Phytochemical and Biological Evaluation of Methanolic Extract of *Boehmeria macrophylla* Leaves

A project submitted

by

Tasin Tahmid

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This work is dedicated to my parents and my sister to whom I owe my achievement.

Certification statement

This is to certify that, this project titled 'Phytochemical and Biological Evaluation of Methanolic Extract of *Boehmeria macrophylla* Leaves' submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.) from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Shejuti Rahman Brishty, Lecturer, Department of Pharmacy, BRAC University and this project is the result of the author's original research and has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the project contains no material previously published or written by another person except where due reference is made in the project paper itself.

Signed,

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Countersigned by the Supervisor

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Acknowledgement

I would like to begin my gratitude to Allah for the help in the completion of this research and preparation of this paper.

I would like to thank my supervisor Shejuti Rahman Brishty, Lecturer, Department of Pharmacy, BRAC University for her continuous provision, guidance and patience since the first day of the project work. As a person, she has continuously inspired and motivated me with her skilled knowledge which made me more passionate about the project when it began. I am really indebted to Professor Dr. Eva Rahman Kabir, Honorable Chairperson, Department of Pharmacy, BRAC University, for her support, encouragement and kind cooperation all through the project.

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Abstract

The present study has been designed to test and establish the scientific basis of the use of *Boehmeria macrophylla* as potential therapeutic agent in traditional medicine in Bangladesh. The methanolic extract of leaves of *Boehmeria macrophylla* was chosen for phytochemical screening and *in vitro* biological evaluation for the first time. The preliminary phytochemical screening revealed the presence of carbohydrates, phenols, phytosterols, glycosides and saponin in the crude methanolic extract of the plant. The leaf extract showed noteworthy antioxidant activities through DPPH free radical scavenging assay, along with its total phenolic content, flavonoid content and antioxidant capacity. Moreover, moderate cytotoxic effect in brine shrimp lethality bioassay was also observed. Antimicrobial activity was evaluated by disc diffusion method and the result showed maximum activity against *Bacillus subtilis*. Increasing concentration of the methanolic extract may help establish its potential against other microorganisms. Additionally, thrombolytic activity was tested using Streptokinase which further presented the plant extract as a mild thrombolytic agent. Future investigations on this plant may help in the development of new drugs and also aid in the research of combination drugs to treat a wide variety of diseases in Bangladesh as well as around the world.

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Abbreviations

AAE - Ascorbic acid equivalent

ASA - Ascorbic acid

BHT - *tert*-butyl-1-hydroxytoluene

DMSO - Dimethyl sulphoxide

DPPH - 1,1-diphenyl-2-picrylhydrazyl

GAE - Gallic acid equivalent

LAF - Laminar Air Flow

ME - Methanolic extract

QE - Quercetin equivalent

SK - Streptokinase

TAC - Total Antioxidant Capacity

TFC - Total Flavonoid Content

TPC - Total Phenolic Content

VS - Vincristine sulphate

Chapter One: Introduction

Chapter One: Introduction

1.1 Rationale and objective of the study

As far back as antiquated circumstances, in scan for safeguard for their sickness, the general population searched for drugs in nature. The beginnings of the restorative plants' utilization were instinctual, just like the case with creatures. In perspective of the way that at the time there was not adequate data either concerning the purposes behind the ailments or concerning which plant and how it could be used as a cure, everything depended on understanding. In time, the explanations behind the utilization of particular restorative plants for treatment of specific infections were being found (Petrovska, 2012).

In the subcontinent there are about 2000 medicinal plants and 450-500 plants are listed in which are growing or available in Bangladesh (Ghani, 2003). Different study shows that almost 700 medicinal plants are there in our county and have variety in diversity. Each of these plants has different properties and Unani, Ayurvedic, Herbal medicines are prepared from these plants (Sadi, 2012).

The main purpose of this study is to know about the constituents of the plants and their use in the medical purpose. About 80 % population of our country depends on drugs and medicinal plants for their cure of diseases (Sadi, 2012). This study will show the effective use of the plant parts and its constituents in curing different diseases. However, the plants may also be used in preparing drugs which will further be helpful in pharmaceutical industry and also in the medical purpose.

1.2 The plant family: Urticaceae

Urticaceae, the nettle family (order Rosales) comprising about 54 genera and 2,625 species of herbs, shrubs, small trees, and a few vines, is distributed primarily in tropical regions. The plant family Urticaceae has variety of leaves which are small and in greenish in color. These plants may grow in herbs, small trees or rarely polygamous. Members of the family Urticaceae have varied leaves and sap that is usually watery. The small greenish flowers often form clusters in the leaf axils. Both male flowers and female flowers may be borne on the same

plant, though some species are dioecious (producing male flowers on one individual and female on another). The curled stamens of the male flowers straighten quickly as the flowers open, releasing the pollen. The dry one-seeded fruit often is enclosed by the outer whorl of the flower cluster. (The Editors of Encyclopedia Britannica, Urticaceae, 2017).

1.3 The Plant genus: *Boehmeria*

Boehmeria is a genus of 47 species of flowering plants in the nettle family Urticaceae. Of the species, 33 are indigenous. It is widely distributed in the tropical and temperate regions. The *Boehmeria* plants are commonly unbranched. The leaves are opposite along the stem and the flowers are small.

1.4 Some reported species

a. *Boehmeria alliphylla*
b. *Boehmeria amarantus*
c. *Boehmeria australis*
d. *Boehmeria caudate*
e. *Boehmeria ramiflora*
f. *Boehmeria rigida*

1.5 Description of *Boehmeria macrophylla*

Boehmeria macrophylla is a shrub which grows up to 2.5 m long. The flowers come from June to September. Color of the flowers may be greenish or yellowish. The plant may grow in the sandy soil, in the shed or semi-shed area. The plant is found near the hill tracks of Chittagong and Sylhet. The plant has different names, somewhere it is called “Jongli chotta” and somewhere it is named “Kankura”. Tribal people call it “Mrangna (Marma)” (*Boehmeria macrophylla* Hornem., Bangladesh ethnobotany online database).

1.6 Traditional use of the plant

There is very few information about the medicinal use of this plant. Currently, this plant is used in the treatment of pathologic conditions like fever, trauma, dermatitis, boils etc.

Figure 1.1: ***Boehmeria macrophylla***

1.7 Taxonomic hierarchy

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Rosales

Family: Urticaceae

Genus: *Boehmeria*

Species: *Boehmeria macrophylla*

1.8 Local name

Bangladesh: Kankura, Jangli chotta

Tribal name: Mrangna (Marma) (Boehmeria macrophylla Hornem.; Bangladesh Ethnobotany online database)

1.9 Chemical constituents

a. Carbohydrate
b. Phytosterol
c. Flavonoids
d. Saponins
e. Glycoside
f. Terpenoids
g. Phenol (Hossain et al., 2016)

Chapter Two: Methodology

Chapter Two: Methodology

2.1 Collection and authentication of plant:

Boehmeria macrophylla plant was chosen for the investigation since no previous experiment was done on any of its properties. The plant *Boehmeria macrophylla* was collected in July 2017 from Sylhet, Bangladesh. Afterwards, the plant was submitted to the National Herbarium of Bangladesh (NHB), Mirpur, Dhaka for the authentication. The plant was identified a few days later (ACCESSION NO. 45299) and authenticated by the taxonomist of National Herbarium of Bangladesh, Mirpur, Dhaka.

2.2 Extraction Procedure

The extraction process can be divided into 2 steps:

- Plant material preparation and drying
- Extraction

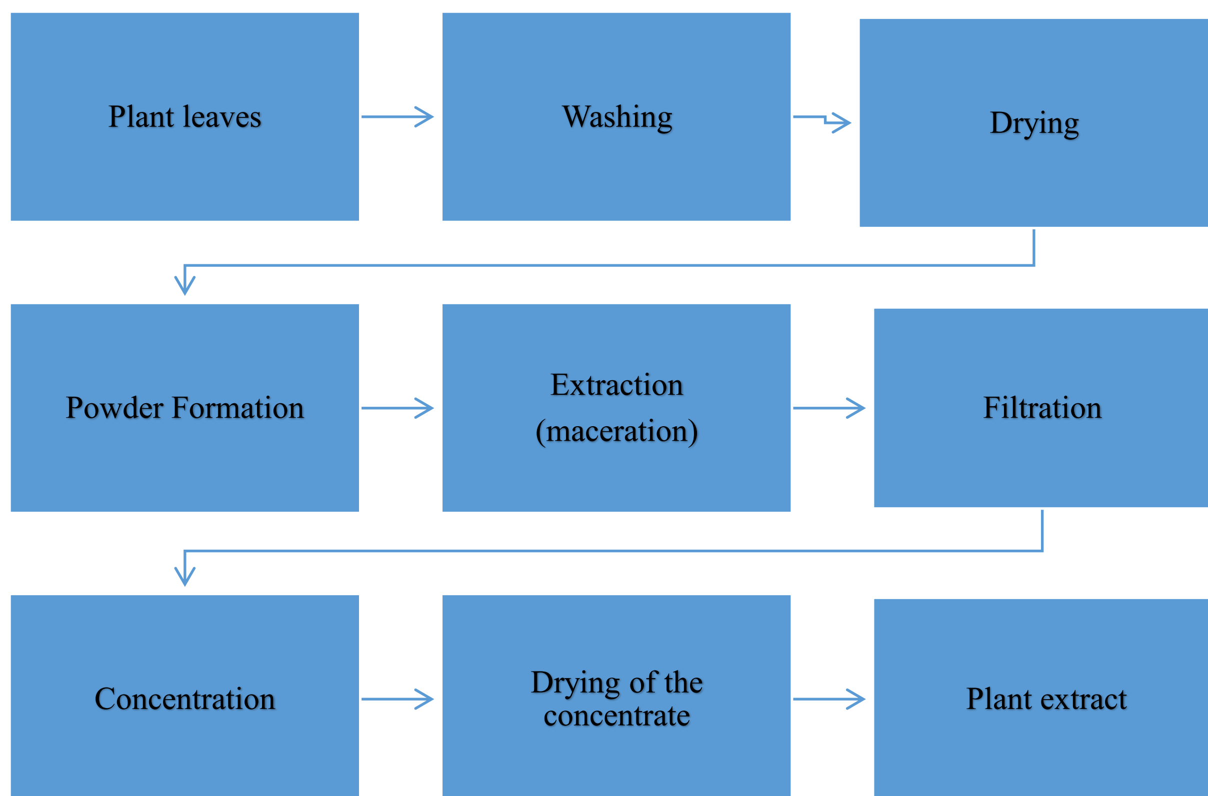

Figure 2.1: Step by step process for extraction of medicinal plant

2.3 Plant leaves preparation and drying

The leaves of the plant were collected from the plant stem and washed properly with clean water in order to remove any kinds of dust particles and debris. The leaves were then dried in the shed for around 7-10 days. After proper drying, the leaves were prepared for the next step.

2.4 Extraction process

2.4.1 Powder formation and weighing

The dried leaves were transformed into powder using a grinding machine. Air tight container was used to keep the powdered extract to prevent it from gaining moisture and any other cross contamination.

The total powder was weighed in the weight machine and the data was recorded in the result part. Necessary labeling was done for the plant weighing and also for the powder.

2.4.2 Extraction

For performing extraction, the process of maceration was employed and as organic solvent methanol was used. The powdered plant material of *Boehmeria macrophylla* contained in amber jar was soaked in 1000 mL of methanol for a time of 10 days at normal room temperature (25°C) with time to time agitation.

2.4.3 Filtration

After 10 days, the content of the amber jar was decanted into the beaker before it was concentrated. For this filtration process, cotton filter was used.

2.4.4 Concentration

The filtrates were concentrated using rotary evaporator (Heidolph) at 100 rpm. The temperature was maintained at 40°C and the process continued until concentrated methanolic extract was produced. After that, the mixture was transferred into a 500 mL beaker and was kept in the normal room temperature (25°C).

2.4.5 Drying

After the concentration was done, it was kept in the normal room temperature for the evaporation of the residual solvents. The evaporation took couple of days. Necessary steps were taken to prevent it from any kinds of microbial growth during drying. After a few days,

extract was collected after drying and evaporation of the solvents, and it was covered with aluminums foil and kept in the room temperature.

2.5 Phytochemical screening

Phytochemical screening was performed on the crude extracts of *Boehmeria macroyphylla* in order to find its qualitative chemical compositions like alkaloids, carbohydrates, tannin, flavonoids, glycosides, phenols, steroids, resins etc.
The following qualitative tests were performed:

2.5.1 Detection of alkaloid

For the detection of alkaloids, two qualitative tests were performed. 0.5 gm of methanolic extract of *Boehmeria macrophylla* was dissolved in 5 mL of 1% Hydrochloric acid, boiled in a water bath followed by filtration. Using the filtrate obtained the following tests were performed:

2.5.1.1 Mayer's test

As per Evans (1997), 10 mL Mayer's reagent was set up by dissolving 0.1358 gm of Mercuric (II) chloride and 0.5 gm of Potassium iodide in 10 mL distilled water.
At that point, from 2 mL of the filtrate, a couple of drops of Mayer's reagent were added the sides of the test tube. The arrangement of a white or creamy precipitate demonstrated the presence of alkaloids.

2.5.1.2 Wagner's test

As per Wagner (1993), 10 mL of Wagner's reagent was prepared by dissolving 0.2 gm of Iodine crystals and 0.6 gm of Potassium Iodide in 10 mL distilled water.
To 2 mL of the filtrate, a few drops of Wagner's reagent were added. Formation of a brownish-black precipitate confirmed the presence of alkaloids in the sample.

2.5.2 Detection of carbohydrates

As per Ramkrishnan et al. (1994), carbohydrate can be detected by weighing out 0.5 gm of methanolic extract of *Boehmeria macrophylla*, dissolving it into 5 mL of distilled water and then filtering the mixture. From the filtrate, two tests were performed:

2.5.2.1 Molisch's test

2 mL of the filtrate obtained which was treated with 2-3 drops of Molisch's reagent to which 2 mL of concentrated Sulfuric acid was pipetted along the sides of the test tube and was allowed to stand for a while. The formation of a violet ring indicated the presence of carbohydrates.

2.5.2.2 Fehling's test

To 2 mL of the filtrate, 1 mL of each of the Fehling's solution A and B were added in a 1:1 ratio and then boiled for a few minutes. Formation of a brick-red precipitation indicated the presence of reducing sugar which further indicated the presence of carbohydrate.

2.5.3 Detection of flavonoids

2.5.3.1 Lead acetate test

The methanolic extract was treated with a few drops of lead acetate solution and the formation of yellow colored precipitate signified the presence of flavonoids.

2.5.3.2 Zinc ribbon test

As per Sindhu et al. (2013), flavonoids presence can be detected by a different method. In a test tube containing 0.5 mL of methanolic extract, 5-10 drops of concentrated Hydrochloric acid and a small piece of Zinc were added. The solution was then boiled for a few minutes and then left to stand. The formation of a red to crimson color solution indicated the presence of flavonoids.

2.5.4 Detection of phenols/phenolic compounds

Ferric chloride test

As per Soni and Sosa (2013), the test was performed by measuring 2 mL of extract into a test tube followed by adding 3-4 drops of 15% (w/v) Ferric chloride solution. The formation of a bluish-black precipitate signified the presence of phenols.

2.5.5 Detection of phytosterols

Libermann Burchard's test

To a small amount of extract, 1 mL of chloroform was added and filtered. The filtrate was then treated with a 2 mL of Acetic anhydride, boiled and cooled. Finally, 1 mL of concentrated Sulfuric acid was added to the solution. Formation of a brown ring at the junction indicated the presence of phytosterols (Soni and Sosa, 2013).

2.5.6 Detection of steroids

Salkowski test

To 1-2 mL of extract, 2 mL of chloroform, 1 mL of Sulfuric acid were added. The appearance of red color indicated the presence of steroids (Ghani, 2003).

2.5.7 Detection of tannins

2.5.7.1 Lead acetate test

To 1 mL of the extract, a few drops of 1% Lead acetate solution were added and the formation of a yellow-colored precipitate indicated the presence of tannins (Tiwari and Bimlesh, 2011).

2.5.7.2 Potassium dichromate test

10% Potassium dichromate solution was prepared by dissolving 1 gm of Potassium dichromate which was dissolved in 10 mL distilled water to prepare this solution. 5 mL of aqueous solution of crude extract was dissolved in 1 mL of 5% Ferric chloride solution and the formation of yellow precipitation indicated the presence of tannin (Ghani, 2003).

2.5.7.3 Ferric chloride Test

5% Ferric chloride solution was prepared by dissolving 0.5 gm of Ferric chloride in 10 mL distilled water. 5 mL aqueous solution of crude extract was dissolved in 1 mL of 5% Ferric chloride solution and the formation of greenish black precipitation indicated the presence of tannin (Ghani, 2003).

2.5.8 Detection of resins

According to Soni and Sosa (2013), presence of resin could be identified by adding 5-10 drops of Acetic anhydride to 2 mL of the extract and heating the solution gently. This was followed by addition of 0.5 mL of Sulfuric acid to the solution. Presence of resin was identified by the formation of a bright purple color.

2.5.9 Detection of glycosides

The methanolic extract of *Boehmeria macrophylla* was hydrolyzed with dilute Hydrochloric acid before subjecting it to Borntrager's test (Selthilkumar, 2013).

Borntrager's test (modified)

To the 5 mL of filtrate, 5 mL of 5% Ferric (III) chloride and 5 mL of dilute Hydrochloric acid were added. This was followed by heating the mixture for 5 min in a boiling water-bath and cooling it down. Then, 5 mL of benzene was added to the mixture and shaken thoroughly. The organic layer was separated by using a separating funnel and an equivalent volume of dilute ammonia solution was added. The formation of a pinkish-red color in the ammoniacal layer signified the presence of glycosides (Kamalakar et al., 2014).

2.5.10 Detection of saponins

Froth test

The extract was diluted with distilled water and the volume was made up to 20 mL. The contents of the cylinder were shaken in a graduated cylinder for about 10-15 min. The formation of a foam layer of about 2 cm in height indicated the presence of saponins (Kokate, 1999).

2.6 Evaluation of antioxidant potential

Various antioxidant studies are performed and among them four of the studies have been selected for the antioxidant study of *Boehmeria macrophylla*.

The four studies are:

- DPPH free radical scavenging assay
- Total Phenolic Content (TPC)
- Total Flavonoid Content (TFC)
- Total Antioxidant Capacity (TAC)

2.6.1 DPPH free radical scavenging assay

DPPH stands for **1,1-diphenyl-2-picrylhydrazyl.** This method which is a free radical scavenging assay was described by Braca et al. (2001) and performed on the methanolic extract of *Boehmeria macrophylla* plant.

Reagents and chemicals for DPPH assay

- DPPH
- Methanol
- L-Ascorbic acid
- *tert*-butyl-1-hydroxytoluene (BHT)

2.6.1.1 Sample and standard preparation

5 mg of the extract was measured and dissolved in 10 mL of methanol. It is the sample stock solution. The sample concentrations were prepared by serial dilution of the sample stock solution to derive 10 serially diluted concentrations starting from 500 µg/mL to 0.997 µg/mL. Two reference standards were used named L-Ascorbic acid and *tert*-butyl-1-hydroxytoluene (BHT), and they were prepared by diluting in the same manner as the sample stock solution was prepared.

2.6.1.2 Preparation of blank solution

3 ml of methanol was the blank solution for this study.

2.6.1.3 Procedure

1 mL of each of the 4 fractions of sample and standards (L-Ascorbic acid and BHT) were taken in test tubes. In each of the test tube, 2 mL of the DPPH solution was added. Then, the test tubes were incubated for 30 minutes at room temperature. The absorbance of the resulting solutions was measured; control (DPPH and methanol) and the solutions were measured at 517 nm against blank (methanol) using a spectrophotometer (U-2910 UV-Vis

Spectrophotometer). The percentage of free radical scavenging activity was calculated from the equation as follows:

$$\text{Free radical scavenging activity} = \frac{(A_0 - A_1) \times 100}{A_1}$$

Where, A_0 = The absorbance of the control

A_1 = The absorbance of the sample/standard

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

2.6.2 Determination of Total Phenolic Content (TPC)

The total phenolic content of the methanolic extract of *Boehmeria macrophylla* was determined by the modified Folin-Ciocalteu method as mentioned by Wolfe et al. (2003).

Reagents and chemicals

- Folin-Ciocalteu Reagent (FCR)
- Gallic acid monohydrate
- Sodium carbonate
- Methanol

2.6.2.1 Reagent and sample preparation

20 mL FCR solution was prepared by taking 2 mL of FCR (10% FCR) in a 20 mL volumetric flask. 100 mL of 7.5% Sodium carbonate was prepared by measuring out 7.5 gm of Sodium carbonate in a 100 mL volumetric flask and then diluting it with distilled water to 100 mL mark.

2.6.2.2 Sample and standard preparation

0.1 mg of the extract was measured and dissolved in 10 mL of methanol. It is the sample stock solution. The sample concentrations were prepared by serial dilution of the sample stock solution to derive 10 serially diluted concentrations: 100 µg/mL to 0 µg/mL.

The standard was Gallic acid and it was prepared by diluting in the same manner as the stock solution was prepared.

2.6.2.3 Preparation of the blank solution

The blank solution contained the same volume of FCR solution and Sodium carbonate which was used in the experiment. 5 ml FCR, 4 ml Sodium carbonate and 1 mL of methanol was used to make volume up to 10 mL.

2.6.2.4 Procedure

1 mL of each of the fraction of sample and standard was taken in test tubes. 5 mL of FCR solution was added into the test tubes. And then 4 mL of Sodium carbonate solution was added. Each of the mixtures were vortexed for 15s and were allowed to stand for 30 min at 40°C in a water bath.

Finally, the absorbance of standard and sample solutions was measured against blank at 765 nm using spectrophotometer.

The total phenolic content, C, for each of the fractions was expressed as Gallic acid equivalent (Gallic acid equivalent)/gm of the extract using the following equation:

$$C = \frac{c \times V}{m}$$

Where,

C = Total content of phenolic compounds

c = Concentration of Gallic acid obtained from calibration curve (mg/mL)

V = Volume of sample solution (mL)

m = Weight of the sample (gm)

2.6.3 Determination of Total Flavonoid Content (TFC)

The total flavonoid contents of the extracts of *Boehmeria macrophylla* was studied according to the method which was described by Kumaran and Karunakaran (2007).

Reagents and chemicals

- Quercetin (standard)
- Methanol
- Potassium acetate
- Aluminium chloride

2.6.3.1 Reagent preparation

100 mL of 10% Aluminium chloride solution was prepared by measuring 10 gm of Aluminium chloride in a 100 mL volumetric flask. The solution was diluted it with distilled water to 100 mL mark.

100 mL of 1M Potassium acetate solution was prepared by measuring 9.815 gm of Potassium acetate in a 100 mL volumetric flask. The solution was distilled with water to make 100 ml.

2.6.3.2 Sample and standard preparation

12 mg of the extract was measured and dissolved in 10 mL of methanol. It is the sample stock solution. The sample concentrations were prepared by serial dilution of the sample stock solution to derive 7 serially diluted concentrations: 1200 μg/mL to 25 μg/mL.

Quercetin was used as the standard solution which was prepared in the same manner as the extract resulting in serially diluted concentrations, ranging from 1200 μg/mL to 25 μg/mL.

2.6.3.3 Preparation of the blank

The blank solution contained 200 μL of 10% Aluminium chloride solution, 200 μL of 1M Potassium acetate solution, 5.6 mL of distilled water and 4 mL of methanol. Total volume of the blank was 10 mL.

2.6.3.4 Procedure

1 mL of each of the sample fractions were taken into the test tubes where 200 μL Aluminium chloride, 200 μL potassium acetate solution was added. Then 3 mL of methanol added and finally 5.6 mL of water was added to the test tube to make it 10 mL in total.

After that the test tubes were incubated in the room temperature for 30 min.

The total flavonoid content, C, for each of the fractions was expressed as Quercetin equivalent (QE) using the following equation:

$$C = \frac{c \times V}{m}$$

Where,

C = Total content of flavonoid compounds

c = Concentration of Quercetin obtained from calibration curve (mg/mL)

V = Volume of sample solution (mL)

m = Weight of the sample (gm)

2.6.4 Determination of total Antioxidant Capacity (TAC)

The total antioxidant capacity of plant extract of *Boehmeria macrophylla* was determined by using the method as described by Prieto et al., (1999).

Reagents and chemicals

- L-Ascorbic acid (Standard)
- Methanol
- Ammonium molybdate
- Concentrated Sulfuric acid
- Sodium triphosphate

2.6.4.1 Reagent preparation

100 mL of 0.6M Sulfuric acid was prepared by measuring 3.28 mL of 98% concentrated Sulfuric acid in a 100 mL volumetric flask. The solution was diluted to a volume of 100 mL.
100 mL of Ammonium molybdate solution was prepared by measuring 4.494 gm of Potassium acetate in a 100 mL volumetric flask.
100 mL of Sodium phosphate solution was prepared by measuring 1.0645 gm of Aluminium chloride in a 100 mL volumetric flask and diluting it with distilled water to 100 mL mark.

2.6.4.2 Sample and standard preparation

12 mg of the extract was measured and dissolved in 10 mL of methanol. It is the sample stock solution. The sample concentrations were prepared by serial dilution of the sample stock solution to derive 4 serially diluted concentrations: 1200 µg/mL to 200 µg/mL.
Ascorbic acid was used as the standard solution which was prepared in the same manner as the extract resulting in serially diluted concentrations, ranging from 1200 µg/mL to 200 µg/mL.

2.6.4.3 Preparation of the blank

3 mL of reagent solution and 300 µL of methanol was used for blank solution.

2.6.4.4 Procedure

300 µL of each of the fraction of the samples were taken into the test tubes where 3 mL of all the reagents were added. Both the sample and the standards were incubated at 95°C at water bath for 90 min. Finally, the absorbance of the samples and the standards were measured at 695 nm in UV spectrophotometer.

The total antioxidant capacity, A, for each of the fractions were expressed as Ascorbic acid equivalent (AAE) using the following equation:

$$A = \frac{c \times V}{m}$$

Where,

A = Total content of antioxidant capacity

c = Concentration of Ascorbic acid obtained from calibration curve (mg/mL)

V = Volume of sample solution (mL)

m = Weight of the sample (gm)

2.7 Antimicrobial study by disc diffusion method

Antimicrobial study can be done by various processes like dilution method, automated antimicrobial susceptibility testing systems, disc diffusion method etc. Among these, the disc diffusion method is rapid, accurate and mostly inexpensive. Testing of antimicrobial susceptibility can be used for various purposes such as drug discovery, epidemiology and prediction of therapeutic outcome (Balouiri et al., 2016).

2.7.1 Apparatus and reagents

- Filter paper discs
- Nutrient agar
- Autoclave
- Petri dishes
- Sterile cotton
- Micropipette
- Laminar Air Flow (LAF) hood
- Incubator
- Refrigerator
- Ethanol
- Inoculating loop
- Sterile forces

- Spirit burner

2.7.2 Test organisms

Both gram positive and gram-negative organisms were taken for the test and they are listed in the table below:

Table 2.1: List of bacteria used in the study

Gram positive bacteria	Gram negative bacteria
Staphylococcus aureus	*Escherichia coli*
Bacillus subtilis	*Vibrio cholerae*

2.7.3 Preparation of medium

Medium was prepared by adding 3.8 mg Mueller Hinton agar in 100 ml distilled water. The mixture was then mixed thoroughly until the agar was completely dissolved in water. After that it was autoclaved for 20 min at 121^0C. The mixture was then cooled up to $(45\text{-}50)^0$C and poured into 4 sterile Petri dishes equally. The pouring into the dishes formed uniform depth in each plate. Finally, the dishes were allowed to cool and solidify at room temperature.

2.7.4 Culturing of bacterial strains

Nutrient Broth medium was prepared by dissolving 0.25 gm Nutrient Broth in 10 ml distilled water. From the collected bacterial strains, the bacteria were then transferred into Nutrient Broth medium and incubated for 24 hours to revive those bacteria. After 24 hours of incubation, new bacterial colony was formed and ready to use.

2.7.5 Preparation of disc

The disc was made of Whatman paper and the size of the disc were 6 mm in diameter. Then solutions of plant extract were added to the disc to soak all the plant extracts and the process took for 10-15 min.

2.7.6 Procedure

- A cotton swab was dipped into the solutions of bacteria. The excess fluid was gently squeezed against the tube.

- The swab was used to streak the bacterial suspension to the nutrient agar plate in one direction and then streaked diagonally.
- The agar plates were allowed to get dry for 5-7 min. Then, the discs containing extract of the plant was placed individually by using forceps, on the surface of the place.
- Kanamycin 30, Amoxicillin 10, Streptomycin 10 were used as the standard and they were placed on the surface of the plates.
- Finally, the Petri dishes were incubated for 24 hours at 37^0C to get the lawn growth of bacteria.

2.8 Brine shrimp lethality bioassay

Brine shrimp lethality bioassay system is a cytotoxicity testing methodology. This method is widely used as it is very quick in process, cheap and requires no special equipment.

2.8.1 Reagents and materials

- Sea salt (NaCl)
- *Artemia salina* leach (brine shrimp egg)
- Small tank with perforated dividing dam to hatch the shrimp
- Lamp to attract shrimps
- Pipettes
- Micropipette
- Glass vials
- Magnifying glass
- Test tubes
- Plant extracts or test samples

2.8.2 Experimental procedure

2.8.2.1 Preparation of seawater

114 gm of sea salt (pure NaCl) was weighed and dissolved into 3 liters of distilled water.

2.8.2.2 Hatching of brine shrimps

Artemia salina leach (brine shrimp eggs) was collected and it was used as the test organism. Into the seawater which was prepared the brine shrimp eggs were poured. 48 hours was

allowed to hatch the shrimp. After 48 hours matured nauplii were formed. Constant oxygen supply and light were carried out through the process.

10-12 living shrimps were added by the Pasteur pipette into the test tubes containing 5ml sea water.

2.8.2.3 Preparation of test samples of the experimental plant

The test sample was taken in vial and dissolved in 100 µL of pure Dimethyl sulfoxide (DMSO) to get stock solutions. This solution was then diluted with the test sample and prepared for a variety of concentrations.

Table 2.2: Test samples with concentration values after serial dilution

Test Tube No.	**Concentration (µg/mL)**
1	400.0
2	200 .0
3	100 .0
4	50 .0
5	25 .0
6	12.5
7	6.25
8	3.125
9	1.5625
10	0.78125

2.8.2.4 Preparation of control group

Control groups are used to validate the test method and ensure that the results obtained are only due to the activity of the test agent and the effects of the other possible factors are nullified. Usually two types of control groups are used:

i) Positive control

ii) Negative control

2.8.2.5 Preparation of the positive control group

Positive control in a cytotoxicity study is a generally recognized cytotoxic representative and the consequence of the test is compared with the result got for the positive control. In the

present review Vincristine sulfate was used as the positive control. Measured amount of the Vincristine sulfate was dissolved in DMSO to get a primary concentration of 20 µg/mL from which serial dilutions are made by using DMSO to get 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.625 µg/mL, 0.3125 µg/mL, 0.15625 µg/mL, 0.078125 µg/mL, 0.0390 µg/mL. The positive control solutions were added to the premarked vials containing ten living brine shrimp nauplii in 5 mL simulated sea water to gain the positive control groups.

2.8.2.6 Preparation of the negative control group

100 µL of DMSO was added to each of three premarked glass vials containing 5 mL of simulated sea water and 10 shrimp nauplii to use as control groups. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compounds.

100 µL of DMSO was added to each of three premarked glass vials containing 5 mL of recreated ocean water and 10 shrimp nauplii to use as control gatherings. In the event that the salt water shrimps in these vials demonstrate a quick death rate, then the test is considered as invalid as the nauplii kicked the bucket because of some reason other than the cytotoxicity of the mixes.

2.8.2.7 Counting of nauplii

After 24 hours, the vials were inspected using a magnifying glass and the number of survivors were counted. The percent (%) mortality was calculated for each dilution. The concentration-mortality data were analyzed statistically by using linear regression method. The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}) value. This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period.

2.9 Thrombolytic activity test

Thrombolytic drugs are utilized to break down (lyse) blood clot (thrombi). Blood clot can happen in any vascular bed; be that as it may, when they happen in coronary, cerebral or pneumonic vessels, they can be instantly hazardous - coronary thrombi are the reason for myocardial infraction, cerebrovascular thrombi produce strokes. Thrombolytic drugs break up blood clumps by actuating plasminogen, which forms a cleaved item called plasmin. Plasmin

is a proteolytic enzyme that is fit for breaking cross-links between fibrin particles, which give structural integrity of blood clusters. In light of these activities, thrombolytic drugs are additionally called "plasminogen activators" and "fibrinolytic drugs" (Klabunde, R. E, Thrombolytic (Fibrinolytic) Drugs).

2.9.1 Collection of blood sample

For the collection of blood sample, two healthy volunteers were chosen who have no previous history of oral contraceptives and anticoagulant drugs. Microcentrifuge tubes were weighed and from the donors 6 mL of blood was taken into that microcentrifuge tubes. They were assigned by the numbers 1, 2, 3 etc. Each participant provided written consent for the donation of the blood sample.

2.9.2 Streptokinase

Commercially available streptokinase was (S-Kinase, 1500000IU, Popular Pharmaceuticals Ltd.) collected and 5 mL of Sodium chloride (0.9%) was added into the vial to make the concentration 30000 IU. From this 100 µL suspension was collected and used as *in vitro* thrombolysis (Fahad Hussain, 2014).

2.9.3 Sample preparation

100 mg of the extracted sample was dissolved into 10 mL of the 0.9% Sodium chloride solution. The solution was then filtered (Fahad Hussain, 2014).

2.9.4 Procedure

The blood samples were permitted to incubate for 45 minutes at 37°C. After clot development, serum was totally evacuated (clot ought not to be irritated) and each tube having clot was again weighed to decide the coagulation weight (clot weight = weight of clot containing tube − weight of tube alone). Each microcentrifuge tube containing clot was legitimately marked, and 100 µL of plant extract, 100 µL of Sodium chloride (0.9%) (as a negative control), 100 µL of 30,000 IU reference streptokinase (as a positive control) were added to tubes with clot. Every one of the tubes were incubated at 37°C for 90 minutes. The liquid left was then precisely removed and the tubes were weighed once more. The distinction in weight prior and then afterward clot lysis was communicated as level of cluster lysis (Biozid, 2015).

Percentage clot lysis = (weight of the clot after lysis by sample and removal of serum/weight of the clot before lysis by sample) ×100

Chapter Three: Results and Discussion

Chapter Three: Results and Discussion

3.1 Determination of percentage yield of the plant extract, *Boehmeria macrophylla*

Table 3.1: Total weight of the powder plant before maceration

Initial weight (beaker)	306 gm
Final weight (powder + beaker)	611 gm
Total weight of the powder plant	305 gm

Table 3.2: Net weight of the plant extract after maceration

Initial weight (beaker)	309 gm
Final weight (extract+ beaker)	330.5 gm
Total weight of the extract	21.5 gm

Interpretation

A total weight of 21.5 gm of extract was produced in the maceration process and after drying of methanolic extract of *Boehmeria macrophylla,* which was carried out in the whole experiment.

Calculation of percentage yield of the extract

Extract yield percentage (%) = $(W_1 \times 100) / W_2$

Where; W_1= Net weight of extract after maceration

W_2=Total weight of powder taken for maceration

Percentage yield in methanol = (21.5×100)/305

= 7.05%

Interpretation

The total weight of the extract after maceration was found 21.5 gm whereas before maceration was 305 gm; therefore, the % yield of *Boehmeria macrophylla was* calculated to be 7.05%.

3.2 Phytochemical screening of *Boehmeria macrophylla*

Table 3.3: Phytochemical analysis of methanolic extract of *Boehmeria macrophylla* leaves

Class of compound	Result
Alkaloid	--
Carbohydrate	++
Flavonoid	++
Phenol	+
Phytosterol	+
Steroid	-
Tannin	-
Glycoside	+
Resin	-
Saponin	+

(+ means present in single test method, ++ means present in two experimental method, - means absent single test method, -- means absent in two experimental method)

Interpretation

The phytochemical screening of methanolic extract of leaves of *Boehmeria macrophylla* showed the presence of carbohydrate, phenol, phytosterol, glycoside and saponin, whilst there was absence of alkaloid, flavonoid, steroid and resin.

3.3 Brine shrimp lethality bioassay

The methanolic extract (ME) of leaves of *Boehmeria macrophylla* was examined for brine shrimp lethality bioassay. The cytotoxicity of the extract to brine shrimp was observed and the results are given in Table 3.5. The lethal concentrations (LC_{50}) of the test samples were determined by plotting the percentage of mortality rate of shrimps against the logarithm of concentration. The curve of regression analysis helps to provide the best-fit line. Vincristine sulfate (VS) was used as positive control and the LC_{50} was found to be 0.43 µg/mL. The LC_{50} of the methanolic extract of leaves of *Boehmeria macrophylla* was 41.40 µg/mL which is much higher than that of Vincristine sulfate (Table 3.6).

Table 3.4: Effect of Vincristine sulfate (positive control) on shrimp nauplii

Conc. (µg/mL)	**Log_{10}Conc.**	**% of mortality**	**LC_{50} (µg/mL)**
0	-	0	
0.039	-1.4089	20	
0.078125	-1.1072	30	
0.15625	-0.8061	30	
0.3125	-0.5051	40	
0.625	-0.2014	60	0.43
1.25	0.09691	80	
2.5	0.39794	80	
5	0.6989	90	
10	1.00	90	
20	1.30102	100	

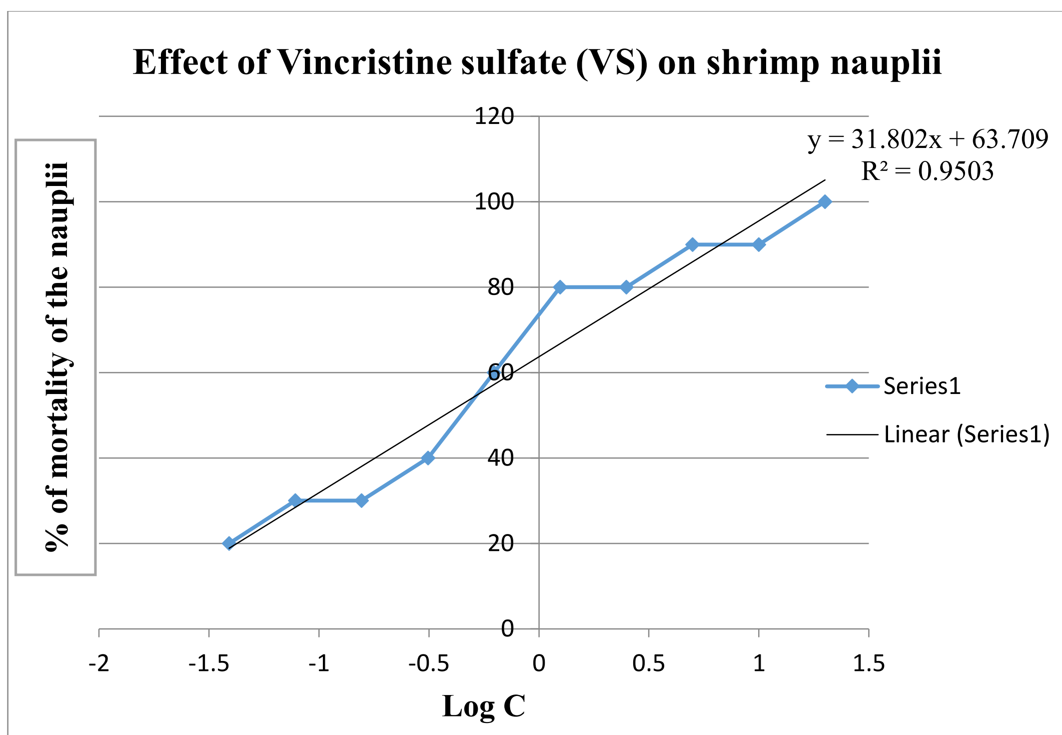

Figure 3.1: Plot of % mortality and predicted regression line of VS

Table 3.5: Effect of the methanolic extract (ME) of leaves of *Boehmeria macrophylla* on shrimp nauplii

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50} (µg/mL)
0	-	0	41.40
0.78125	-1.1072	20	
1.5625	0.19382	30	
3.125	0.49485	30	
6.25	0.79588	40	
12.5	1.09691	50	
25	1.39794	60	
50	1.69897	70	
100	2	80	
200	2.30103	90	
400	2.60206	90	

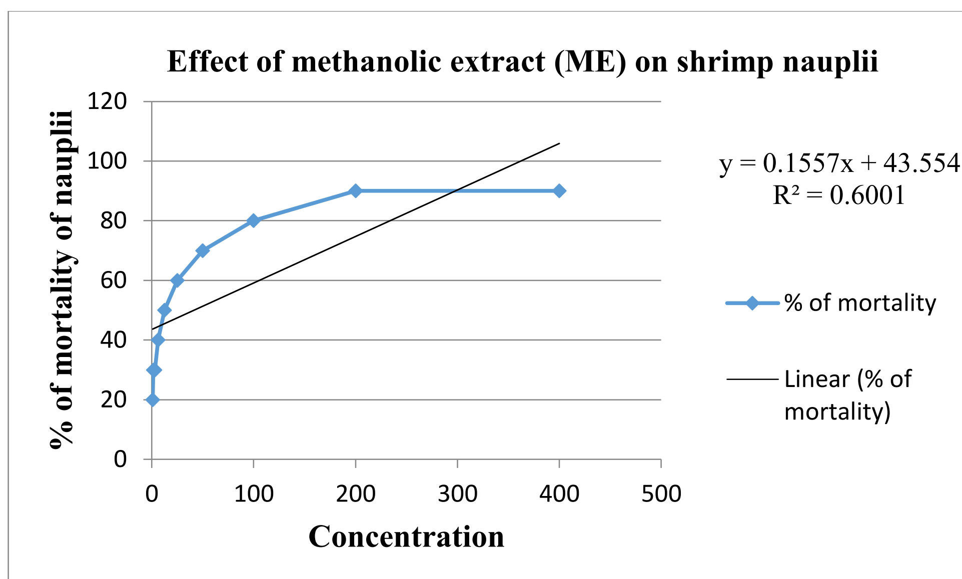

Figure 3.2: Plot of % mortality and predicted regression line of ME of *Boehmeria macrophylla* leaves

Interpretation

It is observed that with the increase in concentration of *Boehmeria macrophylla* from 0 µg/ml to 400 µg/mL, mortality also increased from 30-90% of nuplii. This also indicates a positive correlation existing between concentration and % of mortality.

Table 3.6: LC_{50} values of the test samples (VS and ME)

Test samples	Regression line	Regression coefficient (R^2)	LC_{50} (µg/mL)
Vincristine sulfate (VS)	y = 31.802x + 63.709	0.9503	0.43
Methanolic extract (ME) of *Boehmeria macrophylla* leaves	y = 0.1557x + 43.554	0.6001	41.40

3.4 Determination of antioxidant potential

3.4.1 DPPH free radical scavenging assay

The methanolic extract of the leaves of *Boehmeria macrophylla* (ME) was tested for free radical scavenging activity. Reference standards were Ascorbic acid (ASA) and *tert*-butyl-1-hydroxytoluene (BHT). In this research, methanolic extract (ME) of leaves of *Boehmeria macrophylla* presented an IC_{50} value of 225.654 µg/mL which indicated that the extract has much better free radical scavenging activity compared to the standards (Table 3.10).

Table 3.7: IC_{50} value of Ascorbic acid (ASA)

Absorbance of the blank	Conc. (µg/mL)	Absorbance of the extract	% inhibition	IC_{50} (µg/mL)
0.325	500	0.005	98.46	115.98
	250	0.006	98.15	
	125	0.015	95.38	
	62.5	0.024	92.61	
	31.25	0.068	79.07	
	15.625	0.098	69.84	
	7.813	0.139	57.23	
	3.906	0.186	42.76	
	1.953	0.175	46.15	
	0.977	0.193	40.61	

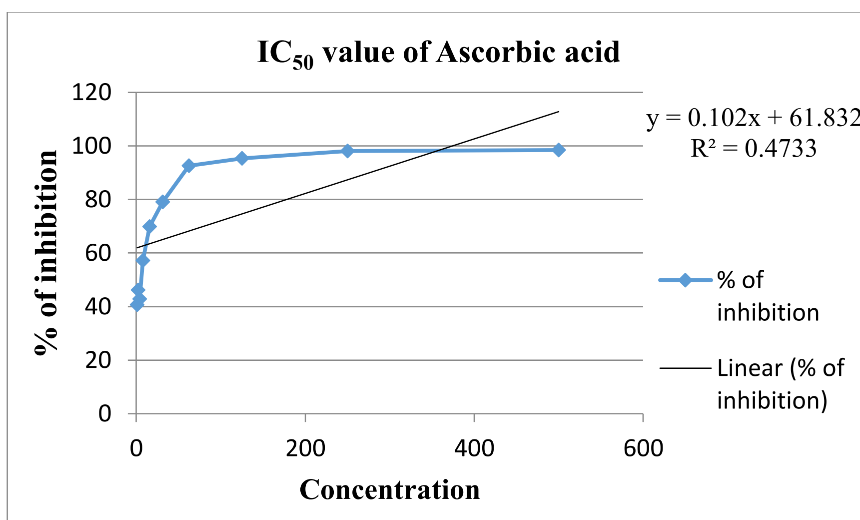

Figure 3.3: IC_{50} value of Ascorbic acid

Table 3.8: IC_{50} value of *tert*-butyl-1-hydroxytoluene (BHT)

Absorbance of the blank	Conc. (µg/mL)	Absorbance of the extract	% inhibition	IC_{50} (µg/mL)
0.325	500	0.018	94.46	96.74
	250	0.068	79.07	
	125	0.097	70.15	
	62.5	0.135	58.46	
	31.25	0.159	51.07	
	15.625	0.175	46.15	
	7.813	0.206	36.61	
	3.906	0.225	30.76	
	1.953	0.238	26.76	
	0.977	0.287	11.69	

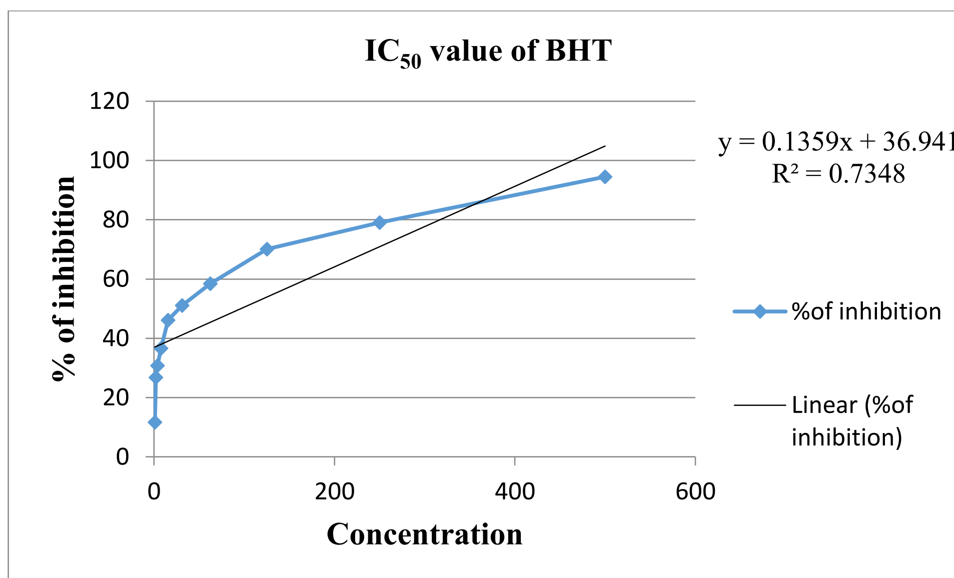

Figure 3.4: IC_{50} value of *tert*-butyl-1-hydroxytoluene (BHT)

Table 3.9: IC_{50} value of methanolic extracts (ME) of *Boehmeria macrophylla* leaves

Absorbance of the blank	Conc. (µg/mL)	Absorbance of the extract	% inhibition	IC_{50} (µg/mL)
0.325	500	0.138	58.18	225.654
	250	0.142	56.96	
	125	0.155	53.03	
	62.5	0.175	46.96	
	31.25	0.188	43.03	
	15.625	0.198	40	
	7.813	0.201	39.09	
	3.906	0.215	34.84	
	1.953	0.222	32.72	
	0.977	0.225	31.81	

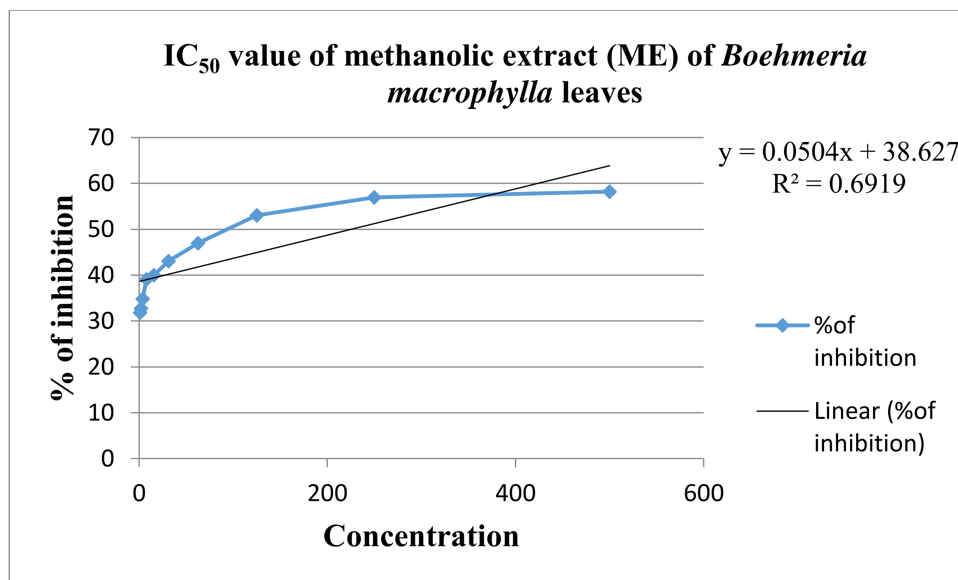

Figure 3.5: IC_{50} value of ME of leaves of *Boehmeria macrophylla*

Table 3.10: IC_{50} values of the standards and methanolic extract of leaves of *Boehmeria macrophylla*

Plant part	Sample code	Test Sample	IC_{50} (µg/mL)
Leaves of *Boehmeria macrophylla*	ME	Methanolic extract	225.654
ASA (Ascorbic acid) (standard)			115.98
BHT (*tert*-butyl-1-hydroxytoluene) (standard)			96.74

Interpretation

The IC_{50} value of dried extract of *Boehmeria macrophylla* signifies that a much higher concentration of 225.654 µg/mL was required for inhibiting 50% of all DPPH free radical scavengers, whilst a small concentration of 115.98 µg/mL was required to achieve the same inhibitory effect for Ascorbic acid and 96.74 µg/mL was required for BHT (*tert*-butyl-1-hydroxytoluene).

3.4.2 Determination of Total Phenolic Content (TPC)

The methanolic extract (ME) of the leaves of *Boehmeria macrophylla* was subjected for total phenolic content determination. Folin-Ciocalteu reagent was used for this test. Depending on the absorbance values of the extract solution, the value of total phenolic content of the extract was determined and differentiated with the standard solutions of Gallic acid (Table 3.11) equivalents. The total phenolic content of the sample is expressed as mg of GAE (Gallic acid equivalent)/gm of extract.

After investigation, the total phenolic content in ME was found to be 45.2 mg of GAE/gm of extract (Table 3.12).

Table 3.11: Standard curve prepration by using Gallic acid

Sl. No.	Conc. of the standard (µg/mL)	Absorbance	Regression line	Regression coefficient (R^2)
1	100	0.41	y = 0.004x + 0.0208	0.956
2	50	0.2		
3	25	0.16		
4	12.5	0.10		
5	6.25	0.09		
6	3.125	0.03		
7	1.5625	0.007		
8	0.78125	0.001		
9	0.3906	0.000		
10	0	0.000		

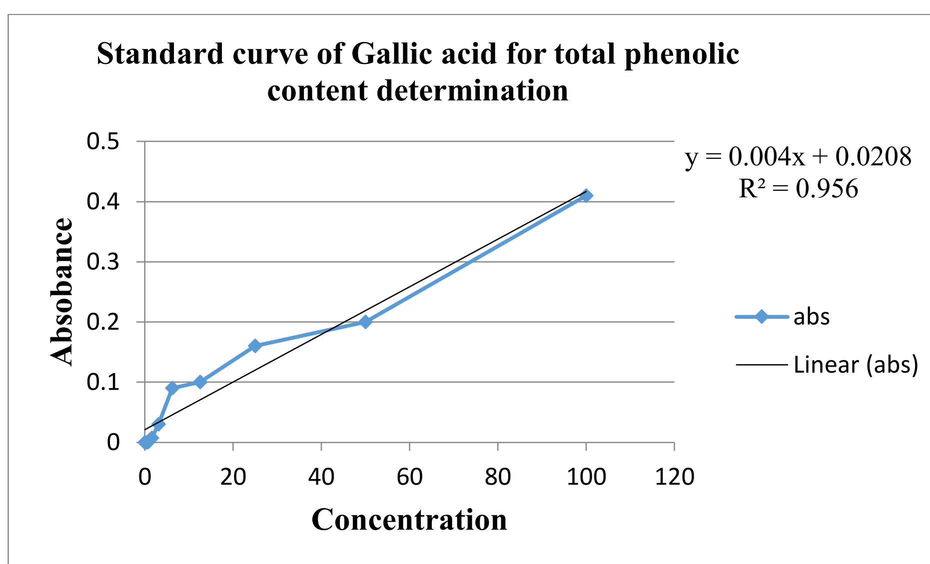

Figure 3.6: Standard curve of Gallic acid for total phenolic content determination

Interpretation

From Table 3.11 it is observed that, as the concentration of *Boehmeria macrophylla* was increased from 0-100 µg/mL, the total phenolic content also increased from 0 to 0.41 mg of Gallic acid per gram of dried extract. Therefore, it indicates that with increase in total phenolic content, the antioxidant activity of plant extract also increased. The equation of the calibration curve of Gallic acid (Figure 3.6) was found to be y = 0.004x - 0.0208 which was used to determine the total phenolic content of methanolic leaf extract of our plant. A regression

coefficient (R^2) of 0.956 was also obtained indicating a good relationship between the concentration and the absorbance.

Table 3.12: Total phenolic content of *Boehmeria macrophylla* (represented as mg of GAE/gm of extract)

Plant part	**Sample code**	**Test Sample**	**Total phenolic content (mg of GAE/gm of extract)**
Leaves of *Boehmeria macrophylla*	ME	Methanolic extract	45.2

3.4.3 Determination of Total Flavonoid Content (TFC)

The methanolic extract (ME) of the leaves of *Boehmeria macrophylla* was subjected to total flavonoid content determination. Quercetin was used for this test as standard. Depending on the absorbance values of the extract solution, the value of total flavonoid content of the extract was determined and differentiated with the standard solutions of Quercetin (Table 3.13) equivalents. The total flavonoid content of the sample is expressed as mg of QE (Quercetin equivalent)/gm of extract, and is given in Table 3.14.

The total flavonoid content in the methanolic extract of leaves of our plant was found to be 993.2 mg of QE/gm of extract.

Table 3.13: Standard curve prepration by using Quercetin

Sl. No.	**Conc. of the standard (µg/mL)**	**Absorbance**	**Regression line**	**Regression coefficient (R^2)**
1	25	0.005	$y = 0.0005x + 0.0724$	0.9252
2	50	0.054		
3	100	0.098		
4	200	0.174		
5	400	0.364		
6	800	0.498		
7	1200	0.569		

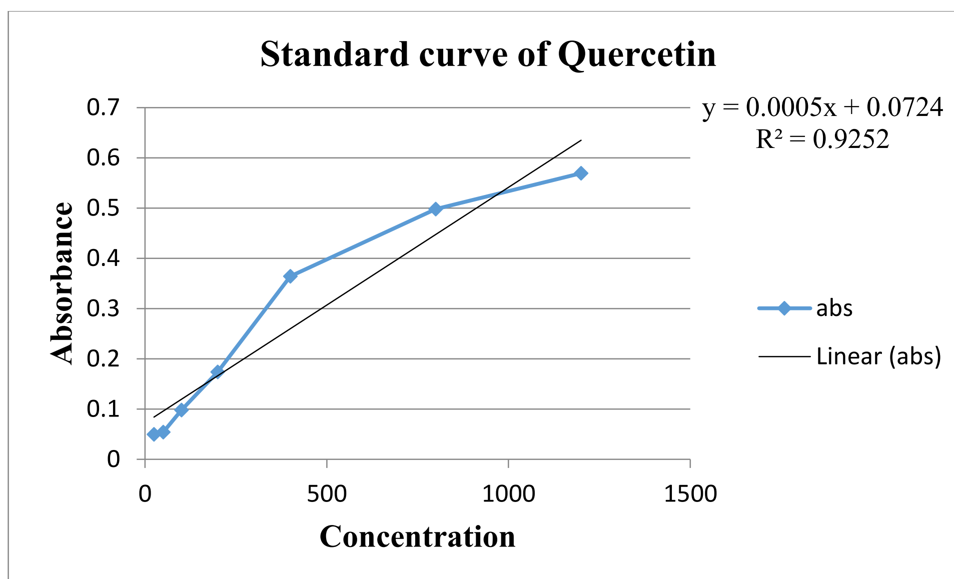

Figure 3.7: Calibration curve of standard Quercetin (at 415 nm) for determining TFC in ME of *Boehmeria macrophylla* leaves

Interpretation: From Table 3.13, it is observed that, as the concentration of *Boehmeria macrophylla* was increased from 25–1200 µg/mL, the total flavonoid content also increased from 0.021 to 1.369 mg of Quercetin per gram of dried extract. Therefore, it indicates that there is as well, a positive correlation between total flavonoid content of *Boehmeria macrophylla* and its antioxidant activity. The equation of the calibration curve of Quercetin was found to be $y = 0.0005x + 0.0724$ (Figure 3.7) which was used to determine the total flavonoid content of *Boehmeria macrophylla* leaf extract. A regression coefficient (R^2) of 0.9252 was also obtained pointing towards a good relationship between the concentration and the absorbance.

Table 3.14: Total flavonoid content of *Boehmeria macrophylla* (represented as mg of QE/gm of extract)

Plant part	Sample code	Test Sample	Total phenolic content (mg of QE/gm of extract)
Leaves of *Boehmeria macrophylla*	ME	Methanolic extract	993.2

3.4.4 Determination of Total Antioxidant capacity (TAC)

The methanolic extract (ME) of the leaves of *Boehmeria macrophylla* was subjected to total antioxidant capacity determination. Ammonium molybdate reagent was used for this test. Depending on the absorbance values of the extract solutions, the value of total antioxidant capacity of the extract was investigated and differentiated with the standard solutions of Ascorbic acid (Table 3.15) equivalents. The total antioxidant capacity of the sample is expressed as mg of AAE (Ascorbic acid equivalent)/gm of extract, and is provided in Table 3.16.

The total antioxidant capacity investigated in the methanolic extract was 205.52 mg of AAE/gm of extract.

Table 3.15: Standard curve prepration by using Ascorbic acid

Sl. No.	Conc. of the Standard (µg/mL)	Absorbance	Regression line	Regression coefficient (R^2)
1	200	0.840	y = 0.0004x + 0.7267	0.9342
2	400	0.886		
3	800	0.99		
4	1200	1.262		

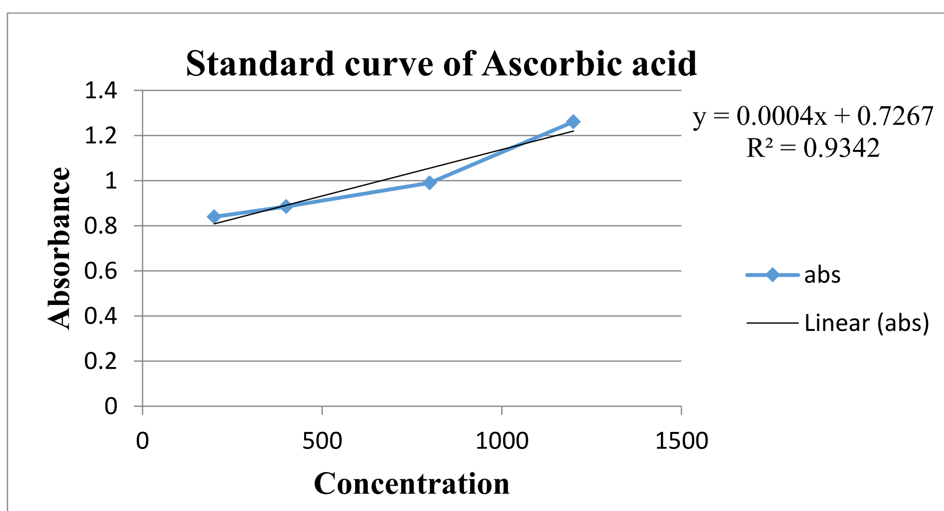

Figure 3.8: Calibration curve of Ascorbic acid (AA) at 695 nm for determining TAC in ME of *Boehmeria macrophylla* leaves

Interpretation

It is observed from Table 3.15 that, as the concentration of *Boehmeria macrophylla* was increased from 200 µg/ml to 1200 µg/mL, the total antioxidant capacity also increased from 1.080-4.122 mg of Ascorbic acid per gram of dried extract. This is indicative of a positive correlation existing between total antioxidant capacity of methanolic extract of *Boehmeria macrophylla* leaves and its antioxidant property. The equation of the calibration curve of Ascorbic acid was found to be $y = 0.0004x + 0.7267$ which was used to determine the total antioxidant capacity of *Boehmeria macrophylla* methanolic leaf extract. A regression coefficient (R^2) of 0.9342 was also obtained which signified a good relationship between the concentration and the absorbance.

Table 3.16: Total antioxidant capacity of *Boehmeria macrophylla* (represented as mg of AAE/gm of extract)

Plant part	**Sample code**	**Test Sample**	**Total phenolic content (mg of AAE/gm of extract)**
Leaves of *Boehmeria macrophylla*	ME	Methanolic extract	205.52

3.5 Antimicrobial Screening

The methanolic extract (ME) of leaves of *Boehmeria macrophylla* was investigated for antimicrobial activity by disc diffusion method with a concentration of 20, 30 and 40 µg/disc. The extract showed moderate antimicrobial activity against different tested microorganisms.

The maximum zone of inhibition exhibited by ME was found to be 20 mm against *Bacillus subtilis* for which Kanamycin was used as standard. The moderate antimicrobial activities were found against *Vibrio cholera* (having zone of inhibition of 16 mm, standard was Amoxicillin) and *Escherichia coli* (zone of inhibition of 15 mm, standard: Cefixime).

The results given in the tables are based on the highest concentration (40 μg/disc) of plant leaf extract that was used in this antimicrobial study.

Table 3.17: Antimicrobial activity of methanolic extract of leaves of *Boehmeria macrophylla*

Test microorganisms	Diameter of zone of inhibition (mm)	
	Sample	Standard
	Gram Positive Bacteria	
Bacillus subtilis	20	27.5 (Kanamycin)
Staphylococcus aureus	10	30 (Cefixime)
	Gram negative bacteria	
Escherichia coli	15	29 (Cefixime)
Vibrio cholerae	16	26.5 (Amoxicillin)

3.6 Thrombolytic activity test

Addition of 100 μL Streptokinase (SK), a positive control (15,00,000 I.U.) to the clots along with 90 minutes of incubation at 37°C, showed 11.4% clot lysis. Clots when treated with 100 μL Sodium chloride (negative control or blank solution) showed only negligible clot lysis (4%). The *in vitro* thrombolytic activity study revealed that the methanolic extract of leaves of *Boehmeria macrophylla* lysed about 3% of blood clot. The effective clot lysis percentage by methanolic extract of *Boehmeria macrophylla*, positive thrombolytic control (Streptokinase) and negative control (Sodium chloride) is presented below.

Table 3.18: Thrombolytic activity test

Serial no.	Weight of the tube (W_1)	Weight of the tube with clot (Before lysis) (W_2)	Weight of the clot (W_3)	Weight of tube with clot (After lysis) (W_4)	$W_5 = (W_4 - W_1)$	Percent of unlysed clot	Percent of clot lysis
1 (Methanolic extract)	0.828	1.471	0.643	1.452	0.624	0.970	3%
2 (Streptokinase)	0.789	1.617	0.828	1.523	0.734	0.886	11.4%
3 (Sodium chloride/blank)	0.786	1.524	0.738	1.495	0.709	0.960	4%

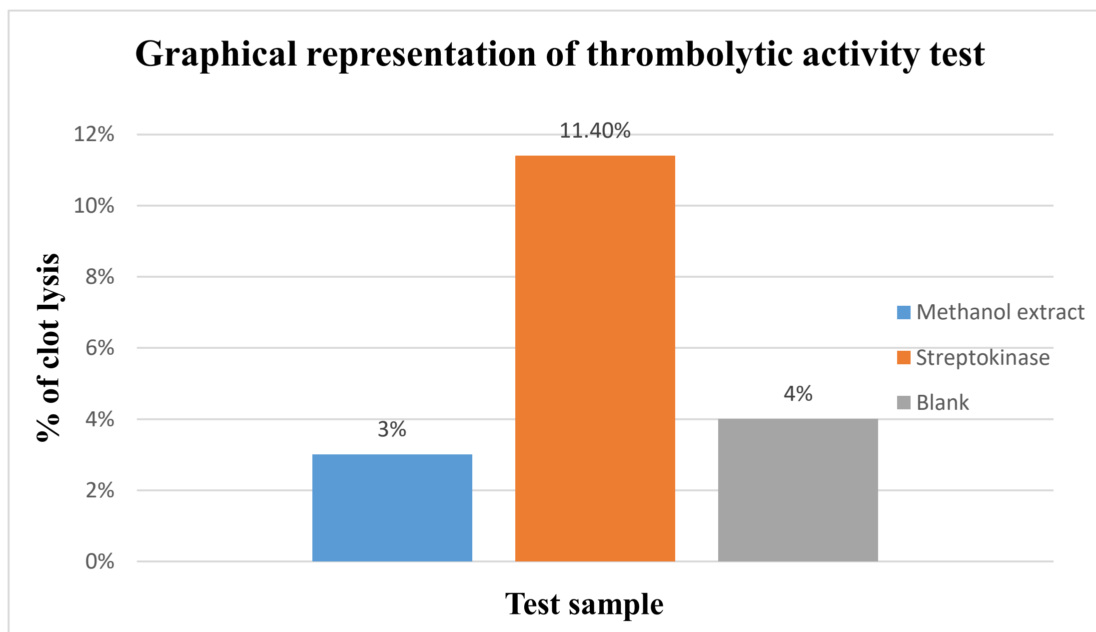

Figure 3.9: Comparison of percent of clot lysis of Streptokinase, blank and methanolic extract of *Boehmeria macrophylla* leaves

Interpretation

Streptokinase, which was employed as positive control caused lysis of blood clot for about 11.4%, whereas the methanolic extract of *Boehmeria macrophylla* leaves lysed about 3% of blood clot. This indicates that methanolic extract has mild thrombolytic activity.

Chapter Four: Conclusion

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The leaves of *Boehmeria macrophylla* were used traditionally for the treatment of fever, dermatitis, trauma etc. This investigation showed that there is presence of glycosides, phenols, phytosterols and saponins in the plant extract which may be exploited for treating specific diseases.

The moderate cytotoxic property exhibited through brine shrimp lethality bioassay is valuable for establishing its cytotoxic potential. The antimicrobial test showed the activity of the methanolic extract against *Bacillus subtilis*. Its antimicrobial potential can be further evaluated by means of determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the plant extract. Preliminary thrombolytic screening confirmed the plant extract as a mild thrombolytic agent. This can be further used as an anticoagulant agent if incorporated in the proper concentration.

Thus, the outcome from the current study on methanolic leaf extract of *Boehmeria macrophylla* gives a positive sign to use this as a medicinal plant. However, studies are needed to be designed to explore other *in vitro*, as well as *in vivo* biological activities. Animal models can be valuable to observe to which extent the plant extract can affect a biological system and show similar therapeutic efficacies as synthetic drugs.

Chapter Five: References

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