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Phytochemical Screening and Antimicrobial Activity of the Ethanol Leaf Extract of *Pavonia hirsuta* (Guill. & Perr.)

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Abstract

The leaf of *Pavonia hirsuta* is traditionally used to treat various diseases in different regions of Nigeria. This study is aimed to evaluate the phytochemical constituents, antibacterial and antioxidant activity studies on the crude leaf extracts of *Pavonia hirsuta*. The air-dried plant sample was powdered, extracted successively with ethanol. The solid matter was separated by filtration and then filtrates were concentrated using a rotary evaporator. The dried extract was subjected to a preliminary phytochemical screening test using standard procedures and the result showed the presence Alkaloids, Tannins, Saponins, Steroids, Terpenoids and Cardiac glycosides in the extract. Flavonoid was found to be absent. Antimicrobial activity of the crude extract was determined by using well diffusion method. The ethanol extract of the leaves of *Pavonia hirsuta* showed a significant inhibition zone on all of the selected bacteria (Staphylococcus aureus, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Bacillus subtillis*) and fungus (*Candila albicans)*. The extract was tested at five different concentrations (200 mg/mL, 100 mg/mL, 50mg/mL, 25 mg/mL and 12.5 mg/mL). It was found to be active against all the tested organisms except *Esherichia coli*. The minimum inhibitory concentration (MIC) of the plant extract against *Staphylococcus aureus*, *Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtillis* and *Candila albicans* was found to be active at concentrations of 50 mg/mL, 100 mg/mL, 50 mg/mL, 12.5 mg/mL and 12.5 mg/mL respectively. The minimum bactericidal concentration (MBC) of the plant extract against *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa* and *Bacillus subtillis* was found to be active at concentrations of 100 mg/mL, 200 mg/mL, 100 mg/mL and 25 mg/mL respectively. The minimum fungicidal concentration (MFC) of the plant extract against *Candila albicans* was found to be active at concentration of 25 mg/mL. *Bacillus subtillis* has the highest zone of inhibition. The extract was isolated and purified by column chromatography and thin layer chromatography 23 fractions were obtained and spotted then compounds having same R_f value were combined together. FAU11 was obtained from the combination of fractions 8, 9, 10 and 11 with an R_f value of 0.46 cm therefore was further analyzed using Fourier Transform Infrared spectroscopy and Gas chromatography-mass spectroscopy for characterization and identification of the bioactive compounds present, five major compounds were isolated which are of biological significance such as antioxidant, hypocholesterolemia, antiandrogenic, anticancer, antimicrobial activities. This study showed that the leaves of *Pavonia hirsuta* constitute phytochemicals that possess antibacterial and antifungal activities and can serve as a potential source in search of plant-based antibiotics.

Keywords: *Pavonia hirsuta,* Phytochemical, Antimicrobial, Chromatography, Isolation.

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Introduction

The traditional knowledge of medical plant if harnessed, can give insight into the vital role medicinal plants play in drug development [1], [2]. The bioactive natural products derived from plants shows substantial structural variability and possess distinctive pharmacological properties. Plant extracts and their bioactive compounds have been utilized for the treatment of several diseases [3; 4]. The presence of phytochemicals in plants helps in inhibiting the growth of bacteria thereby preventing the skins from degradation [5]. Phytochemicals such as alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, resins, cardiac glycosides, coumarins, and phenolic compounds, among others, have a multitude of biological activities, which are effective and could be safe anti-photoaging and photoprotective agents [6]. They also show antimicrobial efficacy against pathogens infecting hair and scalp [7]. They are also sources of potential preventive or therapeutic agents for dermatological indications due to their pharmacological activities including antioxidant, UV absorption and anti-inflammation. [8,9].

Pavonia hirsuta is a plant in the family of Malvaceae. The leaves are traditionally used to treat various diseases in different regions of Nigeria. It is commonly known as Zabiya, Farin tsu, Sarkin bìsa (bìsà): The chief on high of trees (NIGER, HAUSA) [10]. Several species are known as swamp mallows [11]. It is a woody under bush plant, erect to 40 cm or prostrate, it is mostly found in Sandy Sahel, Mali and Nigeria and probably elsewhere in the drier Northern parts of the region and widespread in the drier parts of tropical Africa. The flowers are commonly yellow with a red-purple [12]. Plants of the family are used for food beverages, timber in traditional medicinal and in horticulture [13].

Materials and Methods

Plant sample collection

Fresh leaves of *Pavonia hirsuta* were collected from Saye forest, Zaria local government area, Kaduna state and was identified in the herbarium section of Department of Botany, Faculty of Life Science, Ahmadu Bello University (A.B.U), Zaria. The fresh leaves of the plant were washed with tap water and air dried under shade at room temperature. The dried sample was ground into powder by an analytical mill.

Apparatuses and chemicals

Apparatuses and instruments utilized during the laboratory work include separator funnel, rotary evaporator, Petri-dish, incubator, autoclave, Analytic mill, capillary tube, UV-light, beaker, measuring cylinder, chromatographic glass column, weighing balance. Solvents used for extraction ethanol (analytical grade solvent). Chemicals like hydrochloric acid, sulphuric acid, Ferric chloride, Mayer's reagent, ammonia, barium chloride, acetic anhydride, α-naphthol, iodine, KI, and $HgCl_2$ were used for phytochemical screening.

DMSO and Gentamicin were used for an antibacterial test.

Extraction of plant sample

Dried and powdered leaves of *Pavonia hirsuta* (500 g) was soaked with ethanol (1500 mL) for 72 hrs. with intermittent shaking and then filtered first using cotton plug followed by Whatmann No. 1 filter paper. The filtrates were pooled and concentrated under reduced pressure [14]. The semisolid mass was made to dry at room temperature; then the dried mass was weighed.

Preliminary phytochemical screening

The plant extract was subjected to qualitative standard screening tests for secondary metabolities such as alkaloids, saponins, tannins, steroids, terpenoids, flavonoids and glycosides according to the methods discussed in the literatures [15,16,17,18].

Antimicrobial test

Test organisms

Six bacteria strains (two gram positive): *Staphylococcus aureus* and *Bacillus subtillis*, three gram negative: *Esherichia coli*, *Salmonella typhi,* and *Pseudomonas aeruginosa* with one fungus *Candila albicans* were obtained from microbiology laboratory, of the Nigerian Institute of Leather and Science Technology, Samaru-Zaria, Kaduna State, Nigeria.

Preparation of test solutions

About 0.01 g of ethanol crude extract was dissolved in 10 mL of DMSO separately to test antibacterial activities. Then the resulting concentration was diluted to get the second concentration (1.0 mg/mL, 0.5 mg/mL).

Preparation of inoculums

Bacterial strains from the stock cultures were streaked on Mueller Hinton Agar containing the extracts and incubated at 37°C for 24hr and at 22°C for 48hr for fungus. The broth dilution technique [19] was used to evaluate the antibacterial activity of the plant extracts. Ciprofloxacin, Broad-spectrum antibiotic (Gentamicin) and Fluconazole were used as positive control and the disk soaked with DMSO as a negative control to compare the result of effects of plant extract. After 24 hr the antibacterial activity was evaluated by measuring the diameter of the growth inhabited zone based on the method used by [20] and compared with the standard antibiotic used.

Column Chromatography

Column chromatography was used for the isolation and purification of compounds from the ethanol extract. The process was achieved through the following steps; preparation of the sample, packing of the column, pouring of sample into the column, elution of fractions and lastly analysis of each fractions using thin layer chromatography. A solvent ratio of 8:2 n-hexane: ethyl acetate which served as the mobile phase flowing down the

column with the help of gravity. The sample was dry loaded at the top of the column and the mobile phase was allowed to flow down through the column. About 23 fractions were collected which were labelled as FAU1, to FAU23 [21].

Thin Layer Chromatography

Thin layer chromatography was used to know how many components were in the fractions obtained from column chromatography and also to support the identity of the compound, in the mixture, when the R_f of the compound is compared with the R_f of a known compound [22,23].

FTIR and GC-MS Analysis

Fourier-Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-mass Spectroscopy Analysis (GC-MS) analysis were used for the characterization and identification of the isolated component.

Results and Discussion

Phytochemicals screening result

Phytochemical screening test of the ethanol extract of the leaves of *Pavonia hirsuta* showed the presence of Alkaloids, Tannins, Saponins, Steroids, Terpenoids and Cardiac glycosides and the absence flavonoids (Table 1).

Table 1: Phytochemical Constituents of the Leaves of *Pavonia hirsuta*

Metabolites	Ethanol Extract
Alkaloids	Present
Flavonoids	Absent
Tannins	Present
Saponins	Very much Present
Steroids	Present
Terpenoids	Present
Cardiac glycosides	Present

Plant extracts and their bioactive compounds have been utilized for the treatment of several diseases [3]. This bioactive compounds (phytochemicals) like tannins, saponins and flavonoid have potentially significant applications against bacteria [24]. Different phytochemicals have been found to possess varied medicinal properties; preventive and curative properties for example; saponins,

terpenoids, tannins and steroids have been reported to have anti-inflammatory effects [25,26,27**)**. Some glycosides, flavonoids, and tannins have been reported to have had hypoglycemic activities, tannins show antimicrobial and antioxidant properties [28,29]. Some saponins have also been reported to possess hypochloesterolemic and antidiabetic properties [30,31].

Therefore, the reported traditional medicinal value of the plant is most probably associated with those phytochemicals detected in the plant sample.

Antibacterial activity result

Bacterial growth inhibition data were given in Table 2. The antibacterial activity was determined by measuring the inhibition zone in diameters (mm) and was evaluated according to the parameters suggested by [32; 33]. The ethanol crude extract showed a strong inhibition zone (35 mm) at 1.0 mg/mL concentration against E. coli (Table 2). This is promising as compared to the reference gentamicin (26 mm) on the same pathogen.

Table 2: Determination of Antimicrobial activity of *Pavonia hirsuta* Leaves Extract on Some Test Microbes

	Zone of Inhibition (in mm)			
	Concentration of Extract (in mg/mL)	Control		
Test Microbes	200	CPX	CN	FLU
Staphylococcus aureus	20	43	27	
Esherichia coli	-	38	23	
Salmonella typhi	15	31	24	
Pseudomonas aeruginosa	17	34	22	
Bacillus subtillis	23	45	28	
Candila albicans	22			32

Key: CPX = Ciprofloxacin, CN = Centamycin, FLU = Fluconazole, No zone (-)

Table 3: Minimum Inhibitory Concentration (MIC)

	Concentration of extract (in mg/mL)				
Test Microbes	200	100	50	25	12.5
Staphylococcus aureus	-	-	MIC	+	++
Esherichia coli	ND	ND	ND	ND	ND
Salmonella typhi	-	MIC	+	++	+++
Pseudomonas aeroginosa	-	-	MIC	+	++
Bacillus subtillis	-	-	-	-	MIC
Candila albicans	-	-	-	-	MIC

Key: No turbidity (-), Mild turbidity (+), Low turbidity (++), Moderate turbidity (+++), Not determined (ND)

Table 4: Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

	Concentration of extract (in mg/mL)				
Test Microbes	200	100	50	25	12.5
Staphylococcus aureus	-	MBC	+	++	+++
Salmonella typhi	MBC	+	++	+++	++++
Pseudomonas aeruginosa	-	MBC	+	++	+++
Bacillus subtillis	-	-	-	MBC	+
Candila albicans	-	-	-	MFC	+

Key: Not determined (ND), No growth (-), Mild growth (+), Low growth (++), Moderate growth (+++), High growth (++++)

The test organisms were found to susceptible to the extract of the plant at the concentrations tested. The ethanol extract showed the lowest MIC of 12.5 mg/mL against *B. subtillis* and *C. albicans* with 50 mg/mL for *S. aureus* and *P. aeruginosa*. The MBC for the extract against all the test

organisms were found to be greater than the MIC, indicating that the extract is bacteriostatic. From the result of the antimicrobial activity, it can be seen that the extract has antimicrobial activity against the tested organisms except *E. coli*.

Column Chromatography and Thin Layer Chromatography

Twenty-three (23) fractions were obtained from column chromatography which were spotted on the TLC plate, compounds with the same R_f value were combined; FAU8,9,10 and FAU11 were combined to give FAU11.

TLC image for the separated components as observed with UV light

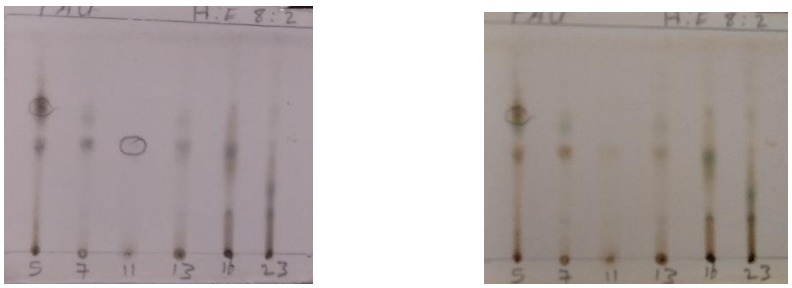


Fig. 1: TLC PLATE OF FAU11

Table 5: TLC Result for components developed with solvent ratio 8:2 of n-hexane: ethyl acetate

S/N	Component	Distance moved by components (X) cm	Distance moved by solvent (y) cm	R_f = x/y
1	FAU11	1.9	4.1	0.46

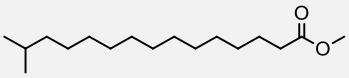
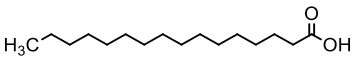
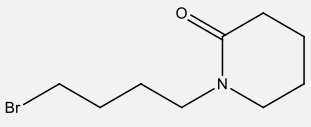
From the TLC table above in Table 5, FAU11 which had a purer compound at an R_f of 0.46 cm was further analyzed using FTIR and GC-MS.

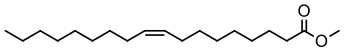
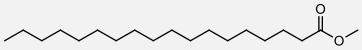
Fourier Transform Infra-Red (FTIR) is a physico-chemical analytical technique used to identify the functional groups of the bioactive components in a plant or other related materials based on the peak value in the region of infrared radiation and as such can be used as a tool for standardization of plant extracts [39]. Various functional groups detected in the spectra (Table 6) signifies the presence of phytochemicals which are responsible for the pharmacological activity and can be used to characterize the extract and fractions.

Table 6: FTIR spectrum interpretation

Absorption (cm^{-1})	Bond	Functional group
3819.18	OH stretch	Carboxylic acid
3240.52	OH stretch	Alcohol
2901.04	C-H stretch	Alkane
2715.86	C-H stretch	Alkane
1689.70	C-H stretch	Alkene
1087.89	C-N stretch	Aliphatic amine
1057.03	C-N stretch	Aliphatic amine
709.83	C-H stretch	Alkane

Table 7: Showing the GC-MS analysis result

Phytochemical Compound / Chemical formula	Mol. Ion mass (g)	Structure	Uses
Pentadecenoic acid,14-methyl, methyl ester $C_{17}H_{34}O_2$	270		Antifungal [34], antioxidant and antimicrobial [35].
n-hexadecanoic acid $C_{16}H_{32}O_2$	256		Antioxidant, hypocholesterolmic, antiandrogenic, hemolytic, 5-alpha-reductase inhibitor [35], nematicide, pesticide, lubricant [36]
2-piperidinone, N-(4-bromo-n-butyl) $C_9H_{16}BrNO$	234		Antimicrobial, antioxidant [37]

9-octadecenoic acid, (Z)-methyl ester $C_{19}H_{36}O_2$	296		Antimycobacterial, [34], antioxidant, anticancer, anti-inflammatory [35], anemiagenic, insectifuge flavour. [36].
Methyl stearate $C_{19}H_{38}O_2$	298		Antimicrobial, nematicide and pesticide [38], cytotoxic [34].

The FTIR method measures the vibrations of bonds in chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic "fingerprint" of the sample [40]. The bond in the compound can be determined through the interpretation of infrared spectrum absorption.

Phytochemicals like triterpenoid, Tannin, saponins and alkaloids isolated from different parts of plants are responsible for cytotoxic [34,41], antibacterial [35,42 and antifungal activities [43] in self-defense mechanisms against microbe, herbivore, and insect predation which makes them an excellent source for substances with antimicrobial properties [44,45]. Oxygenated terpenes have been reported to have antimicrobial activity against gram negative bacteria by causing cell death by loss of cell membrane integrity [46] while saponins have been shown to exhibit antimicrobial activity against Gram positive organisms [47,48]. The antimicrobial activity of the extract against test organisms justifies the ethnobotanical use of the plant in curing kidneys diuretics and also for curing venereal diseases and treating wound infections.

The observed bioactivities of the leaves extract of *Pavonia hirsuta* might be due to the presence of the secondary metabolities like tannins, alkaloids and saponins.

Conclusions

It is evident from the present study that the qualitative phytochemical tests, antimicrobial activities and GC-MS analysis that the leaves of *Pavonia hirsuta* possess biologically active compounds. Important compounds identified in leaf extracts of *Pavonia hirsute* were Pentadecenoic acid,14-methyl, methyl ester, n-hexadecanoic acid, 2-piperidinone, N-(4-bromo-n-butyl), 9-octadecenoic acid, (Z)-methyl ester. These compounds are known to display several diverse activities that may help protect against chronic diseases such as antioxidant, hypocholesterolemia, antiandrogenic, anticancer, antimicrobial activities. These bioactive compounds are useful in the Leather industry, in skin care and final leather finishing.

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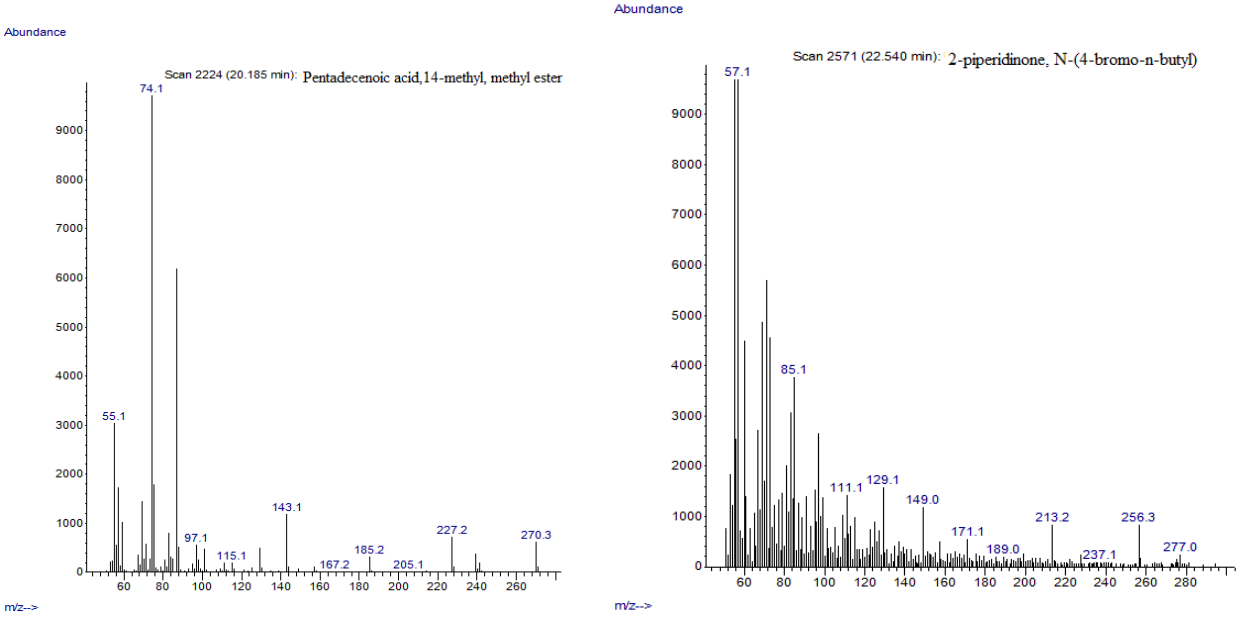
APPENDIX



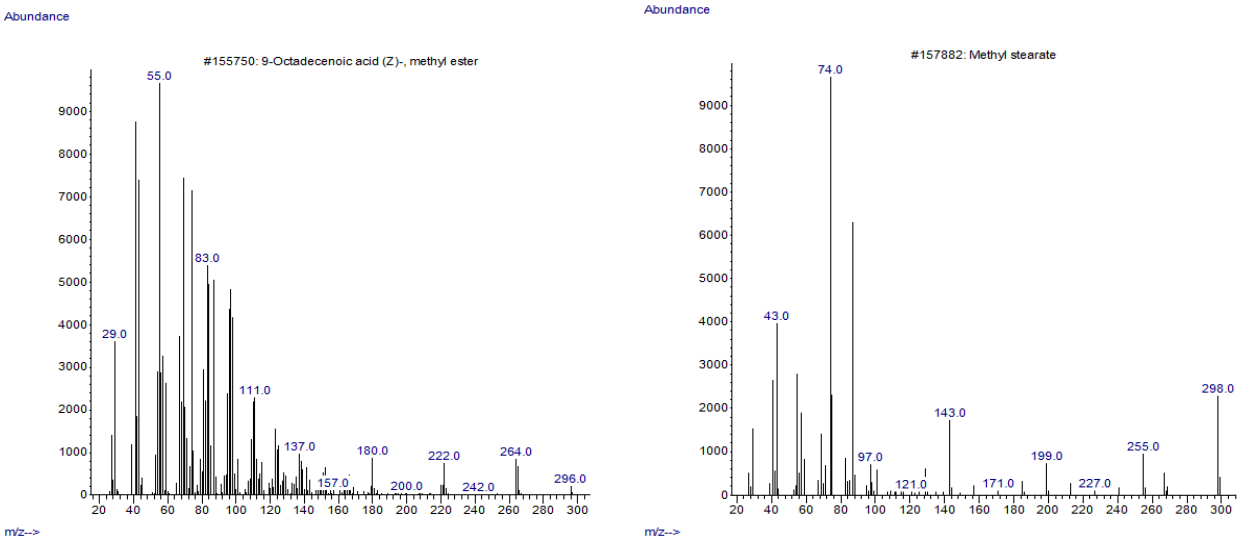


Fig. 2: GC-Ms Result of FAU11.

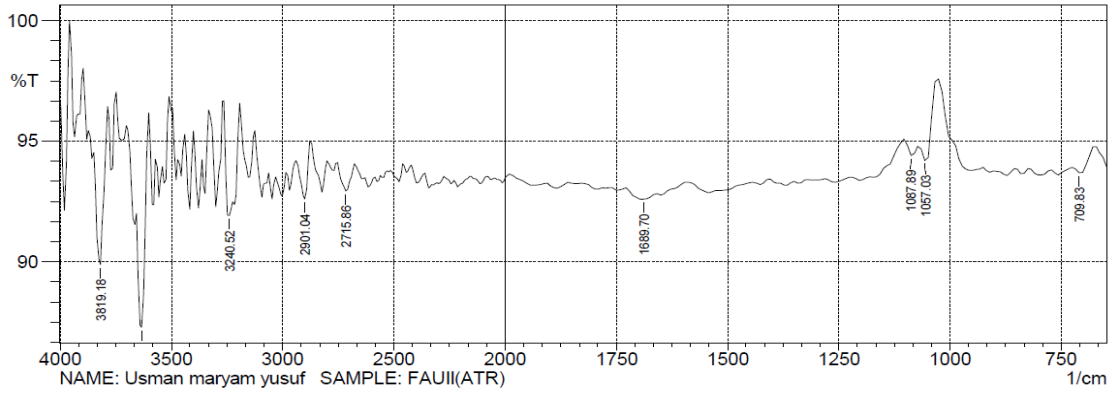


Fig. 3: FTIR Result of FAU11