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GC-MS, Phytochemical and Antimicrobial Properties of Pentaclethra Macrophylla Bark (P.Benth)

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Abstract:
The chemical components of most medicinal plants such as Pentaclethra macrophylla have not been fully documented, to this end, we embarked on the determination of volatile components of stem bark of this plant. The stem bark of Pentaclethra macrophylla was analyzed for its phytochemical and anti-microbial properties with the aid of; Shimazu Japan GC model 5890-11, GC-MS QP 2010 PLUS Shimazu Japan and Jenway digital Spectrophotometer model 6303. The GC-MS analysis of this sample yielded 10 compounds: 1,2,3- benzenetriol (11.53 %), 1, 4 – methoxy -3- (4- methyl phenoxy) phenyl ethanone (2.31 %), 1,2-benzenecarboxylic acid (2.31. %), 3-eicosene (2.31 %), hexadecanoic acid methyl ester, (4.62 %), n-hexadecanoic acid (20.77 %), 6-octadecenoic acid methyl ester (5.38 %), 6-octadecanoic acid, (25.77 %), Octadecanoic acid (13.25 %) and Naphto [-1, 2, 6-] furan -2-one (10.38 %). The phytochemical composition of the sample showed that the alkaloid composition was 0.80 %, with corresponding values of1.36 %,0.71 %,0.018 % and 0.93 % for flavonoids ,saponins, phenols and tannins respectively The extracts showed marked inhibition of the growth of seven selected pathogens; Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Candida albicans, Penicillium and Aspergillus niger with minimum inhibitory concentrations of 6.5 mg/cm^3, 12.5 mg/cm^3 ,6.5 mg/cm^3, 2.5 mg/cm^3,12.5 mg/cm^3, 6.5 mg/cm^3 and 2.5 mg/cm^3 respectively. These various compounds are responsible for the ethnomedicinal uses of the extracts of this plant in the treatment of diseases such as inflammatory disorders, convulsion and dysentery

Keywords: *Pentaclethra macrophylla, alkaloids, saponins, flavonoids, phenols, pathogens*

1. Introduction

In Nigeria some plants are used for food and as medicine. One of such plants is Pentaclethra macrophylla (Benth). This plant commonly known as African oil bean is a leguminous woody plant that belongs to the family mimosoidae (Nwanjo et al 2007). The fermented seeds serve as a delicacy across Ibo land while the leaves and trunk bark serve as important medicinal substances. The seed contains both nutritional and anti-nutritional compounds. Previous studies have revealed that the leaf and seed extracts cause uterine contraction during child labour, (Okorie et al 2009). Pentaclethra macrophylla seed is fermented to give a snack condiment with a meaty taste. The seed is eaten boiled or roasted and is popular in south eastern Nigeria where it is called ugba. (Gurghami and Ezenwanze 1985). It is useful in African veterinary medicine. The ripe fruits are applied externally to heal wounds. The leaf, seed and stem bark extracts have anti-inflammatory and anthemlmintic activities and are used in treating gonorrhea, convulsion and also as analgesic (Bouquet et al 1971), (Cousin and Huffman 2002). The root bark is used as a laxative, an enema against dysentery and as a liniment against itching. The seed, and leaf extract have shown both anti-inflammatory and analgesic properties, (Iwu 1993). Works done by Agbogidi (2010) has shown that the germination of the seed is highly hindered in soils contaminated with spent lubricating oils but this plant shows soil improvement properties. (Akindahunsi 2004.) The tree grows to about 21m in height and about 6m in girth (Kaey, 1989.) The bark is grayish to dark reddish brown; the compound leaves possess a stout angular petiole. The flowering period is between March and April with smaller flushes in June and November. Fruits split by explosive mechanism (Enyiogha and Agbede 2000)

2. Materials and Method

2.1. Plant Materials

The sample was obtained from a farm land in Ezinihitte Mbaise area Imo state, it was identified by Dr. Nmeregini of forestry department Michael Okpala University umudike, the voucher specimen number SE 3426 was deposited in the

Forestry department Herbarium of Michael okpara University Umudike. The sample was washed with distilled water and room dried. The dried samples were milled with a Thomas Wiley machine (model 5 USA) stored in air tight plastic bottles and kept for analysis**.**

2.2. Alkaloid determination

5 g of the sample was weighed into a 250 cm^3 beaker and 200 cm^3 of 20 % 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).

2.3. 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 extracted with 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).

2.4. 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)

2.5. 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)

2.6. 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)

2.7. 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 sample was soaked in ethanol for 48 hour and extracted. The extracts from the soxhlet extracts and that obtained from cold extracts for the sample were combined and re-extracted using chloroform to obtain chloroform soluble extract. This was centrifuged at 10,000 rpm for 20 minutes and the clear supernatant oil was subjected to GC and GC-MS analysis.

2.8. 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 ^oC held at 80 ^oC for I minute, the rate is 5 ^oC/min and at 200 ^oC for 20 minutes. FID Temperature of 300 ^oC, injection temperature of 250 ^oC, carrier gas is Nitrogen at a flow rate of 1 cm^3/min and split ratio of 1: 75. GC-MS Gas chromatography Mass spectrum analysis were conducted using GC-MS QP 2010 Plus Shimazu Japan with injector Temperature at 230 ^oC 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 Spectrometer was also equipped with a computer fed Mass Spectra data bank, HERMCE Z 233 M-Z centrifuge Germany was used. Reagents and solvents such as Ethanol, Chloroform, Diethyl ether, hexane all of analytics grade was obtained from Merck Germany.

2.9. Anti-Microbial Analysis

The Micro Organisms, Pseudomonas aereginosa, Staphylococcus aureus, Escherichia coli, Salmonela typhi, Candida albicans and Aspergillus niger were used for the analysis they are clinical isolates of human pathogens obtained from the Federal Medical Centre Umuahia and were brought to the laboratory and were resuscitated in buffered peptone broth (Secharian chemie) and thereafter into nutrient agar medium and incubated at 37 ^{o}C for 24 hrs (Okigbo and Omodamiro 2006).

2.10 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 discs were used after drying them in an incubator at 40 ^{o}C to remove any trace of solvent Discs were introduced into the surface of the medium. 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.

2.11. Minimum Inhibitory Concentration. (MIC)

The minimum inhibitory concentration of the extract was determined by incorporating constant volume 0.2 cm^3 of each diluent of the extract 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 phytochemical composition of Pentaclethra macrophylla bark are given in table 1 below

Constituent	Bark %
Alkaloid	0.80
Saponins	0.71
Flavonoids	1.36
Tannins	0.93
Phenols	0.018

Table 1: Photochemical Content of Pentaclethra Macrophylla Bark

The stem bark of Pentaclethra Macrophylla contains 0.80 % alkaloid. Alkaloid s 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 samples may be the reason for their use in the treatment of cough, wounds, and rheumatism and skin infections. Most samples containing alkaloid are used in Nigeria for the treatment of malaria and fever. (Adesegun and coker 2001),

Saponins were found to be available at 0.71 %, the saponin content fortifies the use of the extract from this plant 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 fungals and yeast infections. These compounds served as natural antibiotic, helping the body to fight infections and microbial invasion.

The flavonoid content of Pentaclethra Macrophylla bark is 1.36 %, 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 Macrophylla 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 Pentaclethra macrophylla bark was 0.018%. 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 these extracts 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 causes inflammations. They modify the prostaglandin pathways and thereby protect platelets from clumping.

The Tannin content of Pentaclethra macrophylla bark was found to be 3.7 %, Tannins have astringent properties, hastening the healing of wounds and inflamed mucors 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

The anti-microbial activities of the stem bark extract of pentaclethra macrophylla are summarized in table 2;

Organism	**Zone of Inhibition**		**Minimum Inhibition**
	mm.	Cpro	Concentration (mm)
Pseudomonas aeruginosa	13	26	6.5
Staphylococcus aureus	16.5	12	12.5
Escherichia coli	11	16	6.5
Salmonella typhi	12	19	2.5
Candida Albicans	9	22	12.5
Penicillium	10	18	6.5
Aspergillus Niger	9	19	25

Table 2: Antimicrobial Activity of Pentaclethra Macrophylla Bark

3.1. Cpro Ciproflaxin

The following pathogens; Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Candida albicans, Aspergillus niger and Penicillium were tested for the inhibition of their activities. The extract showed marked level of inhibition of the activities of Staphylococcus aureus_(16.5 mm) , Pseudomonas aeruginosa (13 mm) and Escherichia coli (11.0 mm) Salmonella typhi (12 mm) , Candida albican (9 mm) , Penicillium (10 mm) and Aspergillus niger (9 mm) .The observed inhibitory actions on these pathogens explains the reason behind the use of the bark of P.macrophylla in traditional medicine as cough suppressants and in the healing of wounds (Hutchinson et al 1963), the stem bark of Pentaclethra macrophylla contain phytochemicals capable of inhibiting the growth of microbial wound contaminant and accelerate wound healing, . These pathogens are responsible for wounds and inflammatory responses, suggesting that these extracts could be useful in the treatment of these ailments.

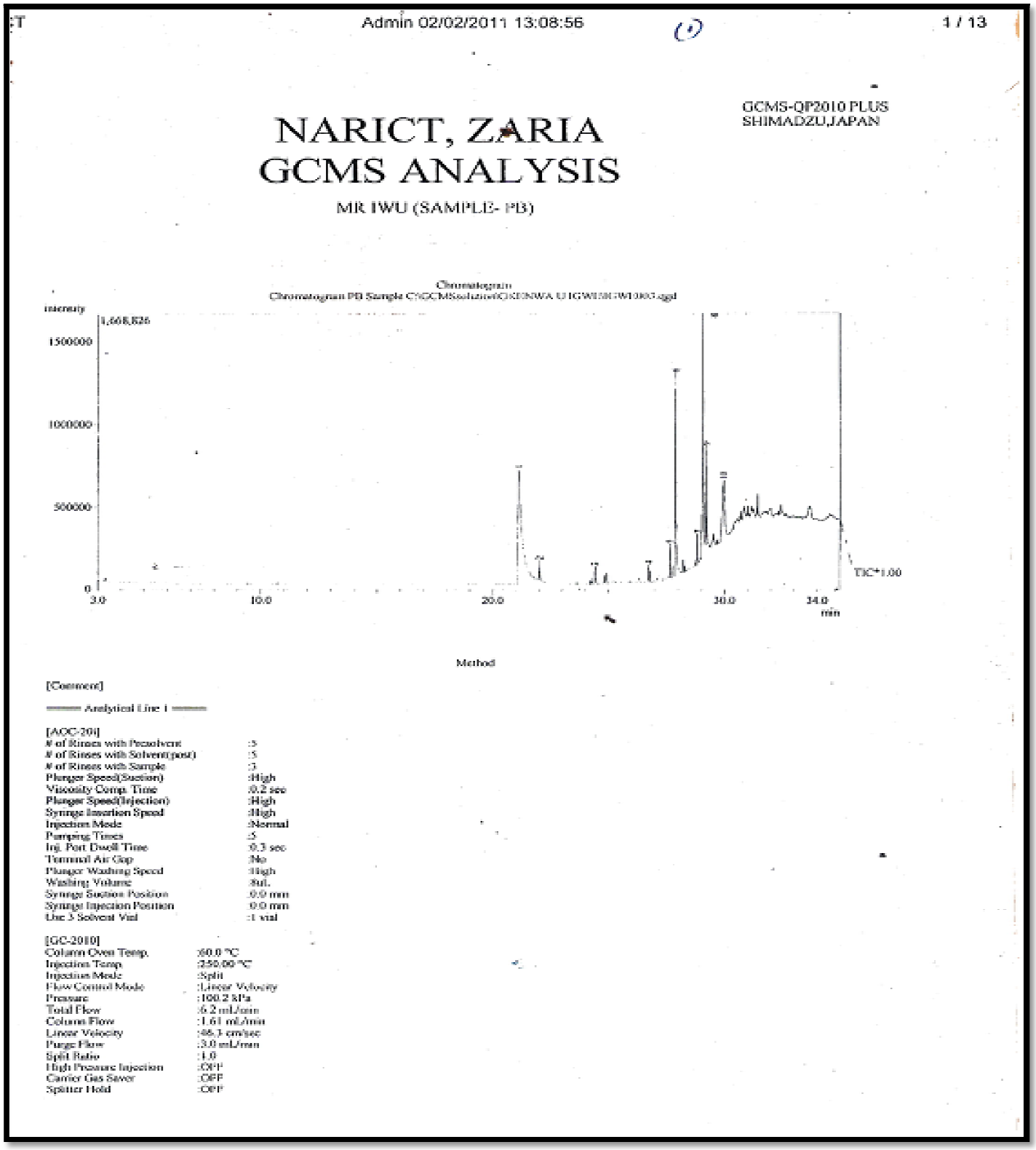

Figure 1: GC of Pentaclethra Macrophylla Stem Bark Extract

The analysis on Pentaclethra Macrophylla stem bark, revealed ten absorption peak fig 1. these peaks are interpreted in (Table 3) Peak 1 occurred at m/z 126 corresponding the molecular formula $C_6H_6O_3$ with 17.53 % content of the oil and is identified as benzentriol [1] a trihydric aromatic alcohol. Peak 2 appeared at m/z 256 with molecular formula $C_{16}H_{16}O_3$ with a percentage of oil content of 3.08 % and identified as 1,4 – Methoxy phenoxy phenyl ethanone [2], an aromatic ketone. Peak 3 appeared at m/z 208, with molecular formula $C_{14}H_{16}O_6$, its % oil content is 2.31 % and its name is Benzene carboxylic acid [3]. Peak 4 occurred at m/z 280 with the formula $C_{20}H_{40,}$ Its % oil content is 2.31% and its name is 3-Eicosene [4]. Peak 5 occurred at m/z 270 with the formula $C_{17}H_{34}O_2$, with 4.62 % oil content and named as Hexadecanoic acid methyl ester [5] a fatty acid.

Peak 6 appeared at m/z 256 with formula $C_{16}H_{30}O_2$ with 20.77 % oil content and named n-Hexadecanoic acid also a fatty acid [6]. Peak 7 occurred at m/z 296 and its formula is $C_{19}H_{36}O_2$, with 5.38 % oil content and is named 6-Octadecenoic acid methyl, [7] Peak 8 occurred at m/z.282 with 25.77 % oil content and is identified as 6-Octadecenoic acid [8] which is also a fatty acid. Peak 9 appeared a m/z 284 its formula is $C_{18}H_{36}O_2$ with 13.85 % oil content and is named Octadecanoic acid, [9]. Peak 10 occurred at m/z 264 with the formula $C_{15}H_{20}O_4$ with 10.38 % oil content and its name is Naphto (1,2, -Furan-2-one The oils of the stem bark of Pentaclethra macrophylla contains fatty acids. Fatty acids always occur in plants. The presence of fatty acids, aromatics, ketones and esters shows the pharmacological properties of this plant. Fatty acid and alcohols in the plant react to produce esters. One or both oxygen atoms in the plant can be replaced by sulphur giving a thio acid or dithio acid respectively. Thio acids react readily with alcohols to form thio ester. Thio esters play important role in the break down and synthesis of lipids and steroids in living tissues. Carboxylic acids are transferred from one enzyme reaction to another as thio esters of the complex thiol, Co enzyme A(CoA-SH.) The thio esters of benzoic acid with Co-enzyme A Is the form in which acetate esters enter the sequence of enzyme catalyzed reaction which results in the synthesis of fatty acids and glycerides (Okwu and Ighodaro 2010)

Chromatographic Peak	Name of Compound	Molecular Formular	Molecular Weight(g)	Retention Time (Sec.)		Fragmentation Peaks
1	1,2,3- Benzenetriol	$C_6H_6O_3$	126	21.1	17.53	27(4.76), 39(14.26) 52(61.90) 68(4.76), 80(57.14) 97(19.06), 108 (38.09), 126 (100)
2.	1, 4 Methoxy -3- (4-methyl Phenoxy) phenyl Ethanone	$C_{16}H_{16}O_3$	256	22.0	3.08	39(9.52), 43 (28.5), 65(14.26), 79(9.52), 91(14.26) 10.5(9.52) 120(9.52), 128(4.76), 141(9.52) 153(9.76) 170(4.76), 185(4.76) 198(4.76), 211(4.76) 226(4.76) 241(100)256(23-80)
3.	1,2 Benzene Carboxylic acid.	$C_{14}H_6O_3$	280	24.4	2.31	27(9.52), 29(42.85), 50(14.28) 65(9.52), 76(28.5) 93(4.76), 104(33.33), 121(4.76), 32(4.76) 149(100 177(33.33), 222(2.38), 235(23.50)
4.	3-Eicosene	$C_{20}H_{40}O$	280	26.7	2.31	27(9.52), 41(85.71), 55(100), 83(71.42) 97(66.66) 111(38.09), 125(14.26), 154(4.76)
5	Hexadecanoic acid Methyl ester	$C_{17}H_{34}O_2$	270	27.7	4.62	57(14.28), 74(100 87(38.09) 101(4.76), 129(4.76)143(14.26) 157(4.76), 171(4.76), 185(4.76) 199(2.38) 227(4.76) 239(4.76), 270(9.52)
6.	n- Hexadecanoic acid.	$C_{16}H_{32}O_2$	256	27.9	20.77	27(23.80), 41(85.71), 43(100), 60(95) ,73(95.7), 85(23.80) 98(10.04) 115(10.04) 129 (38.09) 143(4.76)157(9.52) 171(9.52) 185(9.52) 213(23.80) 227(4.52) 256(4.76)
7.	6-Octadecenoic acid methyl	$C_{19}H_{36}O_2$	296	28.8	5.38	27(19.04) 41(95)55 (100)67(54.14) 74(61.90) 84(45) 98(33.33) 123) (14.26) 137(9.0) 180(9.52) 20(9.52) 264(18.04)
8.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282	29.1	25.77	27(23.80) 41(85.71) 55(100) 69(71.42) 83(61.90) 97(52.38) 98(45),114(14.36), 137(9.52), 222(9.52), 264(25.33)., 282(9.52)
9.	Octadecenoic acid	$C_{18}H_{36}O_2$	284	29.2	13.85	27(14.28, 41(71.42), 43(100), 60(85.71) 73(85.71) 83(23.80)

Table 3 GC-MS Analysis of Pentaclethra Macrophylla Stem Bar

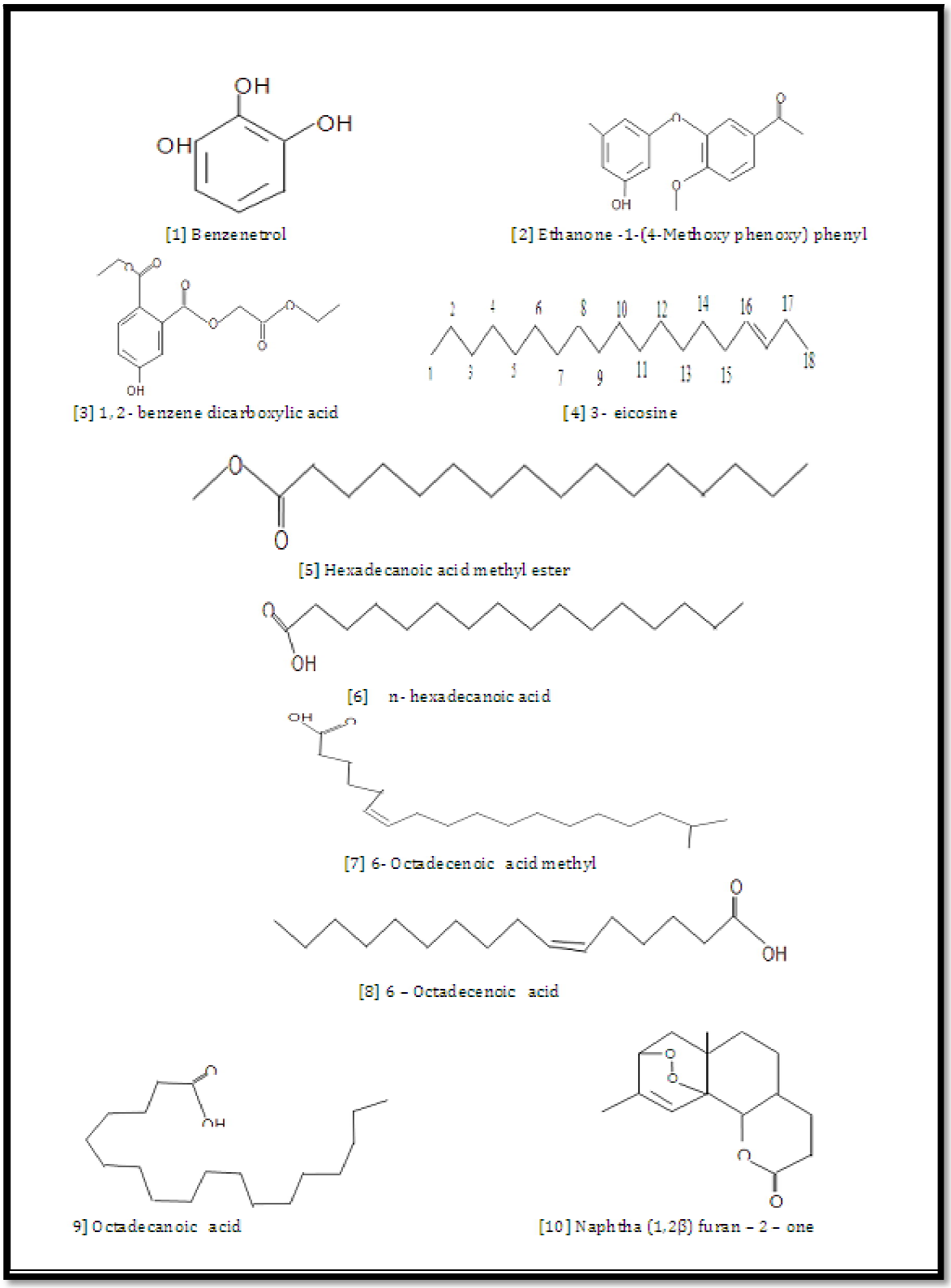

Figure 2: Structure of Compounds Identified from Pentaclethra Macrophylla Bark

4. Conclusion

The stem bark of Pentaclethra macrophylla contain vital chemical compound that have useful pharmacological properties which could be extracted and use as alternatives to synthetic drugs for the treatment of certain diseases, including the treatment of wounds and for anti-inflammatory responds

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