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BENZENETRIOLS AND SATURATED FATTY ACIDS ISOLATED FROM THE STEM BARK EXTRACTS OF *Pentaclethra macrophylla* BENTH.

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ABSTRACT

Pentaclethra macrophylla (African Oil Bean) tree is a plant found in the forest zone of West and Central Africa. In Nigeria the stem bark of the plant is boiled with water and gaggled in the mouth for oral troubles, dental cavies and toothaches.This study attempts to evaluate the antimicrobial activities of the methanol extract of the stem bark of the plant. The result of the phytochemical screening revealed that the extract contained alkaloids, tannins, terpenes, flavonoids, cardiac glycosides, saponins carbohydrates and polyphenols. In the antimicrobial study the extract (100 mg/ml) inhibited *Bacillus subtilis*, *Streptococcus* spp. (clinical isolate), *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* compared to the positive control (1.0mg/ml chloramphenicol). The column chromatography of the extract yielded two samples.The gas chromatography – mass spectrometry (GC-MS) and Fourier Transform – infrared spectroscopy (FT-IR) of these samples afforded three Benzenetriols namely: 1,3,5 – Benzenetriol(phloroglucine),1,2,3-Benzenetriol(Fourrine), 1,2,4-Benzenetriol (Hydroxyhydroquinone) and three saturated fatty acids namely: n-Hexadecanoic acid (palmitic acid), n-tetradecanoic acid (myristic acid) and n-Dodecanoic acid (Lauric acid).

KEYWORDS: Benzenetriols, Saturated fatty acids,Methanol extract, *Pentaclethra macrophylla.*

INTRODUCTION

The therapeutic properties of medicinal plants are characterized by the presence in their organs of active substances which physiologically affect the bodies of humans and animals or which are biologically active in relation to the causative agents of various diseases. Isolation and characterization of pharmacological active constituents from medicinal plants are among the leading research trends of our time.[1]

Pentaclethra macrophylla Benth. (Fabaceae), the Oil bean tree occurs in the humid lowlands of West and Central Africa. In Nigeria, the stem bark of *Pentaclethra macrophylla* is used to traditionally treat skin infections , sores, wound and cuts . The crushed seeds are taken to acquire an abortion. The bark decoction is given to the nursing mother to increase breast milk production. The stem bark is boiled with water and gaggled in the mouth for oral troubles, dental caries and toothache.[2]

Drug resistance to human pathogenic bacteria has been reported all over the world. This accounts for approximately one half of all deaths in tropical countries.[3]

UUThis research therefore, intends to determine the phytochemistry and some of the biological properties of the stem bark extracts of *Pentaclethra macrophylla* Benth. The results of this study may lead to the discovery of drug that could reduce or eliminate drug resistance to human pathogenic bacteria.

The objectives of the Study is to extract the dried pulverized stem bark of the plant with 70% methanol via cold maceration, to evaluate the presence of phytochemicals using standard procedures, to screen for Antimicrobial activity using agar well diffusion method and to isolate the compounds present

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

The stem barks were collected based on ethno-pharmacological information. The barks were harvested in Obot Akara Local Government Area of Akwa Ibom State, Nigeria in April, 2015.

The botanical identification of the plant, its bark and its authenticity were done by Dr. (Mrs.) Margeret Bassey of the department of Botany and Ecological Studies, University of Uyo, Uyo, Akwa Ibom State where a voucher specimen number UUH42(d) was assigned for future references and deposited in the university of Uyo Herbarium, Department of Pharmacognosy and Natural Medicine, faculty of pharmacy, University of Uyo, Uyo, Akwa Ibom State.

The barks were then cut into smaller pieces, sun-dried, pulverized in a mixer-grinder, filtered and the coarse powder was stored in a non-toxic polyethylene bag.

Extraction: The pulverized plant material (1000 g) was weighted and extracted exhaustively with n-hexane at room temperature for 72 hours. . The filtrate obtained was concentrated in volume at 40^0C and the dried extract stored in the refrigerator at -4^0C until required.The dried mass was extracted with ethylacetate and 70% methanol respectively,following the same procedure.

Phytochemical analysis: The above extracts were subjected to preliminary phytochemical screening to determine the presences of bioactive constituents. Thus was carried out using the standard methods of analysis.[4, 5]

CHROMATOGRAPHIC TECHNIQUE

An open glass column (gravity column) was used. 95.213g of silica gel of 50-200 (90%) particle size was constituted into slurry with n-hexane. The slurry was poured into a glass column of 3cm diameter and 53cm long and eluted. 115 eluates were collected.

Eluates 43-45, Rf (0.55) and 58-60 , Rf (0.78) yielded two samples. The samples were then subjected to IR and GC-MS techniques.

ANTI-MICROBIAL STUDIES

The standard strains of the micro-organisms used for the test were obtained from the Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State. The bacteria used include *Staphylococcus aureus, Bacillus subtilis, Streptococcus (clinical isolate).Salmonella typhi*. The fungus used is *candida albican*

Culturing Media: The media used were Nutrient agar (NA), nutrient broth (NB), Sarbouraud Dextrose Agar (SDA) and Sarbouraud Dextrose Broth (SDB). These media were prepared according to the manufacturer's instruction (Biotec^R UK)- NB (8g/L, NA (28g/L), SDB (30g/L) and SDA (62g/L).

Standardization Of Micro-Organism

The tests were characterized and pure isolate obtained. The organism were cultivated overnight in a nutrient both and sarbouraud dextrose broth respectively. The organisms were sustained on slant at 4^0C before use. Exactly 0.2ml of overnight culture of each organism were dispensed into 2ml of sterile Nutrient both (bacterial) and sarbouraud dextrose broth (fungi) and incubated for 3-5hours to standardize the culture to 10^6 cfu/ml. A loopful of the standard culture was used for the antimicrobial assay.

Plate Preparation, InoculationAnd Incubation

1.0 g of the extract each was weighed and the methanol extract reconstituted using sterile distilled water while ethylacetate and n-hexane was reconstituted using tween 80 concentrations of 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml respectively. The bacteria were cultured using nutrient Agar while fungi were cultured with sarbouraud dextrose agar. 25ml of the nutrient agar and sarbouaud dextrose agar each in different plates was seeded with 0.2ml of the test organism respectively by gently swirling for proper mixing of the organism and agar,. The plates were allowed to solidify in an aseptic environment. Then plates divided into quadrant and labeled with the aid of a sterile cork borer of diameter 4mm, five holes were bored at equidistant in the solidified plates.

The anti-microbial activities of the extract and commercial drug (Chloramphenicol and Nystatin) were tested using agar well diffusion technique.Each standard drug 0.2ml was introduced at the respective extract were carefully inoculated to the individual holes in the quadrant using a sterile Pasteur pipette. The prepared plates with the extract and standard drug were allowed to remain at room temperature for one hour to enhance proper diffusion of agents into the medium.

The bacterial cultures were incubated for 24 hours at 37^0C while fungal cultures were incubated at 25^0C for 25 hours before measuring the zones of inhibition in millimeter.[6]

RESULTS

The n-hexane extract weighed 9.199g (1.66 %w/w), Ethylacetate 28.736g (5.22% w/w) Methanol Extract 150.67g (27.39% w/w).

Table: Results of the Inhibition Zone Diameter (mm) of the extracts of *pentaclethra macrophylla* at 100mg/kg.

Test organism	N-hexane extract (100mg/ml)	Ethylacetate extract (100mg/ml)	Methanol Extract (100mg/ml)	Chloramphenicol (1.0mg/ml)	Nystatin (25,000 I.U)
Bacillus Subtilis	4	9	20	22	-
Streptococcus	15	18	21	28	
Staphylococcus Aureus	14	17	19	22	-
Eschiricha coli	13	22	23	30	-
Salmonella	7	18	23	30	-
Candida albicans	11	11	14	-	21

Phytochemical Screening: Phytochemical screening of *Pentaclethra macrophylla* benth extracts showed the presence of alkaloids, steroidal glycosides, cardinolides, flavonoids, cardiac glycosides, saponins carbohydrate and polyphenols.

Table: Result of the preliminary phyto-chemical screening

s/n	Constituents	n-Hexane	Ethylacetate	Methanol
	Alkaloid	+	+++	+++
	Flavonoids	+	+	+
	Tannins	+	+++	+++
	Cardiac glycosides	-	-	++
	Saponins	-	-	+
	Carbohydrates	-	-	+
	Terpenes	+++	+++	+++
	Phlobatanins	-	-	-
	Steroidal glycosides	++	++	+++
	Cardenolide	+	++	++
	Cyanogenic glycoside	-	+	++
	Anthraquinone	-	-	-
	Polyphenols	-	++	++
	Balsam	-	-	-
	Resin	-	-	-

+++ Present in high concentration

++ Present in moderate concentration

+ Present in low concentration

- Absent

GC-MS AND FT-IR ANALYSES OF SAMPLE I

Sample 1 yielded three isolates (isolates 1-3).

ISOLATE 1

The Compound Name is 1, 3, 5- Benzenetriol Common Name is PhloroglucineMolecular Formular is $C_6H_6O_3$, M olecular ion (M^+) is 126 and base peak at 100%. The mass-charge (m/e) ratio and the corresponding relative intensity are as follows:52 (11.90%) ,69 (16.67), 80 (9.52%), 85 (19.05%) and 126(100%).

ISOLATE 2

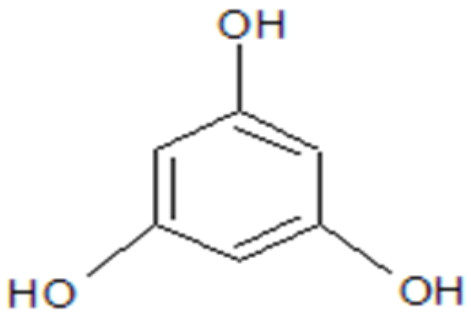
Compound Name is 1, 2, 3 – Benzenetriol , Common Name is Fourrine , Molecular Formular is $C_6H_6O_3$, Molecular ion (M^+) is 126 and the base peak at 100%. The mass-charge (m/e) ratio and the corresponding relative intensity are as follows: 52 (42.86%), 63 (4.76%), 80(42.86%), 97(9.52%), 108 (33.33%) and 126 (100%).

ISOLATE 3

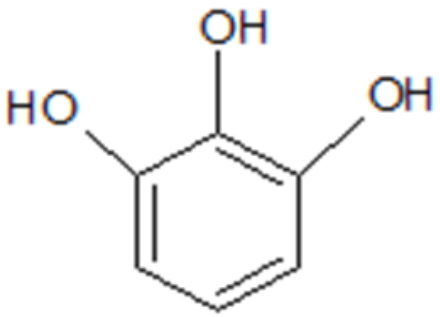
Compound name is 1,2,4 – Benzene triol , Common name is Hydroxyhydroquinon , Molecular Formular is $C_6H_6O_3$, Molecular ion (M^+) is 126 the base peak is 126 at 100%. The mass-charge (m/e) ratio and the corresponding relative intensity are as follows: 26(11.90%), 80(42.86%), 97(23.81%), 124(19.05%) and 126(100%).

IR ANALYSIS FOR SAMPLE I

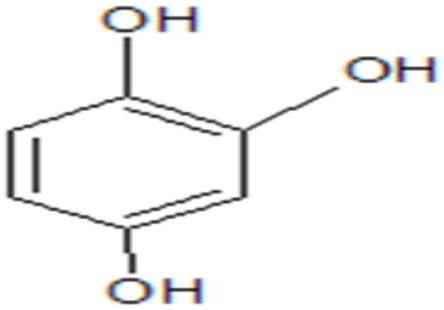
1362.75cm^{-1} to 1446.66cm^{-1} confirmed a H-C-H asymmetric and symmetric stretching frequency. 1531.53cm^{-1} to 1610.61cm^{-1} is due to a C=C conjugate bond present especially when ring is conjugated. 1714.77cm^{-1}confirmed the presence of an Ar=aromatic ring, Ar-CHO or Ar-C=O. 2173.85 cm^{-1} confirmed the presence of a C=C straight chain while 2931.90$cm^{=1}$ indicated the presence of a C-H straight chain. Absorption at 3359.14cm^{-1} was due to an O-H (hydroxy) group stretching frequency.



1, 3, 5 – Benzenetriol (Phloroglucine)



1,2,3-Benzenetriol (Fourrine)



1,2,4-Benzenetriol (Hydroxyhydroquinone).

Sample II yielded three isolates (isolates 4-6)

The GC/MS analysis revealed that the isolated compound may have chemical formulae $C_{12}H_{24}0_2$(Lauric acid),$C_{14}H_{28}O_2$ (Myristic acid) and $C_{16}H_{32}0_2$(Palmitic acid) respectively. [7]

n-Hexadecanoic acid (Palmitic acid)

n-Tetradecanoic acid (Myristic Acid)

n-Dodecanoic acid (Lauric acid)

ISOLATE 4

The Compound Name is n-Hexadecanoic acid , Compound name isPalmitic acid Molecular formular is$C_{16}H_{32}O_2$, Molecular ion (M^+) is 256 and the base peak is 43 at 100%. The masss-charge(m/e) ratio and the corresponding relative intensity are as follows: 41(76.19%), 43(100%), 60(78.57%), 73(90.48%) and 256(16.67%).

ISOLATE 5

Compound Name is n-Tetradecanoic acid , Compound name is Myristic acid Molecular formular is $C_{14}H_{28}O_2$, Molecular ion (M^+) is 228 and base peak is 43 at 100%. The mass-charge(m/e) ratio and the corresponding relative intensity are as follows: 41(90.48%), 60(92.86%), 73(100%), 129(38.10%) and 228(9.52%).

ISOLATE 6

Compound name is n-Dodecanoic acid , Common Name is Lauric acid, Molecular formular is $C_{12}H_{24}O_2$, Molecular ion (M^+) is 200 and the base peak is 60 at 100%. The mass-charge (m/e) ratio and the corresponding relatie intensity are as follows:41(71.43%), 60(100%), 73(97.62%), 129(38.10%) and 200(9.52%).

IR Analysis for Sample II

Absorption observed at 1409.05cm^{-1} was due to a methyl (-CH_3) groups and 1625.08cm^{-1} due to a C=C or C=C conjugate group since 1625.08cm^{-1} is closer to 1600cm^{-1}. 2349.38cm^{-1} sharp revealed that O=C=O (carbondioxide) was neatly present or a carboxylate group.

DISCUSSION

The result of phytochemical screening revealed that the methanol extract contained saponins, alkaloids, tannins, terpenes and steroidal glycosides in high concentrations. The ethylacetate extract had high quantity of alkaloids, tannins and terpenes. On the whole, alkaloids, flavonoids, tannins, terpenes, steroidal glycoside and cardenolides were present in the three extracts. Balsam and Resins were absent in the three extracts.

The GC-MS analysis of sample I revealed the presence of of 1,2,3-Benzenetriol; 1,2,4-Benzenetriol and 1,2,5-Benzenetriol in the methanol extract. Sample II contained three saturated fatty acids (Lauric acid, Palmitic acid and Myristic acid).

Anti-Microbial Analysis

The anti-microbial assay showed that *Bacillus subtilis, Streptococcus* (clinical isolate), *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 10418) and *Salmonella* typhii (NCTC 8571) were susceptible to the extracts and the standard drug (Chloramphenicol). The fungus, *Candida albicans* (clinical isolate), was inhibited by the standard drug (Nystatin) but not by the extracts.

Lauric acid has been found to possess very strong anti- bacteria activity against skin bacteria agents like *Staphylococcus aureus and Staphylococcus epidermidis*[8,9] and *Helicobacterpylori.*[10] Medium chain fatty acids acid has been reported to posses strong antimicrobial properties.[11] Monoalurin is an ester derivative of Lauric acid found to be more effective against gram positive bacteria such as *Staphylococcus and Streptococcus* compared to gram negative bacteria.[12, 13] Some phytochemicals like alkaloids, Flavonoids and tannins[14] are known to posses anti-microbial activity.

CONCLUSION

Based on the finding of this study, it can be concluded as follows

a. That the stem bark extract of *Pentaclethra macrophylla* contain flavonoids, saponins, tannins, alkaloids, cardiac glycosides, carbohydrates and polyphenols.

b. That the extract demonstrated significant anti-microbial activities.

c. That the extract contained Benzenetriols such as phloroglucine, Fourrine and hydroxy hydroquinone. It also contains saturated fatty acids such as Palmitic, myristic and Lauric acid.

d. The phytochemical constituents and the isolated compounds are believed to be responsible for the anti-microbial activity.

RECOMMENDATION FOR FURTHER STUDIES

It is hereby recommended as follows, that

1. Anti-inflammatory, anti-pyretic, anti-nociceptive studies should be carried out on the methanol n-Hexane and ethylacetate bark extracts of this plant.

2. Further chromatographic and spectroscopic studies should be carried out to isolate more compounds from the n-hexane and ethylacetate extracts of the plant.

3. Cellular mechanism of actions of structural components of the isolates in the plant should be elucidated.

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