Cytotoxic, Anti-oxidant and Thrombolytic Activity of Stem Extract of *Boehmeria malabarica* Wedd.

Submitted in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm)

Submitted by

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APPROVAL

This project, "Cytotoxic, Antioxidant and Thrombolytic activity of Stem Extract of *Boehmeria malabarica* Wedd submitted to the Department of Pharmacy, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy and approved as to it style and contents.

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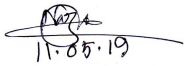

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Dedicated to my Parents and beloved Teachers

ABSTRACT

The present study was carried out to open a new avenues for the improvement of medicinal uses of *Boehmeria malabarica* Wedd primarily for the evaluation of phytochemical screening, in vitro antioxidant and cytotoxicity potential. The phytochemical screening was determined through qualitative analysis which indicates the presence of chemical constituents such as alkaloids, flavonoids, glycosides and tannins. The cytotoxic potential was determined by using brine shrimp lethality bioassay, and showed an LC_{50} of methanol extract and LC_{50} values of standard vincristine. The antioxidant activity was determined by using DPPH (1,1-diphenyl-2-picrylhydrazine). Extract showed DPPH scavenging that was comparable to standard ascorbic acid . The present study demonstrates that methanol extract of *Boehmeria malabarica* Wedd stem has significant antioxidant and cytotoxic effect and thrombolytic effect.

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CHAPTER ONE

INTRODUCTION

1.1 **General Introduction:**

Plants are used for variety of purposes. The history of natural product is relatively old and dates back to the time when the early man became conscious of his environment. Cultured and civilized man is said to have been on earth for some two or three million years and he has struggled for his life during the greater portion of the era .Thousands of years' effort, by examination much has thought him to differentiate between useful and harmful plants. Since then herbs have been used in all cultures as an important source of medicine [1].

The history of human culture and civilization of Egypt, Assyrian, China, and Indies valley, knows that the elders and wise man of those times used medicinal plants to treat many diseases. Information about these medicinal plants is present in the old literature, mythological stories, folklore, medicinal treaties, epic poems and thousand years' old manuscripts, copper plates and palm leaves and other information on these cultures which are preserved even today. The unearth of Shanidar cave in Iraq in 1963 opened the grave of Neanderthal man buried sixty thousand year ago along with so many flowers of his time. The plants present in the grave were later known to have many medicinal properties. The earliest records of the use of medicinal plants are that of Chaulmoogra oil from Hydnocarpus gaertn, which was identified to be effective in the treatment of leprosy. Such a use of medicinal plants for the treatment of leprosy was written in the pharmacopoeia of the Emperor of China between 2730 and 3000 B.C. In the same way, the castor seeds and seeds of opium were found from ancient Egyptian tombs, which confirm their use in that part of Africa as for back as 1500 B.C. The written records existing in "Ebers papyrus" also show the use of medicinal plants at that time in Egypt [2]

The most important factors for the continued use of the traditional medicines are its ready accessibility, cheapness and socio-cultural reasons. A long tradition of the use of herbal remedies exists in some countries and the people especially of the rural areas have more faith in the traditional medicines. The fact that most of the medicinal plants have been used over the ages for treatment of diseases is believable evidence that many of the medicinal plants prescription are realistically safe but scientific toxicological trials are still necessary [3]

1.2 Plant in traditional medicine:

Since ages, humans have relied on nature for their basic needs for the production of foodstuff, shelters, clothing, means of transportation, fertilizers, flavors, and fragrances, and not the least, medicines. Plants have formed the basis of sophisticated traditional systems of medicine that have been in existence for thousands of years and continue to provide humankind with new remedies. [4]

Traditional system of medicine is one of the centuries-old practice and long-serving companion to humankind in the fight against disease and in leading a healthy life. Indigenous people have been using the unique approach of their traditional system of medicine for centuries and among the most renowned are the Chinese, Indian, African systems of medicine. [5]

1.3 Status of medicinal plants in Bangladseh

About 500 medicinal plants have been reported to occur in Bangladesh. Almost 80% of rural population is dependent on medicinal plants for their primary health care. The local people conserve traditional knowledge through their experience and practices, which is handed down orally without any documentation. The over exploitation of wild medicinal plants has become a threat to its extinction. In Bangladesh there is no systemic cultivation process of conservation strategies about medicinal plants. There is no government police or rules and regulation about the medicinal plants cultivation conservation and marketing. There are almost 422 herbal medicinal companies using medicinal plants as raw materials mostly by importing from abroad.

1.4 Medicinal plants in world market

The global market of products derived from plants is estimated at $83 billion US and continues to grow. Furthermore, it is estimated that approximately 25% of modern drugs and as many as 60% of antitumor drugs are derived from natural products [6].

According to the WHO, between 65% and 80% of the populations of developing countries currently use medicinal plants as remedies [1]. The development of new products from natural sources is also encouraged because it is estimated that of the 300,000 plant species that exist in the world, only 15% have been evaluated to determine their pharmacological potential [7].

1.5 Herbal drug research: Bioactivity guided approach:

Books on herbal medicinal practice report numerous medicinal plants, which are still not investigated. These plants can be subjected to pharmacologic screening as per their traditional use to evaluate their utility. In case of significant result, chromatographic and spectroscopic methods can be applied to isolate the responsible agent. Bioactivity guided approach has three characteristic phases of investigation.

First: biological activity is detected in crude material, and a bioassay system is set up to permit the identification of active fractions and discarding the inactive ones.

Second: the crude material is fractionated by the most appropriate chemical procedures, all fractions are tested, and active fractions are further fractionated, and so on, until pure compounds are obtained.

Third: the chemical structure of pure compounds are determined.[8]

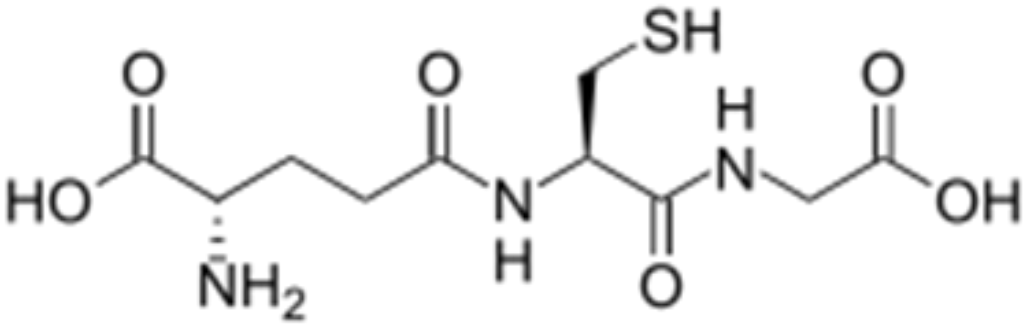
1.6 Modern Herbal Medicine:

The world health organization (who) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary healthcare ("traditional medicine"). Pharmaceuticals are prohibitively expensive for most of the world's population, half of which lived on less than $2 U.S. per day in 2002 (edgar j et al. 2002). In comparison, herbal medicine can be grown from seed or gathered from nature for little or no cost. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, quinine etc. According to the world health organization, approximately 25% of modern drug used in the united states have been derived from plants ("traditional medicine"). At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants.

1.7 Antioxidant

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase), produced internally, or the dietary antioxidants vitamin C, and vitamin E.

The term "antioxidant" is mostly used for two entirely different groups of substances: industrial chemicals that are added to products to prevent oxidation, and naturally occurring compounds that are present in foods and tissue. The former, industrial antioxidants, have diverse uses: acting as preservatives in food and cosmetics, and being oxidation-inhibitors in fuels. [9]



Structure of the antioxidant

All matter consists of atoms, and atoms consist of protons, neutrons, and electrons.

When atoms join forces, they become molecules. The human body is made up of many substances such as DNA, genes, and proteins, all of which are essentially molecules with thousands of atoms linked together. Chemical reactions occur when these molecules are either broken down or built up, and this is our metabolism.

If a molecule in the process of change loses an electron that it shouldn't, this molecule can become a free radical. This is another word you've probably heard before, and it refers to unstable, electrically charged molecules that react and damage other molecules, such as DNA.

1.7.1 Types of antioxidants

This is a list of antioxidants naturally occurring in food. Vitamin C and vitamin E – which are ubiquitous among raw plant foods – are confirmed as dietary antioxidants, whereas vitamin A becomes an antioxidant following metabolism of provitamin A beta-carotene and cryptoxanthin. Most food compounds listed as antioxidants – such as polyphenols common in colorful, edible plants – have antioxidant activity only in vitro, as their fate in vivo is to be rapidly metabolized and excreted, and the in vivo properties of their metabolites remain poorly understood. For antioxidants added to food to preserve them, see butylated hydroxyanisole and butylated hydroxytoluene.

1.8 Cytotoxicity

It is defined as the toxicity caused due to the action of chemotherapeutic agents to the living cells. The Cytotoxicity tests are very important in nanoparticles as they helps in determination of its proposed biomedical use. Cytotoxicity studies are a useful initial step in determining the potential toxicity of a test substance, including plant extracts or biologically active compounds isolated from plants. Minimal to no toxicity is essential for the successful development of a pharmaceutical or cosmetic preparation and in this regard, cellular toxicity studies play a crucial role. The concept of basal cytotoxicity, where deleterious effects are noted on structures and functions common to all human cells, is relevant when considering the relationship between acute toxicity and cytotoxicity. The selectivity index is an important measure to identify substances with promising biological activity and negligible cytotoxicity. Various bioassays and a number of different cell lines have been used to assess cytotoxicity of African medicinal plants. Additionally, extracting solvents ranging in polarity have been used to extract different plant parts, contributing to the wide variety of cytotoxicity results of African plants.

1.9 DPPH

DPPH is a common abbreviation for the organic chemical compound 2,2-diphenyl-1-picrylhydrazyl. It is a dark-colored crystalline powder composed of stable free-radical molecules. DPPH has two major applications, both in laboratory research: one is a monitor of chemical reactions involving radicals, most notably it is a common antioxidant assay. [11]

1.10 Thrombolytic Activity

Thrombolysis is the breakdown (lysis) of blood clots formed in blood vessels, using medication. It is used in ST elevation myocardial infarction, stroke, and very large pulmonary embolisms. The main complication is bleeding (which can be dangerous), and in some situations thrombolysis may therefore be unsuitable. Thrombolysis can also play an important part in reperfusion therapy that deals specifically with blocked arteries. Diseases where thrombolysis is used:

ST elevation myocardial infarction: Large trials have shown that mortality can be reduced using thrombolysis (particularly fibrinolysis) in treating heart attacks. It works by stimulating secondary fibrinolysis by plasmin through infusion of analogs of tissue plasminogen activator (tPA), the protein that normally activates plasmin.

Stroke: Thrombolysis reduces major disability or death when given within 3 hours (or perhaps even 6 hours) of ischaemic stroke onset when there are no contraindications to treatment.

1.11 Objectives of the project Work

The study was undertaken to evaluate the antioxidant and cytotoxic effect of *Boehmeria malabarica* Wedd is most widely used in experimental procedures were followed:

- ✓ To determine the antioxidant and cytotoxic effect activity of *Boehmeria malabarica* stem extract.
- ✓ Seeking for a new antioxidant and cytotoxic compound from *Boehmeria malabarica* plant extract
- ✓ Exploration of possible newer medicinal activities of *Boehmeria Malabarica* Wedd.

CHAPTER TWO

PLANT PROFILE

2.1 Plant review

2.1.1 Introduction of Plant

Scientific Name: *Boehmeria Malabarica*

Synonym: *Boehmeria glomerulifera*

2.1.2 Scientific classification

Kingdom: Plantae

Division: Magnoliphyta

Class: Magnoliopsida

Order: Rosales

Family: Urticaceae

Genus: Boehmeria

Species: Malabarica

Tribal name: Ka Nei Soi, Tha Mang Su, Chang Mang Gri (Marma).

2.1.3 Description of the plant:

Boehmeria malabarica Wedd. is an erect shrub or small tree with spreading branches. Leaves 10-20 cm long, elliptic or ovate to oblong, acuminate, crenulate or serrulate. Flowers minute, in small axillary clusters.

Using information:

In fever of babies, bath with leaf-boiled water is prescribed.

Distribution:

Chittagong, Chittagong Hill Tracts, Sylhet.

2.1.4 Plant part utilized in the study

- ✧ Stem

2.1.5 Image of *Boehmeria malabarica Wedd.*

Figure-1: Image of *Boehmeria malabarica* Wedd

2.2 Literature Review

No literature has found

CHAPTER THREE

MATERIALS AND METHOD

3.1 Preparation of extraction

3.1.1 Plant Collection

The plant was collected from Bangladesh National Herbarium.

3.1.2 Preparation of plant material

The plant materials were collected in fresh condition. Then these are cut into small pieces necessarily to make it suitable for grinding purpose and sun drying. The plant stem was dried by shed drying for about one week and finally the pieces were dried in an oven at 40-45^{o}C for 30 minutes to remove the remaining moisture. The materials are grinded into coarse powder with the help of a grinder and stored in an air tight container and placed in a dark, cool & dry place for further use.

3.1.3 Extraction

A glass made jar with plastic cover was taken and washed thoroughly the jar was rinsed with methanol. Then 300 gram of the dried stem was taken in the jar, and methanol (1500 ml) was poured into the jar up to 1 inch height above the sample surface as it sufficiently covered the sample surface. The plastic cover with Aluminum foil was used to close the jar properly to resist the entrance of air into the jar. The process was performed for 15 days. The jar was shaken on regular basis almost 2-3 times daily for a better extraction.

3.1.4 Filtration

After the extraction process the plant extract was filtered with sterilized cotton and filter paper, the cotton and filter paper was both rainsed with the required solvent and fitted in a funnel. The filtrate was collected in a glass container. And again done the process for refiltration.

3.1.5 Evaporation

The liquid extract was evaporated using rotary evaporator, the evaporation was carried out at 65-67^{0}C temperature. The condensed sample was transferred to a 100 ml beaker. After collecting the pure extract, it was placed directly under fan by covering the beakers with aluminum paper. To evaporate the remaining solvent present in the extract used hair dryer to remove some portion of water remaining in the extract.

3.2 Phytochemical Method

3.2.1 Reagents used for the different chemical group test

The following reagents were used for the different chemical group test (Trease, G.E. and Evans, W.C., 1983).

Mayer's reagent:

1.36 gm mercuric iodide in 60 ml of water was mixed with a solution contains 5 gm of potassium iodide in 20 ml of water.

Dragendroff's Reagent:

1.7 gm basic bismuth nitrate and 20 gm tartaric acid ware dissolved in 80 ml water. This solution was mixed with a solution contains 16 gm potassium iodide and 40 ml water.

Fehling's solution A:

34.64 gm copper sulphate was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water to produce 500 ml.

Fehling's solution B:

176 gm of sodium potassium tartarate and 77 gm of sodium hydroxide were dissolved in sufficient water to produce 500 ml. Equal volume of above solution were mixed at the time of use.

Benedicts Reagent:

1.73 gm cupric sulphate, 1.73 gm sodium citrate and 10 gm anhydrous sodium carbonate were dissolved in water and the volume was made up to 100 ml with water.

Molish Reagent:

2.5 gm. of pure α-naphtha was dissolved in 25 ml of ethanol.

3.2.2 Tests procedure for identifying different chemical groups

The following **t**ests were performed for identifying different chemical groups (Ghana, 1998).

❖ **Test for Carbohydrates**

1) **Molisch Test:**

2ml solution of crude extract was taken in a test tube and 2 drops of freshly prepared 10% alcoholic solution of α-napthol was added to it. Then, sulphuric acid was added to the mixture to the flown side of the inclined tube so that the acid forms a layer beneath the aqueous solution. A red or reddish violet ring would be formed at the junction of the two layers confirming the presence of carbohydrate. Upon standing or shaking, a dark purple solution would be formed.

The test tube was allowed to stand for 2 minutes; dilution of the sample mixture took place with 5ml of distilled water. A dull violet precipitate would be formed immediately confirming the presence of carbohydrate.

2) **Fehling's Test (Standard Test for Reducing Sugars)**

1ml of a mixture of equal volumes of Fehling's solution A and B was added to a 2ml aqueous solution of the crude extract and was boiled for a few minute. Presence of red or brick-red precipitate would be found immediately which would confirm the presence of carbohydrate.

❖ **Test for Flavonoids**

0.5ml of alcoholic solution of the extract of the sample was taken in a test tube and small piece of zinc ribbon or zinc dust with 5-10 drops of concentrated hydrochloric acid was added. The solution was boiled for a few minutes. Development of red to crimson colours would indicate the presence of flavonoids.

❖ **Test for Saponins**

0.5ml of alcoholic solution of the extract of the sample was diluted to 10ml using distilled water and was shaken in a graduated cylinder for 3-5 minutes. Production of persistence frothing would confirm the presence of frothing.

❖ **Tests for Alkaloids**

1) **Mayer's Test**

0.2ml of concentrated Hydrochloric acid was added to 2ml aqueous solution of the crude extract. Then 1ml of Mayer's reagent was added. Formation of yellow colour precipitate would indicate presence of alkaloids.

2) **Dragendroff's Test**

0.2ml of concentrated hydrochloric acid was added to 2ml aqueous solution of the crude extract. Then 1ml of Dragendroff's reagent was added. Formation of orange brown precipitate would indicate the presence of alkaloids.

❖ **Tests for Glycosides**

A small amount of an alcoholic extract of the fresh or dried plant material was taken in 1ml of water. Then, a few drops of aqueous sodium hydroxide were added. A yellow color was considered as an indication for the presence of glycosides.

A small amount of an alcoholic extract of the plant material was taken in water and alcohol and boiled with Fehling's solution. Brick-red precipitate was considered as an indication for the presence of glycosides.

Another portion of the extract was dissolved in water and alcohol and boiled with few drops of dilute sulfuric acid, neutralized with sodium hydroxide solution and boiled with Fehling's solution. Brick red precipitate was considered as an indication for the presence of glycosides.

❖ **Test for Steroids**

1) **Sulphuric acid test**

1 ml solution of chloroform extract was taken and then added1ml Sulphuric acid Red colorindicates the presence of steroid.

❖ **Test for gums**

5 ml solution of the extract was taken and then molish reagent and sulphuric acid were added. Red violet ring produced at the junction of two liquids indicate the presence of gums and carbohydrate

3.3 Antioxidant tests

3.3.1 Quantitative assay

Stock solution of the plant extract was prepared in ethanol from which a serial dilution was carried out to obtain concentration of 1, 5, 10, 50, 100, 500 µg/mL. Diluted solutions (2mL) were added to 3 ml of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 minutes for reaction to occur. The absorbance was determined at 517 nm and from these values corresponding percentage of inhibitions were calculated. Then % inhibitions were plotted against log concentration and from the

graph IC50 was calculated. The experiment was performed 3 times and average absorption was noted for each concentration.

3.3.2 Test Materials

Boehmeria malabarica of stem extract.

3.3.3 Apparatus

- Test tubes and stands
- Beakers
- Pipettes
- Volumetric flasks
- UV spectrophotometer
- Electric balance
- Spatula
- Foil paper
- Sonicator
- Funnel
- Tissue paper
- Marker

3.3.4 Reagents

- Methanol
- 0.004% DPPH (1 , 1 - diphenyl - 2 – picrylhydrazyl - hydrate)

3.4 Preparation 0.004% DPPH solution

4mg of DPPH was measured and dissolved in 100 mL of ethanol thus 0.004% DPPH solution was prepared.

3.4.1 Procedures

- At first 6 volumetric flasks are taken to make 6 different types of concentration (1,5, 10, 50, 100 and 500 µg/mL)
- Test tubes and volumetric flasks are rapped with foil paper.
- In 6 volumetric flaks serial dilution of extract is done and marked them respectively.
- 1ml of sample from each concentration and 3 mL of 0.004% DPPH solution is taken with the help of pipette in 6 test tubes respectively.
- Then solution is kept in dark place for 30 minutes with raping each test tube with foil paper
- In another test tube 3mL 0.004% DPPH & 1ml methanol and Ethyl acetate is taken to prepare blank solution.
- Then absorbance is taken by UV Spectroscopy.
- The percent of inhibition is calculated by using following formula------

$$\%\text{inhibition} = \frac{\text{Blank absorbance} - \text{Solution absorbance}}{\text{Blank absorbance}} \times 100$$

3.5 Cytotoxiccity

The brine shrimp lethality bioassay was first proposed by Michael AS and later developed by Vanhaecke P[75], and Sleet RB.It is based on the ability to kill laboratory – cultured Artemianauplii brine shrimp. The assay is considered an useful tool for preliminary assessment of toxicity, and it has been used for the detection of fungal toxins, plant extract toxicity[62] , heavy metals[63], cyanobacteria toxins[64] pesticides, and cytotoxicity testing of

dental materials.The brine shrimp lethality bioassay can be used as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products. Natural product extracts, fractions or pure compounds can be tested for their bio activity by this method . There is a positive correlation between brine shrimp lethality bioassay and cytotoxicity. This bioassay is indicative of cytotoxicity and a widerange of pharmacological activity of natural products. Generally, the ED_{50} values for cytotoxicity are one-tenth LD_{50} values in brine shrimp test.

3.5.1 Cytotoxic Activity Test

3.5.2 Materials

- Artemiasalina (brine shrimp eggs)
- Petri dish
- Tablet Salt
- Beaker (50mL, 1L)
- Pure NaCl
- Conical flask
- Pipettes
- Magnifying glass
- Micro-pipettes
- 2 liter plastic bottle
- Test tube
- Air stone
- Test tube stand
- Air pump
- Volumetric flask (25 mL)
- Light
- Spoon
- Stand to set bottle

3.5.3 Methodology

3.5.3.1 Preparation if sea water

Pure NaCI 10g and tablet salt 9g was weighed accurately dissolved in distilled water to make one liter and then filtrated of to get clear solution.

3.5.3.2 Construction of hatchery

A beaker of 500ml is taken and washed it carefully. 800ml of prepared sea water is poured in the beaker. Then it is placed on a water bath and temperature is kept at 37◦C. An oxygenator is fitted in the beaker to supply oxygen in water. All the system is placed at restricted area.

3.5.3.3 Hatching of brine shrimp

Sea water is taken in the beaker and shrimps eggs were added. Temperature of the sea water is adjusted to 37°C with water bath. An oxygenator is adjusted to supply oxygen on the sea water. The shrimp were allowed for 18 to 24 hours to hatch and mature as nauplii (larvae). The hatched shrimps were attracted to light to collect them and they were taken for bioassay

3.5.3.4 Preparation of stock solution

4 mg of plant extract is weighed and dissolves in 2mL of Dimethyl sulfoxide (DMSO) to get a solution of 100 µL.

3.5.3.5 Application of test sample and shrimp Nauplii in the testtube

6 Test tubes are taken and mark them according to concentration. Among them one is for control and others for different concentrated samples. All the test tubes are filled with 5ml of sea water . 10 shrimp nauplii are placed in each test tube. Before this different concentrated solution like- 400, 200, 100, 50, 25, 12.5, µg/µL are prepared from the stock solution by serial dilution method. Then with the help of a micro- pipette 20µL of solution is taken from each solution and were added into the 6test tubes. It is given as a manner that every test tube will contain same amount of DMSO and it will not raise than 40µL. After this , the final concentration within the test tube were 400, 200, 100, 50, 25, 12.5, µg/mL respectively & the DMSO was same (20 µL).

3.5.3.6. Counting of nauplii

After 24 hours the test tubes are observed and the number of survived nauplii in each test tube was counted the result were noted. . From this the % of lethality of brine shrimp nauplii was calculated at each concentration for each sample.

3.6 Study of thrombolytic activity test of plant extract

The blood was drawn from healthy volunteers without a history of oral contraceptive or anticoagulant therapy and 1.0 ml of venous blood was transferred to the previously weighed micro centrifuge tubes and was allowed to clot.

The thrombolytic activity of all extractives was evaluated by the method developed by using streptokinase (SK) as the standard substance. The extractive (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 containing the clot was again weighed to determine the clot weight (clot weight = weight of clot contaning tube – weight of tube alone). The ethical clearance for the experiment was obtained from the institutional ethical review committee and was performed by following the safe animal handling protocol.

To each micro centrifuge tube with the pre-weighed clot, 100 µl aqueous solution of different partitionates and crude extract was added separately. Then, 100 µl of streptokinase and 100 µl of distilled water were separately added to the positive and negative control tubes, respectively. All tubes were then incubated at 37° C for 90 minutes and observed for lysis of clot, if any. After incubation, the released fluid was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis as shown below:

% of clot lysis = (wt. of lysis clot /initial clot wt.)×100

CHAPTER FOUR

RESULT AND DISCUSSION

4.1 Yield value determination

Yield value determination of stem extract of *Boehmeria malabarica* Wedd extract:

$$\% \text{ Yield value} = \frac{10 \times 100}{300}$$

$$= 3.33\%$$

4.2 Phytochemical Screening

Table-1: Phytochemical test results of extract of stem of *Boehmeria malabarica* Wedd.

Tested groups	**Methanol extract of *Bohemeria malabarica* Wedd (stem)**
Alkaloids	Presence
Glycosides	Presence
Tannins	Presence
Flavonoids	Presence
Saponins	Presence
Gums	Absence
Carbohydrate	Presence
Steroids	Absence

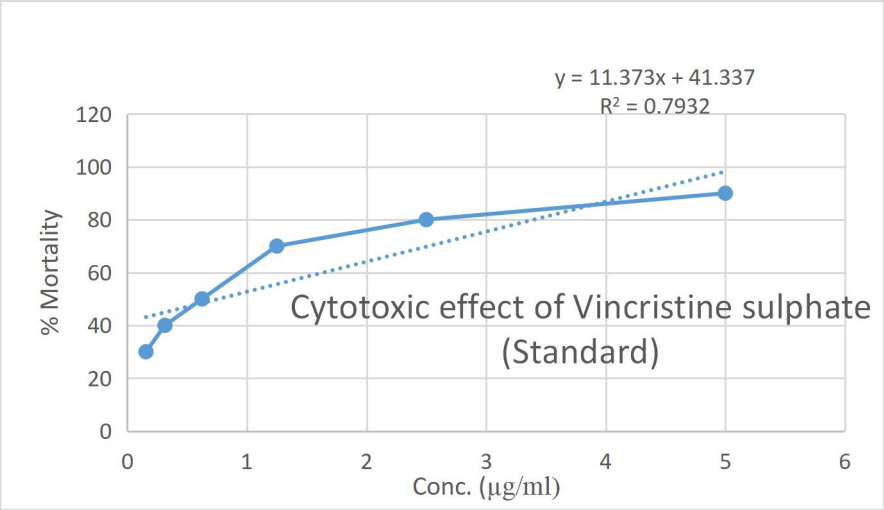
4.3 CYTOTOXIC ACTIVITY

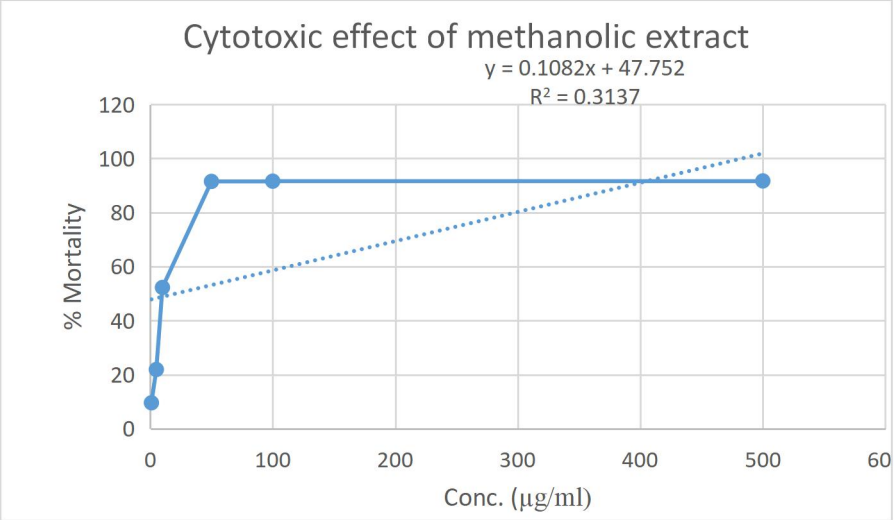
Table-2:-Results of cytotoxic study of *Boehmeria malabarica* stem brine shrimp nauplii-

Serial no.	Control		Standard		Methanol extract	
	No. of Alive	No. of Death	No. of Alive	No. of Death	No. of Alive	No. of Death
01.	10	00	00	10	03	07
02.	08	02	00	10	05	05
03.	08	02	00	10	06	04
04.	08	02	00	10	07	03
05.	08	02	02	08	08	02
06.	08	02	03	07	08	02

The mortality rate of the brine shrimp was found to be increased with the increasing of concentration of the extract and plotting of Concentration versus Response percentage put on the Ldp Line software produced an approximate linear correlation between them. The concentration at which 50% mortality(LC_{50}) of brine shrimp nauplii caused by the test extract were calculated from the graph by extrapolation and was found LC_{50} below in table.

Treatment	Conc.(µg/ml)	No. of nauplii taken	No. of dead nauplii	% Mortality	LC50 (µg/ml)	LC90 (µg/ml)
Methanol extract	640	10	7	70	0.76	4.28
	320	10	5	50		
	160	10	4	40		
	80	10	3	30		
	40	10	2	20		
	20	10	2	20		
Vincristine sulphate	5	10	9	90	355.725	855.73
	2.5	10	8	80		
	1.25	10	7	70		
	0.625	10	5	50		
	0.315	10	4	40		
	0.156	10	3	30		





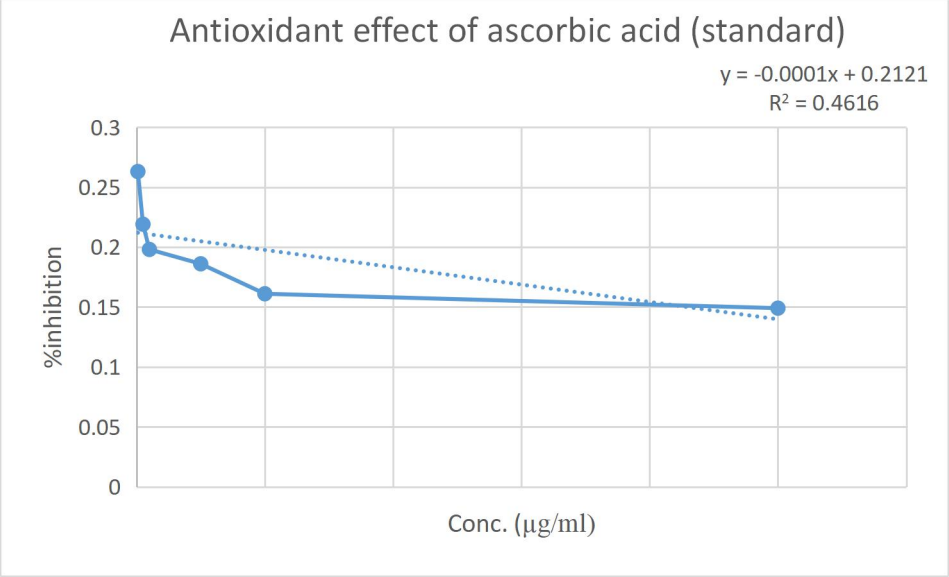
4.3.1 DISCUSSION

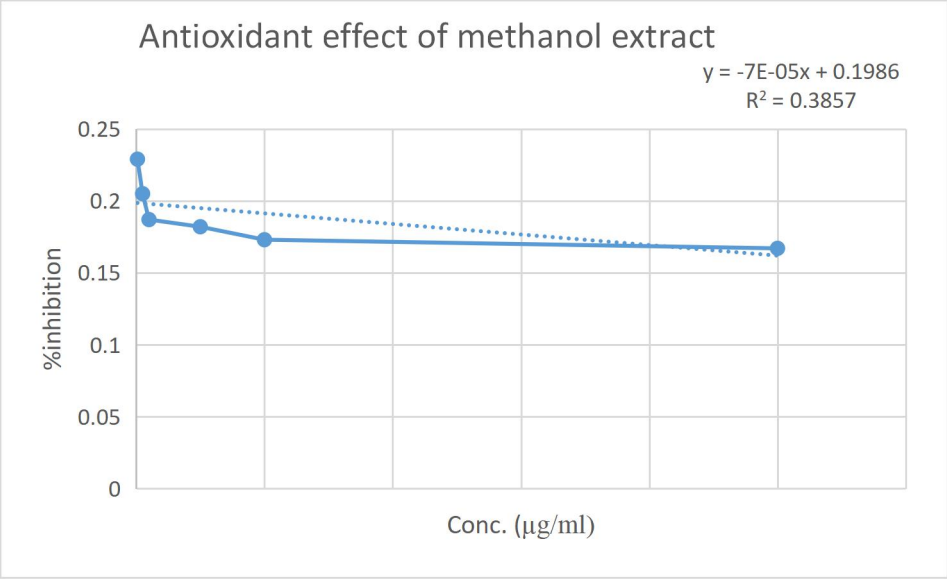
The brine shrimp lethality is a simple, rapid and convenient method for identifying biological activity having cytotoxicity in the crude extract. The methanol crude extract of *Boehmeria malabarica* Wedd showed moderate activity against the brine shrimp nauplii. The LC_{50} value of methanol lextract was 1.625 mg/mL compared with the LC_{50} value of standard (0.0171 mg/mL). Therefore, the response obtained in this assay suggests that the extract may contain cytotoxic compounds. However, this can't be confirmed without further higher and specific tests. So, further investigations are needed to get more information about the activities of the plant.

4.4 Results of Anti-oxidants test

Table-3: for standard (Ascorbic Acid) and extract of *Boehmeria malabarica* **s**tem .

Concentration (µg/mL)	Blank Absorbance (nm)	Standard (Ascorbic acid)		Methanol extract	
		Absorbance (nm)	% of Inhibition	Absorbance (nm)	% of Inhibition
1	0.315	0.263	16.5	0.229	27.30
5		0.219	30.48	0.205	34.92
10		0.198	37.14	0.187	40.63
50		0.186	40.95	0.182	40.95
100		0.161	48.89	0.173	48.89
500		0.149	52.6	0.167	52.69





4.5 Result of thrombolytic activity test

Table-4: Thrombolytic activity (in terms of % clot lysis) of *Boehmeria malabarica* extract

Name	Blank tube weight (gm)	1st clot+ Tube weight	1st clot lysis	2nd clot+ tube weight	2nd clot lysis	% of lysis
Standard (streptokinase)	0.79	1.48	0.69	1.29	0.5	72.4%
Control (Dist. Water)	0.77	1.56	0.79	1.35	0.58	26.58%
***Boehmeria malabarica* Stem extract**	0.81	1.53	0.72	1.23	0.42	41.67%

SK = Streptokinase (positive control)

Blank= Water as negative control.

4.5.1 Discussion

Addition of 100 µl SK, a positive control (30,000 IU), to the clots and subsequent incubation for 90 minutes at 37 °C, showed 72.4% lysis of clot. On the other hand, distilled water was treated as negative control which exhibited a negligible percentage of lysis of clot 26.58. The mean difference of in percentage of clot lysis between positive and negative control was found to be statistically significant. In this study *Boehmeria malabarica* displayed thrombolytic activity 41.67% whereas standard streptokinase showed 72.4%.

CHAPTER FIVE

CONCLUSION

5.1 Conclusion

The experimental findings of from this study showed that the methanol extract of *Boehmeria malabarica* Wedd stem had indicated presence of organic compounds like alkaloids, flavenoids, glycosides and tanins which can extensively responsible for pharmacological activity. The present evaluation also showed good antioxidant activity and cytotoxic activity. This studies was conducted using stem extract of *Boehmeria malabarica* Wedd. Further Advanced studies is highly needed to carry out compound isolation and identification of particular compound that are saddled with the responsibility for specific effect with possible mechanism of action.

CHAPTER SIX

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6.1 References

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