Phytochemical and biological analysis of methanol extract of *Barringtonia acutangula* leaf

DISSERTION

Part of the prerequisites for a Bachelor of Pharmacy (B. Pharm) degree was fulfilled by sending a research paper to the Department of Pharmacy at Daffodil International University.

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The Department of Pharmacy, Faculty of Allied Health Sciences, DIU formally acknowledged this study, " Phytochemical and biological analysis of *Barringtonia acutangula* leaf extract in methanol," as a partial accomplishment of the requirements for the (B. Pharm) degree.

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Acknowledgement

First and foremost, I want to thank Allah for providing me with this opportunity and keeping me well. I also want to thank my family for their care and good will toward me.

My research supervisor at Daffodil International University, Shadhan Kumar Mondal, is a lecturer, and I am appreciative to him for his guidance.

I also want to express my gratitude to Professor Dr. Muniruddin Ahmed, Head of the Pharmacy Department at Daffodil International University, for giving me access to the materials I just needed to build in order to do my research.

I want to express my gratitude to everyone who has helped with our endeavor, whether in person or through their contributions.

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Declaration

I'm Khodaza Nusrat Nova, thus declare that, I am really completing the requirements for the Bachelor of Pharmacy degree. The research project findings have not been distributed to any other educational institution or group with the intention of awarding degrees.

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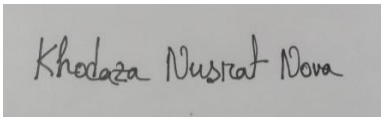

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Certificate

This attestation ensures that the research results applied to this project are original and haven't been fully submitted for consideration toward another degree or certificate that this university accepts. The whole of this paper has been turned in as a project in order to substantially fulfill the requirements for the Bachelor of Pharmacy program.

Shadhan Kumar Mondal

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DEDICATION

Dedicated to my parents, supervisor, loyal friends and all of my well-wishers.

Abstract

Barringtonia acutangula, a member of the Lecythidaceae family, is well-known in many cultures for its traditional medicinal uses. This study attempted to undertake a comprehensive phytochemical analysis in addition to examining the biological activity of an extract from the leaves of *Barringtonia acutangula* both in vitro and in vivo. The traditional medication Loperamide is being contrasted with the anti-diarrheal action. Loperamide considerably reduced the outcome, resulting in a 70.59% decrease. Similarly, at dosages of 200 mg/kg and 400 mg/kg respectively, decreases of 35.29% and 52.94% were seen in response to the extract. Additionally, the proportion of stool output was reduced by the extract dosages. In the thrombolytic assay, the methanol extract of *Barringtonia acutangula* leaves showed an activity level of 58%. And in the anti-oxidant assay, IC50 value for methanol extract of *Barringtonia Acutangula's* leaves at different concentration is 17.546. Therefore, it seems sense to assume that the leaves of *Barringtonia acutangula* have potent thrombolytic, antioxidant, and antidiarrheal properties.

Keywords: *Barringtonia acutangula,* antidiarrheal, anti-oxidant and thrombolytic activities.

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Chapter 1

INTRODUCTION

1.1) General Information:

Barringtonia acutangula L. a prominent medicinal plant with broad-spectrum therapeutic qualities, belongs to the Lecythidaceae family.(Kaur et al., n.d.-a) In Bangladesh, the plant is referred to locally as Hijal and is also known as Indian Oak.

It is commonly found growing on the banks of rivers, ponds, lakes, and low-lying places in the tropical regions of Southeast Asia, Australia, and Africa.(Premila, 2006) Simple, alternating leaves, long pendulous racemes, dark crimson blooms, and ellipsoid to ovoid berries with one ovoid black seed are all features of this evergreen tree.(Padmavathi et al., n.d.) Historically, *Barringtonia acutangula* has been used in folk medicine to treat a wide range of illnesses, including cough, hemiplegia, joint pain, spleenic disorders, stomach disorders, poisoning, anthelmintic, dyspnea, leprosy, periodic fever, eye diseases, and diarrhea .(Yoganarasimhan, S. N. (2000). Medicinal Plants of... - Google Scholar, n.d.) The flavonols in the leaves included quinones, gossypetin, and 3′, 4′-diOMe quercetin.(Daniel & Robin, 2011) As part of the bio reduction process for green synthesis, *B. acutangula* leaf extract was also employed as a reducing agent to create silver nanoparticles.(Bharitkar et al., 2023)

1.2) Justification and goal of the project:

Since the dawn of time, human cultures have maintained a strong relationship with their surroundings and have utilized environmental resources to produce food and medicine.(Jamshidi-Kia et al., 2018) Plants with medicinal properties continue to be an essential part of human beings' therapeutics. For their simplest medical needs, up to 80% of people in poor nations are completely dependent on plants. Furthermore, more than 25% of prescription medications in industrialized nations originate directly or indirectly from plants, even with the notable advancements in synthetic organic chemistry throughout the 20th century.(Hostettmann & Marston, 2002) Several sciences, including medical herbalism, have arisen as a result of the abundance of plant species having therapeutic qualities. In certain nations, this has enabled the widespread advancement of traditional medicine. Every medicinal plant has a historic usage that varies depending on the social group. (Phytomedicine & 2021, n.d.)

Traditional techniques are still widely used by Bangladesh's rural people to treat conditions including dysentery, jaundice, fever, coughing, and headaches etc. The significant of them is *Barrringtonia acutangular*. Historically, a variety of *Barringtonia acutangula* components, including leaves, fruit, roots, and axillary buds, have been used to cure a variety of conditions, including diabetes, liver, spleen, eye disorders, stomach disturbances, blood impurities, colds etc.(Kaur et al., n.d.-b)

Its leaves are well-known for their therapeutic qualities and have been utilized for this purpose in many cultures for a long time. They include bioactive substances that have demonstrated promise

in the treatment of a range of illnesses. The objective of conducting a phytochemical investigation, as well as in vivo and in vitro studies of the leaves extract from *Barringtonia acutangula*, can include a range of scientific goals and practical applications. Here are few possible objectives for doing such a study: I investigated functional group, antidiarrheal, thrombolytic, and antioxidant activities.

1.3) An image of plant:

Fig. 1: Plant

1.4) Review of plant

1.4.1) Introduction of plant

Fig. 2: plant (*Barringtonia Acutangula)*

Scientific Name: *Barringtonia Acutangula*

English Name: Indian Oak, Freshwater Mangrove

Bengali Name: Hijjala

Available locations: Afghanistan, Assam, Bangladesh, Borneo, Cambodia, India, Jawa, Laos, Lesser Sunda Is., Malaya, Myanmar, New Guinea, Pakistan, Philippines, Queensland, Sri Lanka, Sulawesi, Sumatera, Thailand, Vietnam.

1.4.2) Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Ericales

Family: Leythidaceae

Genus: *Barringtonia*

Species: *acutangular*

(Kaur et al., n.d.-c)

1.4.3) Plant portion utilized in this research:

- Leaves

.

1.4.4) The biological functions of the leaves of Barringtonia acutangula:

There are several biological roles that the leaves of *Barringtonia acutangula* are known perform. Potential therapeutic applications are: anti-inflammatory and antioxidant qualities, antitumour activity, anthelmintic activity, antimicrobial activity, antidiarrheal activity, hepatoprotective effects, antifungal activity and antibacterial activity etc. The leaves have bioactive substances that improve general health or assist certain physiological processes, among other positive impacts for human health. Phenolic chemicals are present in good amounts in *the Barringtonia acutangula* leaf extracts.(Daduang et al., n.d.) Terpenes, flavonoids, carbohydrates, tannins, steroids, and glycosides were found in the leaves according to phytochemical analyse.

The leaves of *Barringtonia acutangula* have potential biological functions that beneficial for cosmetics purposes. In cosmetics, the leaves contain compounds with antioxidant properties that help protect the skin from damage caused by free radicals. They also have anti-inflammatory effects that useful in skincare products for soothing and calming the skin.

Chapter 2

LITERATURE REVIEW

(A)

Title: Antimicrobial Investigation of Leaves of *Barringtonia acutangula* Linn.

Author: Satyaranjan Mishra, Sabuj Sahoo, Sagar Kumar Mishra, Kedar Kumar Rout,

Sukant Kumar Nayak, Nabin Kumar Dhal

Abstract:

The leaves of *Barringtonia acutangula* Linn., a member of the Barringtoniaceae family and popularly known as hinjal, were extracted in methanol and water, and their antibacterial activity was assessed against a variety of pathogenic fungi, fish-virulent pathogens, and organisms that cause infections in the gastrointestinal tract and urinary tract. The methanolic extract was analyzed using high performance thin layer chromatography (HPTLC), revealing strong antibacterial activity. For the methanolic extract chromatography, an acetone-hexane (4:6) solvent system was used. Three distinct wave lengths—254, 366, and 650 nm—were used to produce the HPTLC fingerprinting patterns. Using a disc diffusion assay technique at a concentration of 1000 µg/ml, antimicrobial activity was tested against certain strains of bacteria and fungi, and the minimum inhibitory concentration values were determined using a two-fold serial dilution approach within a concentration range of 7.8-1000 µg/ml. For the air-dried plant leaves, the yields (%) of methanolic and aqueous extracts were determined to be 12.6 and 16.1% (w/w), respectively. The extract exhibited strong antimicrobial activity against strains of bacteria and yeast, including Enterococcus faecalis (22.9 ± 0.23 mm, MIC 31.25 µg/ml), Aspergillus niger (27.2 ± 0.15 mm, MIC 62.5 µg/ml), and Candida albicans (23.3 ± 0.87 mm, MIC 31.25 µg/ml), according to an antimicrobial screening.

(B)

Title:

Antinociceptive, antidiarrheal, and neuropharmacological activities of *Barringtonia acutangula*

Author: Mohammad Zafar Imam, Shamima Sultana & Saleha Akter

Context: Folk medicine has utilized *Barringtonia acutangula* (L.) Gaertn. (Lecythidaceae) to treat arthralgia, dysmenorrhea, inflammation, hemorrhoids, diarrhea, and psychiatric disturbances.

Objective: to examine in mice the neuropharmacological, antinociceptive, and antidiarrheal effects of *B. acutangula* leaves and seeds methanol extract.

Materials and methods: The antinociceptive activity of the extracts (200 and 400 mg/kg; p.o.) was assessed using acetic acid-induced writhing, hot plate, and tail immersion models; the antidiarrheal activity was assessed using castor oil and magnesium sulphate-induced diarrheal models, and the neuropharmacological activity was assessed using hole cross and open field models.

Result: In acetic acid and heat-induced pain models, both extracts demonstrated a substantial antinociceptive effect ($p < 0.001$) in a dose-dependent manner. In the hot plate and tail immersion tests, the extracts lengthened the time that the subjects were late to the heat stimuli. In all diarrheal animals, the extracts also significantly reduced defecation ($p < 0.001$, 0.01). Once more, the extracts reduced the spontaneous motor activity in the hole cross and open field tests ($p < 0.001$).

3.1) Gathering of plant specimens:

I gathered the plant portion from my Bangladeshi hometown, Bikrampur.

3.2) Drying and Grinding:

The gathered plant was isolated from unwanted substances, plants, or plant pieces. For a week, they dried in the shadow of the sun. Using an appropriate grinder, the plant components were reduced to a coarse powder. Until analysis started, the powder was maintained in an airtight container in a cold, dry, and dark environment.

3.3) Extraction:

Two liters of methanol were added to a clean, flat-bottomed jar container containing about 500 grams of the powdered substance. Aluminum foil was used to seal the container containing the contents, and it was left for 15 days with periodic shaking and stirring. For better extraction, the pot was shaken about two or three times a day.

3.4) Filtration:

After the plant part of leaves extract was extracted, filter paper was used filter the mixture. The filter paper was placed inside a funnel. The filter was collected in a glass jar. The refiltration process was repeated.

Fig. 3: Filtration (DIU lab)

3.5) Evaporation:

A rotating evaporator operating between 60 and 65 degrees Celsius was used to evaporate the fluid concentration. The concentrated sample was moved into a 100-milliliter container. After the purified concentrate was collected, any residual water in the measuring glasses was removed by covering them with aluminum paper and placing them under regular air. After the purest concentration was collected to remove any remaining amounts of water, the measuring glasses were wrapped in aluminum paper and put in regular air.

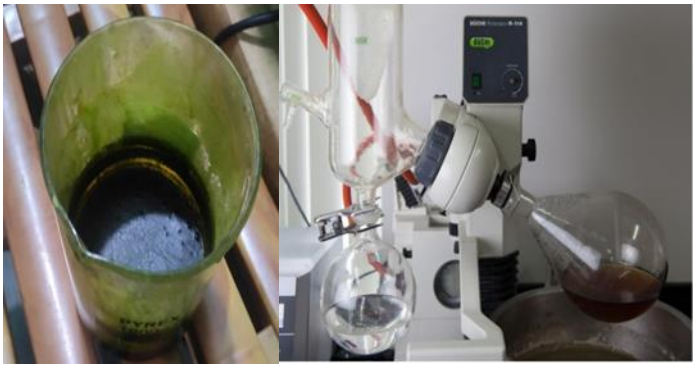
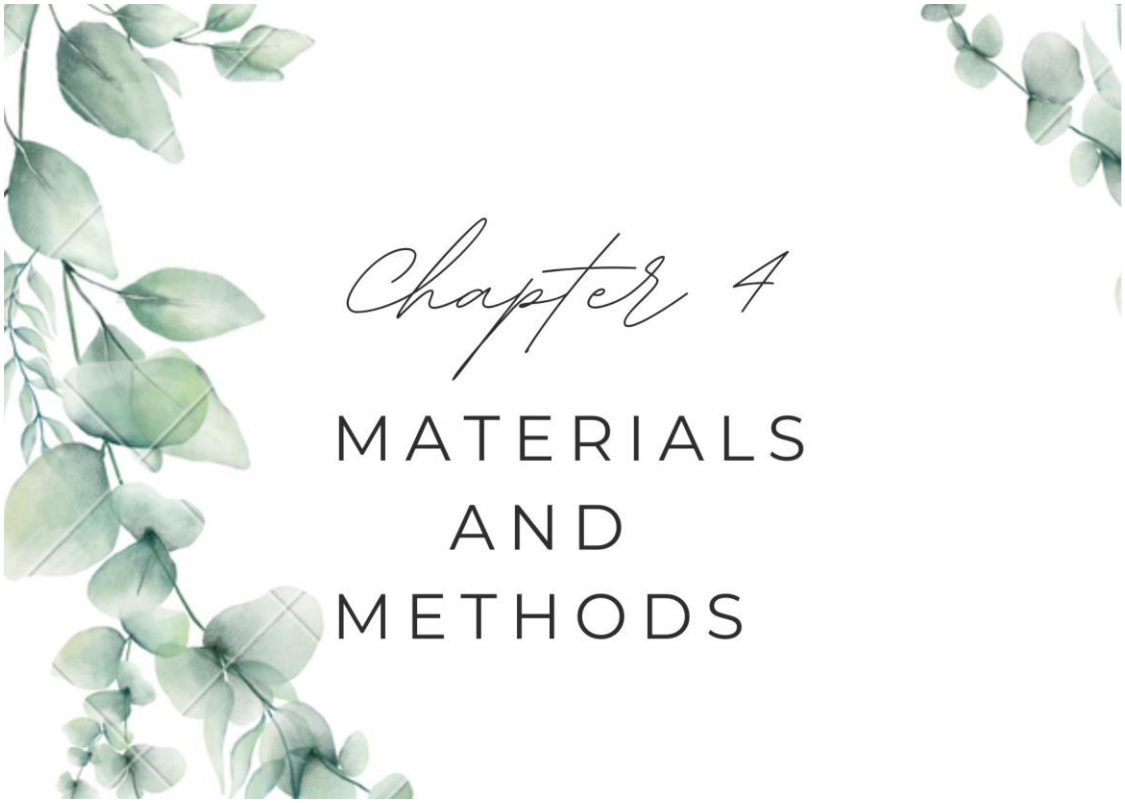


Fig.4: Rotary Evaporator (DIU Lab)

3.6) Methods of Phytochemical Screening:

A minuscule percentage of plants possessing medicinal qualities have been investigated, despite plants being the source of about 50% of pharmaceuticals worldwide.

A medicinal plant's therapeutic efficacy is determined by its phytoconstituents, either separately or in combination. A variety of biological actions are exhibited by significant phytochemicals, including as alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, and terpenes. Phytochemical identification allows for the prediction of a plant's pharmacological action. While several contemporary methods are being used to determine phytochemicals, traditional qualitative analyses are still widely used for plants' first phytochemical screening.(Shaikh et al., n.d.)



4.1. Testing materials:

Extraction of leaves of *Barringtonia acutangula*

4.2. Reagents used in chemical tests:

1. Mayer's reagent
2. Dragendroff"s reagent
3. Hydrochloric acid
4. Sulfuric acid
5. Sodium hydroxide
6. Ferric chloride
7. Benedict's reagent
8. Molish Reagent
9. Distilled water

4.3. Apparatus:

1. Beaker
2. Electric Balance
3. Test tubes and stands
4. Burner
5. Pipettes
6. Volumetric flask
7. Marker
8. Measuring cylinder

4.4. Testing for Alkaloids:

Mayer's test

To a test tube was added 2 milliliters of the extract solution and 0.2 milliliters of diluted hydrochloric acid. Afterwards, 1 milliliter of potassium mercury iodide, or Mayer's reagent, was given in. The precipitate that forms is yellow and presents the alkaloids.

Fig 5: Mayer's Test Result (DIU Lab)

Dragendroff's Test

A test tube was filled with 0.2 ml of diluted hydrochloric acid and 2 ml of the extracted solution. After that, 1 milliliter of the Po-Bismuth-Iodide Solution, or Dragendroff's reagent, was applied. The precipitate that forms is orange brown and presents the alkaloids.

Fig.6: Dragendroff's Test Result (DIU lab)

4.5. Testing for Saponin:

Twenty milliliters of distilled water were added to one milliliter of extract solution. After that, given it a hard shook for 15 minutes, during which time explicit foam will form, showing an existence of saponin. Saponin was detected when a centimeter-thick layer of foam developed.

Fig.7: Saponins Test Result (DIU lab)

4.6. Testing for Steroids:

After dissolving 10 mg of extract in 1 ml of chloroform and adding 1 ml of sulfuric acid, the result revealed A reddish-brown coloration on the chloroform layer indicates the presence of steroids.

Fig.8: Steroids Test Result (DIU Lab)

4.7. Testing for Glycosides:

One milliliter of water was mixed with a tiny quantity of an alcoholic extract. A few drops of aqueous NaOH were added, and the presence of glycoside will shown by the yellow hue of the drops.

4.8. Testing for Flavonoids:

To one milliliter of extract, a few drops of intense hydrochloric acid were applied. The existence of flavonoids had been detected by the rapid production of red hue.

Fig.9: Flavonoids Test Result (DIU Lab)

4.9. Testing for Tannins:

5 milliliter extract solution, was mixed with 1 milliliter of a 5% ferric chloride solution. The presence of tannin had observed by the greenish-black precipitate that has been demonstrated.

Fig.10: Tannins Test Result (DIU lab)

4.10. Testing for Gums & Carbohydrate:

5 ml concentration of the extract was mixed with sulfuric acid and Molisch reagent. The reddish-violet circle that was forms when the two liquids come together indicates the existence of gums and carbohydrate.

Fig. 11: Gums Test Result (DIU lab)

4.11. Animal used in experiments:

In the biological investigation, mice weighing 24–27 grams were used. The source of the sample was Jahangirnagar University. The animals were kept in a typical laboratory setting at a temperature of 25 degrees, with distilled water and standard food.

Fig 12: Animal used in experiments

4.12. Testing of Thrombolytic actions:

The capacity of some chemicals to break up blood clots, or thrombi, is referred to as thrombolytic activity. In order to restore blood flow in obstructed blood arteries, thrombolytic medicines function by dissolving the fibrin mesh that binds the blood clot together. Deep vein thrombosis, heart attacks, and strokes are among the ailments for which these drugs are often utilized. In order to rapidly dissolve blood clots and stop more harm to critical organs, thrombolytic treatment is frequently used in emergency scenarios, such as when a patient is having a heart attack or stroke. Tissue plasminogen activator (tPA), streptokinase, urokinase, and Tenecteplase are common thrombolytic drugs used in medical contexts.

One kind of thrombolytic drug used in medical settings to break up blood clots is streptokinase. It comes from the Streptococcus bacterium and acts by causing the body's plasminogen to become active. This plasminogen then breaks down fibrin, the protein that causes blood clots. This procedure can be life-saving in situations like deep vein thrombosis, heart attacks, and

strokes by helping to restore blood flow in clogged blood veins. Intravenous streptokinase is usually injected in a hospital environment under the guidance of medical specialists.

Current investigations are being conducted to determine whether natural materials, including certain plant extracts or enzymes, have the ability to cause thrombolysis.

4.12.1. Principle:

To evaluate the extract's thrombolytic activity, specimens of blood, streptokinase, and extract gather were employed. The research procedure was authorized by the ethics council of the pharmacy department of Daffodil International University in Dhaka, Bangladesh.

% of lysis- (wt.of lysis clot / frist clot wt.)*100

4.12.2. Apparatus:

1. Blood sample
2. Streptokinase
3. Extract
4. Microcentrifuge tubes
5. Incubator
6. Syringe

4.12.3. Standard:

5 ml of sterilized distilled water was inserted to a publicly accessible lyophilized Streptokinase vial containing 15,00,000 IU, and the mixture was thoroughly mixed. For in vitro thrombolytic research, an amount of 100 microliters (30,000 IU) of this solution was utilized.

4.12.4. Preparation of sample:

Extract Dose: 1000 µg in100 µl

Extract Concentration: Stock solution=100mg/10ml

In other words, 100 milligrams of extract diluted in 10 milliliters of distilled water

Alternatively, 100000 µg of extract dissolved in 10,000 µl of distilled water.

4.12.5. Preparation of blood sample:

Fig 13: Blood collection

Blood samples was collected from me that I had never used an anticoagulant drug or oral contraception before. To form clots, 3 ml of blood was added to the tubes of a microcentrifuge that were already weighted.

4.12.6. Procedure:

1) Calculated the weighted of the 3-blank tube before anything else
2) 1 ml blood from the collected blood is taken in each test tube.
3) I then waited 45 minutes at 37°C to allow a clot to develop.
4) I separated the serum from the blood and the first clot was weight with tube.
5) First clot weight = first clot with tube weight – blank tube weight
6) Then I took a standard of 100 µl (30000 µl) and a controlled of 100 µl. Of pure water and an extract of 100 µl (1000g/100 µl).
7) Waited for clot lysis for ninety minutes at 37 °C incubation.
8) Removed the blood fluid for lysis from the tube.
9) 2nd clot was weight with tube.
10) 2nd clot weight = 2nd clot with tube weight – blank tube weight

11) And weight of Lysis clot = 1st clot weight – 2nd clot weight

12) Finally,

% clot lysis = (Weight of the lysis clot / Weight of clot before lysis) × 100.

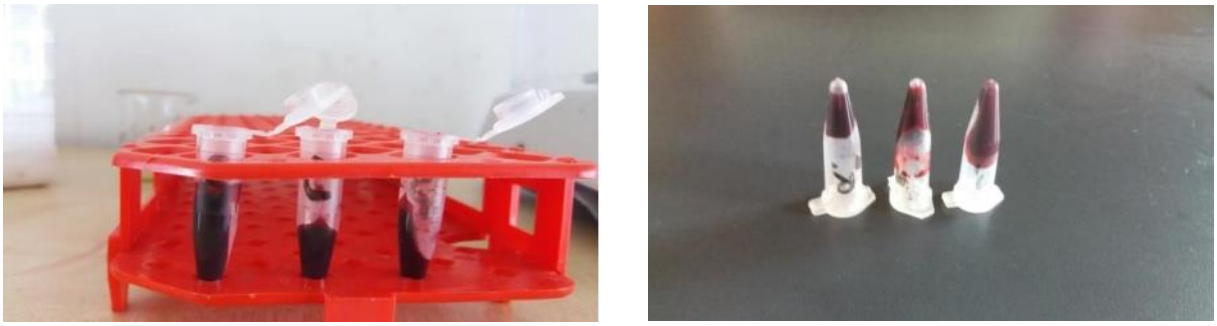


Fig. 14: First and final clot of Blood (DIU lab)

4.13. Testing of Anti -Diarrheal actions:

The term "antidiarrheal activity" describes a substance's capacity to lessen or eliminate diarrheal symptoms. Frequent, loose, or watery bowel movements are the hallmark of diarrhea, a common illness that is also frequently accompanied by bloating, cramping in the abdomen, and dehydration. The way antidiarrheal medication's function is by going after the root causes of diarrhea, which might be infection, inflammation, or increased intestinal motility. There are several kinds of antidiarrheal drugs on the market, such as:

4.13.1. Loperamide: An over-the-counter drug, loperamide acts by slowing down intestinal motility, which promotes increased water absorption and harder stools.

Bismuth subsalicylate: This ingredient, which may be found in Pepto-Bismol and other medicines, has antibacterial and anti-inflammatory qualities that can lessen the symptoms of diarrhea.

4.13.2. Experimental Animal:

Two-month-old mice weighing 24 grams on average were used in the experiment. The test animals were divided into three groups, each consisting of two mice, and were assigned at random. The experimental groups were labeled and accurately weighted. Group 1 or the control was given distilled water. Group 2 was administered an oral suspension of loperamide, the standard anti motility drug, at a dosage of five times their body weight. Plant extract suspension

in the amounts of 250 and 1000 body weight was administered to test group three. After receiving 0.2ml of castor oil, only the mice exhibiting diarrhea were chosen for the trial.

4.13.3. Apparatus:

1. Loperamide
2. Saline
3. Distilled water
4. Castor oil
5. Syringe

4.13.4. Procedure:

Using a feeding needle, test samples, control, and loperamide were administered orally. Forty minutes before the mice were given 0.3 ml of castor oil orally, the animals were fed the samples, control, and loperamide. Throughout the course of a four-hour research following the administration of castor oil, individual animals from each group were housed in cages with absorbent paper below, and every hour they were checked for signs of diarrhea. Over the course of the four hours, the number of feces or any other fluid substance that discolored the adsorbent paper was tallied and recorded for each mouse. Each mice's latent period was also taken into account. Every hour, fresh papers were set aside for the previously used ones. The total amount of feces including diarrheic feces excreted by the animals was counted over a four-hour observation period. Normal stool received a score of 1, while watery stool received a score of 2. The scores were based on the consistency of the feces.

Fig15: Procedure of anti-diarrheal method

4.14. Testing of Antioxidant actions:

Free radical scavenging activity was used to evaluate the antioxidant activity of different extracts; antioxidants that scavenge free radicals are known to be important in the protection of diseases caused by free radicals. The antioxidant qualities of compounds originating from plants are gaining attention because they may be important for both health and illness and because they are prevalent in diet.

4.14.1. DPPH technique for evaluating antioxidants:

A series of dilutions were carried out from the plant extract stock solution in methanol to get concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.95 g/ml. To 3 ml of a 0.004% methanol DPPH solution, diluted solutions (2 ml) were added, mixed, and allowed to stand for 30 minutes to allow the reaction to occur. The absorbance was measured at 517 nm, and these findings were used to calculate the percentage of inhibition. Following a plot of the percent inhibitions against log concentration, the IC50 was calculated using the graph. The average absorption for each concentration was noted after the experiment was conducted three times.

Fig16: Procedure of anti-oxidant method

4.14.2. Test materials:

Barringtonia acutangular's leaves extract.

4.14.3. Apparatus:

1. Test tubes and stands
2. Beakers
3. Pipettes
4. Volumetric flasks
5. UV spectrophotometer
6. Electric Balance
7. Spatula
8. Foil Paper
9. Funnel
10. Tissue paper
11. Marker

4.14.4. Reagents:

1. Methanol
2. 0.004% DPPH (1, 1 - diphenyl -2 – picrylhydrazyl – hydrate)

4.14.5. Preparation 0.004% DPPH solution:

A solution containing 0.004% DPPH was created by measuring and dissolving 4 milligram of DPPH in 100 ml of methanol.

4.14.6. Procedure:

1.Initially, 10 volumetric flasks are used to create 10 distinct concentration kinds.

2.10 volumetric flasks are used for the serial dilution of the extract and are labeled accordingly.

3.Three milliliters of the 0.004% DPPH solution and one milliliter of each concentration sample are pipetted into each of the 10 tubes.

4.After that, each test tube is wrapped in foil paper and the solution is left in a dark area for half an hour.

5. To make the blank solution, 3 milliliters of 0.004% DPPH and 1 milliliter of methanol are placed in another test tube.

6. The next step is to calculate absorption using UV spectroscopy.

7. % Inhibition = (blank absorbance – solution absorbance)/blank absorbance*100.

Chapter 5

RESULT & DISCUSSION

5.1. Phytochemical outcomes:

The subsequent outcomes of a phytochemical analysis of the methanol extract of *Barringtonia acutangula's* leaves were found.

Name of Test	Result
Alkaloids	+
Saponins	+
Steroids	+
Glycosides	+
Flavonoids	+
Tannins	+
Gums and Carbohydrate	+

Table 1: Results of a phytochemical test on leaves extracted from *Barringtonia acutangula*.

Table 1: + denotes the tested group's existence, while - denotes its absence. The studies show that the methanol extract of *Barringtonia acutangula* leaves contains alkaloids, saponins, steroids, flavonoids, tannins, gums and carbohydrates, glycosides.

5.2. Outcomes of Thrombolytic activity:

Streptokinase, water and extract for clot lysis.

Solution	Blank Eppendorf Tube	1st Clot + Blank Eppendorf Tube	1st Clot Weight	2nd Clot + Blank Eppendorf Tube	2nd Clot Weight	Lysis Weight	% of Lysis
Standard	0.81	1.70	0.89	0.97	0.16	0.73	82%
Control	0.80	1.82	1.02	1.67	0.87	0.15	14%
Sample	0.78	1.50	0.72	1.08	0.3	0.42	58%

Table 2: Result of thrombolytic activity test.

Streptokinase had a clot lysis rate of 82% (standard group), according to the data. The research discovered a statistically significant difference in the percentages of clot lysis between the positive and negative control groups, with the negative control group showing an insignificant 14% (control group) dissolution. In this experiment, the methanol extract of *Barringtonia acutangula* leaves showed an activity level of 58% (sample group).

5.3. Outcomes of Anti-Diarrheal activity:

Code no.	Mice no.	No. of diarrheal feces				total no of feces	Average	% Reductio
		1 hour	2 hour	3 hour	4 hour			
CTL	1	3	3	1	0	7	5.67	
	2	0	2	1	0	3		
	3	2	2	0	3	7		
STD	1	0	0	0	1	1	1.67	70.59
	2	0	2	0	0	2		
	3	0	1	1	0	2		
Extract (200mg/ kg)	1	2	1	0	1	4	3.67	35.29
	2	0	3	0	0	3		
	3	1	0	2	1	4		
Extract (400mg/ kg)	1	0	0	1	0	1	2.67	52.94
	2	1	0	2	1	4		
	3	1	1	1	0	3		

Table 3: Result of Anti-diarrheal activity test.

The control group experienced diarrhea for the next four hours after the mice were given castor oil. Loperamide considerably improved the condition; in fact, it reduced it by 70.59%. As shown in the Table, the extract likewise had an impact, resulting in decreases of 35.29% at a dose of 200 mg/kg and 52.94% at a dosage of 400 mg/kg. When the extract was given orally to mice, it considerably postponed the beginning of diarrhea, reduced the number of bowel movements, and produced fewer wet stools than the control group.

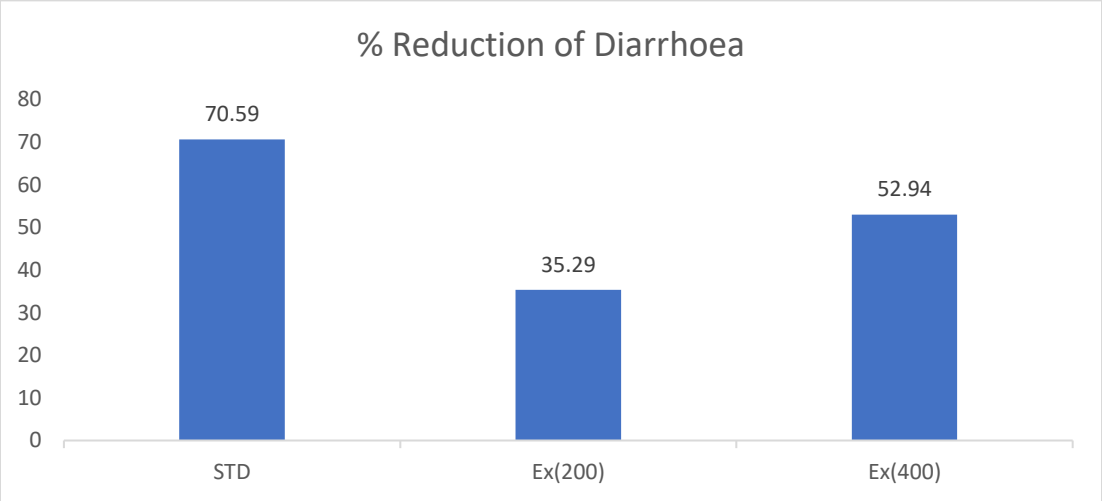


Fig 17: Graphical representation of Anti-diarrheal activity test

5.4. Outcomes of Anti-oxidant activity:

A complete evaluation of the antioxidant activity of different concentrations of *Barringtonia acutangula* leaf extracts is presented in tables 4 and 5, accordingly for BHT and methanol.

Concentration (µg/ml)	% Of Inhibition	IC50 (µg/ml)
1000	90.49	8.261
500	85.71	
250	83.69	
125	77.92	
62.5	70.76	
31.25	62.73	
15.62	54.97	
7.81	46.46	
3.9	40.91	
1.95	38.91	

Table 4: IC50 value for standard (BHT) at different concentration.

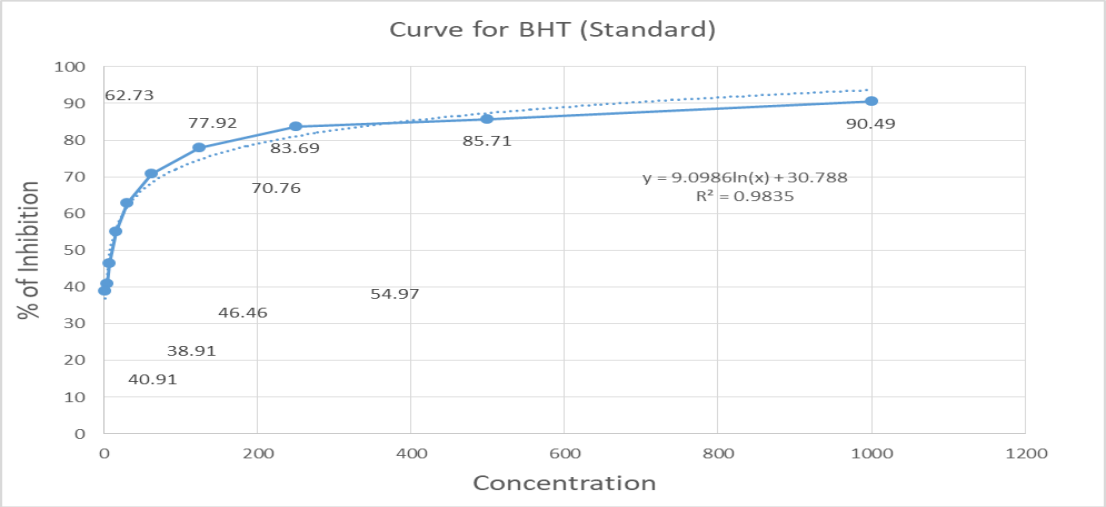


Fig 18: Curve for standard (BHT) at different concentration.

Concentration (µg/ml)	% Of Inhibition	IC50 (µg/ml)
1000	75.22	17.546
500	72.9	
250	70.34	
125	66.82	
62.5	56.78	
31.25	51.42	
15.62	46.31	
7.81	43.67	
3.9	40.72	
1.95	37.54	

Table 5: IC50 value for methanol extract of *Barringtonia Acutangula*'s leaves at different concentration.

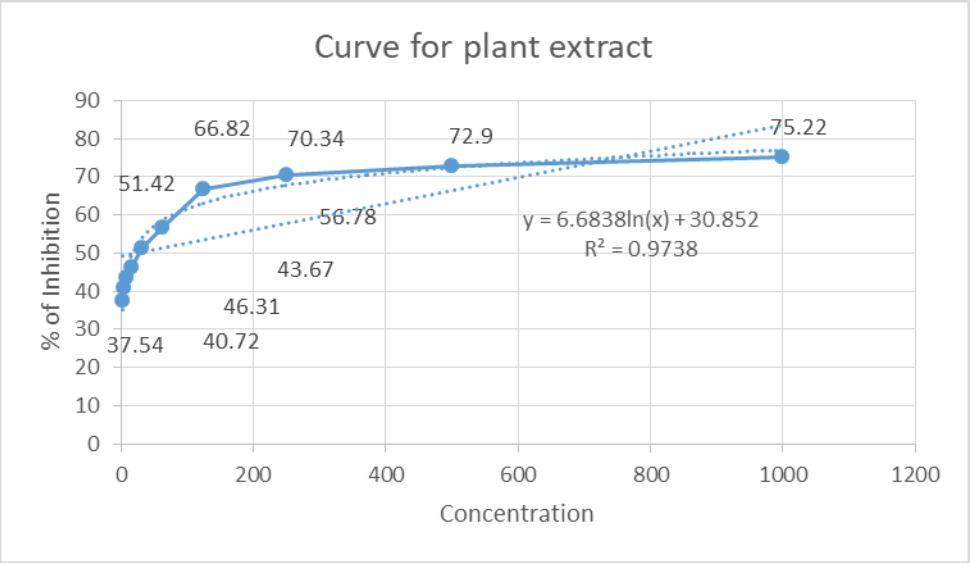


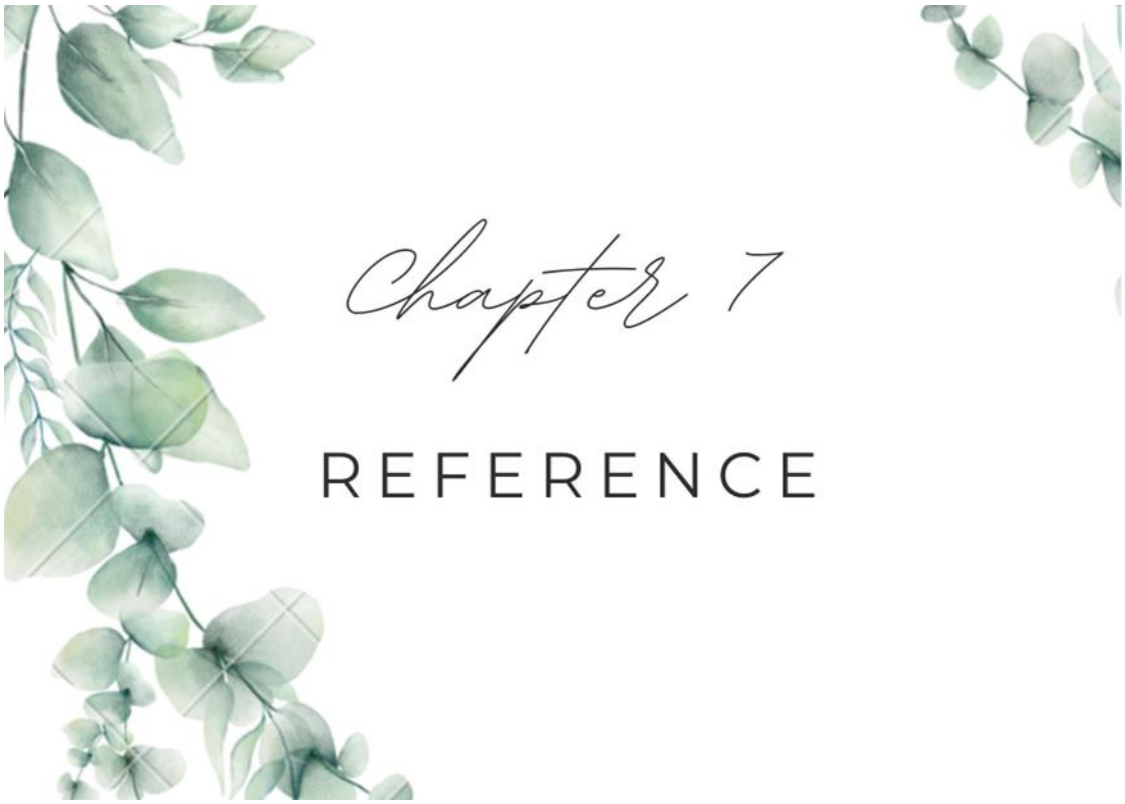
Fig 19: Curve for methanol extract of *Barringtonia Acutangula*'s leaves at different concentration.

Here, IC50 value for standard (BHT) at different concentration is 8.261 and IC50 value for methanol extract of *Barringtonia Acutangula's* leaves at different concentration is 17.546. The leaves of *Barringtonia acutangula* were extracted with methanol, and this result shown the effective activity.

Chapter 6

CONCLUSION

Barringtonia acutangula leaf extract in methanol was subjected to a medicinal plants and bio examination, which concluded that the resulting substance included a wide variety of phytochemical elements that may have medicinal uses. Numerous bioactive substances, including saponins and tannins, as well as alkaloids, flavonoids, and phenol compounds all recognized for their antibacterial, anti-inflammatory, antioxidant, and other therapeutic qualities were found, according to the phytochemical study. These substances could be involved in the biological properties of the extract, such as its anthelminthic, antidiarrheal, and thrombolytic properties. To fully understand these chemicals' unique modes of effect and possible uses in the creation of novel medications or natural cures, more research is required. Overall, the results point to intriguing bioactive characteristics of *Barringtonia acutangula* leaf extract in methanol that merit more research for possible therapeutic use.



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