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GC-MS, Phytochemical and Antimicrobial Analysis of Pentaclethra Macrophylla Leaf

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Abstract:
Pentaclethra macrophylla is a plant used all over the world for its good nutritional qualities as well medicinal importance, the chemical constituents of this plant has not been fully documented. On this basis, it became necessary to probe more into this plant forits medicinal constituents The leaf of Pentaclethra macrophyllawas analyzed for its phytochemical and anti-microbial propertieswith the aid of; Shimazu Japan GC model 5890-11, GC-MS QP 2010 PLUS Shimazu Japan and Jenway digital Spectrophtometer model 6303. The GC-MS analysis of the leaf of Pentaclethra macrophylla gave 8 compounds ; ε, ε -2, 15 –octadecedien -1-ol acetate (6.90 %), hexadecanoic acid (3.14 %), n-hexadecanoic acid (10.69%), 6-octadecenoic acid (13.84 %),octadecanoic acid (8.18 %), (.2H) phenanthrenone (42.14 %) ,Pregnan-2-one (6.29 %) and -4-methylene-1-methyl-2- (z– methyl 1-propene -1- yl) cyclo hexane (9.43 %).The phytochemical compositon of the sample showed that the alkaloid composition was 1.76 %, with corresponding values of 1.35.%, 0.91%, 3.7ppm and 0.86 ppm for flavonoids,saponins, phenols and tannins respectively The extracts showed marked inhibition of the growth of seven selected pathogens; Pseudomonas aeruginosa13 mm, Staphylococcus aureus 13 mm, Escherichia coli17.5 mm, Salmonella typhi16 mm, Candida albican10 mms,Aspergillus niger 13 mm and Penicillium12mm with minimum inhibitory concentrations of 25mg/cm^3, 12.5 mg/cm^3,6.5 mg/cm^3, 25 mg/cm^3,12.5mg/cm^3, 6.5 mg/cm^3 and 25mg/cm^{3}respectively. These finds lend credence to the ethnomedicinal applications of these plant part in the treatment of various ailments

Keywords: *Newbouldia laevis, alkaloids, saponins flavonoids, tannins, phenols, pathogens*

1. Introduction

Pentaclethra macrophylla is a multipurpose tree of West Africa, in Agro forestry and in the tropics. It is recognized by farmers in the south east Nigeria for its soil improvement properties. Its seed is useful as food. The leaves, bark and root are useful medicines while the trunk is used as timber, the tree yields forest products for making· household utensils. The mature dispersed seeds are harvested and sold in the market and may serve as a revenue earner. The seed could serve as protein supplement, (Enujiugha and Agbede ,2000). Its richness in vitamins and minerals makes it a highly sought after food supplement for both local consumption and export. The seed serves as a source of oils for candle making, cooking and soap (Tico, 2005.) The seed shells are decorative and are often used to craft beads which are worn as necklace, rosaries and sometimes for local dancing apparels, (NFT, 1995; ICRAF ,2004). Every part of this multipurpose plant has numerous useful application***, the*** bark of this tree is used to treat leprosy sores and the seed when cooked and fermented is used in preparing delicacies (Asoegwu *et al,* 2006). The seed is rich in alkaloids, saponins, flavonoid phenols and tannins (Okwu and Aluwuo, 2008). The leaf and stem are applied in the treatment of diarrhea while the pod and leaf are used to treat convulsion. The bark, fruits, seed and leaves are used as anthelminitcs, for gonorrhea treatment and for convulsion as well as analgesics. (Bouquet *et al,*1971; Iwu *et al,* 1990.). Works by (Okunrubo *et al* ,2009) has demonstrated that the Methanolic extract and aqueous fractions of the stem bark of *Pentaclethra macrophylla* showed marked antinociceptive activities. The aqueous and ethanolic extract of *Pentaclethra macrophylla*has been found to have antidiarrheal potentials, (Akah *et al* ,1999). The works of (Ugbogu and Akukwe, 2000) on the anti-microbial properties of the oil of *Pentaclethra Macrophylla*, *Chrysophyllium albidum and Persea gratissimum seed on E. Coli, P. mirabilis, P. aeruginosa, S. aureus* and*S. epidermidis* have shown that the oils of Pentaclethra.marcphylla have marked anti-microbial properties as none of the organisms showed complete resistance to the oil.

(Okunrubo *et al,* 2009) carried out investigations into the antinociceptive activities of the methanol and aqueous extracts of the stem bark of *Pentaclethra. macrophylla*using acetic acid induced mouse writing assay and tail immersion assay in mice. Their findings confirm the ethno medicinal usage of Pentaclethra macrophylla stem bark as an antinociceptive agent. The leaves of this plant when boiled with bush pepper produces a liquid given for the treatment of fever. Extracts of the leaves seed and bark are used' to treat itching and pains in animals and man and improving the anti-inflammatory response. (Okorie *et al* ,2006). Oils from the leaves have anti-inflammatory qualities and aid in wound management. The seed when ground into paste/lotion renders antimicrobial effects promoting healing while extracts from the bark are applied to leprosy sores. The rich mineral composition of the fermented seed makes it a low cost source of protein. Increase intake of the seed as food increases the hemoglobin value in test animals, increased oxygenation of tissue, enhances specific hormone and stimulates the production of red blood cells important in proper cardiac function. The plant is a source of dietary estrogens (phytoestrogens) which can be employed in nutritional supplement and pharmaceutical preparation and vitamin supplement in the control of obesity. The seed when crushed and eaten with red ants induces abortion and is used in native African population as a home abortion remedy (Abbiw ,1990; Isawumi ,1993; Tico, 2005)

2. Materials and Method

2.1. Plant Materials

The sample were obtained from a farm land in Ezinihitte Mbaise area of Imo state and was identified by Dr. Nmeregini of forestry department Michael Okpala University umudike, the voucher specimen marked E3625 was deposited in the. Forestry department Herbarium of Michael okpara University Umudike. They were washed with distilled water and room dried. The dried samples were milled with an electric milling machine and stored in air tight plastic bottles and kept for analys**is.**

2.2. Alkaloid Determination

5 g of the sample was weighed into a 250 cm^3 beaker and 200 cm^3 of 29 % acetic acid in ethanol was added and covered to stand for 6hrs. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. The Alkaloid was precipitated out using concentrated ammonium hydroxide which was added drop by drop until precipitation was complete. The solution was allowed to settle and the precipitation was collected by filtration using whatman filter paper, the precipitate was dried and weighed (Obadoni and Ochuko (2001).

Saponin determination 20 g of the sample was weighed into a 250 cm^3 beaker and 200 cm^3 of 20 % ethanol was added and stirred using a glass rod. The mixture was heated over water bath for 4hrs with continuous stirring while the temperature was maintained at 55 °C. The mixture was extracted and the residue was retractedwith 200 cm^3 of 20 % ethanol. The combined extract was reduced to 40 cm^3 over water bath at 90 °C. The concentrated extract was transferred into a 250 cm^3 separation funnel and 20 cm^3 of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. This process was repeated thrice. 60 cm^3 of n-butanol was added. The mixture was washed twice with a10 cm^3 of 5 % sodium chloride. The remaining solution was heated over water bath and the residue dried to constant weight. The saponin content was calculated in percentages (Obadoni and Ochuko 2001).

2.3. Flavonoid Determination

10 g of the plant sample were extracted repeatedly with 100 cm^3 of 80% of aqueous methanol at room temperature. The solution obtained was filtered with whatman filter paper no 45. The filtrates were later transferred into a crucible and evaporated to dryness over a water bath and weighed (Boham and Kocipai ,1994)

2.4. Phenol Determination

2 g of the sample was defatted with 100 cm^3 of diethyl ether using a soxhlet apparatus for two hours. The defatted sample was boiled within 50 cm^3 of ether for 15 minutes, then 5 cm^3 of the extract was pipetted into a 50 cm^3 flask and 10 cm^3 of distilled water was added. 2cm^3 of ammonium hydroxide and 5 cm^3 of amyl alcohol were added. The samples were made up to the mark and left for colour development. The absorbance of the solution was measured using Jenway digital spectrophotometer model 6303 at 505 nm wavelength (Obandoni and Ochuko 2001, Harbone 1973)

2.5. Tannin Determination

0.5 g of the sample was weighed into 250 cm^3 beaker and 50 cm^3 of distilled water was added and stirred vigorously with a glass rod for one hour the solution was filtered into a 50 cm^3 volumetric flask and made up to the mark. 5 cm^3 of the filtrate was pipetted into a test tube and mixed with 3 cm^3 of O.I M $FeCl_3$ in O.IN HCI and 0.008 M Potassium Ferro cyanide. The absorbance was measured with the Jenway digital spectrophotometer model 6303 at 120 nm wave length. The absorbance was compared with those of standard made from tannic acid (Van-Burden and Robinson 1981)

2.6. Preparation of Samples for GC-MS Analysis

Two hundred grams of the sample was repeatedly extracted with ethanol using soxhlet extractor, another 200 g of each sample was soaked in ethanol for 48 hours and extracted. The extracts from the soxhlet extracts and that obtained from cold extracts were combined and they were re-extracted using chloroform to obtain chloroform soluble extract and store in sample bottle which was taken to NARIT, Zaria for GC-MS analysis.

2.7. GC-MS Experimental Procedures

GC- analysis was carried out with SHIMAZU Japan Gas Chromatography 5890-11 with a fused GC column OV 101 coated with polymethyl silicon (0.25 mm x 50 m) and the conditions are as follows: Temperature programming from 80 – 200 ^{o}C held at 80 ^{o}C for I minute, rate 5 ^{o}C/min and at 200 ^{o}C for 20 minutes. FID Temperature of 300 ^{o}C, injection temperature of 250 ^{o}C, carrier gas is Nitrogen at a flow rate of 1 cm^3/min, split ratio 1: 75. Gas chromatography Mass spectrum analysis were conducted using GC-MS QP 2010 Plus Shimazu Japan with injector Temperature at 230 ^{o}C and carrier gas pressure of 100kpa. The column length was 30 m with a diameter of 0.25 mm and the flow rate of 50 ml/min. The eluents were automatically passed into the Mass Spectrometer with a detector voltage set at 1.5 kv and sampling rate of 0.2 seconds. The Mass Spectrum was also equipped with a computer fed Mass Spectra data bank, HERMCE Ƶ 233 M-Ƶ centrifuge Germany was used. Reagents and solvents such as Ethanol, Chloroform, Diethyl ether, hexane all of analytics grade was obtained from Merck Germany.

2.8. Anti-Microbial Analysis

The Micro Organisms*, Pseudomonas aureginosa, Staphylococcus aureus, Escherichia coli, Salmonela typhi, Candida albicans and Aspergillus niger* were used for the analysis. They were obtained from the stock cultures of the Federal Medical Centre Umuahia cultures and were brought to the laboratory and were resuscitated in peptone water and thereafter subcultured into nutrient agar medium and incubated at 37 ^{o}C for 24 hrs (Okigbo and Omodamiro 2006).

2.9. Antibacterial Assay

The test solution of each extract was Prepared by dissolving 0.1 g of the plant extract separately in 1.0 cm^3 of dimethyl sulphoxide (DMSO) to get a concentration of 100 mglcm^3. The antibacterial activity was performed by filter paper disc diffusion technique. Filter paper disc (Watman No 1.6 mm diameter) were placed in glass petridishes and sterilized in hot air oven (Ekundayo and Ezeogu, 2006). The media (10 g nutrient Agar in 200 cm^3 distilled water, auto-claved at 115 ^{o}C for 30 minutes) was cooled to 50^{o}C. The sterile nutrient Agar media were poured into the sterile petridish and allowed to solidify. The bacteria were swabbed with a sterile wire loop. Each disc was impregnated with 0.2 cm^3 of plant extract standard, Ciprofloxacin was used as a control on a disc with DMSO 100 mg/$cm^{3.}$ The disc was used after drying them in an incubator at 40 ^{o}C to remove any trace of solvent. The plates were microbated at 37 ^{o}C for 24 hrs to obtain zones of inhibition. The experiments were repeated three times for each extract and twice for reference antibiotics to minimize error and the average of these values were recorded.

2.10. Minimum Inhibitory Concentration. (MIC)

The minimum inhibitory concentration of the extracts was determined by incorporating constant volume 0.2 cm^3 of each diluent of the extracts into the perforated disc on a seeded nutrient agar plate as described in the anti-microbial susceptibility test section (Okigbo and Omodamiro 2006). 0.1 g of each extract was dissolved in 1cm^3 of DMSO to obtain 100 mg/cm^3. This concentration of DMSO was then double to obtain 50 mg/ml, then double again to obtain 12.5 mg/cm^3 and again to obtain 6.25 mg/cm^3. Each concentration was then used in the method earlier described to obtain zone of inhibition. The least concentration that showed inhibitory zones was taken as the MIC.

3. Results and Discussions

The result of the experimental are stated and discussed as follows:

Constituent	leaf %
Alkaloid	1.76
Saponins	0.91
Flavonoids	1.35
Tannins	3.7ppm
Phenols	18.75ppm

Table 1: photochemical content of Pentaclethra Macrophylla leaf

The leaf of *Pentaclethra Macrophylla* contains 1.76 % alkaloid. Alkaloid rank among the most efficient therapeutically significant plant substance. Pure isolated alkaloids and their synthetic derivatives are used by Etinomedicinal practitioners for their analgesic, antispasmodic and bactericidal effects (Okwu and Okwu 2004). They exhibit marked physiological activity when administered to animals, the high alkaloid content of these sample may be the reason for their use in the treatment of cough, wounds, rheumatism and skin infections. Most samples containing alkaloid are used in Nigeria for the treatment of malaria and fever. (Adesegun and Coker 2001)

Saponins was found to be available at 0.91 % in the leaf of *Pentaclethra Macrophylla*, the saponin content fortifies the use of the extract from this plants in the treatment of wounds. Some of the general characteristic of saponins includes; formation of forms in aqueous solutions, hemolytic activity and cholesterol binding properties (Okwu 2005); Sodipo*et al* (2000). Saponin has the natural tendency to ward off microbes and this makes them good candidates for treating fungal and yeast infections. These compounds serve as natural antibiotic, helping the body to fight infections and microbial invasion.

The flavonoid content of *Pentaclethra Macrophylla*leaf was 1.35 %. flavonoids are distributed group of polycyclic compounds characterized by a common Benzo pyrone ring structure that has been reported to act as antioxidants in many biological systems. The family encompasses flavonoids, flavones, chalcones, catchins, anthocyanidins and isoflavonoids (Okwu and Aluwo 2008). In addition to their free radical scavenging activities, Flavonoids have multiple biological activities including – vasodilatory, anti-carcinogenic, anti-allergic, antiviral, estrogenic effects as well as being inhibitors of phospholpase H_2, cycloxygenase, glutathione reductase and xanthine oxidase. (Saleh *et al* 1995; Del Rio *et al.* 1997; Okwu 2004), they support lactogenecity.These properties therefore support the use of *Pentaclethra Macrophyllaleaf* in cancer therapy. (Asoegwu *et al* .2006). Flavonoids in intestinal tracks lower the risk of heart diseases. As anti-oxidant, favonoids provide anti-inflammatory actions.

The phenolic content of the sample is 18.75ppm,there is a growing interest in polyphenolic compounds as therapeutic agents against many diseases such as cardiac and cerebral ischemic, arteriosclerosis and rheumatic or pulmonary diseases. (Saleh 1995, Middletone and Kandaswani 1992). The activated phagocytic cells are known to produce potentially destructive oxygen species like super oxide anion (O^{2-}), hydrogen peroxide (H_2O_2) and Hypochloric acid (HOCl) during chronic inflammatory disorder. (Okwu and Aluwo 2008). Many polyphenolics are known to exhibit antioxidant properties; they are free radical's scavengers. Phenolic flavonoids are also excellent hydroxyl scavengers. These properties promote health, and prevents certain chronic disorders such as cancer, cardiovascular diseases, diabetics and arthritis. The presence of phenols means that the extract could act as anti-inflammatory, anti-clothing, anti-oxidants, immune enhancers and hormone modulators. Phenols have been the subject of extensive research as disease preventives. (Saleh *et al* 1995, Duke 1992). The have the ability to block specific enzymes that cause inflammations. They modify the prostaglandin pathways and thereby protect platelets from clumping.

The Tannin content of *Pentaclethra Macrophylla* leaf was 0.93 %. Tannins have astringent properties, hastening the healing of wounds and inflamed mucous membrane (Okwu and Okwu 2004). The presence of Tannins in these samples supports their use in treating wounds, varicose ulcers, hemorrhoids, frost bites and burns in herbal medicine.

Organism	Zone of inhibition (mm)	Cipro	Minimum inhibition concentration(mg/cm^3)
Pseudomonas aeruginosa	18	26	25
Staphyloccus aureus	13	12	12.5
Escherichia coli	17.5	16	6.5
Salmonella typhi	16	19	25
Candida Albicans	10	22	12.5
Penicillium	12	18	25
Aspergillus Niger	13	19	6.5

Table 2: Antimicrobial activity of Pentaclethra macrophylla leaf

The role of*Pentaclethra Macrophylla* leaf extract in the inhibiting the activities of *Pseudomonas aureginosa, Staphylococcus aureus, Eschirichia coli, Salmonella typhi, Candida albicans, Aspergillus niger and Pencillum* have beendocumented (table 2.) The leaf extract showed high inhibition of *Pseudomonas Aeruginosa* 18 mm. *Escherichia Coli* 17.5 mm *Staphylococcus aureus* 13 mm and *Candida albicans* 10 mm Penicillum 12 mm and Aspergillus niger 13mm. These zones are considerable when compared to those of standard antibiotic ciproflaxin. This inhibition therefore supports the use of the leaf extract in treatment of convulsion and diarrhoea.Okorie et al (2005). Also the use of this plant part for fever treatment can therefore be understood.

3.1. GC-MS Analysis of Pentaclethra macrophylla leaf

GC-MS analysis of the leaf of*Pentaclethra Macrophylla* leaf showed 8 peaks. Peaks 1 appeared at m/z 308 with formula $C_{20}H_{38}O_2$, its oil content is 6.9% and is named, z, z-1,2,5-Octadecan -1- ol acetate [1].Peak 2 occurred at m/z 270 with molecular formula $C_{17}H_{34}O_2$ with 3.14% oil content and is named Hexadecanoic acid [2].Peak 3 appeared at m/z 256 with the formula $C_{16}H_{32}O_2$, having 10.6% oil content and named n-hexadecanoic acid, [3],Peak 4 occurred at m/z 262, its formula is $C_{18}H_{34}O_2$, with 13.84% oil content and is identified as 6-Octadecanoic acid [4]. Peak 5 occurred at m/z 284 with the formula $C_{18}H_{36}O_2$ its oil contentis 8.18% and its name is Octadecanoic acid [5].Peak 6 appeared at m/z 292 with the formula $C_{18}H_{28}O_3$ having 42,14% oil content and named (2H) Phenantherone [6]. Peak 7 occurred at m/z 334 with the formula $C_{21}H_{34}O_3$ with 6.27% oil content and named Pregnan-20-one [7]. Peak 8 occurred at m/z 204 with formula $C_{15}H_{24}$ and having 9.43% oil content and is identified as cyclo heptan-4-methylene-1-methyl-2 (z-methyl propen-1-yl [8]

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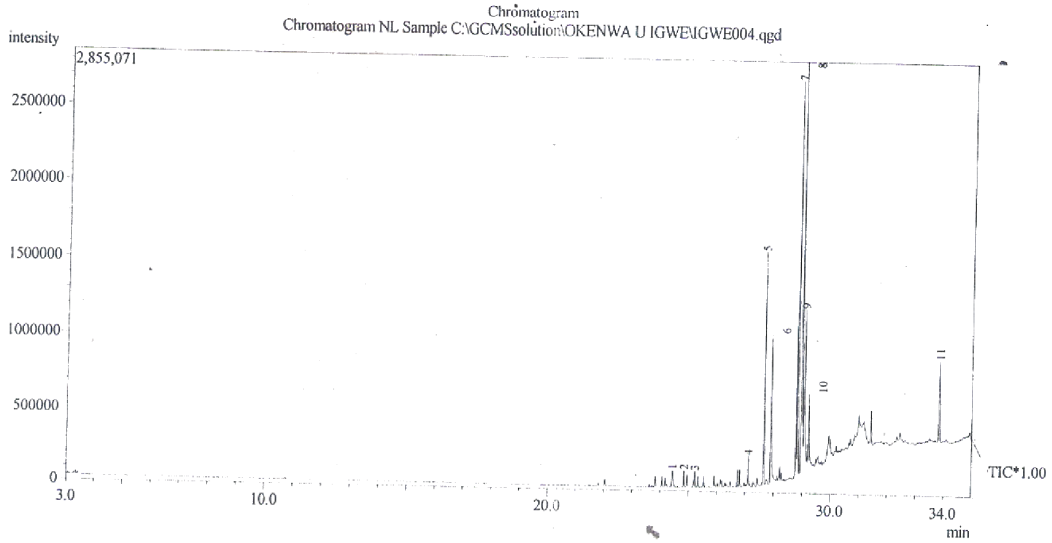
GCMS-QP2010 PLUS
SHIMADZU,JAPAN

NARICT, ZARIA GCMS ANALYSIS

MR IWU (SAMPLE- NL)



Method

[Comment]

Analytical Line 1

[AOC-20i]

# of Rinses with Presolvent	:5
# of Rinses with Solvent(post)	:5
# of Rinses with Sample	:3
Plunger Speed(Suction)	:High
Viscosity Comp. Time	:0.2 sec
Plunger Speed(Injection)	:High
Syringe Insertion Speed	:High
Injection Mode	:Normal
Pumping Times	:5
Inj. Port Dwell Time	:0.3 sec
Terminal Air Gap	:No
Plunger Washing Speed	:High
Washing Volume	:8uL
Syringe Suction Position	:0.0 mm
Syringe Injection Position	:0.0 mm
Use 3 Solvent Vial	:1 vial

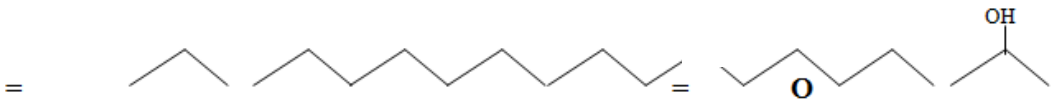
[GC-2010]

Column Oven Temp.	:60.0 °C
Injection Temp.	:250.00 °C
Injection Mode	:Split
Flow Control Mode	:Linear Velocity
Pressure	:100.2 kPa
Total Flow	:6.2 mL/min
Column Flow	:1.61 mL/min
Linear Velocity	:46.3 cm/sec
Purge Flow	:3.0 mL/min
Split Ratio	:1.0
High Pressure Injection	:OFF
Carrier Gas Saver	:OFF
Splitter Hold	:OFF

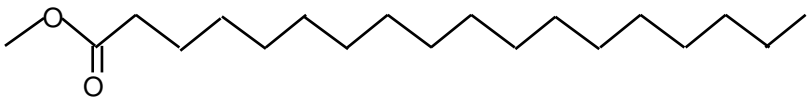
Figure 1: GC-MS of sample

Peak	Name of Compound	Molecular Formular	Molecular Weight	Retention Time (Sec.)	Fragmentation % oil	Peaks
1.	Z, Z - 2, 14 - octa decadien -1-ol actetate	$C_2H_{36}O_2$	308	27.1	6.9	41(90.47) 55(90.4) 68(100) 82(76.19) 96(33.33) 109(28.57) 123(9.52) 135(4.78) 149(4.78) 208(4.78) 248(1428).
2.	Hexadecanoic Acid .	$C_{17}H_{34}O_2$	270	27.7	3.14	27(14.28) 41(33.33) 57(14.28) 74(100) 87(66.66) 101(476) 115(2.38) 127(4.7 143(14.28) 157(2.38) 185(2.38) 199(2.38) 227(9.52) 239(4.76) 270(9.52).
3.	n-Hexadeconoic acid	$C_{16}H_{32}O_2$	256	27.9	10.69	27(19.04) 41(80.95) 43(100) 60(71.42) 73(95) 85(23.80) 98(19.04) 115(19 129(38.09) 143(4.76) 157(9.52) 171(9.52) 185(9.52) 213(23.50) 227(4.76) 256(45).
4.	6-Octadecanoic acid	$C_{16}H_{34}O_2$	282	29.1	13.84	40(4.73) 41(80.85) 53(100) 65(61.90) 83(57.14) 97(38.0) 98(28.57) 112(14.26) 127(9.52) 147(2.38) 189(2.38) 207(2.38) 222(2.38) 235(2.38) 264(9.52) 282(2.38) 302(2.38).
5.	Octadecanoic acid	$C_{16}H_{36}O_2$	284	29.2	8.18	27(14.28) 41(71.45) 43(100) 60(80.95) 73(85.71) 85(23.80) 98(14.26) 115(14.26) 129(28.5) 143(14.76) 171(23.80) 185(14.28) 199(4.76) 213(4.76) 241(14.26) 255(14.28) 284(28.57).
6.	(2H) phenathrenone	$C_{18}H_{28}O_3$	292	30.34	42.14	41(76.19) 43(38.09) 67(33.33) 81(28.57) 93(28.57) 100(37.14) 120(14.24) 134(45) 147(9.52) 159(4.76) 178(42.85) 190(4.72) 217(4.76) 232(100) 291(28.57)
7.	Pregnane -20- one	$C_{21}H_{34}O_3$	334	30.90	6.27	53(71.42) 55(95) 67(85.71)79 91(100) 107(95) 121(52.38) 135(38.05) 173(14.26) 215(57.14) 230(76.19) 255(61.90) 273(33.33) 278(42.85) 288(42.55) 298(4.76) 316(57.14)
8.	cycloheptane4-methylene-1-methyl -2-, (z-methyl propen-1-yl) 1-yl	$C_{15}H_{24}$	204	32.325	9.43	27(14.21) 41(85.71) 55(51.14) 67(71.42) 81(85.71)93(10 107(95) 119(42.85) 133(42.85) 147(23.80) 161(42.85) 176(9.52) 189(23.80) 204(4.76)

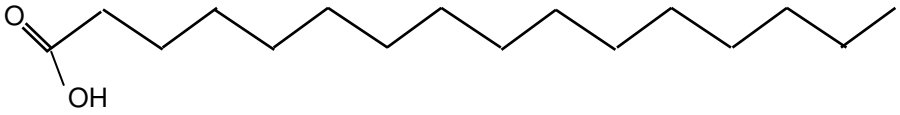
Table 2: constituents of Pentaclethra macrophylla leaf (PL)



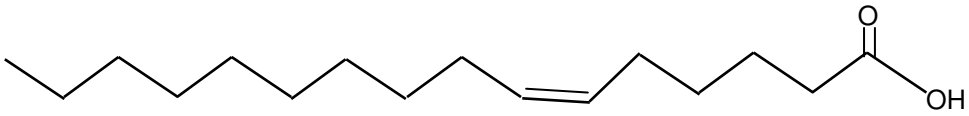
[45]z, z- 2,14-octadecedien-1-ol acetate



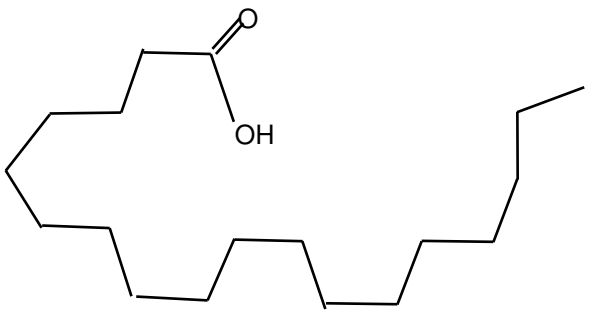
[46]Hexadecanoic acid methyl ester,



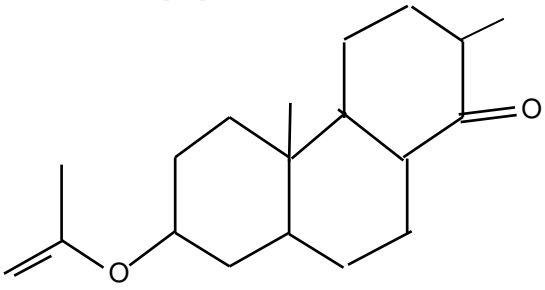
[47] n-hexadeanoic acid



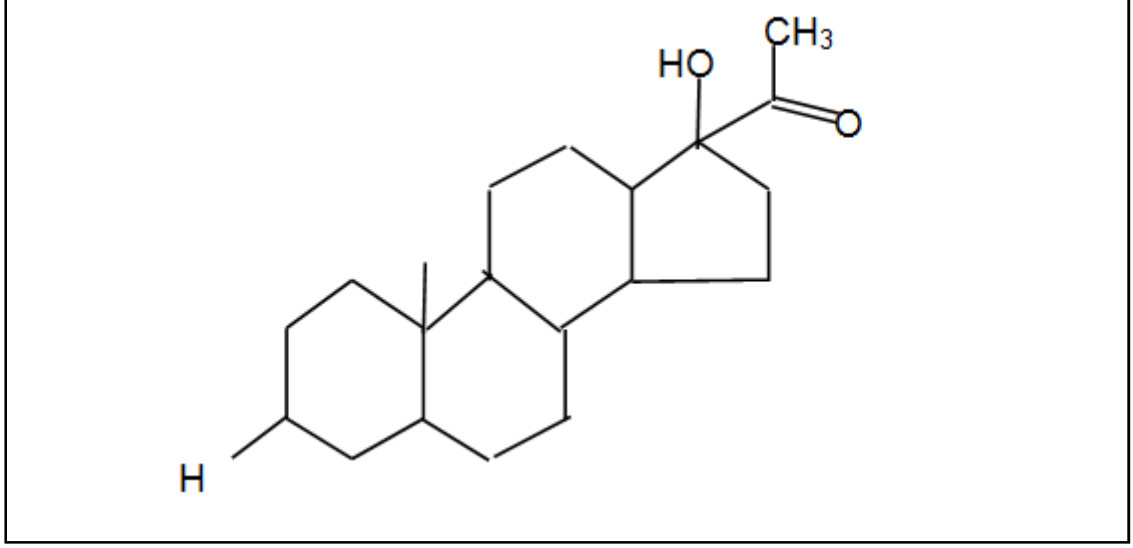
[48]6-octadecanonic acid



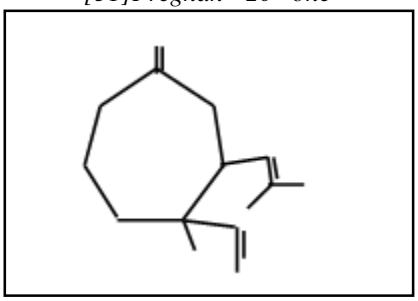
[49]Octadecanoic acid



[50]2- (H) phenanthrenone



[51]Pregnan - 20 –one



[52]cycloheptane – 4 – methylene – 1 – methyl - 2- (~~Z~~- methyl – 1 – propene – 1 – 1 yl)

Figure 2: Structures of compounds identified form Pentaclethramacrophylla

4. Acknowledgements

The authors wish to thanks the staff of NARCIT Zaria Nigeria for carrying out the GC-MS analysis of the sample.

5. References

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