Scientia Africana, Vol. 17 (No. 1), June 2018. Pp 58-68
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MINERAL AND ESSENTIAL OIL COMPOSITION OF JACKFRUIT (*Artocarpus heterophyllus*) LEAVES

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Received: 03-04-18
Accepted:11-05-18

ABSTRACT

The preliminary phytochemical contents of the leaves of Artocarpus heterophyllus were studied using standard methods, the mineral content of the leaves of A. heterophyllus was studied using Atomic absorption spectrophotometry (AAS) while the essential oil components were studied using GC-MS. The qualitative screening of the leaves for phytochemicals showed the presence of alkaloids, flavonoids, carbohydrate, terpenoids, tannins, cardiac glycoside, steroids, saponin, and carboxylic acid. The mineral analysis using Atomic absorption spectrophotometry (AAS) showed the presence of magnesium, potassium, calcium, sodium, phosphorus, zinc, copper, manganese, chromium and iron. The GC-MS analysis of the essential oil revealed eight different compounds; 1H-Indene, 3-methyl-, Neophytadiene, Hexadecanoic acid, methyl ester, Methyl 9-cis 11-trans-octadeca dienoate, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, Phytol, Methyl stearate and Diisooctyl phthalate at a concentration of 2.128%, 1.321%, 27.160%, 11.267%, 30.587%, 19.867%, 5.006% and 2.665% respectively. This study shows that the leaves of A. heterophyllus are rich in bioactive components.

Key Words: *Artocarpus heterophyllus*, essential oil, phytochemical, minerals, antidiabetic

INTRODUCTION

A. heterophyllus is known as jack fruit in English and Kanthal in Bangladesh. It is a fruit in the mulberry family known as Moraceae. It is popularly seen growing wildly in the tropics (Madruga *et al*., 2014). Jack fruit is one of the fruits popularly grown in some parts of Asia such as India, and Malaysia. It is also grown in the Central, Eastern Africa and the Caribbean (Prakash *et al*., 2009). Great economic value is placed on jackfruit; the fruit is prepared in so many ways for consumption and different parts of the plant is used in Ayuverda medicine (Prakash *et al*., 2009) and folkloric medicine in the treatment of different types of diseases and infection. Research has shown that the leaves, roots, stem and fruits of *A. heterophyllus* are rich in plant chemical which in some cases serve as defence for the plant and are also beneficial to man. These phytochemicals include alkaloids, flavonoids, tannins and many others. These plant chemicals have been used over the years in the treatment of different kinds of diseases and infections including cancer, paracetamol-induced toxicity, stroke, diabetes and many others (Onuah *et al*., 2016).

Minerals are chemical substances which exist naturally in the soil, plants and ingested by man as nutrients. Minerals form

a part of food nutrient and are required by animals for normal functioning of the body. Iron is required for the transportation of blood; calcium is needed for the maintenance of strong bones and teeth. Potassium plays a role in skeletal and muscle contraction (Farid and Neda, 2014). Chromium is essential for insulin function and hence improves the ability of insulin to metabolise glucose (Staniek and Krejpcio, 2017). Mineral nutrients are divided into macro and trace minerals. Macro minerals are required in large quantities; they include calcium, phosphorus, magnesium, sodium, potassium, chloride and sulphur. Trace minerals include iron, manganese, copper, iodine, zinc, cobalt, fluoride and selenium. Minerals are important in the protection of the body against different diseases.

Lack of minerals has been linked to different diseases including goitre, osteoporosis, anaemia and many others (Soetan *et al*., 2010)

MATERIALS AND METHODS

Collection and identification of plant material

The leaves of *A. heterophyllus* were collected from Ozuoba, Obio/Akpor Local Government Area of Rivers State, Nigeria. The plant was identified in the Herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt. The Plant sample was washed and air dried under shade. The dried sample was homogenized to fine powder and stored in sterile air tight bottles prior to analyses.

Qualitative determination of the phytochemical contents of the plant

Qualitative phytochemical content of the leaves of *A. heterophyllus* were determined using a standard method according to Harborne, J.B (1998).

Dragendorff's test for alkaloids

About 0.5 g of the plant extract was dissolved in 5ml of 0.1M HCl on steam bath. The filtrate was treated with few drops of Dragendorff's reagent. Formation of orange precipitate showed the presence of alkaloids.

Shinoda test for flavonoids

About 0.2 g of the extracted plant sample was mixed with a chip of magnesium metal, followed by the addition of about 2 drops of concentrated HCl. The formation of orange colour showed the presence of flavonoids

Mollisch test for carbohydrates

About 0.5ml of the diluted plant sample was treated with few drops of alcohol solution of α-naphthol. On addition of concentrated H_2O_4 along the side of the test tube, a purple/violet ring was formed at the junction below the aqueous layer. This showed the presence of carbohydrates.

Ferric chloride test for tannins

About 1.0 g of the powdered plant sample was dissolved in 10 ml of distilled water and filtered (using Whatman No 1 filter paper). About 2ml of ferric chloride reagent was added to the filtrate, formation of dark blue colour indicated the presence of tannins.

Test for steroid

About 0.5 g of the plant extract was dissolved in 3 ml of chloroform and filtered. Concentrated H_2SO_4 was carefully added to the filtrate to form lower layer. Formation of red colour at the interface showed a positive test for steroid.

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Test for Terpenoids

About 2.0 ml of chloroform was added to 5 ml of the aqueous extract of the plant. This was evaporated on the water bath, and then boiled with 3 ml of concentrated H_2SO_4. A grey colour was formed which showed the presence of terpenoids.

Liebermann's Test for Cardiac glycoside

A solution of the extract was made by adding about 0.5g of the extract to 2ml of chloroform. About 2ml of acetic acid was added to the solution and then two drops of concentrated H_2O_4 was also added. The formation of blue colour showed the presence of Cardiac glycoside.

Test for Saponins

Aqueous Crude extract of the powdered plant sample was vigorously mixed with 5.0 ml of distilled water in a test tube. Few drops of olive oil was added and mixed vigorously, the formation of emulsion showed the presence of saponins.

Effervescent test for Carboxylic acid

The plant extract was added to about 2ml of sodium bicarbonate. The presence of effervescent indicated the presence of carboxylic group.

Determination of mineral contents of the plant

The mineral contents of the plant sample were determined using atomic absorption spectrophotometry (AAS) according to the method of AOAC (1980).

GC-MS Analysis of *A. heterophyllus* leaves

The components of the essential oil of the plant sample were analysed using a combined gas chromatograph Model 7890A (GC) and Mass Selective Detector model: 5975C (MSD) by Agilent Technologies. The electron ionization was at a 70v with an ion source temperature at 250 ^{o}C. Highly pure helium gas (99.9% purity) was used as carrier gas, while HP-5 (30mm X 0.25mm X 0.320µm) was used as the stationary phase. The oven temperature was 60 ^{o}C, held for 0.5 minutes and ramped to 140 ^{o} C at the rate of 4 ^{o} C/minute holding for a minute, then ramped to 280 degrees while holding for 5 minutes at the rate of 8 ^{o}C /minute. The milled sample was extracted in dichloromethane after soaking for 5 days. About 10g of the sample was weighed into a well stopper bottle and 20ml of the organic solvent was added. The mixtures were vigorously agitated and were left to stand for 5 days. The crude extract was collected by filtering into a quartz beaker; the process was repeatedly carried out for two more consecutive times. The combined aliquot collected was concentrated on a steam berth to about 5ml. This was purified by passing through a pasture pipette packed with silica gel and anhydrous sodium sulphate on a membrane and air dried to about 2ml for gas chromatographic analysis.

RESULT

Phytochemical content of the leaves of *A. heterophyllus*

The preliminary qualitative screening revealed the presence of alkaloids, flavonoids, carbohydrate, tannins, steroid, terpenoids, cardiac glycoside, saponins, carboxylic acid, aldehyde/ketone, in the leaves of *A. heterophyllus* as shown in Table 1 below.

Table 1: Qualitative Phytochemical content of the leaves of *A. heterophyllus*

Phytochemical	
Alkaloids	++
Flavonoids	++
Carbohydrate	+++
Tannins	+
Steroids	++
Terpenoids	++
Cardiac glycoside	+
Saponin	+
Carboxylic acid	++

+ = positive, - = negative

The macro and micro mineral content of the leaves of A. heterophyllus

Table 2 and 3 below shows the macro micro mineral content of the leaves of *A. heterophyllus.*

Table 2: Macro mineral content of the leaves of *A. heterophyllus*

Minerals	Concentration (ppm)
Magnesium (Mg)	7.150
Poatassuim (K)	34.945
Calcium (Ca)	85.108
Sodium (Na)	47.051
Phosphorous (P)	5.410

Table 3: The trace mineral content of the leaves of *A. heterophyllus*

Minerals	Concentration (ppm)
Zinc (Zn)	8.579
Copper (Cu)	0.640
Manganese (Mn)	1.296
Chromium (Cr)	3.949
Iron (Fe)	40.740

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Essential oil composition of the leaves of *A. heterophyllus* leaves

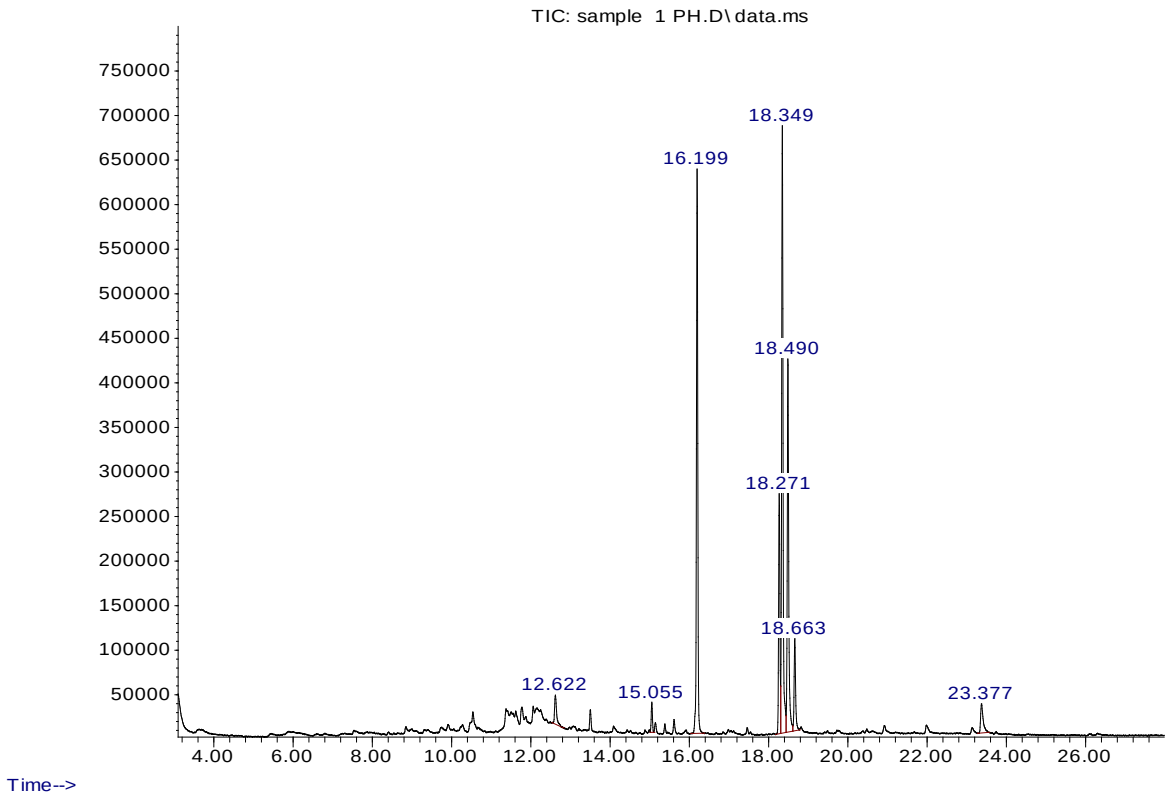

Figure 1: Gas chromatography-mass spectrometry chromatogram of the constituent oil from *A.heterophyllus*

Table 4: Essential oil composition of *A.heterophyllus*

S/N	Compound	Retention Time (min)	Percentage of the total	Molecular formula	Molecular weight (g/mol)
1	1H-Indene, 3-methyl-	12.622	2.128	$C_{10}H_{10}$	130.1864
2	Neophytadiene	15.055	1.321	$C_{20}H_{38}$	278.5157
3	Hexadecanoic acid, methyl ester	16.199	27.160	$C_{17}H_{34}O_2$	270.4507
4	Methyl 9-cis,11-trans-octadeca dienoate	18.271	11.267	$C_{19}H_{34}O_2$	294.4721
5	9,12,15-Octadeca trienoic acid, methyl ester, (Z,Z,Z)-	18.349	30.587	$C_{19}H_{32}O_2$	292.4562
6	Phytol	18.490	19.867	$C_{20}H_{40}O$	296.5310
7	Methyl stearate	18.663	5.006	$C_{19}H_{38}O_2$	298.504
8	Diisooctyl phthalate	23.377	2.665	$C_{24}H_{38}O_4$	390.564

The results of the individual fragmentation of the components of essential oil present in the leaves of *A. heterophyllus* are shown in figures 2A-2H

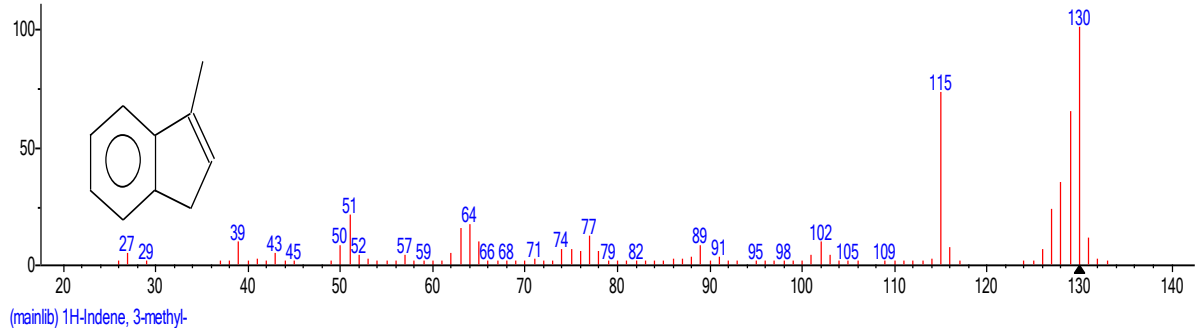

Figure 2A Structure of 1H-Indene, 3-methyl present in the *A.heterophyllus*

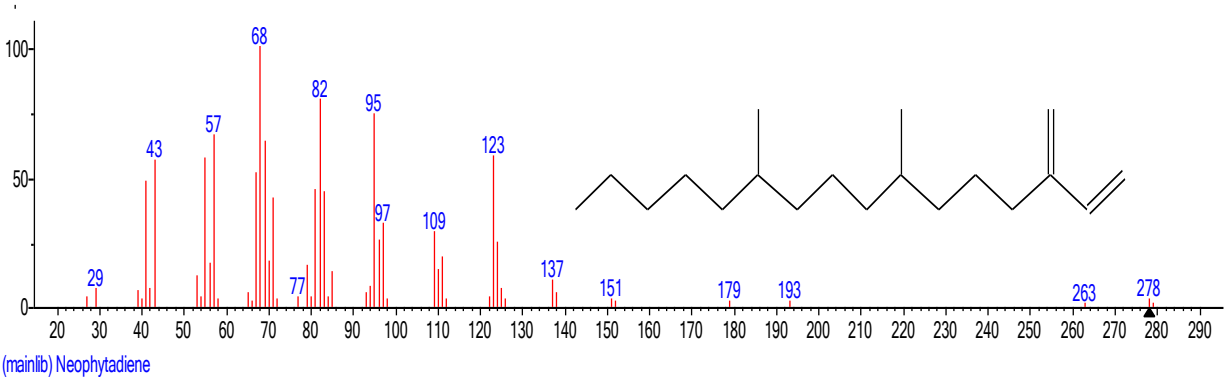

Figure 2B Structure of Neophytadiene present in the *A.heterophyllus*

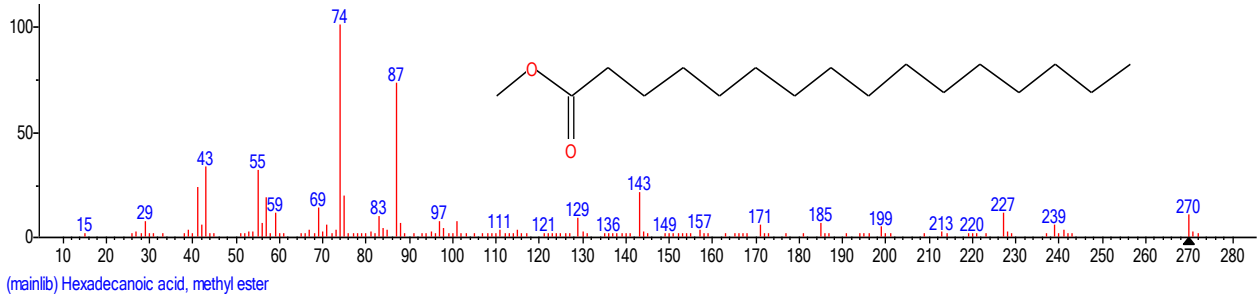

Figure 2C Structure of Hexdecanoic acid, methyl ester present in the *A.heterophyllus*

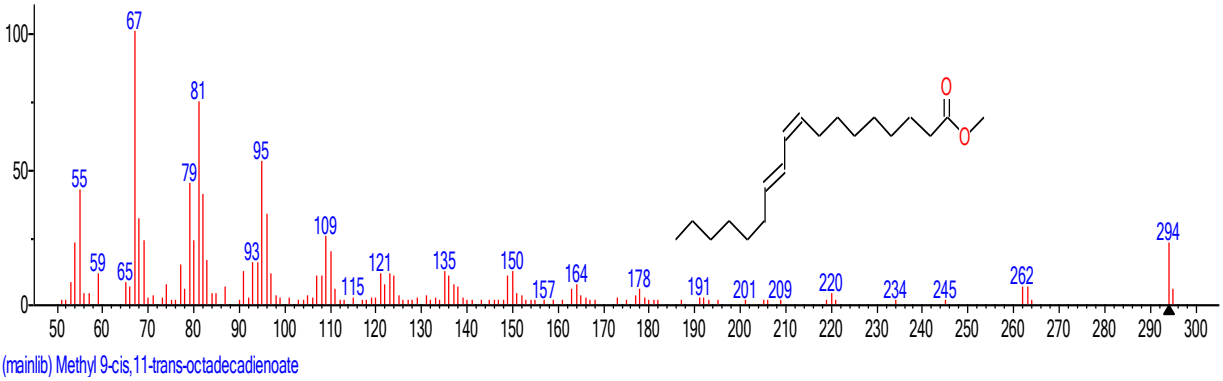

Figure 2D Structure of Methyl 9-cis, 11-trans-octadecadienoate present in the *A.heterophyllus*

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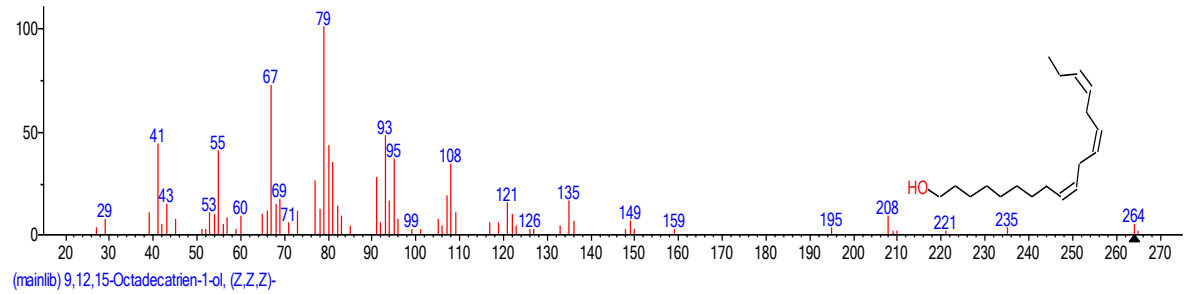

Figure 2E Structure of 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-present in the *A.heterophyllus*

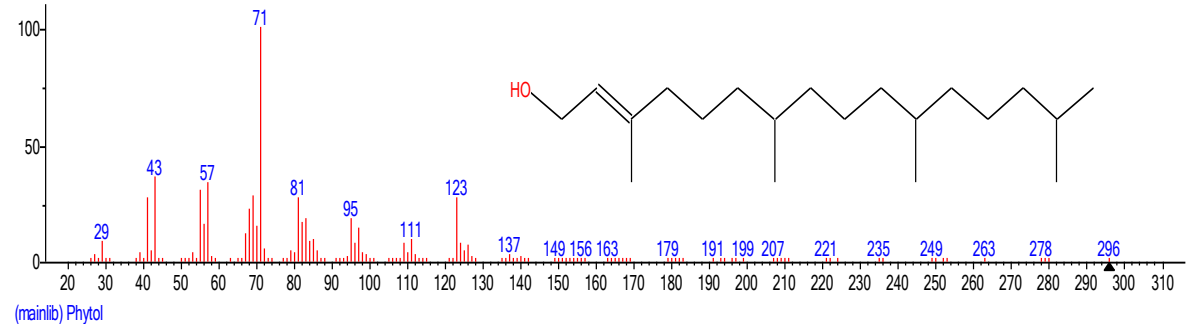

Figure 2F Structure of phytol present in the *A.heterophyllus*

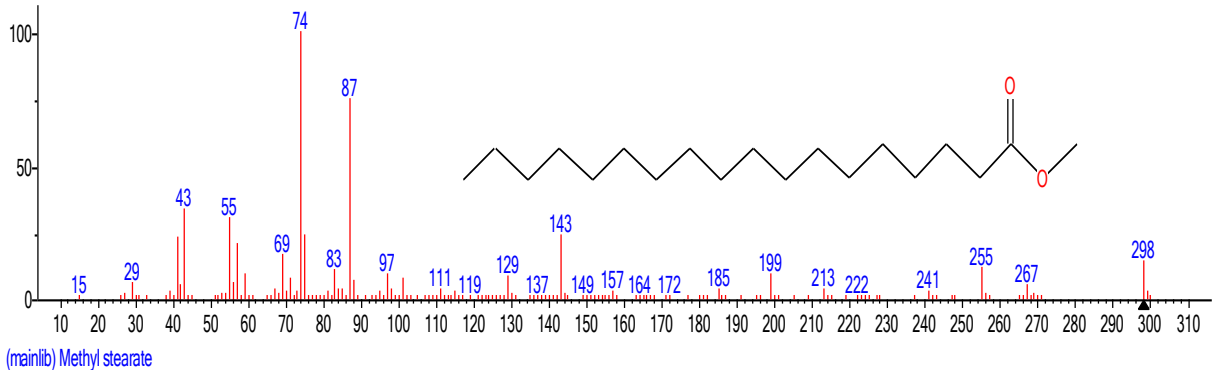

Figure 2G Structure of methyl stearate present in the *A.heterophyllus*

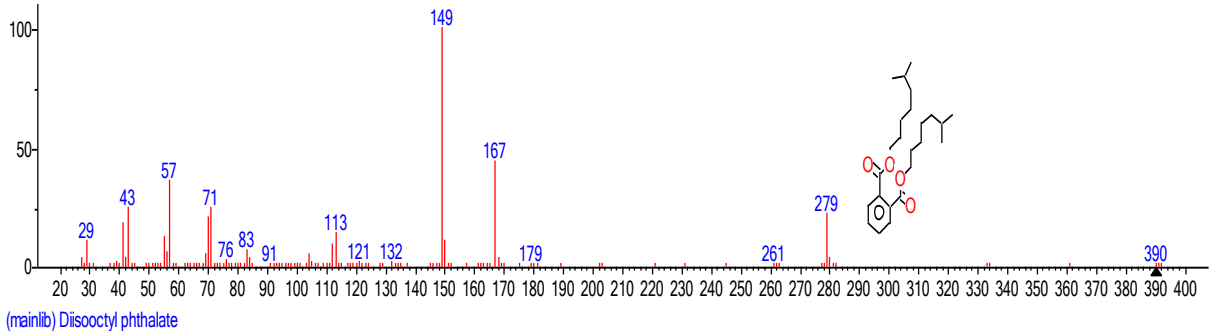

Figure 2H Structure of Diisooctyl phthalate present in the *A.heterophyllus*

Table 6 below shows the Compounds identified from the GC-MS analysis of *A. heterophyllus* leaves and their biological activities

Table 6: Compounds identified from the GC-MS analysis of *A. heterophyllus* leaves and their biological activities

compound	Type of compound	Mode of action	Biological activity
Neophytadiene	Terpenes (sesquiterpenoid)	antioxidant	Gives aroma, flavo and colour to plants. Serves as building blocks of essential oils, used as additive in the production of liquid cigarette (Guerin and Olerich, 1975). Has antifungal activity. Has antibacterial, antioxidant and other prophylactic activities (Raman *et al*., 2012)
Hexadecanoic acid, methyl ester	Palmitic acid	antioxidant	Antibacterial and antifungal, source of vitamin A, (Abubakar and Majinda, 2016)
Methyl 9-cis,11-trans-octadeca dienoate	Conjugated Linoleic acid (omega- 6 fatty acid	antioxidant	A source of vitamin A
9,12,15-Octadeca trienoic acid, methyl ester, (Z,Z,Z)-	Polyenoic fatty acid	antioxidant	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiarthritic, Anticoronary, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic (**Devi and Muthu, 2014**
Phytol	Diterpene alcohol	antioxidant	Anti-cancer, has insulin response in diabetic rats, helps in body building, Anti-inflammatory activity. Used in the preparation of Vit E and K1. effective in the decrease of blood cholesterol levels. Lowers LDL cholesterol (Keawsa-ard *et al*., 2012), (**Devi and Muthu, 2014).**
Methyl stearate	Fatty acid	antioxidant	Anticancer (Phang *et al.*, 2013)
Diisooctyl phthalate	Ester of phthalate		plasticizers in detergent as base and in aerosol spray, (Mohansrinivasan *et al*., 2015). Antifungal activity (Ahsan., 2017)

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DISCUSSION

In the present study, the qualitative phytochemical analysis of the leaves of *A. heterophyllus* shows the presence of alkaloids, flavonoids, carbohydrate, tannins, steroids, terpenoids, cardiac glycoside, saponin, carboxylic acid, and aldehyde/ketone. Antioxidants such as flavonoids, alkaloids, saponin, and tannins are important in neutralizing the effects of reactive oxygen species (hydrogen peroxide, lipid peroxides, hydroxyl and superoxide radicals,). They also help in the maintenance of optimal cellular and systemic health (Karau *et al*., 2015**)**. These antioxidants interact and synergistically stabilize, or deactivate, free radicals before they attack the body cells. These phytochemicals are known for their different biological activities including antihypertensive, antibacterial, antimalarial, anticancer, anti-inflammatory, antidiabetic and antifungal actions. The mineral analysis of the leave s of *A. heterophyllus* showed the presence of both macro and micro minerals. The macro minerals present in the leaves of *A. heterophyllus* include magnesium, potassium, calcium, sodium and phosphorus at a concentration of 7.150, 34.945, 85.108, 47.051 and 5.410 ppm respectively while the micro mineral contents include zinc, copper, manganese, chromium and iron at the concentration of 8.579, 0.640, 1.296, 3.949 and 40.740 ppm respectively. Macro and micro minerals have a lot of health benefits. The deficiency of minerals in the body can lead to different kinds of abnormalities such as stunted growth and neurocognitive deficits (Ward, 2014). The components of essential oil present in the leaves of *A. heterophyllus* were analysed using GC-MS. The spectrum profile of GC-MS confirmed the presence of eight different components including 1H-Indene, 3-methyl-, Neophytadiene, Hexadecanoic acid, methyl ester, Methyl 9-cis 11-trans-octadeca dienoate, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, Phytol, Methyl stearate and Diisooctyl phthalate at concentrations of 2.128%, 1.321%, 27.160%, 11.267%, 30.587%, 19.867&, 5.006% and 2.665% respectively. GC-MS analysis of the leaves of *A. heterophyllus* identified Hexadecanoic acid, methyl ester, Methyl 9-cis,11-trans-octadeca dienoate, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- and phytol as the significant components with the concentration of 27.160ppm, 11.267ppm, 30.587ppm and 19.867ppm respectively. The individual fragmentation of the components is illustrated in Figures 2A-2H. Essential oils have been used so many years in traditional medicine for the treatment of so many illnesses including body pains and rheumatism (Abbas *et al*., 2010). Research also shows that many of these components of essential oils possess different kinds of biological activities including anti-cancer, anti-inflammatory and hypocholesterolemic effects. Hexadecanoic acid a palmitic acid has anti-inflammatory, antioxidant, hypocholesterolemic, anti-androgenic, flavor, hemolytic, 5-alpha reductase inhibitor as well as serve as a potent mosquito larvicide, nematicide, pesticide and also possess anticancer activity (Abubakar and Majinda, 2015), (Keawsa-ard *et al*., 2012). Methyl 9-cis,11-trans-octadeca dienoate is a conjugated linoleic acid (omega- 6 fatty acid) which has anti-cancer and antidiabetic activities in rat models and also helps in body building (Devi and Muthu, 2014*)*. while 9,12,15-Octadecatrienoic acid, a methyl esther of linoleic acid has been reported to have anti-inflammatory, hypocholesterolemic, cancer preventive, 5-Alpha reductase inhibitor

antiandrogenic, antieczemic and anticoronary activities (Devi and Muthu, *2014)*. Phytol a diterpene has been reported to have anticancer activity. The cytotoxicity of phytol against HT-29 human colon cancer cells, MG-63 osteosarcoma cells and AZ-521 gastric cancer cells has been reported (Keawsa-ard *et al*., 2012).
The compounds identified from GC-MS analysis of the leaves and their biological activities are presented in Table 6

The present study has shown that the leaves of *A.heterophyllus* are rich in phytochemicals and minerals. Phytochemicals are known for their different biological activity including free radical scavenging activity, anticancer, anti-diabetic, hypocholesterolemic and anti-lipidemic activity. Minerals are required for the normal functioning of the body. The essential oil components from the leaves of *A.heterophyllus* was analysed using gas chromatogram GC/MS. Eight different components were identified including 1H-Indene, 3-methyl-, Neophytadiene, Hexadecanoic acid, methyl ester, Methyl 9-cis 11-trans-octadeca dienoate, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, Phytol, Methyl stearate and Diisooctyl phthalate. These compounds identified from the essential oil are mainly antioxidants. They have been reported to have anticancer, antidiabetic, Hypocholesterolemic, anti-inflammatory activities and hepatoprotective. The many diverse chemical compounds identified in the essential oil of *A. heterophyllus* make it an important plant for use in Nigerian traditional medicine.

Conflict of interest

All authors declare that there are no conflicts of interest

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