GAS CHROMATOGRAPHY – MASS SPECTROMETRIC ANALYSIS OF THE DICHLOROMETHANE EXTRACT OF THE SEEDS OF *AZANZA GARCKEANA*

BY

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(B.Sc)

DEPARTMENT OF BIOCHEMISTRY

UNIVERSITY OF BENIN

BENIN CITY

SEPTEMBER, 2023.

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A PROJECT REPORT SUBMITTED TO THE DEPARTMNT OF BIOCHEMISTRY, UNIVERISTY OF BENIN IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR IN SCIENCE (B.Sc.) DEGREE IN BIOCHEMISTRY

SEPTEMBER, 2023.

CERTIFICATION OF THESIS

This is to certify that this work was carried out and written by EBONKA SAMUEL with the matriculation number LSC1806274 of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State in partial fulfillment for the award of Bachelor of Sciences, Biochemistry.

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EXTERNAL SUPERVISOR **DATE**

DEDICATION

This report is dedicated to Almighty God who in his infinite mercy has given me the Grace to pass through this stage in my academic career. I also dedicate this report to my amazing family for their encouragement and endless support.

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Foremost, my profound appreciation goes to my project supervisor, Prof. (Mrs.) B.O Agoreyo for her earnest guidance, patience, diligence, kind words and encouragement and also for showing me the right direction throughout the course of my research. I also want to deeply appreciate the project coordinator, Dr. S.I. Ojeaburu for his indispensable guidance and support during my research.

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ABSTRACT

Azanza garckeana also known as snot apple is an important food and medicinal plant, which serves as a good source of herbal medicine in tropical Africa. *Azanza garckeana* fruits were obtained from Tula, Kaltungo Local Government, Gombe State, in the North-Eastern region of Nigeria. This study was carried out in order to identify the bioactive compounds with health-promoting benefits present in the dichloromethane extract of the seeds of *Azanza garckeana*. To characterize diverse bioactive compounds present in the plant, the dichloromethane extract of the seeds of this fruit were subjected to GC-MS analysis. Eighteen (18) bioactive compounds were found in the seeds. According to the result, (Z)-3-(pentadec-8-en-1-yl) phenol had the highest concentration of 69.79% and the longest retention period at 17.987 minutes. This compound was found to have Anti-hyperglycemic and Antioxidant properties. The least component in the seeds extract, pentasiloxane, dodecamethyl, had a concentration of 0.35% and a retention period of 3.430 minutes. Other bioactive compounds present in the dichloromethane extract and their biological activities include; Cyclohexasiloxane, dodecamethyl- (Antibacterial and Antioxidant activities), Cyclododecane (Antimicrobial activity), Cycloheptasiloxane, tetradecamethyl- (Antioxidant and Anti-inflammatory activities), Methyl stearate (Antioxidant activity). According to the findings, *Azanza garckeana* (snot apple) has a lot of potential as a source of naturally occurring bioactive substances utilized as therapeutic agents in the treatment and prevention of different ailments.

CHAPTER ONE

1.0. INTRODUCTION

1.1. BACKGROUND OF STUDY

A balanced and nutritious diet must include fruits and vegetables. Unfortunately, the world as a whole consumes very little fruit, with sub-Saharan Africa having the lowest rate. Insufficient consumption of fruits and vegetables suggests unbalanced and unhealthy diets, which can result in numerous diseases and a higher mortality rate. In most situations, poverty is the main cause of the inadequate intake of fruits and vegetables (Abiola *et al.*, 2022). However, if the abounding wild fruits and vegetables found in several countries across the world are completely utilized and incorporated into the diets of locals, the crisis can be reduced. The use of wild edible plants by early humans provided a variety of advantages, including helping the community members survive times of food scarcity (a practice known as ecosystem-based adaptation) (Jacob *et al.,* 2016). Another crucial component of folk culture that greatly contributes to the resuscitation of traditions is traditional knowledge of wild plant species with culinary and homoeopathic value. In addition to being consumed as food, wild fruits, vegetables, mushrooms, nuts, and grains, several plant species also have highly valuable medicinal properties that are essential for the treatment and management of a variety of health issues. It's interesting to note that majority of these wild foods, both from the farm and the forest, are high in fiber and micronutrients and low in salt and fat. As a result, they might be crucial in reducing the nutritional transition, which raises the risk of cardiovascular illnesses, type 2 diabetes, and obesity (Yuanyuan *et al.*, 2022). According to research on the nutritional value of native fruit-bearing tree species, many are high in sugars, important vitamins, and minerals, while others are high in protein and vegetable oil (Nkafamiya *et al.,* 2016). The long list of advantages, in addition to fruit production and money, also includes firewood, fodder, building material, shade, and medicine, particularly for rural areas. In Northern Nigeria, especially in rural areas where a variety of edible leaves and fruits are abundant, edible wild leaves and fruits are frequently consumed. While some of these fruits are grown on farms, others do so in the wild. *Azanza garckeana* is a suitable illustration of a wild edible fruit with significant nutritional, therapeutic, and economic potential (Abiola *et al.,* 2022).

Figure 1.1. Different parts of *Azanza garckeana.*
Source: Yilni *et al.*, (2020).

Azanza garckeana, also known as *Thespesia garckeana*, is a multifunctional wild fruit tree that is a member of the Malvaceae family. The generic name is thought to be derived from "Azania," which in Zanzibar means "black and surviving." According to Abiola *et al*. (2002), it is also known as Jakjak, Morojwa, Snot Apple, African chewing gum, tree-hibiscus, and slime-apple. It is known as "Goron Tula" in Nigeria, which is a combination of the words "Goron" (kolanut) and "Tula" (the name of the village in Northern Nigeria where the wild fruit trees grow in profusion). *Azanza garckeana* is a semi-deciduous shrub that can reach heights of 3 to 15 meters, depending on the climate, and has a stem diameter of up to 25 centimeters at breast height. The tree has several stems that can be straight, crooked, or occasionally fork from the base. The bark is fibrous with longitudinal fissures and is tough, greyish-black or brown. The branches have woody hairs, and the twigs are initially hairy before becoming smooth with age (Alfred, 2017). The leaves are 8 by 12 cm and have distinctly rounded edges. They are always roundish, basic, and alternating. The leaves contain longitudinal fissures in the midrib and 3 to 5 lobes, each of which is covered with brown star-shaped hairs. The tip of the leaf is typically rounded or bluntly pointed. The heart-shaped base of the leaf has 5–7 nerves. The young, silky leaves are dark in color.

1.2. JUSTIFICATION OF THE STUDY

In order to substantiate the claims of the use of *Azanza garckeana* plant for therapeutic purposes throughout the world, most especially in Africa, this study intends to assess the bioactive compounds by GC-MS analysis.

1.3. AIMS AND OBJECTIVES

Through gas chromatography-mass spectrometric analysis, the bioactive components in the dichloromethane extracts of *Azanza garckeana* (Snot Apple) seeds are the focus of this study.

The objective of the study is to identify:

i. The different bioactive compounds that are present in the seeds using Gas Chromatography-Mass Spectrometric Analysis.

1.4. LITERATURE REVIEW

In underdeveloped nations, due to their accessibility, affordability, and acceptability, plant foods are the most crucial dietary source to satisfy the nutritional needs of the majority of people

(Michael *et al.,* 2015). However, some plant meals continue to have immense potential that is not being utilized. In Nigeria, some commercial goods are rising in price and becoming unaffordable for many households, especially poor ones. It is necessary to investigate certain readily available, inexpensive, and nutrient-rich food crops for the manufacture of commercially viable derivatives. This would diversify the diet and aid in lowering the issues with malnutrition that many individuals in various regions of the nation face (Jacob *et al.,* 2016).

Figure 1.2. *Azanza garckeana* (goron tula)

Source: Joy *et al.* (2022).

1.5. CLASSIFICATION

Botanical Classification of *Azanza garckeana* (Yilni *et al.*, 2020)

Kingdom: Plantae – Plants

Subkingdom: Tracheobionta – Vascular plants

Superdivision: Spermatophyta – Seed plants

Division: Magnoliophyta – Flowering plants

Class: Magnoliopsida – Dicotyledons

Subclass: Dilleniidae

Order: Malvales

Family: Malvaceae – Mallow family

Genus: Azanza

Specie: *Azanza garckeana*

1.6. ECOLOGY

Azanza garckeana naturally grows in thickets, open forests, and miombo grasslands. According to Omosa *et al.* (2016), it is common throughout tropical eastern and southern Africa. *Berchemia discolor, Cassia abbreviata, Cassia singueana, Combretum molle, Dalbergia melanoxylon, Ehretia spp., Grewia mollis,* and *Tamarindus indica* are among the tree species that are frequently connected. The tree is semi-deciduous in freezing climates but evergreen in warmer climates. Although *Azanza garckeana* is drought-resistant, it benefits greatly from an abundance of water during the wet season. It is resilient to light frost (Yuanyuan *et al.,* 2022).

1.7. DISTRIBUTION AND HABITAT

Azanza garckeana is widely distributed in tropical Africa: Malawi, Zambia, Zimbabwe, Nigeria, Sudan and Kenya (Jacob *et al.,* 2016). It is a pioneer tree that grows on termite mounts and in barren areas. In Nigeria, it may have been brought in and naturalized. It has a high light requirement as befits a typical pioneer species. It can withstand light frost and is resistant to drought (Abiola *et al.,* 2022).

1.8. USES OF *AZANZA GARCKEANA PLANT*

About 8.1 kJ/g of energy can be obtained from the fruits of *Azanza garckeana*. The fruits are eaten either fresh or dried, preserved, and used as a taste to porridge. Local markets frequently sell the fruits. Although the wood is easily utilized, it is only appropriate for small building requirements like house frames, poles, and oxen yokes. For smaller things like spoons, carvings, combs, and tool handles, it is highly prized (Lilian *et al.,* 2021). The wood is also used to make charcoal and fuel. The inner bark is utilized to make fiber for rope of the highest caliber. The tree is browsed by cattle that are allowed to roam free and could be used as fodder (Jacob *et al.,* 2016). Decreased digestibility brought on by secondary compounds may make usage as fodder more difficult. The leaves are often steamed to produce salts or made into a relish.

1.9. DIETARY AND MEDICINAL PROPERTIES OF *AZANZA GARCKEANA*

Azanza garckeana's ripe fruit carpels can be eaten and are frequently consumed across the plant range. The jelly or syrup usually obtained from the plant is used as a food ingredient, and it is occasionally dried and then reconstituted (Alfred, 2017). In order to enhance the nutritional, medicinal, and economic security of local communities, *Azanza garckeana* has been selected as one of the few plants that should be included in the domestication process in sub-Saharan African farming systems (Alfred, 2017). *Azanza garckeana's* bark, fruits, leaves, roots, and stems are said to have a variety of medicinal characteristics and are used to treat or manage a variety of illnesses throughout its geographic range. In Malawi, Nigeria, and Zambia, a decoction of *Azanza garckeana's* root and stem bark is taken orally as a treatment for gonorrhea, syphilis

STDs, and several other STDs. In Botswana, Kenya, Malawi, and Nigeria, *Azanza garckeana* leaves, stems, roots, or ripe fruits are consumed orally as a treatment for infertility and liver issues (Yuanyuan *et al.,* 2022).

1.1.0. FLOWERING AND FRUITING HABIT

When growing in warmer climates, trees are evergreen; but, in colder climates, they are semi-deciduous. The tree starts bearing fruit two years after it is planted. Fruits typically ripen in the dry season while flowering typically occurs during the wet season (Armistice, 2021). From the time the flower is fertilized until the fruit is matured, it takes about 6 months. Within the distribution range, flowering times vary by location. In Southern Africa, it blooms from December to May, and to the North, in Sudan, flowering starts in October and finishes in January (Abiola *et al.,* 2022).

1.1.1. PESTS AND DISEASES

In both the nursery and the field, the tree is troubled by leaf insects (Cicadellidae family). Malathion and dichlorophos are used as control measures. The tree should not be planted in areas where cotton is produced because it is a host for the cotton stainer (Omosa *et al.,* 2016).

1.1.2. TRADITIONAL APPLICATIONS OF *AZANZA GARCKEANA*

The most useful portions of the plant are the matured fruits and those which are usually dried for later use. When chewed, the fruit's pleasant mucilage transforms into gelatinous slime. The fruits are either boiled to make porridge or made into a delicious jelly syrup by soaking them in a tiny amount of water (Mahalapbutr *et al.,* 2019). Boils and abscesses can also be treated using a paste made from pounded fruits. The leaves are used to make delicious relish and are cooked and consumed as veggies. Salt can be made from the burnt leaves as well. During the dry season, wild animals and livestock consume them as food, and bees eat them as well (Omosa *et al.,* 2016). The leaves and stem are infused and used as a purgative, as well as a remedy for liver disorders and infertility.

1.1.3. NUTRITIONAL COMPOSITION OF AZANZA GARCKEANA

The nutritional makeup of the various parts of *Azanza garkeana* has been demonstrated by numerous research. The nutritional makeup of *Azanza garckeana* fruits was thoroughly studied by Nkafamiya *et al.* (2015). The research also revealed that the plant's fruit and leaves are rich in critical amino acids, with leucine being the most abundant and glycine being the least. The fruits and other portions under investigation also contain a sizable amount of mineral elements. Mineral elements are present in higher concentrations in the fruits and leaves than in the other sections. In the Kaltungo Local Government Area of Gombe State, the nutritional value of snot apple fruits collected from various Tula locations was examined by Jacob *et al.* (2016). Additionally, the composition in terms of minerals, fats, and carbohydrates was disclosed.

The fruit of the plant is also available in suitable amounts as energy source and important elements, according to numerous sources (Omosa *et al.,* 2016). It is clear from the aforementioned facts that *Azanza garckeana*, which is grown around the world, is a good source of the nutrients needed for a healthy lifestyle. The plant is an excellent source of protein, and eating of the fruits or other plant parts will be beneficial in the treatment of protein shortage, according to the amino acid composition and protein content in the parts analyzed. The iron concentration found in all examined samples is a sign that the plant might be useful in the treatment of aneamia. The plant is a good source of energy due to its starch and carbohydrate content and may be used in the creation of healthy energy beverages (Omosa *et al.,* 2016).

1.1.4. PHYTOCHEMICAL COMPOSITION OF *AZANZA GARCKEANA*

Studies have focused on the non-nutritive compounds found in various sections of *Azanza garckeana*. According to research by Michael *et al.* (2015) on the phytochemical makeup of *Azanza garckeana* seed powder, the seeds contain phenolics, tannins, saponins, flavonoids, carotenoids, alkaloids, and cyanogenic glucosides. In a different study, the non-nutritional components in different plant sections of *Azanza garckeana* were quantitatively estimated using standardized methods. Recent research (Jaime *et al.,* 2015) examined the phytochemical profile of dichloromethane extracts of *Azanza garckeana* fruit from Gombe village in Gombe State, Nigeria. The concentrations of alkaloids, tannins, saponins, phenolics, and flavonoids in the extracts were quantitatively assessed. According to the findings of many research, plant parts are enriched with beneficial natural phytochemicals, which may be the cause of the plant's documented therapeutic applications in the prevention and treatment of a variety of ailments (Pauline and Sagaya, 2016).

1.1.5. PHARMACOLOGICAL POTENTIALS OF *AZANZA GARCKEANA*

The existence of chemical compounds in plants that have physiological effects on the human body is what gives them their medicinal qualities. Secondary metabolites, often known as phytochemicals, are chemical substances with biological activity that are found in various concentrations in higher plants. They have the ability to treat or prevent disease (Nkafamiya *et al.,* 2016). *Azanza garckeana* and its chemical constituents are said to possess a number of pharmacological activities. These potentials include analgesic, antidiabetic, antibacterial, antioxidant, and antimalarial properties.

CHAPTER TWO

2.0. MATERIALS AND METHODS

2.1 EQUIPMENT

Electric oven (Genlabwidness, England)

Soxhlet apparatus (Jinotech, England)

Weighing balance (Mettler analytical balance, USA)

Measuring cylinder

Biuret (Pyrex, England)

Conical Flask, beakers (Pyrex, England)

Glass Jars (Pyrex, England)

Chromatographic Equipment (Agilent Technologies, Santa Clara, CA, USA)

Spectrometer (Agilent Technologies, Santa Clara, CA, USA)

2.2. REAGENTS

Commercially purchased chemicals and reagents were used.

Dichloromethane (DCM)

2.3. FRUIT COLLECTION

The plant fruits of *Azanza garckeana* were purchased from a local market in Gombe State, Nigerian local government areas of Tula Kaltungo on the 26^{th} of June, 2023. Its corporate headquarters are located in Kaltungo, a town in the western part of the region. Local ward councilors and participating families in the neighborhood gave their consent for the collection of fruit samples for the study (Armistice, 2021).

2.4. WEIGHT OF SAMPLE

Before Pulverization

Weight of pulp = 10.5kg

Weight of root = 6.1kg

Weight of seeds = 2.7kg

After Pulverization

Weight of pulp = 9.8kg

Weight of root = 5.7kg

Weight of seeds = 2.1kg

2.5. SAMPLE PREPARATION

Fruits were gathered and sorted by choosing ripe and fresh fruits. The seeds were taken out of the ripe and fresh fruits by cutting them open. According to a recommended method from the Association of Official Analytical Chemistry (AOAC), the seeds were dried for 72 hours at 60 °C in an oven until they reached a constant weight (Armistice, 2021). The dried *Azanza garckeana* seeds were ground into a powder using a miller. 1g of the pulverized seeds and 15ml of solvent (dichloromethane) was used for extraction, filtered and 1µL of the extract was injected into the Gas Chromatograph for analysis.

Plate 1a. Seeds of *Azanza garckeana Azanza*. **Source:** Momodu *et al.,* (2022)

Plate 1b. Pulverized form of the seeds of garckeana. **Source:** Momodu *et al.,* (2022)

2.6. SOLVENT EXTRACTION

The seeds were shade-dried and ground into a fine powder. The solvent dichloromethane was used in the extraction process using a Soxhlet equipment. A 1:15 mixture of pulverized seeds and dichloromethane solvent was utilized and they were periodically agitated. In preparation for use, the extract was vacuum evaporated and kept at 4^{o}C.

2.9. GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS

A technique known as Gas Chromatography-Mass Spectrometry (GC-MS) combines the advantages of mass spectrometry and gas-liquid chromatography to identify various compounds in a test sample. Drug detection, fire investigation, environmental analysis, investigating explosives, and identifying unidentified materials are some applications of GC-MS (Ezekiel *et al.,* 2020). GC-MS can also be used at airport security checkpoints to find drugs on passengers or in their luggage. Additionally, items that were previously believed to have decomposed beyond identification can have trace components found in them. The gas chromatograph and the mass spectrometer are the two main components that make up the GC-MS (Faten and Noah, 2023). The capillary column used by the gas chromatograph depends on the phase properties (such as 5% phenyl polysiloxane) and the column's dimensions (length, diameter, film thickness). As the sample moves down the column, the various molecules in a mixture will get separated due to differences in their chemical characteristics (Pauline and Sagaya, 2016).

Because the molecules exit the gas chromatograph at different rates (this is referred to as the retention period), the molecules can be captured, ionized, accelerated, deflected, and detected individually by the mass spectrometer downstream. This is accomplished by the mass spectrometer by fragmenting each molecule into ionized parts and identifying these parts using their mass to charge ratios. When used combined, these two elements provide a far finer level of substance identification than when employed independently. Gas chromatography or mass spectrometry alone cannot be used to accurately identify a specific chemical (Yuanyuan *et al.,* 2022). While gas chromatography, which uses a conventional detector like a flame ionization detector, detects multiple molecules that happen to have the same retention time (i.e., travel through the column at the same rate), the mass spectrometry process typically requires a very pure sample, which causes two or more molecules to co-elute. In a mass spectrometer (mass spectrum), two distinct molecules may occasionally have a similar pattern of ionized fragments. It is quite improbable that two distinct molecules will react similarly in a gas chromatograph and a mass spectrometer when the two processes are combined. Therefore, it usually contributes to greater confidence that the target analyte is in the sample when an identifying mass spectrum appears at a typical retention time in a GC-MS study (Faten and Noah, 2023).

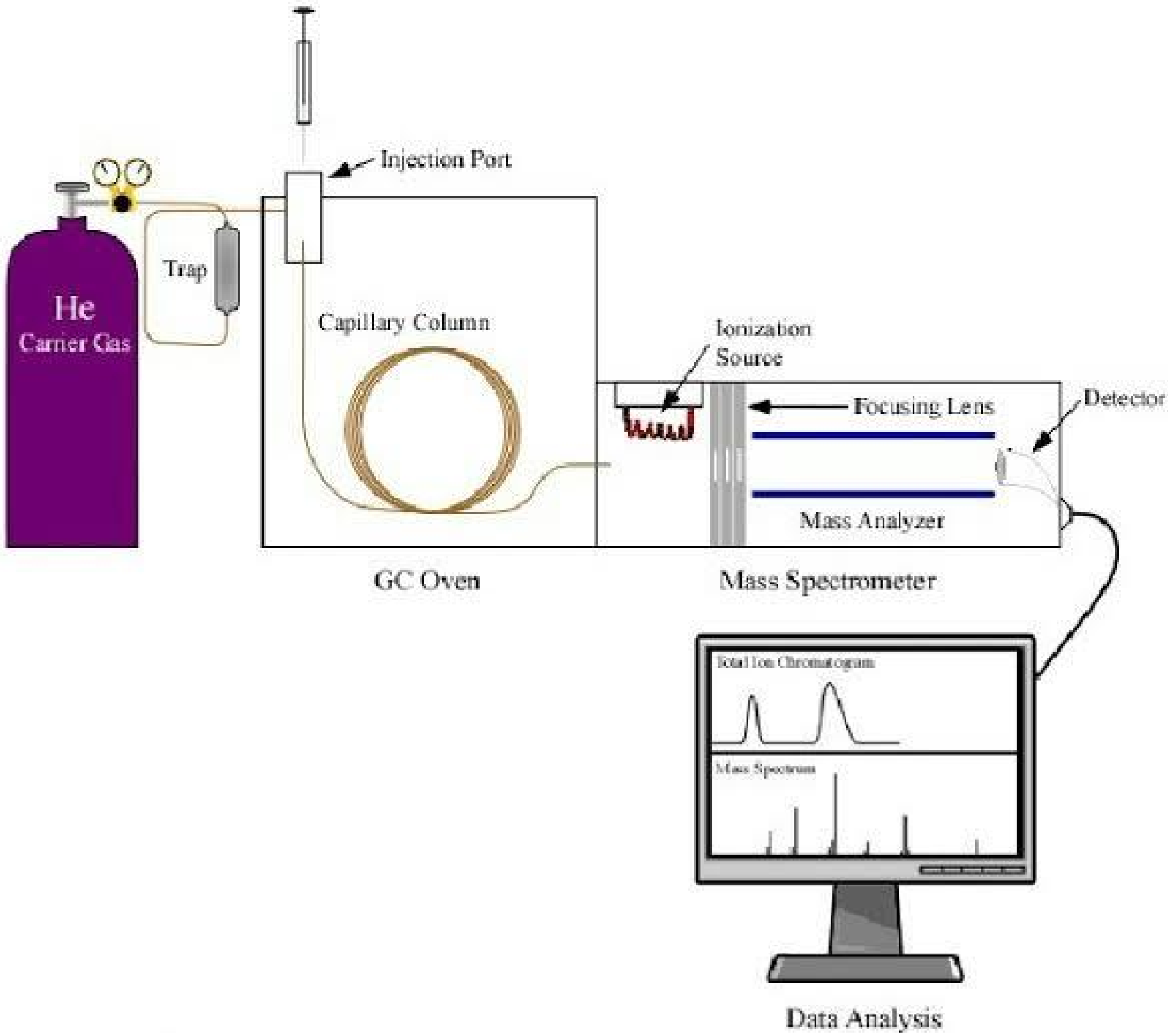


Figure 2.2: Schematic of a GC-MS system.

Source: Ezekiel *et al.*, (2020).

Azanza garckeana pulverized seeds were subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis in order to identify and measure all bioactive compounds present in the seeds (Momodu *et al.,* 2022). The GC-MS analysis was performed utilizing a gas chromatograph (Agilent Technologies' 7890 GC instrument) and an Agilent Technologies' 5975 mass spectrometry detector. Agilent HP5MS column with dimensions of 30 mm in length, 0.320 mm in diameter, and 0.25 m in thickness was employed. 0.5 ml/min of helium gas was employed as the carrier gas. The gas chromatograph was filled with 1µL of the sample. The oven temperature was set to 80 degrees Celsius for 2 minutes, then increased 10 degrees Celsius every minute until it reached 240 degrees Celsius and stayed there for 6 minutes (Momodu *et al.,* 2022). The procedure lasted for about 90 minutes in total. To identify and quantify the compounds, the GC-MS was performed using electron impact ionization at an energy of 70 eV, and the data was analyzed using total ion count (TIC). The data system of the National Institute of Standards and Technology (NIST) library (Gaithersburg, Maryland, United States) data system was used to compare the spectrum of each component with the database of spectra of recognized chemicals (Faten and Noah, 2023).

CHAPTER THREE

3.0. RESULTS

For the GC-MS analysis in this study, *Azanza garckeana* seeds extract in dichloromethane was utilized. About eighteen bioactive compounds were found in *Azanza garckeana* seed according to the GC-MS results. These substances were provided with their molecular weight, composition (%), molecular formulas, and retention times calculated from their peak regions (Figure 3.1). The existence of the main eighteen distinct bioactive compounds in the seeds extract has been demonstrated. The National Institute of Standards and Technology (NIST) library's data system and the spectra of these bioactive compounds were matched. (Z)-3-(pentadec-8-en-1-yl)phenol compound was found to have the highest concentration (69.79%) followed by Phenol, 3-methyl- (16.39%), Phenol, 3-pentadecyl- (3.80%), 9-Octadecenoic acid (Z)-, methyl ester (1.45%), Adamantane, 1-isothiocyanato-3-methyl- (1.32%), p-Cresol (0.86%), 1,4-benzenedicarboxylic acid, mono (1-methylethyl) ester (0.75%), Cycloheptasiloxane, tetradecamethyl- (0.70%), Methyl stearate (0.64%), Pentadecanoic acid, 14-methyl-, methyl ester (0.61%), Cyclohexasiloxane, dodecamethyl- (0.59%), Cyclododecane (0.50%), 9-Octadecene, (E)- (0.50%), Epinephrine, (.beta.)-, 3TMS derivative (0.50%), and several other additional compounds which were discovered in minute quantities (Table 3.1).

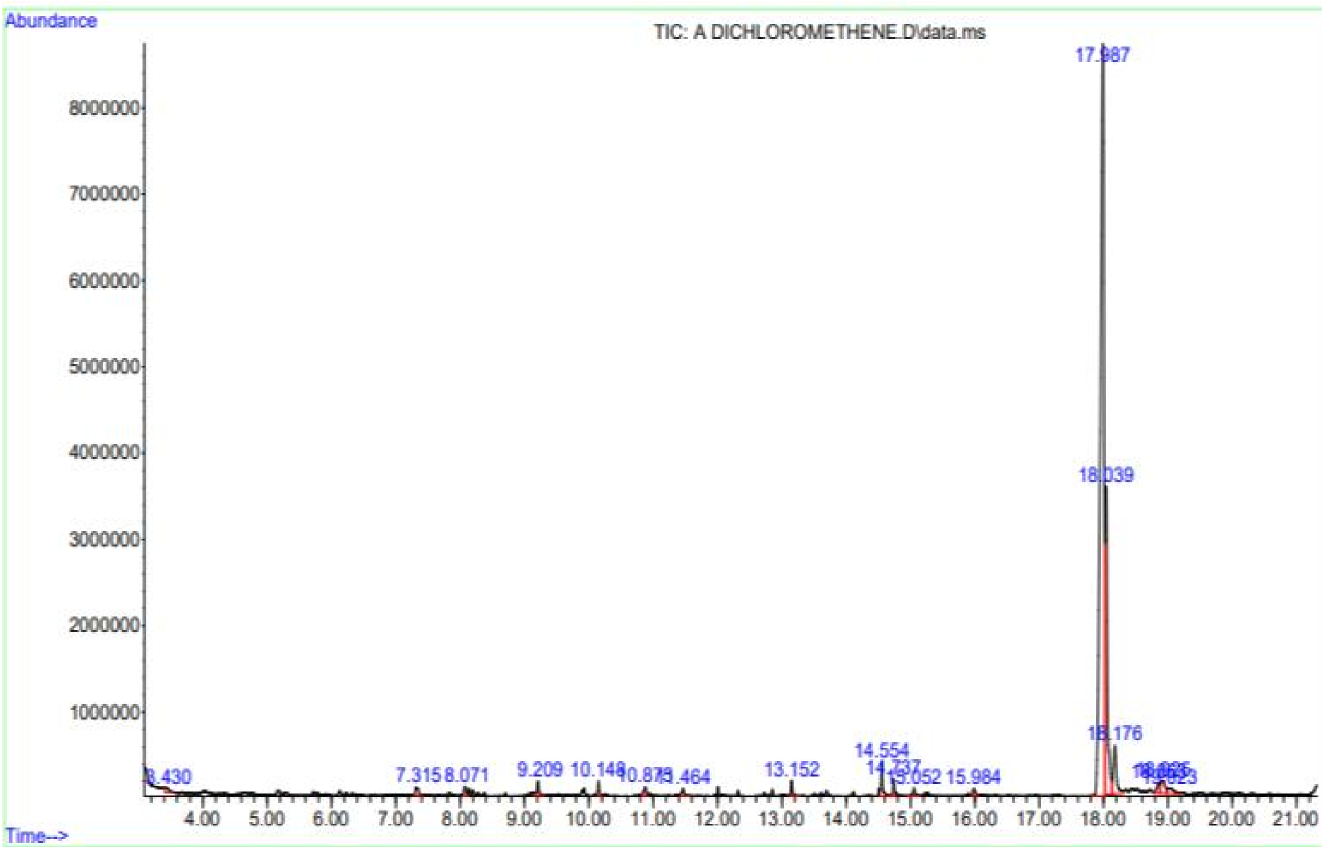
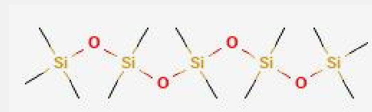
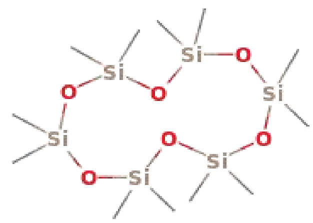
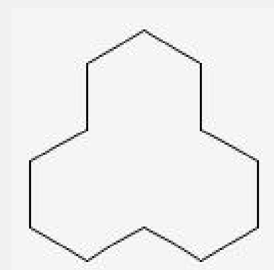
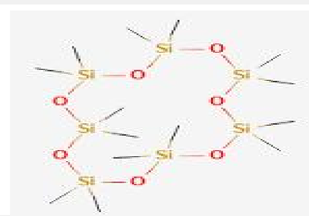
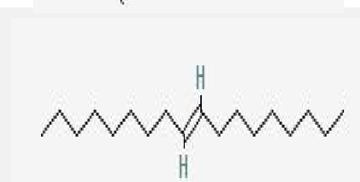
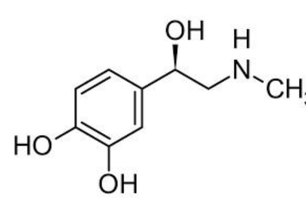
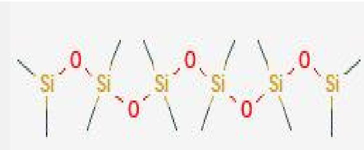
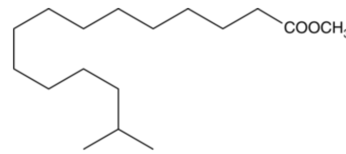
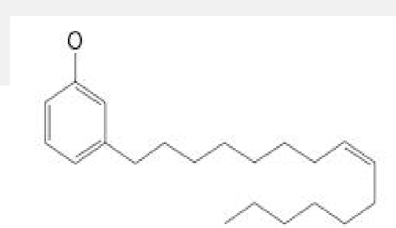


Figure 3.1: GC-MS chromatogram of the dichloromethane extract of the seeds of *Azanza garckeana*

Table 3.1. Compounds identified in the dichloromethane extract of the seeds of *Azanza garckeana*

Peak #	**Retention Time**	**Peak Area %**	**Name**	**Molecular Formula**	**Molecular Weight (g/mol)**	**Structures**
1	3.430	0.35	Pentasiloxane, dodecamethyl-	$C_{12}H_{36}0_{4}Si_{5}$	384.8393	
2	7.315	0.59	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_{6}Si_{6}$	444.9236	
3	8.071	0.50	Cyclododecane	$C_{12}H_{24}$	168.32	
4	9.209	0.70	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{44}0_{7}Si_{7}$	519.0776	
5	10.148	0.50	9-Octadecene, (E)-	$C_{18}H_{36}$	252.4784	
6	10.875	0.50	Epinephrine, (.beta.)-, 3TMS derivative	$C_{9}H_{13}NO_{3}$	131.17	

7	11.464	0.42	Hexasiloxane, 1,1,3,3,5,5,7,7, 9,9, 11,11-dodecamethyl-	$C_{12}H_{38}O_5Si_6$	430.940	
8	13.152	0.61	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270.4507	
9	14.554	1.45	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_9$	296.4879	
10	14.737	0.64	Methyl stearate	$C_{19}H_{38}O_2$	298.5038	
11	15.052	0.45	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.4614	
12	15.984	0.40	3-Tridecylphenol	$C_{19}H_{32}O_2$	276.457	
13	17.987	69.79	(Z)-3-(pentadec-8-	$C_{21}H_{34}O$	302.4941	

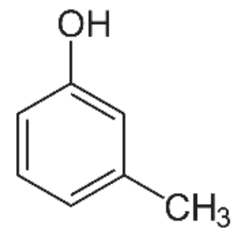
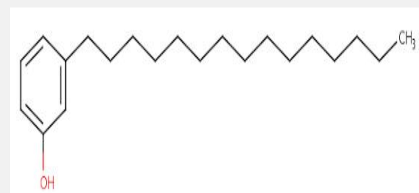
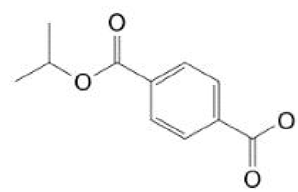
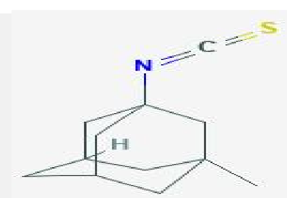
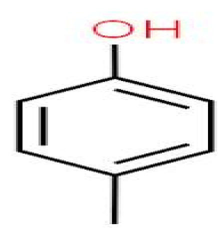
			en-1-yl)phenol			
14	18.039	16.39	Phenol, 3-methyl-	C_7H_8O	108.1378	
15	18.176	3.80	Phenol, 3-pentadecyl-	$C_{21}H_{36}O$	304.510	
16	18.863	0.75	1,4-benzenedicarboxylic acid, mono (1-methylethyl) ester	$C_{11}H_{12}O_4$	208.21	
17	18.925	1.32	Adamantane, 1-isothiocyanato-3-methyl-	$C_{12}H_{17}NS$	207.34	
18	19.023	0.86	p-Cresol	C_7H_8O	108.138	

Table 3.2. Biological activities of the compounds present in the dichloromethane extract of the seeds of *Azanza garckeana*

Compound	Biological Activity	Reference(s)
Cyclohexasiloxane, dodecamethyl-	Antibacterial, Antioxidant	Pauline and Sagaya, (2016)
Cyclododecane	Antimicrobial	Patra and Basak, (2020)
Cycloheptasiloxane, tetradecamethyl-	Antioxidant, Anti-inflammatory	Dikko *et al.,* (2016).
Epinephrine, (.beta.)-, 3TMS derivative	Muscle contraction	Qiaofang *et al*., (2020)
Hexasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11-dodecamethyl-	Antibacterial, Antioxidant	Pauline and Sagaya, (2016)
Pentadecanoic acid, 14-methyl-, methyl ester	Antibacterial, Antifungal	Dikko *et al.,* (2016).
9-Octadecenoic acid (Z)-, methyl ester	Antibacterial, Antifungal	Majumder *et al.,* (2019)
Methyl stearate	Antioxidant	Qiaofang *et al*., (2020)
9-Octadecenoic acid, (E)-	Anti-inflammatory	Pauline and Sagaya, (2016)
3-Tridecylphenol	Antibacterial	Ochokwu *et al.,* (2015)
(Z)-3-(pentadec-8-en-1-yl)phenol	Anti-hyperglycemic, Antioxidant	Ochokwu *et al.,* (2015) and Sunita *et al*., (2017)
Phenol, 3-methyl-	Antimicrobial, Anticancer	Qiaofang *et al.,* (2020)
Phenol, 3-pentadecyl-	Antibacterial	

CHAPTER FOUR

4.0. DISCUSSION

The bioactive compounds in *Azanza garckeana* seeds have a wide range of biological roles. The dichloromethane extract contains a variety of bioactive compounds with several biological activities, as demonstrated by the results of the GC-MS analysis (Patra and Basak, 2020). The seeds extract of *Azanza garckeana* contains substances that are significant in medicine, according to this study. Using references from established databases, the compounds were identified. The GC-MS study was based on the computer evaluation of mass spectra of samples using NIST-based software, direct comparison of peaks and retention times with those for standard substances, and computer matching of multiple peak indices with NIST. According to research (Ochokwu *et al.,* 2015; Sunita *et al*., 2017), the compound (Z)-3-(pentadec-8-en-1-yl) phenol, had the greatest reported functional frequency (69.79%) and was also found to possess anti-hyperglycemic, antioxidant, hypocholesterolemic, anticancer, and anti-inflammatory characteristics. Antioxidants protect cells against the damaging effects of reactive oxygen species otherwise called, free radicals such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynite which results in oxidative stress leading to cellular damage. Since a lot of laboratory evidence from chemical, cell culture, and animal research has indicated that anticancer compounds may slow or possibly prevent the development of cancer, compounds having anticancer action are recognized to help prevent cancer. Phenol, 3-methyl, Cyclohexasiloxane, dodecamethyl, Hexasiloxane, 1,1,3,3,5,5,7,7,9, 11,11-dodecamethyl, Methyl stearate, 1,4-benzenedicarboxylic acid, and mono (1-methylethyl) ester are other components in the dichloromethane extract that have been found to have effects on cancer and have antioxidant properties. In addition to having anti-cancer and antioxidant effects, 9-Octadecenoic acid, (E)- and 9-Octadecene, (E)- have also been discovered to have anti-inflammatory and anti-androgenic action. The dichloromethane extract of the seeds of *Azanza garckeana* contains hexasiloxane, 1,1,3,3,5,5,7,7,9, 11,11-dodecamethyl-, Pentadecanoic acid, 14-methyl-, methyl ester, and 9-Octadecenoic acid (Z)-, methyl ester, all of which have antibacterial activity. These substances, which are free and esterified fatty acids, respectively, Pentadecanoic acid, 14-methyl-, methyl ester, and 9-Octadecenoic acid (Z)-, methyl ester, may provide an anti-bacterial activity by functioning as a barrier to pathogenic bacteria. Bacterial pathogens will have less of an impact on biological habitats if they are killed or have their metabolic activity reduced. Furthermore, by preventing the buildup of germs in the oral environment, these substances help lower the incidence of disorders like caries that are linked to plaque (Ojekale *et al.,* 2016).

The dichloromethane extract include two compounds which were found to have antibacterial properties: pentadecanoic acid 14-methyl-methyl ester and 9-octadecenoic acid (Z)-, methyl ester. Because they are fatty acids and fatty acid ester and can build up in the lipid bilayer of the cell membrane and mitochondria, these bioactive molecules may have antibacterial properties (Patra and Basak, 2020). As a result, they compromise the stability of these microorganisms' cell structure, causing it to loosen up and increase permeability. Internal components of these microorganisms' cells may seep out due to the membrane's enhanced permeability caused by the insertion of unsaturated medium- and long-chain FFAs. This might stunt their growth or possibly lead to their demise (Armistice, 2021). Additionally, increasing membrane permeability can stop some enzymes from working in the membrane or cytoplasm, which is essential for the survival and development of these microorganisms. According to Guan and Liu (2019), this may also be the cause of the antibacterial activity.

The substances Phenol, 3-methyl- and Phenol, 3-pentadecyl-, which were discovered to be the predominant substances in the seeds extract (after (Z)-3-(pentadec-8-en-1-yl) phenol), have been shown to exhibit antibacterial, antimicrobial, anti-inflammatory, and anti-cancer effects (Armistice, 2021). Other compounds identified in the aqueous extract as minor constituents (Pentasiloxane, dodecamethyl-, 9-Octadecenoic acid, (E)-, Epinephrine, (.beta.)-, 3TMS derivative, 3-Tridecylphenol) have various biological activities such as antioxidant, anticancer and anti-inflammatory, antibacterial, antimicrobial activities (Majinda and Abubakar, 2016; Ojekale *et al.,* 2016). The substance, epinephrine, is beneficial in the management of systemic allergic reactions/anaphylaxis and is derived from the dichloromethane extract of *Azanza garckeana* seeds (Sten and Harold, 2021). Both beta (β)- and alpha (α) adrenergic receptors are affected by epinephrine. By stimulating the β_1 receptor, the myocardium contracts more forcefully and beats faster (a positive chronotrope). Due to peripheral vasoconstriction's stimulation of β_1 receptors, which counteracts the vasodilation brought on by the activation of β_2 receptors, systemic vascular resistance is elevated overall. Along with reducing tension in mast cells, these two effects help relax bronchial smooth muscle (Patra and Basak, 2020). Most of the discovered compounds in the dichloromethane extract have been reported to exhibit intriguing biological properties; some of these properties are shared by substances in other solvent extracts, including ethyl acetate, hexane, and methanol extracts (Ojekale *et al.,* 2016).

4.1. CONTRIBUTION TO KNOWLEDGE

The research has advanced knowledge in the following ways:

1. This work presents a GC-MS investigation of the bioactive compounds found in the seeds of *Azanza garckeana.*
2. The biological makeup of the compound extract of the seeds of *Azanza garckeana* suggests that it could be a source of natural antioxidants.

3. Pharmacological potential analysis demonstrates the presence of chemical substances in plants that have physiological effects on the human body.

4.2. CONCLUSION

When compared to manufactured chemicals, plant-based bioactive molecules have an effective dose response with fewer adverse effects. The existence of several bioactive compounds were discovered in the GC-MS analysis of the dichloromethane extract of the seeds of *Azanza garckeana* plant. Its therapeutic actions are due to the presence of these bioactive molecules (secondary metabolites). This study also expresses optimism for the development of many more innovative therapeutic compounds or templates from this plant, which may one day be used to produce synthetically better medicinal molecules.

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