

**ISOLATION AND CHARACTERIZATION OF GLYCOSIDIC
COMPOUNDS FROM THE AQUEOUS EXTRACT OF
SPHENOCENTRUM JOLLYANUM AND EVALUATION OF THEIR
BIOLOGICAL ACTIVITIES**

BY

Okogi favour

BMS2101435

DEPARTMENT OF MEDICAL BIOCHEMISTRY

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

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**A PROJECT SUBMITTED TO THE
DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF
BASIC MEDICAL
SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL
FULFILMENT
OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF
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IN MEDICAL BIOCHEMISTRY**

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CERTIFICATION

This is to certify that this project work was carried out by Okogi favour with matriculation number BMS2101435, of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin city, in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) degree in Medical Biochemistry.

(PROJECT SUPERVISOR)
DR. FIDELIS OLUMESE.

DATE

(Ag. HEAD OF DEPARTMENT)
DR. N.B. AGUEBOR OGIE

DATE

EXTERNAL EXAMINER

DATE

DEDICATION

This work is dedicated to God Almighty, my father Okogi cyril and my mother Okogi happiness

ACKNOWLEDGEMENT

I give God all the glory for my health and vitality, for being with me and seeing me through this academic journey

Many thanks goes to my project supervisor DR FIDELIS OLUMESE for his unending encouragement, constructive criticism throughout the time duration of this research and during my academic journey, I am forever indebted to him, his constant admonishments were instrumental to the completion of this research. I am grateful to my head of department DR AGUEBOR OGIE for creating a conducive environment vital for research and for always looking after the affairs of the students enabling them to achieve academic success

I cannot begin to describe how thankful I am to my parents Mr and Mrs **OKOGI** for their constant support, advice, criticism and always showing me the right path to follow, they've been a constant source of support in my life and I'm forever grateful to have them in my life. I love you very much. To my elder brother Okogi Patrick, I can't begin to say my thanks for your positive impact on my life, you've had an impact everywhere you find yourself and I'm forever grateful to you for all you are and done for the betterment of my life, your sacrifice, words and support are forever appreciated

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ABSTRACT

Sphenocentrum jollyanum is a medicinal plant widely employed in West African ethnomedicine for the management of diabetes mellitus and chronic wounds, yet the specific bioactive constituents responsible for its therapeutic efficacy remain insufficiently characterized. The aim of this study was to profile the phytochemical composition of the root extract to identify bioactive compounds validating its traditional antidiabetic use. The research involved the collection and preparation of root samples, followed by solvent extraction and subsequent analysis using Gas Chromatography-Mass Spectrometry (GC-MS) to separate and identify volatile components based on their retention times, peak areas, and mass spectral fragmentation patterns. The GC-MS analysis revealed the presence of distinct bioactive compounds, with carbohydrate derivatives and glycosides being the most dominant class. Specifically, Inositol, 1-deoxy- was identified as the major constituent, accounting for 43.45% of the total extract, followed by alpha-Methyl Mannofuranoside (3.79%) and the antioxidant D-alpha-Tocopherol (1.13%). The substantial concentration of inositol derivatives, which are known mediators of insulin signal transduction, alongside potent antioxidant agents, scientifically substantiates the traditional application of *Sphenocentrum jollyanum* in the management of diabetes and oxidative stress-related disorders.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Diabetes Mellitus has evolved from a manageable medical condition into one of the most urgent health crises of the 21st century. It is a metabolic disorder characterized fundamentally by chronic hyperglycemia—a state where blood sugar levels remain dangerously high because the body either cannot produce insulin or cannot use the insulin it produces effectively (American Diabetes Association, 2021). The International Diabetes Federation (IDF) estimates that over 537 million adults are currently living with diabetes, a number that is projected to rise to 783 million by 2045 if current trends continue (Sun et al., 2022). This burden is not shared equally; developing nations, including Nigeria, are bearing the brunt of this "silent epidemic" due to rapid urbanization and changing dietary habits.

For decades, the standard approach to managing this disease has relied on synthetic interventions. Patients typically depend on exogenous insulin injections or oral drugs like Metformin and Glibenclamide. While these drugs are effective at lowering blood sugar, they are not perfect solutions. Research has shown that long-term use can lead to adverse effects ranging from gastrointestinal distress and weight gain to more severe issues like hypoglycemia and liver toxicity (Chaudhury et al., 2017). Furthermore, for many people living in rural or low-income communities, the financial cost of these daily medications is simply unsustainable.

This economic and medical gap has sparked a renewed interest in nature's pharmacy. Ethnomedicine is the use of traditional plants for healing which is the primary source of healthcare for nearly 80% of the population in developing countries (Ekor, 2014). One plant

that has stood out in West African folklore is *Sphenocentrum jollyanum* (Menispermaceae), known locally as "Kere-wan-kere." Traditional healers have long used its roots and leaves to treat fatigue, fever, and chronic wounds, claiming it restores vitality to the body (Mbaka et al., 2010).

However, folklore is not enough for modern science. To safely recommend this plant, we must test it rigorously. This study utilizes Streptozotocin (STZ), a compound derived from soil bacteria, to induce diabetes in rats. STZ works by selectively entering and destroying the insulin-producing beta cells of the pancreas, creating a diabetic state that closely mimics the human condition (Ghasemi et al., 2014). By applying this model, we can objectively measure if *Sphenocentrum jollyanum* is merely a placebo or a potent antidiabetic agent.

1.2 STATEMENT OF THE PROBLEM

The core problem is the disconnect between the popularity of herbal medicine and the lack of scientific verification. In Nigeria, market shelves are filled with herbal mixtures containing *Sphenocentrum jollyanum* that promise to "cure" diabetes. These are often consumed without standardized dosages or safety guidelines. This is dangerous because, without empirical data, we do not know the toxic limits of the plant or how it interacts with the liver and kidneys (Olorunnishola et al., 2011).

Additionally, while synthetic drugs manage the symptoms of diabetes, they rarely address the oxidative stress and cellular damage that accompany the disease. There is a critical need for therapeutic agents that not only lower blood sugar but also protect the organs from the "rusting" effect of free radicals. If *Sphenocentrum jollyanum* possesses these dual capabilities, it remains largely undocumented in a comprehensive manner that combines biochemical analysis with histological (tissue) evidence.

1.3 JUSTIFICATION OF THE STUDY

This research is justified by the urgent need for affordable, accessible, and less toxic treatment options for diabetes. *Sphenocentrum jollyanum* is indigenous to West Africa, meaning it is readily available and inexpensive compared to imported synthetic drugs. Validating its efficacy provides a scientific basis for "home-grown" solutions to a global health problem.

Furthermore, exploring the mechanism of this plant contributes to the global search for new drug molecules. Many of our best modern drugs, including Metformin (derived from French Lilac), started as herbal remedies. By investigating the specific effects of this plant on lipid profiles and organ structure, this study moves beyond simple blood sugar testing to provide a holistic view of the plant's therapeutic value.

1.4 AIM AND OBJECTIVE

1.4.1 AIM

The primary aim of this research is to isolate and characterize specific glycosidic compounds from the aqueous root extract of *Sphenocentrum jollyanum* and to evaluate their therapeutic potential,

1.4.2 SPECIFIC OBJECTIVES

To achieve this aim, the study focuses on the following:

To prepare an aqueous extract from the roots of *Sphenocentrum jollyanum* using standard extraction methods like cold maceration

To extract and identify the phytochemical constituents (bioactive compounds) present in the *Sphenocentrum jollyanum* stem

To conduct preliminary qualitative and quantitative phytochemical screening to confirm the presence of glycosides and other secondary metabolites.

To isolate glycosidic fractions or pure compounds from the crude aqueous extract using gas chromatography and mass spectrometry (GC-MS)

To characterize the isolated glycosidic compounds using spectroscopic analysis (such as gas chromatography and mass spectrometry) to determine their structural properties.

1.5 SIGNIFICANCE OF THE STUDY

Advancement from Crude Extracts to Specific Isolates

Most existing literature on *Sphenocentrum jollyanum* focuses on the efficacy of crude extracts. This study is significant because it goes a step further by aiming to isolate and characterize specific glycosidic compounds. Identifying the precise bioactive constituents is a critical step in standardizing herbal formulations. It moves the scientific understanding of this plant from "it works" to "what makes it work," providing a chemical basis for its therapeutic activity

Potential for Novel Drug Discovery

Nature has historically been a primary source of new drug leads; approximately 50% of all modern drugs are gotten from natural products. By isolating glycosides from *sphenocentrum jollyanum*, this research contributes to the global library of potential pharmaceutical agents. If these isolated compounds demonstrate significant hypoglycemic activity, they could serve as

lead compounds for the development of new, more effective antidiabetic drugs with potentially fewer side effects than current synthetic options.

Scientific Validation of Traditional Medicine

Sphenocentrum jollyanum is widely used in West African ethnomedicine ("Aduro kokoo") for managing metabolic disorders. This study provides scientific empirical data to validate these traditional claims. By establishing a correlation between the isolated glycosides and biological activity, the research bridges the gap between folklore and modern science, promoting safer and more rational use of the plant in local communities (Sofowora, 2008).

Economic and Public Health Implications

Diabetes management is a lifelong and expensive endeavor, often placing a heavy financial burden on patients in developing regions. If this study confirms the efficacy of *S. jollyanum* glycosides, it supports the development of cost-effective, locally sourced phytomedicines. This could enhance accessibility to treatment for low-income populations who rely on herbal remedies as their primary source of healthcare

1.6 SCOPE OF THE STUDY

This research is focused on the phytochemical and pharmacological investigation of the aqueous extract of the stem of *Sphenocentrum jollyanum*

CHAPTER TWO

LITERATURE REVIEW

2.1.1 GENERAL INFORMATION

Sphenocentrum jollyanum Pierre is a perennial plant that belongs to the Menispermaceae family. This family is widely recognized in botany for containing a diverse group of plants that exhibit significant biological functions. Geographically, *S. jollyanum* is native to the tropical forest zones of West Africa. Its distribution stretches across the region, with significant populations recorded in countries such as Sierra Leone, Nigeria, Ghana, Côte d'Ivoire, and Cameroon (Olorunnisola et al., 2017).

The plant thrives specifically in the deep shade of dense forest undergrowth. It favors wet tropical biomes and is typically found in regions where the mean annual rainfall exceeds 1800 mm. In terms of local nomenclature, the plant is known by various names depending on the tribe and region. In Ghana, particularly among the Akan and Asante tribes, it is called “Aduro kokoo” (meaning red medicine) or “Okramankote” (meaning dog’s penis). In southwestern Nigeria, the Yoruba people refer to it as “Akerejupon” or “Ajo,” while in the Republic of Benin, it is known as “Oban abe” (Olorunnisola et al., 2017; Mbosso et al., 2012).

2.1.2 DESCRIPTION OF THE PLANT

Morphologically, *Sphenocentrum jollyanum* is defined as a small, erect shrub that is sparsely branched. It typically grows to a height of approximately 1.5 meters. The stems are thinly short-haired when young but tend to become glabrous (smooth and hairless) as the plant matures, with a grey bark texture (Ekpono et al., 2018).

The leaves are a distinctive feature of the plant. They are wedge-shaped or elliptical, measuring about 5 to 12 cm in width and growing up to 20 cm in length. The leaves are

smooth on both sides and usually possess a small-arrowed apex. They are arranged spirally but often appear crowded at the ends of the branches.

The reproductive structures include unisexual flowers. The fruits of *S. jollyanum* occur as clusters of ovoid-ellipsoid drupes. When ripe, these fruits turn a bright yellow or orange color and are fleshy and edible. Each fruit contains a single large, oval-shaped seed. Notably, the roots of the plant are bright yellow, a characteristic that has earned it the common English name “African yellow wood.” These roots are sour when chewed but have a property similar to *Synsepalum dulcificum* where they make food eaten subsequently taste sweet (Nia et al., 2004; Olorunnisola et al., 2017).

2.1.3 TAXONOMY OF THE PLANT

The classification of *Sphenocentrum jollyanum* places it within the order Menispermales. The accepted taxonomic hierarchy is as follows (Olorunnisola et al., 2017; Mbosso et al., 2012):

Kingdom: Plantae

Division: Magnoliophyta

Class: Ranunculopsida

Order: Menispermales

Family: Menispermaceae

Genus: *Sphenocentrum*

Species: *Sphenocentrum jollyanum* Pierre

2.2.1 PROPERTIES OF THE PLANT

The plant is an erect shrub that typically grows to a height of 1.5 meters. It is dioecious, meaning individual plants are distinctly male or female. The stem is sparsely branched and may appear thinly hairy when young but becomes glabrous (smooth) and grey as the plant matures (Mbaka et al., 2010).

2.2.2 ROOT CHARACTERISTICS

The roots are the most commercially and medicinally significant part of the plant. They are deep-rooted with minimal branching. A distinguishing physical feature is the bright yellow to orange coloration of the root interior. The texture is fibrous, and the taste is characteristically bitter due to the presence of specific diterpenes (Olorunnisola et al., 2017).

2.2.3 LEAVES AND FRUIT

Leaves: The leaves are arranged spirally and are often crowded at the tips of branches. They are elliptical or obovate in shape with a wedge-shaped base. The surface is smooth (glabrous) and dark green.

Fruit: The plant produces fruits described as drupes. These are grouped in clusters and change color from yellow to orange upon ripening. Each fruit contains a single, large, oval-shaped seed (Mbaka & Owolabi, 2011).

2.2.4 PHYSICOCHEMICAL PARAMETERS

Standardization parameters are vital for assessing purity. Proximate analysis of the root powder typically yields the following data, though variations occur based on soil composition and harvest time (Ekpono et al., 2018).

Moisture content: Ranges between 3.5% and 6.5%. Low moisture is essential to prevent bacterial and fungal growth during storage.

Ash Value: Total ash values generally range from 3.5% to 7.0%. This measures the amount of inorganic material (minerals and silica) remaining after combustion.

Solubility: The root constituents demonstrate higher solubility in ethanol and water compared to non-polar solvents, indicating the polarity of its bioactive compounds

2.2.5 ETHNOMEDICINAL USE

The ethnomedicinal profile of *Sphenocentrum jollyanum* is extensive and deeply rooted in the traditional healthcare systems of West Africa. Indigenous communities across Nigeria, Ghana, Cameroon, and Côte d'Ivoire utilize various morphological parts of the plant—primarily the roots, leaves, fruits, and stem bark—to manage a diverse spectrum of physiological disorders. The plant is highly regarded for its versatility, serving as a remedy for conditions ranging from sexual dysfunction and chronic wounds to infectious fevers and digestive ailments.

2.2.6 APHRODISIAC AND REPRODUCTIVE HEALTH

The most renowned application of *Sphenocentrum jollyanum* in traditional medicine is its use as an aphrodisiac and a promoter of sexual health. In Ghana, the plant is locally referred to as "Okramankote" (meaning dog's penis) among the Akan people, a name that directly reflects its reputed efficacy in enhancing erectile function and libido. Men in rural and urban communities frequently consume the root extract, often prepared as an alcoholic tincture or "bitters," to sustain penile erection and manage erectile dysfunction. Beyond sexual

performance, the root is also used as a tonic to combat fatigue, which traditional healers believe contributes to improved stamina and vitality (Owiredu *et al.*, 2007; Raji *et al.*, 2006).

2.2.7 WOUND HEALING AND DERMATOLOGICAL CARE

The plant plays a critical role in the management of skin conditions and the healing of wounds. Traditional healers in Nigeria and Ghana apply decoctions made from the roots and leaves to dress chronic wounds, tropical ulcers, and cuts. This practice is particularly significant for patients with delayed wound healing, such as those suffering from diabetes. The application of the aqueous leaf or root extract is believed to prevent microbial infection and accelerate the closure of the wound. In some practices, the crushed leaves are applied directly to sores to stop bleeding and promote tissue regeneration (Adeleke *et al.*, 2021; Nia *et al.*, 2004).

2.2.8 DIGESTIVE AND METABOLIC DISORDERS

Sphenocentrum jollyanum is widely employed to treat gastrointestinal disturbances. The roots are frequently used as chewing sticks, not only for oral hygiene but also to relieve stomach aches, constipation, and loss of appetite. The swallowing of the root sap is considered an effective remedy for improving digestion. In Côte d'Ivoire, a specific traditional formulation involves pulping the root with salt, palm oil, and the fruit of *Aframomum melegueta* (alligator pepper/maniguette) to treat severe abdominal disorders. Furthermore, the plant is used as an emetic (to induce vomiting) and a purgative in cases of suspected food poisoning, highlighting its detoxifying role in folk medicine (Mbaka *et al.*, 2011; Olorunnisola *et al.*, 2017).

2.2.9 RESPIRATORY AND FEBRILE CONDITIONS

The fruits and aerial parts of the plant are integral to treating respiratory issues. A prominent traditional cough remedy involves combining the fruits of *Sphenocentrum jollyanum* with the fruits of *Piper guineense* (West African black pepper) and lime juice. This mixture is reputed to relieve chronic coughs and soothe throat infections. Additionally, the plant is used to manage feverish conditions associated with malaria and jaundice. In the context of sickle cell disease and rheumatism, practitioners often prescribe root extracts to manage pain and inflammation associated with these chronic conditions (Abbiw, 1990; Mbosso *et al.*, 2012).

2.2.10 MANAGEMENT OF CHRONIC DISEASES

Ethnomedicinal surveys indicate that the plant is increasingly used to manage chronic metabolic diseases. Pounded roots are ingested to lower high blood pressure (hypertension). In Ghana, specific preparations of the pulped roots are applied topically or ingested to treat breast tumors, suggesting a traditional awareness of the plant's potential anti-tumor properties. The widespread use of the root for "sweetening" the palate (similar to *Synsepalum dulcificum*) often accompanies its use in making bitter medicines more palatable for patients (Olorunnisola *et al.*, 2017).

2.2.11 ROOTS

The root is the most frequently utilized part of the plant in traditional medicine. In Ghana, it is widely renowned as an aphrodisiac. Men steep the roots in alcohol to create bitters that are believed to enhance sexual performance and sustain penile erection (Owiredu *et al.*, 2007). Beyond sexual health, the roots are used as chewing sticks to relieve stomach aches and constipation. In Nigeria, a decoction of the root is applied topically to dress tropical ulcers, and pounded roots are ingested to manage high blood pressure. Some practitioners also use the root extract to treat sickle cell disease and rheumatism (Abbiw, 1990; Moody *et al.*, 2006).

2.2.12 LEAVES AND AERIAL PARTS

The leaves and leafy twigs are primarily employed for wound healing. A decoction of the leafy twigs serves as a wash to stop bleeding in wounds, sores, and cuts. In some traditional settings, the crushed leaves are ingested to curb the spitting of blood. Furthermore, the aerial parts are often combined with the fruits of *Piper guineense* (West African black pepper) and lime juice to treat chronic coughs and feverish conditions (Raji et al., 2006).

2.2.13 FRUITS

The fruits are edible and are traditionally consumed as an anti-fatigue snack. They are also believed to possess stomachic properties (aiding digestion) and are occasionally used in formulations for treating coughs (Raji et al., 2006).

2.2.14 GENERAL USES

In the Ivory Coast, the plant is considered an effective antidote for poison and is used as an emetic. Traditional healers also apply pulped roots to treat breast tumors in certain regions of Ghana (Olorunnisola et al., 2017)



Leave, seed and stem of *sphenocentrum jollyanum*

2.3.1 PHYTOCHEMISTRY AND BIOACTIVE COMPOUNDS

Various natural products have been identified in *Sphenocentrum jollyanum* (*S. jollyanum*) through the application of conventional extraction and purification protocols (Walsh et al., 2017). Investigations into the methanol extract of the stem bark by Nia et al. (2004) confirmed the presence of secondary metabolites such as tannins, alkaloids, saponins, and terpenes. In a separate study regarding the root essential oil, Aboaba and Ekundayo (2010) utilized GC-MS analysis to characterize 19 distinct compounds. Notable constituents included β -pinene, α -eudesmol, p-cymene, 5-guaiene-11-ol, globulol, δ -3-carene, and camphene. Furthermore, proximate analysis of the seeds indicated a composition of 48.1% crude protein, 48.1% carbohydrates, 16.8% ash, 16.7% moisture, 9.7% crude fat, and 5.5% fiber, contributing to a total energy value of 1460 kcal/100 kg. The isolated fractions were predominantly composed of sesquiterpenoids (56.3%) and monoterpenoids (33.5%), with 10.2% of the content remaining unidentified.

A study by Uka et al. (2022) highlighted a diverse array of bioactive phytochemicals within the plant. The most significant compounds detected included 2,4-di-tert-butylphenol and 3,5-bis(1,1-dimethylethyl) phenol (21.05%); methyl 9,12-heptadecadienoate, Z-8-methyl-9-tetradecenoic acid, and 6-butyl-1-nitro cyclohexene (19.12%); as well as ethyl esters of undecanoic and hexadecanoic acids (7.86%). Phthalates such as bis(2-ethylhexyl) phthalate and diisooctyl phthalate accounted for 7.13%, while cis-11-hexadecenal, oleic acid, and phytol constituted 7.03%. Other identified constituents appearing in smaller quantities (ranging from 1.47% to 6.65%) included (Z, Z)-9,12-octadecadien-1-ol, 9-octadecenoic acid (Z)-, trans-13-octadecenoic acid, L-galactose, n-decanoic acid, n-hexadecanoic acid, and various isomers of octadecadienal and eicosene.

Research by Moody et al. (2005) focused on the fruits of *S. jollyanum*, leading to the isolation of three clerodane diterpenes: fibleucin, isocolumbin, and columbin. For this study, fruits obtained from the University of Ibadan, Nigeria, were authenticated (FHI 105364), dried in the shade, and powdered. A 2 kg sample was subjected to cold percolation with methanol for 72 hours. The resulting filtrate was concentrated to yield 84.2 g of extract. Subsequent purification of 10 g of this extract using preparative thin-layer chromatography and silica gel vacuum liquid chromatography yielded the diterpenes. Bioassay results indicated that columbin possesses significant anti-inflammatory properties, demonstrating 67.08% inhibition at a dosage of 20 mg/kg, which is comparable to the 72.50% inhibition observed with acetylsalicylic acid at 100 mg/kg.

The essential oil of *Sphenocentrum jollyanum* contains camphene as a major bicyclic monoterpene. This compound is known for its utility in insecticide production, camphor synthesis, artificial flavoring, and fragrances, while also offering protection against oxidative stress (Tiwari et al., 2009). Additionally, Angelini et al. (2008) reported that *S. jollyanum* essential oils display broad-spectrum antimicrobial capabilities. They were effective against bacterial strains including *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, and *Listeria innocua*, as well as fungal species such as *Trichoderma* spp., *Chaetomium globosum*, *Aspergillus* spp., and *Penicillium chrysogenum*.

Further phytochemical work by Akinwumi et al. (2020) involved the isolation of five ecdysteroids from the seed extracts: atrotosterone A, 20,26-dihydroxyecdysone, 20-hydroxyecdysone, polypodine B, and pinnatasterone. Seeds harvested from Odo Ona, Ibadan, were authenticated (FHI 110510), shade-dried, pulverized, and extracted with 100% methanol. This extract was fractionated using n-butanol, ethyl acetate, dichloromethane, and n-hexane. Purification of the ethyl acetate fraction via silica gel chromatography yielded

pinnatasterone and atrotosterone A, while the n-butanol fraction was processed using preparative reversed-phase HPLC to isolate the remaining three compounds.

In their assessment of biological activity, Akinwumi et al. (2020) evaluated the antacid and urease-inhibitory potential of these isolated ecdysteroids. Pinnatasterone, 20-hydroxyecdysone, and polypodine B demonstrated potent urease inhibition with IC₅₀ values of $14.1 \pm 0.59 \mu\text{M}$, $13.8 \pm 0.49 \mu\text{M}$, and $7.0 \pm 0.56 \mu\text{M}$, respectively. Conversely, atrotosterone A and 20,26-dihydroxyecdysone displayed moderate inhibitory activity, recording IC₅₀ values of $29.3 \pm 7.45 \mu\text{M}$ and $24.1 \pm 1.21 \mu\text{M}$, respectively. For comparison, the standard drug acetohydroxamic acid exhibited an IC₅₀ of $20.3 \pm 0.43 \mu\text{M}$ at 0.5 mM. Antioxidant Activity

The aqueous extract exhibits proton-donating ability and can serve as a free radical inhibitor. The presence of phenolic glycosides in the water extract contributes to this antioxidant capacity. Studies utilizing the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay show that while the aqueous extract may have slightly lower antioxidant power compared to the ethanolic extract, it still possesses significant scavenging activity against reactive oxygen species (ROS), which helps in protecting tissues from oxidative stress associated with diabetes and inflammation (Olorunnisola *et al.*, 2017; Mbaka *et al.*, 2013).

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1.1. PLANT ACQUISITION AND AUTHENTICATION

Fresh stem samples of *Sphenocentrum jollyanum* were procured from Iwo, Osun State, located in South-West Nigeria. Following collection, the specimens were verified and authenticated at the Herbarium Unit, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin (Benin City, Edo State, Nigeria; P.M.B. 1154, Ugbowo, 300283). The research exclusively utilized the stems of the plant, which were processed for subsequent extraction and assay.

3.1.2. REAGENTS AND SOLVENTS

For the extraction processes, two solvent types were employed: ethanol (95% or absolute grade) acted as the organic solvent, while distilled or deionized water functioned as the aqueous solvent.

3.1.3. APPARATUS

Various apparatuses were employed for sample handling and preparation, including stainless steel knives for sectioning, polypropylene vessels for extraction, and drying trays. Additional items included manual stirrers, spatulas, sieves, and a mortar and pestle. Filtration and safety materials consisted of muslin/cheesecloth, universal bottles, beakers, test tubes, nose masks, nitrile gloves, masking tape, and permanent markers.

3.1.4. INSTRUMENTATION

Major laboratory equipment utilized included washing basins for cleaning, a mechanical grinder for pulverizing the dried stems, and a precision analytical balance. A freezer was used to stabilize samples prior to lyophilization, and a freeze dryer (lyophilizer) was employed for solvent removal.

3.2. METHODS

3.2.1. PROCESSING AND PULVERIZATION

The harvested stems underwent thorough washing with running water to eliminate soil and external contaminants. Once cleaned, the stems were sectioned into smaller fragments and subjected to air-drying at room temperature in a shaded, ventilated area to prevent the thermal degradation of phytochemicals. Upon achieving complete dryness, the material was milled into a coarse powder using a mechanical grinder to maximize surface area for extraction.

3.2.2. EXTRACTION PROCEDURE (COLD MACERATION)

The pulverized stem material was separated into two distinct batches to produce an ethanolic extract and an aqueous extract, utilizing ethanol and distilled water, respectively. A solvent-to-sample ratio of 1 L of solvent to 0.15 g of plant powder was maintained for each batch. The mixtures were macerated for a total of 72 hours. To facilitate the diffusion of phytochemicals and ensure thorough solvent penetration, the mixtures were agitated gently every 2 hours.

3.2.3. FILTRATION

Post-maceration, the mixtures were passed through a muslin cloth to filter out solid plant debris, resulting in clarified liquid extracts.

3.2.4. CONCENTRATION VIA LYOPHILIZATION

The liquid filtrates were frozen and subsequently processed in a freeze dryer. Through sublimation, the solvents were evaporated to yield dry crude extracts. This technique was selected to ensure the preservation of volatile and heat-sensitive bioactive compounds.

3.2.5. PHYTOCHEMICAL SCREENING

For analysis, 1 g aliquots of both the freeze-dried ethanolic and aqueous extracts were weighed and sealed in labeled universal bottles. These samples were dispatched to a certified laboratory in Lagos for Gas Chromatography–Mass Spectrometry (GC–MS) profiling.

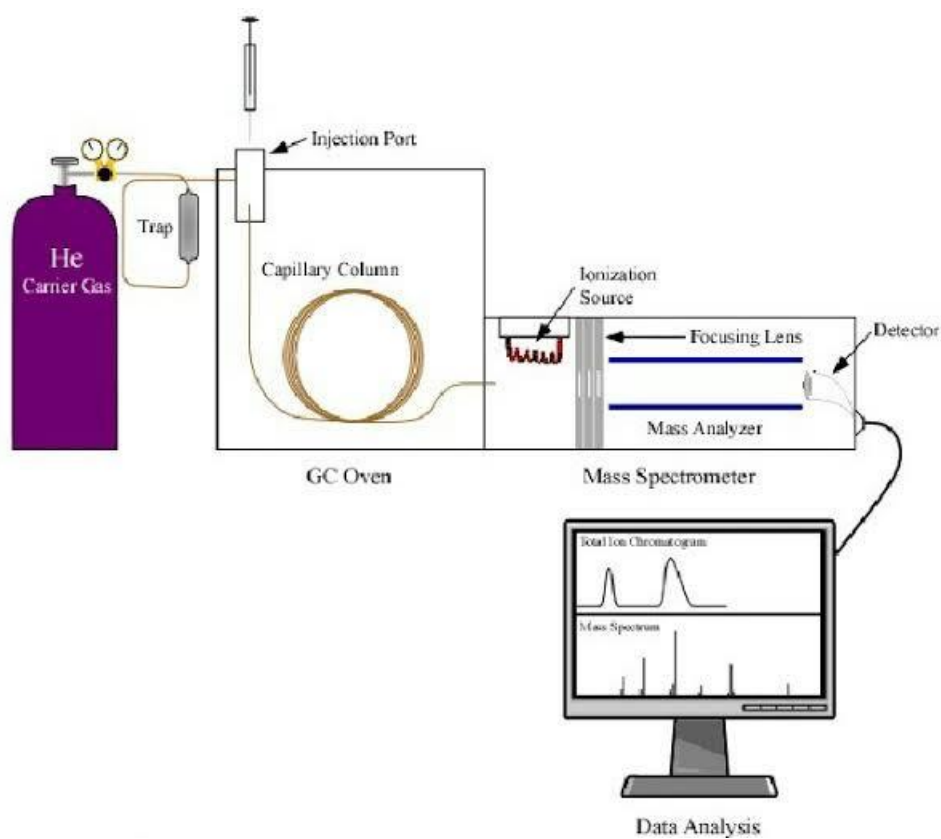
3.2.6. METHODOLOGICAL EVALUATION

The chosen extraction protocol offered distinct advantages: the use of freeze-drying protected heat-labile compounds, frequent agitation improved extraction yield, and the dual-solvent approach allowed for a comparative analysis of phytochemical solubility based on polarity.

3.2.7. GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GC–MS) ANALYSIS

GC–MS analysis was employed to identify phytoconstituents by combining the separation capabilities of Gas Chromatography (GC) with the identification power of Mass Spectrometry (MS). The process involves vaporizing the sample, separating components via a capillary column based on boiling points and stationary phase interactions, and ionizing the

compounds to generate mass spectral fingerprints for identification. Mass spectrometry (MS) is an analytical technique in which gaseous ions formed from the Molecules or atoms of a sample are separated in space or time and detected according to Their mass-to-charge ratio, m/z (Sparkman, 2000). It is usually used for determining masses Of particles, for determining the elemental composition of a sample or molecule, and for Elucidating the chemical structures of molecules, such as phenols, aldehydes, and other Chemical compounds.



Picture of the inner working of the GC-MS machine

The analysis was conducted using a Shimadzu GC–MS–QP2010 system fitted with an AOC-20i autosampler. The specific operating conditions were as follows:

AUTOSAMPLER SETTINGS (AOC-20I): The injection sequence included three pre-solvent rinses, three post-solvent rinses, and two sample rinses. A single solvent vial was utilized. Plunger suction and injection speeds were set to "high," with a viscosity compensation time of 0.2 s. The system operated in normal injection mode with five pumping cycles, a 0.3 s injection port dwell time, and no terminal air gap. Syringe suction and injection positions were set to 0.0 mm, with a high plunger washing speed and an 8 μ L washing volume.

GAS CHROMATOGRAPHY CONFIGURATION (GC-2010): The column oven was initiated at 60°C, with the injection temperature set to 250°C. The system used a splitless injection mode with a 1.00 min sampling time. Flow control was set to pressure mode (100 kPa), achieving a total flow of 4.7 mL/min, a column flow of 0.80 mL/min, and a purge flow of 3.0 mL/min. Linear velocity was maintained at 23.1 cm/s. The split ratio was 1:1, and the carrier gas saver and high-pressure injection features were disabled.

OVEN TEMPERATURE PROGRAM: The thermal profile began with a 1.00 min hold at 60°C. The temperature was then ramped at 13°C/min to 240°C (held for 1.00 min) and further increased at 13°C/min to 300°C, where it was held for 39.70 min.

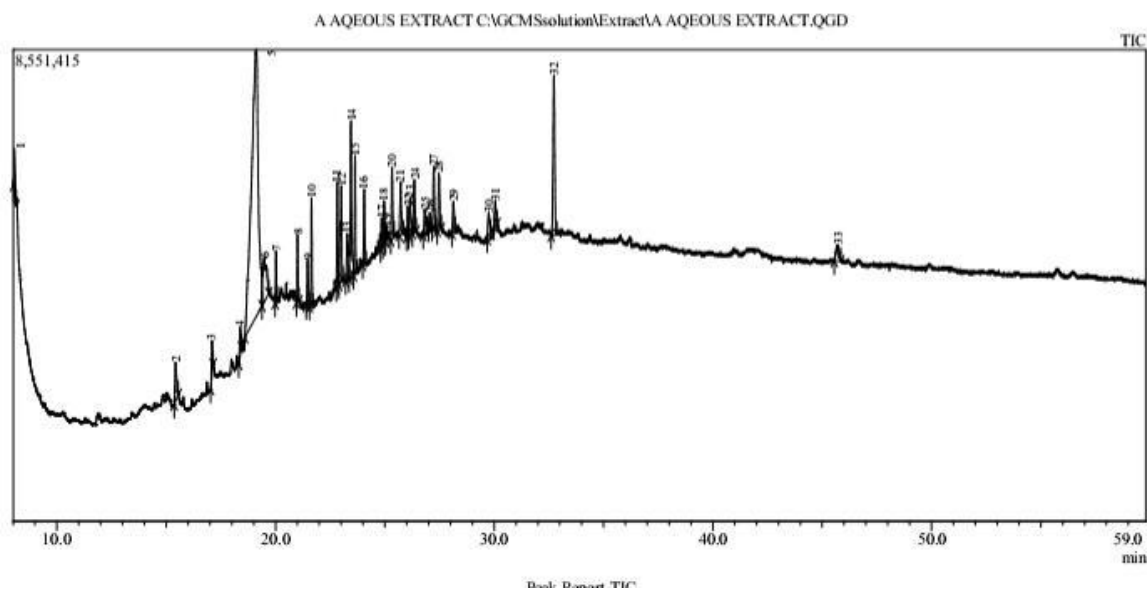
MASS SPECTROMETRY PARAMETERS (MS: QP2010): The ion source and interface temperatures were maintained at 230°C and 250°C, respectively. The solvent cut time was 4.00 min. The detector operated in relative gain mode (1.33 kV) with a threshold of 2000.

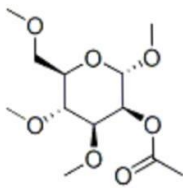
SCAN SETTINGS: Data acquisition occurred between 8.00 min and 59.80 min in scan mode. The parameters included a scan range of m/z 35–500, a scan speed of 1666 amu/s, and an event time of 0.30 s.

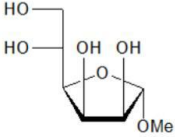
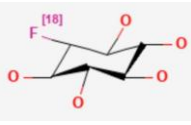
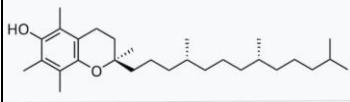
SYSTEM READINESS AND HEATING: Prior to analysis, a comprehensive check was performed to verify the stability of temperatures (oven, injector, interface), pressure, flow rates, and vacuum levels. The autosampler syringe alignment and baseline stability were also confirmed. Upon validation, the heat unit activated the column oven, SPL1 injector, and mass spectrometer, ensuring that SPL1 carrier and purge flows remained active throughout the run.

CHAPTER FOUR

RESULT



S/N	NAME/STRUCTURE	RETENTION TIME	AREA PERCENT	PHYSICAL PROPERTIES/MOLECULAR WEIGHT	BIOLOGICAL IMPORTANCE
1	Methyl (methyl 2-O-acetyl-3,4-di-O-methyl-alpha-D-mannopyranoside)  uronate	17.098 min	0.94	292	Anti inflammatory and detoxifying properties
2	alpha-Methyl mannofuranoside	19.483 min	3.79%	Molecular weight: (194.18)\g/mol Appearance: A white, crystalline solid	It serves as a raw material or intermediate in the

				<p>Solubility: Soluble in solvents like DMSO, water (H_2O), and methanol (MeOH)</p> <p>Melting point: $(114-117)^\circ\text{C}$</p>	<p>synthesis of more complex pharmaceutical compounds</p>
3	<p>Inositol, 1-deoxy-</p> 	19.121 min	43.45	<p>Appearance: White crystalline powder</p> <p>Melting point: $216-217^\circ\text{C}$</p> <p>Solubility: Water-soluble</p> <p>Solubility in organic solvents: Practically insoluble in most organic solvents</p>	<p>It is used as a tracer for imaging inflammation and as biomarkers for certain cancers e.g breast cancer</p>
4	<p>D-alpha-Tocopherol</p> 	45.710 min	1.13	<p>A slightly yellow to amber, clear, viscous oil, insoluble in water but soluble in ethanol and ether. Molecular weight: 430.7 g/mol</p>	<p>It is used for its Antioxidant properties, and is commonly used in supplements, personal care products, and as a food additive</p>

CHAPTER

DISCUSSION

Gas Chromatography-Mass Spectrometry (GC-MS) was employed to profile the bioactive constituents of the *Sphenocentrum jollyanum* extract. This analytical technique is pivotal in phytochemistry because it combines two powerful mechanisms: Gas Chromatography (GC), which separates the complex mixture of plant chemicals based on their volatility and interaction with the column, and Mass Spectrometry (MS), which bombards these separated compounds with electrons to fragment them. By measuring the mass-to-charge ratio (m/z) of these fragments, the system compares the “fingerprint” of each compound against a spectral library (such as the national institute of standards and technology) to identify specific metabolites with high precision.

The chromatogram obtained In this study revealed distinct peaks at various retention times, indicating the presence of diverse phytochemicals. Notably, the analysis highlighted a significant presence of carbohydrate derivatives, glycosides, and vitamins, which provides strong scientific validation for the plant’s ethnomedicinal use in managing metabolic disorders like diabetes.

Analysis of the aqueous extract through gas chromatography and mass spectrometry revealed four glycosidic compounds, starting from Inositol, 1-deoxy- it was the most abundant compound identified, constituting 43.45% of the total extract area. Inositols are carbocyclic sugar alcohols that play a critical role in cellular signal transduction It is relevant to Diabetes because Inositol derivatives are well-documented insulin sensitizers. They act as second messengers in the insulin signaling pathway. The high concentration of a deoxy-inositol derivative strongly suggests that *Sphenocentrum jollyanum* exerts its anti-diabetic effect by mimicking insulin signaling or enhancing the body’s sensitivity to insulin. inositol derivatives

often serve as biomarkers for inflammation. Their presence suggests the plant may help mitigate the systemic low-grade inflammation often associated with chronic diabetes.

The second glycosidic compound is Methyl (methyl 2-O-acetyl-3,4-di-O-methyl-alpha-D-mannopyranoside) uronate. This compound is a complex glycoside derived from mannose (a sugar monomer). The presence of the "uronate" group indicates oxidation of the sugar, which often increases the molecule's reactivity and biological interaction. Mannose-based glycosides are frequently involved in immune modulation. They can bind to lectins on the surface of bacteria or immune cells, potentially preventing infection (antimicrobial) and reducing inflammatory responses. This aligns with the ethnomedicinal use of the plant for wound healing.

The third glycosidic compound is Alpha-Methyl Mannofuranoside, it Occupies 3.79% of the extract, it's pharmacological potential is high mainly because methylated sugars are often more lipophilic than their free sugar counterparts, allowing them to cross cell membranes more easily. This compound likely acts co-operatively with the other glycosides to stabilize the extract and provide metabolic support.

The fourth glycosidic compound is D-alpha-Tocopherol (Vitamin E). Although present in a smaller quantity (1.13%), but it is a very important constituent. D-alpha-Tocopherol is a potent lipid-soluble antioxidant. In the context of diabetes, hyperglycemia induces oxidative stress, which damages pancreatic beta-cells. The presence of Vitamin E suggests that *Sphenocentrum jollyanum* offers a protective mechanism, scavenging free radicals and preventing lipid peroxidation in cell membranes

CONCLUSION

The present study successfully evaluated the phytochemical profile of *Sphenocentrum jollyanum* using GC-MS analysis. The results provide a molecular basis for the plant's traditional application in treating diabetes and inflammatory conditions. Furthermore, the analysis revealed that the extract is rich in bioactive metabolites, dominated by Inositol, 1-deoxy-. The high prevalence of this inositol derivative is a breakthrough finding, as inositols are direct mediators of insulin action. Furthermore, the identification of glycosidic compounds (Mannofuranosides) and antioxidants (vitamin E) suggests its effectiveness in

Metabolic Regulation: via inositol-mediated insulin sensitization. Anti-inflammatory Action: via mannose-based glycosides and Cellular Protection: via the antioxidant activity of Tocopherol.

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