

**ANTI-DIARRHOEA PROPERTIES OF SPHEMOCENTRUM JOLLYNUM LEAF
AQUEOUS EXTRACT IN CASTOL OIL AND MG SULPHATE INDUCED
DIARRHOEA IN MICE**



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FACULTY OF LIFE SCIENCES,
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BENIN CITY**

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**A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE
LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF
BENIN, BENIN CITY.**

**IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF
BACHELOR OF SCIENCE (BSc) DEGREE IN SCIENCE LABORATORY
TECHNOLOGY.**

FEBRUARY, 2025

CERTIFICATION

This is to certify that this final year project work was carried out by EMORDI OGOCHUKWU WILLIAMS (MAT. NO. LSC1907284) of the Department of Science Laboratory Technology, Faculty of life Sciences, University of Benin, Benin City, in partial fulfilment for the award of Bachelor of Science (B. Sc) degree in Science Laboratory Technology (Biotechnology Techniques).

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DEDICATION

This project work is dedicated to God Almighty for his strength; wisdom and understanding that brought this work to fulfilment and to my late dad Mr. Sunday Emordi.

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This research project is a complex entity and emerges from the contribution of many people. First and foremost, praises and thanks to God Almighty, for his showers of blessings and faithfulness throughout the preparation for my undergraduate research project.

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ABSTRACT

Herbal medicine has a long history as part of traditional healthcare systems. *Sphenocentrum jollyanum*, a medicinal plant, has shown promise due to its phytochemical properties and potential health benefits. This study investigated the antidiarrheal efficacy of *Sphenocentrum jollyanum* leaf aqueous extract (SJLAE) in animal models. Fresh leaves of *Sphenocentrum jollyanum* were collected and processed by air-drying and aqueous extraction. The crude aqueous extract was prepared by maceration. Twenty Swiss albino mice were acclimatized and divided into control and experimental groups. Diarrhea was induced using castor oil and magnesium sulfate. The mice received different doses of SJLAE, with loperamide used as a standard reference drug. Defecation frequency, stool weight, and diarrhea onset were monitored for six hours. SJLAE administration significantly delayed stool onset and reduced the total number of stools, the number of diarrhea episodes, and stool weight in a dose-dependent manner compared to the control group. The results suggest that SJLAE's antidiarrheal effects may involve mechanisms such as inhibiting intestinal motility, reducing fluid secretion, and potentially through anti-inflammatory or antioxidant pathways. These findings support the potential of *Sphenocentrum jollyanum* as a source for further research and development of plant-based diarrhea treatments.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The utilization of medicinal plants as an alternative to conventional pharmaceuticals for managing diverse diseases has been on the rise worldwide. This trend is largely attributed to limited access to modern healthcare facilities, the widespread availability of medicinal herbs, economic constraints, and recent discoveries highlighting the presence of bioactive compounds in these plants that exhibit significant biological and pharmacological activities (Olorunnisola *et al.*, 2017).

Herbal medicine, also known as botanical medicine, encompasses the use of herbs and their extracts for medicinal and therapeutic purposes. The practice of utilizing herbs for addressing health issues has been in existence for centuries (Mani, 2016). The World Health Organization (WHO) estimates that approximately 4 billion people globally rely on herbal medicine as part of their primary healthcare. In 1992, the WHO promoted research into Ayurvedic medicine, with the WHO Regional Office for the Western Pacific assembling experts to guide studies aimed at evaluating herbal remedies (WHO, 2022). This expert group emphasized the importance of herbal medicine in global health, noting that while only a few herbal treatments have undergone scientific validation, many are traditionally used to enhance health. Herbal medicine is a key element in the traditional medical practices of indigenous communities and is integral to Ayurvedic, homeopathic, naturopathic, Oriental, and Native American medicine (WHO, 2022). The use of herbal medicine expanded significantly in developed nations during the latter half of the 20th century, supported by organizations such as the European Scientific Cooperative on Phytotherapy (Majeed *et al.*, 2019).

Traditional medicine has been practiced for centuries across civilizations such as China, Japan, India, Mesopotamia, and Rome (Maqbool *et al.*, 2019). In China, approximately 500 items out of a total of 12,000 are commonly utilized by traditional healers (Gaur, 2024). Japan's first pharmacopoeia of traditional medicine, compiled in the ninth century, categorized native herbs (Zhu *et al.*, 2019). Ayurveda, practiced in India for nearly 5,000 years, adopts a holistic approach to disease prevention and treatment, focusing on the interplay of body, mind, and spirit. Despite developing independently, these systems share common conceptual and methodological traits (WHO, 2022). Numerous plants have been identified for their medicinal properties, and their active compounds have been isolated and synthesized using advanced techniques. While synthetic drugs provide notable benefits, medicinal plants remain a vital source of novel drugs, potential candidates, and new chemical entities (Mani, 2016).

Africa possesses a rich diversity of medicinal plants used to treat a wide range of ailments, including bacterial and fungal infections, as well as metabolic and neurological disorders. These plant-based products have also contributed significantly to the development of conventional medicines (Stankovic *et al.*, 2016). This highlights the need for developing natural anti-inflammatory drugs that are effective, safe, biocompatible, and cost-efficient for managing inflammatory diseases (Uddin *et al.*, 2014).

S. Jollyanum, a perennial shrub native to West Africa's tropical forests and part of the Menispermaceae family, typically grows to a height of 1.5 meters. It has wedge-shaped leaves, 5–12 cm wide and up to 20 cm long, with a distinctive yellow root. Known as “*Ibong Isong*” in Ibibio, it is widely found in Sierra Leone, Nigeria, Ghana, Ivory Coast, and Cameroon (Olorunnisola *et al.*, 2020). It is referred to by various names across West Africa, such as “*Aduro kokoo*” (red medicine) in Ghana, “*Oban abe*” in Edo, and “*Okramankote*” in Côte d'Ivoire (Olorunnisola *et al.*, 2018).

The plant is recognized for its extensive medicinal uses. Its root is valued for its ability to stimulate the central nervous system and manage mental disorders, inflammation, pain, and depression. Traditionally, the dried, powdered root is combined with other anti-malarial plants to treat fever and muscular pain. Studies reveal the anti-inflammatory, anti-angiogenic, and analgesic properties of methanol extracts derived from its leaves, roots, and fruits. This underscores the need to scientifically evaluate the anti-inflammatory potential of ethanol extracts from its leaves to substantiate its folkloric medicinal applications (Uka *et al.*, 2021).

Medicinal plants contain phytochemicals, which serve as natural defense mechanisms and protect against diseases. These compounds are categorized as primary constituents, such as chlorophyll and proteins, and secondary compounds, including terpenoids, alkaloids, and phenolic compounds (Bwanbale, 2024; Agidew, 2022). Terpenoids are notable for their diverse pharmacological properties, including anti-inflammatory, anticancer, anti-malarial, antiviral, antibacterial effects, and cholesterol synthesis inhibition (Udoh *et al.*, 2021). Alkaloids, commonly found in medicinal plants, are often used as anesthetics (Agidew, 2022). Plants have long been utilized for both nutrition and medicine, with herbalism being one of the oldest therapeutic practices (Mani, 2016).

Herbal medicine's therapeutic applications are deeply rooted in history, particularly in regions rich in diverse flora, where it is considered a vital natural resource. The growing reliance on herbal formulations is attributed to the perception of their safety, leading to widespread use, particularly in rural areas. However, adverse effects and health risks associated with herbal medicine have been reported among various ethnic communities. Consequently, evaluating the toxicity profiles of these medicinal plants is crucial for ensuring their safe use (Dar *et al.*, 2017; Agidew, 2022).

S. Jollyanum Pierre, native to West Africa, is particularly noted for its medicinal and pharmacological properties. Its bark is used as a purgative and emetic, while its root serves as an aphrodisiac tonic. Root-based preparations are also employed for managing abdominal disorders, high blood pressure, and epileptic seizures. Its leaves are utilized for wound healing, while its fruit is consumed to alleviate fatigue (Ekpono *et al.*, 2018). Research indicates that the plant possesses anti-inflammatory, anti-angiogenic, and analgesic properties and demonstrates efficacy against polio type-2 virus (Olorunnisola *et al.*, 2020; Bwanbale, 2024).

The plant's morphology and reproductive characteristics reflect its adaptive biology. *S. Jollyanum* is a small, evergreen shrub with yellow roots and grey bark. Its flowers, fruits, and seeds display unique structural features that contribute to its ecological resilience (Uka *et al.*, 2021). Known by different names across various African languages, its medicinal uses are diverse and culturally significant. The plant is particularly valued for its efficacy in treating wounds, fevers, coughs, breast tumors, and ulcers (Ekpono *et al.*, 2018).

1.2 AIM AND OBJECTIVES

The aim of this study is to investigate the antidiarrhea properties of *S. Jollyanum* leaf aqueous extract in castor oil- and magnesium sulfate-induced diarrhea models in mice.

The objectives of this study include to;

- To investigate the effects of *Sphenocentrum jollyanum* leaf aqueous extract on the onset and frequency of Castor Oil-Induced diarrhea in mice
- To investigate the effects of *Sphenocentrum jollyanum* leaf aqueous extract on magnesium sulphate-induced diarrhea models in mice.

CHAPTER TWO

LITERATURE REVIEW

2.0. INTRODUCTION

Sphenocentrum jollyanum is a perennial plant native to the tropical forests of West Africa and is part of the Menispermaceae family (Olorunnisola *et al.*, 2017). This modest, upright shrub, which usually grows to about 1.5 meters, is sparsely branched. Its leaves are wedge-shaped, ranging from 5–12 cm in width and up to 20 cm in length, with a smooth texture on both sides, a small pointed tip, and a bright yellow root system (Uka *et al.*, 2021; Ugwu *et al.*, 2023). The plant, commonly known as “*Ibong Isong*” in Ibibio, is found across Sierra Leone, Nigeria, Ghana, Ivory Coast, and Cameroon. It is also referred to as “*Aduro kokoo*” (red medicine) in Ghana, “*Okramankote*” (dog's penis) in some regions, and “*Oban Abe*” or “*Ouse-abe*” in other parts of West Africa (Uka *et al.*, 2021). Recognized for its therapeutic qualities, *S. Jollyanum* is used to treat various ailments, particularly mental health issues, pain, inflammation, and depression, with its root being a key component in stimulating the central nervous system. Additionally, the powdered root extract is often mixed with anti-malarial plants to treat fever and muscle pain. Methanol extracts from its leaves, roots, and fruits have shown anti-inflammatory, anti-angiogenic, and analgesic effects (Uka *et al.*, 2021; Ugwu *et al.*, 2023).

Sphenocentrum jollyanum is a small, evergreen shrub, dioecious in nature, with initially hairy stems that become smooth over time. The bark is gray, and the leaves are arranged in a spiral pattern near the branch tips (Ekpono *et al.*, 2018). Its flowers, which appear singly on older branches, are unisexual and symmetrical. The male flowers feature sessile stamens with numerous erect filaments, while the female flowers have pedicels up to 4 mm long. The fruit is composed of 3–12 ellipsoid drupes, which transition from yellow to orange when ripe. These

fleshy, smooth drupes contain a single seed without endosperm, and during germination, the cotyledons remain within the stone (Ekpono *et al.*, 2018). Locally, *S. Jollyanum* is recognized by various names: “*Akerejupon*” among the Yoruba, “*Ezeogwu*” in Igbo, and “*Oban Abe*” in Edo State, Nigeria, while it is called “*Adurukokoo*” or “Red Medicine” in Ghana, “*Krakoo*” in Asante, “*Dangbo-Pobè-Niaouli*” in the Ewe language, and “*Orji-nkoro*” in the Izzi dialect (Ekpono *et al.*, 2018).

The plant’s therapeutic applications are wide-ranging. The bark is utilized as an emetic and purgative, especially in cases of poisoning, while the root is valued as an aphrodisiac tonic. Additionally, sap from its chewing sticks is believed to help with stomachaches, constipation, and to stimulate appetite and libido. The sour-tasting roots, which leave a sweet aftertaste, are used as a natural sweetener. In Côte d'Ivoire, the roots are combined with salt, *Aframomum melegueta*, and palm oil to treat abdominal issues, and pounded roots are used for managing high blood pressure or as an enema to address epilepsy. In Ghana, the pulped roots are applied to breast tumors, while in Nigeria, they are used to treat tropical ulcers. Leafy twig decoctions are employed to wash wounds, and powdered bark is applied for wound healing. Crushed leaves are ingested to treat haemoptysis (spitting blood), while the fruit is consumed as a remedy for fatigue (Ekpono *et al.*, 2018).

2.1 ETHNO-MEDICINAL USES

Various parts of *Sphenocentrum jollyanum* have long been used in traditional medicine. In Ghana, the root is commonly steeped in alcohol to create a bitter tonic known for enhancing and maintaining penile erection (Olorunnisola *et al.*, 2020). Numerous studies have highlighted the root's effectiveness in stimulating the central nervous system and treating a range of disorders including mental health issues, pain, and inflammation (Olorunnisola *et al.*, 2017;

Ugwu *et al.*, 2023; Akinwumi and Sonibare, 2022). The powdered root, when mixed with other plants, is used for treating fever and muscular pain (Ugwu *et al.*, 2023). The aerial parts, such as leafy twigs and fruits, are used for managing chronic wounds, fevers, and coughs, often in combination with *Piper guineense* and lime juice (Olorunnisola *et al.*, 2020). In Nigeria, chewing the roots is a common remedy for constipation, appetite stimulation, and aiding digestion (Akinwumi and Sonibare, 2022). The roots have also been used in the management of sickle cell disease. Traditional healers in Ghana and Côte d'Ivoire rely on the roots for treating conditions such as high blood pressure, breast tumors, irregular menstrual cycles, and diabetes (Olorunnisola *et al.*, 2017). The powdered roots, when combined with *Aframomum melegueta* and *Elaeis guineensis*, are used to treat abdominal discomfort. The charred fruits are also used in the treatment of fibroids and as an anti-fatigue snack (Ekpono *et al.*, 2018). Leaf decoctions are used traditionally to expel intestinal parasites and stop blood spitting (Olorunnisola *et al.*, 2020; Ugwu *et al.*, 2023).

2.2. PHYTOCHEMICAL AND PROXIMATE ANALYSIS

The pharmacological effects of *Sphenocentrum jollyanum* are primarily attributed to its bioactive compounds. Phytochemical analysis of the plant's root ethanol extract has identified terpenoids and flavonoids, with alkaloids as the predominant constituents (Bwanbale, 2024). A gas chromatography-mass spectrometry (GC-MS) study of the root's essential oil revealed 19 compounds, including α -pinene, guaia-6,9-diene-4 α -ol, globulol, and camphene. The oil consisted of 33.5% monoterpenoids and 56.3% sesquiterpenoids, with 10.2% of the compounds unidentified. Seed extracts also contain flavonoids, alkaloids, and saponins, while phylobatannin and anthraquinone were absent. Proximate analysis revealed 9.65% crude fat, 16.70% moisture, 48.09% protein, 16.79% carbohydrate, 3.26% ash, and 5.51% fiber, with an

energy value of 1460 kcal/100g (Ekpono *et al.*, 2018; Olorunnisola *et al.*, 2020). Flame photometry and atomic absorption spectrophotometry analysis identified essential minerals such as calcium, magnesium, potassium, iron, manganese, zinc, and sodium, highlighting the plant's nutritional potential (Olorunnisola *et al.*, 2020). Previous studies have isolated compounds like columbin, isocolumbine, and fibeucin, as well as the alkaloid protoberberine, from its fruit (Olorunnisola *et al.*, 2020).

2.3. PHARMACOLOGICAL AND BIOLOGICAL ACTIVITIES

2.3.1 Anti-Diabetic Activity

Research on extracts from *Sphenocentrum jollyanum* suggests notable anti-diabetic properties. Petroleum ether seed extracts significantly reduced blood glucose levels in alloxan-induced diabetic rabbits, performing comparably to glibenclamide (Ugwu *et al.*, 2023). Additionally, Alese *et al.* (2014) found that a 200 mg/kg dose of methanol root extract effectively lowered blood glucose in diabetic Wistar rats, supporting the plant's potential as an anti-diabetic agent.

2.3.2 Antioxidant Activity

Several studies have highlighted *S. Jollyanum*'s antioxidant properties. Methanolic stem extracts showed dose-dependent radical scavenging activity, with LC50 values of 13.11 µg/mL for superoxide and 30.04 µg/mL for hydrogen peroxide, which is comparable to ascorbic acid (Olorunnisola *et al.*, 2011). Additionally, research by Olorunnisola and Afolayan (2014) demonstrated that *S. Jollyanum* effectively reduced oxidative stress caused by *Plasmodium berghei* infections in mice, evidenced by decreased liver MDA levels and increased catalase, superoxide dismutase, and glutathione activity. Uka *et al.* (2020) further observed antioxidant activity in various plant organs, with the stem bark showing the highest activity (LC50 of 1.80

µg/mL), followed by the root and leaf. The chloroform fraction of the stem bark exhibited the most potent activity, with an LC50 of 1.54 µg/mL.

.2.3.3. Anti-Inflammatory Properties

In vivo studies examining the anti-inflammatory effects of *S. Jollyanum*'s crude extracts and isolated compounds have been conducted using healthy rats with carrageenan-induced inflammation. The results indicated that the methanol fruit extract, at a dosage of 200 mg/kg, provided the most significant inhibition (79.58%) of oedema in the rats' hind paws, while the root extract exhibited a lower inhibition rate of 53.75%. Furthermore, three furanoditerpenes columbin, isocolumbine, and fibleucin isolated from the methanol fruit extract, demonstrated notable anti-inflammatory effects. Specifically, columbin and a flavonoid-rich fraction, when administered at 200 mg/kg, showed 67.08 and 76.25% inhibition, respectively, comparable to the effects of acetylsalicylic acid (Olorunnisola *et al.*, 2017). Additional studies by Olorunnisola *et al.* (2017) on the in vitro anti-inflammatory effects of the plant's extracts and metabolites further supported its traditional use in treating inflammation-related disorders in West Africa.

2.3.4. Anti-Allergic Activity

The anti-allergic potential of *S. Jollyanum* was assessed using milk-induced leukocytosis and eosinophilia in mice. The ethanolic fruit extracts led to a dose-dependent reduction in eosinophil and lymphocyte counts, highlighting the extract's anti-allergic effects. These effects are believed to be the result of multiple mechanisms involving various phytochemicals (Olorunnisola *et al.*, 2017).

2.3.5. Anti-Malarial Activity

In a study by Olanlokun et al. (2024), the anti-malarial activity of *S. Jollyanum* leaf and root extracts was assessed, revealing the plant's effectiveness against chloroquine-resistant *Plasmodium berghei* in Swiss albino mice. The methanol extracts from both the leaves and roots exhibited significant, concentration-dependent anti-malarial effects.

2.3.6. Anti-Bacterial Activity

Research on the antibacterial properties of essential oils derived from the *S. Jollyanum* root extract demonstrated activity against bacterial strains such as *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus cereus*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*. Notably, the root extract showed inhibition zones of 10 and 9.0 mm against *Bacillus subtilis* and *Pseudomonas aeruginosa* at a concentration of 1000 µg/mL (Olorunnisola *et al.*, 2017). The antimicrobial properties, particularly against *S. Typhi*, also support the traditional use of the plant as a laxative to aid digestion and promote bowel movements (Olorunnisola *et al.*, 2017).

2.3.7. Anti-Viral Activity

According to Olorunnisola et al. (2017), a study by Moody and colleagues (2002) evaluated the antiviral activity of *S. Jollyanum* methanol extracts against poliovirus Types 1, 2, and 3. The results indicated that both leaf and root extracts were effective against poliovirus Type 2. Further testing of hexane and methanol extracts also showed inhibitory effects against mosaic virus in cowpea, suggesting the plant's broad antiviral potential.

2.3.8. Haematological Effects

The hematopoietic effects of *S. Jollyanum* methanol extracts were studied using Wistar mice infected with chloroquine-resistant *P. berghei*. After seven days of oral administration of root and leaf extracts, significant increases in packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and hemoglobin (Hb) levels were observed. Additionally, red and white blood cell counts were elevated, excluding monocytes and neutrophils, indicating that *S. Jollyanum* may stimulate hematopoietic stem cells (Olorunnisola *et al.*, 2017).

2.3.9. Effect on Weight

In a study evaluating the effects of *S. Jollyanum* leaf and root extracts on weight changes in malaria-infected and diabetic rats, results showed a significant ($p < 0.05$) increase in weight gain among the treated animals. A comparison of the treated and untreated control groups revealed that the extracts effectively prevented weight loss in a concentration-dependent manner. This effect was attributed to the extracts' potential to reduce acute fluid loss and mitigate fat and protein breakdown, factors typically responsible for weight reduction (Olorunnisola *et al.*, 2017).

2.3.10. Hepatoprotective and Toxicological Effects

The hepatoprotective properties of *S. Jollyanum* stem bark extract were validated in a study where liver damage induced by CCl₄ in rats was significantly reversed. The extract reduced levels of aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and total bilirubin, while lowering serum protein in a dose-dependent manner (Olorunnisola *et al.*, 2017). Histological analysis revealed no signs of inflammation or

toxicity in the liver tissue. The essential oil's safety was also confirmed through a brine shrimp lethality test, with an LC50 of 84.87 ppm (Olorunnisola *et al.*, 2017).

2.3.11. Hypolipidemic Activity

The hypolipidemic activity of *S. Jollyanum* ethanol root extract was assessed in streptozotocin-induced diabetic rats, showing no significant changes in total cholesterol levels between the extract-treated and glibenclamide-treated groups. However, the extract-treated group displayed a notable improvement in anti-atherogenic index (AAI) and HDL levels (Olorunnisola *et al.*, 2017).

2.3.12. Antidepressant Activity

The antidepressant properties of *S. Jollyanum* were assessed in mice using forced swimming and tail suspension tests. The ethanol root extract (100–1000 mg/kg) significantly improved mobility in both models in a dose-dependent manner, although it was less potent than standard antidepressant drugs like imipramine and fluoxetine, suggesting its mild effects on monoamine modulation (Olorunnisola *et al.*, 2017).

2.3.13. Anxiogenic Activity

The anxiogenic effects of *S. Jollyanum* were evaluated using ethanol extract doses ranging from 100 to 1000 mg/kg. Treated animals displayed increased anxiety-like behavior, similar to the effects of caffeine, contrasting with the anxiolytic action of diazepam. This supports the plant's traditional use as a CNS stimulant and mood enhancer (Olorunnisola *et al.*, 2017).

2.3.14. Anti-Angiogenic Activity

Olorunnisola et al. (2017) reported that the anti-angiogenic properties of *S. Jollyanum* were evaluated by Nia and colleagues in 2004 using a chick chorioallantoic membrane (CAM) assay. The methanol extract from the stem bark demonstrated the most potent anti-angiogenic activity, with an LC50 value of 1.00 µg/mL. The chloroform fraction also showed strong inhibition, supporting its use in traditional tumor prevention.

2.3.15. Antipyretic and Analgesic Activities

Both analgesic and antipyretic activities were observed in petroleum ether and methanol extracts of *S. Jollyanum* leaves, further confirming the plant's analgesic potential.

2.3.16. Reproductive and Sexual Activity

Studies on the reproductive effects of *S. Jollyanum* revealed that its extracts increased testosterone levels, reduced post-ejaculatory latency, and enhanced male sexual performance in animals (Olorunnisola *et al.*, 2017). However, contradictory findings from Raji and colleagues in 2007 showed a decrease in sperm count, motility, and testicular function, suggesting potential adverse reproductive effects that warrant further investigation to assess the balance between its therapeutic benefits and risks.

2.4. PLANT PHYTOCHEMISTRY

Phytochemistry is the exploration of plant-derived chemicals, focusing on a diverse range of bioactive compounds that play essential roles in plant functions and offer considerable therapeutic promise for human health. Secondary metabolites in plants, such as alkaloids, flavonoids, terpenoids, phenolics, and glycosides, are not vital for the basic survival of plants

but are critical for their interaction with the environment, including defense against herbivores, pathogens, and environmental stress (Egbuna *et al.*, 2018; Agidew, 2022).

The study of these compounds is central to understanding the medicinal potential of plants, as many bioactive substances demonstrate antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. Phenolic compounds, including flavonoids and tannins, are particularly known for their strong antioxidant effects, which play an essential role in neutralizing free radicals and mitigating oxidative stress in living organisms (Batiha *et al.*, 2020). Similarly, alkaloids—nitrogenous compounds—are widely researched for their pharmacological effects on the central nervous system, with some serving as analgesics, stimulants, or antimalarials (Oladeji *et al.*, 2019).

2.5. DIARRHEA

Diarrhea is defined by the frequent passage of loose or watery stools, typically more than three times per day, or a stool frequency higher than what is usual for an individual (Obeagu and Obeagu, 2019). It is important to differentiate this condition from the passage of formed stools or from the occasional occurrence of loose stools in breastfed infants. Generally, diarrhea indicates an infection in the gastrointestinal tract, which can be caused by a variety of bacterial, viral, or parasitic pathogens (WHO, 2024). Diarrhea is categorized into three types: acute watery diarrhea, which lasts for a few hours to days and includes conditions such as cholera; acute bloody diarrhea, also known as dysentery; and persistent diarrhea, which lasts for 14 days or longer. These infections are mainly transmitted through the consumption of contaminated food or water or through direct contact between individuals, often due to poor hygiene practices (WHO, 2024).

2.6. MAGNESIUM SULPHATE-INDUCED DIARRHEA

Magnesium sulphate (MgSO_4) is widely known for its laxative properties, which primarily arise from its osmotic effect in the gastrointestinal tract. When ingested orally, MgSO_4 increases the osmotic gradient in the intestinal lumen, leading to water retention and enhanced intestinal motility. This osmotic effect stimulates bowel movements, often resulting in diarrhea when administered in high doses. The mechanism is attributed to magnesium ions, which trigger the release of cholecystokinin (CCK), a hormone that accelerates gastrointestinal motility and fluid secretion (Fenn *et al.*, 2020). MgSO_4 -induced diarrhea is frequently used in experimental models to investigate antidiarrheal agents due to its reproducible effects and predictable onset. However, prolonged exposure to MgSO_4 can disrupt electrolyte balance and cause dehydration, necessitating careful monitoring in therapeutic or research applications (Zhao *et al.*, 2021).

2.7. CASTOR OIL-INDUCED DIARRHEA

Castor oil-induced diarrhea is a classic experimental model widely employed to evaluate the efficacy of antidiarrheal agents. Castor oil is metabolized in the small intestine to ricinoleic acid, an active component that irritates the intestinal mucosa, leading to increased secretion of electrolytes and water into the intestinal lumen (Rao *et al.*, 2019). This secretory diarrhea is mediated by the activation of prostaglandin receptors, particularly prostaglandin E_2 , which enhances intestinal motility and fluid secretion. Due to its predictable and consistent effects, castor oil-induced diarrhea is commonly used in pharmacological studies to assess the potential of natural and synthetic compounds in managing diarrhea. Nevertheless, ricinoleic acid's mucosal irritant properties highlight the need for caution, as excessive use of castor oil may lead to intestinal inflammation and discomfort (Manna *et al.*, 2022).

CHAPTER THREE

MATERIALS AND METHOD

3.0. Plant collection

Fresh leaves of *Sphenocentrum jollyanum* were collected from Ondo State in southern Nigeria. The plant was identified and authenticated by Prof. O. Timothy at the University of Benin, Nigeria. The leaves were carefully washed with clean water to remove debris and any potential contaminants, then air-dried in a shaded environment for two weeks to maintain their phytochemical properties.

3.1. Plant preparation

Fresh roots were cut into smaller pieces and allowed to air dry at a temperature of 36-39°C. Once dried, the roots were ground into a coarse powder using a grinder. A total of 2370 g of the powdered material was processed in batches using a Soxhlet extractor, with water as the solvent, over three cycles lasting approximately 60 hours. The resulting extract was filtered using Whatman No. 4 filter paper. The filtrate was then dried under vacuum conditions at a temperature range of 30-36°C, yielding approximately 54 g of extract. This was stored at 4°C in a refrigerated environment until further use.

3.2. Experimental Animals

A total of 20 adult Swiss albino mice, both male and female, were used for this study. The animals were housed in the animal facility of the Department of Science Laboratory Technology, University of Benin, Nigeria. They were provided with a diet of commercially prepared rodent feed (Ladokun Livestock Feeds Limited, Ibadan, Nigeria) and had unrestricted

access to distilled water. Before the experiment, the mice were acclimatized under standard laboratory conditions for one week.

3.3. Experimental Design

The experiment was structured into two groups of 10 mice each (n = 10 per group). Treatments were allocated as follows: a negative control group receiving 10 mL/kg of distilled water, a positive control group administered loperamide at 2 mg/kg, and three experimental groups treated with the extract at low (250 mg/kg), medium (500 mg/kg), and high doses (1000 mg/kg). All treatments were delivered orally using an oral gavage.

3.4. Acute Toxicity Study

The acute toxicity of the extract was evaluated using Lorke's method. Mice were divided into groups and given various oral doses of the extract (100–1000 mg/kg). Observations for mortality and behavioral changes were recorded over 24 hours to calculate the LD50.

3.5. Induction of Diarrhea

Castor Oil-Induced Diarrhea

Diarrhea was induced by administering 1 mL/kg of castor oil orally. One hour later, the respective treatments were administered. Each mouse was placed in an individual cage lined with non-absorbent white paper to observe and record defecation for 6 hours. The weights of the animals ranged from 18 g – 35 g.

Magnesium Sulfate-Induced Diarrhea

Magnesium sulfate (2 g/kg) was administered orally to induce diarrhea, following the same treatment protocol as above. The weights of the animals ranged from 19 g - 27 g.

3.6. Statistical Analysis

Data were expressed as mean \pm SEM and analyzed using a one-way ANOVA followed by Tukey's post-hoc test. A p-value of < 0.05 was considered statistically significant.

CHAPTER FOUR

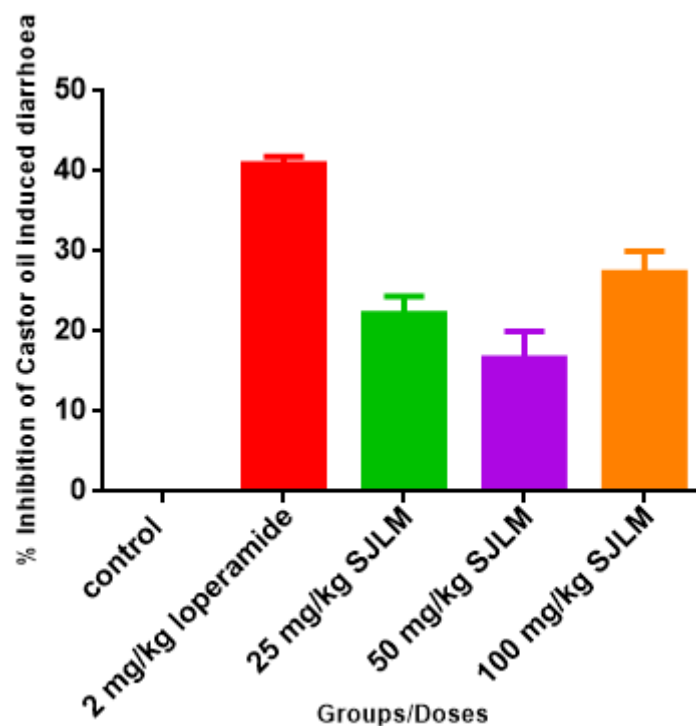
RESULTS

Table 4.1: Antidiarrhoea effect of *Sphenocentrum jollyanum* leave aqueous extract in castor oil induced diarrhoea in mice

Treatment	Dose mg/kg	Mean± SEM Onset of stool (sec)	Mean± SEM Total number of stool	Mean± SEM Number of diarrhea	Mean± SEM Weight of stool (g)
Control	DW	300.00±3.00 ^a	8.50±0.25 ^a	5.50±0.15 ^a	2.56±0.14 ^a
Loperamide	2	5160.00±7.80 ^c	1.50±0.00 ^c	1.00±0.00 ^c	0.31±0.03 ^b
SJLA	25	930.00±39.00 ^b	5.00±0.20 ^b	3.50±0.20 ^b	0.61±0.21 ^b
SJLA	50	960.00±4.20 ^b	4.00±0.10 ^b	4.00±0.00 ^b	0.14±0.02 ^b
SJLA	100	390.00±3.00 ^b	2.50±0.50 ^b	2.50±0.30 ^b	0.63±0.03 ^b

P-value < 0.05, showed the level, DW---- distilled water, SJLA (*Sphenocentrum jollyanum* leaf aqueous extract)

Table 4.1 presents the antidiarrhea effects of *Sphenocentrum jollyanum* leaf aqueous extract (SJLA) in castor oil-induced diarrhea in mice. The extract demonstrated dose-dependent efficacy, as indicated by a significant increase in the onset of stooling time and a reduction in the total number of stools, diarrhea stools, and stool weight compared to the control (distilled water). Loperamide (2 mg/kg) served as the positive control and exhibited the highest inhibitory effect on diarrhea. Among the extract-treated groups, the 50 mg/kg and 100 mg/kg doses showed notable reductions in diarrhea stool frequency and stool weight ($P < 0.05$), suggesting potential antidiarrhea activity.



P-value < 0.05, showed the level, DW---- distilled water, SJLA (*Sphenocentrum jollianum* leaf aqueous extract)

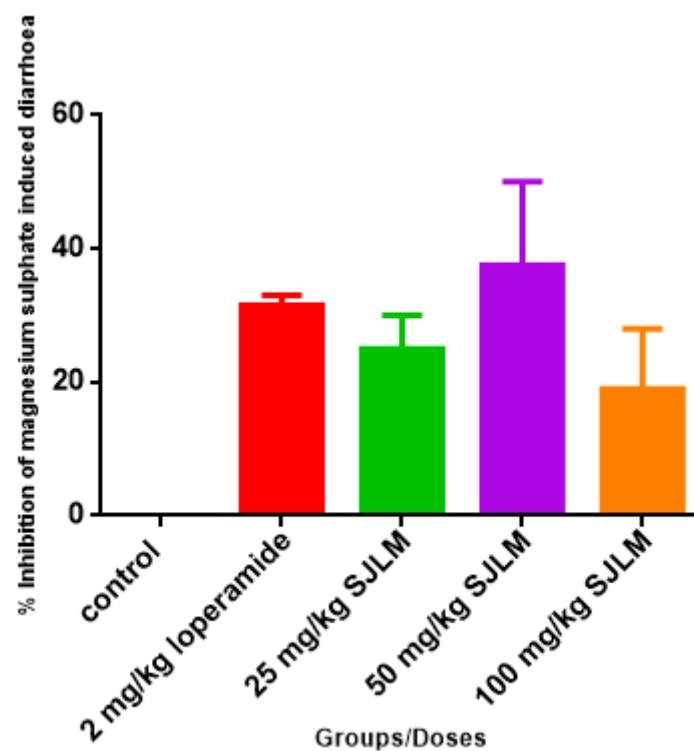
Figure 1: Antidiarrhoea effect of *Sphenocentrum jollianum* leave aqueous extract percentage inhibition in castor oil induced diarrhoea in mice

Table 4.2: Antidiarrhoea effect of *Sphenocentrum jollianum* leave aqueous extract in magnesium sulphate induced diarrhoea in mice

Treatment	Dose mg/kg	Mean± SEM Onset of stool (sec)	Mean± SEM Total number of stool	Mean± SEM Number of diarrhea	Mean± SEM Weight of stool (g)
Control	DW	9.50±30.05 ^a	5.00±0.30 ^a	4.00±0.20 ^a	1.25±0.05 ^a
Loperamide	2	4470.00±20.70 ^c	1.50±0.10 ^c	1.50±0.01 ^c	0.01±0.000 ^a
Extract	25	2490.00±24.90 ^c	2.500±0.05 ^b	2.00±0.20 ^b	0.09±0.00 ^b
Extract	50	4200.00±42.00 ^c	1.50±0.01 ^c	1.00±0.00 ^c	0.13±0.05 ^b
Extract	100	930.00±3.30 ^b	3.00±0.10 ^b	2.50±0.05 ^b	0.01±0.000 ^a

P-value < 0.05, showed the level, DW---- distilled water, SJLA (*Sphenocentrum jollianum* leaf aqueous extract)

Table 4.2 illustrates the antidiarrhea effects of *Sphenocentrum jollyanum* leaf aqueous extract (SJLA) in magnesium sulphate-induced diarrhea in mice. The extract significantly delayed the onset of diarrhea and reduced the total number of stools, diarrhea stools, and stool weight in a dose-dependent manner compared to the control (distilled water). The highest efficacy was observed at 50 mg/kg, which showed comparable effects to Loperamide (2 mg/kg), the positive control. The 100 mg/kg dose also demonstrated a notable reduction in diarrhea stool frequency and stool weight ($P < 0.05$), indicating potential antidiarrhea activity of the extract.



P -value < 0.05 , showed the level, DW---- distilled water, SJLA (*Sphenocentrum jollyanum* leaf aqueous extract)

Figure 2: Antidiarrhoea effect of *Sphenocentrum jollyanum* leave aqueous extract in magnesium sulphate induced diarrhoea in mice

CHAPTER FIVE

DISCUSSION AND CONCLUSION

The antidiarrhea activity of *Sphenocentrum jollyanum* leaf aqueous extract (SJLA) was evaluated using both the castor oil-induced and magnesium sulfate-induced diarrhea models. In the castor oil-induced diarrhea model, SJLA showed a significant dose-dependent effect on various diarrhea parameters, as summarized in Table 4.1. At the 25 mg/kg dose, the onset of stool was significantly delayed (930.00 ± 39.00 sec), compared to the control group (300.00 ± 3.00 sec). This delay suggests that SJLA may inhibit the irritative effects of castor oil on the intestinal wall, which typically leads to rapid diarrhea by disrupting the water and electrolyte balance (Fokam-Tagne *et al.*, 2019). Notably, the 50 mg/kg dose also demonstrated a delayed stool onset (960.00 ± 4.20 sec), though slightly less marked than the 25 mg/kg dose, while the highest dose (100 mg/kg) exhibited a reduced onset of stool (390.00 ± 3.00 sec). This effect, although less pronounced than the standard antidiarrhea agent loperamide (5160.00 ± 7.80 sec), still shows the potential of SJLA in delaying the onset of diarrhea.

The extract's capacity to reduce the total number of stools and the number of diarrhea episodes was also evident in Table 4.1. At the 25 mg/kg dose, the total number of stools was significantly reduced to 5.00 ± 0.20 , compared to the control group, which had 8.50 ± 0.25 stools. The number of diarrhea episodes also decreased from 5.50 ± 0.15 in the control group to 3.50 ± 0.20 in the 25 mg/kg group, suggesting that SJLA effectively reduces intestinal motility. The stool weight was notably reduced in the higher doses of SJLA, especially at the 50 mg/kg dose (0.14 ± 0.02 g), further confirming its antidiarrhea potential. These findings align with the work of Adela *et al.*, (2022) and other ther plant-based studies demonstrating a reduction in stool frequency and weight, indicating a potential mechanism through modulation of gastrointestinal motility and secretion.

In the magnesium sulfate-induced diarrhea model, as shown in Table 4.2, the antidiarrhea effect of SJLA was similarly pronounced. Magnesium sulfate is known to induce diarrhea by enhancing intestinal motility and increasing fluid secretion (Das *et al.*, 2024). The administration of SJLA (25 mg/kg, 50 mg/kg, and 100 mg/kg) significantly delayed the onset of stool, with the 25 mg/kg dose showing a notable improvement (2490.00 ± 24.90 sec) over the control (9.50 ± 30.05 sec). These results are consistent with the hypothesis that SJLA delays gastrointestinal transit, reducing the severity of diarrhea. At higher doses, such as the 100 mg/kg dose, stool weight was reduced to 0.01 ± 0.00 g, comparable to the standard antidiarrhea agent loperamide (0.01 ± 0.00 g), further validating the efficacy of SJLA in mitigating diarrhea. The reductions in stool frequency, stool number, and stool weight in both models suggest that SJLA exerts its antidiarrhea effect via multiple mechanisms. It may modulate intestinal motility, reduce fluid secretion, and potentially act through antioxidant or anti-inflammatory pathways (Wu *et al.*, 2024). The bioactive compounds in SJLA, including alkaloids, flavonoids, and tannins, may contribute to these effects. Alkaloids are known to modulate acetylcholine receptors, which influence intestinal motility. Flavonoids exhibit anti-inflammatory properties, which could help mitigate the inflammation in the intestinal wall, and tannins, with their astringent properties, might reduce fluid loss from the intestines (Mekonnen *et al.*, 2019). The findings of Wu *et al.*, (2024) provide compelling evidence for the antidiarrhea potential of *Sphenocentrum jollyanum* leaf aqueous extract, demonstrating its ability to alleviate diarrhea induced by both castor oil and magnesium sulfate, through mechanisms such as modulation of intestinal motility and fluid secretion.

5.1. CONCLUSION

The study demonstrated that the ethanol extract of *Sphenocentrum jollyanum* leaves (SJLA) exhibited notable antidiarrhea properties in murine models of diarrhea induced by castor oil and magnesium sulfate. The extract prolonged the time before stool onset, decreased stool frequency, and reduced stool weight in a dose-dependent manner, indicating its potential therapeutic application for managing diarrhea. These findings suggest that the presence of bioactive compounds such as alkaloids, flavonoids, and tannins that may be implicated and potent in the antidiarrhea effects.

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