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Phytochemical Screening, Antimicrobial Activities and Functional Groups Determination of Isolate Organic Compound from the Barks of Avocado

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Abstract:- In this research work, the bark of Avocado was collected from Pyin Oo Lwin Township, Mandalay Region. The collected sample was tested by phytochemical screening which gives positive for alkaloid, flavonoid, polyphenol, sterol, terpene, glycoside, saponin, and sugar tests. Moreover, the antimicrobial activities of the bark of Avocado was tested by Agar-well diffusion method on six tested organisms such as *Bacillus subtilis, Staphylococus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and E-*coli* respectively. The ethylacetate extract of the bark of Avocado responds high activity on four tested organisms. In addition, organic compound was isolated from the bark of avocado by using thin layer and Column Chromatographic methods. Pure compound (yellow needle like crystal, 0.1755 mg, 0.65% based upon the EtOAc extract) was obtained from the Column Chromatographic technique. The functional groups present in isolated compound were identified by FT-IR spectral data.

Keywords:- *Avogado, Phytochemical, Antimicrobial, FT-IR, Chromatography.*

I. INTRODUCTION

In most developing countries of the world, many parts of the plant are used in traditional medicines. In Myanmar, most of the people depend on traditional medicinal plants and herbal medicines for the treatment of various disorders. Myanmar traditional practitioners use a variety of medicine mostly containing potent medicinal plants available in Myanmar to cure various diseases depending on their own nature and localities. Avocado is well known traditional medicinal plant in Myanmar. Avocado fruit is used to lower cholesterol levels, decrease hunger, to increase sexual desire, and stimulate menstrual flow. The seeds, leaves, and bark are used for dysentery and diarrhea. (Website - 1) Tree bark has so many amazing medical *benefits*, particularly when it comes to relieving pain, healing damaged and inflamed skin, and reducing the symptoms of colds and flus, and for that reason it is used in many products today. Therefore, this plant is selected to study in this research work. (Website-2)

➢ *Botanical Description*

Fig 1:- The Plant of Avocado

Fig 2:- The Fruits of Avocado

Botanical Name	-	*Persea americana* Mill
Family Name	–	*Laureace*
English Name	-	Avocado
Habit	-	Tree
Local Name	-	Htaw-bat
Part Use	-	Bark

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II. MATERIALS AND METHODS

A. Sample Collection

The bark of Avocado for chemical analysis was collected from Pyin Oo Lwin Towship, Mandalay Region, Myanmar. The sample was cut into small pieces and allowed to air dry. The dried pieces were stored in a well-stoppered bottle and used throughout the experiment.

Fig 3:- The Barks of Avocado

Fig 4:- Dried Pieces of Bark of Avocado

B. Phytochemical Test [1][2][3][4]

Phytochemical tests were performed to detect the presence or absence of organic constituents in the bark of avocado. The results were tabulated in Table 1.

C. Determination of Antimicrobial Activity of Crude Extracts

The antimicrobial activities of crude sample of bark of avocado were examined by using Agar well diffusion method in Development Center of Pharmaceutical Technology, Insein, Yangon. [5]

D. Instrumentation and Materials

➢ *Instrumentation*

The FT-IR spectrophotometer shimadsu, Japan was used for FT-IR measurement. UV lamp was lambed – 40, Pelkin Ehmer Co, England used as colour developer. Common laboratory apparatus and equipments were used through the course of this research work.

➢ *Materials*

Commericial grade reagents and solvents were used by further distillation. Analytical and preparative thin layer chromatography (TLC) was performed by using percolated silica gel plates (Merk Co. lnc. Kiesel gel 60 F_{256}). Silica gel was used for column chromatography. Visualization was taken via UV lamp and iodine vapour.

E. Extraction of the Bark of Avocado

The air dried sample (500g) was percolated with Ethyl alcohol (EtOH) (2L) for two months. The solution was filtered and concentrated. It was extracted with (250ml) Ethyl acetate (EtOAc) and evaporated. Then the residue obtained was checked by TLC. This TLC plate was developed in iodine vapour and more than one spot were observed. Therefore, it was separated by using column chromatography. [6]

F. Isolation of a Pure Organic Compound by Column Chromatographic Separation

The EtOAc extract (2g) was fractionated by column chromatography over silica gel (SiO_2, 30g). The various ratios of n-hexane and EtOAc were used form non-polar to polar for this separation. Each and every fraction was checked by TLC. Three combined fractions were obtained. In the last combined fraction observed yellow needle like crystal. Then, this fraction was washed and recrystallized by EtOAc. The resulting fraction was isolated to obtain pure compound by using next column chromatography. The yield percent of pure known compound is found to be 0.1755mg, 0.65% based upon the EtOAc extract. This compound was identified by the FT-IR spectroscopic studies. [7]

G. Confirmatory Test of Isolated Compound

This isolated pure compound was also rechecked by phytochemical tests which gave rise to positive for flavonoid and glycoside.

Fig 7:- Phytochemical Test of Pure Compound for Glycoside and Flavonoid

III. RESULTS AND DISCUSSION

A. Phytochemical Tests of Barks of Avocado

Barks of Avogado were tested by phytochemical screening and the results were shown in Table 1.

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No	Constituent	Reagent	Observation	Results
1	Glycoside	10% leadacetate	white ppt	+
2	Alkaloid	1% HCl, (i) Dragendroff's solution (ii) Wagner's solution	(i) orange ppt (ii) reddish-brown ppt	+
3	Polyphenol	10% $FeCl_3$, 10% $K_3[Fe(CN)_6]$	bluish green solution	+
4	Flavonoid	Conc:HCl, piecies of Mg	red color solution	+
5	sterol	acetic anhydride, Conc:H_2SO_4, $CHCl_3$	greenish-blue colour solution	+
6	Saponin	Conc:H_2SO_4, vigorously shaken	frothing	+
7	Terpene	aceyianhydride, $CHCl_3$, Conc:H_2SO_4	Purple solution	+
8	Reducing sugar	Benedict's solution	red ppt	+

Table 1:- Phytochemical Screening of Barks of Avocado
(+) presence (-) absence

According to this table, the bark of Avocado extract consists of glycoside, alkaloid, polyphenol, flavonoid, sterol, saponin, terpene and reducing sugar respectively.

B. Results of Antimicrobial Activity of Bark of Avocado

The extracts of bark of Avocado with various solvents such as n-hexane, chloroform, acetone, ethyl acetate and ethanol were taken and examined the antimicrobial activities.

Sample	Organisms						
	Solvents	(1)	(2)	(3)	(4)	(5)	(6)
Avocado bark	n-hexane	-	15mm (++)	-	15mm (++)	17mm (++)	-
	$CHCl_3$	-	15mm (++)	-	15mm (++)	20mm (++)	-
	Acetone	-	18mm (++)	-	20mm (++)	20mm (++)	-
	EtOAc	-	25mm (+++)	-	25mm (+++)	23mm (+++)	25mm (+++)
	EtOH	-	25mm (+++)	-	27mm (+++)	23mm (+++)	-

Table 2:- Antimicrobial Activity of Bark of Avocado

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

Organisms
(1)*Bacillus Subtilis*
(2)*Staphylococusaureus*
(3)*Pseudomonas aeruginosa*
(4)*Bacillus pumilus*
(5)*Candida albicans*
(6)E-*coli*

According to these results, the ethyl acetate extract of the bark of avocado responds high activities on four tests organisms, such as *Staphylococus aureus*, *Bacillus pumilus*, *Candida albicans*, E-*coli* respectively.

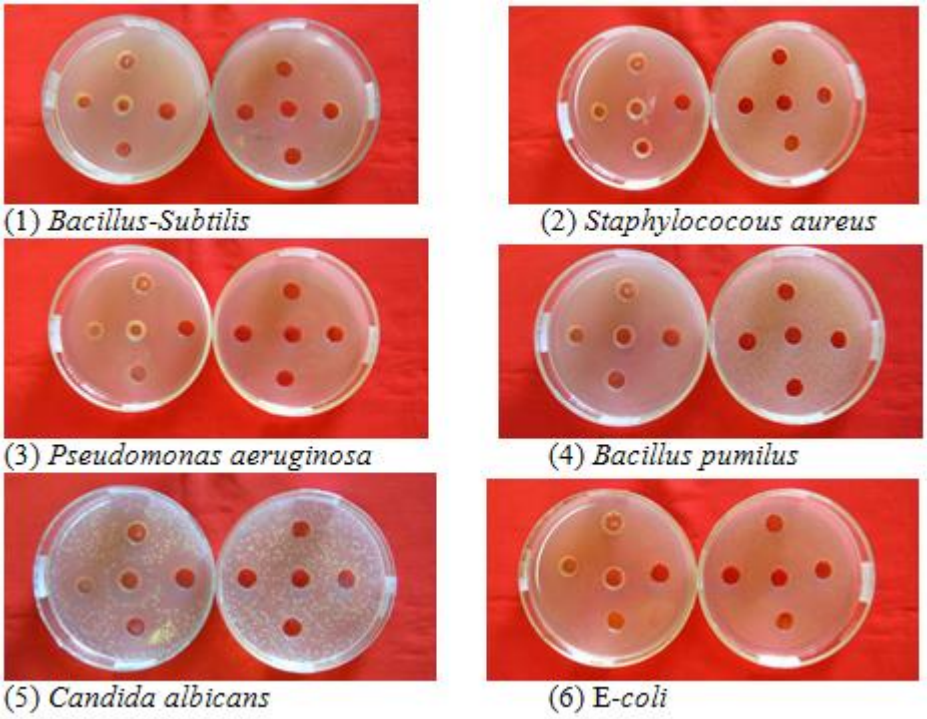

Fig 5:- Antimicrobial activities of Bark of Avacado

C. Studies on FT-IR Spectral Data of Pure Compound

FT-IR spectrum of pure compound was measured at the Department of Chemistry, University of Mandalay. In this FT-IR spectrum, the band appears at 3363.10 cm^{-1} shows the OH stretching vibration of alcohol group. The band which appears at 3100.32cm^{-1} shows the C-H stretching vibration of sp^2 hydrocarbon. Asymmetrical and symmetrical C – H stretching vibration of sp^3 hydrocarbons are observed at 2999.31 cm^{-1} and 2831.50 cm^{-1}.

The shoulder peak at 1684.84 cm^{-1} should be C=O stretching vibration of carbonyl group and the C═C stretching vibration of aromatic ring can be observed at 1637.2 cm^{-1}. The band appears at 1590.22cm^{-1} should be C-H bending vibration of sp^2 hydrocarbons. The peaks at 1392.61 cm^{-1} and 1360.47 cm^{-1} show the –OH bending vibration of alcohol groups. C–C–O stretching vibration of alcohol group could be observed at 1266.30 cm^{-1} and 1234.44 cm^{-1}. The bands appear at 1172.32 cm^{-1}, 1103.28 cm^{-1} and 1025.10cm^{-1} show the C – O – C stretching vibration of ether groups. The C – H out of plane bending vibration of trans or E and cis or Z alkenic groups could be appeared at 971.12 cm^{-1}, 879.54 cm^{-1} and 763.81 cm^{-1}.

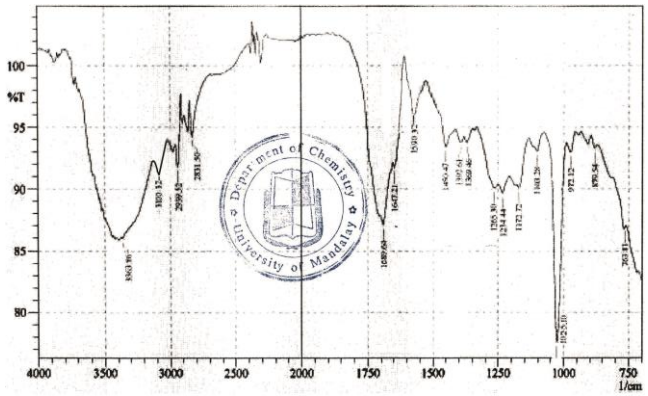

Fig 6:- FT-IR Spectrum of Compound

IV. CONCLUSION

In this research work, one of Myanmar indigenous medicinal plants, Avocado was selected for preliminary phytochemical screening and the results are shown in table 1. In this table, crude extract of bark of Avocado gave positive for alkaliod, flavonoid, polyphenol, sterol, terpene, glycoside, saponin, and sugar tests.

The antimicrobial activity of selected sample was tested by Agar-well diffusion method on six tested organisms, such as *Bacillus subtilis*, *Staphylococcus auerus*, *Pseudomonas aeruginosea*, *Bacillus pumilus*, *Candida albicans*, E-*coli* respectively. As mentioned in table 2, ethyl acetate extract of the bark of Avocado responds high activity on four tested organisms. As description in experimental work, a pure organic compound, yellow needle like crystal (0.1755mg, 0.65%) was isolated from the bark of Avocado by Thin layer and Column Chromatographic methods.

The structure of pure compound was identified by FT-IR spectral data. According to FT-IR spectroscopy, the pure compound consists of alcohol group, carbonyl group, aromatic ring, allylic hydrocarbon, ether group and trans or E and cis or Z alkenic groups respectively.

This isolated pure compound was also rechecked by phytochemical tests which gave rise to positive for flavonoid and glycoside. Thus the isolated compound may be flavonoid type compound.

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ONLINE MATERIALS

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- https://treescience.com.au/blog/exploring-the-medicinal-uses-of-tree-bark/