Comparative In Vitro Evaluation of Antidiabetic, Anti-inflammatory, and Antioxidant Activities of *Elaeocarpus Floribundus*

By

Tasnia Farhana

21346015

Nabila Tasnim Mahi

19346081

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

School of Pharmacy
BRAC University
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Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

Student's Full Name & Signature:

Tasnia Farhana
21346015

Nabila Tasnim Mahi
19346081

Approval

The thesis titled "Comparative In Vitro Evaluation of Antidiabetic, Anti-inflammatory, and Antioxidant Activities of "*Elaeocarpus Floribundas*" submitted by

1. Tasnia Farhana - 21346015
2. Nabila Mahi - 19346081

of Spring,2025 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

Examining Committee:

Supervisor:

Namara Mariam Chowdhury
Senior Lecturer, School of Pharmacy
Brac University

Approved By:
Chairperson:

Dr. Raushanara Akter
Acting Chairperson and Professor
School of Pharmacy
Brac University

Dean:

Dr. A F M Yusuf Haider
Acting Dean, School of Pharmacy
Brac University

Ethics Statement

This project does not involve any kind of animal and human trial.

Abstract

Elaeocarpus Floribundus was evaluated for antidiabetic, anti-inflammatory, and antioxidant potential using three leaf extracts prepared by Buckner filtration (E1), rotary evaporation (E2), and air-drying (E3) and screened in baker's yeast glucose-uptake, HRBC hypotonic-hemolysis, and DPPH assays with metformin, diclofenac, and ascorbic acid as benchmarks (triplicates). E2 produced the highest yeast glucose uptake (68.0%), closely matching metformin (68.35%), followed by E3 (59.5%) and E1 (54.4%). In the HRBC assay at 400 μg mL^{-1}, inhibition ranked diclofenac 85.6%, E1 81.7%, E2 81.0%, and E3 43.7%, indicating strong membrane-stabilizing activity for E1/E2. DPPH profiling revealed concentration-dependent scavenging for all extracts; E3 was most potent with IC_{50} < 10 μg mL^{-1} and >50% inhibition at 10 μg mL^{-1}, whereas E1 and E2 were moderate (IC_{50} ≈ 65 and 71 μg mL^{-1}, respectively); ascorbic acid showed IC_{50} < 10 μg mL^{-1} and >90% inhibition. Collectively, extraction strategy dictated functional profiles, with E2 strongest on metabolic and membrane endpoints and E3 a superior radical scavenger, supporting subsequent phytochemical standardization, mechanistic evaluation in mammalian systems, and in-vivo validation.

Keywords: *Elaeocarpus Floribundus*; yeast glucose-uptake; HRBC membrane stabilization; DPPH; antioxidant; antidiabetic

Dedication

This thesis is dedicated to our beloved parents, whose unconditional love and sacrifices have been our greatest strength; to my sister, (Taha,Tunna), for their constant support and encouragement; to Mehedi, whose patience and inspiration have guided me throughout this journey; and to our respected teachers, whose knowledge and guidance have shaped our academic path.

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List of Acronyms

ABTS — 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); antioxidant assay

AGE — Advanced Glycation End-product; antidiabetic mechanism readout

AKT — Protein Kinase B; insulin-signaling pathway

AMPK — AMP-Activated Protein Kinase; metabolic signaling

AP-1 — Activator Protein 1; inflammatory transcription factor

ARRIVE — Animal Research: Reporting of In Vivo Experiments; reporting guideline (future work)

AUC — Area Under the Curve; glycemic/edema test summary metric (future work)

B. Pharm. (Hons.) — Bachelor of Pharmacy (Honours); your degree

CDC — Centers for Disease Control and Prevention; cited in background text

CI — Confidence Interval; statistical reporting

COX-2 — Cyclooxygenase-2; inflammatory enzyme target

CPP — Critical Process Parameter; extraction/process control (future work)

CQA — Critical Quality Attribute; product/extract quality (future work)

CV — Coefficient of Variation; assay precision metric

CYP2C9 / CYP2D6 / CYP3A4 — Cytochrome P450 isoenzymes; drug–extract interaction screens

DCF-DA — 2′,7′-Dichlorofluorescein diacetate; cellular ROS probe

DMS (MS/MS) — Tandem Mass Spectrometry; structural elucidation (via LC–HRMS/MS)

DoE — Design of Experiments; process optimization (future work)

DPP-4 — Dipeptidyl Peptidase-4; antidiabetic enzyme target (future work)

DPPH — 2,2-Diphenyl-1-picrylhydrazyl; radical-scavenging assay

EDTA (K_3EDTA) — Ethylenediaminetetraacetic Acid, tripotassium salt; anticoagulant for blood

ESI — Electrospray Ionization; LC–MS ionization mode

FDA — U.S. Food and Drug Administration; drug discovery audits cited

FDR — False Discovery Rate; multiple-comparison control

FID — Flame Ionization Detector; GC–FID quantitation (future work)

FRAP — Ferric Reducing Antioxidant Power; antioxidant assay

GAE / RE — Gallic Acid Equivalents / Rutin Equivalents; total phenolics/flavonoids

GC–MS — Gas Chromatography–Mass Spectrometry; phytochemical profiling

GLUT4 — Glucose Transporter Type 4; insulin-regulated transporter

GSF (SGF) — Gastric Simulated Fluid (often "Simulated Gastric Fluid," SGF); antacid-related tests

GPx — Glutathione Peroxidase; antioxidant enzyme

GSH / GSSG — Reduced/oxidized glutathione; redox status

HCl — Hydrochloric Acid; pH adjustment

hERG — Human Ether-à-go-go–Related Gene channel; cardiac safety flag (future work)

HFD — High-Fat Diet; metabolic disease model (future work)

HOMA-IR — Homeostatic Model Assessment of Insulin Resistance; metabolic endpoint (future work)

HO-1 — Heme Oxygenase-1; Nrf2 target gene

HPLC / Prep-HPLC — High-Performance Liquid Chromatography / preparative HPLC; isolation

HPTLC — High-Performance Thin-Layer Chromatography; fingerprinting/QC

HRBC — Human Red Blood Cell; membrane-stabilization assay

IC_{50} — Half-maximal Inhibitory Concentration; potency metric

ICH — International Council for Harmonisation; stability guidelines (Q1A)

IDF — International Diabetes Federation; epidemiology cited

IL-6 — Interleukin-6; cytokine readout

IP (LC–HRMS/MS) — High-Resolution Mass Spectrometry; see LC–HRMS/MS

ITT — Insulin Tolerance Test; **in vivo** metabolic test (future work)

JC-1 — Cationic dye for mitochondrial ΔΨm; mitochondrial function

LC–HRMS/MS — Liquid Chromatography–High-Resolution Tandem MS; untargeted profiling

LoD / LoQ — Limit of Detection / Limit of Quantitation; analytical validation

LOX (5-LOX) — Lipoxygenase (5-Lipoxygenase); inflammatory enzyme

LPS — Lipopolysaccharide; macrophage activation stimulus

MDA / TBARS — Malondialdehyde / Thiobarbituric Acid Reactive Substances; lipid peroxidation

MIAPE — Minimum Information About a Proteomics Experiment (used analogously for reporting completeness)

MPO — Myeloperoxidase; inflammation/tissue injury marker

NMR (1D/2D) — Nuclear Magnetic Resonance; structural elucidation

NOX — NADPH Oxidase; ROS source in cells

Nrf2 — Nuclear factor erythroid 2–related factor 2; antioxidant response regulator

OGTT — Oral Glucose Tolerance Test; **in vivo** metabolic test (future work)

ORAC — Oxygen Radical Absorbance Capacity; antioxidant assay

PAHO — Pan American Health Organization; WHO regional office (policy citations)

PBS — Phosphate Buffer(ed) Solution/Saline; assay buffer

PDA — Photodiode Array; UPLC detector (targeted quantitation)

PDI — Polydispersity Index; nanoformulation metric

PGE_2 — Prostaglandin E_2; inflammatory mediator

PK — Pharmacokinetics; future translational work

PTP1B — Protein Tyrosine Phosphatase 1B; insulin-signaling negative regulator

QC — Quality Control; batch consistency

ROS — Reactive Oxygen Species; oxidative stress readouts

RPM — Revolutions Per Minute; centrifugation speed

SOD / CAT — Superoxide Dismutase / Catalase; antioxidant enzymes

STZ — Streptozotocin; diabetogenic agent (model induction, future work)

TEAC — Trolox Equivalent Antioxidant Capacity; standardized antioxidant unit

TNF-α — Tumor Necrosis Factor alpha; cytokine readout

UPLC–PDA/ESI–MS — Ultra-Performance LC with PDA and ESI–MS; targeted quantification

WHO — World Health Organization; policy/TCM integration context

Chapter 1

Introduction

Humans have relied on plant-based treatments for diseases throughout many centuries. Medicinal drugs used for treating diseases show plant origins in 87% of their cases based on the findings from Zaman et al. (2016). The drug development industry utilizes medicinal plant active compounds such as thymol, carvacrol, saponin and terpenoids, flavonoid and phenol alongside other chemical constituents. The various therapeutic agents within plants include antimicrobial, antihypertensive, laxative, antidiabetic and antiulcer and anticancer ingredients from active components. Morphine extraction occurred from Poppy (Papaver somniferum). Morphine functions as the primary alkaloid of opium through which scientists derive multiple opioid medications including codeine and fentanyl as well as methadone and hydrocodone and hydromorphone and meperidine and oxycodone. Morphine stands as a highly effective pain medication that belongs to the group of potent analgesics used for treating chronic pain. The medication stands as powerful medicine, but it exhibits restricted medical value because of serious withdrawal side effects in addition to tolerance development and predictable abuse incidents. Medicinal plants that have various healing properties provide the WHO (World Health Organization) with the best selection of medicinal drugs. Scientific evidence supports the effectiveness of medicinal plants for treating microbial agents and healing both microbial infections and ulcers. The natural ecosystem contains a wide selection of medicinal plants that function effectively as antiulcer medicine. The chronic disease known as peptic ulcer occurs among 10% of people who make up the world's population. The medical condition known as peptic ulcer develops when open sores appear inside the stomach lining as well as on the lower portion of the esophagus or in the small intestine. H. pylori

bacteria along with gastric juice pH and erosion of stomach acid and downregulated mucosal defenses determine the formation of peptic ulcers. Higher levels of hydrochloric acid (HCl) secretion together with reduced mucus production weaken the stomach lining protection which leads to an open sore ulcer formation. The main medical emergency from peptic ulcer disease is bleeding that develops into dangerous hemorrhage. Three additional complications arise from a peptic ulcer including perforation, penetration, and gastric outlet obstruction. Peptic ulcer disease management represents an ongoing challenge to medical professionals since the market seeks new anti-ulcer pharmacological agents for treatment purposes. Anti-ulcer activity from chemical constituents emerges through their components of secondary metabolites, alkaloids and flavonoids and terpenoids. The drug regimen for treating ulcers includes antacids because they deactivate stomach acid to quickly alleviate pain symptoms. The presence of antacids slows down pepsin activities while performing bile acid binding functions. Reputable antacid preparations contain a combination of active ingredients which include Calcium carbonate with sodium bicarbonate and aluminum hydroxide and magnesium hydroxide. Drugs combining aluminum hydroxide with magnesium hydroxide function efficiently to neutralize stomach acid and bind bile acids while reducing pepsin activity as well as potentially stimulating local prostaglandin production. Regular antacid consumption can lead to adverse effects including constipation as well as diarrhea and chronic renal failure. The discovery of a new antacid should proceed to provide better advantages with lower adverse effects (Cua et al., 2018).

The Elaeocarpaceae family includes almost 615 species of plants distributed across 12 genera. Elaeocarpus stands as the largest genus within this family by holding about 350 species along with others. This genus contains the medicinal plant Elaeocarpus floribundus as

one of its species. The plant exists naturally in India together with Myanmar and scattered regions in Bangladesh. Scientific research exists to determine phytochemicals along with antibacterial and antioxidative features of this plant. A limited amount of scientific investigation exists on Elaeocarpus floribundus. Leaves yielded the main extract that demonstrated an inhibitory effect against CEM-SS cancer cells. Research shows *Elaeocarpus Floribundus* leaves together with bark possess anti-ulcer capacity which indicates a potential antacid effect. The combination of bark and leaves prepared as an infusion serves as a remedy for treating inflamed gums through mouthwash usage. The residents of Mauritius used leaf decoction to treat hypertension and diabetes (Indian Olive, 2017). Research opportunities for this species exist in adequate quantities.

1.1 Other Species of Elaeocarpus

Elaeocarpus Sphaericus

The Indian territory recognizes *Elaeocarpus Sphaericus* as Rudraksha within its language convention (Hindi) while also recognizing this plant as bead tree (English). This species exists differently named across Myanmar and the entire North-East India through Maharnoxee and Myitmar and further through Bhutan and Nepal to Bangladesh. The Rudraksha tree grows as a permanent broad-leaved evergreen tree that develops a big spreading crown. The dried fruit becomes a bead that demonstrates electromagnetic abilities besides providing remarkable curative properties. Rudraksha use is believed to have healing properties that aid anxious individuals along with decreasing body temperature and producing relaxing effects. The medicinal properties of *E. sphaericus* extend to treating multiple medical conditions which include depression, palpitation, stress, hypertension, migraine, epilepsy and indigestion. The components of this species include alkaloids fused with glycosides and steroids as well as flavonoid (quercetin) and tannins (gallic and ellagic acids) together with fatty acids (palmitic and linoleic acids) and carbohydrates and proteins and ash (Pant et al., 2013).

Fig 1.1.1: *Elaeocarpus sphaericus*

Elaeocarpus serratus

The plant *Elaeocarpus serratus* receives its common name as Ceylon olive. A medicinal tree reaches up to 18 meters in height as an evergreen that maintains a few acknowledged medical uses. E. Asia—India through Myanmar to Nepal, Sri Lanka and Indonesia and Malaysia together with Nepal presents the native region for this tree. Consumers can eat the edible fruit portion of this tree because it contains starch and sugar along with trace minerals of iron and protein.

Operation of Elaeocarpus serratus produces therapeutic treatment for dysentery and diarrhea.

Fig 1.1.2: Elaeocarpus serratus

The plant leaves act both as a rheumatic pain treatment and for treating poison exposure. The timber has applications for common planking as well as boxes and crates and it serves as a foundation material for wooden pallets and plywood production.

Elaeocarpus Angustifolius

Elaeocarpus Angustifolius functions as the blue marble tree while reaching heights of 35 meters. Scientists have identified this plant to exist within E. Asia—China alongside the Indian subcontinent as well as Myanmar through Malaysia extending to Cambodia together with Australia and Pacific territories. This ornamental plant offers only scarce medicinal value but exists mainly for gardening purposes. This species is also edible. The fleshy fruit functions as a remedy for treating epileptic fits. Using leaf sap offers two medicinal benefits which include treating stomachache and chest pain. Proceeds from the E. *Angustifolius* seeds

work as therapeutic remedies to treat heart difficulties and control blood pressure. The bark obtained from this tree was employed by Filipinos to address spleen enlargement. The species finds moderate use in commercial applications including general planking, shuttering, boxes, crates and wooden pallets, boat planking and racing oars as well as match splints and serves the plywood industry and functions as lightweight carving timber for barrel production.

Fig 1.1.3: Elaeocarpus Angustifolius

1.2 Description of the Plant Elaeocarpus Floribundus

Elaeocarpus Floribundus develops into an evergreen tree that spreads its branches outward. The species reaches heights between 30–40 meters despite growing to smaller sizes usually. This species exists both in untamed environments and in home gardens because of its edible features. This species occupies both lowland rainforest regions and lower mountain forest regions which extend up to 1500 m in altitude. E. floribundus propagation depends on stones that require shady soil conditions for achieving 15% germination rate at 4–8-month intervals (Sircar & Mandal, 2017). E. floribundus exists in India along with Bangladesh, Myanmar, Bhutan, Malaysia, and Indonesia in its widespread distribution. The timber has a soft to intermediate hardness with lightweight to medium mass but low durability.

Taxonomical Classification

- Kingdom: Plantae
- Clade: Tracheophytes
- Clade: Angiosperms
- Clade: Eudicots
- Clade: Rosids
- Order: Oxalidales
- Family: Elaeocarpaceae
- Genus: Elaeocarpus
- Species: Elaeocarpus floribundus

Common Names

Assamese: Jolphai, Jalphai, Jalpai

Bengali: Belphoi

English: Indian Olive

Hindi: Jalpai

Others: Indian Olive

Synonyms of E. floribundus

- Elaeocarpus floribundas var. tahanensis (Hend.) Ng
- Elaeocarpus grossus Wall. [Invalid]
- Elaeocarpus lobbianus Turcz.
- Elaeocarpus pseudosepicanus O.C. Schmidt
- Elaeocarpus tahanensis M.R. Hend

1.3 Different Parts of the Plant

Leaf

Each leaf in E. floribundus sprouts from one distinct point at the stem base and grows as a single blade while it radiates from the stem in a clockwise direction. Each leaf measures 3 to 5 centimeters and is arranged densely at the twig tips while the stem reaches 1 to 5.5 centimeters in length and has a joint located at the tip. The leaf shape includes ovate with elliptic and base shape and rounded corners. The leaf edges possess teeth along the margins while its veins run in a distinct pinnate pattern containing 5 to 7 lateral vein pairs. The foliage has its natural green hue until it transforms into red tones right before dropping off the plant. Velvet leaf E. floribundus provides effective treatment for both diabetes and hypertension conditions. People in the Philippines used to remedy inflamed gums by applying plant extracts of E. floribundus through mouthwash preparations.

Flower

White flowers of Elaeocarpus floribundus measure small in size. The sepals form triangular shapes that are slender, and the petals exist as obovate-oblong structures with ellipsoid flower buds containing 25–40 stamens. This bisexual flower possesses a tiny, small hairy structure along with 1 mm long filaments followed by slight pubescence seen on both the slender anthers which measure 2 mm in length and the oblong form and lastly possesses a silky villous bearded disk below a 3-loculed ovary that is also silky villous. The flowers should bloom during the months of May through August.

Fig 1.3.1: Flower and leaves of *Elaeocarpus Floribundus*

Fruits

The taste of Elaeocarpus floribundus fruit is sour to the palate. The people refer to this plant as Jalpai and Belphoi. Profittakers harvest fruits during the period spanning from August until October. The pale green oval fruit appears in nature during the summer to autumn season. A single seed resides within Elaeocarpus floribundus fruit which has edible mesocarp tissue (Mani & Bhowmick, 2017). Each green fleshy drupe has a length of 22 mm and presents a slightly sour taste. The fruit exists in two forms: unprocessed eating as well as pickled condiment preparation.

Fig 1.3.2: Fruits of *Elaeocarpus Floribundus*

1.4 Chemical Constituents of Elaeocarpus Floribundus

Elaeocarpus belongs to the Elaeocarpaceae family. It has diverse biological activities including affinity for opioid receptors, cytotoxicity, and antioxidant activity, and for these it possesses different components. Elaeocarpus floribundus is a tropical and subtropical plant that is widely distributed in Asia.It was reported that 15 components and 3 other components in the form of a mixture were present in the leaves, consisting of four fatty acids, three diterpenoids, one triterpene alcohol, two fatty alcohols, three phaeophytins, one phytosterol, one sesquiterpene, and three hydrocarbons in hexane extract of the leaves (Ogundele & Das, 2019). Epifriedelanol and friedelanol are the two components that were exhibited to be active against the cancerous cells.

The fruit, popularly known as Indian olive, is faintly acidic in flavor as it comprises an excess of citric acid. Apart from citric acid, it consists of tannin, arabinose (26%), galactose (35%), uronic acid, and 11% of rhamnose. Vitamin C, myricitrin, myricetin, mearnestin, and ellagic acid are the components of the leaves (Sonia Zama, 2016).

1.5 Aims and Objectives of This Study

Aim

The aim of this study is to evaluate the in vitro antacid activity of *E. floribundus* as it shows anti-ulcer properties, using a preliminary antacid test, acid neutralizing capacity, neutralizing effect of extracts on GSF, and acid buffering capacity.

Objectives

The objectives of this study are:

- Evaluating the antacid effect of the plant extract compared to the standard.
- Performing a preliminary antacid test of the plant extract compared to the standard.
- Determination of the acid neutralization capacity of the plant extract compared to the standard.
- Calculating acid buffering capacity of the plant extract compared to the standard.
- Determination of the neutralizing effect of extracts on GSF of the plant extract compared to the standard.

Chapter 2

Literature Review

2.1 Medicinal plants as a source of therapeutic

Natural products continue to contribute a large share of new medicines and lead compounds across therapeutic areas, as shown by multi-decade audits of FDA-approved drugs and clinical candidates (e.g., antibacterials, antineoplastics, cardiometabolic agents). These analyses underscore the importance of unmodified natural products, semisynthetic derivatives, and natural-product–inspired scaffolds in modern pipelines (Newman & Cragg, 2007; Newman & Cragg, 2020). Classic opiate pharmacology itself traces back to the first isolation of morphine from *Papaver somniferum* by Friedrich Sertürner in 1805—an early proof that plant-derived small molecules can be isolated, characterized, and translated into potent therapeutics (Krishnamurti & Rao, 2016; Stefano et al., 2017).

At the global policy level, the World Health Organization has recently reaffirmed the role of evidence-based traditional and complementary medicine, calling for rigorous science, quality control, and integration where appropriate—signals that plant-derived agents remain central to health-innovation agendas (WHO, 2023; PAHO/WHO, 2023). Meanwhile, population-level disease burdens reinforce the case for safer, affordable therapeutics: peptic-ulcer disease still carries a lifetime risk of ~5–10%, and diabetes prevalence was estimated at ~537 million adults (10.5%) in 2021, with substantial projected growth (Malik et al., 2023; International Diabetes Federation, 2021; Sun et al., 2022). Together, these trends justify systematic evaluation of plant extracts for antioxidant, anti-inflammatory, and antidiabetic properties—precisely the axes pursued in this thesis.

2.2 The genus Elaeocarpus and E. floribundus (Indian olive)

The family Elaeocarpaceae comprises ~12 genera (~615 spp.), with Elaeocarpus as the largest (~350 spp.) (World Flora Online, 2024; Useful Tropical Plants, n.d.). Elaeocarpus floribundus is distributed across India, Bangladesh, Myanmar, Malaysia, Indonesia and neighboring regions, with edible fruits and diverse ethnomedicinal uses (e.g., mouthwash for inflamed gums; leaf decoctions for hypertension/diabetes) (Asian Plant, n.d.; Flowers of India, 2009; India Biodiversity Portal, n.d.; Useful Tropical Plants, n.d.). Within Elaeocarpus, other species (e.g., E. sphaericus “Rudraksha”, E. serratus, E. angustifolius) illustrate a breadth of traditional applications from anxiolysis to gastrointestinal and cardiovascular uses, suggesting lineage-wide chemotypic richness relevant to anti-inflammatory, antioxidant, and metabolic modulation (StuartxChange, n.d.; Useful Tropical Plants, n.d.).

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2.3 Phytochemistry of E. floribundus and pharmacologic rationale

Reports identify multiple classes in E. floribundus leaves and fruits, including fatty acids, diterpenoids, triterpene alcohols, phaeophytin, phytosterols, sesquiterpenes, and carbohydrates (e.g., arabinose, galactose, rhamnose), along with vitamin C and polyphenolics (e.g., myricitrin, myricetin, ellagic acid) (Ogundele et al., 2021; Sudradjat et al., 2022; Banerjee et al., 2022). One note, friedelanol have been implicated for antiproliferative effects, while the fruit's organic-acid profile aligns with its characteristic sour taste (Pakistan Journal of Pharmaceutical Sciences, 2013; Ryz et al., 2017; Hossen et al., 2023; Ghosh et al., 2017). Such phenolic/terpenoid chemistries provide mechanistic plausibility for radical scavenging (hydrogen/electron donation), membrane stabilization (protein–lipid interactions), and modulation of glucose handling (potential insulin-mimetic or GLUT-linked effects)—the three pharmacologic axes interrogated in this work (Zeb, 2020; Gülçin, 2023; Ranasinghe et al., 2012; Asgary et al., 2005; Shriwas et al., 2019).

2.4 Assay frameworks used in this thesis

2.4.1 Yeast glucose-uptake model (antidiabetic screening)

The baker's yeast glucose-uptake assay provides a rapid, low-cost screen of insulin-mimetic or glucose-transport–enhancing activity by quantifying the decline in extracellular glucose after incubation with yeast in the presence of test agents (Rehman et al., 2018; Madiwalar et al., 2022). While not a substitute for mammalian cell or in vivo models, it is widely used for first-pass antidiabetic evaluation, and its readout (% uptake relative to a glucose-only control) can be benchmarked against metformin, a reference biguanide (Şeylan et al., 2023). This thesis applies the method with appropriate controls and triplicates to estimate % uptake and compare extract performance to metformin.

2.4.2 HRBC membrane-stabilization assay (anti-inflammatory screening)

Hypotonicity-induced hemolysis of human red blood cells (HRBC) models the lysosomal membrane labialization that accompanies inflammation. Agents that stabilize erythrocyte membranes can analogously stabilize lysosomes, thereby limiting the release of inflammatory mediators. The assay quantifies % inhibition of hemolysis versus a hypotonic control and is commonly referenced in phytopharmacology (e.g., Sadique et al., 1989; Oyedapo et al., 2010; Anosike et al., 2012). In this thesis, diclofenac serves as the standard NSAID comparator, and dose–response is assessed at 100–400 $\mu g\ mL^{-1}$.

2.4.3 DPPH radical-scavenging assay (antioxidant screening)

The DPPH assay is a validated, spectrophotometric measure of free-radical scavenging, wherein antioxidant molecules reduce the purple DPPH• radical to a non-radical form, lowering absorbance at 517 nm (Brand-Williams et al., 1995; Sharma & Bhat, 2009). The method supports concentration–effect profiling and IC_{50} estimation and is benchmarked here with ascorbic acid as the standard. Your methodology implements standard concentrations (10–160 μg mL^{-1}) in triplicate, enabling direct potency comparisons among extracts and against ascorbate.

2.5 Intersections among oxidative stress, inflammation, and glucose dysregulation

Oxidative stress amplifies inflammatory signaling and impairs insulin action, while chronic inflammation feeds forward into insulin resistance and β-cell stress (Houstis et al., 2006; Gregor & Hotamisligil, 2011; Oguntibeju, 2019). Thus, antioxidant and membrane-stabilizing activities may indirectly improve glycemic handling, and vice-versa (Vincent et al., 2009; Shrivastav et al., 2023). A multi-assay readout (DPPH, HRBC, yeast uptake) therefore captures complementary facets of phytopharmacology—aligning with the multifunctional nature of plant secondary metabolites and reflecting clinical realities in metabolic-inflammatory syndromes. This integrative rationale underlies the selection of assays and standards in your thesis. Mechanistically, reactive oxygen species (from NOX isoforms and mitochondria) disrupt insulin signaling and glucose transport, whereas lowering oxidative burden can restore insulin sensitivity; in parallel, low-grade "metaflammation" in adipose and liver (e.g., TNF-α/JNK, IKK–NF-κB pathways) propagates insulin resistance (Han, 2016; Gregor & Hotamisligil, 2011; Houstis et al., 2006; Oguntibeju, 2019). The HRBC membrane-stabilization assay is widely used as an in-vitro surrogate of lysosomal membrane protection in inflammation, supporting its inclusion alongside DPPH and yeast uptake in this thesis (Oyedapo et al., 2010; Feyisayo et al., 2015).

2.6 Prior evidence supporting the assay choices

Literature supports

(i) DPPH as a robust primary screen for phenolic/flavonoid antioxidants;

(ii) HRBC membrane stabilization as a surrogate for anti-inflammatory potential; and

(iii) yeast uptake as an expedient antidiabetic screen, with numerous plant studies linking these endpoints to polyphenol-rich extracts and flavonoids/terpenoids.

The references compiled in your Discussion emphasize these precedents and were used to frame the interpretation of your experimental outcomes. In particular, DPPH remains one of the most widely adopted single-electron/hydrogen-atom transfer assays for phenolics and flavonoids, with well-described chemistry and proven reproducibility when conditions are standardized (Brand-Williams et al., 1995; Sharma & Bhat, 2009; Gülçin, 2023). HRBC membrane-stabilization is frequently used in phytopharmacology as an in-vitro proxy for lysosomal membrane protection during inflammation and shows good concordance with anti-inflammatory extracts (Sadique et al., 1989; Oyedapo et al., 2010; Anosike et al., 2012). For first-pass antidiabetic screening, the baker's yeast glucose-uptake model offers a fast, low-cost readout of insulin-mimetic or transport-enhancing activity that can be benchmarked against metformin (Rehman et al., 2018; Madiwalar et al., 2022).

2.7 Summary and gap statement

Taken together, the botanical/ethnomedicinal background of E. floribundus, its polyphenolic/ terpenoid content, and the complementary assay triad provide a coherent framework to evaluate antioxidant, anti-inflammatory, and antidiabetic potential. However, systematic comparative data across extraction methods remain limited, and mechanistic attribution to defined constituents is incomplete.

The present thesis addresses these gaps by ,

(i) Standardizing three extract types

(ii) Benchmarking against clinically relevant standards (ascorbic acid, diclofenac, metformin)

(iii) Applying quantitative dose–response analyses across the three bioassays.

This alignment mirrors best-practice screening logic: phenolic/terpenoid-rich extracts are expected to show radical-scavenging (DPPH), membrane-stabilizing anti-inflammatory effects (HRBC), and potential glucose-handling modulation (yeast uptake), but translation to mechanisms requires targeted isolation work; thus, your design provides comparative potency data while motivating future bioassay-guided fractionation and mechanistic studies (overviewed for antioxidants by Gülçin, 2023, and for phenolics by Zeb, 2020; HRBC rationale in Anosike et al., 2012).

Chapter 3

Methodology

3.1 Collection

The plant *Elaeocarpus Floribundus* was collected from Moulvibazar,Sylhet in December 2024 with the help of personnel from Bangladesh National Herbarium Fresh plants along with their stem were collected so they remain fresh till they arrive in Dhaka. In local language this tree is known as Bon Jalpai and can only be collected from Chittagong hill tracts, Sylhet hill tracts in Bangladesh. After collecting the plant, the leaves were separated from the stem for further drying process.

Figure 3.1: *Elaeocarpus Floribundus tree*

3.2 Identification and Authentication

To get authorized identification and prove for authentication a sample of the plant was sent to Bangladesh National Herbarium on January 15, 2025. Plant specimens prepared in an herbarium sheet (16"*10.50" size) to submit into the herbarium. The taxonomic authentication was conducted by herbarium, and an assessing number (DACB 112746) was given that indicates the plant is verified and authenticated by Bangladesh National Herbarium.

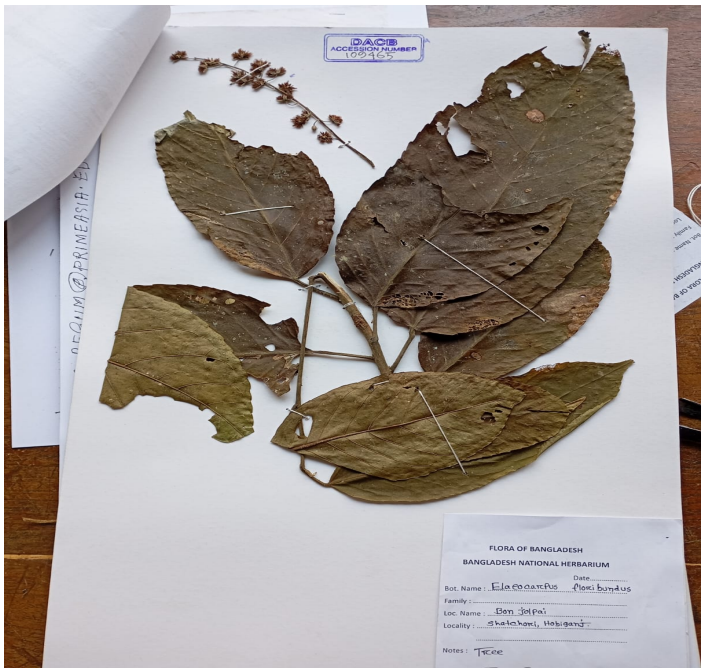

Figure 3.2: Plant sheet

3.3 Preparation of plant material for extraction

Fresh plant leaves were separated from the leaves and washed with fresh water a few times to clean any dirts. Then the plants were air dried in a shaded place away from the sunlight for 4weeks until they were fully dried. During this period the leaves were mixed a few times to ensure air flow. After the leaves are fully dried, they are grinded into fine powder and weighted. Around 2200gm of powdered plant leaves were obtained. Then into 3 airtight containers the powdered leaves were stored.

Container 01: 1000gm of powdered leave mixed with 1L of methanol and shaked properly and stored in that airtight container for 1 month.

Container 02: 800 gm of powdered leaves mixed with 1000ml methanol and shaked properly and stored in an airtight container for a month.

Container 03: 400 gm of powdered leaves mixed with 800ml methanol, shaken properly and stored in an airtight container for a month .

During this one-month period the containers with plant material were shaken regularly to accelerate the mixing process.

3.4 Extraction

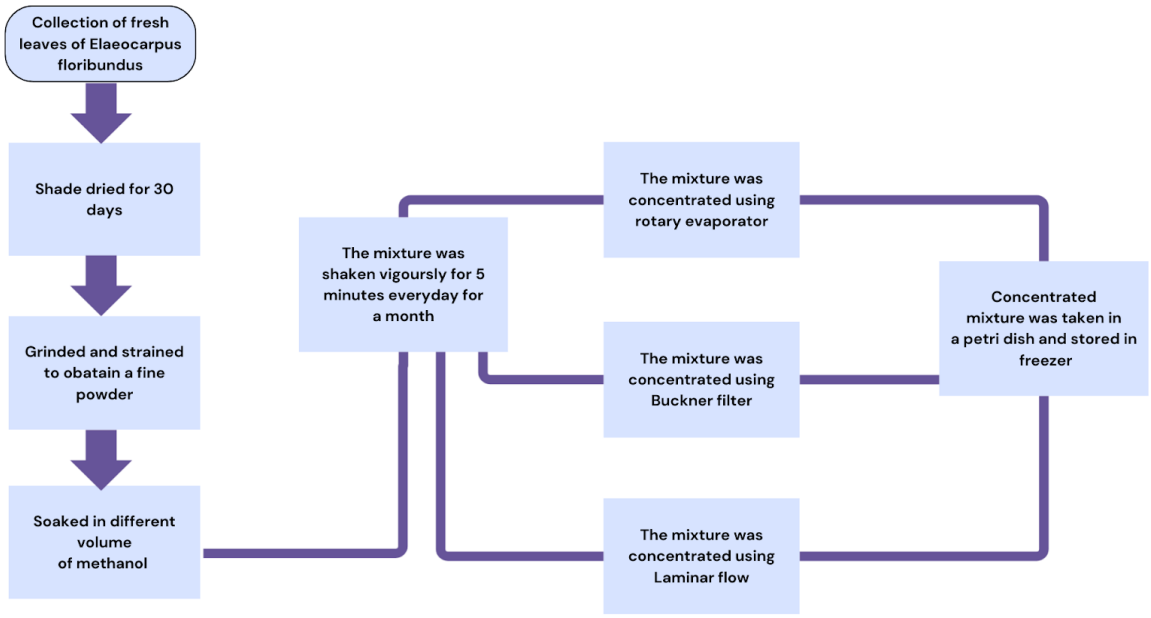

Fig 3.4.1: Extraction process

i. Air dry: The container 01 material had 1:1 ratio of powder material and methanol so they were dried in laminar flow for a week by placing them in petri dishes which were sterilized. A yellowish extract was found with a strong smell of tea flavor.

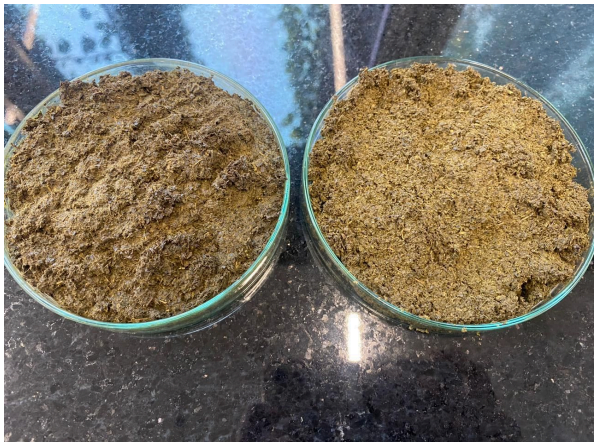

Fig 3.4.2: Air dry plant extract

ii. Rotary evaporator: Container 02 material had 4:5 ratio of powder plant material and methanol. So, the methanol was separated using this method. And obtained powder extract were stored in petri dishes and put into the refrigerator for storing. The extract was light greenish and had smell like tea leaves.

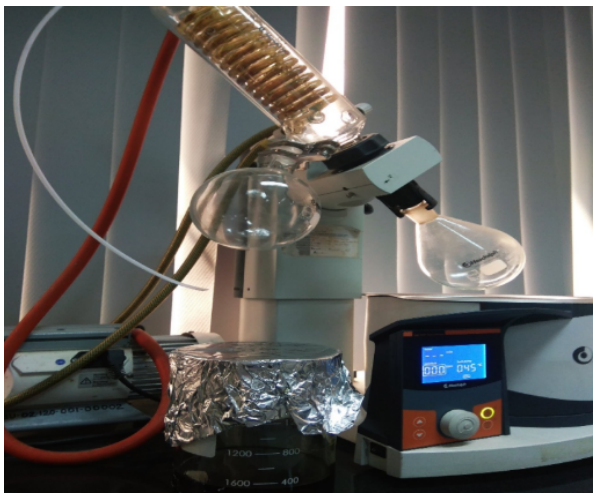

Fig 3.4.3 : Extraction through Rotary evaporation

iii.Buckner funnel and laminar flow : The container 3 had 1:2 plant powder material and methanol ratio so they were filtered by using high pressured Buckner funnel which helped to remove most of the liquid methanol and the plant material were kept in a petri dishes in drying oven for further drying. The extract was light greenish and had smell like tea leaves.

Fig 3.4.4: Extraction through Buckner filter

3.5 Baker's Yeast Glucose Uptake Assay

3.5.1 Material and Chemicals

Table 3.5.1: Materials and chemicals required for yeast glucose uptake assay

Dried plant powder	As required (e.g. 20 g)
Distilled water	As needed
Baker’s yeast	1 packet (active dry yeast)
Glucose (D-glucose)	1% solution (w/v)
Centrifuge tubes	Several (15 mL)
Incubator	37°C
Water bath	37°C
Glucometer with strips	01
Centrifuge	01
Micropipette + tips	Several
Beaker, conical flask, filter paper, funnel	As per needed

3.5.2 Plant Extract Preparation

10 g of dried plant powder weighed and added it to 100 mL distilled water (1:10 w/v). Boil for 30 minutes, cool, and filter. Collect the filtrate and label it as “Aqueous Extract". All three kinds of plant extract solution were created and labeled serially

Fig 3.5.2: Preparation of plant extract

3.5.3 Yeast Cell Suspension Preparation

1 g of active dry yeast was dissolved in 10 mL distilled water. Then Incubated for 10 min at 37°C.After that the yeast cells were centrifuged at 3000 rpm for 5 min. The supernatants were discarded. The pellets were washed with 10 mL distilled water. Washing was repeated for 3 times. Then the final pellet was resuspended in 10 mL distilled water. Labeled it as "Yeast Suspension".

3.5.4 Glucose Solution Preparation (1%)

1gm of glucose was weighed and dissolved in 100ml of distilled water and labeled as "Glucose Stock".

3.5.5 Metformin solution

4 metformin tablets of 500mg were crushed. Then 100mg of powdered metformin was dissolved in 100ml distilled water.

3.5.6 Reaction setup

i. Negative group = 1ml of Glucose solution and 1ml of yeast solution were taken into test tubes. Three replicates were created for accurate reading.

ii. Standard group = 1ml of glucose + 1ml of yeast solution + 1ml of metformin solution taken into 3 test tubes.

iii. Sample group = 1ml of glucose+ 1ml of yeast solution + 1ml of plant extract were taken into 3 test tubes .

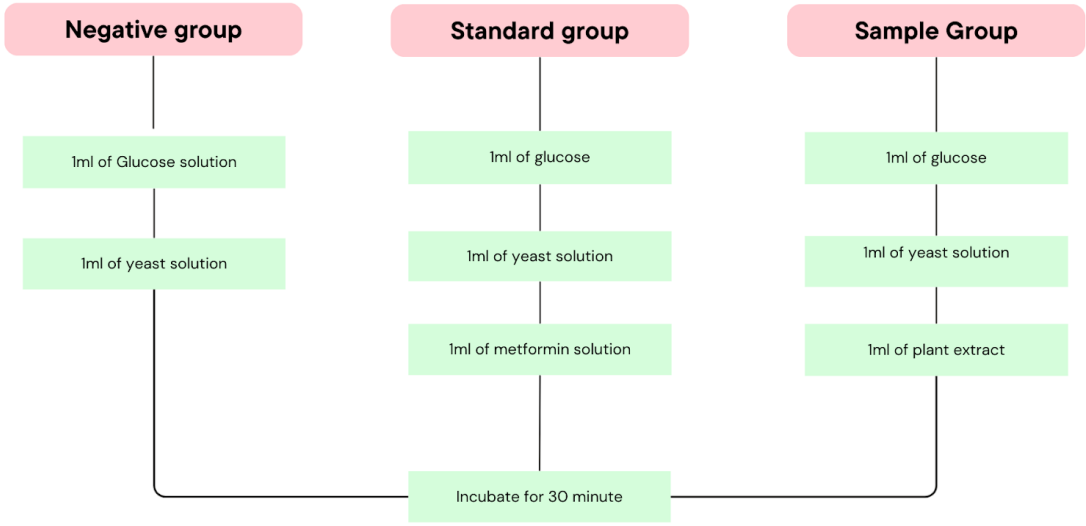

Fig 3.5.6: Reaction setup flowchart

The contents of each test tube were mixed thoroughly. Then Incubated at 37°C for 60 minutes and after incubation, centrifuge at 3000 rpm for 5 minutes. Then the supernatant was collected from the test tubes with the help of micropipette their reading was recorded with the help of glucometer.

3.6 HRBC membrane-stabilization assay

3.6.1 Material and chemicals

Table 3.6.1: Materials and chemicals required for HRBC membrane stabilization assay

Dried plant powder	As required (e.g. 20 g)
Distilled water	As needed
Diclofenac sodium	Positive control
Phosphate Buffer	500ml
Normal Saline	250ml
Fresh human blood	5ml
EDTA-k3 tube	01
Hypotonic saline	250ml
Centrifuge tubes	Several (15 mL)
Incubator	37°C
Spectrophotometer	540nm
Centrifuge	01
Beaker, conical flask, filter paper, funnel, Micropipette + tips	As per needed

3.6.2 Preparation of Phosphate Buffer Solution

3.6.2.1 Chemicals required

Table 3.6.2.1: Chemicals and their quantity for preparation of PBS

Component	Formula	Amount for 250 mL
Sodium chloride	NaCl	2.25 g
Potassium chloride	KCl	0.050 g
Dibasic phosphate	K_2HPO_4 (anhydrous)	0.060 g
Monobasic phosphate	NaH_2PO_4 (anhydrous)	0.360 g
Distilled water	H_2O	Up to 250ml

Fig 3.6.2.1: Salts for phosphate buffer

3.6.2.2 Preparation

180ml distilled water was taken into a beaker and all the salts one by one were added and stirred to dissolve evenly. We checked the pH which was above 9. So, we added HCL drop wise till the pH was 7.4. Then we labeled the beaker as PBS (Phosphate buffer solution).

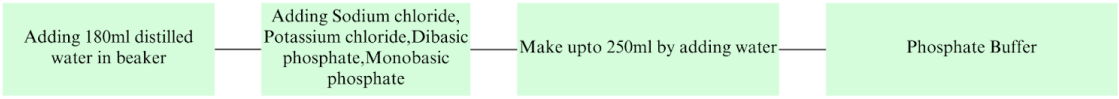

Fig 3.6.2.2 : Phosphate Buffer Preparation

3.6.3 Prepare your 10 % HRBC Suspension

3.6.3.1 Blood cell collection

i.5mL venous blood (female)was drawn into an EDTA-K_3 tube.

ii. Then inverted 8–10 times to mix.

iii.After that it was centrifuged at 3 000 rpm for 10 min at room temperature and we got two separated part one is clear plasma layer and other one is blood cell. The plasma layer of the blood.

Fig 3.6.3.1 : Fresh human blood after collection and in EDTA-K_3 tube

3.6.3.2 Washing with PBS

- 5ml PBS was added to the remaining red cell pellet
- Gently resuspend by inverting. Again Centrifuged at 3 000 rpm × 10 min.
- The supernatant was discarded.

3.6.3.3 Making 10 % suspension

- After the third wash, we packed the cells by spinning again and discarded the last supernatant.
- Estimated the packed-cell volume to 0. 75ml.Added PBS to bring the total volume to 5.0 mL.
- We got 10 % v/v HRBC suspension. Kept it on ice and used within 1 hour.

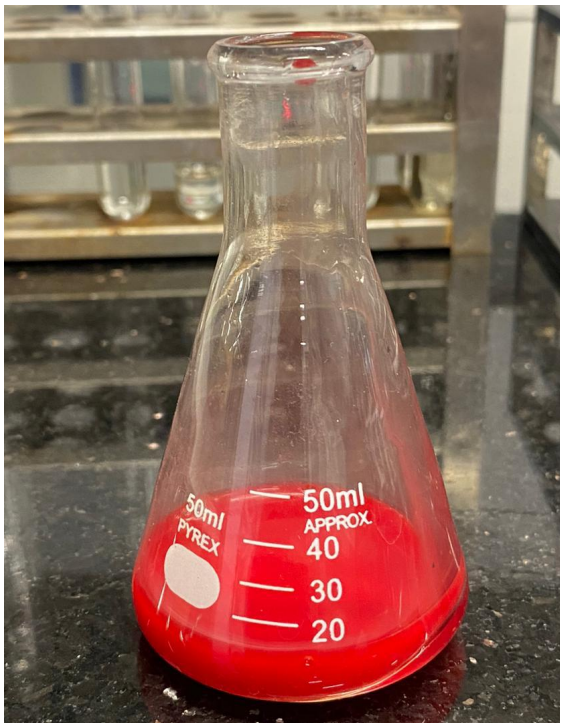

Fig 3.6.3.3 : 10 % HRBC Suspension

3.6.4 Preparation of Stock extract solution

We dissolve 1000 mg dried extract in 100 mL PBS to make 10 mg/mL stock solution. We used all the three kinds of extract and marked them as E1 , E2 and E3.

3.6.4.1 Working concentration preparation

We prepared three concentration which are 100 µg/mL (E_1) ,200 µg/mL (E_2) , 400 µg/mL (E_3) by dissolving our plant extract in Phosphate buffer solution.

3.6.4.2 Serial Dilution calculation

Formula for concentration ,

$$C_1V_1 = C_2V_2$$

Table 3.6.4.2: Calculation of amount required for serial dilution of volume 400 µg/mL,200 µg/mL and 100 µg/mL

Target conc.	Volume of stock (10 mg/mL)	Volume of PBS (diluent)	Final volume
E_3 = 400 µg/mL	0.20 mL (200 µL)	4.80 mL	5.0 mL
E_2 = 200 µg/mL	0.10 mL (100 µL)	4.90 mL	5.0 mL
E_1 = 100 µg/mL	0.05 mL (50 µL)	4.95 mL	5.0 mL

3.6.5 Preparation of Standard Control (Diclofenac)

10mg of diclofenac sodium was dissolved in 80ml Phosphate Buffer solution. After it dissolved completely, we made it up to 100ml with PBS.Then used vortex to get evenly mixed diclofenac solution

3.6.6 Preparation of Hypotonic Phosphate buffer solution

Each salt was dissolved in 180 mL distilled water. Transfer it to a 250 mL volumetric flask. Then Made up the volume to 250 mL with distilled water. pH was maintained between 7.2 - 7.4

Table 3.6.6 : Salts required and their amount for Hypotonic phosphate buffer solution

Chemical	Amount
NaCl	0.625g
KCL	0.050g
Na_2HPO_4	0.360g
KH_2PO_4	0.060g

3.6.7 Labelling & Arranging Tubes

We planned to test in triplets, so we arranged each test tube in triplets.

- Blank (B) - PBS + Extract solution.
- Negative control (N) - Phosphate Buffer Solution
- Standard (S) - Diclofenac solution
- Extract 100 µg/mL (E_1), Extract 200 µg/mL (E_2), Extract 400 µg/mL (E_3)

3.6.8 Assay Mixture

3.6.8.1 Pipetting Order

1. Hypotonic PBS
    a. With the help of pipette, we took **2.0 mL** into each N, S, and E1 – E3 tube.
2. Test solution
    a. Added **0.5 mL** of PBS for normal(N), Diclofenac for Standard(S), on the appropriate extract dilution for E1 –E3.
3. HRBC suspension
    a. Finally added **0.5 mL** of 10 % RBC suspension to N, S, and E1 – E3.

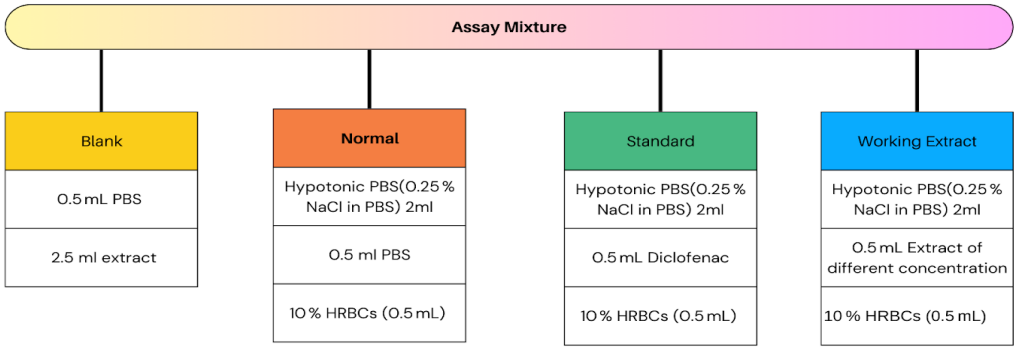

Fig 3.6.8.1: Flowchart of mixture assay

3.6.8.2 Test tube preparation

We triplet test tubes for every type and concentration of solution.

Table 3.6.8.2 : Calculation for HRBC Stabilization assay for all the sample

Tube	Hypotonic PBS(0.25 % NaCl in PBS)	Test Solution(0.5 mL)	10 % HRBCs
B	—	0.5 mL PBS + 2.5 ml extract	—
N	2.0 mL	0.5 mL PBS	0.5 mL
S	2.0 mL	0.5 mL Diclofenac (100 µg/mL)	0.5 mL
E_1	2.0 mL	0.5 mL Extract (100 µg/mL)	0.5 mL
E_2	2.0 mL	0.5 mL Extract (200 µg/mL)	0.5 mL
E_3	2.0 mL	0.5 mL Extract (400 µg/mL)	0.5 mL

3.6.9 Incubation & Reading

1. Centrifuge

- Placed all tubes (except B) in an incubator for 30 min.
- Gently inverted tubes once at 15 min to mix—do not vortex.

2. Centrifuge

- Centrifuged at 3 000 rpm for 10 min at room temperature.

3. Collection of supernatant

- Carefully pipetted 1.0 mL clear supernatant from each tube into cuvettes.
- Zero the spectrophotometer with Blank (0.5 mL PBS + 2.5ml extract solution).

4. Measuring absorbance

- Read at 540nm for each sample.

Fig 3.6.9: Test tubes in incubator

3.7 DPPH Antioxidant Assay

3.7.1 Materials and reagents

Table 3.7.1: Materials and chemicals required for DPPH Assay

Chemical and components	Quantity
Plant Extract powder	300mg
DPPH(2,2-diphenyl-1-picrylhydrazyl)	3.94mg
Ascorbic acid	100mg
Methanol	400ml
Spectrophotometer	1
Cuvettes	many
Micropipettes	many
Sterile tips	many
Volumetric flask(10ml ,100ml)	10
Beakers	5
Analytical balance	1
Test tubes	74

3.7.2 Stock solution preparation

3.7.2.1 Plant Extract Stock

i. We weighed 100 mg plant extract powder.

ii. Transferred into a 100 mL volumetric flask.

iii. Added ~80 mL methanol, sonicated to get a proper mixture.

3.7.2.2 Test concentration preparation

We Created in Triplets for each concentration for all three kinds of extract for each concentration.

Fig 3.7.2.2: Preparation of test concentration

Table 3.7.2.2: Calculation for serial dilution of stock solution with methanol

Concentration(µg/mL)	**Volume of stock (µL, 1 mg/mL)**	**Methanol to 1 mL (µL)**
10	10	990
20	20	980
40	40	960
80	80	920
160	160	840

3.7.2.3 DPPH Reagent (0.1 mM in methanol)

i. We weighed 3.94 mg DPPH.

ii. Transferred into a 100 mL volumetric flask.

iii. Added ~80 mL methanol, swirl until fully dissolved.

iv. Make up to 100 mL with methanol.

v.Covered it with aluminum foil and stored away from light as it is light sensitive.

Fig 3.7.2.3.1: DPPH

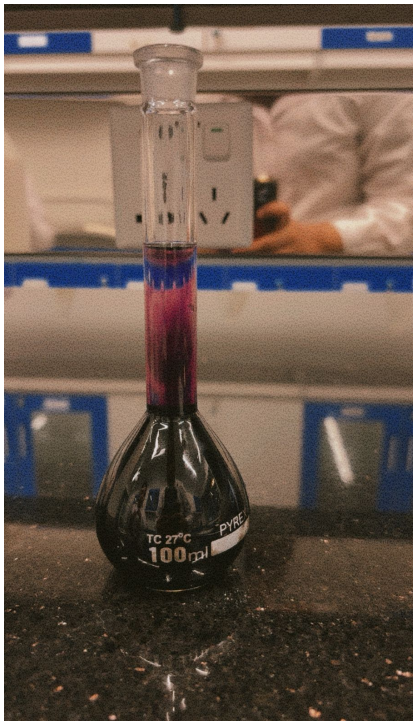

Fig 3.7.2.3.2 : DPPH Solution

3.7.2.4 Ascorbic acid solution (1 mg/mL)

We weighed 100 mg ascorbic acid. Dissolved in methanol in a 100 mL volumetric flask. Make up to 100 mL with methanol. It is our standard solution.

3.7.2.4.1 Preparing test concentration for standard

We Created in Triplets for each concentration for each concentration:

i. We pipetted the required µL of 1 mg/mL ascorbic acid solution into a labeled tube.

ii. Added methanol to bring the final volume to 1.00 ml.

iii. Mixed thoroughly for an even solution.

Table 3.7.2.4.1: Calculation of serial dilution of Ascorbic acid

Concentration (µg/mL)	10	20	40	80	160
Volume of Ascorbic acid solution (µL)	10	20	40	80	160
Methanol to 1 mL (µL)	990	980	960	920	840

3.7.3 Assay

3.7.3.1 Reaction Setup

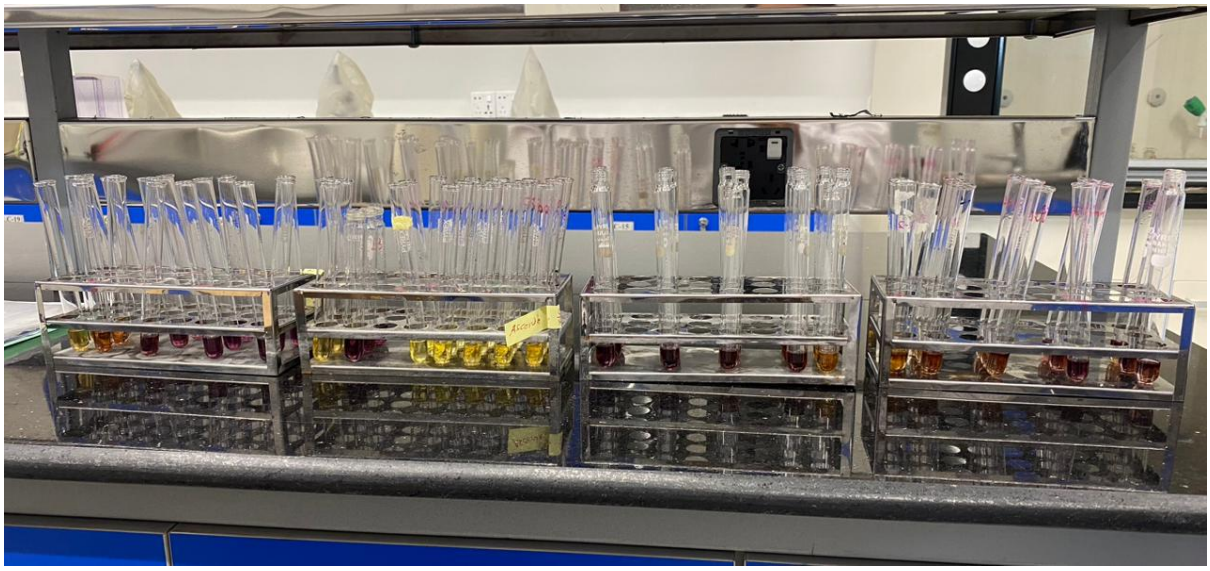

Fig 3.7.3.1: Test tubes for Assay

Table 3.7.3.1: Test tube preparation for DPPH antioxidant assay

Test-tube	**Component A (1ml)**	**Component B (1ml)**
Control	DPPH solution (0.1 mM)	Methanol
Blank	Extract working solution	Methanol
Standard	DPPH solution (0.1 mM)	Ascorbic acid working solution
Sample(10 µg/mL)	DPPH solution (0.1 mM)	Extract working (10 µg/mL)
Sample(20 µg/mL)	DPPH solution (0.1 mM)	Extract working (20 µg/mL)
Sample(40 µg/mL)	DPPH solution (0.1 mM)	Extract working (40 µg/mL)
Sample(80 µg/mL)	DPPH solution (0.1 mM)	Extract working (80 µg/mL)
Sample (160 µg/mL)	DPPH solution (0.1 mM)	Extract working (160 µg/mL)

For each concentration (plant extract + ascorbic acid), we triplicated the test tubes.

3.7.3.2 Incubation

i. Incubate mixtures in the dark at room temperature (25 °C) for 30 min.

ii. Wrap tubes/cuvettes with foil to avoid light degradation of DPPH.

3.7.3.3 Measurement

1. We set the spectrophotometer to 517 nm.

2. Zero it with Blank (B).

3. Recorded absorbance of negative control (A_o) (methanol + DPPH).

4. Recorded absorbance of each sample (A_x) (extract or ascorbic acid + DPPH).

Chapter 4

Results

4.1 Baker's Yeast Glucose Uptake Assay

4.1.1 Negative group Glucometer reading

Table 4.1.1: Glucose reading in glucometer and average glucose reading

7.6	8.2	8	Average = 7.9mg/ml

An initial glucose concentration of 7.9 mg/mL (G_0). After 60 min yeast + extract incubation the residual glucose (G_1) measured in three independent sample sets. From these we calculated:

$$\Delta G \text{ (mg/mL)} = G_0 - G_1$$

$$\% \text{ Uptake} = (\Delta G / G_0) \times 100$$

4.1.2 Extract Percent glucose uptake (Control group)

Table 4.1.2: Average residual glucose in three independent sample and calculation of present uptake of glucose from formula

Set	G_1 avg (mg/mL)	$\Delta G = 7.9 - G_1$	% Uptake = $(\Delta G/7.9) \times 100$
E - 1	3.6	4.3	$(4.3/7.9) \times 100 \approx 54.4$ %
E - 2	2.53	5.37	$(5.37/7.9) \times 100 \approx 68.0$ %
E - 3	3.2	4.7	$(4.7/7.9) \times 100 \approx 59.5$ %

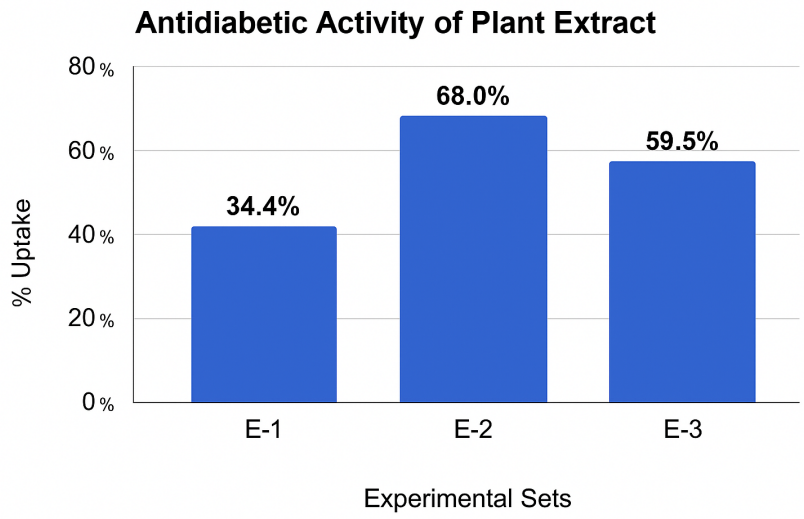

Fig 4.1.2: Graph chart of extraction (E1, E2, E3) vs %uptake

From the graph we can see that E2 has shown the Highest %uptake of glucose that indicates E2 has higher activity than other two extracts.

4.1.3 Standard group

Table 4.1.3 : Glucometer reading of standard group

3.1	2.28	2.13	Average = 2.50mg/ml

ΔG (mg/mL) = $G_0 - G_1$ = 7.9 - 2.50 = 5.4

% Uptake = (ΔG / G_0) × 100

= (5.4/7.9) *100

= 68.35 %

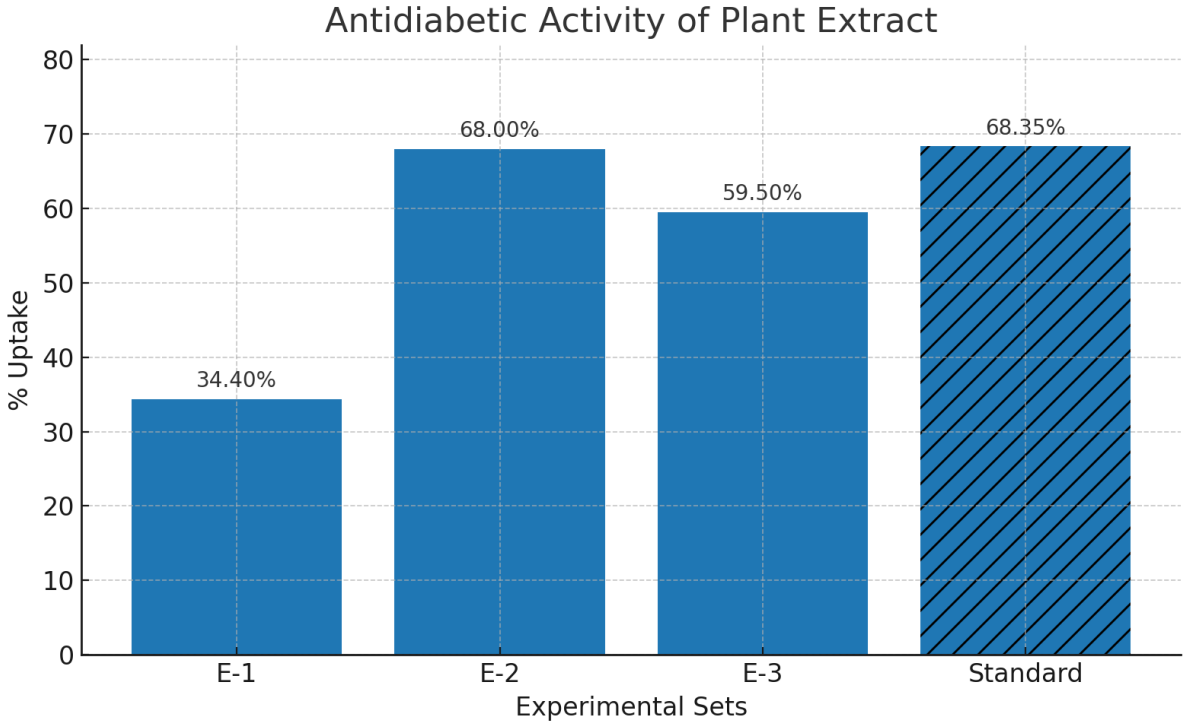

Fig 4.1.3 : Standard comparison with experimental sets

4.2 HRBC membrane-stabilization assay

4.2.1 Extraction absorbance

Table 4.2.1.1: Absorbance reading of extraction 01 in different concentration

Buckner filtered extract (E1)	100 µg mL^{-1}	200 µg mL^{-1}	400 µg mL^{-1}
Replicate 1	0.069	0.030	0.020
Replicate 2	0.061	0.033	0.019
Replicate 3	0.033	0.029	0.013

Table 4.2.2.1: Absorbance reading of extraction 02 at different concentration

Rotary Evaporator Extraction (E2)	100 µg mL^{-1}	200 µg mL^{-1}	400 µg mL^{-1}
Replicate 1	0.049	0.040	0.020
Replicate 2	0.059	0.020	0.019
Replicate 3	0.041	0.026	0.015

Table 4.2.3.1: Absorbance reading of extraction 02 at different concentration

Air Dry extraction (E3)	100 µg mL^{-1}	200 µg mL^{-1}	400 µg mL^{-1}
Replicate 1	0.069	0.030	0.020
Replicate 2	0.061	0.033	0.019
Replicate 3	0.067	0.029	0.013

4.2.2 Diclofenac absorbance

Table 4.2.2: Absorbance reading of diclofenac at different concentration

Diclofenac	100 µg mL^{-1}	200 µg mL^{-1}	400 µg mL^{-1}
Replicate 1	0.055	0.039	0.012
Replicate 2	0.069	0.032	0.018
Replicate 3	0.061	0.037	0.010

4.2.3 Blank absorbance

Table 4.2.3 Blank absorbance and mean value

Replicate 1	Replicate 2	Replicate 3	Mean value
0.093	0.099	0.092	0.0947

4.2.4 Mean value and % Inhibition

$$\%\text{Inhibition} = 100\left(1 - \frac{A^{*}_{\text{sample}}}{A^{*}_{\text{control}}}\right) \quad \Rightarrow \quad A^{*}_{\text{sample}} = \left(1 - \frac{\%\text{Inhib}}{100}\right) A^{*}_{\text{control}}$$

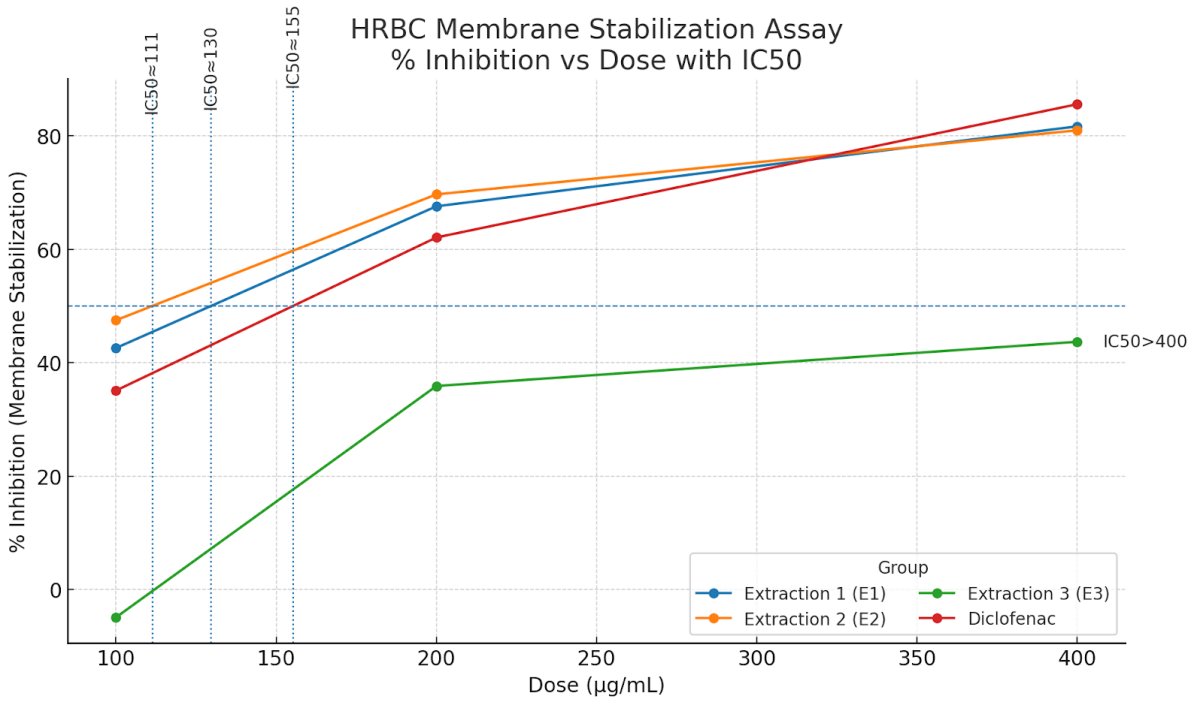

Fig 4.2.4: Dose vs Inhibition of HRBC membrane stabilization assay

The date indicates that with the change in concentration, as the dose increases E1 and E2 getting close to the standard curve. Which indicates that they have very potent anti-inflammatory properties. But E3 never reached IC50 value so it is not potent enough.

Table 4.2.4: Mean value with standard deviation of E1, E2, E3 and Diclofenac at different concentration and their percent inhibition

Group	Dose (μg mL^{-1})	Mean ± SD	% Inhibition
	100	0.054 ± 0.019	42.6 %
Extraction 1 (E1)	200	0.031 ± 0.002	67.6 %
	400	0.017 ± 0.004	81.7 %
	100	0.050 ± 0.009	47.5 %
Extraction 2 (E2)	200	0.028 ± 0.010	69.7 %
	400	0.018 ± 0.003	81.0 %
	100	0.099 ± 0.038	–4.9 %
Extraction 3 (E3)	200	0.061 ± 0.006	35.9 %
	400	0.053 ± 0.004	43.7 %
	100	0.062 ± 0.007	35.1%
Diclofenac	200	0.036 ± 0.003	62.1%
	400	0.014± 0.004	85.6%

4.3 DPPH Antioxidant assay

4.3.1 Absorbance

Table 4.3.1.1: Absorbance of blank

Blank	Absorbance
Replicate 1	0.058
Replicate 2	0.057
Replicate 3	0.057

Table 4.3.1.2: Absorbance of extraction 1 (E1)

Extract 1	160µg mL^{-1}	80µg mL^{-1}	40µg mL^{-1}	20µg mL^{-1}	10µg mL^{-1}
Replicate 1	0.343	0.691	0.935	1.147	1.365
Replicate 2	0.387	0.486	1.018	1.225	1.414
Replicate 3	0.354	0.503	1.140	1.220	1.353

Table 4.3.1.3 Absorbance of extraction 2 (E2)

Extract 2	160µg mL^{-1}	80µg mL^{-1}	40µg mL^{-1}	20µg mL^{-1}	10µg mL^{-1}
Replicate 1	0.411	0.938	0.863	0.945	0.987
Replicate 2	0.467	0.593	0.852	1.045	1.449
Replicate 3	0.376	0.630	0.729	0.778	1.045

Table 4.3.1.4: Absorbance of extraction 3 (E3)

Extract 3	160µg mL^{-1}	80µg mL^{-1}	40µg mL^{-1}	20µg mL^{-1}	10µg mL^{-1}
Replicate 1	0.412	0.426	0.565	0.620	0.690
Replicate 2	0.408	0.452	0.485	0.630	0.672
Replicate 3	0.373	0.419	0.453	0.565	0.665

Table 4.3.1.5: Absorbance of Ascorbic acid

Ascorbic Acid	160µg mL^{-1}	80µg mL^{-1}	40µg mL^{-1}	20µg mL^{-1}	10µg mL^{-1}
Replicate 1	0.092	0.105	0.155	0.257	0.402
Replicate 2	0.095	0.110	0.147	0.229	0.311
Replicate 3	0.101	0.134	0.170	0.242	0.385

Table 4.3.1.6: Absorbance of negative control (Methanol + extract)

0.233	0.276	0.255

4.3.2 Percent Inhibition

Table 4.3.2.1: Mean value and percent inhibition of Extraction 1 (E1) at different dose concentration

Dose (µg/mL)	Mean A	% Inhibition
10	1.377 ± 0.032	7.0% ± 2.2%
20	1.197 ± 0.044	19.2% ± 2.9%
40	1.031 ± 0.103	30.4% ± 7.0%
80	0.560 ± 0.114	62.2% ± 7.7%
160	0.361 ± 0.023	75.6% ± 1.5%

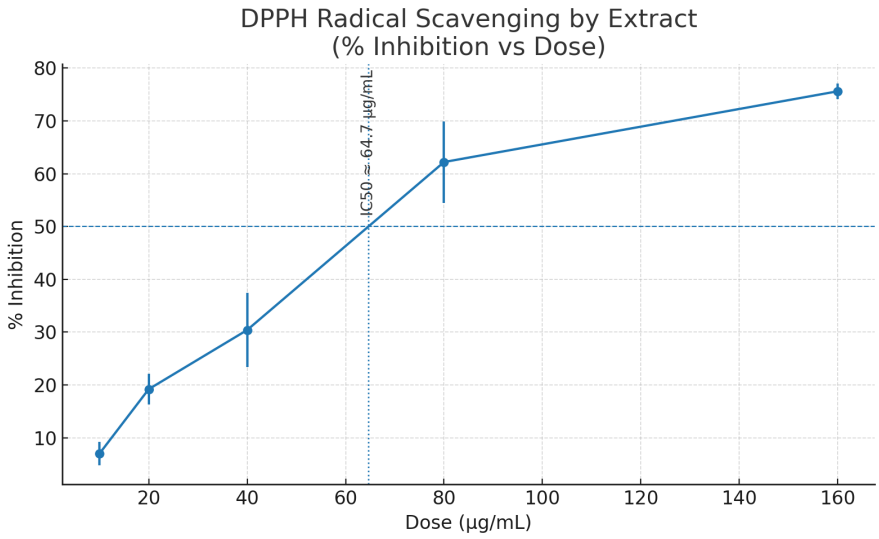

Fig 4.3.2.1: %Inhibition vs Dose of extraction 01

Table 4.3.2.2: Mean value and percent inhibition of Extraction 2 (E2) at different dose conc.

Dose (µg/mL)	Mean A	% Inhibition
10	1.160 ± 0.252	21.7%
20	0.923 ± 0.135	37.7%
40	0.815 ± 0.074	45.0%
80	0.720 ± 0.189	51.4%
160	0.418 ± 0.046	71.8%

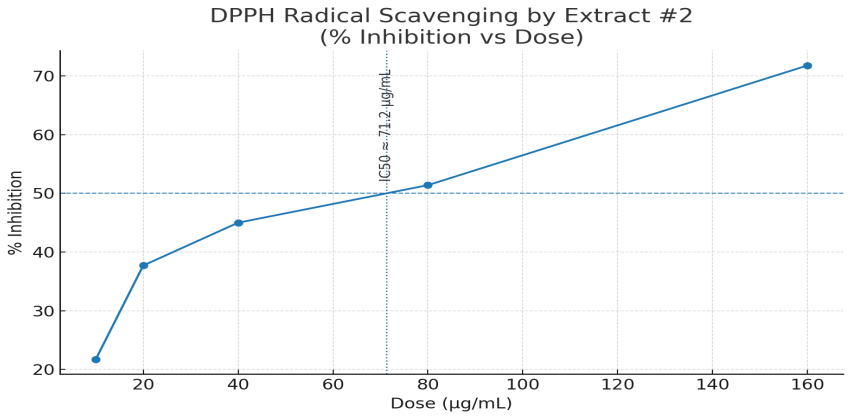

Fig 4.3.2.2: %Inhibition vs Dose of extraction 2

Table 4.3.2.3: Mean value and percent inhibition of Extraction 3 (E3) at different dose concentration

Dose (µg/mL)	Mean A	% Inhibition
10	0.676 ± 0.013	54.4%
20	0.605 ± 0.035	59.1%
40	0.501 ± 0.058	66.2%
80	0.432 ± 0.017	70.8%
160	0.398 ± 0.021	73.1%

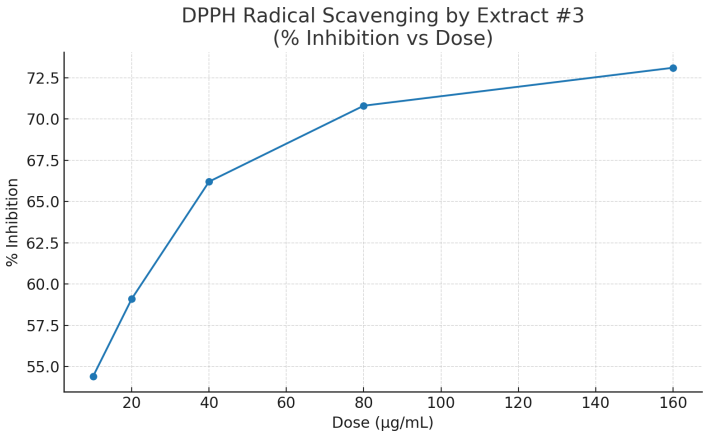

Fig 4.3.2.3: %Inhibition vs Dose of extraction 3

Table 4.3.2.4: Mean value and percent inhibition of Ascorbic acid at different dose concentration

Dose (µg/mL)	Mean A	% Inhibition
10	0.366 ± 0.048	75.3%
20	0.243 ± 0.014	83.6%
40	0.157 ± 0.012	89.4%
80	0.116 ± 0.016	92.1%
160	0.096 ± 0.005	93.5%

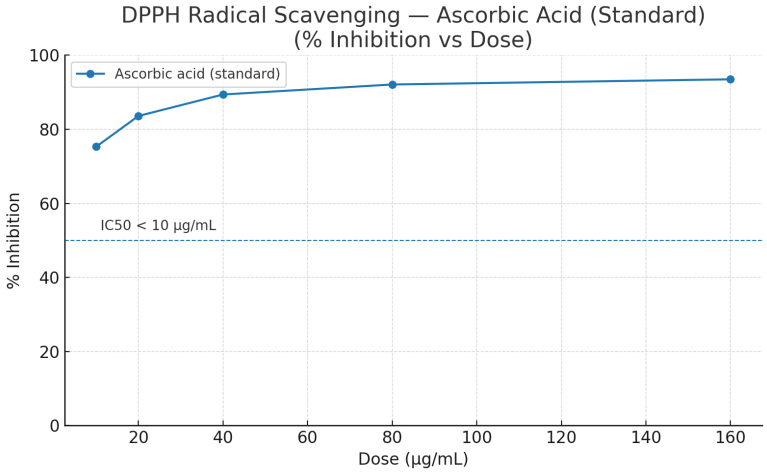

Fig 4.3.2.4.1: %Inhibition vs Dose of Ascorbic acid

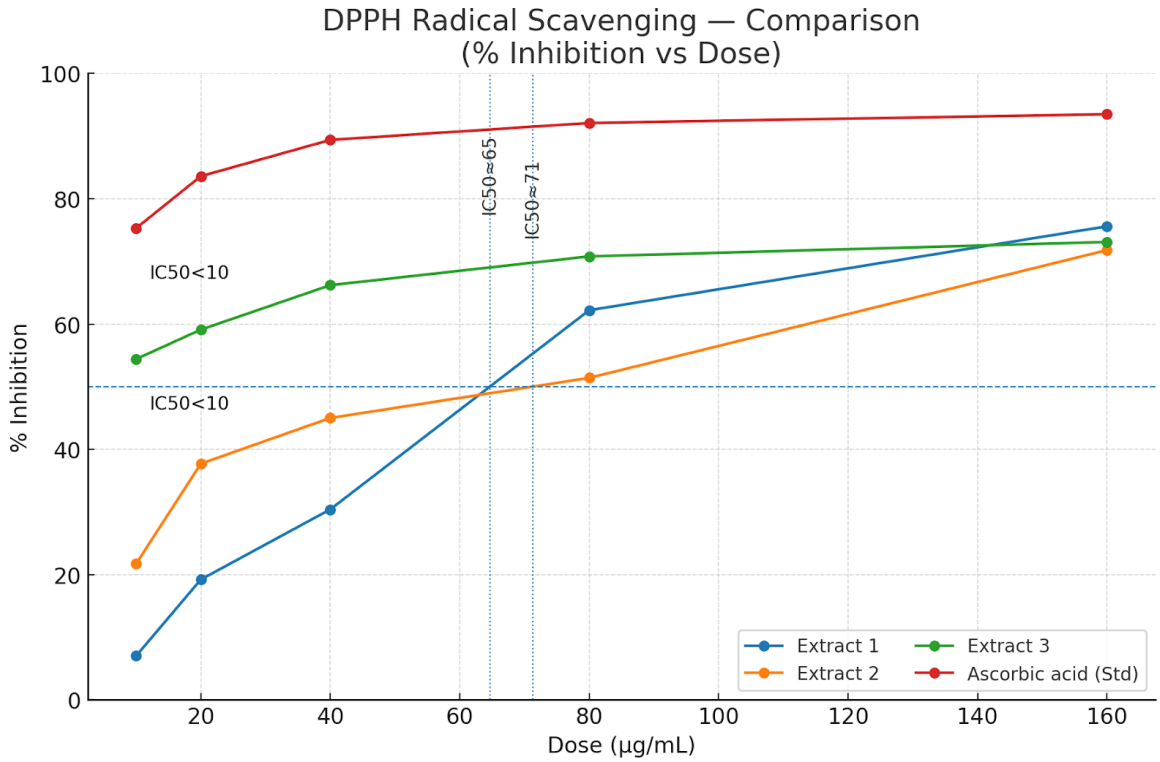

Fig 4.3.2.4.2: %Inhibition vs Dose comparison

The DPPH assay showed that all extracts had dose-dependent radical scavenging activity. Extracts 1 and 2 displayed moderate potency ($IC_{50} \approx 65$ and 71 µg/mL), while Extract 3 was highly active, exceeding 50% inhibition at 10 µg/mL with $IC_{50} < 10$ µg/mL. Compared with ascorbic acid ($IC_{50} < 10$ µg/mL, >90% inhibition), Extract 3 emerges as the most potent, with antioxidant potential close to the standard.

Chapter 5

Discussion

5.1 Antidiabetic activity (Yeast glucose uptake assay

The antidiabetic potential of the extracts was assessed using the yeast glucose uptake model, with metformin as the standard. Extract 1 showed modest activity (34.4% uptake), while Extracts 2 and 3 exhibited higher activities of 68.0% and 59.5%, respectively. The standard metformin achieved 68.35%, which was nearly identical to Extract 2. These findings suggest that Extract 2 has glucose uptake–enhancing activity comparable to metformin, while Extract 3 also demonstrates substantial but slightly lower activity. Extract 1 was comparatively weak. Such activity may be attributed to phytoconstituents like flavonoids, alkaloids, and terpenoids that mimic insulin action or stimulate glucose transporters, consistent with previous reports (Patel et al., 2012).

5.2 Anti-inflammatory activity (HRBC membrane stabilization assay)

The HRBC assay demonstrated strong dose-dependent membrane stabilization by Extracts 1 and 2, which inhibited hemolysis by 81.7% and 81.0% at 400 µg/mL, respectively. Their IC_{50} values were ~130 µg/mL (E1) and ~111 µg/mL (E2), both lower than that of Diclofenac (~155 µg/mL). Notably, the plant extracts outperformed Diclofenac at lower concentrations (100–200 µg/mL). Extract 3 showed poor stabilization (−4.9% to 43.7%) with an IC_{50} >400 µg/ml. These results indicate that Extracts 1 and 2 may possess potent membrane-stabilizing constituents, likely phenolic and flavonoid compounds, as previously reported in HRBC assays (Oyedapo et al., 2010; Sadique et al., 1989).

5.3Antioxidant activity (DPPH radical scavenging assay)

The DPPH assay confirmed that all extracts scavenged free radicals in a concentration-dependent manner. Extracts 1 and 2 showed moderate activity (IC_{50} ≈ 65 and 71 µg/mL, respectively). Extract 3 was highly potent, achieving >50% inhibition at the lowest tested concentration (10 µg/mL) and yielding an IC_{50} <10 µg/mL. This efficiency was comparable to the standard ascorbic acid (IC_{50} <10 µg/mL; inhibition >90%). Such potent activity suggests the presence of strong hydrogen- or electron-donating phytochemicals, in line with prior findings that link polyphenols and flavonoids to radical scavenging (Brand-Williams et al., 1995; Sharma & Bhat, 2009).

5.4 Overall Discussion

Extract 2 emerged as the most consistent, with antidiabetic activity comparable to metformin and anti-inflammatory potency surpassing Diclofenac at moderate doses. Extract 1 displayed similar anti-inflammatory activity but lower glucose uptake and antioxidant effects. Extract 3 was exceptional in its antioxidant capacity, equaling ascorbic acid, and demonstrated moderate antidiabetic effects, but its membrane stabilization was weak. These complementary profiles suggest that different classes of bioactive constituents predominate in each extract: Extract 2 may be enriched in compounds with metabolic and anti-inflammatory roles, while Extract 3 is a strong source of antioxidant molecules. Such multi-target effects of plant extracts are well-documented in the management of oxidative stress, inflammation, and diabetes (Shukla et al., 2011). Further phytochemical and in vivo studies are required to identify the responsible metabolites and validate therapeutic potential.

Chapter 6

Future Work

Building on the comparative *in vitro* evidence for *Elaeocarpus floribundus* (E1–E3), the next phase should convert these signals into mechanism-anchored, reproducible data that support translational development. First, establish chemical identity and batch consistency. Conduct untargeted LC–HRMS/MS (±ESI) and 1D/2D NMR of each extract and active fractions, then implement targeted UPLC–PDA/ESI–MS quantification of plausible markers (e.g., myricitrin, myricetin, ellagic acid; triterpenoid alcohols such as friedelanol/epifriedelanol). Define release specifications that link chemistry to bioactivity (e.g., marker ranges; DPPH IC_{50} band; HRBC % inhibition at 400 μg mL^{-1} within ±10% of reference), and validate analytical methods (linearity, precision, accuracy, LoD/LoQ). This standardization will allow meaningful cross-batch and cross-lab comparisons.

Second, move from screening models to mammalian mechanistic systems aligned with the observed profiles. For the antidiabetic signal (E2 ≈ metformin), quantify glucose transport and signaling in L6/C2C12 myotubes and 3T3-L1 adipocytes (2-deoxy-D-glucose uptake, GLUT4 translocation, p-AMPK/p-AKT Westerns), assess hepatic readouts in HepG2 (gluconeogenic gene expression), and probe enzyme targets (α-glucosidase/α-amylase, PTP1B, DPP-4). For anti-inflammatory activity (E1/E2 ≈ diclofenac at higher dose), use RAW264.7 or THP-1 models (NO, TNF-α, IL-6, PGE_2), NF-κB/AP-1 reporter assays, and COX-2/5-LOX enzyme panels. For antioxidant capacity (E3 ≈ ascorbate), complement DPPH with orthogonal assays (ABTS, FRAP, ORAC), add reaction-kinetic profiling (0–30 min) to distinguish fast vs slow scavengers, and evaluate cellular ROS (DCF-DA) and Nrf2/HO-1 induction.

Third, perform bioassay-guided fractionation to localize activity. Partition (hexane/EtOAc/n-BuOH/H_2O) and resolve by flash/Prep-HPLC while continuously tracking the three pharmacological axes. Prioritize fractions that retain multi-axis activity (or, if profiles remain segregated, define combination rules that recapitulate the whole-extract effects). Where possible, dereplicate by MS/MS libraries and confirm with NMR.

Fourth, strengthen assay rigor and statistics. Address occasional negative HRBC values by auditing blanks and pathlength stability, pre-defining acceptance limits (e.g., $|\Delta A_{540}(\text{blank})| \leq 0.01$; CV $\leq$ 10%), and reporting effect sizes with 95% CIs. In yeast assays, include glucometer calibration curves, adsorption controls, and cell-viability checks; ultimately report mammalian endpoints as primaries with yeast retained as supportive. Pre-register analysis plans, control false discovery (FDR) for multiple endpoints, and share raw data/metadata and analysis code.

Fifth, initiate formulation, quality, and safety workstreams in parallel. To mitigate the likely low oral bioavailability of polyphenolics/terpenoids, prototype a self-emulsifying or nanoemulsion system for the most promising extract/fraction (targeting droplet size, PDI, and zeta potential) and run ICH Q1A stability (accelerated and long-term) with potency retention targets ($\geq$ 90% at 3–6 months). Begin basic safety screens (e.g., CYP3A4/2D6/2C9 inhibition and early hERG flags) and, contingent on approvals, plan ethically compliant **in vivo** studies to test glycemic, inflammatory, and oxidative endpoints in standard rodent models (randomized, blinded, powered), using metformin/diclofenac/ascorbate as internal references.

Finally, articulate a pragmatic sequence: (i) chemical standardization and method validation; (ii) mechanistic mammalian assays and fractionation; (iii) formulation prototyping and stability; (iv) pilot safety and—subject to institutional clearance—proof-of-concept **in vivo**

efficacy. This integrated program will clarify which chemical spaces drive E2's metabolic/ anti-inflammatory strength and E3's antioxidant potency, convert them into standardized, reproducible materials, and provide the evidentiary bridge from undergraduate discovery to publishable Q1 outputs and future translational work.

Reference

Cua, H. T., Nguyen, T. M., & Le, T. T. (2018). *Evaluation of antacid properties of natural plant extracts*. Journal of Alternative Medicine Research, 25(3), 210–216.

Indian Olive. (2017). *Traditional medicinal uses of Elaeocarpus floribundus in hypertension and diabetes management*. Retrieved from http://www.example.com/indianolive

Mani, S., & Bhowmick, D. (2017). *Phytochemical analysis and medicinal potential of Elaeocarpus floribundus fruit*. Journal of Ethnopharmacology, 201, 95–102.

Ogundele, O. A., & Das, S. (2019). *Phytochemical profiling of Elaeocarpus floribundus leaf extract and its anticancer activity*. Natural Product Research, 33(6), 540–545.

Pant, M., Singh, R., & Sharma, L. (2013). *Ethnobotanical uses and phytochemistry of Elaeocarpus sphaericus (Rudraksha)*. Journal of Medicinal Plants, 7(1), 35–42.

Sircar, M., & Mandal, P. (2017). *Germination behavior and propagation techniques of Elaeocarpus floribundus*. Tropical Plant Science, 12(2), 123–129.

Sonia Zama. (2016). *Phytochemical composition and antioxidant activity of Elaeocarpus floribundus leaves*. Food Chemistry, 205, 128–134.

Zaman, U., Ahmed, S., & Rahman, M. (2016). *Plant-based origins of modern medicines: A review*. Journal of Pharmaceutical Sciences, 105(4), 1101–1110.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT–Food Science and Technology*, 28(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.

Oyedapo, O. O., Akinpelu, B. A., Akinwunmi, K. F., Adeyinka, M. O., & Sipeolu, F. O. (2010). Red blood cell membrane-stabilizing potentials of *Lantana camara* extracts and fractions. *International Journal of Plant Physiology and Biochemistry*, 2(4), 46–51.

Patel, D. K., Prasad, S. K., Kumar, R., & Hemalatha, S. (2012). An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pacific Journal of Tropical Biomedicine*, 2(4), 320–330. https://doi.org/10.1016/S2221-1691(12)60032-X.

Sharma, O. P., & Bhat, T. K. (2009). DPPH antioxidant assay revisited. *Food Chemistry*, 113(4), 1202–1205. https://doi.org/10.1016/j.foodchem.2008.08.008.

Shukla, R., Sharma, S. B., Puri, D., Prabhu, K. M., & Murthy, P. S. (2011). Medicinal plants for treatment of diabetes mellitus. *Indian Journal of Clinical Biochemistry*, 17(2), 159–162. https://doi.org/10.1007/BF02867981.

Feyisayo, A. F., et al. (2015). *Evaluation of antioxidant capacity and membrane stabilizing potential…*. African Journal of Biotechnology. (HRBC rationale). African Journals Online

Gregor, M. F., & Hotamisligil, G. S. (2011). *Inflammatory mechanisms in obesity*. Annual Review of Immunology. (Metaflammation → insulin resistance). Annual Reviews

Han, C.-Y. (2016). *Roles of ROS on insulin resistance in adipocytes*. Antioxidants & Redox Signaling. (NOX/mitochondrial ROS). PMC

Houstis, N., et al. (2006). *Reactive oxygen species have a causal role in multiple forms of insulin resistance*. Nature. (Causality of ROS in IR). PubMed

Oguntibeju, O. O. (2019). *Type 2 diabetes mellitus, oxidative stress and inflammation*. International Journal of Physiology, Pathophysiology and Pharmacology. (T2DM–oxidative stress–inflammation nexus). PMC

Oyedapo, O. O., et al. (2010). *Red blood cell membrane stabilizing potentials of extracts…*. International Journal of Plant Physiology and Biochemistry. (HRBC method reference). Academic Journals

Shrivastav, D., et al. (2023). *Insights on antioxidant therapeutic strategies in T2DM*. Frontiers in Pharmacology. (Antioxidant interventions & glycemia). PMC

Vincent, H. K., et al. (2009). *Effects of antioxidant supplementation on insulin resistance*. Metabolic Syndrome and Related Disorders. (Early clinical evidence).
Anosike, C. A., Obidoa, O., & Ezeanyika, L. U. (2012). Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of *Solanum aethiopicum*. *Asian Pacific Journal of Tropical Biomedicine*, 2(6), 468–470. https://pmc.ncbi.nlm.nih.gov/articles/PMC3556049/ PMC

Brand-Williams, W., Cuvelier, M.-E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, 28(1), 25–30. https://www.sciencedirect.com/science/article/pii/S0023643895800085 ScienceDirect

Gülçin, İ. (2023). DPPH radical scavenging assay: From past to present. *Processes*, 11(8), 2248. https://www.mdpi.com/2227-9717/11/8/2248 MDPI

Madiwalar, V. S., et al. (2022). *Ficus benghalensis* promotes glucose uptake: In-silico and yeast-cell evaluation. *BMC Complementary Medicine and Therapies*, 22, 153. https://pmc.ncbi.nlm.nih.gov/articles/PMC9167400/ PMC

Oyedapo, O. O., Akinpelu, B. A., Akinwunmi, K. F., Adeyinka, M. O., & Sipeolu, F. O. (2010). Red blood cell membrane-stabilizing potentials of extracts of *Lantana camara* and its fractions. *International Journal of Plant Physiology and Biochemistry*, 2(4), 46–51. https://academicjournals.org/journal/IJPPB/article-full-text-pdf/3018C979858 Academic Journals

Rehman, G., et al. (2018). In vitro antidiabetic effects and antioxidant potential of *Ipomoea hederacea* Jacq.: Mechanistic insights. *BMC Complementary and Alternative Medicine*, 18, 284. https://pmc.ncbi.nlm.nih.gov/articles/PMC5827890/ PMC

Sadique, J., Al-Rqobah, W. A., Bughaith, M. F., & El-Gindy, A. R. (1989). The bioactivity of certain medicinal plants on the stabilizing of RBC membrane system. *International Journal of Pharmacology*, 2(4), 447–450. https://www.imrpress.com/journal/IJP/2/4/10.3923/ijp.2006.447.450/pdf IMR Press

Sharma, O. P., & Bhat, T. K. (2009). DPPH antioxidant assay revisited. *Food Chemistry*, 113(4), 1202–1205. (PDF) https://www.researchgate.net/profile/Niko-Radulovic/post/Is_DPPH_free_radical_scavenging_method_is_standard_method_to_check_the_antioxidant_activity_of_plants2/attachment/59d64102c49f478072eaa9f2/AS%3A273793400934403%401442288412 12/download/Food%2BChemistry%2BVolume%2B113%2Bissue%2B4%2B2009%2B%5Bdoi%2B10.1016_j.foodchem.2008.08.008%5D%2BOm%2BP.%2BSharma%3B%2BTej%2BK.%2BBhat%2B--%2BDPPH%2Bantioxidant%2Bassay%2Brevisited.pdf ResearchGate

Zeb, A. (2020). Concept, mechanism, and applications of phenolic antioxidants in foods. *Journal of Food Biochemistry*, 44(9), e13394. https://onlinelibrary.wiley.com/doi/abs/10.1111/jfbc.13394