Investigation of Thrombolytic Potential of Combined Approach on Methanolic Extracts from Rosa, Centella asiatica, and Swertia using In-Vitro Technique

PROJECT WORK

THIS IS A PROJECT REPORT THAT FULFILLS SOME OF THE REQUIREMENTS FOR A BACHELOR OF PHARMACY DEGREE

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A project paper investigating the thrombolytic activity of three medicinal plant in combination has been submitted and accepted by the Department of Pharmacy at Daffodil International University for partial fulfillment of a Bachelor of Pharmacy degree.

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DECLARATION

I hereby declare that this project report is done by me under the supervision of **Dr. Ekramul Haque Professor**, Department of Pharmacy, Daffodil International University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy. I am declaring that this Project is my original work. I also declare that neither this project nor any part thereof has been submitted elsewhere for a Bachelor's or any degree award.

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DEDICATED TO MY BELOVED PARENTS, RESPECTED TEACHERS, AND WELL- WISHERS

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ABSTRACT

This study aimed to investigate the phytochemical composition and thrombolytic potential of three plant extracts: *Centella asiatica* leaves, *Rosa* petals, and *Swertia* bark. Phytochemical analysis was conducted using standard constituent tests to investigate the presence of variety of bioactive compounds, but more extensive research is needed to isolate and characterize specific compounds and evaluate their therapeutic potential. Thrombolytic activity was assessed by measuring the percentage inhibition of clot lysis in human blood. The results demonstrated that all three plant extracts exhibited a certain degree of thrombolytic activity with *Rosa* exhibited 35.67% (moderate) thrombolytic activity, while *Swertia and centella asiatica* demonstrated 30.20% and 19.71% (mild) thrombolytic properties respectively. Furthermore, a combination of *Centella asiatica* leaves, *Rosa* petals, and *Swertia* bark extract was evaluated for its thrombolytic efficacy. While the combination exhibited some activity in breaking down blood clots, it did not consistently outperform the standard thrombolytic agent. The highest clot lysis observed was 27.76%, which, although significant, was lower than the 72% lysis achieved by the standard. Despite these findings, the study highlights the potential of these plant extracts as promising sources of natural thrombolytic agents. Additional examination is needed to fully elucidate the underlying mode of working, optimize extract preparation methods, and evaluate their safety and efficacy in clinical trials. Ultimately, these findings could contribute to the development of novel, natural-based therapies for thrombotic disorders.

CHAPTER ONE: INTRODUCTION

1.1 INTRODUCTION

1.1.1. Rose (*Rosa*)

Roses are revered as the epitome of beauty and love, recognized for their diverse species, cultivars, and hybrids cherished for their exquisite appearance and delightful fragrance. With over 200 species originated from Asia, with fewer native to Europe, North America, and North Western Africa, their fruits, flowers, leaves, and bark are utilized across a wide spectrum of product development sectors such as cosmetics, food, pharmaceuticals, and engineering. [1] In China alone, there are 82 species of *Rosa*. [o] *Rosa* plants possess a diverse chemical composition, primarily featuring flavonoids, triterpenes, tannins, phenolic acids, polysaccharides, and vitamins. These compounds contribute to the genus's health benefits, which include anti-inflammatory, antioxidant, antimicrobial, and cardiovascular protective effects. Moreover, some *Rosa* species have demonstrated promising anti-cancer and neuroprotective properties. [12]

1.1.2. Thankuni *(Centella Asiatica)*

Thankuni, also known as 'Goku Kola' in India, is a widely consumed edible plant in Bangladesh. It is an herbaceous perennial native to Bangladesh, India, and several other Asian countries, as well as the wetlands of the Southeastern United States. In English, it is referred to as 'Indian pennywort' or 'Asiatic pennywort'. It is commonly used in home remedies in Bangladesh for treating minor stomach upsets, diarrhea, and dysentery. [2] Phytochemical analysis indicated the presence of tannins, saponins, flavonoids, and other beneficial compounds. In addition, both the aerial parts and roots of the plant are employed for medicinal purposes, and its chemical constituents have numerous therapeutic applications, including antimicrobial, anti-inflammatory, anticancer, neuroprotective, antioxidant, and wound healing effects. [14] [16]

1.1.3. Chirata *(Swertia)*

Chirata is a flowering herbaceous plant found in the northern Himalayan regions of India and Bhutan, growing up to 3 to 4 feet tall. Its tall, upright stem is covered with bark, and its leaves are opposite, lanceolate, and sessile (without stems). The **Swertia** genus, consisting of about 170 species, is predominantly found in temperate, subtropical, and montane tropical regions. Chirata is valued for its various beneficial components, including antioxidants, alkaloids, and glycosides. Chirata is known for its effectiveness in reducing fever caused by colds and flu, as well as its efficacy against malarial fever, hysteria, and convulsions. These plants are rich in phytochemicals such as xanthonoids, flavonoids, and terpenoids, which have diverse biological activities, including anticancer, antidiabetic, antioxidant, and neuroprotective effects. [3][19]

1.2 POSITION OF MEDICINAL PLANTS IN BANGLADESH:

Bangladesh boasts a wide variety of medicinal plants, many of which are endemic to the region. This diversity offers a rich source of potential therapeutic compounds. Many of them have been scientifically studied for their bioactive compounds and potential therapeutic applications. This has led to the development of new drugs and nutraceuticals. The ongoing use of these plants in traditional medicine for treating various health issues has ingrained them into the cultural fabric of the people in Bangladesh. In addition, Plant specimens were collected and identified at the Bangladesh National Herbarium, revealing 119 species from 64 families utilized by traditional medical practitioners for various ailments. The Asteraceae family was the most common, featuring six species, followed by Moraceae, Solanaceae, and Apocynaceae with five species each. Among the species identified, herbs constituted the majority at 38%, followed by trees (32%), shrubs (21%), climbers (7%), and palms (2%). Medicinal plants form the cornerstone of traditional medicine practices in Bangladesh, providing affordable and accessible healthcare to a significant portion of the population. The cultivation, trade, and export of medicinal plants contribute to the country's economy, particularly in rural areas. Both Unani and Ayurvedic traditional medicine systems have deep-rooted traditions in the country and are practiced widely. [4][5]

1.3. OVERVIEW ON THROMBOLYTIC ACTIVITY:

Blood clots are a natural part of healing damaged blood vessels, but excessive clotting can lead to serious health issues. Clots can block blood flow (embolism), causing organ damage or death. Reduced blood flow can cause tissue damage, including heart problems. Brain clots can lead to strokes, causing permanent brain damage. Thrombolysis, a medical procedure, uses medications to dissolve blood clots. Three main types are tPA, streptokinase, and urokinase. Thrombolysis improves blood flow and prevents complications like heart attacks and strokes. Blood clots are a major cause of cardiovascular disease, forming in arteries (heart attacks, strokes) or veins (lung clots). Treatment differs based on clot location: platelet-targeting drugs for arterial clots, and coagulation-affecting drugs for venous clots. Current treatments have bleeding risks, motivating research for new, natural anti-clotting medications. [6][7][8]

CHAPTER TWO: PURPOSE OF INVESTIGATION

2.1. PURPOSE OF INVESTIGATION:

I. To evaluate the thrombolytic property of the sources in human blood using In-vitro method.

II. This study investigates the feasibility of employing a strategically selected combination of three plant for the dissolution of blood clots.

III. The study aims to investigate their combined ability to reduce the formation of thrombi.

IV. This combination interaction suggests that the components within these plants complement and enhance each other's clot-dissolving properties.

V. The ultimate goal is to determine the potential of a plant-based combination to effectively dissolve them. therefore, it could be offered as a valuable alternative or complementary treatment option.

CHAPTER THREE: PLANT OUTLINE

PLANT PREVIEW

3.1. Rosa

Fig 1: Image of Rose

3.1.1. TAXONOMIC CLASSIFICATION: [9]

- **Kingdom:** Plantae;
- **Sub-kingdom**: Tracheobionta;
- **Division**: Magnoliophyta;
- **Sub-division:** Spermatophyta;
- **Class**: Magnoliopsida;
- **Sub class:** Rosidae;
- **Order:** Rosales;
- **Family:** Rosaceae;
- **Sub-families:** Rosoideae, Prunoideae, Maloideae, Spairaeoideae
- **Genus:** Rosa L.;
- **Species:** Papa Meilland

3.1.2. SYNONYMS [10]

- Rosa tetrapetala
- Rosa wallichii
- Rosa polyphylla
- Rosa omeiensis

3.1.3. PLANT CONFIGURATION:

The rose, a tropical plant with unique structural characteristics, is widely admired for its beauty and medicinal properties, making it a popular subject of scientific study. A rose is a perennial plant with woody stems that can grow as upright shrubs, climbers, or groundcover. Its leaves are arranged alternately on the main stem. They are 2 to 6 inches long and pinnately compound, with 5 to 9 leaflets. The leaflets are often serrated and have small prickles on their undersides. These prickles, which are epidermal outgrowths, are typically curved hooks that help the rose climb. Rose flowers are diverse, varying in size, shape, and color, often large and showy. They typically have separate petals and numerous stamens, a feature of the Rose family. The petals are generally urn-shaped and roll outward. Roses readily hybridize, leading to a vast array of garden roses bred for their high petal count. The family yields many edible fruits, including apples, strawberries, cherries, blackberries, and rose hips. [11]

3.1.4. TRADITIONAL USES:

Roses are highly popular ornamental plants with around 200 species and 18,000 cultivars, primarily used for decoration and oil extraction. They are rich in vitamin C and used in food and medicine. Rose oil, especially from Rosa damascena, is one of the most valuable essential oils, requiring 3000 kg of petals to produce 1 kg of oil. In Pakistan, rose oil production is emerging, with cultivation in Punjab and Sindh. Farmers grow scented species like Rosa centifolia and Rosa bourboniana for year-round blooming. Rosa damascena is a key species for producing rose attar, used in perfumery and rose water for flavoring. Other species like R. gallica, R. centifolia, and R. bourboniana are also valued for their fragrance in the perfume industry. [13]

3.2. Centella Asiatica

Fig 2: Image of Thankuni

3.2.1. TAXONOMIC CLASSIFICATION: [14]

- **Kingdom:** Eukaryota
- **Sub-kingdom**: Embryophyta
- **Division**: Embryophyta
- **Sub-division:** Angiospermae
- **Class:** Dicotyledoneae
- **Sub-Class:** Rosidae Superorder Aralianae
- **Order:** Araliales (Umbelliflorae)
- **Family:** Apiaceae or Umbelliferae
- **Sub-family:** Hydrocotyle
- **Genus:** Centella
- **Species:** Centella asiatica

3.2.2. SYNONYMS [14]

- Hydrocotyle asiatica L.
- Hydrocotyle lunata Lam.
- Centella coriacea Nannfd.
- Centella cordifolia (Hooker fil.) Nannfd.
- Centella dusenni Nannfd.
- Centella floridana (C. et R.) Nannfd.
- Centella repanda (Pers.) Small.
- Centella triflora (R. et P.) Nannfd.
- Centella uniflora (Col.) Nannfd

3.2.3. PLANT CONFIGURATION:

Centella, a plant thriving in temperate and tropical wetlands worldwide, is characterized by its slender, creeping stems and long-stalked, rounded leaves. Its roots grow vertically downwards, and its small, white or crimson flowers are borne in rounded clusters near the soil's surface. The plant's fruit is distinctively reticulated, setting it apart from similar species. Centella is a rapidly spreading plant, considered a high-risk invasive species, and is known by various common names in different regions. [15]

3.2.4. TRADITIONAL USES:

The study investigated the nutritional content, phytochemical composition, antioxidant properties, and antimicrobial effects of **Centella asiatica** (Thankuni), a plant frequently used in herbal medication in Bangladesh for various health issues. This plant has been utilized as a vegetable for many years in regions such as China, Southeast Asia, India, Sri Lanka, Oceania, and Africa. In Southeast Asia, Centella asiatica is traditionally employed to address various disorders such as skin diseases, rheumatism, inflammation, syphilis, mental illnesses, epilepsy, hysteria, dehydration, and diarrhea. In India, Gotu Kola is prized for its ability to enhance memory and treat skin and nerve conditions. The medicinal benefits of this plant have been acknowledged in Java and Indonesia for centuries, and it has been recognized in China for over 2,000 years as one of the "miracle elixirs of life." Additionally, Centella asiatica is known for its adaptogenic properties, which help reduce stress responses and provide protection against chronic stress. In India, it is used to treat a variety of ailments, including body aches, headaches, insanity, asthma, leprosy, ulcers, eczema, and for wound healing. [16]

3.3. Swertia

Fig 3: Image of chirata

3.3.1. TAXONOMIC CLASSIFICATION: [17]

- **Phylum:** Tracheophyta
- **Class:** Magnoliopsida
- **Order:** Gentianales
- **Family:** Gentianaceae
- **Genus:** Swertia
- **Species:** Chirata

3.3.2. SYNONYMS [17]

- Charayatah
- Chirata
- Chiarayata
- Bhunimba
- Chiratika
- Nilavembu
- Chirayata
- Chirayita
- Charita
- Cherata

3.3.3. PLANT CONFIGURATION:

The plant is characterized by its relatively short, twisted roots measuring 5-10 centimeter in length and up to 1.25 centimeter in diameter. The stem, ranging from 60-125 cm in length and 6 mm in diameter, transitions from a cylindrical or slightly cylindrical base to a square-shaped top. It exhibits a smoothsurface with a color gradient from rusty brown or purplish brown to greenish-yellow or dark green.The leaves, arranged in opposite pairs, measure 3.5-10 cm in length and 1.5-4 cm in width. They are broadly oval or lance-shaped, with a smooth surface, 3-7 prominent veins, and a color change from dark green to reddish-brown (upper surface) and pale greenish-brown (lower surface) upon drying. The petiole is V-shaped in cross-section with a wide, concave upper surface and three prominent ridges. The central ridge, or midrib, measures approximately 1075-1750 µm horizontallyand 1000-1725 µm vertically. [18]

3.3.4. TRADITIONAL USES:

Swertia species are commonly utilized in traditional medicine in India, China, Bhutan, Nepal, and Japan, especially for their anti-inflammatory and anthelmintic effects. They are traditionally employed to address various health issues, including hepatitis, pneumonia, dysentery, liver disorders, gastric problems, jaundice, diabetes, and malaria. Their widespread medicinal applications highlight their therapeutic potential, emphasizing their importance in ethnomedicine across different cultures. Additional research is needed to explore and harness their complete pharmacological benefits. [19]

3.4. THE SPECIFIC PLANT PART UNDER INVESTIGATION:

Fig 4: Image of Rosa Petals

https://www.confettidirect.co.uk/product/red-rose-petals/

Fig 5: Image of Centella asiatica leaves

https://www.freepik.com/premium-photo/medicinal-thankuni-leaves-indian-subcontinent_21641324.htm

Fig 6: Image of Swertia Bark

https://www.shutterstock.com/image-photo/dry-kiratatikta-swertia-chirata-very-famous-2034906263

Chapter four: literature review

4.1. ROSE (ROSA)

4.1.1. THE ANTI-INFLAMMATORY AND ANTI-OXIDANT ACTIVITY OF ETHANOL EXTRACT FROM ROSE PETALS

Rose petals, often discarded, are abundant in phenolics with known anticancer, antioxidant, and anti-inflammatory benefits. This study examined the anti-inflammatory properties of red rose ethanolic extracts (GRP) on lipopolysaccharide (LPS)-activated RAW 264.7 cells. The findings revealed that pretreatment with GRP significantly lowered nitric oxide (NO) production by reducing the expression of inducible nitric oxide synthase (iNOS) in LPS- stimulated cells. Moreover, red rose petals inhibited NF-κB activation by blocking its translocation to the nucleus and suppressing IκB-α phosphorylation. These results highlight the significant anti-inflammatory potential of red rose petals, positioning them as a promising natural source of anti-inflammatory compounds. [20]

4.1.2. CHEMICAL AND TECHNOLOGICAL PROPERTIES OF ROSE (*ROSA CANINA* L.) FRUITS GROWN WILD IN TURKEY

The study examined the nutritional and technological properties of *Rosa canina* L. fruits (rosehips) from the Konya (Hadim) and Kastamonu regions. Fully ripe rosehips were found to contain a variety of nutrients, including ascorbic acid, crude protein, crude energy, and minerals such as potassium, phosphorus, magnesium, calcium, and iron. Measurements were also taken for technological characteristics like fruit size, weight, bulk density, and skin break resistance. Kastamonu rosehips were shown to have higher levels of dry matter, crude protein, ascorbic acid, and minerals, along with larger fruit size and weight compared to those from Hadim. Both regions' fruits demonstrated similar frictional properties. [21]

4.2. THANKUNI *(CENTELLA ASIATICA)*

4.2.1. ANTINOCICEPTIVE ACTIVITY EVALUATION OF LEAVES OF CENTELLA ASIATICA AND ZIZYPHUS MAURITIANA

This study explored the antinociceptive (pain-relieving) properties of methanolic extracts from *Zizyphus mauritiana* (MEZM) and *Centella asiatica* (MECA) leaves in Swiss albino mice, using an acetic acid-induced pain model. MEZM, given at doses of 50, 100, 200, and 400 mg/kg, significantly decreased abdominal constrictions by 31.2% to 62.5%. MEZM provided greater pain relief compared to aspirin, which reduced pain by 34.4% and 46.9% at doses of 200 and 400 mg/kg. MECA, at the same doses, showed even stronger effects, reducing pain by 46.9% to 71.9%. Dose-for-dose, MECA proved more effective than MEZM, and both extracts surpassed aspirin in pain relief. The findings suggest that these plant extracts, even in their crude form, could serve as affordable and accessible alternatives for pain management. Further research is needed to identify the active compounds responsible for these effects. [22]

4.2.2. EVALUATION OF NUTRITIONAL COMPOSITION, PHYTOCHEMICALS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF CENTELLA ASIATICA

The study investigated the nutritional content, phytochemical composition, antioxidant properties, and antimicrobial effects of Centella asiatica (Thankuni), a plant frequently used in herbal medication in Bangladesh for various health issues. Phytochemical analysis indicated the presence of tannins, saponins, flavonoids, and other beneficial compounds. The plant's nutrient profile revealed 42.96% carbohydrates, 16.44% minerals, and 17.02% crude fiber. Its antioxidant capacity was noteworthy, showing a DPPH value of 33.44 µg/ml, significantly higher than ascorbic acid's 9.22 µg/ml. Furthermore, ethanol extracts displayed considerable antimicrobial activity against Staphylococcus aureus and E. coli, with inhibition zones measuring 11 to 16 mm. These results suggest that Centella asiatica may serve as a viable alternative to synthetic antibiotics and be a valuable addition to a nutritious diet. [23]

4.3. CHIRATA *(SWERTIA)*

4.3.1. EVALUATION OF HYPOGLYCEMIC AND HYPOLIPIDEMIC PROPERTIES OF SWERTIA CHIRATA

The study examined the potential of **Swertia chirata**, sourced from Abbottabad, Pakistan, to reduce blood sugar and cholesterol levels in individuals with type 2 diabetes. Participants were given a powdered form of the plant orally. The results indicated that Swertia chirata significantly lowered fasting blood glucose levels by 14.5%, triglycerides by 10.5%, total cholesterol by 8.6%, and LDL cholesterol by 14.4%. Further research is needed to identify the specific compounds responsible for these beneficial effects. [24]

4.3.2. IN-VITRO THROMBOLYTIC AND ANTI-INFLAMMATORY ACTIVITY OF SWERTIA CHIRATA ETHANOLIC EXTRACT.

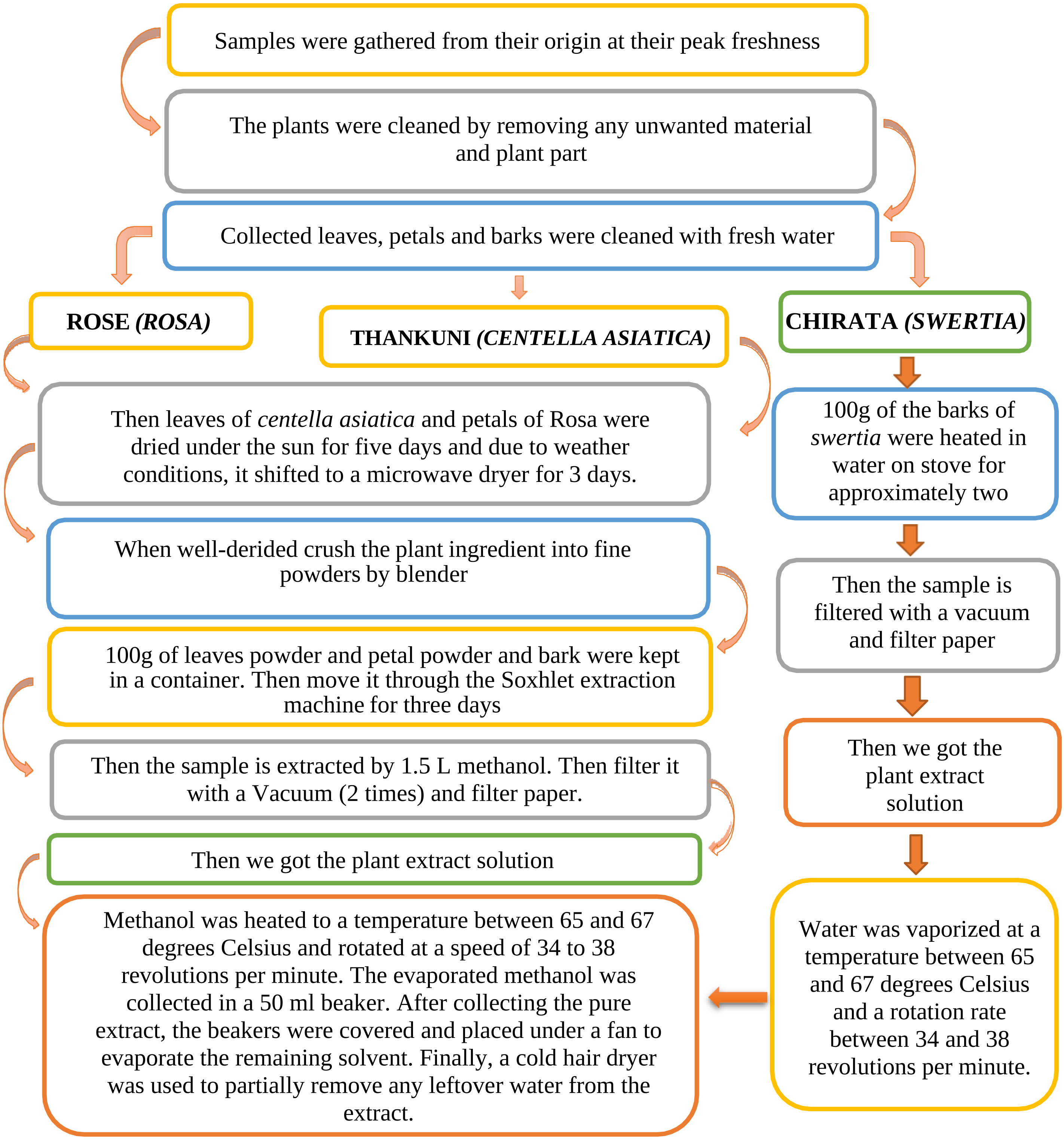
The study examined the ethanol extract of **Swertia chirata** for its thrombolytic and anti-inflammatory effects and included a phytochemical analysis. The anti-inflammatory activity was assessed in vitro using albumin denaturation, with aspirin serving as the standard comparator. The ethanol extract showed a moderate ability to prevent protein denaturation, with a mean inhibition of 45.31%. While the control group exhibited a slightly higher inhibition of 50.00%. In terms of clot-dissolving activity, the ethanol extract demonstrated a significant thrombolytic effect, achieving a maximum clot lysis of 40.38%, which is comparable to the standard drug, streptokinase. These findings suggest that **Swertia chirata** has considerable therapeutic potential for treating thrombolytic and anti-inflammatory conditions.[25]

CHAPTER FIVE:
METHOD AND MATERIAL

5.1 COLLECTION OF PLANT MATERIAL:

The leaves of ***centella asiatica***, petals of ***Rosa***, and bark of ***swertia*** of the experimental plants were gathered locally during the daytime in August 2024.

5.2 PREPARATION OF CRUDE EXTRACT:



5.3 SOXHLET EXTRACTION:

Soxhlet extraction is a method used to extract a specific substance from a mixture using a solvent. It's a continuous process that uses a small amount of solvent to efficiently extract a large quantity of the desired substance. This technique is useful when the desired substance doesn't dissolve well in the solvent. [26] [27]

The powdered leaves of *centella asiatica* and petals of Rosa were filled in two separate water bag and placed inside the thimble which is connected to a flask containing methanol

↓

Due to the attachment of condenser, the solvent is heated until it reaches its boiling point and starts to evaporate

↓

The vapor travels up to a distillation arm and enters the chamber where the thimble is located. The powder material in the chamber gradually filled with warm solvent which enable desired component to dissolve

↓

After approximately three days, when all the extract is collected into the distillation flask. It is then removed from the flask and poured into a beaker for drying

Fig 7: Soxhlet Extraction on our research lab D

5.5. FILTRATION

After the extraction process, the extracts are filtered twice using sterilized cotton and filter paper to ensure they are clean. Suitable solvents are poured through a funnel, and the filtered extract is collected in a glass container. This filtration process is repeated if needed. [28]

Fig 8: Filtration machine (Source of Internet)

https://www.mrclab.com/

5.6. ROTARY EVAPORATION

A rotary evaporator was used to concentrate the extract by removing the liquid. The extract was heated at 64-65°C and rotated at 34-38 RPM, and the liquid that evaporated was collectedin a 100 ml beaker. The concentrated extract was then dried using a fan and a hairdryer. [29] [30]

Fig 9: Rotary evaporation machine Source of our lab

5.7. PLANT CONSTITUENT EXAMINATION

Test Material:

Methanolic extract of leaves of ***centella asiatica***, petals of ***Rosa***, and aqueous extract of bark of ***swertia***

5.7.1. INSTRUMENT / APPARATUS

I. Electronic balance
II. Funnel
III. Filter paper
IV. Glass rod
V. Stand with a clamp
VI. Cotton
VII. Measuring Cylinder
VIII. Test-tube
IX. Spatula

5.7.2. REAGENTS

I. Mayer's reagent
II. Dragendorff's reagent
III. Benedict's reagent
IV. DPPH solution
V. 5.5% of Ferric chloride solution
VI. 10% potassium dichromate solution
VII. Dilute sulfuric acid
VIII. Distilled waters.
IX. Aqueous sodium hydroxide
X. Conc. HCl
XI. Conc. H2SO4.

Sample of leaves of *centella asiatica*, petals of *Rosa*, and bark of *swertia*: The examination of different chemical groups in the extract provides an initial overview of the phytochemical findings. The tests for these chemical groups are performed as described as follows: [31]

5.7.3. TEST FOR ALKALOIDS:

Mayer's reagent: When 2 ml extract was mixed with 0.2 ml of hydrochloric acid and Mayer's Reagent, a yellowish-buff substance formed, which suggests the presence of an alkaloid.

Dragendroff's reagent: A solution was made by combining 2 ml extract with 0.2 ml of hydrochloric acid. Then, Dragendorff's reagent was added to test for alkaloids. An orange-brown substance formed, indicating that alkaloids are present.

Figure 10: **Mayer's Test for Alkaloid**

Figure 11: **Dragendorff's Test for Alkaloid**

5.7.4. TEST FOR GLYCOSIDE:

In a test tube, take Small quantity of dried plant extract was combined with water and sodium hydroxide. The presence of a **yellow color indicated the presence of glycosides in the plant.**

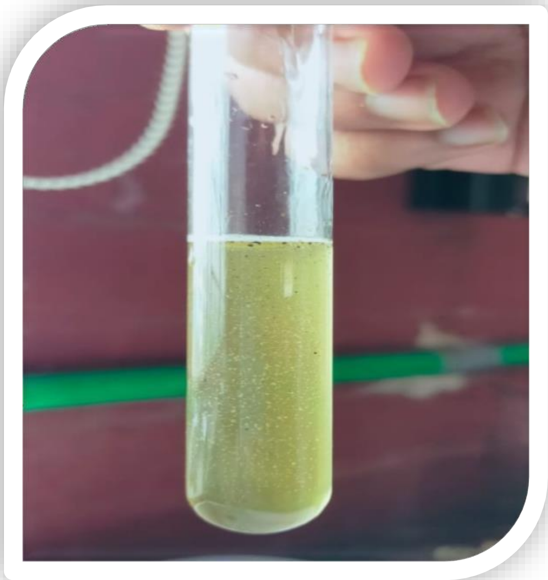


Fig 12: **Glycoside test**

5.7.5. TEST FOR STEROIDS:

In a test tube, take 10 milligrams of the extract were mixed with 1 ml chloroform. The chloroform turned reddish-brown, which suggests that there are steroids present.

Fig 13: **Steroid test**

5.7.6. TEST FOR GUMS:

In a test tube, to 5 milliliters of the extract, add Molisch reagent including few drops of sulfuric acid. Formation of red-violet ring at the interface of the two solution indicates the presence of gum.

Fig 14: **Gum test**

5.7.7. TESTS FOR FLAVONOIDS:

In a test tube, adding a few drops of concentrated hydrochloric acid to 1 milliliter of the ethanolic extract immediately produced a redish color, indicating the existence of flavonoids.

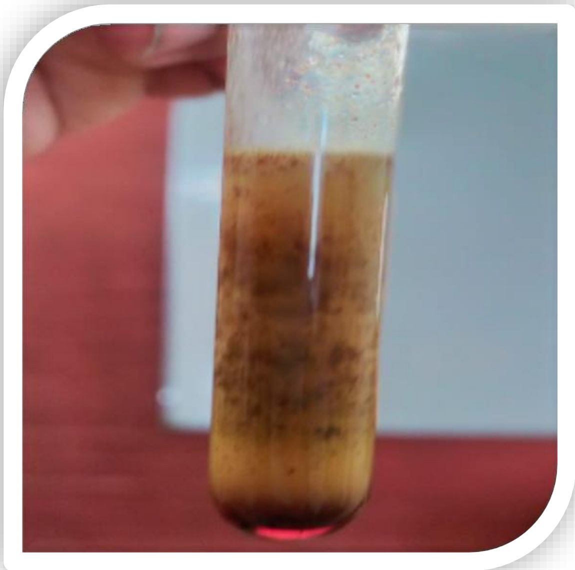


Fig 15: **Flavonoid test**

5.7.8. TESTS FOR SAPONINS:

In a test tube, take 1 ml solution of distilled water was diluted to 20 ml and shaken in a graduated cylinder for 15 minutes. The formation of a foam layer measuring one centimeter indicates the presence of saponins.

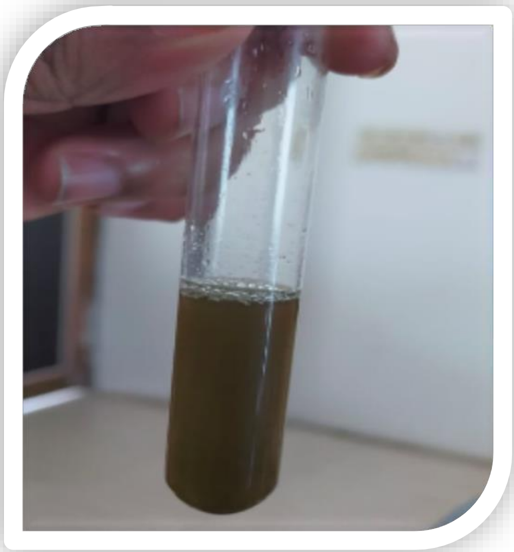


Fig 16: Saponin test

5.7.9. TESTS FOR TANNINS:

In a test tube, take 5 ml solution of the extract was mixed with 1 ml of 5% Ferric chloride solution. The formation of a greenish-black precipitate indicates the presence of tannins.

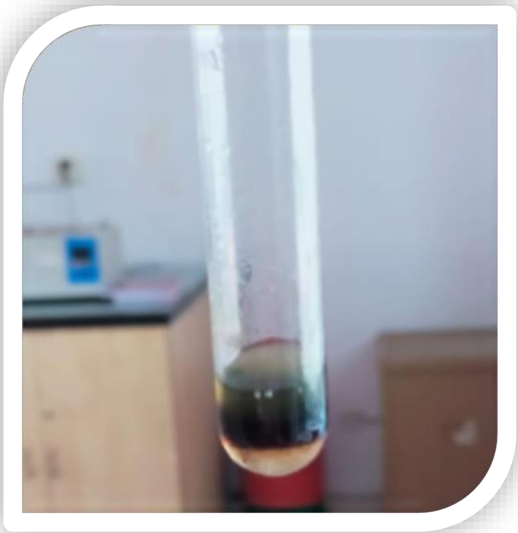


Fig 17: Tannins test

5.7.10. TEST FOR PHENOLS:

In a test tube, take 2 ml solution of extract with 3 or 4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

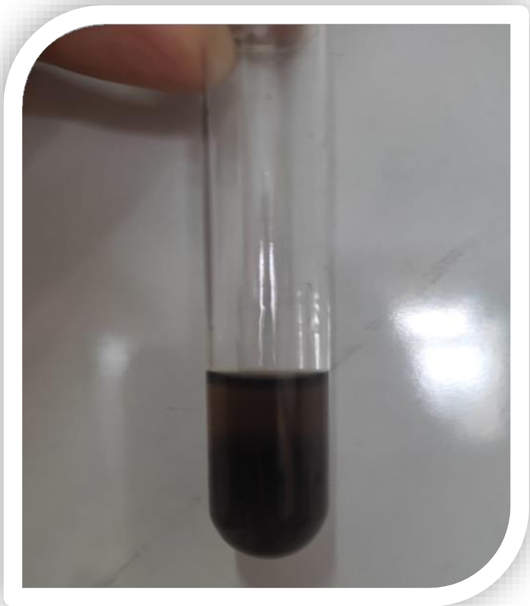


Fig 18: **Phenol Test**

CHAPTER SIX: EXPERIMENTAL DESIGN AND TEST PROCEDURE

6.1. IN-VITRO THROMBOLYTIC MODEL:

To evaluate the clot-dissolving (thrombolytic) properties of methanolic extracts of *Centella asiatica* leaves, petals of *Rosa*, and aqueous extract of *Swertia* bark. [32]

6.2. REAGENTS REQUIRED:

I. Blood (Human)
II. Streptokinase (Standard)
III. Distilled water (Controlled)
IV. Extracted Sample

6.3. PREPARATION OF STANDARD:

Streptokinase is a medication used to dissolve blood clots. It is available in a lyophilized form, which means it is dried and needs to be reconstituted with water before use. In this case, a vial of Streptokinase containing 15,00,000 International Units (I.U.) was reconstituted with 5 ml of sterile distilled water. The resulting suspension was then used as a stock solution, from which 100 microliters (µl) were taken containing 30000 IU streptokinase for an in vitro thrombolysis experiment. [32]

6.4. PREPARATION OF BLANK:

1 ml of blood was taken in a tube and incubated for 45 minutes. Followed by, addition of **100 µl** of distilled water to prepare the controlled solution.

6.5. EXTRACT SOLUTION PREPARATION:

25 mg of Methanolic extract of leaves of *Centella asiatica*, petals of *Rosa*, and aqueous extract of bark of *Swertia* were dissolved in 2.5ml of Distill Water and left to stand for 24 hours. On the following day, the prepared solution was used to carry the Thrombolytic Test.

6.6. TEST PROCEDURE:

A healthy person was chosen for the experiment

5 ml of blood was taken from the person and put into 5 different, pre-weighed, sterile tubes (1ml/tube) after recording the blank tube weight.

The tubes were kept at a temperature of 37 ^{o}C for 45 minutes in an

After the blood clot formed, the blood serum was carefully removed from the tubes without touching the clot.

Each tube containing the clot was weighed again to find out how much the clot weighed by,
1st clot weight = 1stclot with tube weight – blank tube weight

To each tube containing clot after appropriate labeling, followed by addition of 100 µl of methanolic extract of leaves of ***centella asiatica***, petals of ***Rosa***, and aqueous extract of bark of ***swertia***.

Then, addition to positive control tube dose of standard **100 µl Streptokinase** and negative control tube **100 µl distilled water**.

All tubes were incubated at 37 degrees Celsius for 90 minutes and then observed for clot lysis.

Then, serum on top of the clot was carefully removed and the tubes were weighed again to see how much the clot had changed in weight.,
2nd clot weight = 2nd clot with tube weight – blank tube weight

Difference between weight taken before and after clot lysis was plot as ratio to obtain the percent of clot lysis,

$$\% \text{ of lysis} = \frac{\text{weight of Lysis clot } (1^{st} \text{ clot weight} - 2^{nd} \text{ clot weight})}{1^{st} \text{ clot weight}} \times 100$$

[32] [34]

6.7. STANDARD DEVIATION FOR COMBINED PLANT EXTRACT:

Three different plant extracts were combined with the help of a pipette in equal proportions (0.5ml) to evaluate their combined thrombolytic activity. The activity was recorded three times. Finally, their mean was calculated in order to measure the Standard Deviation of the mean value of the combined sample between those three readings obtained.

Formula:

Standard deviation = $\sqrt{(\Sigma (x - \text{mean})^2 / n)}$

Where:

Σ = summation

x = individual value

mean = average of all values

n = total number of values

1. Mean = 24.58%
2. Differences:

   C = (21.03 - 24.58) % = -3.51%

   T = (27.76 – 24.58) % = 3.22%

   R = (24.94 – 24.58) % = 0.16%
3. Squared differences:

   C = $(-3.51\%)^2$ = 12.32%

   T = $(3.22)^2$ = 10.37%

   R = $(0.16)^2$ = 0.4%
4. Average of squared differences:

   (12.32% + 10.37% + 0.16%) / 3 = 7.62%
5. Standard deviation:

   $\sqrt{7.62\%} \approx$ **2.76%**

   Therefore, the standard deviation for the sample is approximately **2.76%.** [33]

CHAPTER SEVEN:
RESULT AND DISCUSSION

7.1 PHYTOCHEMICAL ANALYSIS:

PHYTOCHEMICAL COMPONENTS	RESULT
Alkaloid test	+
Mayer's Test Dragendorff's Test	
Glycoside test	-
Phenol test	+
Gum test	+
Steroids test	+
Flavonoid test	-
Saponins test	+
Tannins test	+

Table 1: Qualitative analysis of Methanolic extract of petals of ***Rosa.***

(+) Implies presence, (-) Implies absence

PHYTOCHEMICAL COMPONENTS	RESULT
Alkaloid test	+
Mayer's Test Dragendorff's Test	
Glycoside test	+
Phenol test	+
Gum test	+
Steroids test	-
Flavonoid test	-
Saponins test	-
Tannins test	+

Table 2: Qualitative analysis of Methanolic extract of leaves of ***centella asiatica***.

(+) Implies presence, (-) Implies absence

PHYTOCHEMICAL COMPONENTS	RESULT
Alkaloid test	+
Mayer's Test Dragendorff's Test	
Glycoside test	+
Phenol test	-
Gum test	+
Steroids test	+
Flavonoid test	-
Saponins test	-
Tannins test	-

Table 3: Qualitative analysis of Methanolic extract of leaves of ***Swertia***.

(+) Implies presence, (-) Implies absence

7.2. INDIVIDUAL ACTIVITY RESULTS:

1 ml of samples were taken from three different plants and thrombolytic activity of each of them were individually observed.

Serial	Tube weight (g)	1st clot weight with tube (g)	Weight of 1st clot (g)	2nd clot with tube (g)	Weight of 2nd clot (g)	% of lysis
Blank	0.672	1.712	1.4	1.532	0.86	17.31
Standard	0.710	1.76	1.5	1.004	0.294	72
Swertia	0.764	1.711	0.947	1.425	0.661	30.20
centella asiatica	0.776	1.674	0.898	1.497	0.721	19.71
Rosa	0.775	1.775	1.0	1.433	0.658	35.67

Table 4: Individual observation on Thrombolytic Activity Test of Methanolic Extract of ***Swertia, centella asiatica & Rosa***

7.3. DISCUSSION ON INDIVIDUAL THROMBOLYTIC ACTIVITY:

This study investigated the thrombolytic potential of three plant-based extract individually. The results demonstrated that all three samples exhibited some degree of thrombolytic activity. Among them, *Rosa* exhibited the moderate thrombolytic activity, while ***Swertia and centella asiatica*** demonstrated mild thrombolytic properties, However, none of the samples reached the potency of the standard thrombolytic agent.

7.4. COMBINATION ACTIVITY RESULTS:

Three different samples from different plant were combined by adding 0.5 ml from each extract solution and thrombolytic activity was observed.

Serial	Tube weight (g)	1st clot weight with tube (g)	Weight of 1st clot (g)	2nd clot with tube (g)	Weight of 2nd clot (g)	% of lysis	Standard Deviation of three Samples (%)
Blank	0.672	1.712	1.4	1.532	0.86	17.31	--
Standard	0.710	1.76	1.5	1.004	0.294	72	--
Swertia* + *centella asiatica* + *Rosa	0.774	1.663	0.889	1.476	0.702	21.03	2.76
	0.865	1.636	0.771	1.422	0.557	27.76	
	0.774	1.636	0.862	1.422	0.647	24.94	

Table 5: Combined observation on Thrombolytic Activity Test of Methanolic Extract of ***Swertia, centella asiatica & Rosa***

7.5. DISCUSSION ON COMBINED THROMBOLYTIC ACTIVITY:

The thrombolytic potential of combination between Swertia, *centella asiatica & Rosa* was evaluated which indicate varying levels of clot lysis activity among the three replicates. The standard deviation of the three samples were calculated to be 2.76%. This suggests a moderate level of variability in the thrombolytic activity of the combined extract, which could be attributedto factors such as natural variations in plant composition or experimental errors. While the combined sample exhibited promising thrombolytic activity in some replicates, it did not consistently outperform the standard thrombolytic agent. The highest clot lysis observed was 27.76%, which, although significant, was lower than the 72% lysis achieved by the standard.

CHAPTER EIGHT: CONCLUSION

8.1 CONCLUSION:

The samples showed varying levels of thrombolytic activity, with methanolic extract of petals of *Rosa* being the most promising, while aqueous barks extract of *Swertia* and methanolic leaves extract of *Centella asiatica* exhibits clement thrombolytic activity. However, none of them matched the standard's potency. The combined extract of these three-sample exhibited varying levels of thrombolytic activity with mean moderate activity, suggesting potential for further investigation as a potential treatment option.

CHAPTER NINE: REFERENCE

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