# Antimicrobial activities and Characterization of Isolated Compound from the Stem Bark of *Couroupita Guianensis* Aubl. (Amyauk-San-Bin)

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DOI: 10.29322/IJSRP.9.07.2019.p9182
http://dx.doi.org/10.29322/IJSRP.9.07.2019.p9182

**Abstract**: In this paper, the Myanmar indigenous medicinal plant, *Couroupita Guianensis* Aubl. (Amyauk-San-Bin), was studied. The phytochemical constituents of *C. Guianensis* was investigated and the antimicrobial activities of n-hexane, dichloromethane, ethyl acetate, ethanol and methanol extracts of *C. Guianensis* was tested by Agar-well diffusion method on six selected organisms *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonos aeruginosa*, *Bacillus pumilus*, *Candida albican* and *E-coli*. The isolation and characterization of organic compounds from the extracts of *C. Guianensis* bark was carried by Thin Layer Chromatography (TLC) and FT IR. The isolated compounds (I, II and III) from ethyl acetate extract of the bark of *C. Guianensis* were studied by Thin Layer and Column Chromatography (TLC). The yield percent of compound I was found to be 1.92% ( 0.05 g) base upon the ethyl acetate crude extract, that of compound II was found to be 0.38% ( 20 mg) and that of compound III was found to be 0.76 % (20 mg) respectively.

***Key words***: *Couroupita Guianensis* Aubl., antimicrobial activities, Thin Layer Chromatography, FT IR, dichloromethane.

## Introduction

Medicinal plants have been in use for treatment of various diseases all over the world. Medicinal plants are used as traditional form of providing relief to several diseases. Presently, millions of adults are depending on medicinal plants for their primary health care needs. Medicinal plants have been a major source in the maintenance of health, as well as in the prevention, improvement or treatment of physical and mental illness.

Herbal medicines certainly have over a millenary history, especially through the Orient. Although, the use of these medicines in the western world is decidedly amplifying, most of them are not yet acceptable scientific evidences to support this conviction. Various medicinal plants have been identified and modern scientific approaches have been used to study their authenticity, safety and efficacy of their therapeutic uses.

*Couroupitaguianensis* Aubl. (Myanmar name Amyauk-San-Bin) is also called as “Cannonball tree” or “Sal tree”. The effects of the Cannon ball tree in medical use are strong. As when using any natural medicine, the correct dosage is vital. In medicinal use, the flowers, leaves, bark and fruit flesh are used. The bark of Cannon ball tree malaria, antibiotic, antifungal, antiseptic and analgesic qualities. The trees are used to cure colds and stomach aches.

**Botanical Description**

| | |
|---|---|
| Scientific name | - *Couroupita Guianensis* Aubl. |
| Family name | - Lecythidaceae |
| Local name | - Amyauk-San-Bin |
| Distribution | - Amazonian Colombia, Northen Venezuela, Guyana, Surinan, French Guiana, Amazonian Ecuador, Amazonian Peru, eastern and southwestern Amazonian Brazil and Myanmar |
| Part of Use | - Bark |
| Medicinal Use | - malaria, antibiotic, antifungal, antiseptic and analgesic qualities |

**Figure1. Plant, flower and bark of *Couroupita Guianensis* Aubl.**

## Experimental

### Instrumentation and Materials

### Instrumentation

The occurrence of UV absorption on TLC plate was checked by UV detector and iodine vapor. The apparatus for extraction and chromatography were used with common laboratory equipments.

### Materials

Before the research work was taken all the commercially available reagent and solvent were distilled. Analytical and preparative thin-layer chromatography was performed by using precoated silica gel plates. Silica gel (Merck-Co., Inc., Kieselgel 60, 70-230 Mesh ACTM) was used for column chromatography.

### Sample Collection and Preparation

Myanmar medicinal plant Amyauk-San-Bin was collected from Magway Township, Myanmar.

### Phytochemical screening

The air-dried powdered sample was subjected to preliminary phytochemical test in order to find out the types of phytochemical constituents such as alkaloid, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, steroid, polyphenol and terpene present in sample according to appropriate reported methods.

### Test for Alkaloid

Dried powdered sample (2 g) was boiled with 1% HCl for about 10 minutes, allowed to cool and then filtered. The filtrate was divided into two portions in test tubes. The first portion was then tested with Dragendorff's reagent and the second with Wagner reagent respectively. The yellow precipitates indicates the presence of alkaloids

### Test for Flavonoid

Dried powdered sample (2 g) was extracted with 95% ethanol (25 $cm^3$) and concentrated alcoholic HCl solution (4 $cm^3$) was treated with a few pieces of magnesium turning and a few drops of concentrated sulphuric acid. The appearance of pink colour solution indicates the presence of flavonoids.

### Test for Steroid

Dried powdered sample (2 g) was introduced into a round-bottomed flask followed by the addition of petroleum ether. The mixture was kept on water-bath under reflux for 15 minutes and filtered.The filtrate was treated with acetic anhydride (3 drops) and a few drops of concentrated sulphuric acid were carefully added and shaken. The mixture was left in a dark place for a few minutes. The appearance of greenish blue colour solution indicates the presence of steroids.

### Test for Terpene

Dried powdered sample (2g) was boiled with ethanol 25 $cm^3$ for about 10 minutes and filtered, 3 drops of acetic anhydride, 1 $cm^3$ of chloroform and one drop of concentrated sulphuric acid were added to ethanol extract and recorded the observed colour. Reddish brown coloration indicates the presence of terpene.

### Test for Glycoside

Dried powdered sample (2 g) was boiled with distilled water (100 $cm^3$) for about 10 minutes, allowed to cool and filtered. The filtrate was treated with a few drops of 10% lead acetate solution. The formation of white precipitates indicates the presence of glycosides.

### Test for Reducing Sugar

Dried powdered sample (2g) was boiled with distilled water for about 10 minutes and then filtered. The filtrate was boiled a few drops of Benedict's solution for about 2 minutes. The formation of brick red precipitate indicates the presence of reducing sugars.

**Test for Polyphenol**

Dried powdered sample (2 g) was extracted with ethanol for 10 minutes. It was alloed to cool and filtered. The filtrate was added to the mixture of 1% $FeCl_3$ (1 mL) and 1% $K_3Fe(CN)_6$ (1 mL). The appearance of greenish blue colour solution indicates the presence of polyphenol.

**Test for Saponin**

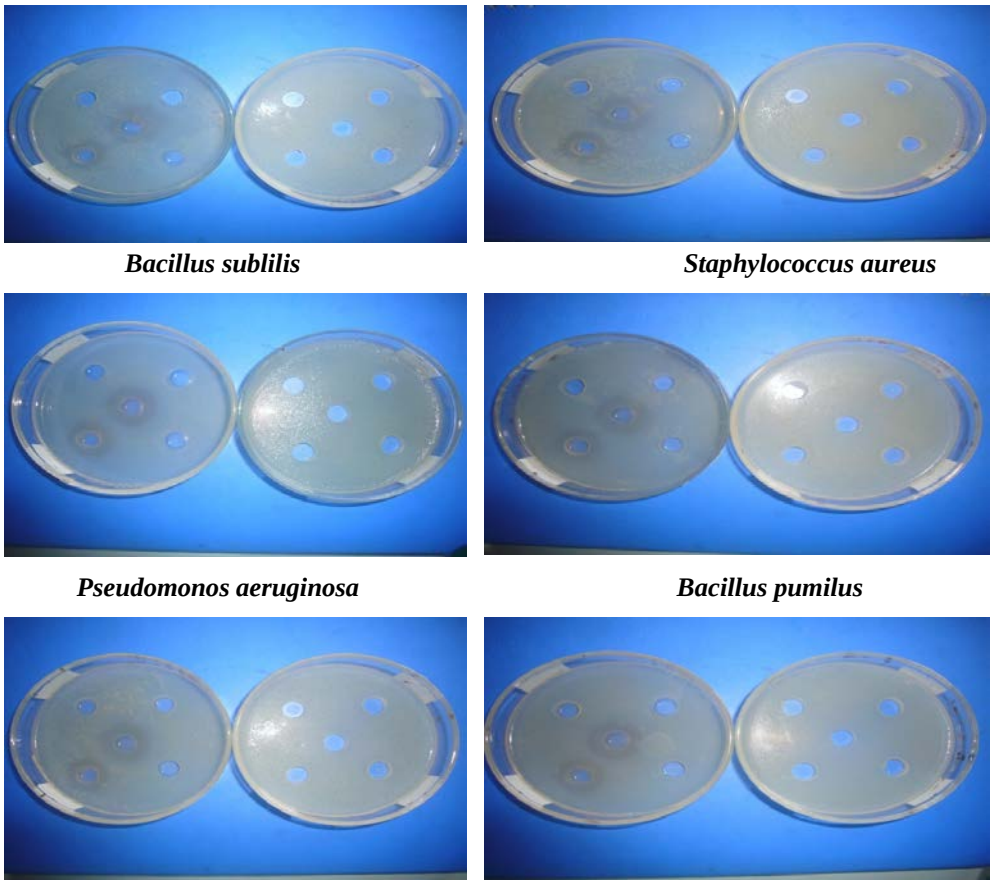
Dried powdered sample (2 g) was put into the test tube followed by the addition of distilled water. The mixture was vigorously shaken for a few minutes, allowed to settle for 10 minutes. Formation of stable foams indicates the presence of saponin.

**Test for Phenolic Compound**

Dried powdered sample (2 g) was boiled with distilled water and filtered. The filtrate was treated with a few drops of 10% ferric chloride solution. The brown colour solution indicates the presence of phenolic groups.

**Determination of Antimicrobial Activity by Agar Well Diffusion Method**

Antimicrobial activities of different crude extracts of Amyauk-San-Bin was screened in vitro by agar well diffusion method on nutrient agar medium. The studies were performed at CRDT (Central Research Development and Technology), Insein, Yangon as shown in Figure (2).



***Bacillus sublilis*** ***Staphylococcus aureus***

***Pseudomonos aeruginosa*** ***Bacillus pumilus***

***Candida albican*** ***E-coli***

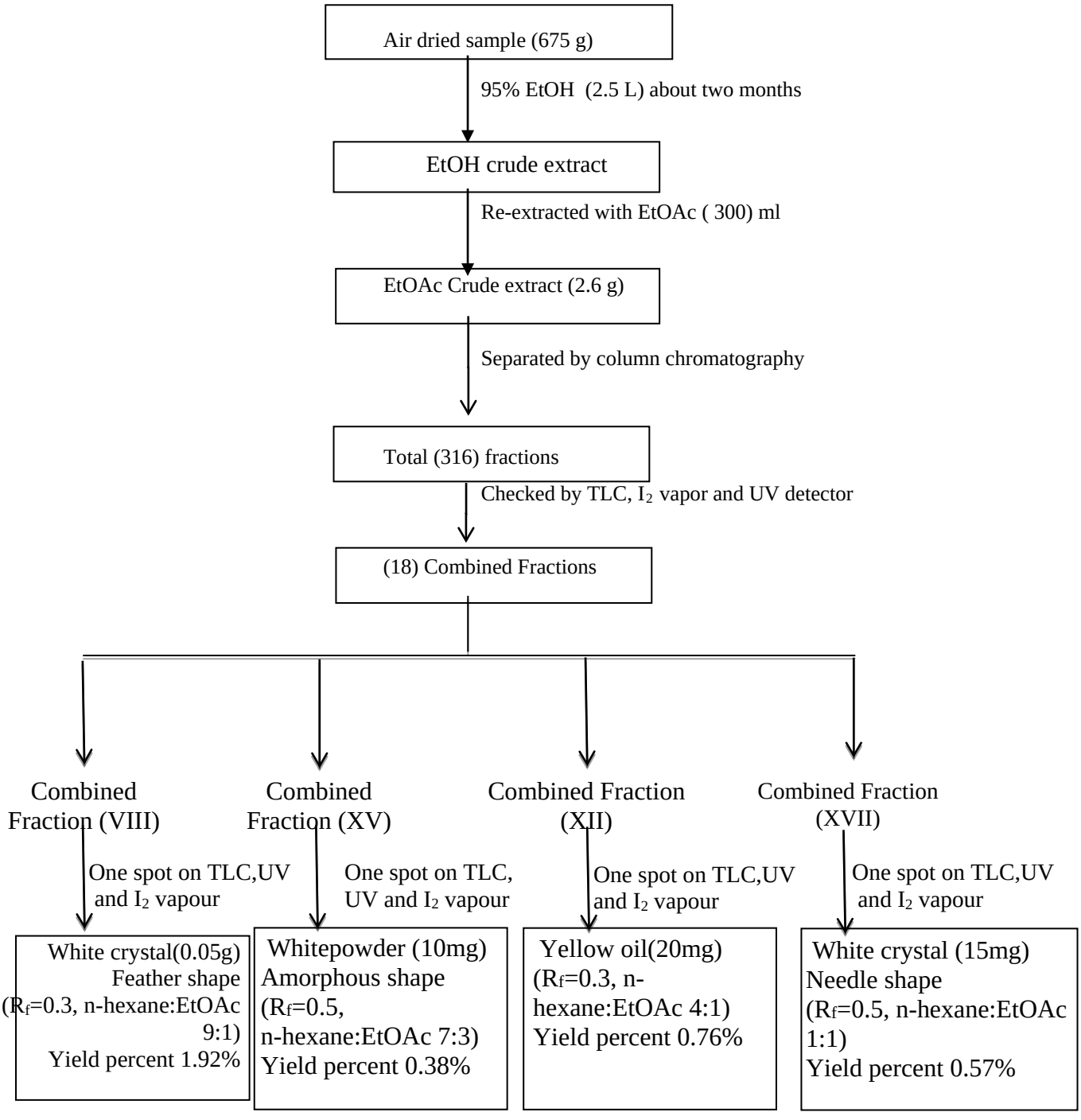
**Figure 2. Antimicrobial Activities of *Couroupita Guianensis* Aubl. (Amyauk-San-Bin)**

**Column Chromatography Separation**

The bark of *Couroupita Guianensis* Aubl.( Lecythidaceae)Myanmar named Amyauk-San-Bin collected from Magway Township, Myanmar.

The air dried bark of *Couroupita Guianensis* Aubl.(675 g) were extracted with 95% EtOH (2.5 L) at room temperature about two months. Ethanol extract was re-extracted with EtOAc (300) ml and then filtered and evaporated. Ethyl acetate crude extract (2.6 g) was separated by column chromatography with adsorbent (silica gel 70-230 mesh) and eluent (n-hexane: EtOAc

various ratio). Total (316) fractions were obtained. Each fractions were checked by TLC, $I_2$ vapor and UV detector. The same $R_f$ value fractions were combined. And then 18 combined fractions were obtained. Among them combined VIII gave white powder 0.5 g. This shape is feather shape with $R_f$ value 0.3. n-hexane and EtOAc ratio is 9:1, yield percent 1.92%. White crystal 10 mg was obtained from combined XV, this shape is amorphous with $R_f$ value 0.5. n-hexane and EtOAc ratio is 7:3, yield percent 0.38% .Combined fraction XII gave yellow oil 20 mg. $R_f$ value 0.3. n-hexane and EtOAc ratio is 4:1, yield percent 0.76%.White crystal 15 mg was obtained from combined XVII, this shape is needle shape with $R_f$ value 0.5. n-hexane and EtOAc ratio is 1:1, yield percent 0.57% .



## Results and Discussion

**Preliminary Phytochemical Test for the bark of *Couroupita guianensis* Aubl.**

**Table 1. Phytochemical Test for the bark of *Couroupita guianensis* Aubl.**

| No. | Constituents | Reagent used | Observation | Results |
|---|---|---|---|---|
| 1. | Alkaloid | Dragendroff's reagent | Yellow ppt | + |
| | | Wagner reagent | Orange ppt | + |
| 2. | Flavonoid | EtOH, Mg ribbon, Conc: HCl | Pink colour | + |
| 3. | Steroid | EtOH, acetic anhydride,$CHCl_3$ Conc: $H_2SO_4$ | Greenish blue colour | + |
| 4. | Terpene | Petether, acetic anhydride, $CHCl_3$, Conc; $H_2SO_4$ | Reddish brown colour | + |
| 5. | Glycoside | 10% lead acetate solution | White ppt | + |
| 6. | Reducing Sugar | Benedict's solution | Brick red ppt | + |
| 7. | Polyphenol | 1% $FeCl_3$, 1% $[K_3Fe(CN)_6]$ solution | Greenish blue colour | + |
| 8. | Saponin | $H_2O$, shaking | Frothing | + |
| 9. | Phenolic | $H_2O$, $\Delta$, 10 min, 10% $FeCl_3$ solution | Brown colour | + |

**Results of Antimicrobial Activities of *Couroupita guianensis* Aubl.**

**Table 2. Antimicrobial Activities of *Couroupita Guianensis* Aubl.**

| No | Type of Solvent | Inhibition Zone | | | | | |
|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | Vi |
| 1 | n-hexane | 12mm (+) | 12mm (+) | 13mm (+) | 13mm (+) | 12mm (+) | 12mm (+) |
| 2 | Di-chloro methane | 11mm (+) | 13mm (+) | 13mm (+) | 12mm (+) | 13mm (+) | 12mm (+) |
| 3 | EtOAC | 14mm (+) | 14mm (+) | 14mm (+) | 14mm (+) | 14mm (+) | 14mm (+) |
| 4 | EtOH | 14mm (+) | 13mm (+) | 20mm (+++) | 15mm (++) | 15mm (++) | 15mm (++) |
| 5 | MeOH | 13mm (+) | 13mm (+) | 12mm (+) | 13mm (+) | 13mm (+) | 13mm (+) |

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

I - *Bacillus subtilis*
II - *Staphylococcus aureus*
III - *Pseudomonos aeruginosa*
IV - *Bacillus pumilus*
V - *Candida albican*
VI - *E-coli*

According to this table, n-hexane, dichloromethane, ethyl acetate, ethanol and methanol extracts of three selected samples were tested by Agar-well diffusion method on six selected organisms. In this study, it can be observed that n-hexane, dichloromethane, ethyl acetate and methanol extracts of ***Couroupita Guianensis*** Aubl**.** gave low activities on six selected organisms. Ethanol extract of this sample gave medium activities on *Bacillus pumilus, Candida albican* and *E-coli* and high activity on *Pseudomonos aeruginosa.*

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

| No. | Wave number of absorption band ($cm^{-1}$) | Assignment of functional group |
|---|---|---|
| 1. | 3303.21 | O – H stretching vibration of alcohol group |
| 2. | 3098.5 | C-H stretching vibration of sp2 hydrocarbon |
| 3. | 2926.11,2852.81 | Assymmetric and symmetric C-H stretching vibration of sp3 hydrocarbon |
| 4. | 1710.92 | C = O stretching vibration of carbonyl group |
| 5. | 1640.51 | C=C stretching vibration of alkenic group |
| 6. | 1459.20 | C-H stretching vibration of allylic hydrocarbon |
| 7. | 1382.04, 1361.79 | C – H bending vibration of sp3 hydrocarbon |
| 8. | 1261.49 | C – C –O stretching vibration of ether group |
| 9. | 1095.60,1033.88 | C – O – C stretching vibration of ether group |
| 10. | 804.34 | C-H out of plane bending vibration |

**Table 4. FT-IR Spectral Data of Compound -2**

| No. | Wave number of absorption band ($cm^{-1}$) | Assignment of functional group |
|---|---|---|
| 1. | 3080.42 | – CH stretching vibration of $sp^2$ hydrocarbon alkene |
| 2. | 2932.86,2868.24 | Asymmetric and symmetric C-H stretching vibration of $sp^3$ hydrocarbon |
| 3. | 1735.99 | C = O stretching vibration of carbonyl group |
| 4. | 1680.05 | C=C stretching vibration of alkenic group |
| 5. | 1456.30 | C-H stretching vibration of allylic hydrocarbon group |
| 6. | 1371.43 | C – H bending vibration of $CH_3$ group |
| 7. | 1041.60 | C – O– C stretching vibration of ether group |
| 8. | 968.30 | C – H out of plane bending vibration of trans or E and cis or Z alkenic group |
| 9. | 883.43 | C – H out of plane bending vibration of cis or Z alkenic group |

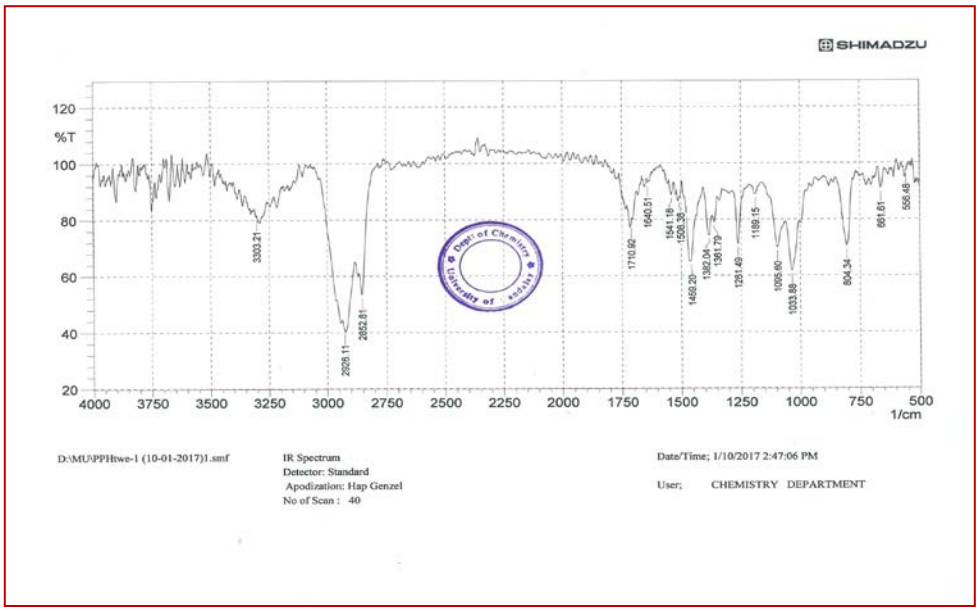



**Figure3. FT IR Spectrum of pure compound -1**

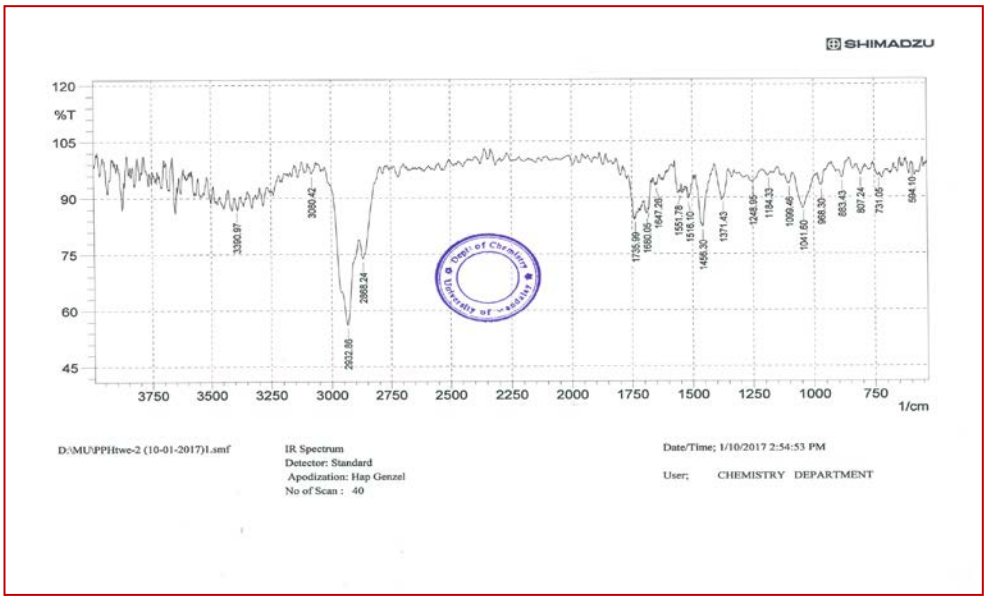



**Figure 4. FT-IR Spectrum of pure compound -2**

**Table 5. FT-IR Spectral Data of Compound -3**

| No. | Wave number of absorption band ($cm^{-1}$) | Assignment of functional group |
|---|---|---|
| 1. | 3004.23 | – CH stretching vibration of $sp^2$ hydrocarbon |
| 2. | 2926.11,2854.74 | Asymmetric and symmetric C-H stretching vibration of $sp^3$ hydrocarbon |
| 3. | 1743.71 | C = O stretching vibration of carbonyl group |
| 4. | 1608.5 | C=C stretching vibration of alkenic group |
| 5. | 1456.30 | C-H stretching vibration of allylic hydrocarbon |
| 6. | 1376.26 | C – H stretching vibration of $CH_3$ group |
| 7. | 1169.87 | C – O– C stretching vibration of ether group |
| 8. | 971.19 | C – H out of plane bending vibration of trans or E and cis or Z alkenic group |
| 9. | 811.09 | C – H out of plane bending vibration of cis or Z alkenic group |

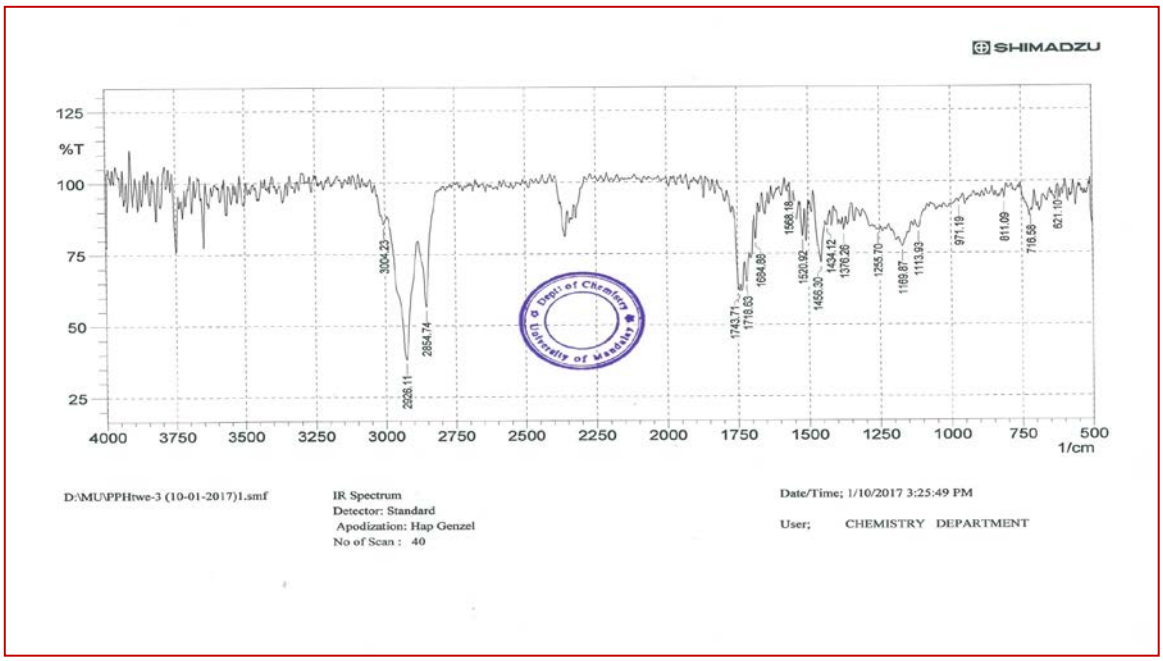



**Figure 5. FT-IR Spectrum of pure compound -3**

## Conclusion

In this paper,Myanmar indigenous medicinal plant was collected from Magway township, Magway Region.In the phytochemical screening, the bark of *Couroupita Guianensis* Aubl.*(* Amyauk-San-Bin) contains alkaloid, flavonoid, steroid, terpene, glycoside, reducing sugar, polyphenol, saponin,and phenolic compound respectively. The antimicrobial activities of n-hexane, dichloromethane, ethyl acetate, ethanol and methanol extracts of *Couroupita Guianensis* Aubl.*(* Amyauk-San-Bin) was tested by Agar-well diffusion method on six selected organisms. In this study, it can be observed that n-hexane, dichloromethane, ethyl acetate and methanol extracts of Lumbini Ingyin gave low activities on six selected organisms. Ethanol extract of this sample gave medium activities on *Bacillus pumilus, Candida albican* and *E-coli* and high activity on *Pseudomonos aeruginosa.*

Furthermore, pure organic compounds 1, 2 and 3 were isolated from ethyl acetate extract of the bark of Couroupita Guianensis Aubl.( Amyauk-San-Bin) by applying advanced separation techniques such as Thin Layer and Column Chromatography. The yield percent of compound -I was found to be 1.92% ( 0.05g) base upon the ethyl acetate crude extract, compound- 2 was found to be 0.38% ( 20mg) and compound -3 was found to be 0.76 %(20mg) base upon the ethyl acetate crude extract .

The FT-IR spectrum of compounds -1, 2 and 3 were measured at the Department of Chemistry, University of Mandalay and the functional groups containing in this pure compounds could be determined by the FT-IR assignments.

## Acknowledgment

Special thanks are extended to Ministry of Education, Department of Higher Education for the permission of the research project. We feel a deep sense of gratitude to Dr SoeWin, Rector, Yenangyaung Degree College for his invaluable encouragement.

We would like to express our profound gratitude to Professor and Head Dr Lin Lin Htun, Department of Chemistry, Yenangyaung Degree College, for her guidance, encouragement and allowing us to use the facilities in the Department.

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### Online Materials

https://en.wikipedia.org/wiki/Couroupita_guianensis

http://www.stuartxchange.org/CannonBallTree.html

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