IN-VITRO THROMBOLYTIC AND ANTICOAGULANT ACTIVITY OF ETHANOLIC EXTRACT OF *Carum copticum* AND *Triconella foenum-graecum*

A Thesis

By

MD. AKTER HOSSEN
Registration No. 1305106
Semester: July-December, 2014
Session: 2013-14

MASTER OF SCIENCE (M.S.)
IN
PHARMACOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

DECEMBER, 2014

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Submitted to the
Department of Physiology & Pharmacology
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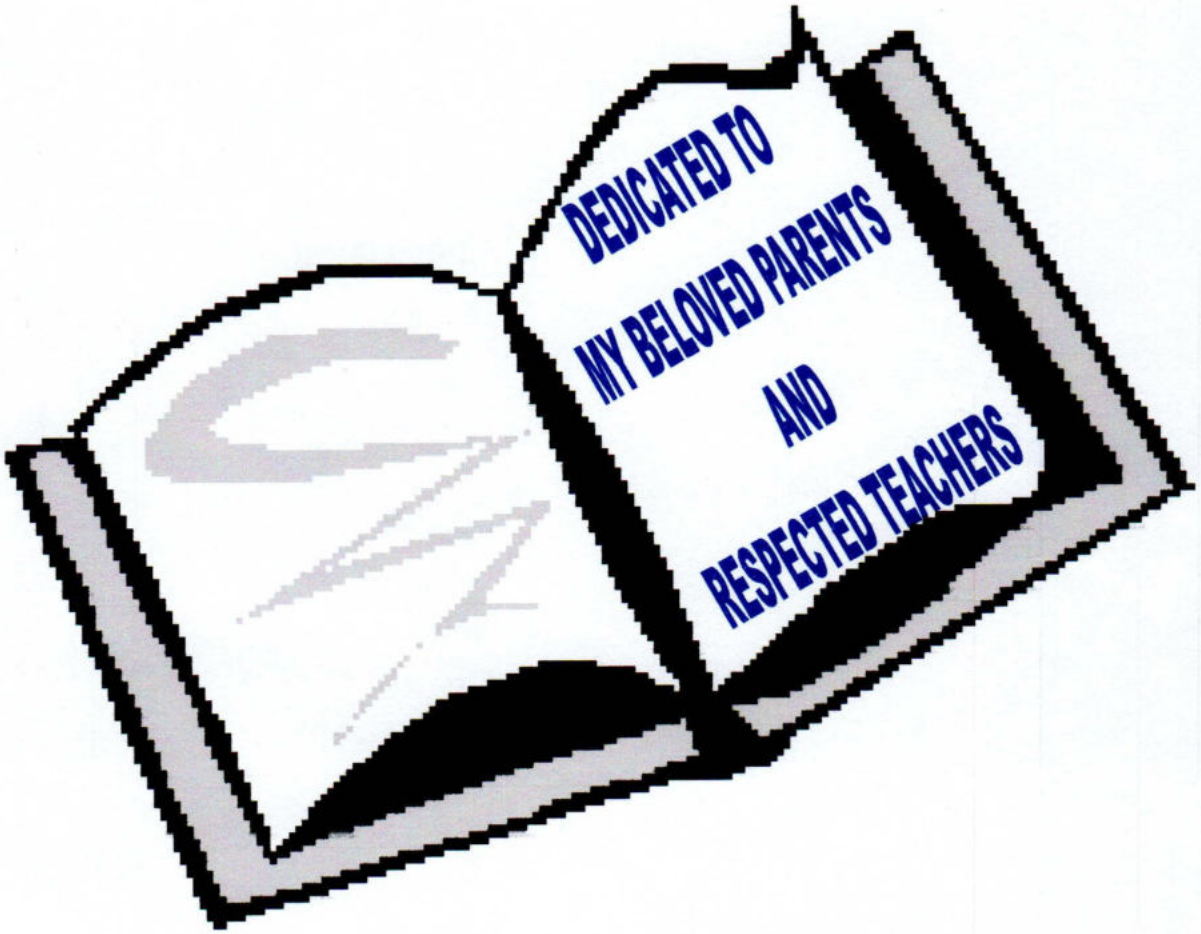
DECEMBER, 2014


DEDICATED TO
MY BELOVED PARENTS
AND
RESPECTED TEACHERS

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And lastly I would like to express my thanks and gratitude to all those, whose names could not be mentioned, but have extended their co-operation and help during my research work.

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ABSTRACT

An experiment was carried out in the laboratory of the department of physiology and pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, during the period of July-December/2014. Some plant and spices were collected from Dinajpur district. Primarily ethanol extract was prepared from those plant and spices. 20 micropipette was taken filled with 0.5 ml human blood and 3 micropipette was taken as excess micropipette for control. These two kind of micropipette were screened for assessment their comparative efficacy of clotted blood lysis. Ethanol extract was used at a dose of 100 micro ml/ micropipette. Then this micropipette was incubated at 37 degree centigrade temperature for 1hr 45 minutes. After incubation I found 4 plant and 2 spices has anticoagulant property. These plant are Fern, Arum, Cannabis Tezpata and spices are Ajawin and Pesta. Finally I was experimented with Ajawin and Methi *in-vitro* thrombolytic and anticoagulant property. Anticoagulant and thrombolytic property was experimented in laboratory compared with Streptokinase (SK). Streptokinase showed 100% efficacy after treatment. Efficacy was recorded by compare among streptokinase control treated extract. Ethanol ajawin extract lysis clotted blood 57.33%.

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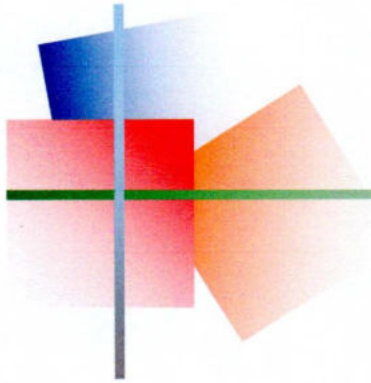
LIST OF ABBREVIATIONS

B.wt.	:	Body weight
BAU	:	Bangladesh Agricultural University
Conc.	:	Concentration
Cu mm	:	Cubic millimeter
d.w.	:	Drinking water
ESR	:	Erythrocyte Sedimentation Rate
et al.	:	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
0^0C	:	Degree centigrade



Chapter 1

INTRODUCTION

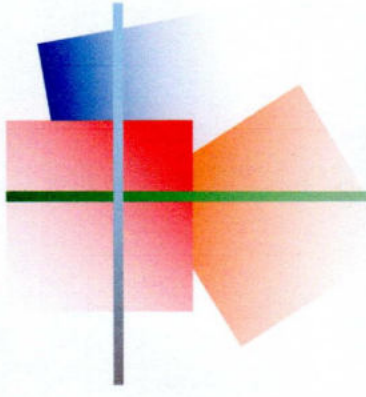
CHAPTER 1

INTRODUCTION

Man has many problem for their life. Man has many diseases such as cardiovascular disease, urogenital disease, nutritional disease etc. When man affected with cardiovascular or myocardial or cerebral infections. Myocardial or cerebral infractions are the serious consequences in Atherothrombotic diseases leading to death and the side effects produced by consecutive use of thrombolytic agent like t-PA, Urokinase and streptokinase to treat these diseases has become a global concern. Thrombolytic agents such as tissue plasminogen activator, urokinase, streptokinase (SK), etc are used to dissolve the already formed clots in the blood vessels . However, these drugs have certain limitations which cause serious and sometimes fatal consequences including hemorrhage, severe anaphylactic reaction, lacked specificity, etc. Moreover, as a result of immunogenicity multiple treatments with SK in a given patient are restricted. Methi and ajawin has anticoagulant and thrombolytic property respectively. The present study was carried out to evaluate the thrombolytic and anticoagulant activity of ethanolic extract of ajawin methi spices by in vitro method. Extraction was carried out using Soxhlet apparatus. The ajawin ethanol extract showed highest thrombolysis than methi extract. But methi showed highest anticoagulant property than ajawin. Concentration of phytochemicals and incubation time were directly proportional to the clot lysis. Application of the present study may be accessible for greater section of the society for treatment of cardiovascular diseases. Now-a-day synthetic drug like streptokinase so expensive has many side effect. As Methi and Ajawin has thrombolytic anticoagulant activity so the extraction of Methi and ajawin used as treatment for cardiovascular disease against t-PA, Urokinase and streptokinase. Which will be cheapest than t-PA, Urokinase and streptokinase.

In concern to that consideration my study was designed for the following objectives

- To observe the performance of thrombolytic activity of spices.
- To observe the performance of anticoagulant activity of spices.



Chapter 2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

Cardiovascular disease serious human diseases, causing millions of deaths every year. Thrombosis is one of the leading cause of thromboembolic disorders affecting million persons worldwide. Several plants used for the treatment of thromboembolic diseases in different systems of traditional medicine have shown anticoagulant/antithrombotic activity and such plants claimed in the traditional system still remain to be scientifically investigated. For more than five decades, anticoagulant drugs consisting of heparins, vitamin K antagonists, and their derivatives have been the major players in the clinical setting. Although their efficacy remains undisputed, the deleterious life-threatening side effects of these drugs have also been well documented. Plants may serve as the alternative sources for the development of new anticoagulant agents due to their biological activities. There is compelling scientific evidences demonstrating that the consumption of dietary anticoagulants or phytochemicals with anticoagulant properties can ultimately reduce or eliminate the risks of thromboembolic diseases. Prothrombin time (PT) is measure of the extrinsic coagulation pathway.

Mohammad Shahadat Hossain, *et al.* (2012) In-Vitro Thrombolytic and Anti-inflammatory Activity of *Swertia Chirata.* Ethanolic extract of *Swertia chirata* was assessed for its thrombolytic, anti-inflammatory activity and phytochemical screening. *In vitro* anti-inflammatory activity was evaluated using albumin denaturation. Aspirin was used as a standard drug for the study of anti-inflammatory activity. The ethanol extract of *Swertia chirata* showed mean inhibition of protein denaturation 45.31±0.000576 whereas, for control group it was found to be 50.00±0.00177. In thrombolytic activity using *in vitro* clot lysis assay method, the crude ethanol extract was found to have significant, thrombolytic test showed a maximum effect of 40.38% while the standard streptokinase showed 69.35ct.

Narjis Hadi Mansoor (2013) In vitro study of the anticoagulant activity of some plant extracts examined by prothombin time, anticoagulant activity, red onion, garlic oil, grape oil.

Parvin, *et al.* (2013) Phytochemical screenings, thrombolytic activity and antimicrobial properties of the leaf extracts of *Lablab parpureus.* In this present study, the leaves extracts of *Lablab parpureus* were subjected to the thrombolytic activities were assessed by using human erythrocyte and the results were compared with standard streptokinase (SK). On the other hand, leaves extracts of *L. parpureus* revealed moderate antibacterial activity against some microorganisms used in the screening. Preliminary phytochemical investigation suggested the presence of reducing sugar group, tannins, saponins and alkaloids.

Y., Sai Sandeep, *et al.* (2012) Evaluation of in vitro thrombolytic activity of phytochemicals in Bacopa monnieri Linn. Myocardial or cerebral infractions are the serious consequences in Atherothrombotic diseases leading to death and the side effects produced by consecutive use of thrombolytic agent like t-PA, Urokinase and streptokinase to treat these diseases has become a global concern. Bacopa monnieri has been used for centuries in Ayurveda system of medicine as a memory vitalizer and as a liver and heart tonic. The present study was carried out to evaluate the thrombolytic activity of ethanolic, methanolic, acetone and aqueous extract of different parts (root, stem and leaf) of B. monnieri by in vitro method. Extraction was carried out using Soxhlet apparatus. The leaf ethanolic extract showed highest thrombolysis followed by aqueous, methanol and acetone extract. Concentration of phytochemicals and incubation time were directly proportional to the clot lysis. Application of the present study may be accessible for greater section of the society for treatment of cardiovascular diseases.

Md. R. Al-Mamun *et al.* (2012), Thrombolytic activity of some spices and plants available in Bangladesh. Thrombolytic activities of some plants, namely *Tamarindus indica* (Fabaceae), *Flemingia congesta* (Fabaceae), *Lawsonia inermis*

(Lythraceae), *Mesua nagassarium* (Clusiaceae, and spices, namely *Coriandrum sativum* (Apiaceae), *Curcuma longa* (Zingiberaceae), *Cinnamomum tamala* (Lauraceae), *Nigella sativa* (Ranunculaceae), *Eugenia aromaticum* (Myrtaceae), available in Bangladesh, were evaluated using an *in vitro* model. The thrombolytic activity in terms of percentage of weight loss of *in vitro* formed clots were found as *C. sativum* 43.25 ± 7.18%, *C. longa* 53.32 ± 4.96%, *C. tamala* 22.10 ± 3.18%, *N. sativa* 28.49 ± 3.72%, *E. aromaticum* 32.18 ± 3.10%, *T. indica* 28.91 ± 2.29%, *F. congesta* 35.27 ± 7.35%, *L. inermis* 62.40 ± 5.04%, *M. nagassarium* bark 39.54 ± 7.15% and *M. nagassarium* leaf 46.75 ± 3.97% with reference to the negative control distilled water 8.37 ± 1.18% and positive control streptokinase 84.63 ± 1.03%. Through our study, it was found that *L. inermis* and *C. longa* possess thrombolytic property that could lyse blood clots *in vitro.*

Md. Al Amin Sikder, *et al.* (2011) Journal of Pharmacy and Nutrition Sciences examined evaluation of ehrombolytic activity of four Bangladeshi medicinalplants, as a possible renewables for thrombolytic Compounds. Four Bangladeshi medicinal plants *Sansevieria trifasciata*, *Justica gendarussa*, *Hydnocarpus kurzii* and *Mesua nagassarium* have been investigated for their *in vitro* thrombolytic activity. The clot lysis activity was assessed by addition of the test material to the pre-clotted blood and incubation for 90 min. at 37oC and was expressed as % lysis of clot. Each of the plant was extracted with methanol at room temperature and the concentrated methanolic extract was fractionated by the modified Kupchan partitioning method to provide pet-ether, carbon tetrachloride, chloroform and aqueous soluble fractions. Among the four plants the aqueous soluble fraction of *M. nagassarium,* carbon tetrachloride soluble fraction of *H. Kurzii* , aqueous soluble fraction of methanolic extract of *S. trifasciata* exhibited highest thrombolytic activity with clot lysis value of 50.86%, 47.50%, and 47.10% respectively. However, the pet ether and carbon tetrachloride soluble fraction of methanolic extract of *J. gendarussa* demonstrated significant thrombolytic activity as evident from 45.93% and 45.47% lysis of clot,

respectively. Standard streptokinase was used as positive control which exhibited 61.50% lysis of clot while the negative control water revealed 2.56% lysis of clot.

J.H. Evangelista *et al.* (2012) examined Preliminary Assessment of In vitro Anticoagulant Activity vs. Heparin 1,000I.U. and Cytotoxicity of Selected Philippine Medicinal Plants. One main cause of mortality in developing countries is thromboembolic disorders such as pulmonary emboli, deep vein thrombosis and heart attacks. Several agents and interventions were available; however, there are still side effects that are acquired through these therapies. Herbal plants are popularly used nowadays in drug discovery due to their ancient medicinal use. Philippines, a tropical country, have a variety of herbal plants. The aim of the study was to assess the anticoagulant activity and cytotoxicity of both flesh and peels/seeds of selected plants available in the Philippines. The plants Allium sativum, Cucurma longa, Ananas comosus and Lycopersicum esculentum were used in the in vitro method using Heparin and water as controls. Percentage clot lysis of the plants were 18.30%, 21.77%, 21.85%, 35.91%, 15.67% and 24.52%, respectively. Only C. longa peel extracts and L. esculentum showed clot lysis beyond the negative control. C. longa peels showed a higher percentage clot lysis as compared with Heparin. Using one-way ANOVA, statistics showed the p value of 0.674574 from the clot lysis activity of all plant extracts. Cytotoxicity of the herbal plants was also determined using brine shrimp lethality assay (BSLA). The LC50 values of the herbal extracts ranges from 6.72 to 31.2µg/mL. A. sativum and C. longa flesh have the lowest values (most potent among the extracts). Descriptively, C. longa peels extract exhibited promising clot lysis activity, however further studies are still needed to strengthen the effectiveness of these Philippine plants as anticoagulant. Furthermore, it was also seen in this study that fruit and vegetable peels have prospective therapeutic application. It will not only benefit the industrial waste products reduction, but it can also serve as an alternative source of pharmacologic agents.

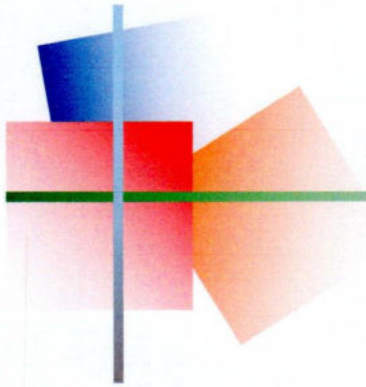
Jannat-e-Zereen and Gwyneth Ingram (2012) A Possible Involvement of *ACR4,* a Receptor Like Kinase, in Plant Defence Mechanism, *Bangladesh Pharmaceutical Journal.*

M Atiar Rahman, Rabeya Sultana, Talha Bin Emran, M Saiful Islam, M Ashiqur Rahman, Joti Sankhar Chakma, Harun-ur Rashid and **Chowdhury Mohammad Monirul Hasan (2013)** Effects of organic extracts of six Bangladeshi plants on *in vitro* thrombolysis and cytotoxicity, *BMC Complementary and Alternative Medicine.*

Md. Rakib Al-Mamun1,2, Nabiha Amrin2, Jahura Begum2 and Md. Abdul Mazid1 (2012) Thrombolytic activity of some spices and plants available in Bangladesh.

Sweta Prasad, Rajpal Singh Kashyap, and Hatim F Daginawala (2012) Effect of *Fagonia Arabica* (Dhamasa) on in vitro thrombolysis, BioMed Central. Atherothrombotic diseases such as myocardial or cerebral infarction are serious consequences of the thrombus formed in blood vessels. Thrombolytic agents are used to dissolve the already formed clots in the blood vessels; however, these drugs have certain limitations which cause serious and sometimes fatal consequences. Herbal preparations have been used since ancient times for the treatment of several diseases. Herbs and their components possessing antithrombotic activity have been reported before; however, herbs that could be used for thrombolysis has not been reported so far. This study's aim was to investigate whether herbal preparations (aqueous extract) possess thrombolytic activity or not.

Y., Sai Sandeep; Panigrahi, Mamata; C., Divya. G.; B., Beena D. (2011) Evaluation of in vitro thrombolytic activity of phytochemicals in Bacopa monnieri Linn, January 2012, Journal of Pharmacy Research;Jan2012, Vol. 5 Issue 1, p100, Academic Journal.



Chapter 3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

The experiment was conducted at the department of Physiology and Pharmacology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, during the period from January to June/2014. To complete the research work following steps were followed:

3.1 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. Plants are Fern, Auram, Tobacco, spices are cardamon, Tezpat, Zira, Zowin, Masted oil, Small alach etc.

3.2 Collection and processing of plant material

Fern, Auram, Tobacco leaves, Cardamon, Tezpat, Zira, Zowin, Masted oil, Small alach were selected for effectiveness as thrombolytic agent on Cardiovascular system. Mature and disease free Fern, Auram, Tobacco leaves, Cardamon, Tezpat, Zira, Zowin, Masted oil, Small alach leaves were collected from HSTU campus.

3.3 Preparation of Ethanol Extract:

3.3.1 Ethanol

Ethanol also called ethyl alcohol pure alcohol, grain alcohol, or drinking alcohol, is a volatile, flammable, colorless liquid with the structural formula CH_3CH_2OH, often abbreviated as C_2H_5OH or C_2H_6O. Ethanol is a psychoactive drug and is one of the oldest recreational drugs still used by humans. Ethanol can cause alcohol intoxication when consumed. Best known as the type of alcohol found in alcoholic beverages, it is also used in thermometers, as a solvent, and as a fuel. In common usage, it is often referred to simply as alcohol or spirits.

Ethanol is a 2-carbon alcohol with the empirical formula C_2H_6O. Its molecular formula is CH_3CH_2OH. An alternative notation is $CH_3–CH_2–OH$, which indicates that the carbon of a methyl group ($CH_3–$) is attached to the carbon of a methylene group ($–CH_2–$), which is attached to the oxygen of a hydroxyl group (–OH). It is a constitutional isomer of dimethyl ether.

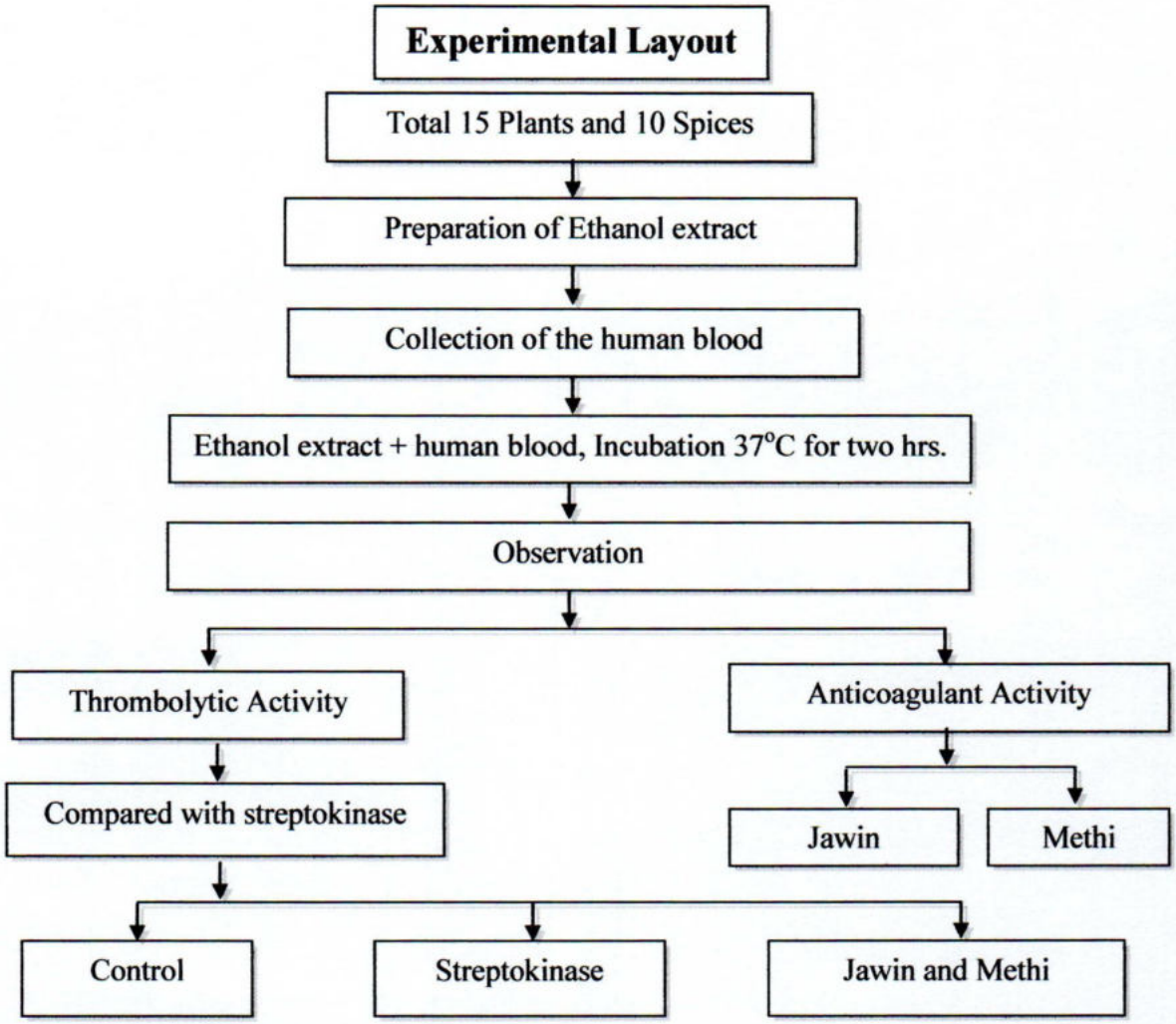


Fig. 3.1 Layout of the experiment

3.3.2 Extract

Extract is a solution of the essential constituents of a herbal agent. It is prepared by boiling the plant material in water and then evaporating the strained decoction to a desired concentration. Extracts harbour the more active principles of the medicinal plants, allowing the less active principles to be removed as a dross. Various solvents may be used including alcohol, water and glycerin. Extracts should always be prepared at the lowest temperature possible that is compatible with good extraction of the healing principles. If care is not taken, some of the lighter and more volatile oils and other important ingredients may be lost. In addition, the vessel in which the extract is prepared should always be tightly covered until such time as the extract has reached a sufficiently cool temperature (i.e., body temperature). Fluid Extract is an alcohol or glycerin preparation of herbal extract containing the active constituents in a definite ratio of plant material to solvent. There are several types of fluid extracts:

- Heated extracts are prepared by boiling (but preferably simmering) a herbal agent in water and then evaporating the strained decoction to a desired concentration — this yields a more concentrated herbal remedy.
- Fluid extract is made by evaporating an already prepared fluid (such as an infusion or decoction) to the desired concentration.
- Cold extract is similar to an infusion. It is prepared by taking twice as much herb as is desired for an infusion and then letting it sit in an enamel or non-metallic pot for eight to twelve hours. It is then strained and taken as one would take an infusion.
- Freeze drying is also used to get active ingredients out of plants and preserve them. The technique is used in the coffee industry as well as for herbs.
- Fresh Plant Extracts are becoming commercially popular. In these cases, fresh plants are used to make the tincture rather than dried material. Sometimes this results in a more potent preparation, other times less potent. The properties of a fresh plant extract will also vary from dried plant extracts, both positively and negatively.

3.3.3 Plant extract

A plant extract must, by definition, be obtained from a solid-liquid extraction. Solid-liquid extraction is defined as an operation to separate elements contained in a solid body

by solubilization with a solvent, and it may be followed by purification. The extract is contained in the solvent. If the solvent is an edible solvent, it is not necessary to dissociate it from the extract. If the solvent is not an edible solvent, separation allows obtaining a dry extract. Selection of raw materials, choice of solvent, process utilization and equipment performance are determining factors. These multiple parameters must be professionally combined. Only their proper combination allows obtaining high-quality plant extracts.

3.3.4 Preparation of leaves extract

For the preparation of extract, the leaves were dried in sun for 10 days and followed by oven at 55-60°C for 2 days. The dried leaves were pulverized with a blender. A 25 (unit) mesh diameter sieve was used to obtain the fine dust, after then dust was preserved in airtight plastic container 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 up to 100ml to make 10% extract.

Figure 1. Preparation of ethanol plant extract

3.3.5 Preservation of Plant Extract

The collected plant material (plant parts: stems, leaves, flowers, roots, bark etc.) is dried in a ventilated oven at 45 °C for 24 H, and subsequently milled to a fine powder by means of an IKA Universal Mixer M20 (or other type). An amount of 20.0 g of the dried plant powder is weighed in an Erlenmeyer of 100 ml to which 70 ml of hexane (purity grade 99 %) is added (the plant sample has to be submerged with solvent) for pre-

extraction. The Erlenmeyer is placed in a sonicator-bath (Branson 8210 or some other type) and sonicated at a temperature 40 °C during 30 minutes. The mixture is filtered using paper filter, followed by washing the Erlenmeyer with 20 ml of hexane and then with 50 ml of hexane. The filtrate is poured in a round-bottomed flask and the solvent is concentrated in vacuo (at about 11 mm Hg) up to 5-10 ml by means of rotavapor, utilizing a water bath at 40°C. This residue is brought in a 30-ml vessel to let the solvent evaporate. The open vessel is left overnight in a well-ventilated hood in order to evaporate the last traces of the solvent in the hexane pre-extract. The solids, collected on the filter, are broken up and dried in the air overnight in the hood. The dried material is extracted in the same way with methanol-water (90:10). The dried material from the filters placed in an Erlenmeyer of 100 ml to which 70 ml of 90 % methanol is added. The mixture is sonicated as above at 40C during 30 minutes, after which it is filtered, followed by washing the Erlenmeyer with 20 ml of 90 % methanol. The filtrate is poured in a round-bottomed flask and the solvent is evaporated in vacuo completely. The dry 90% methanol extract is dissolved in as little as possible 100 % methanol by using the sonicator-bath and poured in a 30-ml vessel to let it evaporate overnight in the hood.

3.4 Collection and management of Human Blood

1oml Human blood were collected from Vein by 10ml syrenge. This blood were kept into 20 micropippet. Each micropippet contain 0.5ml blood. After few minutes blood automatically was cloted and serum was separated from the tube. After then blood containing pippet kept into pippet stand.

3.5 Different plant extract mixing with clotted blood

100 micromililitre ethanol plant extract measured by micropippet syringe and mixed with specific plant extract with specific test tube. Three blood test were preserved for control.

3.6 Streptokinase (SK)

3.6.1 Streptokinase (SK) is an enzyme secreted by several species of streptococci that can bind and activate human plasminogen. SK is used as an effective and inexpensive thrombolysis medication in some cases of myocardial infarction (heart attack) and pulmonary embolism. Streptokinase belongs to a group of medications known as fibrinolytics, and complexes of streptokinase with human plasminogen can hydrolytically activate other unbound plasminogen by activating through bond

cleavage to produce plasmin. There are three domains to Streptokinase, denoted α (residues 1–150), β (residues 151–287), and γ (residues 288–414). Each domain binds plasminogen, although none can activate plasminogen independently.

3.6.2 Mechanism of action

Plasmin is produced in the blood to break down fibrin, the major constituent of blood thrombi, thereby dissolving clots once they have fulfilled their purpose of stopping bleeding. Extra production of plasmin caused by streptokinase breaks down unwanted blood clots, for example, in the lungs (pulmonary embolism). The usual activation of Plasminogen (Plgn) is by proteolysis of the Arg561—Val562 bond. The amino group of Val562 then forms a salt-bridge with Asp740, which triggers a conformational change producing the active protease Plasmin (Pm). When (SK) is present, it binds to Plgn to form a complex (SK. Plgn) that converts substrate Plgn to Pm. Residues 1–59 of SK regulate its capacity to induce an active site in bound Pg by a nonproteolytic mechanism and to activate substrate Pg in a fibrin-independent manner. This complex subsequently rearranges to an active complex although the Arg561–Val562 bond remains intact. Therefore another residue must substitute for the free amino group of Val562 and provide a counterion for Asp740 in this active complex. Two candidates for this counterion have been suggested: Ile1 of streptokinase and Lys698 of Plgn. Deletion of Ile1 of SK markedly inhibits its capacity to induce an active site in plasminogen, which supports the hypothesis that establishment of a salt bridge between Ile1 of SK and Asp740 of plasminogen is necessary for SK to induce an active site in plasminogen by a nonproteolytic mechanism. In contrast with the Ile1 substitutions, the Lys698 mutations also decreased the dissociation constant of the SK complex by 15 to 50 fold. These observations suggest that Lys698 is involved in formation of the initial SK. Plgn complex.

3.7 Plant material collection

Carum copticum (Ajowan) and *Triconella foenum-graecum (Methi/Fenugreek)* was collected from local market Bahadur Bazar at Dinajpur in Bangladesh. The seed were washed with fresh water and dried under shade at room temperature. The seed were sinked into ethanol at two days. The seed were grinded wit morter and pestle and squeezed out the liquid portion with hand and centrifuged at 1000 rpm. Then dried out

the water from the centrifugal material and powdered and stored. 60g of powdered drug was extracted separately with methanol, petroleum ether by continuous hot percolation in soxlet apparatus and with water by cold maceration for 3 days respectively. All the extracts were filtered and evaporated using a rotary evaporator. Dried extracts were stored at 20°c until used.

3.8 Study population

Blood samples obtained from my dear batchmates, were used to assess the anticoagulant effects of *Triconella foenum-graecum (Methi/Fenugreek)*. Participants were 25-26 years old. They had been chosen for this study according to the following criteria: having normal prothrombin time, not suffering from any cardiovascular diseases (hypertension, congestive heart failure, coagulation disorders such as, Hemophilia A or B) or diabetes, not recently using nonsteroidal anti inflammatory drugs, not obese or smokers and free from dyslipidemic disorders.

3.9 Collection of blood samples

The blood samples were obtained from normal individuals by using sterile syringes, withdrawn from vein of right arm of each individual and placed separately in containers containing tri-sodium citrate to prevent the clotting process. Centrifugation (15 minutes at rate 3000 rpm) was carried out to separate the blood cells from plasma in order to obtain pure platelet plasma (ppp) for prothrombin time test. The obtained plasma sample of each individual were poured separately in plane containers using automatic pipette and stored at room temperature.

3.10 Invitro thombolytic activity of *Carum copticum (Ajowan)*

***Blood sample*:** Blood (n=6) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 1ml of blood was transferred to the previously weighed micro centrifuge tubes and was allowed to form clots.

Figure 2. Lysis of the clotted blood (Ajawin)

***Thrombolytic activity*:** The thrombolytic activity of all extracts was evaluated by the method developed by Daginawala (2006) and slightly modified by Kawsar *et al.* (2011) using streptokinase (SK) as the standard.

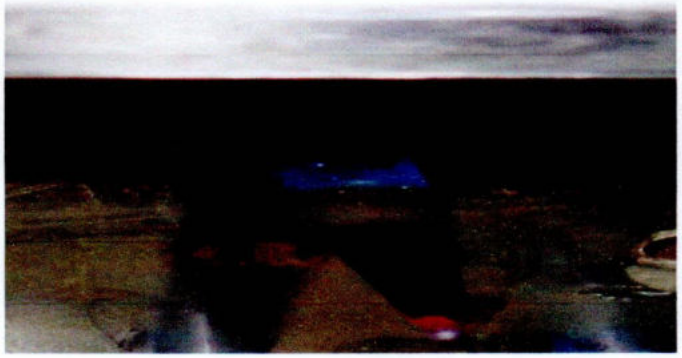


Figure 3. Control

Figure 4. Blood Lysis by Streptokinase

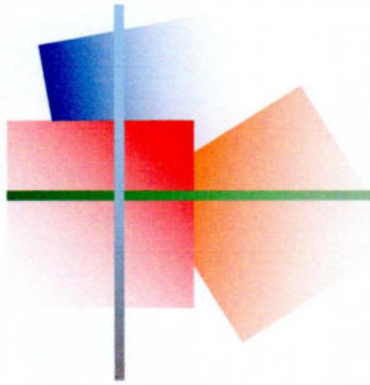
3.11 Invitro anticoagulant activity of *Triconella foenum-graecum (Methi)*

3.11.1 Phytochemical screening

Dried extracts were subjected for the presence of different phytoconstituent like alkaloid, steroid, flavonoid, tannin, glycoside etc.

3.12.2 Collection of blood and Plasma re-calcification

0.2 ml plasma, 0.1 ml of crude extract of different concentration and different volume of $CaCl_2$ (25 *m*M) were added together in a clean fusion tube and incubated at 37^0C in water bath. For control experiment extract solution was replaced by same volume of 0.9% saline water. The clotting time was recorded with stopwatch by tilting the test tubes every 5 seconds. This time is called the prothrombin time.



Chapter 4

RESULTS AND DISCUSSION

CHAPTER 4

RESULTS AND DISCUSSION

This experiment was conducted to study the Thrombolytic activity of ethanol plant extract that may act on cardiovascular thrombus.

This experiment was held under the Department of Physiology & Pharmacology, Faculty of Veterinary and Animal Science.

Finally five plant and spices have thrombolytic activity after first trial. I was carrying on my experiment these five plant and spices such as ajawin, pesta, fern, auram (kochu) and Tobacco plant.

The experimental units were kept in the laboratory. At first I weighted micro test tubes. The weight of each test tube is 0.75g. Fresh and clean water was made available at all the times. The experiment was conducted according to the completely randomized design.

Thrombolytic activity*:** As a part of discovery of cardio-protective drugs from natural sources the extractives of ***Carum copticum (Ajowan) were assessed for thrombolytic activity and the results are presented in Table 1. Addition of 100µl SK, a positive control (30,000 I.U.), to the clots and subsequent incubation for 90 minutes at 37°C, showed 91.67% lysis of clot. At the same time, distilled water was treated as negative control which exhibited negligible lysis of clot (4.74%). In this study, the carbon tetrachloride soluble fraction (CCSF) exhibited highest thrombolytic activity (57.33%).

Table 1: Thrombolytic property of different group

Sl. No.	Weight of Blood Before Lysis (gm) (mean ± SEmean)	Weight of blood (After lysis) (mean ± SEmean)	Level of Significance (P value)
Aajawin	0.3967 b ± 0.03811	0.2200 ± 0.1581	P > 0.001 1% Level of Significance
Methi	0.3967 b ± 0.03811	0.2320 ± 0.01304	
Streptokinase	0.3967 b ± 0.03811	0.0320 ± 0.01095	

Note: Values followed by same superscripts in the same Colum are not statistically significant (P>0.001), 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 lysised blood) gm, Control no change.

Table 1 Revealed that

Group A initial weight 0.3967 final weight 0.2200 weight lose 0.1767.

Group B initial weight 0.3967 final weight 0.2320 weight lose 0.1647.

Group C initial weight 0.3967 final weight 0.0320 weight lose 0.3647.

The thrombolytic activity presents significantly (p>0.001) in *Carum copticum (Ajowan)* than *Triconella foenum-graecum (Methi).*

Table 2: Lysis of clotted blood by Streptokinase at standard Thrombolytic agent

Sl. No.	Weight of Blood Before Lysis (gm)	Weight of blood (After lysis)	Result (amount of lysised blood) gm	Percentage	Average
1st Day	0.39	0.02	0.378	94%	91.67%
2nd Day	0.44	0.04	0.40	90%	
3rd Day	0.45	0.04	0.41	91%	

Table 3: Comparision of Thrombolytic Property between aajawin and methi regarding streptokinase.

Treatment Group	N	Subset for alpha = 0.05	
		1	2
Steptokinase	5	.0320	
Aajawin	5		.2200
Methi	5		.2320
Sig.	1% level of significance		

Note: Values followed by same superscripts in the same Colum are not statistically significant ($p>0.001$), different superscripts indicate that difference is significant ($p<0.001$). In this other tables, A=Total amount of blood, B= Weight of blood (After lysis), C= Result (amount of lysised blood) gm, Control no change.

Table 3 Revealed that

Group A initial weight 0.3680 final weight 0.1480 weight lose 0.2200.

Group B initial weight 0.3860 final weight 0.1540 weight lose 0.2320.

Group C initial weight 0.3820 final weight 0.1600 weight lose 0.2220.

The thrombolytic activity presents significantly ($p>0.05$) in *Triconella foenum-graecum (Methi/Fenugreek.* less than *Carum copticum (Ajowan)*

Table 4: Thrombolytic activity of different fractions of *Carum copticum*

Sample	**Thrombolytic Activity (% of lysis)**
SK	91.67%
Water	4.74%
ECSF	57.33%

SK= Streptokinase, and CCSF= Ethanolic extract of carum capticum fraction of the seed extracts of *Carum copticum (Ajowan)*

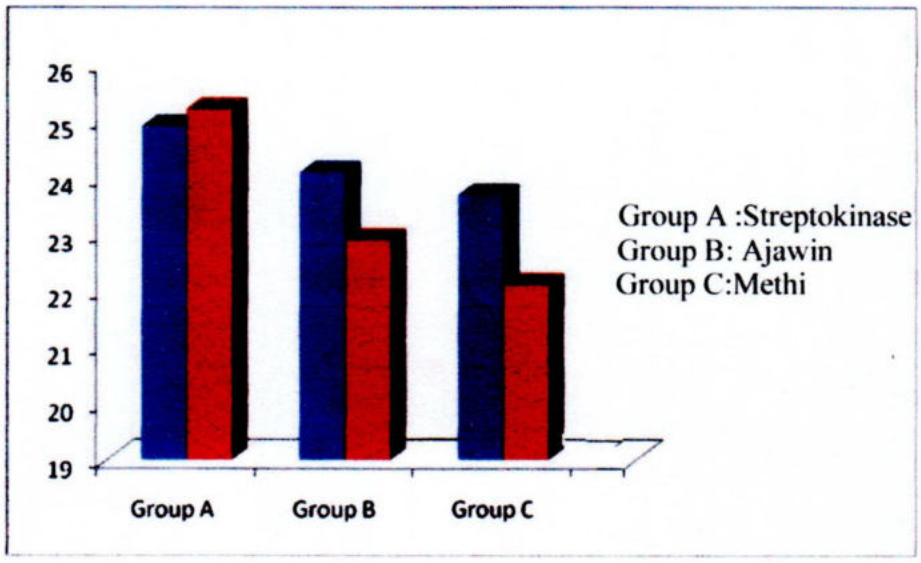


Fig. Thrombolytic activity

Table 5: Anticoagulant Property in Methi

Sl. No.	Clotting time (Minutes)	Control
A=1st Day	56.40 a ±0.8124	68.00 a ±2.2583
B=2nd Day	56.80 a ±0.8602	70.00 a ±0.4472
C=3rd Day	57.80 a ±0.8602	70.00 a ±0.3162
Probability value	0.00	0.00
LSD value	2.603	4.134
CV %	3.31	4.33

Note: Values followed by same superscripts in the same Colum are not 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 lysised blood) gm, Control no change.

Table 5 revealed that

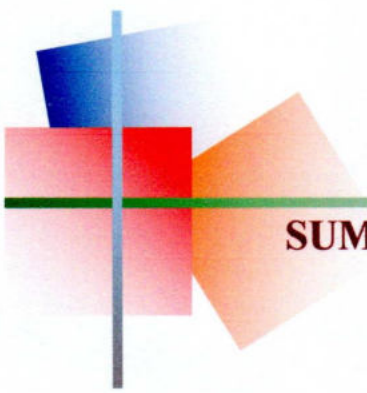
Group A Clotting time (Minutes) 56.40 control 68.00 .

Group B Clotting time (Minutes) 56.80 control 70.00 .

Group C Clotting time (Minutes) 57.80 control 70.00.

The anticoagulant activity presents significantly ($p>0.05$) in *Triconella foenum-graecum (Methi/Fenugreek).*

Anti-coagulant activities of aqueous and methanolic extract of *Triconella foenum-graecum (Methi/Fenugreek)* were carried out. From the present study it is proved that both the extract have remarkable anti-coagulant activity than the control solution. The anticoagulant of methi is 1 hour 3 minutes. Further study is under progress to isolate the pure component fraction.



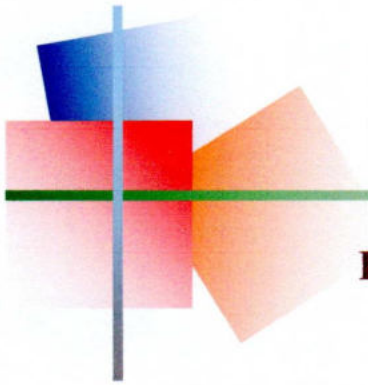
Chapter 5

SUMMARY AND CONCLUSION

CHAPTER 5

SUMMARY AND CONCLUSION

Five tube contain blood in three days experiment carried out in the laboratory in this research work. In this experiment extract of methi and ajawin were studied in terms of thrombolytic and anti-coagulant activity because we showed that this harbal plant or spices are available cost effective and produced treatment free from synthetic drug resudal effect suitable for human. In this research work extract of ajawin produced significant ($p<0.05$) increased of treatment of cardiovascular system in the live body. This experiment also proved that anti-coagulant property of methi increased significantly ($p<0.05$) that would be treated to cardiovascular system. No significant ($p>0.05$) the anti-coagulant property of ajawin. The further studies are necessary to see any adverse effect in relation to histopathology before making a definite conclusion.



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