Original Research Article

Characterization and Antimicrobial Profiling of Ethanol-Toluene Extract of *Pentaclethra Macrophylla Benth Pod*

Abstract

The research reports the characterization and antimicrobial profiling of an ethanol-toluene extract of the *Pentaclethra macrophylla Benth* pod. The sample was powdered and extracted with a solvent system of ethanol-toluene (2:1). The extract was characterized with Fourier transform infrared (FTIR) spectroscopy and Gas Chromatographic-Mass Spectrometric (GC-MS) analysis, as well as a wet analysis investigation. The qualitative and quantitative analysis reveals the presence of flavonoids (0.307), phenols (0.4545), alkaloids (1.7165), tannins (6.118), saponins (5.700), and glycosides (0.2375). The FTIR revealed the presence of 1723cm^{-1}, which was assigned to carbonyl groups, and the peaks within the range of 3416.10–3777.54cm^{-1} were attributed to hydroxyl groups. The GC-MS reveals the presence of 34 molecules in the chromatogram, which is consistent with the mass spectra. The ethanol-toluene extract contains 19 guaiacyl and its derivatives, as well as 15 syringyl and its derivatives. The molecule, propenylsyringol (*cis*)^{b} has the highest yield based on the height of the peak. Other lignin-derived molecules with a good percentage of extraction were vanillin, syringaldehyde and synapylalcohol (*cis*). The antimicrobial analysis of the extract was tested on nine pathogens (*Staphylococcus aureus, Proteus bacilli, Streptococcus pyogenes, Escherichia coli*, *Candida species, Aspergillus Niger, Penicillium spp., Fusarium spp.*, and *Aspergillus fumigates*). The result therefore reveals that the ethanol-toluene extract of PMBP was more effective on *S. pyogenes* (0.9mm), ***P. bacilli*** (0.5 mm), and *Fusarium spp.* (0.9mm) at the MIC of 6.25mg/mL. The standard antimicrobial agents, Focknazol (antifungal) and Ciprotab (antibacterial), have the highest zone inhibition at an MIC of 6.25mg/mL, when compared with the ethanol-toluene extract of *Pentaclethra macrophylla Benth* pod. The ethanol-toluene extract of the *Pentaclethra macrophylla Benth* pod can be used as a source of guaiacyl or syringyl derivatives and also clinically since it is sensitive to some pathogens.

.

Keywords: Antimicrobial, Bacteria, Extract, Fungi, *Pentaclethra macrophylla Benth*,

1.0 Introduction

"In most parts of the world, the growing frequency of bacterial illnesses and antibiotic resistance has become a major problem. Without the use of appropriate antibiotics, modern medical treatments such as organ transplants, chemotherapy, and surgery may become dangerous. Plant research for therapeutic purposes has recently acquired popularity for various reasons, including its simple availability without a prescription, low cost, natural origin, and the potential to reduce the need for synthetic medications with potentially severe side effects" [1,2,3]. "Medicinal plants have long been essential natural factories of phytochemicals that are responsible for their biological actions, such as flavonoids, tannins, phenols, steroids, alkaloids, and terpenoids" [4,5].

“Plant products derived from fruits, flowers, seeds, roots, leaves, pods, and barks are used to make phyto-medicines, and numerous bioactive elements of plants have been discovered and characterized using several conventional analytical methods” [6,7].

“The *Pentaclethra macrophylla Benth* (African oil bean), also known as Ugba in Nigeria, is a tropical tree of the Leguminosae (Mimosoideae) family. It is native to tropical Africa, while the genus has representatives in tropical South and Central America. The tree can reach a height of 21 metres and has a crown-like canopy. The oil bean tree grows wild in West Africa's southern rainforest zone, having never been grown to any significant scale by individuals around homesteads or commercially in plantations” [9, 10,11]. “The flowers are golden and pinkish-white in colour, have a delicious scent, and attract a variety of insects, including the honey bee. The seeds are housed in pods that are about 40–50 cm long and 5–10 cm wide. Each pod contains anything from 6 to 10 flat, flossy brown seeds. The pods are firm, dark brown, and woody in appearance, and they coil up as they dry. When fully ripe, the pods split explosively, dispersing the seeds indiscriminately. The scattered seeds are collected by gathering them from within and outside the tree. The majority of the split pods fall to the ground, but some may remain attached to the tree's stalk. Empty, dried pods litter the surroundings indiscriminately, posing an environmental hazard” [11,12,13].

Recent studies have shown that the pods of *Pentaclethra macrophylla Benth* contain microcrystalline cellulose, which serves as an adsorbent [14] and is used as fuel for cooking and the production of charcoal. Madukasi et al. [15] investigated oil bean pods as an energy source with 3456 kcal/kg. The elemental analysis confirms that *Pentaclethra macrophylla Benth pod* is environmentally benign and serves as a feed substrate for livestock [16]. Oboh et al. [17] argued that its dispersion is usually done in an uncontrolled manner with attendant significant contributions to ozone layer depletion and gross environmental pollution.

“On the other hand, the antibacterial activity of extracts produced from medicinal plants forms the basis of several applications, including the preservation of raw and processed food, pharmaceuticals, and natural remedies” [18, 19]. “For drug-resistant pathogenic bacterial strains, new opportunities to discover and formulate effective antibiotics are presented by studies involving biochemical analysis to identify phytochemical constituents and antimicrobial properties of natural products like extracts and/or *Pentaclethra macrophylla Benth* pod” [20,21,22]. Therefore, the purpose of this research was to characterize and analyze the antibacterial properties of *Pentaclethra macrophylla Benth* pod extracts in ethanol-toluene, comparing them to both commonly used antimicrobials and global reports of similar results.

2.0 Materials and method

2.1 Chemicals and reagents

The natural *Pentaclethra macrophylla benth* pod, Potassium hydroxide (KOH), hydrochloric acid (HCl), ethanol, toluene, acetic acid, sodium chlorite, NaOH and other chemicals were

analytical grade. All the chemicals were used as received. Deionized water was used in all experiments

2.1 Plant material collection

The *pentaclethra macrophylla Benth* pod was gathered in Aku, Igbo Etiti L.G.A, Enugu State, and transported to the department of Industrial chemistry laboratory, University of Science and Technology, Enugu. It was carefully sorted to eliminate foreign material from the sample. To prepare for pulverisation, the sample was rinsed with distilled water, sun-dried for 2-3 weeks, and then chopped with a cutter. To expand the surface area and improve future treatment, the sun-dried chopped pod sample was crushed into fine powder and sieved to particle sizes of 0.07 mm.

2.3 preparation of Plant Extract

The technique used was consistent with Nsude et al. [10]. The powdered sample (100g) was extracted for 6 hours with 375 ml of ethanol and toluene (1:2) using a soxhlet extractor. The extract was then filtrated using Whatman No.1 filter paper and filtrates were concentrated using a rotary evaporator under reduced temperature and pressure; and the hydro-alcoholic extracts were further dried over water bath at 90°C. Crude extracts were then stored in a refrigerator at 4°C for further analyses.

2.4. Phyto-chemical profiling test

Each crude extract was tested for the confirmation of the absence/or presence of some majorc lasses of phytochemicals following the standard qualitative procedures described by Wishart [23] with a slight modification

2.4.1. Tests for flavonoids

“Alkaline reagent test: 2 ml of 2.0% NaOH was mixed with each plant crude extract. An intense yellow colour was formed which turned colourless on the addition of two drops of diluted acid which indicated the presence of flavonoids” [23].

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate also confirmed the positive test of flavonoids.

2.4.2. Test for alkaloids

“The presence of alkaloids was tested using two reagents, Mayer’s and Wagner’s reagents. A fewdrops of the solution were added to the extract solution (0.5 ml). A reddish-brown turbidity orprecipitate demonstrated the positive alkaloids” [24].

2.4.3. Test for tannins (Ferric chloride test)

"To the plant extracts solution (0.5 ml), few drops of 5% ferric chloride were added. Black or blue-green colouration or precipitate was taken as evidence for the presence of tannins" [23].

2.4.4. Test for saponins

"Few drops of $NaHCO_3$ were added to the plant extract solution (0.5 ml) and shaken vigorously tofroth and then allowed to stand for 15–20 min. A height of persistent foam greater than 1 cmindicated the presence of saponins" [25]

.2.4.5. Test for steroids and terpenoids

"Salkowski test: 2 ml of each plant extract was mixed with 2 ml of chloroform followed by the addition of concentrated H_2SO_4 (2 ml) by shaking well. A red colour produced in the lower chloro-form layer indicated the presence of steroids" [23].

2.4.6. Test for carbohydrates Molisch test:

"Plant extracts (2 ml) were treated with a few drops of alcoholic alpha-naphthol. Concentrated sulphuric acid (0.5 ml) was then poured slowly along the sides of the test tube. The appearance of purple to violet colour ring at the junction indicated the presence of carbohydrate" [23].

2.4.7. Detection of phenols

Ferric chloride Test: Extracts were treated with 3–4 drops of ferric chloride solution. Formation of bluish-black colour indicated that the presence of phenols.

2.5.0 Characterization

2.5.1 Gas Chromatography-Mass Spectrometry (GC-MS) Analyses

The extracts were analyzed on a Shimadzu GC-MS-QP2010 instrument (Kyoto,Japan) equipped with split-splitless inlets, a mass spectrometer, and an auto injector.SHRXI-5ms (30m X 0.25 mm I.D., 0.25 µm film thickness) capillary column was used and the carrier gas was helium. The temperature injection was 250 ^{o}C. The oven was temperature-programmed from 50 ^{o}C (1 min) to 320 ^{o}C (10 min) at 10 ^{o}C/min. The National Institute of Standards and Technology (NIST) library was used for identifying components

2.5.2 Fourier Transform Infrared

The IR spectra were obtained from the FTIR-8400S Fourier Transform Infrared spectrophotometer at National arbovirus research center Enugu using an ATR disc. It was used to identify the functional groups

2.6.0 Antimicrobial Study

3.6.1 Pathogen Isolation and Agar Preparation

The pathogens used for the bioactivity of this study were five bacteria strains (*Escherichia coli, Proteus* bacilli, *Staphylococcus aureus, Streptococcus* pyogenes, *Candida species*) and four

fungi strains (*Aspergillus fumigates, Aspergillus niger, Fusarium spp and Penicillium spp*). These pathogens were isolated and confirmed in the Department of Microbiology, University of Port Harcourt.

The nutrition agar was prepared by dissolving 28g of solid nutrient agar in one litre of distilled water. After being homogenized, the resulting agar solution was sterilized in an autoclave at 121°C for 15 minutes, allowed to cool at 47°C, and then poured into Petri plates. The plate's contents were then allowed to harden and cool. The antimicrobial activities of different concentrations the ethanol-toluene extracts were investigated using the well diffusion.

2.6.2 Bacteria Sensitivity Test

The technique of well in agar diffusion followed Orie et al. [25]. The bacteria pathogens were refreshed by streaking on freshly made agar medium and suspended for 30 minutes. The bacteria pathogens were prepared to march 0.5 McFarland standards (10^8 cells). Wells in the media were bored using a sterilized cork-borer. The ethanol-toluene extract was then added to the wells at various quantities. For 24 hours, the inoculated medium was incubated at 37 ^{o}C. Finally, the plates were examined, and millimetre (mm) measurements were made of the diameters of the zone of inhibition surrounding the wells.

2.6.3 Fungal Sensitivity Test

The well in Agar diffusion method with Dextrose Agar was used for this test and is in-line with Don-lawson et al. [36]. The medium was prepared according to the manufacturer's standard. The fungal isolates were refreshed on a freshly medium and incubated at room temperature for 120 hours. The spores were harvested from a well-sporulated colony into a tube of sterile distilled water, introduced into the molten medium, gently agitated to homogenize, poured into sterile Petri dishes and allowed to solidify. Cork borer was used to create wells in the medium and several dilutions of the bioactive compounds prepared with dimethyl sulphoxide DMSO were introduced into the wells. All inoculated plates were incubated at room temperature for 5-7 days. Thereafter, plates were observed for the zone of inhibition around the wells

3.0 Results and discussion

3.1 Preliminary Phytochemical Profiling of Ethanol-Toluene Extract of *Pentaclethra macrophylla Benth* Pod

In this study, the chemical profiles of the crude extracts prepared from PMBP were screened using different reagents to confirm the absence or presence of major phytochemical classes. Accordingly, eight phyto-compound families, namely, flavonoids, phenols, alkaloids, tannins, saponins, steroids, terpenoids, and glycosides, were screened as depicted in Table 1.

Table 1: Preliminary Phytochemical Profiling of Ethanol-Toluene Extract of *Pentaclethra macrophylla Benth* Pod

s/no	**Metabolites**	Qualitative	Quantitative (%)
1.	*Saponins*	+	5.700
2.	*Tannin (Catecholic)*	+	6.118
3.	*Flavonoids*	+	0.307
4.	*Alkaloids*	+	1.7165
5.	*Steroids*	ND	-
6.	*Terpeniods*	+	
7.	*Glycosides*	++	0.2375
8.	*Phenol*	+	0.4545

+ Present in trace concentration, ++Present in moderately high concentration
ND- (not detected)

As it can be seen from Table 1, the obtained preliminary qualitative analysis results revealed that flavonoids, phenols, saponins, alkaloids, and terpeniods were detected at trace concentrations in the ethanol-toluene extract of PMBP. where glycoside was moderately high in ethanol-toluene extracts. According to the previous study reported by Al-Fatimi [27], "an ethanolic extract of aerial parts of the same plant species showed the presence of flavonoids", which is in line with the present result. Also in this study, alkaloids and tannins were observed at trace concentrations in the extract.

On the contrary, steroids were completely absent in all tested crude plant extracts. From the present result, it is possible to say that the ethno-medicinal value of *Pentaclethra macrophylla Benth* pod as reported by researchers might be attributed to the positively screened secondary metabolites such as alkaloids, flavonoids, and tannins (Table 1).

From the analysis of an ethanol-toluene extract of *Pentaclethra macrophylla Benth* pod, glycosides were moderately at high concentration with the value of 0.2375, the tannins were at trace concentration with the highest value of 6.118, whereas the steroid was undetected. This is in line with Gonfa et al. [29], who worked on the effect of extraction solvent on the qualitative and quantitative analysis of major phytoconstituents in *Cadaba rotundifolia Forssk* leaf extracts. Their analysis reveals the presence of tannin content at 0.92, and flavonoids at 0.51 in methanol and ethanol extracts.

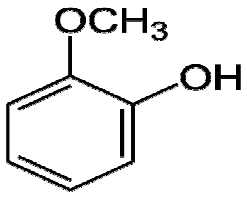
3.1 GC-MS Analysis of Ethanol-Toluene Extract of *Pentaclethra macrophylla Benth* Pod

The constituent of ethanol-toluene extract is revealed in the GC-MS in Table 2. The table contain the lignin-derived molecules, retention time, and the skeletal molecular structure.

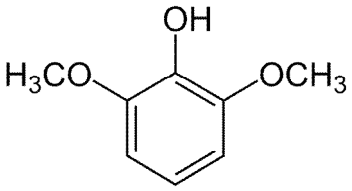
Table 2. Lignin-derived extract of ethanol-toluene from *Pentaclethra macrophylla Benth* Pod

No.	Product	Retention time (min)	Structure
1	Guaiacol	11.8	G
2	Methylguaiacol	14.8	G – C
3	Ethylguaiacol	17.2	G – C – C
4	Vinylguaiacol	18.2	G – C =C
5	Syringol	19.1	S
6	Vanillin	20.5	G – CHO
7	Eugenol	19.3	G – C – C =C
8	Propylguaiacol	19.5	G – C – C – C
9	Methylsyringol	21.4	S – C
10	Isocugenol (*cis*)	21.8	G – C =C – C
11	Homovanillin	22.0	G – C – CHO
12	Isocugenol (*trans*)	22.2	G – C =C – C
13	Acctoguaiacone	22.7	G – CO – C
14	Propioguaiacone[b]	23.1	G – CO – C – C
15	Ethylsyringol[b]	23.4	S – C – C
16	Guaiacylacetone[b]	23.7	G – C – CO – C
17	Vinylsyringol[b]	24.4	S – C =C
18	Propylsyringol[b]	25.6	S – C – C – C
19	2-Methoxy-4-(1-hydroxypropyl)phenol	26.5	G – C – C – C – OH
20	Allylsyringol	26.6	S – C – C =C
21	Syringaldehyde	26.7	S – CHO
22	Propenylsyringol (*cis*)[b]	26.8	S – C = C – C
23	Homovanillic acid	26.8	G – C – COOH
24	Synapylalcohol (*cis*)	27.1	S – C = C – C – OH
25	Synapylalcohol (*trans*)	27.3	S – C = C – C – OH
26	Propenylsyringol (trans)[b]	27.6	S – C = C – C
27	2-Methoxy-4-(prop-1-en-3-one)phenol	27.9	G – CO – C =C
28	Synapylaldehyde (*cis*)	28.2	G – C = C – CHO
29	Acetosyringone	28.3	S – CO – C
30	Coniferylaldehyde (*trans*)	28.4	G – C = C – CHO
31	Propiosyringone[b]	28.6	S – CO – C – C
32	Syringylacetone[b]	29.0	S – C – CO – C
33	Ferulic acid	30.1	G – C = C – COOH
34	Synapylaldehyde (*trans*)	32.7	S – C = C – CHO

The two prominent nuclei that other molecules of lignin-derived compounds are derived were guaiacyl nuclei and syringil nuclei;



Guaiacyl (G)



Syringil (S)

The retention times show that guaiacyl has the smallest molecular weight or has less affinity with the stationary phase of the column of the GC-MS, whereas the lignin-derived synapylaldehyde (*trans*)-lignin has the highest molecular weight and highest affinity.

The GC-MS ion chromatogram of the ethanol-toluene extract is shown in Figure 1. The numbers in the chromatogram of figure 1 are found in Table 2, along with the corresponding molecules, retention time, and structure. The molecule propenylsyringol (*cis*)[b] with the serial number 22 has the highest yield based on the height of the peak. Other lignin-derived molecules with a good percentage of extraction were vanillin (5), syringaldehyde (21), and synapylalcohol (*cis*) (24). From the table, syringil nuclei were found in 15 molecules, whereas guaiacyl nuclei were found in 19 molecules. This is consistent with Al-Fatimi [27] and Ehara et al. [30], who worked on GC-MS of different plant extracts.

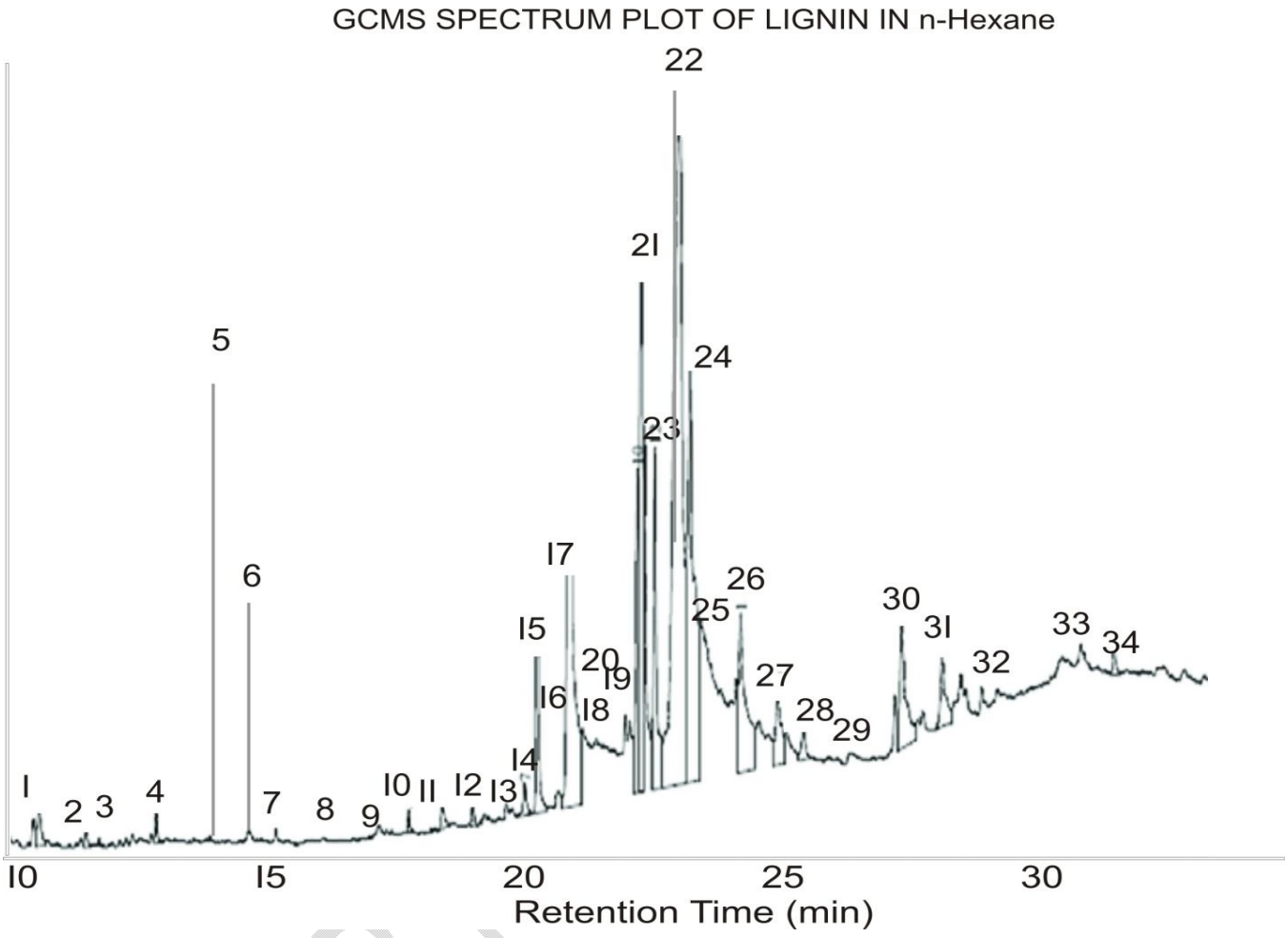


Figure 1: GC-MS Chromatogram of PMBP

3.2 FTIR Analysis of Ethanol-Toluene Extract of *Pentaclethra macrophylla Benth* Pod

The functional group constituent of the ethanol-toluene extract of PMBP is shown in Figure 2. The spectra contain aromatic nuclei that were well represented in the samples as peaks at 1510 cm^{-1} and 1380.00cm^{-1}.The peaks at 1285.06cm^{-1} and 1380 cm^{-1} represented guaiacyl and syringyl nuclei, respectively [25, 29]. These peaks are typical of lignin, and suggest that aromatic nuclei are almost stable during the ethanol-toluene extraction. The peak at 1030cm^{-1}, was assigned to aromatic CH deformation, etc. The peak at 1723cm^{-1} was assigned to carbonyl groups [26-28]. This might be due to the increase of products like 5, 21, 22, 23, and 24 (Table 2). The peak at 678.00cm^{-1} was attributed to —HC=CH— out-of-plane deformation. The peak at 2926.30 cm^{-1} was assigned to aliphatic CH, and the peaks within the range of 3416.10-3777.54 cm^{-1} were attributed to the hydroxyl group [29].

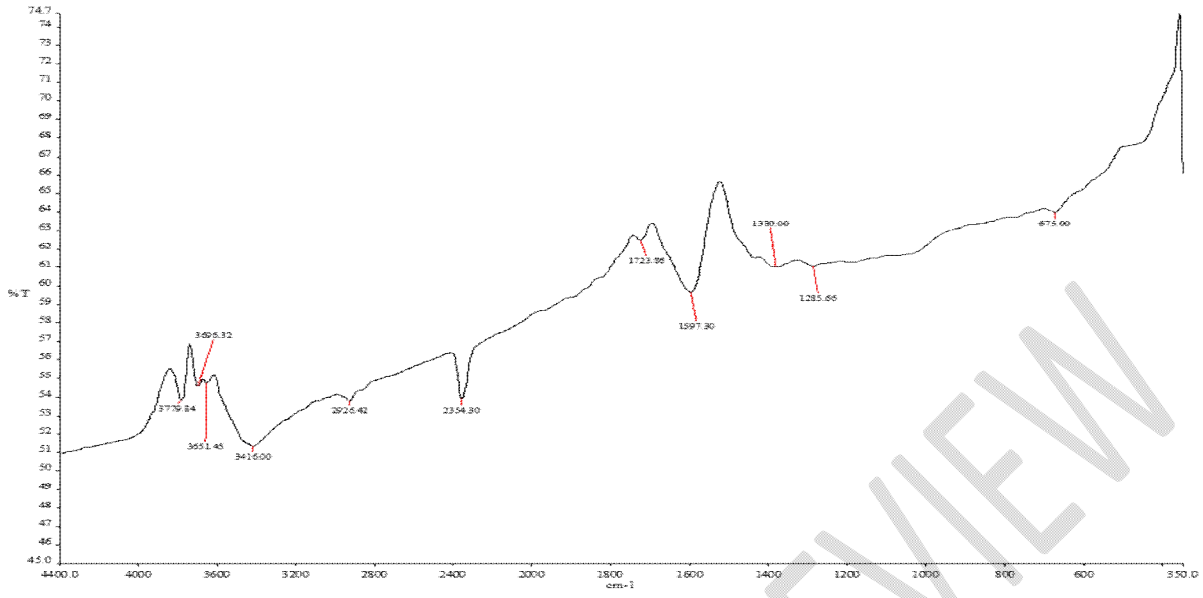


Figure 2: FTIR Spectrum Analysis of ethanol-toluene extract of PMBP

3.3 Antibacteria Activity of Ethanol-Toluene Extract of *Pentaclethra macrophylla Benth* Pod

The antimicrobial activity of an ethanol-toluene extract of *Pentaclethra macrophylla Benth* pods against some bacteria strains were shown in Table 3. Some of the bacterial pathogens tested were *Staphylococcus aureus, Proteus bacilli, Streptococcus pyogenes, Escherichia coli, and Candida species.*

Table 3: Susceptibility ethanol-toluene extract of *pentaclethra macrophylla Benth* pod

Organism/ concentration	*Staphylococcus*	*Proteus*	*Streptococcus*	*E-coli*	*Candida*
1000mg/mL	3.4mm	3.6mm	4.1mm	0.6mm	2.1mm
500mg/mL	2.1mm	2.5mm	4.0mm	0.2mm	1.0mm
200mg/mL	1.6mm	2.1mm	3.5mm	-	0.8mm
100mg/mL	1.2mm	1.6mm	2.2mm	-	0.2mm
50mg/mL	0.9mm	1.2mm	1.3mm	-	-
25mg/mL	0.3mm	1.1mm	1.0mm	-	-
12.5mg/mL	-	0.7mm	1.0mm	-	-
6.5mg/mL	-	0.5mm	0.9mm	-	-
Ciprotab(Standard)	7.2mm				

This result showed that the ethanol-toluene extract of *Pentaclethra macrophylla Benth* pods was susceptible to five bacteria that were investigated in the range of 6.5–1000 mg/mL. The ethanol-toluene extract has the highest zone at 1000mg/mL. The zone inhibition 1000mg/mL of Gram

(+) *Staphylococcus aureus* was 3.5mm, Gram. (-) *Proteus* bacilli was 3.6mm, Gram. (+) *Streptococcus pyogenes* was 4.1mm, Gram (-) *Escherichia coli* was 0.6mm, and *Candida species* was 2.1mm.

The Minimum Inhibitory Concentration (MIC) of Gram (+) *S. aureus* was 25mg/mL (0.3mm), Gram (-) *P. bacilli* was 6.5mg/mL(0.5mm), Gram (+) ***S. pyogenes*** was 6.5mg/mL(0.9mm), Gram (-) *E. coli* was 500mg/mL (0.2mm) and *Candida sp.* was 100mg/mL (0.2mm) [30]. The result therefore reveals that the ethanol-toluene extract of *Pentaclethra macrophylla Benth* pods was more effective on *S. pyogenes* (0.9mm) and *P.bacilli* (0.5mm). The bioactivity of the ethanol-toluene extract of *Pentaclethra macrophylla Benth* pods on the bacteria strains was compared with that of Ciprotab (the standard), and the result thus reveals that the standard was better than the ethanol-toluene extract. This is consistent with the findings of Orie et al. [25], who studied bacterial sensitivity and discovered that the MIC of standard antibacterial agents is higher than that of synthetic compounds.

3.4 Antifungal Activity of ethanol-toluene extracts of *Pentaclethra macrophylla Benth* Pod

The antifungal activity of an ethanol-toluene extract of *Pentaclethra macrophylla Benth* pods against some fungi strains was shown in Table 4. *Aspergillus Niger, Penicillium spp., Fusarium spp.,* and *Aspergillus fumigatus* were some of the fungi pathogens tested.

Table 4: Antifungal activity of ethanol-toluene extract of *Pentaclethra macrophylla Benth* Pod

Concentration/ Organism	*Aspergillus Niger*	*Penicillium spp*	*Fusarium spp*	*Aspergillus fumigates*
1000mg/mL	0.8mm	2.7mm	4.1mm	1.6mm
500mg/mL	0.3mm	1.3mm	4.0mm	1.2mm
200mg/mL	-	0.3mm	2.60mm	0.4mm
100mg/mL	-	-	1.22mm	0.2mm
50mg/ml	-	-	0.73mm	-
25mg/ml	-	-	0.mm	-
12.5mg/ml	-	-	1.60mm	-
6.25mg/ml	-	-	0.9mm	-
Focknazol (standard)	7.2mm			

This result showed that the ethanol-toluene extract of *Pentaclethra macrophylla Benth* pod was susceptible to four fungi that were investigated in the range of 6.5–1000mg/mL. At the 1000mg/mL concentration, *Aspergillus niger, Penicillium spp., Fusarium spp.,* and *Aspergillus fumigatus* have their respective zone inhibitions of 0.8mm, 2.7mm, 4.1 mm, and 1.6mm. The ethanol-toluene extract of *Pentaclethra macrophylla Benth* Pod was more sensitive to *Fusarium*

spp. with a MIC of 6.25mg/mL and zone inhibition of 0.9mm. This is in line with Chedia et al. [21] and Hosseinzadeh et al. [22], who worked on some pathogen sensitivity with plant extracts. The standard antifungal agent Focknazol has the highest zone inhibition at a minimum inhibitory concentration of 6.25mg/mL in all the fungi stains when compared with the ethanol-toluene extract of *Pentaclethra macrophylla Benth* pod.

4.0 Conclusion

The research work on characterization and antimicrobial profiling reveals that the ethanol-toluene extract of *Pentaclethra macrophylla benth* pod contains metabolites like flavonoids, phenols, alkaloids, tannins, saponins, steroids, terpenoids, and glycosides at trace concentrations. The extract on GC-MS analysis contains guaiacyl and syringil derivatives and, with the FTIR affirmation, the functional group of aromatic nuclei and the hydroxyl group.

The result of antimicrobial analysis reveals that the ethanol-toluene extract is most sensitive to *S. pyogenes*, *P. bacilli*, and *Fusarium spp.* at the MIC of 6.25mg/mL, but less active than standard antimicrobial agents Focknazol (antifungal) and Ciprotab (antibacterial). The wide sensitivity of the ethanol-toluene extract implies that it can be used clinically to control some pathogens in the pharmaceutical industry.

References

1. Wright, GD. Antibiotic resistance in the environment: a link to the clinic?. *Current opinion in microbiology*. 2010:*13*(5): 589-594.
2. Laxminarayan R, Duse A, Wattal C, Zaidi A K, Wertheim H F, Sumpradit N, Cars O. Antibiotic resistance—the need for global solutions. *The Lancet infectious diseases.* 2013: *13*(12): 1057-1098.
3. Okocha B I, Orie K J, Duru R U, Ngochindo R I. Analysis of the Active Metabolites of Ethanol and Ethyl Acetate Extract of Justicia carnea. *African Journal of Biomedical Research*. 2023;*26*(1): 111-117.
4. Nsude OP, Orie KJ. Phytochemical constituent and anti-corrosion properties of the root extract of Phyllanthus mellerianus (Nvo-nkwu) plant on mild steel in 1.5 M HCl medium. *Chemical Science International Journal*. 2022; *31*(2); 21-34.
5. Saxena M, Saxena J, Nema R, Singh D, Gupta A. Phytochemistry of medicinal plants. *Journal of pharmacognosy and phytochemistry*. 2013; *1*(6); 168-182.
6. Matic I, Guidi A, Kenzo M, Mattei M, Galgani A. Investigation of medicinal plants traditionally used as dietary supplements: A review on Moringa oleifera. *Journal of public health in Africa*. 2018; *9*(3).
7. Chhikara N, Kaur A, Mann S, Garg MK, Sofi SA, Panghal A. Bioactive compounds, associated health benefits and safety considerations of Moringa oleifera L.: An updated review. *Nutrition & Food Science*. 2021;*51*(2); 255-277.

8. Balogun BI. Evaluation of the nutritional potentials of fermented oil beans seed Pentaclethra macrophyllah Benth. *PAT*. 2013;*9*(2); 73-87.
9. Aremu AK, Ademuwagun AA, Ogunlade CA. Effects of moisture content and loading orientation on some mechanical properties of the african oil bean seed (Pentaclethra Macrophylla Benth). *African Journal of Agricultural Research*. 2014; *9*(49); 3504-3510.
10. Nsude OP, Agboeze E, Ezeh EC, Ike OC, Omuluche OC, Orie KJ, Ogbobe O. Isolation and characterization of cellulose from Pentaclethra macrophylla Benth pod biomass wastes for polymer reinforcement composite. *Journal of Chemical Society of Nigeria*. 2022;*47*(3).
11. Olaifa FE, Ajayi FR, Taiwo VO, Bello OS. Growth response and nutrient utilization of Clarias gariepinus on feeds supplemented with African oil bean (Pentaclethra Macrophylla Benth) seed residues. *Food*. 2012;*6*(1); 44-48.
12. Ajugwo AO, Adias TC, Osuala FN, Aghatise K, Onoriode DO, Anosike AU. In vitro studies of anticoagulation activity of *pentaclethra macrophylla*. *Journal Nutrient Health*. 2013; *1*(2); 15-17.
13. Yusuf-Babatunde AM, Kasim LS, Osuntokun OT. Short Term Toxicological Evaluation of the Pentaclethra macrophylla (Benth) Essential Oil (Oil Bean Seed) on Organs of Normal Male Albino Rats. *Journal of Advances in Medical and Pharmaceutical Sciences*. 2020; *22*(3); 41-48.
14. Nsude OP, Orie KJ. Microcrystalline Cellulose of Oil Bean Pod: Extraction, Physico-chemical, Brunauer–Emmett–Teller (BET), and Flow-ability Analysis. *Asian Journal of Applied Chemistry Research*. 2022;*12*(4);1-12.
15. Madukasi EI, Tojola OB, Oso K, Igwe CC. Thermo-chemical features of coating sludge and codensification of ad alternative energy source. *AASCIT Journal of Environment*. 2015; *1*(2); 28-34.
16. Mhilu CF. Analysis of energy characteristics of rice and coffee husks blends.*International Scholarly Research Notices, 2014*.
17. Oboh G, Bello FO, Ademosun AO, Akinyemi AJ, Adewuni TM. Antioxidant, hypolipidemic, and anti-angiotensin-1-converting enzyme properties of lemon (Citrus limon) and lime (Citrus aurantifolia) juices.*Comparative Clinical Pathology*. 2015; *24*; 1395-1406.
18. Omer SA, Adam, SEI, Mohammed OB. Antimicrobial Activity of Commiphora myrrha Against Some Bacteria and Candida albicans Isolated from Gazelles at King. *Research Journal of Medicinal Plant*. 2011;*5*(1); 65-71.
19. Balakrishnan B, Paramasivam S, Arulkumar A. Evaluation of the lemongrass plant (Cymbopogon citratus) extracted in different solvents for antioxidant and antibacterial activity against human pathogens. *Asian Pacific Journal of Tropical Disease*. 2014; *4*; S134-S139.

20. Bayoub K, Baibai T, Mountassif D, Retmane A, Soukri A. Antibacterial activities of the crude ethanol extracts of medicinal plants against Listeria monocytogenes and some other pathogenic strains.*African Journal of Biotechnology*. 2010;*9*(27); 4251-4258.
21. Chedia A, Ghazghazi H, Brahim H, Abderrazak M. Secondary metabolite, antioxidant and antibacterial activities of Teucrium polium L. methanolic extract. *International Journal of Plant Production*. 2013; *4*; 1790-1797.
22. Hosseinzadeh S, Jafarikukhdan A, Hosseini A, Armand R. The application of medicinal plants in traditional and modern medicine: a review of Thymus vulgaris. *International Journal of Clinical Medicine*. 2015; *6*(09); 635-642.
23. Wishart DS. Advances in metabolite identification. *Bioanalysis*. 2011;*3*(15); 1769-1782.
24. Abdulaziz Al-Hamoud, G., Saud Orfali, R., Sugimoto, S., Yamano, Y., Alothyqi, N., Mohammed Alzahrani, A., & Matsunami, K. (2019). Four New Flavonoids Isolated from the Aerial Parts of Cadaba rotundifolia Forssk.(Qadab). *Molecules*,*24*(11), 2167.
25. Orie KJ, Duru RU, Ngochindo RI. Metal complexes of heterocyclic sulphonamide: synthesis, characterization and biological activity. *Science Journal of Analytical Chemistry*. 2021;*9*(4); 104.
26. Don-Lawson C, Nweneka DO, Okah R, Orie KJ. Synthesis, Characterization and Bioactivity of 1, 1-bis (2-Carbamoylguanidino) furan-2-ylmethane. *American Journal of Analytical Chemistry*. 2020; *11*(06); 261-271.
27. Al-Fatimi M. Ethnobotanical survey of medicinal plants in central Abyan governorate, Yemen.*Journal of ethnopharmacology*. 2019; *241*; 1119-11127.
28. Orie KJ, Ngochindo RI, Abayeh OJ. Synthesis of 1, 1-bis (2-carbamoylguanidino) furan-2-ylmethane. *Journal of Chemical Society of Nigeria*, 2019; *44*(2);263-268
29. Gonfa T, Teketle S, Kiros T. Effect of extraction solvent on qualitative and quantitative analysis of major phyto-constituents and in-vitro antioxidant activity evaluation of Cadaba rotundifolia Forssk leaf extracts. *Cogent Food & Agriculture*. 2020; *6*(1); 1853867.
30. Ehara K, Takada D, Saka S. GC-MS and IR spectroscopic analyses of the lignin-derived products from softwood and hardwood treated in supercritical water. *Journal of wood science*. 2005; *51*; 256-261.