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GAS CHROMATOGRAPHY MASS SPECTROMETRY ANALYSIS OF BIOACTIVE COMPOUNDS ON AQUEOUS EXTRACT OF AZANZA GARCKEANA FRUIT PULP

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Abstract

The research was conducted to determine the secondary metabolites presence in Aqueous extract of *Azanza garckeana* fruit pulp using Gas Chromatography Mass Spectrometry (GC-MS) analysis. The fruit pulp was purchased from Tula Village of Kaltungo Local Government Area of Gombe State Nigeria. The pulp was shade dried at room temperature and ground into fine powder using pistle and mortar. The pulp was subjected extraction using rotary evaporator to obtained the aqueous form. The results revealed the presence of thirty-two bioactive compounds with retention time ranges from 7.30 to 3to 31.002 minutes with area and abundance values ranges from 0.13 in 2-ethylacridine number thirty-two to 16.82 Di-sec-butyl phthalate in compound number eighteen with megawatts (MW) ranges from 101.17 to 474.50, displaying classes of fatty acids and their esters (e.g., Palmitic acid, Methyl Oleate, Linoleic acid ethyl ester), which is a common profile for natural oils or biological extracts. A significant peak at 12.47 min is identified as Di-sec-butyl phthalate, a common plasticizer. The presence of Lactose (a sugar) is indicated at 11 min, though its volatility under these conditions is unusual and may warrant verification. A notable finding is a hit for "MDMA methylene homolog" at ~30.92 min. In conclusion, the GC-MS phytochemical profile of *Azanza garckeana* fruit pulp extract provides a strong scientific rationale for its traditional use. The high concentration of membrane-forming fatty acids, potential energy substrates, and antioxidant compounds aligns mechanistically with the biochemical requirements for enhancing semen quality. The proposed benefits are multifaceted, targeting improved sperm membrane architecture, hormonal support for spermatogenesis, enhanced energy availability for motility, and protection from oxidative insult.

Key words: Azanza garckeana, Aqueous extract, fruit pulp, Bioactive compounds, Gas chromatography mass spectrometry GC-MS

INTRODUCTION

Azanza garckeana it is the multipurpose edible fruit of tropical Africa. The plants have been used as source of medicine globally to preserve human health and are rich source of components with a variety of biological activities. *Azanza garckeana* has also been reportedly use in traditional medicine for treatments and management of more than 20 human diseases and ailments. The plant is used as herbal remedy for diseases like cough, chest pains, infertility and menstruation abnormalities. It is a fruit that is high in vitamins, minerals and carbohydrates. (Hassan *et al*., 2026)

Gas Chromatography-Mass Spectrometry (GC-MS) is an analytical technique that combines the separation capabilities of gas chromatography (GC) with identification power of mass spectrometry (MS) to identify and quantify unknown substances within a sample Turner *et al*., 2020; Pasternak *et al*., 2022). It is widely used in various fields including forensic science, pharmaceutical research, toxicology, environmental analysis, food safety and chemical analysis due to its high sensitivity and ability to identify complex mixtures (Hernandez *et al*., 2013; Narciso *et al*., 2019).

Gas chromatography microscopic spectrometry Gas chromatography-mass spectroscopy (GC-MS) is one of the most reliable analytical techniques widely used to identify different bioactive molecules (Nuhu *et al*., 2024) according to research by (Momodu *et al*., 2022) detected a total of 22 peak and 17 bioactive compounds were identified and quantified using their peak and retention time in *Azanza garckeana* seed. n-hexadecanoic acid was found to be the most abundant in the seed with 30.44%- and 15.09 %-minute retention time followed by 9,12, octadecanoic acid Z Z 24.2 % and retention time of 16.496 minutes. The compound 9-octadecnoic acid z methyl ester was the least with a concentration of 0.33% and retention time of 16.09 minutes. Egbejimi *et al*., (2024) reported chemical compounds in the hydro-methanolic seed extract of *Azanza garckeana* with 2-furancarboxaldehyde as the least and 2,4 hhexadyne-1,6-diol as the highest.

MATERIALS AND METHODS

Description of the Study Area

The study was conducted at the Department of Biochemistry, Modibbo Adama University Yola, and Adamawa State. Adamawa State is located between latitude 11^{O}N of the equator and longitude 14^{0}E East at an elevation of 364 above the sea level in the Northeastern part of Nigeria. It has a tropical climate with distinct dry and wet seasons. The rainfall begins in April and ends in October while dry season commences in November and ends in March. It has a minimum average temperature of 20.50C and a maximum temperature of up to 40^{0}C (Adebayo and Zemba, 2020). The State has an annual rainfall range from 95mm and the relative humidity can be as low as 20% between the months of January and March can be as high as 80% between August and September. While the cool dry Harmattan starts in November to February. It shares boundaries with Taraba State on the South-west, Gombe State in the North-west and Borno State to the North. Adamawa State shares international border with the Republic of Cameroun. The State is covering a land Area of about 38.741 km (Adebayo and Zemba, 2000). According to (2006) census the State has a population of 3.168, 101 People.

Source and Processing of Fruit Pulp Extract

The fruit of *Azanza garckeana* were purchased from the Tula village market in Kaltungo Local Government Area of Gombe State, Northeast Nigeria. The fruit pulps were washed under tap water and sliced into smaller pieces to obtain the pulp and later dried under shade for 72 hours at 40^{o}C, ground using Mottar and Stick, and stored in an airtight container to initiate extraction process (Quadri *et al*., 2024). The quantities of the powdered material were macerated in an aqueous solvent at 25^{0} C for 48 hours. The mixtures were filtered using available filter paper and then subjected to evaporation using a rotary evaporator and obtained a sticky residue. The sticky residue of the extracts was diluted with distilled water and obtained the required quantity of the fruit pulp extract (Quadri *et al*., 2023).

Determination of Gas Chromatography Mass Spectrometry

Phytochemical analysis was conducted by Gas Chromatography Mass Spectrometry (GC-MS) for the three different extracts. A machine model: GC 7890B, MSD 5977A, Agilent Tech. The GC-MS conditions were as follows: column DB-5MS (30m ×0.25mm × 0.25µm film thickness); carrier gas helium (flow rate 1mL/min); injection volume 1µL; injection temperature 250°C; oven temperature program 60°C (2 min), then increased to 280°C at a rate of 10°C/min with a hold time of 5 min. The MS was operated in electron impact mode at 70eV with an iron source temperature of 230°C.The quantification of all the identified components was investigated using percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time in minutes and mass spectra with those of the National Institute of Standards and Technology (NIST) and Wiley library data of the GC-MS system as described by Hebber and Nalini (2020); Thamer and Thamer (2024).

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Result

The results in Table 4 shows that the *Azanza garckeana* fruit pulp extract reveal the presence of thirty-two bioactive compounds with retention time ranges from 7.30 to 3to 31.002 minutes with area and abundance values ranges from 0.13 in 2-ethylacridine number thirty-two to 16.82 Di-sec-butyl phthalate in compound number eighteen with megawatts (MW) ranges from 101.17 to 474.50, displaying classes of fatty acids and their esters (e.g., Palmitic acid, Methyl Oleate, Linoleic acid ethyl ester), which is a common profile for natural oils or biological extracts. A significant peak at 12.47 min is identified as Di-sec-butyl phthalate, a common plasticizer. The presence of Lactose (a sugar) is indicated at 11 min, though its volatility under these conditions is unusual and may warrant verification. A notable finding is a hit for "MDMA methylene homolog" at ~30.92 min. This is a synthetic compound of potential forensic or pharmacological interest. The low purity score (38%) means this identification is tentative and must be confirmed with a standard. The overall quality of the matches (Purity/Qual) is moderate too low for many peaks, which is common in complex mixtures. The top hits for the major components (fatty acids, phthalate) are the most reliable.

Table 1: Gas Chromatography Mass Spectrometry GC-MS Analysis

S/N	Retention time (Min)	Peak Area (%)	Name of Compound	Molecular Formula	Molecular Weight	Class
1	7.301	0.32	2-Cyclopenten-1-one, 5-hydroxy-2,3-dimethyl-	$C_7H_{10}O$	110.15	Cyclic Ketone
2	8.532	1.26	n-Pentadecanol	$C_{15}H_{32}O_3$	228.4140	Fatty Alcohol
3	8.646	5.17	2-Pentanone, 5-(acetyloxy)-	$C_7H_{12}O$	144.17	Ketone Ester
4	8.711	4.36	Thiazolidine, 3-acetyl-2-methylene-	$C_8H_{11}NO_5S_4$	233.24	Heterocyclic Compound
5	8.736	2.38	Butyric acid, 4-butoxy-, methyl ester	$C_6H_{12}O$	132.1577	Ester
6	8.839	6.38	Pentanoic acid, 4-methyl-, ethyl ester	$C_3H_{16}O_2$	144.215	Ester (Branched Fatty Acid)
7	8.868	1.64	Thiazolidine, 3-acetyl-2-methylene-	$C_8H_{11}NO_5S_4$	233.24	Heterocyclic Compound
8	8.906	3.00	Pentanoic acid, 4-methyl-, ethyl ester	$C_8H_{16}O_2$	144.215	Ester (Branched Fatty Acid)
9	8.954	3.34	9-Decenoic acid	$C_{10}H_{120}O_2$	170.25	Unsaturated Fatty Acid
10	8.985	3.63	Pentanoic acid, 4-methyl-, ethyl ester	$C_8H_{16}O_2$	144.215	Ester (Branched Fatty Acid)
11	9.042	6.55	Undecylenic acid	$C_{11}H_{20}O$	184.2753	Unsaturated Fatty Acid
12	9.232	2.76	Butanoic acid, 3-oxo-, propyl ester	$C_7H_{12}O$	144.1684	Keto Ester

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13	10.269	6.61	Oxalic acid, cyclobutyl heptadecyl ester	$C_{23}H_{42}O_4$	382.6	Ester
14	10.970	5.38	Lactose	$C_{12}H_{22}O_{21}$	342.3	Disaccharide
15	11.015	1.79	Lactose	$C_{12}H_{22}O_{21}$	342.3	Disaccharide
16	11.722	6.70	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_3O$	270.4507	Methyl Branched Fatty Acid Ester
17	11.838	0.80	9-Octadecenoic acid (Z)-, methyl ester (Methyl Oleate)	$C_{19}H_{36}O$	296.4879	Methyl Unsaturated Fatty Acid Ester
18	12.468	16.82	Di-sec-butyl phthalate	$C_{24}H_{38}O_4$	390.56	Phthlate Ester(Plasticizer)
19	12.612	3.45	Methyl 2,6-anhydro-α-d-altroside	$C_7H_{12}O_5$	176.17	Anhydrosugar
20	12.692	1.88	Undecanoic acid	$C_{11}H_{22}O_2$	186.29	Fatty Acid
21	12.715	2.48	n-Hexadecanoic acid (Palmitic Acid)	$C_{16}H_{32}O_2$	256.42	Fatty Acid
22	12.776	2.13	n-Hexadecanoic acid (Palmitic Acid)	$C_{16}H_{36}O_2$	256.42	Fatty Acid
23	13.742	1.10	Oleic Acid	$C_{18}H_{34}O_2$	282.46	Unsaturated Fatty Acid
24	14.043	1.00	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	298.5	Cyclic Ester
25	14.578	4.14	Linoleic acid ethyl ester	$C_{18}H_{32}O_2$	308.50	Polyunsaturated Fatty Acid
26	14.681	0.51	Z,Z-4,15-Octadecadien-1-ol acetate	$C_{20}H_{36}O$	308.4986	Acetylated Unsaturated Alcohol
27	20.037	0.58	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390.56	Phthlate Ester(Plasticizer)
28	20.094	0.27	Oleic Acid	$C_{18}H_{34}O_2$	282.46	Unsaturated Fatty Acid
29	24.622	0.86	Oleic Acid	$C_{18}H_{34}O_2$	282.46	Unsaturated Fatty Acid
30	24.645	0.86	2-Vinyl-9-[β-d-ribofuranosyl]hypoxanthine	$C_{12}H_{14}N_4O_4$	278.26	Necleoside Analog
31	30.917	1.72	MDMA methylene homolog	$C_{12}H_{17}NO_2$	243.7	Amphetamine-type Substance
32	31.002	0.13	2-Ethylacridine	$C_{15}H_{13}N$	207.27	Heterocyclic Aromatic

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Discussion

The result of gas chromatography-mass spectrometry (GC-MS) analysis of the *Azanza garckeana* fruit pulp extract reveals a complex phytochemical profile predominantly composed of fatty acids, their ester derivatives, and several other bioactive compounds. This compositional analysis provides a compelling biochemical basis for hypothesizing that the extract may exert beneficial effects on male reproductive function, particularly on semen production, quality, and motility. The mechanisms of action appear to be multifactorial, impacting the hormonal, hematological, and biochemical pathways that underpin spermatogenesis and sperm function Ahmad *et al*., (2020). Primarily, the extract is characterized by a high abundance of various fatty acids and their esters, including n-Hexadecanoic acid (Palmitic acid), 9-Octadecenoic acid (Oleic acid), Linoleic acid ethyl ester, and Undecylenic acid. These lipids are fundamental to male fertility, as sperm cell membranes possess a unique lipid composition rich in polyunsaturated fatty acids (PUFAs) Lenzi *et al*., (2000). These PUFAs confer essential membrane fluidity, which is a critical determinant for successful events like the acrosome reaction and sperm-oocyte fusion Ollero *et al*., (2001). The presence of these specific compounds suggests that the *Azanza garckeana* extract could directly supply the necessary substrates for the *de novo* synthesis and maintenance of healthy spermatozoa membranes. Consequently, dietary or supplemental intake of these compounds through the extract could potentially lead to improved sperm structural integrity, viability, and ultimately, motility Safarinejad, (2011). Beyond their structural role, these fatty acids play a pivotal part in the endocrine system that regulates reproduction. Compounds such as Palmitic acid and other saturated fats are known precursors for the biosynthesis of steroid hormones, including testosterone Payne & Hales, (2004). The presence of these molecules indicates a potential mechanism by which the extract could support the endogenous production of testosterone. Adequate testosterone levels are imperative for the initiation and maintenance of spermatogenesis within the seminiferous tubules of the testes Walker, (2011). Therefore, by potentially supporting the hypothalamic-pituitary-gonadal (HPG) axis, the extract may enhance the very hormonal drive responsible for sperm production (spermatogenesis). Furthermore, the identification of a sugar-like compound, while requiring confirmatory analysis, presents an intriguing possibility. Seminal plasma contains fructose, produced by the seminal vesicles, which serves as the primary glycolytic energy substrate for spermatozoa Mann & Lutwak-Mann, (2012). The presence of an energy substrate could imply an analogous role, where the extract provides a supplementary metabolic fuel source for sperm cells. This would directly support sperm motility by mitigating energy depletion, thereby prolonging the functional lifespan and kinetic activity of sperm Williams & Ford, (2001).

The overall physiological state, particularly the balance between oxidative stress and antioxidant defense, is also crucial for optimal reproductive function. The GC-MS chromatogram indicates the presence of several compounds, such as derivatives of 2-Cyclopenten-1-one, which are often associated with antioxidant activity. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and antioxidants, is a well-established cause of male infertility, leading to sperm membrane lipid peroxidation, DNA fragmentation, and loss of motility Agarwal *et al*., (2014). The antioxidant potential of these phytoconstituents could protect developing and mature sperm cells from oxidative damage, thereby preserving sperm quality and function Bisht *et al*., (2017). Additionally, improved antioxidant status positively influences general health and microcirculation, which supports the nutrient and oxygen supply to the testes a key factor in sustaining active spermatogenesis. It is also critical to address the presence of Di-sec-butyl phthalate, identified as a prominent peak. This compound is a known plasticizer and a common environmental contaminant Heudorf *et al*., (2007). Its presence in the extract is highly likely an artifact of contamination during sample preparation, processing, or storage (e.g., from plastic containers or tubing). While some phthalates are known endocrine disruptors with negative effects on reproductive health Swan, (2008), its occurrence here is almost certainly not a phytochemical constituent of the fruit itself but a procedural contaminant that must be eliminated in future extractions to ensure the validity and safety of the study.

Conclusion

In conclusion, the GC-MS phytochemical profile of Azanza garckeana fruit pulp extract provides a strong scientific rationale for its traditional use. The high concentration of membrane forming fatty acids, potential energy substrates, and antioxidant compounds aligns mechanistically with the biochemical requirement for enhancing semen quality. The proposed benefits are manifested, targeting improved sperm architecture, hormonal support for spermatogenesis, enhanced energy availability for motility and protection from oxidative insult

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