INHIBITORY EFFECT OF ACETONE FRACTION OF *Lonchocarpus cyanescens* ON ALPHA AMYLASE AND ALPHA GLUCOSIDASE

BY

OBAH GIFT ONYINYECHI

BMS2101428

DEPARTMENT OF MEDICAL BIOCHEMISTRY,

SCHOOL OF BASIC MEDICAL SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER, 2025

INHIBITORY EFFECT OF ACETONE FRACTION OF *Lonchocarpus cyanescens* ON ALPHA AMYLASE AND ALPHA GLUCOSIDASE

BY

OBAH GIFT ONYINYECHI

BMS2101428

A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE, BSc (HONS) MEDICAL BIOCHEMISTRY, OF THE UNIVERSITY OF BENIN, BENIN CITY

NOVEMBER, 2025

CERTIFICATION PAGE

We the undersigned hereby certify that Miss. **OBAH GIFT ONYINYECHI** carried out this work, in the department of Medical Biochemistry, University of Benin, Benin city and we approve same as adequate in scope and quality for the awards of Bachelors of Science degree(B.sc) in Medical Biochemistry

....

DR.(Mrs.) N. ELUEHIKE Date

(Project Supervisor)

....

DR. AGUEBOR – OGIE. Date

(Head of Department)

....

External Supervior Date

(Head of Department)

DEDICATION

I dedicate this project to God Almighty my creator, my strong pillar, my source inspiration, wisdom,knowlwdge and understanding. He has been my source of strength throughout this program.

ACKNOWLEDGEMENTS

This research work would not have been possible without encouragement from my parents Mr and Mrs CHRISTOPHER MONYE, and MR. MONYE ISIOMA VICTOR AND MR. ONYEMAI ONYISI CHRISTOPHER. I would also like to acknowledge my siblings Bright, Morris who have played a vital role in my life from birth.

I would not proceed without acknowledging my Supervisors, Dr. Mrs. N. Eluehike whose precision and ideas gave more light to my research.

Finally, I thank my friends, Gbenoba-West Ehiose, Garba Eucharia, Orogbemi Kehinde, stella, Ojo Itohan, among others for their active presence when I needed them to elaborate on one issue or another. Thank you all

TABLE OF CONTENT

LIST OF FIGURES

ABSTRACT

Introduction: Diabetes mellitus is a metabolic disorder characterized by high blood sugar levels. Inhibiting enzymes like alpha-amylase and alpha-glucosidase is a key strategy to control hyperglycemia. *Lonchocarpus cyanescens* is a medicinal plant with potential antidiabetic properties that warrants scientific evaluation. The major aim of this research is to ascertain and provide scientific information on the antidiabetic properties of *Lonchocarpus cyanescens* utilizing alpha amylase and alpha glucosidase inhibitory assay All materials used were of high quality which includes alpha amylase, alpha glucosidase, distilled water, ethanol, acetone, hexane. Methodology involves detrmining the antidiabetic properties of the plant extract utilizing alpha amylase and alpha glucosidase inhibitory assay. The potential for *Lonchocarpus cyanescens* extract to reduce hyperglycemia and perform antidiabetic functions was determined. Alpha-amylase inhibition activity of each fraction was determined by the method of Worthington 1993. An aliquot of 500 microliter of the extract (0.1–0.4 mg/ml) and 500 microliters (0.02M) of sodium phosphate buffer (pH 6.9 with 0.006M NaCl) containing 0.5 mg/ml of alpha-amylase will be mixed together and incubated for 10 min at room temperature.Afterwards, 500 microliters of 1% starch solution prepared with 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) will be added and incubated in a water bath at 25°C for 10 minutes.The reaction mixture will be stopped by adding 1.0 ml (96 mM) of Dinitro salicylic acid.The mixtures in the test tubes will be incubated in boiling water in a water bath for 5 minutes and then cooled for alpha amylase. Then for alpha glucosidase Alpha-glucosidase activity of each fraction will be determined by the method of Apostolidis et al., 2007.The substrate solution, p-nitrophenyl-glucopyranoside (pNPG), was prepared in 0.02M phosphate buffer, pH 6.9. 1000 microliter of alpha-glucosidase was incubated with 500 microliters of different concentrations of the extract for 10 minutes at 25°C. An aliquot (500 microliter) of freshly prepared phosphate-buffered p-nitrophenyl-glucopyranoside (5 mM) solution will be added. The reaction mixture will be incubated at 25°C for 5 minutes and stopped by adding 2 ml of 0.1 Na_2CO_3. The alpha-glucosidase activity was determined by measuring the absorbance at 405 nm using a spectrophotometer. Absorbance reading assay was carried out and statistical analysis was also carried out to checkn for satistical significance. A p-value for alph amylase was found out to be [p=0.0321] and for alpha glucosidase the p –value was recorded to be [p=0.0002] in this assay. In conclusion, Lonchocarpus cyanscens possess antidiabetic properties and can serve for several medical purposes.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Phytochemical investigation of *Lonchocarpus cyanescens* Benth. (Family: Fabaceae) have revealed the presence of diverse bioactive compounds including flavonoids, tannins, steroids, terpenoids, cardiac glycosides, phlobatannins, and saponins (Ogumoyole et al, 2024; Adesina et al, 2024). Analyses of its volatile oils show that leaf oil is dominated by phytol (~65.5%) and hexadecenoic acid (~12.4%), while stem oil contains high amounts of octadecanoic acid (~24.1%) and hexadecenoic acid (~17.2%) (Ejele & Nnoli, 2022).

Furthermore, seed oils are rich in unsaturated fatty acid such as linolenic and oleic acids and also contain sterols, tocopherol, and other unsaponifiable matter (Adeniyi & Odufowora, 2023). These phytochemical constituents are often linked with antioxidant, hypoglycemic, and enzyme inhibitory effects suggesting potential pharmacological activities of the plant (Akinmoladun et al, 2020). However, many of its therapeutic properties remain underexplored.

Diabetes mellitus is a chronic metabolic disease characterized by persistent hyperglycemic resulting from defects in insulin secretion action, or both (American Diabetes Association, 2024). Uncontrolled diabetes can lead to severe complications including cardiovascular diseases, nephropathy, retinopathy, and neuropathy. Type 2 diabetes mellitus, the most prevalent form is strongly associated with obesity, poor diet, and physical inactivity, and its global burden continues to rise. In 2024, the prevalence of diabetes among adults aged 20-79 years was estimated at 9.3% (463 million people), with projections to reach 578 million by 2030 and 700 million by 2045 (International Diabetes Federation, 2024).

One of the major therapeutic challenges in type 2 diabetes management is the regulation of postprandial hyperglycemia---the sharp rise in blood glucose following meals. Pharmacological approaches to mitigate this include inhibition of key digestive enzymes such as -amylase and ---glucosidase, which are responsible for the breakdown of dietary carbohydrate into absorbable glucose (Sales et al., 2024). Synthetic inhibitors such as acarbose, migitol, and voglibose are effective but often produce gastrointestinal side effects like flatulence and diarrhea (Zhou et al., 2024). This has intensified the search for plant derived enzyme inhibitors with potentially fewer adverse effects and wider accessibility, especially in low-resource setting.

Given the traditional use of L. Cyanescens in western Nigeria for managing diabetes (Ogunmoyole et al., 2024), and the presence of bioactive lipophilic compounds such as phytol, unsaturated fatty acids, and sterols, it is plausible that polar fractions (e.g., acetone extract) may exhibit α-amylase and α-glucosidase inhibitory activity. However, there is currently little to no literature specifically addressing the antidiabetic potential of the Acetone fraction of L. Cyanescens, highlighting a critical gap in knowledge.

1.2 Aim of the Study

The aim of this study is to evaluate the inhibitory effect of the Acetone fraction of *Lonchocarpus cyanescens* on α-amylase and α-glucosidase activities, with a view to providing scientific evidence for its potential antidiabetic properties

Diabetes

Diabetes mellitus is a group of metabolic disorders characterized by chronic glycemia resulting from defects in insulin secretion, insulin action or both.

Long term hyperglycemia is associated with damage to multiple organ system (eyes, kidneys, hearts, and vessels).

Diabetes especially type 2 is a leading cause of cardiovascular disease, kidney failure, blindness and lower limb amputation.

Classifications/major types (Clinical categories)

Type 1 Diabetes (T1D); autoimmune beta cell destruction-absolute insulin deficiency. Typically presents in children/young adults but can occur at any age.

Type 2 Diabetes (TD); relative insulin deficiency with insulin resistance, strongly associated with overweight and obesity, sedentary lifestyle and aging, T2D accounts for the majority of diabetic cases.

Treatment goal and general approach.

Goals center on preventing acute metabolic decompensation and long term microvascular and microvascular complications by achieving individualized glycemic targets, managing cardiovascular risk factors and addressing comorbidities, diabetes self-management education, eating healthy, physical activity, and ongoing monitoring are foundational.

Types and treatment (non pharma logical and pharma logic).

Non pharma logical.

Medical nutrition therapy, weight management, increased physical activity, smoking cessation structured education, and psychosocial support, (joining support group.)

CHAPTER TWO

LITERATURE REVIEW

2.1 The Plant *Lonchocarpus cyanescens* Benth.

Is a perennial climbing shrub or small tree belonging to the family Fabaceae (Leguminosae), one of the largest families of flowering plants. It is locally known as “Elu” (Yoruba), “Ebelu” (Edo), “Talaki/Talagi” (Hausa), and “Nji” in other dialects. Internationally, it is referred to as West Africa Indigo or Yoruba Indigo due to its traditional use as a natural indigo dye source.

The plant is widely recognized for its dual role as a medicinal resource and dye plant, with

historical, cultural, and therapeutic significance in West Africa.

Figure 2.1 *Lonchocarpus cyanescens* leaves/plants

Source NMPPDB (Nigeria Medicinal Plants& Products Database)

2.2 Taxonomic Classification

The taxonomic classification of L. Cyanescens is presented below (Burkill, 2022; The Plant List, 2023);

Kingdom; Plantae

Phylum; Tracheophyte

Class; Magnoliopsid

Order; Fabales

Family; Fabaceae (Leguminosae)

Genus; Lonchocarpus

Species; *Lonchocarpus cyanescens* Benth

Synonyms; Lonchocarpus cyaneus (Schumach. & Thonn.) Benth, Macrolobium cyanescens Schumach. & Thonn., Philenoptera cyanescens (Schumach & Thonn) Roberty.

2.3 Geographical Distribution and Habitat

l. cyanescens is indigenous to West and Central Africa with occurrence in Nigeria, Benin, Ghana, Cameroon, Cote d Ivoire, Guinea, Liberia, Sierra Leone, Togo, Equatorial Guinea, Gabon, and the Democratic Republic of Congo (Orishadipe et al., 2022; Burkill, 2022).

In Nigeria, it is especially abundant in the southwestern and south eastern regions, where it is cultivated around homesteads and farm for medicinal purposes and dye production.

Globally, species of the genus Lonchocarpus are also distributed in tropical and subtropical regions of the Americas and Africa, but L. Cyanescens is particularly prominent in West Africa (Akinmoladun et al., 2016).

It thrives in coastal forest, riverine thickets, and wooded savannahs, typically at elevations up to 400 m. the plant prefers well drained sandy=loam soils (ph. 5.5—7.0) and tolerates seasonal flooding (Burkill, 2022).

2.4 Morphological Feature

Lonchocarpus cyanesens display diverse growth habits depending on ecological conditions.

Habit; a perennial climbing shrub or a small tree, reaching heights of 6-15 m. It often twines on other vegetation when growing in forest margins.

Stem/Bark; woody, greyish-brown, smooth when young but fissured with age; branches are slender and sometimes drooping.

Leaves; alternate, compound, pinnate (15-25 cm long), usually with 5-9 ovate-lanceolate leaflets, 4-10 cm in length, dark green and glabrous above, slightly pubescent beneath.

Flowers; small, bluish to purplish (sometimes white to pale violet), fragrant, and arranged in dense axillary or terminal racemes. The species name cyanescens (“bluish”) reflects the flower color

Fruits/seeds; flat, oblong pods (5-10 cm long, 1-2 cm wide), containing 1-5 seeds rich in oil.

Special feature; the leaves contain indicant, which upon hydrolysis and oxidation produces indigo dye, making the plant economically important in textile production (Cardon & Jansen, 2022).

2.5 Ethnomedicinal Use

The plant has a long use of ethnopharmacological applications across West Africa;

Leaves; used for treating malaria, fevers, gastrointestinal disorders, constipation, venereal diseases, skin infections, wounds, rheumatism (Adesina, 2022; Sonibare et al., 2024). Leaf decorations are also employed in managing diabetes mellitus in parts of Western Nigeria (Ogunlana et al., 2024).

Roots; used in Ghana and Nigeria for arthritis, rheumatism, bone pain, postpartum care, and body pains.

Bark; applied for relief of arthritis rheumatism, and parasitic infections.

Traditional Psychotherapy; leaf extracts are used to manage psychosis in Nigeria. Studies confirmed suppression of amphetamine-induced stereotypy in rats, linked to flavonoids like quercetin and kaempferol (Sonibare et al., 2024).

Anthelminthic; in Benin, fresh leaf sap is used against intestinal worms.

Antimicrobial; in Sierra Leone, leaf poultices are applied to ulcers.

Women's Health; root decoctions aid recovery after childbirth, and leaf infusions are used in uterine health.

Beyond medicine, L. Cyanescens plays important cultural and economic roles.

Dye production; leaves yield indigo dye for traditional textiles such as Yoruba "Adire" cloth, dating back to the 11^{th} century.

Ecological; enhances soil fertility through nitrogen fixation, thus contributing to agroforestry systems (Achigan-Dako,2009).

Wood use; its hard and durable timber us used locally for carpentry and small-scale construction.

2.6 Phytochemical Constituent.

Phytochemical screening of different parts of L. Cyanescens has revealed numerous bioactive compounds (Ogunlana et al., 2024; Ojo et al., 2024; Moronkola et al., 2023).

General constituents; Flavonoids (quercetin, kaempferol), tannins, alkaloids, saponins, terpenoids, triterpenes (lupeol), cardiac glycosides, phlobatannins, and Indian (precursor of indigo).

Leaf oil; rich in phytol (~62.5%) and hexadecenoic acid (~12.4%)

Stem oil; contains octadecanoic acid (~24.1%) and hexadecenoic acid (~17.2%)

Seed oil; composed of unsaturated fatty acids (linolenic and oleic acids), sterols, β-tocopherol, and unsaponifiable matter.

These compounds contribute to its medicinal versatility. For instance;

Flavonoids & tannins; antioxidant, anti-inflammatory, antidiabetic, and antimicrobial.

Saponins; anthelmintic, hypoglycemic, and uterine tonic effects.

Terpenoids & sterols; anti-inflammatory and potential enzyme inhibitory activity.

Indicant; provides dye and contributes to antimicrobial properties

2.7 Pharmacological Activities

Several experimental and clinical studies have validated the traditional uses of L. Cyanescens.

Antioxidant activity; strong free-radical scavenging ability due to flavonoids and phenolic compounds (Ajayi et al., 2025).

Antimicrobial activity; active against Staphylococcus aureus and other pathogens (Sonibare et al., 2014; Ojo et al., 2024).

Anti-inflammatory & analgesic effects; roots extracts used for arthritis and rheumatism show significant activity (Akinmoladun et al., 2016).

Antipsychotic effects; leaf decoctions reduce amphetamine-induced stereotypy in animal models (Sonibare et al., 2024).

Antidiabetic potential; reported mainly with methanol and aqueous extracts. These show glucose-lowering activity, though research on non-polar hexane fractions remains limited (Ogunlana et al., 2024).

2.8 Toxicological Profile

Toxicological evaluations show that aqueous extract of the leaves are relatively safe up to 5,000 mg/kg in rats over 28 days, with no significant hepatoxicity (Iyoha & Aisuhuehien, 2023). However;

High doses may cause mild gastrointestinal upset due to saponins

Fresh leaf sap may cause skin irritation in sensitive individuals (Baumgartel et al., 2023).

2.9 Distribution of *Lonchocarpus cyanescens*.

Lonchocarpus cyanescens is a species native exclusively to tropical Africa, with its core range concentrated in West Africa. It is not considered introduced or naturalized elsewhere based on current records from global biodiversity databases.

The plants distribution is influenced by factors such as rainfall patterns (typically 1,000 -2,000 mm annually with a pronounced dry season), soil types (preferring sandy-loamy, well drained soils), and human activities, including traditional cultivation for dye production and medicine. It is not uniformly distributed; densities are higher in coastal and riverine areas, decreasing inland toward drier Sahelian zones. No significant range expansions or contractions have been documented in recent decades, though climate change models predict potential shifts southward due to increasing aridity in northern margins.

The plants distribution spans several countries, primarily those with suitable savanna and woodland habitats. Key countries include;

- West Africa; Nigeria, Ghana, Benin, Togo, Ivory Coast (Cote d Ivoire), Senegal, Guinea, Sierra Leone, and Liberia. This region represents the densest occurrence, where it is commonly found in distributed areas, forest edges, and secondary growth.
- Central Africa; Cameroon, Gabon, Central African Republic, and the and the Democratic Republic of the Congo (DRC). Here, it appears in transactional zones between rainforests and savannas
- Other Tropical African Regions; Sporadic records in Mali, Burkina Faso, and possibly northern Angola, though these are less frequent.

The global occurrence map from sources like GBIF shows over 1,000 documented records, with majority clustered between latitudes 4°N and 12°N and longitudes 15°W and 20°E, aligning with the Guinea savannah and Sudan savanna ecoregions. No significant populations are reported in East or Southern Africa.

2.10 Habitat and Ecological Preferences

This deciduous shrub or small tree (growing 6-15 m tall) thrives in lowland tropical environments at elevations typically below 500 m. Preferred habitats include;

Open woodlands, savannas, and grassy clearings.

Forest margins and secondary vegetation in disturbed sites (e.g., abandoned farmlands).

It tolerates seasonal flooding but not waterlogged conditions

It flowers during the dry season (often October- December) and fruits in the wet season, benefitting from the region's bimodal rainfall patterns (1,000-1,500 mm annually). As a legume (Fabaceae family), it contributes to soil nitrogen fixation, making it ecologically valuable in agroforestry.

2.11 Ethnomedicinal Uses of *Lonchocarpus Cyanescens*

The leaves, roots, stems, and sometimes seeds, of *Lonchocarpus cyanescens* are used by various ethnic groups to treat a wide range of ailments, leveraging its bioactive compounds such as flavonoids, tannins, saponins, and rotenoids. Below is an exhaustive overview of its ethnomedicinal uses, drawing from regional practices, scientific studies, and traditional knowledge systems.

2.12 Ethnomedicinal Uses By Region And Ethnic Group.

The plant is known by various local names, reflecting its widespread cultural significance; elu (Yoruba), anunu (Igbo), talaki (Hausa), sauru (Tiv), ebelu (Edo), and sangara (Mandinka). Its uses are tailored to local needs and vary across communities, primarily in West Africa (Nigeria, Ghana, Senegal, Benin, Côte d'Ivoire) and parts of Central Africa (Cameroon, DRC).

1. Mental Health and Neurological Disorders

Psychosis and Schizophrenia; among the Yoruba of Nigeria, leaf decoctions are traditionally used to manage psychosis and related mental disorders. Aqueous and ethanolic leaf extracts have been studied for their antipsychotic properties, showing efficacy in rodent models by reducing stereotypic behaviors induced by drugs like amphetamine or apomorphine. The mechanism may involve dopamine receptor modulation or antioxidant effects from flavonoids and phenolics. In Ghana, similar preparations are used for calming agitation or "madness."

Convulsions and Seizures; in Senegal and Benin, root infusions are administered to control convulsions, particularly in children, possibly due to sedative compounds like terpenoids.

Sedative Effects; The Igbo in Nigeria use leaf extracts as a mild sedative for anxiety or insomnia, often combined with other herbs like Ocimum gratissimum.

2. Inflammatory and Pain-Related Conditions

Arthritis and Rheumatism; In Nigeria (Edo and Yoruba communities) and Côte d'Ivoire, leaf and stem bark poultices or decoctions are applied topically or ingested to relieve joint pain and inflammation associated with arthritis. Studies confirm anti-inflammatory activity, likely due to flavonoids and glycyrrhizin acid derivatives, which inhibit prostaglandin pathways.

General Pain Relief; In Ghana, root infusion is used for headaches and body aches, sometimes boiled with peppers for enhanced effect.

Swelling and Edema; the Tiv in Nigeria apply crushed leaves to reduce localized swelling, leveraging the plants anti-edematous properties.

3. Gastrointestinal Disorders

Peptic Ulcers; among the Hausa in northern Nigeria, leaf extracts are taken orally to soothe stomach ulcers, attributed to mucosal-protective compounds like tannins and saponins. Preliminary studies suggest gastroprotective effects in animal models,

Diarrhea and Dysentery; in Sierra Leone and Liberia, decoctions of leaves or roots are used to treat diarrhea, possibly due to antimicrobial and astringent properties.

Worm Infestations; in Benin, root preparations are used as an anthelmintic to expel intestinal parasites, likely linked to rotenoid compounds with toxic effects on parasites.

4. Infectious Diseases

Antimicrobial Applications; across West Africa, leaf and stem extracts are used to treat skin infections, wounds, and sores. In vitro studies confirm antibacterial activity against pathogens like staphylococcus aureus and Escherichia coli, attributed to volatile oils (e.g., phytol) and flavonoids. In Cameroon, leaf poultices are applied to abscesses.

Malaria and Fevers; in Guinea and Nigeria, leaf decoctions are used as antipyretics for malaria and other fevers, sometimes combined with Azadirachta indica (neem). While less studied, antimalarial potential may stem from alkaloids or flavonoids

5. Reproductive and Women's Health

Menstrual Pain; in Ghana and Togo, women use leaf infusions to alleviate dysmenorrhea (painful periods), possibly due to anti-inflammatory and muscle-relaxant effects.

Postpartum Recovery; among the Yoruba, leaf decoctions are given to new mothers to promote healing and reduce inflammation after childbirth.

Fertility Support; in some Nigerian communities, root extracts are believed to enhance fertility, though scientific validation is limited.

6. Dermatological and Cosmetic Uses

Wound Healing; across Nigeria and Senegal, crushed leaves or bark pastes are applied to cuts, burns, or ulcers to promote healing and prevent infection, leveraging antimicrobial and astringent properties.

Skin Conditions; in Côte d'Ivoire, leaf extracts treat rashes and fungal infections, with ethnobotanical surveys noting use for eczema-like conditions.

7. Piscicidal and Insect-Repellant Uses

Fish Poisoning; in Nigeria, particularly among the Yoruba and Tiv, the roots and leaves are used as a piscicide (fish poison) to stun fish in small streams, facilitating capture. Rotenoids, such as rotenone, are responsible for this ichthyotoxic effects, disrupting fish respiration. This practice, while effective, raises ecological concerns due to non-selective impacts on aquatic life.

Insect Repellant; in rural Ghana and Benin, dried leaves are burned or scattered to repel mosquitoes and other insects, likely due to volatile compounds like phytol.

8. Other Ethnomedicinal Uses

Respiratory Issues; in Sierra Leone, leaf decoctions are used for coughs and bronchitis, possibly due to expectorant or anti-inflammatory effects.

Blood Disorders; some Nigeria communities use root infusions for anemia or as a blood tonic, though mechanism is unclear.

Snakebites; in rural Togo and Benin, root pastes are applied to snakebites as a first aid measure, potentially neutralizing venom through alkaloid activity, though this is poorly studied.

2.13 Alpha amylase and Alpha glucosidase

Alpha-amylase and alpha-glucosidase are enzymes that digest dietary carbohydrates, with alpha-amylase initiating the breakdown of long chain starches and alpha-glucosidase completing the process by hydrolyzing disaccharides and oligosaccharides into glucose

Both enzymes are crucial in carbohydrate digestion and their activity increases blood glucose levels after a meal.

Inhibiting these enzymes can control postprandial hyperglycemia, making them targets for antidiabetic therapies, which include medications and natural compounds like polyphenols found in plants.

Role in Carbohydrate Digestion

Alpha-amylase; this enzyme begins the breakdown of complex carbohydrates (polysaccharides) like starch into smaller oligosaccharides, such as maltose. It is found in saliva and pancreatic secretions.

Alpha-glucosidase; this enzyme acts as on disaccharides and oligosaccharides, cleaving their bonds and releasing individual glucose molecule that can be absorbed into bloodstream. It works at the brush border of the small intestine and includes enzymes like isomaltose.

Clinical Significance

Controlling Blood Sugar; the primary clinical role of inhibiting these enzymes is to manage type 2 diabetes. By slowing the digestion and absorption of carbohydrates, the enzymes combined effect reduces the rate at which glucose enter the blood, thus controlling postprandial hyperglycemia (high blood sugar after meals).

Therapeutic Targets; alpha-amylase and alpha-glucosidase inhibitors are used in the treatment of diabetes. Examples of commercial alpha-glucosidase inhibitors include acarbose, migitol, and voglibose, though they can cause side effects like flatulence and nausea.

Natural Inhibitors: many natural compounds, particularly polyphenols found in plant extracts, exhibit potent inhibitory activity against these enzymes. Bitter gourd Momordica charantia) and other plant-derived compounds are being studied as potential therapeutic tools for diabetes management.

Factors affecting alpha amylase and alpha glucosidase

PH - both enzymes have optimal activity in neutral to slightly acidic ph. (6.0-7.0)

Extreme Ph values denature the cative site (Gupta et al., 2023)

Temperature – optimal temperature 23°C for mammalian enzymes, microbial enzymes tolerate for higher ranges.

High temperature can also cause denaturation (Gupta et al. 2023)

Enzyme concentration – reaction rate increases with enzyme concentration until substrate saturation is reached. (Butterworth et al. 2022)

Inhibitors – polyphenols, alkaloids, flavonoids, and synthetic drugs like acarbose inhibit enzymatic activities (Tadera et al. 2024)

Metal ions – calcium ion stabilizes alpha amylase, heavy metals e.g., mercury, copper inhibits activity by interacting with active site residues (Gupta et al. 2023)

Mechanism of inhibitions of Alpha amylase and Alpha glucosidase

Therapeutic

Postprandial hyperglycemia (rapid rise in blood glucose after meals) is a major problem in type 2 diabetes. Inhibiting Alpha amylase and Alpha glucosidase slows starch digestion and delays glucose absorption, leading to lower post blood sugar peaks (Lebovitz, 2022; Sales et al. 2024.)

Mechanism of Inhibitions

Alpha amylase inhibitors – they bind to enzymes active site, blocking access to polysaccharides structure and completely inhibits.

Alpha glucosidase inhibitors – e.g., Acarbose and miglitol

They mimic the oligosaccharides' structure and competitively occupy the catalytic site on intestinal Alpha glucosidase enzymes. (Kim et al., 2022).

The results of both is a slower hydrolysis of starch, reduced glucose availability and moderated glycemic response (Sales et al. 2024).

Natural inhibitions

Phytochemicals such as flavonoids, tannins, saponins and terpenoids form medicinal plants exhibits Alpha amylase and alpha glucosidase inhibitions.

These compounds often interact with enzymes active sites through hydrogen bonding or hydrophobic interactions, stabilizing inactive enzymes conformations, (Tandera et al., 2024)

Analytical profiling

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is an important analytical technique employed to identify groups present in plant extracts by measuring infrared absorption patterns corresponding to molecular vibrations. It provides insight into the possible chemical constituents responsible for biological activity such as hydroxyl, carbonyl, and amine groups (Rani et al., 2020). In studies involving *Lonchocarpus cyanescens*, FTIR analysis of the hexane fraction can reveal the presence of compounds such as flavonoids, terpenoids, and phenolic derivatives, which may contribute to α-amylase and α-glucosidase inhibition.

Gas chromatography – mass spectrophotometry (GC – MS)

In vitro assays, including α-amylase and α-glucosidase inhibition tests, are biochemical models used to simulate the mechanism of post – prandial glucose regulation. They measure the capacity of plant extracts or fractions to interfere with carbohydrate-digesting enzymes, thereby delaying glucose release in the body (Kazeem – Adamson et al., 2023). Such enzyme-based in vitro evaluations are fundamentals in assessing potential antidiabetic agents before in vivo confirmation.

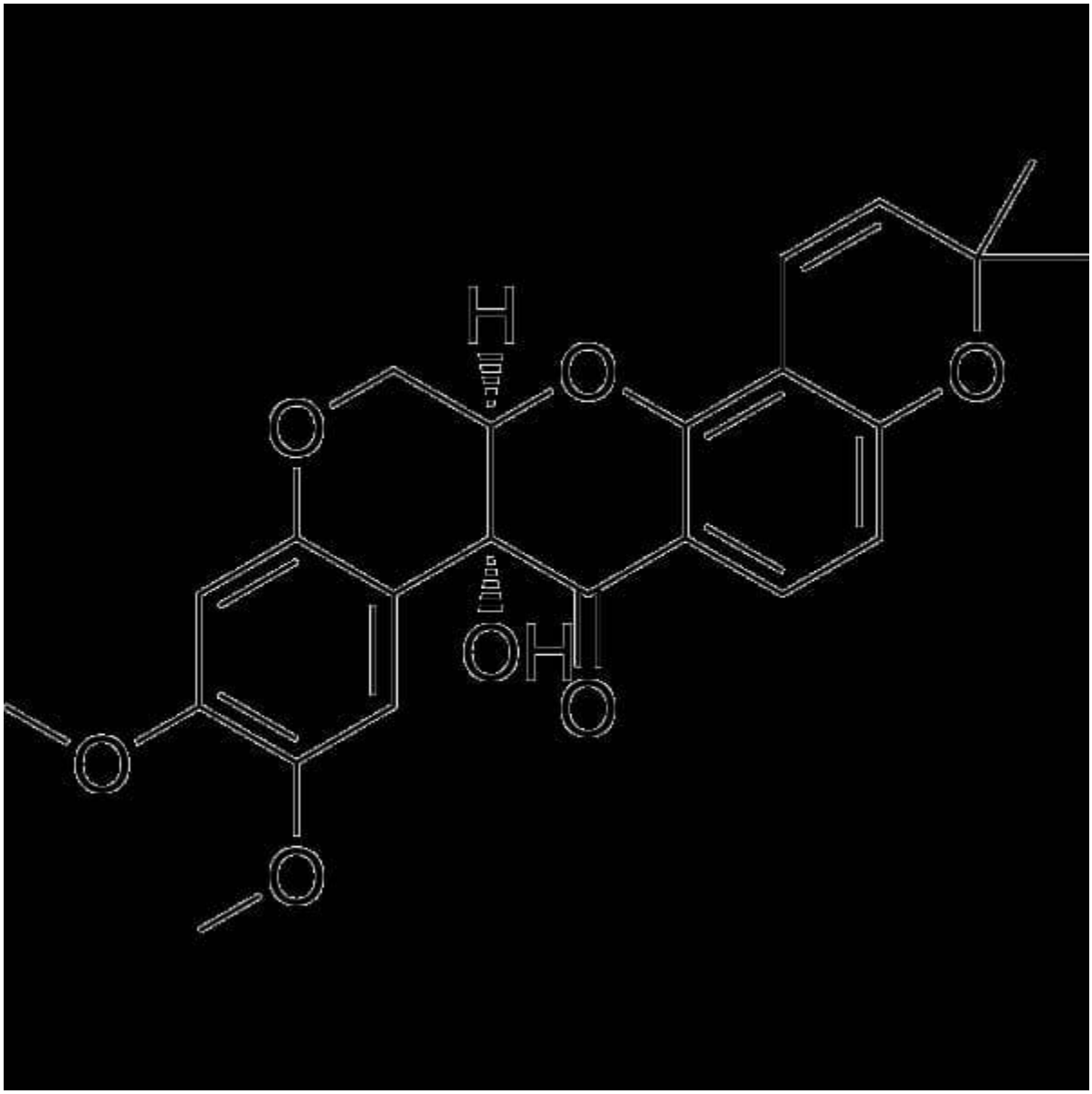

Figure 2.2 . Duegelin

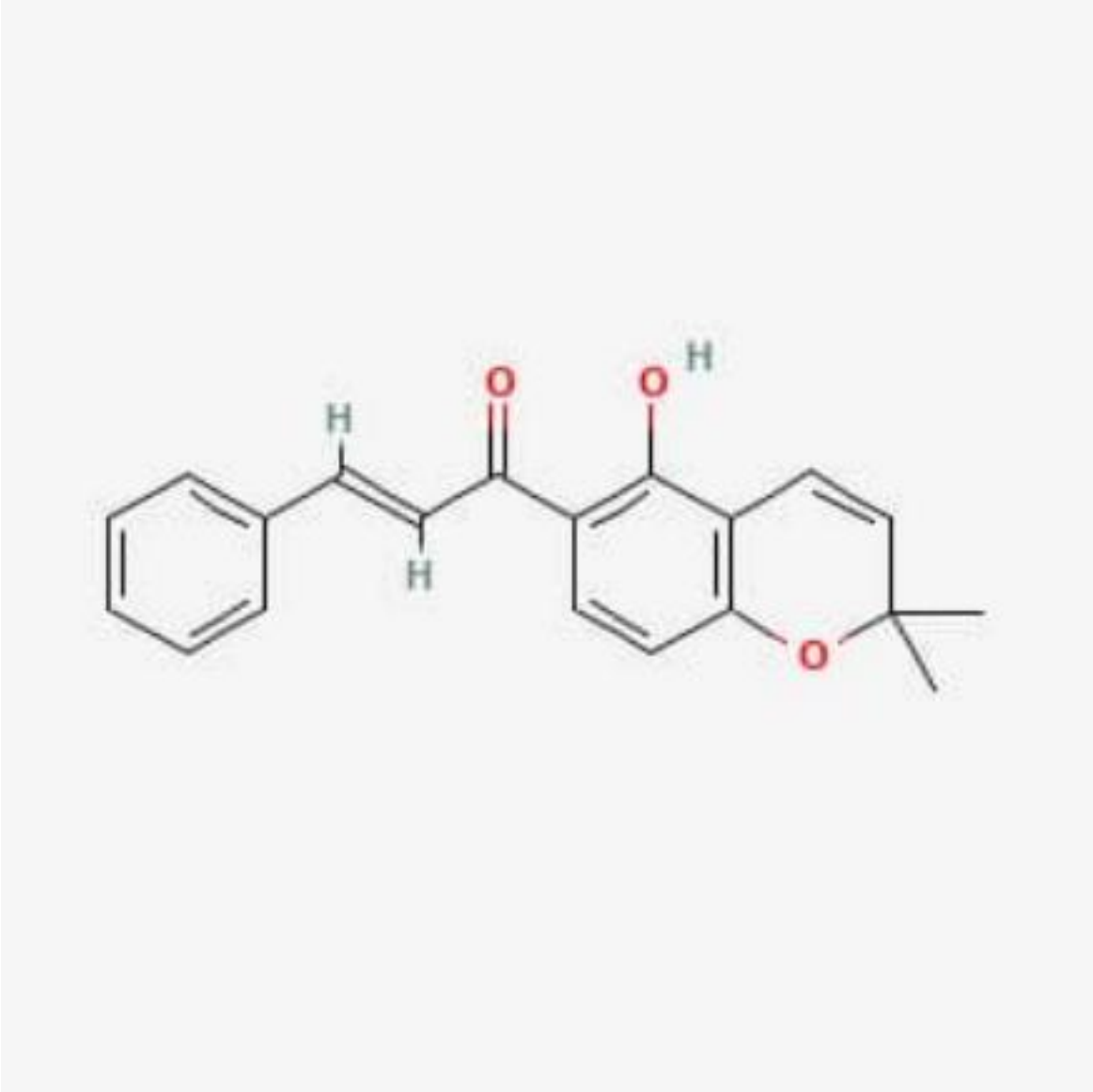

Fig. 2.3 Lanchocarpin

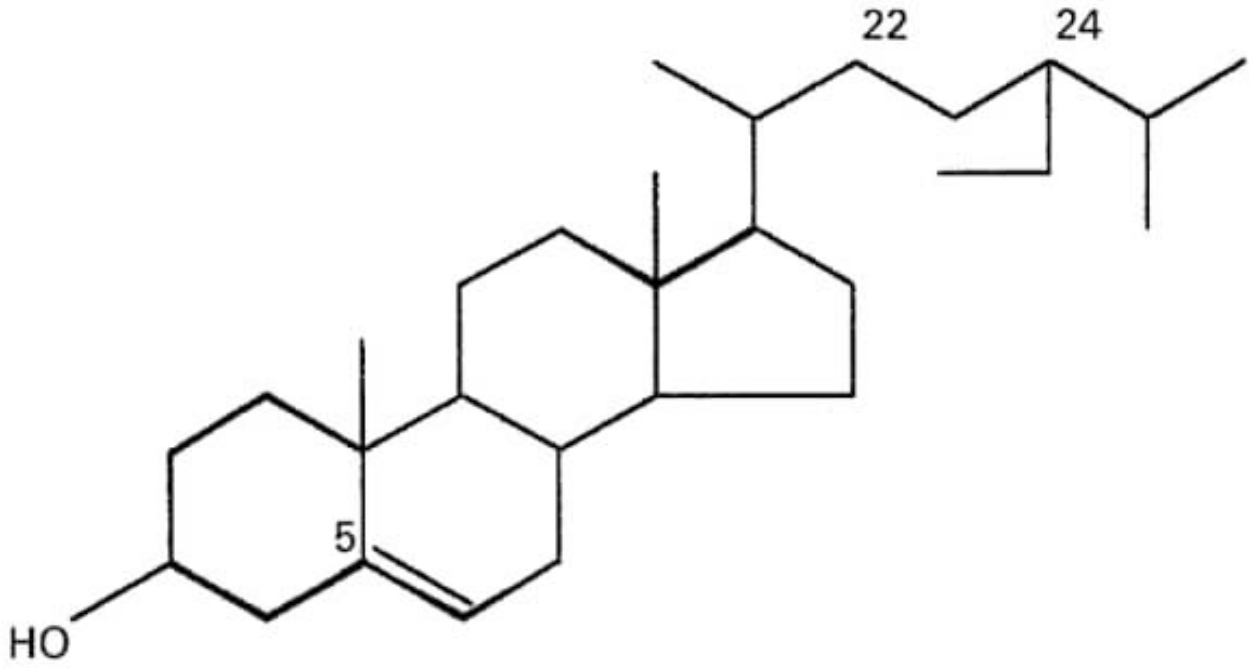

Fig. 2.4. Beta sitosterol

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant Materials

Fresh leaves of the *Lonchocarpus cyanescens* otherwise known as Yoruba indigo, west African indigo, gara.

It was gotten from a herb dealer from Lagos state, Nigeria.

The plant with voucher number UBHf029, was authenticated at the department of plant biology and Biotechnology, University of Benin, Edo State.

The leaves were air dried under shade at room temperature for 3 weeks (21 days).

The dried leaves were then pulverized into fine powder using a clean, mechanical grinder and was kept at room temperature until extraction was carried out.

3.2 Chemical and Reagents

All chemical and Reagents used in the study were of analytical reagents grade and of the highest quality available and were purchased from reliable firms and institutions.

They include.

Distilled water

Ethanol

N-hexane

Acetone

3.3 Major equipment used in the study includes;

Fractionating column

Reagent's bottle

Stirrer

Muscin cloth

Aluminum foil

White handkerchief

Face mask

Graduated cylinder

Measuring cylinders

Micro pipette

Beakers 500ml – 1000ml (Pyrex, England).

Freeze dry machine

Centrifuge

Conical flask

Test tubes and Racks

Separating funnel

Retort stand.

Mechanical grinder

3.4 Sample collection

Large amount of *Lonchocarpus cyanescens* were collected from a local herb dealer in Lagos state Nigeria. They were air dried under shade at room temp for 21 days.

The 155g powdered plant was then kept in a transparent container and kept away from moisture at room temp.

3.5 Preparation of plant extract.

Method

The ground *Lonchocarpus cyanescens* was weighed on the measuring scale to get the required 155g for the process.

Which was;

Solute: 155g of the ground Yoruba Indigo Leaves (*Lonchocarpus cyanescens*)

Solvent: Immersed in 10Litres of Ethanol(solvent), mixed thoroughly!

To form a *Lonchocarpus cyanescens* x ethanol solution.

Total Ground *Lonchocarpus cyanescens* in Grams = 4171.823g

1g of 2500g in 10,000 milliliters = 4g

It was then stored in a dry cool place for 3 days, after that it was the filtered, the *Lonchocarpus cyanescens* residue was dissolve again for another 24 hours. This continued until there was an exhaustive reaction.

The filtrate was taken to An Energy center for drying so as to get an extract that was used in further analysis.

250ml of acetone, add the 50g of dried filtrate into it, pour dissolved solvent into the fractionating column and shake vigorously.

After about 20 minutes, it separates into 3 lays with the filtrate at the bottom.

Use a muscin cloth to receive the extract into a beaker, that is poured into a glass bottle.

Repeat the process until 7.5litres of Acetone is finished

3.6 Enzyme Inhibition Assay

3.6.1 Alpha-amylase Inhibition Activity

Alpha-amylase inhibition activity of each fraction will be determined by the method of Worthington 1993.

An aliquot of 500 microliter of the extract (0.1–0.4 mg/ml) and 500 microliters (0.02M) of sodium phosphate buffer (pH 6.9 with 0.006M NaCl) containing 0.5 mg/ml of alpha-amylase will be mixed together and incubated for 10 min at room temperature.

Afterwards, 500 microliters of 1% starch solution prepared with 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) will be added and incubated in a water bath at 25°C for 10 minutes.

The reaction mixture will be stopped by adding 1.0 ml (96 mM) of Dinitro salicylic acid.

The mixtures in the test tubes will be incubated in boiling water in a water bath for 5 minutes and then cooled.

Absorbance of samples and reference control (reaction without sample) will be measured at 540 nm using a spectrophotometer.

The control was prepared using the same procedure but replacing the extract with DMSO, while the activity of the standard was tested by replacing the extract with acarbose.

The alpha-amylase inhibitory activity was calculated as percentage inhibition, thus:

% Inhibition = (A control − A extract) / A control × 100

3.7 Alpha-glucosidase Inhibition Activity

Alpha-glucosidase activity of each fraction will be determined by the method of Apostolidis et al., 2007.

The substrate solution, p-nitrophenyl-glucopyranoside (pNPG), was prepared in 0.02M phosphate buffer, pH 6.9.

1. 1000 microliter of alpha-glucosidase was incubated with 500 microliters of different concentrations of the extract for 10 minutes at 25°C.

2. An aliquot (500 microliter) of freshly prepared phosphate-buffered p-nitrophenyl-glucopyranoside (5 mM) solution will be added.

3. The reaction mixture will be incubated at 25°C for 5 minutes and stopped by adding 2 ml of 0.1 Na_2CO_3.

The alpha-glucosidase activity was determined by measuring the absorbance at 405 nm using a spectrophotometer.

The control was prepared using the same procedure but replacing the extract with DMSO, while the activity of the standard was tested by replacing the extract with acarbose.

The alpha-glucosidase inhibitory activity was calculated as percentage inhibition, thus:

% Inhibition = (A control − A extract) / A control × 100

Reagents	0.1mg/ml	0.2mg/ml	0.3mg/ml	0.4mg/ml	Control	Standard
Extract	500ml	500ml	500ml	500ml	Nil	Nil
Phosphate buffer (0.02ml, PH 6.9)	500ul	500ul	500ul	500ul	500ul	500ul
Alpha Amylase	500ul	500ul	500ul	500ul	500ul	500ulin

Incubated in water bath for 25-degree Celsius

1% starch solution	500ul	500ul	500ul	500ul	Control	Standard

Incubate in Watergate for 25 degree Celsius for 10 minutes.

To stop DNS reagent (0.1ml)	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml

Incubated in Watergate for minutes and then coded

DMSO	Nil	Nil	Nil	Nil	DMSO	Nil
Acarbose	Nil	Nil	Nil	Nil	Nil	Acarbose

Absorbance is 540mm

3.8 Procedure for Alpha Glucosidase For *Lonchocarpus Cyanescens*

Reagents	0.1mg/ml	0.2mg/ml	0.3mg/ml	0.4mg/ml	Control	Standard
Extract	500ul	500ul	500ul	500ul	Nil	Nil
Alpha Glucosidase (1000ul)	1000ul	1000ul	1000ul	1000ul	1000ul	1000ul

Pre incubation at 25-degree Celsius for 10 minutes

P-nitrophenyl-glucopyranoside	500ul	500ul	500ul	500ul	500ul	500ul

Incubation at 25-degree Celsius for 5 minutes

NA2CO3 (To stop)	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml

DMSO	Nil	Nil	Nil	Nil	Nil	Nil
Acarbose	Nil	Nil	Nil	Nil	Nil	Nil

Absorbance is 405mm

3. 10 Statistical Analysis

The graph pad prism software, inc, (Version 9) was used to analyze and obtain the mean, SEM.

Using Independent samples ‘t’ test, the level of significant was taken as $P < 0.05$.

CHAPTER FOUR

RESULTS AND STATISTICAL ANALYSIS

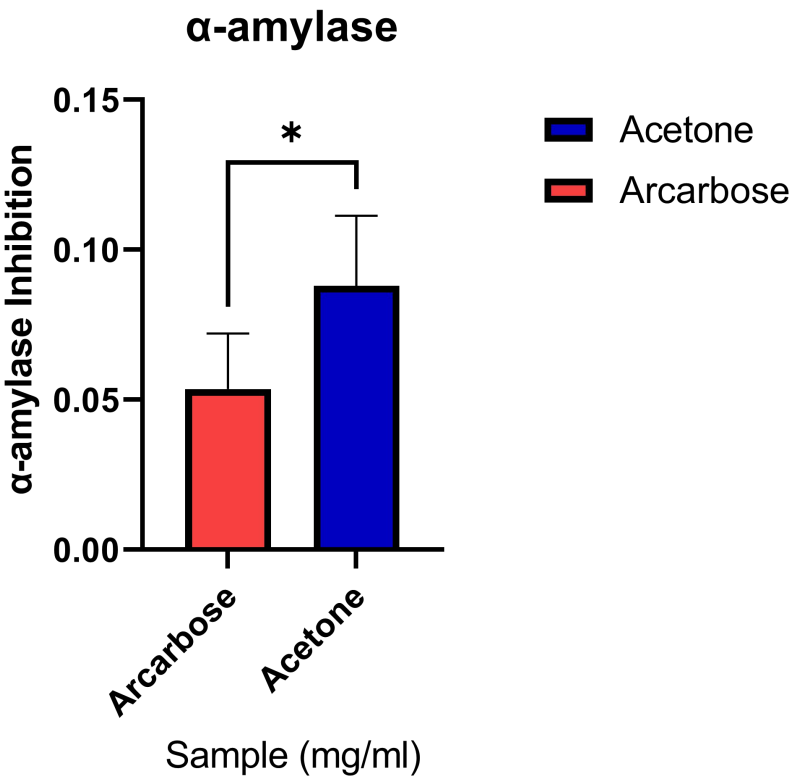

Figure 4.1 Effect of Acetone faction of Lonchocarpus cyanescens on α-amylase activity

Figure 4.1 shows that the acetone extract produced a higher α-amylase inhibitory effect than acarbose at the same concentration. The acetone sample reached about 0.10 inhibition, while acarbose reached around 0.06. The asterisk indicates that this difference is statistically significant, (P= 0.0321) which indicate the acetone extract inhibited α-amylase more effectively than acarbose in this test.

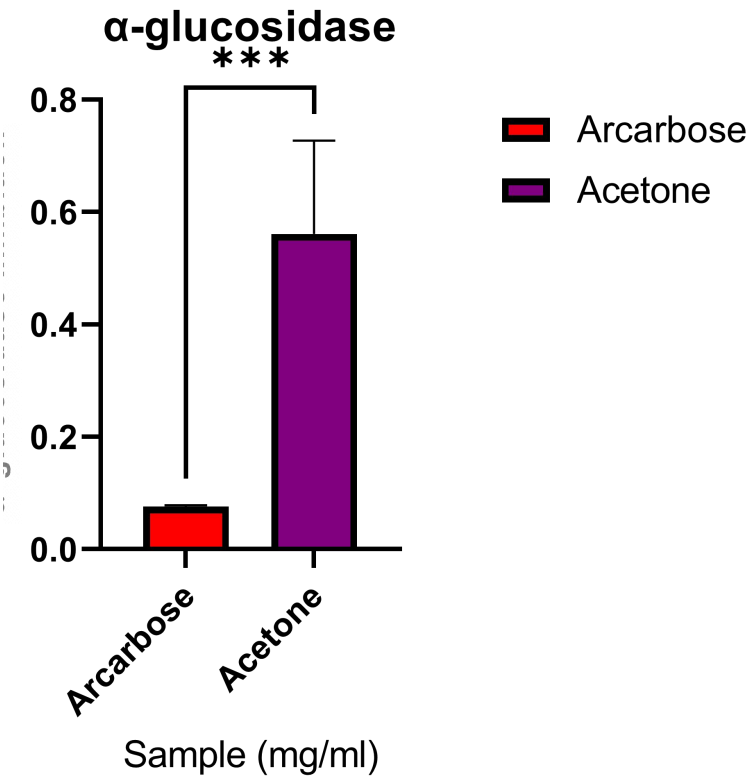

Figure 4.2 Effect of Acetone faction of Lonchocarpus cyanenscens on α-glucosidase activity

Figure 4.2 shows that the acetone extract produced a much stronger α-glucosidase inhibitory effect than acarbose at the same concentration. While acarbose showed only a small level of inhibition, the acetone extract reached well above 0.5. The triple asterisks (***) indicate that this difference is highly significant (P = 0.0002), meaning the acetone extract was far more effective at inhibiting α-glucosidase in this assay.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Alpha amylase and alpha glucosidase assay is used to check antidiabetic properties of various substances [e.g. drugs or plant extract]. Antidiabetic agents are agents with the exception of insulin have been approved for hyperglycemic treatment in type 2 diabetics mellitus. diabetes is a gathering of metabolic issue normal for hyperglycemia over a significant stretch of time [world health organization,2014]

It shows as slowed healing, unexplained weight loss, increased thirst, blurred visions, polyuria. When left untreated, it can lead to increased risk of infections, skin problems, kidney damage [nephropathy]. Intense inconveniences can turn into severe hypoglycemia, Hyperosmolar hyperglycemic state [HHS], Diabetic ketoacidosis [DKA] or passing.

Alpha amylase starts the process of breaking down carbohydrate by hydrolyzing large starch molecules [polysaccharides] into smaller units like maltose, it is secreted in saliva and by the pancreas

Alpha glucosidase completes digestion in the small intestine. It breaks down the resulting disaccharides and oligosaccharides into individual glucose molecules. The final glucose is then absorbed into the bloodstream, raising blood sugar levels.

The plant extract of Lonchocarpus cyanscens after been subjected to alpha amylase and alpha glucosidase assay generated absorbance reading was further subjected to statistical analysis. A p-value of less than 0.05 was obtained from the two assay carried out, when compared against both their respective standard, indicating a statistical significant difference.

5.2 Conclusion

Diabetes is a disease with quite a lot of complications.by inhibiting these carbohydrate digesting enzymes, the *Lonchocarpus cyanescens* effectively delays the digestion and absorption of dietary carbohydrates thereby reducing th sudden spike in blood glucose[postprandial hyperglycemia]that is characteristic of type 2 diabetes. The plant is a potential synthetic anti diabetic drugs with few side effects.

Research are being carried out all over the world trying to discover the new drugs, plants or other substances that will be valuable in curbing the issue of diabetes. This include agents that will help clear up excess blood glucose, and cause the release of insulin.

My research was carried out on the plant and its extract *Lonchocarpus cyanescens* to test for its anti-diabetic effect using alpha amylase and alpha glucosidase assay.

From the results of the assay and all the biochemical literature used, it is seen that Lonchocarpus cyanscens possess potentials to prevent diabetes and hyperglycemia.

It is therefore recommended that the plant be put into more scientific use for further discoveries in the medical field.

REFERENCES

Adeniyi, T. A. and Odufowora, E. O. (2023). Chemical composition and nutritional quality of seed oil from *Lonchocarpus cyanescens*. *African Journal of Plant Science, 7*(12), 421–427.

Adesina, A., Adeoye, A. and Ayeni, O. (2024). Phytochemical profiling and therapeutic potentials of *Lonchocarpus cyanescens* extracts. *Journal of Medicinal Plant Research, 13*(4), 55–62.

Ajani, E. O., Sabiu, S., Odufuwa, K. T., Ibrahim, T. B. and Salau, B. A. (2025). *Evaluation of lens aldose reductase inhibitory and free radical scavenging potential of fractions of Lonchocarpus cyanescens leaves: potential for cataract remediation*. *Pharmacognosy Journal,* **9**(1), 62–69

Akinmoladun, A. C., Olaleye, M. T., Farombi, E. O. and Akindahunsi, A. A. (2020). Antioxidant and enzyme inhibitory activities of selected Nigerian medicinal plants. *Journal of Applied Pharmaceutical Science, 10*(3), 45–52.

American Diabetes Association. (2024). Standards of Medical Care in Diabetes—2024. *Diabetes Care, 42*(1), S1–S193

American Diabetes Association. (2024). Standards of medical care in diabetes—2024. *Diabetes Care, 42*(Suppl. 1), 192-195.

Arowona, I. T., Sonibare, M. A. and Umukoro, S. (2014). Antipsychotic property of solvent-partitioned fractions of *Lonchocarpus cyanescens* leaf extract in mice. *Journal of Basic and Clinical Physiology and Pharmacology, 25*(2), 235–240

Bioassay-guided investigation of *Lonchocarpus cyanescens* Benth. leaves extracts for antioxidant activities. (2014). *African Journal of Biotechnology / Academic Journals* 1(1):23-29.

Burkill, H. M. (2022). *The Useful Plants of West Tropical Africa* (2nd ed., Vol. 1). Royal Botanic Gardens, Kew. Available at Cabi Digital Library+2SCIRP+2. Accessed 27th July, 2025

Butterworth, P. J., Warren, F. J. and Ellis, P. R. (2022). Human α-amylase and starch digestion: An unfinished story. *Trends in Food Science and Technology,* 22(8), 398–409.

Ejele, A. E. and Nnoli, M. C. (2022). Volatile oil constituents of *Lonchocarpus cyanescens* leaves and stem bark. *International Journal of Biological and Chemical Sciences, 4*(6), 215–222.

Gupta, R., Gigras, P., Mohapatra, H., Goswami, V. K. and Chauhan, B. (2023). Microbial α-amylases: A biotechnological perspective. *Process Biochemistry,* 38(11), 1599–1616.

International Diabetes Federation. (2024). *IDF Diabetes Atlas* (9th ed.). Brussels, Belgium: International Diabetes Federation. Retrieved from https://www.diabetesatlas.org. SCIRP+1. Accessed 27th August, 2025

Isyaka, S. M., Olayemi, O. A. and Abdullahi, M. (2025). Phytochemistry and medicinal uses of Lonchocarpus species: Systematic review of a promising medicinal plant. *South Asian Research Journal of Natural Products, 8*(1), 32–44.

Kim, Y. M., Wang, M. H. and Rhee, H. I. (2022). Acarbose-like inhibitory effects of flavonoids on α-glucosidase. *Nutrition Research, 26*(8), 583–588.

Lebovitz, H. E. (2022). Postprandial hyperglycemia: The importance of its treatment. *Diabetes Research and Clinical Practice, 89*(1), 1–8.

Moronkola, D. O. (2023). Chemical compositions of *Lonchocarpus cyanescens* Benth. (Fabaceae) — Case study of its volatile oils and two triterpenoids. *American Journal of Plant Sciences, 4*(8), 1653–1659.

Moronkola, D. O. and Oladosu, I. A. (2023). *Chemical compositions of Lonchocarpus cyanescens Benth. (Fabaceae)—case study of its volatile oils and two triterpenoids*. *American Journal of Plant Sciences,* 4(8), 35-68

Ogunmoyole, T., Oboh, G., Fagbemi, T. N. and Ademiluyi, A. O. (2024). Phytochemical screening and antidiabetic potentials of *Lonchocarpus cyanescens* extracts. *Nigerian Journal of Natural Products and Medicine, 16*(1), 98–104.

Sales, P. M., Souza, P. M., Simeoni, L. A., Magalhães, P. O. and Silveira, D. (2024). α-Amylase inhibitors: A review of raw material and isolated compounds from plant source. *Journal of Pharmacy & Pharmaceutical Sciences, 15*(1), 141–183.

Sales, P. M., Souza, P. M., Simeoni, L. A., Magalhães, P. O. and Silveira, D. (2024). α-Amylase inhibitors: A review of raw material and isolated compounds from plant source. *Journal of Pharmacy and Pharmaceutical Sciences, 15*(1), 141–183.

Sonibare, M. A., Umukoro, S. and Shonibare, E. T. (2024). Antipsychotic property of aqueous and ethanolic extracts of *Lonchocarpus cyanescens* (Schumach. and Thonn.) Benth. (Fabaceae) in rodents. *Journal of Natural Medicines, 66*(1), 127–132

Tadera, K., Minami, Y., Takamatsu, K. and Matsuoka, T. (2024). Inhibition of α-glucosidase and α-amylase by flavonoids. *Journal of Nutritional Science and Vitaminology, 52*(2), 149–153.

U.S. National Library of Medicine, LiverTox: Acarbose (2021). Acarbose produces malabsorption and common GI adverse effects (flatulence, diarrhea, bloating). Available at https://www.ncbi.nlm.nih.gov/books/NBK548181/. Accessed 27th October, 2025

Zhou, J., Chen, L. and Chen, X. (2024). Clinical efficacy and gastrointestinal effects of α-glucosidase inhibitors in type 2 diabetes management. *Journal of Diabetes Research, 2024*, 1–12