

IN-VITRO THROMBOLYTIC ACTIVITY OF ETHANOLIC EXTRACT OF SOME TRADITIONAL PLANTS

**A THESIS
BY**

**SHAMIM AHMED
STUDENT ID: 1504048
SEMESTER: JANUARY-JUNE, 2024**

**MASTER OF SCIENCE (M.S.)
IN
PHARMACOLOGY**



**DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY,
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*Submitted to
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Hajee Mohammad Danesh Science and Technology University, Dinajpur,
In Partial fulfillment of the requirements
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JUNE, 2024

DEDICATED TO
MY
BELOVED PARENTS
&
RESPECTED TEACHERS

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All praises are to the Almighty Allah, who kindly enables the author to complete the present research work successfully and to submit the thesis leading to Master of Science (MS) degree in Pharmacology.

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The Author

June, 2024

ABSTRACT

Atherothrombosis or thrombosis is a major cause of global life threatening heart and cerebral diseases. For the treatment of myocardial infarction, many thrombolytic agents are used. Among them, streptokinase is remarkable and widely used. But it is noted that all available thrombolytic agents still have significant deficiencies, including the necessity of large doses to be maximally effective, limited fibrin specificity and a significant associated bleeding tendency and high cost. Herbal agent or thrombolytic plants can solve those problems. Consider this, present study was designed to investigate thrombolytic activity of some traditional plants. Some medicinal plants and spices were collected from Dinajpur district. Primarily ethanol extract was prepared from those plant and spices. 12 micro pipettes were taken filled with human blood and 6 micropipettes were taken as excess micropipette for positive and negative control. These two kinds of micropipette were screened for assessment their comparative efficacy of clotted blood lysis. Ethanol extract was used at a dose of 100 micro ml/micro pipette. Then this micro pipette was incubated at 37 degree centigrade temperature for 90 minutes. After incubation I found 2 plants and 2 spices have thrombolytic activity. These plants are *Blumea lacera* (Kukurmutha), *Persicaria hydropiper* (Bishkatali) and spices are *Trigonella foenum* (methi) and *Carum copticum* (Ajawin). Finally, I experimented those plants in laboratory compared with Streptokinase (SK) as positive control and water as negative control. Streptokinase showed 75.18% efficacy after treatment water showed 2.28% efficacy after treatment. Efficacy was recorded by compare among streptokinase control treated extract. Ethanol extract of Ajawin, methi, Kukurmutha and Bishkatali lysis clotted blood 57.25%, 41.31%, 45.00%, 35.52% respectively. In those result show that the efficacy of ajawan extract is more efficient than other extracts.

Keywords: *Thrombolytic activity, streptokinase, thrombus, plant extract. Blumea lacera, Persicaria hydropiper, Trigonella foenum Carum copticum,*

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LIST OF ABBREVIATIONS

B.wt.	:	Body weight
BAU	:	Bangladesh Agricultural University
Conc.	:	Concentration
Cu mm	:	Cubic millimeter
d.w.	:	Drinking water
ESR	:	Erythrocyte Sedimentation Rate
<i>et al.</i>	:	Associates
Fig.	:	Figure
Gm	:	Gram
Hb	:	Hemoglobin
i.e.	:	That is
J.	:	Journal
Kg	:	Kilogram
Lit	:	Liter
Ltd.	:	Limited
Mg	:	Milligram
ml	:	Milliliter
mm ³	:	cubic millimeter
No.	:	Number
PBS	:	Phosphate Buffer Solution
PCV	:	Packed Cell
PM	:	Population Mean
SE	:	Standard Error

LIST OF ABBREVIATIONS (contd.)

SM	:	Sample Mean
TEC	:	Total Erythrocyte Count
Vol.	:	Volume
µg	:	Microgram
%	:	Percent
&	:	And
@	:	At the rate of
<	:	Less than
>	:	Greater than
±	:	Plus minus
0°C	:	Degree centigrade

CHAPTER ONE

INTRODUCTION

CHAPTER ONE

INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomena of the symbiosis. Natural products from plants, animals and minerals have been the basis of the treatment of human disease. About 500 plants with medicinal use are mentioned in ancient literature and around 800 have been used in indigenous system of medicine. Bangladesh is a vast repository of medicinal plants that are used in traditional medical treatment.

Thrombus (blood clot) can develop in blood circulatory system because of homeostasis which causes vascular blockage that can be responsible to health threatening results. Atherothrombotic diseases like cerebral or acute myocardial infarction can also even lead to death. Thrombolysis is a complex mechanism which interacts with clot components and surrounding plasma. In this interaction, plasmin, plasminogen, plasminogen activator and fibrin are involved. The thrombolytic activity of plasma is physiologically very important. Usually, thrombolytic agents are anistreplase, streptokinase, alteplase, TPA (tissue plasminogen activator) and urokinase. Although there are several thrombolytic drugs with those obtained by recombinant DNA technology, but side effects related to some of these drugs that lead to further difficulties have been reported (Baruah *et al.*, 2006; Gallus *et al.*, 1998; Wardlaw *et al.*, 2004 and Capstick *et al.*, 2005).

All available thrombolytic agents still have significant shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity and bleeding tendency. Due to shortcoming of accessible thrombolytic drugs, studies are underway to design new improved recombinants variants regimen. Aspirin and Heparin are significantly effective for activation of lysis and prevention of reocclusion. The selective antiplatelet agents and thrombin inhibitor are most potent though safety is yet a main concern. The new studies and investigations in this area will give new imminent that encourage the advancement of the ideal thrombolytic therapy. The use of herbal for treatment of disease has been in practice since ancient times. Herbal medicines are considered safer due to their natural activity. It has been reported from studies that herbal products showing their thrombolytic activity significantly. With the advancement in phytochemistry and identification of new plants compounds having significant efficacy

against certain diseases, it has been proved by research conducted on herbal medicines. The anti-thrombotic activity of herbs and their natural compound has reported previously.

Recently blood clot formation has been a severe problem of blood circulation. Thrombus or embolus hinders the blood flow by blocking the blood vessel therefore depriving tissues of normal blood flow and oxygen. These consequence yield necrosis of the tissue in that area. Thrombin formed blood clot from fibrinogen and is lysed by plasmin, which is activated from plasminogen by tissue plasminogen activator (tPA). The purpose of a fibrinolytic drug is to dissolve thrombin in acutely occluded coronary arteries thereby to restore blood supply to ischemic myocardium, to limit necrosis and to improve prognosis (Laurence *et al.*, 1992).

For the treatment of myocardial infarction, many thrombolytic agents are used. Among them, streptokinase is remarkable and widely used. Moreover, Tissue-type Plasminogen activator is more effective and safer than either urokinase or streptokinase type activators. It is noted that all available thrombolytic agents still have significant deficiencies, including the necessity of large doses to be maximally effective, limited fibrin specificity and a significant associated bleeding tendency. Therefore, steps are taken to develop improved recombinant variants of these drugs in order to minimize deficiencies of the available thrombolytic drugs (Adams *et al.*, 1991; Nicolini *et al.*, 1992; Lijnen *et al.*, 1991; Marder 1993; Wu *et al.*, 2006).

The present study was carried out to investigate thrombolytic activity of three plants available in Bangladesh. Streptokinase (SK), a known thrombolytic drug is used as a positive control (Prasad *et al.*, 2007). Water, on the other hand, was designated as a negative control. The comparison of positive control with negative control clearly confirmed that clot dissolution occurs in negligible when water was added to the clot. By comparing with this positive & negative control, a significant thrombolytic activity was observed after treating the clots with *Carum copticum*, *Trigonella foenum*, *Bluea lacera*, *Persicaria hydropiper* extracts.

Recognizing the thrombolytic activity of *Trigonella foenum* (methi), *Carum copticum* (Ajawin) *Blumea lacera* (Kakurmutha) and *Persicaria hydropiper* (Bishkatali), this study aimed to evaluate the ethanolic extracts of these spices using the in vitro method. The extraction process utilized a Soxhlet apparatus. Results indicated that the ajawin ethanol extract exhibited superior thrombolysis compared to the methi extract,

Kakurmutha extract and Bishkatali extract. Moreover, the concentration of phytochemicals and incubation time correlated directly with clot lysis. The implications of this study suggest that the application of methi, ajawin, kakurmutha, Bishkatali extracts could provide accessible and cost-effective treatment options for cardiovascular diseases, potentially replacing expensive and side-effect-laden synthetic drugs like streptokinase, t-PA, and Urokinase.

The study was designed with the following objectives:

- To observe the thrombolytic activity of spices and plants extract.

CHAPTER TWO
REVIEW OF LITERATURE

CHAPTER TWO

REVIEW OF LITERATURE

Cardiovascular diseases are significant health issues, leading to millions of deaths annually. Thrombosis stands out as a primary cause of thromboembolic disorders, impacting millions world wide. Various plants utilized in traditional medicine for treating thromboembolic diseases have displayed anticoagulant or antithrombotic properties, yet their scientific investigation remains incomplete. Anticoagulant medications such as heparins and vitamin K antagonists, along with their derivatives, have dominated clinical treatment for over five decades, despite well-documented life-threatening side effects. Plants offer potential as alternative sources for developing new anticoagulant agents due to their biological activities. Scientific evidence supports the notion that consuming dietary anticoagulants or phytochemicals with anticoagulant properties can potentially reduce or eliminate the risks associated with thromboembolic diseases.

Mohammad Shahadat Hossain, *et al.* (2012) The ethanol extract of *Swertia chirata* was evaluated for its thrombolytic and anti-inflammatory activity, as well as phytochemical screening. In vitro anti-inflammatory activity was assessed using albumin denaturation, with aspirin as the standard drug. The extract showed a mean inhibition of protein denaturation at 45.31 ± 0.000576 , compared to 50.00 ± 0.00177 for the control group. In the thrombolytic activity assay, the crude ethanol extract exhibited significant clot lysis with a maximum effect of 40.38%, while standard streptokinase showed 69.35% effectiveness.

Md. R. Al-Mamun *et al.* (2012) Using an in vitro model, we evaluated the thrombolytic activities of several plants and spices available in Bangladesh. Results indicated significant thrombolytic activity in certain species. The percentage of weight loss of in vitro formed clots were as follows: *Lawsonia inermis* (Lythraceae) $62.40 \pm 5.04\%$, *Curcuma longa* (Zingiberaceae) $53.32 \pm 4.96\%$, *Coriandrum sativum* (Apiaceae) $43.25 \pm 7.18\%$, *Mesua nagassarium* leaf (Clusiaceae) $46.75 \pm 3.97\%$, and others. Notably, *Lawsonia inermis* and *Curcuma longa* demonstrated thrombolytic properties capable of lysing blood clots in vitro.

Evangelista *et al.*, (2012) The study explored the preliminary assessment of in vitro anticoagulant activity compared to Heparin 1, 000 I.U. and cytotoxicity of selected medicinal plants from the Philippines. Thromboembolic disorders, a leading cause of mortality in developing nations, necessitate effective treatments with minimal side effects. Herbal plants, with their historical medicinal uses, are increasingly prominent in drug discovery. The Philippines, boasting a rich variety of herbal plants, provided the focus for this research. The aim was to evaluate both flesh and peels/seeds of *Allium sativum*, *Curcuma longa*, *Ananas comosus*, and *Lycopersicum esculentum*. In vitro methods, with Heparin and water as controls, were employed to assess percentage clot lysis, which ranged from 15.67% to 35.91%. Notably, only *C. longa* peel extracts and *L. esculentum* showed clot lysis beyond the negative control, with *C. longa* peels demonstrating higher clot lysis compared to Heparin. Statistical analysis using one-way ANOVA yielded a p-value of 0.674574 for clot lysis activity. Cytotoxicity was evaluated using the brine shrimp lethality assay (BSLA), revealing LC50 values ranging from 6.72 to 31.2µg/mL. *A. sativum* and *C. longa* flesh displayed the lowest values, indicating their potency among the extracts. Descriptively, *C. longa* peel extract exhibited promising clot lysis activity, although further studies are necessary to confirm the effectiveness of these Philippine plants as anticoagulants. Additionally, the study highlighted the potential therapeutic application of fruit and vegetable peels, offering benefits in both waste reduction and as alternative sources of pharmacologic agents.

Narjis Hadi Mansoor *et al.*, (2013) The anticoagulant activity of some plant extracts was examined in vitro using prothrombin time. The extracts tested included red onion, garlic oil, and grape oil.

Md.AIAminSikder *et al.* (2011) The thrombolytic activity of four Bangladeshi medicinal plants was investigated to explore their potential as renewable sources for thrombolytic compounds. *Sansevieria atrifasciata*, *Justicia gendarussa*, *Hydnocarpus kurzii*, and *Mesua nagassarium* were examined for their ability to lyse clots in vitro. The evaluation involved adding the test material to pre-clotted blood and incubating it for 90 minutes at 37°C, with clot lysis expressed as a percentage. Each plant was methanol-extracted at room temperature, and the concentrated methanolic extract was fractionated using the modified Kupchan partitioning method to obtain pet-ether, carbon tetrachloride, chloroform, and aqueous soluble fractions. The study found that the aqueous soluble fraction of *Mesua nagassarium*, the carbon tetrachloride soluble fraction

of *Hydnocarpus kurzii*, and the aqueous soluble fraction of the methanolic extract of *Sansevieria trifasciata* exhibited the highest thrombolytic activity, with clot lysis values of 50.86%, 47.50%, and 47.10% respectively. Notably, the pet ether and carbon tetrachloride soluble fractions of the methanolic extract of *Justicia gendarussa* also demonstrated significant thrombolytic activity, with clot lysis values of 45.93% and 45.47% respectively. Standard streptokinase served as a positive control, exhibiting 61.50% clot lysis, while water, the negative control, showed 2.56% clot lysis.

Prasad *et al.* (2012) Exploring the Impact of *Fagonia arabica* (Dhamasa) on In Vitro Thrombolysis, A BioMed Central Investigation Atherothrombotic diseases like myocardial or cerebral infarction pose significant risks due to blood vessel thrombosis. While thrombolytic agents dissolve existing blood clots, their efficacy is limited by potential adverse effects, sometimes resulting in severe or fatal outcomes. Herbal remedies, with a long history of use, have been employed for treating various ailments. Although some herbs and their components exhibit antithrombotic properties, none have been identified specifically for thrombolysis. This study aims to assess whether herbal preparations, specifically aqueous extracts, demonstrate thrombolytic activity.

Parvin *et al.*, (2013) The study investigated the phytochemical compositions, thrombolytic activities, and antimicrobial properties of *Lablab purpureus* leaf extracts. Thrombolytic activities were evaluated using human erythrocytes and compared with standard streptokinase (SK). Additionally, the leaf extracts of *L. purpureus* exhibited moderate antibacterial activity against select microorganisms. Preliminary phytochemical analysis indicated the presence of reducing sugar groups, tannins, saponins, and alkaloids.

Rahman *et al.* (2013) The study investigated the effects of organic extracts from six Bangladeshi plants on in vitro thrombolysis and cytotoxicity. It was conducted as part of BMC Complementary and Alternative Medicine.

Ratnasooriya *et al.*, (2008) observed the thrombolytic potential of black tea [*Camellia sinensis* (L) O. Kuntze.] in vitro, using Sri Lankan high grown Dust grade No: 1 black tea and goat blood. Different concentrations of tea were made in isotonic saline (2.5, 5, 10 and 20 mg/mL) and added to preclotted blood and the clot lysis activity was determined (in terms of weight and expressed as % of lysis) following 90 minute incubation at 37 °C. The results showed that tea induced mild to moderate (121-520%)

and significant ($p < 0.05$) clot lysis activity. This effect was dose-related. However, the clot lytic activity of black tea was markedly lower than streptokinase (0.1 mg/mL). It is concluded that Sri Lankan black tea possesses fast acting mild to moderate thrombolytic activity in vitro when tested on goat blood the thrombolytic potential of black tea [*Camellia sinensis* (L) O. Kuntze.] in vitro, using Sri Lankan high grown Dust grade No: 1 black tea and goat blood. Different concentrations of tea were made in isotonic saline (2.5, 5, 10 and 20 mg/mL) and added to preclotted blood and the clot lysis activity was determined (in terms of weight and expressed as % of lysis) following 90 minute incubation at 37 o C. The results showed that tea induced mild to moderate (121-520%) and significant ($p < 0.05$) clot lysis activity. This effect was dose-related. However, the clot lytic activity of black tea was markedly lower than streptokinase (0.1 mg/mL). It is concluded that Sri Lankan black tea possesses fast acting mild to moderate thrombolytic activity in vitro when tested on goat blood the thrombolytic potential of black tea [*Camellia sinensis* (L) O. Kuntze.] in vitro, using Sri Lankan high grown Dust grade No: 1 black tea and goat blood. Different concentrations of tea were made in isotonic saline (2.5, 5, 10 and 20 mg/mL) and added to preclotted blood and the clot lysis activity was determined (in terms of weight and expressed as % of lysis) following 90 minute incubation at 37 o C. The results showed that tea induced mild to moderate (121-520%) and significant ($p < 0.05$) clot lysis activity. This effect was dose-related. However, the clot lytic activity of black tea was markedly lower than streptokinase (0.1 mg/mL). It is concluded that Sri Lankan black tea possesses fast acting mild to moderate thrombolytic activity in vitro when tested on goat blood the thrombolytic potential of black tea [*Camellia sinensis* (L) O. Kuntze.] in vitro, using Sri Lankan high grown Dust grade No: 1 black tea and goat blood. Different concentrations of tea were made in isotonic saline (2.5, 5, 10 and 20 mg/mL) and added to pre-clotted blood and the clot lysis activity was

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QuratU Ai et al. (2022) investigated the anticoagulant and thrombolytic activities of *Mangifera indica* in smokers. Methods: The thrombolytic and anticoagulant (in vitro and in vivo) activities, phytochemical screening and high performance liquid chromatography of aqueousmethanolic (70:30) leaf extract of *M. indica* have been investigated. Results: For *in vitro* experiment *M. indica* displayed a noteworthy ($p < 0.05$) increment in prothrombin time, clotting time and activated partial thromboplastin time while *in vivo* experiment noteworthy ($p < 0.05$) increase in clotting time, bleeding time, activated partial thromboplastin time and prothrombin time in a dose dependent manner in rabbits after one week of treatment while heparin being taken as a positive and distilled water being taken as negative control. For *in vitro* experiment, aqueous-methanolic extract in a dose dependent manner displayed noteworthy ($p < 0.05$) clot lysis while streptokinase being taken as a positive and distilled water being taken as negative control. Conclusion: HPLC showed the presence of mangiferin, quercetin and isoquercetin which down regulate the activity of factor Xa as well as act on antithrombin and thrombin respectively, in coagulation cascade.

Phytomed et al., (2014) The thrombolytic activity of *A. bilimbi*, *C. viscosum* and *D. quercifolia* extracts were determined as a part of exploration of cardio protective drugs from plants reserves, and the conclusion are conferred in. It presents 41.05 % lysis of clot in a process of subsequent incubation for 90 minutes at 37°C, where 100µl SK was added as a positive control (30, 000 I.U.). On the other side, it showed negligible percentages of lysis of clot (3.31 %), when we use distilled water as a negative control.

CHAPTER THREE
MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1 Site of the Experiment:

The experiment was conducted at the department of Physiology and Pharmacology, Hajee Mohammad Danesh Science & Technology University, Dinajpur.

Some plants are collected from the HSTU campus, Dinajpur and spices sample was collected from Bahadur Bazar Market, Dinajpur. *Carum copticum* (Ajawin) and *Trigonella foenum-graecum* (Methi) seeds were purchased from the local market Bahadur Bazar in Dinajpur, Bangladesh. *Blumea lacera* (Kakurmutha) and *Persicaria hydropiper* (Bishkatali) were collected from local farm land.

3.2 Experimental duration:

This study was conducted during one month from collection plants and spices in February to March, 2024.

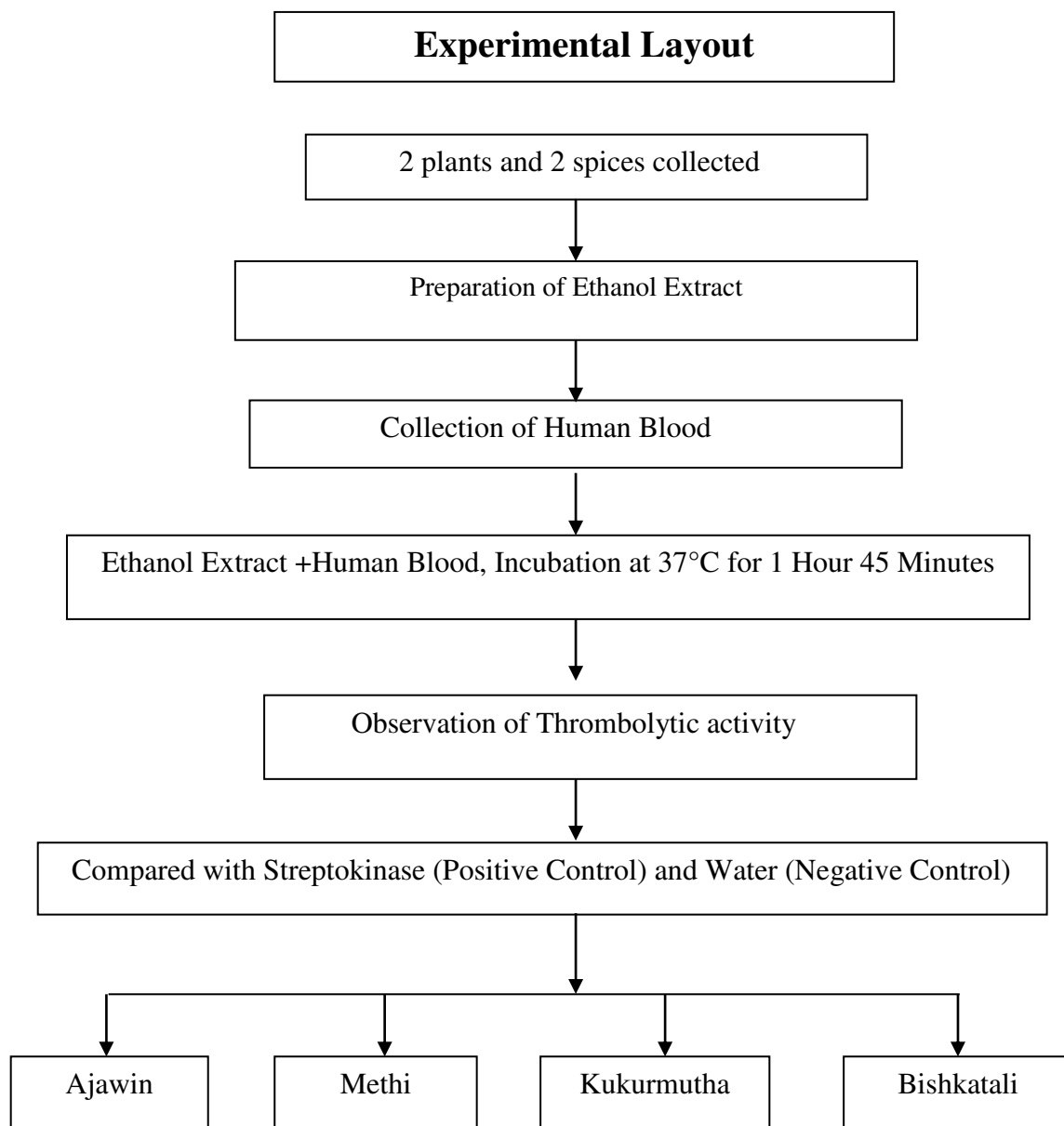


Figure 1: Experimental Layout

To complete the research work following steps were followed:

3.3 Collection of the plant and spices

Some plants are collected from the HSTU campus, Dinajpur and spices sample was collected from Bahadur Bazar Market, Dinajpur. *Carum copticum* (Ajawin) and *Trigonella foenum* (Methi) seeds were purchased from the local market Bahadur Bazar in Dinajpur, Bangladesh. *Blumea lacera* (kakurmutha) and *Persicaria hydropiper* (Bishkatali) were collected from local farm land.

3.4 Preparation of Plants Extract

Preparation of plants: The plants were washed with fresh water and dried under shade at room temperature and dried in open sun for 10 days and followed by oven 55-60°C for 2days. The dried leaves were pulverized with a blender. A 25 (unit) mesh diameter sieve was used to obtain the fine dust, after the dust was preserved in air tight plastic contain until they were directly used for screening and preparation of water extract. 10g each leaves powder was added to 70ml of distilled water and was shaking overnight at room temperature, filtered and distilled water was added upto 100 ml to make 10% extract.

- **Ehanol Treatment:** The dried seeds were soaked in ethanol for two days.

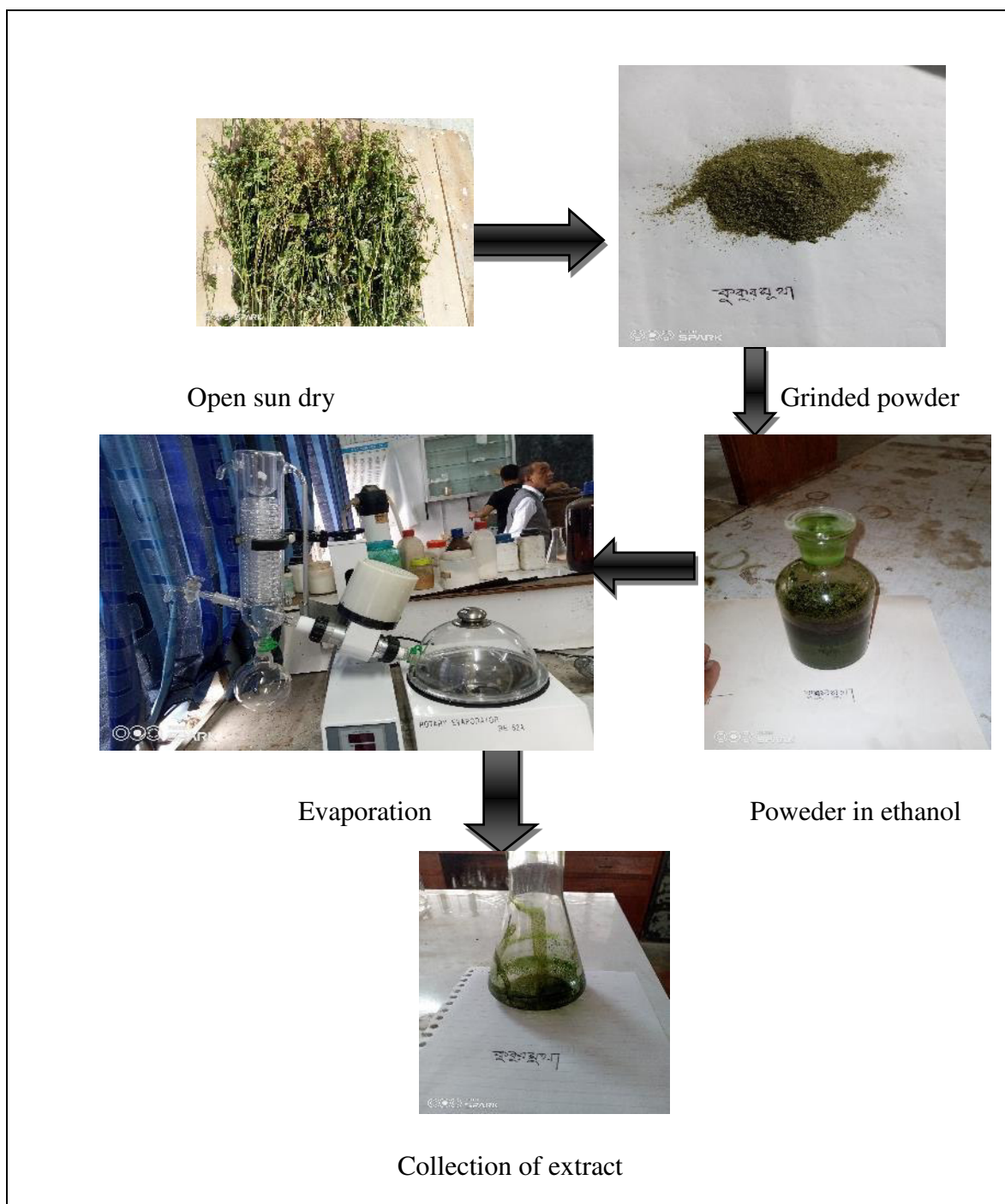


Figure 2: Process of Ehtanolic Extract of *Blumea lacera* (Kukurmutha)

- **Grinding and Extraction:** The seeds were ground using a mortar and pestle, and the liquid portion was squeezed out by hand. The resulting mixture was centrifuged at 1000 rpm to separate the components. Water from the centrifugal material was dried out, and the remaining material was powdered and stored.

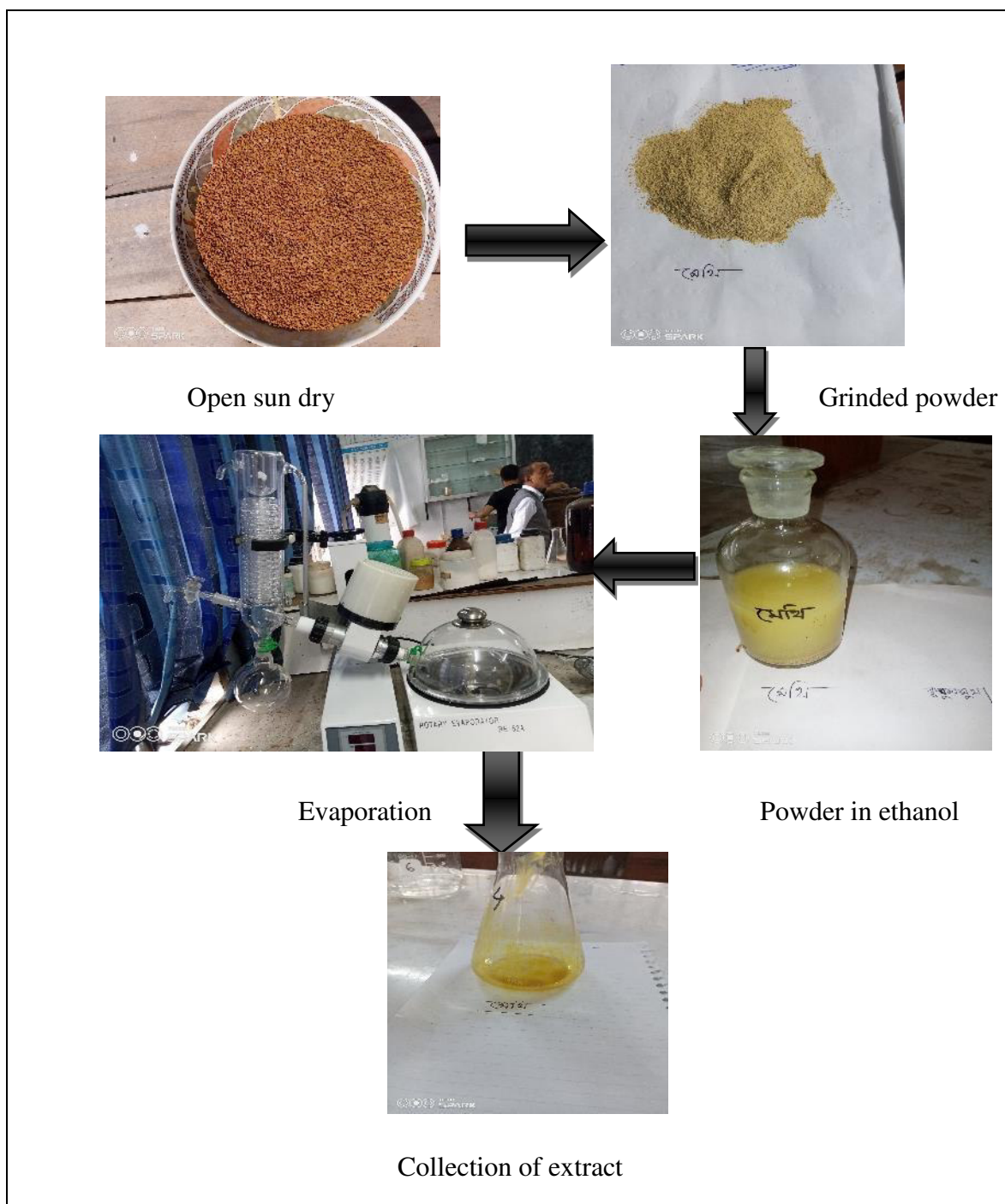


Figure 3: Pictorial View of Ehtanolic Extract of *Trigonella foenum* (Methi)

- **Extraction with Different Solvents:** 60g of powdered drug was separately extracted with ethanol (using continuous hot percolation in a Soxlet apparatus), and water (by cold maceration for 3 days)
- **Filtration and Evaporation:** All the extracts were filtered, and the solvents were evaporated using a rotary evaporator.
- **Storage:** The dried extracts were stored at 20°C until further use. This process describes the extraction and preparation of *Carum copticum* and *Trigonella foenum-graecum* seeds for further analysis and use.

3.5 Streptokinase (SK)

Streptokinase (SK) is an enzyme secreted by several species of streptococci. It belongs to a group of medications known as fibrinolytics. SK binds and activates human plasminogen, forming complexes that hydrolytically activate other unbound plasminogen to produce plasmin. SK is used as a thrombolysis medication in cases of myocardial infarction and pulmonary embolism due to its effectiveness and affordability. It consists of three domains: α (residues 1–150), β (residues 151–287), and γ (residues 288–414), each of which binds plasminogen but cannot activate it independently.

3.5.1 Mechanism of action

1. Plasmin is produced in the blood to break down fibrin, the major constituent of blood thrombi
2. Extra production of plasmin caused by streptokinase breaks down unwanted blood clots, like in pulmonary embolism.
3. Plasminogen (Plgn) activation: Arg561Val562 bond undergoes proteolysis.
4. Amino group of Val562 forms a salt-bridge with Asp740, triggering conformational change to produce active protease Plasmin (Pm)
5. Streptokinase (SK) binds to Plgn forming a complex (SK.Plgn) converting Plgn to Pm
6. Residue 159 of SK regulates its capacity to induce an active site in Plgn
7. Two candidates for counterion in active complex: Ile1 of SK and Lys698 of Plgn
8. Deletion of Ile1 of SK inhibits its capacity to induce an active site in Plgn

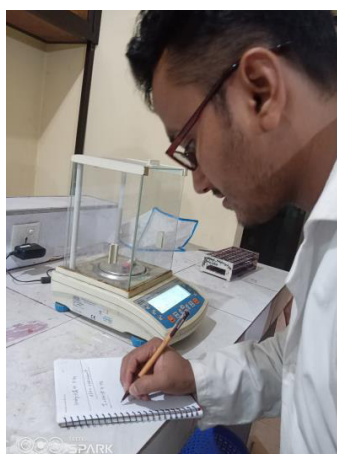
9. Lys698 mutations decrease dissociation constant of SK complex by 15 to 50 fold.
10. Lys698 is involved in the formation of the initial SK.Plg complex

3.6 Study population

- **Selection Criteria:** Participants aged 21-23 years were selected.
- **Criteria for selection included:** Normal prothrombin time. Absence of cardiovascular diseases (hypertension, congestive heart failure) or coagulation disorders (such as Hemophilia A or B). Not suffering from diabetes. Not recently using nonsteroidal anti-inflammatory drugs. Not obese or smokers. Free from dyslipidemic disorders.
- **Blood Sample Collection:** Blood samples were obtained from the selected junior and batchmate for the study.
- **Assessment of thrombolytic activity and Anticoagulant Effects:** The thrombolytic activity and anticoagulant effects of *Trigonella foenum* (Methi), *Carum copticum* (Ajawin), *Persicaria hydropiper* (Bishkatali) and *Blumea lacera* (kakurmutha) were assessed using these blood samples.

3.7 Preparation of clot

10 milliliters of blood was withdrawn from healthy human volunteers (n=9) irrespective of gender following the guidelines set up by the local ethical committee. The blood was distributed into 18 previously weighed microcentrifuge tubes (5ml to each centrifuge tube) to form clots and they were then centrifuged at 2000 rpm for 5 minutes to let serum separate above for easy removal from the centrifuge tube. The centrifuge tubes were then incubated in simulated body temperature i.e. at 37°C for 45 min in heat controlled incubator.



3.8 Preparation of extract and streptokinase solution

Using streptokinase (SK) as the standard substance. In the dry extract (100 mg) from each plant was suspended in 10 ml of distilled water and it was kept overnight. Then the soluble supernatant was decanted and filtered through a 0.22-micron syringe filter. For clot lysis venous blood drawn from healthy volunteers was distributed in different pre weighed sterile microcentrifuge tube (1 ml/tube and incubated at 37°C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube- weight of tube alone).



Figure 4: Preparation of extract and streptokinase solution

3.9 Clot lysis

The thrombolytic activity in terms of in vitro clot lysis was carried as earlier. Briefly 10 ml of blood was withdrawn from healthy volunteers. The blood was distributed into 18 pre weighed centrifuge tube (5ml to each centrifuge tube) and they are then centrifuged 2000 rpm for 5 min. Then the centrifuged tubes were incubated in simulated body temperature i.e. at 37°C for 45 min in heat controlled incubator. After 45 min blood clot was formed at the bottom of each centrifuge tube. The serum was removed from each centrifuge tube above the clot carefully and completely without disrupting the clot. After removing the serum, the tube containing clot was weighed again. One hundred microliter of each aqueous extractive was added in each microcentrifuge tubes, where

streptokinase and distilled water were applied as positive and negative control respectively. All the 18 centrifuge tubes were then again incubated 37°C for 90 minutes to see their dissolving capacity. After 90 min the centrifuge tubes were taken out the incubator and the dissolved clot along with applied agent (extract solution or streptokinase preparation or distilled water) were removed carefully and completely from the centrifuge tubes. Then the centrifuge tubes that contained undissolved clot were weighed. The weights of the clot before and after the clot lysed by the applied extract solutions, streptokinase and distilled water were calculated from the difference in the weights. The weight loss due to thrombolytic activity of each applied agent was calculated in percentage. The ability of the extracts to dissolve clot in percentage of weight loss were compared with that of standard and blank. The equivalent doses of streptokinase for the highest activity showed by the planta and spices were also measured. For those streptokinase was added at concentration of 15000000 IU to the clot. The clot lysis activity was measured according to the procedure described above % Of clot lysis= $(\text{wt of released clot}/\text{clot wt})\times 100$

3.9.1 In-vitro Thrombolytic Activity :

Before study of clot



After study of clot



Figure 5: Before and after study of clot by Ajawin extract

Before study clot



After study clot



Figure 6: Before and after study of clot by methi extract

Before study of clot



After study of clot



Figure 7: Before and after study of clot by kukurmutha extract

Before study of clot

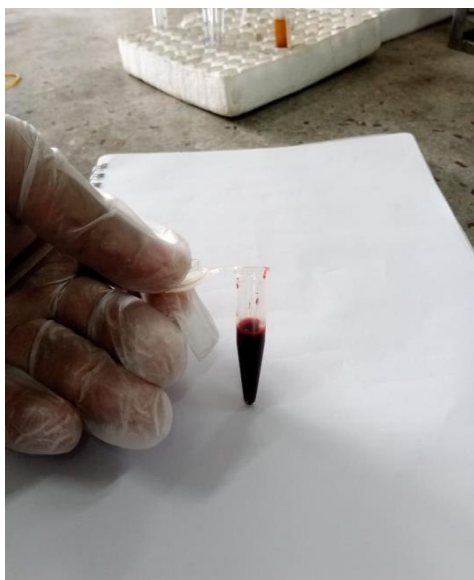


After study clot



Figure 8: Before and after study of clot by Bishkatali extract

Before study of clot



After study of clot



Figure 9: Before and after study of clot by positive control (streptokinase)

Before study of clot



After study of clot

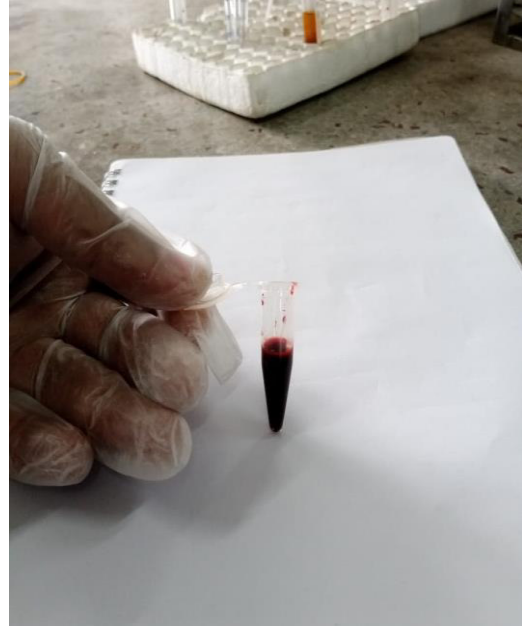


Figure 10: Before and after study of clot by negative control (water)

CHAPTER FOUR
RESULTS AND DISCUSSION

CHAPTER FOUR

RESULTS AND DISCUSSION

This experiment, conducted under the Department of Physiology & Pharmacology, Faculty of Veterinary and Animal Science, aimed to study the thrombolytic activity of ethanol plant extract on cardiovascular thrombus. Two plants and two spices—ajawin, methi, kakurmutha, Bishkatali plant—showed thrombolytic activity after the first trial. The experimental units were maintained in the laboratory, and micro-test tubes were weighed initially, each weighing 0.75g. Fresh and clean water was continuously provided. The experiment followed a completely randomized design.

In the pursuit of discovering cardio-protective drugs from natural sources, the thrombolytic activity of *Carum copticum* (Ajawin) extractives was evaluated, and there results are presented in Table 1 A positive control, 100 µl SK (150000IU.), was added to the clots, leading to 75.18% clot lysis after 90 minutes of incubation at 37°C. Conversely, distilled water, serving as the negative control, displayed minimal clot lysis (2.28%). The *Carum copticum* (ajawin) extract exhibited the highest thrombolytic activity in this study, at 57.25%.

Table 1: Lysis of clotted blood by *Trigonella foenum (methi)* extract, *Carum copticum* (ajawin) extract, *Blumea lacera* (kukurmutha) extract, *Persicaria hydropiper (bishkatali)* extract, Streptokinase and Water at standard Thrombolytic agent

Treated Group	Sl. No.	Weight of Blood Before Lysis (gm)	Weight of blood (After lysis)	Result (amount of lysed blood) gm	Percentage	Average
<i>Trigonella foenum</i> extract	1 st	0.64	0.38304	0.25696	40.15%	41.32%
	2 nd	0.65	0.38025	0.26975	41.50%	
	3 rd	0.67	0.38659	0.28341	42.30%	
<i>Carum copticum</i> extract	1 st	0.53	0.23161	0.29839	56.30%	57.25%
	2 nd	0.54	0.23139	0.30861	57.15%	
	3 rd	0.57	0.23769	0.33231	58.30%	
<i>Blumea lacera</i> extract	1 st	0.85	0.4760	0.3740	44.00%	45.00%
	2 nd	0.87	0.4785	0.3915	45.00%	
	3 rd	0.86	0.4643	0.3957	46.01%	
<i>Persicaria hydropiper</i> extract	1 st	0.78	0.6119	0.1681	21.55%	35.52%
	2 nd	0.75	0.435	0.3150	42.00%	
	3 rd	0.79	0.4503	0.3397	43.00%	
Streptokinase	1 st	0.91	0.23525	0.674765	74.15%	75.18%
	2 nd	0.93	0.23157	0.69843	75.10%	
	3 rd	0.94	0.22278	0.71722	76.30%	
Water	1 st	0.75	0.733875	0.016125	2.15%	2.28%
	2 nd	0.78	0.763308	0.016692	2.14%	
	3 rd	0.79	0.769776	0.020224	2.56%	

Table 2: Thrombolytic activity of different fractions of *Trigonella foenum (methi)*

Sample	Thrombolytic activity (% of lysis)
SK	75.18%
Water	2.28%
EETF	41.32%

Note: SK = Streptokinase, EETF= Ethanolic extract of *Trigonella foenum (methi)*

Table 3: Thrombolytic activity of different fractions of *Carum copticum* (ajawin)

Sample	Thrombolytic activity (% of lysis)
SK	75.18%
Water	2.28%
EECC	57.25%

Note: SK = Streptokinase, EECC= Ethanolic extract of *Carum copticum* (ajawin)

Table 4: Thrombolytic activity of different fractions of *Blumea lacera* (kukurmutha)

Sample	Thrombolytic activity (% of lysis)
SK	75.18%
Water	2.28%
EEBL	45.00%

Note: SK = Streptokinase, EEBL= Ethanolic extract of *Blumea lacera* (kukurmutha)

Table 5: Thrombolytic activity of different fractions of *Persicaria hydropiper* (bishkatali)

Sample	Thrombolytic activity (% of lysis)
SK	75.18%
Water	2.28%
EEPH	35.52%

Note: SK = Streptokinase, EEPH= Ethanolic extract of *Persicaria hydropiper* (bishkatali)

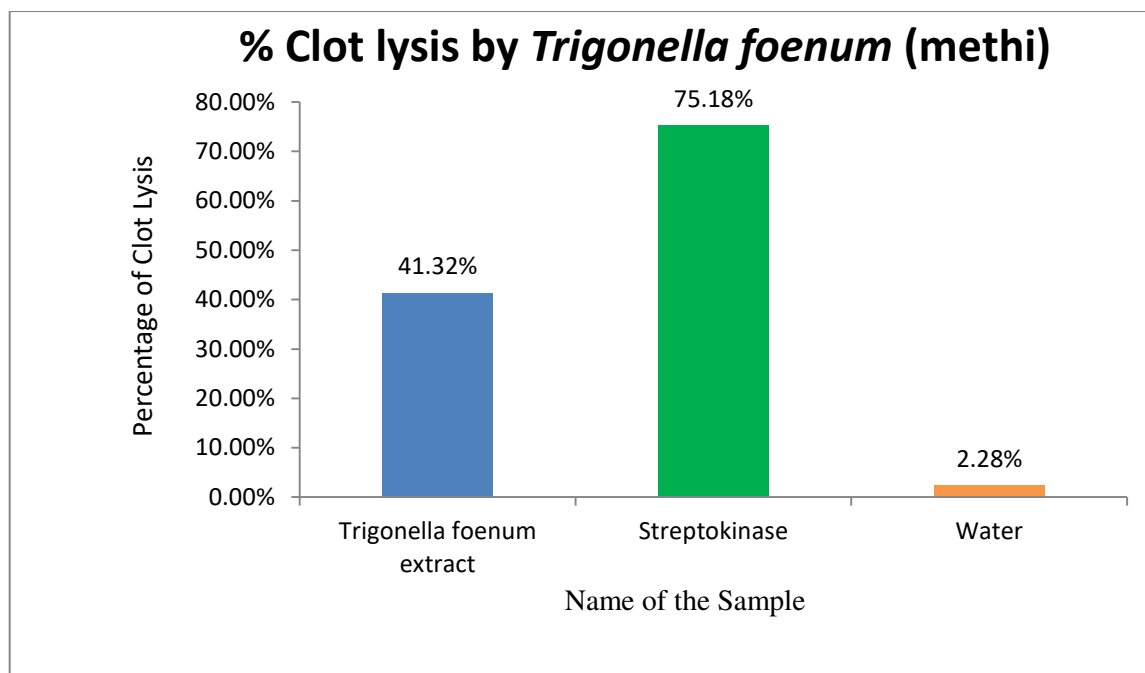


Figure 11: Thrombolytic activity of *Trigonella foenum* (methi)

This graph shows that clot lysis by *methi* is 41.32 % and by streptokinase is 75.18% and by water is 2.28% .

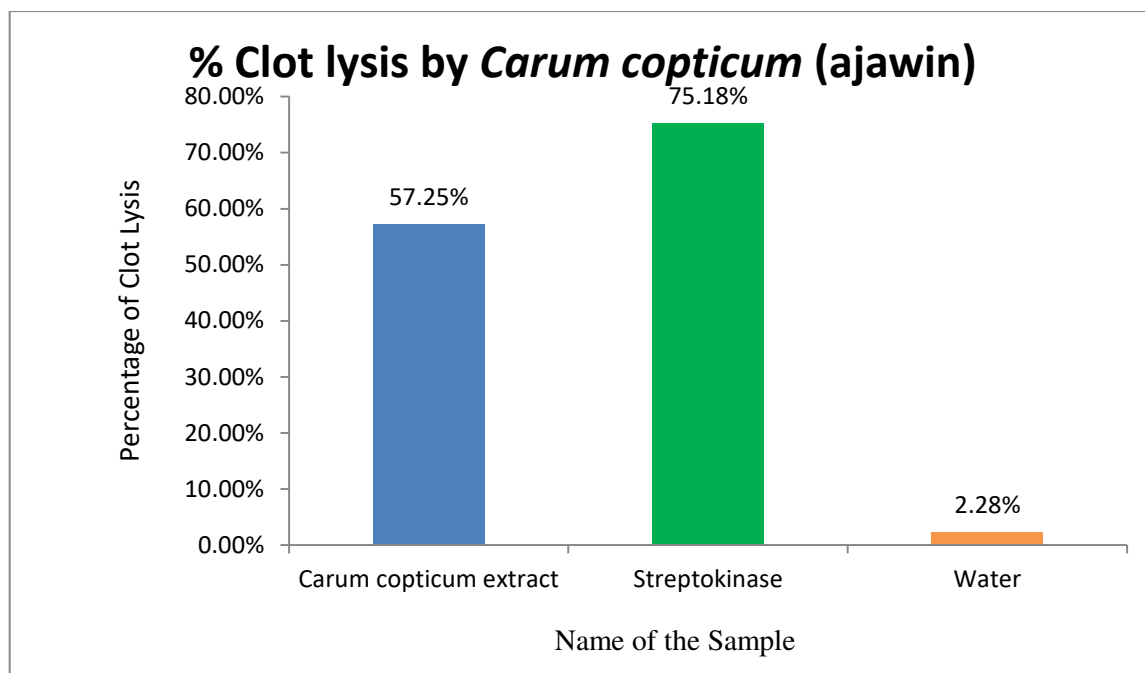


Figure 12: Thrombolytic activity of *Carum copticum* (ajawin)

This graph shows that clot lysis by ajawin is 57.25% and by streptokinase is 75.18% and by water is 2.28% .

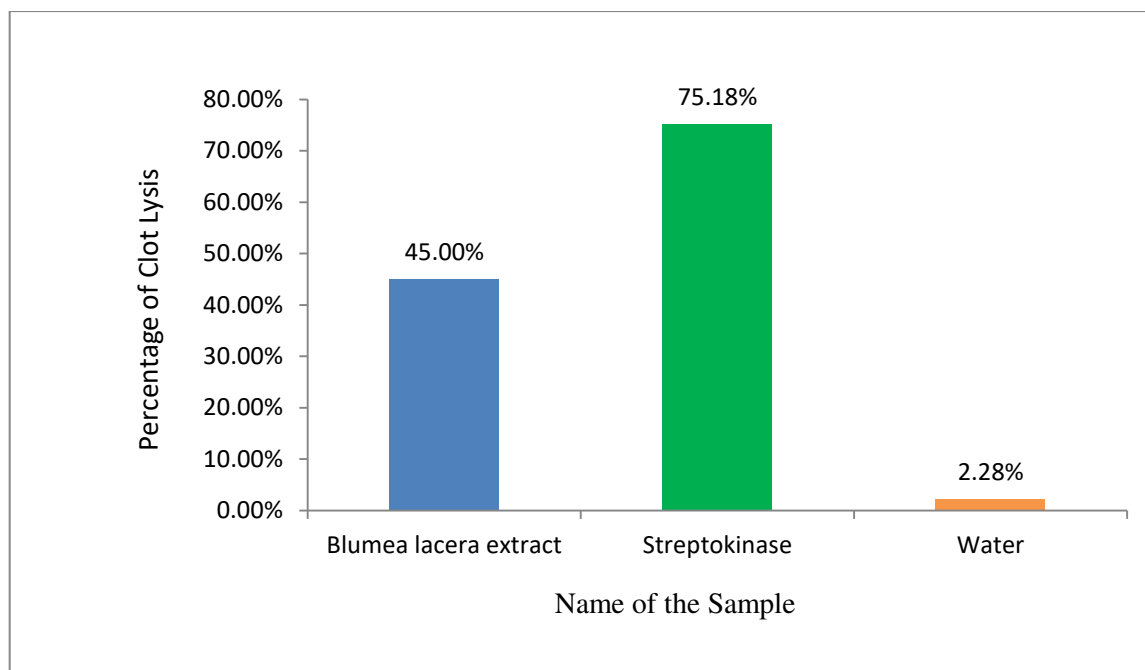


Figure 13: Thrombolytic activity of *Blumea lacera* (kukurmutha)

This graph shows that clot lysis by *kukurmutha* is 45 % and by streptokinase is 75.18% and by water is 2.28%.

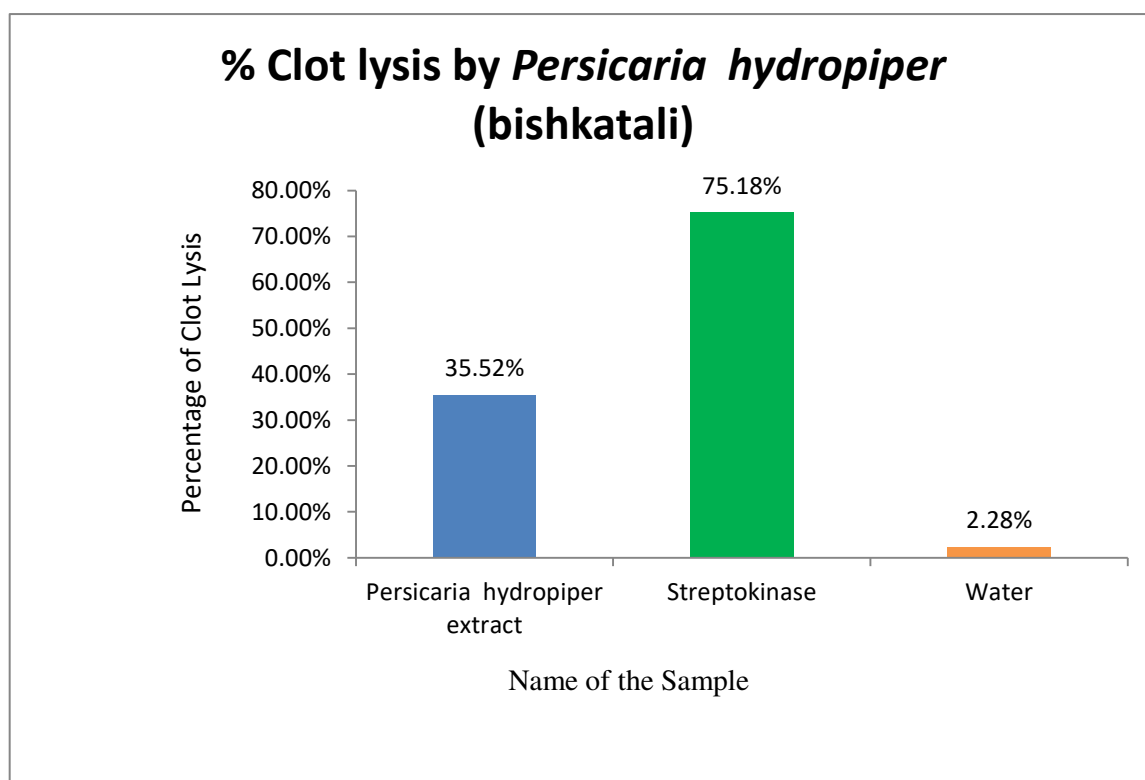


Figure 14: Thrombolytic activity of *Persicaria hydropiper* (bishkatali)

This graph shows that clot lysis by *bishkatali* is 35.52 % and by streptokinase is 75.18% and by water is 2.28%

Table 6: Lysis of clotted blood by *Trigonella foenum* extract, *Carum copticum* extract, *Blumea lacera* extract, *Persicaria hydropiper* extract

Treated Group	A (mean $\pm$ SEmean)	B (mean $\pm$ SEmean)	C (mean $\pm$ SEmean)
<i>Trigonella foenum</i> extract	0.6523 ^d $\pm$ 0.0088	0.3832 ^d $\pm$ 0.0018	0.2710 ^e $\pm$ 0.0076
<i>Carum copticum</i> extract	0.5460 ^e $\pm$ 0.0120	0.2335 ^e $\pm$ 0.0021	0.3131 ^c $\pm$ 0.0100
<i>Blumea lacera</i> extract	0.8557 ^b $\pm$ 0.0058	0.4730 ^c $\pm$ 0.0044	0.3869 ^b $\pm$ 0.0066
<i>Persicaria hydropiper</i> extract	0.7736 ^c $\pm$ 0.0120	0.4991 ^b $\pm$ 0.0566	0.2743 ^d $\pm$ 0.0536
Streptokinase	0.9267 ^a $\pm$ 0.0088	0.2297 ^f $\pm$ 0.0037	0.6968 ^a $\pm$ 0.0123
Water	0.7734 ^c $\pm$ 0.0120	0.7556 ^a $\pm$ 0.0110	0.0177 ^f $\pm$ 0.0013
Probability Value	0.0112	0.0201	0.0345
LSD	3.876	7.777	2.659
CV	2.3901	4.8204	10.4781

Note: Values followed by same superscripts in the same Column are statistically significant ($p < 0.05$), different superscripts indicate that difference is significant ($p < 0.05$). In this other tables, A=Total amount of blood, B= Weight of blood (After lysis), C= Result (amount of lysed blood) gm, Control no change.

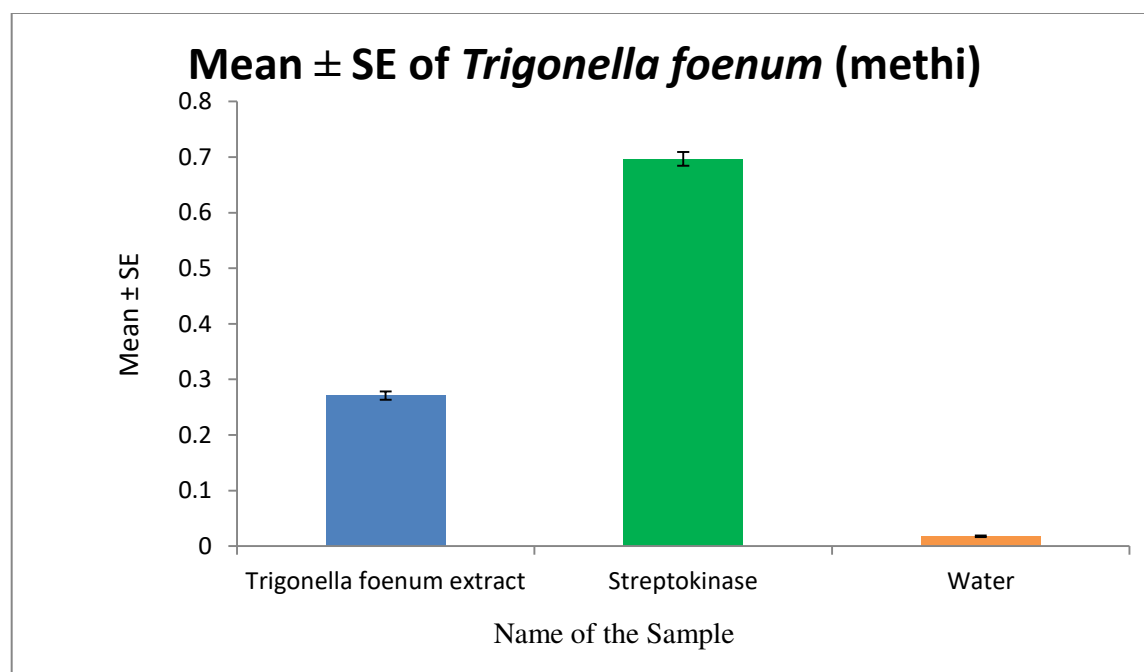


Figure 15: Thrombolytic activity of Mean $\pm$ SE of *Trigonella foenum* (methi)

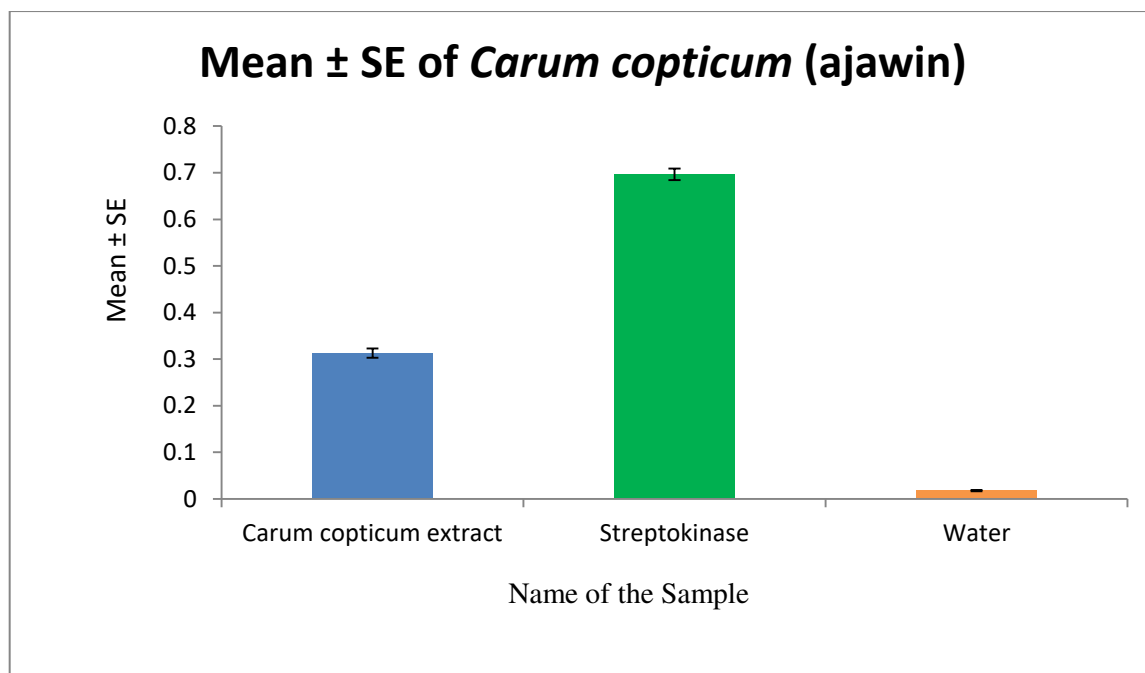


Figure 16: Thrombolytic activity of Mean $\pm$ SE of *Carum copticum* (ajawin)

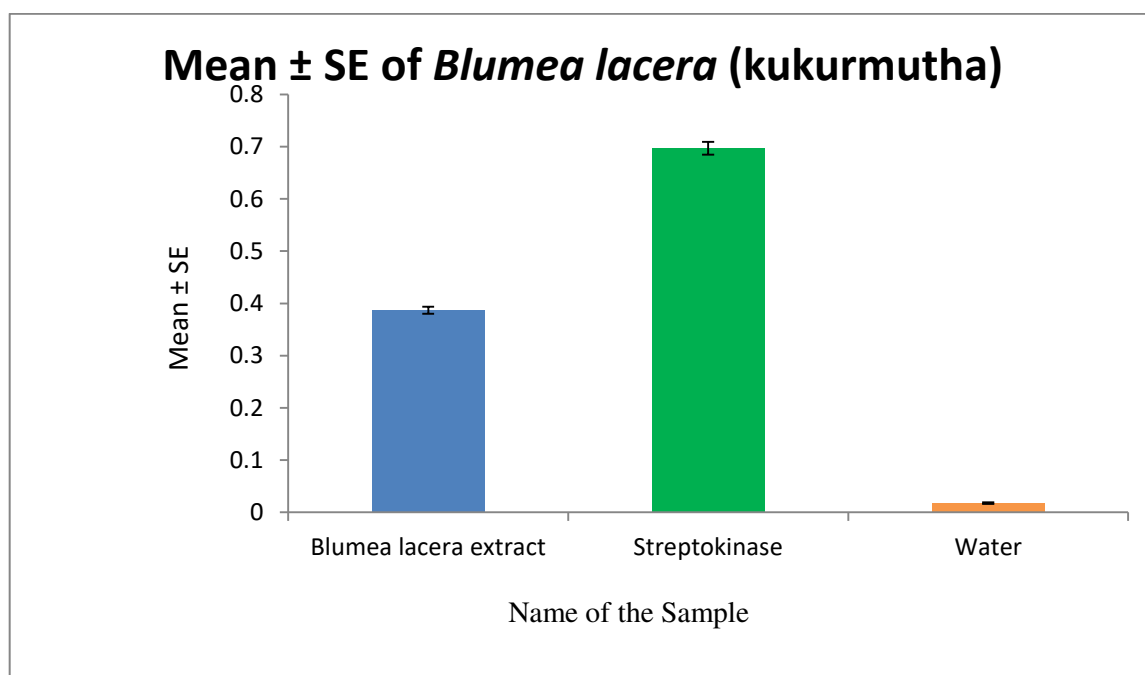


Figure 17: Thrombolytic activity of Mean $\pm$ SE of *Blumea lacera* (kukurmutha)

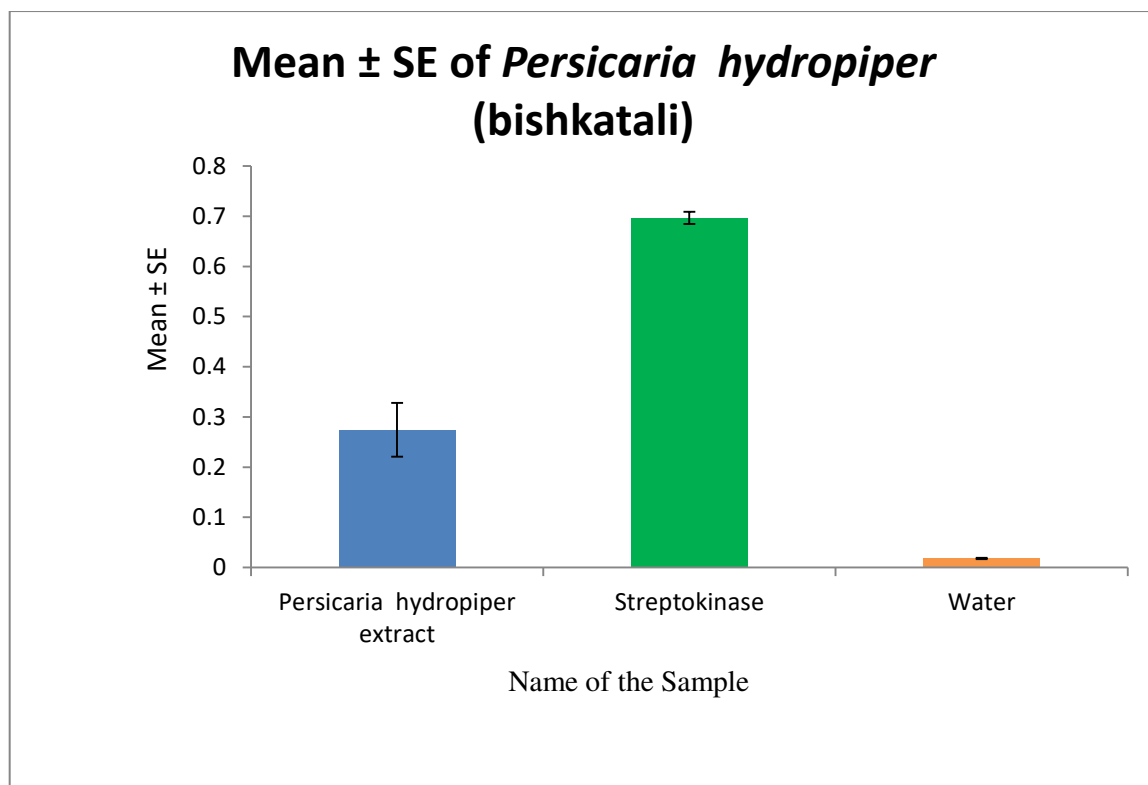


Figure 18: Thrombolytic activity of Mean $\pm$ SE of *Persicaria hydropiper* (bishkatali)

CHAPTER FIVE

SUMMARY AND CONCLUSION

CHAPTER FIVE

SUMMARY AND CONCLUSION

From my findings it is observed that all the plants revealed remarkable thrombolytic activity. Therefore, steps should be taken to observe *in vitro* clot dissolving potential extracts in comparison of thrombolytic activity, Streptokinase showed 75.18% as a standard agent, where ehtanolic ajawin extract 57.25% and ethanolic kukurmutha extract also showed 45%, ehtanolic methi extract 41.32% and ethanolic Bishkatali extract 35.52% significant result. In conclusion from my recorded data, it can be demonstrated that my findings may have significant implications in cardiovascular health. In addition, this finding may indicate the possibility of developing novel thrombolytic activity of extracts. Further studies are necessary to in-vivo analysis and characterize the compounds responsible for thrombolytic activity.

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