Nig. J. Biotech. Spec. Edtn. BSN-SW 1: 25-38 (May, 2022)
ISSN: 0189 1731
Available online at http://www.ajol.info/index.php/njb/index
and https://bsn.org.ng
DOI: https://dx.doi.org/10.4314/njb.v38i.3S

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Gas Chromatography – Mass Spectrometry Identification of Bioactive Compounds in Methanol and Aqueous Seed Extracts of *Azanza garckeana* Fruits

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Abstract
***Azanza garckeana* is a tropical fruit plant found in Africa. Its edible fruit is used as food or herbal medicines, while the seeds are discarded. In an attempt to turn waste to wealth and obtain useful substances from it, this study was carried out to identify the bioactive compounds with nutritional and health-promoting benefits present in methanol and aqueous extracts of the seeds of this fruit. Freshly harvested few fruits of *A. garckeana* containing the seeds were obtained from Tula in Kaltungo LGA, Gombe State, Nigeria. They were identified and authenticated at the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The pulverized seeds were used to obtain methanol and aqueous extracts and subjected to GC-MS analysis. The results of the study revealed the presence of thirty-eight bioactive compounds which have been reported to have anticancer, antioxidant, antimicrobial, anti-androgenic, anti-inflammatory and hepatoprotective properties. Among these compounds are the following with reported biological functions namely: Pentadecanoic acid, Octadecadienoic acid, Hexadecanoic acid, Tetradecanoic acid, Heneicosane, 2-Methyltetracosane and Methyl stearate. There concentrations range from 0.33% for 9-Octadecenoic acid-methyl ester (Methyl oleate) to 24.3% for 9,12–octadecadienoic acid-(z-z) (Linoleic acid) in methanol extract. In the aqueous extract, the bioactive components measured 0.18% (lowest) for Dodecane, 2,6,11-trimethyl- and 2-Methyltetracosane (Isopentacosone) to 7.34% (highest) for 9-Octadecenoic acid (Z)-(Oleic acid). Other compounds without known reported functions are 10,13-Octadecadienoic acid, 9-Octadecenoic acid 12-hydroxy-methyl ester [R-(Z)]- and 1,13-Tetradecadiene among others. The seeds of *A. garckeana* have useful constituents that can be exploited for health benefits.**

Keywords: *Azanza garckeana,* Bioactive compounds, GC-MS analysis, Seeds

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Introduction

Medicinal plants are a valuable gift of nature and have been used since time immemorial by man for the treatment of various ailments. They are also used as food supplements, where there is poor nutrition (Nwachukwu et al., 2010; Mensah et al., 2019). Currently, one of the central themes of scientific research is the emphasis on the use of naturally occurring substances with therapeutic potentials for the treatment of ailments. These naturally occurring substances are majorly from plant sources and are of great interest in the research world, especially for pharmaceutical purposes because of their high availability, low cost and sometimes, because of the severe side effects associated with conventional drugs or pharmacological treatments (Arvind, 2016; Ugboko et al., 2020).

Plants and plant extracts have been shown scientifically to be excellent sources of

bioactive substances that act as therapeutic agents with healing relevance to humans (Kamboj, 2000; Mgbeahuruike et al., 2017). These bioactive substances are present in plants as secondary metabolites and they are also called phytochemicals. They primarily play defensive roles against plant pathogens and herbivores. Bioactive substances have been shown from several studies to have therapeutic effects which are beneficial to man and these therapeutic effects include antimicrobial, anti-inflammation and neutralization of harmful effects of free radicals (Nascimento et al*.,* 2000; Edoga et al*.,* 2005; Forni et al., 2019; Masyudi et al., 2022).

Advancement and increased sensitivity of modern methods of analytical techniques for the identification and quantification of these bioactive compounds in plants have enabled a wealth of information to be gathered about the therapeutic potential of medicinal plants and have also led to the discovery of new bioactive compounds. One of such modern methods of analytical techniques adapted for the screening of these plants is the Gas Chromatography-Mass Spectrometry (GC-MS). This technique is a powerful tool and has in recent times been found useful in the study of medicinal plants (Elaiyaraja and Chandramohan, 2016).

The plant *Azanza garckeana* is a tropical fruit plant found in Africa and commonly called "Goron Tula" (Cola of Tula) in Northern Nigeria, West Africa. It is also called "Morajwa" in Botswana, South Africa and snot Apple in English (Mojeremane and Tshwenyane, 2004; Orwa et al., 2009; Ochokwu et al., 2014). The plant is a member of the Malvaceae family which includes other well-known plants of economic importance such as okra, cotton, cacao and durian. The genus name "*Azanza*" is probably derived from the word "Azania" an old word in Zanzibar Tanzania in East Africa, meaning black and the specie *garckeana* was named after the German botanist Christian August Friedrich Garcke (Schmidt et al., 2002).

Azanza garckeana can be found in Botswana, Malawi, Zambia and Zimbabwe in South Africa. It is also present Burundi, Kenya and Tanzania in East Africa. It can also be found in Democratic Republic of Congo in Central Africa, Sudan in North Africa and Nigeria in West Africa (Ochokwu et al., 2014). In Nigeria, *Azanza garckeana* is found in Tula in Kaltungo Local Government Area of Gombe State and also in Michika Local Government area of Adamawa State (Ochokwu et al., 2015).

Azanza garckeana is a deciduous shrub which sheds its leaves at the end of the growing season. The plant has a height of about 3-13m and diameter of up to 25cm. The fruits of *Azanza garckeana* are rough with hairy bark. The fruits are also reddish-brown in colour when ripe and green when unripe; they are fibrous with longitudinal fissures (Glew et al*.,* 2005; Maroy, 2017). The outer coverings of the fruits are fleshy but a bit hard and generally eaten as a good source of proteins, minerals, fibres, vitamins and also used for ethnomedicinal purposes. The fruits can clearly be divided into five segments with each segment containing seeds (Orwa et al., 2009). The seeds are hemispherical in shape and are covered with brownish woolly floss and are usually discarded as wastes.

The bark, fruits, leaves, roots and stems of *Azanza garckeana* are reportedly used for diverse medicinal purposes such as treatment of menstrual disorder, chest pains and cough in Nigeria, Zimbabwe and Kenya (Mshelia et al., 2016). They are also used for the treatment of sexually transmitted diseases such as gonorrhoea and syphilis in Malawi and Nigeria (Nkafamiya et al., 2015; Maroy, 2017; Bioltif et al., 2020). However, there is no mention of the use of the seeds of *Azanza garckeana* for any medicinal purpose across all reviewed literatures. Hence, in an attempt to turn waste to wealth and obtain useful substances from such waste, this study was carried out to evaluate and quantify the bioactive compounds with health-promoting benefits, present in methanol and aqueous extracts of the seeds of *Azanza garckeana* fruits with the aid of Gas Chromatography-Mass Spectrometry (GC-MS) Technique.

Materials and Methods

Plant Material

Freshly harvested fruits of *Azanza garckeana* containing the seeds were obtained from Tula in Kaltungo LGA, Gombe State. The plant was identified and authenticated at the Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria where an Herbarium Voucher specimen number FHI-112621 was deposited in its reference.

Preparation of pulverized sample

The pulps (gummy outer coats) of the fruits of

Azanza garckeana were removed and the seeds were oven-dried at 40°C for 24 hours (Plate 1a) and ground using mortar and pestle. The pulverized sample (Plate 1b) was wrapped in aluminium foil and stored in an airtight container at room temperature (26°C ± 2°C) before analyses. Aqueous extract of the sample was prepared by mixing 250 mL distilled water with 50 g of the pulverized seeds. Methanol extract was made likewise by substituting the solvent for distilled water. The extracts were subjected to GC-MS analysis.

(a) (b)

Plate 1: (a) The seeds of *Azanza garckeana* and (b) The Pulverized form of the seeds of *Azanza garckeana*

Gas chromatography–Mass Spectrometry (GC-MS) Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was carried out using methanol and aqueous extracts of the pulverized samples of *Azanza garckeana*, to identify and quantify all bioactive compounds present in the samples.

The GC-MS analysis was carried out using Gas Chromatograph (Agilent technologies, 7890 GC system) coupled with a Mass Spectrometry detector (Agilent technologies 5975). The column used was Agilent H5MS column measuring 30 mm in length, 0.320 mm in diameter and 0.25 µm in thickness. Helium gas was used as carrier gas at a flow rate of 0.5 ml/min. A sample volume of 1 µL was injected into the gas chromatograph. The oven temperature was programmed initially at 80°C for 2 minutes with a gradual increase of 10°C per minute until a final temperature of 240°C was reached and maintained for 6 minutes. The total run time was 90 minutes. The GC-MS was carried out using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectra of various components were compared with the database of spectra of known compounds stored in the data system of the National Institute of Standards and Technology (NIST) library (Gaithersburg, Maryland, United State).

Results

The total ion chromatogram (TIC) showing the peaks of the various bioactive compounds that were found in the methanol extract of *Azanza garckeana* seeds are shown in Figure 1. A total of 22 peaks were detected and 17 bioactive compounds were identified and quantified using their peaks and retention times. The compound n-hexadecanoic acid (without known reported function) was found to be the most abundant in the methanol extract of the seeds of *Azanza garckeana* with a concentration of 30.44% and a retention time (RT) of 15.09 min, followed by 9,12-Octadecadienoic acid (Z, Z) (24.32% and RT of 16.496 min). The compound: 9-Octadecenoic acid (Z)-methyl ester was the least with a concentration of 0.33% and retention time of 16.09 min**.**

The compounds found in the methanol extract of the seeds of *A. garckeana*, along with their biological activities, retention time (RT), molecular formula, molecular weight and concentration (% Area) are presented in the Table 1.

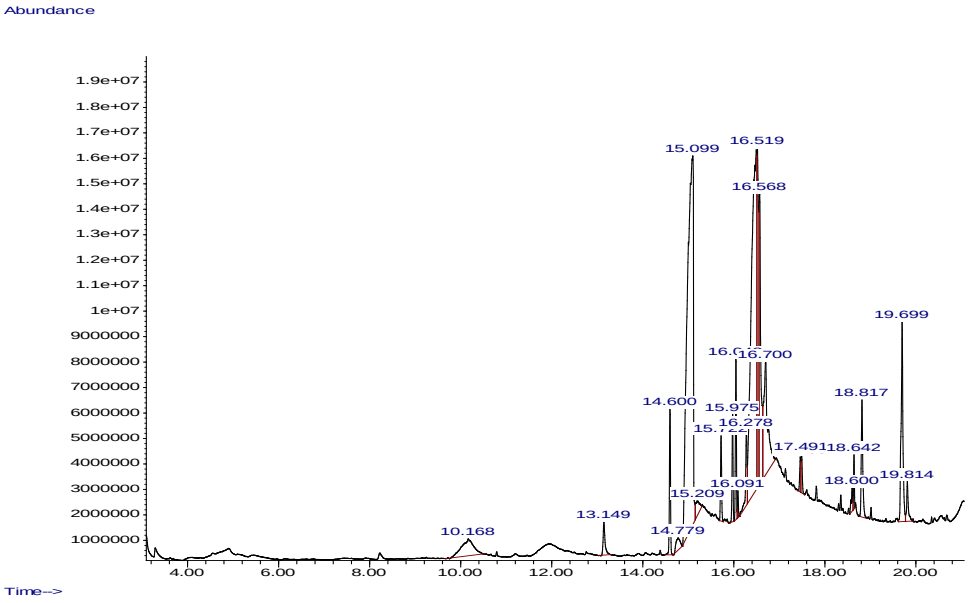
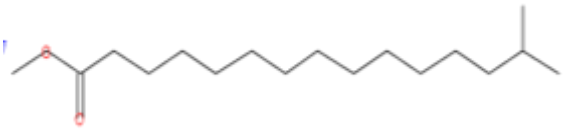
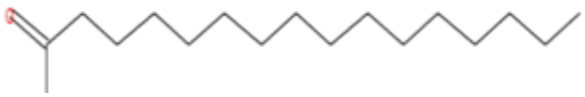
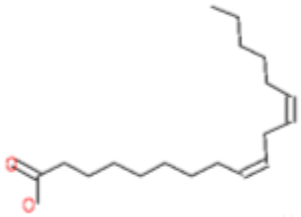
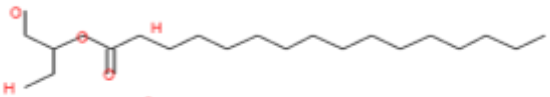
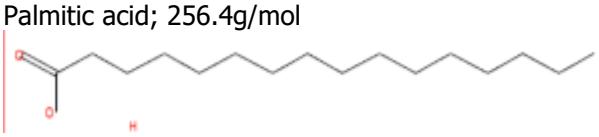
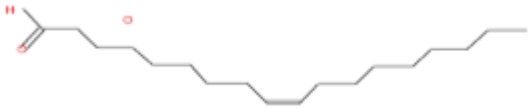
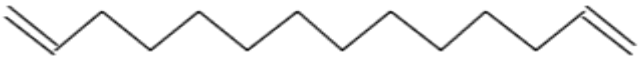
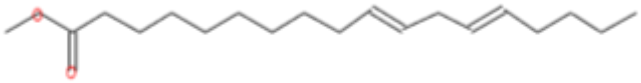
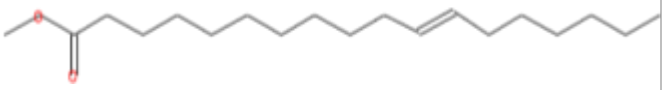
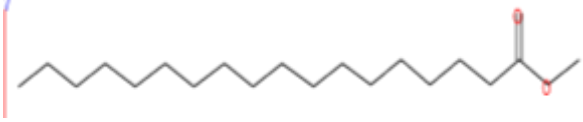
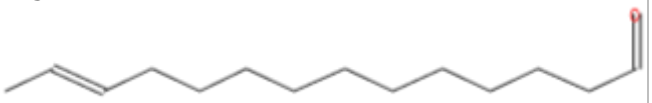


Figure 1. Total ion chromatogram (TIC) of the methanol extract of *Azanza garckeana* seeds

Table 1: GC-MS compounds found in the methanol extract of *Azanza garckeana* seeds

Compound name; chemical formula	Retention time (min)	Area (%)	Common name; Molecular weight; Chemical structure; Biological activity
Pentadecanoic acid, 14-methyl-methyl ester $C_{17}H_{34}O_2$	14.601	2.18	Methyl isohexadecanoate; 270.5g/mol  Antifungal, Antimicrobial (Bashir et al., 2012).
9,12-Octadecadienoic acid (Z,Z)-, methyl ester $C_{19}H_{34}O_2$	15.973	1.42	Methyl linoleate; 294.5g/mol  Anticancer (Majinda and Abubakar, 2016).
9-Octadecenoic acid, methyl ester $C_{19}H_{36}O_2$	16.092	0.33	Methyl oleate; 296.5g/mol  Antioxidant activity, anti-hypertensive, anticarcinogenic activity, exist in human red blood cells and serve as endogenous peroxisome proliferator-activated receptor ligand, dermatigenic flavour, increase HDL and decreases LDL (Akpuaka et al., 2013).
Hexadecanoic acid $C_{16}H_{32}O_2$	15.211	0.76	Palmitic acid; 256.4g/mol  Antioxidant, antimicrobial, anti-inflamatory, haemolytic-5-α reductase inhibitor and antiandrogenic

			activities (Majinda and Abubakar, 2016; Ojekale et al. 2016; Zulbayu et al., 2021).
9,12 – Octadecadienoic acid-(z-z) $C_{18}H_{32}O_2$	16.497	24.3	Linoleic acid; 280.5g/mol  Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic, anticancer and anti-inflammatory properties (Majinda and Abubakar, 2016; Sunita et al., 2017).
Tetradecanoic acid $C_{14}H_{28}O_2$	13.149	0.854	Myristic acid; 228.4g/mol  Antifungal, antioxidant, hypocholesterolemic, cancer preventive, nematicide, lubricant* Zulbayu et al. (2021).
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester $C_{19}H_{38}O_4$	18.816	2.37	Palmitin, 2-mono-; 330.5g/mol  Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic activities, haemolytic-5-α reductase inhibitor*
n-hexadecanoic acid $C_{16}H_{32}O_2$	15.211	0.76	Palmitic acid; 256.4g/mol  Antioxidant, anti-inflammatory properties, antitumor* Zulbayu et al. (2021).
Oleic acid $C_{18}H_{34}O_2$	16.568	7.04	282.5g/mol  Antifungal activity *
Undecylenic acid $C_{11}H_{20}O_2$	17.463	0.51	10-undecenoic; 184g/mol  Antifungal activity*
9, 17 Octadecadienal (Z) $C_{18}H_{32}O$	19.701	4.97	264.5g/mol  Antimicrobial, anti-inflammatory*
Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester $C_{21}H_{42}O_4$	19.816	0.88	Stearin, 2-mono-; 358.56g/mol  Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic, anticancer,

			anticoronary, antiacne and anti-inflammatory properties* (Majinda and Abubakar, 2016).
1,13-Tetradecadiene $C_{14}H_{26}$	15.72	1.24	194g/mol  NF
10,13-Octadecadienoic acid, methyl ester $C_{19}H_{34}O_2$	15.97	1.42	294.5g/mol  NF
11-Octadecenoic acid, methyl ester $C_{19}H_{36}O_2$	16.05	2.15	297g/mol  Antibacterial, antifungal and antioxidant (Asghar and Choudahry, 2011).
Methyl stearate	16.28	1.40	Stearic acid; 298.511g/mol  White crystal semi-solid ester, flavour component in food, lubricant, used in the manufacture of pharmaceuticals, cosmetics and soaps, surfactant and softening agent (Enas and Duha, 2014).
E-12-Tetradecenal $C_{14}H_{26}O$	17.49	0.66	210g/mol  NF

*Source: Duke (2016)

Legend: NF: Biological activity not found

Peaks of the GC-MS compounds detected in the aqueous extract of *A. garckeana* seeds are shown below in the total ion chromatogram (Figure 2). A total of 21 peaks corresponding to 21 bioactive compounds were identified and quantified using their peak and retention time (RT). A 9-Octadecenoic acid 12-hydroxymethyl ester (R-(Z))- was found to be the most occurred in the aqueous extract with 28.042% in content and RT of 17.472, followed by 9,12-Octadecadienoic acid methyl ester (11.78% in content and RT 15.99) while 2,6,11 -trimethyl dodecane was found to be the least with 0.18% and RT of 8.187 (Table 2). The compounds found in the aqueous extract of the seeds of *Azanza garckeana*, along with their biological activities, retention time (RT), molecular formula, molecular weight and concentration (% Area) are presented in the Table 2.

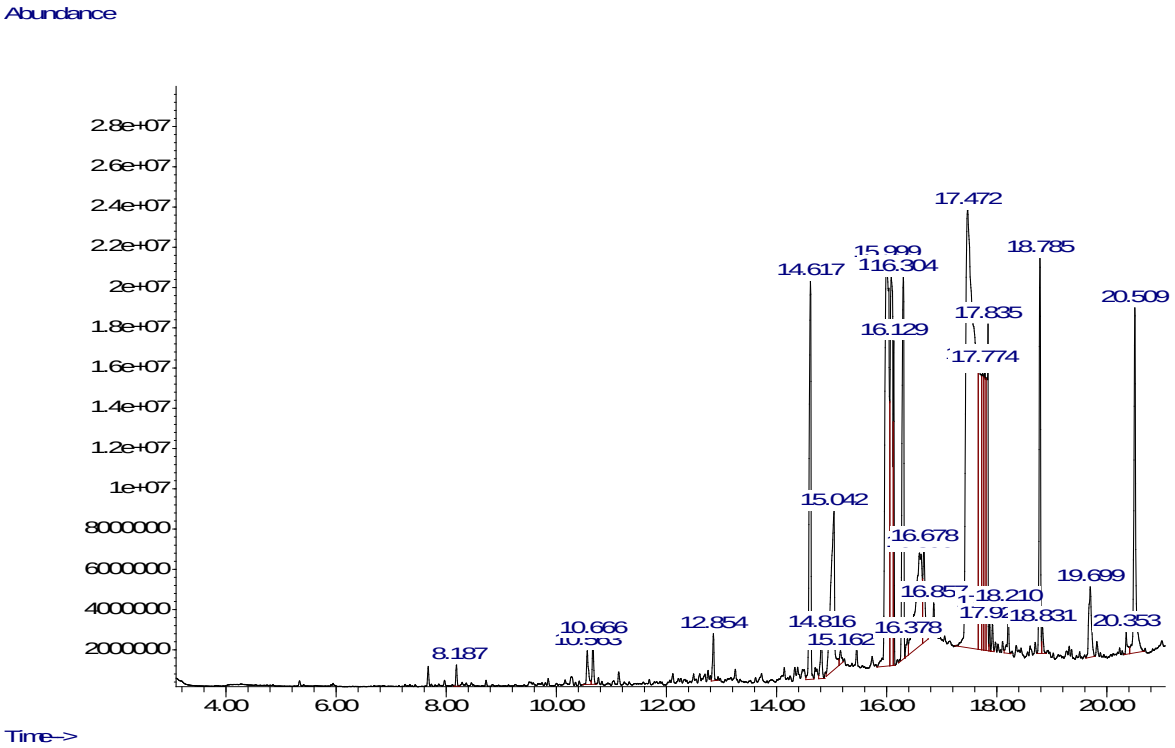
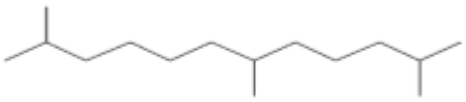
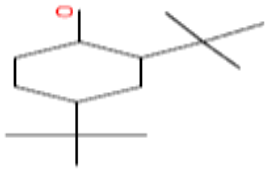
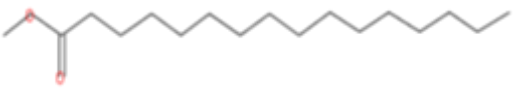
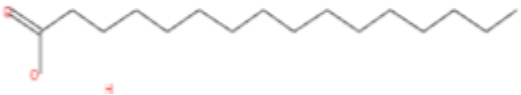
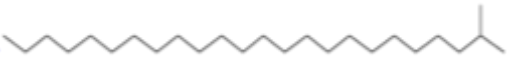
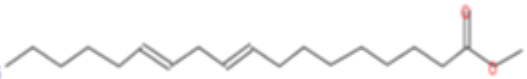
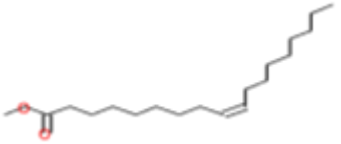
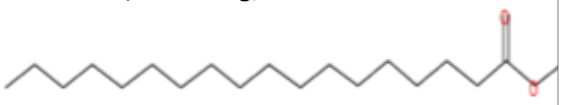
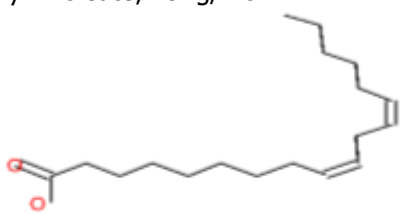
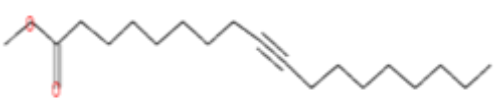
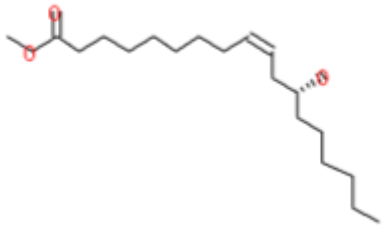
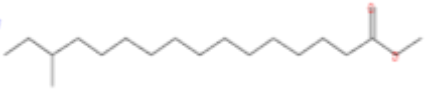
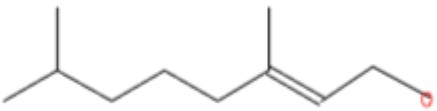
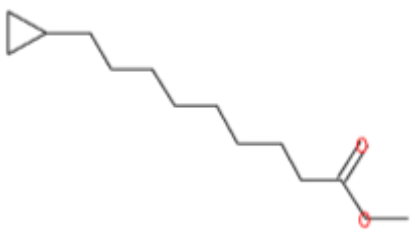


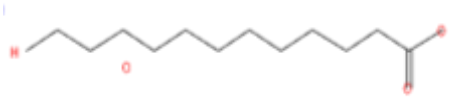
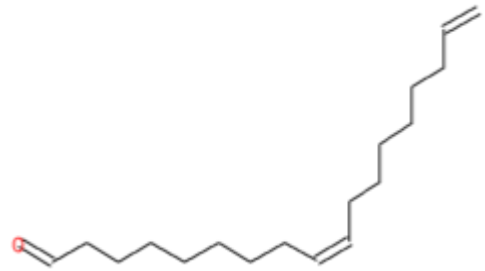
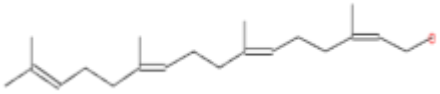
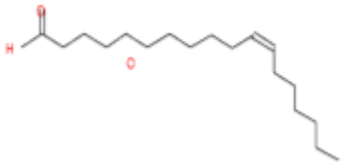
Figure 2: Total ion chromatogram (TIC) of the aqueous extract of *Azanza garckeana* seeds

Table 2: GC-MS compounds of the aqueous extract of *Azanza garckeana* seeds

Compound name; chemical formula	Retention time (min)	Area (%)	Common name; Molecular weight; Chemical structure; Biological activity
Dodecane, 2,6,11-trimethyl- $C_{15}H_{32}$	8.187	0.18	212.41g/mol  Antibacterial activity*
2,4-Di-tertbutylphenol $C_{14}H_{22}O$	10.563	0.44	206.32g/mol  NF
Heneicosane $C_{21}H_{44}$	14.816	0.55	296.57g/mol  Antiasthmatics, urine acidifiers and antimicrobial*

Hexadecanoic acid, methyl ester $C_{17}H_{34}O_2$	14.616	5.03	Methyl palmitate; 270.453g/mol  Antioxidant, antimicrobial, anti-inflamatory, haemolytic-5-α reductase inhibitor and antiandrogenic activities (Majinda and Abubakar, 2016; Ojekale et al., 2016).
n-Hexadecanoic acid $C_{16}H_{32}O_2$	15.044	4.25	Palmitic acid; 256.4241g/mol  Antioxidant, hypocholesterolmic, nematicide, pesticide, lubricant, antiandrogenic, flavour, hemolytic and 5-Alpha reductase inhibitor activities*
2-Methyltetracosane $C_{25}H_{52}$	15.163	0.18	Isopentacosone; 352.7g/mol  Drug for dermatological disorders, antibacterial and urinary problem*
9,12-Octadecadienoic acid, methyl ester $C_{19}H_{34}O_2$	15.996	11.8	Linoleic acid, methyl ester; 294.472g/mol  Antibacterial*
9-Octadecenoic acid (Z)-, methyl ester $C_{19}H_{36}O_2$	16.087	7.34	Oleic acid, methyl ester; 296g/mol  Cancer preventive (Simin et al., 2000), flavour (Omolosa and Vagi, 2001), antimicrobial activity (Wagh et al., 2007) and anti-inflammatory (Yunfeng et al., 2007)
Methyl stearate $C_{19}H_{38}O_2$	16.306	4.82	Stearic acid; 298.511g/mol  White crystal semi-solid ester, flavour component in food, lubricant, used in the manufacture of pharmaceuticals, cosmetics, soaps, surfactant and softening agents (Enas and Duha, 2014).
9,12-Octadecadienoic acid (Z, Z); $C_{19}H_{34}O_2$	16.596	4.29	Methyl linoleate; 294g/mol  Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic, anticancer

			and anti-inflammatory properties (Majinda and Abubakar, 2016).
9-Octadecynoic acid, methyl ester $C_{19}H_{34}O_2$	16.38	0.24	294.5g/mol  NF
Cyclopropane octanal, 2-octyl- $C_{19}H_{36}O$	16.86	0.28	280.5g/mol  NF
9-Octadecenoic acid, 12-hydroxy- methyl ester, [R-(Z)]- $C_{19}H_{36}O_3$	17.47	28.0	Methyl ricinoleate; 312.5g/mol  NF
2-Heptadecenal $C_{17}H_{32}O$	17.87	0.20	252.4g/mol  NF
Hexadecanoic acid, 14-methyl-, methyl ester $C_{18}H_{36}O_2$	17.93	0.22	Methyl 14-methylhexadecanoate; 284.5g/mol  NF
2-Octen-1-ol, 3,7-dimethyl- $C_{10}H_{20}O$	18.21	0.46	156.26g/mol  NF
Cyclopropane nonanoic acid, methyl ester $C_{13}H_{24}O_2$	18.79	4.04	Methyl 9-cycopropylnonanoate; 212.33g/mol  NF

12-Hydroxydodecanoic acid $C_{12}H_{24}O_3$	18.83	0.27	12-hydroxylauric acid; 216.32g/mol  NF
9,17-Octadecadienal, (Z)- $C_{18}H_{32}O$	19.70	1.34	264.4g/mol  NF
Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl- $C_{20}H_{34}O$	20.35	0.27	290.5g/mol  NF
cis-Vaccenic acid $C_{18}H_{34}O_2$	20.511	4.48	Asclepic acid; 282.5g/mol  NF

*Source: Duke (2016)

Legend: NF: Biological activity not found

Discussion

The result of the GC-MS analysis of the methanol extract of *Azanza garckeana* seeds revealed several compounds with varying biological activities. The highest occurring (24.32%) documented functional, compound: 9,12-Octadecadienoic acid (Z, Z) is known to have hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic, anticancer and anti-inflammatory properties (Majinda and Abubakar, 2016; Sunita et al., 2017). Compounds with anticancer activity are known to help prevent cancer, as considerable laboratory evidence from chemical, cell culture and animal studies have shown that anticancer may slow or possibly prevent the development of cancer. Other compounds in the methanol extract that were found to have effects on cancer and have antioxidant properties include: 9-Octadecenoic acid methyl ester, Tetradecanoic acid, 11-Octadecenoic acid methyl ester, Hexadecanoic acid, 2 hydroxyl-1-(hydroxymethyl) ethylester.

Aside from anticancer and antioxidant properties, hexadecanoic acid has also been found to have anti-inflammatory properties, anti-androgenic and 5 alpha reductase (5AR) inhibitory activity. Hexadecanoic acid, 2-hydroxy 1- (hydroxymethyl) ethyl ester found in the methanol extract of the seeds of *Azanza garckeana* has anti-androgenic activity. The compounds: Hexadecanoic acid and Hexadecanoic acid 2-hydroxy 1-(hydroxymethyl) ethyl ester, which are free and esterified fatty acids respectively, may elicit this anti-androgenic effect by acting as inhibitors of the enzyme 5 alpha reductase (5AR) (Liang and liao, 1992; Jean-pierre et al., 2002; Tulika and Mala, 2017). The enzyme 5AR catalyzes the conversion of Testosterone into the much more potent 5 alpha

Dihydrotestosterone (5a-DHT) linked to enlarged prostate and prostate cancer, Androstenedione to androsterone, Cortisol to allo-tetrahydrocortisol among others. Elevated 5AR activity is associated with obesity and insulin resistance in both men and women (Tomlinson and Finney, 2008). In women, increased 5AR activity is also associated with polycystic ovary syndrome (PCOS); a hormonal disorder associated with elevated androgens and hirsutism, a condition in women that results in excessive growth of dark or coarse hair in a male-like pattern in the face, chest and back, which often arise also from excess androgens (Vassiliadi and Barber, 2009). In men, elevated 5AR activity is associated with benign prostatic hypertrophy (BPH); a common non-cancerous enlargement of the prostate gland that can cause difficulty in urination and premature baldness (Vassiliadi and Barber, 2009; Selma et al., 2019; Salisbury and Tadi, 2021), therefore inhibition of 5AR may be desirable in conditions like those listed above where 5AR activity is seen to be elevated. Natural 5AR inhibitors like Hexadecanoic acid can provide effective and moderate inhibition without shutting down 5AR altogether, as seen with 5AR inhibiting drugs.

The compounds Oleic acid, Undecylenic acid, Stearin 2-mono, 9, 17-Octadecadienal Pentadecanoic acid 14-methyl-methyl ester identified in the methanolic extract were reported to have antimicrobial activities. The antimicrobial effects of these bioactive compounds may be due to the fact that they are fatty acids, fatty acid ester and aliphatic chains (long chain alkanes and alkenes) and they can accumulate in the lipid bilayer of the cell membrane and mitochondria. Consequently, they disturb the integrity of the cell structure of these microbes and cause it to become more fluid thus increasing its permeability (Solorzano-Santos and Miranda-Novales, 2012). The increased permeability of the membrane by the insertion of unsaturated medium- and long-chain FFAs can allow internal contents to leak from the cells of these microbes, which can cause growth inhibition or even death. Also, increasing membrane permeability can inhibit enzyme activity in the membrane or cytosol that is crucial for survival and growth of these microbes. This could also account for the antimicrobial activity (Zheng et al., 2005; Guan and Liu, 2019). Stearin 2 mono, in addition to its antimicrobial activity, it has hepatoprotective and hypocholesterolemic properties. Hexadecanoic acid methyl ester inhibits cyclooxygenase II enzymes and thus, produces a selective anti-inflammatory action (Hema et al., 2011; Smith and Malkowski, 2019). Several of these bioactive compounds have more than one biological function.

The compounds namely: 9,12-octadecadienoic acid methyl ester and 9-octadecenoic acid methyl ester found to be major compounds in the aqueous extract (after 9-Octadecenoic acid, 12-hydroxy- methyl ester, [R-(Z)]-) have been reported to have antibacterial, antimicrobial, anti-inflammatory and anti-cancer activities (Simin et al., 2000; Omolosa and Vagi, 2001; Wagh et al., 2007; Yunfeng et al. 2007; Duke, 2016). Other compounds identified in the aqueous extract as minor constituents (n-hexadecanoic acid, hexadecanoic acid methyl ester, methyl stearate, methylinoleate, 12-hydroxydodecanoic acid, 2,6,11-trimethyldodecane) have various biological activities such as antioxidant, hepatoprotective, hypocholesterolemic, antihistaminic, antieczemic, anticancer and anti-inflammatory, antibacterial, antimicrobial, haemolytic-5-α reductase inhibitor, antiandrogenic activities (Enas and Duha, 2014; Duke, 2016; Majinda and Abubakar, 2016; Ojekale et al., 2016).

Most of the identified compounds in the aqueous extract have been reported to possess interesting biological activities, some of which are similar to the compounds found in the methanol extract. The compounds Dodecane 2,6,11-trimethyl, heneicosane, hexadecanoic acid and hexadecanoic acid methylester have antimicrobial activity and may carry out this function as described previously for compounds that were found in the methanol extract with similar biological activity. Hexadecanoic acid and hexadecanoic acid methylester found in the aqueous extract also have antioxidant and anti-inflammatory properties.

Conclusion

The GC-MS analysis of the methanol and aqueous extracts of the seeds of *Azanza garckeana* identified several useful bioactive compounds, which have been reported to have health-promoting benefits and could be exploited for their benefits through the use of

biotechnology.

Acknowledgement
The authors gratefully acknowledge the University of Benin, Nigeria and Prof. B. O. Agoreyo's Lab in the University for providing some of the facilities needed.

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