

**EFFECT OF VARYING CONCENTRATION OF AQUEOUS *PIPER GUINEENSE*
EXTRACT ON THE SURVIVAL RATE AND IRON REGULATORY PROTEIN (IRP1)
GENE IN
DROSOPHILA MALANOGASTER**

BY

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BMS2001160

**DEPARTMENT OF MEDICAL LABORATORY SCIENCE,
SCHOOL OF BASIC MEDICAL SCIENCES,
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BENIN CITY.**

OCTOBER, 2025.

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**BEING A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL
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CERTIFICATION

This is to certify that this work was carried out by **EROMOSELE, JEFFREY** with the matriculation number **BMS2001160** under the supervision of **DR J.O. OSAKUE** and is being submitted to the department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City in partial fulfillment of the requirement for the award of Bachelor of Medical Laboratory Science degree

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DEDICATION

I dedicate this work to God Almighty for His guidance, grace, and strength. I also dedicate it to my amazing family for their love, care, and support throughout my journey in this study

.

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TABLE OF CONTENTS

COVER PAGE	i
TITLE PAGE	ii
CERTIFICATION	iii
DEDICATION	iv
ACKNOWLEDGEMENT	Error! Bookmark not defined.
TABLE OF CONTENTS	vi
LIST OF TABLES	ix
LIST OF FIGURES	x
ABSTRACT	xi
CHAPTER ONE	1
INTRODUCTION	1
1.1. Background of Study	1
1.2. Statement of Problem	4
1.3. Justification of Study	5
1.4 Aim of the Study	5
1.5 Specific Objectives	5
1.6 Research Questions	6
1.7. Research Hypothesis	6
1.7.1 Null Hypothesis (HO)	6
1.7.2. Alternate Hypothesis (HA)	6
1.8 Significance of the Study	6
1.9. Scope of Study	7
CHAPTER TWO	8
LITERATURE REVIEW	8
2.1. Plant of Study (<i>Piper Guineense</i>)	8
2.1.1 Scientific Classification of <i>Piper Guineense</i> (Taxonomy)	9
2.1.2 Plant Morphology and Plant Description	9
2.1.3 Origin, Habitat and Distribution of <i>Piper Guineense</i>	12
2.2 Ethnomedicinal Uses of <i>Piper Guineense</i>	12
2.3 Phytochemical Composition of <i>Piper guineense</i>	15

2.3.1 Nutritional Value of <i>Piper Guineense</i>	17
2.4 Biological Activities of <i>Piper guineense</i>	17
2.4.1 Antimicrobial activities	17
2.4.2 Anti-parasitic Potential	18
2.4.3 Anti-inflammatory potential	18
2.4.4 Antioxidant activity	19
2.4.5 Fertility Effect	19
2.4.8 Insecticidal and Repellent Properties	20
2.4.9 Larvicidal Property	20
2.4.11 Bioactive constituents of special interest	21
2.5 Organism of study (<i>Drosophila Melanogaster</i>)	21
2.3.3 Morphology	23
2.3.4. Life Cycle of <i>Drosophila melanogaster</i>	23
2.3.5. <i>Drosophila</i> Culture and Media	25
2.3.6. <i>Drosophila</i> as a Model Organism to Study Human Diseases	25
2.4 Genome and Genetic Conservation with Humans	26
2.4.1 Genomics in <i>Drosophila melanogaster</i>	26
2.5 Iron Metabolism in <i>Drosophila melanogaster</i>	28
2.5.1 Iron Metabolism in Aging and Disease Models	29
2.6.1 Structure	30
2.6.3 IRP1 in <i>Drosophila</i>	31
2.6.4 Physiological Roles and Phenotypes	31
2.6.6 Research Methods in <i>Drosophila</i> Models	32
2.6.7 Molecular Interactions with Phytochemicals	32
CHAPTER THREE	33
MATERIALS AND METHODS	33
3.1. Study Location	33
3.2. Study Population Description	34
3.3.1 Collection and Identification of <i>Uziza</i> Leaves	34
3.3.2 Processing of <i>Piper guineense</i> Leaves	34
3.3.3 Preparation of Plant Extract	35
3.4 Animal Care	35

3.4.1 Inclusion criteria	35
3.4.2 Exclusion criteria	35
3.5.1 Feed Formulation of <i>Drosophila melanogaster</i>	36
3.5.2 Procedure for Feed Preparation	36
3.6 Study Design	36
3.7 Extract Dosing of Piper Guineense	37
3.8. Experiment 1: Survival Assay	38
3.9 Tissue Homogenate Preparation for Biochemical Assay	39
3.10 Preparation of Reagent	40
3.11 Iron Regulatory Protein-1 (IRP1) mRNA Assay	40
3.11.2 Isolation of Total RNA	40
3.11.3. Complementary DNA (cDNA) Conversion	40
3.11.4 PCR amplification and agarose gel electrophoresis	41
3.11.5. Statistical Analysis	41
CHAPTER FOUR	43
RESULTS	43
CHAPTER FIVE	52
5.1. DISCUSSION	52
5.2. CONCLUSION	56
5.3. RECOMMENDATIONS	57
REFERENCES	58
APPENDIX	71

LIST OF TABLES

Table 2.2 Ethnomedicinal Uses of various parts of <i>Piper guineense</i>	14
Table 2.3: Photochemical Composition of Various Parts of <i>Piper guineense</i>	16
Table 4.1. Percentage of survival at different concentrations of <i>Piper guineense</i>	42

LIST OF FIGURES

Fig 2.1: <i>Piper guineense</i> seeds and leaves	11
Figure 2.4 Life cycle of <i>Drosophila Melanogaster</i>	24
Figure 4.1. Survival rates of <i>Drosophila melanogaster</i> administered different concentrations of <i>Piper guineense</i> at day 7.	43
Figure 4.2. Survival rates of <i>Drosophila melanogaster</i> administered different concentrations of <i>Piper guineense</i> at day 14.	44
Figure 4.3. Survival rates of <i>Drosophila melanogaster</i> administered different concentrations of <i>Piper guineense</i> at day 21.	45
Figure 4.4. Survival curve of <i>Drosophila melanogaster</i> administered different concentrations of <i>Piper guineense</i> over 21 days.	47
Figure 4.5: PCR and agarose gel analysis of Iron Regulatory Protein 1 (IRP1) gene	49

ABSTRACT

Iron regulation is a vital aspect of hematological function, ensuring adequate iron supply for erythropoiesis while preventing oxidative toxicity from excess iron. The Iron Regulatory Protein 1 (IRP1) gene plays a central role in maintaining intracellular iron balance by modulating iron uptake, storage, and utilization. This study evaluated the effect of varying concentrations of aqueous *Piper guineense* (Uziza) leaf extract on the survival rate and IRP1 gene expression in *Drosophila melanogaster*, a recognized model for human iron metabolism. The aim was to determine how phytochemicals in *Piper guineense*, such as alkaloids, tannins, and flavonoids, influence iron homeostasis at the molecular level. Adult flies were divided into five groups: a control group and four treatment groups administered 100 mg/ml, 200 mg/ml, 300 mg/ml, and 400 mg/ml of *Piper guineense* extract through their diet. Survival was monitored over 21 days, and IRP1 expression was analyzed using semi-quantitative PCR. Results showed that the 100 mg/ml group recorded the highest survival rate ($80 \pm 2.65\%$) compared to the control ($71.33 \pm 1.76\%$), indicating mild protective effects at low doses. Conversely, 200 mg/ml ($75.67 \pm 3.38\%$), 300 mg/ml ($72.33 \pm 2.33\%$), and 400 mg/ml ($73.33 \pm 2.03\%$) exhibited slightly higher but statistically insignificant survival relative to control, suggesting that higher concentrations may induce mild stress or toxicity. Gene expression analysis revealed that IRP1 mRNA levels were significantly reduced in the 200 mg/ml (1.55 ± 0.05), 300 mg/ml (1.05 ± 0.15), and 400 mg/ml (0.85 ± 0.05) groups compared with control (2.30 ± 0.10) ($p < 0.05$), while the 100 mg/ml (1.80 ± 0.10) group maintained a relatively higher expression, implying upregulation of iron metabolism at lower doses. These findings demonstrate a dose-dependent effect of *Piper guineense*, where lower dosage intake enhances iron upregulation and increased survival rate, but increased dosage downregulates IRP1 expression and reduces the survival rate. The study concludes that controlled consumption of *Piper guineense* may potentially support hematological health, and further research on its molecular mechanisms and safe therapeutic dosage is recommended.

CHAPTER ONE

INTRODUCTION

1.1. Background of Study

Plants are rich in bioactive compounds that contribute significantly to human health, nutrition, and industry (Walia *et al.*, 2019). These bioactive properties stem largely from the production of secondary metabolites, including alkaloids, flavonoids, phenolic acids, tannins, terpenoids, saponins, and glycosides, which are synthesized by plants as defense mechanisms against pathogens, herbivores, and environmental stressors (El Ramady *et al.*, 2022). In humans, these compounds exhibit a broad spectrum of biological activities such as antimicrobial, anti-inflammatory, antioxidant, anticancer, antihypertensive, and immunomodulatory effects, making them highly valuable in both traditional and modern medicine (Dar *et al.*, 2023). For instance, alkaloids like quinine and morphine have long been used as antimalarial and analgesic agents, while flavonoids and phenolics are increasingly recognized for their role in reducing oxidative stress and lowering the risk of chronic diseases such as diabetes, cardiovascular disorders, and neurodegenerative conditions (Kaushik *et al.*, 2021). Beyond their therapeutic relevance, bioactive compounds from plants are widely utilized in the nutraceutical, cosmetic, and food industries for their preservative, flavour-enhancing, and health-promoting properties (Kumari *et al.*, 2024). With increasing concerns over antibiotic resistance, lifestyle-related diseases, and the safety of synthetic chemicals, research into plant-based bioactives has intensified, leading to the identification of novel compounds with pharmaceutical potential (Mouneir *et al.*, 2022).

One of such plants with vast medicinal properties is *Piper guineense*. *Piper guineense*, commonly known as West African black pepper or “Uziza” in Nigeria, is a medicinal and culinary plant widely used across West and Central Africa for its nutritional, therapeutic, and preservative

properties (Abd *et al.*, 2022). Belonging to the family Piperaceae, the plant produces seeds and leaves that are rich in bioactive compounds such as alkaloids, flavonoids, tannins, saponins, essential oils, and terpenoids, which are responsible for its characteristic pungent aroma and diverse biological activities (Ademuyiwa *et al.*, 2023). Traditionally, *Piper guineense* is incorporated into local diets not only as a spice to enhance flavor but also as a natural remedy for ailments such as cough, fever, gastrointestinal disorders, infertility, and microbial infections (Alagbe *et al.*, 2021). Scientific studies have confirmed its antimicrobial, antioxidant, anti-inflammatory, antihypertensive, and aphrodisiac properties, showing its potential in both ethnomedicine and modern pharmacology (Ademuyiwa *et al.*, 2023). In addition, the seeds and leaves are used in postpartum care to aid uterine contraction and promote lactation (Alagbe *et al.*, 2021). Some of the phytochemicals present in *Piper guineense*, such as tannins and flavonoids, are known to influence iron absorption and utilization in the body, thereby establishing a potential link between the plant's bioactive properties and iron metabolism (Isikhuemen *et al.*, 2020).

Iron metabolism refers to the complex processes that regulate the absorption, transport, storage, and utilization of iron in the body to maintain a delicate balance between iron sufficiency and toxicity (Yiannikourides *et al.*, 2019). Iron is an essential micronutrient required for vital biological functions, including oxygen transport through haemoglobin, electron transfer in cellular respiration, and DNA synthesis (Obeagu, 2025). Dietary iron is absorbed in the duodenum primarily in two forms: heme iron from animal sources and non-heme iron from plant sources, with absorption influenced by enhancers such as vitamin C and inhibitors such as phytates, tannins, and polyphenols (Dasa and Abera, 2018). Once absorbed, iron is transported in the bloodstream bound to transferrin and delivered to tissues for metabolic needs or stored in

ferritin and hemosiderin within the liver, spleen, and bone marrow. Regulation of systemic iron homeostasis is tightly controlled by hepcidin, a hormone produced in the liver, which modulates intestinal absorption and macrophage release of iron (Yiannikourides *et al.*, 2019). Disruptions in iron metabolism can lead to disorders such as iron-deficiency anaemia, iron overload (hemochromatosis), or impaired cellular energy production, showing its role in health and disease. Iron metabolism is carried out so as to always maintain iron homeostasis in the body (Lal, 2020).

Iron homeostasis is the regulation of iron levels in the body to ensure that sufficient iron is available for essential physiological processes while preventing excess accumulation that could lead to toxicity (Gozzelino and Arosio, 2016). Since the body lacks a robust mechanism for excreting iron, maintaining balance depends largely on controlling intestinal absorption, storage, and recycling (Wallace, 2016). Central to this regulation is the hormone hepcidin, produced by the liver, which acts by binding to ferroportin, the only known iron exporter on enterocytes, hepatocytes, and macrophages (Nemeth and Ganz, 2021). By causing ferroportin degradation, hepcidin effectively reduces dietary iron absorption and the release of stored iron into circulation. Other proteins, such as ferritin, transferrin, and divalent metal transporter 1 (DMT1), also play critical roles in iron uptake, transport, and storage, ensuring that iron is delivered where it is needed while avoiding free iron toxicity caused by reactive oxygen species generation (Daher *et al.*, 2017). Understanding the molecular control of iron homeostasis is essential, and one of the key regulatory factors at the cellular level is Iron Regulatory Protein 1 (IRP1) (Galy *et al.*, 2024). Iron Regulatory Protein 1 (IRP1) is a key regulator of intracellular iron metabolism, functioning as a molecular sensor that maintains cellular iron balance through post-transcriptional control of iron-related genes. It exerts its action by binding to iron-responsive elements (IREs), which are

conserved stem-loop structures located in the untranslated regions (UTRs) of target mRNAs (Hernández-Gallardo and Missirlis, 2020). Under conditions of iron deficiency, IRP1 binds strongly to IREs in the 5' UTR of ferritin mRNA, thereby inhibiting its translation and reducing iron storage. At the same time, it binds to IREs in the 3' UTR of transferrin receptor 1 (TfR1) mRNA, stabilizing it and enhancing the synthesis of transferrin receptors that facilitate iron uptake into the cell (Garza *et al*, 2020). This coordinated mechanism ensures that when iron is scarce, cells increase absorption and conserve available iron rather than storing it (Hernández-Gallardo and Missirlis, 2020). However, in iron-rich conditions, IRP1 incorporates a [4Fe-4S] cluster, which induces a conformational change that abolishes its RNA-binding activity and converts it into a functional cytosolic aconitase enzyme, linking iron regulation to energy metabolism through the citric acid cycle (Huynh *et al.*, 2019). This double role as both a regulatory RNA-binding protein and an enzymatic aconitase highlights the versatility of IRP1 as a master regulator of iron homeostasis. In *Drosophila melanogaster*, IRP1 is the major iron regulatory protein and has been widely studied as a genetic model for understanding iron metabolism (Garza *et al*, 2020).

1.2. Statement of Problem

Iron metabolism is tightly regulated to maintain a balance between adequate iron supply for essential biological processes and the prevention of excess iron that can cause toxicity (Yiannikourides *et al.*, 2019). At the cellular level, this regulation is largely controlled by Iron Regulatory Protein 1 (IRP1), which modulates the expression of genes involved in iron uptake, storage, and utilization. Dysregulation of IRP1 has been linked to conditions such as anaemia, iron overload, and neurodegenerative diseases (Hernández-Gallardo and Missirlis, 2020). Piper guineense, a medicinal plant rich in bioactive compounds like tannins and flavonoids (Abd *et al.*,

2022), is widely used for its therapeutic properties and is known to influence iron absorption and utilization. However, little is known about its effect on iron metabolism at the molecular level, particularly on the regulation of IRP1. This gap highlights the need to investigate the effect of *Piper guineense* leaf extract on IRP1 gene expression using *Drosophila melanogaster* as a model organism.

1.3. Justification of Study

Piper guineense is widely consumed in West Africa for its nutritional and medicinal benefits, yet its potential effect on genes regulating iron homeostasis remains poorly understood. Since dysregulation of iron metabolism is associated with serious health conditions such as anemia, iron overload, and neurodegenerative disorders, exploring natural plant products that may influence this pathway is of scientific and therapeutic importance. *Drosophila melanogaster* provides an excellent model system due to its conserved iron regulatory mechanisms and genetic tractability, making it suitable for investigating the impact of *Piper guineense* on IRP1 gene regulation. This study is therefore justified as it may provide novel insights into the molecular effects of *Piper guineense*, bridging the gap between traditional medicinal knowledge and modern molecular biology, with potential implications for the management of iron-related disorders.

1.4 Aim of the Study

To investigate the effect of *Piper guineense* leaf extract on survival rate and expression of Iron Regulating Protein [IRP1] gene in *Drosophila melanogaster*.

1.5 Specific Objectives

1. To assess the survival rate of *Drosophila melanogaster* treated with varying concentrations of *Piper guineense*.

2. To assess the IRP1 gene activity in *Drosophila melanogaster* treated with varying concentrations of *Piper guineense*.
3. To compare the survival rate of *Drosophila melanogaster* in treatment groups with the control group.
4. To compare IRP1 gene activity in *Drosophila melanogaster* in the treatment groups with the control group

1.6 Research Questions

1. Does *Piper guineense* extract have any effect on the expression of the IRP1 gene in *Drosophila melanogaster*?
2. Do varying concentrations of *Piper guineense* extract have any effect on the survival rate, lifespan of *Drosophila melanogaster*?

1.7. Research Hypothesis

1.7.1. Null Hypothesis (H₀)

Piper guineense does not have any effect on the survival rate of *Drosophila melanogaster*.

Piper guineense does not have any effect on IRP1 gene activity in *Drosophila melanogaster*.

1.7.2. Alternate Hypothesis (H_A)

Piper guineense has an effect on the survival rate of *Drosophila melanogaster*.

Piper guineense has an effect on IRP1 gene activity in *Drosophila melanogaster*.

1.8 Significance of the Study

This study investigates how *Piper guineense* influences iron regulation through the IRP1 gene in *Drosophila melanogaster*, providing insights into potential therapies for human iron disorders

such as anemia. It also lays the groundwork for future research on plant-based supplements for treating iron imbalances.

1.9. Scope of Study

This study was designed to cover the effect of *Piper guineense* on the Iron regulatory gene parameter in *Drosophila melanogaster*. Laboratory techniques, including gene expression analysis, *Drosophila* culture and treatment, and quantitative real-time PCR [qRT-PCR], will be used.

CHAPTER TWO

LITERATURE REVIEW

2.1. Plant of Study (*Piper Guineense*)

Piper guineense Schumach. & Thonn., commonly known as West African black pepper, is a climbing perennial vine belonging to the family Piperaceae, which consists of about 3600 species of tiny trees, tropical shrubs, and herbs, many of which are used as spices, flavourings, and medications globally. It is closely related to *Piper nigrum* (the common black pepper), but distinguished by its unique phytochemical composition and ethnobotanical relevance in West and Central Africa (Salehi *et al.* 2019). Indigenous to West and Central Africa, the plant is extensively used for culinary, medicinal, and insecticidal purposes (Nwachukwu *et al.* 2020). The plant is regarded as a climbing plant and can grow to a length of 20 metres. The plant has seeds which are smooth and oval and are referred to as West African black pepper, Uziza in Igbo, and Iyere in Yoruba. In Nigeria, the leaves and seeds are typically offered as an edible medicinal herb or as a flavouring and additional scent for cuisine. *Piper guineense* contains bioactive compounds such as alkaloids, flavonoids, and essential oils, which contribute to its aroma and medicinal properties. These natural compounds, which include volatile oils, ligans, amides, alkaloids, flavonoids, and polyphenols, are abundant in the fruits (Ohemu, T.L., Bello, H.O., Datok, T., & Dafam, D.G., 2024).

The leaves and seeds of *Piper guineense* are commonly used in West African culinary practices to give a pungent, peppery, and slightly bitter flavor to soups, stews, and sauces. In Nigeria, the leaves are used in preparing traditional dishes such as pepper soup and vegetable soups, serving not only as a spice but also as preservatives (Akinmoladun *et al.* 2016). Traditionally, the seeds of *Piper guineense* are administered to women after childbirth as it is believed to stimulate

uterine contractions (Chinedu Imo, Ogochukwu J. Onukwugha,2018). The seed and leaves are used in alleviating ailments like cough, bronchitis, rheumatism, and digestive disorders due to its carnitive and anti-inflammatory properties (Mgbeahuruike, E.E.,2019). The seeds can also be used to treat infertility in both males and females, and extracts of the plant are used as an aphrodisiac (by OA Alagbe,2021).

2.1.1 Scientific Classification of *Piper Guineense*(Taxonomy)

***Piper guineense* is scientifically classified as follows:**

Kingdom: Plantae

Clade: Tracheophytes (Vascular plants)

Clade: Angiosperms (Flowering plants)

Clade: Magnoliids

Order: Piperales

Family: Piperaceae

Genus: Piper

Species: P. Guineense

2.1.2 Plant Morphology and Plant Description

The morphological features of *Piper guineense* are typical of the Piperaceae family, but with distinct traits that differentiate it from closely related species. It is a vine plant that is a member of the family Piperaceae, genus Piper, Order Piperales, Division Magnoliophyta, Class Magnoliopsida, and Kingdom Plantae. It is commonly referred to as West African Black pepper, Benin pepper, Guinea pepper, Ashanti pepper, and false cubeb. Local names include Iyere in Yoruba, uziza in Igbo, Ebe-ahinhi in Edo, Etinkene in Efik/Ibibio and Urhie in Urhobo (Besong et al., 2016; Katzer,2015). *Piper guineense* is a dioecious perennial climbing vine or shrub that

can grow up to 20m with slender stems that often cling to supports. The plant is propagated by seeds or stem cuttings (Chinedu Imo,2018). The leaves are simple, alternate, heart-shaped, and can be quite large, up to 40cm in length and 12 to 15cm in width, they have a pinnate venation and an acute apex (Tolonen,2018). The flowers are greenish-yellow and grow in a spiral pattern along a pedicelled flower spike that is 3-6cm long, the flowers are small, radially symmetrical and have a small bract at their base. The fruits are small green globular berries about 3-5mm in diameter, they turn reddish brown or orange when ripe and black when dried. Each fruit contains a single seed and is characterized by its pungent aroma due to the presence of essential oils (MC Ojinnaka,2016). The roots are fibrous, aiding the plant's climbing habit and absorption of nutrients from various soil.

Fig 2.1: *Piper guineense* seeds and leaves



2.1.3 Origin, Habitat and Distribution of Piper Guineense

Piper guineense is native to tropical regions of Central and West Africa, particularly countries like Nigeria, Ghana, and the Republic of Congo. It thrives in shaded, humid, and wet environments like rainforests, forest clearings, forest edges, and in gallery forests along rocky rivers, at elevations between 750 and 1,650m. It is semi-cultivated in farmlands and home gardens. Its distribution extends widely across Central and West Africa, from Senegal through Nigeria, Ghana, Cameroon, and Uganda to the Democratic Republic of Congo, Zambia, and Tanzania (Akinmoladun *et al.* 2016).

2.2 Ethnomedicinal Uses of Piper Guineense

Piper guineense holds significant ethnomedicinal value across various African communities. Traditional healers and local populations have long utilized different parts of the plant, such as stem bark, leaves, roots, and seeds, to address a range of health conditions. The Roots and leaves are commonly employed in decoctions used for treating coughs, bronchitis, chest pains, colds, and asthma (Gbeklel *et al.* 2017). In some parts of Nigeria, the seeds are commonly administered to women after childbirth to enhance uterine contraction, facilitating the expulsion of the placenta and residual matter from the womb (N Mudonhi,2022). They are also added to the diets of nursing mothers during postpartum recovery, as they are believed to promote the rapid return of the uterus to its normal size (Mgbeahuruike *et al.*, 2019). The leaves and seeds act as carminatives and are used in soups or extracts applied in treating intestinal diseases, stomachaches, diarrhea, dyspepsia, and inflammation (TC Onwudiwe,2021). The leaves and seeds are used to help control body weight, possibly due to their metabolic effects. The seeds possess aseptic qualities and are used to relieve flatulence, with leaves acting as carminatives to reduce gas and bloating. The seeds are used as an adjuvant in the treatment of rheumatic pains,

lumbago, and other inflammatory conditions (Kabiru *et al.* 2016). Extracts of the leaves and fruits are applied to treat fungal diseases like skin rashes, oral thrush, and candidiasis. In Cameroon, West African pepper leaves are mixed with leaves of *Pentas shimperana* spp. to make a yellow soup that is used to treat diarrhea [Nguyenang Murielle *et al.*, 2019]

Table 2.2 Ethnomedicinal Uses of various parts of *Piper guineense*

Morphological part of the plant	Medicinal Uses
Leaves	Respiratory infections, rheumatism, syphilis To relieve flatulence As aperitif, carminative and eupeptic
Fruit	To treat epilepsy As an aphrodisiac
Seed	The adjuvant in the treatment of rheumatism and as an anti-asthmatic Control of weight As an aphrodisiac
Root	As an aphrodisiac

2.3 Phytochemical Composition of *Piper guineense*

Various parts of the plant have undergone analysis, with their chemical profiles identified. Phytochemical screening of *Piper guineense* has identified several bioactive compounds: Alkaloids, Glycosides, Tannins, Flavonoids, and Terpenes. Phytochemicals represent bioactive substances in plant-based foods that collaborate with essential nutrients and fiber to safeguard against illnesses. Flavonoids offer benefits such as antioxidant effects, reduction of inflammation, tumor suppression, allergy relief, and inhibition of blood clotting, alongside the capacity to decrease cholesterol levels (Gao et al., 2022). The alkaloids, which are naturally occurring in *P. guineense* and feature nitrogen-containing ring structures, demonstrate efficacy against malaria, high blood pressure, irregular heart rhythms, and cancer. Additionally, they function as central nervous system stimulants, effective analgesics, and local anesthetics in eye care, among other applications. Tannins, which interact with proteins rich in proline, prevent iron uptake in the digestive tract, thereby limiting its availability in the body and aiding in the treatment of conditions like intestinal inflammation, stomach irritation, and esophageal issues. Plants rich in tannins exhibit astringent qualities, making them valuable for controlling diarrhea, dysentery, and irritation of mucosal tissues (Wasswa, R. & Busani, L., 2023). Saponins contribute to anti-cancer effects and likely support the plant's anti-malarial capabilities (Khan M.I. et al., 2022). *P. guineense* harbors substantial levels of cardiac glycosides, which are beneficial for addressing cardiac-related disorders. It also includes compounds like dillapiol, elemicin, 10% myristicin, and safrole. Secondary metabolites such as aromadendrene, 1,6,10-dodecatriene, 7,11-dimethyl-3-methylene, piperine, and piperidine have also been identified in *Piper guineense* (Alagbe et al., 2021).

Table 2.3: Photochemical Composition of Various Parts of Piper guineense

Parts	Composition
Leaves	Alkaloids include piperine, wisamine, dihydrowisamine, dihydropiperine. Flavonoids, Saponins, Tannins, Resins. Essential oils include dillapiol, elemicin, myristicine, and safrole.
Seeds	Alkaloids, Flavonoids, Saponins, Tannins, Terpenes, Resins, Steroids, Essential oil includes dillapiole, elemicin, myristicine and safrole, Cardiac glycosides

2.3.1 Nutritional Value of *Piper Guineense*

Piper guineense possesses both nutritional and non-nutritional elements that contribute to its distinctive aroma, taste, and ability to act as a natural preservative. Proximate assessments indicate that the plant is a source of crude protein, fats, carbohydrates, vitamins, and minerals. (Uhegbu *et al.* 2015). Its essential oils are present at levels ranging from 0.1% to 5%, with notably low peroxide values and free fatty acid concentrations, suggesting good oxidative stability (Akinmoladun, F.O., Komolafe, T.R., Farombi, E.O., 2021). Research reveals high ash content, suggesting rich stores of minerals like calcium, zinc, magnesium, copper, and potassium (Ogungbenle *et al.*, 2017). Uziza leaves exhibit elevated crude fiber levels, which can enhance digestion, facilitate intestinal water absorption, and mitigate constipation (Ogungbenle H. N. *et al.*, 2017; Ebere Chidiebere Favour, 2021). With relatively low fat levels, Uziza leaves can fit into dietary plans aimed at weight management. Their protein profile positions them as a valuable plant-based protein option, while the elevated carbohydrate content supplies essential energy for routine bodily functions (Adeyeye E. I. & Olaleye A. A., 2020). Studies highlighted significant vitamin C in *P. guineense*, which supports dental and gingival health while facilitating wound recovery; the plant also features vitamin A, along with minor quantities of vitamins B1 and B2, all contributing to overall well-being. Additionally, vitamin E serves a key antioxidant function.

2.4 Biological Activities of *Piper guineense*

2.4.1 Antimicrobial activities

Piper guineense possesses notable antimicrobial potential, demonstrating both antifungal and antibacterial effects against Gram-positive and Gram-negative organisms (O. C. Anika *et al.*, 2017; Nwiyi P. O. *et al.*, 2022). Studies have shown that extracts from its leaves and seeds inhibit

the growth of various pathogenic bacteria and fungi. The methanolic extracts, in particular, are rich in secondary metabolites such as alkaloids, glycosides, tannins, flavonoids, and terpenes, which play a crucial role in its antimicrobial action (Alagbe *et al.*, 2021). Furthermore, bioactive constituents like piperine and piperlongumine have been linked to its antifungal activity, especially in suppressing strains such as *Candida albicans* (Mgbeahuruike *et al.*, 2019).

2.4.2 Anti-parasitic Potential

Traditionally, *Piper guineense* has been employed in managing parasitic infections, like malaria. Its extracts exhibit significant anti-parasitic effects, largely attributed to bioactive compounds such as alkaloids and flavonoids, which disrupt parasite life cycles and reduce their survival (Ohemu *et al.*, 2024). Beyond human health, aqueous extracts of the plant have also proven effective in crop protection. For instance, they achieved complete larval mortality of *Plutella xylostella* in brassica crops, reduced egg viability of pests like *Clavigralla tomentosicollis* and *Maruca vitrata* in cowpea (*Vigna unguiculata*), and acted as repellents against the banana weevil (*Cosmopolites sordidus*) in banana and plantain fields (Juliana A.U *et al.*, 2020; Akinbuluma D. E. *et al.*, 2025.)

2.4.3 Anti-inflammatory potential

The plant's extracts have been shown to inhibit inflammatory mediators, thereby reducing inflammation. This activity is attributed to the presence of compounds such as betacaryophyllene, which has been investigated as an anti-inflammatory agent (Younis and Mohamed, 2019). Asthma and rheumatoid arthritis are two inflammatory disorders for which *P. guineense* is one of the top plants in an ethnobotanical list of plants and plant recipes (Okafor *et al.*, 2022).

2.4.4 Antioxidant activity

Piper guineense demonstrates strong antioxidant properties, which play an important role in reducing the risk of oxidative stress-related disorders. Its extracts are rich in flavonoids and phenolic compounds that neutralize free radicals, thereby safeguarding cells from oxidative injury (Alagbe *et al.*, 2021). The high levels of polyphenols present in the plant account for its notable reducing ability and radical-scavenging effects (Adeniyi *et al.*, 2017). Furthermore, these compounds help maintain the body's antioxidant defense system by delaying low-density lipoprotein (LDL) oxidation and prolonging its lag phase (Nwachukwu *et al.*, 2025).

2.4.5 Fertility Effect

The influence of *Piper guineense* on reproductive health has been investigated, with findings indicating both positive and adverse effects. Prolonged intake of its seeds has been associated with structural damage in male reproductive tissues, including atrophied seminiferous tubules, degenerated myoid and spermatogenic cells, intestinal fibrosis, and areas of necrosis within the testes. Research using female rodents suggests that the plant may act as an abortifacient or inhibit conception by preventing follicular development (Aporiku & Nwogu, 2018). On the other hand, some reports indicate that its extracts may modulate hormonal balance and potentially enhance reproductive function, though further research is required to clarify these outcomes (Ohemu *et al.*, 2024).

2.4.6 Cardiovascular and Antihypertensive Activity

P. guineense seed extracts inhibit angiotensin-converting enzyme (ACE), a key target in hypertension treatment. (Ifeanyi I.E *et al.*, 2024.) The flavonoids and alkaloids in the plant reduce oxidative stress in the cardiovascular system, lower blood pressure, and improve vascular

function. This suggests potential for the development of natural antihypertensive therapies.

2.4.7 Anticancer Activity

Preliminary studies indicate that *P. guineense* extracts may exert cytotoxic and antiproliferative effects on cancer cell lines (Iweala et al., 2015). The bioactive compounds, especially piperamides and flavonoids, may induce apoptosis, inhibit angiogenesis, and block tumor growth pathways. While further clinical investigations are necessary, current findings points to the plant's promising potential in cancer chemoprevention.

2.4.8 Insecticidal and Repellent Properties

The essential oils from *P. guineense* have shown insecticidal and mosquito repellent effects, making them useful in vector control. Compounds such as dillapiol and eugenol interfere with insect nervous systems, reducing mosquito bites and malaria transmission. This aligns with its ethnobotanical use in fumigation and pest control in African communities.

2.4.9 Larvicidal Property

Piper guineense has demonstrated larvicidal properties, particularly against mosquito larvae at high concentrations. The Larvicidal action increases with plant extract concentration. Because they are widely available, reasonably priced, environmentally benign, and biodegradable, botanical larvicides made from this plant may be used instead of synthetic ones. This suggests its potential use in controlling vector populations responsible for diseases like malaria (Owoeye J.A. et al., 2016).

2.4.10 Effect on Iron Metabolism

Piper guineense has been shown to influence iron absorption and utilization in the body. The spice can enhance non-heme iron bioavailability by reducing the chelating effect of dietary phytates, thereby improving iron absorption (Aké Claude Bernard et al., 2019). This effect is

partly attributed to its alkaloids and essential oils, which may modify gastrointestinal enzyme activity. Additionally, the antioxidant properties of *P. guineense* protect red blood cells and hemoglobin from oxidative damage, reducing the risk of iron deficiency anemia.

2.4.11 Bioactive constituents of special interest

Piper guineense contains high amounts of iron, making it a potentially valuable dietary source for managing iron-deficiency anemia. One proximate analysis revealed that *P. guineense* seeds contain iron concentrations of about 68.75 mg/dl, with the levels being significantly higher in the seeds compared to the leaves (Okeke *et al*, 2015). Piperine is a major bioactive constituent influencing iron metabolism. It is widely recognized for enhancing the bioavailability of curcumin and has also been shown to improve the absorption of micronutrients, including iron. Piperine stimulates intestinal microvilli, increasing the uptake of iron, which in turn improves hemoglobin and packed cell volume in animal models. Piperine also exhibits antioxidant activity that helps counteract oxidative stress associated with excess free iron, this effect supports the stability of iron-containing proteins and maintains metabolic balance (Cho, S., Jung, Y., Rho, S.-J., & Kim, Y.-R. 2025). Flavonoids and Phenols provide strong antioxidant protection against oxidative damage. Polyphenols can sometimes inhibit non-heme iron absorption; the overall effect of *P. guineense* appears favorable due to its high iron content and synergistic compounds. Tannins are present in measurable amounts and can bind to iron and reduce its absorption; however, their impact is moderated by the preparation method and the presence of absorption-enhancing compounds in the plant (Akinmoladun *et al.*, 2018).

2.5 Organism of study (*Drosophila Melanogaster*)

2.5.1 Introduction

Drosophila melanogaster, commonly called the fruit fly, has served as a fundamental model organism in genetic research for more than a century. Its importance lies in advancing knowledge of inheritance, developmental biology, and human disease processes, owing to its considerable genetic similarity to humans (Imoleayo O. & Oyeniran O., 2019). The name *Drosophila Melanogaster* comes from the Greek words. *Drosophila* comes from *drósos* and *phília*, meaning “dew-loving.”, while *Melanogaster* comes from *mélas* and *gastér*, meaning "black-belly". These flies are often referred to as fruit flies, vinegar flies, wine flies, or pomace flies and belong to the family *Drosophilidae*. A primary characteristic is their attraction to ripening or decaying fruits. Several species, such as the Mediterranean fruit fly, are notorious agricultural pests capable of infesting and damaging crops by depositing eggs in ripening fruits through their ovipositors (Abd-Elgawad, M. M., 2021). In addition, *D. melanogaster* is considered a domestic species, frequently living in close association with humans.

2.3.2. Scientific Classification of *Drosophila melanogaster*

Kingdom: Animalia

Subkingdom: Bilateria

Superphylum: Ecdysozoa

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Family: *Drosophilidae*

Genus: *Drosophila*

Species: *D.melanogaster*

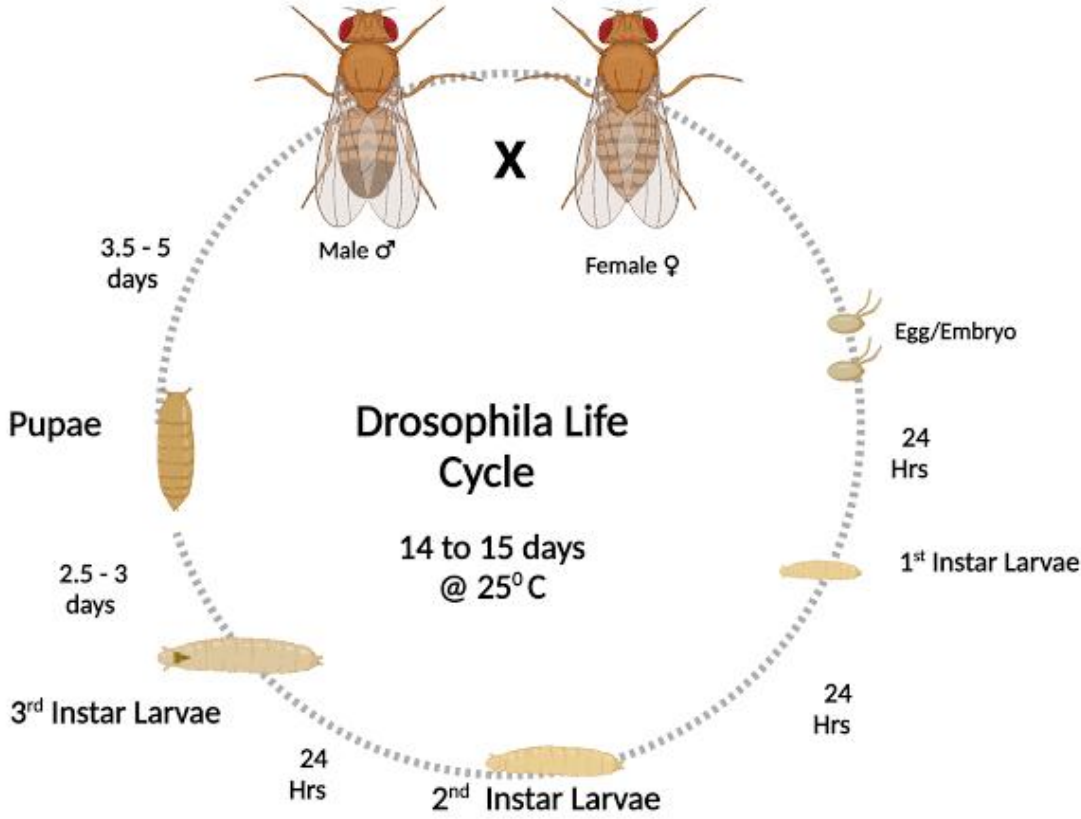
2.3.3 Morphology

Adult *Drosophila melanogaster* are typically 3–4 mm long with a yellowish-tan body and black abdominal stripes. Their body is divided into a head, thorax, and abdomen (Wu, Yin, & Zhou, 2022). The head features large, red compound eyes, smaller ocelli, and short, feathery antennae (Jürgens, K.J., & Paululat, A., 2024). A pair of transparent wings on the mesothorax and small, balancing halteres on the metathorax facilitate flight (Rauscher, M.J., & Fox, J.L., 2024). Six segmented legs extend from the three segments of the thorax. (Basumatary, D., & Deka, N., 2024). Females are usually larger with a pointed abdomen that displays prominent dark, transverse stripes, while males have a smaller, rounded abdomen with heavier black pigmentation at the posterior end (Bier, E. & De Robertis, E.M., 2015). Males also possess a distinct cluster of bristles, known as a "sex comb," on their forelegs (Alam, M.S., & Shahjahan, R.M., 2020). The larval stage is a legless maggot with an indistinct head and segmented body (Basumatary, D., & Deka, N., 2024).

2.3.4. Life Cycle of *Drosophila melanogaster*

The life cycle spans approximately 10 to 14 days at optimal conditions (25°C): Females lay fertilized eggs on moist surfaces and these eggs hatch into little, white first instar larvae after 12 to 15 hours at 25°C (77°F). These larvae then consume food and grow for about four days. They then hatch and undergo two more moults becoming second and third instar maggots, respectively. They consume microorganisms present on rotting and decaying fruit breeding resources as well as on fruits sugar, vegetable matter and yeasts when they are in larval stage (Brian L. Sidransky, 2015). Metamorphosis occurs within 4–5 days into the pupa stage. Fully formed flies emerge from the puparium and becomes ready to reproduce within 8–12 hours.

Figure 2.4 Life cycle of *Drosophila Melanogaster*



2.3.5. Drosophila Culture and Media

Drosophila is cultured on a nutrient-rich medium composed of cornmeal, molasses, yeast, and agar. Antifungal agents are sometimes added to prevent mold contamination. *Drosophila melanogaster* primarily feeds on the yeasts that grow on fruit rather than the fruit itself. The standard culture medium contains cornmeal, yeast, and sugar as nutrient sources, agar for solidification, and antimicrobials such as propionic acid to inhibit microbial growth. This medium supports *Drosophila* breeding, with agar providing the right firmness to prevent fly entanglement while still allowing larval feeding. Flies are typically bred in glass vials or jars containing this culture medium, which are sealed with cotton wool or foam stoppers to prevent escape and contamination by mites or other pests (Chaudhary *et al*, 2024).

2.3.6. Drosophila as a Model Organism to Study Human Diseases

The fruit fly is instrumental in studying neurodegenerative diseases (e.g., Alzheimer's), metabolic disorders (e.g., diabetes), and cancer. These disease models are generated through precise genetic modifications (Surya Banerjee *et al.*, 2020). Owing to its conserved metabolic and oxidative stress pathways, *Drosophila* is also employed to assess the therapeutic potential and toxicity of plant-based compounds, including antioxidant screenings and toxicological assays that measure survival and behavior (Lopez-Ortiz *et al.*, 2023). With approximately 75% of its genes homologous to humans, *Drosophila melanogaster* has become a standard model in developmental biology and disease research (David J. McKay, 2023). Its ease of genetic manipulation and short life cycle further enhance its utility.

2.4 Genome and Genetic Conservation with Humans

2.4.1 Genomics in *Drosophila melanogaster*

The adult *Drosophila melanogaster* genome is one of the best-characterized animal genomes, providing a foundation for modern genetics and biomedical research. The complete genome spans approximately 165 million base pairs and encodes about 13,600 protein-coding genes, along with thousands of non-coding RNAs, regulatory elements, and repetitive sequences (Samuelson & Hanlon, 2021). Despite being smaller than the human genome, it shares strong conservation, with nearly 75% of human disease-related genes having fly orthologs (Ugur Ugur et al., 2016). This makes the fly a powerful system for studying gene function, developmental biology, and disease mechanisms.

The genomic material is arranged on four pairs of chromosomes: the X and Y sex chromosomes, two large autosomes (chromosomes 2 and 3), and a tiny fourth chromosome. Chromosomes 2 and 3 contain the bulk of functional genes, while chromosome 4 is largely heterochromatic and gene-poor (Hoskins *et al.*, 2015). The Y chromosome, although gene-sparse, carries fertility factors essential for male reproduction.

Adult flies show distinct genomic compartmentalization between euchromatin and heterochromatin. Euchromatin is rich in protein-coding genes and regulatory DNA, whereas heterochromatin is composed mainly of repetitive elements and transposable sequences. This organization provides insight into chromatin dynamics, gene silencing, and epigenetic regulation (Swenson et al., 2016). One unique aspect of *Drosophila* genomic organization is the presence of polytene chromosomes in the salivary glands of larvae. These oversized chromosomes arise from repeated rounds of DNA replication without cell division (endoreplication), producing distinct banding patterns that make it possible to map genes cytologically. Polytene chromosomes have

historically been instrumental in gene mapping and studying chromosomal rearrangements (Strashnyuk, V.Y., Shakina, L.A., & Skorobagatko, D.A., 2023). The adult *Drosophila* genome also exhibits functional compartmentalization, with clusters of co-expressed genes, conserved regulatory networks, and extensive use of non-coding RNAs. Advances in sequencing technologies and genetic tools, such as CRISPR-Cas9, P-element transposons, and balancer chromosomes, further enhance the ability to manipulate and analyze its genome with high precision (Hoskins *et al.*, 2015).

2.4.2 Similarities between Genomics in *Drosophila Melanogaster* and Humans

The genomics of *Drosophila melanogaster* shares remarkable similarities with humans. Despite its smaller size, approximately 60% of the fly genome is conserved with humans, and nearly 75% of human disease-associated genes have homologs in *Drosophila* (Casas-Tintó, S. (2024). Signaling pathways such as Notch, Wnt, Hedgehog, insulin/TOR, and JAK/STAT are present in both flies and humans, governing development, growth, and metabolism (Perrimon, N., & Langley, R., 2018). This conservation extends to pathways critical for health and disease, including iron metabolism, oxidative stress responses, and apoptosis. For instance, *Drosophila* encodes homologs of transferrin (Tsf1), ferritin, and iron regulatory protein (IRP1), which parallel mammalian systems in regulating iron absorption, transport, and storage (Marra, A., & Lemaitre, B., 2021). Similarly, antioxidant defenses mediated by superoxide dismutase (SOD), catalase, and glutathione peroxidase are conserved, enabling flies to serve as models for human oxidative stress-related diseases (Pal, A., & Mishra, N., 2023).

Apoptosis in flies also relies on conserved molecules such as caspases and inhibitors of apoptosis proteins (IAPs), reflecting mechanisms found in mammals and providing insights into cancer and neurodegeneration (Shinoda, N., & Miura, M., 2024). Furthermore, both organisms utilize non-

coding RNAs (miRNAs, lncRNAs, siRNAs) to regulate gene expression, revealing deep evolutionary parallels in genomic regulation (Marco, A. (2017). Epigenetic processes like dosage compensation and Polycomb/Trithorax regulation are similarly preserved, ensuring stable gene expression patterns across cell generations (Lakhota, S.C., 2015).

These genetic similarities enable *Drosophila* to effectively model human disease conditions, including iron dysregulation, metabolic abnormalities, and neurodegenerative disorders. Notably, modern genetic technologies such as RNAi and CRISPR-Cas9 are applicable in both flies and humans, strengthening the translational value of discoveries made in the fly model (Bier, E. (2021).

2.5 Iron Metabolism in *Drosophila Melanogaster*

Iron metabolism in *Drosophila melanogaster* is a tightly regulated process that encompasses absorption, storage, transport, and sensing. The midgut serves as the primary site for dietary iron uptake, where specific transport proteins ensure efficient absorption. Malvolio (Mvl), a homolog of mammalian DMT1, transports ferrous iron (Fe^{2+}) into enterocytes, and loss of this transporter leads to severe iron deficiency (Wu, S., Yin, S., & Zhou, B., 2022). In addition, multicopper oxidases such as MCO1 and MCO4 function as ferroxidases, converting ferrous to ferric iron (Fe^{3+}) for systemic trafficking (Xiao *et al.*, 2019). Specialized “iron cells” in the midgut also play a critical role in maintaining homeostasis by accumulating ferritin in response to excess dietary iron (Zhou, B. (2019).

Drosophila employs ferritin as its major iron transporter. This ferritin is composed of two subunits, Fer1HCH (heavy chain) and Fer2LCH (light chain), and is secreted via the endoplasmic reticulum Golgi pathway into the hemolymph, where it delivers iron to essential tissues such as the fat body and brain (Zhao, M., Wang, H.L.2019). Mutations in either ferritin

subunit result in embryonic or larval lethality, highlighting the importance of ferritin-mediated iron transport (González-Morales *et al.*, 2015). Although ferritin is central, other proteins also contribute. For example, Transferrin 1 (Tsf1) helps transport iron from the gut to the fat body, while ZIP13 ensures delivery of ferrous iron to the Golgi apparatus for ferritin assembly (Xiao *et al.*, 2019). Similarly, mitoferrin (mfrn) imports iron into mitochondria for heme and iron-sulfur cluster biosynthesis, and its mutation results in male sterility, linking mitochondrial iron metabolism to spermatogenesis (Xiao *et al.*, 2019; Wu *et al.*, 2022).

At the regulatory level, *Drosophila* employs the Iron Regulatory Protein (IRP)/Iron Responsive Element (IRE) system to balance cellular iron availability. Under low iron conditions, IRP-1A binds IREs on ferritin mRNA, blocking its translation to conserve iron, while high iron levels convert IRP-1A into cytosolic aconitase, preventing its binding to IREs and allowing ferritin synthesis (Muckenthaler *et al.*, 2017).

2.5.1 Iron Metabolism in Aging and Disease Models

Iron plays a central role in aging and the progression of several diseases, and *Drosophila melanogaster* has proven to be an effective model for investigating these processes. Iron imbalance increases with age and contributes to diseases such as neurodegeneration, cardiovascular disease, diabetes, and cancer (Brooks, J., *et al.*, 2019). Since the body lacks active excretion, aging weakens intestinal regulation, leading to tissue iron accumulation (Yuan, Z. *et al.*, 2022). Excess iron catalyzes the Fenton reaction, producing reactive oxygen species (ROS) that drive oxidative stress and cellular injury (Anderson & Frazer, 2017). Mitochondria are particularly vulnerable, as iron overload disrupts heme and Fe-S cluster synthesis, impairing energy production (Lill & Mühlenhoff, 2008). Declines in proteolysis and chronic inflammation further elevate hepcidin, trapping iron in storage cells and worsening anemia of aging (Terman &

Brunk, 2006; Weiss & Ganz, 2019). Neurodegenerative disease models show that iron accelerates protein aggregation in Alzheimer's and Parkinson's (Zhang, J., Li, W., 2025). Similarly, cardiac models reveal that both iron deficiency and overload impair heart and vascular function (Lakhal-Littleton, 2019). In diabetes, excess iron damages pancreatic β -cells and promotes insulin resistance (Gao, Q., Yuan, Z., 2025). Cancer models demonstrate that iron-driven oxidative stress causes DNA damage and tumor progression (Kroemer, G., & Tang, D., 2021). Notably, interventions such as iron chelators and dietary restriction have shown protective effects in experimental models, offering therapeutic potential (Kawabata, H. (2019).

2.6 Iron Regulatory Protein 1 (IRP1)

Iron Regulatory Protein 1 (IRP1) is a central post-transcriptional regulator of cellular iron metabolism. IRP1 can exist in two interconvertible biochemical forms: an aconitase enzyme containing a [4Fe-4S] cluster that participates in the citric acid cycle, and an RNA-binding protein that specifically recognizes iron-responsive elements (IREs) located in the untranslated regions (UTRs) of target mRNAs. When cellular iron is low, the [4Fe-4S] cluster is lost, and IRP1 adopts the RNA-binding form; this binding stabilizes or represses translation of mRNAs bearing IREs (e.g., ferritin, transferrin receptor), thereby adjusting iron uptake, storage, and utilization to cellular needs (Grandemange, S., & Monari, A. (2025)).

2.6.1 Structure

IRP1 is a 100 kDa cytosolic protein whose activity depends on reversible assembly of an iron-sulfur cluster. The structural switch between aconitase and IRE-binding activities is a classic example of a metalloregulatory protein that integrates iron and redox signals. The aconitase conformation has enzymatic activity (aconitase catalyzes isomerization of citrate to isocitrate),

while the apo-form lacks enzymatic activity but binds IRE folding motifs with high specificity (Rouault, T.A. (2020)).

2.6.2 Regulation

IRP1 activity is regulated by intracellular iron availability and oxidative stress. Low iron or oxidative disruption of the cluster favours the IRE-binding state; high iron levels favour cluster assembly and aconitase activity. Additionally, IRP1 is influenced by cellular redox conditions, nitric oxide, and iron–sulfur cluster biogenesis pathways (Pantopoulos, K. 2022). This dual functionality allows IRP1 to act as a rapid sensor that links iron metabolism to mitochondrial function and cellular metabolism.

2.6.3 IRP1 in Drosophila

Drosophila encodes IRP homologs (s) (commonly referred to as IRP-1A and IRP-1B) that conserve the IRE/IRP regulatory logic observed in vertebrates. Functional studies in flies demonstrate that manipulating IRP1 activity alters translation or stability of iron-related mRNAs and impacts systemic iron distribution, development, and stress responses (Zhou, B. (2022) Yan, M. (2023)). Because flies lack some vertebrate-specific layers (for example, mammalian hepcidin), the IRP/IRE system in Drosophila provides a cleaner genetic background to dissect cell-intrinsic iron sensing and its downstream effects.

2.6.4 Physiological Roles and Phenotypes

IRP1 controls the expression of ferritin (iron storage) and iron importers by binding IREs in their mRNAs. In the RNA-binding state, IRP1 represses ferritin translation (reducing storage) and stabilizes mRNAs like transferrin receptor (increasing iron uptake), thereby promoting iron acquisition under deficiency. Perturbations of IRP1 in flies cause misregulated ferritin levels, altered iron distribution, mitochondrial dysfunction, developmental defects and sensitivity to

oxidative stress phenotypes that model aspects of human iron-related disorders (Zhou, B. (2022), Masson, F.,2025).

2.6.5 Relevance to human disease models

In humans, IRP1 (and its paralog IRP2) are implicated in disorders of iron metabolism, neurodegeneration, and mitochondrial disease. Dysregulation of IRP activity contributes to conditions such as anemia of chronic disease, iron overload pathologies, and may worsen neurodegenerative cascades where iron-catalyzed oxidative damage plays a role (Rouault, 2020; Zhang *et al.*, 2025). *Drosophila* IRP studies provide mechanistic insight translatable to mammalian biology because the IRP/IRE post-transcriptional network is conserved.

2.6.6 Research Methods in *Drosophila* Models

IRP1 activity is typically investigated using genetic manipulation approaches such as mutant generation, RNAi-mediated silencing, or overexpression, alongside biochemical assays for aconitase activity. Its RNA-binding function can be evaluated with electrophoretic mobility shift assays (EMSAs). For studies involving *Piper guineense*, assessing IRP1 gene expression together with ferritin and *Tsfl* levels, in addition to aconitase activity, would give comprehensive insight into whether the phytochemicals influence iron sensing or the functional balance between aconitase and IRE-binding forms.

2.6.7 Molecular Interactions with Phytochemicals

Phytochemicals can influence cellular redox status and iron-sulfur cluster stability, potentially switching IRP1 between aconitase and IRE-binding forms. Antioxidant activity may protect the [4Fe-4S] cluster, promoting aconitase activity, whereas pro-oxidant circumstances destabilise the cluster and promote IRP1 RNA binding. *Piper guineense*, the cluster, directs IRP1 towards RNA

interaction. *Piper guineense* extracts may thus alter IRP1 via cellular redox balance or direct iron binding (Rouault, 2020; Zhang *et al.*, 2025).

CHAPTER THREE

MATERIALS AND METHODS

The research methodology details the strategies and procedures that will be utilized to achieve the research objectives and evaluate the hypotheses. This study proposes the following:

3.1. Study Location

The study took place in a specialized laboratory facility—BIOTOXCS Research Laboratory, part of the Central Biomedical Research unit—situated at the University of Benin, Benin City, Edo State, Nigeria. This laboratory was equipped with the appropriate infrastructure and equipment for rearing and handling *Drosophila melanogaster*. The controlled environment of the laboratory provided optimal conditions for maintaining the flies and conducting the experimental procedures.

3.2. Study Population Description

The study utilized *Drosophila melanogaster* (fruit flies) as the experimental model. The flies were obtained from the *Drosophila* Laboratory, Department of Biochemistry, University of Ibadan, Oyo State, Nigeria. Before the feeding and transfer procedures commenced, the flies were allowed sufficient time to acclimatize to the laboratory conditions. *Drosophila melanogaster* was chosen due to its well-characterized genome, short life cycle, ease of maintenance, and established relevance in genetic and biochemical research.

3.3.1 Collection and Identification of Uziza Leaves

Dried Uziza leaves were sourced from New Benin Market, Benin City, Edo State. It was identified as *Piper guineense* at the herbarium of the Department of Plant Biology and Biotechnology (PBB), Faculty of Life Sciences, University of Benin, Nigeria, with the voucher number UBH-P094. The leaves were separated from the stem, sorted to remove the debris, washed using clean tap water, and they were air-dried at room temperature for about a month in the laboratory.

3.3.2 Processing of *Piper guineense* Leaves

The preparation of *Piper guineense* leaves involved first removing any damaged or unhealthy leaves to ensure the quality of the sample. The selected healthy leaves were then thoroughly washed under running water to eliminate dust and other contaminants, followed by draining to remove excess water. To preserve the bioactive compounds and minimize nutrient loss, the leaves were air-dried at room temperature. Once completely dried, they were ground into a fine powder using a 1000A high-speed industrial grinder. The powdered leaves were subsequently stored in an airtight container under appropriate conditions until needed for experimental use.

3.3.3 Preparation of Plant Extract

The dried leaves of *Piper guineense* were further air-dried at room temperature for seven days and then ground into a fine powder using a commercial blender. Fifty grams (50g) of the powdered leaves were soaked in 450 ml of distilled water and left at room temperature for 48 hours to allow maceration. The extracts were filtered multiple times using Whatman filter paper [nitrocellulose] to separate the aqueous extracts from the plant residue, which was then discarded. The filtrate was concentrated into a semi-solid paste in an incubator, set at 37 °C for five days to obtain the crude extract. Finally, the extract was stored in Falcon tubes under refrigeration at 4 °C until it was needed for the environment.

3.4 Animal Care

The *Drosophila melanogaster* used in this study were cultured under controlled laboratory conditions in clear plastic vials containing a cornmeal-agar medium enriched with yeast. The vials were kept in the *Drosophila* laboratory at a stable room temperature of $25 \pm 2^\circ\text{C}$.

3.4.1 Inclusion criteria

- Male and female *Drosophila melanogaster* hatched within 38-72 hours.
- Healthy flies with no visible signs of disease or malformations

3.4.2 Exclusion criteria

- Flies with physical abnormalities or signs of disease.

- All male and female flies above 72 hours of life.

3.5.1 Feed Formulation of *Drosophila melanogaster*

The flies were provided with a complete standard cornmeal-based diet consisting of cornmeal (52 g), brewer's yeast (5 g), glucose (3.5 g), agar (7.5 g), nipagin (1 g), and 1 mL of alcohol, all prepared using distilled water. Flies aged 1–3 days were randomly selected from the culture vials for the study. Careful handling was observed during counting, using a soft-bristled brush to minimize stress and avoid harm to the flies.

3.5.2 Procedure for Feed Preparation

To prepare the feed, 850 mL of distilled water was measured, reserving 150 mL for mixing with the cornmeal. The remaining 700 mL was brought to a boil, and a small portion was set aside to dissolve the yeast. Agar was then added to the boiling water and stirred continuously until fully dissolved, after which the pre-dissolved cornmeal was gradually incorporated with constant stirring. Glucose was added next, and the mixture was stirred thoroughly to ensure even distribution. The mixture was gently boiled for about two minutes before adding the dissolved yeast. Nipagin, dissolved in ethanol, was incorporated to prevent microbial growth. Finally, the feed was removed from heat, dispensed into clean vials, and allowed to cool before being used for rearing *Drosophila melanogaster*.

3.6 Study Design

A total of 525 *Drosophila melanogaster* were utilized in this study and allocated into five experimental groups: Group A (Control), Group B, Group C, Group D, and Group E. Each group

contained 35 flies, with experiments conducted in triplicate, resulting in 105 flies per group. The Control group (Group A) was maintained on a standard cornmeal-agar diet with distilled water. Groups B, C, D, and E were fed the same medium supplemented with *Piper guineense* extract at concentrations of 100 mg/mL, 200 mg/mL, 300 mg/mL, and 400 mg/mL, respectively.

Flies were randomly chosen from vials prepared within the past 17 days and anesthetized by chilling in a freezer at 4°C for 5 minutes. Gentle handling using a soft-bristled brush minimized stress during counting.

Flies in each group were reared under identical environmental conditions, including temperature, humidity, and lighting. Additionally, the duration of exposure to *Piper guineense* extracts was standardized across the treatment groups to ensure valid and comparable results.

3.7 Extract Dosing of Piper Guineense

Group A: The Control group received only the Cornmeal diet and distilled water

Group B: was administered 100 mg/ml *Piper guineense leaf* extract

Group C: was administered 200 mg/ml *Piper guineense leaf* extract

Group D: was administered 300 mg/ml *Piper guineense leaf* extract

Group E: was administered 400 mg/ml *Piper guineense leaf* extract

Dose of extract = mg/ml

To prepare these concentrations, we use the formula:

Mass (mg) = Concentration (mg/ml) × Volume of solvent (ml)

Group B (100 mg/ml)

Using 4 ml solvent:

Mass (mg) = 100 × 4 = 400mg = 0.4g.

So, dissolve 0.4 g of extract in 4 ml of distilled water → 100 mg/ml

Group C (200 mg/ml)

Using 4 ml solvent:

$$\text{Mass (mg)} = 200 \times 4 = 800\text{mg} = 0.8\text{g}.$$

So, dissolve 0.8 g of extract in 4 ml of distilled water → 200 mg/ml

Group D (300 mg/ml)

Using 4 ml solvent:

$$\text{Mass (mg)} = 300 \times 4 = 1200\text{mg} = 1.2\text{g}.$$

So, dissolve 1.2 g of extract in 4 ml of distilled water → 300 mg/ml

Group E (400 mg/ml)

Using 4 ml solvent:

$$\text{Mass (mg)} = 400 \times 4 = 1600\text{mg} = 1.6\text{g}.$$

So, dissolve 1.6 g of extract in 4 ml of distilled water → 400 mg/ml

3.8. Experiment 1: Survival Assay

For the survival assay, 1–2-day-old male and female flies were assigned to five groups, each comprising three vials. Each vial housed 100 flies. Four of the groups were exposed to different concentrations of *Piper guineense extract*, while the Control group received only the standard cornmeal medium supplemented with 0.2 mL of distilled water.

Group A: Control group received only the Cornmeal diet and distilled water

Group B: 100 mg/ml *Piper guineense leaf* extract/cornmeal diet

Group C: 200 mg/ml *Piper guineense leaf* extract/cornmeal diet

Group D: 300 mg/ml *Piper guineense leaf* extract/cornmeal diet

Group E: 400 mg/ml *Piper guineense leaf* extract/cornmeal diet

The survival assay was performed in triplicate for each concentration. The flies' diet was refreshed every five days throughout the experiment. Daily observations were made to record the number of live and dead flies for all groups and concentrations. At the conclusion of the 21 days, the collected data were compiled, converted into survival percentages, and the survival rates were compared across all experimental groups.

3.9 Tissue Homogenate Preparation for Biochemical Assay

Another group experiment was done to evaluate the biochemical test. In this experiment, flies (both genders) less than 17 days old were separated into five groups, with each group having three treatment vials. Each group contained varying doses of *Piper guineense leaf* extract. 35 flies were placed in each treatment vial and the experiment was monitored for 5 days.

GROUP 1(A): Control flies fed on normal standard 9.8g commeal diet.

GROUP 2(B): Flies fed on 100mg/kg *Piper guineense* Leaf Extract + 9.8g Commeal diet.

GROUP 3(C): Flies fed on 200mg/kg *Piper guineense* Leaf Extract + 9.8g Cornmeal diet.

GROUP 4(D): Flies fed on 300mg/kg *Piper guineense* Leaf Extract + 9.8g Commeal diet.

GROUP 5(E): Flies fed on 400mg/kg *Piper guineense* LE + 9.8g Commeal diet.

At the end of the treatment period, the flies were anesthetized on ice, weighed, and homogenized in cold 0.1 M phosphate buffer, pH 7.0 (1:10 w/v). They were then centrifuged at 3500g for 5 minutes at 4°C (Allegra X-15R Centrifuge; Beckman Coulter, USA). The supernatants were separated into labeled Eppendorf tubes and used for biochemical assays. The experiments were conducted in three replicates for each of the five groups.

3.10 Preparation of Reagent

Homogenising buffer (0.1M Phosphate buffer, pH 7.0) was prepared. 2.76g of Na₂HPO₄ was dissolved in 100mL of distilled water to make a solution A. 2.893g of NaH₂PO₄ was then dissolved in 100mL of distilled water to make a solution B. 39.7mL of solution A was added to 61mL of solution B to create the buffer, which was then stored at room temperature.

3.11 Iron Regulatory Protein-1 (IRP1) mRNA Assay

3.11.1. RNA Extraction and Semi-Quantitative Polymerase Chain Reaction (PCR)

RNA was isolated from *Drosophila melanogaster* with Trizol Reagent (Thermofisher Scientific) in an Eppendorf tube and converted to cDNA using ProtoScript II first-strand technology (New England Biolabs). PCR amplification of the IRP1 gene was done using One Taq®2X Master Mix (NEB).

3.11.2 Isolation of Total RNA

Total RNA was isolated from whole *Drosophila* samples with the Quick-RNA MiniPrep Kit (Zymo Research). The DNA contaminant was removed following DNase I (NEB, Cat: M0303S) treatment. The RNA was quantified at 260nm, and the purity confirmed at 260nm and 280nm using an A&E Spectrophotometer (A&E Lab.UK).

3.11.3. Complementary DNA (cDNA) Conversion

One microgram (1 µg) of DNA-free total RNA was converted into complementary DNA (cDNA) by reverse transcriptase reaction using the PhotoScript II First-Strand cDNA Synthesis Kit (New England BioLabs, USA), in a 3-step reaction: 65°C for 5 min, 42°C for 1 hour, and 80°C for 5minutes (Olugbemon *et al.*, 2020).

65°C for 5 minutes for primer annealing and denaturation of secondary structures,
42°C for 1 hour for reverse transcription and cDNA synthesis,

80°C for 5 minutes to inactivate the enzyme reverse transcriptase, effectively stopping the reaction.

3.11.4 PCR amplification and agarose gel electrophoresis

Polymerase chain reaction (PCR) for the amplification of the gene of interest was carried out with OneTaqR2X Master Mix (NEB) using the following primers (Inqaba Biotec, Hatfield, South Africa): set:> NM_078544.3 *Drosophila melanogaster* IRP1, Forward primer GCATTTCCACCAGGATCTATT Reverse primer AGCAAAGGAAGTACGGATTG, > NM_080094.4 *Drosophila melanogaster* Glycerol-3-phosphate dehydrogenase (Gpdh), Forward primer TCGGAGTGCGTAGACACTAGA Reverse primer AGCGCCATCTATGTAAGGATGT.

PCR amplification was performed in a total of 25 µl volume reaction mixture containing cDNA, primer (forward and reverse), and Ready Mix Taq PCR master mix. Under the following condition: Initial denaturation at 95 °C for 5 min, followed by 30 cycles of amplification (denaturation at 95 °C for 30 s, annealing for 30 s, and extension at 72 °C for 60 s) and ending with final extension at 72 °C for 10 min. The amplicons were resolved on a 1.0% agarose gel. The GAPDH gene was used to normalize the relative level of expression of each gene, and quantification of band intensity was done using “ImageJ” software (Elekofehinti *et al.*, 2020).

3.11.5. Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 21.0 (IBM Corp., Armonk, NY, USA) was used to present and analyze the data from this study. For continuous variables, such as the mRNA expression levels of the IRP1 gene, Analysis of Variance (ANOVA) was used to identify statistically significant differences between treatment groups. Tukey's Honest Significant Difference (HSD) post hoc test was applied, where a significant difference was observed in the

ANOVA. The patterns of mRNA gene expression were displayed using bar charts. A p-value of less than 0.05 was considered statistically significant.

CHAPTER FOUR

RESULTS

Table 4.1 shows the percentage survival rate of *Drosophila melanogaster* exposed to different concentrations of *Piper guineense* over a period of 21 days. At Day 7, the control group had a survival rate of (88.67 ± 3.22) . The group administered 100 mg of the extract had the highest survival rate of (94.67 ± 0.88) , followed by those given 400 mg with (91.67 ± 2.91) , 200 mg with (89.33 ± 2.60) , and 300 mg with (85.67 ± 3.18) . Statistical analysis showed no significant difference across groups ($p = 0.183$). At Day 14, the control group recorded a survival rate of (84.33 ± 2.33) . The group administered 100 mg again showed the highest survival rate of (89.33 ± 2.33) . This was followed by 200 mg at (83.00 ± 2.52) , 400 mg at (81.67 ± 2.33) , and 300 mg at (78.33 ± 2.19) . Differences among the groups approached significance but did not reach the threshold ($p = 0.075$). At Day 21, survival rates declined across all groups. The control group had the lowest value of (71.33 ± 1.76) . The group administered 100 mg maintained the highest survival rate of (80.00 ± 2.65) , followed by 200 mg at (75.67 ± 3.38) , 400 mg at (73.33 ± 2.03) , and 300 mg at (72.33 ± 2.33) . No statistically significant difference was observed among the groups ($p = 0.184$).

Table 4.1. Percentage of survival at different concentrations of *Piper guineense*

Time Point	Group A (Control) (%)	Group B (100 mg) (%)	Group C (200 mg) (%)	Group D (300 mg) (%)	Group E (400 mg) (%)	F	p value
Day 7	88.67 ± 3.22	94.67 ± 0.88	89.33 ± 2.60	85.67 ± 3.18	91.67 ± 2.91	1.927	0.183
Day 14	84.33 ± 2.33	89.33 ± 2.33	83.00 ± 2.52	78.33 ± 2.19	81.67 ± 2.33	2.955	0.075
Day 21	71.33 ± 1.76	80.00 ± 2.65	75.67 ± 3.38	72.33 ± 2.33	73.33 ± 2.03	1.916	0.184

Values are shown as Mean ± SEM. $p < 0.05$ is considered significant.

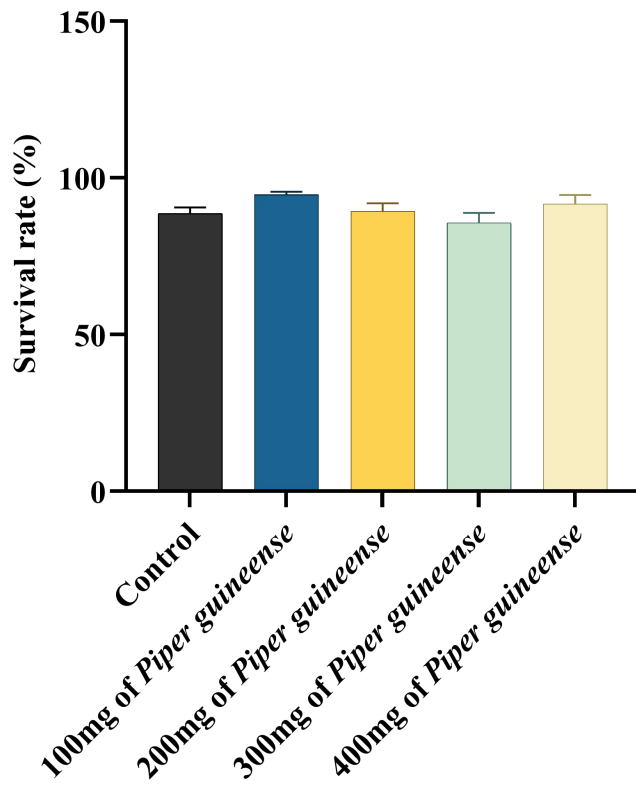


Figure 4.1. Survival rates of *Drosophila melanogaster* administered different concentration of *Piper guineense* at day 7. Values plotted represent mean percentages while error bars represent the standard error of the mean (SEM).

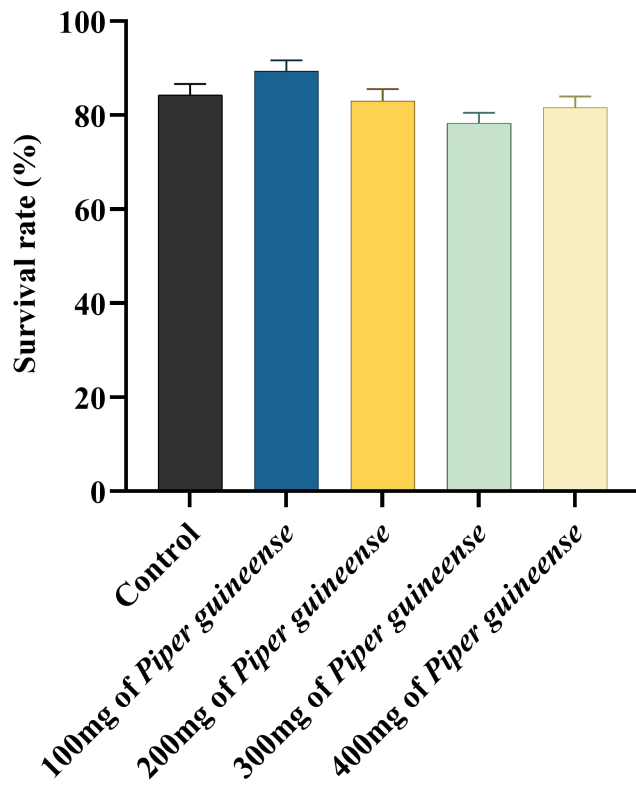


Figure 4.2. Survival rates of *Drosophila melanogaster* administered different concentration of *Piper guineense* at day 14. Values plotted represent mean percentages while error bars represent the standard error of the mean (SEM).

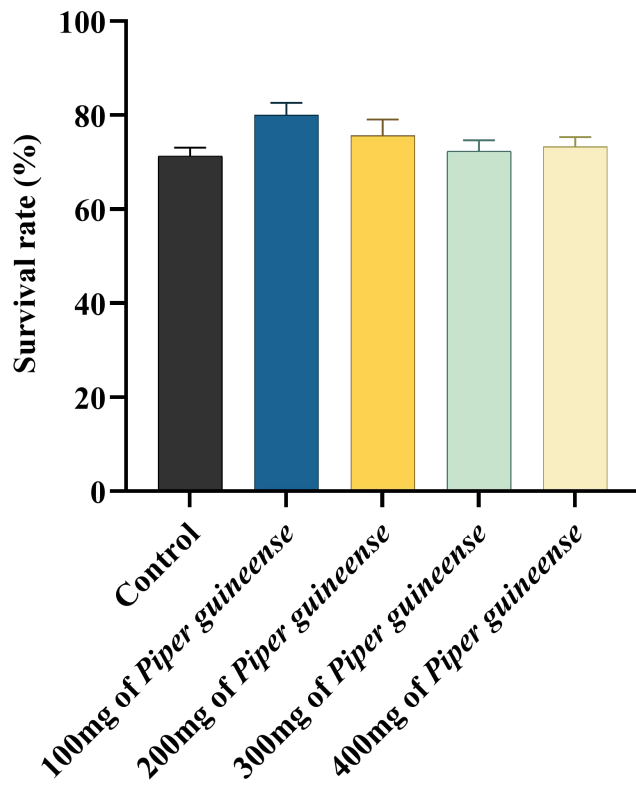


Figure 4.3. Survival rates of *Drosophila melanogaster* administered different concentration of *Piper guineense* at day 21. Values plotted represent mean percentages while error bars represent the standard error of the mean (SEM).

Figure 4.4. shows the percentage of survival over a period of 21 days. All treatment groups, including the Control and the four dosage groups showed a reduction in survival over the study duration. Initially, all groups showed a survival rate of 100%. The 100mg group consistently had the highest survival rate throughout the study. However, the Control, 300mg, and 400mg groups showed the most significant reduction in survival. The 200mg group maintained a survival rate intermediate to the high- and low-performing groups.

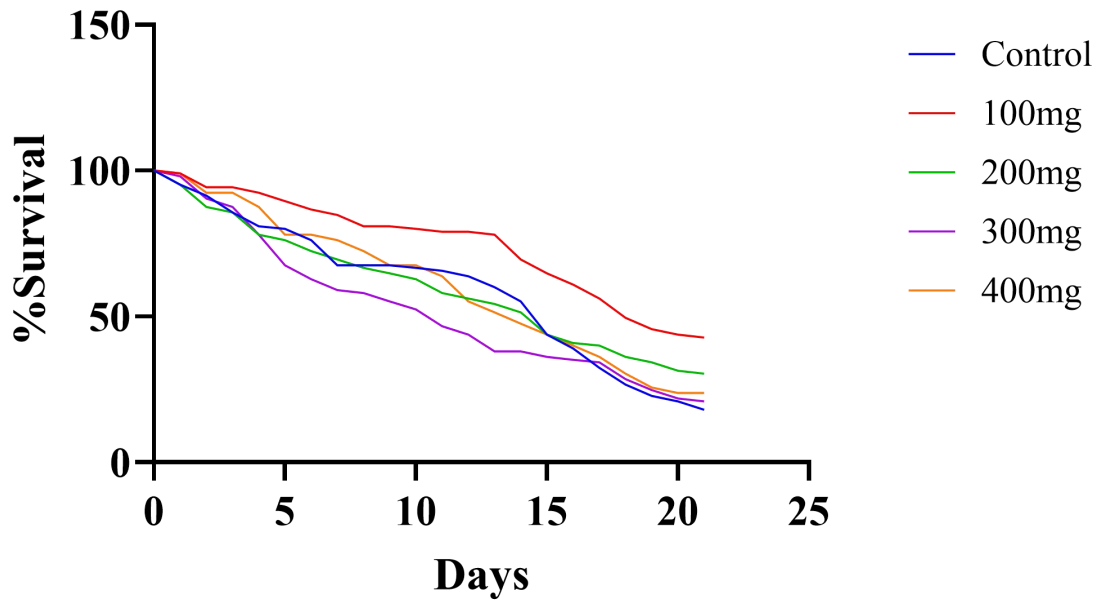


Figure 4.4. Survival curve of *Drosophila melanogaster* administered different concentrations of *Piper guineense* over 21 days.

Figure 4.5 shows the mRNA expression of Iron regulatory protein 1 (IRP1) of five groups A to E, representing control, 100mg of *Piper guineense*, 200mg/kg of *Piper guineense*, 300mg/kg of *Piper guineense*, and 400mg of *Piper guineense* administered to *Drosophila melanogaster*, each represented on different bars on the bar chart. IRP1 mRNA expression was significantly lower in the groups administered 200mg of *Piper guineense*(1.55 ± 0.05), 300mg of *Piper guineense*(1.05 ± 0.15), and 400mg of *Piper guineense* (0.85 ± 0.05) when compared to the control group (2.30 ± 0.10) ($p < 0.05$). The group administered 100mg of *Piper guineense* (1.80 ± 0.10) had significantly higher expression of IRP1 when compared to the groups administered 300mg of *Piper guineense* and 400mg of *Piper guineense*($p < 0.05$), while the group administered 200mg of *Piper guineense* had higher expression of IRP1 when compared to the group administered 400mg of *Piper guineense*($p < 0.05$).

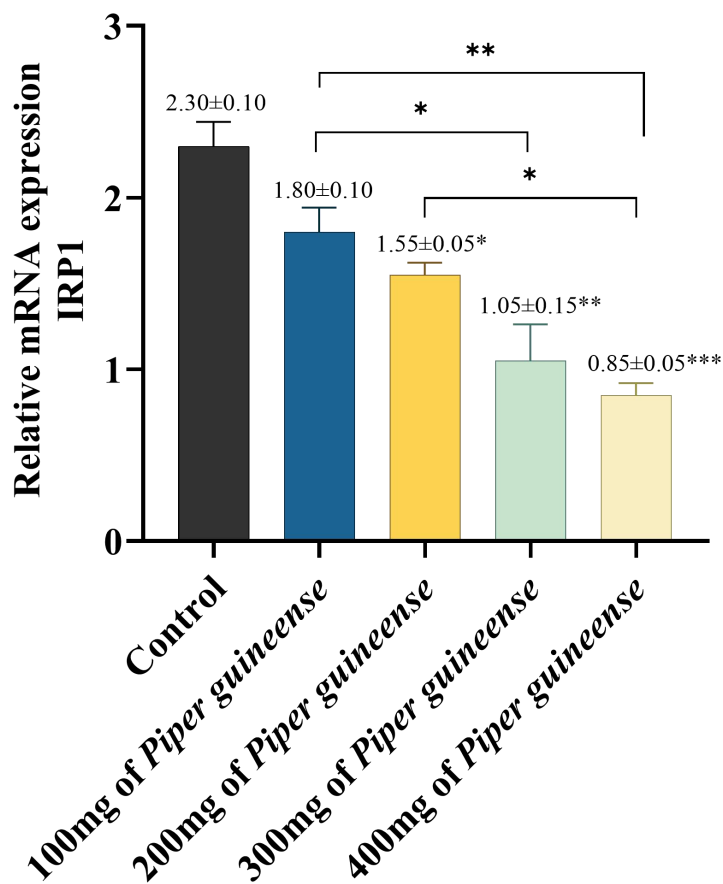


Figure 4.5: PCR and agarose gel analysis of Iron Regulatory Protein 1 (IRP1) gene. Error bar represents mean±SEM. Statistical significance represented by (p<0.05, p<0.01, p<0.001).

CHAPTER FIVE

5.1. DISCUSSION

The present study investigated the effect of *Piper guineense* leaf extract on the IRP1 gene in *Drosophila melanogaster*. The choice of *Drosophila* as a model organism is well justified, given its genetic similarity to higher eukaryotes and its wide use in toxicological studies (Missirlis, F.2017). The IRP1 gene plays a major role in iron homeostasis, acting as a regulatory protein that influences cellular iron metabolism, oxidative stress balance, and mitochondrial function (Khalimonchuk, O. (2021)). Natural products such as *Piper guineense*, which are rich in bioactive phytochemicals including alkaloids, flavonoids, and tannins, have been reported to modulate oxidative stress and influence gene expression (Carsono *et al.*, 2022). Therefore, evaluating the interaction between *Piper guineense* extract and the IRP1 gene provides

information on the molecular mechanisms underlying the medicinal potential or possible toxicity of this plant.

The survival analysis of *Drosophila melanogaster* exposed to varying concentrations of *Piper guineense* extract over 21 days showed that at Day 7 the group administered 100 mg of the extract had the highest survival rate compared to the control and other treatment groups. This suggests that at lower concentrations, *Piper guineense* may possess bioactive compounds with protective effects that promote survival. These effects are likely linked to the plant's rich phytochemical composition, including flavonoids and phenolic compounds, which are known antioxidants that mitigate oxidative stress and enhance cellular resilience (Ademuyiwa *et al.*, 2023). Studies have shown that phytochemicals can improve survival in *Drosophila* by modulating stress response pathways and reducing free radical damage (Belyi, A.A., Alekseev, A.A., 2020). The slight increase in survival observed at 400 mg on Day 7 compared to 200 mg and 300 mg may indicate that certain higher concentrations can still confer some level of protection, although not as consistently as the 100 mg group. By Day 14, survival rates declined across all groups, which aligns with the natural aging process of *Drosophila* and stress from extract exposure (Jo and Imm, 2017). The 100 mg group continued to maintain the highest survival rate, while the control group recorded slightly lower values. This further supports the notion that *Piper guineense* at moderate doses may exert a protective effect. Flavonoids have been reported to extend lifespan and improve stress tolerance in *Drosophila* models (Fedintsev, A.Y. *et al.*, 2020), which may explain why the 100 mg group consistently outperformed the control. The groups treated with 200 mg and 400 mg showed moderate survival, whereas the 300 mg group showed the lowest survival. This pattern suggests a possible dose-dependent biphasic response, often described as hormesis, where low doses of bioactive compounds are beneficial,

but higher doses can become harmful (Kujawska, M. (2020). At Day 21, survival rates dropped across all groups, consistent with the expected life span of *Drosophila melanogaster* under experimental conditions (Missirlis, F., 2017). The control group had the lowest survival, while the 100 mg group maintained the highest rate, which is further indication that the extract may have provided some long-term protective benefits as observed at day 7 and day 14. This prolonged effect may be attributed to sustained antioxidant activity, reduced lipid peroxidation, and stabilization of mitochondrial function (Kurnia, D. et al., 2022). However, the gradual reduction in survival rate seen in the 200 mg, 300 mg, and 400 mg groups could suggest that higher concentrations exert mild toxic effects over time. Previous studies on plant extracts in *Drosophila* have reported similar findings, where higher concentrations led to reduced survival due to the accumulation of phytochemicals that may interfere with normal metabolism (Adedara *et al.*, 2016).

The expression profile of the IRP1 gene revealed a clear dose-dependent response to *Piper guineense* extract in *Drosophila melanogaster*. The control group had the highest mRNA expression OF IRP1, which represents the normal physiological state of iron regulation in untreated flies. Administration of 100 mg of the extract did not suppress IRP1 expression. Rather, this group maintained a relatively higher expression compared to the higher dose groups, showing that a small amount of *Piper guineense* may enhance or preserve IRP1 activity. This finding aligns with the survival data, where the 100 mg group consistently demonstrated the highest survival across the study period, suggesting that optimal IRP1 activity could be linked to improved cellular iron metabolism and reduced oxidative burden (Bresgen and Eckl, 2015). The groups administered 200 mg, 300 mg, and 400 mg of the extract showed progressively lower IRP1 expression, with the most suppression observed at 400 mg. This shows a potential

inhibitory effect of *Piper guineense* on IRP1 expression at higher concentrations. IRP1 is a dual-function protein that acts as an iron regulatory protein when bound to iron-responsive elements (IREs) and as cytosolic aconitase when containing an iron-sulfur cluster (Kuhn, 2015). Its expression and activity are tightly linked to iron availability and oxidative stress levels (Milczarek *et al.*, 2017). Suppression of IRP1 at higher doses of *Piper guineense* may show the chelating ability of phytochemicals such as flavonoids and tannins present in the extract. These compounds can bind iron and reduce its availability, thereby lowering the requirement for IRP1 activity (Anise, E.O. 2020). Similar observations have been reported in studies where flavonoid-rich extracts downregulated IRP1 to maintain iron balance and prevent iron-mediated oxidative damage (Sulaimon, L.A.,2020; Ojimelukwe, P.C., 2023).

The significant difference between the 100 mg group and the 300 mg and 400 mg groups suggests a threshold effect. At lower doses, *Piper guineense* may provide antioxidant protection that indirectly supports IRP1 expression by reducing oxidative stress and maintaining the stability of iron-sulfur clusters. This protective effect is consistent with previous reports showing that dietary antioxidants in *Drosophila* can enhance the expression of genes involved in metabolic homeostasis (Kim, S.K. *et al.*, 2021). However, at higher doses, the pro-oxidant and cytotoxic properties of phytochemicals may dominate, leading to oxidative disruption of the iron-sulfur cluster in IRP1 and subsequent suppression of its expression. This biphasic response is characteristic of hormesis, where low doses of bioactive compounds are beneficial, but higher doses exert detrimental effects (Calabrese *et al.*, 2017). The decrease in IRP1 expression from the group administered 200 mg to and the group administered 400mg further shows the idea that prolonged or excessive exposure to *Piper guineense* may disturb iron homeostasis. Reduced IRP1 activity at higher doses could compromise ferritin translation and transferrin receptor

regulation, leading to impaired cellular iron handling (Fox, J.L.,2021). In mammalian models, disruption of IRP1 has been associated with altered iron metabolism, mitochondrial dysfunction, and oxidative stress (Gu, W., Wilkinson,2025). Therefore, the reduction in IRP1 expression in flies exposed to higher extract concentrations could partly explain their lower survival compared to the 100 mg group.

The observation that the 200 mg group had significantly higher IRP1 expression compared to the 400 mg group also points to a dose-sensitive mechanism. While moderate doses begin to exert suppressive effects, extreme concentrations appear to strongly downregulate IRP1. This is consistent with findings by Adedara *et al.* (2016), where exposure of *Drosophila* to certain plant-derived compounds resulted in gene expression changes that varied sharply with dose, reflecting a balance between adaptation and toxicity. Taken together, these results suggest that *Piper guineense* modulates IRP1 expression in a dose-dependent manner.

5.2. CONCLUSION

The present study demonstrated that *Piper guineense* extract influences both Survival analysis showed that while all groups experienced a natural decline over the 21-day period, the 100 mg group consistently maintained the highest survival rate, suggesting a protective effect of the extract at lower concentrations. The increase in dosage from 200mg to 300mg to 400mg showed a gradual reduction in survival rate, suggesting that higher concentration exerts mild toxic effects on the fly.

IRP1 mRNA levels were significantly downregulated in the 200mg, 300mg, and 400mg groups compared to the control, whereas the 100mg group showed relatively higher upregulation of

IRP1 expression. Suggesting that at lower doses, *Piper guineense* may provide antioxidant protection that indirectly supports IRP1 expression by reducing oxidative stress and maintaining the stability of iron-sulfur clusters. However, at higher doses, the pro-oxidant and cytotoxic properties of phytochemicals may dominate, leading to oxidative disruption of the iron-sulfur cluster in IRP1 and subsequent suppression of its expression.

5.3. RECOMMENDATIONS

- 1 Further studies should be conducted to investigate the molecular pathways through which *Piper guineense* influences IRP1 expression and iron metabolism.
- 2 Additional experiments using varying doses and longer exposure periods are needed to better define the threshold between beneficial and toxic effects of the extract.
- 3 Phytochemical profiling of *Piper guineense* leaves is recommended to identify the specific compounds responsible for the observed effects on survival and gene expression.
- 4 Comparative studies in other organisms are advised to validate the translational relevance of these findings.
- 5 Future research should also assess other genes involved in oxidative stress and iron regulation to provide a broader understanding of the extract's impact.
- 6 Prolonged use of *Piper guineense* should be done with caution, while further research should be done.

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APPENDIX

MATERIALS AND REAGENTS USED

MATERIALS USED

Centrifuge

Incubator

Automated Pipettes

Eppendorf tubes

Piper guineense leaf extract

Homogenising stick

Measuring cylinders

Digital weighing scale

Falcon tubes

Funnel and Whatman's filter paper

Laboratory coat and gloves

REAGENTS USED

Trizol reagents

Phosphate buffer

Distilled water

Primers used were synthesized by Inqaba Biotec, South Africa.

DNA extraction kit

Agarose gel



Drosophila cornmeal diet



Treatment vials for survival assay