EFFECT OF PLANT FIBERS (*Irvingia gabonensis, Hunteria umbellata*, and *Abelmoschus esculentus*) ON DISACCHARIDASES AND ALKALINE PHOSPHATASE IN THE INTESTINAL MUCOSA OF RATS

BY

Joy Imade OBANOR (Miss)

PG/LSC2110419

DEPARTMENT OF BIOCHEMISTRY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

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BY

Joy Imade OBANOR (Miss)

(PG/LSC2110419)

A THESIS WRITTEN IN THE DEPARTMENT OF BIOCHEMISTRY AND SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (M.Sc) IN BIOCHEMISTRY OF THE UNIVERSITY OF BENIN, BENIN CITY.

MARCH, 2026.

CERTIFICATION

We certify that this work was carried out by Miss. **Joy Imade OBANOR** with matriculation number **PG/LSC2110419** in the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City.

__________________________ __________________________

Prof. (Mrs.) R.I Nimenibo-Uadia Date

(Project Supervisor)

__________________________. __________________________

Prof. E.C. Onyeneke Date

(Head of Department)

CERTIFICATION OF THESIS

We the undersigned attest and declare that the thesis of Miss. Joy Imade OBANOR with matriculation number **PG/LSC2110419** titled: Effect of plant fibers (*Irvingia gabonensis, Hunteria umbellata,* and *Abelmoschus esculentus*) on disaccharidases and alkaline phosphatase in the intestinal mucosa of wistar rats has successfully passed the anti-plagiarism test and does not violate any copywrite regulations.

__________________________ __________________________

Prof. (Mrs.) R.I Nimenibo-Uadia Date

(Project Supervisor)

___________________________. ___________________________

Prof. E.C. Onyeneke Date

(Head of Department)

DEDICATION

This thesis is dedicated to God Almighty who made it possible and my beloved family members for their support.

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ABSTRACT

The interaction between plant fibers and digestive enzymes is a critical aspect of gastrointestinal physiology, and plant fibers from traditional medicinal plants have attracted attention for their potential effects on digestive processes and metabolic health. However, the specific effects of *Irvingia gabonensis* (bush mango), *Hunteria umbellata* (osu), and *Abelmoschus esculentus* (okra) fibers on key small intestinal enzymes in a mammalian model are not fully elucidated. This study, therefore, investigated the effects of these plant fibers on the activities of disaccharidases (maltase, lactase, sucrase) and alkaline phosphatase, as well as total protein content and body weight, in the small intestinal mucosa of Wistar rats.

Fifty-four (54) male wistar rats were divided into nine (9) groups of six (6) animals each, Control 1- fiber-free group, Control 2 Fybogel 1.5 %, Fybogel 3.0 %, Bush mango 5.0 %, Bush mango 10.0 %, Okra 5.0 %, Okra 10.0 %, Osu 5.0 %, and Osu 10.0 %. The animals were fed formulated diets containing different concentrations of each plant fiber for 36 days. The primary data collected were measurements of enzymatic activity, total protein content, and body weight gain. Enzyme activities (maltase, lactase, sucrase, and alkaline phosphatase) were assessed using spectrophotometric assays on the small intestinal mucosal homogenates. Statistical analysis was performed to determine significant variations in enzymatic activity, total protein, and body weight gain across the different dietary groups, with a threshold of $p < 0.05$ defining statistical significance.

The findings demonstrated significant ($p < 0.05$) variations in enzymatic activity and metabolic parameters across the fiber-supplemented groups. Lactase activity was significantly elevated (122% increase) in the *I. gabonensis* 10.0 g group but significantly reduced by both doses of *A. esculentus* and the *H. umbellata* 10.0 g dose. Sucrase activity was consistently reduced across all plant fiber treatments, with *H. umbellata* showing the greatest reduction (44% decrease). Notably, alkaline phosphatase activity was significantly ($p < 0.05$) elevated in all fiber-supplemented diets compared to the fiber-free control (ranging from 105% to 353% increase). All plant fibers also significantly ($p < 0.05$) increased total protein content (up to 76% increase) and significantly ($p < 0.05$) reduced body weight gain (up to 35% decrease) compared to the control. These results suggested that supplementation with these plant fibers significantly modulated intestinal enzyme

activities and influenced metabolic processes. The significant alterations in enzyme activities and weight control highlighted the potential therapeutic benefits of *I. gabonensis, H. umbellata,* and *A. esculentus* fibers in improving gastrointestinal health and metabolic function.

CHAPTER ONE

INTRODUCTION

1.0 Introduction

The interaction between plant fibers and digestive enzymes represents a crucial area of research in nutritional biochemistry and gastrointestinal physiology. Plant fibers, particularly those derived from traditional medicinal plants, have garnered increasing attention for their potential effects on digestive processes and metabolic health. Understanding the complex relationship between dietary plant fibers and intestinal enzymes, specifically disaccharidases and alkaline phosphatase, provides valuable insights into nutrient absorption, metabolism, and overall gastrointestinal function (Xu *et al.,* 2021).

1.1 Background of the Study

The small intestinal mucosa serves as a primary site for nutrient digestion and absorption, featuring a sophisticated array of enzymes that facilitate these processes. Among these enzymes, disaccharidases and alkaline phosphatase play pivotal roles in carbohydrate metabolism and maintaining intestinal homeostasis. Recent research has highlighted the significance of these enzymes in not only digestive processes but also in maintaining gut barrier integrity and modulating immune responses (Wu *et al*., 2022).

Disaccharidases, including maltase, lactase, and sucrase, are essential brush border enzymes responsible for the final stages of carbohydrate digestion. These enzymes hydrolyze dietary disaccharides into their constituent monosaccharides, making them crucial for efficient nutrient absorption. Studies have demonstrated that alterations in disaccharidase activity can significantly

impact carbohydrate metabolism and overall digestive efficiency (Julio-Gonzalez *et al.,* 2023). The regulation of these enzymes is particularly important as their dysfunction can lead to various metabolic disorders and nutritional deficiencies.

Alkaline phosphatase, particularly intestinal alkaline phosphatase (IAP), has emerged as a critical enzyme in maintaining intestinal homeostasis. Recent investigations have revealed its multifaceted roles beyond simple phosphate hydrolysis, including the regulation of nutrient absorption, maintenance of gut barrier function, and modulation of inflammatory responses (Adedibu *et al.*, 2024). The enzyme's activity serves as an important marker of intestinal health and function, with its expression patterns closely linked to various physiological and pathological conditions.

Plant fibers have historically been recognized for their beneficial effects on gastrointestinal health. Traditional medicinal plants such as *Irvingia gabonensis* (bush mango), *Hunteria umbellata* (osu), and *Abelmoschus esculentus* (okra) have been utilized in various cultures for their therapeutic properties. Modern research has begun to elucidate the mechanisms through which these plant fibers interact with intestinal enzymes and influence digestive processes (Maji and Banerji, 2016).

Recent studies have demonstrated that dietary components, including plant fibers, can significantly modulate the activity of intestinal enzymes. For instance, research has shown that certain plant compounds can affect the expression and activity of disaccharidases in the small intestinal mucosa (Moré and Vandenplas, 2018). These interactions have important implications for nutrient absorption and metabolism, potentially influencing various aspects of health and disease.

The complex interplay between plant fibers and intestinal enzymes extends beyond simple mechanical interactions. Evidence suggests that bioactive compounds present in plant fibers can influence enzyme expression and activity through various molecular mechanisms. Studies have shown that these interactions can lead to changes in brush border enzyme activities, potentially affecting nutrient digestion and absorption patterns (Messina *et al*., 2019).

Furthermore, research has indicated that the effects of plant fibers on intestinal enzymes may be concentration-dependent and specific to certain fiber types. This specificity has important implications for therapeutic applications and dietary recommendations. Studies examining various plant sources have demonstrated differential effects on enzyme activities, suggesting the need for detailed investigation of specific plant fiber sources (Gu *et al*., 2016).

The impact of plant fibers on intestinal enzyme activity has been shown to have broader physiological implications. Research has demonstrated that modulation of enzyme activity can influence various aspects of gastrointestinal function, including nutrient absorption, barrier integrity, and immune responses. These effects may contribute to the observed health benefits associated with plant fiber consumption (Chen *et al.*, 2023).

1.2 Justification of the Study

The investigation of plant fiber effects on intestinal enzymes is justified by several critical factors. First, there is a growing need to understand the mechanisms through which dietary components influence digestive processes and metabolic health. With the increasing prevalence of metabolic disorders and gastrointestinal diseases, identifying natural compounds that can modulate digestive enzyme activity has become increasingly important (Pan *et al.,* 2003).

Additionally, traditional medicinal plants have long been used for their therapeutic properties, yet the mechanisms underlying their effects often remain poorly understood. Scientific investigation of these plants' effects on intestinal enzymes can provide valuable insights into their traditional uses and potential therapeutic applications. This research can help bridge the gap between traditional knowledge and modern scientific understanding (Fang *et al*., 2016).

Furthermore, the study of plant fiber effects on disaccharidases and alkaline phosphatase has important implications for developing dietary interventions and therapeutic strategies. Understanding these interactions can lead to more effective nutritional recommendations and potentially new therapeutic approaches for various gastrointestinal and metabolic disorders (Grant *et al*., 2015).

The focus on specific plant fibers from *Irvingia gabonensis, Hunteria umbellata,* and *Abelmoschus esculentus* is particularly relevant given their traditional use and potential therapeutic applications. These plants represent important sources of dietary fiber and bioactive compounds, yet their effects on intestinal enzyme activity remain incompletely characterized.

1.3 Aim of Study

The primary aim of this study is to investigate the effects of plant fibers derived from *Irvingia gabonensis*, *Hunteria umbellata*, and *Abelmoschus esculentus* on the activities of disaccharidases (maltase, lactase, and sucrase) and alkaline phosphatase in the small intestinal mucosa of rats.

1.4 Objectives of Study

The specific objectives of this study are to:

1. Evaluate the effects of different concentrations of *Irvingia gabonensis*, *Hunteria umbellata,* and *Abelmoschus esculentus* formulated diet on maltase, lactase, and sucrase activities in rat small intestinal mucosa.
2. Determine the impact of these formulated diets on intestinal alkaline phosphatase activity.
3. Compare the relative effects of the formulated diets on the investigated intestinal enzyme activities.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction to Plant Fibers and Intestinal Enzymes

The complex relationship between plant fibers and intestinal enzymes represents a fundamental aspect of digestive physiology and nutritional biochemistry. Recent advances in understanding this interaction have revealed intricate mechanisms through which dietary components influence gastrointestinal function and overall health. The study of specific plant fibers and their effects on digestive enzymes has emerged as a crucial area of research, particularly in the context of traditional medicinal plants and their potential therapeutic applications (Moré and Vandenplas, 2018).

The small intestine serves as the primary site for nutrient digestion and absorption, featuring a sophisticated enzymatic machinery that facilitates these processes. Among these enzymes, disaccharidases and alkaline phosphatase play essential roles in maintaining optimal digestive function and intestinal homeostasis. Understanding how plant fibers modulate these enzyme activities provides valuable insights into both traditional medicine and modern therapeutic approaches (Wu *et al*., 2022).

2.1 Overview of the Selected Plant Samples (*Irvingia gabonensis*, *Hunteria umbellata* and *Abelmoschus esculentus*)

2.1.1 *Irvingia gabonensis* (Bush Mango)

Taxonomy

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Malpighiales*

Family: *Irvingiaceae*

Genus: *Irvingia*

Species: *Irvingia gabonensis* (Chen *et al*., 2023).

Irvingia gabonensis belongs to the family *Irvingiaceae*, a group of tropical plants known for their ecological and economic importance. The species was first described by Aubry-Lecomte ex O'Rorke Baill, and its classification reflects its distinct characteristics within the genus *Irvingia*. The taxonomic placement of *I. gabonensis* has been well-established through both morphological and molecular studies, confirming its position within the broader context of tropical forest species (Chen *et al*., 2023).

2.1.1.1 Plant Description

Irvingia gabonensis presents as a large tree species, typically reaching heights of 25-40 meters in mature specimens. The tree is characterized by its distinctive buttressed trunk and dense, evergreen canopy. The leaves are alternately arranged, simple, and elliptic to ovate-oblong in shape, with a dark green glossy surface. The fruit is a large drupe, containing a hard stone surrounding an edible kernel. The tree's architecture and morphological features reflect its adaptation to tropical forest environments, with specialized structures supporting its role in both ecological systems and human use (Maji and Banerji, 2016).

2.1.1.2 Medicinal Uses

2.1.1.2.1 Traditional and Scientific Perspectives on *I. gabonensis*

The medicinal significance of *Irvingia gabonensis*, commonly known as African mango, has been widely recognized in traditional African medicine for centuries. Various parts of the plant, including its seeds, bark, and leaves, have been utilized in treating ailments ranging from gastrointestinal disorders to metabolic dysfunctions and inflammatory conditions. In West and Central Africa, traditional healers have used *I. gabonensis* in managing infections, wound healing, and digestive issues. Recent scientific research has provided substantial evidence supporting its therapeutic applications, particularly in metabolic health, anti-inflammatory processes, and digestive functions (Pan *et al*., 2003).

One of the most notable aspects of *I. gabonensis* is its high fiber content and bioactive compounds, which contribute to its broad spectrum of health benefits. These compounds interact with the body’s metabolic and enzymatic systems to regulate blood sugar, reduce inflammation, and promote digestive health (Xu *et al*., 2021). The combination of traditional knowledge and

modern pharmacological studies highlights the potential of *I. gabonensis* as a functional medicinal plant with wide-ranging therapeutic properties.

2.1.1.2.2 Effects on Digestive Health

The role of *I. gabonensis* in digestive health has been widely studied, particularly in relation to its fiber content and interaction with digestive enzymes. The plant's seeds and bark contain significant amounts of soluble and insoluble fiber, which enhance gut motility, regulate bowel movements, and improve overall gastrointestinal function (Doniec *et al*., 2023).

One of the key ways *I. gabonensis* supports digestive health is by modulating enzymatic activity in the digestive tract. Research has demonstrated that its bioactive compounds can influence enzymes responsible for breaking down macronutrients, thereby improving nutrient absorption and reducing the risk of metabolic disorders (Julio-Gonzalez *et al*., 2023). Additionally, the plant's fiber content provides prebiotic benefits, promoting the growth of beneficial gut bacteria and preventing dysbiosis (Chen *et al*., 2023). This modulation of gut microbiota contributes to improved digestion, enhanced immune function, and a reduced risk of gastrointestinal infections (Moré and Vandenplas, 2018).

Moreover, *I. gabonensis* has been shown to have gastroprotective effects, particularly in the management of conditions such as acid reflux, gastritis, and irritable bowel syndrome (IBS). The plant's high mucilage content helps form a protective barrier over the mucosal lining of the stomach, reducing irritation caused by excessive stomach acid and promoting the healing of damaged tissues (Hu *et al*., 2018). These properties make *I. gabonensis* an effective natural remedy for digestive health.

2.1.1.2.3 Metabolic Health and Blood Sugar Regulation

One of the most well-documented medicinal properties of *I. gabonensis* is its ability to regulate blood sugar levels and improve metabolic function. Traditional medicine has long utilized the plant as a remedy for diabetes, and scientific studies have provided substantial evidence supporting its antihyperglycemic effects (Pan *et al*., 2003).

The hypoglycemic properties of *I. gabonensis* stem from its ability to influence carbohydrate digestion and glucose absorption. Studies have shown that extracts from the plant can inhibit key digestive enzymes, such as α-amylase and α-glucosidase, which are responsible for breaking down complex carbohydrates into simple sugars (Sukumaran *et al*., 2016). By slowing down carbohydrate metabolism, *I. gabonensis* helps prevent rapid spikes in blood sugar levels, making it a valuable natural therapy for managing diabetes.

Additionally, *I. gabonensis* has been found to enhance insulin sensitivity, a critical factor in maintaining metabolic health. Research suggests that the plant's bioactive compounds can improve glucose uptake by cells and reduce insulin resistance, thereby lowering the risk of type 2 diabetes (Omiyale *et al*., 2023). These findings highlight the potential of *I. gabonensis* in formulating functional foods and nutraceuticals for metabolic health management.

2.1.1.2.4 Lipid-Lowering and Cardiovascular Benefits

In addition to its effects on blood sugar regulation, *I. gabonensis* has demonstrated significant lipid-lowering properties, making it beneficial for cardiovascular health. The plant's fiber-rich seeds have been shown to reduce cholesterol absorption in the intestines, leading to lower levels of total cholesterol and low-density lipoprotein (LDL) (Pleić *et al*., 2022).

Studies indicate that *I. gabonensis* can influence lipid metabolism by modulating enzyme activity involved in fat breakdown and storage. The plant's bioactive compounds, including flavonoids and polyphenols, have been linked to improved lipid profiles, reduced triglyceride levels, and increased high-density lipoprotein (HDL) levels (Wu *et al*., 2022). These effects contribute to cardiovascular health by reducing the risk of atherosclerosis, hypertension, and other metabolic complications associated with obesity (Grant *et al*., 2015).

Furthermore, the anti-inflammatory properties of *I. gabonensis* play a crucial role in cardiovascular protection. Chronic inflammation is a major contributor to heart disease, and research suggests that the plant's bioactive compounds can reduce inflammatory markers such as C-reactive protein (CRP) and interleukins (Nciri and Cho, 2018). By reducing inflammation and oxidative stress, *I. gabonensis* helps maintain healthy blood vessels and reduces the risk of cardiovascular events.

2.1.1.2.5 Anti-Inflammatory and Antioxidant Properties

The anti-inflammatory and antioxidant properties of *I. gabonensis* have been widely studied, with evidence supporting its role in reducing systemic inflammation and protecting against oxidative damage (Xu *et al*., 2021). Chronic inflammation is a key driver of many diseases, including arthritis, diabetes, and neurodegenerative disorders, and *I. gabonensis* has been shown to mitigate inflammatory responses by modulating cytokine production and inhibiting pro-inflammatory pathways (Moré and Vandenplas, 2018).

The plant's high antioxidant content, including polyphenols and flavonoids, contributes to its ability to neutralize free radicals and reduce oxidative stress (Wu *et al*., 2022). Oxidative stress is a significant factor in aging and the development of chronic diseases, and regular consumption of

antioxidant-rich foods such as *I. gabonensis* has been linked to improved cellular health and longevity (Adedibu *et al*., 2024).

Additionally, *I. gabonensis* has demonstrated neuroprotective properties, with research suggesting that its bioactive compounds can enhance cognitive function, protect against neurodegeneration, and support overall brain health (Julio-Gonzalez *et al*., 2023). These findings highlight the potential of *I. gabonensis* as a functional food for promoting long-term health and preventing age-related cognitive decline.

2.1.1.2.6 Therapeutic Potential and Future Applications

As scientific research continues to explore the medicinal properties of *I. gabonensis*, its potential applications in both preventive and therapeutic medicine are expanding. The plant's diverse health benefits, including its effects on digestion, metabolism, inflammation, and cardiovascular health, position it as a promising candidate for integrative medical approaches.

Further studies are needed to fully elucidate the molecular mechanisms underlying its therapeutic effects and to optimize formulations for clinical use. However, the growing body of evidence supporting its medicinal properties suggests that *I. gabonensis* could be utilized in functional foods, pharmaceuticals, and nutraceuticals to address a range of health concerns.

The medicinal applications of *I. gabonensis* are extensive and well-supported by both traditional knowledge and modern scientific research. Its ability to enhance digestive health, regulate blood sugar levels, reduce inflammation, and support cardiovascular function highlights its importance as a functional food with therapeutic potential. As research continues, *I. gabonensis* may play a crucial role in the development of dietary and medicinal strategies aimed at promoting overall health and disease prevention.

2.1.2 *Hunteria umbellata (Osu)*

Taxonomy

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Gentianales*

Family: *Apocynaceae*

Genus: *Hunteria*

Species: *Hunteria umbellata* (Nciri and Cho, 2018).

Hunteria umbellata is classified within the family *Apocynaceae*, a diverse group of flowering plants known for their medicinal properties. The genus *Hunteria* encompasses several species of medical importance, with *H. umbellata* being particularly significant in traditional medicine. Its taxonomic classification reflects evolutionary relationships within the *Apocynaceae* family, contributing to understanding its biochemical properties and potential therapeutic applications (Nciri and Cho, 2018).

2.1.2.1 Plant Description

H. umbellata manifests as a medium-sized tree, typically reaching heights of 15-25 meters. The plant exhibits distinctive morphological features, including a well-developed root system and characteristic branching patterns. The leaves are simple, opposite, and broadly elliptic, with a

leathery texture and prominent venation. The inflorescence is umbellate, giving rise to the species epithet, and produces fruits that are paired follicles containing numerous seeds. The plant's structural organization reflects adaptations to its native habitat while supporting its various ecological and medicinal functions (Messina *et al*., 2019).

2.1.2.2 Medicinal Uses

2.1.2.2.1 Traditional and Contemporary Perspectives o*n H. umbellata*

The medicinal significance of *H. umbellata* has been recognized for centuries in traditional healing practices, particularly in the management of gastrointestinal disorders, metabolic imbalances, and inflammatory conditions. The plant has been widely used in ethnomedicine to treat ailments such as indigestion, diarrhea, and parasitic infections, often prepared as herbal infusions or decoctions. Recent scientific investigations have begun to validate these traditional claims, uncovering the bioactive compounds responsible for the plant's therapeutic effects (Fang *et al*., 2016).

A significant aspect of *H. umbellata*'s medicinal relevance lies in its influence on digestive enzyme activity, which plays a fundamental role in nutrient breakdown and absorption (Doniec *et al*., 2023). Studies have shown that extracts from *H. umbellata* can modulate enzymatic function, thereby enhancing digestion and potentially alleviating conditions linked to enzyme deficiencies or malabsorption syndromes. This has opened new avenues for its use in both preventive and therapeutic applications, particularly in addressing metabolic disorders such as diabetes and obesity (Gu *et al*., 2016).

2.1.2.2.2 Effects on Digestive Health

One of the most well-documented uses of *H. umbellata* is in the promotion of digestive health. The plant's bioactive compounds have been found to exert a protective effect on the intestinal epithelium, reducing the risk of damage from dietary toxins and microbial infections (Babot *et al*., 2021). Research indicates that *H. umbellata* extracts can enhance gut barrier integrity, thereby improving overall gastrointestinal function and reducing susceptibility to enteric diseases (Messina *et al*., 2019).

Furthermore, the plant has demonstrated significant effects on gut microbiota composition. Studies suggest that its phytochemical constituents support the proliferation of beneficial gut bacteria while inhibiting pathogenic strains, leading to a more balanced microbial environment (Chen *et al*., 2023). This is particularly beneficial in cases of dysbiosis, where an imbalance in gut flora contributes to conditions such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). The modulation of gut microbiota by *H. umbellata* has been linked to improved digestion, enhanced nutrient absorption, and reduced intestinal inflammation (Hu *et al*., 2018).

The influence of *H. umbellata* on digestive physiology is further underscored by its modulation of intestinal enzyme kinetics. Research indicates that extracts from the plant significantly alter the activity of brush border enzymes, which serve as the primary mediators for nutrient hydrolysis and absorption within the small intestine (Julio-Gonzalez *et al*., 2023). By regulating these critical catalysts, *H. umbellata* emerges as a viable therapeutic agent for addressing metabolic disorders characterized by enzymatic insufficiency, including lactose intolerance and the malabsorptive challenges associated with celiac disease (Moré & Vandenplas, 2018).

2.1.2.2.3 Metabolic Benefits and Blood Sugar Regulation

Beyond its effects on digestive processes, *H. umbellata* has garnered attention for its potential role in metabolic regulation, particularly in the management of diabetes and obesity. Research has shown that the plant's extracts can influence carbohydrate metabolism by modulating enzyme activity related to glucose absorption and insulin sensitivity (Pan *et al*., 2003). This is particularly relevant in the context of diabetes, where impaired glucose regulation leads to elevated blood sugar levels and associated complications.

The antihyperglycemic effects of *H. umbellata* have been attributed to its ability to inhibit certain digestive enzymes involved in carbohydrate breakdown, such as α-glucosidase. By reducing the rate of carbohydrate digestion and absorption, the plant helps maintain stable blood glucose levels, thus offering a natural approach to glycemic control (Sukumaran *et al.*, 2016). These findings align with traditional uses of *H. umbellata* in managing diabetes-related symptoms, further supporting its potential as a complementary therapy in metabolic disorders.

Additionally, *H. umbellata* has demonstrated potential benefits in lipid metabolism. Some studies indicate that its bioactive compounds may help regulate cholesterol levels and prevent lipid accumulation, reducing the risk of obesity-related complications (Pleić *et al*., 2022). The plant's ability to influence metabolic pathways suggests that it may play a role in weight management, making it a promising candidate for inclusion in functional foods or dietary supplements aimed at supporting metabolic health.

2.1.2.2.4 Anti-Inflammatory and Immune-Boosting Properties

The therapeutic applications of *H. umbellata* extend beyond digestion and metabolism to include notable anti-inflammatory effects. Chronic inflammation has been implicated in a wide range of diseases, including autoimmune disorders, cardiovascular conditions, and neurodegenerative diseases. Studies have shown that *H. umbellata* extracts possess significant anti-inflammatory activity, which may be mediated through their ability to modulate cytokine production and oxidative stress markers (Xu *et al*., 2021).

The plant's anti-inflammatory properties are particularly relevant in the context of gastrointestinal health, where excessive inflammation can contribute to disorders such as ulcerative colitis and Crohn's disease (Nciri and Cho, 2018). By reducing inflammation in the intestinal lining, *H. umbellata* may help alleviate symptoms associated with these conditions, improving overall gut function and quality of life for affected individuals.

Furthermore, the immunomodulatory effects of *H. umbellata* have been highlighted in studies examining its influence on immune cell function. Certain bioactive compounds in the plant appear to enhance the body's natural defense mechanisms, supporting immune resilience against infections and inflammatory diseases (Omiyale *et al*., 2023). This suggests that *H. umbellata* could be useful not only as a treatment for existing conditions but also as a preventive measure to bolster immune health.

2.1.2.2.5 Neuroprotective and Cognitive Benefits

Emerging research has also pointed to potential neuroprotective effects of *H. umbellata*, particularly in the context of cognitive function and neurodegenerative diseases. The plant's anti-inflammatory and antioxidant properties have been suggested to play a role in protecting

neuronal integrity, reducing oxidative stress, and mitigating neuroinflammatory responses (Wu *et al*., 2022). These mechanisms are of particular interest in the study of conditions such as Alzheimer's disease and Parkinson's disease, where chronic inflammation and oxidative damage contribute to neuronal degeneration.

Additionally, preliminary evidence suggests that *H. umbellata* may influence neurotransmitter activity, potentially offering benefits in mood regulation and mental health. Certain plant-derived compounds have been found to interact with neurochemical pathways involved in stress response and cognitive function, raising the possibility of its use in managing anxiety, depression, and age-related cognitive decline (Adedibu *et al*., 2024).

2.1.2.2.6 Potential for Therapeutic Development

The growing body of research on *H. umbellata* has underscored its potential for further therapeutic development. As scientific understanding of its bioactive compounds deepens, there is increasing interest in exploring its applications in pharmaceutical and nutraceutical formulations. The plant's diverse medicinal properties, spanning digestive health, metabolic regulation, anti-inflammatory activity, and neuroprotection, position it as a valuable candidate for integrative medicine (Adedibu *et al*., 2024).

Further research is needed to elucidate the precise mechanisms underlying its therapeutic effects and to optimize extraction and formulation techniques for clinical use. However, the convergence of traditional knowledge and modern scientific validation suggests that *H. umbellata* holds significant promise in the development of novel treatments for a range of health conditions (Grant *et al.,* 2015).

The medicinal applications of *H. umbellata* are extensive and well-supported by both traditional practices and emerging scientific evidence. Its influence on digestive enzymes, metabolic regulation, inflammatory responses, and immune function highlights its potential as a versatile therapeutic agent. As research continues to advance, *H. umbellata* may find increasing integration into modern medical and dietary approaches, offering natural solutions for managing digestive disorders, metabolic diseases, inflammatory conditions, and neurodegenerative disorders (Omiyale *et al*., 2023).

2.1.3 *Abelmoschus esculentus* (Okra)

Taxonomy

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Malvales*

Family: *Malvaceae*

Genus: *Abelmoschus*

Species: *Abelmoschus esculentus* (Doniec *et al*., 2023).

Abelmoschus esculentus, commonly known as okra or lady's finger, belongs to the family *Malvaceae*. The taxonomic history of this species reflects several revisions, having been previously classified under the genus *Hibiscus*. This reclassification was based on detailed morphological studies and molecular evidence, highlighting the distinct characteristics that

separate *Abelmoschus* from related genera. The current taxonomic placement provides important insights into the plant's evolutionary relationships and biochemical properties (Doniec *et al*., 2023).

.2.1.3.1 Plant Description

A. esculentus is characterized as an annual, erect herb reaching heights of 1-2 meters. The plant exhibits a robust stem structure with large, palmate leaves displaying distinct lobing patterns. The flowers are conspicuous, with five petals typically showing a pale yellow color with a dark center. The fruit, which is the most economically important part, is an elongated capsule containing numerous seeds arranged in longitudinal rows. The plant's morphological features, particularly its fruit structure, contribute to its significance in both nutritional and medicinal applications (Kokou *et al*., 2016).

2.1.3.2 Medicinal Uses

2.1.3.2.1 Traditional and Scientific Perspectives on *A. esculentus*

The medicinal significance of *A. esculentus* (okra) has been widely recognized in traditional medicine systems, particularly in the treatment of digestive ailments, metabolic disorders, and inflammatory conditions. Various cultures have utilized its mucilaginous properties to soothe gastrointestinal distress, regulate blood sugar levels, and promote overall health. Modern scientific investigations have begun to validate these traditional claims, revealing bioactive compounds in *A. esculentus* that contribute to its therapeutic effects (Babot *et al*., 2021).

One of the defining characteristics of *A. esculentus* is its high mucilage content, which plays a crucial role in digestive health. This gelatinous substance, rich in polysaccharides, interacts with

the gut lining to enhance nutrient absorption and protect against irritation and inflammation (Doniec *et al*., 2023). In addition, the plant's fiber composition has been associated with improved gut motility, reduced cholesterol absorption, and enhanced microbial balance, all of which contribute to its broad therapeutic potential (Chen *et al*., 2023).

2.1.3.2.2 Effects on Digestive Health

The digestive benefits of *A. esculentus* stem from its ability to regulate enzymatic activity, enhance intestinal integrity, and modulate gut microbiota. Studies have demonstrated that okra components can influence digestive enzymes responsible for breaking down macronutrients, thereby improving nutrient utilization and reducing the risk of malabsorption disorders (Julio-Gonzalez *et al*., 2023).

Furthermore, the mucilage in *A. esculentus* serves as a natural demulcent, forming a protective layer over the mucosal lining of the digestive tract. This property is particularly beneficial in the management of conditions such as gastritis, acid reflux, and peptic ulcers, where excessive stomach acid can cause irritation and erosion of the stomach lining (Hu *et al*., 2018). The protective effect of okra on the gastrointestinal tract has been linked to its ability to enhance mucus production, neutralize harmful substances, and support the healing of damaged tissues (Messina *et al*., 2019).

Additionally, *A. esculentus* plays a role in gut microbiota modulation. Its fiber content provides a substrate for beneficial gut bacteria, fostering a balanced microbial environment and preventing dysbiosis (Chen *et al*., 2023). By promoting the growth of probiotics such as Lactobacillus and Bifidobacterium, okra contributes to improved digestion, enhanced immune function, and reduced susceptibility to gastrointestinal infections (Moré and Vandenplas, 2018).

2.1.3.2.3 Blood Sugar Regulation and Metabolic Benefits

The role of *A. esculentus* in metabolic health has been widely studied, particularly in relation to its effects on blood sugar regulation and lipid metabolism. Traditional medicinal practices have long utilized okra as a natural remedy for diabetes, and recent scientific research has provided evidence supporting its antihyperglycemic properties (Pan *et al*., 2003).

One of the primary mechanisms through which *A. esculentus* exerts its blood sugar-lowering effects is by modulating carbohydrate digestion and absorption. Studies have shown that okra extracts can inhibit α-glucosidase and α-amylase enzymes, which are responsible for breaking down carbohydrates into simple sugars (Sukumaran *et al*., 2016). By slowing the rate of glucose release into the bloodstream, okra helps maintain stable blood sugar levels and reduces postprandial hyperglycemia, making it a valuable dietary component for diabetes management.

Additionally, *A. esculentus* has demonstrated beneficial effects on lipid metabolism. Research indicates that its fiber-rich composition can reduce cholesterol absorption in the intestines, thereby lowering total cholesterol and low-density lipoprotein (LDL) levels (Pleić *et al*., 2022). This lipid-lowering effect contributes to cardiovascular health by reducing the risk of atherosclerosis, hypertension, and other metabolic complications associated with obesity and diabetes.

Moreover, the bioactive compounds in *A. esculentus* have been found to enhance insulin sensitivity, a key factor in the prevention and management of type 2 diabetes. The presence of flavonoids, polyphenols, and polysaccharides in okra has been linked to improved glucose uptake by cells and reduced insulin resistance, further supporting its role in metabolic regulation (Omiyale *et al*., 2023).

2.1.3.2.4 Anti-Inflammatory and Antioxidant Properties

Inflammation is a central factor in the development of many chronic diseases, including diabetes, cardiovascular disease, and neurodegenerative conditions. *A. esculentus* has been widely studied for its anti-inflammatory and antioxidant properties, which contribute to its therapeutic potential in various inflammatory disorders (Xu *et al*., 2021).

The anti-inflammatory effects of okra are attributed to its ability to modulate cytokine production and inhibit pro-inflammatory signaling pathways. Research has shown that okra extracts can reduce the expression of inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), thereby mitigating inflammatory responses in conditions such as arthritis and inflammatory bowel disease (Nciri and Cho, 2018).

Furthermore, *A. esculentus* contains high levels of antioxidants, including flavonoids, polyphenols, and vitamin C, which help neutralize free radicals and reduce oxidative stress (Wu *et al*., 2022). Oxidative stress plays a key role in aging and the development of chronic diseases, and the consumption of antioxidant-rich foods such as okra has been associated with improved cellular health and longevity (Adedibu *et al*., 2024).

The antioxidant activity of *A. esculentus* also extends to cardiovascular protection. Studies suggest that its bioactive compounds can improve endothelial function, reduce oxidative damage to blood vessels, and lower the risk of hypertension and atherosclerosis (Grant *et al*., 2015). These findings highlight the potential of okra as a functional food for cardiovascular health maintenance.

2.1.3.2.5 Neuroprotective and Cognitive Benefits

Emerging research suggests that *A. esculentus* may also play a role in neuroprotection and cognitive health. The plant's anti-inflammatory and antioxidant properties have been linked to reduced neuroinflammation, improved neuronal function, and enhanced cognitive performance (Wu *et al*., 2022).

Chronic inflammation and oxidative stress are key contributors to neurodegenerative diseases such as Alzheimer's and Parkinson's. The bioactive compounds in *A*. *esculentus* have been found to protect against neurotoxic damage by inhibiting pro-inflammatory markers and enhancing antioxidant defenses in brain tissues (Adedibu *et al*., 2024). These effects suggest that okra could be beneficial in delaying the onset or progression of age-related cognitive decline.

Moreover, some studies have indicated that *A. esculentus* may influence neurotransmitter activity, potentially contributing to mood stabilization and mental well-being. Certain phytochemicals in the plant have been found to interact with serotonin and dopamine pathways, which are critical for regulating mood, anxiety, and stress response (Julio-Gonzalez *et al*., 2023). This raises the possibility of *A. esculentus* as a dietary supplement for supporting mental health and cognitive function.

2.1.3.2.6 Therapeutic Potential and Future Applications

As scientific research continues to explore the medicinal properties of *A. esculentus*, its potential applications in both preventive and therapeutic medicine are expanding. The plant's diverse health benefits, including its effects on digestion, metabolism, inflammation, and cognitive health, position it as a promising candidate for integrative medical approaches (Wu *et al.,* 2022).

Further studies are needed to fully elucidate the molecular mechanisms underlying its therapeutic effects and to optimize formulations for clinical use. However, the growing body of evidence supporting its medicinal properties suggests that *A. esculentus* could be utilized in functional foods, pharmaceuticals, and nutraceuticals to address a range of health concerns (Omiyale *et al*., 2023).

The medicinal applications of *A. esculentus* are vast and well-supported by both traditional practices and modern scientific research. Its ability to enhance digestive health, regulate blood sugar levels, reduce inflammation, and support cognitive function highlights its importance as a functional food with therapeutic potential. As research continues, *A. esculentus* may become an integral component of dietary and medicinal strategies aimed at promoting overall health and disease prevention (Kokou *et al.,* 2016).

2.2 Small Intestinal Enzymes

The small intestinal mucosa represents a complex enzymatic environment crucial for nutrient digestion and absorption. The orchestrated activities of various enzymes, particularly disaccharidases and alkaline phosphatase, facilitate efficient nutrient processing and maintain intestinal homeostasis. Understanding these enzyme systems is essential for appreciating how plant fibers might influence digestive processes and overall gastrointestinal health (Grant *et al*., 2015).

2.2.1 Disaccharidases

The role of disaccharidases in carbohydrate digestion represents a fundamental aspect of nutritional physiology. These enzymes, located in the brush border membrane of intestinal epithelial cells, catalyze the final steps in carbohydrate digestion, converting disaccharides into

their constituent monosaccharides for absorption. Research has demonstrated that their activity can be modulated by various dietary components, including plant-derived substances (Julio-Gonzalez *et al.*, 2023).

2.2.1.1 Maltase

Maltase plays a crucial role in starch digestion, hydrolyzing maltose into glucose molecules. Recent studies have shown that maltase activity can be significantly influenced by dietary components, including plant fibers and associated bioactive compounds. The regulation of maltase activity has important implications for glucose absorption and overall carbohydrate metabolism. Research has demonstrated that various plant-derived substances can modulate maltase activity, potentially affecting glucose homeostasis and metabolic health (Pleić *et al.*, 2022).

Studies investigating the effects of plant fibers on maltase activity have revealed complex interactions that may contribute to their therapeutic properties. For instance, research has shown that certain plant fiber components can influence maltase expression and activity through both direct and indirect mechanisms. These findings have important implications for understanding how traditional medicinal plants might influence carbohydrate digestion and metabolism (Sukumaran *et al.*, 2016).

2.2.1.2 Lactase

Lactase, responsible for hydrolyzing lactose into glucose and galactose, represents another crucial disaccharidase in the small intestine. Research has shown that lactase activity can be modulated by various dietary factors, including plant-derived compounds. Understanding these

interactions is particularly important given the prevalence of lactose intolerance and the potential role of plant-based interventions in managing this condition (Moré and Vandenplas, 2018).

Recent investigations have revealed that plant fibers can influence lactase activity through multiple mechanisms. These effects may involve direct enzyme interactions, changes in gene expression, or alterations in the intestinal environment. The modulation of lactase activity by plant components has important implications for both nutritional science and therapeutic applications (Wu *et al*., 2022).

2.2.1.3 Sucrase

Sucrase, essential for the digestion of dietary sucrose, has been shown to respond to various dietary components, including plant-derived substances. Research has demonstrated that sucrase activity can be modulated by specific plant compounds, potentially affecting sugar digestion and absorption. These findings have important implications for understanding how traditional medicinal plants might influence carbohydrate metabolism (Xu *et al*., 2021).

2.2.2 Alkaline Phosphatase

Intestinal alkaline phosphatase (IAP) represents a crucial enzyme in maintaining gastrointestinal homeostasis and function. This multifaceted enzyme plays essential roles beyond its classical phosphate-hydrolyzing activity, contributing to various aspects of intestinal health and metabolic regulation. Recent research has significantly expanded our understanding of IAP's functions and its interactions with dietary components, particularly plant-derived substances (Adedibu *et al*., 2024).

Studies have demonstrated that IAP activity can be modulated by various dietary factors, including plant fibers and their associated bioactive compounds. The enzyme's expression and activity patterns respond to changes in the intestinal environment, making it an important target for dietary interventions. Research has shown that plant-derived compounds can influence IAP activity through multiple mechanisms, potentially contributing to their therapeutic effects (Wu *et al*., 2022).

The significance of IAP in intestinal barrier function and inflammatory regulation has been increasingly recognized. Studies have shown that the enzyme plays crucial roles in maintaining gut barrier integrity and modulating local immune responses. These functions make IAP an important consideration in understanding how plant fibers might influence intestinal health beyond their direct effects on nutrient digestion (Omiyale *et al*., 2023).

2.3 Plant Fibers and Enzyme Interactions

2.3.1 Mechanisms of Plant Fiber Effects on Intestinal Enzymes

The interaction between plant fibers and intestinal enzymes involves complex mechanisms that extend beyond simple physical effects. Research has revealed multiple pathways through which plant fibers can influence enzyme activity and expression. These mechanisms include direct enzyme-substrate interactions, modulation of gene expression, and alterations in the intestinal microenvironment (Chen *et al*., 2023).

Studies investigating the molecular basis of these interactions have shown that specific plant compounds can bind to enzyme proteins, potentially affecting their catalytic activity or stability. Additionally, research has demonstrated that plant fibers can influence enzyme expression through various signaling pathways, leading to changes in enzyme levels and activity patterns.

These findings provide important insights into how traditional medicinal plants might achieve their therapeutic effects (Messina *et al*., 2019).

2.3.2 Structural Changes in Intestinal Mucosa

Plant fibers have been shown to influence the structural organization of the intestinal mucosa, potentially affecting enzyme distribution and activity. Research has demonstrated that exposure to certain plant fibers can lead to changes in brush border architecture and enterocyte differentiation. These structural modifications may contribute to altered enzyme activities and digestive functions (Gu *et al*., 2016).

Studies examining the effects of plant fibers on intestinal morphology have revealed significant impacts on villus height, crypt depth, and epithelial cell differentiation. These structural changes can affect the distribution and accessibility of brush border enzymes, potentially influencing their functional capacity. Understanding these structural modifications is crucial for appreciating how plant fibers might influence digestive processes (Kokou *et al*., 2016).

2.3.3 Enzyme Activity Modulation

The modulation of enzyme activity by plant fibers involves multiple mechanisms that can vary depending on the specific fiber type and concentration. Research has shown that different plant fibers can exhibit distinct effects on enzyme activities, potentially leading to varied physiological outcomes. These differences may explain the diverse therapeutic applications of different medicinal plants (Fang *et al*., 2016).

Studies have demonstrated that plant fibers can influence enzyme kinetics through both direct and indirect mechanisms. Direct effects may involve physical interactions between fiber

components and enzyme proteins, while indirect effects might occur through changes in the intestinal environment or signaling pathways. Understanding these mechanisms is essential for optimizing the therapeutic use of plant-based interventions (Pan *et al*., 2003).

2.4 Physiological Implications

2.4.1 Nutrient Absorption

The effects of plant fibers on intestinal enzyme activities have important implications for nutrient absorption and metabolism. Research has shown that modulation of enzyme activities can influence the efficiency of nutrient digestion and uptake, potentially affecting overall nutritional status. Understanding these effects is crucial for developing targeted dietary interventions (Julio-Gonzalez *et al*., 2023).

Studies investigating the impact of plant fibers on nutrient absorption have revealed complex relationships between enzyme activity modulation and nutrient uptake patterns. These interactions can affect various aspects of nutrient metabolism, including glucose absorption, lipid processing, and protein digestion. The implications of these effects extend beyond simple nutritional considerations to include potential therapeutic applications (Sukumaran *et al*., 2016).

2.4.2 Intestinal Barrier Function

The relationship between plant fibers, intestinal enzymes, and barrier function represents a critical aspect of gastrointestinal health. Research has demonstrated that enzyme activities, particularly those of alkaline phosphatase, play essential roles in maintaining barrier integrity. Plant fibers have been shown to influence these processes through multiple mechanisms, potentially contributing to their therapeutic effects (Moré and Vandenplas, 2018).

Studies investigating the impact of plant fibers on intestinal barrier function have revealed significant effects on tight junction proteins and epithelial integrity. The modulation of enzyme activities by plant fibers can influence these barrier components, potentially affecting intestinal permeability and immune responses. These findings have important implications for understanding how traditional medicinal plants might influence gastrointestinal health (Wu *et al*., 2022).

Recent research has highlighted the role of intestinal alkaline phosphatase in maintaining barrier function through its effects on local inflammation and microbial interactions. Plant fibers have been shown to influence these processes, potentially contributing to their beneficial effects on intestinal health. Understanding these mechanisms is crucial for developing targeted therapeutic approaches (Adedibu *et al*., 2024).

2.4.3 Metabolic Effects

The influence of plant fibers on intestinal enzyme activities has broader implications for metabolic health. Research has demonstrated that modulation of enzyme activities can affect various metabolic processes, including glucose homeostasis, lipid metabolism, and energy balance. These effects may contribute to the observed metabolic benefits of plant-based interventions (Xu *et al*., 2021).

Studies examining the metabolic impact of plant fiber-enzyme interactions have revealed complex relationships between enzyme activity modulation and systemic metabolism. These interactions can influence various aspects of metabolic regulation, including insulin sensitivity, lipid profiles, and energy expenditure. Understanding these relationships is essential for developing effective therapeutic strategies (Pan *et al*., 2003).

The role of intestinal enzymes in metabolic regulation extends beyond their direct effects on nutrient processing. Research has shown that these enzymes can influence metabolic signaling pathways and hormone responses, potentially contributing to the systemic effects of plant fiber consumption. These findings highlight the importance of considering enzyme-mediated effects in understanding the therapeutic potential of plant-based interventions (Chen *et al*., 2023).

2.5 Clinical Applications

2.5.1 Therapeutic Potential

The understanding of plant fiber effects on intestinal enzymes has important implications for therapeutic applications. Research has demonstrated potential benefits in various conditions, including metabolic disorders, inflammatory conditions, and gastrointestinal diseases. The modulation of enzyme activities by plant fibers offers promising approaches for developing targeted interventions (Messina *et al*., 2019).

Studies investigating the therapeutic applications of plant fiber-enzyme interactions have revealed potential benefits in managing various health conditions. These applications extend from traditional uses to novel therapeutic approaches based on modern understanding of enzyme regulation. The development of targeted interventions requires careful consideration of specific fiber types and their effects on particular enzyme systems (Grant *et al*., 2015).

Research has shown that different plant fibers can exhibit distinct therapeutic effects through their influence on enzyme activities. Understanding these specific effects is crucial for optimizing therapeutic applications and developing personalized interventions. The combination of traditional knowledge and modern scientific understanding offers promising approaches for treating various conditions (Gu *et al*., 2016).

2.5.2 Dietary Considerations

The implementation of plant fiber-based interventions requires careful consideration of dietary factors and individual responses. Research has demonstrated that the effects of plant fibers on enzyme activities can vary depending on various factors, including fiber type, concentration, and individual physiological conditions (Kokou *et al*., 2016).

Studies examining dietary applications have highlighted the importance of considering both direct and indirect effects of plant fibers on enzyme activities. These considerations include potential interactions with other dietary components, timing of consumption, and individual metabolic responses. Understanding these factors is essential for developing effective dietary recommendations (Fang *et al*., 2016).

The development of dietary interventions based on plant fiber-enzyme interactions requires careful attention to dosage, timing, and individual factors. Research has shown that optimal benefits may require personalized approaches considering specific health conditions and therapeutic goals. These considerations highlight the importance of evidence-based approaches in developing dietary recommendations (Julio-Gonzalez *et al*., 2023).

2.6 Future Directions and Research Needs

The understanding of plant fiber effects on intestinal enzymes continues to evolve, revealing new opportunities for research and therapeutic development. Current evidence suggests several promising directions for future investigation, including:

The need for detailed mechanistic studies examining specific interactions between plant fiber components and enzyme systems has been highlighted by recent research. Understanding these

mechanisms could lead to more effective therapeutic applications and improved dietary recommendations (Sukumaran *et al*., 2016).

Investigation of individual variations in response to plant fiber interventions represents an important area for future research. Understanding factors that influence individual responses could help optimize therapeutic approaches and improve treatment outcomes (Babot *et al*., 2021).

The development of targeted interventions based on specific plant fiber-enzyme interactions offers promising opportunities for therapeutic advancement. Research in this area could lead to more effective treatments for various health conditions while providing scientific support for traditional medicinal applications (Doniec *et al*., 2023).

Integration of traditional knowledge with modern scientific understanding continues to reveal new insights into plant fiber effects on health. This approach offers valuable opportunities for developing evidence-based interventions while preserving traditional medicinal knowledge (Omiyale *et al*., 2023).

The investigation of novel plant sources and their effects on intestinal enzymes represents an ongoing opportunity for discovery. Research in this area could lead to the identification of new therapeutic agents and improved understanding of traditional medicines (Pleić *et al*., 2022).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and Reagents

The following are the chemicals and reagents used for this study, as well as their manufacturer, country name and percentage purity, stated respectively.

- Diethyl ether (TKM Pharma, India), ≥ 99.0 %

- Sodium chloride (Weifang Xinchang Chemical, China), ≥ 99.5 %

- Sodium phosphate dibasic heptahydrate (Sigma-Aldrich, US), ≥ 98.0 %

- Sodium phosphate monobasic monohydrate (Sigma-Aldrich, US), ≥ 98.0 %

- Perchloric acid (Sigma-Aldrich, US), 60.0 % – 70.0 %

- Hydrochloric acid (Sigma-Aldrich, US), 36.5 % – 38.0 %

- 3,5-Dinitrosalicylic acid (Sigma-Aldrich, US), ≥ 98.0 %

- Sodium acetate (Sigma-Aldrich, US), ≥ 99.0 %

- Acetic acid (Sigma-Aldrich, US), ≥ 99.7 %

- Tris-HCl (Sigma-Aldrich, US), ≥ 99.0 %

- Eco-Pak glucose reagent (S/N: GL-2009-03-001, Accurex Biomedical PVT. LTD, India) containing:

- Phosphate buffer (pH 7.0, 120 mmol/l)
- Glucose oxidase (≥ 5000 IU/l)
- Peroxidase (1050 IU/l)
- 4-aminoantipyrine (0.20 mmol/l)
- Phenol (11 mmol/l)

- Total Protein reagent (ChemCHEK AGAPPE, India)
- Alkaline phosphatase reagents:
  - p-Nitrophenyl phosphate (PNPP) (Sigma-Aldrich, US), ≥ 99.0 %
  - Carbonate buffer (Sigma-Aldrich, US)
  - Sodium hydroxide (Sigma-Aldrich, US), ≥ 97.0 %

Substrates:

- Maltose (Sigma-Aldrich, US), ≥ 98.0 %
- Lactose (Sigma-Aldrich, US), ≥ 99.0 %
- Sucrose (Sigma-Aldrich, US), ≥ 99.5 %

3.1.2 Equipment

The following are the equipments with their model name, manufacturer and country name, that were utilised during this study.

- Analytical balance (Sartorius BSA224S-CW, Germany)

- Digital pH meter (Mettler Toledo SevenCompact™, Switzerland)

- Drying oven (DHG-9030A) from the Department of Biochemistry, University of Benin.

- Grinder (Vikino) from the Department of Pharmacognosy, University of Benin.

- Laboratory refrigerator (Thermo Scientific™, USA)

- Magnetic stirrer with hot plate (Stuart™ SB162, UK)

- Refrigerated Centrifuge (Eppendorf 5810R, Germany) from the Department of Biochemistry, University of Benin.

- Spectrophotometer (UV-Visible Spectrophotometer, Shimadzu UV-1800, Japan)

- Temperature-controlled Water bath (Memmert WNB14, Germany)

- Vortex mixer (IKA® Vortex 3, Germany)

3.1.3 Animals

Male Wistar albino rats (weight range 27-40 g, 54 in number), were obtained from the institutional animal facility, the department of Biochemistry, University of Benin. The animals were housed in individual cages under standard laboratory conditions with 12-hour light/dark cycles. They were acclimatized for three days on standard growers pellet feed before the experimental period. Animal handling and experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals.

3.1.4 Plant Material

The following plant materials, Bush mango (*Irvingia gabonensis*), Osu (*Hunteria umbellata*), and Okra (*Abelmoschus esculentus*) were obtained from a market in Evbuotubu village, Edo State, thoroughly washed with running water, sliced into tiny pieces and spread to air-dry for 21 days.

The plant samples were identified at the Department of Plant Biology and Biotechnology in the University of Benin and voucher numbers were assigned to each of the plant samples as stated below:

- Bush mango (*Irvingia gabonensis*): UBH-1153

- Osu (*Hunteria umbellata*): UBH-H637

- Okra (*Abelmoschus esculentus*): UBH-A399

3.2 METHODS

3.2.1 Animal Grouping and Experimental Design

The rats were randomly divided into nine (9) groups of six (6) animals each:

C1 – Control 1 (Fiber free group)

C2 – Control 2 (Fybogel at level of 1.5 %)

C2 – Control 2 (Fybogel at level of 3.0 %)

Bush mango – 5.0 %

Bush mango – 10 .0 %

Osu – 5.0 %

Osu – 10.0 %

Okra – 5.0 %

Okra – 10.0 %

The animals were fed ad libitum on their respective diets for 36 days following the 3-day acclimatization period.

3.2.2 Diet Preparation

Formulation of the compounded diet

The formulated diet contained 10 % sucrose/100 % and 10 % protein (egg albumin)/100 %. The fiber source was incorporated at 5 %/100 % and 10 %/100 % for each of the plant samples, bush mango, osu and okra respectively. Other dietary ingredients such as vitamin mixture, mineral mixture, and oil) were added as per standards for nutritional studies (AIN And Hoc Committee, 1977). Each of the components for the respective groups were measured by multiplying each of their percentage composition by a factor of 20, except for the carbohydrate source which was measured upon addition of all the components concentrations, and then subtracted from 2000 to yield the value for each of the respective carbohydrate source, so as to obtain a total mixture of 2000 % as represented in the table below:

For group 5.0 % Bush mango, starch (carbohydrate source) was calculated as:

= Total % of formulated diet - (% protein + % oil + % vitamin + % mineral + % fiber source + % sucrose)

= 2000 - (% egg albumin + % palm oil + % vitamin mixture + % mineral mixture + % fiber source + % sucrose)

= 2000 - (200 + 200 + 20 + 80 + 100 + 200)

= 2000 - (800)

= 2000 - 800

= 1200 %

1200 % of starch (carbohydrate source) was incorporated in the diet. The same calculation was applied for the other groups.

Compounded Diet Formulation Table

Components (%)	C1 Fiber free	C2 Fybogel 1.5	C2 Fybogel 3.0	Bush mango 5.0	Bush mango 10.0	Okra 5.0	Okra 10.0	Osu 5.0	Osu 10.0
Egg Albumin	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00
Starch	1,300.00	1,270.00	1,240.00	1,200.00	1,100.00	1,200.00	1,100.00	1,200.00	1,100.00
Palm Oil	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00
Vitamin mixture	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Mineral mixture	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00
Fiber source	-	30.00	60.00	100.00	200.00	100.00	200.00	100.00	200.00
Sucrose	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00
TOTAL (%)	2,000.00	2,000.00	2,000.00	2,000.00	2,000.00	2,000.00	2,000.00	2,000.00	2,000.00

3.2.3 Sample Preparation

The mixed plant samples were transported to the Lish Pellet Factory in Benin City, Edo State, for processing. There, the plant material was compressed into uniform pellets to improve palatability and facilitate easier digestion for the rats.

3.2.4 Proximate Analysis of the Plant materials

The samples were oven dried at 60°C for 24 hours and allowed to cool, and was processed for proximate content determination by passing the sample through a grinder a sufficient number of times to obtain a uniform mixture.

Determination of moisture content

The moisture content of plant samples were determined using A.O.A.C (2000) method (The gravimetric method).

Principle

The moisture content is determined from the difference in weight after complete evaporation of moisture.

Procedure

One gram of each of the plant samples were weighed in crucibles and oven dried at 105°C to a constant weight. The samples were cooled in a dessicator and weighed.

Moisture loss = initial weight– final weight (weight after drying)

Calculation

$$\%moisture = \frac{\text{loss in weight (g) x 100}}{\text{weight of sample (g)}}$$

Determination of crude protein content

Nitrogen was determined using the micro-Kjeldahl method (A.O.A.C 2000) and crude protein content was subsequently calculated by multiplying the nitrogen content by a factor of 6.25.

Principle

Proteins and other food components are digested with sulphuric acid in the presence of catalysts. Ammonium sulphate is produced from the total organic nitrogen in the food. Alkali is then used to neutralize the acid digest to produce ammonium which is steam distilled directly into a hydrochloric acid containing the indicator methylene red. Hydrochloric acid and ammonium react to produce ammonium chloride which is then titrated with sodium hydroxide. A blank determination is run to determine the nitrogen content of the reagents. The percentage nitrogen is calculated. The percent nitrogen multiplied by the conversion factor for that particular food produces the percent protein in a food.

Procedure

One millilitre of 4 % $CuSO_4$, H_2SO_4, and 0.8 g of K_2SO_4 were placed in the micro kjeldahl flask. One gram of each plant samples was added to the reagents in micro Kjeldahl flask. It was digested first at low temperature until frosting ceases, then at a high temperature, until the solution was clear, pale yellow or light blue. The flask was left to cool, and 4 ml of distilled water was gradually added and the content were distilled using a Kjeldahl distillation apparatus. Ten millilitres of 30 % NaOH was used to liberate during distillation of ammonium. Ammonium was collected in 0.01 M HCl (a drop of methylene red was added to the HCl). The distillate was titrated with 0.01 M NaOH. Nitrogen content of samples was calculated from the volume of HCl neutralized by NaOH.

Calculations

$$\%\text{nitrogen} = \text{titre value (blank)} - \frac{\text{titre value (distillate)} \times 0.14}{\text{weight of sample (g)}}$$

Protein = % nitrogen x 6.25

$$\% \text{ protein} = \frac{\text{protein x 100}}{\text{Initial weight (g)}}$$

Where 0.14 = dilution factor

6.25 = protein conversion factor

Determination of lipid content

Crude fat was determined using Soxhlet extraction A.O.A.C (2000).

Principle

The free lipid content consists of neutral fats (triglycerides) and free fatty acid was determined by extracting the dried and ground material with diethyl ether in a continuous extraction apparatus (Soxhlet extractor).

Procedure

The weight of an empty flask was determined. One gram of sample was wrapped and placed in an extraction thimble. The thimble was plugged with cotton wool to avoid loss of sample. The thimble was placed in the extractor. An already weighed, clean and dry soxhlet extractor flask was attached to bottom of the extractor. Petroleum ether (500 ml) was poured into dry soxhlet flask and the heating mantle switched on so that the petroleum ether boiled. Heating continued for eight hours after which the solvent was siphoned completely into flask and taken to dryness by distillation. The flask was removed dried to a constant weight. Then cooled, weighed and the amount of extracted lipids was calculated from the difference between the weight before and after extraction.

Calculation:

Weight of empty porous thimble (g) = w_o

Weight of thimble + ground sample (g) = w_1

Weight of ground sample (g) = $w_1 - w_o$

Weight of empty extraction flask (g) = w_2

Weight of extraction flask + ether (g) = w_3

$$\%lipid = \frac{w_3 - w_2}{w_1 - w_o} \times 100$$

Determination of Ash Content

Ash content was determined using the method of A.O.A.C (2000).

Principle

The total ash content is estimated by complete removal of organic material using ignition.

Procedure

One gram of each plant samples was weighed in crucibles and ignited in a furnace at 500-600 °C for 3 hours until it ashes completely. It was then cooled in a dessicator, cooled and weighed immediately at room temperature.

Calculation:

$$\%Ash = \frac{w_2 - w_0\,(g)}{w_1 - w_0\,(g)} \times 100$$

Determination of Dietary Fibre

This was determined by enzymatic-gravimetric method as described by A.O.A.C (2000).

Principle

The samples are first rendered fat-free by treatment with petroleum ether. The defatted sample is treated with boiling H_2SO_4 and later with boiling NaOH, and the residue left after subtraction of the ash is taken as the fibre.

Procedure

One gram of each plant samples (w_0) was boiled in 200 ml of sulphuric acid for thirty minutes. The boiled sample were filtered through a muslin cloth which was rinsed with hot distilled water. 200 ml of sodium hydroxide was added to the residue and allowed to boil for 30 minutes, it was then rinse with hot distilled water and also rinsed with hydrochloric acid. It was finally rinsed three times with petroleum ether, allowed to drain, dried in the oven, allowed to cool in a dessicator and weighed (w_1). the samples were ashed at 500 °C, for 90 minutes in a muffle furnace cooled in a dessicator and weighed (w_2).

Calculation:

$$\% \text{ crude fibre} = \frac{w_1 - w_2 \text{ (g)} \times 100}{w_0 \text{ (g)}}$$

Determination of Carbohydrate Content

Carbohydrate content was determined by obtaining the difference after adding up the % protein content, % fibre content, % ash content, % moisture content and % lipid, then subtracting from one hundred.

Calculation:

% carbohydrate= 100 - % lipid +% protein + % ash + % fibre + % moisture

3.2.5 Animal Sacrifice, Tissue Collection and Preparation

The small intestine was collected following a 36-day feeding period. Animals were lightly anesthetized with diethyl ether, and the abdominal cavity was accessed through a mid-line incision. The small intestine was carefully separated at both the pyloric sphincter and ileocaecal junction. The tissue was then gently lifted while cutting the mesentries, and all membranes were cleaned off. Ice-cold saline (9 g/L) was used to flush the entire intestine to remove all debris, with care taken to prevent freezing. The intestine was slit open while being washed and cleaned with tissue paper, of all ruminal contents using iced saline. The segment was then gently blotted dry, and the mucosa was carefully scraped off using a glass blade and weighed. The intestinal mucosa was subsequently homogenized using a mortar and pestle, placed on ice, combining 1 g of intestinal mucosa with 9 ml of normal saline. The homogenate was centrifuged at 3000 g for 10 minutes at 4 °C to separate solid debris from the liquid fraction containing the enzymes, and the supernatant was collected for enzyme activity measurements.

3.2.6 Biochemical Assays

3.2.6.1 Total Protein Determination

Total protein of the intestinal mucosa was determined by the Biuret method using ChemCHEK Total Protein Kit (AGAPPE) (Lubran, 1978).

Principle:

The determination of total protein was based on the formation of peptide bonds between proteins and copper ions under alkaline conditions. When treated with cupric ions in alkaline solution, proteins formed a characteristic blue-colored complex. The intensity of this blue color was directly proportional to the protein concentration present in the sample, allowing for quantitative measurement at a wavelength of 546 nm.

Procedure:

Three (3) reaction vessels were prepared: a blank, a standard, and a sample tube. To each tube, 1000 µl of the protein reagent was added. The standard tube received 20 µl of the protein standard solution, while the sample tube received 20 µl of the homogenised intestinal mucosa. After thorough mixing, all tubes were incubated at 37 °C for a period of 10 minutes. Following incubation, the absorbance of both the standard and sample were measured spectrophotometrically at 546 nm, using the reagent blank as a reference.

Calculation:

The total protein concentration was then calculated using the ratio of sample to standard absorbance, multiplied by the standard concentration.

$$\text{Total protein concentration (g/dL)} = \frac{\text{Absorbance of sample} \times 6}{\text{Absorbance of standard}}$$

Where 6 = Standard concentration

3.2.6.2 Alkaline Phosphatase Assay

Alkaline phosphatase activity was determined by the p-Nitrophenyl phosphate (PNPP) hydrolysis method using Fortress Alkaline phosphatase Kit (FORTRESS KIT) (Schumann *et al.,* 2011).

Principle:

Alkaline phosphatase activity was measured through its catalytic action on p-Nitrophenyl phosphate (PNPP), which resulted in the formation of p-Nitrophenol and inorganic phosphate. Under alkaline conditions, p-Nitrophenol developed a yellow color, and the rate at which this colored product formed, measured at 405 nm, corresponded directly to the enzyme's activity level in the sample.

Procedure:

Initially, 0.2 ml of 2 mM PNPP substrate was mixed with 0.2 ml of 0.1 M carbonate buffer at pH 10.2. To this mixture, 0.05 ml of the homogenised mucosa was added to initiate the reaction. The solution was then maintained at room temperature for a 10-minute incubation period. Following incubation, 1.0 ml of 0.5 M sodium hydroxide was introduced to stop the reaction. The absorbance was measured spectrophotometrically at 405 nm, and readings were taken for both fresh and stored samples.

Calculation:

The enzyme activity was calculated due to the conversion of PNPP to p-Nitrophenol, with results expressed in μM/min after averaging triplicate measurements.

$$\text{Alkaline phosphatase activity} = \frac{4.27 + 5.6 + 11.2 \times (10^{-3})}{3} = 7.02 \times 10^{-3}\ \mu M/min$$

3.2.6.3 Maltase Activity Assay

Maltase activity was determined by the Glucose oxidase coupled assay system (Viigand *et al.*, 2016).

Principle:

Maltase activity was determined by measuring the enzyme's capacity to hydrolyze maltose into glucose molecules. The assay employed p-nitrophenyl-α-D-glucopyranoside as a substrate, and the rate of its hydrolysis by maltase was monitored through the release of p-nitrophenol measured at 500 nm. The quantity of product formed correlated directly with enzyme activity, developing a purple colour as a result of quinoneimine formation.

Procedure:

A volume of 850 μl maltase buffer and 50 μl of diluted maltase protein in 5 ml tubes were preincubated. This mixture was warmed in a water bath at 37 °C for 5 minutes. The enzymatic reaction was initiated by introducing 100 μl of 500 mM sucrose solution prepared in maltase buffer. After mixing, the reaction proceeded for 20 minutes at 37 °C. Following incubation, 50μl homogenized mucosa were withdrawn and transferred to tubes containing 150 μl of stopping solution (200 mM Tris-HCl buffer, pH 8.3). These tubes were heated at 96 °C for 5 minutes in a

water bath to terminate the reaction completely. The samples were then cooled on ice, after which 800 µl of Eco-Pak Glucose Reagent was added and mixed thoroughly. The mixture was incubated at 37 °C with open lids for 5 minutes, during which the solution developed a purple color due to quinoneimine formation. The absorbance of the purple product was measured at 500 nm using a spectrophotometer, with readings taken against a blank control (reagent blank).

100 µl of 500 mM sucrose solution prepared in maltase buffer:

$$= 500 \times \frac{\text{molecular weight of sucrose in 1 L}}{1000} \text{ of maltase buffer}$$

Molecular weight of sucrose = 342.3 kg/mol

$$= \frac{500 \times 342.3 \text{ in 1 L}}{1000} \text{ maltase buffer}$$

$$= \frac{171150 \text{ in}}{1000} \text{ 1 L}$$

= 171.15g/L

$$\text{That is, } \frac{171.15 \text{ g/1000 ml}}{10}$$

Therefore, 500 mM sucrose= 17.115 g/100 ml.

Calculation

$$\text{Maltase activity (E)} = \frac{OD_{500} \text{ (1 min)}}{(\varepsilon \text{ x [c]})}$$

OD_{500} (1 min) is absorbance (optical density) change of the reaction mixture per 1 min measured at 500 nm wavelength.

[c] is concentration of the maltase protein (mg/ml) in the reaction mixture.

(ε) is the extinction coefficient withdrawn from glucose calibration curve.

3.2.6.4 Lactase Activity Assay

Lactase activity was determined by the Glucose oxidase coupled detection system (Craven *et al.*, 1965).

Principle:

The lactase activity assay measured the enzyme's ability to break down lactose into its component sugars, glucose and galactose. The method utilized o-nitrophenyl β-D-galactopyranoside as a substrate, which released o-nitrophenol, a yellow colour upon enzymatic cleavage at 400 nm. The rate of o-nitrophenol production, measured at 400nm, directly reflected lactase activity.

Procedure:

A volume of 0.9 ml phosphate buffer (0.1 M, pH 7.0) was combined with an equal volume of intestinal homogenate. To this mixture, 4 ml of substrate solution, prepared by dissolving 1 g of lactose in 100 ml of distilled water, was added. The reaction mixture was incubated in a water bath maintained at 37 °C for 15 minutes. The enzymatic reaction was terminated by adding 1 ml of 4 % perchloric acid. From this stopped reaction, 1ml portions were transferred to tubes containing 9 ml of Eco-Pak glucose reagent. After allowing the yellow color to develop for 10 minutes, 0.5 ml of 5 N hydrochloric acid was added to stabilize the reaction. The final absorbance measurements were taken at 400 nm after a 5-minute waiting period.

Calculation:

The specific activity of lactase was calculated based on the rate of lactose hydrolysis, with one unit defined as the amount of enzyme that hydrolyzed 1.0 micromole of lactose per minute.

Specific activity of lactase = $\frac{\text{Rate of lactose hydrolysis (micromoles/min)}}{\text{Total protein (mg)}}$

Where,

Rate of lactose hydrolysis is the number of micromoles of lactose hydrolyzed per minute (in micromoles/min).

Total Protein is the total amount of protein in the enzyme preparation (in mg)

3.2.6.5 Sucrase Activity Assay

Sucrase activity was determined by Dinitrosalicylic acid method (Birkhed *et al*., 1974).

Principle:

Sucrase activity was evaluated by monitoring the enzyme's ability to catalyze the hydrolysis of sucrose into glucose and fructose. The assay relied on detecting the released glucose and fructose measured at 540 nm using a colorimetric method, which resulted in a pink colour. The amount of monosaccharides generated over time directly indicated the sucrase activity level in the sample.

Procedure:

A volume of 0.2 ml of the enzyme-containing homogenate was combined with 1 ml of 0.3 M sucrose solution and 0.05 ml of 0.05 M sodium-acetate buffer at pH 4.7. This reaction mixture was maintained at 30 °C for a 2-hour incubation period. The enzymatic reaction was stopped by introducing 3,5-dinitrosalicylic acid reagent. Color development was achieved by heating the tubes at 100 °C for 5 minutes, followed by dilution with 1.87 ml of water. The intensity of the pink color developed was compared against standards prepared using 5 mM glucose plus 5 mM fructose, with absorbance measurements taken at 540 nm using a spectrophotometer.

$$5\text{ mM fructose} = 5 \times \frac{\text{molecular weight of fructose}}{1000} \text{ in 1 litre of distilled water}$$

$$= 5 \times \frac{180.18 \text{ in 1L}}{1000}$$

$$= 0.9009\text{g/L}$$

That is, $= \frac{0.9009 \text{ g/1000 ml}}{10}$

Therefore, 5 mM fructose = 0.09009 g/100 ml

Since, molecular weight of glucose = 180.18 g/mol

5 mM glucose= 0.09009 g/100 ml.

Calculation

$$\text{Sucrase activity} = \frac{\Delta A \times \text{Total volume (L)}}{\text{Incubation time (min)} \times \text{Dilution factor}}$$

ΔA is the change in absorbance measured at 540 nm after the color development.

Total Volume (L) is the final volume of the reaction mixture after dilution.

Incubation Time (min) is the total time the reaction was allowed to proceed, which is 120 minutes (2 hours).

Dilution Factor accounts for the dilution performed after the enzymatic reaction.

3.3 Statistical Analysis

All experimental data were analyzed using Statistical Package for Social Sciences (SPSS) version 21.0. Results were presented as mean ± SEM of three replicates. Statistical significance among groups was determined using one-way analysis of variance (ANOVA), with $p \leq 0.05$ considered significant. Duncan's multiple range test was employed to separate homogeneous groups.

CHAPTER FOUR

RESULTS

4.1 Proximate Composition of Plant Samples

Table 4.1 presents the proximate composition analysis of the plant fibers used in this study. The fresh sample moisture content varied significantly among the three plant samples. Okra (*Abelmoschus esculentus*) had the highest moisture content (79.86 ± 0.46%), followed by Osu (*Hunteria umbellata*) (75.18 ± 0.04%) and Bush mango (*Irvingia gabonensis*) (62.27 ± 0.45%).

Regarding ash content, Osu showed the highest percentage (9.90 ± 0.62%), followed by Okra (9.23 ± 1.65%) and Bush mango (6.17 ± 1.40%). For crude fiber, Okra demonstrated the highest content (24.37 ± 0.65%), followed by Osu (19.80 ± 1.56%) and Bush mango (15.57 ± 1.10%).

Bush mango had the lowest fat content (1.03 ± 0.05%) compared to Okra (1.14 ± 0.10%) and Osu (0.93 ± 0.11%). Regarding protein content, Bush mango showed the highest value (3.01 ± 0.34%), followed by Osu (2.49 ± 0.36%) and Okra (1.17 ± 0.06%).

Carbohydrate content was highest in Bush mango (58.86 ± 1.73%), followed by Osu (55.35 ± 2.93%) and significantly lower in Okra (38.19 ± 0.77%).

Table 4.1: Proximate Composition Analysis of Plant Samples (Dry Weight Basis)

Parameter	Bush mango (*Irvingia gabonensis*)	Okra (*Abelmoschus esculentus)*	Osu (*Hunteria umbellata*)
Fresh sample Moisture Content (%)	15.37 ± 0.45[c]	25.90 ± 0.46[a]	11.53 ± 0.04[b]
Ash (%)	6.17 ± 1.40	9.23 ± 1.65	9.90 ± 0.62
Crude Fiber (%)	15.57 ± 1.10	24.37 ± 0.65	19.80 ± 1.56
Fat (%)	1.03 ± 0.05	1.14 ± 0.10	0.93 ± 0.11
Crude Protein (%)	3.01 ± 0.34	1.17 ± 0.06	2.49 ± 0.36
Carbohydrate (%)	58.85 ± 1.73	38.19 ± 0.77	55.35 ± 2.93

Values are expressed as mean ± SEM of three replicates. Different superscript letters within a column indicate significant differences ($p < 0.05$) between groups. The superscripts "a" represents the highest value in the column while the superscripts "b", and "c", represent intermediate values that are significantly lower than "a".

4.2 Body Weights of Rats

Table 4.2 presents the initial and final body weights, as well as body weight differences of rats in each experimental group. Initial body weights ranged from 26.70 g to 55.70 g across all groups. The Osu 5.0 group had the highest average initial body weight (44.83 ± 4.61 g), while the Okra 10.0 group had the lowest average initial weight (34.55 ± 3.44 g). There were no statistically significant differences in initial body weights among the experimental groups at the beginning of the study ($p > 0.05$).

Final body weights ranged from 38.61 g to 86.52 g across all groups. The fiber-free control group had the highest average final body weight (86.52 ± 3.44 g), followed closely by the Osu 10.0 group (83.92 ± 2.35 g). The Okra 10.0 group maintained the lowest average final weight (38.61 ± 2.18 g). Significant differences in final body weights were observed between the groups ($p < 0.05$).

The body weight differences (weight gain) over the experimental period were highest in the fiber-free control group (42.89 ± 3.57 g) and the Osu 10.0 group (41.92 ± 4.26 g), with no significant difference between these two groups.

Weight gain was significantly reduced ($p < 0.05$) in all fiber-supplemented groups relative to the fiber free control. The most pronounced suppression of weight gain was observed in Bush mango, specifically the Bush mango 10.0 group (0.83 ± 5.96 g). Similarly, within the Okra treatments, the Okra 10.0 group exhibited the lowest increase (4.06 ± 4.08 g).

Table 4.2: Initial and Final Body Weights of Rats in Different Experimental Groups (grams)

Groups	Initial Body Weight (g)	Final Body Weight (g)	Percentage weight gain (%)
C1 (Fiber free)	43.63 ± 0.97^{a}	86.52 ± 3.44^{a}	98.3%
C2 (Fybogel 1.5 %)	44.95 ± 2.13^{a}	71.59 ± 3.41^{a}	59.3%
C2 (Fybogel 3.0 %)	42.42 ± 3.19^{a}	65.63 ± 4.22^{ab}	54.7%
Bush mango 5.0 %	37.03 ± 3.99^{a}	38.70 ± 5.30^{c}	4.5%
Bush mango 10.0 %	44.67 ± 3.07^{a}	45.50 ± 5.09^{bc}	1.9%
Okra 5.0 %	36.37 ± 3.55^{a}	46.22 ± 1.97^{bc}	27.1%
Okra 10.0 %	34.55 ± 3.44^{a}	38.61 ± 2.18^{c}	11.8%
Osu 5.0 %	44.83 ± 4.61^{a}	76.13 ± 6.32^{a}	69.8%
Osu 10.0 %	42.00 ± 3.55^{a}	83.92 ± 2.35^{a}	99.8%

Values are expressed as mean ± SEM of three determinants. Different superscript letters within a column indicate significant differences ($p < 0.05$) between groups. No. of animals (n) = 6. The superscripts "a" represents the highest value in the column, "c" represent intermediate values that are significantly lower than "a". The shared superscripts "ab" means that the value is statistically similar to groups marked with "a" and groups marked with "b", while "bc" means that the value is statistically similar to groups marked with "b" and groups marked with "c".

4.3 Disaccharidase Activities in Small Intestinal Mucosa

4.3.1 Lactase Activity

The lactase activity in the small intestinal mucosa of rats across different experimental groups is presented in Table 4.3. The Bush mango 10.0 % group showed the highest lactase activity (8.36 ± 4.71 µmol/min/g/tissue), which was significantly higher than most other groups ($p < 0.05$). This was followed by the fiber-free control group (3.76 ± 0.58 µmol/min/g) and the Osu 5.0 % group (3.21 ± 0.56 µmol/min/g/tissue). The Fybogel 3.0 % group exhibited the lowest lactase activity (1.43 ± 0.26 µmol/min/g/tissue). Both doses of Bush mango increased lactase activity compared to the fiber-free control, with the higher dose (10.0 %) showing more pronounced effects.

Table 4.3: Lactase Activity in Small Intestinal Mucosa

Groups	Lactase Activity (μmol/min/g/tissue)
C1 (Fiber free)	3.76 ± 0.58^{ab}
C2 (Fybogel 1.5 %)	2.94 ± 0.68^{ab}
C3 (Fybogel 3.0 %)	1.43 ± 0.26^{b}
Bush mango 5.0 %	3.90 ± 1.81^{ab}
Bush mango 10.0 %	8.36 ± 4.71^{a}
Okra 5.0 %	2.83 ± 0.66^{ab}
Okra 10.0 %	1.81 ± 0.42^{b}
Osu 5.0 %	3.21 ± 0.56^{ab}
Osu 10.0 %	2.21 ± 0.59^{b}

Values are expressed as mean ± SEM of three determinants. Different superscript letters within a column indicate significant differences ($p < 0.05$) between groups. n = 6
The superscripts "a" represents the highest value in the column while the superscripts "b" represent intermediate values that are significantly lower than "a". Shared Superscripts "ab" means that the value is statistically similar to groups marked with "a" and groups marked with "b".

4.3.2 Sucrase Activity

As shown in Table 4.4, sucrase activity varied considerably among the experimental groups. The Fybogel 1.5 % group showed the highest sucrase activity (24.82 ± 4.65 μmol/min/g/tissue), followed by the fiber-free control group (19.82 ± 3.16 μmol/min/g/tissue). The Osu 5.0 % group exhibited the lowest sucrase activity (11.31 ± 4.67 μmol/min/g/tissue). Sucrase activity was lower in all plant-treated groups than in the fiber-free control, with the most substantial decrease observed in the Osu samples.

Table 4.4: Sucrase Activity in Small Intestinal Mucosa

Groups	Sucrase Activity (µmol/min/g/tissue)
C1 (Fiber free)	19.82 ± 3.16^{ab}
C2 (Fybogel 1.5 %)	24.82 ± 4.65^{a}
C3 (Fybogel 3.0 %)	21.53 ± 4.92^{ab}
Bush mango 5.0 %	15.19 ± 2.06^{abc}
Bush mango 10.0 %	14.21 ± 2.72^{abc}
Okra 5.0 %	19.58 ± 5.59^{ab}
Okra 10.0 %	13.76 ± 2.49^{abc}
Osu 5.0 %	11.31 ± 4.67^{bc}
Osu 10.0 %	11.04 ± 2.56^{c}

Values are expressed as mean ± SEM of three determinants. Different superscript letters within a column indicate significant differences ($p < 0.05$) between groups. n = 6

The superscripts "a" represents the highest value in the column while the superscripts "c" represent intermediate values that are significantly lower than "a". The shared superscripts "ab" means that the value is statistically similar to groups marked with "a" and groups marked with "b", while "bc" means that the value is statistically similar to groups marked with "b" and groups marked with "c". The shared superscripts "abc" indicates that the value is not significantly different from groups marked with "a", groups marked with "b", or groups marked with "c".

4.3.3 Maltase Activity

Table 4.5 presents the maltase activity across all experimental groups. The Fybogel 1.5 % group showed the highest maltase activity (125.96 ± 18.38 µmol/min/g/tissue), while the Okra 10.0 % group exhibited the lowest activity (57.02 ± 3.58 µmol/min/g/tissue). Maltase activity was numerically lower across all plant sample groups compared to the fiber-free control (85.93 ± 13.81 µmol/min/g/tissue), however, these differences, including the 81.23 ± 19.17 µmol/min/g/tissue recorded for the 10.0 % Bush mango group did not reach statistical significance ($p > 0.05$).

Table 4.5: Maltase Activity in Small Intestinal Mucosa

Groups	Maltase Activity (µmol/min/g/tissue)
C1 (Fiber free)	85.93 ± 13.81[a]
C2 (Fybogel 1.5 %)	125.96 ± 18.38[a]
C3 (Fybogel 3.0 %)	85.93 ± 16.21[a]
Bush mango 5.0 %	59.82 ± 15.70[a]
Bush mango 10.0 %	81.23 ± 19.17[a]
Okra 5.0 %	69.76 ± 11.08[a]
Okra 10.0 %	57.02 ± 3.58[a]
Osu 5.0 %	86.58 ± 21.75[a]
Osu 10.0 %	73.94 ± 17.66[a]

Note: No significant differences were observed between groups for maltase activity

Values are expressed as mean ± SEM of three determinants. Similar superscript letters within a column indicate insignificant differences ($p > 0.05$) between groups. n = 6

The superscripts "a" represents the highest value in the column

4.4 Alkaline Phosphatase Activity

The alkaline phosphatase (ALP) activity in the small intestinal mucosa is presented in Table 4.6. The Bush mango 10.0 % group demonstrated significantly ($p < 0.05$) higher ALP activity (20.62 ± 11.92 µM PNp) compared to the fiber-free control group (4.77 ± 1.64 µM PNp) and Fybogel groups ($p < 0.05$). All plant sample treatments at both doses increased ALP activity compared to the fiber-free control group, with Okra 5.0 % showing the highest enhancement (21.60 ± 4.26 µM PNp), followed by Osu 10.0 % (13.45 ± 3.03 µM PNp).

Table 4.6: Alkaline Phosphatase Activity in Small Intestinal Mucosa

Groups	ALP Activity (µM PNp)
C1 (Fiber free)	4.77 ± 1.64[c]
C2 (Fybogel 1.5 %)	3.73 ± 1.86[c]
C3 (Fybogel 3.0 %)	7.58 ± 3.24[bc]
Bush mango 5.0 %	12.85 ± 3.16[ab]
Bush mango 10.0 %	20.62 ± 11.92[a]
Okra 5.0 %	21.60 ± 4.26[a]
Okra 10.0 %	17.53 ± 2.59[a]
Osu 5.0 %	12.24 ± 1.50[ab]
Osu 10.0 %	13.45 ± 3.03[ab]

Values are expressed as mean ± SEM of three determinants. Different superscript letters within a column indicate significant differences ($p < 0.05$) between groups. n = 6

The superscripts "a" represents the highest value in the column while the superscripts "c" represent intermediate values that are significantly lower than "a". The shared superscripts "ab" means that the value is statistically similar to groups marked with "a" and groups marked with "b", while "bc" means that the value is statistically similar to groups marked with "b" and groups marked with "c".

4.5 Total Protein Content

Table 4.7 presents the total protein content in the small intestinal mucosa across all experimental groups. The Osu 10.0 % group had the highest total protein content (5.11 ± 0.13 g/dL), while the fiber-free control group showed the lowest protein content (2.91 ± 0.05 g/dL). All plant sample treatments significantly increased the total protein content compared to the fiber-free control group ($p < 0.05$), with more pronounced effects observed at higher doses (10.0 %) for each plant fiber. Among the three plant fibers, Osu demonstrated the most significant increase in protein content, followed by Okra and Bush mango.

Table 4.7: Total Protein Content in Small Intestinal Mucosa

Groups	Total Protein (g/dL)
C1 (Fiber free)	2.91 ± 0.05^{e}
C2 (Fybogel 1.5 %)	3.10 ± 0.04^{de}
C3 (Fybogel 3.0 %)	3.17 ± 0.05^{d}
Bush mango 5.0 %	4.05 ± 0.13^{c}
Bush mango 10.0 %	4.53 ± 0.09^{b}
Okra 5.0 %	4.85 ± 0.09^{ab}
Okra 10.0 %	4.85 ± 0.07^{ab}
Osu 5.0 %	4.84 ± 0.05^{ab}
Osu 10.0 %	5.11 ± 0.13^{a}

Values are expressed as mean ± SEM of three determinants. Different superscript letters within a column indicate significant differences ($p < 0.05$) between groups. n = 6. The superscripts "a" represents the highest value in the column while the superscripts "b", "c", and "d" represent intermediate values that are significantly lower than "a" but may be significantly higher than "e". Superscript "e" represent the lowest value in the column. The Shared superscripts "ab" means that the value is statistically similar to groups marked with "a" and groups marked with "b" while superscript "de" means that the value is statistically similar to groups marked with "d" and groups marked with "e".

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The present study investigated the effects of three indigenous plant fibers *Irvingia gabonensis* (Bush mango), *Abelmoschus esculentus* (Okra), and *Hunteria umbellata* (Osu) on disaccharidases and alkaline phosphatase activities in the small intestinal mucosa of rats. The findings reveal significant variations in the proximate composition of these plant fibers and their differential effects on intestinal enzyme activities compared to both fiber-free and commercial fiber controls, which could have important implications for gut health, nutrient absorption, and metabolism.

The proximate composition analysis demonstrated substantial differences in the nutritional profiles of the three plant fibers. Bush mango (*Irvingia gabonensis*) exhibited the lowest moisture content (62.27 ± 0.45 %) which was moderate compared to the other samples. The plant showed the highest carbohydrate content (58.86 ± 1.73 %) and moderate crude fiber (15.57 ± 1.10 %) among the three fibers. These findings represent a departure from previous research on *Irvingia gabonensis* seeds, which reported different compositional profiles (Nciri and Cho, 2018), suggesting potential differences between the fiber component and whole seed composition.

Okra (*Abelmoschus esculentus*) demonstrated the highest crude fiber content (24.37 ± 0.65 %) and the lowest carbohydrate value (38.19 ± 0.77 %), which differs from the previous assessment (Gemede *et al*., 2015). This fiber-rich composition might explain some of the effects observed on disaccharidase activities. Notably, Okra also showed the highest moisture content in both wet

and dry states (79.86 ± 0.46 % and 25.90 ± 0.80 %, respectively), which may influence its hydration properties and physiological effects.

The significant mineral content in Osu (reflected in an ash value of 9.90 ± 0.62 %) and the protein levels in Bush mango (3.01 ± 0.34 %) suggest that the varied physiological effects of these fibers on intestinal function may be driven by their unique nutrient profiles. These compositional differences potentially play a more substantial role in modulating enzyme activity than previously documented (Airaodion *et al*., 2019).

The body weight data revealed striking differences in weight gain patterns among the experimental groups when compared to controls.

Comparison with fiber-free control (C1): The fiber-free control group exhibited the highest weight gain (42.89 ± 3.57 g), which was significantly greater than all plant fiber groups except Osu 10.0 % (41.92 ± 4.26 g). This finding suggests that most plant fibers, particularly Bush mango, may have anti-obesogenic properties compared to a fiber-free diet.

Bush mango demonstrated the most dramatic contrast with controls: Both Bush mango doses showed remarkably low weight gains (5.0 % : 1.67 ± 6.61 g; 10.0 % : 0.83 ± 5.96 g) representing 96 % and 98 % low weight gain respectively compared to the fiber-free control. Even when compared to commercial fiber controls, Bush mango showed substantial reductions (Fybogel 1.5 % : 26.64 ± 4.01 g vs. Bush mango groups). This dramatic weight suppression despite adequate food consumption indicates possible interference with nutrient absorption or metabolism that is unique to Bush mango fiber.

Okra showed moderate anti-obesogenic effects: Okra treatments resulted in significant low weight gain compared to the fiber-free control (5.0 % : 9.85 ± 4.06 g vs. 42.89 ± 3.57 g, representing a 77 % low weight gain; 10.0 % : 4.06 ± 4.08 g vs. 42.89 ± 3.57 g, representing a 91% low weight gain). Compared to Fybogel controls, Okra groups still showed lower weight gains, though less dramatic than Bush mango.

Osu exhibited dose-dependent effects: The lower dose (5.0 %) showed moderate lower weight gain (31.30 ± 7.83 g vs. 42.89 ± 3.57 g control, 27 %), while the higher dose (10.0 %) maintained weight gain comparable to the fiber-free control (41.92 ± 4.26 g vs. 42.89 ± 3.57 g). This contrasting effect with Osu 10.0 % suggests a fiber-specific effect rather than a general consequence of dietary fiber consumption, as it performed similarly to the fiber-free control but differently from commercial fiber controls.

As noted by Igile *et al*., (2013), plant fibers should not be viewed as a homogeneous group. The distinct reduction in sucrase activity observed in the Osu-treated group compared to the Okra-treated group in this study confirms that structural and chemical diversity results in varied physiological outcomes compared to the fiber free control group.

Lactase Activity: Bush Mango Enhancement vs. Other Treatments

The investigation of disaccharidase activities revealed complex and enzyme-specific effects when plant fibers were compared to controls.

Lactase activity comparison with fiber-free control: Bush mango 10.0 % demonstrated dramatic enhancement (8.36 ± 4.71 μmol/min/g/tissue vs. 3.76 ± 0.58 μmol/min/g/tissue control,

representing a 122 % increase), suggesting that Bush mango fiber might particularly benefit lactose digestion compared to a fiber-free diet (3.76 ± 0.58 μmol/min/g/tissue,, 15 % increase).

Contrasting effects among plant fibers: While Bush mango enhanced lactase activity, both Okra and Osu treatments showed reductions compared to the fiber-free control. Okra 10.0 % significantly reduced lactase activity (μmol/min/g/tissue vs. μmol/min/g/tissue, control, 52% reduction), and Osu 10.0 % also showed reduction (2.21 ± 0.59 μmol/min/g/tissue vs. 3.76 ± 0.58 μmol/min/g/tissue control, 41 % reduction).

Comparison with commercial fiber controls: Interestingly, Fybogel treatments showed intermediate effects (1.5 % : 2.94 ± 0.68 μmol/min/g/tissue; 3.0 % : 1.43 ± 0.26 μmol/min/g/tissue), with the higher Fybogel dose showing the lowest lactase activity overall.

These differential effects highlight the specificity of plant fiber-enzyme interactions, where Bush mango uniquely enhanced lactase activity while other fibers, including commercial preparations, generally reduced it.

Sucrase Activity: Universal Reduction by Plant Fibers

Comparison with fiber-free control: Contrasting with lactase findings, sucrase activity was reduced across all plant fiber treatments compared to the fiber-free control (19.82 ± 3.16 μmol/min/g/tissue, 16 % reduction). Osu showed the most pronounced reduction at both doses (5.0 %: 11.31 ± 4.67 μmol/min/g/tissue 43 % reduction; 10.0 %: 11.04 ± 2.56 μmol/min/g/tissue, 44 % reduction). Bush mango treatments showed moderate reductions (5.0 % : 15.19 ± 2.06 μmol/min/g/tissue, 23 % reduction; 10.0 %: 14.21 ± 2.72 μmol/min/g/tissue, 28 % reduction),

while Okra showed variable effects (5.0 % maintained similar levels: 19.58 ± 5.59 µmol/min/g/tissue; 10.0 % showed reduction: 13.76 ± 2.49 µmol/min/g/tissue 31 % reduction).

Unique response to commercial fiber: Interestingly, Fybogel 1.5 % showed the highest sucrase activity overall (24.82 ± 4.65 µmol/min/g/tissue), representing a 25 % increase compared to the fiber-free control, while Fybogel 3.0 % maintained levels similar to control (21.53 ± 4.92 µmol/min/g/tissue). This suggests that commercial fiber preparations may have fundamentally different effects on sucrase activity compared to indigenous plant fibers.

Maltase Activity: Minimal but Consistent Effects

Comparison across all treatments: Maltase activity exhibited less pronounced changes compared to other disaccharidases, with no statistically significant differences among groups. However, numerical trends showed interesting patterns.

Compared to fiber-free control (85.93 ± 13.81 µmol/min/g/tissue, 16 % decrease): Bush mango 5.0 % showed reduction (59.82 ± 15.70 µmol/min/g/tissue, 30 % decrease), while 10.0 % maintained similar levels (81.23 ± 19.17 µmol/min/g/tissue). Okra treatments both showed reductions (5.0 %: 69.76 ± 11.08 µmol/min/g/tissue, 19 % decrease; 10.0 %: 57.02 ± 3.58 µmol/min/g/tissue, 34 % decrease). Osu 5.0 % maintained similar levels (86.58 ± 21.75 µmol/min/g/tissue) while 10.0 % showed reduction (73.94 ± 17.66 µmol/min/g/tissue, 14 % decrease).

Commercial fiber comparison: Notably, Fybogel 1.5 % showed the highest maltase activity (125.96 ± 18.38 µmol/min/g/tissue, 47 % increase vs. fiber-free control), while Fybogel 3.0 % matched the control level exactly (85.93 ± 16.21 µmol/min/g/tissue). This differential response

between indigenous and commercial fibers suggests distinct mechanisms of action (Oboh *et al*., 2010).

Alkaline Phosphatase activity:

One of the most striking findings was the marked enhancement of alkaline phosphatase (ALP) activity by all plant fiber treatments compared to controls.

Comparison with fiber-free control: All plant fibers significantly increased ALP activity compared to the fiber-free control (4.77 ± 1.64 μM PNp, 105 % increase). Bush mango showed dose-dependent increases (5.0 % : 12.85 ± 3.16 μM PNp, 169 % increase; 10.0 %: 20.62 ± 11.92 μM PNp, 332% increase). Okra demonstrated the highest enhancement at the lower dose (5.0g: 21.60 ± 4.26 μM PNp, 353% increase; 10.0g: 17.53 ± 2.59 μM PNp, 267 % increase). Osu showed consistent enhancement at both doses (5.0 %: 12.24 ± 1.50 μM PNp, 157 % increase; 10.0.%: 13.45 ± 3.03 μM PNp, 182 % increase).

Limited effect of commercial fiber: In stark contrast, commercial fiber controls showed substantially less enhancement. Fybogel 1.5 % showed minimal change (3.73 ± 1.86 μM PNp, 22 % decrease vs. fiber-free control), while Fybogel 3.0 % showed moderate increase (7.58 ± 3.24 μM PNp, 59 % increase). This dramatic difference between indigenous plant fibers and commercial fiber highlights unique properties of the traditional plant materials.

The substantial elevation in ALP activity suggests potent stimulatory effects of indigenous plant fibers on intestinal barrier function and detoxification processes (Oboh *et al*., 2010). Intestinal alkaline phosphatase (IAP) plays crucial roles in gut homeostasis, including detoxification of bacterial endotoxins, regulation of intestinal pH, reduction of inflammation, and modulation of

gut microbiota (Wu *et al*., 2022). The enhancement of ALP activity by plant fibers, especially at higher doses, suggests potential protective effects against intestinal inflammation and dysbiosis that are not achieved by commercial fiber preparations.

Total Protein activity:

Universal enhancement compared to fiber-free control: The total protein content in the small intestinal mucosa was significantly enhanced by all plant fiber treatments compared to the fiber-free control (2.91 ± 0.05 g/dL, 4 % increase). Bush mango showed dose-dependent increases (5.0 % : 4.05 ± 0.13 g/dL, 39 % increase; 10.0 %: 4.53 ± 0.09 g/dL, 56 % increase). Okra treatments showed substantial and consistent enhancement (both doses: 4.85 ± 0.09 and 4.85 ± 0.07 g/dL respectively, 67 % increase for both). Osu demonstrated the highest enhancement, particularly at the higher dose (5.0 %: 4.84 ± 0.05 g/dL, 66 % increase; 10.0 %: 5.11 ± 0.13 g/dL, 76 % increase).

Modest effects of commercial fiber: Commercial fiber controls showed much smaller increases compared to indigenous plant fibers. Fybogel 1.5 % showed minimal enhancement (3.10 ± 0.04 g/dL, 7 % increase vs. fiber-free control), while Fybogel 3.0 % showed slightly higher increase (3.17 ± 0.05 g/dL, 9 % increase). The dramatically greater protein enhancement by indigenous plant fibers (39-76 % increases) compared to commercial fiber (7-9 % increases) suggests profound stimulatory effects on protein synthesis in the intestinal mucosa that are unique to traditional plant materials (Oboh *et al*., 2010).

This substantial increase suggests profound stimulatory effects on protein synthesis, indicating improved enterocyte proliferation, increased brush border protein expression, or enhanced

secretion of protective proteins such as mucins. The correlation between increased protein content and enhanced ALP activity, particularly in the Osu and Okra groups, suggests coordinated stimulation of various aspects of intestinal mucosal function (Oboh *et al*., 2010).

The dose-dependency observed for most parameters, with higher doses generally eliciting stronger effects, supports direct mechanistic links between the plant fibers and observed changes. Bush mango showed clear dose-dependent effects for lactase activity, ALP activity, and total protein content. Okra demonstrated optimal effects at the lower dose for ALP activity but dose-dependent effects for other parameters. Osu showed the most consistent dose-dependent responses across all measured parameters.

Comparison between indigenous and commercial fibers: The differential responses between indigenous plant fibers and commercial fiber (Fybogel) at equivalent or higher doses highlight the importance of fiber source and composition. While Fybogel showed some effects on enzyme activities, these were generally less pronounced and sometimes opposite to those of indigenous plant fibers, particularly for lactase and ALP activities.

The contrasting effects observed between plant fibers and controls, as well as among different plant fibers, suggest multiple mechanisms of action rather than simple bulk effects. Direct enzyme interaction mechanisms might involve phytochemicals present in plant fibers that directly modulate enzyme activities through binding and conformational changes. Fermentation-based mechanisms could involve differential fermentation patterns producing distinct short-chain fatty acid profiles that influence gene expression in enterocytes. Physical interaction mechanisms might involve unique structural properties of each plant fiber that induce specific local signaling pathways (Oboh *et al*., 2010).

The universal enhancement of ALP activity and protein content by all indigenous plant fibers, contrasted with minimal effects from commercial fiber, suggests that bioactive compounds beyond simple fiber content are responsible for these effects. The enzyme-specific effects (e.g., Bush mango enhancing lactase while reducing sucrase) indicate sophisticated regulatory mechanisms that differ fundamentally from the more uniform effects typically associated with commercial fiber preparations.

Comparison with literature

Similarities

- ***Irvingia gabonensis* on metabolic health:** The observed anti-obesogenic effects of Irvingia gabonensis, characterized by a marked reduction in weight gain, aligns with the findings of Pan *et al*., (2003) and Xu *et al*., (2021). These authors attribute such metabolic benefits to the plant's substantial fiber content and its role in blood glucose modulation. Consequently, the data presented in this study further substantiate the therapeutic viability of Irvingia gabonensis in the treatment of metabolic disorders.
- **Plant fibers and intestinal enzymes:** From this study, it is evident that plant fibers play a crucial role in modulating intestinal enzyme activity, a result that is consistent with Moré and Vandenplas (2018), Gu *et al*. (2016), and Messina *et al*. (2019) research, which establishes that dietary components, including plant fibers, can significantly alter the expression and activity of disaccharidases and alkaline phosphatase.

Contradictions

- **Proximate Composition of *Irvingia gabonensis*:** The study found that *Irvingia gabonensis* had the highest carbohydrate content (58.86 ± 1.73 %) and a moderate crude fiber content (15.57 ± 1.10 %). This contradicts previous research on *Irvingia gabonensis* seeds by Nciri and Cho (2018), which reported a different compositional profile.
- **Lactase Activity:** The study's results for lactase activity were complex and contradictory. While *Irvingia gabonensis* significantly enhanced lactase activity, both *Abelmoschus esculentus* and *Hunteria umbellata* treatments reduced it. This highlights a contrast in the effects of different plant fibers and differs from a simple, uniform effect suggested by some general literature on fiber and enzyme interactions (Oboh *et al*., 2010).
- **Sucrase Activity:** The study found that all three indigenous plant fibers (*Irvingia gabonensis, Hunteria umbellata,* and *Abelmoschus esculentus*) consistently reduced sucrase activity compared to the fiber-free control. This is particularly noteworthy as commercial fiber (Fybogel) at a lower dose (1.5 %) increased sucrase activity, suggesting a fundamental difference between indigenous and commercial fibers (Onyechi *et al*., 1998)

Targeted therapeutic applications: The enzyme-specific effects suggest potential applications in managing specific conditions. Bush mango's lactase enhancement could benefit lactose intolerant individuals, while Osu's sucrase reduction might help modulate postprandial glycemic responses in glucose intolerance. The universal ALP enhancement suggests applications in conditions with compromised intestinal barrier function.

Weight management implications: The dramatic weight gain suppression by Bush mango compared to both controls suggests potential obesity management applications, though mechanisms require further investigation. The differential weight effects among plant fibers indicate that fiber choice matters significantly more than previously recognized.

5.2 Conclusion

The study concluded that supplementing the diets of Wistar rats with fibers from *Irvingia gabonensis, Hunteria umbellata,* and *Abelmoschus esculentus* significantly influenced intestinal enzyme activities and body weight. The results confirm that these plant fibers modulate digestive processes, with effects being specific to the type of fiber and the enzyme in question. *Irvingia gabonensis* demonstrated a strong anti-obesogenic effect, likely due to its unique interaction with nutrient metabolism. *Hunteria umbellata* and *Abelmoschus esculentus* also reduced weight gain, but to a lesser degree than *Irvingia gabonensis.*

5.3 Findings

The following are the findings from the study:

1. All plant samples (*Irvingia gabonensis, Abelmoschus esculentus, Hunteria umbellata*) reduced maltase activity, though not statistically significant ($p > 0.05$), and Sucrase activity reduced significantly ($p < 0.05$) across all treatments compared to the fiber-free control.
2. All three indigenous plant samples (*Irvingia gabonensis, Hunteria umbellata,* and *Abelmoschus esculentus*) significantly ($p < 0.05$) increased intestinal ALP activity across different doses indicating a positive impact on gut health and nutrient absorption.

3. *Irvingia gabonensis* enhanced lactase activity, both *Abelmoschus esculentus* and *Hunteria umbellata* treatments showed reductions compared to the fiber-free control, indicating the specificity of plant fiber-enzyme interactions ($p < 0.05$).

5.4 Contribution to Knowledge

The study has contributed to knowledge in the following ways:

1. Revealed that *Irvingia gabonensis* (bush mango) greatly increases the activity of lactase, an enzyme that helps digest milk sugar, while *Abelmoschus esculentus* (okra) and *Hunteria umbellata* (osu) reduce the activity of this enzyme.
2. Showed that the samples do not work in the same way and affect digestion differently. All three samples reduced the activity of sucrase, an enzyme that digests sugar, but not by the same amount. *Hunteria umbellata* reduced it the most.
3. The plant samples helped reduce weight gain in the animals. *Irvingia gabonensis* was especially effective. This means these plants may help in controlling body weight.

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APPENDICES

Body weights of the Rats

Initial body weight (gram)

C1 Fiber free	C2 Fybogel 1.5	C2 Fybogel 3.0	Bush mango 5.0	Bush mango 10.0	Okra 5.0	Okra 10.0	Osu 5.0	Osu 10.0
47.30	47.80	47.90	33.80	34.10	50.60	43.40	55.70	43.50
42.30	39.20	45.60	49.50	47.40	33.20	29.70	45.20	46.20
43.40	53.90	47.70	44.60	48.30	36.70	47.30	50.20	39.40
45.10	41.90	42.50	38.10	44.20	39.60	28.10	48.60	39.10
41.60	43.20	43.40	26.70	51.60	28.40	29.40	40.90	31.10
42.10	43.70	27.40	29.50	42.40	29.70	27.40	28.40	52.70

Final body weight (gram) of the rats

C1 Fiber free	C2 Fybogel 1.5	C2 Fybogel 3.0	Bush mango 5.0	Bush mango 10.0	Okra 5.0	Okra 10.0	Osu 5.0	Osu 10.0
72.47	76.92	64.19	54.25	61.15	45.35	40.22	88.71	76.24
88.75	80.04	65.24	28.90	36.64	39.76	41.73	71.11	91.12
88.27	58.18	64.09	47.15	30.83	51.13	30.20	90.49	82.35
92.49	72.84	68.25	29.07	46.79	38.35	37.53	58.18	81.71
91.36	76.84	52.75	34.11	42.92	46.52	41.76	72.17	88.18
85.77	62.07	79.26		55.15		40.20		74.27

Calculation of the Percentage (%) weight gain of rats

To calculate the % weight gain:

$$\% \text{ weight gain} = \frac{\text{Final body weight - Initial body weight}}{\text{Initial body weight}} \times 100$$

Therefore;

$$C1 = \frac{86.52 - 43.63}{43.63} \times 100 = 98.3\%$$

$$C2 \text{ (Fybogel 1.5g)} = \frac{71.59 - 44.95}{44.95} \times 100 = 59.3\%$$

$$C2 \text{ (Fybogel 3.0g)} = \frac{65.63 - 42.42}{42.42} \times 100 = 54.7\%$$

$$\text{Bush mango 5.0g} = \frac{38.70 - 37.03}{37.03} \times 100 = 4.5\%$$

$$\text{Bush mango 10.0g} = \frac{45.50 - 44.67}{44.67} \times 100 = 1.9\%$$

$$\text{Okra 5.0g} = \frac{46.22 - 36.37}{36.37} \times 100 = 27.1\%$$

$$\text{Okra 10.0g} = \frac{38.61 - 34.55}{34.55} \times 100 = 11.8\%$$

$$\text{Osu 5.0g} = \frac{76.13 - 44.83}{44.83} \times 100 = 69.8\%$$

$$\text{Osu 10.0g} = \frac{83.92 - 42.00}{42.00} \times 100 = 99.8\%$$

Formulation of the compounded diet

Compounded Diet Formulation Table									
Components (%)	**C1 Fiber free**	**C2 Fybogel 1.5**	**C2 Fybogel 3.0**	**Bush mango 5.0**	**Bush mango 10.0**	**Okra 5.0**	**Okra 10.0**	**Osu 5.0**	**Osu 10.0**
Egg Albumin	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00
Starch	1,300.00	1,270.00	1,240.00	1,200.00	1,100.00	1,200.00	1,100.00	1,200.00	1,100.00
Palm Oil	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00
Vitamin mixture	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Mineral mixture	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00	80.00
Fiber source	-	30.00	60.00	100.00	200.00	100.00	200.00	100.00	200.00
Sucrose	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00
TOTAL (%)	2,000.00	2,000.00	2,000.00	2,000.00	2,000.00	2,000.00	2,000.00	2,000.00	2,000.00

PROXIMATE ANALYSIS TABULAR REPRESENTATION OF RESULTS

SAMPLE	Wt of C	Wt of S	Wt of C+S	Wt of C+S after	30mins	Interval	Moisture Content
ID	(g)	(g)	(g)	3hrs dry (105°C)	1	2	%
A1	9.755	1.00	10.755	10.612	10.608	10.608	14.70
A2	10.141	1.00	11.141	10.982	10.981	10.980	16.00
A3	10.404	1.00	11.404	11.254	11.252	11.250	15.40
B1	9.480	1.00	10.480	10.229	10.229	10.229	25.10
B2	9.953	1.00	10.953	10.689	10.686	10.686	26.70
B3	17.890	1.00	18.890	18.640	18.632	18.631	25.90
C1	9.765	1.00	10.765	10.649	10.646	10.644	12.10
C2	9.031	1.00	10.031	9.932	9.932	9.931	9.90
C3	9.481	1.00	10.481	10.357	10.355	10.355	12.60

SAMPLE	Wt of C	Wt of S	Wt of C+S	Wt of C+Ash @500°C for 3hrs	ASH Content
ID	(g)	(g)	(g)	(g)	%
A1	45.081	1.00	46.081	45.132	5.10
A2	24.331	1.00	25.331	24.388	5.70
A3	29.608	1.00	30.608	29.685	7.70
B1	29.611	1.00	30.611	29.718	10.70

B2	36.656	1.00	37.656	36.730	7.40
B3	42.254	1.00	43.254	42.350	9.60
C1	37.215	1.00	38.215	37.321	10.60
C2	42.236	1.00	43.236	42.330	9.40
C3	37.205	1.00	38.205	37.302	9.70

SAMPLE	Wt of C	Wt of S	Wt of C+S	Wt of C+	Crude fibre Content
ID	(g)	(g)	(g)	Fibre (g)	%
A1	42.228	1.00	43.228	42.385	15.70
A2	40.014	1.00	41.014	40.185	16.60
A3	29.608	1.00	30.608	29.752	14.40
B1	39.996	1.00	40.996	40.240	24.40
B2	45.078	1.00	46.078	45.315	23.70
B3	36.634	1.00	36.634	36.405	25.00
C1	37.204	1.00	38.204	37.405	20.10
C2	32.269	1.00	33.269	32.450	18.10
C3	29.607	1.00	30.607	29.819	21.20

SAMPLE	Wt of Flask	Wt of S	Wt of Flask+Oil	Crude Fat
ID	(g)	(g)	(g)	%
A1	161.3463	1.00	161.3566	1.03
A2	161.3463	1.00	161.3561	0.98

A3	161.3463	1.00	1613570	1.07
B1	161.3463	1.00	161.3567	1.04
B2	161.3463	1.00	1613579	1.16
B3	161.3463	1.00	161.3586	1.23
C1	161.3463	1.00	161.3546	0.83
C2	161.3463	1.00	161.35.54	0.91
C3	161.3463	1.00	161.3568	1.05

SAMPLE			Crude Protein
ID	Wt of sample (g)	Abs @ 520nm	%
A1	1.00	0.120	2.64
A2	1.00	0.141	3.10
A3	1.00	0.150	3.30
B1	1.00	0.053	1.17
B2	1.00	0.051	1.12
B3	1.00	0.056	1.23
C1	1.00	0.106	2.33
C2	1.00	0.132	2.90
C3	1.00	0.102	2.24

% Carbohydrate = 100 – (%Moisture + %Ash + %Crude Fibre + %Crude Fat + %Crude Protein)

= 100 – (14.70 + 5.10 + 15.70 + 1.03 + 2.64)

= 100 – 39.17

A1= 60.83

SAMPLE	Moisture Content	ASH Content	Crude fibre Content	Crude Fat	Crude Protein	Carbohydrate
ID	%	%	%	%	%	%
A1	14.70	5.10	15.70	1.03	2.64	60.83
A2	16.00	5.70	16.60	0.98	3.10	57.62
A3	15.40	7.70	14.40	1.07	3.30	58.13
B1	25.10	10.70	24.40	1.04	1.17	37.59
B2	26.70	7.40	23.70	1.16	1.12	36.59
B3	25.90	9.60	25.00	1.23	1.23	37.04
C1	12.10	10.60	20.10	0.83	2.33	54.04
C2	9.90	9.40	18.10	0.91	2.90	58.78
C3	12.60	9.70	21.20	1.05	2.24	53.24

KEY:

A = BUSH MANGO

B = OKRO

C = OSU

C1 - Control 1 (Fiber free group)

C2 - Control 2 (Fybogel at level of 1.5g)

C2 - Control 2 (Fybogel at level of 3.0g)

Bush mango - 5.0g

Bush mango - 10.0g

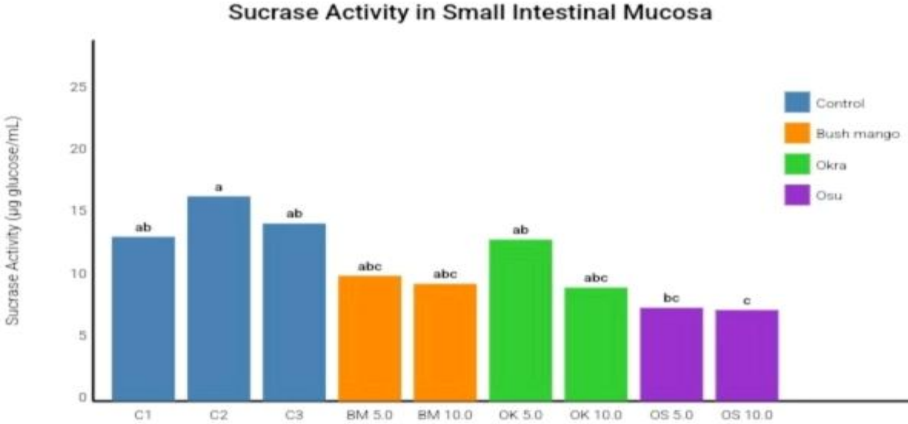
Okra - 5.0g

Okra - 10.0g

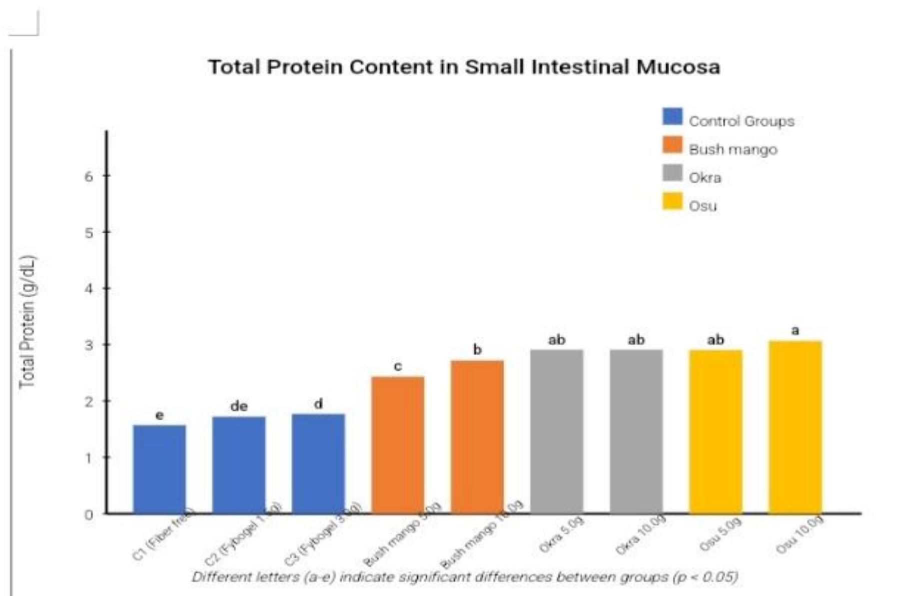
Osu - 5.0g

Osu - 10.0g

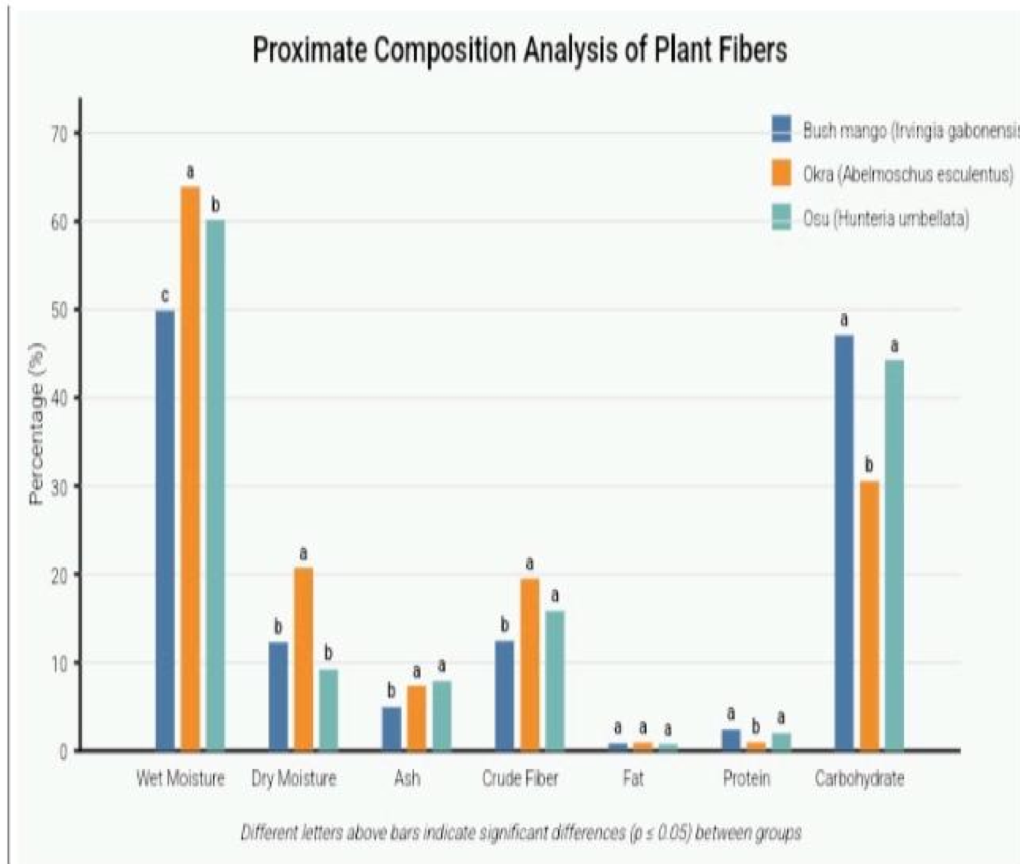
BAR CHARTS FOR THE VARIOUS PARAMETERS



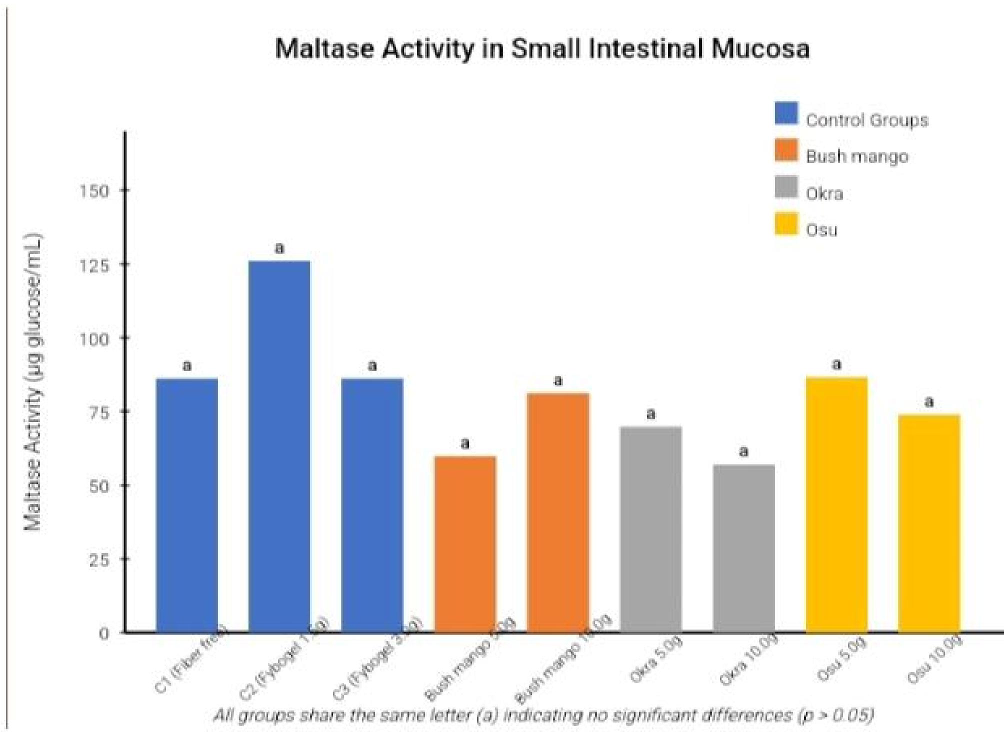
Sucrase Activity in Small Intestinal Mucosa



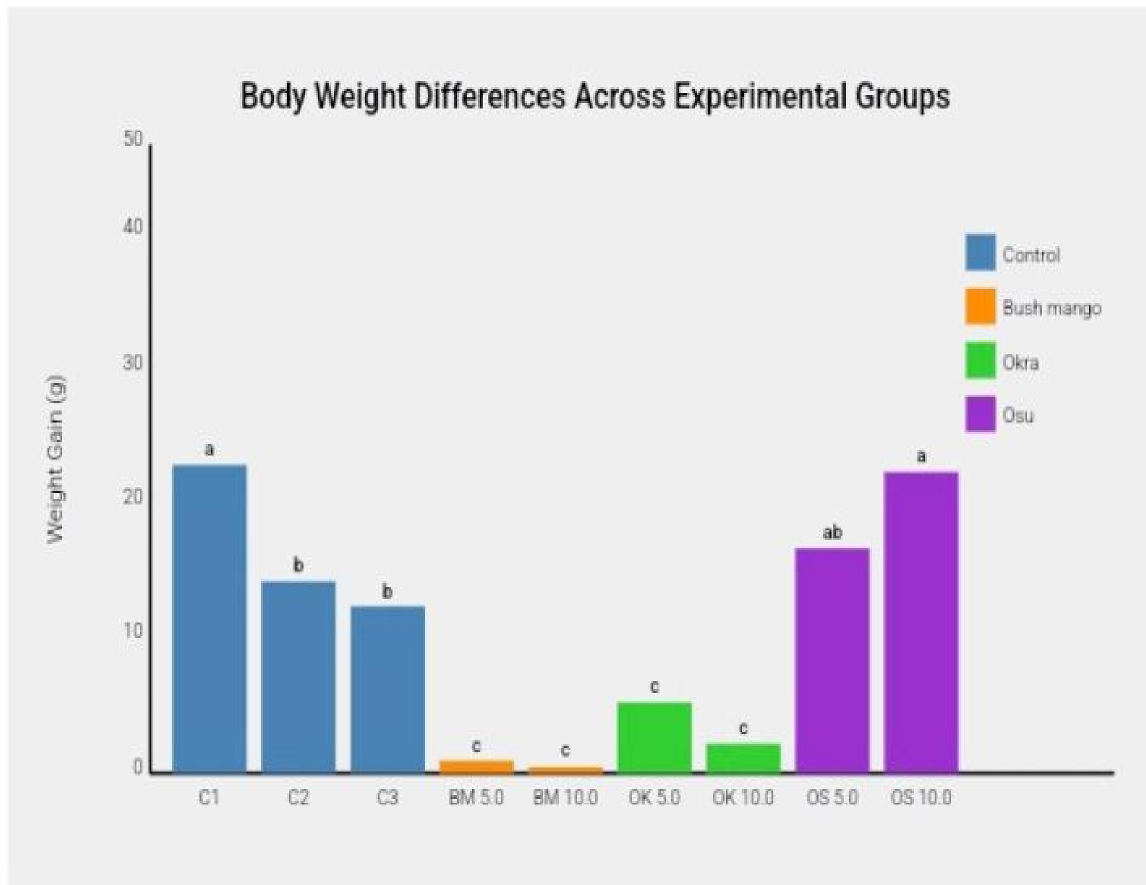
Total Protein Content in Small Intestinal Mucosa



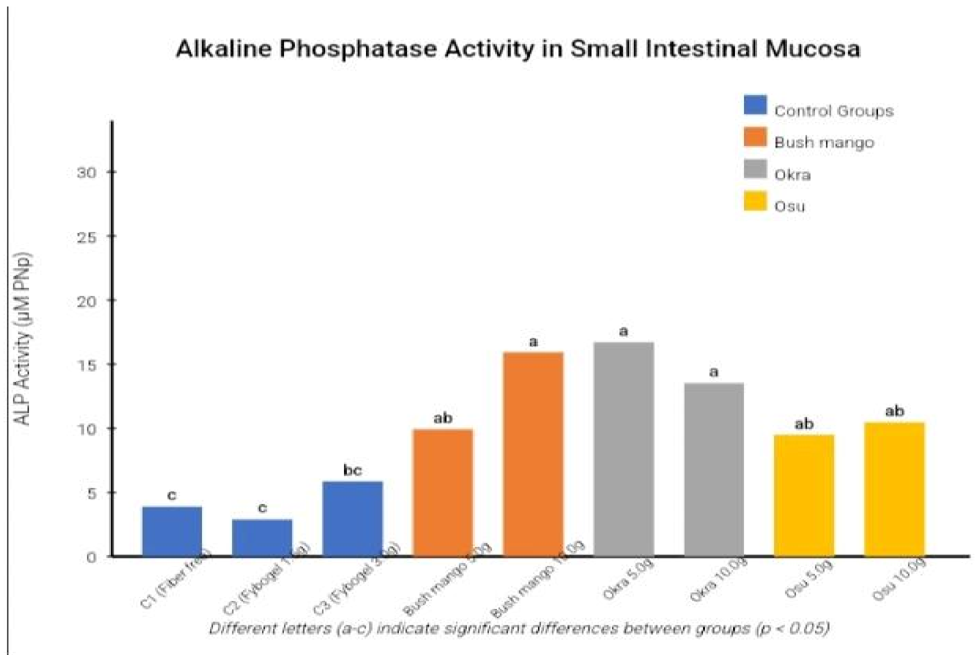
Proximate Composition Analysis of Plant Samples (Dry Weight Basis)



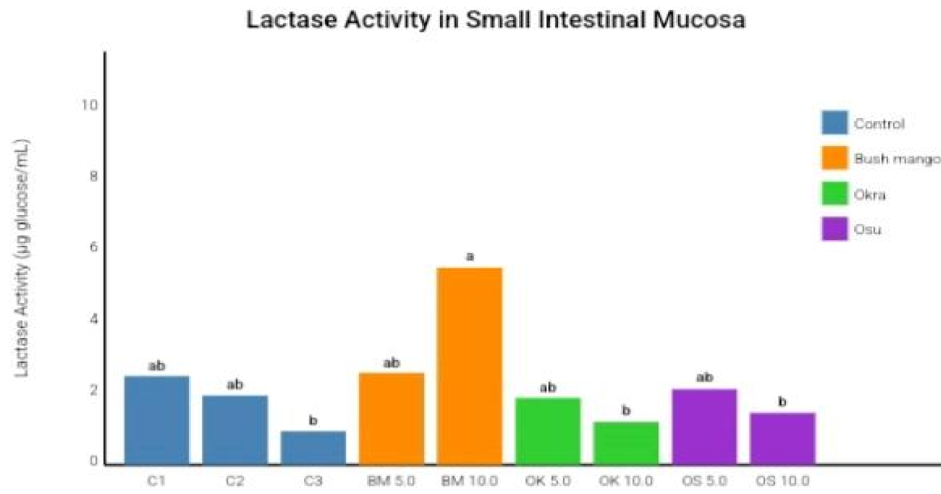
Maltase Activity in Small Intestinal Mucosa



Differences in Body Weights of Rats in Different Experimental Groups (grams)



Alkaline Phosphatase Activity in Small Intestinal Mucosa



Lactase Activity in Small Intestinal Mucosa

PREPARATION OF REAGENTS

A.) Maltase activity assay

Preparation of reagents:

Maltase buffer–100 mM potassium-phosphate buffer (pH 6.5) with 0.1 mM EDTA

a. 1 M stock solution was prepared for potassium phosphate dibasic (K2HPO4) and potassium phosphate monobasic (KH_2PO_4) salts in MilliQ quality water (MQ) water.

b. 0.5 M EDTA solution was prepared in MQ water.

c. For 400 ml 100 mM maltase buffer (pH 6.5) 14 ml of 1 M K_2HPO_4 (alkaline component) and 26 ml of 1 M KH_2PO_4 (acidic component) was mixed. The buffer was then prepared using pre-calculated amounts of acidic and alkaline components of the same molarity to obtain the desired pH value of the buffer (Gomori, 1955)

d. 80 µl of 0.5 M EDTA was then added

e. It was then completed to 400 ml with (MQ) water

f. And then filtered through a 0.2 µm cellulose acetate membrane filter and stored at 4 °C

Preparation of 100µl of 500mM sucrose solution prepared in maltase buffer:

a. First, the mass of sucrose required for 100 µL (0.1 mL) of a 500 mM solution was calculated as;

100µl of 500mM sucrose solution prepared in maltase buffer:

$$= \frac{500 \times \text{molecular weight of sucrose in 1L}}{1000} \text{ of maltase buffer}$$

Molecular weight of sucrose ($C_{12}H_{22}O_{11}$) = 342.3 g/mol

$= \frac{500 \times 342.3}{1000}$ in 1L maltase buffer

$= \frac{171150}{1000}$ in 1L

= 171.15g/L

That is, $\frac{171.15g/1000ml}{10}$

Therefore, 500mM sucrose= 17.115g/100ml.

17.115 mg of sucrose was utilised

b. 17.115mg was weighed using a weighing balance

c. 50 µL of maltase buffer was added to the weighed sucrose and gently mixed until the sucrose was dissolved in the solution

d. After the sucrose is dissolved, maltase buffer was added to make up the total volume to 100 µL.

Calculation

$$\text{Maltase activity (E)} = \frac{OD_{500}\ (1\ \text{min})}{(\varepsilon \times [c])}$$

OD_{500} (1 min) is absorbance (optical density) change of the reaction mixture per 1 min measured at 500 nm wavelength; [c] is concentration of the maltase protein (mg/ml) in the reaction mixture and (ε) is the extinction coefficient withdrawn from glucose calibration curve.

B.) Lactase activity assay

Preparation of reagents:

a. Phosphate buffer (0.1M, pH 7.0)

100mL (0.1L) of 0.1M buffer was prepared by utilising a combination 0.12 M NaH_2PO_4 and 0.08 M NaH_2PO_4 to achieve pH 7.0.

Molar mass:

Na_2HPO_4 = 141.96 g/mol

NaH_2PO_4 = 119.98 g/mol

For Na_2HPO_4:

mass = 0.12 × 0.1 × 141.96 = 1.70g

For NaH_2PO_4:

mass = 0.08 × 0.1 × 119.98 = 0.96g

1.70g of Na_2HPO_4 and 0.96g of $NaH_2PO_4$was dissolved in distilled water. The pH was then adjusted with HCl to reach pH 7.0 with a pH meter and the final volume was adjusted to 100ml with distilled water.

b. Substrate Solution (1 g of Lactose in 100 mL):

1 g of lactose was weighed and dissolved in distilled water. Distilled water was added to reach a final volume of 100mL, and the solution was stirred until it completely dissolved.

c. Perchloric acid solution (4%)

To prepare 100 mL of 4% (w/v) perchloric acid:

Calculate;

$$\text{mass} = \text{concentration (g)} \times \text{volume (mL)} = \frac{4 \times 100}{100} = 4\text{g}$$

(mL)

4 g of perchloric acid was then diluted in distilled water to 100 mL total volume.

C.) Sucrase activity assay

Preparation of reagents:

a. Sucrose Solution (0.3 M)

First, the volume required was determined: 1 mL of 0.3 M sucrose solution.

The amount of sucrose needed was calculated as follows::

Molar mass of sucrose = 342.3 g/mol.

For 0.3 M:

mass = concentration × volume × molar mass

$\text{mass} = 0.3 \times 0.1 \times 342.3 = 10.27\text{g}$

Therefore, for 100 mL of 0.3 M sucrose solution, 10.27g of sucrose was weighed dissolved in distilled water and the final volume was adjusted to 100 mL.

b. Sodium Acetate Buffer (0.05 M, pH 4.7): 100 mL

The amount of sodium acetate needed was determined:

Molar mass of sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$) = 136.08 g/mol.

For 0.05 M:

mass (g) = 0.05 × 0.1 × 136.08 = 0.68g

Therefore, 0.68g of sodium acetate trihydrate was weighed.

To prepare the buffer solution:

0.68g of sodium acetate trihydrate was dissolved in distilled water, adjusted to a pH of 4.7 by using NaOH using a pH meter. The final volume was made up to 100mL with distilled water.

c. 3,5-Dinitrosalicylic Acid (DNS) Reagent: 100 mL

3g of dinitrosalicylic acid was weighed and dissolved in 90 mL of distilled water, then 30 mL of 0.5 M sodium hydroxide was added as follows:

Molar mass of NaOH = 40.00 g/mol.

For 0.5 M:

mass (g) = 0.5 × 0.03 × 40.00 = 0.6g

0.6g of NaOH was dissolved in distilled water and then mixed with DNS solution. The volume was made up to 100mL with distilled water.

d. Glucose and Fructose Standard Solution (5 mM each):

First, the amount of glucose and fructose required was calculated as;

For a 5 mM concentration in 10 mL each (for standards):

Molar mass of glucose ($C_6H_{12}O_6$) = 180.18 g/mol and fructose ($C_6H_{12}O_6$) = 180.18 g/mol.

$$5\text{mM fructose} = 5 \times \frac{\text{molecular weight of fructose in 1 litre of distilled water}}{1000}$$

$$= 5 \times \frac{180.18 \text{ in } 1\text{L}}{1000}$$

$$= 0.9009\text{g/L}$$

That is, $= \frac{0.9009\text{g}/1000\text{ml}}{10} = 0.09009\text{g}/100\text{ml}$

Therefore, 5mM fructose = 0.09009g/100ml, which is equivalent to 9.01mg (0.09009 × 100)

Since, molecular weight of glucose = 180.18 g/mol

5mM glucose= 0.09009g/100ml, which is equivalent to 9.01mg (0.09009 × 100).

9.01 mg of glucose and 9.01 mg of fructose each was then weighed and dissolved in separate small volumes of distilled water, after which both were combined into a final volume of 10 mL resulting in 5 mM for both.

The fruits of *Irvingia gabonensis* (Self, 2024).

Hunteria umbellata fruits (Self, 2024).

Abelmoschus esculentus fruits (Self, 2024).

University of Benin

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London)
Faculty of Life Sciences,
Department of Plant Biology and Biotechnology,
P. M. B. 1154 Ugbowo, 300283 Benin City,
Edo State, Nigeria.

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *Irvingia gabonensis* Aubry-Lecomte ex O'Rorke

Family: Irvingiaceae

Common Name: Bush Mango, Wild Mango, African Mango

Voucher Number: UBH-I153

Student Name: Joy Imade Obanor

Plant Identification and Voucher Number Issued By:

____________________ 19/08/2025_

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, LMBOSON, MNES; Nigeria)

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London)
Faculty of Life Sciences,
Department of Plant Biology and Biotechnology,
P. M. B. 1154 Ugbowo, 300283 Benin City,
Edo State, Nigeria.

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *Hunteria umbellata* (K. Schum.) Hallier f.

Family: Apocynaceae

Common Name:

Voucher Number: UBH-H637

Student Name: Joy Imade Obanor

Plant Identification and Voucher Number Issued By:

19/08/2025

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, LMBOSON, MNES; Nigeria)

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London)
Faculty of Life Sciences,
Department of Plant Biology and Biotechnology,
P. M. B. 1154 Ugbowo, 300283 Benin City,
Edo State, Nigeria.

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *Abelmoschus esculentus* (L.) Moench.

Family: Malvaceae

Common Name: Ladies finger, Okro, Okra

Voucher Number: UBH-A399

Student Name: Joy Imade Obanor

Plant Identification and Voucher Number Issued By:

19/08/2025

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, LMBOSON, MNES; Nigeria)