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GAS CHROMATOGRAPHY-MASS SPECTROSCOPY (GC-MS) ANALYSIS OF THE METHANOL EXTRACTS OF THE STEM BARK, LEAVES AND ROOTS OF *Nauclea pobeguinii*

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ABSTRACT This study was aimed at identifying and quantifying the biologically active components present in the methanol extracts of the stem bark, leaves and roots of the plant. The stem bark, leaves and roots of the plant were pulverized, extracted with methanol and the biologically active components were evaluated using Gas chromatography - mass spectroscopy (GC-MS). The mass spectrums of the bioactive compounds that were present in the methanol extracts of the stem bark, leaves and roots of the plant were matched with the database of known compounds of National Institute of Standards and Technology (NIST) for identification. The results obtained revealed the presence of 17, 16, and 10 bioactive compounds in the methanol extracts of the stem bark, leaves, and roots, respectively. These bioactive compounds ranged from fatty acid methyl esters, alkanes and aromatic diesters to triterpenoids. These bioactive compounds also possessed antidiabetic, antioxidant, anticancer, antibacterial and other biological activities, which justify the use of the plant, *Nauclea pobeguinii* for the treatment of various diseases.

Keywords: GC-MS, methanol extract, *Nauclea pobeguinii*and Text classification.

Introduction

Plants have been utilized as food by man as far back as the primordial era. In addition to being used as food, some plants are also used as medicine for treating ailments and are known as medicinal plants. These medicinal plants contain one or more bioactive compounds that can be used to treat disease conditions or used as startup material for the development of useful drugs (Doughari, 2012). Medicinal plants also owe their therapeutic ability to the presence of phytochemicals (Peteros and Uy, 2010). These phytochemicals also known as phytonutrients have vast arrays of non-nutritive secondary metabolites that are secreted and deposited in different parts of the plant, where they sometimes act as defense against plant pathogens and herbivores (Eze and Obinwa, 2014). The plant *Nauclea pobeguinii* belong to the Family Rubiaceae and is a deciduous plant common to the West African region. The stem, leaves and root parts are used as herbal remedy for the treatment of diverse diseases by traditional healers in Nigeria and across parts of West and Central Africa. In Cameroun, among the people of the upper Nyong valley, the bark preparation of this plant is used against threatened abortion (Jiofack *et al*., 2009), while in Gabon the traditional healers used the plant as a treatment for diabetes with or without hypertension. The leaf infusion is also used to treat fever and the stem bark maceration is used as a treatment for urogenital infections (Agnaniet *et al*., 2016). In the Democratic Republic of Congo, the bark decoction is used as a treatment for malaria (Mesia *et al*., 2005), while the decoction of the bark of *Nauclea pobeguinii* and *Symphonia globulifera* (Boar wood) is taken against sexual asthenia (sexual weakness). A bark preparation is equally applied as a suppository (drug inserted into the body cavity where it melts at body temperature) against epilepsy. In Senegal, the powdered bark is taken against intestinal pain and diarrhoea (Brink, 2012).

In Nigeria, the root preparation has been reported to be used as treatment for gonorrhea. In Uromi, Edo state, Nigeria, the root preparation is equally used by traditional healers to treat high blood pressure as well as migraine. A preparation of the roots of *Nauclea pobeguinii* and *Anthocleista djalonesis* is also used to treat waist pain and dysentery. In the local community of Abraka, Nigeria, the aqueous extract of the leaves is used as a remedy for jaundice (Kadiri *et al*., 2007). Scientific investigations, particularly on malaria and cytotoxicity (Mesia *et al*., 2005; Mesia *et al*., 2010; Kuete *et al*., 2015) have also shown that this plant is indeed potent in treating malaria and equally has the potential of killing cancer cells.

Gas chromatography – mass spectroscopy (GC-MS) studies, in recent years have been increasingly applied for the analysis of medicinal plants. GC-MS technique has proved to be a valuable method for the analysis of non-polar components, essential oil, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds present in medicinal plants (Knovalova *et al*., 2013; Vetha *et al*., 2016). The association of chromatographic and spectroscopic methods

in GC-MS has also proved to be essential in medicinal plants analyses as it offers high sensitivity and selectivity. Additionally, GC-MS has immense value in further facilitating insights into the medicinal applications of medicinal or herbal plants (Elangovan *et al*., 2015).
This study was therefore aimed at identifying the bioactive compounds present in the stem bark, leaves and roots of *Nauclea pobeguinii* using GC-MS, in order to provide scientific basis for the numerous claims and therapeutic abilities of this plant and by extension, help in identifying novel compounds that may have therapeutic values that could be used for drug development and discovery.

MATERIALS AND METHODS

Plant sample collection and identification

Fresh stem bark, leaves, and roots of *Nauclea pobeguinii* were collected from a forest in Uromi, Esan North East Local Government Area (LGA) of Edo State, Nigeria. The plant parts were identified by Mr. K. Adeniji, Herbarium Officer at the Forest Research Institute of Nigeria (FRIN), Ibadan and voucher specimen with registration number FHI 111259 was deposited at the same Institute.

Preparation of plant extract

The stem bark, leaves, and roots of the plant were washed under running water, air dried for two weeks and pulverized using an electric grinder. Two (2) grams of the dried pulverized sample of each plant part was placed in a conical flask to which 30 ml of methanol was added. The mixture was placed on a shaker and allowed to mix vigorously for 30 minutes. The conical flask was transferred to an ultrasonic bath at 37 ˚C and extraction was allowed to take place for 2 hours. A filter column was prepared by packing a burette with cotton wool, anhydrous sodium sulphate and silica gel. The extracted sample was then poured through the filter column and the percolate collected and used for the GC-MS analysis.

GC-MS Analysis of plant extract

GC-MS analysis of the stem bark, leaves and roots extracts of the plant were carried out using Gas Chromatograph (Agilent technologies, 7890 GC system) directly coupled to Figure 1 below shows the Gas chromatograph displaying the peaks of the 17 compounds from the methanol extract of the stem bark of *Nauclea pobeguiniii.*

a Mass Spectroscopy detector (Agilent technologies 5975). Agilent H5MS column measuring 30 m in length, 0.32 mm in diameter and 0.25 µm in thickness was used for the analysis. Helium gas was used as carrier gas at a flow rate of 0.5 ml/min. One (1) µl sample injection volume was used for analysis. The oven temperature was programmed initially at 80˚C for 2 min with a gradual increase of 10˚C per minute until a final temperature of 240˚C for 6 min. Total run time was 90 min. The analysis was done using electron impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of components were compared with the database of spectrums of known compounds stored in the GC-MS library of the National Institute of Standards and Technology (NIST). Measurements of peak areas and data processing were carried out by Turbo-mass OCPTVS-demo SPL software.

RESULTS AND DISCUSSION

The total ion chromatogram (TIC) revealed 17, 16 and 10 peaks for the stem bark, leaves and roots, respectively as shown in Figures 1 to 3. The peaks were resolved and crossed checked with the database of NIST to identify the individual compounds. The compounds as revealed are presented in Tables 1, 2 and 3. The biological activities of some of the compounds were determined after a review of scientific literature and they are presented in Tables 4 to 6.

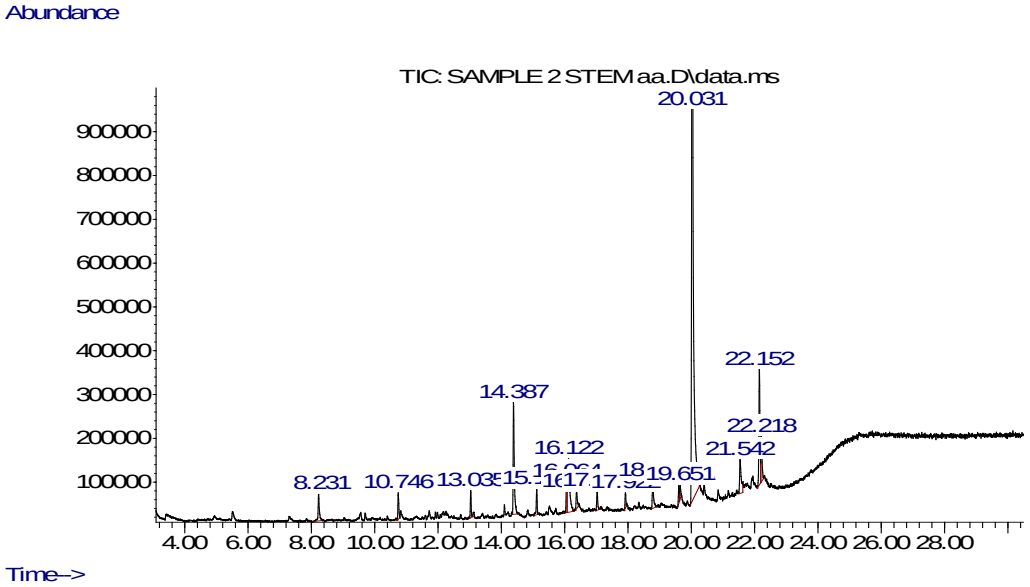

Figure 1: Gas chromatogram of the methanol extract of the stem bark of *Nauclea pobeguinii*

Each peak represents a distinct compound. The area covered by each peak corresponds to the relative abundance of that compound. The identified compound corresponding to each peak is presented in Table 1

The 17 compounds identified in the methanol extract of the stem bark of *Nauclea pobeguinii* are shown in Table 1 below.

Table 1: Phyto-components Identified in the Methanol Extract of the Stem bark of *Nauclea pobeguinii*

Peak	Retention time (min)	Area (%)	Compound Name	Compound nature	Molecular formula	Molecular weight
1	8.231	1.74	Tetradecane	Alkane	$C_{14}H_{30}$	198
2	10.749	1.23	Hexadecane	Alkane	$C_{16}H_{34}$	226
3	13.037	1.09	Octadecane	Alkane	$C_{18}H_{38}$	254
4	14.388	6.27	Hexadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270
5	15.120	1.27	Eicosane	Alkane	$C_{20}H_{42}$	282
6	16.064	1.34	8,11 octadecadienoic acid, methyl ester	Fatty acid methyl ester	$C_{19}H_{34}O_2$	294
7	16.122	5.86	9-octadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{19}H_{36}O_2$	296
8	16.379	0.97	Heptadecanoic acid 16-methyl methyl ester	Fatty acid methyl ester	$C_{19}H_{38}O_2$	298
9	17.026	0.98	Tritetracontane	Alkane	$C_{43}H_{88}$	605
10	17.924	0.63	Tricosane	Alkane	$C_{23}H_{48}$	324
11	1.788	1.84	Eicosane	Alkane	$C_{20}H_{42}$	282
12	19.606	0.69	Eicosane	Alkane	$C_{20}H_{42}$	22
13	19.656	0.60	Carbonic acid 2-ethyl hexyl octadecyl ester	Ester	$C_{20}H_{42}$	426
14	20.030	64.32	Bis (2-ethylhexyl) phthalate	Aromatic diester	$C_{27}H_{54}O_3$	390
15	21.540	2.70	1,3-Benzenedicarboxylic acid bis 2-ethylhexyl) ester	Aromatic Ester	$C_{24}H_{38}O_4$	390
16	22.153	6.10	Supraene	Triterpene	$C_{30}H_{50}$	410
17	22.216	2.36	Didecan-2-yl phthalate	Aromatic diester	$C_{28}H_{46}O_4$	446

The peak is as identified from the Gas chromatogram in figure 1. The Area (%) of each peak represents the relative abundance of each compound

Figure 2 below shows the Gas chromatograph displaying the peaks of the 16 compounds from the methanol extract of the leaves of *Nauclea pobeguinii*.

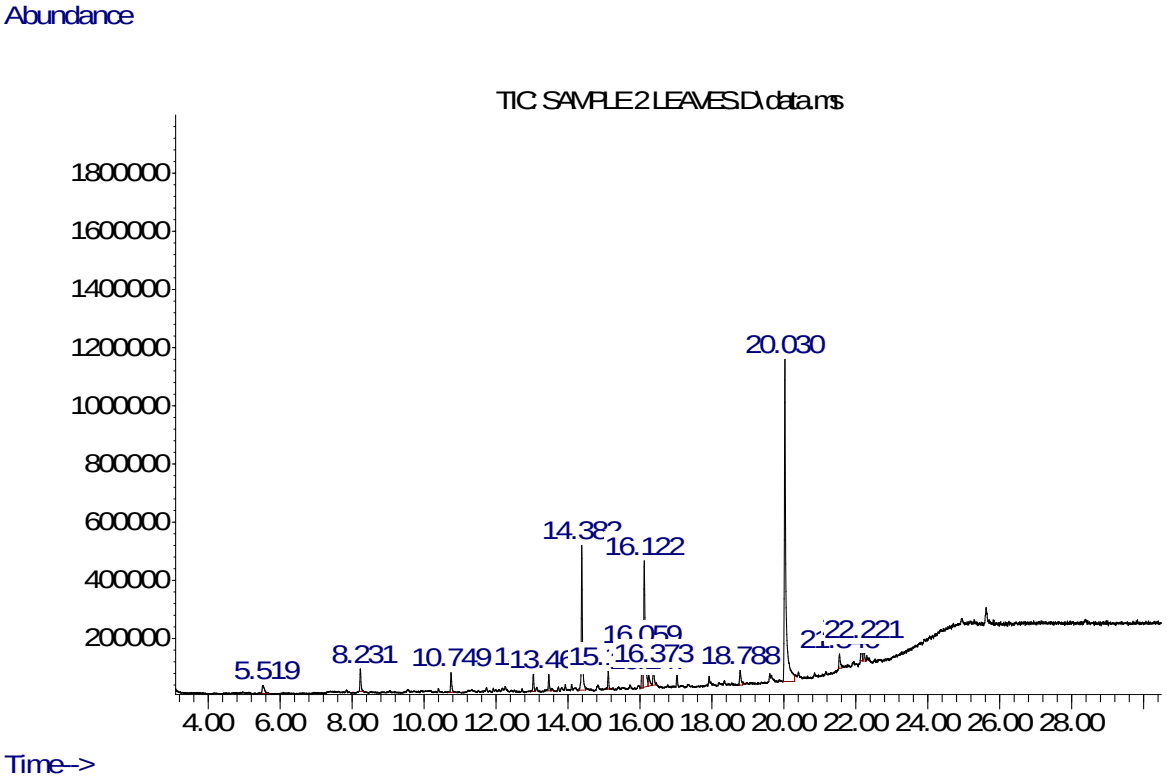

Figure 2: Gas chromatogram of the methanol extract of the leaves of *Nauclea pobeguinii*

Each peak represents a distinct compound. The area covered by each peak corresponds to the relative abundance of that compound. The identified compound corresponding to each peak is presented in Table 2

The 16 compounds identified in the methanol extract of the leaves of *Nauclea pobeguinii* are shown in Table 2 below.

Table 2: Phyto-components Identified in the Methanolic Extract of the Leaves of *Naucleapo beguinii.*

Peak	Retention time (min)	Area (%)	Compound Name	Compound Nature	Molecular formula	Molecular weight
1	5.519	1.66	Tetradecane	Alkane	$C_{14}H_{30}$	198
2	8.231	2.20	Dodecane	Alkane	$C_{12}H_{26}$	170
3	10.749	2.04	Hexadecane	Alkane	$C_{16}H_{34}$	226
4	13.037	1.72	Octadecane	Alkane	$C_{18}H_{38}$	254
5	13.467	1.42	Neophytadiene	Alkane	$C_{20}H_{38}$	278
6	14.382	14.43	Hexadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270
7	15.114	1.49	Eicosane	Alkane	$C_{20}H_{42}$	282

8	16.059	3.99	9,12-octadecadienoic acid (Z,Z), methyl ester	Fatty acid methyl ester	$C_{19}H_{34}$	294
9	16.122	16.31	9,12,15-octadecatrienoic acid methyl ester	Fatty acid methyl ester	$C_{19}H_{32}O_2$	292
10	16.247	1.48	Phytol	Diterpene Alcohol	$C_{20}H_{40}O$	296
11	16.373	1.93	Hepatadecanoic acid, 14-methyl methyl ester	Fatty acid methyl ester	$C_{19}H_{38}O_2$	298
12	18.788	1.91	Octadecane	Alkane	$C_{18}H_{38}$	254
13	20.030	44.19	Bis (2-ethyl hexyl) phthalate	Aromatic diester	$C_{24}H_{33}O_4$	390
14	21.546	1.69	1,3 Benzenedicarboxylic acid, bis 2-ethyl hexyl ester		$C_{24}H_{38}O_4$	390
15	22.152	1.87	Indolizine 2-(4-methyl phenyl)		$C_{15}H_{13}N$	207
16	22.221	1.68	Phthalic acid monocyclohexyl ester		$C_{14}H_{16}O_4$	248

The peak is as identified from the chromatogram in figure 2. The Area (%) of each peak represents the relative abundance of each compound

Figure 3 below shows the Gas chromatograph displaying the peaks of the 10 compounds from the methanol extract of the roots of *Nauclea pobeguinii*.

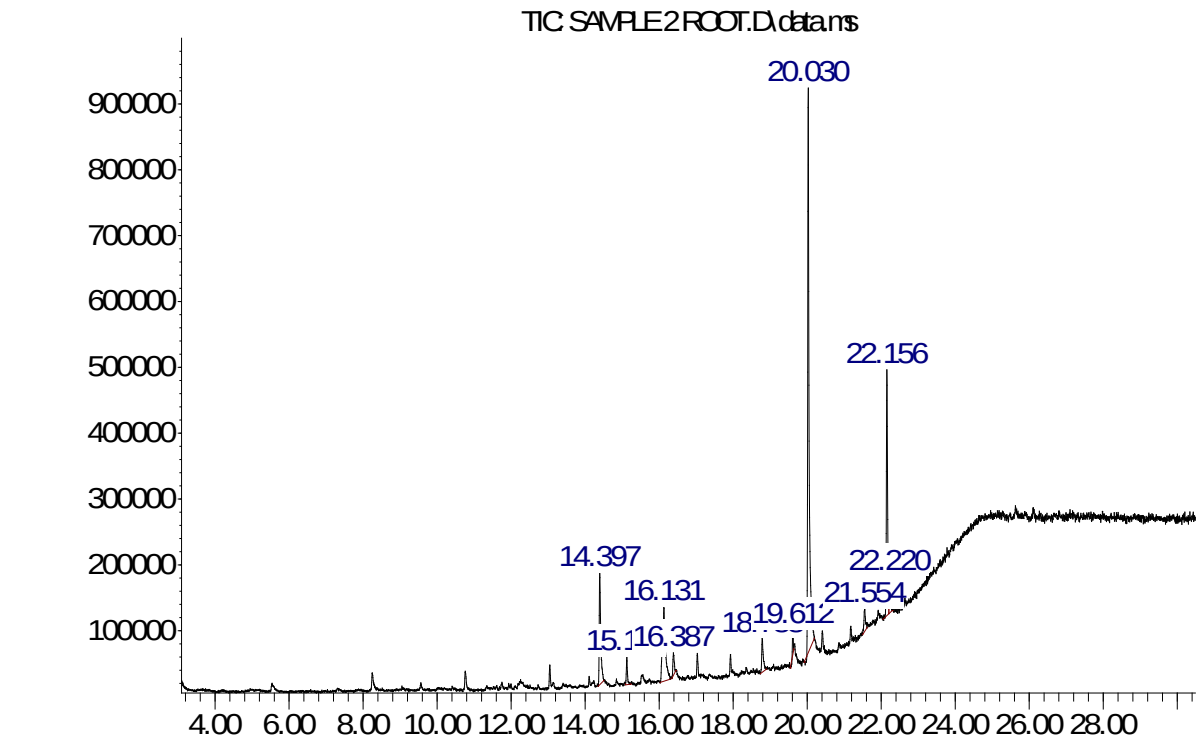

Figure 3: Gas chromatogram of the methanol extract of the roots of *Nauclea pobeguinii*
Each peak represents a distinct compound. The area covered by each peak corresponds to the relative abundance of that compound. The identified compound corresponding to each peak is presented in Table 3.
The 10 compounds identified in the methanol extract of the leaves of *Nauclea pobeguinii* are shown in Table 3 below.

Table 3: Phyto-components Identified in the Methanol Extract of the Roots of *Nauclea pobeguinii*

Peak	Retention time	Area %	Compound Name	Compound nature	Molecular formular	Molecular weight
1	14.399	9.84	Hexadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270
2	15.126	1.85	Eicosane	Alkane	$C_{20}H_{42}$	282
3	16.133	12.06	11 octadecenoic acid, methyl ester	Fatty acid methyl ester	$C_{19}H_{36}O_2$	296
4	16.385	1.67	Heptadecanoic acid, 16-methyl ester	Fatty acid methyl ester	$C_{19}H_{38}O_2$	298
5	18.788	3.17	Tetracosane	Alkane	$C_{24}H_{50}$	338
6	19.612	0.94	Octadecane	Alkane	$C_{18}H_{30}$	254
7	20.030	47.97	Bis (2-ethyl hexyl)phthalate	Aromatic diester	$C_{24}H_{38}O_4$	390

8	21.552	2.09	4-methyl-2-trimethylsilyloxyacetophenone		$C_{12}H_{18}O_2Si$	222
9	22.158	17.82	Squalene	Triterpene	$C_{30}H_{50}$	410
10	22.221	2.57	Benzo(h) quinoline, 2,4-dimethyl		$C_{15}H_{13}N$	207

The peak is as identified from the chromatogram in figure 3. The Area (%) of each peak represents the relative abundance of each compound

The biological activities of the compounds identified in the methanol extract of the stem bark of *Nauclea pobeguinii* are shown in Table 4 below

Table 4: Biological Activities of Phyto-components identified in the Methanol Extract of the Stem Bark of *Nauclea pobeguinii*

Compound Name	Biological Activities
Tetradecane	Antifungal activity against *Candidiasis albicans*, antibacterial (Smiline *et al*., 2014)
Octadecane	Antifungal activity against *Candidiasis albicans*, antibacteria (Smiline *et al*., 2014)
9-octadecenoic acid methyl ester	Antioxidant activity, anticarcinogenic activity, Exist in human blood and serve as endogenous peroxisome proliferator-activated receptor ligand, dermatigenic, flavor (Akpuaka *et al*., 2013)
Heptadecanoic acid, 16 methyl methyl ester	Antioxidant, Antimicrobial (Vetha *et al*., 2016)
Eicosane	Antifungi, antibacterial, antitumour and cytotoxic (Akpuaka *et al*., 2013)
Bis (2-ethylhexyl)phthalate	Antimicrobial, antifungi, antioxidant, anticancer (Habib and Karim, 2009; Singaravadivel and Santhanaraj, 2016)
Tricosane	Antibacteria (Knovalova *et al*., 2013)
Hexadecanoic acid methyl ester	Antioxidant, Antifungi, hypocholesterolemic agent, Nematicide, pesticide, antiandrogenic agent, flavour, hemolytic agent, 5-Alpha reductase inhibitor, potent antimicrobial activity (Akpuaka *et al*., 2013).

The biological activities of the compounds identified in the methanol extract of the leaves of *Nauclea pobeguinii* are shown in Table 5 below

Table 5: Biological Activities of the Phyto-components identified in the Methanol Extract of the Leaves of *Nauclea pobeguinii*

Compound Name	Biological Activities
Bis (2-ethylhexyl)phthalate	Antimicrobial, antifungi, antioxidant, anticancer (Habib and Karim, 2009; Singaravadivel and Santhanaraj, 2016)
Neophytadiene	Antipyretic, anagelsic, anti-inflammatory, antimicrobial, antioxidant (Singaravadivel and Santhanaraj, 2016)
Phytol	Antiinflammatory, antimicrobial, anticancer, antidiuretic, immunostimulatory and antidiabetic (Singaravadivel and Santhanaraj, 2016)
Eicosane	Antifungi, antibacterial, antitumour and cytotoxic (Akpuaka *et al*., 2013)
Tetradecane	Antifungal activity against *Candidiasis albicans*, antibacterial (Smiline *et al*., 2014)

Octadecane	Antifungal activity against *Candidiasis albicans*, antibacteria (Smiline *et al*., 2014)
Hexadecanoic acid methyl ester	Antioxidant, Antifungi, hypocholesterolemic agent, Nematicide, pesticide, antiandrogenic agent, flavour, hemolytic agent, 5-Alpha reductase inhibitor, potent antimicrobial activity (Akpuaka *et al*., 2013).
9,12 Octadecadienoic acid methyl ester	**Anticancer (Abubakar and Majinda, 2016)**
9,12,15octadecatrienoic acid, methyl ester	**Anticancer, antimicrobial, antioxidant and hypercholesterolemic (Akpuaka *et al*., 2013)**
Dodecane	**Enhances antifungi activity (Akpuaka *et al*., 2013)**

The biological activities of the compounds identified in the methanol extract of the roots of *Nauclea pobeguinii* are shown in Table 6 below

Table 6: Biological Activities of Phyto-components identified in the Methanol Extract of the Roots of *Nauclea pobeguinii*

Compound Name	Biological Activities
Hexadecanoic acid methyl ester	Antioxidant, Antifungi, hypocholesterolemic agent, Nematicide, pesticide, antiandrogenic agent, flavour, hemolytic agent, 5-Alpha reductase inhibitor, potent antimicrobial activity (Akpuaka *et al*., 2013).
Eicosane	Antifungi, antibacterial, antitumour and cytotoxic (Akpuaka *et al*., 2013)
Heptadecanoic acid, 16 methyl methyl ester	Antioxidant, Antimicrobial (Vetha *et al*., 2016)
Tetracosane	Cytotoxic against AGS,MDA-MB-231,HT29 and NIH3T3 cells (Uddin *et al*., 2012; Casuga *et al*., 2016)
Octadecane	Antifungal activity against *Candidiasis albicans*, antibacteria (Smiline *et al*., 2014)
Bis (2-ethylhexyl)phthalate	Antimicrobial, antifungi, antioxidant, anticancer (Habib and Karim, 2009; Singaravadivel and Santhanaraj, 2016)
Squalene	Antimicrobial, antioxidant, anticancer, neutralize different xenobiotics, anti-inflammatory, antiatherosclerotic and antineoplastic. Role in skin aging and pathology and adjuvant (Singaravadivel and Santhanaraj, 2016)

In spite of the advent of modern drug discovery and screening techniques, the traditional knowledge systems have always laid the blueprint for the discovery of valuable drugs. Knowledge of the traditional uses of medicinal plants, therefore has over time guided the search for new drugs. Also, with the sensitivity of spectroscopic techniques, an in depth qualitative and quantitative analyses of these medicinal plants can be achieved towards identifying potential compounds for drug development.

The spectroscopic technique, GC-MS that was used to analysis the medicinal plant *Nauclea pobeguinii* revealed the presence of valuable potential compounds that can be used for drug development. The methanol extract of the stem bark, root and leaves of *Nauclea pobeguinii* revealed the presence of diverse compounds ranging from alkanes (Neophytadiene, dodecane, tetradecane, Hexadecane, octadecane, eicosane), Fatty acid methyl esters (Heptadecanoic acid, 14-methyl ester), Aromatic diester (Bis-(2-ethylhexyl) phthalate), diterpene alcohol (phytol) to triterpenes (squalene). These compounds have been reported to have several biological activities as shown in Tables 4 to 6. Few of the compounds such as Benzo(h)quinoline-2,4-dimethyl, didecane-2-yl-phthalate, 1,3-benzenedicarboxylic acid, bis2-ethyl hexyl ester, 4-methyl-2-trimethylsilyloxyacetophenone, indolizine 2(4 methyl) phenyl, carbonic acid 2-ethyl hexyl octadecyl ester and phthalic acid monocyclohexyl ester however, have no reported biological activities found for them.

The GC-MS results also revealed that the stem bark of the plant, *Nauclea pobeguinii* had the highest amount of compounds, while the roots had the least number of compounds out of the three plant parts, namely stem bark, leaves and roots that were used for the analysis. Furthermore, the compound Bis (2-ethyl hexyl) phthalate, which has been reported to have antimicrobial, antifungi, antioxidant and anticancer activities (Habib and Karim,

2009; Singaravadivel and Santhanaraj, 2016) was found in all the three plant parts however, it had the highest peak area of 64.32 % (indicating a high concentration) in the stem bark (Figure 1 and Table 1). It was also observed that this compound, Bis (2-ethyl hexyl) phthalate was found to have the highest peak area of all the compounds identified from all the three plant parts (Figures 1 to 3 and Tables 1 to 3). This compound, Bis (2-ethyl hexyl) phthalate probably may be one of the compounds that confers one of the most therapeutic abilities of this plant. In addition, Eicosane, octadecane and hexadecanoic acid methyl ester were also found to be present in all the three plant parts with hexadecanoic acid methyl ester and octadecane having the highest peak areas of 14.43% and 1.72%, respectively in the leaves (Table 2). This implies that these compounds, hexadecanoic acid methyl ester and octadecane, with antifugal, antibacterial activities among other biological activities had the highest abundance in the leaves than in the other two plant parts (Tables 1 to 3).

The presence of these bioactive compounds in the methanol extracts of the stem bark, leaves and roots of *Nauclea pobeguinii* with their various biological activities justifies the use of this plant for the treatment of various diseases by traditional healers. Therefore, based on the findings from this study, it can be concluded that the stem bark, leaves and root of *Nauclea pobeguinii* contain constituents of pharmacological importance. Since the plant is of phyto-pharmacological importance, the individual compounds with therapeutic properties in this plant can be isolated and characterized for drug development.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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