GAS CHROMATOGRAPHY MASS SPECTROMETRY OF PHENOLIC COMPOUNDS AND ANTIOXIDANT RELATED CONSTITUENTS IN THE AQUEOUS EXTRACT OF *SPHENOCENTRUM JOLLYANUM*

BY

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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 DEPARMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE AWARD OF BACHELOR OF SCIENCE, B.Sc. (HONS) MEDICAL BIOCHEMISTRY, OF THE UNIVERSITY OF BENIN, BENIN CITY

DATE:

(NOVEMBER, 2025)

CERTIFICATION

This is to certify that this project work was carried out by Jennifer Eloghosa OSARIYEKEMWEN with the matriculation number BMS2101450, of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin city, in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) degree in Medical Biochemistry.

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DEDICATION

This work is dedicated to the almighty God, and my parents, Mr and Mrs Osariyekemwen.

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With a hear full of gratitude, I give thanks to the **Almighty God** for his grace, strength, and continuous guidance throughout the period of this project and my academic journey.

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ABSTRACT

The study focused on the Gas Chromatography Mass Spectrometry of phenolic compounds and antioxidant related constituent in the aqueous extract of *Sphenocentrum jollyanum* stem, a medicinal plant commonly used in traditional healthcare across Nigeria. The stem samples were sourced from Iwo Market in Osun State. The analysis was carried out using Gas Chromatography–Mass Spectrometry (GC–MS) at LEEDEX Laboratory in Lagos to identify the major bioactive constituents responsible for antioxidant activity. A total of 33 compounds were detected in the aqueous extract. Among these, four major compounds were identified due to their relevance to phenolic and antioxidant properties. Peaks 2 and 4 were confirmed as phenolic compounds—Phenol, 2,6-dimethoxy- and Phenol, 3,4,5-trimethoxy—indicating the presence of significant phenolic content in the extract. Peaks 32 and 33 were identified as Squalene and di-α-Tocopherol, respectively. Although non-phenolic, both compounds are well-documented antioxidants that contribute to the overall antioxidant capacity of the extract. The presence of both phenolic and non-phenolic antioxidant compounds suggests that the aqueous extract of *Sphenocentrum jollyanum* possesses strong antioxidant potential. These findings support the medicinal value of the plant and align with its traditional use for managing oxidative stress-related conditions. The results highlight the importance of further research to explore additional therapeutic properties, optimize extraction methods, and understand the stability of its bioactive constituents.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Medicinal plants have long played a central role in health care for centuries, providing compounds used in both traditional remedies and the development of modern drugs. In many developing countries like Nigeria, traditional plant-based remedies remain a major part of primary health care due to their affordability, availability and the limited access to modern medicines (Lifongo *et al*., 2014). Among the numerous medicinal species found in West Africa is *Sphenocentrum jollyanum* Pierre, commonly known as Akerejupon in Yoruba language in Nigeria, a perennial shrub belonging to the family **Menispermaceae**. The shrub *Sphenocentrum jollyanum* naturally grows within the humid rainforest environments of West Africa and is found in nations such as Nigeria, Ghana and Côte d'lvoire (Olorunnisola *et al*.,2017). In traditional West Africa medicine, different parts of *Sphenocentrum jollyanum* commonly known as Akerejupon are employed to treat conditions including fever, wound, coughs, hypertension, constipation, and enhance sexual function (Olorunnisola *et al*., 2017). The wide range of traditional applications has encouraged researchers to investigate the plant's chemical and biological properties.

Although research has been carried out on the roots and leaves of *S. jollyanum,* there remains limited information on its stem. Previous studies on *S. jollyanum,* shown that it contains a variety of bioactive phytochemicals such as alkaloids, flavonoids, annin, terpenoids and saponins, which may underlie its traditional medicinal activities (Olorunnisola *et al*., 2017). Animal studies indicate that the root extracts of *S. jollyanum* reduce blood glucose levels, improve lipid profiles, and exhibit antioxidant activity, supporting their potential use in antidiabetic and antioxidant therapies (Alese *et al*., 2014; Olorunnisola *et al*., 2017). However, the stem of *S. jollyanum* has not been well characterised chemically, even though different parts of a plant often contain unique compounds with distinct pharmacological potential. This knowledge gap makes further investigation into the stem's composition necessary.

Phenolic compounds are among the most important phytochemicals in plants due to their strong antioxidant properties. These molecules typically consist of aromatic rings structure bearing hydroxyl or methoxy groups, and they have the ability to donate hydrogen atoms, donate electrons, and scavenge free radicals (Shahidi and Ambigaipalan, 2015). Beyond phenolics, plants also contain other antioxidant-associated constituents, such as tocopherol and coenzyme Q10, selenium that contribute to their overall therapeutic potential (Liguori *et al*., 2018). Understanding the chemical composition of these compounds is essential for evaluating the medicinal value of plant materials.

Gas Chromatography-Mass Spectrometry (GC-MS) provides a sensitive analytical approach to characterize volatile and semi-volatile compounds, enabling identification of bioactive constituents that may contribute to observed pharmacological effects. The technique provides information on molecular structure, retention time, and relative abundance making it suitable for profiling bioactive constituents, including certain phenolic derivative and antioxidant-related compounds like 2,6-dimethoxyphenol and 3,4,5-trimethoxyphenol, squalene and tocopherols. Previous GC-MS studies have confirmed its effectiveness in identifying methoxy-substituted phenols and antioxidant terpeniods commonly present in medicinal plants (Wang *et al*.,2020).

In this study, GC-MS analysis was applied to the aqueous stem extract of *S. jollyanum* to determine the presence and relative amounts of phenolic compounds and other antioxidant-related constituent. Unlike conventional colourimetric assays that measure total phenolic content or overall antioxidant activity, GC-MS allows for compound-specific identification. The GC-MS analysis revealed key phenolic compounds that are also classified as antioxidant compounds - 2,6-dimethoxyphenol and 3,4,5-trimethoxyphenol. Further, the analysis also identified non-phenolic constituents, including squalene and di-alpha-tocopherol (vitamin E), that are also considered as antioxidant compound.

1.2 Justification of the Study

Medicinal plants continue to play an important role in primary healthcare, especially in many developing regions, which makes it necessary to scientifically validate their chemical and therapeutic potential. Although *S.jollyanum* is commonly used in West African traditional medicine, most existing research has focused mainly on its roots and leaves. The stem, however,

has received very little scientific attention. Since different parts of a plant can contain different phytochemicals, studying the stem may reveal additional bioactive compounds that have not yet been documented.

Identifying the specific phenolic compounds and antioxidant-related constituents in the stem is especially important, as these molecules are known to help mitigate oxidative stress and contribute to a range of pharmacological effects. Rather than relying solely on general measures of total phenolic or broad antioxidant assays, GC-MS enables precise identification and quantification of individual bioactive compounds, offering more concrete chemical evidence to support the plant's traditional use

This study is therefore warranted, as it represents a comprehensive GC-MS profiling of phenolic and antioxidant-related compounds in the aqueous extract of *Sphenocentrum jollyanum,* helping to scientifically substantiate its ethnomedicinal applications.

1.3 Aim of the Study

The aim of this study is to identify and quantify phenolic compounds and antioxidant related constituents present in the aqueous extract of *Sphenocentrum jollyanum*, using Gas Chromatography-Mass Spectrometry (GC-MS).

1.4 Objective of the Study

1. To identify the phenolic compound present in the aqueous extract of *Sphenocentrum jollyanum.*
2. To determine the antioxidant-related constituents, including non-phenolic compounds in the stem aqueous extract.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Plant: Sphenocentrum jollyanum

Sphenocentrum jollyanum Pierre is a **perennial understorey shrub** in the **Menispermaceae** family. It naturally grows in the shaded forest undergrowth of West Africa tropical rainforests, including countries such as Nigeria, Ghana, Côte d'lvoire, Cameroon, and Sierra Leone, and is commonly used in traditional medicine in these regions (Olorunnisola et.al., 2017; Akinwumi, I., and Sonibare, M. 2022). The plant is employed in the treatment of fever, malaria, gastrointestinal disorders, and other ailments, reflecting its wide-ranging ethnomedicinal applications across local communities.

Sphenocentrum jollyanum Pierre holds significant cultural and medicinal value and is known by various local names. Among the Yoruba of Southwestern Nigeria, it is referred to as **'Akerejupon',** while in Ghana it is called **'Aduro kokoo',** meaning "red medicine", reflecting its characteristic yellowish-orange root and its therapeutic importance (Olorunnisola *et al*., 2017). Nearly all parts of the plant including the root, stem, leaves, and fruits are employed in traditional medicine for a range of health conditions.

The bright yellow root of *Sphenocentrum jollyanum* is the most commonly used part in traditional medicine. It is prepared and administered to treat conditions such as fever, hypertension, muscular pains, wound and as an aphrodisiac (Olorunnisola *et al*., 2017; Nafui, 2008;). In addition, the leave and stem are utilized by folk healers to manage inflammatory dissorders and other chronic ailments (Uka *et al*., 2021; Akinwumi, I., and Sonibare, M. 2022). The extensive use of all plant parts in ethnomedicine continues to drive scientific research into its pharmacological potential.

Recent scientific investigations have validated and expanded these traditional applications. For example, root extracts have been shown to reduce blood glucose in diabetic animal models, while root and leaf extracts exhibit strong antioxidant activity both in vitro and in vivo (Adeleke *et al*., 2024). The leaf extract demonstrates anti-inflammatory, mitochondrial-protective, and apoptosis-modulating effects in diabetic models (Omoyajowo *et al*., 2025). The stem bark has

been reported to possess antioxidant and anti-angiogenic properties (Nia *et al*., 2004). Furthermore, studies have documented the protective effects of leaf extracts on pancreatic beta-cell morphology in alloxan-treated rabbits (Mbaka and Adeyemi, 2012).

Sphenocentrum jollyanum typically reaches about 1.5 m in height and has few branches, growing under the shade of West Africa forests (Olorunnisola, *et al*., 2017). Its leaves are wedge-shaped, shaped, smooth, and can grow up to 20 cm long, tapering to a small, arrow-like point (Olorunnisola *et al*., 2017). The fruit forms in clusters, each berry being fleshy and containing a single large seed; when fully ripened, the fruit turns bright yellow to orange colour (Olorunnisola *et al*., 2017). These characteristics help distinguish *S. jollyanum* from other rainforest shrubs.

Phytochemical investigations show that *Sphenocentrum jollyanum* harbours a variety of secondary metabolites, including alkaloids, phenolics, tannins, flavonoids, steroids, and terpenoids (Olorunnisola, *et al*., 2017; Ekpono *et al*., 2018). Among the compounds isolated from the plant are diterpenes such as columbin, isocolumbin, fibleucin, atrotosterone A and 20-hydroxyecdysone (Akinwumi, I., and Sonibare, M. 2022). These bioactive constituents likely contribute to the pharmacological efficacy of the plant.

Research on *Sphenocentrum jollyanum* has shown that its extracts exhibit several biological activities, including anti-inflammatory, antioxidant, properties (Uka *et al*., 2021; Mbaka and Owolabi, 2011; Mbaka *et al*., 2019). These experimentally demonstrated properties support many of the plant's documented uses in traditional medicine across West Africa. However, while some plant parts have been extensively examined, others-such as the stem – have received comparatively limited scientific attention, indicating the need for further phytochemical studies.

2.1.1 General Information

The Scientific name is *Sphenocentrum jollyanum* Pierre.

The Common names or vernacular names are Akerejupon (Yoruba, Nigeria), Adurokokoo or "red medicine" (Ghana)

It belongs to the Family of **Menispermaceae.**

The Plant is a Perennial, evergreen shrub (often ~1-1.5 m tall) that grows naturally in humid, shaded parts of tropical forest environments.

Planting or flowering months: *Sphenocentrum jollyanum* flowers and bears fruit either irregularly or continuously throughout the year.

Origin or native range: Originate from the tropical forest zone of West Africa – recorded in Nigeria, Ghana, Côte d'Ivoire, Cameroon, Sierra Leone and neighbouring countries.

Availability: The plant is available in West Africa

2.1.2 Taxonomical classification

Kingdom – Plantae

Phylum – Streptophyta

Class – Equisetopsida

Subclass – Magnoliide

Order – Ranunculales

Family – Menispermaceae

Genus – Sphenocentrum

Species – *Sphenocentrum jollyanum*

2.1.3 Description of the plant (morphological and ethnobotanical)

The roots are bright yellow roots, widely recognized as the most commonly used part of the plant. Frequently studied for phytochemicals and bioactivity

The Stem, in its early stage is covered with fine short hairs, which becomes hairless as it matures, becoming smooth.

Leaves are described as wedge-shaped leave, about 5-12 cm wide, that can grow up to 20 cm long having a small-arrowed apex. The leave is smooth on both side and are often noted to be adapted to shaded tropical forest environments.

Flowers are generally solitary, regular; the arrangement of the sepals are more or less spiral arrangement and unisexual (male and female separate). It is of the family of **Menispermaceae**.

Fruits are fleshy, orange-yellow in colour and edible when ripe. The fruit has an ovoid-ellipsoid shape.

The Seed usually consist of one large seed enclosed within the fleshy fruits and it has an oval-shape.

The Habitat for this species is the humid tropical rainforest zones. The species grows in a moist, shaded forest locations.

The Distribution for *Sphenocentrum jollyanum* is to the West African rainforest belt. It is reported in countries like Nigeria, Ghana, Cameroon, Côte d'lvoire and neighbouring regions.

The plant is used traditionally for the treatment of fever, malaria, hypertension, wounds, digestive problems, and reproductive health.

The Phytochemical contained in the plant includes alkaloids (palmatine, jatrorrhizine), saponins, flavonoids, tannins, terpenoids, phenolic compounds.

The studies of the plant show its Pharmacological activities or Bioactivity and this includes antioxidant, anti-inflammatory, antimicrobial, antimalarial, antidiabetic, and aphrodisiac properties. These findings are gotten from the result of both animal (in vivo) and in-vitro experiments.

2.1.4 Botanical Description of *Sphenocentrum jollyanum*

Sphenocentrum jollyanum Pierre (Menispermaceae) is a small, evergreen understorey shrub native to the dense rainforests of West Africa. It typically grows to about 1.5 m in height, with sparse branching. Its leaves are alternate and wedge-shaped (cuneate), ranging from approximately 5-12 cm in width and up to around 20 cm in length; both surfaces of the leaves are smooth, and the apex is somewhat arrow-shaped.

The flowers are unisexual, and they tend to appear singly on older branches or along the stem between leaves. Some sources describe the sepals as being spirally arranged. Fruit development results in clusters of fleshy fruits that are ovoid-ellipsoid in shape. When ripe, the fruits turn bright yellow to orange, and each fruit contains a single large, oval seed.

The roots of *Sphenocentrum jollyanum* are notably bright yellow and have a sour, acidic taste; in traditional medicine, consumption of the root is said to make subsequent foods taste sweet. Ecologically, the plant thrives most in moist, shaded forest habitats, occurring in the understorey of tropical rainforests across West African countries such as Nigeria, Ghana, Côte d'lvoire, Sierra Leone, and Cameroon

Figure 2.1 – *Sphenocentrum jollyanum* Seed (from Olorunnisola *et al*., 2017).

Figure 2.2 – *Sphenocentrum jollyanum* Leaves (from Olorunnisola *et al*., 2017)

2.1.5 Ethnomedicinal Uses of *S. jollyanum*

Sphenocentrum jollyanum is well recognised in many West African communities for its wide range of traditional uses. It is used as a multipurpose medicinal plant, with different parts of the plant applied depending on the illness being treated. The **root** is the most commonly used and is prepared in various forms to manage fever, malaria, constipation, hypertension, wounds and issues related to sexual weakness (Nafui 2008; Olorunnisola *et al*., 2017). The **leaves** are also

used traditionally for relieving inflammation, treating malaria, and also involving in antimicrobial activities (Akinwumi and Sonibare, 2022). In some local practice, the plant is additionally employed as a purgative and managing symptoms such as cough, menstrual pain and certain forms of swelling; inflammatory conditions. These long-standing uses highlight the medicinal relevance of the plant and support the need for further scientific investigation into its bioactive components.

2.1.6 Biological and Pharmacological activities

Previous research has shown that *S. jollyanum* exhibits a wide range of biological and pharmacological activities. These include antidiabetic, antioxidant, hepatoprotective, anti-inflammatory, antimalarial, anti-allergic, antimicrobial, and antidepressant effects. The plant has also been reported to possess gastroprotective and hematological benefits and is used in managing benign prostatic hyperplasia (BPH) (Akinwumi and Sonibare, 2022).

2.1.6.1Antioxidant Activity

Several studies have demonstrated the antioxidant potential of *Sphenocentrum jollyanum*, highlighting differences across plant parts and extract types. A study done reported that the methanol stem extract effectively scavenged superoxide and hydrogen peroxide radicals, recording IC_{50} values of 13.11 µg/mL and 30.0 µg/mL, respectively. These results were comparable to ascorbic acid, which showed slightly higher IC_{50} values of 15.34 µg/mL and 35.44 µg/mL for the same assays (Olorunnisola *et al*., 2011).

Earlier evaluations using the 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging assay revealed varying levels of activity among plant parts (Nia *et al*., 2004). The leaf extract showed relatively weak antioxidant action (half-maximal inhibitory concentration IC_{50} = 4.35 µg/mL), followed by the root bark (IC_{50} = 3.50 µg/mL). In contrast, the chloroform fraction of the stem bark demonstrated the strongest activity, with an IC_{50} of 1.54 µg/mL, though still less potent than the reference antioxidant ascorbic acid (IC_{50} = 0.80 µg/mL).

Further evidence of its antioxidant capacity was provided when the ethanol leaf extract was examined alongside its phytochemical profile and acute toxicity (Uka *et al*., 2020). The extract contained alkaloids, saponins, tannins, flavonoids, and cardiac glycosides, with total phenolic, flavonoid, and gallic acid equivalents of 31.49, 29.98, and 1215.80 mg/mL, respectively. Toxicity tests in albino mice indicated a median lethal dose above 4743.42 mg/kg b.w., suggesting low toxicity. Antioxidant assays showed IC_{50} values of 164.214, 72.410, and 167.202 mg/mL for DPPH scavenging, ferric reducing power, and iron chelation, respectively, all of which outperformed ascorbic acid under the same conditions (IC_{50} = 248.081, 155.134, and 270.703 mg/mL) (Uka *et al*., 2020).

Overall, these studies indicate that *S. jollyanum*, particularly its leaves and stem, contains bioactive compounds capable of exerting antioxidant effects through different mechanisms, supporting its traditional medicinal applications.

2.1.6.2 Antidiabetic Activity

Research on the petroleum ether seed extract of *S. jollyanum* has shown that it possesses measurable antidiabetic effects in both hyperglycemic and alloxan-induced diabetic rabbits. A research done reported that administering 1 g/kg body weight (b.w.) of the extract to hyperglycemic animals lowered blood glucose by 20%, whereas the standard drug glibenclamide (10 mg/kg b.w.) produced a greater reduction of 43.8% relative to untreated controls (Mbaka *et al*., 2010).

In diabetic rabbits induced with alloxan, the extract continued to demonstrate activity, with significant ($p < 0.05$) decreases in blood glucose observed from the third day of treatment onward. The response was dose-dependent: extract doses of 300, 600, and 1200 mg/kg b.w. achieved maximum glucose reductions of 12.3%, 29.2%, and 32.7%, respectively. As expected, the glibenclamide-treated group exhibited the strongest effect, with a 51.9% decline in blood glucose levels (Mbaka *et al*., 2010).

2.1.6.3 Anti-inflammatory Activity

The anti-inflammatory properties of *Sphenocentrum jollyanum* have been demonstrated across several in vivo and in vitro studies (Moody *et al*., 2006). A research was carried to investigate methanol crude extracts using the carrageenan-induced hind paw oedema model in albino rats (Moody *et al*., 2005). At 200 mg/kg, the methanol fruit extract produced 79.58% inhibition of edema, outperforming the root extract, which showed 53.75% inhibition at the same dose. The standard reference drug, acetylsalicylic acid (100 mg/kg), achieved 72.5% inhibition. Further purification of the fruit extract yielded three clerodane diterpenoids—columbin, isocolumbin, and fibleucin. Among these, columbin exhibited the greatest activity, producing 67.08% inhibition at a dose of 20 mg/kg ($p < 0.05$).

Complementary in vitro evidence was provided when the leaf extract was accessed using membrane stabilization, proteinase inhibition, and anti-lipoxygenase of red blood cell (Samuel *et al*., 2018). Both the aqueous extract and the saponin-rich fraction showed strong dose-dependent erythrocyte membrane stabilization. The aqueous extract also demonstrated more pronounced lipoxygenase inhibition (IC_{50} = 637 µg/mL) compared to diclofenac (IC_{50} = 52 µg/mL). In the proteinase inhibition test, the ethanol and tannin-rich fractions were most effective, with IC_{50} values of 840 and 1810 µg/mL, respectively, while indomethacin exhibited an IC_{50} of 246 µg/mL.

Overall, these findings validate the traditional use of *S. jollyanum* for inflammatory conditions and suggest that its activity is closely linked to its phytochemical constituents.

2.1.6.4 Anti-Malarial Activity

During the course of a research it was demonstrated that methanol extracts from the leaves and roots of *S. jollyanum* possess notable anti-plasmodial properties (Olorunnisola, and Afolayan, 2011). Their study used Swiss albino mice infected with chloroquine-resistant *Plasmodium berghei* NK67, and both extracts produced significant, dose-dependent suppression of parasitemia when administered either alone or in combination. These treatments also improved survival outcomes in the infected mice.

At a dose of 200 mg, the leaf extract produced 74.7% inhibition of parasite growth, while the root extract achieved 54.1% inhibition. In comparison, the reference drug artemether–

lumefantrine (5 mg) recorded an inhibition of 81.4%. Beyond parasitemia reduction, the extracts also promoted favorable changes in body weight and hematological indices.

Overall, these findings support the ethnomedicinal use of *S. jollyanum* for malaria treatment and indicate that its extracts are active even against chloroquine-resistant strains.

2.1.6.5 Anti-Allergy Activity

Allergic conditions—including eczema, asthma, allergic rhinitis, and inflammatory bowel disease—result from exaggerated immune responses to non-infectious triggers and affect more than 300 million people worldwide, with approximately one in every 250 deaths attributed to allergy-related complications (Olorunnisola *et al*., 2017)**.** In their investigation of *S. jollyanum*, they evaluated the anti-allergic effects of the fruit extract using a mouse model of milk-induced eosinophilia and leukocytosis (Olorunnisola *et al*., 2017)**.**

The ethanol fruit extract produced a dose-dependent decrease in eosinophil and lymphocyte levels, a pattern similar to that seen with dexamethasone (Olorunnisola *et al*., 2017)**.** These outcomes indicate that the plant's anti-allergic activity may operate through multiple mechanisms associated with its diverse phytochemical constituents **(**Olorunnisola *et al*., 2017)**.**

2.1.6.6 Haematological Activity

A study done using ethanol root extract of *S. jollyanum* on *P. berghei*–infected mice, treatment at doses of 200, 400, and 800 mg/kg body weight produced a **dose-dependent and significant ($p < 0.05$)** restoration of PCV, hemoglobin, RBC and WBC counts — all of which had been significantly depressed by the infection. The highest dose (800 mg/kg) restored these parameters to levels comparable with the standard control group (Ekpono *et al*., 2019).

This finding and other studies into haematological activities of *S. jollyanum* lend support to the potential of *S. jollyanum* root extract in alleviating anaemia associated with malaria infection.

2.1.7 Phytochemistry of *Sphenocentrum jollyanum*

Studies of *S. jollyanum* have revealed that the plant harbors a variety of bioactive secondary metabolites as well as essential oils — findings that help explain many of its reported pharmacological effects **(**Olorunnisola *et al*., 2017**)**. Phytochemical screening of methanol extracts from the stem bark of *S. jollyanum* indicated the presence of terpenes (terpenoids), saponins, alkaloids, and tannins (Olorunnisola *et al*., 2017**)**. Similarly, analysis of the root — especially via ethanol extracts — has shown that terpenoids, flavonoids, alkaloids and other phenolic compounds are among the dominant chemical constituents (Olorunnisola *et al*., 2017).

Gas chromatography–mass spectrometry (GC-MS) analysis of essential oil obtained from the root of *S. jollyanum* identified 19 chemical constituents (Olorunnisola *et al*., 2017**)**. These compounds include monoterpenoids and sesquiterpenoids — with the overall oil composition comprising roughly **33.5% monoterpenoids**, **56.3% sesquiterpenoids**, and about **10.2% of constituents remaining unidentified**. Among the identified compounds are **α-pinene, globulol, guaiene-11-ol, α-eudesmol, camphene, p-cymene, and δ-3-carene**, among others (Olorunnisola *et al*., 2017)**.**

Proximate-analysis data for *S. jollyanum* seed extracts indicate that the seeds contain substantial nutritional constituents — including protein (48.09%), fat (9.65%), carbohydrate (16.79%), ash (3.26%), fiber (5.51%), and moisture (16.70%) (Olorunnisola *et al*., 2017)**.** This suggests that beyond pharmacologically active secondary metabolites, the plant's seeds may offer nutritive value as well. From the seeds of *S. jollyanum*, researchers have successfully isolated a clerodane-type furano-diterpene, **columbin** (named "DO6" in the original study), using vacuum-liquid chromatography (VLC) followed by HPLC purification. This isolation confirms that *S. jollyanum* contains structurally-defined diterpenes, which may contribute to some of its observed biological activities.

2.2 Phenolic Compounds and Their Importance

2.2.1 Definition and Classification of Plant Phenolics

Phenolic compounds are a diverse group of secondary metabolites widely distributed in plants, they are characterised by at least one aromatic ring bearing one or more hydroxyl groups (Balasundram *et al*., 2006). They play important roles in plant growth and reproduction, offering

defense against pathogen and herbivores, additionally, they influence the colour and sensory qualities of fruits and vegetables (Balasundram *et al*., 2006). Plant phenolics are broadly classified into phenolic acids, flavonoids, tannins, stilbenes and lignins, with flavonoids and phenolic acids being the most commonly encountered in plant extracts (Shahidi and Yeo, 2018). These compounds occur in both free and bound forms, depending on how they are linked within the plant matrix, which influences their extractability and total measurable content (Zhenyu Wang *et al*., 2020).

2.2.2 Biological Significance of Phenolics

Phenolic compounds are recognised for their strong antioxidant properties, which primarily arises from their ability to neutralize reactive species through free-radical scavenging and by chelating pro-oxidant metal ions (Soobrattee *et al*., 2005). In a study it is seen that polyphenol which is a phenolic compound that contains multiple phenol structural unit with antioxidant properties reduce oxidative damage caused by reactive oxygen species (ROS) in biological systems, contributing to the protective role phenolics play in plant defense and animal health (Stiller *et al*., 2021). Because oxidative stress is associated with chronic illnesses, including cancer, diabetes, and inflammatory disorders, phenolics have gained significant scientific interest for their potential protective effects (Stiller *et al*., 2021). Therefore, quantifying phenolic content in medicinal plants provides insight into their health-promoting properties and supports scientific validation of their ethnomedicinal uses.

2.3 Extraction of Phenolic Compounds in Plants

2.3.1 Extraction Methods with Emphasis on Aqueous Solvents

Extraction is a critical step in determining the phenolic composition of plant materials. Phenolics are generally polar compounds (owing to their hydroxyl and other polar functional group) and are best extracted using polar solvents such as water, ethanol, methanol or their aqueous mixtures (Mohammed *et al*., 2022). Although organic solvents often yield higher amounts of phenolics, aqueous extraction remains relevant where traditional practices involve water-based preparations such as decoctions or infusions. In many plants, phenolic compounds exist in both free (soluble) and bound (insoluble) forms; bound phenolics are often covalently linked to cell wall polymers

and are not efficiently released by simple aqueous extraction alone and to release bound phenolic compound, hydrolysis is frequently applied under controlled conditions (Shahidi and Yeo, 2016). This has implications that, as the aqueous extract of *Sphenocentrum jollyanum* stem may not reflect the full phenolic potential of the plant, yet it represents the form consumed traditionally.

2.3.2 Factors Affecting Extraction

Extraction efficiency values are influenced by solvent to sample ratio, extraction time, temperature, the solvent type and the number of repeated extractions of the sample (Khoddami *et al*., 2013). Studies have shown that base hydrolysis treatments increase measurable phenolics by releasing bound forms, whereas mild extraction conditions may underestimate total content (Qinzhang jiang *et al*., 2023).

2.4 Antioxidant-Related Constituent

2.4.1 Antioxidants Functions

Antioxidants are substances that can delay or prevent oxidative damage to biomolecules by scavenging free radicals or reactive oxygen species. Phenolic compounds exhibit antioxidant properties through several mechanisms, including radical scavenging, metal chelation and prooxidant activities (Dia and Muimper, 2010).

2.4.2 Antioxidant-Related Non-Phenolic Compounds in Plants

In many plant extracts, phenolic compounds as well as non-phenolic compounds contribute substantially to antioxidant activity. For example, non-phenolic compounds like

- Tocopherol which is a derivative of vitamin E is a lipid-soluble molecule that has antioxidants function that play a role in protecting the cell membrane by scavenging lipid-derived radicals and preventing oxidative damage to membrane lipids (Brigelius-Flohé and Traber,1999).
- Squalene which is a triterpenoid hydrocarbon has been reported to exhibit antioxidant effects. In eukaryotic cells, squalene plays essential roles as an intermediate within the

sterol-forming pathway and its production is carried out the squalene synthase, a membrane bound enzyme in the endoplasmic reticulum (Micera *et al*., 2020).

2.5 GC-MS Analysis of phytochemicals

Gas Chromatography-Mass Spectrometry (GC-MS) is a widely employed technique for analyzing and identifying various phytochemicals in plant extracts, along with their structural characteristics. Its strong separation capability ensures highly precise and accurate chemical profiling. Furthermore GC-MS, in conjunction with mass spectral databases, can generate quantitative data that are crucial for establishing links between bioactive compounds and their pharmacological uses (Thamer and Thamer, 2023).

2.6 Previous GC-MS Studies on *Sphenocentrum jollyanum*

Several studies on *Sphenocentrum jollyanum* have analyzed the chemical composition of its roots and leaves, often using methanolic or ethanolic extraction methods. In such analyses, researchers have reported the presence of terpenes, alkaloids, flavonoid, phenolic derivatives (Alese *et al*., 2014).

However – as far as the literature reveals there is no published GC-MS analysis of the stem, especially for aqueous extract (water-based) extracts of *S. jollyanum.* Most existing studies focus on non-polar or semi-polar solvents rather than water. Consequently, the composition of water-soluble constituents in the stem remains largely uncharacterized – representing a clear gap in the phytochemical profiling of this species.

Thus, a GC-MS investigation targeting the aqueous stem extract of *S. jollyanum* would likely yield novel and valuable data, particularly about antioxidant-related phenolic compounds, and non-phenolic compound with antioxidant constituent.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

This study was carried out using materials, apparatus, equipment, and chemical reagents obtained from the Medical Biochemistry Laboratory, University of Benin. All materials were confirmed to be in proper working condition and suitable for experimental use at the time of analysis.

3.1.1 Apparatus

- Weighing balance (B. Bran Scientific, 80-2, England)
- Weighing boat
- Volumetric flasks (Technico, England)
- Measuring cylinders (Pyrex, England)
- Pestle and mortar
- Beakers (Pyrex, England)
- Test tubes (Pyrex, England)

- Cotton wool
- Gloves
- Funnels
- Glass rod
- Cheese cloth
- Sieve
- Bowls
- Knife (for cutting the stem)
- Universal bottles
- Spatula
- Permanent markers

3.1.2 Equipment

- Rotary evaporator
- Freeze dryer
- Freezer
- Mechanical grinder
- GC-MS system (AOC-20i autosampler, GC-2010, GCMS-QP2010)

3.1.3 Chemical Reagents

- Distilled water
- Absolute ethanol (British Drug House)

3.2 Methods

3.2.1 Collection and Identification of Plant Material

The stem of *Sphenocentrum jollyanum* was purchased from Iwoh Market in Osun State, Nigeria. The sample was transported to the University of Benin for proper botanical identification.

Authentication was carried out at the Herbarium Unit, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin. The plant was confirmed as *Sphenocentrum jollyanum*, commonly known as **Akerejupon** in the Yoruba language, belonging to the family **Menispermaceae**, with voucher number **UBH-S449**.

3.2.2 Preparation of Plant Sample

After authentication, the stem was taken to the Medical Biochemistry Laboratory, University of Benin, for processing. The stem was manually broken into smaller pieces using a knife and washed thoroughly under running water to remove dirt and surface impurities. The cleaned stem was air-dried at room temperature until adequately dehydrated. It was then pulverized using a mechanical grinder to obtain a fine powdered sample. The powder was stored in a labeled universal bottle and kept under laboratory conditions until extraction.

3.2.3 Extraction Procedures

Two extraction methods were used: **aqueous extraction** and **ethanolic extraction**. All procedures were carried out using standard laboratory materials including gloves, funnels, beakers, cheese cloth, bowls, sieves, measuring cylinders, volumetric flasks, spatula, glass rods, and cotton wool.

A. Aqueous Extraction

A total of **0.15g** of the powdered stem was soaked in **1 L** of distilled water in a polypropylene bowl. The mixture was stirred thoroughly with a glass rod every 4 hours and kept covered after each stirring. The sample was left to stand for **72 hours and stirred at every 2 hours interval** to enhance solvent penetration and facilitate phytochemical diffusion.

After maceration, the mixture was filtered using a sieve to remove coarse residues, followed by filtration through cheese cloth to produce a clearer extract. The resulting aqueous extract was collected into clean beakers and transferred into labeled universal bottles.

B. Ethanolic Extraction

Another **0.15g** of the powdered stem was soaked in **1 L** of absolute ethanol. Stirring was performed at 2-hour intervals, and the mixture was covered after each stirring. The extraction was allowed to proceed for **72 hours** to enhance solvent penetration and facilitate phytochemical diffusion.

Following maceration, filtration was done using a sieve, and cheese cloth, similar to the aqueous extract. The final ethanolic filtrate was poured into clean, labeled universal bottles.

3.2.4 Concentration of the Extracts

The filtered **ethanolic extract** was concentrated using a rotary evaporator operated under controlled temperature and pressure to remove ethanol. The **aqueous extract**, which did not require solvent evaporation, was taken directly for freeze-drying.

Both concentrated extracts were stored in a freezer at low temperature before lyophilization.

3.2.5 Freeze-Drying (Lyophilization)

The concentrated aqueous and ethanolic extracts were taken to the National Center for Energy and Environment, Energy Commission of Nigeria, University of Benin, for freeze-drying. The freeze-drying process involved the use of a system called Freeze-dryer consisting of:

- Drying chamber
- Condenser
- Vacuum system
- Refrigeration system
- Control system

Freeze-drying procedure:

1. The concentrated extracts were transferred into labeled universal bottles.
2. The samples were frozen in a freezer until completely solid.

3. The frozen samples were placed into the freeze dryer.
4. Under vacuum, sublimation occurred, where frozen water or solvent evaporated directly from solid to vapor.
5. The resulting dry extracts were collected as light, dry powders.
6. The powders were sealed, labeled appropriately (aqueous or ethanolic), and stored for further analysis.

The freeze-dried extracts were transported to **LEEDEX Laboratories, Lagos State, Nigeria**, for GC-MS analysis.

3.2.6 Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

Gas Chromatography–Mass Spectrometry (GC-MS) is an advanced analytical technique that separates compounds based on their volatility (GC) and identifies them using their mass-to-charge ratio (MS). This technique is widely applied in the identification of phytochemicals in plant extracts.

At LEEDEX Laboratories, the aqueous and ethanolic extracts were analyzed using the following instruments:

- **AOC-20i autosampler**
- **GC-2010 gas chromatograph**
- **GCMS-QP2010 mass spectrometer**

Figure 3.1- Gas Chromatography - Mass Spectrometry – QP2010 SE

(from Shimadzu Excellence in Science)

A. GC-MS Parameters for the Aqueous Extract

Autosampler (AOC-20i) Conditions

- Presolvent rinses: 3
- Post-solvent rinses: 3
- Sample rinses: 2
- Plunger suction speed: High
- Viscosity compensation time: 0.2 sec
- Plunger injection speed: High
- Syringe insertion speed: High
- Injection mode: Normal
- Pumping times: 5
- Injection port dwell time: 0.3 sec
- Terminal air gap: None
- Plunger washing speed: High
- Washing volume: 8 µL
- Syringe suction and injection positions: 0.0 mm
- Solvent vial used: 1 vial

GC-2010 Conditions

- Column oven temperature: 60°C
- Injection temperature: 250°C
- Injection mode: Splitless
- Sampling time: 1.00 min
- Flow control mode: Pressure
- Pressure: 100 kPa
- Total flow: 4.7 mL/min
- Column flow: 0.80 mL/min
- Linear velocity: 23.1 cm/sec

- Purge flow: 3.0 mL/min
- Split ratio: 1.1
- Carrier gas saver: Off

Oven temperature program:

- 60°C for 1.00 min
- Increase at 13°C/min to 240°C, hold 1.00 min
- Increase at 13°C/min to 300°C, hold 39.70 min

GC-MS-QP2010 Conditions

- Ion source temperature: 230°C
- Interface temperature: 250°C
- Solvent cut time: 4.00 min
- Detector gain mode: Relative
- Detector gain: 1.33 kVs
- Threshold: 2000

MS Scan Table

- Start time: 8.00 min
- End time: 59.80 min
- Acquisition mode: Scan
- Event time: 0.30 sec
- Scan speed: 1666
- m/z range: 35–500

B. GC-MS Parameters for the Ethanolic Extract

Autosampler (AOC-20i) Conditions

- Presolvent rinses: 3
- Post-solvent rinses: 3

- Sample rinses: 2
- Plunger suction speed: High
- Viscosity compensation time: 0.2 sec
- Plunger injection speed: High
- Syringe insertion speed: High
- Injection mode: Normal
- Pumping times: 3
- Injection port dwell time: 0.3 sec
- Terminal air gap: None
- Plunger washing speed: High
- Washing volume: 8 µL
- Syringe suction and ejection positions: 0.0 mm
- Solvent vial used: 1 vial

GC-2010 Conditions

- Column oven temperature: 60°C
- Injection temperature: 250°C
- Injection mode: Splitless
- Sampling time: 1.00 min
- Flow control mode: Pressure
- Pressure: 100 kPa
- Total flow: 47 mL/min
- Column flow: 0.80 mL/min
- Linear velocity: 23.81 cm/sec
- Purge flow: 3.0 mL/min
- Split ratio: 1.1

Oven temperature program

- 60°C for 1.00 min
- Increase at 13°C/min to 240°C, hold 1.00 min

- Increase at 13°C/min to 300°C, hold 39.70 min

GC-MS-QP2010 Conditions

- Ion source temperature: 230°C
- Interface temperature: 250°C
- Solvent cut time: 4.00 min
- Detector gain mode: Relative
- Detector gain: 1.33 kVs
- Threshold: 2000

MS Scan Table

- Start time: 8.00 min
- End time: 59.80 min
- Acquisition mode: Scan
- Event time: 0.30 sec
- Scan speed: 1666
- m/z range: 35–500

3.2.7 Compounds Identified from GC-MS Analysis

- **Aqueous extract:** 33 compounds were identified.

    1. 1,3-propanediol
    2. Phenol, 2,6-dimethoxy-
    3. Methyl(methyl 2-O-acetyl-3,4-di-O-methyl-al)
    4. Phenol, 3,4,5-trimethoxy-
    5. Inositol, 1-deoxy-
    6. alpha-Methyl mannofuranoside
    7. Phytol, acetate
    8. Hexadecanoic acid, methyl ester
    9. 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien

10. Hexadecanoic acid, ethyl ester
11. 9-Octadecenoic acid (Z)-, methyl ester
12. Methyl stearate
13. 2,8,2-Trioxa-5-aza-1-silabicyclo(3,3,3)undeca
14. Ethyl Oleate
15. Octadecanoic acid, ethyl ester
16. Hexadecanamide
17. 1-Buten-1-amine, N,N-dipropyl-
18. Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester
19. Eicosanoic acid, methyl ester
20. Octadecanoic acid, 10-hydroxy-, methyl ester
21. Octadecanoic acid, 3-hydroxypropyl ester
22. Cyclononasiloxane, octadecamethyl-
23. 9-Octadecenamide, (Z)-
24. 2-Naphthalenemethanol, decahydro-alpha...al
25. Octanoic acid, 2-dimethylaminoethyl ester
26. 3-Cyclopentylpropanoic acid, 2-dimethylaminoethyl ester
27. 13-Octadecenal, (Z)-
28. Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester
29. Di-n-octyl phthalate
30. 16-Trimethylsilyloxy-9-octadecenoic acid, methyl ester
31. 1,3,5-Trisilacyclohexane
32. Squalene
33. di-alpha.-Tocopherol

- **Ethanolic extract:** 34 compounds were identified.

1. Octanoic acid, ethyl ester
2. Cyclohexasiloxane, dodecamethyl-
3. 3-Butoxy-1,1,7,7,7-hexamethyl-3,5,5-trist(trimethylsiloxy)tetrasiloxane
4. Cyclooctasiloxane, hexadecamethyl-
5. Dodecanoic acid, ethyl ester

6. Cyclohexasiloxane, dodecamethyl-
7. Tetradecanoic acid, ethyl ester
8. Phytol, acetate
9. Hexadecanoic acid, methyl ester
10. 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien
11. Hexadecanoic acid, ethyl ester
12. 9-Octadecenal, (Z)-
13. d-Mannitol, 1-decylsulfonyl
14. 9-Octadecenoic acid (Z)-, methyl ester
15. Methyl stearate
16. 2,8,9-Trioxa-5-aza-1-silabicyclo(3,3,3)undeca
17. (E)-9-Octadecenoic acid ethyl ester
18. Octadecanoic acid, ethyl ester
19. Cyclononasiloxane, octadecamethyl-
20. 1-Buten-1-amine, N,N-dipropyl-
21. Hexadecanoicacid,1-[(2-aminoethoxy)hydroxyphosphinyl]oxy-3-octadecoxypropan-2-yl ester
22. 10-Undecenoic acid, methyl ester
23. Docosanoic acid, ethyl ester
24. Cyclononasiloxane, octadecamethyl-
25. 9-Octadecenamide, (Z)-
26. 1-Naphthalenecarboxylic acid, 5-[2-(3-furanyl)ethenyl]octahydro-2-oxo-
27. Octadecanoic acid, 2,3-dihydroxypropyl ester
28. 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (Z,Z,Z)-
29. Octadecanoic acid, 2-hydroxy-1,3-propanetriyl ester
30. Di-n-octyl phthalate
31. Cyclononasiloxane, octadecamethyl-
32. Eicosanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester
33. Cyclononasiloxane, octadecamethyl-
34. Squalene

3.2.8 Identification and Estimation of Phenolic Compound with Antioxidant properties

Two compounds identified from the GC-MS analysis where phenolic compound with antioxidant properties due to their phenolic structure:

- **Phenol, 2,6-dimethoxy- (Peak 2)**
- **Phenol, 3,4,5-trimethoxy- (Peak 4)**

These phenolic constituents were used in the interpretation of the extract's phenolic composition.

To get the estimate of the phenolic compound concentration with antioxidant properties amongst the other compound identified during the GC-MS analysis the formula below is used:

$$Relative\ \%\ of\ compound = \frac{Peak\ area\ of\ compound}{Total\ peak\ area\ of\ all\ compounds} \times 100$$

The above formula is used to get the estimate of a compound relative abundance or concentration and it is also called **Area Percentage.**

3.2.9 Identification and Estimation of Non-Phenolic Antioxidant Constituent

Two non-phenolic compounds contributed significantly to the antioxidant capacity was identified in the GC-MS analysis and they include:

- **Squalene (Peak 32)**
- **di-alpha-Tocopherol (Peak 33)**

The estimation of the concentration or relative abundance of non-phenolic compound with antioxidant properties amongst other identified compound from the GC-MS analysis has the formula:

$$Relative\ \%\ of\ compound = \frac{Peak\ area\ of\ compound}{Total\ peak\ area\ of\ all\ compounds} \times 100$$

Both compounds are known for their antioxidant activity and supported the antioxidant potential of the *Sphenocentrum jollyanum* stem extract.

CHAPTER FOUR

RESULTS

4.1 GC–MS Chromatogram of the Aqueous Extract

The aqueous extract of *Sphenocentrum jollyanum* stem was examined using Gas Chromatography–Mass Spectrometry (GC–MS). The chromatogram generated from the analysis showed **33 distinct peaks**, each representing a compound that eluted at different retention times.

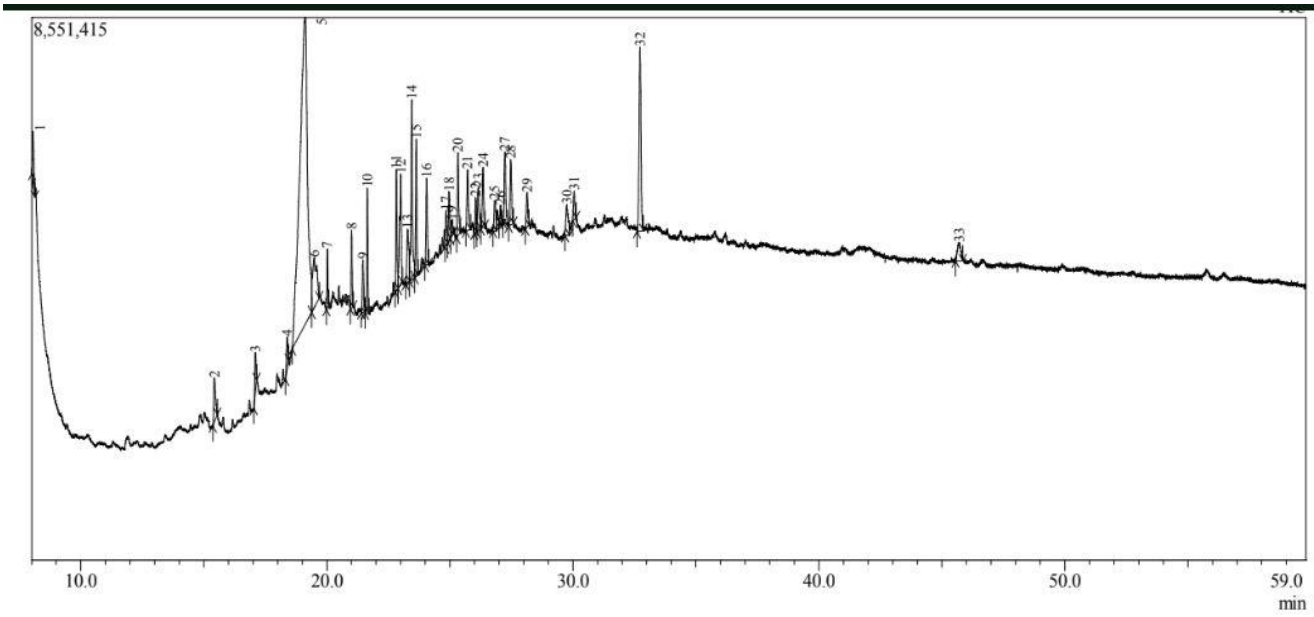

Figure 4.1- GC-MS Chromatogram of Aqueous Extracts of *Sphenocentrum jollyanum* with x-axis as Time and y-axis as Intensity Spectral represent 33 peaks representing 33 compounds

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	8.051	2827941	1.16	774289	2.06	3.65	1,3-Propanediol
2	15.434	3523796	1.44	688430	1.83	5.12	Phenol, 2,6-dimethoxy-
Peak#	**R.Time**	**Area**	**Area%**	**Height**	**Height%**	**A/H**	**Name**
3	17.098	2306675	0.94	631129	1.68	3.65	Methyl(methyl 2-O-acetyl-3,4-di-O-methyl-.al
4	18.387	2045157	0.84	508606	1.35	4.02	Phenol, 3,4,5-trimethoxy-
5	19.121	106284171	43.45	4832241	12.86	21.99	Inositol, 1-deoxy-
6	19.483	9273991	3.79	785567	2.09	11.81	.alpha.-Methyl mannofuranoside
7	20.021	2445064	1	923449	2.46	2.65	Phytol, acetate
8	21.006	3275213	1.34	1228078	3.27	2.67	Hexadecanoic acid, methyl ester
9	21.467	2960731	1.21	825561	2.2	3.59	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien
10	21.645	5685868	2.32	1972950	5.25	2.88	Hexadecanoic acid, ethyl

							ester
11	22.826	6202908	2.54	1898009	5.05	3.27	9-Octadecenoic acid (Z)-, methyl ester
12	23.004	8642725	3.53	1748191	4.65	4.94	Methyl stearate
13	23.281	4720563	1.93	836660	2.23	5.64	2,8,9-Trioxa-5-aza-1-silabicyclo(3.3.3)undeca
14	23.448	11571245	4.73	2831698	7.53	4.09	Ethyl Oleate
15	23.635	6798900	2.78	2161983	5.75	3.14	Octadecanoic acid, ethyl ester
16	24.056	4533286	1.85	1367719	3.64	3.31	Hexadecanamide
17	24.847	1970470	0.81	542901	1.44	3.63	1-Buten-1-amine, N,N-dipropyl-
18	24.967	2778847	1.14	771207	2.05	3.6	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-eth
19	25.085	1012762	0.41	237366	0.63	4.27	Eicosanoic acid, methyl ester
20	25.327	5010347	2.05	1302637	3.47	3.85	Octadecanoic acid, 10-hydroxy-, methyl ester
21	25.718	4293464	1.76	951962	2.53	4.51	Octadecanoic acid, 3-hydroxypropyl ester
22	26.046	1692526	0.69	544718	1.45	3.11	Cyclononasiloxane, octadecamethyl-
23	26.151	3277763	1.34	672622	1.79	4.87	9-Octadecenamide, (Z)-
24	26.35	3884681	1.59	949886	2.53	4.09	2-Naphthalenemethanol, decahydro-.alpha.,.al
25	26.821	2411742	0.99	479442	1.28	5.03	Octanoic acid, 2-dimethylaminoethyl ester
26	27.056	927554	0.38	297517	0.79	3.12	3-Cyclopentylpropionic acid, 2-dimethylamino
Peak#	**R.Time**	**Area**	**Area%**	**Height**	**Height%**	**A/H**	**Name**
27	27.239	4750873	1.94	1125826	3	4.22	13-Octadecenal, (Z)-
28	27.475	4428146	1.81	1025543	2.73	4.32	Octadecanoic acid, 2-hydroxy-1,3-propanediyl
29	28.139	2288537	0.94	546178	1.45	4.19	Di-n-octyl phthalate
30	29.754	3108905	1.27	488619	1.3	6.36	16-Trimethylsilyloxy-9-octadecenoic acid, me
31	30.053	1830807	0.75	441970	1.18	4.14	1,3,5-Trisilacyclohexane
32	32.731	15096402	6.17	2906208	7.73	5.19	Squalene
33	45.71	2758340	1.13	290294	0.77	9.5	dl-.alpha.-Tocopherol

Table 4.1 – GCMS-Analysis showing the 33 Compounds, Peaks, Retention.time, Area, Area Percentage, Height, Height Percentage, A/H, and Names of the Aqueous Extract of *S. jollyanum.*

4.2 Major peaks Identified in the Extract

Out of the 33 compounds detected in the GC-MS analysis of the aqueous extract of *S. jollyanum* stem four compounds with different peaks where highlighted due to their relevance to phenolic chemistry and antioxidant-related activity. These include Peaks **2**, **4**, **32**, and **33**. Their retention times and compound classifications based on the GC–MS results are described below.

Peak 2 (Retention Time ≈ 15.43 min)

This peak represents a **phenolic compound** called Phenol, 2,6-dimethoxy. Phenolic compounds are commonly associated with antioxidant activity, and Peak 2 indicates the presence of one such phenolic component in the extract.

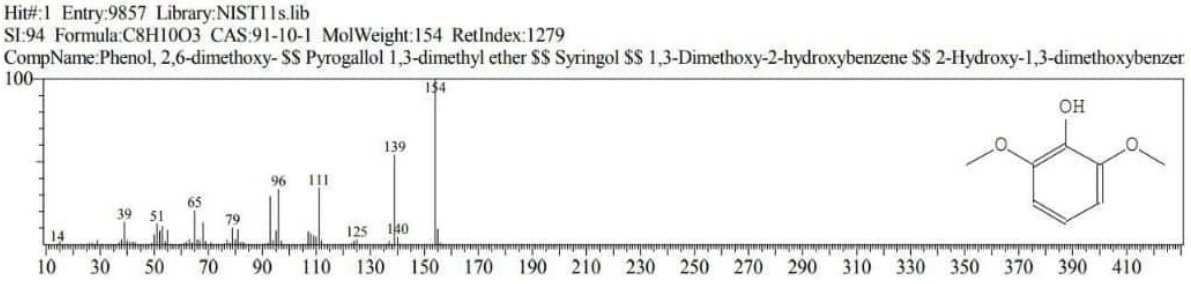

Figure 4.2 – Chromatogram Section Showing Peak 2

Peak 4 (Retention Time ≈ 18.38 min)

Peak 4 is also identified as a **phenolic compound** called Phenol, 3,4,5-trimethoxy-, confirming the presence of multiple phenolic constituents in the aqueous extract. Phenolic compounds are known contributors to natural antioxidant capacity.

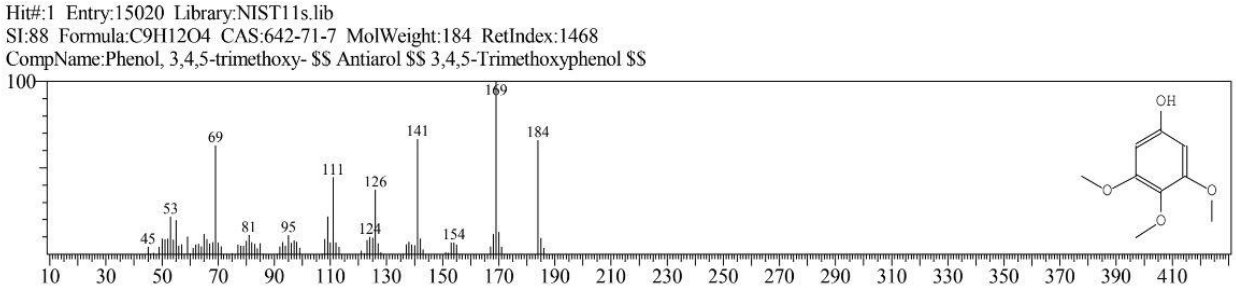

Figure 4.3 – Chromatogram Section Showing Peak 4

Peak 32 (Retention Time ≈ 32.73 min)

Peak 32 corresponds to a **non-phenolic compound** called Squalene. However, based on the GC–MS identification and your findings, the compound shows **antioxidant activity** even though it does not belong to the phenolic group.

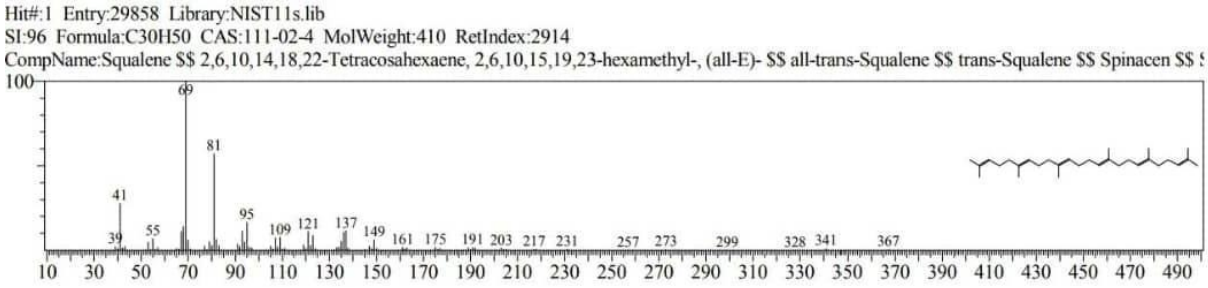

Figure 4.4 – Chromatogram Section Showing Peak 32

Peak 33 (Retention Time ≈ 33.73 min)

This peak represents another **non-phenolic compound** called di-alpha.-Tocopherol. Similar to Peak 32, it exhibits **antioxidant-related activity**, despite being classified outside the phenolic group.

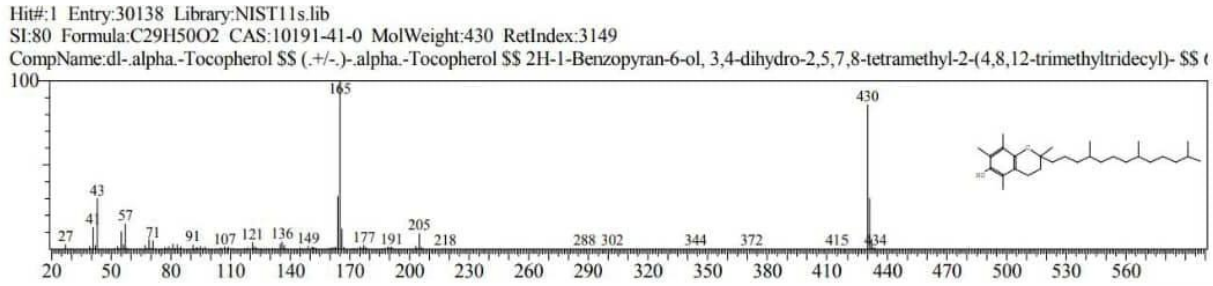

Figure 4.5 – Chromatogram Section Showing Peak 33

4.3 Summary of Relevant Peaks

Peak#	Name of Compound	R. Time	Area%	Compound Type	Antioxidant Capacity	MolWeight & Formula,	Function / Biological Activity

2	Phenol, 2,6-dimethoxy-	15.434	1.44	Phenolic	Yes	MolWeight: 154 Formula: $C_8H_{10}O_3$	Commonly known as Syringol. It is a naturally occurring aromatic compound often found in wood smoke. It has antioxidant properties and is used in the synthesis of pharmaceuticals and as a flavor/fragrance agent.
4	Phenol, 3,4,5-trimethoxy-	18.387	0.84	Phenolic	Yes	MolWeight: 184 Formula: $C_9H_{12}O_4$	Also known as Antiarol. It is a derivative of phenol found in various plants. It exhibits antimicrobial and antioxidant activities and is often studied for its potential anti-inflammatory properties.
32	Squalene	32.731	6.17	Non-phenolic	Yes	MolWeight: 410 Formula: $C_{30}H_{50}$	A triterpene that is a key precursor for the synthesis of all steroids (like cholesterol) in plants and animals. It is widely used in cosmetics for skin hydration, as an adjuvant in vaccines, and has potential chemo-preventive (anti-cancer) effects

33	di-.alpha.-Tocophero	45.710	1.13	Non-phenolic	Yes	MolWeight: 430 Formula: $C_{29}H_{50}O_2$	This is a form of Vitamin E. It is a powerful lipid-soluble antioxidant that protects cell membranes from oxidative damage. It supports immune function and skin health, and is crucial for preventing lipid peroxidation

Table 4.2 – The summary of Peak 2,4,32 and 33

4.4 Summary of GC–MS Results

The GC–MS analysis identified 33 compounds in the aqueous extract of *Sphenocentrum jollyanum* stem. Among these, Peaks 2 and 4 indicate the presence of **phenolic compounds**, while Peaks 32 and 33 correspond to **non-phenolic compounds** that also possess antioxidant activity. These four peaks represent the major antioxidant-related constituents detected in the extract.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The present study investigated the aqueous stem extract of *Sphenocentrum jollyanum* using Gas Chromatography–Mass Spectrometry (GC–MS) in order to determine the phenolic constituents and other antioxidant-related compounds present in the plant. GC–MS was selected because it is a widely accepted analytical technique capable of identifying compounds based on their mass spectra and retention characteristics, while also providing quantitative insight through peak area measurements.

A total of 33 compounds were detected in the chromatographic profile of the extract. Of these, four compounds were considered particularly important due to their known biological activities. Two of these compounds are Phenol, 2,6-dimethoxy- and Phenol, 3,4,5-trimethoxy and they were confirmed to be phenolic compounds. Phenolics are an important group of plant metabolites characterized by aromatic ring structures containing hydroxyl or methoxy substitutions. They are well documented for their ability to neutralize free radicals, chelate metal ions, and contribute to a plant's defensive mechanisms. Their presence in the stem extract confirms that *Sphenocentrum jollyanum* contains bioactive phenolic molecules, which is consistent with earlier reports indicating that the plant possesses significant phytochemical content.

Apart from the phenolics, two additional compounds which are Squalene and di-α-Tocopherol were identified as non-phenolic antioxidant-related components. Squalene, a naturally occurring triterpene, is known for its strong singlet oxygen-quenching capacity and has been associated with protection against oxidative stress in biological systems. Di-α-Tocopherol, a major form of Vitamin E, is a potent lipid-phase antioxidant that helps maintain membrane integrity by preventing lipid peroxidation. Their detection suggests that the stem extract contains antioxidant agents from multiple structural classes, not limited to phenolics.

Quantitative interpretation based on peak area percentages showed that Squalene (Peak 32) had one of the highest relative abundances, indicating it is a dominant compound in the extract. Although the phenolic compounds (Peaks 2 and 4) were present in smaller amounts, this does not diminish their biological relevance, as phenolics often exhibit strong antioxidant properties even at low concentrations. The coexistence of both phenolic and non-phenolic antioxidants points to a broad-spectrum antioxidant profile, which may help explain why the plant has traditionally been used to manage ailments associated with oxidative stress.

Overall, the GC–MS results highlight that the aqueous stem extract of *Sphenocentrum jollyanum* contains a combination of phenolic compounds and non-phenolic antioxidant-related constituents, providing chemical evidence that supports the plant's traditional medicinal applications.

5.2 Conclusion

The GC–MS analysis of the aqueous stem extract of *Sphenocentrum jollyanum* led to the identification of 33 distinct compounds, including two phenolic compounds which include Phenol, 2,6-dimethoxy- and Phenol, 3,4,5-trimethoxy and two non-phenolic antioxidant-related compounds**,** Squalene and di-α-Tocopherol. These four major bioactive constituents form a significant portion of the extract and highlight the chemical diversity present in the stem.

The findings demonstrate that the aqueous stem extract possesses important phenolic constituents**,** alongside antioxidant-active non-phenolics. This combination strengthens the scientific basis for the traditional use of *Sphenocentrum jollyanum*, particularly in managing oxidative stress and related health conditions.

To enhance the understanding of the plant's medicinal value, it is recommended that future research should:

Isolate and structurally characterize the major compounds identified,

Investigate their pharmacological properties using appropriate biological assays, and

Explore improved extraction techniques to enhance compound yield and stability.

Overall, this study provides essential GC–MS data that serve as a foundation for advancing phytochemical research, drug development, and the therapeutic application of *Sphenocentrum jollyanum*.

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