COMPARATIVE GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS OF UNSATURATED FATTY ACID DERIVATIVES IN AQUEOUS AND ETHANOLIC STEM EXTRACTS OF *SPHENOCENTRUM JOLLYANUM*

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CERTIFICATION

We the undersigned hereby certify that Oghenemaro Ephrame John-Mark(BMS2101420) carried out this research in the Department of Medical Biochemistry, University of Benin, Benin City, and thereby approve same as adequate in scope and quality for the awards of Bachelor of science (B.sc) in Medical Biochemistry

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DEDICATION

With a heart full of appreciation and gratitude, I dedicate this project to God almighty and my family, who has been my strength and support all the way from my time as a fresher till now

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I acknowledge with gratitude the grace of God who granted me strength and wisdom throughout my academic journey. I thank him for his mercy, love and protection which guided through every step of my study.

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ABSTRACT

Sphenocentrum jollyanum (Menispermaceae) is a medicinal plant traditionally used to treat fever, inflammation, jaundice, tumors, and metabolic disorders. Its pharmacological potential is thought to arise from bioactive secondary metabolites, yet comprehensive profiling of stem extracts remains limited. This study aimed to compare the phytochemical composition of aqueous and ethanolic stem extracts using gas chromatography–mass spectrometry (GC–MS).

Dried stem material of *S. jollyanum* was extracted sequentially with water and ethanol. Extracts were analyzed using GC–MS, and compounds were identified by matching mass spectra to the NIST library. Retention times, relative peak areas, and compound identities were documented, with particular attention to unsaturated fatty acid derivatives.

GC–MS detected 33 and 34 compounds in the aqueous and ethanolic extracts, respectively. The aqueous extract was dominated by inositol, 1-deoxy- (43.45%), along with α-methyl mannofuranoside, phytol acetate, and five unsaturated fatty acid derivatives, including methyl and ethyl oleate, oleamide, and 9-octadecenal. The ethanolic extract contained a higher proportion of lipophilic compounds, including triolein (13.46%), squalene (7.35%), methyl and ethyl oleate, oleamide, and 10-undecenoic acid methyl ester. Overall, unsaturated fatty acids and their esters accounted for over 30% of the total peak area in both extracts.

S. jollyanum stems are a rich source of pharmacologically relevant UFAs (Unsaturated fatty acids), sugar alcohols, and bioactive lipids. The aqueous and ethanolic extracts provide complementary phytochemical profiles, supporting the plant's traditional medicinal applications and highlighting its potential for anti-inflammatory, antioxidant, and antimicrobial activities. Further studies are warranted to isolate individual compounds and validate their therapeutic effects.

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the Study

Human societies have relied on plants for medicinal purposes since ancient times. This reliance encouraged early humans to explore their natural environment and experiment with various plants, animal products, and minerals, ultimately leading to the development of a wide range of therapeutic agents (Uka *et al*., 2017). The World Health Organization now emphasizes the importance of integrating safe and effective traditional medicine practices into universal health coverage, promoting the inclusion of herbal remedies in both self-care and formal healthcare delivery (Ipsos, 2008). It has been reported that more than four billion people worldwide currently depend on herbal medicine as their primary source of healthcare. Medicinal plants, therefore, remain a major source of valuable organic compounds, and *Sphenocentrum jollyanum* is one notable example (Uka *et al*., 2017).

S. jollyanum Pierre belongs to the family Menispermaceae, a diverse group of plants known for their significant biological activities. The species is a shrub native to tropical forests of West Africa and is widely distributed across Sierra Leone, Nigeria, Ghana, the Ivory Coast, and Cameroon (Nia *et al*., 2004). It is widely used in traditional medicine to manage various ailments. Local names for the plant include “Aduro kokoo” (red medicine), “Okramankote” (dog’s penis), and “Krakoo” among the Akan and Asante tribes of Ghana. In South-Western Nigeria, it is commonly referred to as Ajo or Akerejupon, while in the Republic of Benin and Côte d’Ivoire, it is known as Oban abe and Ouse-abe, respectively (Amidu, 2008). *S. jollyanum* holds strong medicinal value in several West African communities, where different parts of the plant are used to treat malaria, erectile dysfunction, gastrointestinal disorders, and other illnesses. Powdered root extracts have shown broad pharmacological activities, including analgesic, antidepressant, and anti-inflammatory effects. Alcoholic extracts are traditionally administered as aphrodisiacs, particularly for managing penile erection problems in Ghana. Studies have also reported antiviral and antibacterial activities in leaf extracts (Olorunnisola *et al*., 2017). Additionally, the fruits and leaf twigs are used, either alone or in combination therapies, across Nigeria, Ghana, and the

Ivory Coast for their antimalarial, antinociceptive, antihypertensive, antitumor, antidiabetic, and wound-healing properties. Phytochemical analyses have shown that *S. jollyanum* leaves contain saponins, tannins, alkaloids, and flavonoids, which contribute to their diverse biological activities (Omoyajowo *et al*., 2025).

Fatty acids perform several essential functions in living organisms. They act as energy stores, form key structural components of biological membranes, and serve as signaling molecules that regulate numerous physiological processes. Based on the presence or absence of double bonds in their hydrocarbon chains, fatty acids are classified as either saturated or unsaturated (Czumaj and Śledziński, 2020). Many natural food sources, particularly plant-derived materials, contain high levels of unsaturated fatty acids (UFAs). Plant seeds are particularly rich in unsaturated fatty acids (UFAs), especially the predominant 18-carbon species—18:1 (oleate), 18:2 (linoleate), and 18:3 (α-linolenate). In this notation, *m:n* indicates a molecule with *m* carbon atoms and *n* double bonds. UFAs are aliphatic carboxylic acids containing one or more double bonds, usually in the cis configuration, and they are essential to the functioning of higher organisms. These compounds play multiple critical roles and are closely associated with plant responses to biotic and abiotic stress. In addition to serving as major membrane components and regulators in glycerolipids and as carbon and energy reserves in triacylglycerols, C18 UFAs act as intrinsic antioxidants and precursors to various bioactive molecules. These include the stress hormone jasmonic acid and extracellular structural materials such as cutin and suberin (He *et al*., 2020).

Despite the pharmacological relevance of *S. jollyanum*, limited information exists on the distribution of UFA derivatives in its stem, particularly across different extraction media. Comparative gas chromatography–mass spectrometry (GC–MS), a preferred analytical method for profiling small-to-medium bioactive compounds, including fatty acids and their esters, can offer insight into solvent-specific differences in the plant's chemical composition, supporting

both scientific validation and optimized extraction strategies for therapeutic and industrial applications. Therefore, the aim of this study was to compare the unsaturated fatty acid derivatives present in aqueous and ethanolic stem extracts of *S jollyanum* using GC–MS.

1.2. Problem Statement

Despite the widespread use of *S. jollyanum* in West African ethnomedicine for conditions such as malaria, inflammation, erectile dysfunction, and various gastrointestinal disorders, the chemical constituents responsible for many of these therapeutic effects have not been fully elucidated. Previous research has primarily focused on alkaloids, saponins, flavonoids, tannins, and other phenolic compounds found in the plant's roots, leaves, and fruits (Olorunnisola *et al*., 2017). These studies have reported significant antiviral, antibacterial, anti-inflammatory, analgesic, and antidepressant activities. However, the role of UFAs and their derivatives, bioactive compounds known to function as antioxidants, membrane components, precursors to signaling molecules, and modulators of physiological stress responses (He *et al*., 2020; Czumaj and Śledziński, 2020), remains poorly understood in *S. jollyanum*.

UFAs such as oleate, linoleate, and α-linolenate are abundant in many plant species and significantly influence biological activity; however, their presence and distribution in *S. jollyanum* stem extracts have not been systematically examined. Most available phytochemical studies on the plant overlook lipid-based constituents, leaving a major gap in the understanding of its complete chemical profile. Furthermore, aqueous and ethanolic extracts are frequently used in both traditional medicine and laboratory analyses, yet no comparative profiling of their UFA derivatives has been conducted.

This lack of detailed lipidomic information restricts the interpretation of pharmacological findings, limits the ability to connect specific compounds to therapeutic outcomes, and hinders efforts to standardize plant-based preparations derived from *S. jollyanum*. Without such data, the plant's full medicinal potential cannot be assessed, optimized, or reliably reproduced in scientific, clinical, or industrial applications.

1.3. Justification of the Study

A comparative GC–MS analysis of aqueous and ethanolic stem extracts of *Sphenocentrum jollyanum* is justified for several reasons:

1. Solvent-dependent extraction variability must be understood.
   The polarity of extraction solvents strongly influences the types and quantities of phytochemicals recovered from plant materials. Water tends to extract polar compounds, while ethanol extracts a broader range of constituents, including lipid-based molecules such as UFAs. Since aqueous and ethanolic preparations are commonly used in both traditional and scientific contexts, a comparative analysis will clarify how solvent choice affects the recovery of UFA derivatives and related bioactive compounds.

2. UFAs play vital biological and therapeutic roles.
   UFAs function as membrane stabilizers, precursors of stress-related signaling molecules such as jasmonic acid, intrinsic antioxidants, and modulators of biotic and abiotic stress responses (He *et al*., 2020). They also possess known anti-inflammatory, antimicrobial, cardioprotective, and metabolic regulatory properties (Czumaj & Śledziński, 2020). Identifying and comparing these compounds in *S. jollyanum* extracts may help explain some of the plant's documented medicinal activities (Olorunnisola *et al*., 2017).

3. Existing data on the plant's lipid composition are extremely limited. While extensive work has been conducted on alkaloids, flavonoids, and phenolics, research on lipid constituents of *S. jollyanum* is largely absent. Comprehensive profiling will therefore fill a major gap in the current literature and broaden the scientific understanding of the plant's full phytochemical spectrum.

4. GC–MS provides a robust platform for identifying fatty acid derivatives. GC–MS offers high sensitivity, accuracy, and selectivity for detecting and characterizing fatty acids, even at low concentrations. Its suitability for analyzing thermally stable, volatile derivatives makes it an ideal tool for UFA profiling.

5. Improved chemical characterization supports standardization and product development. Reliable identification of UFA derivatives will enhance quality control measures, support reproducibility in future pharmacological studies, and guide the development of standardized herbal formulations, functional foods, and potential therapeutic agents derived from *S. jollyanum*.

Overall, this study addresses a critical knowledge gap as it enables the evaluation of the UFA composition of *S. jollyanum* stem extracts and the examination of solvent-dependent variations. The findings have the potential to support improved scientific validation, pharmaceutical exploration, and sustainable utilization of this important medicinal plant.

1.4. Aim and Objectives

The primary aim of this study was to compare the UFA derivatives present in aqueous and ethanolic stem extracts of *S. jollyanum* using GC–MS.

Specific objectives include:

1. To prepare aqueous and ethanolic extracts from the stem of *S. jollyanum*.
2. To perform GC–MS analysis on both extracts for the identification of UFA derivatives.
3. To compare the qualitative and quantitative profiles of these compounds between the two extraction solvents.
4. To provide baseline data that can support pharmacological research, standardization, and formulation of *S. jollyanum* stem extracts.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. *Sphenocentrum jollyanum* Pierre

S. jollyanum Pierre, a member of the Menispermaceae family, is a West African shrub commonly found in countries such as Sierra Leone, Nigeria, Ghana, the Ivory Coast, and Cameroon. The plant is widely used in traditional medicinal practices, and its local names vary across regions, including "Aduro kokoo," "Okramankote," and "Krakoo" in Ghana, as well as "Ajo" or "Akerejupon" in South-Western Nigeria. In Benin and Côte d'Ivoire, it is known as Oban abe and Ouse-abe. Considerable medicinal value has been attributed to the plant in many West African communities, where different parts of the shrub are used to manage conditions such as malaria, erectile dysfunction, and gastrointestinal disorders. Powdered root extracts have been associated with analgesic, antidepressant, and anti-inflammatory effects, while alcoholic preparations are traditionally taken as aphrodisiacs, especially for erection-related issues. Antiviral and antibacterial properties have been reported for leaf extracts, and the fruits and leaf twigs are commonly used alone or in combination therapies for antimalarial, antihypertensive, antitumor, antidiabetic, and wound-healing purposes. Phytochemical studies have identified saponins, tannins, alkaloids, and flavonoids in the leaves, which are believed to contribute to the plant's wide-ranging biological activities (Nia *et al*., 2004; Amidu, 2008; Olorunnisola *et al*., 2017).

2.1.1. Scientific Classification

According to Plants of the World Online (2025), the plant is scientifically classified as:

- Kingdom: Plantae

- Phylum: Streptophyta
- Class: Equisetopsida
- Subclass: Magnoliidae
- Order: Ranunculales
- Family: Menispermaceae
- Genus: *Sphenocentrum*

The Menispermaceae family is a diverse group of mostly climbing woody plants within the order Ranunculales. Members of this family are predominantly found in tropical and subtropical regions worldwide, with a strong representation in Africa, Asia, and South America. The family is taxonomically recognized for its distinctive morphological traits, including twining stems, peltate leaves, and highly curved or "moon-shaped" seeds, features that have earned it the common name *"moonseed family"* (Heywood *et al*., 2007). Phytochemically, Menispermaceae species are notable for their rich alkaloid content, particularly isoquinoline alkaloids, many of which exhibit potent pharmacological properties. Historically, several species have been used in traditional medicine, and some served as sources of arrow poisons such as *curare* in South America. These bioactive compounds have contributed to the family's significance in ethnomedicine, drug discovery, and natural product research (Barbosa-Filho *et al*., 2000).

2.1.2. Botanical Description

S. jollyanum Pierre (Menispermaceae) is a perennial shrub that typically reaches about 1.5 m in height and grows in regions with approximately 1800 mm of annual rainfall, mean minimum

temperatures around 20°C, and mean maximum temperatures near 29°C (Iwu, 1993; Olorunnisola *et al*., 2017). The plant has few, sparsely distributed branches. Its leaves are wedge-shaped, smooth on both surfaces, and measure approximately 5–12 cm in length, tapering to a small arrow-like apex, as shown in Figure 1A (Iwu, 1993; Olorunnisola *et al*., 2017).

The fruit consists of clusters of 3–12 drupes, each ellipsoid and transitioning from yellow to orange as it ripens. The drupes are smooth and fleshy, with a single seed. The seed has a very thin seed coat, lacks endosperm, and contains a straight, ellipsoid embryo measuring 15–18 mm by 8–9 mm. Seedlings possess plano-convex cotyledons that remain enclosed within the stone (Figure 1B) (Moody *et al*., 2005). The roots are distinctly bright yellow and have a sour, acidic taste that can make foods consumed afterward taste sweeter (Iwu, 1993; Nia *et al*., 2004; Olorunnisola *et al*., 2017).

The plant bears solitary flowers on mature branches or along the stem between the leaves (Figure 1C). The flowers are unisexual, with regular sepals arranged spirally and increasing in size toward the center (Ekpono *et al*., 2018). They are cream-colored. Male flowers are sessile and bear 15–21 sepals: the outer sepals are triangular to ovate-oblong and short-hairy, while the inner sepals are obovate and glabrous externally, measuring 0.5–6.5 mm by 0.5–4 mm. The stamens, numbering 16–31, are erect, free, and measure about 1.5–2.5 mm in length, with inflated filaments. Female flowers are sessile or borne on pedicels up to 4 mm long, with 9–11 sepals (Nia *et al*., 2004). The young stems are thin and covered with fine hairs, becoming smooth and glabrous as they mature; the bark is grey (Figure 1D) (Moody *et al*., 2005). *S. jollyanum* is native to the tropical forest zones of West Africa and is widely distributed across Nigeria, Sierra Leone, Ghana, Cameroon, and the Ivory Coast (Nia *et al*., 2004).

Figure 1. Overview of parts of the *Sphenocentrum jollyanum* plant. (A) Leaves (from Olorunnisola *et al*., 2017); (B) Fruits (from Olorunnisola *et al*., 2017); (C) Flowers (from Pl@ntNet, 2025); and (D) Bark (from Pl@ntNet, 2025).

2.1.3. Ethnomedicinal Uses

Different parts of the *S. jollyanum* plant have been utilized in traditional medicine (Olorunnisola *et al*., 2017). In Southwestern Nigeria, for instance, the fruit and root are commonly used to manage gastric ulcers; the powdered forms are mixed with pap or water before consumption (Akinwumi and Sonibare, 2019). Root decoctions are also traditionally used to treat malaria. The

root is particularly valued as an aphrodisiac; it is soaked in alcohol for several days, and the resulting extract is consumed to enhance male sexual performance (Burkill, 1995). This extract is taken as a bitter tonic and is reported to have long-lasting effects. Dried, pulverized roots are also combined with other antimalarial plants to alleviate muscular pain and fever (Akinwumi and Sonibare, 2022).

The aerial parts of the plant are traditionally mixed with *Piper guineense* Schumach. & Thonn. (Piperaceae) and lime juice to treat coughs, chronic injuries, and fever. Literature further indicates that the root stimulates the central nervous system and is used in managing mental and inflammatory disorders, pain, and depression (Oke and Hamburger, 2002). In Nigeria, chewing the roots can stimulate appetite, relieve constipation, and improve digestion. Various plant parts are also incorporated into treatments for sickle cell disease (Akinwumi and Sonibare, 2022). In Ghana and Côte d'Ivoire, the roots are used in herbal medicine to manage hypertension, irregular menstrual flow, breast tumors, and diabetes mellitus (Odugbemi, 2006). Traditional practitioners in the Ivory Coast also regard the root as having stomachic and hemostatic properties, and it is used as an emetic in cases of poisoning (Nafiu *et al*., 2008). Across Sub-Saharan Africa, *S. jollyanum* is valued as an aphrodisiac and for treating sexual dysfunction (Ajao *et al*., 2019).

2.1.4. Biological and Pharmacological Activities

Extensive studies have reported the biological and pharmacological activities of *S. jollyanum*, including antidiabetic, antioxidant, hepatoprotective, anti-inflammatory, antimalarial, anti-allergic, antimicrobial, and antidepressant activities, as well as its gastroprotective and hematological effects, and for treating benign prostatic hyperplasia (BPH) (Akinwumi and Sonibare, 2022).

2.1.4.1. Antidiabetic Activity

The antidiabetic potential of *S. jollyanum* petroleum ether seed extracts has been evaluated in hyperglycemic and alloxan-induced diabetic rabbits. Administration of the seed extract (1 g/kg body weight [b.w.]) and the standard drug glibenclamide (10 mg/kg b.w.) resulted in blood glucose reductions of 20% and 43.8%, respectively, compared with those in untreated controls. In alloxan-induced diabetic animals, treatment with the extract significantly ($p < 0.05$) lowered blood glucose levels in a dose-dependent manner from the 3rd day of daily treatment through the end of the study. Post-treatment with extract doses of 300, 600, and 1200 mg/kg b.w. produced maximum reductions in blood glucose of 12.3%, 29.2%, and 32.7%, respectively, while the glibenclamide-treated group showed a 51.9% reduction (Mbaka *et al*., 2010).

2.1.4.2. Treatment of Benign Prostatic Hyperplasia

BPH is one of the most common urinary disorders in older men, often causing lower urinary tract symptoms. Age is a major risk factor, with prevalence increasing with advancing age (Csikos *et al*., 2021). Mbaka *et al*. (2019) investigated the effects of *S. jollyanum* seed extract on BPH, which frequently leads to bladder outlet obstruction, and found that prostate weight, a reliable indicator of BPH progression, was reduced in a dose-dependent manner by 79.3% and 89.7% at extract doses of 300 and 600 mg/kg, respectively, compared with the 68% reduction achieved with the standard drug finasteride (0.1 mg/kg). Testosterone levels, elevated to 5.7 ng/mL in animals with BPH, decreased dose-dependently in the extract-treated groups to 1.8 ng/mL (68.4%) and 1.7 ng/mL (70.2%) at 300 and 600 mg/kg, respectively, while finasteride produced a 70.2% reduction (1.7 ng/mL). Histomorphological examination revealed that the petroleum

ether seed extract caused a notable reduction in prostate weight, with disintegration of stromal and epithelial cells and minimal epithelial involutions of glandular tissue.

2.1.4.3. Antioxidant Activity

The antioxidant activity of different parts of *S. jollyanum* has been assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Nia *et al*., 2004). Among the extracts tested, leaf extract exhibited weak activity (half-maximal inhibitory concentration [IC_{50}] = 4.35 µg/mL), followed by the root bark (IC_{50} = 3.50 µg/mL), while the chloroform fraction of the stem bark was the most active (IC_{50} = 1.54 µg/mL). For comparison, the standard antioxidant ascorbic acid had an IC_{50} of 0.80 µg/mL.

Olorunnisola *et al*. (2011) further investigated the *in vitro* antioxidant activity of the stem extract for superoxide and hydrogen peroxide radical scavenging (Olorunnisola *et al*., 2011). The methanol stem extract showed IC_{50} values of 13.11 µg/mL and 30.0 µg/mL for superoxide and hydrogen peroxide radicals, respectively, while ascorbic acid had corresponding IC_{50} values of 15.34 µg/mL and 35.44 µg/mL, respectively.

To further validate its therapeutic potential, Uka *et al*. (2020) examined the phytochemical composition, acute toxicity, and antioxidant activity of the ethanol leaf extract. Phytochemical screening revealed the presence of alkaloids, saponins, tannins, flavonoids, and cardiac glycosides, with total phenolic, flavonoid, and gallic acid equivalents measured at 31.49, 29.98, and 1215.80 mg/mL, respectively. Acute toxicity studies in albino mice indicated a median lethal dose greater than 4743.42 mg/kg b.w., confirming low toxicity. The leaf extract exhibited IC_{50} values of 164.214, 72.410, and 167.202 mg/mL for DPPH radical scavenging, ferric reducing antioxidant power, and iron chelating activity, respectively. These values compare favorably

with those of ascorbic acid, which had IC_{50} values of 248.081, 155.134, and 270.703 mg/mL in the same assays.

Collectively, these findings showed that *S. jollyanum* leaves are rich in antioxidant compounds with multiple mechanisms of action, providing a scientific basis for their traditional medicinal use (Uka *et al*., 2020).

2.1.4.4. Hepatoprotective Activity

Olorunnisola *et al*. (2011) evaluated the hepatoprotective potential of *S. jollyanum* using methanol crude extracts administered orally at doses of 50, 100, and 200 mg/kg to Wistar rats, with hepatotoxicity induced by 30% carbon tetrachloride (1.0 mL/kg b.w.), using olive oil (1.0 mL/kg b.w.) as the control. They found that the extract effectively prevented depletion of total protein and antioxidant markers in the liver and restored the activity of marker enzymes to normal levels, showing significant hepatoprotective activity likely owing to its strong antioxidant properties.

2.1.4.5. Anti-Inflammatory Activity

Moody *et al*. (2005) evaluated the *in vivo* anti-inflammatory activity of methanol crude extracts of *S. jollyanum* using carrageenan-induced hind paw edema in albino rats. At a dose of 200 mg/kg, the methanol fruit extract inhibited edema by 79.58%, while the root extract produced 53.75% inhibition. The standard drug, acetylsalicylic acid (100 mg/kg), showed 72.5% inhibition. The fruit extract was further purified to yield three clerodane diterpenoids—columbin, isocolumbin, and fibleucin—among which columbin exhibited the highest activity, with 67.08% inhibition at 20 mg/kg ($p < 0.05$).

In another study, Samuel *et al*. (2015) assessed the anti-inflammatory potential of the leaf extract using *in vitro* assays, including erythrocyte membrane stabilization, trypsin inhibition, and lipoxygenase inhibition. The aqueous extract and the saponin-rich fraction showed the strongest dose-dependent membrane-stabilizing activity. The aqueous extract exhibited stronger lipoxygenase inhibition (IC_{50} = 637 µg/mL) than diclofenac did (IC_{50} = 52 µg/mL). In proteinase inhibition assays, ethanol and tannin-rich fractions showed the highest inhibitory activity (IC_{50} = 840 and 1810 µg/mL, respectively), while indomethacin had an IC_{50} of 246 µg/mL. These results provide scientific support for the traditional use of *S. jollyanum* in the management of inflammation.

Uka *et al*. (2021) evaluated the ethanol leaf extract *in vivo* using carrageenan-, egg albumin-, and xylene-induced edema models in mice at doses of 474.34, 948.68, and 1423.03 mg/kg b.w. The extract significantly reduced paw thickness in a dose- and time-dependent manner. At the highest dose, the extract inhibited carrageenan- and egg albumin-induced edema by 34.49% and 36.71%, respectively, values comparable to those of acetylsalicylic acid (34.69% and 31.54%). Xylene-induced ear edema was reduced by 44% at 1423.03 mg/kg, approaching the effect of dexamethasone (48% at 4 mg/kg). These findings corroborate the traditional use of *S. jollyanum* leaf extract for anti-inflammatory purposes, likely linked to its phytochemical content.

2.1.4.6. Anti-Malarial Activity

The anti-plasmodial activity of methanol extracts from *S. jollyanum* leaves and roots was reported by Olorunnisola and Afolayan (2011). Using Swiss albino mice infected with chloroquine-resistant *Plasmodium berghei* NK67, both extracts showed significant, dose-dependent anti-plasmodial activity, individually and in combination, resulting in improved

survival rates. The standard drug, artemether-lumefantrine (5 mg), achieved 81.4% inhibition. The leaf extract at 200 mg produced 74.7% inhibition, while the root extract at 200 mg achieved 54.1%. Additionally, the extracts positively influenced animal body weight and hematological parameters. These results validate the traditional use of *S. jollyanum* in the management of malaria and confirm its effectiveness against chloroquine-resistant strains.

2.1.4.7. Haematological Activity

Mbaka *et al*. (2010) investigated the hematological effects of the methanol extracts of *S. jollyanum* (leaf and root) using Wistar mice infected with chloroquine-resistant *P. berghei* NK67. The extracts were administered daily for 7 days, resulting in increased packed cell volume (PCV) and mean corpuscular volume. Red and white blood cell counts also rose, except for neutrophils and monocytes, indicating stimulation of haematopoietic stem cells. Similarly, Ekpono *et al*. (2019) reported that treatment of *P. berghei*-infected mice with the ethanol root extract of *S. jollyanum* significantly reduced parasitemia ($p < 0.05$). Infection with *P. berghei* resulted in a significant decline ($p < 0.05$) in PCV, hemoglobin, red blood cell count, and white blood cell count compared with normal controls. However, administering the ethanol root extract at 200, 400, and 800 mg/kg body weight resulted in a dose-dependent, significant ($p < 0.05$) restoration of these hematological parameters, approaching values observed in the standard control group, particularly at the highest dose of 800 mg/kg. These findings suggest that the ethanol root extract of *S. jollyanum* may be effective in managing anemia associated with *P. berghei* infection.

2.1.4.8. Anti-Allergy Activity

Allergic disorders arise from the immune system's hypersensitivity to non-infectious stimuli, leading to conditions such as eczema, asthma, allergic rhinitis, and inflammatory bowel disease. These disorders affect over 300 million people globally, with one in every 250 fatalities linked to allergies (Olorunnisola *et al*., 2017). The anti-allergic potential of *S. jollyanum* fruit extract was evaluated in mice with milk-induced eosinophilia and leukocytosis. Both the ethanol fruit extract and dexamethasone demonstrated dose-dependent reductions in eosinophil and lymphocyte counts, suggesting the plant's anti-allergic activity may involve multiple mechanisms mediated by its phytochemical constituents (Olorunnisola *et al*., 2017).

2.1.4.9. Antimicrobial Activity

The methanol leaf extract of *S. jollyanum* has been reported to show significant antifungal activity against *Fusarium* species isolated from human and plant samples. Using modified agar diffusion methods, the extract produced mean inhibition zone diameters of 31.97 ± 0.66 mm and 29.03 ± 0.97 mm for human and plant isolates, respectively, outperforming standard antifungals such as voriconazole and fluconazole (Udoh *et al*., 2021). The extract also showed a low minimum inhibitory concentration of 0.0679 µg/mL, indicating potent antimicrobial activity. Additionally, the crude extract inhibited *Salmonella typhi* in agar well diffusion assays, showing broad-spectrum antibacterial activity (Koleosho *et al*., 2013).

2.1.4.10. Antidepressant Activity

Woode *et al*. (2009) evaluated the antidepressant activity of ethanol extracts from S. jollyanum roots using the forced swimming test (FST) and tail suspension test (TST) in animal models. The

extract reduced immobility time in a dose-dependent manner in both FST (median effective dose [ED_{50}] = 296.20 ± 53.97 mg/kg) and TST (ED_{50} = 203.90 ± 39.01 mg/kg), with standard drugs imipramine and fluoxetine used for comparison. These results suggest that *S. jollyanum* possesses effective antidepressant properties with potential for further development as a therapeutic agent.

2.1.5. Phytochemistry

Several natural compounds have been reported from *S. jollyanum* using traditional extraction and purification techniques (Walsh *et al*., 2017). Phytochemical analysis of the methanol extract of the stem bark revealed the presence of terpenes, saponins, alkaloids, and tannins (Nia *et al*., 2004). Aboaba and Ekundayo (2010) analyzed the root essential oil using GC–MS, identifying 19 compounds, including camphene, δ-3-carene, globulol, 5-guaiene-11-ol, p-cymene, α-eudesmol, and β-pinene. Proximate analysis of the seed extract showed crude protein (48.1%), moisture (16.7%), carbohydrates (48.1%), ash (16.8%), crude fat (9.7%), fiber (5.5%), and an energy value of 1460 kcal/100 kg. The isolated compounds were composed of monoterpenoids (33.5%) and sesquiterpenoids (56.3%), with the remaining 10.2% unidentified.

Uka *et al*. (2022) reported the presence of several bioactive phytochemicals. The major compounds identified were 2,4-di-tert-butylphenol and phenol, 3,5-bis(1,1-dimethylethyl) (21.05%), cyclohexene, 6-butyl-1-nitro, Z-8-methyl-9-tetradecenoic acid, and methyl 9,12-heptadecadienoate (19.12%), hexadecanoic acid ethyl ester and undecanoic acid ethyl ester (7.86%), diisooctyl phthalate and bis(2-ethylhexyl) phthalate (7.13%), as well as phytol, oleic acid, and cis-11-hexadecenal (7.03%). Other notable constituents include 6,9,12-octadecatrien-1-ol, ethanol, 2-(9,12-octadecadienyloxy)-, (Z, Z)- (6.65%), 5-eicosene, (E)-, 3-eicosene, (E)-, 1-

octadecene (4.63%), 9,17-octadecadienal (Z)-, 2-methyl-Z, Z-3,13-octadecadienol, and cis-7, cis-11-hexadecadien-1-yl acetate (4.24%), among others. These compounds were detected in smaller proportions, ranging from 4.09–1.47%, including n-hexadecanoic acid, n-decanoic acid, L-galactose, trans-13-octadecenoic acid, 9-octadecenoic acid (Z)-, and 9,12-octadecadien-1-ol (Z, Z)-.

Moody *et al*. (2005) isolated three clerodane diterpenes—columbin, isocolumbin, and fibleucin—from the fruits of *S. jollyanum*. The fruits, collected from the University of Ibadan, Nigeria, were authenticated at the Forest Herbarium Ibadan (FHI 105364), shade-dried, and pulverized. They extracted 2 kg of the powdered fruits with methanol using cold percolation for 72 h, filtered, and evaporated under reduced pressure to yield 84.2 g. The methanol extract (10 g) was further purified using silica gel vacuum liquid chromatography and preparative thin-layer chromatography, isolating the three diterpenes. Columbin, specifically, showed significant anti-inflammatory activity, with 67.08% inhibition at 20 mg/kg, compared with 72.50% inhibition for acetylsalicylic acid (100 mg/kg).

Camphene, a major bicyclic monoterpene in *S. jollyanum* essential oil, is used in fragrances, as an artificial food flavoring, for camphor production, and for insecticide manufacturing. It also exhibits protective effects against oxidative stress (Tiwari *et al*., 2009). Essential oils from *S. jollyanum* have shown broad-spectrum antibacterial activity against strains such as *Listeria innocua*, *Listeria monocytogenes*, *Bacillus cereus*, and *Staphylococcus aureus*, as well as antifungal activity against *Penicillium chrysogenum*, *Aspergillus* species, *Chaetomium globosum*, and *Trichoderma* species, demonstrating their potential as natural antimicrobial and antioxidant agents (Angelini *et al*., 2008).

Akinwumi *et al*. (2020) further isolated five known ecdysteroids—pinnatasterone, polypodine B, 20-hydroxyecdysone, 20,26-dihydroxyecdysone, and atrotosterone A—from ethyl acetate and n-butanol fractions of *S. jollyanum* seeds. The seeds were collected from Odo Ona, Apata, Ibadan, Nigeria, authenticated at FHI (110510), shade-dried for 3 weeks, and pulverized. The powdered seeds were extracted with 100% methanol and fractionated with n-hexane, dichloromethane, ethyl acetate, and n-butanol. The ethyl acetate fraction was purified using silica gel chromatography and preparative thin-layer chromatography, yielding atrotosterone A and pinnatasterone. The n-butanol fraction yielded polypodine B, 20-hydroxyecdysone, and 20,26-dihydroxyecdysone after preparative reversed-phase high-performance liquid chromatography.

Akinwumi *et al*. (2020) also investigated the urease-inhibitory and antacid properties of these ecdysteroids. Polypodine B, 20-hydroxyecdysone, and pinnatasterone exhibited significant urease inhibition (IC_{50}: 7.0 ± 0.56, 13.8 ± 0.49, and 14.1 ± 0.59 μM, respectively). Meanwhile, 20,26-dihydroxyecdysone (IC_{50}: 24.1 ± 1.21 μM) and atrotosterone A (IC_{50}: 29.3 ± 7.45 μM) showed moderate activity, compared to acetohydroxamic acid (IC_{50}: 20.3 ± 0.43 μM) at 0.5 mM.

2.2. Fatty Acids in Living Systems

Fats and oils serve as storage lipids and are found in nearly all living organisms (Nelson *et al*., 2008). According to the Dietary Guidelines for Americans, total fat intake should account for 20–35% of daily caloric intake (Nelson *et al*., 2008). These lipids are composed of fatty acids, which are carboxylic acids with hydrocarbon chains ranging from four to 36 carbon atoms (Figure 2). Fatty acids are not only key components of fats but also of membrane lipids, including phospholipids and sphingolipids, the latter of which are abundant in neural tissues (Nelson *et al*., 2008).

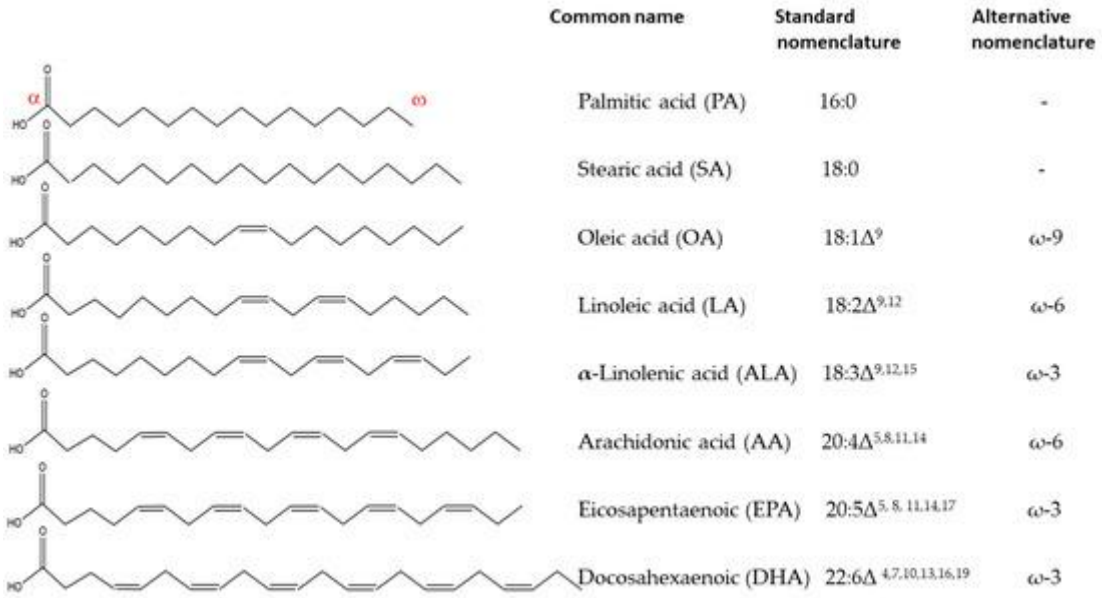

Figure 2. Important fatty acids for human health (Coniglio *et al*., 2023).

Saturated fatty acids have hydrocarbon chains without double bonds, whereas UFAs (unsaturated fatty acids) contain one or more double bonds. The nomenclature of fatty acids indicates the total number of carbon atoms in the chain, followed by the number of double bonds after a colon, with the position of double bonds designated by Δ and the number of carbons from the carboxyl (α-) carbon. Polyunsaturated fatty acids (PUFAs) contain double bonds at specific positions from the methyl (ω-) end of the chain, such as between the third and fourth or the sixth and seventh carbons. These PUFAs, commonly referred to as ω-3 and ω-6 fatty acids, are particularly important for human health, as their physiological functions are closely linked to the position of the double bond relative to the methyl end (Figure 2) (Coniglio *et al*., 2023).

Humans require the ω-3 PUFA α-linolenic acid (ALA, 18:3Δ9,12,15) to produce other essential PUFAs but cannot synthesize it owing to the absence of specific enzymes. Consequently, ALA (α-linolenic acid) must be obtained from dietary plant sources. Once ingested, ALA can be

metabolically converted into longer-chain ω-3 PUFAs, including eicosapentaenoic acid (EPA, 20:5Δ5,8,11,14,17) and docosahexaenoic acid (DHA, 22:6Δ4,7,10,13,16,19) (Nelson *et al*., 2008). Additionally, EPA and DHA can be directly acquired from fish and other seafood (Oliver *et al*., 2020).

2.2.1. Biosynthesis of Essential Fatty Acids in Plants

In plants, fatty acid biosynthesis occurs in plastids, the photosynthetic organelles of plant cells. These fatty acids are subsequently utilized as components of plastid and endoplasmic reticulum membrane phospholipids, storage lipids, or extracellular waxes (Zhukov *et al*., 2020). Major plastid lipids are initially synthesized using 16:0 and 18:1 acyl groups, after which additional double bonds are introduced by fatty acid desaturases. These desaturases insert double bonds into fatty acid hydrocarbon chains, modulating lipid and membrane fluidity (Cerone and Smith, 2022). Phospholipid membranes rich in saturated fatty acids are more rigid, potentially causing physiological problems under cold stress owing to membrane solidification. In contrast, UFAs enhance membrane flexibility, improving tolerance to chilling stress (Los and Murata, 2004; Murata *et al*., 1997).

Accumulation of UFAs such as ALA in membranes is a common response to abiotic stress, increasing membrane fluidity and preventing rigidification. Beyond modulating membrane properties, C18 PUFAs also act as intrinsic antioxidants. The double bonds in these fatty acids make them susceptible to reactive oxygen species (ROS), which are often generated during stress. Overexpression of ω-3 fatty acid desaturases is a general plant defense mechanism to trigger stress responses. Excessive ROS can cause peroxidation of C18 PUFAs, leading to the accumulation of malondialdehyde. At moderate levels, malondialdehyde serves as a signaling

molecule that facilitates stress perception, but excessive peroxidation can lead to oxidative damage and DNA degradation. Plants thus maintain a tightly regulated balance of UFAs and reactive species (Zhang *et al*., 2003; He *et al*., 2018).

2.2.1.1. Unsaturated Fatty Acid Synthesis

Key plant UFAs include oleic acid (OA, 18:1Δ9), linoleic acid (LA, 18:2Δ9,12), and ALA (18:3Δ9,12,15) (He *et al*., 2020). These fatty acids play critical roles in stress responses and serve as precursors for plant hormones. Their synthesis is regulated by central phytohormones such as abscisic acid, auxin, and jasmonic acid, which coordinate plant growth, development, and defense mechanisms (Shahid *et al*., 2019). A comprehensive database of plant fatty acids and their structures is available on PlantFAdb (Ohlrogge *et al*., 2018).

OA is synthesized *de novo* in plastids from acetyl-CoA. First, saturated stearic acid (18:0) is produced by fatty acid synthase and acetyl-CoA carboxylase, after which stearoyl-ACP desaturase introduces a double bond at the 9th carbon position, forming OA (He *et al*., 2020). Humans can synthesize OA endogenously, so it is not considered essential (Piccinin *et al*., 2019). In plants, C18 PUFAs are coupled with membrane lipid synthesis; OA is incorporated into phospholipids such as phosphatidic acid or phosphatidylcholine, which undergo further desaturation via either prokaryotic (chloroplast) or eukaryotic (endoplasmic reticulum) pathways (Wu *et al*., 2009).

The biosynthesis of LA(linoleic acid) and ALA depends on Δ12 (ω-6) and Δ15 (ω-3) fatty acid desaturases, which are exclusive to photosynthetic organisms. Genes encoding these ω-6 and ω-3 fatty acid desaturases are present in the genomes of many staple crops, and their expression is generally upregulated under cold stress conditions (Yu *et al*., 2009; Lee *et al*., 2005). Humans

lack these enzymes, rendering ω-3 and ω-6 fatty acids essential components of the human diet (Tvrzicka *et al*., 2011).

2.2.2. Dietary Plant Sources of Fatty Acids

Plant oils and seeds serve as rich dietary sources of essential fatty acids. Flaxseed (linseed) oil is particularly high in ALA, while LA is abundant in safflower oil. Genomic analyses of safflower (*Carthamus tinctorius*) have identified tandem duplications of an ω-6 fatty acid desaturase gene, specifically expressed in seeds, contributing to the crop's high LA content (Wu *et al*., 2021). Efforts to enhance the production of 18:3 fatty acids through genetic modification in model plants such as tobacco and *Arabidopsis* have successfully increased both their abiotic stress tolerance and 18:3 fatty acid content (Shi *et al*., 2018; Yin *et al*., 2018). These advances hold promise for breeding stress-tolerant crops with improved nutritional oil quality (Coniglio *et al*., 2023).

OA, although non-essential for humans, is widely synthesized in nutritionally valuable plants such as olive (*Olea europaea*), Brassica species, and peanut (*Arachis* species). Genome-editing approaches using CRISPR-Cas9 to disrupt ω-6 desaturase have been applied in rice (*Oryza sativa*) and soybean (*Glycine max*), producing oils enriched in OA (Abe *et al*., 2018; Do *et al*., 2019). Similarly, high-oleic and high-stearic oils have been produced in cotton via hairpin RNA-mediated post-transcriptional silencing of the ω-6 desaturase (Liu *et al*., 2004). These oils, with enhanced thermal stability, have potential as healthier alternatives to saturated fats and hydrogenated oils in cooking applications (Coniglio *et al*., 2023).

Many common cooking oils contain substantial amounts of saturated fatty acids. For instance, palmitic acid (16:0) comprises approximately 44% of palm oil, 26% of cocoa butter, 8–20% of

olive oil, and 10–12% of soybean oil (Carta *et al*., 2015). However, novel plant varieties can be developed with elevated PUFA levels and reduced saturated fat content. For example, genetically modified soybean lines with early termination of palmitoyl-acyl carrier protein thioesterase show reduced palmitic acid content, offering a pathway to healthier oil production (Carrero-Colon *et al*., 2022). Beyond seafood, plants can also significantly contribute to EPA and DHA supply. Microalgae cultivated on glucose-rich media, as well as transgenic plants like *Camelina*, can be engineered to accumulate ω-3 EPA and DHA, providing a sustainable alternative to fish-based sources (Oliver *et al*., 2020; Valenzuela *et al*., 2015; Napier *et al*., 2015).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Plant Collection and Identification

Fresh samples of *S. jollyanum* were collected from Iwo, Osun State, in South-West Nigeria. The plant material was subsequently 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 give Voucher Number for the plant is UBH-S449. The study was focused specifically on the plant stems, which were prepared for extraction and further analysis.

3.1.2. Chemical Reagents

Ethanol of 95% or absolute grade served as the organic extraction solvent, while distilled or deionized water was used as the aqueous extraction solvent.

3.1.3. Apparatus

The apparatus used throughout the sample preparation and extraction procedures included stainless steel cutting knives, drying trays, polypropylene extraction vessels, manual stirrers or spatulas, muslin or cheesecloth, sieves, universal bottles, nose masks, nitrile gloves, masking tape, beakers, test tubes, spatulas, permanent markers, and a mortar and pestle. All items were utilized as needed during various stages of sample handling.

3.1.4. Laboratory Equipment

The laboratory equipment included washing basins, a mechanical grinder for pulverizing dried stem samples, a freeze dryer (lyophilizer) for removing solvents, weighing boats, an analytical weighing balance, and a freezer for stabilizing samples before freeze-drying.

3.2. Methods

3.2.1. Pre-treatment and Pulverization

Fresh stems were washed thoroughly under running water to remove soil, debris, and surface contaminants. The cleaned stems were then cut into smaller pieces and air-dried at room temperature in a shaded and well-ventilated environment to prevent degradation of heat-sensitive phytochemicals. After complete drying, the stem pieces were ground into coarse powder using a mechanical grinder to increase surface area and enhance extraction efficiency.

3.2.2. Extraction (Cold Maceration)

The powdered stem material was divided into two batches for extraction: an ethanolic extract, in which the powder was soaked in ethanol, and an aqueous extract, in which the powder was soaked in distilled water. Each batch was prepared with a solvent-to-sample ratio of 1 L solvent per 0.15 g plant powder. The samples were macerated for 72 h, with gentle agitation every 2 h to enhance solvent penetration and facilitate phytochemical diffusion.

3.2.3. Filtration

Following maceration, each mixture was filtered through muslin cloth to remove plant residues and obtain clarified solvent extracts.

3.2.4. Concentration (Freeze-Drying)

The filtrates were frozen and subsequently placed in a freeze dryer, where the solvents were removed through sublimation. This process preserved volatile and heat-labile phytochemicals, yielding dry crude extracts.

3.2.5. Phytochemical Analysis

A 1 g portion of each freeze-dried extract, both ethanolic and aqueous, was accurately weighed and stored in labeled universal bottles. These samples were transported to a certified laboratory in Lagos for GC–MS analysis to determine their phytochemical constituents.

3.2.6. Evaluation of the Procedure

The extraction method had several strengths because freeze-drying preserved heat-labile bioactive compounds, regular agitation enhanced solvent extraction efficiency, and the use of both organic and aqueous solvents enabled comparison of polarity-dependent solubility profiles of phytochemicals.

3.2.7. Gas Chromatography–Mass Spectrometry

GC–MS is an analytical technique that combines GC for separating volatile components with MS for identifying compounds based on their mass-to-charge ratios. It operates by first vaporizing the sample and carrying it through a capillary column using an inert gas such as helium. As the vaporized constituents travel through the column, they separate based on

differences in boiling points and their interactions with the column's stationary phase. Once separated, each compound enters the mass spectrometer, where it is ionized and fragmented. The resulting mass spectra serve as distinct chemical fingerprints, enabling accurate identification of the individual components present in the sample.

For this study, the GC–MS analysis of the extracts was performed using a Shimadzu GC–MS-QP2010 system equipped with an AOC-20i autosampler, operated under specified analytical conditions:

1. **Autosampler (AOC-20i) Conditions:** The autosampler operated with three pre-solvent rinses, three post-solvent rinses, and two sample rinses. Both the plunger suction and injection speeds were set to high, with a viscosity compensation time of 0.2 s. The injection mode was normal, with five pumping cycles and an injection port dwell time of 0.3 seconds. No terminal air gap was applied. The plunger washing speed was high, the washing volume was 8 μL, and both the suction and injection positions of the syringe were set to 0.0 mm. A single solvent vial was used throughout the process.
2. **Gas Chromatography Conditions (GC-2010):** The GC system operated with a column oven temperature of 60°C and an injection temperature of 250°C. The injection mode was splitless, with a sampling time of 1.00 min. Flow control was maintained under pressure mode with a carrier gas pressure of 100 kPa, a total flow of 4.7 mL/min, a column flow of 0.80 mL/min, a 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 carrier gas saver were disabled.

3. **Oven Temperature Program:** The oven program began with a hold at 60°C for 1.00 min, followed by heating at 13°C/min to 240°C with a 1.00-minute hold, and continued at 13 °C/min to 300 °C with a final hold of 39.70 min.
4. **Mass Spectrometric Conditions (MS: QP2010):** The mass spectrometer operated with an ion source temperature of 230°C and an interface temperature of 250°C. The solvent cut time was set to 4.00 min. The detector was run in relative gain mode at 1.33 kV with a threshold of 2000.
5. **Mass Spectrometry Scan Program:** The scan program began at 8.00 min and ended at 59.80 min. The acquisition mode was set to scan, using an event time of 0.30 s, a scan speed of 1666 amu/s, and a scan range of m/z 35–500.
6. **Ready Check Parameters:** Before initiating the GC–MS run, the system underwent a readiness check to ensure optimal performance. The column oven temperature, injector temperature, and interface temperature were verified to be stable at their set values. The carrier gas flow and pressure were confirmed to be within the programmed limits. The autosampler syringe was inspected for proper alignment, cleanliness, and plunger function. Baseline stability was assessed to ensure the detector exhibited minimal noise. The system vacuum level was also checked to confirm adequate ionization conditions. Only after all parameters met the required specifications was the instrument deemed ready for sample analysis.
7. **Heat Unit:** All heating components, including the column oven, split/splitless injector 1 (SPL1), and the mass spectrometer, were activated.
8. **Injection Flow:** The injection flow settings ensured that both SPL1 carrier and purge flows remained active throughout the analysis.

CHAPTER FOUR

4. RESULTS

4.1. Gas Chromatography–Mass Spectrometry Analysis of the Aqueous Extract of *Sphenocentrum jollyanum*

The GC–MS analysis of the aqueous extract of *S. jollyanum* stems revealed the presence of 33 phytochemical compounds, as shown in Table 1, which lists the retention times, relative peak areas, and compound identities. The GC–MS chromatogram (Figure 3) illustrates the separation and relative abundance of the detected compounds. Among the identified constituents, five UFA derivatives were observed, each of which is of particular biological significance. These include 9-Octadecenoic acid (Z)-, methyl ester (Peak #11, R.T. 22.826 min, 2.54% area; Figure 4), Ethyl Oleate (Peak #14, R.T. 23.448 min, 4.73% area; Figure 5), 9-Octadecenamide, (Z)- (Peak #23, R.T. 26.151 min, 1.34% area; Figure 6), 13-Octadecenal, (Z)- (Peak #27, R.T. 27.239 min, 1.94% area; Figure 7), and 16-Trimethylsilyloxy-9-octadecenoic acid, methyl ester (Peak #30, R.T. 29.754 min, 1.27% area; Figure 8). The relative abundance of these UFA derivatives in the aqueous extract indicates the plant's potential for contributing bioactive lipid compounds that may support anti-inflammatory, antioxidant, and other pharmacological activities.

Table 1 also shows that other major compounds detected in the aqueous extract include Inositol, 1-deoxy- (Peak #5, 43.45% area), .alpha.-Methyl mannofuranoside (Peak #6, 3.79% area), Phytol acetate (Peak #7, 1.00% area), and Hexadecanoic acid derivatives (Peaks #8 and #10, 1.34% and 2.32% area, respectively), which collectively contribute to the overall phytochemical profile of the extract. These findings underscore the diversity of chemical constituents in the aqueous extract and provide a basis for understanding the bioactivity and traditional medicinal uses of *S. jollyanum*.

Table 1. GC–MS–identified phytochemical constituents in the aqueous extract of *Sphenocentrum jollyanum*.

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-
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	1.00	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	2.20	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	3.60	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	26.350	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
27	27.239	4750873	1.94	1125826	3.00	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	1.30	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	45.710	2758340	1.13	290294	0.77	9.50	dl-.alpha.-Tocopherol
		244620400	100.00	37589456	100.00		

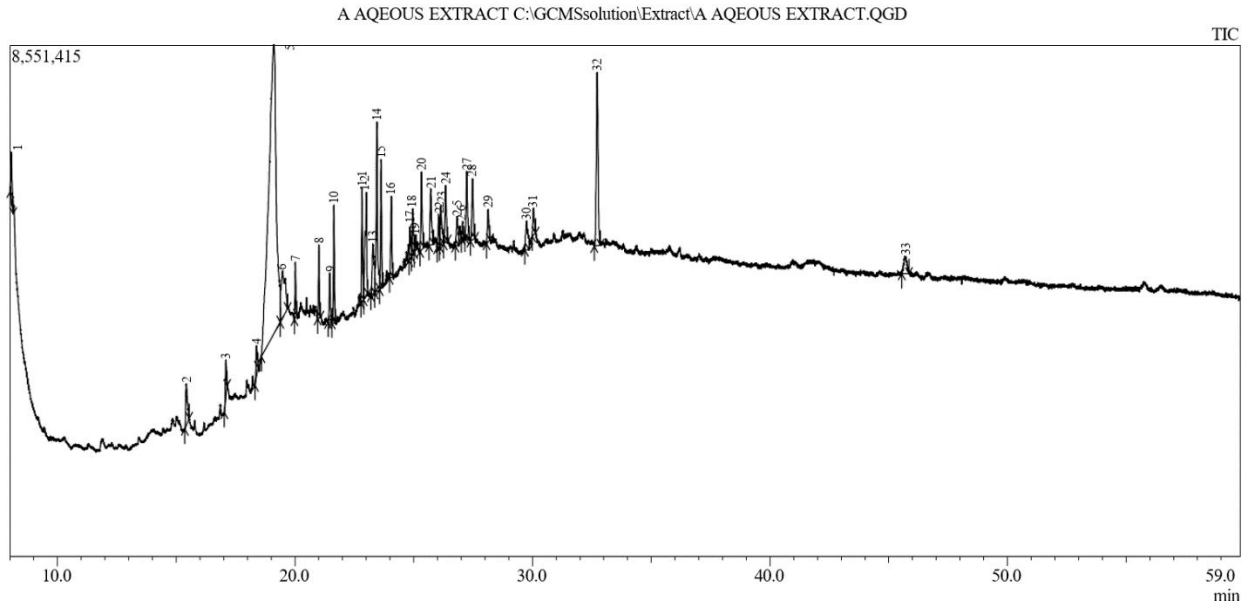

Figure 3. GC–MS chromatogram of aqueous extract of *Sphenocentrum jollyanum*

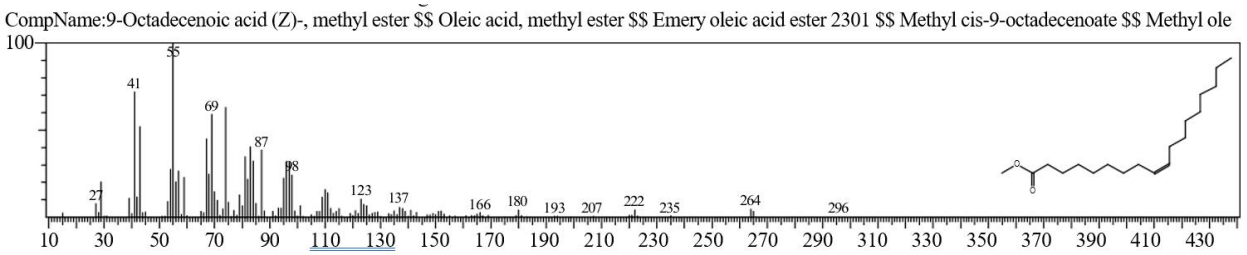

Figure 4. GC–MS spectra of 9-Octadecenoic acid (Z)-, methyl ester

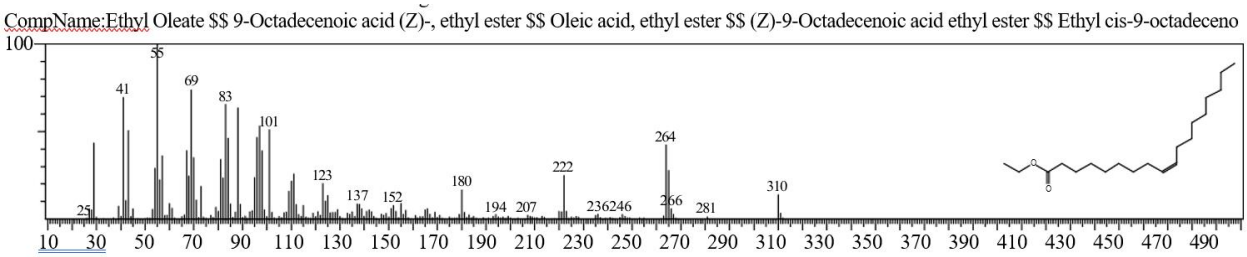

Figure 5. GC–MS spectra of Ethyl Oleate

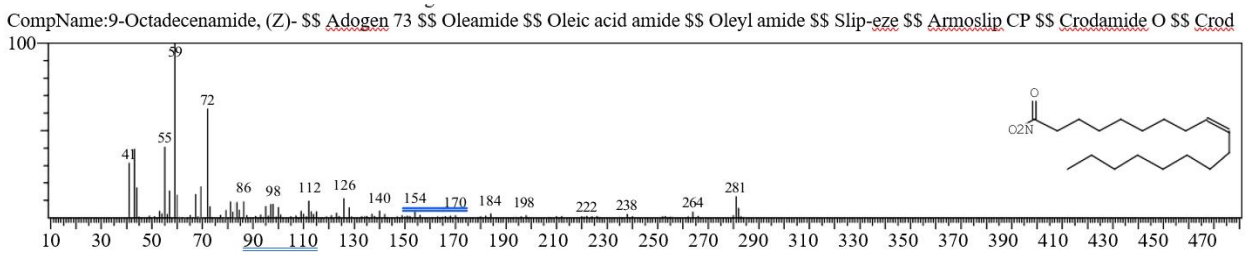

Figure 6. GC–MS spectra of 9-Octadecenamide, (Z)-

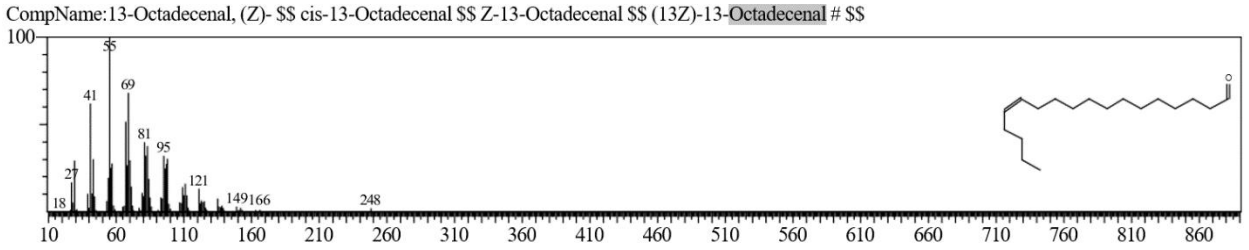

Figure 7. GC–MS spectra of 13-Octadecenal, (Z)-

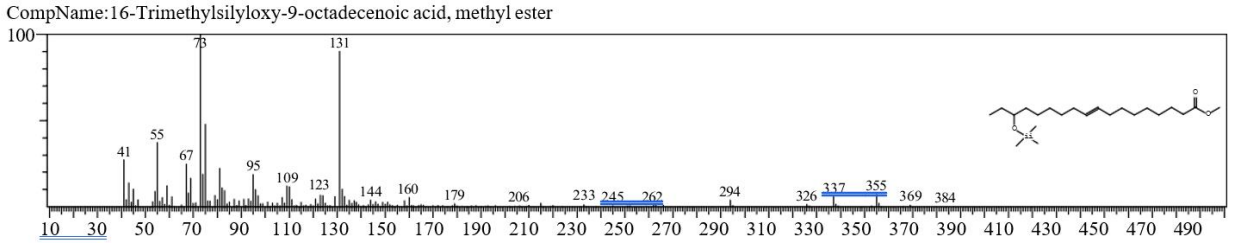

Figure 8. GC–MS spectra of 16-Trimethylsilyloxy-9-octadecenoic acid, methyl ester

4.2. Gas Chromatography–Mass Spectrometry Analysis of the Ethanolic Extract of *Sphenocentrum jollyanum*

The GC–MS analysis of the ethanolic extract of *S. jollyanum* stems revealed 34 phytochemical compounds, as summarized in Table 2. The GC–MS chromatogram (Figure 9) displays the retention times and relative abundance of the detected compounds, highlighting the chemical diversity of the extract. Among the identified constituents, six UFA derivatives were identified, which are particularly notable for their biological relevance. These include 9-Octadecenal, (Z)- (Peak #12, R.T. 21.971 min, 0.61% area; Figure 12), 9-Octadecenoic acid (Z)-, methyl ester (Peak #14, R.T. 22.826 min, 4.53% area; Figure 4), (E)-9-Octadecenoic acid ethyl ester (Peak #17, R.T. 23.446 min, 9.93% area; Figure 11), 10-Undecenoic acid, methyl ester (Peak #22, R.T. 25.060 min, 1.18% area; Figure 10), 9-Octadecenamide, (Z)- (Peak #25, R.T. 26.146 min, 1.40% area; Figure 6), and 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- (Peak #28, R.T. 27.233 min, 13.46% area; Figure 13). The combined area percentage of these UFAs indicates their substantial contribution to the extract's lipid profile and potential pharmacological activities.

Other prominent compounds detected include Hexadecanoic acid derivatives (Peaks #9, #11, and #21), Phytol acetate (Peak #8), and Squalene (Peak #34, 7.35% area), reflecting the presence of both saturated and unsaturated bioactive lipids in the ethanolic extract. These findings provide a comprehensive chemical profile, underscoring the potential of *S. jollyanum* as a source of bioactive UFAs and other secondary metabolites with possible health benefits and pharmacological applications.

Table 2. GC–MS–identified phytochemical constituents in the ethanolic extract of *Sphenocentrum jollyanum*.

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	12.765	375195	0.52	120718	0.60	3.11	Octanoic acid, ethyl ester
2	13.350	329502	0.46	161579	0.80	2.04	Cyclohexasiloxane, dodecamethyl-
3	15.354	774310	1.08	342947	1.70	2.26	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(tri
4	17.125	1689821	2.36	538152	2.67	3.14	Cyclooctasiloxane, hexadecamethyl-
5	17.569	1107769	1.55	449706	2.23	2.46	Dodecanoic acid, ethyl ester
6	18.628	485484	0.68	243517	1.21	1.99	Cyclohexasiloxane, dodecamethyl-
7	19.647	656185	0.92	263358	1.30	2.49	Tetradecanoic acid, ethyl ester
8	20.028	1141800	1.60	381326	1.89	2.99	Phytol, acetate
9	21.012	709655	0.99	280121	1.39	2.53	Hexadecanoic acid, methyl ester
10	21.485	680066	0.95	175785	0.87	3.87	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien

11	21.648	2288319	3.20	823085	4.08	2.78	Hexadecanoic acid, ethyl ester
12	21.971	438328	0.61	160855	0.80	2.72	9-Octadecenal, (Z)-
13	22.645	685357	0.96	76946	0.38	8.91	d-Mannitol, 1-decylsulfonyl-
14	22.826	3239169	4.53	965967	4.78	3.35	9-Octadecenoic acid (Z)-, methyl ester
15	23.008	2922567	4.09	754834	3.74	3.87	Methyl stearate
16	23.258	2081075	2.91	431254	2.14	4.83	2,8,9-Trioxa-5-aza-1-silabicyclo(3.3.3)undeca
17	23.446	7101875	9.93	2010745	9.96	3.53	(E)-9-Octadecenoic acid ethyl ester
18	23.631	6417798	8.97	2000335	9.91	3.21	Octadecanoic acid, ethyl ester
19	24.778	1247381	1.74	444680	2.20	2.81	Cyclononasiloxane, octadecamethyl-
20	24.843	836129	1.17	252507	1.25	3.31	1-Buten-1-amine, N,N-dipropyl-
21	24.970	2914947	4.08	885634	4.39	3.29	Hexadecanoic acid, 1-[[[(2-aminoethoxy)hydr
22	25.060	840803	1.18	247510	1.23	3.40	10-Undecenoic acid, methyl ester

23	25.774	682026	0.95	203926	1.01	3.34	Docosanoic acid, ethyl ester
24	26.053	1175938	1.64	455025	2.25	2.58	Cyclononasiloxane, octadecamethyl-
25	26.146	999962	1.40	292033	1.45	3.42	9-Octadecenamide, (Z)-
26	26.340	2013141	2.81	522371	2.59	3.85	1-Naphthalenecarboxylic acid, 5-[2-(3-furanyl
27	26.798	858360	1.20	239700	1.19	3.58	Octadecanoic acid, 2,3-dihydroxypropyl ester
28	27.233	9625278	13.46	2244662	11.12	4.29	9-Octadecenoic acid, 1,2,3-propanetriyl ester,
29	27.470	8529876	11.92	2148162	10.64	3.97	Octadecanoic acid, 2-hydroxy-1,3-propanediyl
30	28.134	1465747	2.05	425187	2.11	3.45	Di-n-octyl phthalate
31	29.209	749065	1.05	234505	1.16	3.19	Cyclononasiloxane, octadecamethyl-
32	30.031	590332	0.83	175607	0.87	3.36	Eicosanoic acid, 2,3-bis[(trimethylsilyl)oxy]pr
33	31.257	624020	0.87	181328	0.90	3.44	Cyclononasiloxane, octadecamethyl-

34	32.724	5254490	7.35	1053421	5.22	4.99	Squalene
		71531770	100.00	20187488	100.00		

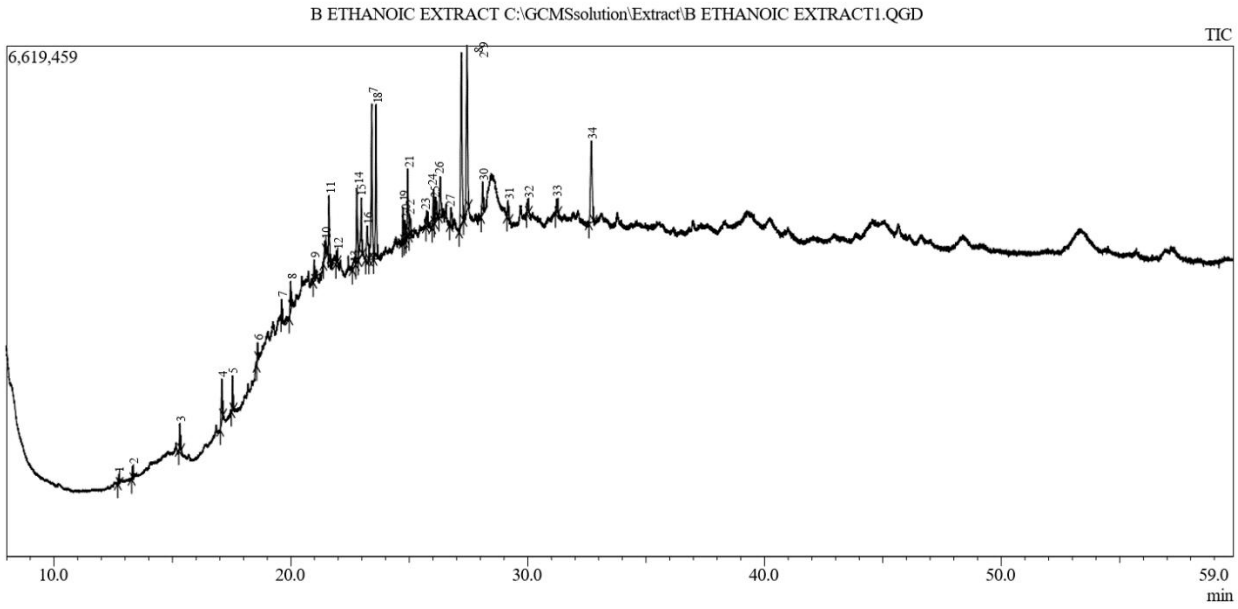

Figure 9. GC–MS chromatogram of ethanolic extract of *Sphenocentrum jollyanum*

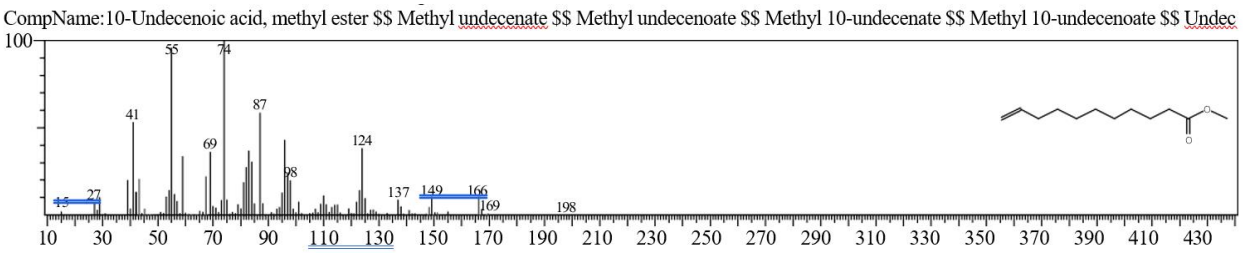

Figure 10. GC–MS spectra of 10-Undecenoic acid, methyl ester

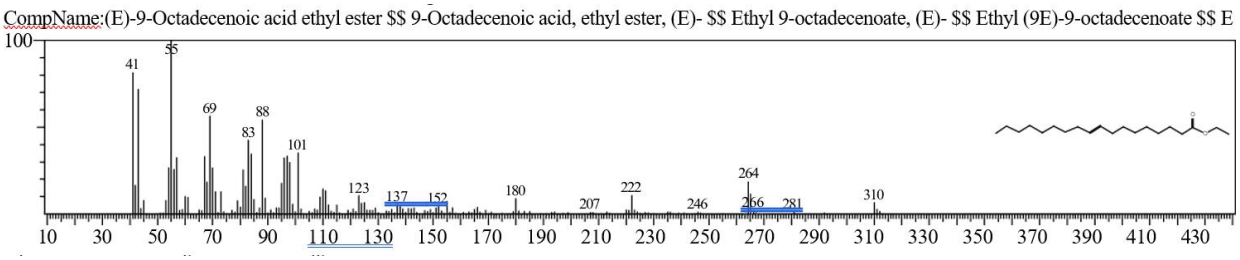

Figure 11. GC–MS spectra of (E)-9-Octadecenoic acid ethyl ester

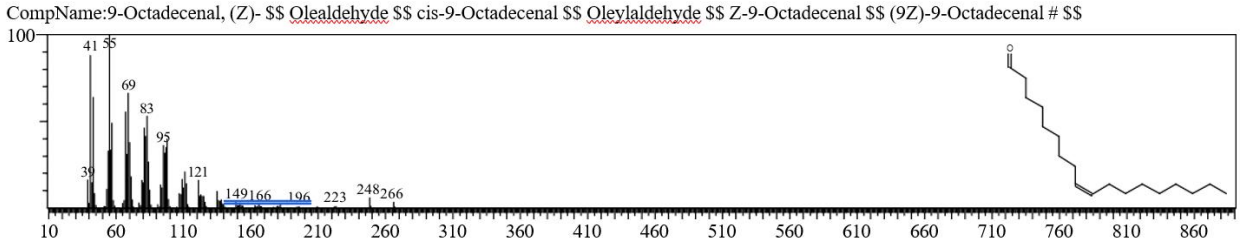

Figure 12. GC–MS spectra of 9-Octadecenal, (Z)-

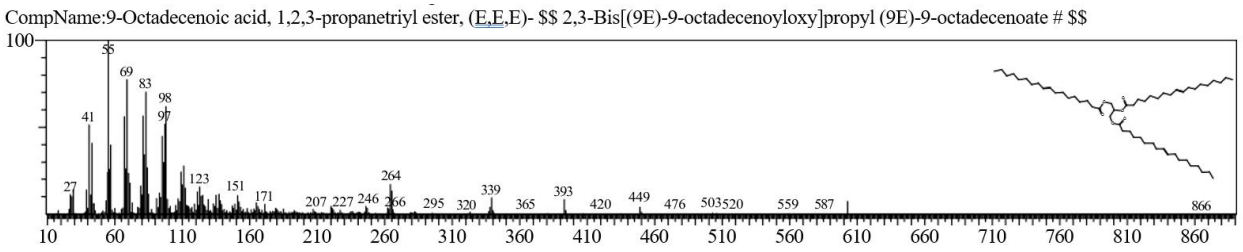

Figure 13. GC–MS spectra of 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-

CHAPTER FIVE

5. DISCUSSION AND CONCLUSIONS

5.1. Summary and Implications of Findings

The GC–MS profiles of *S. jollyanum* stem extracts revealed a rich mixture of fatty acids, esters, and other bioactive metabolites. In the aqueous extract, 33 compounds were detected. Remarkably, the major constituent (43.45% of total area) was identified as inositol, 1-deoxy- (a sugar alcohol) (Modi *et al*., 2021). Other abundant components included α-methyl mannofuranoside (3.8%) and phytol acetate (1.0%). In particular five UFA derivatives were present in noteworthy amounts: methyl oleate (9-octadecenoic acid (Z)- methyl ester, 2.54%; see Figure 4), ethyl oleate (4.73%), oleamide (9-octadecenamide, Z-, 1.34%), 9-octadecenal (Z) (1.94%), and a silylated octadecenoate (16-trimethylsilyloxy-9-octadecenoic acid methyl ester, 1.27%), consistent with previous reports (Oladukun, 2022; Olorunnisola *et al*., 2017). The presence of these UFAs indicates that the aqueous extract is unusually rich in lipophilic bioactives despite using water as a solvent. In addition, saturated lipids such as hexadecanoic acid (palmitic acid) derivatives (peaks 8 and 10) were detected (1.34% and 2.32%).

The ethanolic extract showed a similarly complex profile with 34 compounds. Six UFA derivatives were identified, several of which overlapped with the aqueous extract but showed some differences. Ethyl oleate and methyl oleate again appeared (4.53% methyl oleate), but new lipids emerged: notably triolein (9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-, 13.46%) and a 9-octadecenal (Z) isomer (0.61%). An (E)-9-octadecenoic acid ethyl ester (ethyl oleate form) was found at 9.93%. Additional UFAs included oleamide (1.40%) and 10-undecenoic acid methyl ester (1.18%). Prominent non-UFA constituents were squalene (7.35%) and multiple hexadecanoic acid derivatives (peaks 9, 11, and 21). Phytol acetate also appeared. In summary,

the ethanol extract had a larger fraction of long-chain lipids (including a triglyceride and squalene) whereas the aqueous extract was dominated by a single sugar alcohol and smaller esters (Oladukun, 2022; Olorunnisola *et al*., 2017). The combined UFA content (over 30% total area) underscores that *S. jollyanum* stems are a rich source of UFAs.

These findings are significant because many of the identified UFAs are pharmacologically significant. For example, methyl oleate and ethyl oleate (OA esters) are known bioactive lipids, and oleamide is a biologically active fatty amide. The high percentage of inositol 1-deoxy (a sugar alcohol) is unusual – a similar result was found in *Tinospora cordifolia* stems, where inositol, 1-deoxy- appeared as the predominant GC–MS peak (Modi *et al*., 2021). Phytol acetate is a diterpenoid alcohol ester often linked to antioxidant activity. Overall, the stem extracts contain a diverse array of fatty acids (both saturated and unsaturated) and other metabolites, supporting their potential bioactivity.

The findings of this study align closely with those of Uka *et al*. (2022) in several aspects, highlighting the rich phytochemical profile of *S. jollyanum* and supporting its traditional medicinal uses. While Uka *et al*. focused on ethanol leaf extracts and identified 45 bioactive compounds representing almost the entire extract, both aqueous and ethanolic stem extracts were analyzed in the present study, which resulted in the identification of 33 and 34 compounds, respectively, with notable UFA derivatives such as methyl oleate, ethyl oleate, oleamide, and 9-octadecenal. Similar to Uka *et al*., hexadecanoic acid derivatives and phytol acetate were detected, indicating that both leaves and stems of *S. jollyanum* are rich in bioactive lipids and other secondary metabolites. The compounds identified in both studies, including fatty acids, esters, and alcohols, are known to possess anti-inflammatory, antioxidant, antimicrobial, and potential anticancer activities, which corroborates the plant's traditional applications in treating

jaundice, tumours, fibroids, and other health conditions (Uka *et al*., 2022; Modi *et al*., 2021). The present study adds to these findings by showing that aqueous extracts, often considered less efficient for lipid recovery, still contain significant levels of UFAs and sugar alcohols, such as inositol and 1-deoxy-, suggesting that different solvents can extract complementary bioactive profiles. Furthermore, the identification of triglyceride derivatives such as 9-octadecenoic acid, 1,2,3-propanetriyl ester in the ethanolic stem extract indicates the presence of more complex lipophilic molecules, which were not reported in Uka *et al*.'s leaf study. Collectively, these results reinforce the medicinal potential of *S. jollyanum* across plant parts and extraction methods, while also emphasizing the need for further studies to isolate, characterize, and evaluate the pharmacological activities of these compounds in medical biochemistry contexts.

From a medical biochemistry perspective, the identified compounds have plausible therapeutic roles. UFAs and their esters, which were abundant in the plant extracts used in the present study, often exhibit anti-inflammatory, antioxidant, and membrane-modulating effects. For instance, the (Z)-9-octadecenoic acid (OA) esters detected (methyl and ethyl oleate) have been reported to possess antioxidant and cytotoxic (anti-cancer) activities (Mostofa *et al*., 2024). In cell and molecular studies, OA derivatives can modulate inflammatory pathways and have been linked to reduced cardiovascular risk (as in the Mediterranean diet). Oleamide (cis-9-octadecenamide) is a known neuromodulator and has shown potent anti-inflammatory activity *in vitro* and *in vivo* (Moon *et al*., 2018). Moon *et al*. showed that oleamide strongly suppresses lipopolysaccharide-induced production of inflammatory mediators (such as tumor necrosis factor-alpha and interleukin-1β) in macrophages and reduces edema in rats, suggesting anti-arthritic potential. Therefore, the presence of oleamide in *S. jollyanum* stems may partly explain its traditional use in treating fever, swelling, or pain.

Squalene, found at 7.35% in the ethanol extract, is another noteworthy compound. Squalene is a triterpene widely distributed in plants and animals; it has been documented to have antioxidant and anti-inflammatory effects (Du *et al*., 2024). Du *et al*. reviewed that squalene protects against oxidative stress and can modulate immune responses. In pharmacological terms, squalene may contribute to tissue-protective effects, including hepatoprotection and improved skin health, as well as lowering oxidative damage markers, which align with possible anti-inflammatory or cardioprotective benefits. The saturated fatty acid identified, hexadecanoic acid (palmitic acid) and its derivatives, also have bioactivities. Palmitic acid has been reported to show antimicrobial and antioxidative effects in several studies. Nabi *et al*. (2022) reported that *n*-hexadecanoic acid (and its methyl ester) appears in many plant extracts and has antibacterial and antioxidant properties. Moreover, some in silico work suggests palmitic acid can inhibit inflammatory enzymes (phospholipase A2). Thus, even the saturated lipids in the extracts may support health effects, especially antimicrobial ones, which could complement the anti-infective uses of *S. jollyanum*, such as treating coughs or fevers. The high level of inositol, 1-deoxy- (a sugar alcohol) in the aqueous extract is intriguing. Inositols are implicated in cellular signaling (such as inositol phosphates, insulin signaling). While the specific 1-deoxy isomer's bioactivity is not well-studied, its abundance suggests that *S. jollyanum* stem stores significant polyol/carbohydrate reserves. A previous comparative analysis by Oluronnisola *et al*. (2017) showed that *T. cordifolia* stems also accumulate 1-deoxy-inositol. In general, plant inositols and related cyclitols can have anti-diabetic or osmoprotective roles. Thus, the presence of this sugar alcohol may partly underlie reports of *S. jollyanum's* effects on metabolism or "blood cleansing" in folk medicine.

In summary, the phytochemical profile suggests that *S. jollyanum* stems could exert anti-inflammatory and antioxidant effects through multiple constituents (OA esters, oleamide, squalene, phytol derivatives, inositols). These synergistic lipid and phenolic compounds are expected to modulate inflammatory signaling (e.g., the NF-κB pathway), scavenge free radicals, and support membrane health. The significant UFA content especially aligns with known anti-inflammatory plant extracts (Mostofa *et al*., 2024; Modi *et al*., 2014). These biochemical implications are consistent with the plant's traditional uses (e.g., treating fever, swelling, and hypertension).

Our findings agree with and extend prior phytochemical studies of *S. jollyanum* and related species. For example, Ugwu *et al*. (2023) performed GC–MS on ethanol extracts of *S. jollyanum* root and also reported hexadecanoic acid (palmitic acid) and OA methyl esters among the major peaks. In their root study, they identified these fatty acids in both crude extract and fractions, supporting our observation that UFAs are characteristic of this species (both stems and roots). Similarly, Olorunnisola *et al*. (2017) noted in their review that Menispermaceae plants often contain fatty acid esters and terpenoids, which likely underlie anti-malarial and anti-inflammatory properties. In other medicinal plants, analogous GC–MS profiles have been reported. For instance, Modi *et al*. (2021) found that *T. cordifolia* stems (a climber also used in Ayurveda) contain over 50% inositol, 1-deoxy- by area, paralleling our result in *S. jollyanum*. Likewise, Nabi *et al*. (2022) reported that root extracts of *Skimmia anquetilia* (a Himalayan shrub) contained squalene, palmitic acid, and related UFAs with known antioxidant/antimicrobial activities. Thus, the pattern of abundant long-chain fatty acids and sugar alcohols is common among diverse medicinal plants.

Notably, the relative extract differences observed between both extracts in the present study (aqueous vs. ethanolic) are consistent with general solvent-polarity effects. Many studies report that alcoholic extracts of plants yield higher amounts of nonpolar lipids (triglycerides, sterols, squalene) than do water extracts. For example, Uchegbu *et al*. (2016) reported that the ethanolic extraction of *Costus afer* stems yielded mostly hexadecanoic acid and related fatty acids. In the present study, the ethanol extracted large UFAs and squalene (nonpolar), whereas water extracted mostly polar or mid-polar components (inositol, glycosides, plus smaller esters). This trend aligns with expectations and underscores that using both solvents reveals complementary phytochemical profiles.

5.2. Limitations

Despite the study's notable significance, some limitations that qualify the results should be acknowledged. First, GC–MS analysis only detects volatile or derivatizable compounds. Non-volatile constituents, including most alkaloids, tannins, and large polyphenols, will not be seen. Thus, important bioactives (many alkaloids are known in Menispermaceae) could be missed. Second, all identifications are tentative library matches; without standards or tandem mass spectrometry confirmation, some assignments (especially isomers) remain uncertain. For example, the "16-trimethylsilyloxy-" peak likely reflects a derivatization artifact, so its biological identity is unclear. Third, only relative peak areas, not absolute concentrations, were reported. The percentage areas assume similar response factors, which may not hold across very different chemistries (for example, sugars vs. lipids). Fourth, only two solvents and a single plant part (stem) were utilized in this study. Other parts (leaves, roots, seeds) or extraction methods (such as supercritical carbon dioxide, hexane, acid hydrolysis) might reveal additional

compounds. Fifth, no bioactivity assays were performed. Pharmacological potential was inferred from the known properties of the compounds, but the actual effects of these extracts require biochemical validation (cell or animal tests). Lastly, sample preparation (drying, grinding, and derivatization) could alter the composition of some compounds. In summary, while the GC–MS fingerprint provides useful chemical insights, further analytical (e.g., MS for polar metabolites) and functional studies are needed to fully characterize *S. jollyanum's* pharmacology.

5.3. Conclusions

The GC–MS analysis of aqueous and ethanolic stem extracts of *S. jollyanum* revealed a diverse phytochemical profile dominated by UFA derivatives, sugar alcohols, and other bioactive lipids. The aqueous extract was rich in polar constituents, notably inositol, 1-deoxy-, whereas the ethanolic extract contained larger amounts of lipophilic compounds such as triolein, squalene, and long-chain UFAs. These metabolites, including methyl and ethyl oleate, oleamide, and hexadecanoic acid derivatives, have well-documented anti-inflammatory, antioxidant, antimicrobial, and potential anticancer properties, providing a biochemical rationale for the plant's traditional medicinal uses. The comparative solvent extraction approach revealed complementary phytochemical profiles, highlighting the importance of extraction polarity in capturing the full spectrum of bioactives. The findings of this study was limited to volatile and derivatizable compounds in the stems; however, the results support further isolation, characterization, and pharmacological evaluation of *S. jollyanum* metabolites for therapeutic applications across diverse fields.

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