‘‘Phytochemical Screening and Pharmacological Studies of methanolic extract of *Flacourtia inermis* plant(leaves)”

This dissertation is presented to the Department of Pharmacy at Daffodil International University to meet the partial requirements for earning a Bachelor of Pharmacy degree.

PROJECT WORK

Submitted by

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Submission Date

APPROVAL

The project titled “Phytochemical Screening and Pharmacological Studies of methanolic extract of *Flacourtia inermis* plant (leaves)’’ has been submitted to the Department of Pharmacy at Daffodil International University. This document has been evaluated and recognized as part of the requirements for earning a bachelor’s degree in pharmacy. The research presented here is the result of my personal investigation, and I have cited the language, ideas, or writings I have referenced appropriately.

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DECLARATION

The project titled "Phytochemical Screening and Pharmacological Studies of methanolic extract of *Flacourtia inermis* plant (leaves)'' has been submitted as a partial requirement for the Bachelor of Pharmacy degree at Daffodil International University, Faculty of Health & Life Sciences. It has been approved concerning its academic format and scientific substance.

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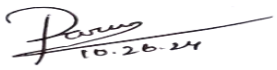

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ACKNOWLEDGMENT

I am immensely thankful to Allah, the Almighty, for granting me the strength, patience, and determination needed to successfully complete this research. Throughout this journey, my inspiration has stemmed from the limitless blessings of God. I owe a deep debt of gratitude to my parents for their steadfast emotional support, endless affection, and the countless sacrifices they have made to ensure my success. Their encouragement has always been a source of motivation for me. May Allah generously reward each of these individuals for their contributions.

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The Author

Mahmuda Akber

DEDICATION

I respectfully dedicate this work to my cherished family, whose limitless love has continuously inspired me, and to all my supporters, whose presence has been invaluable throughout this journey.

ABSTRACT

This study explores the phytochemical makeup, thrombolytic properties, and antioxidant actions of *Flacourtia inermis*, a plant known for its traditional medicinal applications. The research starts with a phytochemical analysis to identify essential bioactive compounds such as flavonoids, tannins, phenols, and alkaloids, which are recognized for their various pharmacological benefits. The thrombolytic capability was measured in vitro to evaluate the plant's effectiveness in dissolving blood clots, an important feature for promoting cardiovascular health. Moreover, antioxidant capacity was assessed through free radical scavenging tests to gauge the plant's potential to alleviate oxidative stress, a contributor to chronic diseases. Findings reveal that *Flacourtia inermis* exhibits considerable thrombolytic and antioxidant effects, likely due to its abundant phytochemical constituents, thereby reinforcing its potential in natural treatments for cardiovascular issues and conditions related to oxidative stress. This research lays the groundwork for further investigation into the therapeutic uses *of Flacourtia inermis* and its bioactive compounds within pharmacology and integrative medicine.

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

1.1.1 General Introduction

A strong connection has always existed between humans and plants. Since the dawn of civilization, medicinal plants have played a crucial role in promoting the well-being of humanity, with their use for alleviating suffering being as old as civilization itself. Despite the transformative impact of advancements in science, technology, and medical practices on healthcare, people around the globe still rely on herbal remedies for their essential health needs. Remarkably, most medications prescribed in developed countries originate from plant sources, and the characteristics of these compounds are modified to ensure cultural acceptability, compatibility with the human body, and minimal side effects. [1,2,4,5,6,7,8,9]

Plants are a crucial source of medication for both treating and preventing various diseases, playing a vital role in global health. The World Health Organization estimates that over 75% of people worldwide use botanical medications as part of their regular primary healthcare routine. Recently, there has been a renewed interest in plant-derived compounds, driven by their wide-ranging applications and the enduring belief in the healing properties of plants. For a long time, medicinal plants have provided biologically active compounds that alleviate human illnesses, serving as intermediaries in pharmaceuticals, nutraceuticals, and traditional healing practices. A particular focus has been placed on phytochemicals due to their therapeutic and preventive benefits. In ancient medical practices, medicinal plants contributed not only to the rich diversity of colors, scents, and aromas in nature but also offered remedies. The bioactive compounds found in these plants are what give them their healing potential, as they trigger physiological reactions in humans. As research into nature continues, plants remain at the forefront of drug discovery in the quest for new therapeutics.

1.1.2 Status of Medicinal Plants in Bangladesh

Bangladesh, as a sub-tropical nation, has an extensive supply of naturally available medicinal plants. In the early 1980s, botanical medicine companies in Bangladesh sourced 80 percent of their plant needs from the country's forests, with the remaining 20 percent imported. However, this dynamic has shifted, and now 80% of local demand is fulfilled through imports, while only 20% comes from domestically grown medicinal plants. According to the Bangladesh Agricultural Research Institute (BARI), the nation is home to 722 varieties of medicinal plants, which is significantly fewer than the 4,000 species available in neighboring India. In Bangladesh, producers of Ayurvedic and Unani medicines make use of 255 out of the 700 plants that are regarded for their medicinal properties.

1.1.3 Importance of Medicinal Plants

Medicinal plants are readily accessible and are well-known for their bioactive effects. Traditional medicine plays a crucial role in the healthcare frameworks of many developing nations. These plants are viewed as a significant natural asset, crucial for creating both traditional and contemporary treatments, and they provide vital healthcare services to rural communities. The pharmaceutical, cosmetic, agricultural, and food sectors have all gained from the utilization of medicinal plants. Medical research has revealed that the healing abilities of these plants arise from the active compounds they contain. In the healthcare arena, medicinal plants remain vital, offering natural and sustainable solutions to a wide range of health concerns. They deliver cost-effective healthcare alternatives, support sustainable development, and help in preserving biodiversity, especially in marginalized areas. The ongoing research into their potential in drug discovery and development highlights the importance of medicinal plants in tackling global health issues.

1.1.4 Phytochemicals

Plants contain naturally occurring compounds called phytochemicals that play a role in various biological functions, enhancing the plant's defenses. These compounds, which include flavonoids, alkaloids, terpenoids, saponins, tannins, and glycosides, are not deemed essential for human nutrition, yet they have shown potential health benefits. Phytochemicals possess a range of medicinal properties, such as antimicrobial, anti-inflammatory, anticancer, and cardioprotective effects. Because of their bioactive characteristics, phytochemicals are crucial in traditional medicine and represent a vital area of research in contemporary pharmacology, aiding in the creation of new medications and therapies. The production of phytochemicals is a result of secondary metabolism in plants.

1.1.5 Thrombolytic Activity

"Thrombolytic activity" denotes the capacity of a substance to break down or fragment thrombi, or blood clots, found in the vascular system. Thrombolytic medications activate the body's fibrinolytic system, which transform plasminogen into plasmin, an enzyme that subsequently breaks down fibrin, the main protein involved in blood clotting. This process aids in restoring normal blood flow to arteries and vessels blocked by obstructions, thereby reducing the risk of serious complications such as myocardial infarction (heart attack), stroke, and pulmonary embolism. Thrombolytic agents, such as streptokinase, alteplase, and urokinase, are commonly used to treat conditions associated with emboli formation. Recent research has shifted focus toward natural products, especially plant extracts, because of their bioactive compounds that may promote thrombolytic effects.

Evaluating thrombolytic activity can help identify new therapeutic opportunities with reduced risk of adverse effects for preventing and treating clot-related conditions by comparing plant extracts to synthetic drugs. [51,52,53,54,55,56]

The thrombolytic activity of a substance is commonly assessed through in-vitro tests, like the thrombus lysis assay. In this process, the extent of thrombus dissolution is evaluated against that of established thrombolytic drugs. When comparing plant extracts to synthetic treatments, the evaluation of thrombolytic activity may help identify potential novel therapeutics with fewer adverse effects for preventing and treating clot-related conditions. The thrombolytic activity of a substance is commonly assessed through in-vitro tests, like the thrombus lysis assay. In this process, the extent of thrombus dissolution is evaluated against that of established thrombolytic drugs.

[20,21,22,23,24,25,26,27,28,29,30,31,32,33,34]

1.1.6 Antioxidant Activity

Antioxidants are potent compounds that assist in safeguarding cells from damage triggered by free radicals—unstable molecules that can harm cells, contributing to aging and illness. Our bodies naturally generate some antioxidants, but we can also obtain them from our diet, particularly from fruits, vegetables, nuts, and grains. Well-known antioxidants comprise vitamins C and E, beta-carotene, and selenium.

By counteracting free radicals, antioxidants are crucial in alleviating oxidative stress, which has been associated with several health problems such as heart disease, cancer, and cognitive decline. While they are advantageous for maintaining health, achieving a balanced intake of antioxidants through a diverse diet is typically more effective than depending solely on supplements.

CHAPTER 2

THE STUDY OBJECTIVE

2.1.1 Purpose of Study

The aim of this study was to investigate the effects of the plant *Flacourtia inermis* by analyzing its phytochemical components and conducting targeted pharmacological evaluations. The rationale for using this plant is supported by several reasons, including:

**Analysis of Chemical Components

**This research will assess a wide range of phytochemical activities to identify the presence or absence of various phytochemicals.

**Investigation of Pharmacological Activities

**The project seeks to understand the scientific principles behind the plant's medical uses.

**Assessment of Physiological and Biochemical Changes

**The study will evaluate the potential of the extract to alleviate physiological and biochemical alterations in animal models. The primary focus of my current research was to study the effects of extracts derived from the leaves of *Flacourtia inermis*. The specific activities under investigation are:

1.Thrombolytic Activity

2. Antioxidant Activity

CHAPTER 3

LITERATURE REVIEW

3.1.1 Evaluation of Thrombolytic Activity of *Flacourtia Inermis* In-Vitro

Assessing the thrombolytic (clot-dissolving) potential of Flacourtia inermis, an ethnomedicinal species recognized for its various pharmacological effects, involves examining its ability to dissolve blood clots. Below is a standard methodology and anticipated results for evaluating in-vitro thrombolytic activity:

Methodology

Sample Preparation:

Create an extract of Flacourtia inermis using solvents like methanol, ethanol, or water. Concentrate and filter the extract before diluting it to different concentrations to evaluate dose-dependent effects.

Blood Sample Collection:

Obtain blood samples (typically from a healthy donor, with ethical approval), and allow for clot formation in a controlled laboratory setting. The blood is generally incubated at 37°C for 45 minutes to an hour to facilitate clotting.

Thrombolytic Assay:

Distribute blood clots into separate tubes, with each receiving either the *Flacourtia inermis* extract (test sample), streptokinase (the positive control), or saline (the negative control).

Introduce the test solutions (extract, streptokinase, or saline) to each tube and incubate for a designated period (commonly 90 minutes at 37°C).

Observation of Clot Lysis:

After incubation, carefully remove the liquid, leaving only the residual lysed clot.

Assess the reduction in weight or volume of each clot. The difference will indicate clot lysis, with a greater reduction in clot mass reflecting higher thrombolytic activity.

Calculation:

Determine thrombolytic activity as a percentage of clot lysis in relation to the original clot weight.

The formula to use is: {% Clot Lysis} = (Initial Clot Weight) – (Final Clot Weight)/(Initial Clot Weight) *100

Potential Outcomes:

Significant Thrombolytic Activity: Suggests that *Flacourtia inermis* has notable clot-dissolving abilities, potentially on par with established thrombolytics like streptokinase.

Moderate to Low Activity: Indicates a limited capacity for thrombolytic action, suggesting that while the extract may influence clot breakdown to some degree, it might not match the potency of conventional thrombolytic agents.

No Activity: Indicates that the plant extract does not demonstrate thrombolytic effects.

3.1.2 Evaluation of Antioxidant Activity of *Flacourtia Inermis* In-Vitro

To assess the antioxidant properties of *Flacourtia inermis* in vitro, one generally measures its capacity to counteract free radicals or inhibit oxidative harm. Below is a detailed guide on how to conduct this assessment, including commonly employed assays and possible results:

Methodology

Preparation of Plant Extract:

Collect and dry the Flacourtia inermis plant material (like leaves or fruit). Use appropriate solvents (such as methanol, ethanol, or water) to extract through methods such as maceration or Soxhlet extraction.

Filter the extract and concentrate on it. Create stock solutions in varying concentrations for dose-response evaluation.

Common Antioxidant Assays: Several in-vitro assays can be utilized to assess the antioxidant activity of Flacourtia inermis. Some frequently utilized methods include:

DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay:

The DPPH assay evaluates the capability of antioxidants to neutralize the stable DPPH radical.

Combine various concentrations of the plant extract with a DPPH solution and allow it to incubate for 30 minutes in the dark.

Using a UV-Vis spectrophotometer, measure the absorbance at 517 nm. A reduction in absorbance signifies the scavenging activity.

Determine the percentage of radical scavenging activity with the following formula: Radical Scavenging Activity (%) = (Absorbance of Control - Absorbance of Sample)/ (Absorbance of Control) * 100.

CHAPTER 4

PLANT PREVIEW

4.1.1 Plant Introduction

Flacourtia inermis, also known as Governor's Plum or Indian Plum, belongs to the Salicaceae family and is a flowering plant. This species is native to the tropical areas of Southeast Asia, commonly found in Indonesia, the Philippines, and certain regions of India*. Flacourtia inermis* is noted for its small, round fruits that turn reddish-purple when they ripen. The plant typically grows as a deciduous shrub or small tree, reaching heights of up to 15 meters, and features a visually appealing, dense crown with branches that lack thorns.

The fruits of *Flacourtia inermis* are abundant in vitamins, especially vitamin C, and possess a range of beneficial phytochemicals such as phenolic compounds, flavonoids, and antioxidants. Due to these compounds, the plant has garnered interest in the field of ethnomedicine for its traditional applications in treating various health issues, including gastrointestinal problems, inflammation, and infections. Notably, the plant's antioxidant and antibacterial properties are significant, presenting potential therapeutic advantages that are being further explored in scientific studies.

In addition to its medicinal properties, *Flacourtia inermis* holds cultural and economic importance. The fruit can be eaten fresh or incorporated into various culinary creations like jams and preserves. Furthermore, the plant serves an ecological purpose by offering habitat and nourishment for local wildlife and is utilized in agroforestry practices as a source of shade and soil enhancement.

This overview underscores *Flacourtia inermis* as a valuable candidate for research, given its abundance of phytochemicals, traditional medicinal uses, and potential applications in the food and pharmaceutical sectors.

4.1.2.1 Scientific Classification

4.1.2.2 Scientific Classification of *Flacourtia Inermis*

Kingdom: Plantae

Phylum: Tracheophy

Class: Magnoliopsida

Order: Malpighiales

Family: Salicaceae

Genus: Flacourtia

Fig-01: *Flacourtia Inermis* Plant

4.1.3 Common vernacular Names

4.1.3.1 Common vernacular Names of *Flacourtia inermis*

Governor's Plum – English

Indian Plum – English

Batoko Plum – English

Lovi-lovi – Indonesia and the Philippines

Tome-Tome – Indonesia

Bubi or Bubinga – West Africa

Pau Mulato – Brazil

Prune de Governour – French-speaking areas

Lamio – Tamil (India and Sri Lanka)

Pulung-pung – Tagalog (Philippines)

Damatad – Sinhalese (Sri Lanka)

Luk-Luki Fol – Bangladesh

4.1.4 Habitat and Distribution

Flacourtia inermis flourishes in tropical and subtropical climates, with its origins in Southeast Asia, specifically Indonesia and the Philippines. However, due to its adaptability and human introduction, it has now been widely distributed across South Asia, Africa, and certain areas of Central and South America.

This species favors warm, moist environments and is typically found in lowland tropical forests, along forest edges, and in open woodlands. It is commonly located in:

Disturbed regions such as secondary forests, where they can flourish in a variety of soil types.

Cultivated areas and gardens, as it is occasionally grown for fruit or for ornamental purposes.

Coastal and riverbank regions, where it can withstand periodic flooding and thrive in moderately saline soils.

The plant is resilient, able to grow in different soil types, from sandy to clay-rich environments. It favors well-drained soil and demonstrates moderate drought resistance once it is established.

Flacourtia inermis is native to:

Southeast Asia: Widely found in Indonesia, the Philippines, and Malaysia.

South Asia: Present in certain regions of India, Sri Lanka, and Bangladesh.

Pacific Islands: Cultivated and introduced in Fiji and other tropical islands.

Africa: Dispersed throughout various tropical regions, usually in climates similar to those of its native habitat.

Central and South America: Introduced to regions in Brazil and surrounding countries.

With its extensive distribution*, Flacourtia inermis* is well-suited to tropical agroforestry systems and is appreciated for its resilience and ecological benefits.

4.1.5 Phytochemistry and Pharmacological Potential of *Flacourtia Inermis*

Flacourtia inermis, commonly known as Governor's Plum, is a species noted for its wide range of bioactive compounds, which contribute to its traditional medicinal applications and increasing interest in pharmacological exploration. Its phytochemical composition includes various secondary metabolites, such as phenolic compounds, flavonoids, terpenoids, and saponins, that provide the plant with therapeutic properties.

Phytochemistry

The bioactive constituents found in *Flacourtia inermis* are responsible for numerous biological activities. Important phytochemicals include:

Phenolic Compounds: The leaves and fruits of *F. inermis* are abundant in phenolic acids, which possess potent antioxidant capabilities. These metabolites are essential in minimizing oxidative stress and neutralizing free radicals.

Flavonoids: The plant contains considerable amounts of flavonoids, including quercetin and kaempferol. These compounds have been found to offer protective effects against diseases related to the heart, cancer, and inflammation by modulating various cellular signaling pathways.

Tannins: Recognized for their astringent quality, the tannins in *F. inermis* enhance its antimicrobial and anti-inflammatory properties, aiding in wound healing and infection management.

Terpenoids and Saponins: Both groups of compounds exhibit promising anti-inflammatory, antimicrobial, and antidiabetic effects. In particular, terpenoids are thought to contribute to the anticancer potential of the plant.

Vitamins and Minerals: *Flacourtia inermis* fruits are notable for their high vitamin C content and essential minerals, increasing their nutritional benefits and boosting antioxidant effectiveness.

Pharmacological Potential

Thanks to its varied phytochemical makeup, *Flacourtia inermis* shows a wide range of pharmacological effects, including:

Antioxidant: The abundant phenolic and flavonoid content provides the plant with significant antioxidant capabilities, which assist in tackling oxidative stress. This effect is beneficial in protecting cells from damage related to aging and degenerative conditions.

Anti-inflammatory: Extracts derived from *F. inermis* have demonstrated promise in alleviating inflammation, likely owing to the presence of tannins, saponins, and terpenoids. This positions the plant as a candidate for developing anti-inflammatory medications, potentially aiding those with inflammatory illnesses.

Antimicrobial: The extracts from the plant have shown efficacy against various pathogens, including E. coli and Staphylococcus aureus, revealing potential uses for infection treatments.

Antidiabetic: Initial research suggests that *F. inermis* may assist in the regulation of blood sugar levels. Bioactive compounds like flavonoids and saponins may improve insulin sensitivity and facilitate glucose metabolism.

Anticancer: Some research indicates that *Flacourtia inermis* might possess anticancer properties due to its antioxidant and anti-inflammatory actions. Certain compounds in the plant, particularly terpenoids, have been linked to cytotoxic effects on specific cancer cell lines in laboratory settings.

4.1.6 Uses

1. Culinary Applications

Fruit Use: The small, plum-like fruits of Flacourtia inermis are eaten fresh, often appreciated for their sweet and tangy taste. They are also high in vitamin C, making them a nutritious addition to local diets.

Food Products: The fruits are processed into preserves, jams, jellies, and syrups, taking advantage of their natural pectin content and zesty flavor. They can also be incorporated into desserts and drinks.

2. Traditional Medicine

Digestive Support: In traditional healing practices, the fruits and leaves of Flacourtia inermis are utilized to relieve digestive problems like constipation and indigestion.

Antimicrobial Use: Extracts from the plant are applied to minor cuts, infections, and skin irritations due to their natural antimicrobial properties.

Anti-inflammatory and Pain Relief: The leaves and fruits are occasionally used to address inflammation and pain, beneficial for ailments such as arthritis and muscle soreness.

Blood Sugar Management: In some regions, Flacourtia inermis is employed to help control blood sugar levels, as it may enhance glucose metabolism.

3. Pharmacological Exploration

Flacourtia inermis has attracted interest in scientific studies for its potential in drug development. Its bioactive compounds—flavonoids, phenolics, tannins, and saponins—exhibit promise for creating antioxidants, anti-inflammatory agents, and antimicrobial drugs.

Natural Health Products: Extracts from F. inermis are being investigated for their potential in nutraceuticals and natural supplements that aim to boost immune function, lower oxidative stress, and regulate blood sugar levels.

4. Environmental and Agricultural Applications

Agroforestry: Flacourtia inermis is occasionally incorporated into agroforestry practices as a shade-giving tree, contributing to improved soil fertility and moisture retention.

Erosion Prevention: The plant's extensive root structure helps to secure the soil and can assist in preventing erosion, especially in sloped or degraded regions.

Habitat for Wildlife: The plant provides shelter and nourishment for various bird species and small animals, promoting biodiversity in areas where it is grown or found naturally.

5. Ornamental Characteristics

With its attractive, dense foliage and vibrant fruits, Flacourtia inermis is sometimes cultivated as an ornamental plant in home gardens and public areas. Its moderate size and manageable growth make it ideal for urban landscaping.

6. Timber and Wood

While not commonly used for construction, the wood from Flacourtia inermis is sometimes used for small building projects, tool handles, and firewood, particularly in rural communities where it is easily accessible.

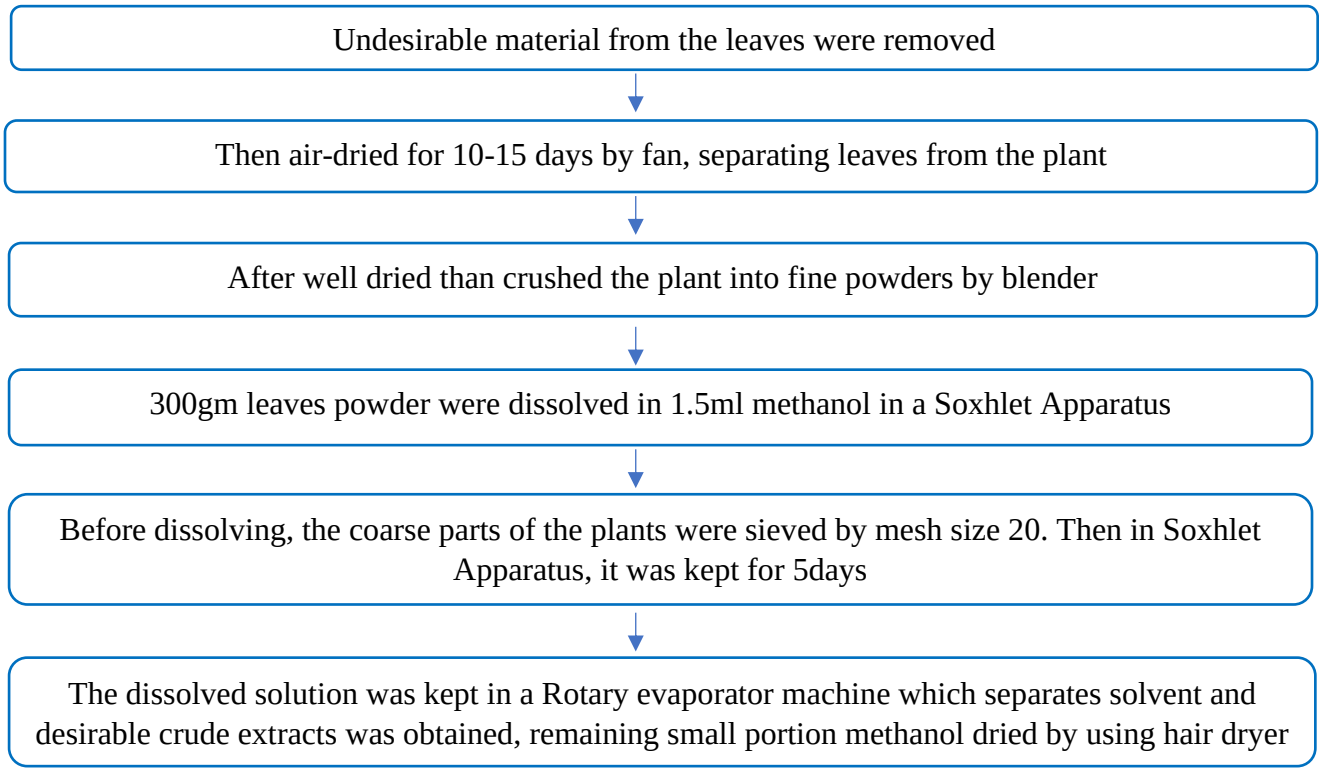
CHAPTER 5

MATERIALS AND METHOD

5.1.1 Plant Collection & Identification

The parts of the experimental plant *Flacourtia inermis (stem, bark, leaves) were* collected from the regional area of Sylhet. Those plants were collected in July 2024 in the daytime. During the plant part collection of *Flacourtia inermis*, leaves, roots, and stems, any chance of chemical degradation, hydrolysis, and adulteration was avoided.

5.1.2 Preparation of Plant for Test



5.1.3 Cotton Filtration, Rotary Evaporation, Drying

The plant material was extracted by passing it through sterilized cotton and filter paper. Initially, the powders were soaked in the appropriate solvent, then set in a funnel, following the methanolic extraction method. The resulting filtrate was gathered using glass equipment. The mixture was then filtered once more through a clean, white piece of cotton for initial filtration. It was filtered again using Whatman filter paper. The collected filtrates were stored in a glass container.

The liquid extracts were evaporated at a rotational speed of 30 rpm and at temperatures between 64 and 65 degrees Celsius with the aid of a rotary evaporator. The condensed

sample was moved to a 100 ml beaker. The beakers were wrapped in aluminum foil, and the purified extract was quickly placed under a fan. The remaining solvent in the extract was evaporated, and a hair dryer was used to eliminate excess moisture from the extract. The non-polar, dried extract is refrigerated for future use.

5.1.4 Phytochemical Screening for Identifying Different Chemical Groups

Phytochemical analysis of the plant was performed to detect the functional groups as outlined.

1. Test for Alkaloids

Mayer's test

In a test tube, 2 ml of the extract solution combined with 0.2 ml of diluted hydrochloric acid was prepared. Then, 1 ml of Mayer's reagent (Potassium Mercuric Iodide) was added. The appearance of a yellow precipitate indicates the presence of alkaloids.

Dragendroff's Test

2 ml of the extract solution and 0.2 ml of diluted hydrochloric acid were mixed in a test tube. Subsequently, 1 milliliter of Potassium Bismuth Iodide Solution, known as Dragendroff's reagent, was introduced. The formation of an orange-brown precipitate shows that alkaloids are present.

Wagner's Test

1 ml of Wagner's reagent (KI) and 2 ml of the extract solution were combined. The presence of alkaloids is suggested by the occurrence of a brown or reddish precipitate.

2. Flavonoid Test

To test flavonoids, mix 1 ml of the extract with a few drops of concentrated HCl. The immediate appearance of a red color indicates the presence of flavonoids.

3. Tannin Test

For the tannin test, combine 0.5 ml of the extract with 5 ml of distilled water in a test tube, then add 1% ferric chloride. A deep green color signifies the presence of tannins.

4. Saponin Test

Dilute 1 ml of the extract in 20 ml of distilled water and shake vigorously for 15 minutes. The formation of clear foam indicates the presence of saponins.

5. Steroid Test

Dissolve 1 ml of the aqueous solution in 2 ml of chloroform. Carefully layer concentrated sulfuric acid to create a lower layer. A reddish-brown color at the interface confirms the presence of a steroid ring.

6. Cardiac Glycosides Test

Treat 0.5 ml of the extract with 0.2 ml of glacial acetic acid, then add 3.5% ferric chloride dropwise. Finally, add 1 ml of concentrated sulfuric acid. The formation of a reddish-brown ring confirms the presence of cardiac glycosides.

7. Reducing Sugar Test

a) Benedict's Test

In a test tube, combine 0.5 ml of the plant extract with 5 ml of Benedict’s solution and boil for 5 minutes. Allow to cool afterwards.

b) Fehling’s Test

Take 2 ml of the aqueous extract in a test tube and add 1 ml of a mixture of equal volumes of Fehling’s solution A (blue copper (II) sulfate solution) and B (clear alkaline sodium potassium tartrate solution).

Results Interpretation:

Blue: reducing sugars are not present (no color change).

Yellow: low amounts of reducing sugars.

Brick-red precipitate: high amounts of reducing sugars.

8. Phenol Test

Mix 2 ml of the extract with 3 or 4 drops of ferric chloride solution. A bluish-black color indicates the presence of phenols.

5.1.5 Thrombolytic Activity Test

Working Procedure

1. Dose preparation

A 50 mg of each extract was dissolved individually in 5 ml of distilled water. For combine dose preparation 25mg taken from both extract and dissolved in 5ml. After that, all were placed in a vortex mixer and shaken to make a better solution. After 24 hours, the soluble supernatant was decanted, it was filtered through a syringe filter paper. The solution was prepared for In-vitro test.

2. Streptokinase Solution as Standard

The standard solution was available commercially 1500000 I.U. lyophilized Streptokinase alpine tube Beacon Pharmaceutical Ltd. The streptokinase vial was then filled with 5 water and thoroughly mixed. For in vitro thrombolysis, 100µl (30,000 I.U.) of this suspension was utilized.

3. Eppendorf Tube Preparation

Five tubes were washed with distilled water, dried, and sterilized and weighed those tubes then labelled with permanent marker to distinguish the distinct tubes. Then 4 ml blood was withdrawn from healthy person. Then transferred 1ml of blood to each of the Eppendorf tube previously weighed sterile Eppendorf tubes to form clot.

4. The blood tubes were incubated at 37 degrees Celsius for 45 minutes in order to facilitate the formation of clots.
5. The serum was taken out of the tube and weighed using the tube to take wight of the first clot. Weight (first clot) = first clot weight (with tube) - blank tube weight
6. Apply the usual dose of 100µl (Streptokinase) and extract 100µl, while controlling with 100µl of distilled water and again done a 90-minute clot lysis incubation at 37°C.
7. Separate the lysis blood fluid from each tube and measured the 2nd clot with tube weight.

   2nd clot weight = 2nd clot with tube weight – blank tube weight

8. At last, Lysis clot weight = weight of first clot – weight of second clot
9. Finally, clot of lysis = (lysis clot weight / clot weight before lysis) × 100%

5.1.6 Antioxidant Activity Test

Procedure

When solid test samples are used, they should be dissolved in an appropriate solvent, such as methanol or ethanol, to create a stock solution. The concentration must be suitable for the testing process.

Preparation of the DPPH solution involves adding the measured DPPH powder to a clean, dry container. Then, methanol is poured into the container, ensuring that it fully dissolves the DPPH. The DPPH solution should be stored in an amber or dark glass bottle to reduce light exposure. A 0.4mM methanol solution of DPPH is prepared. Since the solution is sensitive to light, it should be handled in a dim or dark environment.

For the working method, 10mg of the extract is used to create a 1ml solution, which is then diluted to concentrations of 500µg/ml, 100µg/ml, 50µg/ml and 25µg/ml. From each prepared test solution/sample, 2ml is taken, placed into a test tube, and mixed with 2ml of DPPH solution, allowing it to stand for 30 minutes. The reaction mixture is incubated to permit the antioxidants in the samples to interact with the DPPH radicals.

Absorbance measurements are performed at the maximum wavelength of 517 nm using a UV-Vis spectrophotometer. To eliminate any absorbance variation caused by the solvent, a blank containing the solvent and DPPH solution should be included.

To determine antioxidant activity, the percentage of inhibition against DPPH radicals for each concentration of the sample solution can be calculated using the formula:

Inhibition (%) = 1 - (Absorbance of sample ÷ Absorbance of control) × 100.

CHAPTER 6

RESULT & DISCUSSION

6.1.1 Result of Phytochemical Screening with Discussion

Phytochemical constituents	*Flacourtia inermis*
Alkaloids	+
Flavonoids	+
Tannins	-
Saponin	-
Glycosides	+
Reducing Sugar	+
Phenol	-

Table-01: Results of phytochemical screening of methanolic extract

(+) indicate presence, (-) indicate absence

6.1.2 Result of Thrombolytic Activity with Discussion

Solutions	Blank tube weight (gm)	1st clot + tube weight (gm)	1st clot weight (gm)	2nd clot + tube weight (gm)	2nd clot weight (gm)	% Of lysis
Standard (previously reported)	0.781	1.694	0.913	1.334	0.553	39.43%
Control	0.767	1.575	0.808	1.342	0.575	28.837%
Flacourtia inermis	0.840	1.471	0.88	1.350	0.0.64	27.27%

Table 02: Thrombolytic Activities on The Basis Of % Clot Lysis

Streptokinase used as the standard which was previously reported 39.43% clot lysis. The clot lysis rate in distilled water (Control) was 28.837%, methanolic extract of *Flacourtia inermis* was 27.27% in research.

The result of thrombolytic activity of methanolic extract of *Flacourtia inermis* showed partially positive result when compared to standard (SK). According to my research, all extracts showed mild thrombolytic activity.

6.1.2 Result of Antioxidant activity

Dose	Blank Absorbance (Methanol+ DPPH)	Plant Sample Absorbance (With DPPH)	Percentage of inhibition (%) Plant	Ascorbic Acid Absorbance (With DPPH)	Percentage of inhibition (%) Ascorbic Acid
500	0.233	0.032	86.27	0.011	95.28
100	0.233	0.090	61.37	0.046	80.26
50	0.233	0.119	48.93	0.090	61.37
25	0.233	0.137	41.20	0.110	57.79
10	0.233	0.169	27.47	0.127	45.49

The combined outcomes from these assays suggest that *Flacourtia inermis* has strong antioxidant capabilities, likely due to its abundant polyphenolic and flavonoid compounds. These compounds not only neutralize free radicals but also display reducing and metal-chelating properties. This varied antioxidant activity indicates that *Flacourtia inermis* may be beneficial in efforts to address oxidative stress, such as in functional foods, nutraceuticals, or as a natural preservative in cosmetics and pharmaceuticals.

The presence of various antioxidant activities also indicates the possibility that extracts from *Flacourtia inermis* could have synergistic effects with other antioxidants, making it a suitable candidate for additional in-vivo research to validate these results in living systems.

CHAPTER 7

CONCLUSION

Conclusion

The studies conducted on *Flacourtia inermis* have underscored its potential as a source of bioactive substances with notable antioxidant and thrombolytic effects. In vitro testing has revealed that extracts from *Flacourtia inermis* possess effective free radical scavenging and clot-dissolving capabilities, which are crucial for addressing oxidative stress and promoting cardiovascular wellness. These results align with the traditional medicinal applications of the plant and suggest its possible role in preventing and treating issues associated with oxidative stress, including inflammation, heart disease, and aging

The observed antioxidant properties could be linked to elevated levels of phenolic compounds, flavonoids, and various other phytochemicals found in the plant, while the thrombolytic properties indicate its potential as a natural alternative or supplement to synthetic thrombolytic drugs. Nevertheless, additional research, such as in vivo studies and clinical trials, is required to confirm these findings and evaluate safety, efficacy, and mechanisms of action in physiological settings.

To sum up, *Flacourtia inermis* stands out as a promising candidate for the development of natural therapeutic agents. Its bioactive characteristics make it an appealing target for further investigation in areas like nutraceuticals, functional foods, and pharmaceuticals that aim to enhance cardiovascular and overall health.

CHAPTER 8

REFERENCES

References

1. https://www.sciencedirect.com/science/article/abs/pii/S0963996914002051

2. https://link.springer.com/article/10.1007/s13197-015-1937-6

3. https://www.researchgate.net/profile/Srinivasan-Nagarajan-3/publication/371579587_Phytochemical_Profiling_and_Pancreatic_Lipase_Inhibitory_Activity_of_Flacourtia_inermis_Roxb_Fruits/links/648d43f78de7ed28ba30d3bb/Phytochemical-Profiling-and-Pancreatic-Lipase-Inhibitory-Activity-of-Flacourtia-inermis-Roxb-Fruits.pdf?origin=journalDetail&_tp=eyJwYWdlIjoiam91cm5hbERldGFpbCJ9

4. https://www.tandfonline.com/doi/abs/10.1080/14786419.2011.586638
5. https://www.researchgate.net/profile/Shibu-George-3/publication/205989588_Antifungal_activity_of_acetonic_extract_of_Flacourtia_inermis_against_oppertunistic_pathogenic_fungi/links/5c2dd0c3a6fdccfc707964a8/Antifungal-activity-of-acetonic-extract-of-Flacourtia-inermis-against-oppertunistic-pathogenic-fungi.pdf
6. https://en.wikipedia.org/wiki/Flacourtia_inermis#:~:text=Flacourtia%20inermis%2C%20known%20commonly%20as,lovi%2C%20and%20lobi%2Dlobi.
7. https://www.nparks.gov.sg/florafaunaweb/flora/2/9/2920

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