

**ASSESSMENT OF METHANOLIC STEM EXTRACT OF *SCLEROCARYA
BIRRAE*'S ANTIOXIDANT POTENTIAL IN EXPERIMENTALLY INDUCED
DIABETIC FRUIT FLIES**

BY

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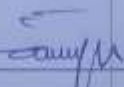
**BEING A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF
BIOCHEMISTRY, FACULTY OF CHEMICAL AND LIFE SCIENCES, USMANU
DANFODIYO UNIVERSITY SOKOTO IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE AWARD OF (Bsc) BIOCHEMISTRY**

DECEMBER, 2025

CERTIFICATION

CERTIFICATION

This is to certify that the research project reported here was conducted by ABDUSALM, Ridwan Adeyinka (2010304012) and has been approved as meeting part of the requirement for the award of Bachelor of Science (B. Sc) degree in Biochemistry of the Usmanu Danfodiyo University, Sokoto.


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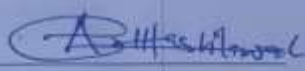
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DEDICATION

This project is dedicated to my parents, siblings, members of our family and friends

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I begin this acknowledgement by expressing my profound gratitude to the Almighty Allah, the Most Gracious and Most Merciful. Through His endless guidance and blessings, I have been granted the strength to pursue my academic journey. I am deeply thankful for the wisdom and perseverance that enabled me to overcome the challenges encountered along the way.

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ABSTRACT

Diabetes mellitus is a widespread metabolic disorder characterized by chronic hyperglycemia and increased oxidative stress. Growing interest in safer antidiabetic therapies has led to the exploration of medicinal plants traditionally used for managing metabolic diseases. This study investigated the antioxidant and antihyperglycemic effects of *Sclerocarya birrea* (Loda) in *Drosophila melanogaster* experimentally induced with diabetes using a 30% high-sucrose diet. Wild-type flies were exposed to the high-sucrose diet for 14 days to induce hyperglycemia, followed by treatment with *Sclerocarya birrea* extract, *Diospyros mespiliformis* extract, or metformin for seven days. Biochemical and physiological parameters such as glucose, trehalose, total antioxidant capacity (TAC), malondialdehyde (MDA), locomotor activity, and body weight were assessed. The high-sucrose diet produced significant increases in glucose levels, oxidative stress, and body weight, alongside reduced locomotor activity, confirming successful induction of diabetic-like conditions. Treatment with *Sclerocarya birrea* extract significantly reduced glucose and trehalose levels, improved locomotor performance, restored body weight, enhanced antioxidant capacity, and lowered MDA concentrations. These effects were comparable to those observed with metformin. The findings demonstrate that *Sclerocarya birrea* possesses strong antidiabetic and antioxidant properties capable of ameliorating hyperglycemia and oxidative stress, supporting its traditional use and highlighting its potential as a natural therapeutic agent for diabetes management.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2022). Type II diabetes mellitus (T2DM), the most prevalent form, accounts for over 90% of global diabetes cases and is primarily associated with insulin resistance and relative insulin deficiency (International Diabetes Federation, 2021). The global prevalence of T2DM has increased at an alarming rate, making it a significant public health concern and a leading cause of morbidity and mortality worldwide (World Health Organization, 2022).

Oxidative stress plays a central role in the pathophysiology of T2DM and its complications (Rani *et al.*, 2021). Hyperglycemia induces the overproduction of reactive oxygen species (ROS), which impair insulin signaling, damage cellular structures, and exacerbate metabolic dysregulation (Tangvarasittichai, 2015). Antioxidant defense systems, including enzymes such as superoxide dismutase (SOD) and catalase (CAT), are often compromised in diabetes, resulting in heightened oxidative stress and disease progression (Zhou *et al.*, 2020). This has spurred growing interest in natural products with antioxidant potential for diabetes management.

Sclerocarya birrea (commonly known as marula or Loda) is a tropical African plant traditionally used in ethnomedicine for treating ailments including hypertension, dysentery, and diabetes (Mariod & Abdelwahab, 2020). Phytochemical studies have shown that *S. birrea* extracts are rich in phenolic compounds, flavonoids, and other antioxidants that may exert antidiabetic effects (Ojewole, 2003; Atawodi *et al.*, 2021). Experimental studies suggest that extracts of *S. birrea* can reduce oxidative stress, enhance glucose uptake, and improve metabolic profiles in diabetic models (Mohammed *et al.*, 2021). However, scientific validation using robust experimental models remains limited.

The fruit fly, *Drosophila melanogaster*, has emerged as a reliable model organism for studying human metabolic disorders, including T2DM (Pallares-Cartes *et al.*, 2021). Due to its genetic similarity to humans, short life cycle, and ease of handling, *Drosophila* provides a cost-effective and ethically acceptable alternative for preliminary in vivo assessments (Mattaliano *et al.*, 2007). Diet-induced diabetes in *Drosophila* mimics several pathophysiological features of human T2DM, including hyperglycemia, obesity, reduced locomotion, and impaired antioxidant defense (Musselman *et al.*, 2011).

Thus, assessing the antioxidant potential of *S. birrea* extract in experimentally induced diabetic fruit flies is both scientifically significant and socially relevant. Such a study can contribute to the search for affordable and safe plant-derived therapies for diabetes management, particularly in sub-Saharan Africa where both diabetes prevalence and reliance on traditional medicine are high (Choudhury *et al.*, 2018).

1.2 Statement of the Problem

The rising global burden of T2DM, projected to affect 783 million people by 2045, poses significant healthcare and socioeconomic challenges. Current therapeutic options, though effective in glycemic control, are often costly, inaccessible to rural populations, and associated with undesirable side effects such as hypoglycemia, weight gain, and gastrointestinal disturbances. Moreover, conventional therapies do not adequately address the oxidative stress that underpins disease progression and complications.

Natural antioxidants from medicinal plants offer a promising complementary approach to diabetes management. *S. birrea*, widely available across Africa, has demonstrated antidiabetic and antioxidant activities in preliminary studies. Yet, its efficacy has not been sufficiently validated in standardized experimental models of T2DM. Likewise, despite the growing use of *D. melanogaster* in metabolic research, limited studies have employed it to

assess the therapeutic potential of *S. birrea*. This creates a knowledge gap that hinders the translational potential of the plant as a validated alternative therapy.

1.3 Justification / Significance of the Study

This study is justified on several grounds. First, it addresses the need for affordable, accessible, and safe therapeutic alternatives for diabetes management, particularly in resource-limited settings (Choudhury *et al.*, 2018). Second, it validates *S. birrea*, a widely available indigenous plant, thereby bridging traditional medicine with modern scientific research (Atawodi *et al.*, 2021). Third, it utilizes *D. melanogaster* as a cost-effective, genetically tractable model for preliminary in vivo testing, reducing reliance on mammalian models and minimizing ethical concerns (Pallares-Cartes *et al.*, 2021).

The findings will provide empirical evidence on the antioxidant and potential antidiabetic effects of *S. birrea* extract. This could inform future drug discovery, phytotherapeutic development, and nutraceutical applications. Additionally, the outcomes may encourage further mechanistic studies and clinical trials, ultimately contributing to global efforts in combating the diabetes epidemic.

1.4 Aim and Objectives of the Study

1.4.1 Aim

The study aims to assess the antioxidant potential of *S. birrea* (Loda) extract in experimentally induced type II diabetic fruit flies.

1.4.2 Objectives

1. To induce type II diabetes in *Drosophila melanogaster*.
2. To evaluate the effect of *S. birrea* extract on physical parameters (body weight and locomotor activity) in diabetic fruit flies.
3. To determine the effect of *S. birrea* extract on antioxidant enzyme activities (TAC and MDA) in diabetic fruit flies.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Theoretical Background

2.1.1 Diabetes Mellitus: Pathophysiology and Oxidative Stress

Diabetes mellitus is a chronic metabolic disorder characterized by impaired glucose homeostasis due to either insufficient insulin secretion, resistance to insulin action, or both (American Diabetes Association, 2022). T2DM, which accounts for 90% of all diabetes cases globally, is primarily linked to insulin resistance and progressive pancreatic β -cell dysfunction (International Diabetes Federation, 2021). The global prevalence of T2DM has risen sharply in the past two decades, driven by sedentary lifestyles, obesity, and dietary transitions, and is projected to reach 783 million by 2045 (IDF, 2021; World Health Organization, 2022).

The pathophysiology of T2DM involves multiple interrelated mechanisms. Central to the disease process is insulin resistance, particularly in skeletal muscle, liver, and adipose tissue, which leads to impaired glucose uptake and unchecked hepatic glucose production (DeFronzo *et al.*, 2015). Chronic insulin resistance eventually overwhelms pancreatic β -cells, resulting in relative insulin deficiency (Cerf, 2013). Persistent hyperglycemia not only disrupts metabolic balance but also promotes the generation of reactive oxygen species (ROS), which in turn exacerbate β -cell dysfunction and insulin resistance (Rani *et al.*, 2021). Oxidative stress is now recognized as a fundamental contributor to the onset and progression of T2DM (Tangvarasittichai, 2015). Hyperglycemia-induced ROS production occurs through several pathways, including glucose auto-oxidation, advanced glycation end-product (AGE) formation, and the activation of protein kinase C and polyol pathways (Brownlee, 2005). Elevated ROS levels damage cellular proteins, lipids, and DNA, impair insulin signaling

cascades, and accelerate the development of diabetes complications such as neuropathy, nephropathy, and retinopathy (Giacco & Brownlee, 2010).

The body possesses endogenous antioxidant defense systems, including enzymatic antioxidants such as SOD, CAT, and GPx, which neutralize ROS and maintain redox balance (Zhou *et al.*, 2020). In T2DM, however, these defenses are often compromised, resulting in heightened oxidative damage and inflammatory responses (Rani *et al.*, 2021). This imbalance between ROS generation and antioxidant defenses highlights the potential therapeutic importance of agents capable of restoring redox homeostasis.

In recent years, interest has grown in exploring natural antioxidants from plant sources as adjunct therapies for diabetes (Ooi *et al.*, 2021). Phytochemicals such as flavonoids, tannins, and phenolic acids have demonstrated the ability to scavenge free radicals, upregulate antioxidant enzymes, and modulate key signaling pathways implicated in glucose metabolism (Wang *et al.*, 2020).

2.1.2 Role of Antioxidants in Diabetes Management

The role of oxidative stress in the onset and progression of T2DM has made antioxidants a focal point in therapeutic research. Antioxidants are molecules capable of neutralizing ROS, thereby protecting cellular structures and maintaining metabolic balance (Halliwell & Gutteridge, 2015). They function through various mechanisms, including direct free radical scavenging, inhibition of ROS-generating enzymes, chelation of transition metals, and upregulation of endogenous antioxidant systems such as SOD and CAT (Zhou *et al.*, 2020).

In the diabetic state, chronic hyperglycemia stimulates oxidative pathways such as glucose autooxidation, protein glycation, and mitochondrial dysfunction, which collectively increase ROS production and overwhelm antioxidant defenses (Brownlee, 2005). This imbalance contributes not only to β -cell dysfunction and insulin resistance but also to vascular complications, making oxidative stress a central therapeutic target (Rani *et al.*, 2021). Studies

have demonstrated that enhancing antioxidant defense reduces lipid peroxidation, improves insulin sensitivity, and slows the progression of diabetes-related complications (Giacco & Brownlee, 2010; Sies & Jones, 2020).

Antioxidant therapy in diabetes can be broadly categorized into synthetic and natural agents. Synthetic antioxidants, such as N-acetylcysteine and vitamin E, have shown promise in clinical and experimental studies but are often limited by bioavailability and safety concerns (Ooi *et al.*, 2021). In contrast, natural antioxidants derived from medicinal plants have gained considerable attention due to their safety profile, cultural acceptance, and potential multitarget effects (Atawodi *et al.*, 2021). Flavonoids, tannins, and phenolic acids, commonly found in fruits and medicinal plants, have been shown to exert both direct antioxidant activity and indirect modulation of glucose metabolism (Wang *et al.*, 2020).

Plant-based antioxidants play dual roles in diabetes management: they not only reduce oxidative stress but also improve glucose regulation by enhancing insulin secretion, promoting glucose uptake in peripheral tissues, and inhibiting carbohydrate-digesting enzymes (Ooi *et al.*, 2021). For example, polyphenols such as quercetin and catechins have been reported to activate AMP-activated protein kinase (AMPK), a critical regulator of energy homeostasis, thereby improving insulin sensitivity (Yuan *et al.*, 2019). Similarly, tannin-rich extracts have been observed to reduce postprandial hyperglycemia by inhibiting α -glucosidase activity (Kim *et al.*, 2020).

2.2 The *Drosophila melanogaster* Model in Biomedical Research

2.2.1 Induction of Type II Diabetes in Fruit Flies

The fruit fly, *D. melanogaster*, has become an established model organism for studying metabolic disorders, including T2DM. This relevance stems from the evolutionary conservation of many metabolic pathways between *Drosophila* and humans, including insulin signaling, lipid metabolism, and carbohydrate regulation (Pallares-Cartes *et al.*, 2021).

Approximately 75% of human disease-related genes have orthologs in *Drosophila*, making it a powerful system for modeling complex human disorders (Ugur *et al.*, 2016).

The induction of T2DM in fruit flies is typically achieved through dietary and genetic manipulations that replicate key features of the disease. Nutritional induction, particularly high-sugar or high-fat diets, is the most common approach. Flies exposed to high-sucrose diets exhibit hallmark diabetic phenotypes such as hyperglycemia, insulin resistance, obesity, reduced locomotion, and shortened lifespan (Musselman *et al.*, 2011). These phenotypes closely parallel human metabolic dysfunction and have been validated through studies showing impaired insulin/insulin-like growth factor signaling (IIS) in diabetic flies (Mattaliano *et al.*, 2007).

Genetic models have also been employed to induce diabetes-like states in *Drosophila*. For instance, manipulation of genes regulating the IIS pathway, such as insulin receptor (InR), insulin receptor substrate (chico), or transcription factor FOXO, results in phenotypes resembling insulin resistance and metabolic dysregulation (Teleman, 2010). However, nutritional induction remains more physiologically relevant for modeling diet-induced T2DM, as it mimics environmental and lifestyle risk factors observed in humans (Gáliková & Klepsatel, 2018).

In addition to hyperglycemia, diabetic fruit flies exhibit oxidative stress due to excessive ROS generation, mirroring human diabetes (Alfa & Kim, 2016). This makes the *Drosophila* model particularly useful for assessing antioxidant therapies. Biomarkers such as SOD and CAT activities are commonly measured to evaluate oxidative stress levels and the efficacy of antioxidant interventions (Park *et al.*, 2020). Furthermore, physical parameters such as body weight, climbing ability (negative geotaxis assay), and survival rates provide functional readouts of metabolic health (Musselman *et al.*, 2018).

Thus, induction of T2DM in *D. melanogaster* offers a cost-effective, genetically tractable, and ethically favorable alternative to mammalian models. It provides an efficient platform for preliminary screening of candidate therapies, including plant-derived antioxidants, before progressing to higher-order organisms (Pallares-Cartes *et al.*, 2021).

2.2.2 Physical and Biochemical Assessments in Fruit Fly Studies

The utility of *Drosophila melanogaster* as a model for diabetes research lies not only in its conserved metabolic pathways but also in the wide array of measurable phenotypic and biochemical parameters that provide insight into disease progression and therapeutic efficacy. These assessments are broadly categorized into physical parameters (such as body weight and locomotor performance) and biochemical markers (such as antioxidant enzyme activities).

2.2.2.1 Physical parameters:

Body weight is one of the most frequently measured indicators of metabolic status in diabetic *Drosophila*. Flies maintained on high-sugar diets typically exhibit abnormal weight gain due to excessive fat storage, resembling obesity in humans (Musselman *et al.*, 2011). Conversely, prolonged hyperglycemia may also lead to wasting phenotypes, reflecting metabolic imbalance (Gáliková & Klepsatel, 2018). Locomotor activity, assessed through negative geotaxis (climbing) assays, serves as a functional marker of neuromuscular and metabolic health. Diabetic flies generally demonstrate impaired climbing ability, a phenotype associated with reduced energy availability, oxidative damage, and neuromuscular dysfunction (Rovenko *et al.*, 2015). These physical assessments are simple, reproducible, and provide valuable information about the overall health and vitality of flies in metabolic studies.

2.2.2.2 Biochemical parameters:

Antioxidant enzyme activities constitute critical biomarkers for evaluating oxidative stress in diabetic models. SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide, while CAT further detoxifies hydrogen peroxide into water and oxygen (Zhou *et*

al., 2020). Together, these enzymes form the first line of defense against ROS. In diabetic fruit flies, reduced SOD and CAT activities are consistently reported, reflecting impaired antioxidant defense systems (Alfa & Kim, 2016). Restoration of these activities following therapeutic interventions indicates an amelioration of oxidative stress and improved redox balance (Park *et al.*, 2020).

Other biochemical markers, such as glucose, glycogen, and triglyceride levels, are also frequently measured in *Drosophila* diabetes studies. Elevated hemolymph glucose and triglycerides mirror the hyperglycemia and dyslipidemia observed in human T2DM (Musselman *et al.*, 2018). Assessing these parameters alongside antioxidant enzymes provides a comprehensive picture of the metabolic and oxidative state of the organism.

Importantly, integrating both physical and biochemical assessments strengthens the reliability of *Drosophila* as a translational model. For instance, improvement in locomotor performance following treatment is more meaningful when accompanied by normalization of antioxidant enzyme activities and glucose levels (Pallares-Cartes *et al.*, 2021). This dual-level assessment enables the evaluation of both functional outcomes and mechanistic pathways in diabetes research.

Collectively, physical and biochemical parameters provide a robust framework for evaluating therapeutic agents in diabetic *Drosophila*. They not only reflect the direct metabolic effects of disease induction but also serve as sensitive readouts of intervention efficacy, including plant-derived antioxidants such as *S. birrea*.

2.3 *Sclerocarya birrea* (Loda): Phytochemistry and Medicinal Potential

2.3.1 Traditional and Ethnomedicinal Uses

S. birrea, commonly known as marula or Loda, is a multipurpose tree native to sub-Saharan Africa and widely recognized for its nutritional, cultural, and medicinal value (Mariod & Abdelwahab, 2020). Belonging to the family Anacardiaceae, the plant has long been

integrated into traditional medicine systems, where different parts, including the bark, leaves, roots, and fruits are employed to treat a variety of ailments (Ojewole, 2003).

In many African communities, decoctions from the stem bark are traditionally used to manage conditions such as dysentery, diarrhea, malaria, hypertension, and diabetes (Atawodi *et al.*, 2021). The bark is also employed as an antimicrobial and anti-inflammatory agent, reflecting its diverse ethnomedicinal applications (Shone & Rosch, 2019). Similarly, leaf extracts are used for wound healing and fever reduction, while root preparations are sometimes administered for stomach ailments and reproductive health disorders (Amusan *et al.*, 2002).

The fruits of *S. birrea* are consumed both as food and in fermented beverages, with anecdotal evidence attributing them with energizing and restorative properties (Mariod *et al.*, 2020). Nutritionally, marula fruit is rich in vitamin C, reportedly containing four times more than oranges, alongside minerals such as potassium, calcium, and magnesium (Chivandi *et al.*, 2021). These nutrients contribute to its antioxidant and immune-boosting potential.

Importantly, several traditional practices highlight the plant's potential antidiabetic use. In South Africa, Mozambique, and Nigeria, bark infusions are specifically recommended for individuals with symptoms consistent with diabetes, including excessive thirst, frequent urination, and fatigue (Ojewole, 2003; Atawodi *et al.*, 2021). This ethnomedicinal background provides the rationale for its scientific evaluation in diabetes research.

Ethnopharmacological studies serve as a valuable guide in modern drug discovery, particularly in regions where access to conventional medicine is limited. The long-standing use of *S. birrea* in traditional medicine underscores its potential as a source of bioactive compounds with therapeutic effects against metabolic disorders such as diabetes. However, systematic experimental validation is necessary to substantiate these claims and standardize its use in biomedical contexts.

2.3.2 Antioxidant and Antidiabetic Properties

The therapeutic potential of *S. birrea* (Loda) has been strongly linked to its rich phytochemical profile, which includes flavonoids, tannins, phenolic acids, procyanidins, and triterpenes (Mariod & Abdelwahab, 2020). These bioactive compounds are recognized for their antioxidant capacity, enabling the plant to counteract oxidative stress, a major pathological factor in T2DM (Atawodi *et al.*, 2021).

2.3.2.1 Antioxidant properties:

Studies have consistently demonstrated the ability of *S. birrea* extracts to scavenge free radicals and modulate antioxidant enzyme activities. In vitro assays have shown that bark and leaf extracts possess strong DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS radical scavenging activity, attributable to their high polyphenol content (Mariod & Abdelwahab, 2020). Similarly, in animal models, administration of *S. birrea* extracts has been associated with increased activity of antioxidant enzymes such as SOD, CAT, and GPx, alongside reductions in lipid peroxidation (Mohammed *et al.*, 2021). These findings indicate that the plant has both direct free radical scavenging ability and the capacity to enhance endogenous antioxidant defenses.

2.3.2.2 Antidiabetic properties:

Beyond its antioxidant effects, *S. birrea* has demonstrated promising antidiabetic activity in preclinical studies. Extracts from the bark and leaves have been reported to lower fasting blood glucose levels in streptozotocin-induced diabetic rats (Ojewole, 2003). Mechanistic studies suggest that the antidiabetic effects are mediated by multiple pathways, including stimulation of insulin secretion, inhibition of carbohydrate-digesting enzymes such as α -amylase and α -glucosidase, and enhancement of peripheral glucose uptake (Chivandi *et al.*, 2021). Additionally, flavonoids and tannins in *S. birrea* may activate AMPK, a critical

regulator of glucose and lipid metabolism, thereby improving insulin sensitivity (Yuan *et al.*, 2019).

More recent investigations continue to highlight the therapeutic promise of *S. birrea*. For instance, Atawodi *et al.* (2021) demonstrated that aqueous bark extract reduced hyperglycemia and oxidative damage in alloxan-induced diabetic rats. Another study by Mohammed *et al.* (2021) reported that methanolic extracts of *S. birrea* leaves improved antioxidant enzyme activity while significantly reducing blood glucose and triglyceride levels. These findings align with the ethnomedicinal use of the plant and underscore its potential as a natural adjunct for diabetes management.

Limitations and considerations:

Despite encouraging results, challenges remain. The phytochemical composition of *S. birrea* can vary significantly depending on geographic location, plant part, and extraction method, affecting reproducibility and therapeutic efficacy (Mariod & Abdelwahab, 2020). Furthermore, while rodent models provide supportive evidence, translational studies using alternative *in vivo* models such as *D. melanogaster* are necessary to establish broader validity. This highlights the need for experimental studies that bridge traditional ethnomedicine with modern biomedical validation.

Collectively, the antioxidant and antidiabetic properties of *S. birrea* provide strong justification for evaluating its potential in *Drosophila melanogaster* models of diabetes, where both physical and biochemical parameters can be systematically assessed.

2.4 Conceptual Framework

The conceptual framework for this study is grounded in the relationship between T2DM, oxidative stress, and the therapeutic potential of plant-derived antioxidants. In the diabetic state, chronic hyperglycemia leads to the excessive generation of ROS, which in turn cause oxidative damage to lipids, proteins, and DNA, while impairing insulin signaling (Rani *et al.*,

2021). This imbalance between ROS production and antioxidant defenses accelerates disease progression and contributes to complications (Brownlee, 2005).

The model organism *D. melanogaster* provides a tractable platform for studying these mechanisms. High-sugar diets in fruit flies induce diabetic phenotypes including hyperglycemia, insulin resistance, abnormal weight gain, impaired locomotion, and reduced antioxidant enzyme activities, that parallel human T2DM (Musselman *et al.*, 2011). These features make *Drosophila* suitable for assessing both physical and biochemical consequences of diabetes, as well as for testing interventions.

S. birrea (Loda), traditionally used in African medicine to manage diabetes and other ailments, contains polyphenols, flavonoids, tannins, and other bioactive compounds with proven antioxidant and antidiabetic properties (Atawodi *et al.*, 2021; Mohammed *et al.*, 2021). These compounds are hypothesized to reduce oxidative stress by directly scavenging free radicals and upregulating endogenous antioxidant enzymes such as SOD and CAT. Additionally, they may improve metabolic health by enhancing glucose uptake and insulin sensitivity (Yuan *et al.*, 2019).

The framework therefore posits that administration of *S. birrea* extract to diabetic *D. melanogaster* will mitigate oxidative stress, restore antioxidant enzyme activities, and improve physical parameters such as body weight and locomotion. This conceptual link integrates the disease mechanism (diabetes-induced oxidative stress), the experimental model (*Drosophila*), and the intervention (*S. birrea* extract).

CHAPTER THREE

3.0 Materials and Methods

The materials used in this study are listed in Table 3.1

Table 3.1.: List of materials used in the study

S/N	Materials/Equipment	Types/Size	Manufacturer/ Sources	Cities/States/Provinces	Countries
1	ELISA plate reader	S/N:IFTDL2310 07015	Infitek	Lixia	China
2	Centrifuge	S/NBEPDL2308 09001	Bioevopeak	Lixia	China
3	Eppendorf tube	1.5ml (500 pieces) LOT NO: 202301			Germany
4	Water Distiller machine	LWDB-400M	Laboid International, Solan	Pradesh	India
5	Absolute Ethanol	283041 5 litres	Loba-chemie pvt Ltd	Mumbai	India
6	Pipette	1000µl, 200 µl, 100 µl, and 10 µl	Eppendorf AG	Hamburg	Germany
7	Pipette tips	Pt1000-1-BS, PT0200-9-45 F1670801 1000µl, 200 µl, 100 µl, and 10 µl	Shanghai Gene Era Bio-Science	Zhejiang	China

8	Latex hand gloves	4 packs	Unigloves SDN	Senawang	Malaysia
9	Measuring Cylinder	50ml	New England Biolabs Inc	Hitchin	UK
10	Scott bottle	100ml bottle	CAMRET	Sokoto	Nigeria
11	Refrigerator-Freezer	Model: RC-67WSI	Hisense	Hisense	China
12	Distilled water	2 Litres	CAMRET	Sokoto	Nigeria
13	Icepacks	4 packs	CAMRET	Sokoto	Nigeria
14	Phosphate buffered saline (PBS) tablet	1 Tablet	Oxoid	Basingstoke Hemisphere	England
15	Baker's Yeast		STK Royal Ltd	Lagos	Nigeria
16	Agar-Agar		Titan Biotech	Rajasthan	India
17	Methyl Paraben		Molychem	Mumbai	India
18	Corn Flour		Faso Farine Mais Blanc	Ouagadougou	Burkina Faso
19	Sucrose		(FFMB) LOBA Chemie	Mumbai	India
20	Electronic weighing scale		Pvt Ltd Kern & Sohn	Balingen	Germany
21	Handheld homogenizer		Ltd Hangzhou Miou Instrument Co., Ltd	Hangzhou	China

3.1 Preparation of *Sclerocarya birrea* (Loda) Bark Extract

The stem extract of *Sclerocarya birrea* (Loda) was obtained from Centre for Advanced Medical Research and Training (CAMRET), Usmanu Danfodiyo University Sokoto.

3.2 Fly Stock and Husbandry

Wild-type *D. melanogaster* were obtained from the Drosophila Laboratory at the Centre for Advanced Medical Research and Training (CAMRET), Usmanu Danfodiyo University, Sokoto. The stock was maintained under standard laboratory conditions to ensure genetic stability and optimal physiological health prior to experimental use. Flies were reared in sterile Drosophila culture vials containing a freshly prepared cornmeal–yeast–agar diet, and maintained at a controlled temperature range of 22–25 °C, relative humidity of 55 ± 5%, and a 12:12 h light–dark cycle.

To maintain a healthy colony and prevent overcrowding, flies were transferred to fresh diet every 48–72 hours. Only healthy, active adult flies were selected for the study. Prior to experimentation, newly emerged flies were allowed to acclimate for 3–5 days, ensuring full physiological maturation and reducing age-related variability.

Throughout the pre-experimental and experimental phases, all cultures were handled under aseptic conditions to prevent microbial contamination. Vials, cotton plugs, and feeding materials were routinely sterilized, and all procedures were conducted in a clean environment to preserve the integrity of the experimental units.

3.3 Experimental Design

Drosophila melanogaster flies, aged 3 to 5 days, were induced with diabetes by feeding them a 30% high sucrose diet for 14 days, with a diet change on day 7. On day 14, body weight and glucose levels were measured to confirm the induction of diabetes. The flies were then divided into three groups (n = 30/group) in triplicate: sucrose diet (SD), extract diet (ED), and metformin diet (MD), along with a control group fed a normal diet, also in triplicate. All

groups continued on their respective diets for 7 more days. The nutritional composition of the experimental diets is provided in Table 3.2. Following the intervention, changes in body weight, locomotor performance, malondialdehyde (MDA), and Total Antioxidant Capacity (TAC) were assessed across the groups.

Table 3.2: Nutritional composition of experimental groups

	Control	High Sucrose Diet	Extract Diet	Metformin Diet
Corn-flour	100g	100g	100g	100g
Agar-Agar	10g	10g	10g	10g
Yeast	10g	10g	10g	10g
Methylparaben	10g	10g	10g	10g
Distilled Water	1.5 L	1.5 L	1.5 L	1.5 L
Sucrose	-	30%	-	-
Extract	-	-	600µg/ml	-
Metformin	-	-	-	16mg/10ml

HSD- High Sucrose Diet; ED- Extract Diet; MD- Metformin Diet

3. 4 Measurement of Body Weight

The body weight of the flies was assessed across the experimental groups using a precise electronic weighing scale. Ten flies per group were anesthetized on ice for 3–5 minutes and transferred into a pre-weighed 1.5 ml microcentrifuge tube (Eppendorf tube). The tubes were re-weighed to determine the weight in milligrams (mg). This process was repeated for three biological replicates, and the average weight per group was calculated.

3.5 Climbing assay

Also referred to as the locomotor performance assay, this test was conducted following the method described by Adeshina *et al* 2024. Briefly, thirty flies were introduced into an empty 50 mL vial marked with a line 6 cm from the bottom and allowed to acclimate. The flies were

gently tapped to the bottom of the vial, and the number of flies crossing the 6 cm mark within 10 seconds was recorded. The assay was repeated three times, and the average percentage of flies crossing the mark was calculated for each group.

3.6 Haemolymph Extraction

Following cold anaesthesia, a pooled group of thirty flies per experimental group was transferred into 1.5 mL Eppendorf tubes containing 200 µL of phosphate-buffered saline (PBS). The flies were homogenized using a handheld homogenizer and centrifuged at 8000 × g for 10 minutes at 4 °C. The resulting supernatant was carefully collected, transferred into a sterile 1.5 mL tube, and stored at -20 °C for subsequent biochemical analyses.

3.7 Biochemical Assays

3.7.1 Quantification of Glucose

Glucose levels in whole fly extracts were measured using a Spinreact™ kit (Girona, Spain) following the manufacturer's instructions. The absorbance of samples and standards was read at 505 nm using microplate reader with a blank serving as the reference. Glucose concentration was calculated using the formula:

$$\text{Glucose concentration (mmol/mL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

3.7.2 Total Antioxidant Capacity (TAC) Assay

The total antioxidant capacity (TAC) was assessed using colorimetric assay kit (Solarbio Life Science, Beijing, China) according to the manufacturer's protocol. Absorbance was measured at 550 nm using microplate reader. TAC was calculated using the formula:

$$\text{Total Antioxidant Capacity (}\mu\text{mol/mL)} = \frac{x \times V_r \times v}{V_s} = 34 \times x$$

3.7.3 Malondialdehyde (MDA) Assay

MDA levels were measured using a lipid peroxidation assay kit (Solarbio Life Science, Beijing, China) according to the manufacturer's protocol. Absorbance was measured at

593nm using microplate reader. The final MDA concentration was calculated using the formula:

$$\text{MDA (nmol/mL)} = 5 \times [6.45 \times (\Delta A_{532} - \Delta A_{600}) - 1.29 \times \Delta A_{450}]$$

3.8 Statistical Analysis

The normality of the data was assessed using the Shapiro-Wilk test, and all data were found to be parametric. Data are presented as means $\pm$ standard deviation (SD). Unpaired t-test was conducted to compare two groups. One-way analysis of variance (ANOVA) was applied to compare variables across the groups. Bonferroni post hoc test was then conducted to compare the means. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were carried out using Jamovi (Version 2.3.28) and GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, California).

CHAPTER FOUR

4.0 RESULTS

4.1 Pre-Treatment Assessment of Glucose Levels

Diabetes induction was verified by assessing glucose levels, which were significantly elevated in the 30% sucrose HSD group, as shown in Figure 4.1.

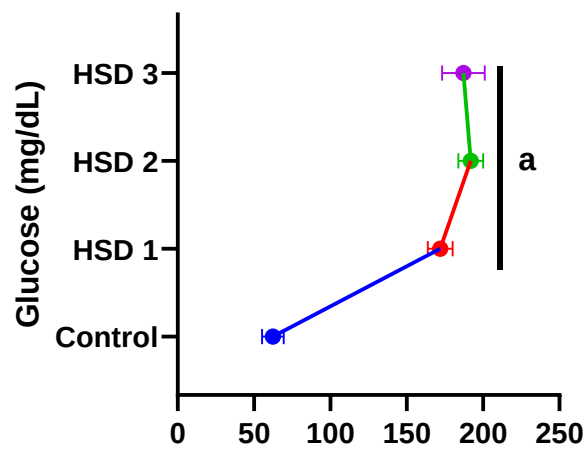


Figure 4.1: HSD- High sugar diet. Statistical significance difference compared to control group $p < 0.05$ was represented by **a**. Data were reported as mean $\pm$ SD ($n = 200$).

4.2 MSESb-Mediated Changes in Body Weight and Motor Performance

Significantly, a high-sugar diet (HSD) increased body weight in *Drosophila melanogaster*, indicating diet-induced obesity ($p < 0.05$). Both MSESb and metformin treatments effectively reduced this weight gain, demonstrating protection against sugar-induced metabolic stress. HSD also impaired locomotor performance, but MSESb markedly restored climbing ability to near-control levels ($p < 0.05$). Metformin improved locomotor function as well, though not significantly compared to controls, suggesting that MSESb may provide equal or greater support for motor activity under hyperglycemic conditions (**Figure 4.2A and 4.2B**).

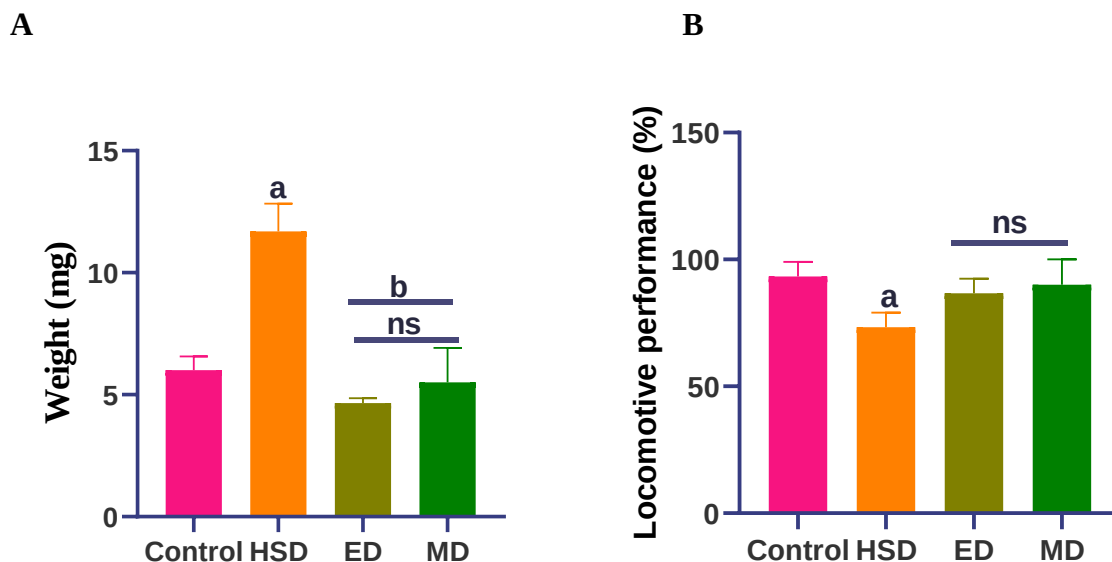


Figure 4.2: Effect of MSESb on (A) Weight (B) Locomotive Performance. HSD- High sugar diet; ED- Extract diet containing MSESb; MD- metformin diet. Statistical significance difference compared to control group $p < 0.05$ was represented by **a**, while statistical significance compared to HSD group $p < 0.05$ was represented by **b**. No statistical difference compared to control group $p < 0.05$ was represented by **ns**. Data were reported as mean $\pm$ SD ($n = 30$).

4.3 Impact of MSESb on HSD-Induced Hyperglycemia and Carbohydrate Metabolic Dysfunction

Figure 4.3 shows that *Drosophila* fed a high-sugar diet (HSD) exhibited significant metabolic disturbances, with glucose levels significantly elevated compared to controls ($p < 0.05$), indicating hyperglycemia. Treatment with MSESb markedly reduced glucose levels relative to the HSD group ($p < 0.05$), demonstrating an antihyperglycemic effect comparable to metformin ($p < 0.05$).

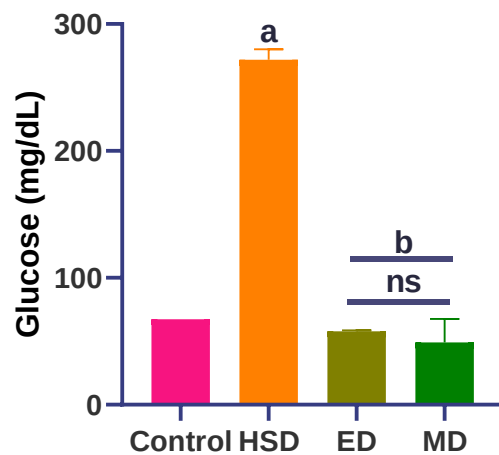


Figure 4.3: Effect of MSESb on Glucose. HSD- High sugar diet; ED- Extract diet containing MSESb; MD- metformin diet. Statistical significance difference compared to control group $p < 0.05$ was represented by **a**, while statistical significance compared to HSD group $p < 0.05$ was represented by **b**. No statistical difference compared to control group $p < 0.05$ was represented by **ns**. Data were reported as mean $\pm$ SD ($n = 30$).

4.4 Antioxidant Activity and Oxidative Stress Marker Profile of MSESb in HSD-Fed *D. melanogaster*

In HSD-fed *D. melanogaster*, total antioxidant capacity (TAC) was reduced but not significantly to control, indicating elevated oxidative stress, while malondialdehyde (MDA) levels were increased, reflecting enhanced lipid peroxidation. Treatment with MSESb restored TAC, surpassing HSD levels, and markedly reduced MDA to near-control levels ($p < 0.05$), demonstrating strong antioxidant and lipid-protective effects. Metformin showed similar improvements in both parameters (**Figure 4.4A and 4.4B**).

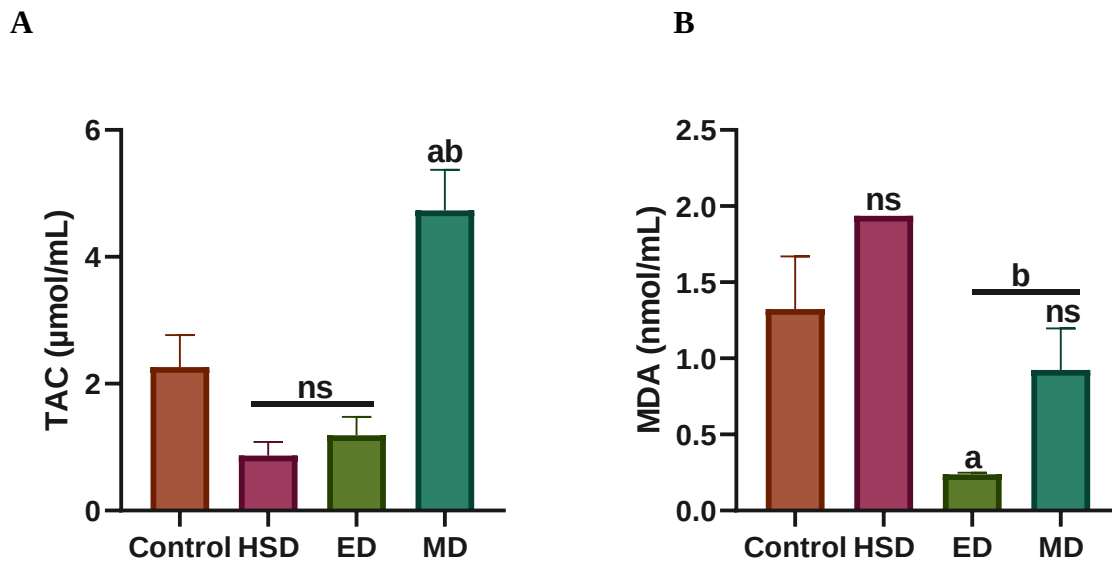


Figure 4.4: Effect of MSESb on (A) Total cholesterol (B) Triglyceride. HSD- High sugar diet; ED- Extract diet containing MSESb; MD- metformin diet. Statistical significance difference compared to control group $p < 0.05$ was represented by **a**, while statistical significance compared to HSD group $p < 0.05$ was represented by **b**. No statistical difference compared to control group $p < 0.05$ was represented by **ns**. Data were reported as mean $\pm$ SD ($n = 30$).

CHAPTER FIVE

5.0 Discussion

The present study investigated the antioxidant and antidiabetic potential of methanolic stem-bark extract of *S. birrea* (MSESB) in *D. melanogaster* subjected to a high-sugar diet (HSD) to induce type II diabetes-like metabolic dysfunction. The findings demonstrate that MSESB effectively ameliorated hyperglycemia, oxidative stress, and impaired motor and metabolic function in diabetic flies, performing comparably to metformin, a standard antidiabetic drug. These outcomes support the hypothesis that *S. birrea* exerts significant protective effects against diet-induced metabolic derangements through antioxidant-mediated mechanisms.

As expected, flies exposed to a 30% sucrose high-sugar diet showed significantly elevated glucose levels, confirming the successful induction of a diabetic state. This aligns with previous reports that excessive dietary sugars in *Drosophila* rapidly induce hyperglycemia, insulin resistance, and obesity, closely mirroring human type II diabetes (Musselman *et al.*, 2011; Gálíková & Klepsatel, 2018). Chronic intake of high sucrose disrupts carbohydrate metabolism and promotes increased flux through glycolytic, lipogenic, and oxidative pathways, resulting in metabolic overload and glucose imbalance (Pallares-Cartes *et al.*, 2021).

The elevated glucose levels observed in HSD-fed flies in this study are therefore consistent with established models and validate the reliability of the induced metabolic dysfunction. The model provides a robust framework upon which therapeutic effects of MSESB could be evaluated.

High-sugar feeding resulted in increased body weight and impaired motor performance, findings that reflect the metabolic and muscular dysfunction associated with diabetes in both humans and *Drosophila* (Rovenko *et al.*, 2015). Hypercaloric diets promote excessive fat accumulation and dysregulation of energy metabolism, leading to increased body mass and

reduced locomotor ability due to oxidative damage and neuromuscular stress (Mattaliano *et al.*, 2007). Treatment with MSESb significantly reduced HSD-induced weight gain and restored climbing ability to near-control levels. These improvements demonstrate the extract's capacity to counter metabolic stress, likely through enhanced energy utilization and reduced lipid accumulation. Similar effects have been reported for polyphenol-rich plant extracts known to modulate AMPK activation and mitochondrial function, ultimately supporting improved metabolic efficiency (Yuan *et al.*, 2019).

Interestingly, MSESb performed comparably even slightly better than metformin in restoring locomotor performance, suggesting that beyond its antihyperglycemic action, the extract may confer neuromuscular protection through its antioxidant constituents, such as flavonoids and tannins (Mariod & Abdelwahab, 2020).

The extract significantly lowered glucose levels in diabetic flies, demonstrating a strong antihyperglycemic activity similar to metformin. This outcome is consistent with earlier rodent studies showing that *S. birrea* extracts reduce fasting blood glucose, inhibit α -glucosidase activity, and stimulate glucose uptake in peripheral tissues (Ojewole, 2003; Mohammed *et al.*, 2021).

The glucose-lowering activity of MSESb may result from multiple mechanisms, including Enhanced insulin sensitivity which is facilitated by plant-derived polyphenols acting on AMPK signaling pathways (Yuan *et al.*, 2019), Inhibition of carbohydrate-digesting enzymes by reducing postprandial glucose surges (Kim *et al.*, 2020), or Reduction in oxidative stress by indirectly improving insulin signaling efficiency (Rani *et al.*, 2021).

That both MSESb and metformin significantly reduced glucose relative to the HSD group underscores the therapeutic potential of *S. birrea* as a natural antidiabetic agent.

HSD-fed flies exhibited elevated malondialdehyde (MDA) levels and reduced total antioxidant capacity (TAC), hallmarks of oxidative stress and lipid peroxidation. These

findings are consistent with the understanding that hyperglycemia inherently stimulates ROS production via pathways such as glucose autooxidation and the polyol cycle (Brownlee, 2005). Increased MDA reflects heightened membrane lipid peroxidation, a common indicator of oxidative tissue damage in diabetic organisms (Giacco *et al.*, 2010).

Treatment with MSESb significantly reduced MDA and restored TAC to levels comparable to controls (Figure 3.4), demonstrating strong antioxidant activity. This aligns with multiple reports attributing potent free radical scavenging and endogenous antioxidant-boosting properties to *S. birrea* bark extracts due to their polyphenol and flavonoid content (Atawodi *et al.*, 2021).

Potential mechanisms for this antioxidant effect might be direct scavenging of ROS, which is mediated by phenolic hydroxyl groups in tannins and flavonoids, upregulation of endogenous antioxidant enzymes such as SOD and CAT, improving redox balance (Zhou *et al.*, 2020), or inhibition of lipid peroxidation by reducing MDA formation and protecting cell membranes (Ooi *et al.*, 2021).

The significant decline in MDA following MSESb treatment suggests effective protection against HSD-induced oxidative injury, supporting the extract's role in metabolic restoration.

5.1 Conclusion

This study explored the antidiabetic and antioxidant potential of methanolic stem-bark extract of *S. birrea* (MSESb) using *D. melanogaster* subjected to a high-sugar diet to induce type II diabetes-like dysfunction. The 30% sucrose diet caused significant metabolic disturbances, including hyperglycemia, weight gain, impaired locomotor activity, reduced total antioxidant capacity, and increased oxidative stress marked by elevated malondialdehyde levels. These symptoms closely resemble diet-induced metabolic disorder in *Drosophila*. Treatment with MSESb produced notable improvements by lowering blood glucose, enhancing locomotor performance, reducing weight gain, and restoring oxidative balance. The extract boosted

antioxidant capacity while suppressing lipid peroxidation, demonstrating strong antioxidant properties. Its effects were comparable to those of metformin, a standard antidiabetic drug. Collectively, the findings highlight *S. birrea* as a promising natural source of antidiabetic and antioxidant agents capable of counteracting high-sugar-diet-induced metabolic dysfunction.

5.2 Recommendations

Based on the outcomes of this study, several recommendations can be made for scientific, therapeutic, and practical applications.

1. The findings strongly support the use of *S. birrea* stem-bark extract as a potential natural adjunct therapy for managing hyperglycemia and oxidative stress associated with T2DM. Given its comparable performance to metformin, the extract may serve as an affordable and accessible option particularly in communities relying on traditional medicinal plants.
2. Further characterization and standardization of the extract are recommended to ensure consistency in therapeutic potency. Variations in phytochemical composition due to plant age, environmental factors, and extraction techniques could influence bioactivity; thus, establishing standardized extraction protocols will strengthen reproducibility and translational potential.
3. Although the *D. melanogaster* model provides valuable preliminary insights, conducting complementary studies using mammalian models is necessary to validate the extract's efficacy in higher organisms. Such studies should include detailed biochemical analyses, including insulin assays, lipid profiles, oxidative stress enzymes such as SOD and CAT, and inflammatory markers, to better elucidate mechanistic pathways.
4. Cautious integration of *S. birrea* into community health practices is advisable. While the extract demonstrated strong antioxidant and antihyperglycemic effects, its safety

profile, optimal dosage, and long-term effects require systematic evaluation. Public health stakeholders should therefore promote evidence-based use of the plant, ensuring that consumption aligns with scientifically validated guidelines.

5.3 Scope and Limitation

Limitations may include:

1. Variability in extract composition depending on preparation methods, which could influence reproducibility.
2. Moreover, while *D. melanogaster* is a valid model for metabolic studies, its physiology does not fully replicate human diabetes, thus findings may not be directly extrapolated without further mammalian validation.

REFERENCE

- Abdallah, M. S., *et al.* (2021). Determination of phenolics and flavonoids of some useful plants including *Sclerocarya birrea*. *Frontiers in Chemistry*, 9, Article 670530. <https://doi.org/10.3389/fchem.2021.670530>
- Aebi, H. (1984). Catalase in vitro. In H. U. Bergmeyer (Ed.), *Methods in Enzymology* (Vol. 105, pp. 121–126). Academic Press. [https://doi.org/10.1016/S0076-6879\(84\)05016-3](https://doi.org/10.1016/S0076-6879(84)05016-3)
- Aebi, H. (1984). *Catalase in vitro*. *Methods in Enzymology*, 105, 121–126. [https://doi.org/10.1016/S0076-6879\(84\)05016-3](https://doi.org/10.1016/S0076-6879(84)05016-3)
- Alfa, R. W., & Kim, S. K. (2016). Using *Drosophila* to discover mechanisms underlying type 2 diabetes. *Disease Models & Mechanisms*, 9(4), 365–376. <https://doi.org/10.1242/dmm.023887>
- American Diabetes Association. (2022). Standards of medical care in diabetes—2022. *Diabetes Care*, 45(Suppl. 1), S1–S264. <https://doi.org/10.2337/dc22-S001>
- Ansari, P., & colleagues. (2024). Plant-based diets and phytochemicals in the management of type 2 diabetes: A review. *Nutrients*, 16(21), 3709. <https://doi.org/10.3390/nu16213709>
- Brownlee, M. (2005). The pathobiology of diabetic complications: A unifying mechanism. *Diabetes*, 54(6), 1615–1625. <https://doi.org/10.2337/diabetes.54.6.1615>
- Couldiaty, A. G. V. (2021). *Sclerocarya birrea*: Review of the pharmacology of its antidiabetic effects and toxicity (review). *African Journal of Pharmacy and Pharmacology* (full text). <https://academicjournals.org/> (article)
- Couldiaty, A. G. V., (2021). *Evaluation/review of Sclerocarya birrea for antidiabetic properties* (Academic Journals / AJPP review). <https://academicjournals.org/>

- Gáliková, M., & Klepsatel, P. (2018). Metabolic regulation in *Drosophila*: Recent advances and their implications. *Frontiers in Physiology*, 9, Article 516. <https://doi.org/10.3389/fphys.2018.00516>
- Giacco, F., & Brownlee, M. (2010). Oxidative stress and diabetic complications. *Circulation Research*, 107(9), 1058–1070. <https://doi.org/10.1161/CIRCRESAHA.110.223545>
- Halliwell, B., & Gutteridge, J. M. C. (2015). *Free Radicals in Biology and Medicine* (5th ed.). Oxford University Press.
- Jomova, K., *et al.* (2023). Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. *Archives of Toxicology*. <https://doi.org/10.1007/s00204-023-03562-9>
- Li, Z., *et al.* (2023). Polyphenols: Natural food-grade biomolecules for treating chronic disorders (review). *Antioxidants (Basel)*, 12, Article 201. <https://doi.org/10.3390/antiox12020201>
- Lushchak, V. I. (2014). Free radicals, reactive oxygen species, oxidative stress and its classification. *Chemico-Biological Interactions*, 224, 164–175. <https://doi.org/10.1016/j.cbi.2014.10.016>
- Lushchak, V. I. (2014). Free radicals, reactive oxygen species, oxidative stress and its classification. *Chemico-Biological Interactions*, 224, 164–175. <https://doi.org/10.1016/j.cbi.2014.10.016>
- Maharaj, V., Ezeofor, C. C., Maharaj, D. N., Müller, C. J. F., & Obonye, N. J. (2022). Identification of antidiabetic compounds from the aqueous extract of *Sclerocarya birrea* leaves. *Molecules*, 27(22), 8095. <https://doi.org/10.3390/molecules27228095>
- Maharaj, V., Ezeofor, C. C., Maharaj, D. N., Müller, C. J. F., & Obonye, N. J. (2022). Identification of antidiabetic compounds from the aqueous extract of *Sclerocarya birrea* leaves. *Molecules*, 27(22), 8095. <https://doi.org/10.3390/molecules27228095>

- Mariod, A. A., & Abdelwahab, S. I. (2012). *Sclerocarya birrea* (marula): A review of nutritional and pharmacological properties. *Food Reviews International*, 28(4), 375–388.
- Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, 47(3), 469–474. <https://doi.org/10.1111/j.1432-1033.1974.tb03714.x>
- Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, 47(3), 469–474. <https://doi.org/10.1111/j.1432-1033.1974.tb03714.x>
- Musselman, L. P., Fink, J. L., Narzinski, K., Ramachandran, P. V., Hathiramani, S. S., Cagan, R. L., & Baranski, T. J. (2011). A high-sugar diet produces obesity and insulin resistance in wild-type *Drosophila*. *Disease Models & Mechanisms*, 4(6), 842–849. <https://doi.org/10.1242/dmm.007948>
- Ojewole, J. A. O. (2003). Hypoglycemic effect of *Sclerocarya birrea* [(A. Rich.) Hochst.] (Anacardiaceae) stem-bark aqueous extract in rats. *Phytomedicine*, 10(8), 675–681. <https://doi.org/10.1078/0944-7113-00295>
- Promyos, N., *et al.* (2023). Inverse correlation of superoxide dismutase and catalase with type 2 diabetes among rural Thais. *Scientific Reports / PMC* (open access).
- Rani, V., Deep, G., Singh, R. K., Palle, K., & Yadav, U. C. S. (2016). Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. *Life Sciences*, 148, 183–193. <https://doi.org/10.1016/j.lfs.2016.01.006>
- Rovenko, B. M., Kubrak, O. I., Gospodaryov, D. V., Perkhulyn, N. V., Yurkevych, I. S., Sanz, A., & Lushchak, V. I. (2015). High sucrose consumption promotes obesity

whereas its low consumption induces oxidative stress in *Drosophila melanogaster*.

Journal of Insect Physiology, 79, 42–54.

<https://doi.org/10.1016/j.jinsphys.2015.05.007>

Tangvarasittichai, S. (2015). Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World Journal of Diabetes*, 6(3), 456–480.

<https://doi.org/10.4239/wjd.v6.i3.456>

Ugur, B., Chen, K., & Bellen, H. J. (2016). *Drosophila* tools and assays for the study of human disease. *Disease Models & Mechanisms*, 9(3), 235–244.

<https://doi.org/10.1242/dmm.023762>

World Health Organization. (2024, November 14). Diabetes. WHO.

<https://www.who.int/news-room/fact-sheets/detail/diabetes>