FTIR ANALYSIS AND ANTIMICROBIAL EVALUATION OF AQUEOUS ETHANOL LEAF EXTRACT AND FRACTIONS OF PAVONIA SENEGALENSIS (CAV.) LIESTNER (MALVACEAE)

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ABSTRACT

The leaves of *Pavonia senegalensis* have been used in African traditional medicine in treatment of ailments such as bone and wound infections. In this study the FTIR analysis and antimicrobial evaluation of the extracts and fractions were carried out so as to provide a chemical fingerprint for the standardization of the plant and the scientific basis for the use of the plant in treatment of wounds and bone infections. FTIR analysis was conducted by scanning the samples in the mid-IR region (650-4000 cm^{-1}). The *in-vitro* antimicrobial activity of the leaf extract and fractions of plant was evaluated using *S. aureus, E. coli, P. aeruginosa, S. Typi, S. pyrogens* and Vancomycin reisitant *enterooccci* (VRE) as test organisms. The FTIR spectra of the plant extract and fractions revealed the presence various functional groups which include alkanes, alkenes, alkynes, aldehydes, ketones and alcohols/phenols. In the antimicrobial studies, the extract and fractions of the plant inhibited the growth of all the test organisms used in the study and were found to be bacteriostatic. In conclusion, the result of the study provide a biochemical fingerprint for the standardization of the plant and also justifies the ethnobotanical use of the plant in treatment of bone and wound infections.

Keywords: fingerprint, standardization, inhibition, bacteriostatic, concentration

INTRODUCTION

Plants possess an almost limitless ability to combine aromatic compounds, mostly phenols, ketones, polyphenols, flavonoids, terpenoids, alkaloids, ketones, and essential oils, in self-defense mechanisms against microbe, herbivore, and insect predation which makes them an excellent source for substances with antimicrobial properties (Shin et al., 2018). Numerous bioactive plant-derived compounds, called phytochemicals, have been evaluated and are comparatively safer than synthetic alternatives, exerting multiple therapeutic benefits associated with their high efficacy (Cowan, 1999). 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 (Johnson et al., 2012).

In this study the Fourier transform infra-red (FTIR) spectroscopy analysis and the evaluation of the antimicrobial properties of *Pavonia senegalensis* were carried out so as to provide a chemical fingerprint for the standardization of the plant and the scientific basis for the use of the plant in treatment of wounds and bone infections.

Pavonia senegalensis (Cav.) Liestner synonyms *P. hirsuta* Gull. & Perr., *P.*

arabica Hoschst ex Steud and *P. argentina* Gurke is from the family Malvaceae. It is called *Tsu* in Hausa and found in drier parts of tropical Africa on sandy-clay soils, humid sands and sometimes on rocky screen in savannah areas; often near villages; in woodland with *Grewia*, *Terminalia*, etc.; along rivers and in seasonally dry riverbeds; usually in light shade (Burkill, 1997). *P. senegalensis* is usually an annual plant, but occasionally lives longer. A spreading, short-lived perennial with semi-prostrate to ascending branches, up to 1.25 m. (Heywood, 1979).

The roots are put into cold water to draw and the infusion is taken as a remedy for diarrhoea in South and East Africa (Neuwinger, 2000). The powdered seed is taken with milk and used as a contraceptive in Sokoto North-west Nigeria (Adebisi and Alebiosu, 2014). Infusion of the roots is used in antenatal care for general wellbeing in Katsina North-west Nigeria and the maceration of the leaves is used in Zaria North-west Nigeria to treat wounds and bone infections (Kankara *et al.*, 2015). A cold-water infusion of the dry roots is taken to induce labour in Botswana, particularly if the onset is being delayed (Burkill, 1997).

The acute toxicity study of the aqueous ethanol extract of *P. senegalensis* leaves showed that the extract is non-toxic when given orally over a short period but the sub-chronic (28 days) toxicity study showed that the extract is nephrotoxic and slightly hepatotoxic in rats (Shehu et al., 2019a). The hydro-alcoholic leaf extract and fractions (n-hexane, ethyl acetate and n-butanol fractions) of the plant were shown to be effective against both acute and chronic inflammation in a dose related manner in rats (Shehu et al., 2019b).

MATERIALS AND METHODS

Plant Collection, Preparation and Identification

Plant samples, consisting leaves and flowers, of *P. senegalensis* were collected from Rafin Yashi, Giwa Local Government Area of Kaduna State in November, 2018. The plant was identified and authenticated by U.S Gallah at National Research Institute for Chemical Technology (NARICT), Zaria, Kaduna State Nigeria and assigned a voucher number NARICT 24011.

The collected leaves were air dried at room temperature for seven days and powdered using pestle and mortar.

Extraction and Solvent-Solvent Partitioning of Plant Material

One (1) kilogram of the powdered leaves was weighed and macerated in 70 % aqueous ethanol for 5 days with occasional shaking. It was subsequently filtered and the filtrate was evaporated under reduced pressure using a rotary evaporator at 65° C. The dried extract was kept in a desiccator until used.

Partitioning of the extract was carried out according to the method described by Woo *et al.*, (1980). Portion of the extract (80 g) was suspended in water (400 ml) and then successively partitioned with n-hexane (2×200 ml), ethyl acetate (2×200 ml), and n-butanol (2×200 ml). The fractions were collected, concentrated, coded and kept in a desiccator for further use.

Phytochemical Screening

The extract and fractions were subjected to phytochemical screening test for the presence of secondary metabolites such as flavonoids, tannins, alkaloids, anthraquinones, saponins, steroids/terpenoids, and cardiac glycosides using methods described by Kokate *et al.*, (2016).

Fourier Transform Infra-Red (FTIR) Analysis

The FTIR spectrum of the extract and fractions (2 mg) was recorded in the mid-IR region (650-4000 cm^{-1}) at resolution 8 cm^{-1} with 16 scans using FTIR Carry 630 Agilent technologies. All experiments were performed in triplicates and the mean values were used for the analysis. The spectra were then interpreted and similarity between the sample was determined by superimposing the various spectra.

In-vitro Antimicrobial Activity Testing

Test Microorganisms

Both standard and clinical isolates were used in the study. The standard isolates were obtained from the Department of Pharmaceutical Microbiology, Ahmadu Bello University Zaria while the clinical isolates were obtained from Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria. The standard isolates were *Staphylococcus aureus* (ATCC021001), *Escherichia coli* (ATCC11775), *Pseudomonas aeruginosa* (ATCC10145) and *Salmonella Typhi* (ATCC3345) while the clinical isolates were *Streptococcus pyrogens*, *Vancomycin Resistant enterococci (VRE)* and *Streptococcus pyogenes*. All the isolates were authenticated, purified and maintained in nutrient agar slant.

Culture Media

Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB) culture media were used. All media were prepared according to manufacturer's instruction.

Preparation of Inocula of Test Organisms

The McFarland turbidity standard scale 0.5 was used to standardize the organisms. The scale was prepared by adding 1 % sulphuric acid (9.95 mL) to 1 % Barium Chloride (0.05 mL). The volume of the prepared stock solution was mixed according to the McFarland nephelometer standard. The organisms were suspended in sterile distilled water and compared with the McFarland turbidity standard, until the opacity matched with the scale number 0.5, which corresponds to 1.5 x 10^8 bacterial suspension/mL (CLSI, 2014).

Sensitivity Test

Agar-in-well diffusion method as described by the Clinical and Laboratory Standards Institute was used (CLSI, 2014). The antimicrobial activities of the extract and the fractions of *P. senegalensis*, at concentrations of 100, 50, 25 and 12.5 mg/mL, were determined. The standardized inocula of the isolates were uniformly streaked unto freshly prepared Mueller Hinton agar plates with the aid of a sterile swab stick. Using a sterile cork borer (6 mm in diameter), 5 appropriately labeled wells were bored into each agar plate. Aliquot (0.2 mL) of the extract and fractions were placed in each well and then allowed to diffuse into the agar. Ciprofloxacin (10 µg/mL) was used as a standard antimicrobial drug. The plates were incubated at 37^{o}C for 24 h. The antimicrobial activities were expressed as diameter (mm) of inhibition zones produced by the plant extract and fraction.

Determination of Minimum Inhibitory Concentration (MIC)

The determination of minimum inhibition concentrations of the extract and fractions were carried out on the test microbes using the broth dilution method as outlined by the Clinical and Laboratory Standards Institute (CLSI, 2014). Mueller Hinton broth was prepared; 10 mL was dispensed into tubes and was sterilized at 121° C for 15 minutes and allowed to cool. The McFarland turbidity standard scale 0.5 was prepared to give

turbidity solution. Normal saline was prepared, 10 mL was dispensed into sterile test tube and the test microbes was inoculated and incubated at 37° C for 24 hours. Dilution of the test microbes was done in the normal saline until the turbidity marched that of the Mc-Farland turbidity scale by visual comparison at this point the test microbe had a concentration of about $1.5x10^8$ cfu/mL. Two-fold serial dilution of the extract and fractions in the sterilized broth were made to obtain the concentrations of 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 mg/mL. Having obtained the different concentrations of the extract and fractions in the sterile broth, 0.1 mL of the standard inoculum of the test microbe in normal saline was then inoculated into the different concentrations. Incubation was carried out at 37 for 24 h, after which each test tube of the broth was observed for turbidity (growth). The lowest concentration of the extract in which the broth shows no turbidity was recorded as the Minimum Inhibition Concentration (MIC).

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration of the extract and fractions were determined as outlined by the CLSI (2014) on the nutrient agar plates. Minimum bactericidal concentrations were determined by assaying the test tube contents of the MIC determinations. A loopful of the content of each tube was inoculated by streaking on a solidified nutrient agar plate and then incubated at 37° C for 24 h. After which it was observed for microbial growth. The lowest concentration of the subculture with no growth was considered as the minimum bactericidal concentration.

RESULTS

Phytochemical Screening

The result of phytochemical screening (Table 1) detected saponins, flavonoids, tannins and steroids/terpenes in the aqueous ethanol extract of the plant while the n-hexane and ethyl acetate fractions were found to contain steroids/ triterpenoids and the n-butanol fraction contains flavonoids, tannins, saponins and steroids/triterpenoids. Alkaloids, cardiac glycosides and anthraquinones were absent in both the extract and the fractions.

Table 1: Phytochemical screening of the Aqueous Ethanol leaf extract and fractions of *P. senegalensis*

Phytochemical Class	Aqueous ethanol extract	n-Hexane fraction	Ethyl acetate fraction	n-Butanol fraction
Flavonoids	+	-	-	+
Alkaloids	-	-	-	-
Tannins	+	-	-	+
Anthraquinones	-	-	-	-
Cardiac glycosides	-	-	-	-
Saponins	+	-	-	+
Steroids/Triterpenoids	+	+	+	+

(+) - present, (-) - absent

Fourier Transform Infra-Red (FTIR) Result of the Aqueous Ethanol Leaf Extract and Fractions of *P. senegalensis*

The superimposed FTIR spectra of the extract and fractions is shown in figure 1 while the various functional groups identified in the spectra are showed in Table 2.

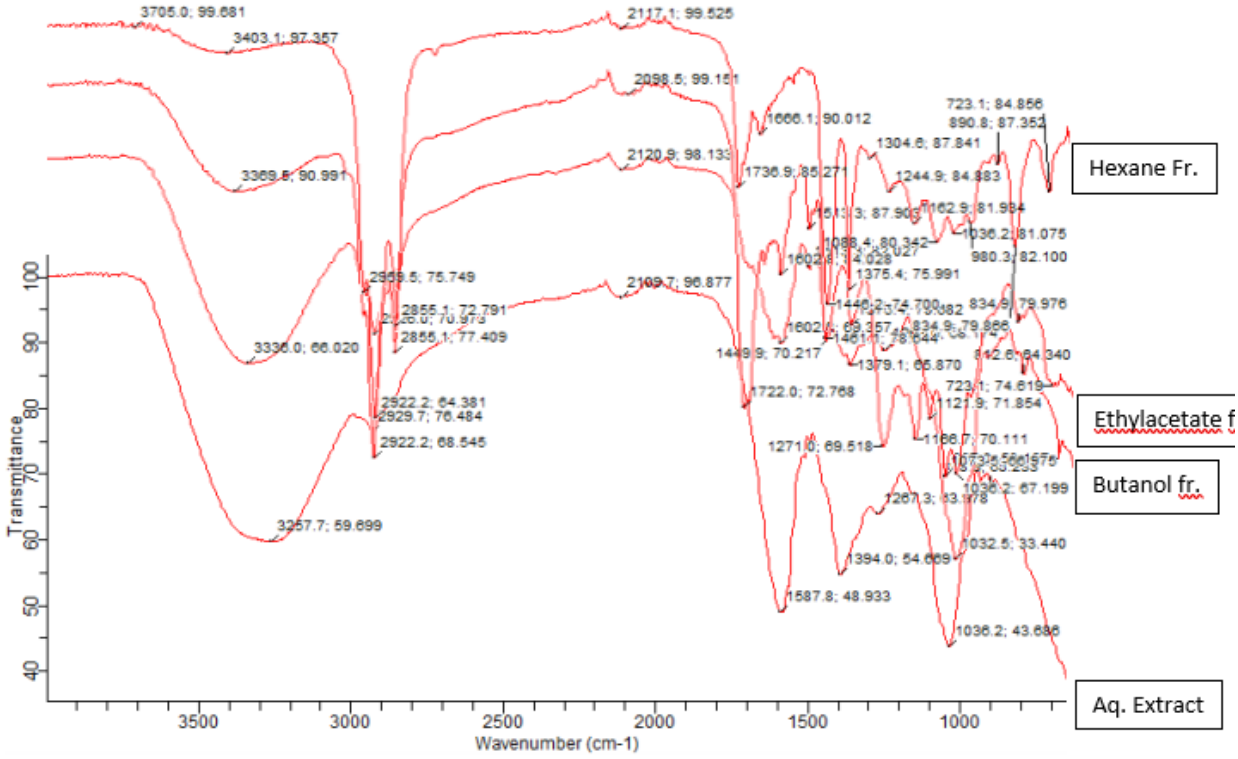

Figure 1: superimposed FTIR Spectra of the aqueous ethanol leaf Extract and fractions of *P. senegalensis*

Table 2: Interpretation of FTIR spectra of Aqueous Ethanol Leaf Extract and Fractions of *P. senegalensis*

Sample	Absorption (cm^{-1})	Functional Groups
Aqueous ethanol Extract	3257	OH
	2929	CH stretching
	2109	C≡C
	1587	C=O
	1036	C-O
n-Butanol Fraction	3336	OH
	2926	CH stretching
	2120	C≡C
	1449	C=C
	1032	C-O

Ethyl acetate Fraction	3369	OH
	2922	CH stretching
	2855	CH stretching
	2098	C≡C
	1722	C=O
	1602	C=C
	1271	C-O
n-Hexane Fraction	3403	OH
	2922	CH stretching
	2855	CH stretching
	2117	C≡C
	1440	C=C

In-vitro Antimicrobial Activity of *P. senegalensis* Leaf Extract and Fractions

Sensitivity (Zones of Inhibition)

The sensitivity of the aqueous ethanol leaf extract and fractions of *P. senegalensis* are shown in Tables 3.

Table 3: Zone of Inhibition (mm) of Aqueous Ethanol Leaf Extract and Fractions of *P. senegalensis* against Test Organism

Sample (mg/mL)	**Zone of Inhibition (mm) ± SEM**					
Test organisms	**SA**	**SP**	**VRE**	**ST**	**EC**	**PA**
AEPS100	19 ± 0.00	17 ± 0.10	15 ± 0.00	16 ± 0.12	16 ± 0.20	16 ± 0.10
AEPS50	16 ± 0.20	13 ± 0.10	13 ± 0.00	14 ± 0.10	14 ± 0.30	14 ± 0.10
AEPS25	14± 0.10	0	0	12 ± 0.10	12 ± 0.10	12 ± 0.10
AEPS12.5	12± 0.30	0	0	0	0	0
HFPS100	22 ± 0.00	14 ± 0.10	12 ± 0.10	23 ± 0.20	12 ± 0.10	12 ± 0.00
HFPS50	20 ± 0.10	12 ± 0.10	0	20 ± 0.10	0	0
HFPS25	18 ± 0.20	0	0	18 ± 0.10	0	0
HFPS12.5	16 ± 0.00	0	0	16 ± 0.13	0	0
EFPS100	25 ± 0.00	17 ± 0.10	14 ± 0.00	12 ± 0.10	13 ± 0.10	14 ± 0.20
EFPS50	20 ± 0.00	14 ± 0.00	12 ± 0.10	0	0	12 ± 0.11
EFPS25	18 ± 0.10	0	0	0	0	0
EFPS12.5	16 ± 0.00	0	0	0	0	0
BFPS100	20 ± 0.00	18 ± 0.00	13 ± 0.00	14 ± 0.10	18 ± 0.10	17 ± 0.10
BFPS50	18 ± 0.10	15 ± 0.00	0	12 ± 0.10	14 ± 0.10	15 ± 0.10

BFPS25	16 ± 0.00	12 ± 0.00	0	0	12 ± 0.10	12 ± 0.02
BFPS12.5	13 ± 0.10	0	0	0	0	0
CPX 10 µg/ml	30 ± 0.10	13 ± 0.00	26 ± 0.10	32 ± 0.10	36 ± 0.10	35 ± 0.10

AEPS- Aqueous ethanol leaf extract of *P. senegalensis,* HFPS- n-Hexane fraction of aqueous ethanol leaf extract of *P. senegalensis*, EFPS- Ethyl acetate fraction of aqueous ethanol leaf extract of *P. senegalensis*, BFPS- n-Butanol fraction of aqueous ethanol leaf extract of *P. senegalensis*, CPX- Ciprofloxacin. SA- *S. aureus*, SP- *S. pyrogens*, VRE- Vancomycin resistant enterococci, ST- *S. Typhi*, EC- *E. coli*, PA- *P. aeruginosa.* SEM-standard error of mean. n = 3

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the aqueous ethanol leaf extract of *P. senegalensis* and its fractions against test organisms determined are shown in Tables 4 and 5.

Table 4: Minimum Inhibitory Concentration (MIC) of the Aqueous Ethanol Leaf Extract and Fractions of *P. senegalensis* against Test Organisms

Test organisms	MIC (mg/mL)			
	AEPS	**HFPS**	**EFPS**	**BFPS**
S. aureus	25	6.25	6.25	12.5
S. pyrogens	50	100	25	25
VRE	50	100	100	100
S. Typhi	50	6.25	100	100
E. coli	50	100	100	25
P. aeruginosa	50	100	100	50

AEPS- Aqueous leaf extract of *P. senegalensis*, HFPS- n-Hexane fraction of aqueous ethanol leaf extract of *P. senegalensis*, EFPS- Ethyl acetate fraction of aqueous ethanol leaf extract of *P. senegalensis*, BFPS- n-Butanol fraction of aqueous ethanol leaf extract of *P. senegalensis*. VRE- Vancomycin resistant enterococci

Table 5: Minimum Bactericidal Concentration (MBC) of the Aqueous Ethanol Leaf Extract and Fractions of *P. senegalensis* against Test Organisms

Test organisms	MBC (mg/mL)			
	AEPS	**HFPS**	**EFPS**	**BFPS**
S. aureus	100	50	25	50
S. pyrogens	100	>100	50	50
VRE	100	>100	>100	>100
S. Typhi	100	25	>100	>100
E. coli	100	>100	>100	50
P. aeruginosa	100	>100	>100	100

AEPS- Aqueous leaf extract of *P. senegalensis*, HFPS- n-Hexane fraction of aqueous ethanol leaf extract of *P. senegalensis*, EFPS- Ethyl acetate fraction of aqueous ethanol leaf extract of *P. senegalensis*, BFPS- n-Butanol fraction of aqueous ethanol leaf extract of *P. senegalensis.* VRE- Vancomycin resistant enterococci.

DISCUSSION

High level of similarity in phytochemical contents between the extract and the fractions was detected as revealed by the superimposed spectra in the functional group region 3500-1600 cm^{-1} while the differences were shown in the fingerprint region 1600-650 cm^{-1} (Figure 1). The various functional groups detected in the spectra (Table 2) signifies the presence of phytochemicals which are responsible for the pharmacological activity and can be used to characterize the extract and fractions. 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 (Oladunmoye et al., 2018). The bond in the compound can be determined through the interpretation of infrared spectrum absorption.

The test organisms were found to susceptible to both the extract and fractions of the plant at the concentrations tested. The crude extract showed the lowest MIC of 25 mg/mL against *S. aureus* with 50 mg/mL for the remaining test organisms. The n-hexane fraction showed the lowest MIC of 6.25 mg/mL against *S. aureus* and *S. Typhi* while the ethyl acetate fraction also showed the MIC of 6.25 mg/mL against *S. aureus*. The MBC for the extract and fractions against all the test organisms were found to be greater than the MIC, indicating that the extract and fractions are bacteriostatic. From the result of the antimicrobial activity, it can be seen that fractionation of the extract increases the antimicrobial activity against some organisms while in others the activity was reduced. Differences in chemical compositions recorded between the extract and some fractions may explain their different degrees of antimicrobial properties. Also, the amount of the active components in the crude extract may be diluted and fractionation may have increased their concentrations, thus the activities in the fractions (Babayi et al., 2004; Tamokou et al., 2008).

From the phytochemical screening of the plant extract and fractions, flavonoids and tannins have been reported to possess antimicrobial activities (Aguilar-Galvez et al., 2014; Waltrich et al., 2015). Antimicrobial activity of the flavonoids is probably related to its capacity in complexing extracellular and soluble proteins such as structures from bacterial cell walls (Scervino et al., 2005). The antimicrobial properties of tannins could be associated with the hydrolysis of ester linkage between gallic acid, and multiple esters with D-glucose, which affects the biosynthesis steps in the syntheses of cell membrane and cell walls, causing a decrease in cell volume, by the disjunction of cell membrane from the cell wall (Cowan, 1999; Suraya and Darah, 2002). Oxygenated terpenes have been reported to have antimicrobial activity against gram negative bacteria by causing cell death by loss of cell membrane integrity (Guimarães et al., 2019) while saponins have been shown to exhibit antimicrobial activity against Gram positive organisms (Soetan et al., 2006). The antimicrobial activity of the extract and fractions against test organisms justifies the ethnobotanical use of the plant in the treatment of bone and wound infections.

CONCLUSION

The FTIR spectra of the plant extract and fractions revealed the presence of various

functional groups which include alkanes, alkenes, alkynes, aldehydes, ketones and alcohols/phenols which can serve as a biochemical fingerprint for the standardization of the plant while in the antimicrobial studies, the extract and fractions of the plant inhibited the growth of all the test organisms used in the study and were found to be bacteriostatic justifying the ethnobotanical use of the plant for the treatment of bone and wound infections.

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