GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF FATTY ACIDS AND ESTERS IN THE AQUEOUS EXTRACT OF *SPHENOCENTRUM JOLLYANUM*

BY

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DATE

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CERTIFICATION

We the undersigned hereby certify that Miss Ikhamateh Divine Omoarukhe (BMS2101413) carried out this work, in the Department of Medical Biochemistry, University of Benin, Benin City and we approve same as adequate in scope and quality for the reward of Bachelors of Science Degree (B.Sc.) in Medical Biochemistry.

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DEDICATION

This work is dedicated to the Holy Spirit my consistent guide, and to me.

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With a heart full of joy and gratitude, I give all the glory to God for seeing me through my time in school. For his consistent guidance, provision, protection, understanding, wisdom, grace and love he has showered me, holding my hands in the dark days, I am grateful.

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ABSTRACT

Sphenocentrum jollyanum Pierre is a medicinal plant widely used across West Africa for the treatment of various ailments, yet the chemical constituents responsible for many of its reported therapeutic effects remain underexplored in scientific literature. This study aimed to identify the major fatty acids and ester compounds present in the aqueous stem extract of *S. jollyanum* using Gas Chromatography-Mass Spectrometry (GC-MS), with the goal of contributing to phytochemical profiling and supporting the plant's ethnomedicinal applications. Fresh stems were cleaned, air-dried, pulverized, and extracted by cold maceration. The resulting filtrates were freeze-dried and the crude aqueous extract was subjected to GC-MS analysis under optimized chromatographic and mass spectrometric conditions. The GC-MS scan revealed a spectrum of bioactive constituents including compounds known for antimicrobial, anti-inflammatory, antioxidant, and metabolic regulatory activities. This study provides scientific support for the medicinal uses of *S. jollyanum* and establish a biochemical basis for its reported bioactivities. It further highlights the importance of GC-MS as a robust analytical tool for identifying volatile and semi-volatile compounds in medicinal plant extracts. Overall, the results strengthen the pharmacognostic understanding of *S. jollyanum* and lay groundwork for future studies on its biological mechanisms, safety, and potential drug development applications.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The oldest kind of medicine, medicinal plants have been used in traditional medicine in many nations throughout the world for millennia. The beneficial effects of these plants were empirically known and passed down through generations of people. Natural products serve as a crucial source of medicinal chemicals, and at present, several contemporary medications used in modern pharmacotherapy are based on traditional herbal medicine. In order to investigate the bioactive compounds from plant sources, the extraction process is an essential step. Phytochemical research was greatly enhanced by the creation of sophisticated technologies for the qualitative and quantitative analysis of phytochemicals, such as gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC). However, the biological characteristics of many plant species that have historically been used in conjunction with their bioactive compounds have been identified up to this point. The identification of a large number of bioactive phytochemicals was made possible by the traditional bioassay-guided natural drug discovery process. Medicinal plants continue to have a promising future because the phytochemical makeup and possible health benefits of several species have not yet been investigated or need further investigation (Marrelli, 2021).

Herbal remedies are excellent tonics and body balancers that assist in controlling certain bodily functions. It can be used to promote the body's metabolic processes and provide certain additional nutrients that the body is unable to obtain because of a bad diet or environmental deficiencies in the soil (Ugwu e*t al*., 2021).

Menispermaceae is a large and varied plant family to which *Sphenocentrum jollyanum* Pierre belongs. They are well known for their many vital biological roles. *S. jollyanum* is native to the tropical forest regions of West Africa. Traditional medicine uses *Sphenocentrum jollyanum* Pierre as an aphrodisiac and as a treatment for fever, coughs, high blood pressure, breast tumor, constipation, and wounds. Anti-diabetic, anti-inflammatory, anti-bacterial, anti-viral, anti-malarial, angiogenic, and anxiogenic are just a few of the pharmacological actions.

In order to identify and quantify the chemicals found in a plant sample, gas chromatography-mass spectrophotometry (GC-MS) is a combination of analytical methods used. It is a crucial tool in the phytochemical analysis and chemotaxonomic investigation of medicinal plants with biologically active compounds. GC-MS is one of the most accurate methods for isolating and identifying different secondary metabolites found in plant extracts, such as fatty acid esters, phenyl propane, fatty alcohol, aliphatic alcohol, etc., is GC-MS (Uka *et al*., 2022).

1.2 Justification of the Study

Sphenocentrum jollyanum is widely used to treat pain, inflammation, metabolic illnesses, sexual dysfunction, and a number of chronic diseases. Despite the fact that many of its pharmacological effects have been documented, the precise bioactive chemical components that cause these effects are still not fully understood, particularly those found in its aqueous extract. Scientific verification of the plant's therapeutic uses is hampered by this knowledge gap.

Representing a significant category of bioactive phytochemicals, fatty acids and their esters are well known for a wide range of therapeutic uses, such as anti-inflammatory, antioxidant, antimicrobial, flavoring. It is now more widely accepted that these substances play a role in the pharmacological actions of several medicinal plants. The fatty acid and ester profile of

Sphenocentrum jollyanum, on the other hand, has little data at present, and there has been no systematic GC–MS analysis performed specifically on the aqueous extract of the stem.

Gas Chromatography–Mass Spectrometry (GC–MS) is still one of the most accurate and trustworthy analytical methods for detecting volatile and semi-volatile substances like fatty acids and esters in plant matrices. By using this method to the aqueous extract of *Sphenocentrum jollyanum*, we can generate precise, replicable, and complete chemical profiles, which will aid in the evidence-based validation of the plant's conventional applications. Identifying these compounds might also help us understand how the plant works, guide us in future pharmacological testing, and aid in the creation of consistent herbal preparations.

This work is thus justified because it fills a critical knowledge gap, aids in the scientific validation of a key medicinal plant, and advances our overall understanding of the function of fatty acids and esters in the pharmacology of natural products. The results will serve as the basis for future research on toxicology, therapy, and the possibility of phytopharmaceutical development.

1.3 Aim of the Study.

The aim of this study is to identify and analyze the presence of fatty acids and esters in an aqueous extract sample of the stem of the plant, *Sphenocentrum jollyanum* using gas chromatography-mass spectrometry (GC-MS) techniques.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Medicinal Plants

As a secure therapeutic method, medicinal plants have been employed in various cultures throughout the globe for millennia. Medicinal plants operate on the basis of the extensive empirical knowledge accumulated over generations, transferred through ancestral traditions, healer-to-healer apprenticeship, and long-term practical experience. This body of knowledge has remained unaltered despite cultural transitions and modernization. Traditional medical systems such as allopathic, homeopathic, Ayurvedic and Chinese medicine are well developed. Many nations have also developed their own Materia Medica documenting data of plants used for therapeutic purposes. The integration of this extensive natural pharmacopoeia with advances in different fields of medical science provides the platform for innovation and potential transformation in present healthcare systems (Marrelli, 2021).

2.2 The Plant; Sphenocentrum jollyanum

Sphenocentrum jollyanum Pierre of the family Menispermaceae, is a perennial plant that attains an average height of 1.5m and thrives in regions characterized by an annual rainfall of around 1800 mm. It has mean minimum and maximum temperatures of about 20 °C and 29 °C, respectively. The plant generally develops few, sparsely distributed branches. Its leaves are wedge-shaped, measuring approximately 5–12 cm in length, and exhibit a smooth texture on both surfaces, terminating in a narrow, arrow-like apex (Olorunnisola *et al.,* 2017).

Sphenocentrum jollyanum is a well-known shrub in the tropical forest zones of West Africa and is spread through out Nigeria, Ghana, Ivory Coast, Sierra Leone and Cameroon. It is widely used

in the treatment of various diseases (Olorunnisola *et al.,* 2017). *Sphenocentrum jollyanum* is known in Nigeria by the Yorubas, as "Akerejupon" or "Ajo", by the Igbos as "Ojienyi" and by the Ibibios as "Ibong Isong". The plant is locally known as "Aduro kokoo" (red medicine), "Okramankote" (dog's penis), and "Krakoo" amongst the Akan and Asante tribes of Ghana. It is known as "Oban abe" in Republic of Benin, and "Ouse-abe" in Côte d'Ivoire (Olorunnisola *et al.,* 2017, Uka *et al.*, 2022).

Figure 1: *Sphenocentrum jollyanum* plant

Image source: IITA Forest Center.

2.2.1 Taxonomy

Table 1: Taxonomy of *Sphenocentrum jollyanum* (Olorunnisola *et al*., 2017).

Kingdom	Plantae
Division	Magnoliophyta (Cronquist)
Subdivision	Magnoliophyta (Frohne and Jensen)
Class	Ranunculopsida (Brongn)
Subclass	Ranunculidae (Takht)
Suborder	Ranunculanae (Takht)
Order	Menispermales (Bromhead)
Family	Menispermaceae (Juss)
Genus	Sphenocentrum (Pierre)
Species	Jollyanum

2.2.2 Morphology of the Plant

Sphenocentrum jollyanum, a dioecious plant that is short and evergreen, is frequently seen in dense tropical woodlands, where it grows well in the deep shade from sea level to an altitude of around 400 meters. The plant is often 1.5 meters tall and has few branches. Young stems have a thin coating of short hairs that disappear as they mature. The roots are clearly bright yellow and have a distinctive sour, acidic flavor that can momentarily improve the sweetness of foods consumed after them (Olorunnisola *et al*., 2017; Akinwumi and Sonibare, 2022), while the bark is usually grey. The leaves have a spiral arrangement and are mostly concentrated towards the ends of the branches. Their shape is wedge-like, with a smooth surface on both sides, generally 5

to 12 cm wide and up to 20 cm long, with a pointed arrow-like tip (Akinwumi and Sonibare, 2022).

Figure 2: *Sphenocentrum jollyanum* stem cuts

Image source: Herbal Stroke Specialist

The reproductive system is just as unique. Flowers can be found alone on older branches or along the stem in between leaves. Their sepals are organized spirally and grow in size toward the center; they are unisexual and have a consistent structure. Male flowers are sessile, have 15–21 sepals, and 16–31 free, erect stamens. Female flowers usually have 9–11 sepals and may be sessile or borne on pedicels up to 4 mm long (Ekpono *et al*., 2018).

Figure 3: Reproductive system of *Sphenocentrum jollyanum.*

Image source: Google Photos.

The fruit is composed of 3–12 ellipsoid drupes, which grow in dense clusters. The drupes are fleshy, smooth, and change color from brilliant yellow to orange as they mature. The fruit has a

single, big ellipsoid seed that is around 15–18 mm long and 8–9 mm wide. The seed has a very thin seed coat and a straight embryo, but it lacks endosperm. During the early stages of development, seedlings retain their plano-convex cotyledons, which are still enclosed within the hard endocarp (Ekpono *et al*., 2018; Akinwumi and Sonibare, 2022).

2.2.3 Traditional Uses of the Plant

Sphenocentrum jollyanum, also known as "African yellow wood" has a well-established history of utilization in traditional therapeutic practices across Africa (Ekpono *et al*., 2018). The plant's many parts and sections, notably its roots, have long been used for their curative qualities in traditional African medicine. The plant's alleged health benefits, which are regularly handed down through the generations, are what give it its ethnobotanical significance. The plant parts (e.g. roots, stems, leaves) are prepared by drying (removing moisture), crushing and soaking in solvents especially alcohol for the creation of extracts (tinctures for alcohol). This is then applied topically or taken orally. *Sphenocentrum jollyanum's* extensive phytochemical makeup gives it a variety of therapeutic uses (Bwanbale, 2024).

Sphenocentrum jollyanum's ethanol root extract has great potential as a supplemental and alternative treatment for malaria. When used with medications like chloroquine or artemisinin-based combination treatments (ACTs), its capacity to restore hematological indicators and its possible anti-parasitic properties could improve overall recovery and patient outcomes (Bwanbale, 2024). The extract may lessen the negative effects of traditional antimalarial medications, such as neurotoxicity or gastrointestinal problems, when combined with regular therapies. Another possible treatment option is herbal medicine, particularly in areas where traditional antimalarial are more difficult to obtain or have resistance problems (Emejalu and Nwachukwu, 2023).

It is used in the treatment of gastrointestinal illnesses (Omoyajowo *et al.*, 2025). Particularly if there is suspicion of poisoning, the plant, primarily the bark, is used as an emetic and laxative. It is thought that chewing sticks produced from the root's sap cures constipation and stomach pain (Ekpono *et al*., 2019).

The root is employed as an aphrodisiac tonic for men (Bwanbale, 2024). The effects of the plant in relation to its sexual stimulating functions have been documented by a number of scientific studies (Uka *et al*., 2022). Chewing sticks produced from the root increases sexual desire (Ekpono *et al*., 2018). It is used to treat erectile dysfunction (Omoyajowo *et al*., 2025).

The root is used as a sweetener; it has a sour flavor, but it sweetens the food that is consumed after it. In Côte d'Ivoire, the root is crushed into a paste with salt, maniguette fruit, and palm oil, and the resulting mixture is used to treat stomach ailments. High blood pressure is treated with pounded roots. The boiled or pulped roots are administered as a draught or enema to treat epileptic seizures. In Ghana, the pulped roots have been used to treat breast tumors. In Nigeria, a decoction of the root is used to dress tropical ulcers. A decoction of the leafy twigs is used as a wash to stop bleeding of wounds, sores, and cuts; the wounds are also covered with the powdered bark. Crushed leaves are ingested to reduce blood spitting. The fruit is consumed since it is healthy and combats weariness (Ekpono *et al.*, 2018).

The aqueous extracts of *Sphenocentrum jollyanum* root and leaves exhibit significant antidiabetic effects, particularly through modulation of inflammatory pathways associated with diabetes. Their study showed that treatment with the plant extract enhanced wound healing in diabetic rats by regulating pro-inflammatory cytokines, promoting fibroblast activity, and supporting vascular endothelial repair. These findings suggest that the bioactive components identified through GC-

MS profiling may contribute to the extract's ability to improve tissue regeneration under diabetic conditions (Adeleke *et al.,* 2022).

The antioxidant properties of *Sphenocentrum jollyanum* have been demonstrated across different plant parts, with extracts showing notable free-radical scavenging activity. Studies indicate that both root and seed extracts possess strong activity against DPPH and hydrogen peroxide radicals, comparable in some cases to standard antioxidants. Phytochemical analyses attribute these effects to the presence of flavonoids, phenolics, and other redox-active compounds, which collectively enhance the plant's ability to neutralize reactive oxygen species and reduce oxidative stress. Overall, these findings support the plant's traditional use in managing oxidative-stress-related conditions (Akinwumi and Sonibare, 2022).

Numerous scientific studies have documented the plant's effects on tumor treatment, jaundice treatment, breast engorgement associated with the menstrual cycle, wound healing, antiviral and anti-inflammatory actions, hepatoprotective effects, antidiabetic, antioxidant, and analgesic properties. The charred fruit is utilized in traditional Nigerian medicine for the treatment of fibroids, while the root hair is employed in conjunction with other anti-malaria plants as a cure for fevers, body aches, and rheumatism (Uka *et al*., 2022).

Extracts of *Sphenocentrum jollyanum* have demonstrated notable anti-allergic properties. Studies show that administration of the fruit extract significantly reduced eosinophil and leukocyte counts in milk-induced allergic reactions, indicating suppression of allergic inflammatory responses (Olorunnisola *et al*., 2017). The ethanol root extract produced effects comparable to dexamethasone in decreasing eosinophils and lymphocytes, suggesting that the anti-allergic activity may be mediated through multiple phytochemical constituents.

The plant exhibits antimicrobial activity against resistant bacterial and fungal strains. Crude methanol extracts inhibited various clinical isolates, showing measurable zones of inhibition and significant antifungal and antibacterial effects. The extracts also demonstrated low minimum inhibitory concentrations, indicating strong antimicrobial potency relative to some standard drugs (Akinwumi and Sonibare, 2022).

Ethanol extracts of *Sphenocentrum jollyanum* roots show antidepressant activity in validated behavioral models. In forced swim and tail suspension tests, the extracts significantly reduced immobility time in a dose-dependent manner, exhibiting effects comparable to standard antidepressants such as imipramine and fluoxetine. These findings support the plant's potential for developing novel antidepressant agents (Akinwumi and Sonibare, 2022).

2.3 Bioactivity of Fatty Acids and Esters

Fatty acids have a wide range of biological features. Fatty acids (FA), which are among the monocarboxylic acids, are made up of extended hydrocarbon chains. The cleavage of fats and oils from natural sources, such as triglycerides or phospholipids, results in the formation of saturated or unsaturated oils. The length of the aliphatic tails of the FAs determines the categorization: short chain fatty acids (SCFA) have aliphatic tails with five or fewer carbon atoms, medium chain fatty acids (MCFA) have aliphatic tails with six or more carbon atoms, and long chain fatty acids (LCFA) have aliphatic tails with fourteen or more carbon atoms (Józwiak *et al*., 2020). In addition to their role in membrane function, fatty acids are thought to play a significant role in the brain and retina. They have a wide range of industrial applications, including fuels, surfactants, and catalysts. Furthermore, simple transformations, such as the reaction of the carboxylic moiety to produce a stable ester or amide bond, can be used to turn them into precursors for biologically active substances. These bioactive compounds, branched

and cyclic chain FAs, have a variety of biological effects, including anti-inflammatory, antibacterial, and antioxidant capabilities (Taghreed *et al*., 2023). Gas-chromatography mass-spectrometry (GC-MS) is a technique used for the analysis of fatty acids (Jitkunya *et al*., 2021).

Fatty acids and their ester derivatives play crucial roles in biological systems and have been widely recognized for their broad spectrum of functional and therapeutic activities. Recent advances in lipid chemistry have revealed that both naturally occurring and synthetically produced esters exhibit distinct structure–property profiles that strongly influence their biological behavior. Plant-derived rare fatty acids and lipids possess significant bioactivity and contribute to the unique biochemical composition of many plant oilseeds. These lipids frequently participate in antimicrobial, anti-inflammatory, metabolic, and antioxidant mechanisms, highlighting their relevance in pharmacological research (Avato and Tava, 2022).

Fatty acid methyl esters (FAMEs), a major class of lipid-derived molecules, have been shown to exhibit considerable biological activity. In the study of citrus wax, Cruz *et al*. (2020) demonstrated that the production of fatty acid methyl esters not only contributes to renewable energy and industrial applications but also yields bioactive compounds with potential biological effects. Their work indicates that methylated fatty acids can influence cellular function, modulate biological pathways, and act as components of biologically relevant mixtures derived from plant waxes (Cruz *et al*., 2020). This underscores the dual role of FAMEs as both industrially valuable molecules and compounds with significant bioactivity.

Furthermore, sucrose fatty acid esters (SFAEs) represent a class of biologically active esters with diverse applications in food, pharmaceutical, and biochemical systems. Teng *et al.* (2021) emphasized that the emulsifying capacities and biological activities of sucrose fatty acid esters

are largely determined by the structural relationship between the carbohydrate moiety and the attached fatty acid chain. Their review highlights that SFAEs exhibit notable antimicrobial activity, improved emulsification in biological environments, and functions connected to structure–property interactions (Teng *et al*., 2021). Because of these properties, sucrose fatty acid esters have become valuable bio-based molecules with applications ranging from drug delivery to nutraceutical formulations.

Another important group of bioactive esters includes branched fatty acid esters of hydroxy fatty acids (FAHFAs). FAHFAs possess distinct biological activities that vary across different isomeric families. Their findings demonstrate that subtle structural variations among FAHFA isomers can produce markedly different biological outcomes, emphasizing the specificity and functional diversity of these compounds (Aryal *et al*., 2021). FAHFAs have been implicated in glucose regulation, anti-inflammatory processes, and metabolic responses, highlighting their potential in metabolic disease management.

The convergence of evidence from these studies demonstrates that fatty acids and esters exhibit broad, multifaceted biological activities. FAMEs, as noted by Cruz *et al*. (2020), can participate in biochemical pathways relevant to plant-derived bioactive mixtures. Sucrose fatty acid esters, as discussed by Teng *et al.* (2021), exhibit structure-dependent emulsifying and biological properties that support their role in health and food sciences. FAHFAs, detailed by Aryal *et al.* (2021), showcase the unique metabolic signaling potential of esterified fatty acids. Meanwhile, Avato and Tava (2022) highlight the importance of rare fatty acids and lipids in plant oilseeds, demonstrating that uncommon lipid structures often possess exceptional bioactive profiles.

Collectively, the bioactivity of fatty acids and esters is determined by their structural diversity, degree of esterification, positions of branching, and functional group modifications. These molecular characteristics influence antioxidant capacity, anti-inflammatory potential, metabolic modulation, antimicrobial properties, and interactions with biological membranes. As research progresses, the work of Cruz *et al.* (2020), Teng *et al*. (2021), Aryal *et al*. (2021), and Avato and Tava (2022) provides substantial insight into the bioactive nature of these lipid-based molecules, demonstrating their relevance in modern biochemical, nutritional, and pharmacological science.

3.4 Analytical Techniques for Fatty Acids and Esters

The analysis of fatty acids and esters requires highly sensitive, selective, and precise analytical methods due to the structural diversity and functional complexity of these lipid molecules. Modern analytical chemistry has developed a wide range of techniques capable of detecting free fatty acids, esterified fatty acids, branched derivatives, and specialized lipid conjugates across biological samples, plant materials, and food matrices. According to Kokotou (2020), the determination of fatty acid esters of hydroxy fatty acids (FAHFAs) in biological samples, plants, and foods relies heavily on advanced analytical methods that allow both qualitative and quantitative characterization. These methods include chromatographic separation, spectral identification, and mass-based structural elucidation, all of which provide accurate insight into lipid composition (Kokotou, 2020).

One of the most widely used approaches for the analysis of fatty acids and esters is gas chromatography, particularly when coupled with sensitive detectors. Nikonova *et al.* (2020) demonstrated the effectiveness of gas–liquid chromatography for the determination of free and esterified fatty acids in hydroceles with varying polyunsaturated fatty acid content. Their study highlights the ability of gas–liquid chromatography to differentiate between free fatty acids and

esterified forms, making it especially valuable for lipid profiling in samples rich in polyunsaturated fatty acids (Nikonova *et al*., 2020). The technique provides sharp peak resolution, reproducible retention times, and sensitivity necessary for analyzing complex fatty acid mixtures.

Another essential analytical domain is the assessment of sucrose fatty acid esters (SFAEs). Teng *et al.* (2021) noted that the structural diversity and emulsifying capacities of sucrose fatty acid esters require analytical approaches capable of linking chemical structure to structure–property profiles. Their review underscores the importance of techniques that allow molecular characterization of SFAEs in order to understand their functional behavior in food and biological systems (Teng *et al.*, 2021). These analytical techniques typically include chromatographic separation, nuclear magnetic resonance, and mass spectrometry, all of which enable detailed examination of esterified sugar–lipid structures.

In studies involving esterified bioactive molecules, such as short-chain fatty acid resveratrol esters, analytical techniques play a pivotal role in both synthesis validation and bioactivity assessment. Tain *et al.* (2021) synthesized a range of short-chain-fatty-acid resveratrol esters and demonstrated that the antioxidant properties of these compounds depend on accurate chemical characterization. Analytical techniques, including chromatographic purification and structural confirmation, were essential in identifying specific ester forms and evaluating their antioxidant potential (Tain *et al*., 2021). Their work further emphasizes the need for precise analytical strategies in determining the functional properties of esterified fatty acids.

Overall, the analytical techniques used for fatty acids and esters combine chromatographic methods such as gas-liquid chromatography with advanced detection systems capable of high-

resolution identification. As shown by Kokotou (2020), FAHFA analysis depends on methods that can detect low-abundance esters in complex matrices, while Nikonova *et al*. (2020) demonstrated the value of gas–liquid chromatography for distinguishing free and esterified fatty acids. Teng *et al*. (2021) provided insight into the structural characteristics of sucrose fatty acid esters, and Tain *et al*. (2021) highlighted the importance of analytical confirmation in synthesizing esterified antioxidant compounds. Together, these studies show that modern analytical chemistry provides comprehensive tools for accurately characterizing fatty acids and esters across biological, nutritional, and synthetic contexts.

3.5 Solvent Effects on Extraction of Fatty Acids and Esters

The extraction of fatty acids and esters is strongly influenced by the polarity, chemical characteristics, and extraction conditions of the solvent system employed. Differences in solvent polarity can significantly alter lipid yield, fatty acid composition, and the overall efficiency of ester formation during extraction or transesterification processes. According to Zarrinmehr *et al*. (2022), the polarity of solvents plays a crucial role in determining the micro algal lipid yield, fatty acids profile, and biodiesel properties. Their findings show that solvents with different polarities selectively extract specific classes of fatty acids, thereby altering the total lipid composition and influencing biodiesel characteristics such as viscosity, oxidative stability, and ester content (Zarrinmehr *et al*., 2022). This demonstrates that solvent choice is a key determinant in the extraction of fatty acids and their ester derivatives.

The effect of solvents is also highlighted in Soxhlet extraction systems. Liao *et al*. (2025) reported that solvent effects in Soxhlet extraction of source rocks lead to variations in the chemical composition of extracted materials. Solvents with higher polarity may extract more polar hydrocarbons, whereas nonpolar solvents favor hydrophobic lipid molecules and ester-

linked compounds. Their study emphasizes that solvent selection not only affects extraction yield but also the structural profile of recovered compounds (Liao *et al*., 2025). This supports the idea that solvent–matrix interactions directly shape the qualitative and quantitative recovery of fatty acids and esters.

In the production of fatty acid esters, solvent and reaction conditions also determine ester selectivity. Nguyen *et al.* (2020) investigated biodiesel production from wet microalgae paste through in-situ transesterification and demonstrated that reaction parameters strongly influence the selectivity of fatty acid esters. Their work shows that variations in solvent system, reaction temperature, and transesterification conditions determine the formation of specific fatty acid esters, thereby affecting biodiesel yield and ester composition (Nguyen *et al*., 2020). This underscores how solvent characteristics affect both extraction and esterification efficiency during biofuel production.

Additionally, the extraction of plant-based oils further supports the importance of solvent polarity. Jisieike and Betiku (2020) showed that solvent polarity, extraction time, and solid–solvent ratio significantly influences rubber seed oil yield and quality. Their findings indicate that more polar solvents may extract different lipid fractions compared to nonpolar solvents, directly affecting the concentration of fatty acids and esterifiable lipids recovered during extraction (Jisieike & Betiku, 2020). This demonstrates that extraction variables collectively determine the quality and composition of fatty acid-rich oils.

Overall, the studies of Zarrinmehr *et al.* (2022), Liao *et al*. (2025), Nguyen *et al*. (2020), and Jisieike and Betiku (2020) collectively emphasize that solvent polarity, extraction technique, reaction parameters, and solvent–sample interactions play fundamental roles in determining the

yield, composition, and ester profile of fatty acids. Solvent selection ultimately determines the efficiency of lipid extraction, the types of fatty acids recovered, the formation of fatty acid esters, and the suitability of the extracted materials for applications such as biodiesel production.

3.6 Why GC-MS analysis and aqueous extract?

The phytochemical composition of *Sphenocentrum jollyanum* has received increasing scientific attention due to its wide range of biological and pharmacological activities. Recent studies have shown that both the leaf and root extracts of this plant contain numerous bioactive compounds detectable through Gas Chromatography–Mass Spectrometry (GC–MS), a highly sensitive and accurate analytical technique widely applied for identifying volatile constituents, fatty acids, esters, and other secondary metabolites.

GC–MS profiling enables researchers to establish the chemical fingerprint of plant materials by separating individual components and matching their mass spectra with known library data. According to Uka *et al*. (2022), the GC–MS analysis of ethanol leaf extracts of *Sphenocentrum jollyanum* revealed a wide range of bioactive compounds with significant biological activities. Their findings demonstrated that even polar extracts of the plant contain fatty acid–related structures and ester-like compounds, suggesting that the aqueous fraction may similarly retain such constituents due to the partial solubility of some fatty acid derivatives in water (Uka *et al*., 2022).

Similarly, the work of Ugwu *et al*. (2023) on the ethanol root extract and fractions of *Sphenocentrum jollyanum* demonstrated that GC–MS offers a robust platform for identifying anti-nutritional factors alongside various organic compounds including fatty acids, esters, and related metabolites. Their study revealed that fractions obtained from polar solvents still show

the presence of lipid-associated molecules, which supports the idea that aqueous extracts, though primarily hydrophilic, can still retain measurable quantities of medium-chain fatty acids and water-dispersible esters (Ugwu *et al*., 2023). Hence, the aqueous extract may exhibit a similar trend in terms of chemical diversity.

Further chemical characterization of *Sphenocentrum jollyanum* was carried out by Moronkola *et al*. (2021), who isolated compounds such as columbin and novel clerodane furanoditerpenes. Their GC–MS and antimicrobial analyses of the essential oils from the plant highlight the richness of volatile constituents typically associated with fatty acid decomposition or esterification pathways (Moronkola *et al*., 2021). Essential oils often contain methyl esters and long-chain fatty acid derivatives, providing additional evidence that the plant's biochemical matrix naturally supports ester formation. Even though essential oils are hydrophobic, the metabolic profile presented by these authors guide expectations regarding the fatty acids and esters extractable in aqueous preparations.

In addition to these studies on *Sphenocentrum jollyanum*, the relevance of GC-MS as a tool for lipid and fatty acid analysis is reinforced by the work of Olajide *et al*. (2025) who investigated lipid content in microalgae using GC–MS. Their approach demonstrates the reliability of GC–MS for detecting saturated and unsaturated fatty acids, methyl esters, and structurally related compounds in biological samples (Olajide *et al*., 2025). This methodological relevance directly supports the suitability of GC–MS for profiling fatty acids and esters in aqueous extracts of *Sphenocentrum jollyanum*, particularly when targeting complex mixtures with both polar and semi-polar constituents.

When applied to aqueous extracts of *Sphenocentrum jollyanum*, GC–MS would likely reveal several classes of fatty acids such as palmitic acid, linoleic acid, oleic acid, and their corresponding methyl or ethyl esters. Although aqueous extracts may contain these compounds in lower concentrations than ethanol or essential oil extracts, the presence of hydrophilic fatty acid conjugates, short-chain esters, and glycosylated derivatives is highly plausible. The findings of Uka *et al*. (2022) and Ugwu *et al*. (2023) collectively suggest that the plant contains diverse chemical groups across all its extractable matrices, including those obtained with water as the primary solvent.

Furthermore, the consistent detection of diterpenes, furanoditerpenes, and lipid-associated compounds across multiple studies (Moronkola *et al*., 2021; Uka *et al*., 2022; Ugwu *et al*., 2023) supports the hypothesis that fatty-acid–related molecules contribute significantly to the chemical profile of *S. jollyanum*. Since GC–MS is capable of ionizing and identifying even minute concentrations, the aqueous extract can be examined thoroughly for fatty acids, long-chain esters, hydroxy-fatty acid derivatives, phytosterol esters and other lipid-related metabolites.

GC–MS analysis of fatty acids and esters in the aqueous extract of *Sphenocentrum jollyanum* is strongly supported by prior research emphasizing the plant's chemical richness. Studies by Moronkola *et al.* (2021), Uka *et al*. (2022) and Ugwu *et al.* (2023), collectively demonstrate that this species contains complex mixtures of volatile and semi-volatile compounds detectable by GC–MS across various extraction methods. The analytical approach described by Olajide *et al*. (2025) further validates the sensitivity of GC–MS for identifying lipid components. Therefore, comprehensive GC–MS profiling of the aqueous extract is expected to reveal a diverse array of fatty acids, esters, and structurally related compounds that contribute to the plant's biological and pharmacological properties.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Apparatus and Equipment

The apparatus and equipment utilized during the sample preparation and extraction processes includes:

1. Weighing balance (B. Bran Scientific, 80-20, England).
2. Gloves.
3. Funnel.
4. Rod.
5. Cheese cloth.
6. Sieve.
7. Bowls.
8. Freeze dryer.
9. Freezer.
10. Mechanical grinder.
11. Universal bottle.
12. Spatula.
13. Permanent markers.
14. Knives.
15. Drying trays.
16. Masking tape.

17. Freeze dryer (lyophilizer).
18. Washing basins.

3.1.2 Chemical Reagents

1. Distilled water
2. Absolute ethanol (British Drug House).

3.2 Methods

3.2.1 Plant Collection and Identification

From Iwo, Osun State, in southwestern Nigeria, fresh samples of *Sphen*ocentrum jollyanum Pierre were obtained. The plant material was identified and authenticated at the Herbarium Unit of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, P. M. B. 1154, Ugbowo, 300283, Benin City, Edo State, Nigeria. The plant's voucher number is (UBH-S449).

3.2.2 Plant Preparation and Pulverization

Soil, debris, and surface pollutants were removed from the fresh stems by washing them carefully and diligently under running water. To avoid deterioration of heat-sensitive phytochemicals, the washed stems were then air-dried at room temperature in a well-ventilated area after they were chopped into smaller pieces. After the stems were fully dried, they were crushed mechanically using a mechanical grinder to maximize surface area and improve the efficiency of the extraction process.

3.2.3 Cold Maceration Extraction Process

The powdered stem was partitioned into two portions for extraction: one designated for ethanolic extraction, where the powder was immersed in ethanol, and another for aqueous extraction, in which the powder was soaked in distilled water.

For the aqueous extraction, 1.5kg of the plant powder was macerated in 1L of distilled water for 72 hours, stirring intermittently every two hours. For the ethanolic extraction, 1.5kg of the plant powder was macerated in 1L of absolute ethanol for 72 hours, stirring intermittently every two hours. The container is usually covered to prevent the evaporation of the ethanol. The intermittent stirring is to promote solvent penetration and improve phytochemical diffusion. For each extraction, the solvent is always kept at least 2cm above the plant powder.

3.2.4 Filtration Process

Upon completion of the maceration, the preparation was filtered four successive times using clean cheese cloths to ensure effective separation of the liquid extract from the plant residues and to obtain a clear, particle-free filtrate containing the solvent extracts.

3.2.5 Freeze-drying Process

The filtrates were frozen in a freezer and then subjected to freeze-drying, during which the solvent was removed by sublimation. This process ensured the preservation of volatile and heat-sensitive phytochemicals, resulting in the production of dry extracts.

3.2.6 Phytochemical Analysis

A 1 g portion of each freeze-dried extract including both ethanolic and aqueous extract was precisely weighed and stored in appropriately labeled universal bottles. The samples were then transported to a certified analytical laboratory in Lagos for GC–MS analysis and profiling to identify and characterize their phytochemical constituents.

The extraction protocol demonstrated several methodological advantages. The freeze-drying process effectively preserved heat-sensitive bioactive compounds, while periodic agitation enhanced solvent penetration and improved extraction efficiency. Additionally, employing both

organic and aqueous solvents allowed for comparative assessment of phytochemical solubility based on polarity differences.

3.2.7. Gas Chromatography–Mass Spectrometry

Gas Chromatography–Mass Spectrometry (GC–MS) is a powerful analytical method that integrates the separation capabilities of gas chromatography with the structural identification strength of mass spectrometry. In Gas chromatography, the sample is first vaporized and transported through a capillary column by an inert carrier gas, typically helium. As the vapour travels along the column, its individual constituents separate according to differences in boiling points and interactions with the column's stationary phase. Each separated component then enters the mass spectrometer, where it undergoes ionization and fragmentation. The mass spectra produced serve as characteristic fingerprints, allowing precise identification of the compounds present in the sample.

For this research, GC–MS profiling of the extracts was carried out using a Shimadzu GC–MS QP2010 system equipped with an AOC-20i autosampler. The analytical conditions were as follows:

1. Autosampler (AOC-20i) Settings:

The autosampler operated with three pre-solvent rinses and three post-solvent rinses, alongside two internal sample rinses. Both the plunger suction and injection speeds were kept on high, with a viscosity compensation time of 0.2 seconds. Injections were performed in normal mode with five pumping cycles and an injection port dwell time of 0.3 seconds. No terminal air gap was applied. The plunger washing speed remained high, with a washing volume of 8 µL. Suction and injection positions were set to 0.0 mm, and a single solvent vial was used throughout the run.

2. Gas Chromatography Conditions (GC-2010):

The GC oven was maintained initially at 60°C, while the injector temperature was set at 250°C. A splitless injection mode was used, with a retention time of 1.00 minute. Flow regulation operated under pressure-control mode, with a carrier gas pressure of 100 kPa, total flow of 4.7 mL/min, column flow of 0.80 mL/min, linear velocity of 23.1 cm/s, and a purge flow of 3.0 mL/min. The split ratio was 1:1, and both high-pressure injection and the gas saver function were disabled.

3. Oven Temperature Program:

The temperature program began with a 1-minute hold at 60°C, followed by heating at 13°C/min to 240°C with a brief 1-minute hold. Heating continued at the same rate to 300°C, where the temperature was maintained for 39.70 minutes.

4. Mass Spectrometer (QP2010) Conditions:

The ion source temperature was kept at 230°C, while the interface temperature was set at 250°C. A solvent cut time of 4.00 minutes was applied. The detector operated in relative gain mode at 1.33 kV, with a signal threshold of 2000.

5. Mass Spectrometry Scan Parameters:

Scanning began at 8.00 minutes and ended at 59.80 minutes. The MS operated in full-scan mode using an event time of 0.30 seconds, a scan speed of 1666 amu/s, and a mass range of m/z 35–500.

6. Readiness Check:

Prior to analysis, the system underwent a standard readiness verification. The column oven, injector, and interface temperatures were confirmed to be stable. Carrier gas pressure and flow rates were checked against programmed values. The autosampler syringe was inspected for alignment, cleanliness, and smooth plunger movement. Baseline stability was evaluated to ensure minimal electronic noise, and vacuum levels were reviewed to guarantee proper ionization conditions. The instrument was used for sample analysis only after all parameters met operational standards.

7. Heat Unit Activation:

Heating components—including the column oven, split/splitless injector (SPL1), and the mass spectrometer—were all activated and maintained at their designated temperatures throughout the run.

8. Injection Flow:

During analysis, the injection flow settings ensured continuous operation of both the SPL1 carrier and purge flows.

CHAPTER FOUR

RESULTS

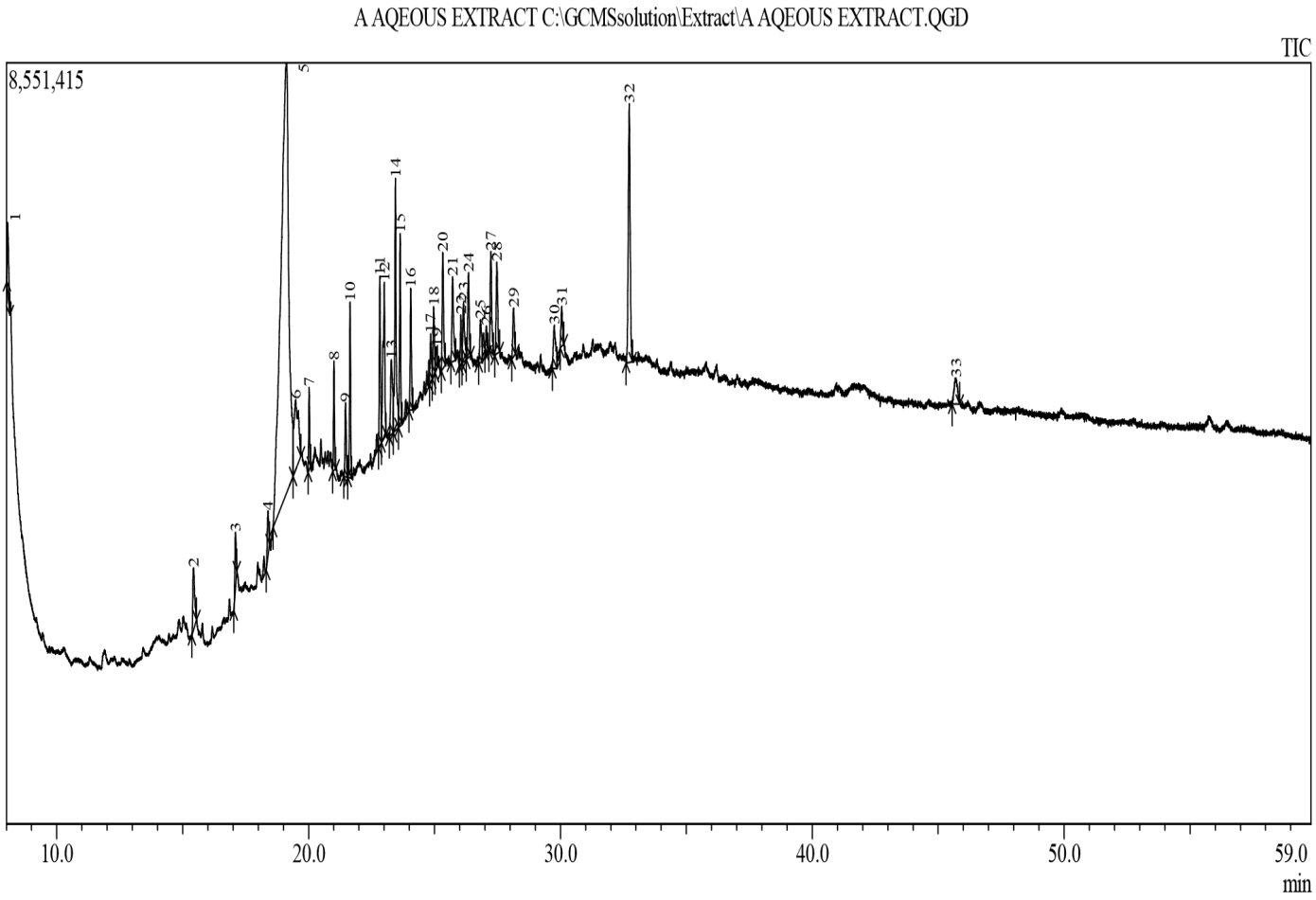
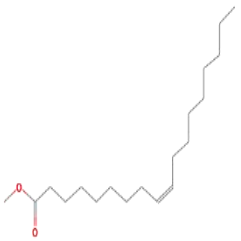
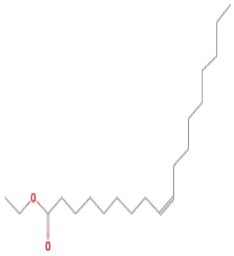
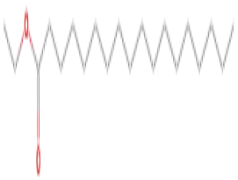
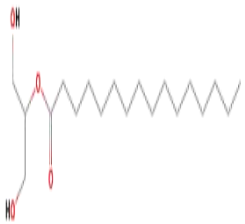
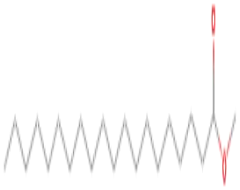
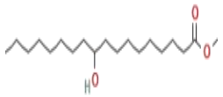
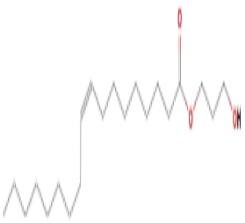
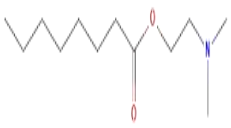


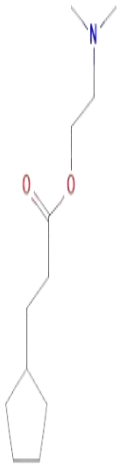
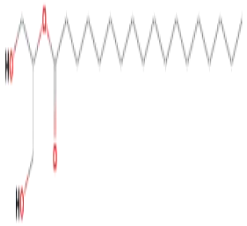
Table **2:** GC-MS Results.

S/N	COMPOUND NAME	STRUCTURE	RETENTION TIME (min)	AREA PERCENT (%)	MOLECULAR WEIGHT/PHYSICAL PROPERTIES	USES/BIOLOGICAL IMPORTANCE
1.	Hexadecanoic acid, methyl ester. Other names: *Palmitic acid, methyl ester *n-Hexadecanoic acid methyl ester *Metholene *Methyl hexadecanoate	C17H34O2 	21.006	1.16	270g Density: 0.852g/mL Melting point: 30.5-35°C Boiling point: 185°C Colorless liquid with fatty odour. Insoluble in water, soluble in organic solvents.	Exhibit anti-inflammatory, antibacterial and antioxidant property. Used in detergent production. A component of biodiesel fuel. Used as food and cosmetic additive.
2.	Hexadecanoic acid, ethyl ester Other names: *Palmitic acid, ethyl ester. *Ethyl palmitate. *Ethyl n-hexadecanoate	C18H26O2 	21.645	2.32	284g Density:0.857g/mL Melting point: 22-26°C. Boiling point: 192-193°C. Colorless crystals insoluble in water and soluble in organic solvents. Fruity odour.	Used as fragrance. Serves as flavoring agent. Potential antioxidant, anti-inflammatory and antimicrobial agent.

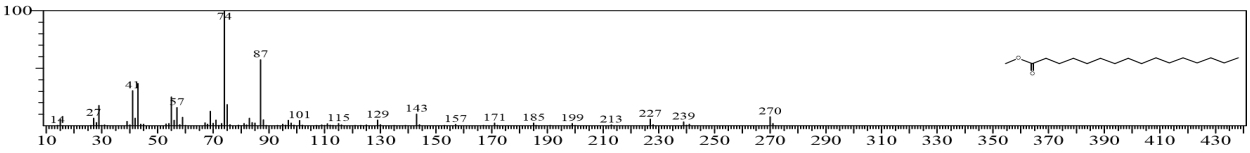
3.	9-Octadecanoic acid (Z)-, methyl ester Other names: *Oleic acid, methyl ester. *Emery oleic acid ester. *Methyl cis-9-octadecenoate.	C19H36O2 	22.826	2.54	296g Density: 0.874g/cm³ Melting point: -20°C. Boiling point: 168-218°C in a vacuum. 351°C at atmospheric pressure. Colourless to pale yellow oil with fatty odour. Insoluble in water, soluble in organic solvents.	Major component of biodiesel production. Used in lubricant production.
4.	Ethyl Oleate Other names: *9-Octadecenoic acid (Z)-, ethyl ester. *Oleic acid, ethyl ester. *(Z)-9-Octadecenoic acid ethyl ester.	C20H38O2 	23.448	4.73	310g Density: 0.87g/cm³ Melting point: -32°C Boiling point: 216-218°C Colourless oily liquid. Insoluble in water, soluble in organic solvents.	Used as a food additive and flavoring agent. Used as a lubricant. Used as a lipid component in culture media.
5.	Octadecanoic acid, ethyl ester Other names: *Stearic acid, ethyl ester *Ethyl n-hexadecanoate *Ethyl octadecanoate *Ethyl stearate.	C20H40O2 	23.635	2.78	312g Density: 0.9g/cm³ Melting point: 34-38°C Boiling point: 213-215°C White crystalline solid with waxy odour. Insoluble in water, soluble in organic solvents.	Used as flavoring agent and food additive. Used in lubricant production. Used as a solvent carrier for active drug ingredients.

6.	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester Other names: *Palmitin-1,2-di- *Dipalmitin *Glycerol 1,2-dipalmitate *1,2-Dipalmitin	C35H68O5 	24.967	1.14	568g Density:0.93g/cm³ Melting point: 66-74°C Boiling point: 620.8-628.6°C White to off-white solid. Insoluble in water, soluble in organic solvents.	Used as stabilizer and lubricant in creams. Used as a raw material in pharmaceuticals.
7.	Eicosanoic acid, methyl ester Other names: *Methyl arachisate *Methyl eicosanoate *Arachiaic acid methyl ester *Kemester	C21H42O2 	25.085	0.41	326g Density: 0.862g/cm³. Melting point: 45-48°C Boiling point:375°C White crystalline solid. Sparingly soluble in water, soluble in organic solvents.	Used in biodiesel production. Used as fragrance and flavoring agent. Used in polymer production.
8.	Octadecanoic acid, 10-hydroxyl-methyl ester Other names: *Methyl 10-hydroxystearate *Methyl 10-hydroxyoctadecanoate.	C19H38O3 	25.327	2.05	314g Density: 0.915g/cm³ Melting point: 37-53°C Boiling point: 400°C Off-white or clear liquid with a mild odour. Slightly soluble in water, soluble in organic solvents.	Used in lubricant production. Used in cosmetic production.

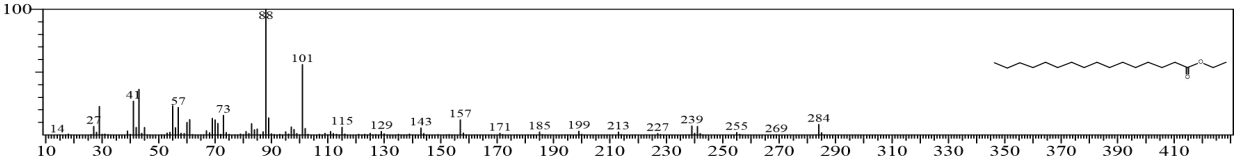
9.	Octadecanoic acid, 3-hydroxypropyl ester Other names: *Stearic acid, 3-hydroxypropyl ester *3-hydroxypropyl stearate	C21H42O3 	25.718	1.76	342g Density: 0.91g/cm³. Melting point: 50-70°C Boiling point: 416.3°C Waxy solid insoluble in water, soluble in organic solvents.	Used in cosmetic production. Used as a lubricant. Can be used to improve the solubility of drugs.
10.	Octanoic acid, 2-dimethyl amino ethyl ester.	C12H25NO2 	26.821	0.99	215g Colourless liquid. Slightly pungent and slightly fruity smell. Slightly soluble in water, soluble in organic solvents. No specific data on density, boiling point and melting point.	Used as a monomer in polymer synthesis.
11.	3-Cyclopentylpropionic acid, 2-dimethyl amino ethyl ester	C12H23NO2	27.056	0.38	213g Colourless liquid. Insoluble in water, soluble in chloroform and methanol. No specific density, boiling point and melting point.	A research pharmaceutical compound likely possessing antioxidant and antibacterial properties.

						
12.	Octadecanoic acid, 2-hydroxy-1,3, propanediyl ester Other names: *Stearin, 1,3-di- *Glycerin 1,3-distearate. *Glyceryl 1, 3-distearate	C39H76O3 	27.475	1.81	624g Density: 0.9g/cm³ Melting point: 67-72°C Boiling point: 410.96°C White solid in beaded, flaky or powdery form. Odorless and sweet tasting. Insoluble in water, soluble in organic solvents.	Used as a stabilizer in cosmetic production. Used as a thickening agent in food industry. Used a s a lubricant in drug manufacturing. Used in lubricant production.
13.	16-Trimethylsilyloxy-9-octadecenoic acid, methyl ester.	C22H44O3Si	29.754	1.27	384g Waxy volatile compound. Insoluble in water. Soluble in organic solvents.	Used as a reference compound and intermediate in biomedical research.

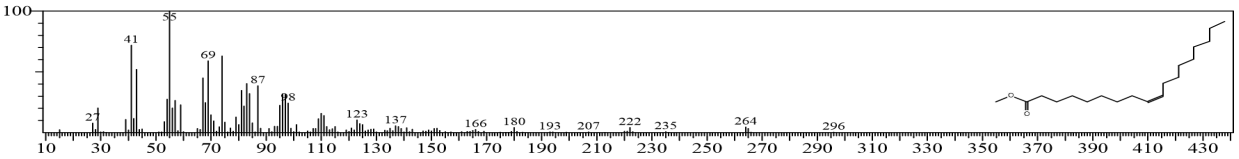
1. Hexadecanoic acid, methyl ester.



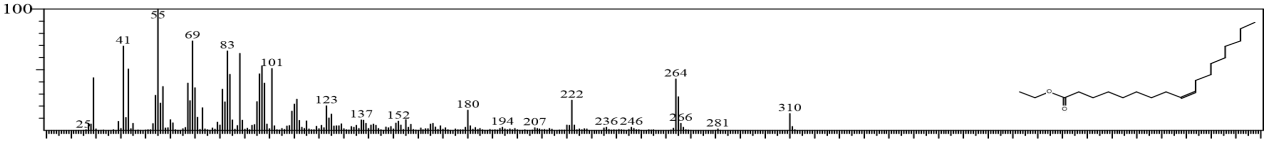
2. Hexadecanoic acid, ethyl ester.



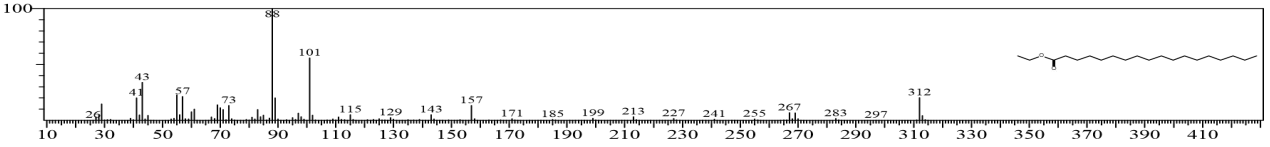
3. 9-Octadecanoic acid (Z)-, methyl ester.



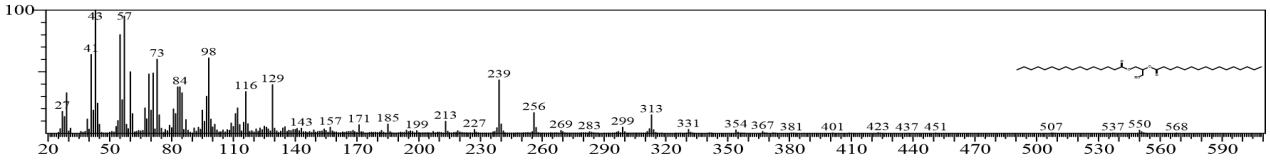
4. Ethyl Oleate



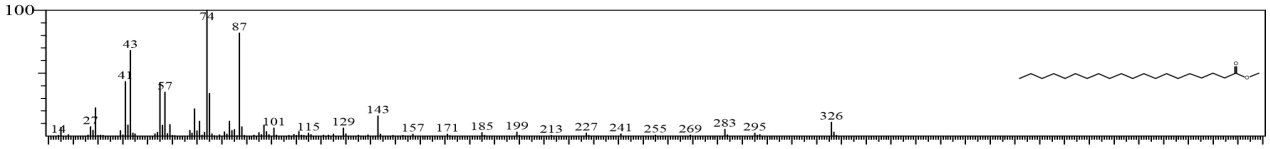
5. Octadecanoic acid, ethyl ester



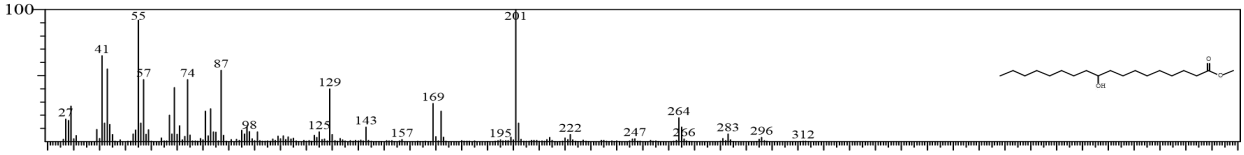
6. Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester



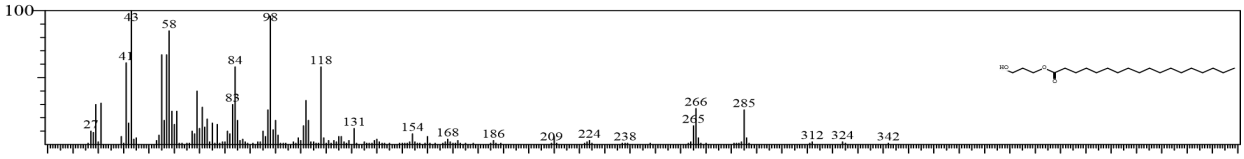
7. Eicosanoic acid, methyl ester



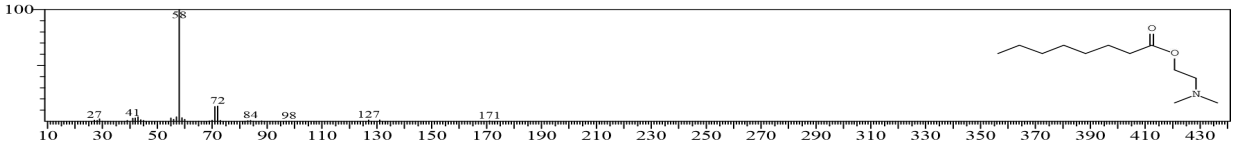
8. Octadecanoic acid, 10-hydroxyl-methyl ester



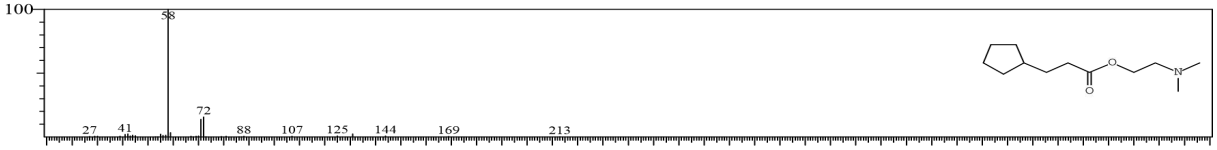
9. Octadecanoic acid, 3-hydroxypropyl ester



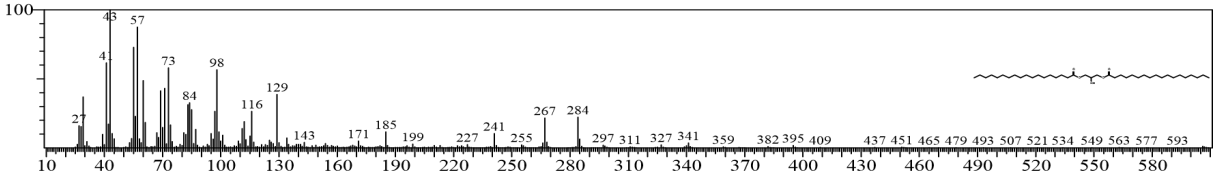
10. Octanoic acid, 2-dimethyl amino ethyl ester



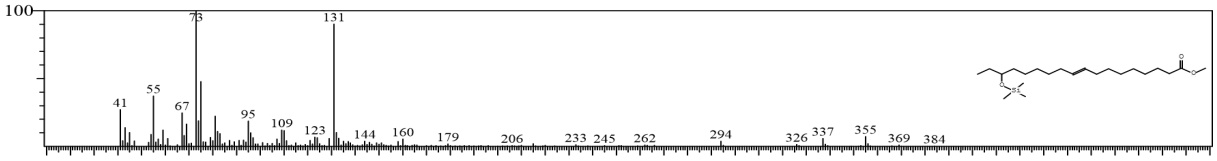
11. 3-Cyclopentylpropionic acid, 2-dimethyl amino ethyl ester



12. Octadecanoic acid, 2-hydroxy-1,3, propanediyl ester.



13. 16-Trimethylsilyloxy-9-octadecenoic acid, methyl ester



CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

This study investigates the fatty acids and esters present in the aqueous stem extract of *Sphenocentrum jollyanum* using Gas Chromatography–Mass Spectrometry (GC–MS). The plant is widely used in traditional medicine for treating pain, inflammation, infections, and malaria. The focus on aqueous extraction reflects indigenous preparation methods and may influence the solubility and recovery of semi-polar lipid constituents. GC–MS profiling revealed a chemical composition dominated by unsaturated fatty acid derivatives, particularly octadecadienoic acid esters.

Key compounds detected include methyl palmitate and ethyl palmitate, both of which possess anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, and potential anticancer activities. They also have broad industrial relevance as lubricants, emulsifiers, stabilizers, and intermediates in cosmetic, food, and pharmaceutical formulations. Methyl oleate and ethyl oleate were also abundant; these esters are widely used as solvents, skin-conditioning agents, plasticizers, and chemical precursors, and have documented antioxidant, anti-inflammatory, and cytotoxic properties. Additional identified esters such as derivatives of stearic, eicosanoic, and hydroxylated long-chain fatty acids serve roles in controlled-release drug formulations, cosmetic emulsions, industrial lubricants, and as standards or intermediates in analytical and biochemical research. Some compounds, including substituted stearate esters, have been reported to exhibit antimicrobial or anti-inflammatory activity and are being investigated for pharmaceutical development.

The predominance of unsaturated fatty acids, collectively representing approximately 73% of the extract, underscores the plant's rich lipid profile and supports previous reports on related Menispermaceae species. These compounds are pharmacologically relevant due to their established roles in modulating inflammatory mediators, influencing lipid metabolism, enhancing membrane fluidity, and inducing apoptosis in certain cancer cell models. Their presence in aqueous extracts suggests partial solubility of lipophilic components, potentially facilitated by endogenous emulsifiers or thermal extraction conditions.

Overall, the GC–MS findings provide biochemical support for the ethnomedicinal applications of *S. jollyanum* and highlight the therapeutic potential of its lipid constituents. Further comparative studies involving aqueous and organic solvent extracts, alongside targeted biological assays, are warranted to clarify the functional relevance, bioavailability, and synergistic interactions of these fatty acids and esters in disease modulation.

5.2 Conclusion

This study provides scientific support for the identification and application of fatty acids and esters found in the plant, *S. jollyanum*. The findings confirm that the plant contains a diverse profile of bioactive lipid-derived compounds based on the GC-MS analysis. Overall, the study contributes valuable analytical data to the limited body of literature on the constituents of the plant's stem. The identification of these compounds forms a basis for future investigations aimed at isolating specific bioactive molecules, elucidating mechanisms of action, and assessing their potential therapeutic effects.

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