of dosage and contact time. From this study, different initial concentrations were ranged from 50 ppm to 250 ppm of metal (II) ions solution. It was found that the percent of removal decreased with the increased in initial concentration of metal (II) ions solution. The optimum initial concentration was chosen as 200 mg L^{-1}. From the dosage method, the amount of dose increased with increasing removal percent ranging from 90.468% to 99.107% of Pb (II) ion and 96.915% to 99.998% of Cd (II) ion. In the contact time method, the removal percent was found to be 61.981% to 69.1684% of Pb (II) ion and 96.467% to 99.773% of Cd (II) ion. The larger the contact time, the more the amount of removal percent was observed. The observed data was fitted with adsorption isotherm. According to the Freundlich isotherm, the value of "n" which is related to the distribution of ions on the adsorbent surface was found to be greater than one. So the adsorption of lead (II), and cadmium (II) ions were favourable adsorbed on rice husk ash-alginate beads.

KEYWORDS: *rice husk ash, rice husk ash-alginate beads, moisture, pH, bulk density*

1. INTRODUCTION

There are many hazardous pollutants and materials, which get mixed in the water and create water problems. It is assumed that water pollution is the main cause of worldwide diseases. The presence of heavy metal ions in both surface and groundwater is a major concern. The removal of various heavy metals from contaminated wastewater has been one of the most important research. Many effective conventional separation technologies methods including precipitation, separation, reverse osmosis, ultrafiltration, ion exchange, evaporation, sedimentation, adsorption etc. (Xu, *et al*., 2013) have been developed to remove heavy metals from aqueous solution. Out of these, adsorption has been widely used and is a most efficient method to eliminate heavy metals from contaminated water. The removal of heavy metals using agricultural waste such as rice straw (Guiso, 2012), rice husk ash (Li, 2017), rice hulls (Rao, 2012), green adsorbent (Augusto, 2009) have been reported to be useful in the removal of heavy metal ions from aqueous system.

1.1Scientific Classification of Rice Husk

Scientific name : *Oryza Sativa* L

Family : Poaceae

English name : Paddy

Myanmar name : Saba

Part used : Husk

Occurrence :Asia, Myanmar, Bangladesh, Laos, Indonesia

1.2 Properties of Rice Husk

Rice husk is a potential material, which is amenable for value addition. Most of the husk from the milling is either burnt or dumped as waste in open fields and a small amount is used as fuel for boilers, electron generation, bulking agents for composting, of animal manure, etc (Asvapisit, *et al*., 2005). The chemical composition of rice husk is similar to that of many common organic fibers and it contains of cellulose 40-50 percent, lignin 25-30 percent, ash 15-20 percent and moisture 8-15 percent (Hwang, *et al*., 1997). After burning, most evaporable components are slowly lost and the silicates are left. No other plant except paddy husk is able to retain such a huge proportion of silica in it.

1.3 Sodium alginate

"Alginate" is the term usually used for the salts of alginic acid, but it can also refer to all the derivatives of alginic acid and alginic acid itself. Alginate is present in the cell walls of brown algae as the calcium, magnesium and sodium salts of alginic acid. Alginate molecules are long chains that contain two different

acidic components, abbreviated here for simplicity to M and G. The way in which these M and G units are arranged in the chain and the overall ratio, M/G of the two units in a chain can vary from one species of seaweed to another. So some seaweeds may produce an alginate that gives a high viscosity when dissolved in water, others may yield a low viscosity alginate.

1.4 Uses of alginate

Alginate absorbs water quickly, which makes it useful as an additive in dehydrated products such as slimming aids and in the manufacture of paper and textiles. It is also use for waterproofing and fireproofing fabrics. Sodium alginates is used in reactive dye printing as a thickener for reactive dye in textile screening printing. Alginate do no reactive with reactive dyes and wash out easily, unlike starch-base thickeners (Aizpurua, 2016).The uses of alginates are based on three main properties. The first is their ability, when dissolved in water, to thicken the resulting solution. The second is their ability to form gels. The third property of alginates is the ability to form films of sodium or calcium alginate and fibers of calcium alginates.

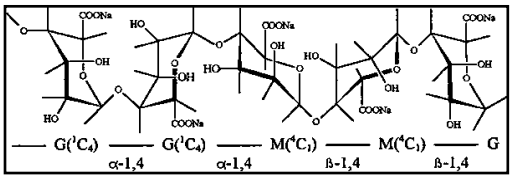

Figure 1.1 Structure of sodium alginate

- Hwang, et al. (1997).
- Asvapisit, et al.,(2005)
- Augusto (2009)
- Guiso (2012)
- Rao (2012)
- Xu, et al. (2013)
- Aizpurua (2016).
- Li (2017)

2. Materials and Methods

In all analytical procedure of the experiments, recommended standard methods and techniques were applied. Various conventional modern techniques instruments were used throughout the experimental procedures. All experimental data were computed on the statistical basis. The apparatus consists of both conventional glassware and modern equipment. The chemicals were used from British Drug House (BDH). Rice huak (Thai 90) was collected from rice mail at Malazali Kone Township in Hinthada District. The collected sample which were sifted by removing unwanted dirt and other matters. Physical properties such as: pH, ash content, moisture content and bulk density were also determined. The ash content and moisture content were determined by AOAC method. The bulk density was determined by conventional method. And then the ash was modified by sodium alginate to form rice husk- ash alginate beads. The physicochemical properties of the prepared beads were also determined. The prepared beads were be used to remove heavy metals from aqueous solutions. The various parameters such as amount of dosage, concentrations and contact times were carried out. And then the resultants data were celebrated by adsorption isotherm.

3. RESULTS AND DISSCUSSION

3.1 Preparation of Rice Husk Ash and Rice Husk Ash-Alginate Beads

In the preparation of rice husk ash-alginate beads, sodium alginate concentration and the concentration of rice husk ash are significant parameters. If the concentration of sodium alginate is very low, the solution is clear homogeneous and the beads are not formed and if the solution alginate concentration is very high, the solution is very viscous. Similarly the more amount of rice husk ash is added to sodium alginate solution, the more precipitate of the mixture solution and the lesser the extent of homogeneous mixture is obtained. So, the optimum condition was selected by mixing 1g of rice husk ash and 6 % w/v of sodium alginate and the mixture was added drop by drop into 2 % calcium chloride solution to obtain rice husk ash-alginate beads. It was shown in Figure 3.1.

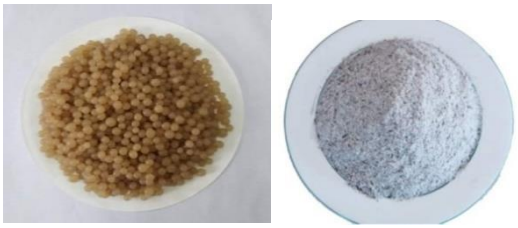

Figure 3.1 Figures of rice husk ash and rice husk ash-alginate beads

3.2 Physicochemical Properties of Rice Husk Ash and Rice Husk Ash-Alginate Beads

Table (3.1) shows physicochemical properties of rice husk ash and rice husk ash -alginate beads.. It was found that ash percent (23.65 %) of dried rice husk ash was obtained. The moisture content of rice husk ash and rice husk ash-alginate beads were (2.46 %) and (93.82 %) respectively. The pH of rice husk ash and rice husk ash-alginate beads were 7.73 and 6.84. The bulk density of rice husk ash (0.2926 g cm^{-3}) was observed. The results were shown in Table 3.1.

Table 3.1 Physicochemical Properties of Rice Husk Ash and Rice Husk Ash- Alginate Beads

Sample	Ash content (%)	Moisture content (%)	pH	Bulk density (g/cm^3)
RHA	23.65	2.46	7.73	0.2926
RHA-A beads		93.82	6.84	

RHA = Rice Husk Ash,
RHA-A beads = Rice Husk Ash- Alginate Beads

3.3 Effect of concentration on the adsorption of metal (II) ions

Effect of initial metal concentration on the adsorption of Pb (II) and Cd (II) ions by rice husk ash alginate beads were shown in Figure 3.2 (a) and Figure 3.2 (b). The optimum dosage was chosen as 1 g. Adsorption percent of Pb (II) and Cd (II) ions from various initial metal concentrations ranging from 50 mg L^{-1} to 250 mg L^{-1} were investigated. It was observed that removal percent of metal (II) ions were decreased with increased in metal ions concentration. The optimum condition for the removal of metal (II) ions (200 mg L^{-1}) of initial metal ions concentration was selected. As the concentration of metal (II) ions increase, more and more surface sites are covered due to higher concentration of metal ions. Therefore, the lower the initial concentration, the more active sites on the surface of adsorbent and the higher the removal percent of metal ion. These results were shown in Table 3.2 (a) and Table 3.2 (b).

Table 3.2 (a) Optimum Concentration of Pb (II) Ion for Adsorption of Rice Husk Ash-Alginate Beads

Initial con: C_i (mg/L)	Final con: C_f (mg/L)	Removal percent (%) $\frac{C_i - C_f}{C_i}$ x 100
50	0.6812	98.63
100	5.1062	94.89
150	16.5000	89.00
200	37.0188	81.49
250	48.1000	80.76

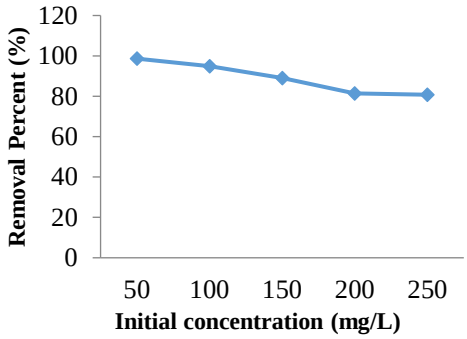

Figure 3.2(a) Adsorption of various concentrations of Pb (II) ion by rice husk ash-alginate beads

Table 3.2 (b) Optimum Concentration of Cd (II) Ion by Adsorption of Rice Husk Ash - Alginate Beads

Initial con: C_i (mg/L)	Final con: C_f (mg/L)	Removal percent (%) $\frac{C_i - C_f}{C_i}$ x 100
50	2.5051	94.98
100	12.7140	87.28
150	22.4355	85.04
200	32.0240	83.98
250	41.9670	83.21

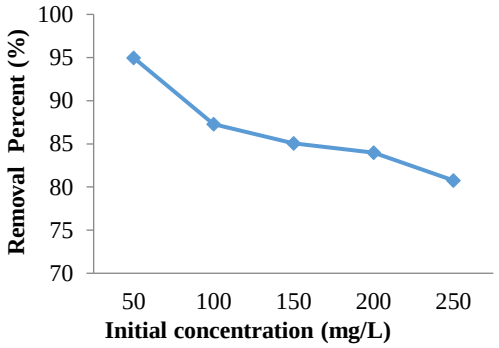

Figure 3.2 (b) Adsorption of various concentrations of Cd (II) ion by rice husk ash-alginate beads

3.4 Effect of dosage on the adsorption of metal (II) ions

Effect of dosage on the adsorption of Pb (II) and Cd (II) ions on rice husk ash-alginate beads were investigated. It was observed that the adsorption percent of Pb (II) from 200 mg L^{-1} of Pb (II) model solution increased from 79.97% to 99.99% on rice husk ash- alginate beads. The dosage of adsorbent rice husk ash- alginate beads were range from 1 g L^{-1} to 5 g L^{-1}. From the investigation, the effect of adsorbent dose on Cd (II) solution was ranged from 78.99% to 99.85% were obtained. From the experimental results, the removal percent of metal (II) ions were increased with increased in adsorbent dose, but metal up take capacity was decreased. This may be attributed to reduction of total surface area of adsorbent, due to probably by aggregation during adsorption of rice husk ash- alginate beads on the experimental conditions. The results were presented in Figure 3.3 (a) and Figure 3.3 (b).

Table 3.3 (a) Optimum Dosage of Pb (II) Ion for Adsorption of Rice Husk Ash-Alginate Beads

Dosage C_a (mg/L)	Final con: C_f (mg/L)	Removal percent (%) $\frac{C_i - C_f}{C_i}$ x 100
1	40.0668	79.97
2	20.0606	89.97
3	18.0486	90.98
4	0.0422	99.98
5	0.0120	99.99

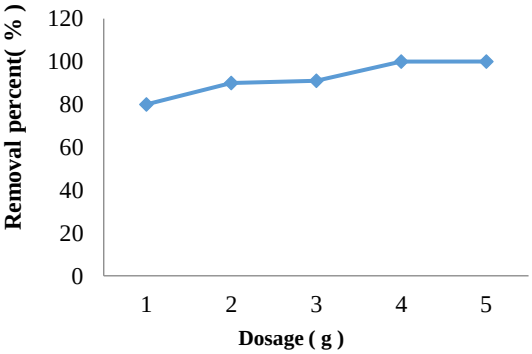

Figure 3.3 (a) Adsorption of various adsorbent dose on Pb (II) ion

Table 3.3 (b) Optimum Dosage of Cd (II) Ion for Adsorption of Rice Husk Ash-Alginate Beads

Dosage C_a (mg/L)	Final con: C_f (mg/L)	Removal percent (%) $\frac{C_i-C_f}{C_i}$ x 100
1	42.0328	78.99
2	21.7424	89.13
3	19.0648	90.47
4	0.6776	99.66
5	0.2904	99.85

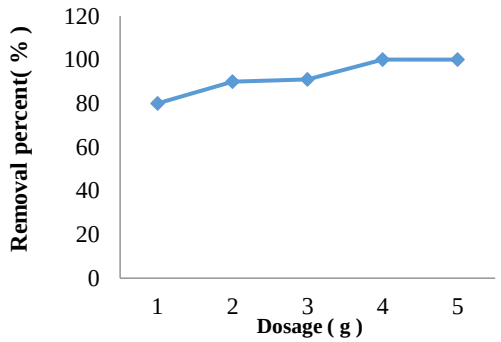

Figure 3.3 (b) Adsorption of various adsorbent dose on Cd (II) ion

3.5 Effect of contact time on the adsorption of metal (II) ions

The effect of contact time on the adsorption of Pb (II) and Cd (II) ions on the surface of rice husk ash-alginate beads were investigated. The adsorbed dose 1 g was chosen. Table 3.4 (a) and Figure 3.4 (a) showed that the adsorption of Pb (II) ion on rice husk ash- alginate beads with various contact time 20 min to 100 min. From the results, it was found that the maximum adsorption was occurred within 100 min for Pb (II) and Cd (II) ion on the rice husk ash- alginate beads. It was observed that the adsorption increased with increasing contact time. The percent removal of metal ion was nearly constant within 80 min to 100 min. Similarly, Table 3.4 (b) and Figure 3.4 (b) showed that the adsorption of Cd (II) by rice husk ash-alginate beads by various contact times. The complete adsorption was observed within 100 min. So the experiments were carried out for the adsorption of Pb (II) and Cd (II) by rice husk ash- alginate beads for 100 min contact time in contact with metal solution and prepared adsorbent samples. At the adsorption equilibrium, the maximum adsorption percent of Pb (II) ion by rice husk ash-alginate beads was (69.12%) and the maximum adsorption percent of Cd (II) ion by rice husk ash alginate beads was (99.75%).

Table 3.4 (a) Optimum Contact Time of Pb (II) Ion for Adsorption of Rice Husk Ash-Alginate Beads

Time (min)	Final con: C_f (mg/L)	Removal percent (%) $\frac{C_i-C_f}{C_i}$ x 100
20	68.0298	65.9900
40	66.0232	66.9900
60	65.8754	67.0600
80	63.8620	68.0700
100	61.7632	69.1200

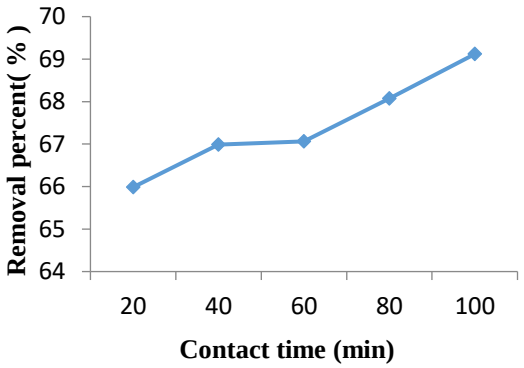

Figure 3.4 (a) Effect of contact time on Pb (II) ion by rice husk ash- alginate beads

Table 3.4 (b) Optimum Contact Time of Cd(II) Ion for Adsorption of Rice Husk Ash-Alginate Beads

Time (min)	Final con: C_f (mg/L)	Removal percent (%) $\frac{C_i-C_f}{C_i}$ x 100
20	7.0654	96.4700
40	4.3942	97.8000
60	2.6440	98.6800
80	0.6448	99.6800
100	0.4928	99.7500

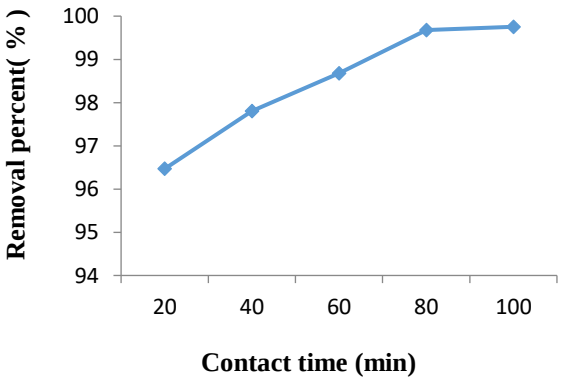

Figure 3.4 (b) Effect of contact time on Cd (II) ion by rice husk ash- alginate beads

3.6 Adsorption isotherm

The linear regression is performed and used to determine whether the isotherms are fitted with experimental adsorption data or not by knowing the R-square values. In this study, Freundlich adsorption isotherms were employed for the treatment of the equilibrium data. Figure 3.5 (a) and 3.5 (b) were shown that the Freundlich adsorption isotherms for rice husk ash-alginate beads where R square values 0.947 and 0.952 respectively. These isotherms are also fitted well with experimental data according to equation;

$\log (C_i - C_f) = (\frac{1}{n}) \log C_a + \log K_f$ (Gomez, 2007).

Here, K_f is the equilibrium constant. According to the Freundlich isotherms, the value of "n" which is related to the distribution of ions on the adsorbent surface.

Table 3.5 (a) Freundlich Adsorption Isotherm of Pb (II) Ion for Rice Husk Ash-Alginate Beads

Dosage, C_a (g)	$C_i - C_f$ (mg L^{-1})	log (C_i-C_f)	log C_a
1	159.9672	2.2040	0.0000
2	179.9394	2.2551	0.3010
3	181.9514	2.2600	0.4771
4	199.9578	2.3009	0.6021
5	199.9880	2.3010	0.6990

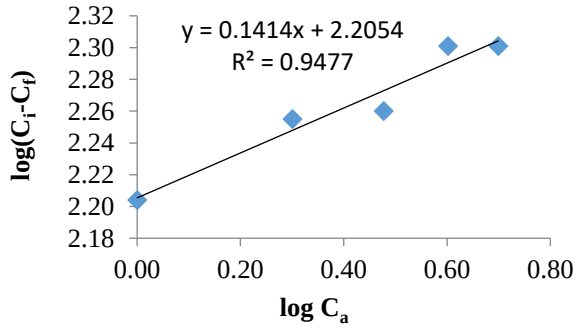

Figure 3.5 (a) Isotherm for adsorption of Pb (II) ion by rice husk ash-alginate beads

Table 3.5 (b) Freundlich Adsorption Isotherm of Cd (II) Ion for Rice Husk Ash- Alginate Beads

Dosag,C_a (g)	$C_i - C_f$ (mg L^{-1})	log (C_i-C_f)	log C_a
1	157.9672	2.1985	0.0000
2	178.2576	2.2510	0.3010
3	180.9352	2.2575	0.4771
4	199.3224	2.2995	0.6021
5	199.7096	2.3004	0.6990

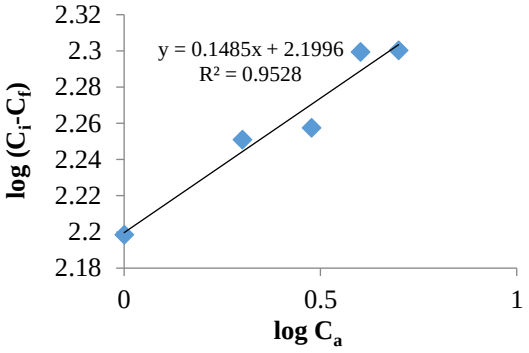

Figure 3.5 (b) Isotherm for adsorption of Cd (II) ion by rice husk ash-alginate beads

4. CONCLUSIONS

The results of the investigation revealed that the rice husk ash-alginate beads can be successfully used for the removal of metal (II) ion from aqueous solution. In the present study, it was observed that rice husk ash-alginate beads which showed the best removal capacity of lead and cadmium ions from aqueous solution. Physicochemical properties of rice husk ash and rice husk ash-alginate beads were also investigated. The moisture percent of rice husk ash and rice husk ash-alginate beads were found to be (2.46 %) and (93.82 %). The pH of rice husk ash and rice husk ash-alginate beads were be nearly the neutral. The bulk density of rice husk ash was 0.2926 g cm^{-3} and ash contact was 23.65% were observed. The adsorption properties was studied by using different parameters such as initial concentration, dosage and contact times.

From this study, the percent removal was found to be ranging from 98.63% to 80.76% of Pb (II) ion and 99.98% to 83.25% of Cd (II) ion by different initial concentration. It was observed that the percent removal was decreased with increasing concentration of metal (II) ion solution. From the different of adsorbed dose, the removal percent was found to be ranged from 79.97% to 99.99% by Pb (II) ion and 78.99% to 99.85% of Cd (II) ion. In the contact time method, the removal amount of was increased with increasing contact time ranging from 20 min to 100 min. The results obtained clearly showed that the maximum amount were found to be 69.12 % (100 min) by Pb (II) ion and 99.75% by Cd (II) ion. According to these equilibrium data, adsorbent of rice husk ash-alginate beads were more fitted for Freundlich model. According to the Freundlich isotherm, the value of "n" which is related to the distribution of ions on the adsorbent surface was found to be greater than one. So the adsorption of lead (II), and cadmium (II) and were favourable adsorbed on rice husk ash-alginate beads.

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REFERENCES

[1] Asvapisit, S. and N. Ruengrit. (2005). "The Role of RHA Blended Cement in Stabilizing Metal – Containing Wastes". *Cem – Coner. Compos* **27**, 782-787

[2] Augusto, D., M. Zaia, R.Ventura and A. Alberto. (2009). "Uses of Rice Straw as Biosorbent for Removal of Cd(II) and Pb(II) Ions in Industrial Effluents", *J.Hazard.Mater*. **166**, 383-388

[3] Aizpurua-Olaizola; Oier, N.P.,A., Vallejo, M.E. Olivares and A. Usobiaga. (2016). "Microencapsulation and Storage Stability of Polyphenols from Vitis ViniferaGrape Wastes". *Food Chemistry***.190,** 614-621

[4] Guiso, M.G., G. Alberti., G. Emma and MPesasvento. (2012). "Removal Through Untreated Rice Husk ; Thermodynamics and Kinetics". *Anal. Sci*, **28**, 993-999

[5] Gomez, V., M.S. Larrechi and M.P. Callao.(2007). "Kinetic and Adsorption Study of Acid dye Removal Using Activated Carbon". *Chemosphere.***69**(7), 1151-1158

[6] Hwang, C.L. and S. Chandra. (1997). "The Use of Rice Husk Ash in Concrete". *Waste Material Used in Concrete Manufacturing. Edited: Chandra, S., NoyesPublication*, USA, **198**

[7] Li, W.C., F.Y. Law and Y.H.M Chan. (2017). "Biosorption Studies on Lead (II) and Cadmium (II) Using Pretreated Rice Straw and Rice Husk, **24**, 8903-8915

[8] Rao, R.A.K. and M. Kashifuddin. (2012). "An Excellent Adsorbent for the Removal of fromAqueous Solution". *Chin. J. Geochem*. **31**, 136-146

[9] Xu, M. P. Yin, X., Liu, Q., Tang, R. Qu and Q. Xu. (2013). "Utilization of Rice Husks Modified by Organomultiphosphonic Acid as Low-cost Biosorbents for Enhanced Adsorption of Heavy Metal Ions", *Bioresour. Technol*. **149**, 420-424

QUANTITATIVE DETERMINATION OF TANNINS IN DIFFERENT EXTRACTIONS OF *Camellia sinensis* L. (GREEN TEA)

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Abstract - In this work, the preliminary phytochemical tests, the qualitative determination of tannins, the conformity of extract tannins and reference tannic acid and quantitative determina-tion of tannins were made for the extracts of various types of Tea Leaves. The preliminary phytochemical tests showed that the leaves contain alkaloids, glycosides, phenolic compounds, tannins, saponins, polyphenols, flavonoids and steroids. Then, the test with 2 % gelatin solution shows the presence of tannins. For conformity of tannins, the tests with FT IR and TLC were carried out. According to the results of FT IR and TLC, it is assumed that extract tannins is identical to reference tannic acid because of the same R_f value and FT IR spectrum. For quantitative determination of tannins in various types of tea leaves, Folin-Denis method was used spectrophotometrically. Results show that young leaves has the greatest content of tannins than the other moderate and old leaves.

Keywords: **tannins, Folin-Denis, *Camellia sinensis*, Tea leaves, phytochemical constituents**

1. INTRODUCTION

Plants produce a wide range of active compounds which provide pharmacological effect. In the world, parts of the plants such as root, stem, leaf, bark, branch, fruit and flower are used in traditional medicine. Not only the plant constituents are used directly as therapeutic agent but also as starting materials for the synthesis of drugs. Nowadays, people are preferring the medicines made from natural herbs, rather than the modern drugs based on chemical compounds which have can cause serious side effects.

Traditional system of medicine is found to have utilities as many accounts. Due to population rise, inadequate supply of drug and high cost of treatment in side effect along with drug resistance has been encountered in synthetic drugs, which lead to an elevated emphasis for the use of herbal plants to treat human diseases.

Tannins also called tannic acid is any of pale-yellow to light-brown amorphous substance in the form of powder, flake, or a spongy mass, widely distributed in plants. The best known human dietary sources of tannins are tea and wine. Tannins have been used for immediate relief of sore throats, diarrhea, dysentery, skin ulcers and cicatrices, inhibitors of HIV replication. (Nanaka, 1990) Tannin can also be effective in protecting the kidneys. Hydrolyzable tannins have shown also potential antibacterial effects against *Helicobacter pylori.* (Furatogawa, 2004)

Green Tea is one of the most ancient and popular therapeutic beverages consumed around the world. It can also be prepared as a drink. Tea is the most consumed drink in the world after water. In addition, its content of certain minerals and vitamins increases the antioxidant potential of this type of tea. (Pan, 2003)

Green Tea is obtained from the tea plant *Camellia sinensis* belongs to the family Theaceae. It is native to mainland China, South and Southeast Asia, but it is today cultivated in tropical and subtropical regions across the world.

Green Tea contains nearly 4,000 bioactive compounds of which one third is contributed by polyphenols. Other compounds are alkaloids, amino acids, carbohydrates, proteins, chlorophyll, volatile organic compounds, fluoride, aluminium, minerals and trace elements. Polyphenols found in tea are mostly flavonoids. (Mukhtar, 2000)

This research work is an attempt to determine the content of tannins in various types of Tea Leaves. The tannins in various types of Tea Leaves was extracted with distilled water, ethanol and acetone and the tannins contents were determined spectrophotometrically.

1.1 Botanical Description

Family - Theaceae
Genus - *Camellia*
Species - *C.sinensis*
Botanical name - *Camellia sinensis* L.
English name - Tea Plant
Myanmar name - Letphet
Parts used - Leaves

Figure 1. Tea leaves

1.2 Medicinal uses of Green Tea Leaves

The health benefits of Green Tea Leaves are Anti-Aging Activity, Anti-Alzheimer Activity, Antistroke Activity, Anticancer Activity, Antidiabetic Activity, Antiparkinson Activity, Cardiovascular Diseases, Anticaries Activity, Skin Disorders and Obesity and Weight Loss. (Cabrera, 2006)

2. MATERIALS AND METHODS

2.1 Materials

The chemicals used in this research were the common analytical grade reagents. The reagent were Drgendorff's reagent, Wagner's reagents, Benedict's solution, lead acetate, ferric chloride, magnesium ribbon, concentrated sulphuric acid, concentrated hydrochloric acid, acetic anhydride, chloroform, $K_3[Fe(CN)_6]$, sodium carbonate, ethanol, acetone, standard tannic acid and Folin-Denis reagent.

The apparatus were a spectrophotometer (JENWAY, 630), centrifuge, an electrical balance, a reflux condenser and common laboratory apparatus were used throughout this work.

2.2 Methods

The contents of tannins in various extractions of *Camellia sinensis* L. were determined by Folin-Denis method.

2.3 Sample Collection

Various types of tea leaves were collected from Homalin Township, Sagaing Region. The sample was dried in air and ground to powder. It was stored in well stoppered bottle and used throughout the experiment.

2.4 Extraction

2.4.1 Extraction of Tea leaves with distilled water

Exactly 1 g of dry powdered sample (young leaves) was boiled with 100 mL distilled water in a 200 mL conical flask for 30 minutes. Then, the mixture was centrifuged at 2000 rpm for 30 minutes. The supernatant was decanted in a 100 mL volumetric flask and diluted with distilled water up to the mark of the flask.

Exactly 1 g of dry powdered sample (moderate leaves) was boiled with 100 mL of distilled water in a 200 mL conical flask for 30 minutes. Then, the mixture was centrifuged at 2000 rpm for 30 minutes. The supernatant was decanted in a 100 mL volumetric flask and diluted with distilled water up to mark of the flask.

Exactly 1 g of dry powdered sample (old leaves) was boiled with 100 mL of distilled water in a 200 mL conical flask for 30 minutes. Then, the mixture was centrifuged at 2000 rpm for 30 minutes. The supernatant was decanted in a 100 mL volumetric flask and diluted with distilled water up to the mark of the flask.

2.4.2 Extraction of Tea leaves with Ethanol

Exactly 1 g of dry powdered sample (young leaves) was boiled with 100 mL ethanol in a 200 mL conical flask for 30 minutes. Then, the mixture was centrifuged at 2000 rpm for 30 minutes. The supernatant was decanted in a 100 mL volumetric flask and diluted with ethanol up to the mark of the flask.

Exactly 1g of dry powdered sample (moderate leaves) was boiled with 100 mL of ethanol in a 200 mL conical flask for 30 minutes. Then, the mixture was centrifuged at 2000 rpm for 30 minutes. The supernatant was decanted in a 100 mL volumetric flask and diluted with ethanol up to mark of the flask.

Exactly 1 g of dry powdered sample (old leaves) was boiled with 100 mL of ethanol in a 200 mL conical flask for 30 minutes. Then, the mixture was centrifuged at 2000 rpm for 30 minutes. The supernatant was decanted in a 100 mL volumetric flask and diluted with ethanol up to the mark of the flask.

2.4.3 Extraction of Tea leaves with acetone

Exactly 1 g of dry powdered sample (young leaves) was boiled with 100 mL of acetone in a 200 mL conical flask for 30 minutes. Then, the mixture was centrifuged at 2000 rpm for 30 minutes. The supernatant was decanted in a 100 mL volumetric flask and diluted with acetone up to the mark of the flask.

Exactly 1 g of dry powdered sample (moderate leaves) was boiled with 100 mL of acetone in a 200 mL conical flask for 30 minutes. Then, the mixture was centrifuged at 2000 rpm for 30 minutes. The supernatant was decanted in a 100 mL volumetric flask and diluted with acetone up to mark of the flask.

Exactly 1 g of dry powdered sample (old leaves) was boiled with 100 mL of acetone in a 200 mL conical flask for 30 minutes. Then, the mixture was centrifuged at 2000 rpm for 30 minutes. The supernatant was decanted in a 100 mL volumetric flask and diluted with acetone up to the mark of the flask.

2.5 Preliminary phytochemical tests

Phytochemical investigation of sample was carried out according to the test tubes method.

2.6 Qualitative Determination of Tannins

Exactly 5 g of dried powder sample was boiled with 100 mL of distilled water for about 30 minutes and then cooled and filtered. 2 mL of 2 % gelatin solution was added to 5 mL of filtrate. A curdy white precipitate was observed which shows the presence of tannins.

2.7 Conformity of Extract Tannin by FT IR and TLC

FT IR and TLC tests for extract tannins were carried out by using a reference (standard) tannic acid at Research Centre of Monywa University. In TLC, R_f values of extract tannins and tannic acid were calculated by the following expression.

$$R_f = \frac{\text{Distance travelled by the solute from the point of origin}}{\text{Distance travelled by the solvent from the point of origin}}$$

2.8 Quantitative Determination of Tannin by Folin-Denis Method

Principle

Tannins reduce phosphotungstomolybdic acid in alkaline condition and produce a blue coloured complex. The optical density of this complex was measured at 660 nm wavelength.

2.8.1 Determination of tannins contents in distilled water extracted samples

1 mL of distilled water extracted sample solution (young leaves) and 75 mL distilled water were added in a 100 mL volumetric flask. 10 mL of sodium carbonate solution and 5 mL of Folin-Denis reagent were added into the flask. The flask was diluted with distilled water up to the mark of the flask. The flask was shaken well and allowed to stand for 30 minutes.

A blank solution was prepared when 1 mL of distilled water was used instead of 1 mL of distilled water extracted sample solution (young leaves). The blank solution contains all the compositions except sample extracted with distilled water.

The remaining distilled water extracted sample solutions (moderate leaves, old leaves and a mixture of leaves) and their blank solutions were prepared in the same as above distilled water extracted sample solution (young leaves).

Then, the absorbance of each solution at λ_{max} was measured. In this case, the spectrophotometer was also calibrated before measuring the absorbance of solution. (Buzarbarua, 2000)

2.8.2 Determination of tannins contents in ethanol extracted samples

1 mL of ethanol extracted sample solution (young leaves) and 75 mL distilled water were added in a 100 mL volumetric flask. 10 mL of sodium carbonate solution and 5 mL of Folin-Denis reagent were added into the flask. The flask was diluted with distilled water up to the mark of the flask. The flask was shaken well and allowed to stand for 30 minutes.

A blank solution was prepared when 1 mL of ethanol was used instead of 1 mL of ethanol extracted sample solution (young leaves). The blank solution contains all the compositions except sample extracted with ethanol.

The remaining samples (moderate leaves and old leaves) extracted with ethanol and their blank solutions were prepared in the same as above ethanol extracted sample solution (young leaves).

Then, the absorbance of each solution at λ_{max} was measured. In this case, the spectrophotometer was also calibrated before measuring the absorbance of solution.

2.8.3 Determination of tannins contents in acetone extracted samples

10 mL of acetone extracted sample solution (young leaves) and 75 mL distilled water added in a 100 mL volumetric flask. 10 mL of sodium carbonate solution and 5 mL of Folin-Denis reagent were added into the flask. The flask was diluted with distilled water up to the mark of the flask. The flask was shaken well and allowed to stand for 30 minutes.

A blank solution was prepared when 10 mL of acetone was used instead of 10 mL of acetone extracted sample solution (young leaves). The blank solution contains all the compositions except sample extracted with acetone.

The remaining samples (moderate leaves and old leaves) extracted with acetone and their blank solutions were prepared in the same as above acetone extracted sample solution (young leaves).

Then, the absorbance of each solution at λ_{max} was measured. In this case, the spectrophotometer was also calibrated before measuring the absorbance of solution.

3. RESULTS AND DISCUSSIONS

3.1 Results of Phytochemical Tests for Tea Leaves

The preliminary phytochemical tests for tea leaves were done. The results are shown in Table 1.

Table 1. Results of Phytochemical Tests for Tea Leaves

No.	Tests	Extract used	Test Reagent	Observation	Remark
1	Alkaloids	1% HCl	Wagner's reagent Dragendorff's reagent	Reddish brown ppt. Orange ppt.	+ +
2	Glycosides	Distilled water	10% lead acetate	White ppt.	+
3	Phenolic compounds	Distilled water	10%$FeCl_3$	Greenish blue colour soln	+
4	Reducing sugars	Distilled water	Benedict's solution	No deep colour	–
5	Tannins	Distilled water	10%$FeCl_3$, dil H_2SO_4	Bluish black colour soln	+
6	Saponins	Distilled water	Water, heat	Frothing	+
7	Polyphenols	Ethanol	1%$FeCl_3$, 1%$K_3Fe(CN)_6$	Greenish blue colour soln	+
8	Flavonoids	Ethanol	Mg ribbons, conc:HCl	Pink colour soln	+
9	Steroids	Ethanol	conc:H_2SO_4	Green colour soln	+
10	Terpenes	Ethanol	Acetic anhydride, $CHCl_3$, conc:H_2SO_4	No red colour soln	–

(+) = present (-) = absent

According to this Table, sample contains alkaloids, glycosides, phenolic compounds, tannins, saponins, polyphenols, flavonoids and steroids.

3.2 Result of the Qualitative Determination of Tannin

When aqueous extract of sample was treated with 2 mL of 2 % gelatin solution, a curdy white precipitate was formed. This precipitate is shown in Figure 2.

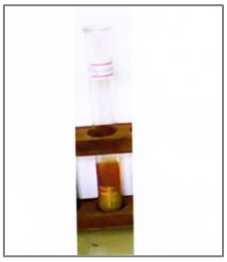

Figure 2. A curdy white precipitate

A curdy white precipitate shows that the extract of tea leaves contains tannins.

3.3 Results for Conformity of Extract Tannin

3.3.1 Result for conformity of extract tannins by FT IR

FT IR spectra of extract tannins and reference tannic acid are shown in Figures 3 and 4.

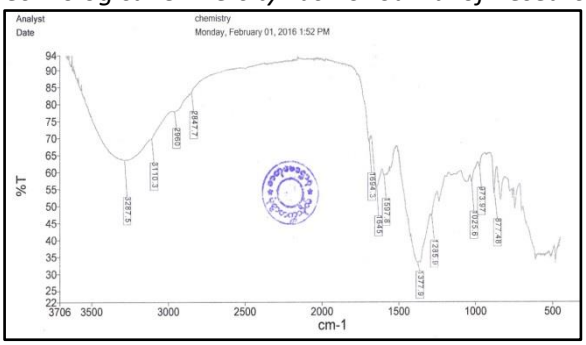

Figure 3. FT IR spectrum of extract tannins

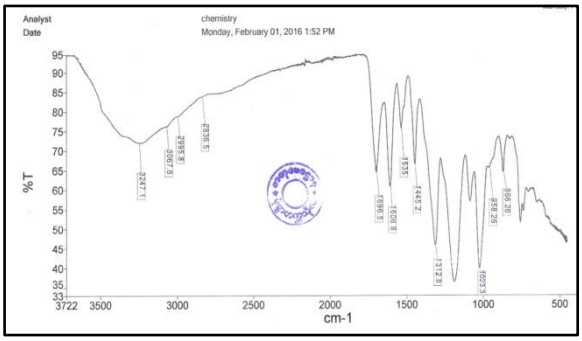

Figure 4. FT IR spectrum of (reference) tannic acid

The measured peaks of extract tannins and reference tannic acid are summarized in Table 2.

Table 2. Results of Comparable Peaks for Extract Tannin and Reference Tannic Acid

No	Parameters	Extract tannins (cm^{-1})	Reference tannic acid (cm^{-1})
1	OH Stretching vibration of alcohol group	3287	3247
2	C-C stretching vibration of asymmetric and symmetric sp^3 hydrocarbon	2960 2847	2995 2836
3	C-H stretching vibration of alkenic group	3110	3067
4	C=O stretching vibration of carbonyl group	1694	1696
5	C-H stretching vibration of aromatic ring skeleton	1645, 1597	1606, 1535
6	C-H bending vibration of methyl group	1377	1445
7	C-C bending vibration of sp^2 hydrocarbon	1285	1312
8	C-O-C stretching vibration of ether group	1025	1023
9	C-H bending vibration of trans or E alkenic group	973	958
10	C-H bending vibration of cis or Z alkenic group	877	866

According to these FT IR spectra, extract tannins is similar to that of reference tannic acid.

3.3.2 Result for conformity of extract tannins by TLC

TLC plates of extract tannins and tannic acid are given in Figure 5.

Figure 5. TLC plates of extract tannins and tannic acid

From TLC, the mobilities of extract tannins and tannic acid are found to be the same and R_f value is 0.12. R_f value is the same, it may be assumed that extract tannins and reference tannic acid are likely to be identical.

3.4 Results of Quantitative Determinations of Tannin by Folin-Denis Method

3.4.1 Results for determination of wavelength of maximum absorptivity

The measured absorbance of standard tannic acid solution at various wavelength values between 400 nm to 730 nm are given in Table 3 and absorption spectrum is shown in Figure 6.

Table 3. Results of Absorbance and Various Wavelengths of Tannic Acid

Wavelength (nm)	Absorbance (A)	Wavelength (nm)	Absorbance (A)
400	0.015	570	0.025
410	0.015	580	0.025
420	0.016	590	0.026
430	0.016	600	0.026
440	0.017	610	0.027
450	0.018	620	0.028
460	0.017	630	0.028
470	0.019	640	0.029
480	0.018	650	0.029
490	0.020	660	0.030
500	0.021	670	0.029
510	0.021	680	0.029
520	0.022	690	0.028
530	0.023	700	0.027
540	0.023	710	0.026
550	0.024	720	0.026
560	0.025	730	0.025

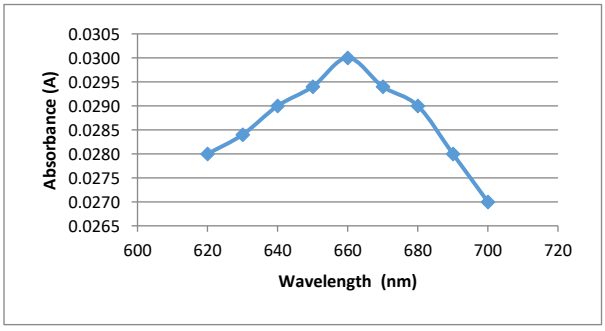

Figure 6. Plotting the absorbance versus wavelength

According to these results, the wavelength of maximum absorptivity λ_{max} is 660 nm.

3.4.2 Results for Calibration Curve of Standard Tannic acid

The measured absorbance at various concentrations of tannic acid in Table 4 and the calibration curve which is plotted the absorbance versus concentration in Figure 7 are presented.

Table 4. Relation between the Concentrations of Tannic Acid with Absorbances at λ_{max} =660 nm

No	Concentrationx 10^5 (g/mL)	Absorbance (A)
1	30	0.201
2	36	0.223
3	42	0.251
4	48	0.278
5	54	0.312
6	60	0.342

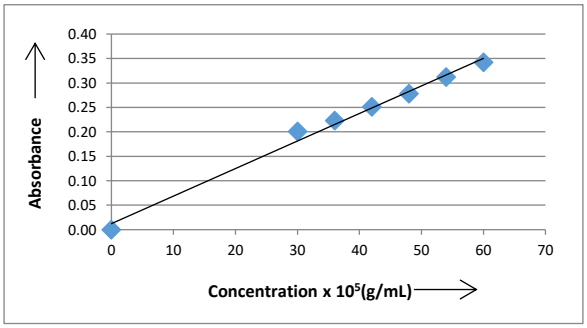

Figure 7. The calibration curve which is plotted the absorbance versus concentration

3.4.3 Results for determination of tannins in distilled water extracted samples

The results obtained from the determination of tannins contents in distilled water extracted samples are shown in Table 5.

Table 5. Results from Measured Absorbances of Distilled Water Extracted Samples

No	Distilled water extracted samples (Tea leaves)	Absorbance(A)	Concentration x 10^5 (g/mL)
1	Young leaves	0.319	55.2
2	Moderate leaves	0.286	48.6
3	Old leaves	0.216	34.8

From this curve, the contents of tannins in distilled water extracts are found to be 55.2 mg/g in young leaves, 48.6 mg/g in moderate leaves and 34.8 mg/g in old leaves, respectively.

3.4.4 Results for determination of tannins in ethanol extracted samples

The results obtained from the determination of tannins contents in ethanol extracted samples are shown in Table 6.

Table 6. Results from Measured Absorbances of Ethanol Extracted Samples

No	Ethanol extracted samples (Tea leaves)	Absorbance(A)	Concentration x 10^5 (g/mL)
1	Young leaves	0.305	52.8
2	Moderate leaves	0.234	39.0
3	Old leaves	0.225	36.6

From this curve, the contents of tannins in ethanol extracts are found to be 52.8 mg/g in young leaves, 39.0 mg/g in moderate leaves and 36.6 mg/g in old leaves, respectively.

3.4.5 Results for determination of tannins in acetone extracted sample

The results obtained from the determination of tannins content in acetone extracted samples are shown in Table 7.

Table 7. Results from Measured Absorbance of Acetone Extracted Samples

No	Acetone extracted samples (Tea leaves)	Absorbance(A)	Concentration x 10^5 (g/mL)
1	Young leaves	0.298	51.6
2	Moderate leaves	0.229	37.8
3	Old leaves	0.205	33.0

From this curve, the contents of tannins in acetone extracts are found to be 5.16 mg/g in young leaves, 3.78 mg/g in moderate leaves and 3.30 mg/g in old leaves, respectively.

Among the various types of Tea Leaves, young leaves have the highest content of tannins from the experimental results. Using different solvents, distilled water is more suitable than the two others for the extraction of tannins.

4. CONCLUSIONS

In this research work, tea leaves were collected from Homalin Township, Sagaing Region. Firstly, the phytochemical tests were performed. This revealed that the phytochemical constituents of tea leaves are alkaloids, glycosides, phenolic compounds, tannins, saponins, polyphenols, flavonoids and steroids. Then, tannins were extracted from tea leaves samples by using distilled water, ethanol and acetone as the solvents.

In additions, the qualitative test on extract sample was carried out by using 2 % gelatin solution. By adding 2 % gelatin solution, a curdy white precipitate shows the presence of tannins. Moreover, the tannins extracted from tea leaves were confirmed using a reference tannic acid by FT IR and TLC. By the comparison of two FT IR spectra, extract tannins is similar to that of reference tannic acid. According to the results of TLC, it may be assumed that extract tannins is identical to reference tannic acid because of the same R_f value.

Finally, the tannins contents in each of every extracts were determined spectrophotometrically by Folin-Denis

method. In this method, the contents of tannins in young leaves were found to be 55.2 mg/g for distilled water extract, 52.8 mg/g for ethanol extract and 5.16 mg/g for acetone extract.

The contents of tannins in moderate leaves were found to be 48.6 mg/g for distilled water extract, 39.0 mg/g for ethanol extract and 3.78 mg/g for acetone extract.

The contents of tannins in old leaves were found to be 34.8 mg/g for distilled water extract, 36.6 mg/g for ethanol extract and 3.3 mg/g for acetone extract.

Hence, distilled water is the best solvent compared to other solvents for tannins extraction from tea leaves. It may be seen that distilled water extracts gave the highest amount of tannins. Among the various types of tea leaves, young leaves extracted with different solvents is found to have the highest content of tannins.

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REFERENCES

[1] Bate-Smith and Swain (1962). "Flavonoid compounds". *Comparative biochemistry III*. New York: Academic Press.pp.75-809.

[2] Buzarbarua, A., (2000),"A Textbook of Practical Plant Chemistry", S.Chand and Co.Ltd., 7361, Ran Nagar, New Delhi.

[3] Cabrera, C., R. Artacho, and R. Gimenez, (2006). "Beneficial effects of green tea a review". *J.Am. Coll. Nutr*., **25**(2):79-99.]

[4] Clifford, M. N., and J. R. Ramirez-Martinez (1991), "Tannins in wet-processed coffee beans and coffee pulp." *Food Chemistry*, **40**(2):191-200.

[5] Funatogawa, K., S. Hayashi, and H. Shimomura, (2004). "Antibacterial activity of hydrolysable tannins derived from medicinal plants against *Helicobacter pylori*" *Microbiol Immunol*. **48**(4):251-61.

[6] Hagerman, A. E., (1998), "High molecular weight plant polyphenolics (tannins) as biological antioxidants." *J.Agric Food Chem*, **46**:1887-1892.

[7] Haslam, E., (1989), "*Plant Polyphenols, Vegetable Tannins" Revisited*. Cambridge University Press, Cambridge, UK

[8] Mukhtar, H., and N. Ahmad., (2000). "Tea polyphenols: Prevention of cancer and optimizing health." *Am.J.Clin.Nutr*., **71**(6): 16985-1702.

[9] Nanaka, G., and M. Nishizawa., (1990), "Anti-AIDS agents, 2:1 inhibitory effects of tannins an HIV reverse transcriptase and HIV replication in H, lymphocyte cells." *J.Nat, Prod. I*, **53**(3): 587-95.

[10] Pan, T., and J. Jankovic., (2003). "Potential therapeutic properties of green tea polyphenols in Parkinson's disease." *Drugs Aging*, **20**(10):711-21.

[11] Tariq, M., and A. Naveed., (2010). "The morphology, characteristics and medicial properties of *Camellia sinensis*." *J.Med. Plants Res*., **4**(19):2028-33.

PRODUCTION AND IDENTIFICATION OF CELLULOSE NITRATE FROM THE SAWDUST OF *Cephalostachyum Pergracile* Munro. (BAMBOO)

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ABSTRACT*:* In this paper, the sawdust of the tinwa bamboo was collected from the building material work of bamboo in Mahaaungmyae Township, Mandalay Region. The elemental analysis of the sawdust was carried out by EDXRF method. The cellulosic pulp was prepared by using 15 % sodium hydroxide. In order to know the effect of time on the yield of cellulosic pulp, refluxing periods were varied (1hr, 2hr, 3hr). The mixture of concentrated sulphuric acid, concentrated nitric acid and distilled water (14:9:2, V/V) was used as the nitrating reagent of cellulose. The cellulosic pulp of the sawdust samples was nitrated by using nitrating reagent. The yield percents of the cellulosic pulp and cellulose nitrate of the sawdust sample from different conditions were determined. The cellulose cloth, cellulose cotton and cellulose paper were also nitrated. The cellulose nitrate samples were characterized by burning test, floating test, diphenylamine test and manganese(IV) oxide test. The cellulose nitrate from sawdust sample was confirmed by FTIR spectroscopic method.

KEYWORDS: *cellulose, tinwa bamboo, cellulose nitrate, sawdust, EDXRF, FTIR*

1. INTRODUCTION

Bamboo is very special. It is a fast growing natural resource whose rate of biomass generation is unsurpassed in the plant kingdom. It is one of the most important non-wood forestry products and one of the most important agricultural plants in the world. There are about 1500 bamboo species in the world. Tinwa bamboo is one of many different bamboos. Tinwa bamboo is a clump-forming, evergreen bamboo with short rhizomes. The erect, thin-walled culms can be 10-30 meters tall and 50-75mm in diameter with internodes 30-45 cm long.

Tinwa bamboo is a self-regenerating natural resource, new shoots that appear annually ensure production after individual culms are harvested. In developing countries, it is a basic raw material with numerous traditional uses. Tinwa bamboo has many applications in rural industries (handicrafts, furniture, utensils and houses) and it is also widely used in modern wood and paper industries. Governments, research institutions and private enterprises are taking increased interest in the environmental and economic possibilities of bamboo. In the last decade, there has been a boom of manufacturing industries utilizing bamboo world-wide.

Sawdust as the name implies, consists of fine particles of wood produced from cutting with a saw. It is the main product of sawmills, a by-product from agricultural, forestry or architectural activities and can serve many useful purposes such as a mulch, fuel and sorbent material. Despite the highlighted utilities, if not adequately utilized, sawdust may constitute an environmental hazard.

Cellulose is one of many polymers found in nature. Wood, paper, and cotton all contain cellulose. Cellulose is an excellent fiber. Wood, cotton and hemp rope are all made of fibrous cellulose. Cellulose is made of repeat units of the monomer glucose. This is the same glucose which our bodies metabolize in order to live, but we can't digest it in the form of cellulose. Because cellulose is built out of a sugar monomer, it is called a polysaccharide.

Nitrocellulose is a highly flammable compound formed by nitrating cellulose through exposure to nitric acid or another powerful nitrating agent. When used as a propellant or low-order explosive, it was originally known as guncotton. Partially nitrated cellulose has found uses as a plastic film and in inks and wood coatings. The aim of this research is to produce and identify the cellulose nitrate from the sawdust of *Cephalostachyum pergracile* Munro.

2. THEORETICAL BACKGROUND

2.1 Sample Collection

The sawdust of tinwa bamboo was collected from the building material work of bamboo in Mahaaungmyae Township, Mandalay Region. The collecting sawdust was sieved by using 40 and 60 mesh size sieves. The sawdust sample (between 40 and 60 mesh size sieves) was stored in plastic bag and used throughout the research.

2.2 Determination of Elemental Compositions of Sawdust from Tinwa Bamboo

Elemental analysis of sawdust from tinwa bamboo was measured at Department of Physics, University of Mandalay, by applying EDXRF (Energy Dispersive X-ray Fluorescence Spectroscopy) method.

2.3 Identification of Cellulose Nitrate from Sawdust of Tinwa Bamboo by Fourier Transform Infrared Spectrum

The infrared spectra of sawdust of tinwa bamboo and cellulose nitrate from sawdust were measured by FTIR instrument at the Department of Chemistry, University of Mandalay.

3. EXPERIMENT

3.1 Preparation of Cellulosic Pulp from Sawdust of Tinwa Bamboo

The cellulosic pulp was extracted from the sawdust of tinwa bamboo under three conditions by using sodium hydroxide solution with various refluxing periods. It was refluxed three conditions with 15 % NaOH, for 1h, 2h and 3h respectively.

3.1.1 Procedure of cellulosic pulp from sawdust of tinwa bamboo

10g of sawdust sample and 100ml of 1% sodium sulphite solution were placed in a flat bottomed flask and refluxed for 1 hour and then cooled and filtered. 100ml of 15% sodium hydroxide solution was added to the residue and refluxed for 1 hour and then cooled and filtered. 100ml of 5% potassium hydroxide solution was added to the residue and shaken for 1 hour with 250 rpm and filtered. The residue was mixed with 100ml of 5% sodium hypochloride solution for 24 hours to bleach and filtered. The residue was washed with acetic acid and then distilled water until neutral to get the cellulosic pulp. Similarly, the above procedure was refluxed with 15% NaOH, for 2h and 3h.

3.2 Preparation of Cellulose Nitrate from Sawdust, Cellulose Cloth, Cellulose Cotton and Cellulose Paper

3.2.1 Procedure of cellulose nitrate from sawdust

1.5g of cellulosic pulp of sawdust and 15ml of nitrating reagent were placed into beaker in ice bath for 30 minutes. The mixture was shaken for 1 hour with 250 rpm and allowed to stand for 24 hours and then was poured into the beaker containing distilled water and allowed to stand for 24 hours and filtered. The residue was washed with distilled water until neutral and dried to get the cellulose nitrate.

3.2.2 Procedure of cellulose nitrate from cellulose cloth

5g of cellulose cloth and 25ml of nitrating reagent were placed into beaker in ice bath for 30 minutes. The mixture was shaken for 1 hour with 250 rpm and allowed to stand for 24 hours and then was poured into the beaker containing distilled water and allowed to stand for 24 hours and filtered. The residue was washed with distilled water until neutral and dried to get the cellulose nitrate. Similarly, the above procedure was repeated for cellulose cotton and cellulose paper.

3.3 Characterization of Cellulose and Cellulose Nitrate Samples by Physiochemical Properties

3.3.1 Burning test for the prepared cellulose nitrate

Burning of 1 g of cellulose nitrate of sawdust gave yellow flame without residue. Similarly, the above procedure was repeated for cellulose cloth and cellulose nitrate from cloth, cellulose cotton and cellulose nitrate from cotton and cellulose paper and cellulose nitrate from paper.

3.3.2 Floating test for sawdust, cellulose pulp and cellulose nitrate

For the floating test, each 0.5g of sawdust, cellulose pulp and cellulose nitrate was added into a test tube containing 2 mL of trichloroethylene. The flotation of sawdust and cellulose pulp can be seen. But, the cellulose nitrate was found to be sunk in trichloroethylene.

3.3.3 Diphenylamine test for cellulose nitrate from sawdust

For the diphenylamine test, the 9 mL of concentrated sulphuric acid, 1 mL of distilled water and 0.5 g of diphenylamine were mixed to get the mixture. 5 drops of mixture were added to 0.05 g of cellulose nitrate from sawdust. Blue colour was observed. Similarly, the above procedure was repeated for cellulose cloth and cellulose nitrate from cloth, cellulose cotton and cellulose nitrate from cotton and cellulose paper and cellulose nitrate from paper.

3.3.4 Manganese(IV) oxide test for cellulose nitrate from sawdust

For the manganese(IV) oxide test, the 5 drops of concentrated sulphuric acid and a few amount of potassium permanganate were placed in a petridish to get freshly prepared manganese(IV) oxide. 0.1 g of cellulose nitrate from sawdust was added immediately. Burning occurred with flame. Similarly, the above procedure was repeated for cellulose cloth and cellulose nitrate from cloth, cellulose cotton and cellulose nitrate from cotton and cellulose paper and cellulose nitrate from paper.

4. Results and Discussion

4.1 Determination of Elemental Compositions of Sawdust of Tinwa Bamboo

Elemental analysis of sawdust from tinwa bamboo was done at Department of Physics, University of Mandalay, by applying EDXRF (Energy Dispersive X-ray Fluorescence Spectroscopy) method as shown in Table 1.

Table 1 The Results of Elemental Compositions of Sawdust

No.	Elements	Relative abundance (%)
1.	Silicon	1.020
2.	Potassium	0.358
3.	Aluminum	0.242
4.	Chlorine	0.198
5.	Calcium	0.138
6.	Iron	0.126
7.	Phosphorus	0.065
8.	Titanium	0.016
9.	Sulphur	0.012
10.	Manganese	0.010

According to Table 1, the sawdust of tinwa bamboo was found to contain silicon, potassium, aluminum, chlorine, calcium, iron, phosphorus, titanium, sulphur and manganese. The content of silicon was the highest in sawdust.

4.2 Preparation of Cellulosic Pulp from Sawdust of Tinwa Bamboo

The cellulosic pulp was extracted from the sawdust of tinwa bamboo under three conditions using sodium hydroxide solution with various refluxing periods. The results are shown in Table 2.

Table 2 Yield Percent of Cellulosic Pulp from Sawdust Refluxed with 15% NaOH at Different Refluxing Periods

Sample	Refluxing Period (h)	Weight of Pulp (g)	Yield Percent of Pulp(%)
Sawdust (10 g)	1	5.7861	57.861
	2	5.9643	59.643
	3	6.0545	60.545

Among the refluxing period, the higher the refluxing time, the greater the yield percent of cellulose pulp was obtained.

4.3 Preparation of Cellulose Nitrate from Cellulosic Pulp

The cellulose nitrate was prepared from the cellulosic pulp under three conditions using sodium hydroxide solution with various refluxing periods. The results are shown in Table 3.

Table 3 Yield Percent of Cellulose Nitrate from Cellulosic Pulp Refluxed with 15% NaOH at Different Refluxing Periods

Sample	Refluxing Period (h)	Weight of Cellulose Nitrate (g)	Yield Percent of Cellulose Nitrate (%)
Cellulosic Pulp (1.5 g)	1	0.5526	36.8400
	2	0.6758	45.0533
	3	0.7126	47.5067

Among the refluxing period, the higher the refluxing time, the greater the yield percent of cellulose nitrate was obtained.

4.4 Characterization of Cellulose Nitrate Samples by Physicochemical Properties

The physicochemical properties of cellulose nitrate samples were studied by burning test, floating test, diphenylamine test and manganese(IV) oxide test. The results are shown in Table 4.

Table 4 Physicochemical Properties of the Various Cellulose Nitrate Samples

No.	Sample	Physical Properties		Chemical Properties	
	-	Burning Test	Floating Test	Diphenyl amine Test	Manganese (IV) Oxide Test
1.	Sawdust	Flame	Float	-	-
2.	Cellulose Nitrate of sawdust	Yellow flame	Sunk	Blue colour	Flame
3.	Cellulose cloth	Flame	-	-	-
4.	Cellulose nitrate of cloth	Yellow flame	-	Blue colour	Flame
5.	Cellulose cotton	Flame	-	-	-
6.	Cellulose nitrate of cotton	Yellow flame	-	Blue colour	Flame
7.	Cellulose paper	Flame	-	-	-
8.	Cellulose nitrate of paper	Yellow flame	-	Blue colour	Flame

From the burning test, they are found that sawdust, cellulose cloth, cellulose cotton and cellulose paper gave flame. And cellulose nitrate of sawdust, cellulose nitrate of cloth, cellulose nitrate of cotton and cellulose nitrate of paper gave yellow flame without residue.

From the floating test, the flotation of sawdust can be seen and the cellulose nitrate was found to be sunk.

From the diphenylamine test, cellulose nitrate of sawdust, cellulose nitrate of cloth, cellulose nitrate of cotton and cellulose nitrate of paper observed blue colour.

From the manganese(IV) oxide test, cellulose nitrate of sawdust, cellulose nitrate of cloth, cellulose nitrate of cotton and cellulose nitrate of paper occurred flame.

4.5 Identification of Cellulose Nitrate from Sawdust of Tinwa Bamboo by FTIR Spectrum

The infrared spectra of sawdust of tinwa bamboo and cellulose nitrate from sawdust were taken by FTIR instrument at the Department of Chemistry, University of Mandalay. The results obtained are illustrated in Table 5 and Table 6 and the spectrums are shown in Figure 1 and Figure 2.

Table 5 Characteristic Absorption of FTIR of Sawdust of Tinwa Bamboo

No.	υ_{max} (cm^{-1})	Assignments (Functional group)
1.	3225	-OH stretching vibration
2.	3080	-CH stretching vibration of sp^2 hydrocarbon
3.	2914	-CH stretching vibration of sp^3 hydrocarbon
4.	1739, 1705	C=O stretching vibration of carbonyl group of esters

5.	1510	C=C stretching vibration
6.	1458	-C-H bending vibration
7.	1120, 1043	C-C stretching vibration
8.	1250, 1100	C-O-C stretching vibration of ethers
9.	594	C-C bending vibration

From the FTIR spectrum of sawdust, it was found that the sawdust of tinwa bamboo contains –OH functional group, C=O functional group, C-O-C functional group, alkenic functional group, sp^2 hydrocarbon and sp^3 hydrocarbon.

Table 6 Characteristic Absorption of FTIR of Cellulose Nitrate from the Sawdust of Tinwa Bamboo

No.	υ_{max} (cm^{-1})	Assignments (Functional group)
1.	3464, 3400	-OH stretching vibration
2.	2922, 2858	-CH stretching vibration of sp^3 hydrocarbon
3.	3101, 3001	-CH stretching vibration of sp^2 hydrocarbon
4.	1728, 1641	N-O stretching vibration of NO_2 group
5.	1539	C=C stretching vibration
6.	1346, 1282	C-C stretching vibration
7.	1111	C-O-C stretching vibration of ether
8.	866, 758	N-O bending vibration of NO_2 group

From the FTIR spectrum of cellulose nitrate from sawdust of tinwa bamboo, it was found that the cellulose nitrate contains –OH functional group, C-O-C functional group, alkenic functional group, NO_2 group, sp^2 hydrocarbon and sp^3 hydrocarbon.

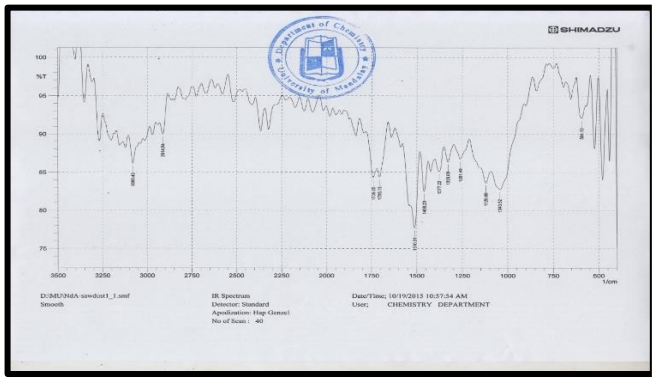

Figure 1 FTIR Spectrum of sawdust

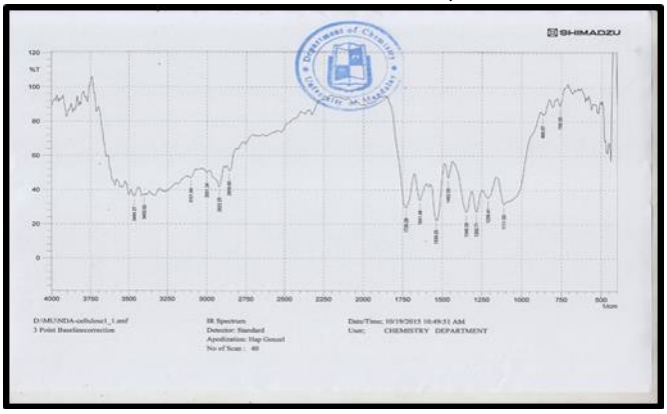

Figure 2 FTIR Spectrum of cellulose nitrate

5. CONCLUSIONS

In this research work, the sawdust of tinwa bamboo was collected from the building material work of bamboo in Mahaaungmyae Township, Mandalay Region. From EDXRF analysis, the sawdust contains silicon, potassium, aluminum, chlorine, calcium, iron, phosphorus, titanium, sulfur and manganese. The content of silicon was the highest.

The cellulosic pulp was prepared by using 15 % sodium hydroxide. Refluxing with 15 % sodium hydroxide for 1h gave the cellulosic pulp in 57.624-57.861 %. Refluxing with 15 % sodium hydroxide for 2h gave the cellulosic pulp in 58.437-59.643 %. Refluxing with 15 % sodium hydroxide for 3h gave the cellulosic pulp in 59.824-60.545 %. In order to know the effect of time on the yield of cellulosic pulp, various refluxing periods were used. When refluxing time increases, the yield percent of cellulosic pulp also increases.

The cellulosic pulp of the sawdust of tinwa was nitrated by using sulphuric acid and nitric acid mixture as nitrating reagent. The yield of the cellulose nitrate depends on the amount of sodium hydroxide used and refluxing period for the preparation of cellulosic pulp. The more amount of sodium hydroxide used, the greater yield of cellulose nitrate obtained. The longer the refluxing period, the more yield of cellulose nitrate obtained. The cellulose cloth, cellulose cotton and cellulose paper were also nitrated.

The cellulose nitrate samples were characterized by burning test, floating test, diphenylamine test and manganese(IV) oxide test. The cellulose nitrate from sawdust sample was confirmed by FTIR spectroscopic method.

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REFERENCES

[1] *I.M Adekunle. (2010). "Production of Cellulose Nitrate Polymer from Sawdust", Abeokuta, Nigeria, Vol.7 (3), pp. 709-716.*

[2] *Klemm, Dieter; Heublein, Brigitte; Fink, Hans-Peter; Bohn, Andreas (2005). "Cellulose: Fascinating Biopolymer and Sustainable Raw Material", Angew. Chem. Int. Ed. Vol.44 (22), pp. 3358–3393.*

[3] Okoro A, Ejike E N and Anunso C, (2007), "Journal of Engineering and Applied Sciences", Vol.2(2), pp. 282-285.

[4] *Source: Adams, C. (n.d.). (2003), "Bamboo Architecture and Construction with Oscar Hidalgo", Vol.5.*

Preparation and Characterization of Soap from Alkaline of Waste Millet Stalks by Traditional Method

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ABSTRACT*:* In this paper, soap plays an essential role in our daily life. It is an important material in dealing with the health of human society. The aim of this study was to prepared soap that is highly quality with minimal damage to human skin without chemical. By utilizing traditional method, millet stalks ash were used to extract alkaline. The extract of alkaline has been used to produce soap by the use of traditional method. In the study works, to determine the chemical analysis of palm oil for soap making process such as saponification value (205.87 mgKOH/g), iodine value (54.54I_2/100g), acid value (0.091 mgKOH/g), and free fatty acid 6.6%. And then, the resulting soap was subjected to physiochemical test, moisture content (3.21%), pH determination (10.2), texture (hardness), reaction of soap with metals, test for acidity/alkalinity of soap, foam ability test (1.2 cm) and reaction of soap with hard water. According to the result, the soap produced by palm oil, that is indicated a very good properties.

KEYWORDS: ***traditional method. millet stalks ash, alkaline, Palm oil, , Saponification, Soap***

1. INTRODUCTION

Soap is common cleaning agent well known to everyone. . Many authors defined soap different ways Warra[1]. It was regarded as any cleaning agent manufactured in granules, bars, flakes or liquid form obtained from by reaction salt of sodium or potassium of various fatty acids that are of natural origin (salt of non-volatile fatty acids). Soap can be also said to be any water-soluble salt of fatty acids containing eight or more carbon atoms. Soaps are produced for varieties of purpose ranging from washing, bathing, medication etc [1].

Today, caustic soda (KOH or Alkali) is an essential ingredient to produce soap. Alkali, which is water soluble base, are usually hydroxide or oxide of potassium or sodium. Alkali can be produced locally from ashes as "potash" by extraction with water. It is generally believed that the highest soluble metal is potassium. However, potassium depends on the species of the plant material and the type of soil where the plant grows [2].

Oil palm (Elaeisguineensis), African tree in the palm family (Arecaceae), cultivated as a source of oil. The oil palm is grown extensively in its native West and Central Africa, as well as in Malaysia and Indonesia [3]. Palm oil is an edible vegetable oil derived from the mesocrap (reddish pulp) of the fruit of the oil palms, primarily the African oil palm Elaeisguineensis [4]. Palm oil is used in making soaps, cosmetics, candles, toothpaste, shampoo, biofuels and lubricating greases and in processing tinplate and coating iron plates. Palm kernel oil from the seeds is used in manufacturing such edible products as margarine, ice-cream, chocolate, confections, cookies and bread as well as many pharmaceuticals. The cake residue after kernel oil is extracted is a cattle feed. The plant is also grown as an ornamental in many subtropical areas [3].

2. MATERIALS AND METHODS

2.1 Samples Collection

The waste millet stalks samples were collected from a farm of Kyaik Pha Nae Estate, Mawlamyine Township, Mon State. Palm oil was collected from Padon Mar Soap Factory, Paung Township, Mon State. These samples were shown in figure 1and figure 2.

Figure 1. Palm Oil

Figure 2. Waste Millet Stalks

2.2 Chemical Analysis of Palm Oil

2.2.1 Determination of saponification value in Palm Oil

Accurately weighed oil sample (Ca. 5g) was taken in a round-bottomed flask (250 cm^3) and alcoholic potassium hydroxide solution (25 cm^3) was then added and refluxed gently in water bath using an air condenser for one hour with occasional shaking. When the sample was saponified completely, the inside of the condenser was washed down with a little distilled water. The condenser was added and the excess alkali was titrated with standard hydrochloric acid (0.5 M) until pink colour just disappeared. The saponification value can be calculated from the equation [5].

Saponification value = $28.05 \times (V_b - Vs)/W$

Where V_b = Volume of hydrochloric acid in blank titration

Vs = Volume of hydrochloric acid in sample

W = Weight of the sample

2.2.2 Determination of Iodine Value in Palm Oil

Accurately weighed oil sample (Ca. 0.3 g) was taken in a conical flask (500 cm^3) with stopper. It was dissolved in carbon tetrachloride (20 cm^3). The Wijs solution (25 cm^3) was pipetted into the flask and the flask was stoppered and swirled to ensure intimate mixing. The flask was then stored in a dark place for 1 hour and 15 % potassium iodide solution (20 cm^3) was added to the solution followed by distilled water (100 cm^3).

The solution was then titrated with standard sodium thiosulphate solution (0.097M) using a few drops of 1 % starch indicator until the blue colour just disappeared. A blank titration was also carried out simultaneously using the same procedure but amitting the oil. The iodine value is given by the expression [5].

Iodine Value = $(V_b - Vs) \times M \times 12.69/W$

Where V_b = Volume of sodium thiosulphate solution required for the titration of the blank

V_s= Volume of sodium thiosulphate used for determination

M= Molar concentration of sodium thiosulphate solution

W= Weight of sample

2.2.3. Determination of Acid Value in Palm Oil

10g of oil was weighed into conical flask and 50 ml of denatured alcohol (ethanol and diethyl ether in ratio 1:1) was added and shaken, 2 drops of phenolphthalein indicator was added, it was then titrated against 0.1 N sodium hydroxide. The acid value can be calculated by using the formula [6].

Acid Value = $100 \times 2.82 \times V/W \times 1000 \times 1$

V = Volume of NaOH used in sample titration

W = Weight of sample

2.2.4 Determination of Free Fatty Acid in Palm Oil

1g of oil was boiled with 50 ml of ethanol and allows to cool, 2 drops of phenolphthalein indicator was added, it was then titrated against 0.1 N NaOH until pink colour was obtained. The free fatty acid was calculated by using formula [6].

% FFA = $T \times 2.82/W$

Where W = Weight of sample

T = Volume of NaOH

2.3. Extracting of Alkaline from Millet Stalk Ash by Analysis of Traditional Method

The millet stalk sample was dried in a laboratory dry oven, (ESCO, OFA-54-8, USA) at 40 ℃ to a constant weight. After then, which they were burned into ashes using muffle furnace at 550 ℃ for 5 hours [5].

100g of millet stalk ash were taken using calabash siever that was placed on cleaned receiving bucket. Water was then added, and then, set up was allowed to stand until the filtrates dried out. Addition of water continues until the volume of filtrate was up to 2 liters. The filtrate was then concentrated on a cleaned cooking pot, to about one – quarter of its original volume. After millet stalk were burned the ash contains potassium oxide K_2O. When this K_2O is mixed with water, the strong base potassium hydroxide (KOH) is formed [7].

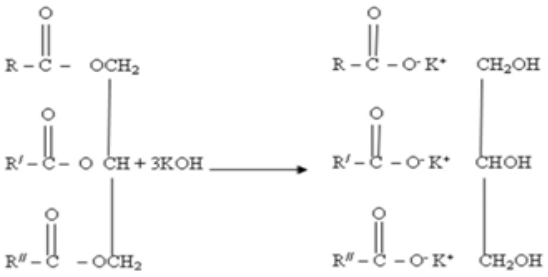
$$K_2O + H_2O \longrightarrow 2KOH$$

Equation for the Reaction.

2.4 Preparation of Soap from Alkaline of millet stalk Ash

50ml of the palm oil was added slowly into the concentrated solution and resulting mixture was stirred vigorously to avoid sedimentation process. 10 ml of the palm oil was added after each interval of 10 mins for four times. The stirring continued until all the surface of the mixture was covered with foam and the lather volume kept increasing until it reached its peak. Heating process was stopped and the soap was allowed to cool into a solid mass [8].

Figure 3. Prepared Soap



Triglycerides (Fat or Oil) — A mixture of potassium carboxylates and glycerol (soap)

Figure 4. A mixture of potassium carboxylates and glycerol (soap)

From the above equation, a triglyceride reacts with KOH to form a mixture of potassium carboxylates and glycerol. In the equation soap is a salt composed of mixture of carboxylate anions and univalent cation. A mixture of anions is formed because each triglyceride molecule contains a variety of fatty acid residue and because of particular fat or oil itself is a mixture of molecules. Potassium soaps are more soluble than sodium soaps and readily produce lather [8].

2.4.1 Moisture Content in Prepared Soap

Moisture content was determined by drying 10 g of the sample to a constant weigh at 105 ℃according to AOCO[9].It was allowed to cool and then reweighed. The % moisture content was calculated from the formula.

% of Moisture Content = W_1/W_2 x 100%

Where

W_1 = weight of soap after drying

W_2 = weight of soap before drying

2.4.2 Determination of pH in Prepared Soap

The pH of soap prepared was determined using a pH meter (pH 51), 10g of the soap shaving was weighed and dissolved in distilled water in a 100 cm^3 volumetric flask. This is made up to prepare 10% soap solution. The electrode of the pH meter was inserted into the solution. The pH was recorded as described by [10].

2.4.3 Reaction of soap with metals

5ml of the soap, 2ml of 4% NaCl, NH_4Cl, and $FeCl_3$ were added in test tubes and each of the test tube was shaken steadily. So that, the formation of precipitation was observed [11].

2.4.4 Teat for Acidity / Alkalinity of Soap

5ml of soap in a test tube and a few drop of phenolphthalein indicator was added and colour change was observed [11].

2.4.5 Foam Ability Test

About 2.0g of the soap (shavings) was added to a 500cm^3measuring cylinder containing 100cm^3 of distilled water. The mixture was shaken vigorously so as to generate foams. After shaking for about 2 minutes the cylinder was allowed to stand for about 10minutes. The height of the foam in the solution was measured and recorded [12].

2.4.6 Reaction of Soap with Hard Water

Soap is a good cleaning agent, its cleaning capacity is reduced when used in hard water. Hardness of water is due to the presence of sulphates, chlorides or carbonate salts of Ca^{2+} or Mg^{2+} ions. Soaps are sodium or potassium salts of long chain fatty acid. When soap is added to hard water, the Ca^{2+} and Mg^{2+} ions present in hard water react with soap. The sodium salts present in soaps are converted to their corresponding calcium and magnesium salts which are precipitated (white orgrey scum)[13].

3. RESULTS AND DISCUSSIONS

3.1. Results

Table.1. Result of chemical Analysis in palm oil and FAO Standard

S/N	Parameters	Palm Oil	FAO Standard
1	Saponification value (mg/KOH/g)	205.87	189-199
2	Iodine value (I_2/100g)	54.54	50-55
3	Acid value (mg/KOH/g)	0.091	-
4	Free fatty acid (%)	6.6	2-7

Table 2. Physicochemical Parameter of Prepared Soap

S/N	Parameters	Observations
1	pH	10.2
2	Color of soap solution	White
3	Texture	Hard and rough
4	Foam height (cm)	1.2
5	moisture content (%)	3.21
6	NaCl	White precipitate observed with foam
7	NH_4Cl	White precipitate observed with foam
8	$FeCl_3$	Thick brown precipitate observed

9	Phenolphthalein	Deep pink colour observed
10	Reaction with hard water	White or grey scum

3.2. Discussions

As a result of the calculation, the saponification value (205.87mgKOH/g) and iodine value (54.54) have been found in the raw material (palm oil). Therefore; the lower iodine value, the higher the saturated fatty acid (palmitic acid). Saponification value reveals that fats and oils are suitable to use in soap making, it must have saponification value greater than 200. This is because the saturated fatty acids can render hardness in soap and high detergent qualities. Palm oil is the best choice for soap-making.

In this work, acid value was observed to be 0.091 mgKOH/g. Higher acid value materials allow faster appearing but less stable suds creation. Lower acid value materials allow slower to appear but more stable suds formation [14]. Lower acid value in soap indicates good cleaning capacity.

In this study free fatty acid value was found to be 6.6 %. Free fatty acid content is one of the important parameter in palm oil industry. Oil which are high in free fatty acids content have poor quality of oil and suffer significant losses during refining process. Low free fatty acid content in palm oil produced good physicochemical properties of palm oil products and could be used for industrial applications.

The content of moisture 3.21%, it have been freshy prepared.

The pH of prepared soap is 10.2 signifying that the soap is strongly basic in nature. This is basic properties for friendly with skin.

Due to salting process, soap is maintained as white colour. Removal of glycerol through the process of salting out, the texture of soap was hardness and rough.

The lower iodine value in palm oil contributes to hardness.

The foam height was observed to be 1.2cm which is lower in comparism in cotton oil (4.5) and castrol oil (1.6).

Sodium and potassium salts in common soaps are soluble in water but Ca^{2+} and Fe^{3+} the metal cations are insoluble complexs with (soap) and hard water. This is one of the disadvantages of the soap over detergent. However, NaCl, and NH_4Cl do not foam insoluble complexes with soap. The precipitating depends on the degree of precipitating agent used. The soap made with cold process is better quality and it is recommended to use in hard water compared to other soap samples.

Pink colouration alkaline property, is observed on addition of phenolphthalein to the soap sample. The pink colour was as a result of the interaction between the OH^- ions form solution and H^+ ion forms the indicator. In the soap solution, the OH^- ion combines with H^+ ion from phenolphthalein to obtained unionized water molecule.

Reaction of soap with hard water the Ca^{2+} and Mg^{2+} ions present in hard water react with soap.The potassium salts present in soaps are converted to their corresponding calcium and magnesium salts which are precipitated (white orgrey scum).The insoluble scum sticks on the clothes and so the cleaning capacity of soap is reduced.

4. CONCLUSIONS

In this research, to prepare the minimal damage human skin soap the chemical properties of raw material (palm oil) was analyzed before the preparation of soap. In this results, it has higher saponification value, lower iodine value, high percent of fatty acid, produced stable creamy leather with soap and its ability to add hardness. the raw material revealed that palm oil could be used as good oil for soap making.

The prepare soap was analyzed by testing its moisture content, reaction of soap with metal, acidity/alkalinity, hardness, pH, phenolphthalein and foam height. According to the data of the analysis, it indicated that the prepared soap is good quality for bathing purposes.

Therefore, traditional method is more effective for preparation of soap without chemical (KOH).KOH (alkaline), which is produced by waste millet stalksash prevents human skin to damage.

It is recommended to wash hands with soap several times aday for 20 seconds to protected against germs.

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REFERENCES

[1]Warra, A. A. (2013) Soap making in Nigeria using indigenous technology and raw materials, African Journal of Pure and Applied Chemistry, 7(4):139-145

[2] Onyegbado, C, O, Lyagha, D. T. and Offor, O. J. (2002) Solid Soap production using plantain peels ash as source of alkali Journal of Applied Science and Enviromental Management, 6: 73-77

[3]Thomas Alfred 'Fats and Oiley V C Hils". Ullmann'sEncyclopedia of Industrial Chemistry, WeinheimWiley.VCH. (2002).

[4] Unknown, https; // enem.wikipedia.org > wiki.

[5] Aung Win Kyaw Thesis, "Soap Quality of Locally Available and Imported Soap Sample with Respect to Total Fatty Matter", University of Mawlamyine 1997.

[6] Atiku, F.A, "Production of Soap Using Locally Available Alkaline Extract from Millet Stalk: A Study on Physical and Chemical Properties of Soap", September 2014.

[7] Sani, N.A. and Hassan, L.G. Preparation and Analysis of the Physical and chemical properties of from Lageneria siceraria (bottle ground) Seed Oil. Nomoda Tech. Scope Journal. 7 (1), 23 -32, (2007).

[8] Schuman, K. and Siekman, K. Soap in Ullmanna's. Encyclopedia of industrial Chemistry. Wiley-VCH, Weinheim, (2005).

[9]AOAC (2000) Official Method 981.11, Oils and FatsPrepation of test sample, 17th edition.

[10]Umar,M.A.Cosmetics,Soaps, Detergents and NASDAC's Regulatory Requirements. A paper presented at training workshop for small and medium scale enterprises organzed by UNDP/JCSL and Ministry of Commerce and Industry, Maiduguri, Borno State, Nigeria. Nigeria (2002).

[11]Mabrouk, S.T,Marking useable quality, opaque or transparent soap, Journsal of Chemical Education. 18(10); 1534-1537, (2005).

[12] Warra, A.A., Hassan, L.G., Gunu, S.Y. and Jega, S.A, "Cold Process Synthesis and Properties of Soaps Prepared from Different Triacylglycerol Sources

[13] Sahil, In the search if sincerity, "Cleaning Capacity of Soap with Hard and Soft water (Theory) : Class 10 Chemistry", Feb 28, 2017.

[14] Luis Spitz," in Soap Manufacturing Technology".(2nd Edition) 2016