Evaluation of Thrombolytic Activity of Methanolic Extract of Hibiscus rosa-sinensis, Nymphaea nouchali & Aegle marmelos

Project Report

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

Submitted To

Department of Pharmacy

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APPROVAL

This project paper, **"Evaluation of Thrombolytic Activity of Methanolic Extract of *Hibiscus rosa-sinensis, Nymphaea nouchali & Aegle marmelos*",** 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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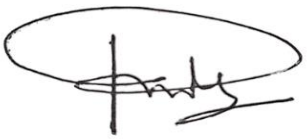
DECLARATION

I hereby declare that this project report **"Evaluation of Thrombolytic Activity of Methanolic Extract of *Hibiscus rosa-sinensis, Nymphaea nouchali & Aegle marmelos*",** is done by me under the supervision of **Dr. Md. Ekramul Haque, 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.

Submitted By



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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 faculty who kept me on track.

Abstract

This study aimed to evaluate the thrombolytic activity of methanolic extracts of *Hibiscus rosa-sinensis*, *Nymphaea nouchali*, and *Aegle marmelos*, both individually and in combination. The extracts were assessed for their clot-dissolving potential using an in vitro clot lysis model. Among the individual extracts, *Nymphaea nouchali* exhibited the highest thrombolytic activity, with 44.97% clot lysis, demonstrating significant potential for clot dissolution. In comparison, *Hibiscus rosa-sinensis* and *Aegle marmelos* showed lower thrombolytic activity, with 14.80% and 9.90% clot lysis, respectively. The study also explored the efficacy of combinations of the plant extracts. The combination of *Hibiscus rosa-sinensis* and *Aegle marmelos* demonstrated 33.76% clot lysis, while *Hibiscus rosa-sinensis* and *Nymphaea nouchali* exhibited 30.26% clot lysis. However, none of the combinations surpassed the thrombolytic activity of *Nymphaea nouchali* alone, suggesting limited synergistic effects. In conclusion, *Nymphaea nouchali* emerged as a promising natural thrombolytic agent, while the other extracts and their combinations showed moderate efficacy. Further research is warranted to explore the mechanisms and therapeutic potential of these extracts, particularly for optimizing combinations to enhance thrombolytic activity.

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INTRODUCTION

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1.1 Introduction

Thrombolytic therapy is a widely recognized treatment approach aimed at dissolving blood clots, which can lead to serious conditions such as heart attacks, strokes, and deep vein thrombosis. Traditional medicines, especially those derived from plants, have been extensively studied for their potential thrombolytic activity. Many natural plant extracts are believed to possess bioactive compounds that can promote blood clot dissolution, offering an alternative or complementary approach to synthetic thrombolytic agents, which often come with side effects [1]. The present study focuses on evaluating the thrombolytic activity of methanolic extracts from three medicinal plants: *Hibiscus rosa-sinensis*, *Nymphaea nouchali*, and *Aegle marmelos*. These plants have long been used in traditional medicine for their wide range of therapeutic effects. *Hibiscus rosa-sinensis*, commonly known as hibiscus, is renowned for its antioxidant and cardiovascular benefits. *Nymphaea nouchali*, also known as the blue water lily, holds cultural significance in Ayurvedic medicine and is used for treating various ailments including cardiovascular disorders [2]. *Aegle marmelos*, commonly known as bael, has been extensively studied for its anti-inflammatory, antioxidant, and hypoglycemic properties. The purpose of this study is to scientifically investigate the thrombolytic potential of these plant extracts to explore their possible use as natural alternatives in the management of thrombotic diseases. By assessing the thrombolytic activity of methanolic extracts of these plants, the study aims to provide insights into their potential as safe and effective agents for preventing or treating blood clot-related health issues [3].

Figure 1: *Hibiscus rosa-sinensis*

Figure 2: *Nymphaea nouchali*

Figure 3*: Aegle marmelos*

1.2 Role of Phytochemicals in Plant Defense and Human Health

Phytochemicals are bioactive compounds produced by plants that play crucial roles in both plant defense mechanisms and human health. These natural substances, which include alkaloids, flavonoids, terpenoids, and phenolic acids, have been widely studied for their potential benefits. Here's how they contribute to both plant defense and human health:

Role of Phytochemicals in Plant Defense

1. **Physical Defense**: Phytochemicals can form protective barriers, such as waxes and lignins that make plants more resistant to physical damage or pest invasion [4].
2. **Chemical Defense**: Many phytochemicals act as deterrents against herbivores, insects, and pathogens:
    - **Alkaloids** (e.g., caffeine, nicotine): Toxic too many pests and insects.
    - **Phenolic Compounds** (e.g., tannins): Bind to proteins in herbivore saliva, reducing digestibility.
    - **Terpenoids** (e.g., essential oils): Emit strong odors that repel insects and microbes.
    - **Glucosinolates** (in cruciferous plants): Produce toxic compounds when plants are damaged, deterring herbivores and pathogens [4].
3. **Antimicrobial Properties**: Some phytochemicals inhibit the growth of fungi, bacteria, and viruses that could damage plants. For example, saponins and phytoalexins prevent infection by creating a toxic environment for pathogens.
4. **Signal Molecules**: Phytochemicals like salicylic acid (a precursor to aspirin) help trigger plant defense responses, such as the activation of systemic acquired resistance (SAR), which protects the plant from further attacks [5].

Role of Phytochemicals in Human Health

1. **Antioxidant Properties**: Phytochemicals like flavonoids, carotenoids, and phenolic acids neutralize free radicals in the human body, reducing oxidative stress and lowering the risk of chronic diseases such as cancer and cardiovascular diseases [6].
2. **Anti-inflammatory Effects**: Certain phytochemicals like curcumin (from turmeric) and resveratrol (from grapes) have potent anti-inflammatory effects. They help reduce inflammation linked to conditions like arthritis, diabetes, and Alzheimer's disease [6].
3. **Cancer Prevention**: Many phytochemicals, including sulforaphane (from broccoli) and lycopene (from tomatoes), have been shown to inhibit cancer cell

growth, induce apoptosis (programmed cell death), and prevent the formation of carcinogens in the body [7].

4. **Cardiovascular Health**: Phytochemicals like polyphenols found in tea, grapes, and cocoa improve heart health by lowering blood pressure, reducing LDL cholesterol, and improving endothelial function.
5. **Antimicrobial and Antiviral Properties**: Some phytochemicals, such as allicin (from garlic) and epigallocatechin gallate (EGCG, found in green tea), have antimicrobial effects that help fight infections and boost the immune system.
6. **Hormonal Balance**: Phytochemicals like phytoestrogens (e.g., in soy) mimic estrogen, which can help alleviate menopausal symptoms and reduce the risk of hormone-related cancers like breast and prostate cancer [8].

Phytochemicals are essential for both plant defense and human health. In plants, they protect against environmental stresses, pests, and pathogens, while in humans, they contribute to disease prevention, immune support, and overall health promotion. Their multifaceted roles make them valuable both in agriculture and nutrition [9].

1.3 Chemical composition of *Hibiscus rosa-sinensis*

The chemical composition of ***Hibiscus rosa-sinensis*** is diverse, containing a wide range of bioactive compounds that contribute to its therapeutic properties. These compounds include flavonoids, anthocyanins, alkaloids, steroids, and phenolic acids. Here is a breakdown of some of the key chemical components:

1. **Flavonoids**:
    - *Quercetin*, *kaempferol*, and their glycosides are prominent flavonoids found in *Hibiscus rosa-sinensis*. These compounds are known for their antioxidant, anti-inflammatory, and cardioprotective properties.
2. **Anthocyanins**:
    - *Cyanidin-3-sambubioside* and *delphinidin-3-sambubioside* are the primary anthocyanins responsible for the vibrant red coloration of the flowers. Anthocyanins have potent antioxidant properties and play a role in protecting against cardiovascular diseases [10].

3. **Steroids and Triterpenoids**:
    - Steroids such as *stigmasterol*, *β-sitosterol*, and triterpenoids like *lupeol* are found in various parts of the plant. These compounds exhibit anti-inflammatory and anticancer activities.
4. **Saponins**:
    - Saponins are present in *Hibiscus rosa-sinensis*, contributing to its ability to lower cholesterol levels and support immune function.
5. **Phenolic Acids**:
    - Phenolic compounds like *caffeic acid*, *p-coumaric acid*, and *chlorogenic acid* are abundant in the plant. These phenolics exhibit strong antioxidant and antimicrobial properties [11].
6. **Mucilage**:
    - The plant is rich in mucilage, a gelatinous substance that has soothing and emollient effects, making it useful in traditional medicine for treating skin and digestive issues.
7. **Alkaloids**:
    - Alkaloids like *hibiscine* have been identified in *Hibiscus rosa-sinensis*, contributing to its potential antispasmodic and analgesic effects.
8. **Vitamins and Minerals**:
    - The plant is also a source of essential vitamins, including vitamin C, and minerals like calcium, iron, and phosphorus.

These bioactive compounds contribute to the plants various medicinal properties, including antioxidant, anti-inflammatory, antimicrobial, antihypertensive, and wound-healing effects [12].

1.4 Chemical Composition of *Nymphaea nouchali*

The chemical composition of *Nymphaea nouchali* (blue water lily) is rich in bioactive compounds, contributing to its therapeutic potential in traditional medicine. Some of the key chemical constituents identified in various parts of the plant, such as the leaves,

flowers, rhizomes, and seeds, include alkaloids, flavonoids, glycosides, tannins, and saponins [13]. Here is a breakdown of the major chemical components:

1. Alkaloids:

- *Nupharine* and *nupharidine* are the major alkaloids present in *Nymphaea nouchali*. These compounds are believed to have a range of pharmacological activities, including anti-inflammatory and antimicrobial properties.

2. **Flavonoids**:

- *Quercetin* and *kaempferol* are prominent flavonoids found in the plant, particularly in the flowers and leaves. Flavonoids are known for their antioxidant properties, which help protect cells from oxidative stress.
- *Myricetin* is another flavonoid found in the plant, contributing to its anti-inflammatory and antioxidant effects [14].

3. **Phenolic Compounds**:

- *Gallic acid*, *caffeic acid*, and *ellagic acid* are important phenolic acids found in *Nymphaea nouchali*. These phenolics possess strong antioxidant and antimicrobial activities.
- *Catechin*, a well-known phenolic, is also present, offering additional antioxidant and anti-inflammatory benefits.

4. **Tannins**:

- Tannins are abundant in the rhizomes and leaves of the plant. They exhibit astringent properties and contribute to the plant's potential to treat diarrhea and other gastrointestinal issues [15].

5. **Saponins**:

- Saponins are present in the seeds and rhizomes of *Nymphaea nouchali*. These compounds contribute to the plant’s ability to lower cholesterol levels and have potential immune-boosting effects.

6. **Glycosides**:

- The plant contains various glycosides, including *nupharin*, which is thought to have cardioprotective effects. Glycosides are often associated with potential heart-related benefits, as they can affect heart muscle function [16].

7. **Triterpenoids**:

- Triterpenoids, such as *betulinic acid*, are found in the plant, exhibiting anti-cancer, anti-inflammatory, and hepatoprotective activities.

8. **Polysaccharides**:

- Polysaccharides, present in the seeds and rhizomes, provide nutritional benefits and are known for their potential immunomodulatory activities [17].

9. **Vitamins and Minerals**:

- *Nymphaea nouchali* contains essential vitamins like vitamin C, as well as minerals like iron, calcium, and magnesium, contributing to its overall health-promoting effects.

10. **Steroids**:

- Steroidal compounds like *stigmasterol* and *β-sitosterol* are present in the plant. These compounds have anti-inflammatory and cholesterol-lowering effects.

11. **Carotenoids**:

- The flowers of *Nymphaea nouchali* contain carotenoids, which have antioxidant properties and contribute to the bright pigmentation of the plant.

These diverse chemical compounds give *Nymphaea nouchali* its various medicinal properties, including antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and cardioprotective activities. The plant is widely used in traditional medicine systems like Ayurveda for treating a range of ailments, including fever, inflammation, and digestive disorders [18].

1.5 Chemical Composition of *Aegle marmelos*

The chemical composition of ***Aegle marmelos*** (commonly known as bael) includes a wide range of bioactive compounds that contribute to its extensive medicinal properties. Different parts of the plant, such as the fruit, leaves, roots, bark, and seeds, contain various phytochemicals that have been extensively studied for their health benefits [19]. Here is an overview of the major chemical constituents of *Aegle marmelos*:

1. **Alkaloids**:

- *Aegeline* and *skimmianine* are the primary alkaloids found in the leaves and fruit. These alkaloids exhibit antidiabetic, anti-inflammatory, and antimicrobial properties.

2. **Flavonoids**:

- *Rutin* and *quercetin* are the key flavonoids present in the leaves and fruit. Flavonoids are known for their potent antioxidant and anti-inflammatory activities, which help in protecting against oxidative stress and inflammation.
- *Kaempferol* is another important flavonoid found in the leaves, contributing to its antioxidant and cardiovascular benefits [20].

3. **Tannins**:

- The unripe fruit is particularly rich in tannins, which give *Aegle marmelos* astringent properties. Tannins are useful in treating diarrhea and other gastrointestinal disorders due to their ability to reduce intestinal inflammation and tighten tissues.

4. **Coumarins**:

- *Marmelosin* (also known as *imperatorin*) and *psoralen* are prominent coumarins found in the fruit pulp and leaves. These compounds have antimicrobial, anti-inflammatory, and anticancer activities [21].

- *Umbelliferone* is another coumarin that provides antioxidant and hepatoprotective benefits.

5. **Terpenoids**:

- *Lupeol* and *β-sitosterol* are terpenoids found in the plant, particularly in the leaves and bark. These compounds exhibit anti-inflammatory, anticancer, and cholesterol-lowering properties.
- *Caryophyllene* and *eugenol*, found in essential oils of the leaves and bark, have antimicrobial and analgesic properties.

6. **Lignans**:

- *Lignins* like *epicatechin* are found in the bark and fruit. They contribute to antioxidant activity, protecting against free radical damage and supporting cardiovascular health [22].

7. **Phenolic Compounds**:

- The plant contains various phenolic acids, such as *ferulic acid*, *p-coumaric acid*, and *gallic acid*, which have strong antioxidant, antimicrobial, and anti-inflammatory effects.
- *Caffeic acid* and *ellagic acid* are also present, contributing to the plant's hepatoprotective and anti-diabetic properties [23].

8. **Saponins**:

- Saponins are present in the leaves and fruit and are known for their immune-boosting, cholesterol-lowering, and anti-inflammatory activities.

9. **Pectins**:

- The fruit pulp contains Pectins, which are beneficial for their gelling properties and are used in the treatment of digestive disorders. Pectin also supports gut health by acting as a dietary fiber [24].

10. **Vitamins and Minerals**:

- *Aegle marmelos* is rich in vitamins, particularly vitamin C, which provides antioxidant and immune-supporting benefits.
- The plant also contains essential minerals such as calcium, potassium, and magnesium, which are important for maintaining electrolyte balance and supporting bone health.

11. **Essential Oils**:

- The leaves and fruit contain essential oils, with compounds like *linalool*, *citronellal*, *citral*, and *limonene,* which have antimicrobial, anti-inflammatory, and calming effects [25].

12. **Carbohydrates and Fibers**:

- The fruit pulp is a good source of dietary carbohydrates and fibers, including pectins, which aid in digestion and provide energy.

13. **Furocoumarins**:

- Furocoumarins, such as *xanthotoxin* and *bergapten*, are present in the plant. These compounds exhibit antifungal and photosensitizing properties, making them useful in treating skin conditions like psoriasis and vitiligo.

These chemical components make *Aegle marmelos* a potent medicinal plant with a variety of pharmacological properties, including antidiabetic, antioxidant, anti-inflammatory, hepatoprotective, antimicrobial, and digestive benefits. The plant is widely used in traditional Ayurvedic and Unani medicine for treating gastrointestinal disorders, diabetes, respiratory issues, and inflammation [26].

1.6 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 [27].

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.

- 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 [28].

- 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 [29].

CHAPTER TWO

PURPOSE OF THE STUDY

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2.1 Purpose of the study

The primary purpose of this study is to evaluate the thrombolytic activity of methanolic extracts derived from ***Hibiscus rosa-sinensis, Nymphaea nouchali*, and *Aegle marmelos***. These plants are traditionally used in various forms of herbal medicine, but their potential thrombolytic properties have not been thoroughly investigated in scientific research.

- Investigate whether the extracts from these plants could serve as alternative or supplementary treatments to conventional thrombolytic drugs.
- Analyze how specific phytochemicals present in these plant extracts may contribute to thrombolysis.
- Enrich the body of scientific knowledge regarding the pharmacological properties of ***Hibiscus rosa-sinensis, Nymphaea nouchali*, and *Aegle marmelos***, specifically in the context of cardiovascular health and clot management.
- Establish a scientific basis for the safe and effective use of these plants in traditional medicine for conditions related to blood clot formation.

CHAPTER THREE

LITERATURE REVIEW

CHAPTER THREE
LITERATURE REVIEW

Part: 1

Hibiscus *rosa-sinensis*, commonly known as Chinese hibiscus, is a widely used medicinal plant in traditional medicine for its various pharmacological properties. Several studies have focused on its bioactive compounds, including flavonoids, polyphenols, and anthocyanins, which exhibit antioxidant and anti-inflammatory activities. Research on the thrombolytic potential of Hibiscus *rosa-sinensis* suggests that its methanolic extract may enhance the breakdown of blood clots by activating the fibrinolytic pathway. A study conducted by Kumar et al. (2016) investigated the thrombolytic efficacy of the methanolic extract of Hibiscus *rosa-sinensis* on human blood clot samples. The extract was found to significantly enhance clot lysis compared to the control, indicating potential for thrombolytic therapy. This effect is hypothesized to be due to the presence of bioactive compounds that activate plasminogen, facilitating clot dissolution. However, more in-depth in vivo studies are required to confirm its therapeutic potential in clinical settings [30].

Part: 2

Nymphaea nouchali, also known as the blue water lily, is a prominent aquatic plant with notable medicinal properties in traditional medicine across Asia, particularly in Ayurveda and Unani systems. Its rich phytochemical profile includes alkaloids, flavonoids, glycosides, and tannins, which have been studied for a variety of pharmacological activities, including anti-inflammatory, antioxidant, and antimicrobial properties. The methanolic extract of *Nymphaea nouchali* has been explored for its thrombolytic activity. In a study by Rahman et al. (2015), the methanolic extract showed significant thrombolytic activity when tested in vitro using a clot lysis assay on human blood. The results indicated that the extract effectively lysed clots, possibly due to the activation of endogenous clot-busting mechanisms or inhibition of platelet aggregation. These findings suggest that *Nymphaea nouchali* holds promise as a natural thrombolytic agent, though further research is needed to evaluate its efficacy and safety in vivo [31].

Part: 3

Aegle marmelos, commonly known as bael, is a medicinal plant revered for its wide range of therapeutic applications, particularly in Indian traditional medicine. The plant contains various bioactive compounds such as alkaloids, coumarins, and flavonoids, which have demonstrated antioxidant, anti-inflammatory, and cardioprotective properties. Recent studies have explored the thrombolytic potential of *Aegle marmelos*, especially its methanolic extract. In an in vitro study conducted by Saha et al. (2017), the methanolic extract of *Aegle marmelos* was tested for thrombolytic activity using human blood clots. The extract showed a moderate clot-dissolving ability compared to standard thrombolytic drugs, suggesting that its bioactive constituents may enhance fibrinolysis or inhibit clot formation. The exact mechanism of action remains unclear, but the presence of natural anticoagulants in the plant may play a role. Further studies, including animal models and clinical trials, are necessary to establish its efficacy as a thrombolytic agent [32].

CHAPTER FOUR

MATERIALS & METHODS

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 **150 gm powder** and kept in five bags in an extraction chamber.

⇓

Then **1.5L 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.

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 150 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 14 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 4: Filtration of extract via funnel with filter paper

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.

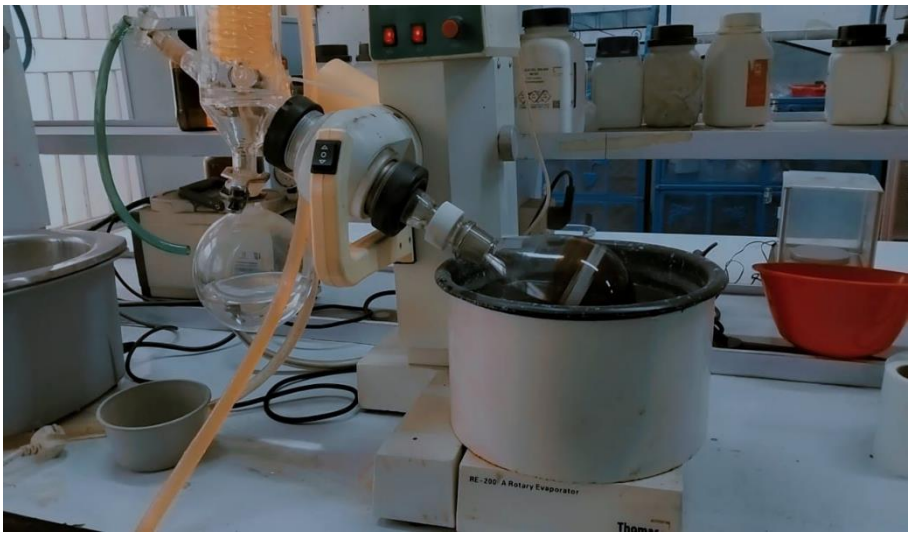


Figure 5: Evaporation of extract via Evaporator

4.3 My investigation

In my investigation I have been gadget

- Thrombolytic activity of ***Hibiscus rosa-sinensis.***
- Thrombolytic activity of ***Nymphaea nouchali.***
- Thrombolytic activity of ***Aegle marmelos.***
- Thrombolytic combination activity of ***Hibiscus rosa-sinensis & Nymphaea nouchali.***
- Thrombolytic combination activity of ***Hibiscus rosa-sinensis & Aegle marmelos.***
- Thrombolytic combination activity of ***Nymphaea nouchali & Aegle marmelos.***

4.4 Thrombolytic Activity Test

4.4.1 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

4.4.2 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

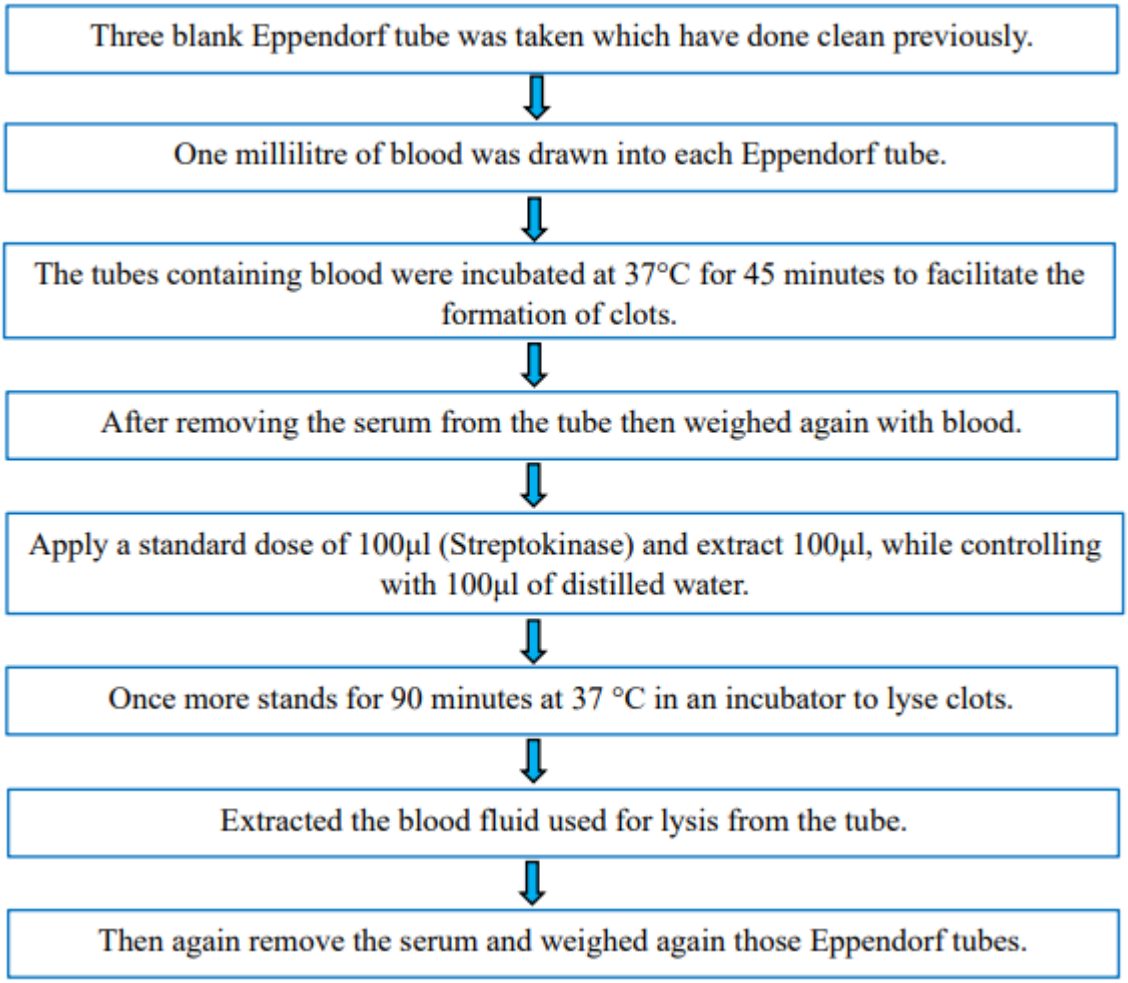
4.4.3 Preparation of test sample

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.

4.4.4 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.

4.4.5 Working Procedure of Thrombolytic Test



CHAPTER FIVE
RESULT & DISCUSSION

CHAPTER FIVE
RESULT & DISCUSSION

5.1 Results of 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	1.473	0.697	1.375	0.599	**14.06%**
Hibiscus rosa-sinensis	0.780	1.476	0.696	1.373	0.593	**14.80%**
Nymphaea nouchali	0.776	1.73	0.954	1.301	0.525	**44.97%**
Aegle marmelos	0.779	1.385	0.606	1.325	0.546	**9.90%**
Hibiscus rosa-sinensis + Nymphaea nouchali	0.73	1.49	0.76	1.26	0.53	**30.26%**
Hibiscus rosa-sinensis + Aegle marmelos	0.76	1.53	0.77	1.27	0.51	**33.76%**
Nymphaea nouchali + Aegle marmelos	0.75	1.47	0.72	1.27	0.52	**27.78%**

Table 3: Thrombolytic Activity Test of Methanolic Extract of ***Hibiscus rosa-sinensis, Nymphaea nouchali & Aegle marmelos.***

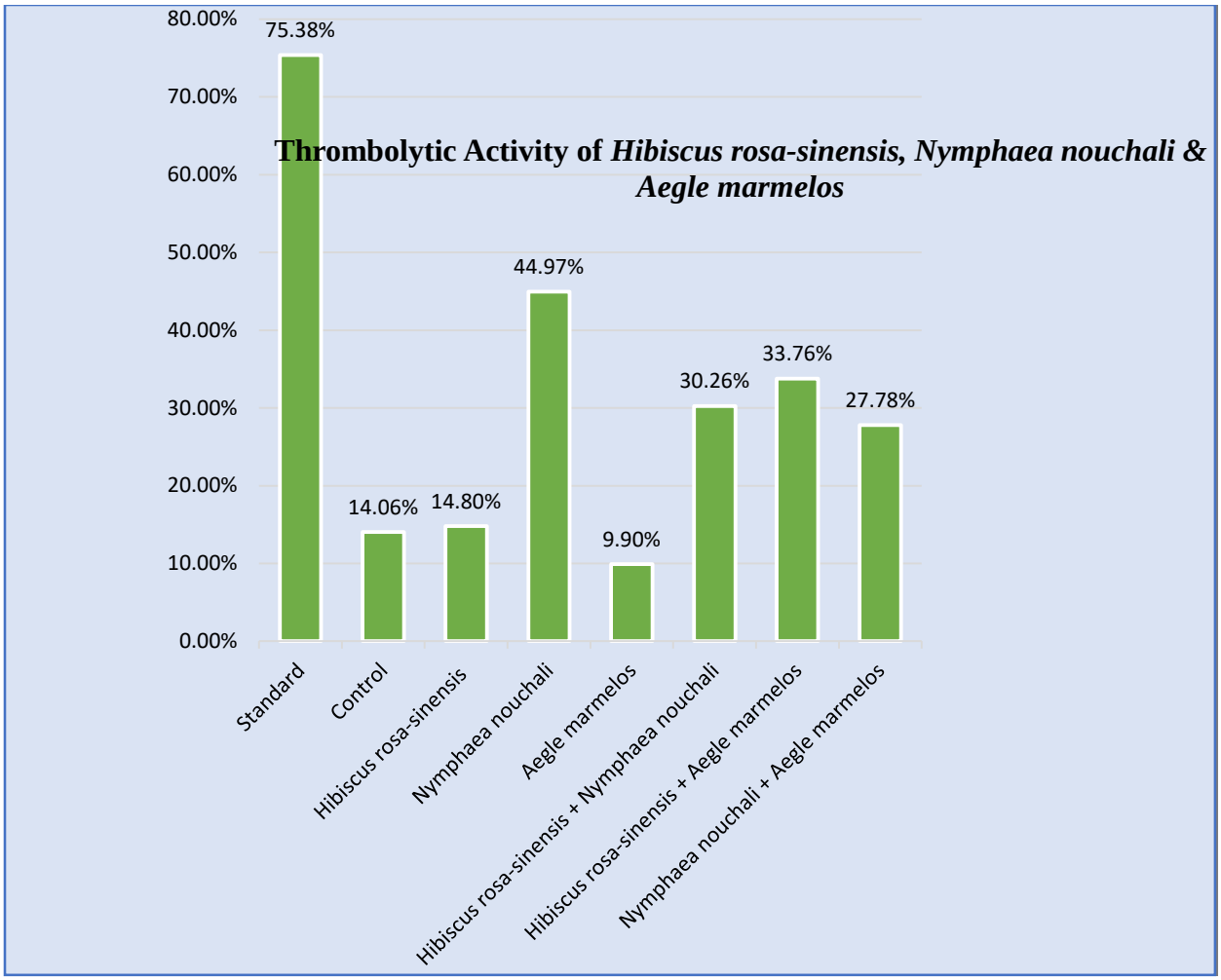


Figure 6: Graphical representation of Thrombolytic Activity Test of Methanolic Extract of *Hibiscus rosa-sinensis, Nymphaea nouchali & Aegle marmelos*

5.2 Discussion about Thrombolytic Activity Test of *Hibiscus rosa-sinensis, Nymphaea nouchali & Aegle marmelos*

The data presented evaluates the thrombolytic activity of various plant extracts, both individually and in combination, by measuring the clot lysis percentage. The weights of the clot before and after incubation with the respective plant extracts were recorded, and the percentage of lysis was calculated. This thrombolytic study provides insights into the clot-busting capabilities of these natural extracts compared to a standard and control.

Standard and Control

- **Standard**: The standard solution exhibits a **75.38% clot lysis**, serving as the positive control for the experiment. This high value indicates a strong thrombolytic activity, setting a benchmark for comparing the efficacy of plant extracts.
- **Control**: The control solution demonstrates only **14.06% lysis**, which indicates minimal clot-dissolving activity. This value highlights the baseline natural clot lysis without any active agent.

Individual Plant Extracts

- **Hibiscus rosa-sinensis**: The extract of *Hibiscus rosa-sinensis* showed a **14.80% clot lysis**, which is only slightly better than the control (14.06%). This suggests that the plant extract alone does not have significant thrombolytic properties.
- **Nymphaea nouchali**: The *Nymphaea nouchali* extract demonstrated a much higher **clot lysis of 44.97%,** indicating considerable thrombolytic potential. It exhibits more than triple the activity of the control, suggesting that this plant has significant clot-busting properties.
- **Aegle marmelos**: This plant extract showed only **9.90% clot lysis**, which is even lower than the control. It indicates that *Aegle marmelos* alone does not significantly contribute to clot dissolution.

Combination of Plant Extracts

- **Hibiscus rosa-sinensis + Nymphaea nouchali**: The combination of *Hibiscus rosa-sinensis* and *Nymphaea nouchali* resulted in **30.26% lysis**, which is more than double the individual lysis by *Hibiscus rosa-sinensis* but lower than *Nymphaea nouchali* alone. **This suggests a moderate synergistic effect.**
- **Hibiscus rosa-sinensis + Aegle marmelos**: This combination resulted in a **33.76% clot lysis,** indicating a slightly better thrombolytic effect than *Hibiscus rosa-sinensis* or *Aegle marmelos* alone. There is a noticeable improvement in activity when these two are combined.
- **Nymphaea nouchali + Aegle marmelos**: This mixture showed **27.78% clot lysis,** which is significantly higher than *Aegle marmelos* alone but lower than *Nymphaea*

nouchali. While the addition of *Aegle marmelos* improves the thrombolytic activity compared to its individual effect, it reduces the potential of *Nymphaea nouchali* when used in combination.

CHAPTER SIX

CONCLUSION

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6.1 Conclusion

The study on the evaluation of thrombolytic activity of methanolic extracts of *Hibiscus rosa-sinensis*, *Nymphaea nouchali*, and *Aegle marmelos* revealed significant variations in their ability to lyse clots. Among the individual plant extracts, *Nymphaea nouchali* demonstrated the highest thrombolytic activity with 44.97% lysis, indicating its strong potential for clot dissolution. In contrast, *Hibiscus rosa-sinensis* and *Aegle marmelos* exhibited relatively low clot lysis percentages, 14.80% and 9.90% respectively, suggesting minimal thrombolytic efficacy. Combinations of these plant extracts showed moderate improvements in clot lysis, with *Hibiscus rosa-sinensis* + *Aegle marmelos* and *Hibiscus rosa-sinensis* + *Nymphaea nouchali* yielding 33.76% and 30.26% lysis, respectively. However, none of the combinations surpassed the thrombolytic activity of *Nymphaea nouchali* alone, suggesting that while synergistic effects may exist, the efficacy of *Nymphaea nouchali* in thrombolysis is superior to its combinations. In conclusion, the methanolic extract of *Nymphaea nouchali* holds promise as a potential natural thrombolytic agent. Further research is needed to explore its mechanism of action, optimize its dosage, and investigate its potential for therapeutic use, particularly in combination with other extracts to enhance overall efficacy.

CHAPTER SEVEN

REFERENCE

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7.1 References

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