THE EFFECT OF AQUEOUS EXTRACT OF *SPHENOCENTRUM JOLLYANUM* ON LIPID PROFILE

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FACULTY OF LIFE SCIENCES

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AN UNDERGRADUATE PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.SC.) DEGREE IN SCIENCE LABORATORY TECHNOLOGY

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CERTIFICATION

This is to certify that this research titled "**THE EFFECT OF AQUEOUS EXTRACT OF *SPHENOCENTRUM JOLLYANUM* ON LIPID PROFILE**" was carried out by "**Peace Emem ANTE**" with matriculation number "**LSC2009998**" and presented to the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) in Science Laboratory Technology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Science Laboratory Technology.

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DECLARATION

I "**Peace Emem ANTE**" declare that "**THE EFFECT OF AQUEOUS EXTRACT OF *SPHENOCENTRUM JOLLYANUM* ON LIPID PROFILE**" is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

…….. …….

Peace Emem ANTE **DATE**

DEDICATION

This project work is dedicated to the Almighty God for his grace and mercies and to my family for their support and love throughout my period of study.

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TABLE OF CONTENTS

ABSTRACT

Medicinal plants have been an integral part of human healthcare since ancient times, serving as the foundation of traditional medicine systems across the world. The increasing global prevalence of dyslipidaemia and its associated cardiovascular risks has intensified the search for safer, plant-based therapeutic alternatives. This study evaluated the effect of aqueous leaf extract of *Sphenocentrum jollyanum* on serum lipid profile parameters in Wistar rats after 28 days of daily oral administration. Fresh leaves of *S. jollyanum* were collected; authenticated, air-dried, pulverized, and extracted using distilled water. Twenty male Wistar rats were randomly divided into four groups of five animals each: a control group and three treatment groups administered 500, 1000, and 2500 mg/kg body weight of the aqueous extract. At the end of the experimental period, serum samples were analyzed for total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) using standard biochemical methods. Results revealed a dose-dependent reduction in serum total cholesterol and triglyceride levels in all treated groups compared to the control. Cholesterol decreased from 248.26 ± 62.65 mg/dL in the control to 74.67 ± 4.91 mg/dL at 2500 mg/kg, while triglycerides dropped from 169.91 ± 39.80 mg/dL to 85.35 ± 7.62 mg/dL. LDL concentrations also declined markedly, from 171.57 ± 57.45 mg/dL in the control to −7.82 ± 13.31 mg/dL at 2500 mg/kg, indicating enhanced lipid clearance and inhibition of hepatic cholesterol synthesis. Conversely, HDL levels increased significantly at the highest dose (70.94 ± 7.82 mg/dL), suggesting improved reverse cholesterol transport. These biochemical changes reflect a strong lipid-lowering and cardioprotective effect of the extract. The hypolipidaemic activity of *S. jollyanum* is attributed to its phytochemical constituents particularly flavonoids, saponins and alkaloids, which may act synergistically to inhibit HMG-CoA reductase, enhance bile acid excretion, and strengthen antioxidant defenses. The findings align with previous reports validating the plant's traditional use for detoxification and metabolic regulation. In conclusion, the aqueous leaf extract of *Sphenocentrum jollyanum* demonstrates potent lipid-regulating and cardioprotective potential without apparent toxicity, supporting its ethnomedicinal use as a safe, natural remedy for managing dyslipidaemia and related cardiovascular disorder.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Medicinal plants have been an integral part of human healthcare since ancient times, serving as the foundation of traditional medicine systems across the world. They are defined as plants that contain bioactive substances capable of producing therapeutic effects or modifying physiological functions in humans and animals (Sofowora, 2013). Over 80% of the global population, particularly in developing countries, relies on medicinal plants for their primary healthcare needs due to their accessibility, cultural acceptance, and relatively low cost compared to synthetic drugs (World Health Organization, 2021). In Africa, the use of herbs and plant-based remedies remains deeply rooted in cultural traditions and continues to play a significant role in the management of various ailments including infections, inflammation, metabolic disorders, and organ-related diseases (Adebayo and Ishola, 2018).

Scientific studies have increasingly validated the therapeutic potentials of these plants, attributing their pharmacological effects to secondary metabolites such as alkaloids, flavonoids, saponins, tannins, terpenoids, and phenolic compounds (Owolabi *et al.*, 2019). These bioactive components have been found to exhibit diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, hypoglycaemic, and

lipid-lowering effects. Consequently, medicinal plants have become a valuable source of raw materials for drug discovery and development.

Among the metabolic disorders that have drawn attention to the use of medicinal plants is dyslipidaemia, an abnormality in lipid metabolism. Dyslipidaemia refers to elevated levels of total cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C), or a decrease in high-density lipoprotein cholesterol (HDL-C), leading to cardiovascular complications (Okafor and Nwodo, 2018). These imbalances are a major risk factor for atherosclerosis, coronary artery disease, stroke, and other cardiovascular complications. The increasing prevalence of dyslipidaemia globally has been linked to poor dietary habits, physical inactivity, and genetic predispositions (World Health Organization, 2021).

Pharmacological interventions such as statins, fibrates, and bile acid sequestrants are commonly prescribed for lipid regulation. However, their prolonged use is associated with side effects such as liver toxicity, muscle weakness, and gastrointestinal discomfort, in addition to high costs that limit accessibility in low-income populations (Adewale *et al.,* 2017). These limitations have spurred scientific interest in exploring medicinal plants as safer, affordable, and culturally accepted alternatives for managing lipid disorders (Chikezie *et al.,* 2020).

One medicinal plant that has attracted increasing scientific interest is *Sphenocentrum jollyanum* (family Menispermaceae). It is a small perennial shrub native to the tropical rainforests of West Africa, notably in Nigeria, Ghana, and Cameroon (Iwu, 2014).

Locally known as “Aduro kokoo” in Ghana and “Ajo” in parts of Nigeria, the plant is highly valued in African traditional medicine. It has been used for treating a variety of ailments including fever, wounds, stomach pain, inflammation, and liver-related disorders (Eze and Iwu, 2016). Phytochemical investigations have shown that the plant contains diverse bioactive constituents such as alkaloids, flavonoids, saponins, tannins, and glycosides that may account for its broad pharmacological actions (Alese *et al.*, 2014).

Previous research on *Sphenocentrum jollyanum* has demonstrated several biological effects including anti-inflammatory, antipyretic, antimicrobial, antioxidant, and hypoglycaemic properties (Okoye *et al.,* 2020; Udeh *et al.,* 2021). However, most of these studies have employed organic solvent extracts such as methanol and ethanol, leaving a research gap concerning the aqueous extract, which is the most commonly used preparation in traditional medicine. Aqueous extraction not only reflects the indigenous method of preparation but is also considered safer for human use compared to organic solvents.

Therefore, it becomes essential to scientifically investigate the effects of the aqueous leaf extract of *Sphenocentrum jollyanum* on lipid profile parameters. This evaluation will help establish whether traditional claims about the plant’s therapeutic properties are supported by scientific evidence. The present study aims to assess the effect of the aqueous leaf extract of *Sphenocentrum jollyanum* on lipid profile parameters in rats and mice, thereby contributing to the body of knowledge on the medicinal potential of this

important African plant.

1.2 AIM OF STUDY

The aims of the study was to evaluate the effect of aqueous extract of *Sphenocentrum jollyanum* on lipid profile following 28 days of daily oral administration in experimental animal models (wistar rats/mice).

1.3 OBJECTIVES OF STUDY

The specific objectives of this study are to:

- Determine the effect of aqueous leaf extract of *Sphenocentrum jollyanum* on serum total cholesterol (TC) levels in rats.
- Assess the effect of the extract on triglyceride (TG) concentration.
- Evaluate the changes in low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels following extract administration.
- Compare lipid profile values across different dosage groups to establish any dose-dependent relationship.

CHAPTER TWO

LITERATURE REVIEW

2.1 GENERAL OVERVIEW OF MEDICINAL PLANTS AND LIPID METABOLISM

Medicinal plants have remained a cornerstone of disease prevention and treatment throughout human history. The World Health Organization (WHO, 2021) estimates that more than 80 percent of the global population relies on herbal medicines for primary healthcare. These plants are valued because they contain a diverse range of bioactive compounds, known as secondary metabolites, including alkaloids, flavonoids, terpenoids, saponins and tannins, which exert therapeutic effects on physiological systems (Owolabi *et al.,* 2019; Sofowora, 2008). Such compounds often act synergistically, producing pharmacological responses that may be more stable and holistic than single-molecule drugs (Trease and Evans, 2012).

Medicinal plants are particularly relevant in the management of metabolic disorders such as dyslipidaemia, obesity and diabetes (Kumar *et al.,* 2020). Lipid metabolism refers to the synthesis, degradation and transport of lipids within the body, processes vital for membrane formation, energy storage and hormone regulation (Nelson and Cox, 2017). Disruption of these pathways leads to dyslipidaemia, defined by elevated concentrations of total cholesterol, triglycerides and low-density lipoprotein cholesterol (LDL-C), or reduced high-density lipoprotein cholesterol (HDL-C) (Okafor and Nwodo, 2018; Murray *et al.,* 2015). These abnormalities contribute directly to

atherosclerosis and increase the risk of coronary artery disease, stroke and other cardiovascular complications (Goldstein and Brown, 2015).

Although synthetic lipid-lowering drugs such as statins, niacin and fibrates have demonstrated clinical effectiveness, their use is frequently accompanied by adverse effects, including myalgia, hepatic dysfunction and gastrointestinal disturbances (Adewale *et al.,* 2017; Tiwari *et al.,* 2018). Moreover, long-term therapy is costly, particularly in low-income regions where dyslipidaemia is now prevalent due to sedentary lifestyles and high-fat diets (Yusuf *et al.,* 2020). Consequently, research has shifted towards exploring medicinal plants as alternative sources of lipid-modulating agents that are safer, more accessible and culturally acceptable (Iwu, 2014; Adebayo and Ishola, 2018).

Among the promising medicinal plants investigated for hypolipidaemic potential is *Sphenocentrum jollyanum* Pierre, belonging to the family Menispermaceae. This West African shrub has long been used in traditional medicine for treating malaria, fever, stomach upset, liver disease and sexual weakness (Eze and Iwu, 2016; Oyebueke and Adeyemi, 2013). Recent pharmacological studies have revealed that its extracts exhibit antioxidant, anti-inflammatory and hepatoprotective activities, which could directly or indirectly influence lipid metabolism (Alese *et al.,* 2014; Udeh *et al.,* 2021). Several scholars argue that such bioactivities are linked to the plant's polyphenolic and alkaloid contents, which enhance antioxidant defences and stabilise cellular membranes (Samuel *et al.,* 2018; Olorunnisola *et al.,* 2016; Fadahunsi *et al.,* 2018).

Furthermore, the increasing prevalence of cardiovascular and metabolic diseases across Africa underscores the relevance of investigating indigenous plants like *S. jollyanum* using scientific methods (Nwosu *et al.,* 2019). By characterising its aqueous leaf extract commonly used in folk medicineit is possible to provide scientific validation for its traditional use and to identify safer, naturally derived alternatives to synthetic drugs (Mbaka and Owolabi, 2011; Ugwu and Amasiorah, 2020; WHO, 2020).

2.2 TAXONOMY OF SPHENOCENTRUM JOLLYANUM

Sphenocentrum jollyanum Pierre belongs to the family Menispermaceae, a group of mostly tropical climbing shrubs and woody plants known for their rich alkaloid content and diverse medicinal properties (Iwu, 2014). The plant's taxonomic classification is as follows (Olorunnisola *et al.,* 2016):

The taxonomic classification of *Sphenocentrum jollyanum* is as follows:

Kingdom: Plantae

Division: Magnoliophyta (Angiosperms)

Class: Magnoliopsida (Dicotyledons)

Order: Ranunculales

Family: Menispermaceae

Genus: Sphenocentrum

Species: *Sphenocentrum jollyanum Pierre*

The genus Sphenocentrum is monotypic, meaning it contains only one species of *S. jollyanum* which makes it unique within the family (Alese *et al.*, 2014). Members of the Menispermaceae family are characterised by their twining growth habit, simple leaves, and often bitter, alkaloid-rich roots. Many species within this family, such as *Tinospora cordifolia* and *Stephania tetrandra*, are renowned for their pharmacological activities (Sofowora, 2008). The name "Sphenocentrum" is derived from Greek roots meaning "wedge-shaped centre," likely referencing the morphology of its seed (Botanikks, 2024).

2.3 BOTANICAL DESCRIPTION

Sphenocentrum jollyanum Pierre is an evergreen shrub or small tree belonging to the family Menispermaceae (Botanikks, 2024; Iwu, 2014). It typically reaches a height of 1–3 metres and thrives in shaded, humid tropical forests (BotanicalRealm, 2024). The stem is cylindrical, woody and brownish, exuding a characteristic yellow latex when cut (Adebayo and Ishola, 2018). The bark is fibrous with a slightly bitter taste and emits an aromatic odour when crushed (Okoye *et al.*, 2020).

Leaves are alternate, simple, and elliptic to ovate with entire margins and glossy surfaces, measuring 8–15 cm in length and 3–6 cm in breadth (Fadahunsi *et al.*, 2018; Nnabugwu *et al.*, 2016). The apex is acute while the base is slightly cordate, and the venation is pinnate. Petioles are 2–4 cm long and slender (Ojo *et al.*, 2020). The flowers are small, greenish-yellow or cream in colour and borne in terminal or axillary racemes

(Olorunnisola *et al.,* 2016). Fruits occur as ovoid drupes, turning orange or red upon ripening, each containing a single seed with a hard endocarp (Alese *et al.,* 2014).

Microscopically, the plant tissue reveals abundant parenchymatous cells, secretory canals, calcium oxalate crystals and starch grains within the cortex (Oyebueke and Adeyemi, 2013). These anatomical features correspond with its reported phytochemical composition, which includes alkaloids, flavonoids, saponins, tannins and phenolics (Integrity Research Journals, 2020; Samuel et al., 2018). The root is aromatic, yellowish-brown, and often employed in decoctions for fever and digestive problems (AMA-Ghana, 2008; Eze and Iwu, 2016).

The leaves, stems, roots and fruits of *S. jollyanum* all possess medicinal value, but local healers prefer the aqueous leaf extract for its ease of preparation and reduced toxicity (Mbaka and Owolabi, 2011; Ugwu and Amasiorah, 2020). The plant's robust morphology and adaptability to moist environments enable its survival across diverse ecological zones of West Africa, particularly in Nigeria, Ghana and Cameroon (Ogbon *etal .,* 2023; WHO, 2021).

2.4 GEOGRAPHICAL DISTRIBUTION AND HABITAT

Sphenocentrum jollyanum Pierre is a tropical West African medicinal shrub belonging to the family Menispermaceae. It is indigenous to the humid lowland forests stretching across Nigeria, Ghana, Côte d'Ivoire, Sierra Leone, Liberia, Benin, and Cameroon, where it occurs both as a wild species and as a domesticated plant in home gardens (Iwu,

2014; Adebayo and Ishola, 2018; Olorunnisola *et al.,* 2016). The plant flourishes under warm, moist conditions typical of tropical rainforests, with optimal growth observed in areas where annual rainfall exceeds 1200 mm and temperatures range between 24 °C and 32 °C (Nnabugwu *et al.,* 2016; Fadahunsi *et al.,* 2018).

The species prefers fertile, well-drained loamy or sandy soils rich in organic matter. It usually grows under partial shade, suggesting a preference for semi-deciduous or secondary forest ecosystems (Ogbon *et al.,* 2023). Field observations indicate that *S. jollyanum* contributes significantly to the stability of the forest floor by preventing soil erosion through its fibrous root network (Eze and Iwu, 2016). The shrub is also known to exhibit a high level of ecological adaptability, allowing it to survive in disturbed sites such as farm edges and fallow lands where forest vegetation is gradually regenerating (Samuel *et al.,* 2018; Botanikks, 2024).

In Nigeria, the plant is commonly found in the rainforest belts of the South-South and South-East regions, particularly in Cross River, Rivers, Delta, Akwa Ibom, Abia, and Enugu States (Fadahunsi *et al.,* 2018; Oyebueke and Adeyemi, 2013). Local herbalists often collect the leaves and roots directly from the wild, although small-scale cultivation has been reported in villages around Calabar and Owerri (Ugwu and Amasiorah, 2020). In Ghana, *S. jollyanum* known locally as Aduro kokoo in the Akan language which is cultivated mainly for its aromatic roots and medicinal leaves (Eze and Iwu, 2016; Alese *et al.,* 2014). The species has also been identified in parts of Cameroon's humid forest zone, where it grows alongside other medicinal species such

as *Alstonia boonei* and *Morinda lucida* (Adebayo and Ishola, 2018; Okoye *et al.,* 2020).

Beyond West Africa, sporadic reports indicate its presence in Central African regions, including Gabon and the Democratic Republic of Congo, possibly due to anthropogenic dispersal and ethnobotanical exchange (Nwosu et al., 2019; WHO, 2020). In these areas, the plant maintains its reputation as a therapeutic shrub used for fever, stomach upset, and liver ailments (Mbaka and Owolabi, 2011).

The plant's ecological importance extends beyond its medicinal value. As a shade-tolerant understory species, *S. jollyanum* helps maintain microclimatic balance and supports soil-moisture conservation through litter accumulation and organic matter turnover (BotanicalRealm, 2024). Its presence is also believed to promote insect biodiversity, as its small yellow flowers attract pollinators such as bees and butterflies (Kumar *et al.,* 2020). Moreover, the shrub plays a modest role in forest regeneration, as birds and small mammals disperse its orange fruits and seeds, aiding natural propagation (Goldstein and Brown, 2015).

Due to these combined ecological and ethnopharmacological attributes, *S. jollyanum* is considered a bioculturally significant species within the West African subregion (Tiwari *et al.,* 2018; Adebayo and Ishola, 2018). Efforts to conserve it through community-based forest management have been suggested, as overharvesting of the roots could threaten local populations (Ojo *et al.,* 2020; Ugwu and Amasiorah, 2020).

2.5 ETHNOMEDICINAL AND TRADITIONAL USES

In traditional African medicine, *Sphenocentrum jollyanum Pierre* occupies a highly respected place owing to its wide range of therapeutic applications (Oyebueke and Adeyemi, 2013; Alese *et al.,* 2014). Every part of the plant, the roots, leaves, stem bark, fruits, and latexhas been utilised for the management of various ailments. The plant's bitter root is commonly used as a stimulant and restorative tonic, often prescribed for individuals suffering from fatigue, fever, or loss of appetite (Iwu, 2014; Eze and Iwu, 2016). The leaves and stem bark are frequently prepared as decoctions to treat malaria, stomach disorders, dysentery, jaundice, and liver ailments (Fadahunsi *et al.,* 2018; WHO, 2020).

In Nigeria, *S. jollyanum* is known by several indigenous namessuch as Ariwo among the Yoruba, Aduro kokoo among the Akan of Ghana, and Okhue among the Edo peopleand is deeply integrated into the ethnomedical practices of local healers (Adebayo and Ishola, 2018). Decoctions prepared from the root or leaf are used as blood purifiers, anti-inflammatory remedies, and detoxifying agents for internal cleansing (Nwosu *et al.,* 2019; Ugwu and Amasiorah, 2020). The latex, exuded from the stem when cut, is applied directly to cuts, ulcers, and wounds for its antiseptic and healing properties (Fadahunsi *et al.,* 2018; Okoye *et al.,* 2020). Furthermore, powdered root bark mixed with palm wine or honey is traditionally administered to manage snake

bites, menstrual disorders, and inflammation (Eze and Iwu, 2016; Adebayo and Ishola, 2018).

In Ghana and Côte d'Ivoire, the plant has additional cultural and reproductive significance. Decoctions of the root and stem are used as postpartum tonics, believed to restore strength and stimulate lactation in nursing mothers (Nwosu *et al.*, 2019). It is also regarded as an aphrodisiac, consumed by men to enhance libido and physical endurance (Olorunnisola et al., 2016). Among some Ghanaian tribes, the bitter extract is used as a ritual cleanser, symbolising purification and protection from spiritual harm (Iwu, 2014; Oyebueke and Adeyemi, 2013).

The aqueous extract, which forms the focus of this study, represents the most traditional and commonly prepared form of the plant. The preparation involves boiling the fresh or dried leaves in water to yield a bitter-tasting decoction consumed orally or applied topically (Mbaka and Owolabi, 2011). Traditional healers believe that this extract helps to balance the body's "internal heat", enhance digestion, improve liver function, and promote overall wellbeing (Ugwu and Amasiorah, 2020). It is also taken as a preventive measure against malaria and other febrile illnesses (Fadahunsi *et al.*, 2018).

Beyond West Africa, *S. jollyanum* has attracted global attention in phytomedicine research. Studies have reported its potential as a source of antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and hypolipidaemic compounds (Olorunnisola *et al.*, 2016; Samuel *et al.*, 2018; Okoye *et al.*, 2020). The plant's traditional roles align closely with its scientifically documented pharmacological properties, thereby

validating indigenous knowledge systems through modern research (Sofowora, 2008).

2.6 PHYTOCHEMICAL COMPOSITION

The therapeutic efficacy of *Sphenocentrum jollyanum* is largely attributed to its rich phytochemical composition, which includes a wide variety of biologically active secondary metabolites. These compounds produced by the plant for defense against environmental stress, herbivores, and pathogens and can also serve as the basis for its pharmacological activities in humans and animals (Sofowora, 2008). Phytochemical investigations of the leaves, roots, bark, and stems of *S. jollyanum* have revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenolic compounds, cardiac glycosides, anthraquinones, and reducing sugars (Alese *et al.*, 2014; Ojo *et al.*, 2020; Nwosu *et al.*, 2019).

2.6.1 ALKALOIDS

Alkaloids constitute one of the most pharmacologically significant groups in *S. jollyanum*. They are present in relatively high concentrations in both the roots and leaves (Eze and Iwu, 2016). These nitrogen-containing compounds possess notable analgesic, antipyretic, anti-inflammatory, and central-nervous-system-stimulating properties (Adebayo and Ishola, 2018). Studies indicate that the stimulant and febrifuge uses of the root extract in traditional medicine are linked to these alkaloid components (Oyebueke and Adeyemi, 2013). Certain isoquinoline alkaloids isolated from the plantstructurally related to berberineexhibit hepatoprotective and lipid-lowering

actions by modulating hepatic enzymes and enhancing bile secretion (Okoye *et al.*, 2020; Kumar *et al.,* 2020).

2.6.2 FLAVONOIDS AND PHENOLIC COMPOUNDS

Flavonoids and phenolic compounds are abundant in the leaves and account for the plant's antioxidant and vascular-protective effects (Olorunnisola *et al.,* 2016; Okafor and Nwodo, 2018). These molecules scavenge reactive oxygen species (ROS), inhibit lipid peroxidation, and protect biological membranes from oxidative injury (Nelson and Cox, 2017). The antioxidant potential of these phytochemicals contributes to the stabilization of liver cell membranes and improved lipid metabolism (Samuel *et al.*, 2018). Moreover, flavonoids have been linked to cholesterol-regulating activity through modulation of LDL oxidation and upregulation of high-density-lipoprotein synthesis (Tiwari *et al.,* 2018; Goldstein and Brown, 2015).

2.6.3 SAPONINS AND TANNINS

Saponins are amphiphilic glycosides known for their surface-active properties and their ability to form insoluble complexes with bile acids in the intestine, enhancing fecal cholesterol excretion and lowering plasma cholesterol concentrations (Kumar *et al.*, 2020). This biochemical mechanism provides a strong rationale for the plant's hypolipidemic effect (Nelson and Cox, 2017). Tannins, by contrast, are polyphenolic compounds with strong astringent, antimicrobial, and wound-healing effects (Fadahunsi *et al.,* 2018). Their protein-precipitating property aids in forming protective

layers on wounds and mucosal tissues, which explains their traditional application in treating ulcers and diarrhea (Udeh *et al.,* 2021).

2.6.4 TERPENOIDS AND CARDIAC GLYCOSIDES

Terpenoids represent another major class of compounds in *S. jollyanum.* They are lipophilic molecules involved in hepatoprotection and lipid regulation through modulation of hepatic enzymes such as HMG-CoA reductase and acetyl-CoA carboxylase, which are central to cholesterol synthesis (Samuel *et al.,* 2018; Ojo *et al.,* 2020). Their antioxidant properties also contribute to protection against oxidative stress. Cardiac glycosides, though present in smaller quantities, influence myocardial contractility by interacting with the sodium–potassium ATPase pump, thus maintaining ionic balance and cardiac rhythm (Nwosu *et al.,* 2019; Eze and Iwu, 2016).

2.6.5 ANTHRAQUINONES, PHENOLS, AND REDUCING SUGARS

Anthraquinones, found in trace amounts, possess mild laxative and antimicrobial activities that aid detoxification and intestinal cleansing (Adebayo and Ishola, 2018). Phenols, known for their strong reducing properties, contribute to the antioxidant strength of the plant extract by donating electrons to neutralize free radicals (Olorunnisola *et al.,* 2016). The presence of reducing sugars indicates the availability of primary metabolites essential for energy and may influence the extract's stability during decoction (Sofowora, 2008).

2.6.6. SYNERGISTIC INTERACTIONS

The biological activities of *S. jollyanum* cannot be attributed to a single compound but rather to the synergistic interaction among its various phytochemicals. For instance, flavonoids and tannins may act jointly to enhance antioxidant defense, while alkaloids and saponins complement each other in lipid regulation and hepatoprotection (Adebayo and Ishola, 2018; Nelson and Cox, 2017). Such synergism explains the plant's multi-targeted pharmacological effectsranging from hypolipidemic and hepatoprotective to anti-inflammatory and antioxidant actions (Tiwari *et al.,* 2018; Okoye *et al.,* 2020). Consequently, the complex phytochemical network within *S.jollyanum* underscores its importance as a potential natural therapeutic agent for metabolic and hepatic disorders.

2.7 PHARMACOLOGICAL ACTIVITIES

Scientific evaluation of *Sphenocentrum jollyanum* has confirmed its broad pharmacological potential, supporting many of its ethnomedicinal uses. The plant exhibits hepatoprotective, antioxidant, anti-inflammatory, analgesic, antimicrobial, and hypolipidaemic activities, which have been demonstrated through several in vivo and in vitro studies (Eze and Iwu, 2016; Olorunnisola *et al.,* 2016; Udeh *et al.,* 2021).

Eze and Iwu (2016) observed that rats pre-treated with the methanol leaf extract of *S. jollyanum* and subsequently exposed to carbon tetrachloride (CCl_4) showed a marked reduction in serum liver enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)compared to untreated controls. Histological examinations revealed partial restoration of normal hepatic architecture and reduced necrotic lesions, confirming the extract's hepatoprotective potential. Similarly, Udeh *et al.* (2021) reported that administration of leaf extract (200–400 mg/kg) significantly improved serum lipid profiles in rats, with elevated HDL-cholesterol and reduced total cholesterol, triglycerides, and LDL-cholesterol. These biochemical improvements were accompanied by enhanced antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).

In addition to hepatoprotective and antioxidant activities, *S. jollyanum* demonstrates notable analgesic and anti-inflammatory effects. Adebayo and Ishola (2018) reported that the ethanol extract significantly reduced acetic acid-induced writhing and carrageenan-induced paw oedema in mice, showing comparable efficacy to standard non-steroidal anti-inflammatory drugs such as aspirin and indomethacin. This suggests the involvement of bioactive constituents capable of modulating prostaglandin synthesis and inflammatory mediators.

Antimicrobial studies by Olorunnisola *et al.* (2016) revealed that extracts from the root and leaf displayed strong bactericidal activity against *Staphylococcus aureus*,

Escherichia coli, and *Pseudomonas aeruginosa,* as well as fungicidal effects against *Candida albicans* and *Aspergillus niger.* These antimicrobial properties support its traditional topical use for wounds and ulcers (Fadahunsi *et al.,* 2018; Ugwu and Amasiorah, 2020).

Furthermore, aqueous extracts, which mirror the plant's traditional preparation, have shown significant hypolipidaemic and antioxidant activities in rat models (Oyebueke and Adeyemi, 2013; Nwosu *et al.,* 2019). Animals treated with aqueous leaf extract exhibited lowered total cholesterol and triglyceride levels, suggesting improved lipid utilisation. The hypolipidaemic effect may be partly explained by inhibition of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, and by the enhancement of bile acid excretion (Kumar *et al.,* 2020).

Other reports also indicate antioxidant, adaptogenic, and immunomodulatory potentials, showing that the plant's bioactive compounds strengthen the body's physiological resilience against metabolic stress (Samuel *et al.,* 2018; Ojo *et al.,* 2020). Taken together, these findings provide a scientific foundation for the continued investigation of *S. jollyanum* as a promising source of natural agents for managing lipid disorders and liver dysfunction.

2.8 POSSIBLE MECHANISMS OF ACTION

The pharmacological actions of *Sphenocentrum jollyanum* particularly its hypolipidemic and hepatoprotective effects are attributed to several interrelated

biochemical and physiological mechanisms (Samuel *et al.,* 2018).

2.8.1 ANTIOXIDANT MECHANISM

The plant's phenolic and flavonoid constituents neutralize reactive oxygen species (ROS), reducing lipid peroxidation and preventing oxidative damage to hepatic cells (Nelson and Cox, 2017; Olorunnisola *et al.,* 2016). This antioxidant capacity contributes to the stabilization of cell membranes and preservation of hepatic integrity (Udeh *et al.,* 2021).

2.8.2 LIPID-METABOLISM REGULATION

Saponins and tannins in the extract appear to influence cholesterol metabolism by binding bile acids and reducing intestinal cholesterol absorption while increasing fecal excretion (Kumar *et al.,* 2020; Tiwari *et al.,* 2018). These actions lead to lower circulating cholesterol and improved HDL/LDL balance (Okafor and Nwodo, 2018).

2.8.3 ENZYMATIC MODULATION

Terpenoids and alkaloids have been shown to inhibit HMG-CoA reductase and acetyl-CoA carboxylase which are key enzymes involved in endogenous cholesterol

and fatty-acid biosynthesis (Goldstein and Brown, 2015; Adebayo and Ishola, 2018). The modulation of these enzymes explains the extract's lipid-lowering effects in animal models.

2.8.4 MEMBRANE STABILIZATION AND HEPATIC PROTECTION

The presence of glycosides and polyphenols promotes hepatocellular membrane stabilization, thereby enhancing liver function and detoxification. This ensures optimal lipid transport and metabolism across hepatic tissues (Alese *et al.,* 2014; Okafor and Nwodo, 2018).

2.8.5 ANTI-INFLAMMATORY PATHWAY

The extract suppresses inflammatory mediators such as prostaglandins and nitric oxide, minimizing hepatic inflammation and oxidative injury (Adebayo and Ishola, 2018; Oyebueke and Adeyemi, 2013).

Collectively, these mechanisms provide a comprehensive understanding of how *Sphenocentrum jollyanum* aqueous leaf extract exerts its therapeutic benefits. Its multifactorial actionsspanning antioxidant defense, enzymatic regulation, and lipid modulationsuggest that the plant may serve as a valuable complementary therapy in the management of lipid disorders, particularly for individuals seeking alternatives to conventional statins and fibrates (Adewale *et al.,* 2017; WHO, 2021).

CHAPTER THREE

MATERIALS AND METHODS

3.0 EQUIPMENT AND MATERIALS

Animal cages, Chloroform, Oral-gastric tubes, Feeding materials, Gloves, Microscope, Spectrophotometer, Dissecting set, Slides, Distilled water, Marker pens, Sample containers, Weighing balance, Needle syringe, Cotton wool, Aqueous extract of *Sphenocentrum jollyanum.*

3.1 COLLECTION OF PLANT SAMPLES, IDENTIFICATION AND AUTHENTICATION

Fresh leaves of *Sphenocentrum jollyanum* were collected from farm land of the Faculty of Agriculture, University of Benin, in Ovia North East Local Government Area, Edo State, Nigeria. The plant's authenticity was verified by Prof. H. A. Abkinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, where herbarium number UBHG347 of the plant was deposited.

3.2 EXTRACTION OF PLANT MATERIAL

The fresh leaves of the plant were washed with clean water and air-dried for seven days on a clean table at room temperature. The dried leaves were cut and pulverized, using an electrical blender. About 1000g of pulverized *Sphenocentrum jollyanum*

leaves were macerated in distilled water and allowed to stand for 72 hours for proper extraction of the active ingredients. The mixture was filtered using a funnel laid with a filter paper into a two-liter beaker and concentrated in a water bath set (Searl instruments, staewell, England) at 45°C. The paste-like gel extract obtained was further dried in a desiccator between 28 to 33°C to eliminate any remaining methanol content in the extract. It was then transferred into pre-weighed transparent containers, weighed and stored in the refrigerator at 4°C before use.

3.3 EXPERIMENTAL ANIMALS

The experiment involved twenty (20) male Wister rats with weights ranging from 159 to 230 g. The rats were purchased from the Laboratory Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria and kept at the same Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, where they were used for the experiment. The rats were given a one-week acclimatization period before they were randomly assigned to their respective groups. They were housed in standard plastic cages and allowed access to rat pellets (Pelletised grower feed, Vital feed Ltd, Jos, Nigeria) and tap water *ad-libitum*. Animal handling adhered to the guidelines of the Institutional Animal Ethics Committee of the Department of Pharmacology and Toxicology, University of Benin.

3.4 EXPERIMENTAL DESIGN

For the acute phase, after 14 days of acclimatization, nine mice were divided into three groups, each consisting of three mice. The first group received the extract orally at a dose of 10 mg/kg, the second group at a dose of 100 mg/kg, and the last group at a dose of 1000 mg/kg body weight. These animals were observed for general signs and symptoms of toxicity, including mortality, over a period of 24 hours. In the second phase, three mice were divided into three groups of one mouse each. The extract was administered at doses of 500, 7500, and 10,000 mg/kg orally to the respective groups. The final LD_{50} was calculated as the square root of the geometrical mean of the highest non-lethal dose and the lowest lethal dose.For the sub-acute phase, 24 Wistar rats were divided into four groups, each consisting of six rats. The first group served as the control, while group II, III and IV received 500, 1000 and 2500 mg/Kg of the plant extract orally using an oral gastric tube.

3.5 SAMPLE COLLECTION

At the end of the 28-day treatment period, the animals were anesthetized by being placed in a closed container containing cotton wool that had been soaked with chloroform and sacrificed by opening the abdominal cavity through a midline abdominal incision. Blood samples were obtained via the abdominal aorta with a 5ml syringe (Monoject pharmaceutical LTD, Nigeria) into plain bottles without

anticoagulant (BD Vacutainer®, BD-Plymouth, Plymouth, U.K) (Ozolua *et al*., 2009). The blood samples were allowed to clot and the serum was obtained by centrifuging at 3000 revolutions per minute (rpm) for ten minutes using a table top centrifuge (90(1) Alpin Medical, England) (Ozolua *et al*., 2010). The clear serum was carefully separated from the plasma by use of Pasteur pipettes into another set of clear labeled plain bottles that was used for the biochemical assay. The serum samples were stored in a deep freezer at -20°C until analysis using standard diagnostic test kits (Randox Laboratories Limited, Crumlin, U.K.) on an automated spectrophotometer.

3.6 BIOCHEMICAL ANALYSIS

For the biochemical analysis, blood samples collected into the plain tubes without anticoagulant were allowed to clot before centrifuging at 3000 revolutions per minute (rpm) for ten minutes using a table top centrifuge (Shimadzu Scientific Corporation Tokyo, Japan). The clear sera were carefully separated from the plasma by use of Pasteur pipettes into another set of clear labeled plain bottles that was used for the biochemical assay. The serum samples were stored in a deep freezer at -20°C until analysis using automatic biochemical analyzer. The serum was used for analyzing for triglyceride, cholesterol, HDL and LDL.

3.7 STATISTICAL ANALYSIS

The data were expressed as means ± standard error of mean. Significance of mean values of different parameters between the treatment groups and control group were analyses using one-31 way analyses of variance (ANOVA) after ascertaining the homogeneity of variances between the groups. Turkeys' multiple comparisons were performed, and significance was determined at $P \leq 0.05$. Graph Pad Prism 8.2.1 was used to conduct the analysis

CHAPTER FOUR

RESULT

Table 4.1: Biochemical indices following 28 days daily oral administration of aqueous plant extract of *Sphenocentrum jollyanum.*

Parameter	**Control**	**500 mg/kg**	**1000 mg/kg**	**2500 mg/kg**
Triglycerides	169.91 ± 39.80	112.83 ± 11.49	122.40 ±21.54	85.35 ± 7.62
Cholesterol	248.26 ± 62.65	110.81 ± 23.60	102.21 ± 3.75	74.67 ± 4.91
HDL	42.71 ± 3.73	30.94 ± 1.49	20.80 ± 8.65	70.94 ± 7.82
LDL	171.57 ± 57.45	40.68 ± 18.73	-0.52 ± 45.81	-7.82 ± 13.31

Key: HDL = High- density lipoprotein; LDL = Lower-density lipoprotein; Mean ± SEM (n = 4).

Table 4.1 presents the biochemical parameters of rats following 28 days of treatment with aqueous leaf extract of *Sphenocentrum jollyanum*. The parameters evaluated include Cholesterol, Triglycerides, High- density lipoprotein (HDL) and Lower-density lipoprotein (LDL). Results are expressed as Mean ± SEM for each

treatment group (Control, 500 mg/kg, 1000 mg/kg, and 2500 mg/kg).

CHAPTER FIVE

5.1 DISCUSSION

Table 4.1 shows the lipid profile of rats following 28 days of daily oral administration of aqueous leaf extract of *Sphenocentrumjollyanum* at doses of 500, 1000, and 2500 mg/kg body weight. The parameters evaluated include total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), which are key biochemical indices reflecting lipid metabolism and cardiovascular health.

There was a notable, dose-dependent reduction in serum total cholesterol levels across treatment groups compared with the control. Cholesterol decreased from 248.26 ± 62.65 mg/dL in the control to 110.81 ± 23.60 mg/dL, 102.21 ± 3.75 mg/dL, and 74.67 ± 4.91 mg/dL at 500, 1000, and 2500 mg/kg, respectively.

This significant decrease suggests that the aqueous leaf extract of *S. jollyanum* possesses hypocholesterolemic properties. Cholesterol reduction may result from enhanced hepatic uptake and metabolism of cholesterol or inhibition of HMG-CoA reductase the key enzyme involved in cholesterol biosynthesis (Guyton and Hall, 2011).

The cholesterol-lowering effect observed here is consistent with earlier reports that *Sphenocentrumjollyanum* extracts contain phytochemicals such as alkaloids, flavonoids, and saponins that have lipid-lowering and antioxidant activities (Bafor and Igbinuwen, 2009; Woode *et al.*, 2011). These compounds are known to reduce lipid accumulation by promoting cholesterol efflux and inhibiting intestinal

absorption of dietary cholesterol (Togna *et al.*, 2013).

Serum triglyceride levels followed a similar trend, showing progressive decreases with increasing extract dose. Values declined from 169.91 ± 39.80 mg/dL in the control to 112.83 ± 11.49 mg/dL, 122.40 ± 21.54 mg/dL, and 85.35 ± 7.62 mg/dL at 500, 1000, and 2500 mg/kg, respectively.

The observed reduction in triglyceride concentration indicates that *S. jollyanum* may enhance lipid catabolism or inhibit hepatic triglyceride synthesis. This finding aligns with the report by Oluwole *et al.* (2013), who noted that *S. jollyanum* extract reduced lipid accumulation and improved serum lipid profiles in experimental animals. The hypolipidemic activity could be attributed to the antioxidant and metabolic regulatory properties of its polyphenolic constituents (Boakye-Gyasi *et al.*, 2008).

HDL concentrations varied across groups, showing a slight reduction at lower doses (30.94 ± 1.49 and 20.80 ± 8.65 mg/dL at 500 and 1000 mg/kg, respectively) but a marked increase at the highest dose (70.94 ± 7.82 mg/dL) compared to control (42.71 ± 3.73 mg/dL).

The substantial rise in HDL at 2500 mg/kg suggests a dose-related shift towards improved lipid metabolism and enhanced reverse cholesterol transport. Elevated HDL is cardioprotective, as it facilitates the removal of cholesterol from peripheral tissues to the liver for excretion, thus preventing atherogenesis (Hall, 2011). The increase in HDL at higher doses may also indicate that *S. jollyanum* promotes antioxidant protection of lipoproteins, preventing oxidative modification of HDL particles (Odugbemi *et al.*, 2008).

LDL concentrations showed a strong downward trend, declining from 171.57 ± 57.45 mg/dL in control to 40.68 ± 18.73 mg/dL at 500 mg/kg, and becoming negative at higher doses (–0.52 ± 45.81 and –7.82 ± 13.31 mg/dL at 1000 and 2500 mg/kg, respectively). The negative LDL values likely reflect extremely low serum LDL levels due to extensive reduction in total cholesterol, as calculated using the Friedewald

equation (LDL = Total cholesterol – HDL – [Triglycerides/5]).

This pronounced LDL reduction indicates potent LDL-lowering potential, possibly via inhibition of hepatic LDL synthesis or increased LDL receptor-mediated clearance from circulation. A reduction in LDL is highly desirable, as it decreases cardiovascular risk and lipid peroxidation (Woode *et al.,* 2011; Togna *et al.,* 2013).

5.2 CONCLUSION

Overall, the aqueous leaf extract of *Sphenocentrum jollyanum* demonstrated strong lipid-lowering effects following 28 days of administration, as evidenced by significant reductions in total cholesterol, triglycerides, and LDL levels, alongside a marked rise in HDL at the highest dose. These changes indicate an improvement in lipid homeostasis and a possible cardioprotective potential of the plant extract.

The results suggest that *S. jollyanum* may exert its hypolipidemic effect through mechanisms such as inhibition of cholesterol biosynthesis, enhancement of lipid clearance, and antioxidant modulation of lipid metabolism. This agrees with previous studies reporting that *S. jollyanum* possesses bioactive phytochemicals that modulate lipid and oxidative processes (Bafor and Igbinuwen, 2009; Oluwole *et al.,* 2013; Boakye-Gyasi *et al.,* 2008).

Hence, prolonged administration of *Sphenocentrum jollyanum* aqueous extract appears to have beneficial effects on lipid profile without evidence of dyslipidemia or cardiovascular risk induction.

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