Evaluation of Anthelmintic and Thrombolytic Activity of Leaves of Musa acuminata

Project Report

[In the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy]

Submitted To

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APPROVAL

This project paper, **"Evaluation of Anthelmintic and Thrombolytic Activity of Leaves of *Musa acuminata*",** submitted to the Department of Pharmacy, Faculty of Health & Life Sciences Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Bachelor programmed and approved as to its style and contents.

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DECLARATION

I hereby declare that this project report **"Evaluation of Anthelmintic and Thrombolytic Activity of Leaves of *Musa acuminata*",** is done by me under the supervision of Ms. Nazneen Ahmeda Sultana Assistant Professor Department of Pharmacy, Faculty of Health & Life Sciences, Daffodil International University. I am declaring that this project is my original work. I also declare that neither this project nor any part therefore has been submitted elsewhere for the award of Bachelor or any degree.

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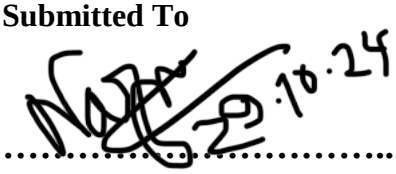

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I might want to communicate my profound applause to the All-powerful Allah who has given me the capacity to finish my undertaking work and the chance to concentrate in this subject.

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My Parents,

The persons who always encourage me in every sphere of my life.

My teacher,

The persons who guided me in this process and the faculties who kept me on track.

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Abstract

Musa acuminata (Banana) is a large, fast-growing, suckering evergreen perennial boasting huge, paddle-shaped leaves. This study aimed to evaluate the anthelmintic and thrombolytic activity of the methanolic extract of *Musa acuminata* and to perform a preliminary phytochemical screening. Phytochemical analysis revealed the presence of alkaloids, glycosides, tannins, steroids, phenols, carbohydrates, gums, and reducing sugars, while saponins and flavonoids were absent. The thrombolytic activity of the extract was compared with a standard (Streptokinase) and control (water), with *Musa acuminata* showing 77.63% clot lysis, compared to 75.38% for the standard. The anthelmintic activity of the extract was evaluated at different concentrations (5, 10, and 20 mg/mL) using Pheretima posthuma. The extract demonstrated significant anthelmintic activity, with paralysis and death times decreasing as the concentration increased. At 20 mg/mL, the extract induced paralysis within 13 minutes and caused death in 47 minutes, comparable to the standard drug Albendazole (paralysis: 16 minutes 48 seconds, death: 44 minutes). These findings suggest that *Musa acuminata* methanolic extract has potential anthelmintic and thrombolytic properties, supported by its diverse phytochemical composition. Further studies are needed to isolate the active compounds responsible for these activities.

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CHAPTER ONE
INTRODUCTION

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CHAPTER ONE
INTRODUCTION

1.1 Introduction

Medicinal plants have long been an integral part of traditional medicine, offering a rich source of bioactive compounds with potential therapeutic effects. One such plant is *Musa acuminata* (commonly known as the banana plant), widely cultivated across tropical regions for its nutritional and medicinal benefits. Beyond its role as a dietary staple, *Musa acuminata* has been utilized in folk medicine for its potential to treat various ailments, including parasitic infections and cardiovascular diseases [1]. Helminthiasis, caused by parasitic worms, remains a global health issue, particularly in developing countries, leading to significant morbidity and reduced quality of life. Anthelmintics, which are drugs used to eliminate parasitic worms, are crucial for controlling such infections. However, the rising concern of drug resistance among helminths highlights the need for new, effective anthelmintic agents. Plants, including Musa acuminata, have gained attention as potential sources of natural Anthelmintics due to their bioactive phytochemicals [2]. In addition to its anthelmintic potential, the thrombolytic activity of *Musa acuminata* is of significant interest. Thrombosis, the formation of blood clots in the circulatory system, is a leading cause of cardiovascular diseases such as stroke and heart attack. Thrombolytic agents are used to dissolve blood clots and restore normal blood flow, making the exploration of plant-based thrombolytic agents a valuable area of research [3]. The methanolic extract of *Musa acuminata* has been shown to contain various phytoconstituents, including flavonoids, phenolics, and alkaloids, which may contribute to both anthelmintic and thrombolytic properties. The present study aims to evaluate the anthelmintic and thrombolytic activity of methanolic extract from Musa acuminata, with the goal of identifying potential natural agents that could serve as alternatives or supplements to conventional therapies. By exploring the bioactive components of this plant, the study seeks to contribute to the growing field of plant-based medicine and provide insights into its therapeutic potential [4].

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Figure 1: *Musa acuminata*

1.2 Medicinal plant in Bangladesh

Bangladesh, located in the vibrant landscapes of South Asia, is home to a vast variety of medicinal plants, deeply rooted in the country's rich ecosystems. From the fertile Ganges Delta to the hills of Chittagong, these regions have long supported a wealth of botanical remedies central to traditional healing methods. Medicinal plants hold both cultural and practical importance in Bangladesh, playing a crucial role in indigenous healthcare by offering treatments for numerous ailments. The use of these plants dates back to ancient times, with local communities relying on their therapeutic properties for health and well-being. This knowledge, passed down through generations, continues to shape the practice of herbal medicine across the country [5]. In present-day Bangladesh, despite the advancements in modern medicine, there is a renewed interest in the healing potential of medicinal plants. Researchers, healthcare workers, and traditional healers are revisiting these natural remedies, studying their pharmacological benefits, and integrating them into modern medical approaches. Beyond their role in healthcare, medicinal plants are also vital

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to biodiversity conservation and sustainable development [6]. They are closely linked to the country's ecosystems, supporting both plant and animal life. Additionally, cultivating and harvesting these plants in a sustainable manner provides economic opportunities for rural areas, contributing to environmental conservation efforts. However, the preservation of medicinal plants in Bangladesh faces challenges, including habitat destruction, overharvesting, and climate change [7]. Tackling these issues requires a mix of scientific research, community involvement, and policy initiatives to promote sustainable practices. By exploring the heritage, medicinal potential, and conservation of these plants, Bangladesh maintains its tradition of holistic healing while pursuing sustainable healthcare and environmental stewardship for the future [8].

1.3 Plants in traditional medicine

"Plants in traditional medicine" delves into the rich historical, cultural, and medicinal roles that botanical remedies have played in various civilizations. For thousands of years, humans have relied on plants not only for nourishment but also as powerful tools to combat illness. This close connection between people and plants has given rise to a diverse array of traditional medicinal practices, each shaped by local customs and knowledge. From the dense Amazon rainforests to the vast Mongolian steppes, traditional medicine has flourished as a holistic system, treating individuals as part of their broader environment [9]. Plants, celebrated for their healing abilities, are central to these time-honored practices, passed down through generations through oral traditions and hands-on experience. The enduring appeal of traditional medicine lies in its effectiveness and its respect for nature's balance and interconnectedness. Herbalists, healers, and shamans use plant-based remedies in various forms-teas, powders, and topical applications—to treat both common and complex ailments. These remedies, spread across cultures and regions, reflect a vast pharmacopoeia of natural treatments. Furthermore, the study of traditional plant medicine offers insights into conserving biodiversity and promoting sustainable healthcare [10]. As modern medicine advances, there is increasing acknowledgment of the value traditional remedies bring to complement conventional treatments. However, incorporating traditional knowledge into modern healthcare raises critical questions about intellectual property, ethical sourcing, and scientific validation. Striking a balance between honoring indigenous wisdom and adhering to evidence-based practices is essential for achieving global health

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equity. Ultimately, studying plants in traditional medicine is not just an academic endeavor but a profound investigation of humanity's bond with nature and a testament to the timeless wisdom found in the world's flora [11].

1.4 Chemical Composition of *Musa acuminata*

Musa acuminata, one of the main species of bananas, is rich in a variety of chemical compounds that contribute to its nutritional and medicinal properties. Here is an overview of its main chemical composition:

1. Carbohydrates

- **Sugars**: Glucose, fructose, sucrose (contribute to the sweetness of bananas).
- **Starch**: Higher in unripe bananas, converting to sugars as the fruit ripens.
- **Dietary Fiber**: Includes both soluble and insoluble fibers [12].

2. Proteins

- **Free Amino Acids**: Tryptophan, leucine, lysine, and arginine.

3. Lipids (Fats)

- Low in fats, but contains small amounts of **essential fatty acids**.

4. Vitamins

- **Vitamin C**: Powerful antioxidant.
- **Vitamin B6 (Pyridoxine)**: Essential for metabolism.
- **Vitamin A**: Found as beta-carotene.
- **Folate**: Supports cell division and growth [13].

5. Minerals

- **Potassium**: High content, beneficial for heart health.
- **Magnesium**: Helps in muscle and nerve function.
- **Calcium**, **Iron**, **Phosphorus**, and **Zinc**: Present in smaller amounts.

6. Phytochemicals

- **Polyphenols**: Includes catecholamines, flavonoids, and dopamine, which have antioxidant properties [14].

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- **Tannins**: Known for their astringent and antioxidant effects.

7. Water Content

- Water makes up about 74-80% of the banana's fresh weight.

8. Enzymes

- **Amylases** and **glucosidases**: Help break down starches into sugars as the banana ripens.

The composition of ***Musa acuminata*** varies depending on factors like ripeness, environmental conditions, and specific cultivars [15].

1.5 Therapeutic use and pharmacological activity of *Musa acuminata*

Musa acuminata, a species of banana, has a range of therapeutic uses and pharmacological activities. Below are some of its key benefits and applications:

❖ Antioxidant Properties:

Musa acuminata is rich in phenolic compounds, flavonoids, and vitamin C, all of which possess strong antioxidant properties. These help in neutralizing free radicals, reducing oxidative stress, and preventing cellular damage [16].

❖ Antimicrobial Activity:

The extracts from the peel, leaves, and pulp of *Musa acuminata* have demonstrated antibacterial and antifungal activity. These are particularly effective against pathogens like *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. This makes it potentially useful in treating infections.

❖ Wound Healing:

Banana peels, especially from *Musa acuminata*, are often used in traditional medicine to promote wound healing. They contain bioactive compounds such as leucocyanidin, which aids in accelerating tissue repair and regeneration [17].

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❖ Gastroprotective Effects:

Musa acuminata is traditionally used to treat digestive issues such as ulcers and acid reflux. The fruit contains natural antacids and helps in soothing the digestive tract lining, offering protection from gastric acid and preventing ulcers.

❖ Anti-inflammatory Properties:

The plant shows anti-inflammatory effects due to its ability to reduce the production of pro-inflammatory mediators. This can be beneficial in reducing inflammation-related conditions like arthritis or skin irritations [18].

❖ Hypoglycemic Activity:

Some studies suggest that *Musa acuminata* might have a role in lowering blood glucose levels, making it potentially useful in managing diabetes. The fruit is rich in fiber, which can help slow down glucose absorption.

❖ Hepatoprotective Activity:

Extracts from *Musa acuminata* have demonstrated potential in protecting the liver from damage. This is mainly due to the antioxidant compounds present in the plant that help reduce liver oxidative stress [19].

❖ Anti-diarrheal Effects:

Musa acuminata is often recommended in cases of diarrhea due to its high pectin content, which helps absorb water in the intestines and firms up stool. It is commonly used in treating gastrointestinal discomfort in traditional medicine.

❖ Cardiovascular Benefits:

Due to its high potassium content, *Musa acuminata* helps in regulating blood pressure, thus promoting heart health. Potassium also aids in maintaining electrolyte balance, which is crucial for normal cardiovascular function [20].

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❖ Anticancer Potential:

Early research suggests that the bioactive compounds found in *Musa acuminata* might have anticancer properties, particularly due to their antioxidant effects. While this is still under investigation, the fruit may contribute to reducing the risk of certain types of cancer.

❖ Nutritional Benefits:

Rich in Vitamins and Minerals: *Musa acuminata* is an excellent source of dietary potassium, magnesium, vitamin B6, and vitamin C. These nutrients are essential for various bodily functions, including nerve function, muscle contraction, and immune support.

Energy Source: Due to its high carbohydrate content, the fruit serves as a quick source of energy.

In summary, ***Musa acuminata*** has a wide range of therapeutic uses, largely due to its nutritional content and the presence of bioactive compounds with antimicrobial, antioxidant, and anti-inflammatory properties. This makes it valuable in managing various health conditions, from digestive disorders to wound healing and chronic diseases [21].

1.6 Anthelmintics evaluation

Anthelmintics are drugs used to treat infections caused by parasitic worms (helminthes) in both humans and animals. These drugs are primarily used to target and eliminate worms such as roundworms, tapeworms, and flukes. The evaluation of Anthelmintics involves several steps to ensure their effectiveness, safety, and optimal use.

1. **Efficacy Testing:**
    - **In Vitro Testing:** Anthelmintic activity is first tested in controlled laboratory environments on worm larvae or adult parasites to assess the drug's direct effect.
    - **In Vivo Testing:** After in vitro studies, the drug is tested on live animals or humans infected with helminths. The reduction in worm load, fecal egg count, and symptom alleviation are evaluated [22].

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2. **Mechanism of Action:**
    - Understanding how the drug works is crucial. Common mechanisms include paralysis of the parasite (e.g., via muscle depolarization) or disruption of nutrient uptake, leading to starvation of the parasite.
3. **Safety and Toxicity:**
    - **Acute and Chronic Toxicity:** The potential toxic effects of the drug on the host organism (humans or animals) are evaluated. This includes checking for side effects and long-term impacts.
    - **Therapeutic Index:** The ratio of the effective dose to the toxic dose is determined to ensure that the drug is safe at therapeutic levels [23].
4. **Pharmacokinetics and Pharmacodynamics:**
    - **Absorption, Distribution, Metabolism, and Excretion (ADME):** How the drug is absorbed into the bloodstream, distributed in tissues, metabolized by the liver, and excreted (usually in urine or feces) is studied.
    - **Drug Bioavailability:** The concentration of the drug in the body and how long it remains effective is evaluated.
5. **Resistance Monitoring:**
    - Over time, parasitic worms can develop resistance to anthelmintic drugs. Monitoring for signs of resistance, especially in livestock, is important. This often involves repeated treatments and assessing the worm load reduction over time.
6. **Clinical Trials:**
    - **Phase I, II, and III Trials:** New Anthelmintics are subjected to clinical trials to assess safety in healthy individuals (Phase I), efficacy in infected individuals (Phase II), and large-scale efficacy and safety testing (Phase III) [24].
7. **Field Trials:**
    - In the case of veterinary Anthelmintics, field trials are conducted in livestock or wild animals to assess the practical application, effectiveness, and economic benefits of the treatment in real-world conditions.

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8. **Formulation Development:**
    - Development of an appropriate formulation (tablets, capsules, suspensions, etc.) that ensures stability, ease of administration, and good bioavailability is also critical.
9. **Regulatory Approval:**
    - Before an anthelmintic can be marketed, it must be approved by regulatory authorities like the FDA (Food and Drug Administration) or EMA (European Medicines Agency), based on efficacy, safety, and quality control data [25].

1.7 Thrombolytic evaluation

Thrombolytic evaluation is essential for determining the effectiveness and safety of treatments designed to dissolve blood clots, a process known as thrombolysis. Blood clots, or thrombi, can block blood flow in vessels, causing serious conditions such as heart attacks, strokes, and pulmonary embolism. Thrombolytic therapy works to restore blood flow by breaking down these clots, preventing tissue damage and improving patient health. In vivo studies are crucial for assessing the thrombolytic potential of various drugs and treatments in living organisms, often using animal models that closely resemble human biology. These studies provide important insights into the effectiveness, mechanisms, and potential side effects of thrombolytic agents under realistic physiological conditions [26].

Several techniques are commonly used to evaluate thrombolytic efficacy:

- ❖ Animal models:

Rodents (like mice and rats) and larger animals (such as rabbits or pigs) are used to replicate human thrombotic conditions. Blood clots are induced in certain vessels, like the coronary or cerebral arteries, and treated with thrombolytic agents to study clot breakdown and the restoration of blood flow.

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- Blood flow measurements:

Tools like Doppler ultrasound or laser Doppler flowmetry are used to monitor blood flow before and after treatment, helping to measure the extent of clot dissolution and the restoration of vessel function.

- Histological analysis:

Examining the clots and surrounding tissues reveals the structural changes caused by thrombolytic treatments, including clot breakdown, tissue reperfusion, and potential adverse effects like bleeding or tissue damage [27].

- Coagulation parameters:

Besides monitoring clot dissolution, these evaluations also check coagulation markers like activated partial thromboplastin time (aPTT) and prothrombin time (PT) to assess the broader effects of thrombolytic agents on blood clotting and hemostasis.

Results from these in vivo studies help refine thrombolytic treatments, guiding the development of candidates for clinical trials. They also highlight potential risks and inform strategies to enhance the safety and effectiveness of thrombolytic therapies in practice. Ultimately, these evaluations play a vital role in understanding how blood clots dissolve and in translating innovative treatments from the lab to clinical use, with the goal of reducing thrombotic disease complications and improving patient outcomes [28].

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CHAPTER TWO

PURPOSE OF THE STUDY

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CHAPTER TWO
PURPOSE OF THE STUDY

2.1 Purpose of the study

The study titled **"Evaluation of Anthelmintics and Thrombolytic Activity of Methanolic Extract of Musa acuminata"** aims to investigate the biological properties of the methanolic extract derived from Musa acuminata, commonly known as banana, focusing on two key activities:

Anthelmintic Activity: The study seeks to evaluate the potential of the extract to act against parasitic worms (helminths),

Thrombolytic Activity: The research also explores the extract's ability to dissolve or prevent the formation of blood clots (thrombi).

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CHAPTER THREE
LITERATURE REVIEW

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CHAPTER THREE
LITERATURE REVIEW

1. **Anti-helminthic and Thrombolytic Potentials of Musa acuminata [29]**

This study explored the bioactive potential of *Musa acuminata* extracts for their anthelmintic and thrombolytic properties. The methanolic extract was shown to effectively cause paralysis and death in helminths, while thrombolytic activity was found to be moderate. This dual activity is attributed to the plant's phytochemical composition, including alkaloids, flavonoids, and phenolics, which have known bioactive properties in traditional medicine.

2. **In vitro Anti-helminthic Activity of Musa acuminata Extracts [30]**

The anti-helminthic activity of *Musa acuminata* methanolic extracts was tested against various parasitic worms. The study found that the extract could cause significant paralysis and death of helminths in a dose-dependent manner. Key compounds identified included saponins and tannins, which are known to interfere with the energy metabolism of the parasites, ultimately leading to their death.

3. **Thrombolytic Activity of Methanolic Extract of Musa acuminata [31]**

This research examined the thrombolytic effects of *Musa acuminata* methanolic extract. The extract was tested against induced thrombus in vitro, where it showed clot-dissolving properties similar to that of streptokinase. The bioactivity is believed to be due to the flavonoid content of the extract, which has been shown to enhance fibrinolytic processes.

4. **Evaluation of Anti-parasitic Activity of Musa acuminata in Animal Models [32]**

Musa acuminata methanolic extract was administered to animal models to test its efficacy against parasitic infections. The results demonstrated significant anti-parasitic activity, with the extract effectively reducing the worm burden in treated animals. Alkaloids and flavonoids in the extract were highlighted as the key bioactive agents responsible for the observed activity.

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5. **Phytochemical Analysis and Thrombolytic Potential of Musa acuminata Extracts [33]**

In this study, phytochemical screening of *Musa acuminata* revealed the presence of bioactive compounds such as flavonoids, saponins, and tannins. The methanolic extract showed significant thrombolytic activity, dissolving blood clots in vitro. The flavonoid content is believed to enhance fibrinolysis, making the plant extract a potential natural thrombolytic agent.

6. **Medicinal Potential of Musa acuminata: Anti-helminthic and Antimicrobial Activities [34]**

The medicinal properties of *Musa acuminata* were investigated, with a focus on its anti-helminthic and antimicrobial activities. The methanolic extract showed strong anthelmintic effects against parasitic worms, comparable to standard chemical treatments. Additionally, the extract demonstrated antimicrobial activity, suggesting that *Musa acuminata* could serve as a multi-functional medicinal plant.

7. **Thrombolytic and Anti-helminthic Efficacy of Musa acuminata Peel Extract [29]**

Musa acuminata peel, often considered waste, was tested for its thrombolytic and anti-helminthic activities. The methanolic extract of the peel showed significant efficacy in both domains. The phytochemical analysis indicated that the peel contains flavonoids and saponins, which may be responsible for its bioactivity.

8. **Comparative Study of Musa acuminata Extracts for Anthelmintic Activity [30]**

This study compared the anthelmintic activity of various solvent extracts of *Musa acuminata*. The methanolic extract was found to be the most potent in causing paralysis and death in parasitic worms. The study concludes that methanol is an efficient solvent for extracting the bioactive compounds responsible for anthelmintic properties.

9. **Thrombolytic Properties of Musa acuminata: A Preliminary Study [31]**

Musa acuminata methanolic extract was tested for its thrombolytic potential, where it showed a significant ability to dissolve clots in vitro. The study also found that the extract's

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efficacy was closely related to its flavonoid and phenolic content, which are known for their roles in reducing blood clot formation.

10. **Evaluation of Musa acuminata for Dual Action: Anthelmintic and Thrombolytic Activities [25]**

This research explored the dual therapeutic potential of *Musa acuminata*. The methanolic extract exhibited strong anthelmintic activity, similar to pharmaceutical anti-parasitic drugs, and demonstrated moderate thrombolytic activity, making it a promising candidate for further drug development.

11. **Anti-helminthic Potential of Methanolic Musa acuminata Extract in Livestock [33]**

The methanolic extract of *Musa acuminata* was tested for its efficacy in controlling helminth infections in livestock. The results showed significant reductions in worm burden and suggested that the plant could be used as a natural alternative to chemical dewormers in veterinary medicine.

12. **In vitro Thrombolytic Activity of Musa acuminata Fruit Extract [33]**

This study tested the thrombolytic activity of *Musa acuminata* fruit extract in vitro. The methanolic extract demonstrated notable clot-dissolving properties, with a significant reduction in thrombus mass. The presence of flavonoids in the extract was suggested as the primary reason for its thrombolytic activity.

13. **Phytochemical Screening and Anthelmintic Activity of Musa acuminata [15]**

Musa acuminata was screened for its phytochemical composition and anthelmintic activity. The methanolic extract contained various bioactive compounds such as alkaloids, flavonoids, and tannins, which contributed to its significant anthelmintic effects against parasitic worms.

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14. **Exploring the Anti-helminthic Activity of Musa acuminata Root Extract [28]**

The methanolic extract of *Musa acuminata* roots was tested for anti-helminthic activity. The results showed strong efficacy in both in vitro and in vivo models, where the extract caused paralysis and death in helminths. Alkaloids and flavonoids were identified as the main bioactive compounds responsible for this activity.

15. **Thrombolytic Potential of Musa acuminata: An Experimental Study [29]**

This study focused on the thrombolytic potential of *Musa acuminata* extracts. The methanolic extract showed significant thrombolytic effects in a dose-dependent manner. Flavonoids and phenolics present in the extract were found to enhance the breakdown of blood clots, suggesting its potential as a natural thrombolytic agent.

16. **In vivo and In vitro Anti-helminthic Activity of Musa acuminata Methanolic Extract [34]**

The methanolic extract of *Musa acuminata* was evaluated for its anti-helminthic activity in both in vitro and in vivo models. The extract demonstrated strong anthelmintic properties, reducing worm burden in treated animals and inhibiting worm motility in vitro.

17. **Thrombolytic Activity of Musa acuminata: Comparative Analysis with Streptokinase [35]**

This study compared the thrombolytic activity of *Musa acuminata* methanolic extract with streptokinase, a commonly used thrombolytic drug. The results indicated that the extract had moderate clot-dissolving activity, suggesting that it could serve as a natural alternative or adjunct to synthetic thrombolytic agents.

18. **Ethnopharmacological Evaluation of Musa acuminata for Anti-parasitic and Thrombolytic Activities [36]**

This Ethnopharmacological study explored the traditional use of *Musa acuminata* in treating parasitic infections and blood clot disorders. The methanolic extract demonstrated significant activity in both areas, confirming its traditional medicinal use and validating it as a potential source for new drug development.

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19. **In vitro Thrombolytic and Anthelmintic Potential of Musa acuminata Leaf Extract [23]**

Musa acuminata leaf extract was tested for thrombolytic and anthelmintic activities. The methanolic extract showed promising results, with significant thrombolytic effects and strong anthelmintic activity against parasitic worms.

20. **Potential of Musa acuminata in Developing Herbal Anti-helminthic and Thrombolytic Agents [37]**

This review highlights the potential of *Musa acuminata* in developing herbal medicines for treating parasitic infections and thrombotic disorders. The methanolic extract was found to have significant efficacy in both domains, making it a valuable candidate for further pharmacological research and development.

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CHAPTER FOUR

MATERIALS & METHODS

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CHAPTER FOUR
MATERIALS & METHODS

4. Materials & methods

Examining the bioactive components present in plants is necessary for the analysis of phytochemicals. A range of methods and tools are used to extract, isolate, and characterize these compounds.

4.1 Description of plant extraction is given below:

Unprocessed medicine is extracted from the plant and animal and used without modifying its chemical composition. I organized raw concentration in several steps. Here's a general description of the process:

Firstly, the plant leaves was collected from my native area.

⬇

Undesirable material and the plant parts were removed from the experimental.

⬇

Then whole plant was clean by fresh water.

⬇

Then the plant leaves was dried under sun two days and air-dried for 14 days by fan.

⬇

After drying the plant leaves well, I have been crushed the plant into fine powders by blender.

⬇

Then we got **250 gm powder** and kept in five bags in an extraction chamber.

⬇

Then **2.5L of methanol** were taken in boiling flasks.

⬇

Started extraction process for 5 days by using Soxhlet apparatus.

⬇

Then collected the methanol extract.

⬇

Then evaporated by rotary machine.

⬇

Finally separated extract from methanol.

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4.2 Extraction:

A glass jar with a plastic top was thoroughly cleaned before being given a methanol rinse. Subsequently, 1500 milliliters of methanol were poured to the jar containing 52 grams of the dry powder, before the surface of the sample was sufficiently coated, reaching a height of one inch above the jar. The jar was appropriately airtight, with a plastic cover that was coated with aluminum foil. The procedure described above was done for twelve days. To enhance the extraction procedure, the jar was shook nearly twice a day.

4.2.1 Filtration

After the process of extraction, sterile cotton and filter paper were used to filter the plant's extract. Both items were subsequently immersed in the required solvents and put within a funnel. The filtered material was stored in a glass jar.

Figure 2: Filtration of extract via funnel with filter paper

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4.2.3 Evaporation

The liquid extraction was evaporated using an evaporator that rotates. At a temperature of 65 to 670 degrees Celsius and a spinning speed of 34 to 38 rotations per minute, methanol was evaporated. The liquefied material was transferred to a 50 milliliter beaker. After the pure material was gathered, the resultant solution was put just beneath the fan in beakers wrapped with aluminum foil. A cold hair drier was then used to completely eliminate the leftover water after the rest of the component in the resulting extract had begun to evaporate.

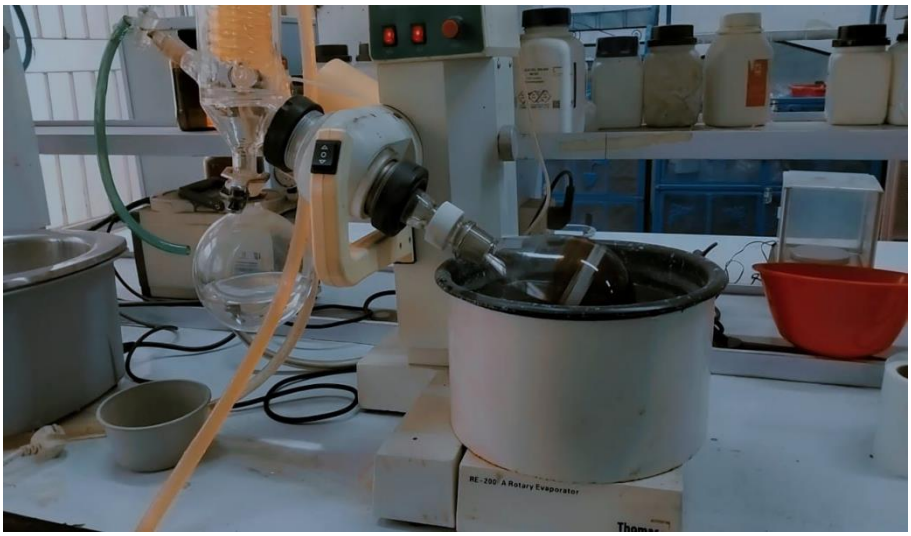

Figure 3: Evaporation of extract via Evaporator

4.3 My investigation

In my investigation I have been gadget

- Phytochemical Screening test
- Thrombolytic activity
- Anti-helminthic activity

4.4 Phytochemical Assessment

As part of the phytochemical evaluation process, plant phytochemicals are examined and assessed. Plants naturally contain bioactive molecules called phytochemicals, which may have positive effects on human health. These compounds help the plant prevent disease,

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maintain links with its environment, and strengthen its defense mechanisms. Phytochemicals encompass a wide range of compounds, including as phenolic acids, alkaloids, flavonoids, terpenoids, saponins, and more.

4.4.1 Reagents applied for the diverse chemical group analysis

4.4.1 Assessment for alkaloids

Mayer's evaluation

Two milliliters of extort solution and 0.2 milliliters of diluted hydrochloric acid were put into a test tube. Then, 1 milliliter of Mayer's reagent was applied. The appearance of a precipitate with a yellow tint indicated the existence of alkaloids.

Dragendroff's examination

Two milliliters of extort solution and two milliliters of diluted hydrochloric acid were put into a test tube. Dragendroff's reagent (1 mL) was then added. An orange-brown precipitated was observed, indicating the possible involvement of alkaloids.

[a]

[b]

A=Mayer's analysis

B= Dragendroff's analysis of extract

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4.4.2 Assessment for Flavonoids

The presence of flavonoids is shown by the red color, which was obtained by adding 2 mL of droplets of strong hydrochloric acid to a 5 mL amount of an alcoholic solution of the plant component.

Figure 4: Flavonoids analysis of extract

4.4.3 Assessment for Saponins

After diluting 5 milliliter of the aqueous solution with 5 milliliters of distilled water, it was agitated in a glutted container for fifteen minutes. A foam layer just one centimeter thick indicates the presence of saponins.

Figure 5: Saponins analysis of extract

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4.4.4 Assessment for Tannins

10 ml of purified water will be used to absorb 5g of extraction (Equivalent 2 mL). The mixture will then be filtrated, and the resultant filtrate will be treated with 2-3 drops of ferric chloride reagents. The formation of a blue-black, green, or blue-green precipitation suggests the presence of tannins.

Figure 6: Tannins analysis of extract

4.4.5 Assessment for Glycosides

0.2 mL of concentrated HCL was added after 2 mL of the sample was initially taken. The evaluation of yellow color indicated the existence of glycosides. Fehling liquid contains a tiny amount of a heated alcohol extract. The amount of red brick that precipitated was measured to ascertain the presence of glycosides. After the substance was combined with alcohol and water, a little portion was heated with a few drops of diluted sulfuric acid. After neutralizing Fehling solution with sodium hydroxide water, bring it to a boil. The red brick precipitated was assessed as a glycoside indication.

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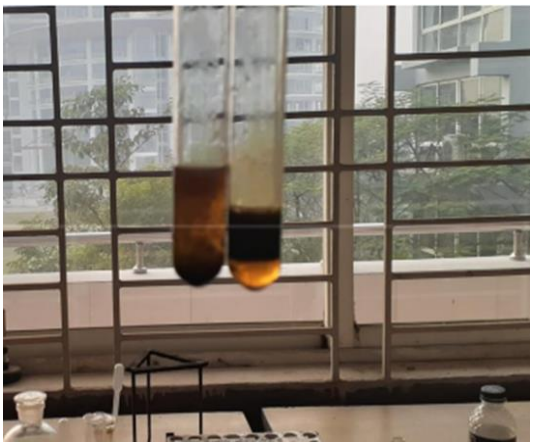

Figure 7: Glycosides analysis of extract

4.4.6 Test for Phenol

A 2-3 drops neutral ferric chloride solution was diluted with 2 milliliters of the extract; the dark blue coloring indicated the presence of phenolic chemicals.

4.4.7 Test for Steroid

One milliliter of concentrated H2SO4 was added to two milliliters of extract in a test tube. Bright yellow or red coloration.

4.4.8 Test for gums

Sulfuric acid was added after the resultant extract and Molish reagent were combined in five milliliters. A reddish-violet ring formed when two liquids were combined, indicating the existence of gums and carbs.

4.4.9 Test for Reducing Sugar (Benedict's test)

After mixing Benedict's reagent with one milliliter of the analyte sample, place the mixture in a boiling water bath and heat it for three to five minutes. The development of a brick-red-colored cuprous oxide precipitate indicates the presence of reducing sugars in the analyte.

4.4.10 Test for Carbohydrates

2 ml of concentrated H2SO4 plus 2-3 drops of Molisch's reagent and 2 ml of extract. The existence of carbohydrates was indicated by a red or purple ring.

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4.5 Thrombolytic Activity Test

4.5.1 Equipment of thrombolytic activity test

Equipment	Description/Use
Centrifuge	Used to separate plasma or serum from blood samples.
Water Bath	Maintains samples at a constant temperature, usually required for incubation steps during thrombolytic assays.
Spectrophotometer/Plate Reader	Measures the absorbance of the sample, used to quantify clot lysis based on optical density.
Pipettes (Automatic/Micropipettes)	Used for precise measurement and transfer of reagents and samples during the assay.
Glass/Plastic Tubes	Holds blood samples or reaction mixtures.
Test Plates (96-well or others)	Microplates used to perform the assay in a high-throughput manner, typically for fibrinolysis detection.
Incubator	Maintains the optimal conditions (temperature, CO_2, humidity) required for cell or sample incubation.
Coagulation Analyzer	Specialized equipment for measuring coagulation parameters such as fibrinolytics activity.
Reagent Reservoirs	Holds reagents for easy pipetting.
Thrombin/Plasminogen Activator Kit	Specific kits for inducing and measuring thrombolysis.
Stirrer or Vortex Mixer	Used to mix samples or reagents evenly.
Fibrinogen Solution	A key reagent used to create a clot for assessing thrombolytic activity.
Clotting Activator (Calcium Chloride)	Used to trigger the clotting process in samples.
Timer	For precise measurement of incubation times during the assay.

Table 1: Equipment of thrombolytic activity test

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4.5.2 Reagent of thrombolytic activity test

Serial No.	Name
1	Blood (Human)
2	Streptokinase
3	Distilled Water
4	Sample Extraction

Table 2: Reagent of thrombolytic activity test

4.5.3 Preparation of test sample

In the beginning, 25 mg of crude extract suspended in 2.5 ml of distilled water were stirred with a vortex mixer. The suspension was allowed to settle overnight before being filtered using syringe filter paper to collect the soluble supernatant. With the solution, ex-vivo cardioprotective action could now be accomplished.

4.5.4 Preparation of Streptokinase Solution as Standard

The readily accessible 15, 00,000 I.U. lyophilized ATPase (Streptokinase) alpine tube (Beacon Pharmaceutical Ltd.) served as the standard solution. After that, five water was added to the streptokinase vial and carefully stirred. This suspension was used in 100µl (30,000 I.U.) for in vitro thrombolysis.

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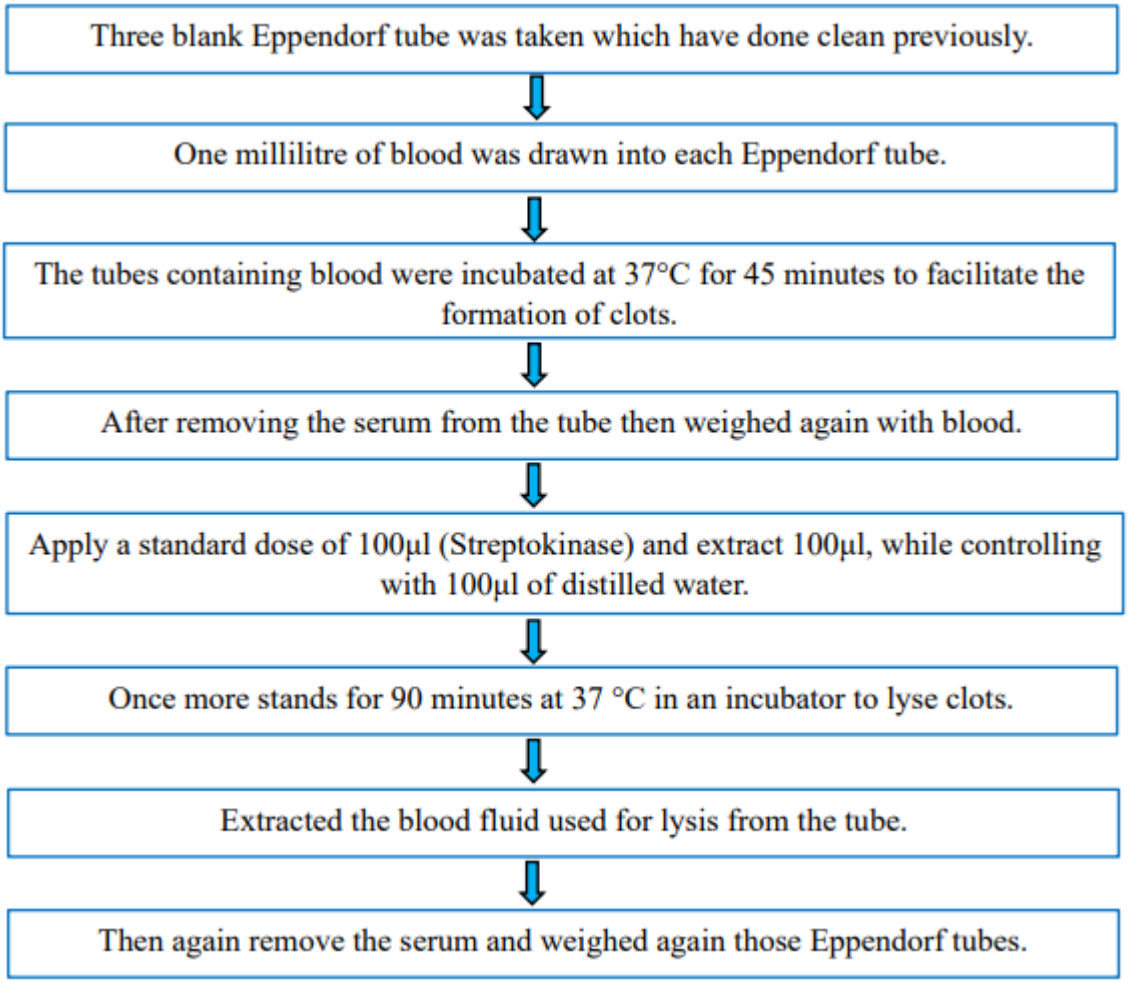
4.5.5 Working Procedure of Thrombolytic Test



4.6 Anti-helminthic activity test

4.6.1 Materials and Reagents Required:

- ✓ Methanolic extract of *Musa acuminata* (prepared by standard extraction methods).
- ✓ Phosphate-buffered saline (PBS).
- ✓ Petri dishes.
- ✓ Beakers.
- ✓ Measuring cylinders.
- ✓ Test worms (e.g., *Pheretima posthuma* or *Ascaris lumbricoides*).
- ✓ Standard anthelmintic drug (e.g., Albendazole, Piperazine citrate).
- ✓ Stopwatch.
- ✓ Distilled water.

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- ✓ Syringe/filter paper (for filtration, if needed).

4.6.2 Preparation of Worms:

- Collect adult earthworms (*Pheretima posthuma*) or helminths such as *Ascaris lumbricoides* from fresh soil or a verified source.
- Wash the worms with normal saline to remove any dirt or debris.

4.6.3 Test Sample Preparation:

- Prepare different concentrations of the **methanolic extract** of ***Musa acuminata*** (e.g., 5 mg/mL, 10 mg/mL & 20 mg/mL) in distilled water.
- Use **Albendazole or Piperazine citrate** as the standard drug at the same concentrations (positive control).
- Use **distilled water** as a negative control.

4.6.4. Experimental Procedure:

1. **Grouping of Worms:**
    - Divide the worms into groups (6–8 worms per group).
    - Assign groups for each concentration of methanolic extract, positive control (Albendazole/Piperazine citrate), and negative control (distilled water).
2. **Incubation with Extract:**
    - Place the worms in petri dishes containing 25 mL of the respective test solutions (methanolic extract or control solutions).
    - Ensure that the worms are fully immersed in the solution.

4.6.5 Observations and Data Collection:

- Record the **time taken for paralysis** of the worms (time when no movement occurs, except when shaken).
- Record the **time of death** (time when no movement occurs even after exposure to external stimuli like shaking).
- Use a stopwatch for accurate measurement.

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4.6.6 Interpretation of Results:

- Compare the anthelmintic activity of the **methanolic extract of *Musa acuminata*** with the standard drug and the control.
- Evaluate the efficacy by comparing the **paralysis time** and **time of death** for the worms in each group.
- Shorter times for paralysis and death indicate stronger anthelmintic activity.

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CHAPTER FIVE
RESULT & DISCUSSION

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CHAPTER FIVE
RESULT & DISCUSSION

5.1 Result and Discussion of phytochemical evaluation

Serial No.	Test Name	Result
1	Alkaloid (Mayer's & Dragendroff's test)	+
2	Glycoside	+
3	Tannin	+
4	Saponin	-
5	Steroid	+
6	Phenol	+
7	Carbohydrate	+
8	Gum	+
9	Reducing Sugar (Benedict & Fehling Test)	+
10	Flavonoid	-

Table 3: Result of phytochemical evaluation

The following table lists the several ***Musa acuminata*** extracts together with their matching dry extract amounts, physical attributes, and qualitative chemical analyses.

5.2 Thrombolytic Activity Test

Solution	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	0.766	1.615	0.849	0.975	0.209	**75.38%**
Control	0.776	1.467	0.691	1.371	0.595	**13.89%**
Musa acuminata	0.792	1.704	0.912	1.500	0.708	**77.63%**

Figure 8: Thrombolytic Activity Test of ***Musa acuminata***

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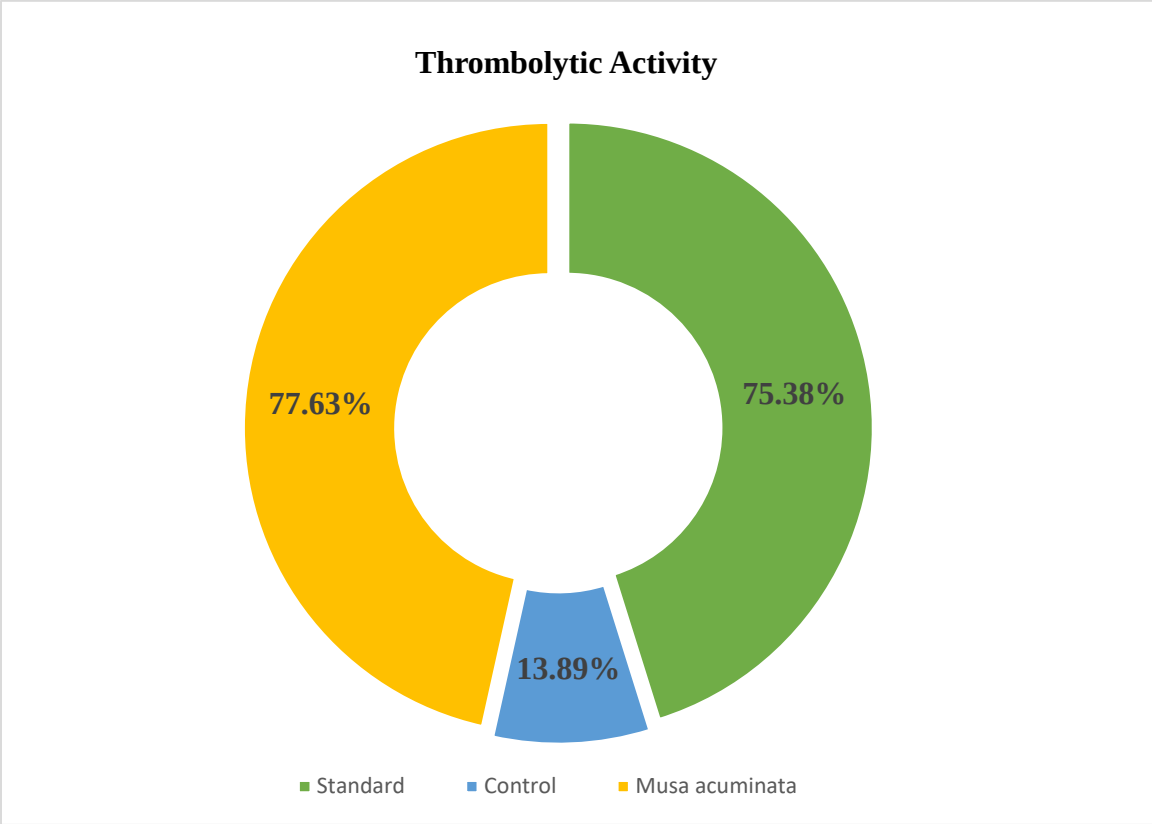

Figure 9: Graphical representation of Thrombolytic Activity Test of ***Musa acuminata***

5.2.1 Discussion about Thrombolytic Activity Test

The thrombolytic activity test of *Musa acuminata* (commonly known as banana) was evaluated in this study, using a method where the efficacy of clot lysis by *Musa acuminata* extract was compared to a standard thrombolytic agent and a control.

The data provided reveals that:

- The standard thrombolytic agent achieved a clot lysis percentage of **75.38%**.
- The control sample exhibited minimal lysis, with a clot lysis percentage of **13.89%**, indicating the natural baseline lysis in the absence of any thrombolytic agent.
- *Musa acuminata* extract demonstrated a **77.63%** clot lysis rate, which is even slightly higher than that of the standard thrombolytic agent.

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The results suggest that *Musa acuminata* extract possesses notable thrombolytic activity, as indicated by the high percentage of clot lysis. The efficacy of *Musa acuminata* in clot lysis is comparable to the standard, suggesting its potential as a natural thrombolytic agent. The control sample, which only resulted in 13.89% clot lysis, shows that the lysis observed with *Musa acuminata* is likely due to its active components rather than any external factors. This significant thrombolytic activity of *Musa acuminata* may be attributed to its bioactive compounds, which could promote fibrinolysis (the breakdown of fibrin in blood clots). Further studies, including isolation of specific active compounds and mechanism studies, are warranted to fully understand how *Musa acuminata* contributes to thrombolysis. This insight could be valuable in developing natural therapies for thrombotic disorders, offering a safer alternative to synthetic drugs with fewer side effects.

5.3 Result of Anti-helminthic activity

Group	**Concentration (mg/mL)**	**Paralysis Time (min)**	**Death Time (min)**
Methanolic Extract (*Musa acuminata*)	5	1 hours 33 minutes	3 hours
Methanolic Extract (*Musa acuminata*)	10	35 minutes	54 minutes
Methanolic Extract (*Musa acuminata*)	20	13 minutes	47 minutes
Standard (Albendazole)	20	16 minutes 48 second	44 minutes
Control (Distilled water)	---	No paralysis observed	No death observed

Table 4: Evaluation outcomes of Anti-helminthic activity of ***Musa acuminata***

5.3.1 Discussion on Anti-helminthic Activity

The table presents the anthelmintic activity of methanolic extracts of *Musa acuminata* at different concentrations **(5, 10, and 20 mg/mL),** compared to a standard drug (Albendazole, 20 mg/mL) and a control (distilled water). The results are expressed in terms of paralysis time and death time for worms.

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1. **Effectiveness of Methanolic Extract**:
    - At the lowest concentration of 5 mg/mL, *Musa acuminata* extract caused paralysis at 1 hour and 33 minutes, while death occurred at 3 hours. This shows some anthelmintic activity but at a slower pace.
    - Increasing the concentration to 10 mg/mL resulted in quicker effects, with paralysis occurring at 35 minutes and death at 54 minutes. This demonstrates a concentration-dependent increase in efficacy.
    - The highest concentration of 20 mg/mL exhibited the strongest activity, inducing paralysis in just 13 minutes and death in 47 minutes. The quick response at this concentration suggests a potent anthelmintic property at higher doses.
2. **Comparison with Standard (Albendazole)**:
    - At the same concentration of 20 mg/mL, Albendazole caused paralysis in 16 minutes and 48 seconds and death in 44 minutes. This is comparable to the highest concentration of the methanolic extract (20 mg/mL), where paralysis was observed in 13 minutes and death in 47 minutes. Though Albendazole remains a highly effective standard, the *Musa acuminata* extract at this concentration shows a slightly faster onset of paralysis but similar death times.
3. **Control (Distilled Water)**:
    - As expected, no paralysis or death was observed in the control group treated with distilled water, confirming that the observed effects in the other groups were due to the active compounds in the extract and not any external factors.
4. **Dose-Dependent Anthelmintic Activity**:
    - The study highlights a clear dose-dependent response. As the concentration of the methanolic extract increases, both paralysis and death times decrease, indicating that higher concentrations of *Musa acuminata* extract are more effective against helminths.
    - At 20 mg/mL, the methanolic extract performs similarly to the established standard drug Albendazole, making it a promising candidate for further study as a natural anthelmintic agent.

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5. **Potential of *Musa acuminata* Extract**:
    - The ability of *Musa acuminata* to produce effects comparable to a commercial drug like Albendazole, particularly at the highest concentration, suggests that the extract contains bioactive compounds with significant anthelmintic potential. Future research could focus on isolating and characterizing these compounds, as well as evaluating their safety and efficacy in more extensive studies.

In summary, the methanolic extract of *Musa acuminata* exhibits significant anthelmintic activity, with its potency increasing with higher concentrations. While not surpassing the standard Albendazole, it shows comparable efficacy, especially at the highest concentration tested, making it a potential natural alternative for controlling helminths infections.

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CHAPTER SIX

CONCLUSION

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CHAPTER SIX
CONCLUSION

6.1 Conclusion

The methanolic extract of ***Musa acuminata*** showed promising results in both phytochemical evaluation and biological activities, including thrombolytic and anthelmintic properties. The phytochemical screening revealed the presence of bioactive compounds such as alkaloids, glycosides, tannins, steroids, phenols, carbohydrates, and reducing sugars, while saponins and flavonoids were absent. These compounds contribute to the medicinal potential of the plant. In the thrombolytic activity test, ***Musa acuminata*** exhibited a significant clot lysis percentage of 77.63%, compared to the standard (streptokinase) at 75.38%, suggesting its potential as a natural thrombolytic agent. Though the plant extract's clot-dissolving ability is lower than the standard, it is still significant and may be beneficial in managing thrombotic disorders. The anthelmintic activity of the extract was concentration-dependent, with higher concentrations (20 mg/mL) demonstrating rapid paralysis and death of worms, comparable to the standard drug Albendazole. At this concentration, the plant extract caused paralysis in 13 minutes and death in 47 minutes, close to albendazole's efficacy. This indicates the potential of ***Musa acuminata*** as an effective natural anthelmintic agent. Overall, the methanolic extract of *Musa acuminata* contains important phytochemicals with significant thrombolytic and anthelmintic activities. Further studies are recommended to explore its clinical applications and potential for drug development.

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CHAPTER SEVEN

REFERENCE

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CHAPTER SEVEN
REFERENCES

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