

**GC-MS COMPARISON OF FATTY ACID ESTER CONTENT IN
SPHENOCENTRUM JOLLYANUM AQUEOUS AND ETHANOIC
EXTRACTS**

BY

**MUSA THERESA GIFT
(BMS2101425)**

**DEPARTMENT OF MEDICAL BIOCHEMISTRY
SCHOOL OF BASIC MEDICAL SCIENCES
UNIVERSITY OF BENIN, BENIN CITY**

NOVEMBER, 2025

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**PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL
BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES IN
PARTIAL FULFILMENT FOR THE AWARD OF BACHELOR OF
SCIENCE, B.Sc (HONS.) MEDICAL BIOCHEMISTRY, OF THE
UNIVERSITY OF BENIN, BENIN CITY**

NOVEMBER, 2025.

CERTIFICATION

We the undersigned hereby certify that Miss Musa Theresa Gift (BMS2101425) carried out this work, in the Department of Medical Biochemistry, University of Benin, Benin city and we approved same as adequate in scope and quality for the reward of Bachelor of Science Degree (B.Sc.) in Medical Biochemistry.

.....
Prof. F. E. Olumese.
(Project supervisor)

.....
Date

.....
Dr N. B. Aguebor-Ogie
(Ag. Head of department)

.....
Date

.....
External Examiner

.....
Date

DEDICATION

To God, whose infinite mercy has been keeping me throughout my stay in school. And also to my parents, for their unwavering support and belief in me.

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My profound gratitude first goes to the Almighty God, the source of all wisdom and knowledge, for His grace, strength, and divine enablement throughout the duration of this research and my entire academic journey.

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ABSTRACT

Using Gas Chromatography-Mass Spectrometry (GC-MS), the fatty acid ester (FAE) profiles in aqueous and ethanolic extracts of *Sphenocentrum jollyanum* roots were compared in this study. The goal was to investigate the effect of solvent polarity on the yield and variety of FAEs, which are bioactive molecules with pharmacological significance. Compared to the aqueous extract, the ethanolic extract had a considerably higher yield and a more intricate fatty acid ester profile. Methyl palmitate, ethyl linoleate, and methyl stearate are some of the major esters found in the ethanolic extract; these compounds are known for their antibacterial and anti-inflammatory properties. Conversely, the aqueous extract had a lower concentration of fewer of these esters. The findings demonstrate unequivocally that ethanol is a superior solvent for extracting lipophilic molecules, such fatty acid esters, from *S. jollyanum*. For the purpose of standardizing extraction methods intended at utilizing the plant's full therapeutic potential for medicinal uses, this discovery is essential.

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CHAPTER ONE

INTRODUCTION

Since the beginning of their existence, human communities have maintained close ties with their environments and utilized the components of the environment to produce food and medicine. Little by little, through experimentation, people learned to satisfy their needs using their environment. Information about medicinal plants, which has been passed down gradually and from one generation to the next, has made us more aware of and able to use plants for cooking and treating diseases. With the establishment of cities and the availability of more amenities, this body of human knowledge has gradually grown. Medicinal plants serve as a therapeutic tool in the majority of cultures.

Just lately, the safety, quality, and efficacy of herbal remedies and medicinal plants have become a major concern in both wealthy and emerging nations. Herbal medicines may aid in the development of a new era of the healthcare system that will treat human illnesses in the future by standardizing and assessing the health of active plant-derived substances.

Traditional knowledge on medicinal plants can be essential to identifying and utilizing natural plant resources.

Due to rising poverty and population, and high cost of pharmaceuticals, it is estimated that 70–80% of the developing world relies on traditional plant-based remedies (Yaniv and Bachrach, 2005). As a result of growing binomial awareness, there has been greater participation in herbal remedies across the world, which is believed to be the "Green Wave. " The use of therapeutic plants has increased by twofold in the west. In order to satisfy demands, the quantity of health foods and plant-derived pharmaceuticals has gradually increased (Grabley and Sattler, 2003).

The plant family Menispermaceae includes the African Yellow Wood, also known as *Sphenocentrum jollyanum* Pierre. These plants are a varied collection that are well-liked for their significant biological functions. Sierra Leone, Nigeria, Ghana, Ivory Coast, and Cameroon are all places where the tree species *Sphenocentrum jollyanum*, which is native to the tropical forest regions of West Africa, is extensively planted. It is frequently used in folk medicine to treat a variety of ailments. The Akan and Asante peoples of Ghana refer to "*Krakoo*," "*Okramankote*" (the dog's penis), and "*Aduro kokoo*" (red medicine), respectively, as the common local names for "*Sphenocentrum jollyanum*." The plant is traditionally called *Akerejupon* or *Ajo* in South-Western Nigeria, *Oban abe* in the Republic of Benin, and *Ouse-abe* in Côte d'Ivoire (Amida, 2008).

Folklore medicine has traditionally used different portions of *Sphenocentrum jollyanum*. The root of the plant is frequently employed as an aphrodisiac by Ghanaian males. It is steeped in alcohol for a few days to extract some ingredients, after which it is consumed as bitters to enhance penile erection, and this effect is well known to be long lasting. Several literary studies have shown that the root is used for its ability to treat pain, depression, and mental and inflammatory diseases by activating the central nervous system (CNS). When mixed with lime juice and *Piper guineense* (West African pepper), the dried, powdered root is utilized as a cure for fever and muscle pain, along with some anti-malarial plants. The aerial parts of the plant (leafy branches and fruits) are widely used with *Piper guineense* and lime juice (Ablow, 1990) to treat persistent wounds, febrile conditions, and coughs.

In traditional Nigerian medicine, the roots are chewed to treat constipation, improve appetite, and aid digestion. The roots of *Sphenocentrum jollyanum* have been proven by traditional healers in Ghana and Côte d'Ivoire to have medicinal effects against diabetes mellitus, high blood pressure,

breast tumor, and irregular menstruation cycle, and all of its morphological organs are crucial components in the management of sickle cell disease (SCD). In addition, the powdered roots are used in conjunction with salt, *Elaeis guineensis* (African oil palm), and *Aframomum melegueta* (alligator pepper) seeds to treat abdominal pain (Ghana herbal Pharmacopeia, 1992). The burnt fruits are used in the treatment of fibroids and as a palatable anti-fatigue snack (Neuwinger, 1996). According to Kayodo *et al.* (2009), the leaf decoctions are also used to treat intestinal parasites and to prevent blood from being coughed up.

Analysis reveals that *Sphenocentrum jollyanum's* high phytochemical makeup is responsible for its biological and pharmacological characteristics. Terpenoids, flavonoids, tannins, lipids, and saponins are the major ingredients in alkaloids. A study of the roots' essential oils found monoterpenoids and sesquiterpenoids, which are vital to several biological processes. The fruit has also yielded additional medically significant substances like protoberberine alkaloids and furanoditerpenes.

The field of phytochemical analysis is expanding quickly. It starts with optimizing extraction methods because they are essential to the outcomes, with the main objective being to isolate bioactive compounds from the complex natural matrix without breaking them down. This initial extraction is crucial because it is necessary to obtain the desired components for subsequent analyses with various goals, such as the determination of the total bioactive compound content, their quantitative and qualitative analyses, or their structural analysis.

Phytochemical analysis is essential for determining the fatty acid makeup of plants, which allows oils to be made to fit their intended purpose. The oils found in plants are both a great source of energy and have a lot of nutritional value. An oil's fatty acid profile is mostly responsible for its quality, nutritional value, and processing features. While unsaturated fatty acids typically have

beneficial effects on human health, saturated fatty acids are known to be associated with weight gain and cardiovascular illnesses. Vegetable oils are a naturally occurring plant product made up of glycerol esters with fatty acid chains that are typically 14 to 20 carbon atoms long and have varying degrees of unsaturation. The fatty acid composition of these oils determines their physical and chemical characteristics.

Due to their varying retention times, gas chromatography is a crucial technique for identifying and quantifying chemical substances like fatty acids. The sample is cleaved during this procedure to prevent overloading the GC system.

1.1 AIMS AND OBJECTIVES

The main aim of study is to analyze the GC-MS comparison of fatty acid ester content in *Sphenocentrum jollyanum* aqueous and ethanoic extracts.

1.1.1 Objectives

The specific objectives of this study were to

- 1) collect and verify *Sphenocentrum jollyanum* stems.
- 2) perform aqueous and ethanolic extraction on stems.
- 3) prepare samples for GC–MS injection.
- 4) identify and quantify fatty acid esters from chromatograms.
- 5) compare extraction efficiency of both solvents(ethanoic and aqueous).

CHAPTER TWO

LITERATURE REVIEW

2.1 BACKGROUND INFORMATION ON *SPHENOCENTRUM JOLLYANUM*

Pierre's *Sphenocentrum jollyanum* is known as *Akerejupon* in Yoruba, in southwest Nigeria. Because of its diverse biological functions, *S. jollyanum* is frequently used in traditional medicine to treat a wide range of ailments. However, a comprehensive examination of the traditional, pharmacological, and phytochemical characteristics of *S. jollyanum* is still lacking. Traditional medicinal practitioners employ different plant organs as aphrodisiacs, treatments for chronic illnesses such as coughs and ulcers, and treatments for malaria. This plant's pharmacological activities include anti-diabetic, antioxidant, hepatoprotective, anti-inflammatory, antimalarial, anti-allergy, and antimicrobial properties, among others. Some of the substances isolated from this plant are columbin, isocolumbin, fibleucin, atrostosterone A, pinnatasterone, polypodine B, and 20-hydroxyecdysone. The goal of the current review is to provide an updated summary of documents sourced from recent publications regarding the ethnomedicinal uses, pharmacology, and phytochemistry of *S. jollyanum* in order to explore this plant for further research and to know its potential effect towards pharmaceuticals.

2.2 INTRODUCTION TO *SPHENOCENTRUM JOLLYANUM*

Sphenocentrum jollyanum (African Yellow Wood) is a tiny dioecious, evergreen shrub with few branches that grows to a maximum of 1.5 meters. When young, the stems are sparsely hairy before becoming smooth; the roots are bright yellow, and the bark is grey.

2.3 DESCRIPTION OF *SPHENOCENTRUM JOLLYANUM*

A little dioecious, evergreen shrub that reaches a maximum height of 1.5 meters, *Sphenocentrum jollyanum* (African Yellow Wood) has few branches. The roots are brilliant

yellow, the bark is gray, and the stems are thinly covered with hairs when they are young before becoming smooth.

2.3.1 Leaves of *Sphenocentrum jollyanum*

The leaves are simple, lack stipules, and have a petiole that is (3–)4. 5–9(–10) cm long and swollen at both ends, are spirally arranged but crowded at the ends of branches, and have a leathery, pinnately veined blade that is 15–26 cm by 6–17(–22) cm in size and has a cuneate or obtuse base, a long-acuminate apex, acute to acuminate lobes, and is entire or shallowly to deeply pinnately lobed, oblong to elliptical or ovate in outline. The petiole is (3–)4. 5–9(–10) cm long, has 8–12 pairs of lateral veins, and is flattened or has a shallow groove above and two shallow lateral grooves.

2.3.2 Flower, Seed and Seedlings of *Sphenocentrum jollyanum*

Unisexual, regular, cream-colored flowers, either solitary on older branches or on the stem between the leaves, with petals that are more or less spirally arranged and get larger as they approach the center.

The male flowers have 15–21 tepals, are sessile, the outer tepals are triangular to ovate-oblong, short-hairy, the inner tepals are obovate, glabrous outside, and measure 0. 5–6. 5 mm by 0. 5–4 mm. There are (13–)16–31 stamens, which are 1. 5–2. 5 mm long, erect, free, and have inflated filaments.

The female flowers are sessile or with pedicel up to 4 mm long, tepals 9–11, early falling, 2–4 outer tepals triangular to circular, 1 mm × 1 mm, short-hairy to glabrous outside, 7 inner tepals obovate to oblong, the innermost spoon-shaped, (2.5–)3–7 mm × 2–4.5 mm, glabrous outside, staminodes up to 14(–17), club-shaped, 1–2 mm long, usually falling with the tepals. Ovaries are

superior, consisting of (3–) 9–12(–15) ovoid carpels c. 3 mm × 1.5 mm, densely short-hairy, stigma large, sessile, horse-shoe shaped, lobed.

Fruit composed of 3–12 drupes, each drupe ellipsoid, (11–)18–26 mm × 10–16 mm, yellow to orange at maturity, smooth, fleshy, 1-seeded. Seed with very thin seed coat; endosperm absent; embryo straight, ellipsoid, 15–18 mm × 8–9 mm.

Seedling with plano-convex cotyledons remaining inside the stone



Figure 2.1: The fruits of the *Sphenocentrum jollyanum* Pierre

Source: Burkill, H. M. (1985). The useful plants of West Tropical Africa (Vol. 1). Kew: Royal Botanic Gardens.



Figure 2.2: *Sphenocentrum jollyanum* shrub

Source: Researchgates

2.3.3 Roots of *Sphenocentrum jollyanum*

The roots are easily recognizable by their distinct bright yellow color, both inside and out. They have a strong sour and acidic taste. This strong flavor gives the plant its most well-known quality, which is that it contains chemicals that momentarily block the sensation of sourness, making foods eaten later taste bland or neutral. This is one of the main reasons why its local name, which means sweet-maker, is so popular.



Figure 2.3: Roots of the *Sphenocentrum jollyanum*

Source: Google photos

2.4 SCIENTIFIC CLASSIFICATION

2.4.1 Kingdom: Plantae

The bulk of the kingdom of Plantae is made up of eukaryotic organisms that are photosynthetic, multicellular, have cellulose cell walls, and are crucial to maintaining ecological balance and oxygen production (Raven *et al.*, 2020; Campbell Reece, 2019).

2.4.1 Phylum: Tracheophyta

It is a significant plant division that has vascular tissue (xylem and phloem) for carrying water and nutrients.

2.4.2 Division: Angiospermae

Flowers, endosperm development, and highly specialized reproductive structures are features of flowering plants known as Angiospermae, which generate seeds that are encased inside of fruits (Campbell and Reece, 2019; Judd *et al.*, 2016).

2.4.3 Class: Eudicotyledon

Eudicotyledons (or eudicots) are a major clade of flowering plants distinguished by having two seed leaves (cotyledons), pollen with three openings (tricolpate pollen), and typically net-like leaf venation (Campbell and Reece, 2019; Judd *et al.*, 2016).

2.4.4 Order: Ranunculale

Ranunculales are an early-diverging order of flowering plants that includes mostly herbaceous species and some shrubs or vines, characterized by simple or compound leaves, numerous free floral parts, and often the presence of alkaloid secondary metabolites (Judd *et al.*, 2016; Soltis *et al.*, 2018).

2.4.4 Family: Menispermaceae

Menispermaceae is a family within Ranunculales, primarily consisting of climbing plants and lianas, many of which produce alkaloids and are used medicinally (Forman and Labiak, 2002).

2.4.5 Genus: Sphenocentrum

Sphenocentrum is a genus of tropical climbing or shrubby plants in the family Menispermaceae, known for its medicinal properties and bioactive compounds (Keay, 1989).

2.4.6 Species: *S. jollyanum*

The scientific name for the African Yellow Wood plant.

2.5 DISTRIBUTION OF SPHENOCENTRUM JOLLYANUM

It is widely distributed across West Africa especially in Sierra Leone, Nigeria, Ghana, Ivory Coast, and Cameroon (Nia, 2004).

2.6. NUTRITIONAL COMPOSITION OF THE *SPHENOCENTRUM JOLLYANUM*

The seed extract's proximate analysis revealed that it contained 9.65% crude fat, 16.70% moisture, 48.09% crude protein, 16.79% carbohydrates, 3.26% ash, and 5.51% fiber, with an energy value of 1460 kcal per 100 kg. According to Bironde and Olushola, 2010, this indicates that the fruits are a significant source of energy and nutrition. The mineral element content of the seeds was found to have a significant concentration of both macro and micro elements necessary for human and animal development when analyzed using flame photometry and atomic absorption spectrophotometry (AAS) (Bironde and Olushola, 2010). Significant amounts of minerals were found, including calcium (8.92 mg/L), magnesium (0.44 mg/L), potassium (4.26 mg/L), iron (0.22 mg/L), manganese (0.19 mg/L), zinc (1.38 mg/L), and sodium (4.70 mg/L). According to Bironde and Olushola, 2010, this demonstrates the plant's significance in maintaining healthy bones, generating energy, and facilitating certain metabolic processes in the body.

2.7. PHYTOCHEMICALS IN THE *SPHENOCENTRUM JOLLYANUM*

A detailed constituents (Amidu *et al.*, 2008). Phytochemical investigation by Nia *et al.* (Nia *et al.*, 2004) revealed the presence of tannins,

The chloroform fraction was found to be the most active of all fractions, and it tested positive to the test of flavonoids and alkaloids.

Phytochemical analysis of the ethanol root extract of *S. jollyanum* revealed that it contains compounds such as terpenoids and flavonoids while alkaloids are reported to be the most dominant chemical saponins, terpenes, and alkaloids in the different fractions of methanol extracts of the stem bark.

Previous studies in the article by Al-Rubaye *et al.* (2017), on the GC-MS analysis of bioactive compounds in ethanol leaves extract of *Sphenocentrum Jollyanum*, possessed certain phytochemicals including:

2.7.1 Phenols: compounds with a phenol ring structure, known for antioxidant, antimicrobial, and anti-inflammatory activities.

- 2,4-Di-tert-butylphenol (Phenol, 3,5-bis(1,1-dimethylethyl)) - 21.05%
- Dibutyl phthalate (1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester)
- Diisooctyl phthalate (Bis(2-ethylhexyl) phthalate) - Often listed as a contaminant but can be plant-derived.
- 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester

2.7.2 Fatty Acids: A large group that includes straight-chain acids, their esters, and alcohols. They are fundamental to cell membranes and have reported antimicrobial and anti-inflammatory properties. Examples

- n-Hexadecanoic acid (Palmitic acid) and its ethyl ester
- 9-Octadecenoic acid (Z)- (Oleic Acid) and its esters
- n-Decanoic acid (Capric acid)
- Undecanoic acid, ethyl ester
- Z-8-Methyl-9-tetradecenoic acid

- Methyl 9,12-heptadecadienoate - 19.12%

2.7.3 Alcohols and Aldehydes: Oxidative derivatives of fatty acids. They often play roles in plant signaling and defense, and can have biological activity. Some of them includes:

- 1-Hexadecanol
- 9,12-Octadecadien-1-ol, (Z,Z)
- 6,9,12-Octadecatrien-1-ol
- 8-Dodecen-1-ol, (Z)-

2.7.4 Terpenes and Terpenoids: A major class of natural products derived from isoprene units, widely known for their diverse pharmacological activities.

- Phytol - A diterpene alcohol, a precursor for vitamins E and K1, with antimicrobial and anticancer properties.
- 9-Oxabicyclo(6.1.0)nonane (and its cis- isomer) - A monoterpene derivative.

2.7.5 Aliphatic Hydrocarbons: Long-chain alkanes and alkenes. Major components of plant waxes can have minor biological activities or serve as biomarkers.

- Cetene (1-Hexadecene)
- 1-Octadecene
- 5-Eicosene, (E)-
- 3-Eicosene, (E)-
- 1-Eicosene

2.7.6 Steroids and Sterol Derivatives: 9,17-Cyclohexene, 6-butyl-1-nitro-. This complex structure suggests a possible degraded sterol or terpenoid derivative.

2.7.7 Carbohydrate Derivatives: The L-Galactose, 6-deoxy - A deoxy sugar, a component of various glycosides and polysaccharides.

2.7.8 Miscellaneous / Synthetic Contaminants

- Trifluoroacetic acid, (9,12-octadecadienyloxy)-, (Z,Z)- - This is highly unusual and may be an artifact of the extraction or derivatization process using trifluoroacetic anhydride.
- Ethanol, 2- - Likely a solvent artifact.
- cis-7,cis-11-Hexadecadien-1-yl acetate - Classified as a fatty acid ester or a pheromone-like compound.

The presence of these various bioactive compounds confirms the application of *Sphenocentrum jollyanum* leaves for various ailments by traditional practitioners which includes antibacterial, antioxidant, antitumor and antifungal activities.

2.8. PHARMACOLOGICAL AND HEALTH BENEFITS OF THE *SPHENOCESTRUM JOLLYANUM*

Sphenocentrum jollyanum has various health benefits due to its antioxidant, and anti diabetic properties.

2.8.1. Anti-Diabetic Activity:

Investigation of the different extracts of morphological organs of *S. jollyanum* indicated its blood glucose lowering potential. The study also reported the anti-hyperglycemic activity of the extract on alloxan-induced diabetic animals. The results of these studies validate the traditional claim of the blood glucose lowering activity of the plant, and thus may serve as a potential source of potent anti-diabetic compounds.

2.8.2 Anti malarial action:

The *Sphenocentrum jollyanum* has a lot of promise as a supplementary and alternative treatment for malaria. When combined with medications such as chloroquine or artemisinin-based combination treatments (ACTs), its capacity to restore hematological indicators and its possible

anti-parasitic benefits may help patients recover more completely and experience better outcomes. Side effects from traditional antimalarials, such as gastrointestinal upset or neurotoxicity, may be lessened by combining them with regular therapies. Herbal medicine is another possible treatment, particularly in areas where traditional antimalarial medications are less available or have resistance problems (Emejulu and Nwachukwu, 2023). It might aid in the fight against malaria resistance by offering an alternative or complementary strategy to current antimalarial drugs. In resource-constrained environments, accessibility and affordability might also be increased. Future research routes include clinical trials, combination studies, mechanistic studies, standardization and quality control, long-term safety studies, medication interaction studies, as well as accessibility and implementation. In people, the safety, efficacy, and ideal dosage of the ethanol root extract will be assessed in Phase I and II trials, while the safety of prolonged usage of the extract in various populations and under different health circumstances will be evaluated in long-term safety studies. The potential for incorporating the extract into national malaria control programs and public health programs to enhance malaria management and outcomes will be evaluated through public health integration.

2.8.3 Anti-angiogenic attribute:

It has an effect in preventing the development of new blood vessels. Marchesan *et al.* (1998) noted these consequences. Possible targets for the search for organic compounds that could block angiogenesis may include the plant itself as well as its bioactive components, such as flavonoids and alkaloids. It has been reported that flavonoids play a particular role in this (Paper, 1998). The plant *Sphenocentrum jollyanum* is employed as a potent wound healer, cough treatment, and anti-tumor therapy (Iwu, 1993; Dalziel. 1985).

The positive effects of the plant against inflammatory and tumor-related diseases may be related to the anti-angiogenic potential of *sphenocentrum jollyanum* and the discovery of flavonoids and alkaloids in the most active fraction. From the plant, earlier scientists have also identified and characterized isoquinoline alkaloids like palmatine, columbamine, and certain bitter-tasting diterpenes (Iwu, 1993).

2.8.4 Antioxidant qualities:

Phytochemicals' antioxidant potential in biological systems is determined by their capacity to chelate metals, activate antioxidant enzymes, scavenge radicals, and block oxidases (Kulkarni *et al.*, 2004). The leaf of *S. jollyanum* demonstrates the capacity to reduce and chelate metals, as well as scavenge radicals. Additionally, African yellow wood trees have a high concentration of flavonoid and phenol. Consequently, it is possible to conclude that the phenolic and flavonoid content of the extract may be responsible for its antioxidant properties. Due to their antioxidant capabilities, the consumption of leafy plant vegetables that contain phenolic and flavonoid chemicals has been shown by numerous epidemiological studies to be linked to a lower risk of cardiovascular disorders, cancer, diabetes, and neurodegenerative illnesses (Le Marchand, 2002; Adebooye *et al.*, 2008; Jiang *et al.*, 2010). Flavonoids have antioxidant capabilities via a variety of distinct mechanisms, such as the chelation of metal ions like iron and copper, the scavenging of free radicals, and the suppression of enzymes that produce free radicals (Benavente *et al.*, 1997).

2.8.5 Capacity to heal wounds:

The leaves of *Sphenocentrum jollyanum* are combined with stem bark to treat wounds in the Southern region of Nigeria. Empirical and statistical data demonstrated that the claimed potency of mixed leaves and stem, as utilized by traditional healers, had no basis aside from additive

effects, although extracts of *S. jollyanum* had promising wound healing properties, confirming the main folkloric application of the plant.

2.8.6 Toxicological and Hepatoprotective Research:

A scientific investigation of the hepatoprotective potential of stem bark extract demonstrated that it markedly improved or prevented liver injury in rats subjected to carbon tetrachloride (CCL4). In a concentration-dependent manner, the extract significantly reduced the elevated aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine amino transferase (ALT), and total bilirubin while simultaneously lowering the total serum protein level. With respect to the in vitro test, *S. jollyanum* is incapable of inducing mutation.

2.8.7 Anti-Bacterial Actions:

The root of *Sphenocentrum jollyanum* yields an essential oil with antibacterial activity against a variety of disease-causing microorganisms. It has been demonstrated to be effective against strains like *Salmonella typhi* (Koleosho *et al.*, 2010), *Staphylococcus aureus*, *Bacillus cereus*, and *Proteus mirabilis*, as well as *Bacillus subtilis* and *Pseudomonas aeruginosa*. Its historical usage as a laxative, where it is thought to help maintain a healthy digestive system and bowel movements by combating microbial imbalances, is supported by this wide range of antibacterial activity.

2.8.8 Anti-Viral Effects:

Sphenocentrum jollyanum's many components, notably the leaves and roots, have antiviral capabilities. Plant extracts have been proven to be effective against the poliovirus and have also been shown to have an inhibitory effect on the mosaic virus in plants.

2.8.9 Hematological Activities:

Sphenocentrum jollyanum's root and leaf extracts have notable hematopoietic activity, which means they can promote blood cell production. The administration of these extracts has been

demonstrated to have a notable impact on important hematological indices, such as pack cell volume (PCV), hemoglobin (Hb) concentration, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). Additionally, this exercise increases levels of both red and white blood cells. These findings imply that the plant has the capacity to increase blood cell synthesis by activating hematopoietic stem cells.

2.8.10 Reproductive and Sexual Behavior:

Sphenocentrum jollyanum extracts have been found to affect hormone levels and sexual activity in men. It has been noted that the plant boosts the sexual drive in male animals, which results in more instances of them mounting women. It also increases the length of ejaculation and reduces the refractory period between sexual encounters. Additionally, it lowers the time between ejaculation and intromission, two important measures of sexual performance and pleasure.

On the other hand, contradictory reports suggest that the plant may also have negative impacts on male reproductive capacity. These effects include asthenozoospermia, which is characterized by a significant reduction in the total sperm count, a deterioration in the sperm cells' ability to fertilize, and a decline in sperm motility. Additionally, evidence of increased superoxide dismutase activity in the testes and degeneration of the seminiferous tubules points to the possibility that *S. jollyanum* may impair reproductive function by adversely impacting the quality, quantity, and structure of the sperm.

2.9 BIOCHEMISTRY OF FATTY ACID ESTERS

2.9.1 Lipids :

The majority of definitions describe lipids as biological compounds that are insoluble in water but soluble in nonpolar solvents (Bruice, 2004). This is a helpful definition since it includes a wide range of chemical space, including several compounds that are otherwise difficult to

categorize. This definition has two flaws. Most organic compounds might fit within this scope if the hydrocarbon (hydrophobic) portion is sufficiently large. Secondly, several of the traditional lipids, such as the fatty acids, are very soluble in water.

Lipids can be defined more narrowly by simply dividing them into fatty acids and their metabolites, while treating other naturally occurring compounds made of hydrocarbons separately. A fatty acid is a carboxylic acid with a long hydrocarbon chain. Acylglycerol esters, wax esters, and alcohols like sterols are examples of fatty acid derivatives. Other acid derivatives are carbohydrates (glycoglycerolipids) or phosphates (glycerophospholipids).

2.9.2 Esters :

The class of compounds known as esters is structurally related to carboxylic acids, but the hydrogen atom in the carboxyl group (the $-\text{COOH}$ group) has been substituted by a hydrocarbon group, giving rise to a $-\text{COOR}$ structure (where R is the hydrocarbon). Therefore, esters are created by the condensation of acids (which contain a $-\text{COOH}$ group) and alcohols (which have an $-\text{OH}$ group). They usually have powerful and sometimes pleasant aromas.

2.9.3 Fatty Acids:

The simplest lipids are fatty acids. They have a polar hydrophilic head region connected to a lengthy hydrophobic tail. Although several lipids are employed for energy storage—including the fats—the majority are utilized to create lipid/protein membranes, which divide intracellular compartments and separate the cell from its environment. Although oleic acid and palmitic acid are the most prevalent fatty acids in plants, there are more than a hundred different kinds. Palmitic acid is an example of a saturated hydrocarbon chain, while oleic acid is an example of an unsaturated one. The main differences between fatty acids are the length of their chain and the placement of their many bonds. Other nomenclature systems may also be used (Davidson and

Cantrill, 1985). For example, palmitic acid (16 carbons, saturated) is represented by 16:0, whereas oleic acid, which has 18 carbons with one cis double bond at carbon 9, may be represented by 18:1. Unless stated differently, double bonds are assumed to be cis. In cells and tissues, only trace amounts of fatty acids exist in their free-acid form, despite being used as the fundamental building blocks of the saponifiable lipids. These are typically found in many bound forms and might make up as much as 7% of the weight of dried leaves. Some generalizations may be made regarding the various fatty acids found in higher plants. The most prevalent have an even number of carbons between C14 and C22. Oleic acid (C18) is one of the most prevalent unsaturated fatty acids in higher plants. The melting point of unsaturated fatty acids is lower than that of their saturated equivalents. Non-essential fatty acids are those that are not deemed necessary in the diet since the human body can produce them, according to Cunnane (2003). It seems that the non-essential fatty acids are easier to replace in tissue lipids than the essential ones.

Linoleic acid and α -linolenic acid are two vital fatty acids that the body is unable to create from scratch. These fatty acids are essential for the synthesis of long-chain polyunsaturated fatty acids (such as arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid), as well as for the production of eicosanoids (thromboxanes, leukotrienes, and prostaglandins), the proper development and function of the brain and nervous system, and the formation of healthy cell membranes.

Partially hydrogenated vegetable oil, dairy products, and meats include trans fatty acids. Since trans-fatty acids are linked to a higher risk of coronary heart disease, there is data to support lowering intake (Wilson *et al.*, 2001). The use of a non-nutritive synthetic fat replacement, such as Olestra (a combination of hexa-, hepta-, and octa-fatty acid esters of sucrose), is one way to

lower dietary fat consumption. However, its usage has been linked to decreased bioavailability of lipophilic vitamins (Schlagheck *et al.*, 1997) and gastrointestinal discomfort (Barlam and McCloud, 2003).

2.10 GAS CHROMATOGRAPHY- MASS SPECTROMETRY (GC-MS)

Gas Chromatography-Mass Spectrometry (GC-MS) is a potent tool for the separation, identification, and quantification of bioactive chemicals originating from plants. One of the fundamental separation methods is chromatography, in which a mobile phase transports a mixture across a stationary phase that absorbs selectively, resulting in the separation of components according to their differing affinities. The complex functions of GC-MS are based on this concept. Chromatography is essential for the quality control and standardization of phytotherapeutics, which are plant-based pharmaceuticals that require consistent chemical profiles in order to be safe and effective, in addition to pure analytical science.

The treatment of complicated, multicomponent mixtures like essential oils, hydrocarbons, and a variety of solvents makes use of gas chromatography. The usage of adjustable temperature programs is a major technological advantage since it enables the precise separation of chemicals that may behave similarly under isothermal circumstances. The ability of GC to conduct quantitative analysis on materials present at trace-level concentrations is made possible by the combination of this with the use of highly sensitive detectors like the Flame Ionization Detector (FID) and the Electron Capture Detector (ECD). Most bioactive qualities in medicinal plants, such as antibacterial, antioxidant, and anti-inflammatory effects, originate from secondary metabolites, which include a wide variety of structural types, such as alkaloids, terpenoids, flavonoids, and phenolic compounds (separated in gas chromatography). These metabolites are a rich and mostly untapped resource.

Prior to gas-liquid chromatography, the sample is vaporized, which necessarily restricts GC-MS to molecules that are either thermally stable or volatile, or may be rendered so by derivatization. This vaporized sample is then introduced into a chromatographic column and carried by an inert gaseous mobile phase, such as helium or nitrogen. The column is made up of a liquid stationary phase that is adsorbed onto an inert solid. The separation is based on a combination of adsorption and partition, in which the components of the sample are distributed between the mobile gas phase and the liquid stationary phase according to their volatility and polarity, resulting in their separation along the length of the column.

GC is transformed from a simple separation method into a potent identification instrument by its integration with a mass spectrometer (MS). The MS functions as a detector by breaking down the eluting chemicals into distinctive ions, creating a mass spectrum that acts as a molecular fingerprint. With the use of comprehensive spectral libraries, this fingerprint enables the accurate identification of compounds. The review claims that GC-MS has grown to become one of the most popular and reliable chromatography-based methods utilized worldwide. Its usefulness extends beyond plant analysis, as seen by its application in space probes and its crucial roles in monitoring organic pollutants in the environment, anti-doping testing in sports, and even in astrochemistry. GC-MS, as a unique and potent synergistic technology, offers an unparalleled opportunity for the characterization and identification of both naturally occurring and synthetically derived chemicals..

2.11 APPLICATIONS OF GC-MS

GC-MS is applied across diverse fields, demonstrating its status as a cross-disciplinary tool.

A detailed knowledge of the chemical constituents of plants is crucial not only for the discovery of new therapeutic agents but also for validating traditional folkloric remedies and disclosing

new sources of valuable chemical precursors. Higher plants are presented as dominant players in maintaining human health, with many green plants representing reservoirs of effective, biodegradable, and often less toxic chemotherapeutants. The emergence of pharmacognosy and the standardization of herbal drugs is a critical scientific discipline. In this context, GC-MS has become an increasingly applied technique for the analysis of medicinal plants. It is particularly valuable for profiling non-polar components, volatile essential oils, fatty acids, lipids, and certain classes of alkaloids. GC-MS is used to identify bioactive compounds in plants like *Artemisia annua*, *Quercus infectoria*, *Nigella sativa*, and *Ocimum basilicum*, linking their chemical profiles to observed antimicrobial activities (Hameed *et al.*, 2016; Hussein *et al.*, 2016)

2.11.1 Forensic Science and Criminalistics:

GC-MS is considered the gold standard in forensic science. In order to establish a link between a crime scene and the particles taken from a suspect's attire or possessions, it is utilized in the analysis of those particles. The American Society for Testing and Materials (ASTM) fire debris analysis standard allows for the identification and detection of accelerators using GC-MS. Its function in forensic toxicology is also crucial, as it is employed to analyze biological samples (blood, urine, tissue) from suspects, victims, or the deceased for a variety of chemicals, including poisons, narcotics, and steroids.

2.11.2 Environmental Monitoring:

The use of GC-MS for monitoring pollution has increased as the technology has grown more dependable and affordable. In environmental protection, GC-MS is essential. It is employed to measure chlorophenols in water and soil, Polycyclic Aromatic Hydrocarbons (PAHs) produced by burning, dioxins, dibenzofurans, organochlorine pesticides, herbicides, and phenols. It is capable of identifying sulfur compounds in air and analyzing pesticides in foods such spinach. In

treated wastewater, the method's sensitivity also enables the monitoring of the breakdown products of medicines like carbamazepine.

2.11.3 The Food, Drink, and Flavor Industry:

Many aromatic substances, both naturally occurring and process-derived, are found in foods and beverages. Quality control, authenticity verification, and safety are all widely performed in this industry using GC-MS. It can identify and quantify pollutants, spoilage markers, and adulteration in goods like butter, ghee, and oil. It is used to analyze the compositional profiles of essential oils, such as spearmint, lavender, lemon, and peppermint oil, as well as specific flavor compounds like piperine in black pepper. It is also necessary for enforcing governmental regulations regarding food safety and labeling.

2.11.4 Chemical Warfare Defense and Security:

The usage of GC-MS in national security is crucial. GC-MS-based explosive detection systems are now commonplace in American airports. Additionally, the military and homeland security organizations have created portable, robust GC-MS devices for field usage in the almost real-time identification of chemical warfare agents (CWAs), such as sarin, soman, and VX, which are essential for public health preparedness and response.

2.11.5 Geochemical and Astrophysical Research:

The extraordinary versatility and resilience of GC-MS technology is on display in this application. The Huygens probe, which brought a GC-MS to Titan, Saturn's moon, carried out atmospheric and surface analysis. In a similar way, the Philae lander of the Rosetta mission carried a GC-MS instrument to study the makeup of a comet, 67P/Churyumov-Gerasimenko. GC-MS is useful in terrestrial geochemistry for identifying the organic makeup of geological

materials, such as low-volatility waxes, for analyzing complex hydrocarbon mixtures, and for offering distinctive isotope ratio data.

2.11.6 Medical and Clinical Uses:

The use of GC-MS in clinical settings has transformed the way newborns are screened for inherited metabolic disorders. It can detect and quantify substances in urine samples that are unusual or found in odd amounts, which allows for the early identification of many metabolic illnesses that are inherited. By using isotopic labeling (such as ^{13}C), GC-MS, when paired with an Isotope Ratio Mass Spectrometer (IRMS), can be used to investigate metabolic pathways and fluxes in live systems. The method is also employed in the analysis of pharmaceutical products, such as identifying the presence of certain oils in creams and ointments.

2.11.7 Biological and Pesticide Analysis:

This is a large area where GC-MS is used to bioanalyze human and animal samples. It is regularly used to identify the presence of residual solvents, alcohols, narcotics, barbiturates, and a wide variety of medications (such as anesthetics, anticonvulsants, and antihistamines) in blood and urine. Furthermore, headspace analysis methodologies employ it as the primary instrument for identifying organochlorinated pesticides in environmental samples such as river water, drinking water, and even soft drinks.

2.11.8 Petrochemical and hydrocarbon analysis:

GC-MS is used extensively by the petrochemical industry to characterize complicated hydrocarbon mixtures. The approach is essential because of its capacity to produce improved molecular ions and structurally important fragmentation patterns for a wide range of substances, including gaseous hydrocarbons and high molecular weight waxes, such as $\text{C}_{74}\text{H}_{150}$. It is used

to analyze a wide range of petrochemical products and geochemical samples, including crude oil, gasoline, kerosene, diesel fuel, biodiesel, naphthenic acids, and other materials.

2.11.9 Industrial Applications and Clinical Toxicology:

Because of its speed, sensitivity, and wide compound range, GC-MS is a key instrument in clinical toxicology for detecting poisons, venoms, and other toxins in biological specimens. Its uses in industry are varied, including monitoring pollutants in industrial solvents like styrene and xylene, analyzing allergens in cosmetics, and assuring the quality of raw materials like acetic acid, a crucial industrial intermediary.

2.11.10 Academic Research, Fuel, and Energy:

In the energy industry, the GC-MS is utilized to evaluate the fuel composition, including pollutants, sulfur compounds, and aromatic solvents, in a variety of fuel kinds and polymers like polyethylene. Lastly, it emphasizes the tool's fundamental role in academic research across pure and applied sciences such as chemistry, polymer science, nanotechnology, and biotechnology, where it generates essential information for research publications and basic breakthroughs.

GC-MS is depicted as a crucial element in every step of drug development and manufacturing, including quality assurance (QA), quality control (QC), analytical research and development (RandD), and production. Its unique duties include method and process development, drug stability testing, and the crucial work of impurity profiling in Active Pharmaceutical Ingredients (APIs). The technology spans a wide range of pharmaceutical disciplines, from medicinal chemistry (where it describes synthetic molecules) to pharmacognosy (where it discovers bioactive plant components), pharmaceutical analysis, and biotechnology.

Al-Rubaye, Hameed, and Kadhim (2017) essentially portray GC-MS as a fundamental technology that has permeated almost every area of contemporary analytical science, rather than

simply as a piece of laboratory apparatus. GC-MS is an essential tool for research, industry, and public safety due to its capacity to distinguish, identify, and quantify chemical substances with great sensitivity and selectivity, ranging from the depths of space to the complexities of human metabolism, from a crime scene to a pharmaceutical manufacturing line.

GC-MS is a preeminent method for analyzing bioactive natural compounds from plants, providing the chemical data necessary to connect traditional herbal medicine with contemporary evidence-based science.

CHAPTER THREE

MATERIALS AND METHODS

3.1 COLLECTION, AUTHENTICATION AND PREPARATION OF PLANT EXTRACT

Fresh and matured whole plant, *sphenocentrum jollyanum* were bought from the local market in Iwoh, Osun State, Nigeria. It includes the leaves and stem. The plant was identified and verified by a taxonomist in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State. The voucher number (UBH-S449), the specimen was deposited at the herbarium Unit. The plant specimen leaves were plucked out from the stem.

3.2. Materials and Reagents

3.2.1 Equipment used

- Sieves
- Cutting knives
- Universal plain bottles
- Masking tape
- Beakers
- Test tube
- Spatula
- Permanent marker
- Mortar and pestle
- Nose mask and surgical gloves for precautions against contamination

3.2.2 Chemical Reagents

- Ethanol (likely 95% or absolute) as the organic solvent
- Distilled/Deionized water as the aqueous solvent

3.2.3 Apparatus

- Cutting tools (stainless steel knives)
- Drying trays
- Extraction vessels (polypropylene bowl)
- Manual stirrers or spatula
- Muslim cloth/cheesecloth

3.2.4. Laboratory Equipment

- Washing basins
- Mechanical grinder for pulverizing samples
- Freeze dryer (lyophilizer) for solvent removal
- Weighing boat
- Weighing balance
- Freezer

3.3. Methodology

3.3.1. Sample Preparation

To eliminate soil, debris, and surface pollutants, the fresh stems were washed well under running water. To prevent the degradation of heat-sensitive phytochemicals, the cleaned stems were then air-dried at room temperature in a well-ventilated, shaded environment after being chopped into smaller pieces. To maximize surface area and improve extraction efficiency, the stem pieces were mechanically ground into coarse powder after they had dried fully.

3.3.2. Extraction (Cold Maceration)

The pulverized stem material was divided into two categories for extraction: an aqueous extract, in which the powder was soaked in distilled water, and an ethanolic extract, in which the powder was steeped in ethanol. The solvent-to-sample ratio utilized in the production of each batch was 1 L of solvent for every 0.15 g of plant powder. To encourage phytochemical diffusion and solvent penetration, the samples were macerated for 72 hours, and the mixture was stirred every 2 hours.

3.3.3. Filtration

Each mixture was strained via muslin cloth after maceration in order to remove plant debris and produce clear solvent extracts.

3.3.4. Concentration (Freeze-Drying)

After being frozen, the filtrates were transferred to a freeze dryer, where the solvents were sublimated. This approach yielded dry crude extracts that retained volatile and heat-labile phytochemicals.

3.3.5. Phytochemical Investigation

Each freeze-dried extract—both aqueous and ethanolic—was accurately measured and kept in 1 g batches in labeled universal bottles. In order to determine the phytochemical makeup of these samples, they were sent to a licenced laboratory in Lagos for GC–MS analysis.

3.3.6. Procedure Evaluation

The extraction method offered a number of advantages since it utilized both organic and aqueous solvents to compare the polarity-dependent solubility profiles of phytochemicals, freeze-drying to preserve heat-labile bioactive compounds, and regular agitation to enhance the efficiency of solvent extraction.

3.3.7. Mass spectrometry-gas chromatography

Combining GC, which separates volatile components, with MS, which identifies compounds based on their mass-to-charge ratios, is the analytical method known as GC–MS. The sample is initially vaporized and then transported via a capillary column using an inert gas like helium. The vaporized components separate as they move through the column according to variations in their boiling points and their interactions with the column's stationary phase. After being separated, each substance enters the mass spectrometer, where it is ionized and broken apart. Because the mass spectra that result are unique chemical fingerprints, it is possible to precisely identify the individual components of the sample.

A Shimadzu GC–MS-QP2010 system with an AOC-20i autosampler, running under specific analytical parameters, was used to conduct the GC–MS analysis of the extracts in this study.

1. Autosampler (AOC-20i) Conditions: The autosampler performed two sample rinses, three pre-solvent rinses, and three post-solvent rinses. The viscosity compensation time was 0.2 seconds, and the injection and plunger suction rates were both set to high. With five pumping cycles and an injection port dwell time of 0.3 seconds, the injection mode was typical. There was no air gap at the terminal. The syringe's suction and injection locations were both set at 0.0 mm, and the plunger washing speed and volume were both 8 μ L. The process employed just one vial of solvent.

2. Gas Chromatography Conditions (GC-2010): The GC system's column oven temperature was 60°C, and the injection temperature was 250°C. With a sampling time of 1.00 minute, the injection mode was splitless. The pressure mode was used to maintain flow control, 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 the carrier gas saver and high-pressure injection were both turned off.

3. The oven temperature program started with a 1.00-minute hold at 60°C, then increased the temperature at a rate of 13°C per minute to 240°C, where it was held for 1.00 minute before continuing to 300°C at the same pace with a 39.70-minute hold at the end.

4. Mass Spectrometric Conditions (MS: QP2010): The mass spectrometer ran at an ion source temperature of 230°C and an interface temperature of 250°C. The solvent cut time was set to 4.00 minutes. At a threshold of 2000, the detector was operated in relative gain mode at 1.33 kV.

5. Mass Spectrometry Scan Program: The scan program ran from 8:00 to 59:80 minutes. The acquisition mode was configured to scan with an event time of 0.30 s, a scan speed of 1666 amu/s, and a scan range of m/z 35–500.

6. Parameters for the Ready Check: The instrument underwent a readiness check prior to beginning the GC-MS run to confirm that it was operating at its best. The column oven, injector, and interface temperatures were all confirmed to be consistent with their predetermined settings. The pressure and flow of the carrier gas were found to be within the set range. The autosampler syringe's cleanliness, alignment, and plunger movement were all examined. To guarantee that the detector displayed the least amount of noise, the baseline stability was evaluated. The system vacuum level was also monitored to ensure suitable ionization conditions. The device was not considered ready for sample analysis until all parameters satisfied the necessary criteria.

7. Heat Unit: Every heating element, including the mass spectrometer, split/splitless injector 1 (SPL1), and column oven, was turned on.

8. Injection Flow: The injection flow parameters guaranteed that the SPL1 carrier and purge flows were still active during the whole experiment.

CHAPTER FOUR

RESULTS

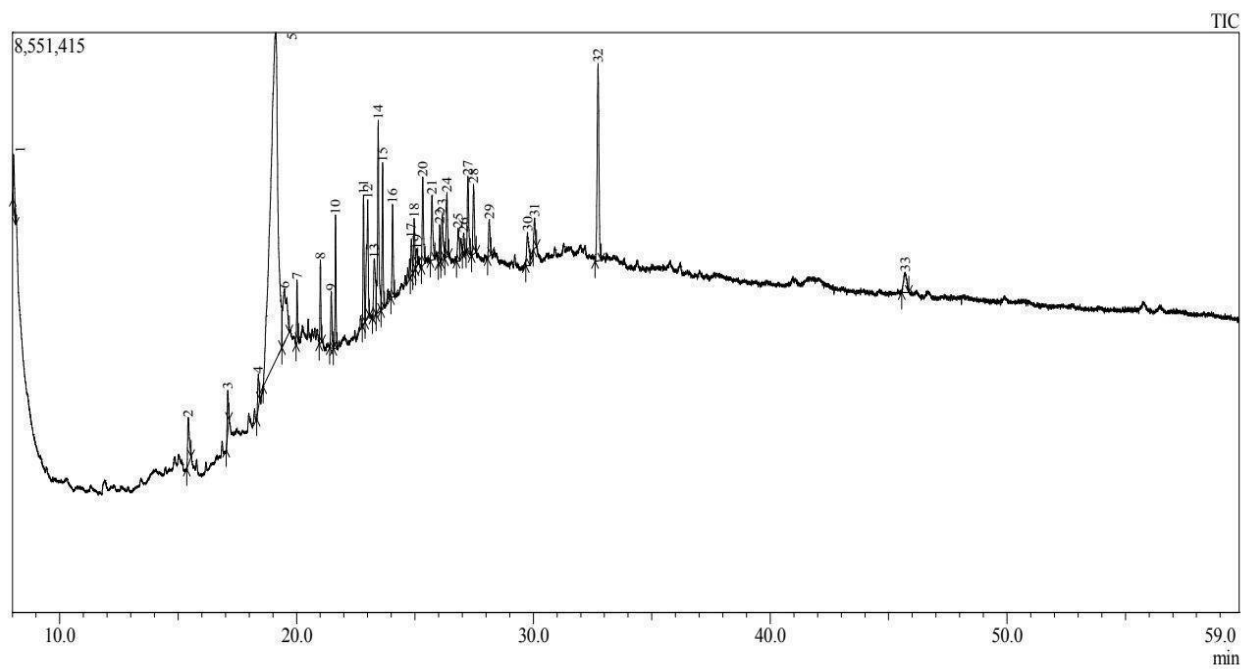


Figure 4.1: GC-MS results for the Aqueous extract of *Sphenocentrum jollyanum*

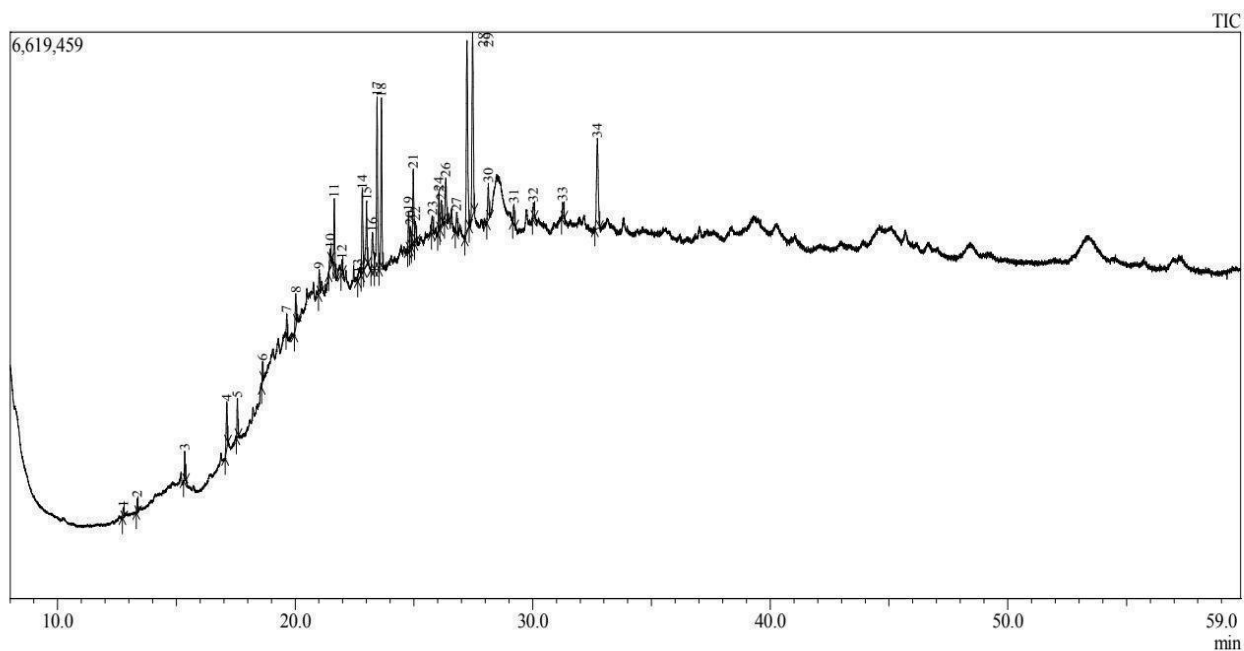


Figure 4.2: GC-MS results for the ethanoic extract of *Sphenocentrum jollyanum*.

Different secondary metabolites are separated and identified using the highly precise technique of gas chromatography-mass spectrometry (GC-MS). In this study, GC-MS analysis and the NIST database were used to ascertain the chemical composition of an aqueous stem extract and an ethanol stem extract from *Sphenocentrum jollyanum*.

According to the GC-MS chromatogram, aqueous extract had 33 peaks and ethanoic extract had 34 peaks, totaling 67 chemical components that made up 99.98% of the whole extract. These compounds include fatty acid esters, phenylpropanes, aliphatics, fatty alcohols, and other varieties.

The retention time of the aqueous sample varies from 8.051 to 45.710 minutes, and the peak area ranges from 0.38% to 43.45%.

The retention time for the ethanol extract ranged from 12.765 to 32.724 minutes, with peak areas ranging from 0.46% to 13.46%.

Based on the optimized parameters, we were able to detect the presence of fatty acids that are abundant in plant extract both in ethanoic extract and aqueous extract.

Table 4.1: Fatty esters present in aqueous and ethanoic extract of *Sphenocentrum jollyanum*

| S/N | Compound Name | Molecular formula | Retention time (min) | Peak Area % (aqueous) | Peak Area % (ethanoic) |
|-----|--|-------------------|----------------------|-----------------------|------------------------|
| 1 | Hexadecanoic acid, methyl ester | C17H34O2 | 21.006 | 1.34 | 0.99 |
| 2 | Hexadecanoic acid, ethyl ester | C18H36O2 | 21.645 | 2.32 | 3.20 |
| 3 | 9-octadecenoic acid(z)-methyl ester | C19H36O2 | 22.826 | 2.54 | 4.53 |
| 4 | Methyl stearate | C19H38O2 | 23.004 | 3.53 | 4.09 |
| 5 | Ethyl oleate | C20H38O2 | 23.448 | 4.73 | ND |
| 6 | Octadecenoic acid, ethyl ester | C20H38O2 | 23.635 | 2.78 | 8.97 |
| 7 | Eicosanoic acid, methyl ester | C21H42O2 | 25.085 | 0.41 | ND |
| 8 | Octadecenoic acid, 10-hydroxy-, methyl ester | C19H36O3 | 25.327 | 2.05 | ND |
| 9 | Octadecenoic acid, 3-hydroxypropyl ester | C21H40O3 | 25.718 | 1.76 | ND |
| 10 | Octadecenoic acid, 2- | C21H40O4 | 27.475 | 1.81 | ND |

| | | | | | | |
|----|---|------------|--------|------|-------|--|
| | hydroxy-1,3-propanediyl | | | | | |
| | ester | | | | | |
| 11 | 16-Trimethylsilyloxy-9- Octadecenoic acid, methyl ester | C22H44O3Si | 29.754 | 1.27 | ND | |
| 12 | Octanoic acid, ethyl ester | C10H20O2 | 12.765 | ND | 1.52 | |
| 13 | Dodecanoic acid, ethyl ester | C14H28O2 | 17.569 | ND | 1.55 | |
| 14 | Tetradecanoic acid, ethyl ester | C16H32O2 | 19.647 | ND | 0.92 | |
| 15 | Phytol, acetate | C22H42O2 | 20.028 | ND | 1.60 | |
| 16 | (E)-9-Octadecanoic acid, ethyl ester | C20H38O2 | 23.446 | ND | 9.93 | |
| 17 | 10-Undecenoic acid, methyl ester | C12H22O2 | 25.060 | ND | 1.18 | |
| 18 | Decosanoic acid, ethyl ester | C22H44O2 | 25.774 | ND | 1.40 | |
| 19 | Octadecenoic acid 2,3- dehydroxypropyl ester | C21H38O4 | 26.798 | ND | 1.20 | |
| 20 | 9-Octadecenoic acid,1,2, 3-propanetriyl ester (z,z,z)- | C57H104O6 | 27.223 | ND | 13.46 | |
| 21 | Octadecanoic acid, 2- | C21H42O4 | 27.740 | ND | 11.92 | |

| | | | | | |
|----|---|--|--------------|--------------|--------------|
| | hydroxy-1, 3-propanediyl | | | | |
| | ester | | | | |
| 22 | Eicosanoic acid, 2,3- bis(trimethylsilyl)oxy propyl ester | C ₂₉ H ₆₂ O ₄ Si ₂ | 30.031 | ND | 2.05 |
| | | | | | |
| | | | TOTAL | 24.54 | 68.51 |

*ND: None detected

CHAPTER FIVE

DISCUSSION

Based on the GC-MS data provided, the conclusion is drawn from a direct comparison of the two sets of data.

It is noticed that the total of the both peak areas equal 96.34%. The most abundant compounds are Ethyl oleate and 9-octadecenoic acid,1, 2 3-propanetriyl ester, (z,z,z)- in aqueous extract and ethanoic extract respectively. The ethanoic extract produces a richer and more diverse profile of ester compounds than the aqueous extract. This is due to the chemical nature of the solvent.

Ethanol actively participate in transesterification and esterification reactions with fats and free fatty acids present in the source material, generating new ethyl esters during the extraction process, and as a less polar solvent compared to water, ethanol is far more effective at dissolving and extracting the wide range of naturally occurring non-polar and moderately polar esters (e.g., methyl esters, glycerides) from the sample matrix. In contrast, distilled water is highly polar and can promote the hydrolysis of esters, breaking them down into fatty acids and alcohols, thereby reducing their concentration in the final extract. This factor makes ethanol extract not only more effective at preserving existing esters but also at generating new ones, making it the richer source for this class of compounds.

Amongst the identified esters, Hexadecanoic acid (palmitic acid) methyl ester is reported to have anti-inflammatory, cancer-preventive, hepatoprotective, antiarthritic, and anticoronary properties. Palmitic acid and its derivatives are hepatoprotective and have anticancer activity.

9-Octadecanoic acid methyl ester belongs to a family known as oleic acid. They are known to have anti-inflammatory effects, preventing chronic diseases such as arthritis, gastritis, and hepatitis, by inhibiting the production of nitric oxide (NO) optimally in lipopolysaccharide

(LPS)-induced RAW264.7 macrophages. Methyl stearate, an octadecenoic acid which is a saturated fatty acid can inhibit the detrimental effects of global cerebral ischemia (i.e. cardiac arrest).

Phytol acetate is a valuable essential oil (EO) used as a fragrance and a potential candidate for a broad range of applications in the pharmaceutical and biotechnological industry. Evidence shows that Phytol acetate may play a crucial role in the development of pathophysiological states. It demonstrates anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects.

Decosanoic acid exhibited profound anti-tumor effects on human HCC through the suppression of HGF/c-Met signaling cascades in vitro and in vivo assessments.

Undecanoic acid is a medium-chain fatty acid (MCFA) that can influence immune cell function, particularly through its interaction with the GPR84 receptor, which is expressed on macrophages and neutrophils, enhancing proinflammatory responses.

Eicosanoids are a large family of oxygenated C₂₀ fatty acids with cis, methylene interrupted bonds, which include prostaglandins (PGs), thromboxanes (TX), and leukotrienes (LX). They act mainly as local hormones and regulate biological processes like inflammation, bronchoconstriction, bronchodilation, and smooth muscle contraction. The most common precursor for the synthesis of eicosanoids is arachidonic acid.

5.1 CONCLUSION

The outcome of the GC-MS analysis of *Sphenocentrum jollyanum* stem extract in ethanol and aqueous identified several secondary metabolites, fatty esters being amongst them. They each contribute to a range of biological effects. These findings validate the traditional use of the plant's leaves in treating conditions such as bacterial and fungal infections, tumors, and congestive diseases. Further research is recommended to isolate these phytochemicals and explore their potential for new drug development.

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