PHYTOCHEMICAL ANALYSIS OF STEM BARK EXTRACTS OF PTEROCARPUS MILDBRAEDII (HARMS) USING ADVANCED GAS-CHROMATOGRAPHY-MASS SPECTROSCOPIC TECHNIQUES

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ABSTRACT

Since time immemorial, man utilized herbs for their medicinal and nutritive value. Traditionally, the stem bark extract of Pterocarpus mildbraedii are used in the treatment of Malaria and digestive disorder in some West African countries including Nigeria. They are believed to possess anti-inflammatory and antioxidant as well as anti-microbial properties. This research aims to phyto-chemically characterize the Ethyl-Acetate stem bark extract of Pterocarpus mildbraedii using GC-MS (Model-QP 2010 Plus Spec). Phyto-compounds were identified using NIST Ver. 2.0 Year 2005 Library. The recorded bioactivity were based on Dr Duke`s Phytochemical and ethno-botanical databases. The GC-MS identified Phyto-chemical principles such as Triacetin, Dodecanoic acid, Isoelemicin, n-Hexadecanioc acid, 9, 12-Octadecadienoic acid, Oleic acid and Stigmasterol. Presence of these bioactive phyto-compounds can serve as a finger print for extended research.

Keywords: Pterocarpus mildbraedii, GC-MS, Phytochemical, anti-oxidant, anti-microbial, anti-inflammatory.

INTRODUCTION

By serendipity, before the advent of orthodox medicines, man was able to distinguish between beneficial and harmful herbs. In recent time, various regions of the world have developed its own traditional medicine Pharmacopoea especially China and India, since this development is highly supported by the World Health Organization. The World Health Organization estimates that about 80% of the global population rely mostly on traditional medicine for their primary health needs. Pterocarpus mildbraedii is an evergreen or semi deciduous tree with a small rounded crown. It is known as Oha tree amongst the Igbo speaking region of Nigeria. This plant grows freely throughout the Eastern Nigeria and down to the Cameroons. The leaves are popularly used in cooking soup by the natives. The bark is smooth, gray and exudes red gum. Leaves are usually light green in colour, the leaf stalk consist of 5-9 leaflets that are ovate to elliptic, acuminate, glabrous and glossy [Ezekwesili et al. 2016]. In West African countries, the stem barks are used to treat cases of Malaria and dysentery. Decoctions of the leaves have also been used to control fever in some eastern parts of Nigeria [Adegbite et al. 2015]. They are used by rural dwellers to fortify local complimentary food low in fibre content. It is a low sodium vegetable which is beneficial for hypertensive persons. It can actually control blood pressure through its mechanistic sodium ion and potassium ion regulation [Mruna 2012]. The presence of phyto-compounds such as flavonoids, Saponins, alkaloids and tannings in Pterocarpus mildbraedii provides some biochemical basis for the ethno-Pharmacological uses of the plant [Usunomena and Igwe, 2016]. Present research highlighted the stem bark characterization of Pterocarpus mildbraedii extract using instrumental method as well as phyto-chemical screening to evaluate and profile

the phyto-chemical principle present in the extract for possible `lead` optimization and justification of their ethno-medicinal uses by the natives.

Materials and Methods

Chemicals

Ethyl-acetate and n-Hexane used were of analytical grade and purchased from Sigma Aldrich (St.Louis, MO, USA).

Plant Material Extraction

The stem bark of Pterocarpus mildbraedii tree was collected in a small farm located in Umulemia village in Isiala Mbano Local Government Area, Imo State, Nigeria in the month of April, 2019. The plant was authenticated by Pharm. Osuala of the Department of Pharmacognosy, Faculty of Pharmacy, Madonna University, Elele, Rivers State, Nigeria. A voucher specimen (MUPH 269502) of the stem bark sample was deposited in the herbarium of the Department. The dried stem bark of P.mildbraedii was milled to fine powder using Willey Mill (Thomas Willy mills, Swedesboro, NJ, USA). 800 g of pulverized stem bark was defatted by macerating with n-Hexane, (3 L) for 72h, filtered and the marc obtained was air dried before macerating in Ethyl-acetate (2 L) for 72 h and subsequently filtered. The filtrate obtained were concentrated under vacuum and reduced pressure using, Buchi, Rotary-evaporator, R-205, BUCHI Labortechnik AG CH-9230, F/Switzerland at 45^0C.

Gas-Chromatographic – Mass Spectrometric Analysis

The Ethyl-acetate extract was re-dissolved in the extracting solvent, vortexed and filtered through 0.45 µm sintered glass funnel. A 1.0 µl aliquot solution of the sample was injected into the above mentioned analytical equipment.

Instrument and Chromatographic Conditions

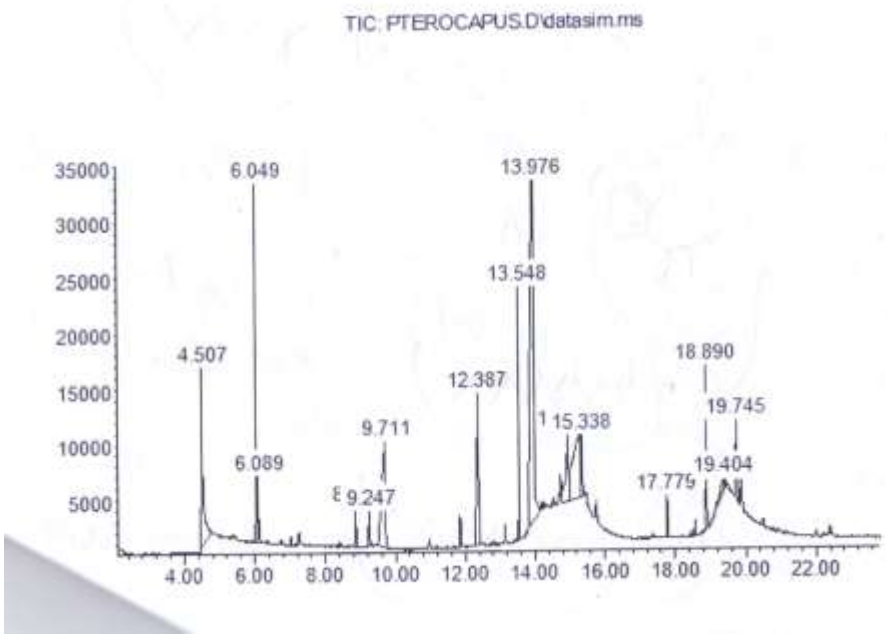
Instrumental analysis was carried out on a Model 7890A gas chromatograph in tandem with Model 7000B (TQ) Mas-Spectrometer with (EI) source equipped with a model 7693 auto sampler (Product of Shimadzu, Tokyo, Japan). The capillary column used here is V5 5 ms fused micro silica capillary column of 30 m length, 0.25 mm diameter and 0.25 µm film thickness. Helium (99.99%) was employed as the carrier gas at a constant flow rate of 1.58 ml/min. The injector and mass transfer line temperature were set at 240 to 200^{o}C respectively, and the injection volume of 1 µl was maintained. The MS operating condition were as follows: ionization energy, 70 eV: ion source temperature, 200 ^{0}C, Solvent cut time, 2.5 min, Scan mode is 1666 µ/sec: Scan range is 40-800 µm, the interface temperature is 250 ^{o}C. Overall running time was 30 min. The percentage peak area and peak ratio was expressed.

Compound Identification

The percentage composition of each Phyto-chemical entity was calculated by comparing its average peak area to the total areas. The identification employed the NIST (National Institute of Standard and Technology) Ver. 2.0 Year 2005 Library. The compound biological activity prediction is based on Dr Duke`s Phytochemical and Ethno-botanical Databases by Dr. Jim Duke of the Agricultural Research Service/ USDA [Shyamala and Manikandan, 2019]. Interpretation of GC-MS was conducted using the database of the NIST having more than 62,000 patterns. The name and molecular weight of the phyto-compounds of the test materials were highlighted.

Results

Gas Chromatogram of ethyl-acetate leaf extract of Pterocapus

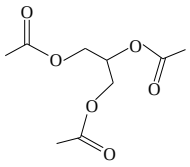


The Gas Chromatogram of ethyl-acetate leaf extract of Pterocapus shows 12 prominent peaks corresponding to 12 different Phyto-chemical compounds.

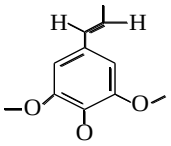
Phyto-components identified in the Ethyl-acetate extract of Pterocarpus. by GC-MS

S/N	**Retention time (min)**	**Name of Compound**	**Molecular Formula**	**Molecular Weight (gmol-1)**	**Peak area (%)**
1	6.09	Triacetin	$C_9H_{14}O_6$	218.2	0.41
2	9.71	Dodecanoic acid	$C_{12}H_{24}O_2$	200.3	3.96
3	10.97	Isoelemicin	$C_{12}H_{16}O_3$	208.25	0.38
4	12.38	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	3.54
5	13.98	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	19.23
6	15.34	9,12-Octadecadienoic acid (Linoleic acid)	$C_{18}H_{32}O_2$	280.5	45.04
7	17.78	Oleic acid	$C_{18}H_{34}O_2$	282.47	0.26
8	19.33	Cis-Valenic acid		222.37	0.64
9	19.40	Octadecenoic acid	$C_{18}H_{34}O_2$	282.68	0.11
10	19.98	Squalene	$C_{29}H_{48}O$	412.68	0.96
11	23.46	Stigmasterol	$C_{28}H_{48}O$	400.69	3.47

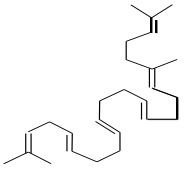
Some Chemical Structures of Phytochemical Principles revealed from the stem bark extracts of Pterocarpus



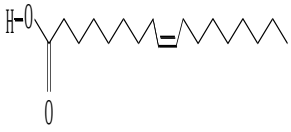
Triacetin



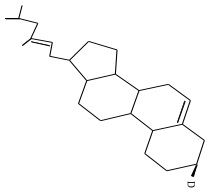
Isoelemicin



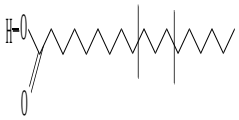
Squalene



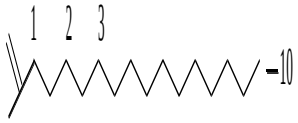
Oleic Acid



Stigmasterol



9, 12- Octadecadienoic acid



n-Hexadecanoic acid

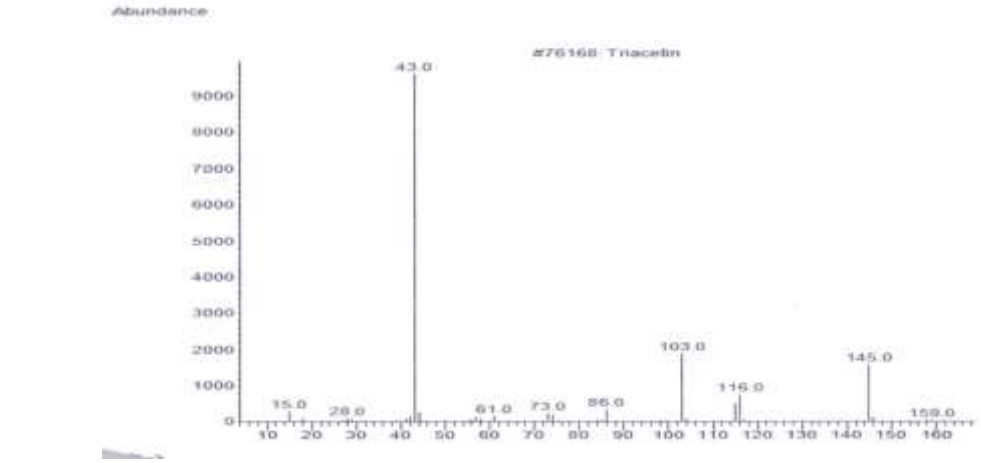
Pharmacological activities of some Phytochemical Compounds revealed from the extract of Pterocarpus

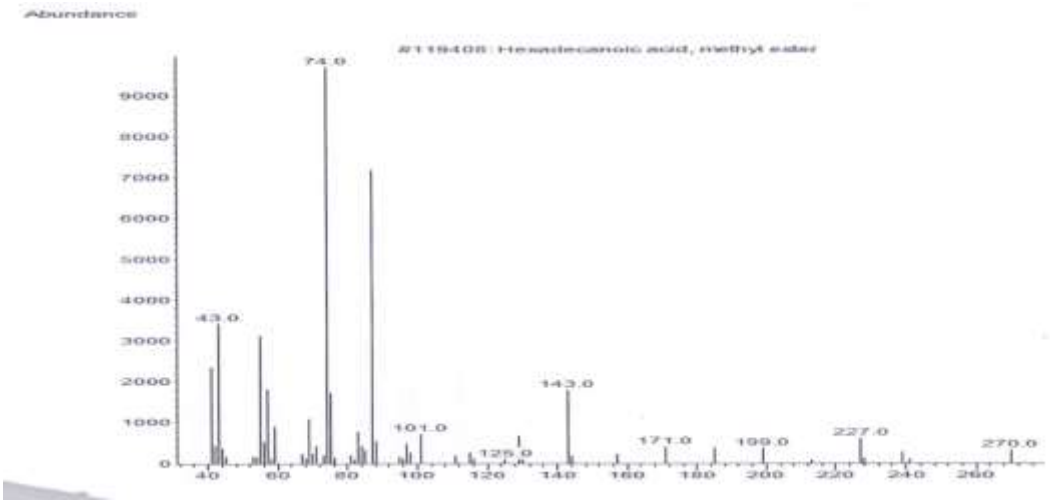
Phyto-Components.	Nature of Compounds	Biological Activities ***
Triacetin	Oily liquid	Used as excipients in Pharmaceutical products, Flavoring agent.
Dodecanoic acid	Fatty acids	Antimicrobial
Isoelemicin		Pharmaceutical Adjuvant
Tetradecanoic acid	Fatty acids	Flavoring agent
n-Hexadecanoic acid	Saturated fatty acid	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Anti-androgenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor
9,12-Octadecadienoic acid (Linoleic acid)	Unsaturated fatty acid	5-Alpha reductase inhibitor, antiandrogenic, Antiarteriosclerotic, Anticoronary, Antifibrinolytic, Antihistaminic, Antiinflammatory, antileukotrienic, anti prostatic, hepatoprotective, carcinogenic, immunomodulator, antieczemic, hypocholesteramine.
Oleic acid	Unsaturated fatty acid	5-Alpha-Reductase-Inhibitor,Allergenic, Alpha-Reductase-Inhibitor, Anemiagenic, Antialopecic, Antiandrogenic,

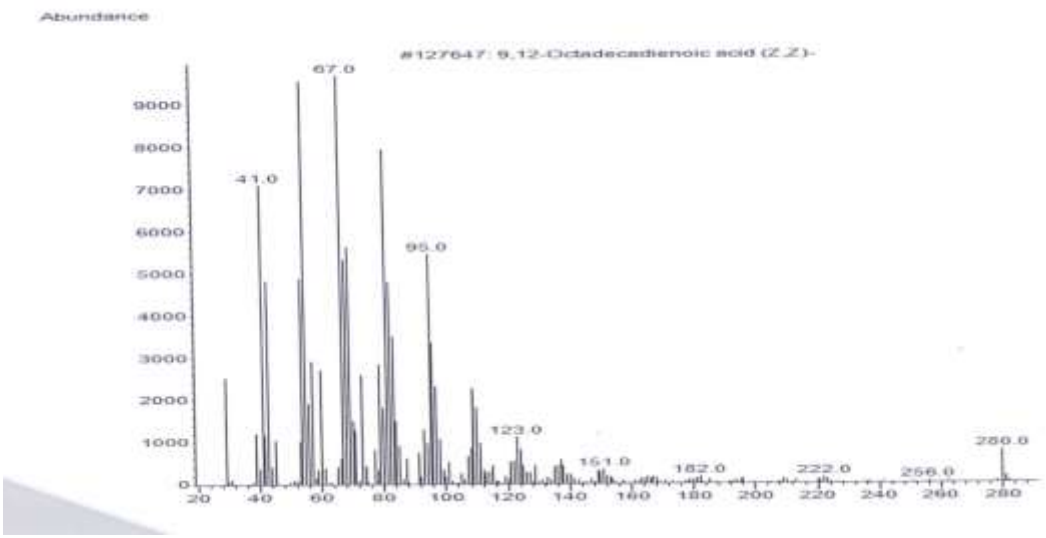
		Antiinflammatory, Antileukotriene, Cancer-Preventive, Choleretic, Dermatitigenic FLavor, Hypocholesterolemic. Insectifuge, Irritant, Percutaneostimulant, Perfumery Propecic
Squalene	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer-Preventive, Chemopreventive, Immunostimulant, Lipoxygenase-Inhibitor, Perfumery, Pesticide, Sunscreen
Stigmasterol	Phytosterol	Reduction of Atherosclerosis, Cardioprotective,Cholesterolytic agent.

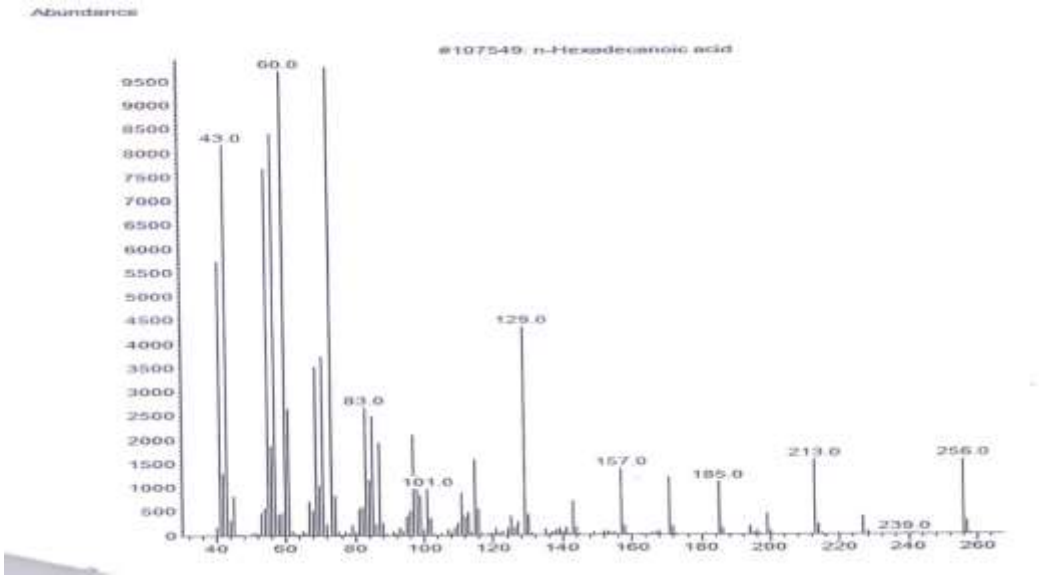
** Source Dr. Duke's Phytochemical and Ethnobotanical Databases [Online database]. ***.

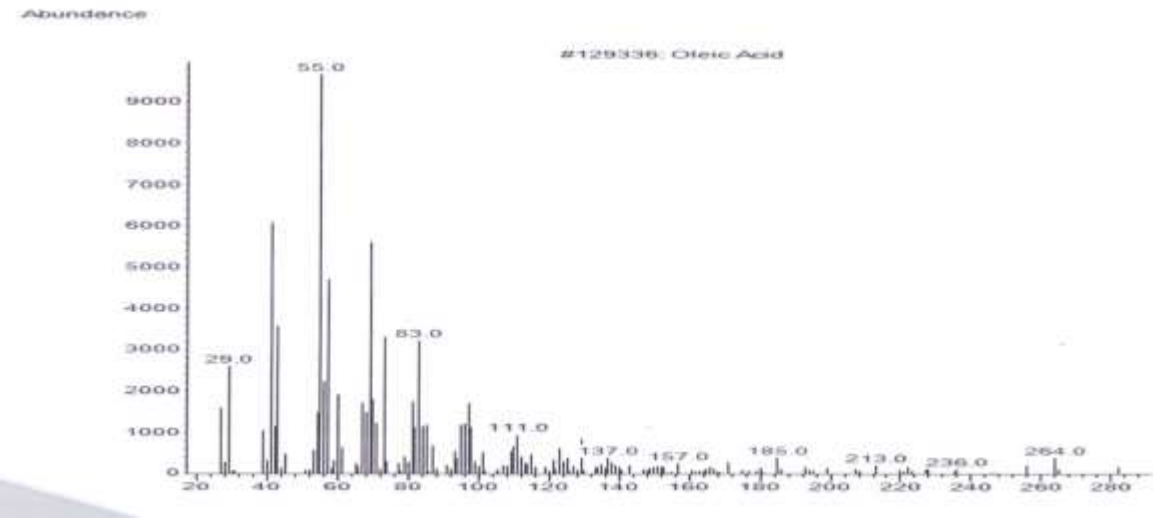
GC-MS Spectra of Some Phytochemical compounds revealed from extracts of Pterocarpus

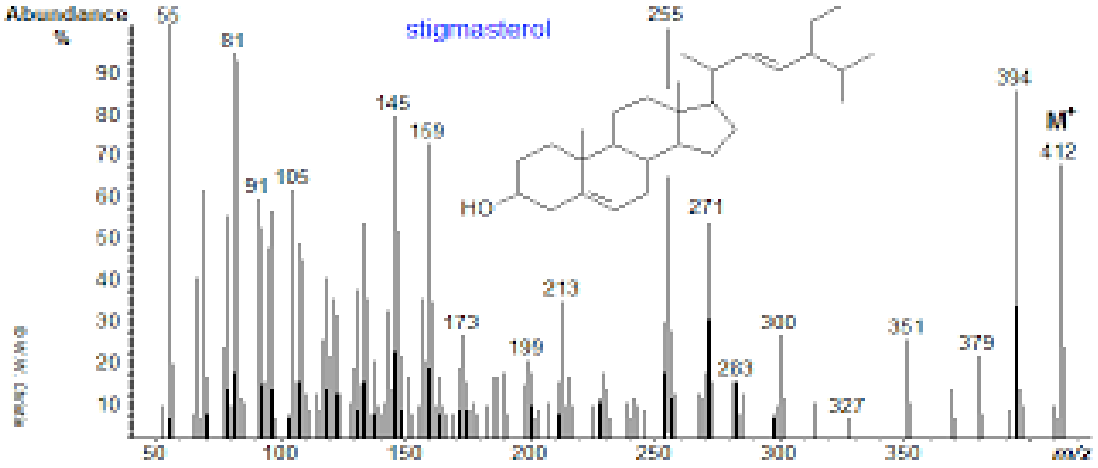





Abundance
#127647: 9,12-Octadecadienoic acid (Z,Z)-
67.0
41.0
95.0
123.0
151.0
182.0
222.0
256.0
280.0


Abundance
#107549: n-Hexadecanoic acid
60.0
43.0
129.0
83.0
101.0
157.0
185.0
213.0
239.0
256.0





DISCUSSION

The use of herbs and other medicinal plants as remedies for diseases in both animals and humans is as old as man himself. Through the structural elucidation of active components of these plants; novel bioactive compounds have been discovered and subsequently modified structurally to improve activity or reduce adverse effects. (Ezealisiji *et al.*, 2020)

The Gas chromatography-Mass spectroscopic analysis of the ethyl-acetate stem bark extract of *Pterocarpus mildbraedii* showed 12 prominent peaks which corresponds to 12 different phytochemical compound.

Triacetin is a triglyceride produced when the three hydroxyl group of glycerol is acetylated. It is used as a food additive serving as a solvent in flavouring, as a humectant and plasticizer in the production of pharmaceuticals. It has also found use in the synthesis of biodegradable phospholipid get system used in the dissemination of cancer drug paclitaxel. (Chen T *et al.*, 2017). Studies have also shown that it can be applied directly in the treatment of glioblastoma due to its ability to mediate acetate supplementation thereby inhibiting cell growth process of gliobastima (Long *et al.*, 2015)

Dodecanoic acid is a 12 carbon saturated medium chain fatty acid also known as lauric acid. It is a white solid with slight odour of bay oil commonly found in coconut and kernel oil.

Biologically, it serves as a membrane stabilizer, energy source, energy storage and nutrient. It has been shown to possess antimicrobial property. Due to its very low toxicity, its salt form, sodium laurate is used in many shampoos and soaps. (Anneken *et al.*, 2006)

Isoelemicin also called trans-isoelemicin belongs to the anisoles class of organic compound. It is an extremely weak olefinic base with a molecular weight of 208.25g /mol. Isoelemicin is commonly used a pharmaceutical adjuvant.

N-hexadecanoic acid also called palmitic acid is a saturated fatty acid readily found in plants, animals and even microorganisms. It is the major constituent of oil gotten from the fruit of oil palm. It has found application in soap, cosmetics, industrial mold release agent and as an inexpensive additive to processed food to improve its "mouth feel". It been shown to possess antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, anti-androgenic, hemolytic, 5-Alpha reductase inhibitor properties. It is also used in the formulation of long-acting release carrier medium such as in the use of oily palmitat ester in the synthesis of paliperidone palmitate. The observed 9, 12- Octadecadienoic acid (Linoleic acid) is a poly unsaturated omega-6 fatty acid which is colourless and insoluble in water. It is one of the two important fatty acids in human, mostly obtained from diet. It is useful in the manufacturing quick-drying oils most commonly used in oil paints and vanishes (Diezel *et al.*, 1993). It is also used in cosmetics due to its beneficial effect on the skin. Its lipid redical is harnessed in demonstrating the antioxidant property of polyphenols and natural phenols. (Peyrat-Mailard *et al.*, 2003) Studies have shown that possess 5-Alpha reductase inhibitor, antiandrogenic, antiarteriosclerotic, anticoronary, antifibrinolytic, antihistaminic, antiinflammatory, antileukotrienic, antiprostatic, hepatoprotective, carcinogenic, immunomodulator, antieczemic, and hypocholesteramic properties.

Oleic acid is a mono-saturated omega-9 fatty acid found naturally in various animals and vegetable fat and oils. Ideally, it is usually colourless and odourless. Oleic acid in its triglyceride form is used as a component in many foods. Its sodium salt is used readily used as an emulsifying agent and emollient in soap and in the formulation of aerosol products. It has also found use in the solution phase synthesis of nanoparticles where it acts as a kinetic knob to aid the control of size and morphology of nanoparticles.

Squalene is a triterpene and a hydrocarbon compound. It is produced by all plants and animals including human, as a biochemical intermediate for the synthesis of all plant and animal sterols including steroid hormones cholesterol in human. It has pharmacological, cosmetic and nutritional potentials. Squalene is used in combination with surfactant in adjuvant formulation for vaccine; it reduces skin damage caused by UV radiation, cholesterol and LDL in the blood. (WHO advisory committee on vaccine safety)

Stigmasterol is an unsaturated plant sterol found in the fat and oil of most herbs. Due to its known ability to reduce atherosclerosis, and its cardioprotective and cholesterolytic properties, it is used as food additive and as food constituent to increase the phytosterol content and LDL cholesterol lowering ability of such food. It serves as a precursor for the large scale manufacture of semisynthetic progesterone, an important human hormone. Stigmasterol is also used a precursor for boldenone, commonly used to growth in cattle. (Hogg J. *et al.*, 1992)

CONCLUSION

The GC-MS analysis of ethyl-acetate stem bark extract of *Pterocarpus mildbraedii* revealed the presence of 12 peaks which indicated the presence of chemicals such as isoelemicin, oleic acid, palmitic acid, squalene, stigmasterol and others diverse phytochemicals. These chemicals have marked pharmacological, cosmetic, nutritional and even economic benefits to humanity. Hence, further research is required to establish *Pterocarpus mildbraedii* as a concrete source of these chemicals. These chemicals can also be isolated and purified for use as a useful hits, leads and novel drugs for use in human and animals.

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