Phytochemical and Biological Investigation of *Persicaria glabra*

A project submitted

by

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This work is dedicated to my parents and my sibling to whom I owe my achievements.

Certification statement

This is to certify that, this project titled ‘Phytochemical and Biological Investigation of *Persicaria glabra* submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Ashis Kumar Podder, Lecturer, Department of Pharmacy, BRAC University and that appropriate credit is given where I have used the language and ideas of writings.

Signed,

_____

Countersigned by the Supervisor

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Acknowledgement

First of all, I am thankful and expressing my gratefulness to the Almighty who overs me his divine blessings, patient, mental and physical strength to complete this project work. I would like to thank my supervisor Ashis Kumar Podder, Lecturer, Department of Pharmacy, BRAC University for his continuous provision, guidance and patience since the first day of the project work. As a person, he has continuously inspired and motivated me with his skilled knowledge which made me more passionate about the project when it began. I am really indebted to our chairperson, Dr. Eva Rahman Kabir, Chairperson, Department of Pharmacy, BRAC University, for her support, encouragement and kind cooperation all through the project.

Finally, I would like to thank the laboratory officers and assistants for their continuous guidance and cooperative attitude.

Abstract

Present study was performed to establish the scientific basis of the use of *Persicaria glabra* as one of the curing and therapeutic agents of traditional practices in Bangladesh. The methanolic extract of the leaves was subjected to in vitro biological investigation for antioxidant test (Total phenolic content and DPPH assay), antimicrobial test (Disc diffusion method) and cytotoxicity test (Brine shrimp lethality bioassay). The plant extract showed potent cytotoxic activity nearly equivalent to standard vincristine sulfate compound. The study also confirmed presence of good amount of total phenolic content and free radical scavenging activity by DPPH method. Antimicrobial screening also done by the extract but it showed low antimicrobial activity compared to standard antimicrobial agent (kanamycin). However, more investigation is needed to identify in vitro anticancer activity on human cell line for the justification of its traditional use for squamous cell carcinoma and high cytotoxic activity of the leaf extract.

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Abbreviations

DPPH - 1, 1-diphenyl-2-picrylhydrazyl

ASA - Ascorbic Acid

DMSO - Dimethylsulphoxide

GAE - Gallic Acid Extract

VS - Vincristine Sulphate

TPC - Total Phenol Content

WHO - World Health Organization

ME - Methanolic extract

Chapter 1: Introduction

1.1 Rationale and objective of the work:

From the sunrise of civilization, medicinal plants are part and parcel of human society to fight diseases commonly used in treating and preventing particular diseases. Medicinal plants are always playing a beneficial function in health care. Medicinal plants re essential part of traditional medicines. The traditional drug is the collection of the knowledge, practices, and skills which are actually constructed on the theories, experiences and beliefs of indigenous to various cultures, whether explainable or not, used to keep good health as well as in the diagnosis, prevention, improvement or treatment of mental and physical illness. Traditional medicines use parts like leafs or roots to treat different diseases. Nature is the basic source of 87% of drugs used to treat all type of human diseases. About 25% of recommended drugs made from the plant. In developing countries, around 80% people depend on traditional based medications for their wellbeing (Khatun, 2004). In Bangladesh, more than 500 medicinal plants found in this region and many of them have an unknown therapeutic effect. To discover those unknown effects of various medicinal plants we need to utilize the knowledge of modern science and phytopharmacology is one of them. Phytopharmacology was designed by the Russian researcher David Macht in the 1930s. Since then, this term has changed its importance to become a well-known field of drug research. In this project, I have focused on traditional plants used in Bangladesh to discover some unknown pharmacological effect of those plants like antioxidant, antimicrobial and cytotoxicity to discover a new source of drugs to treat diseases efficiently and cost effectively.

The estimated amount of higher plants mainly angiosperms and gymnosperms on earth are 250,000 (Ayensu and De Filipps, 1978), with an upper level as high as 500,000 (Trippo *et al*., 1997 and Schultes, 1972). More than 6% of the medicinal plants have been screened for biological activity. Whereas, phytochemical investigations have been run on 15% plants. (Verpoorte, 2000). Furthermore, use of original structures of medicines based on the nearby accessible raw materials and medicinal plants have always been highlighted by the World Health Organization. It would be exceptionally beneficial in lessening consumption of synthetic drugs and antibiotics. Moreover, the use of expensive intermediary chemicals will also fell down (Ali, 2010).

Nowadays the medicinal plants are not only being used by pharmaceutical industry but also by makers of cosmetics, detergents, dyes, insecticides, foods and paints etc. (Sharmin, 2004).

Treatment of diseases from medicinal plant dates back an ancient period of time. Our ancestors were compelled to use medicinal plants to ease the sufferings from illnesses of chronic and acute origin, physical discomforts or injuries like wounds and in some cases terminal diseases. Still, this tradition is unchanged in our society. In a variety of developing countries, ancient medicines are still used as a lifeline of health care. In the developed countries, a significant number of individuals are also taking seasoned (herbal) and Unani remedies these days and most of the factory-made essential medications are being extracted from medicinal plants (Motaleb, 2011). WHO claims that almost one-third of all medicines are plant based and if bacteria and fungi are also included, approximately 60% of pharmaceuticals are from plant origin (Ali, 2010).

It's a well-known fact that many plants especially that are being used by traditional practitioners, do produce pharmaceutically active compounds which pose antiviral, antibacterial, antifungal, anti-inflammatory, anti-helminthic and antioxidant activity (Latifou *et al*., 2011). Bangladesh is enriched with a wide variety of plants. More than 5,700 angiosperm species, 1,700 species of pteridophytes and 3 species of gymnosperms prevail in Bangladesh, among which a total of 24 plants are in various degrees of the threat of extinction (Ali Reza, 2002). Sylhet, Dhaka, Chittagong, and Rajshahi division contains a high amount of medicinal plants (Ghani, 1998). So far, the number of medicinal plants enlisted as growing in Bangladesh is more than 500 of species (Motaleb, 2011). Costly imported drugs, as well as unreachability to western health care system disclose that, the only inexpensive and accessible form of maintaining human health is the traditional method of health care.

Activities that I have assessed in the project are:

- Antioxidant activity using Total Phenolic and DPPH method: This property prevents the reactive or oxidant species and is useful in the aging process. Apart from that, oxidative stress like liver toxicity and cell necrosis can be treated.
- Cytotoxic activity using Brine Shrimp method: It shows potency as an anti-cancer drug.

- Antimicrobial activity test: I have used disc diffusion method (in-vitro) to see antimicrobial activity on both Gm (+ve) and Gm (-ve) bacteria.

1.2 Plant Family: Polygonaceae:

Polygonaceae comprise approximately 40 to 50 genera and about 800 to 1000 species. Growth form within the family varies from herbs and shrubs to woody vines and trees. Stems with swollen nodes that are surrounded by sheathing stipules (ocrea) are unique to Polygonaceae and are common within the family. Polygonaceae usually have fascicles of perfect flowers (although there are a few species with imperfect flowers) surrounded by a bract called an ocreola. The perianth is 3 to 6 lobed and often persistent in fruit. A characteristic unique to the family is the quincuncial aestivation of the perianth. In this arrangement, the flowers have five tepals in two whorls with the fifth tepal representing a fusion between the inner and outer whorls of the perianth. The ovary is superior with 2–3 styles and the stamens in Polygonaceae number from four to twelve. Fruit within the family is an achene.

1.3 Plant genus: *Persicaria:*

Persicaria is a genus of blossoming plants in the knotweed family Polygonaceae. Plant of this genus is commonly recognized as smartweeds. It has a cosmopolitan distribution, with species present all over the world. The genus was isolated from Polygonum. The plant list contains around 354 scientific plant names of species rank for the genus *Persicaria*. Among these 66 are known species names. The genus comprises annual and perennial herbs with taproots or fibrous root systems, and sometimes rhizomes or stolons. The stems are often erect but may be prostrate along the ground, and some species are prickly. The stems are self-supporting or twining and climbing. The leaves are consecutively settled, deciduous, and variously designed. The brownish or reddish ochrea may be leathery to papery. The inflorescence may be a panicle or a spikelike or headlike arrangement of fascicles of flowers. The flower is white, greenish, reddish, or purple, with the tepals somewhat fused together along the bases. The fruit is an achene which can take different shapes, including a disc or a sphere.

1.4 Description of *Persicaria glabra:*

In Bengali its called Bihagni or Lal Kukri and in Chakma language its called Ejadar. The common name of this plant is dense flower knotweed, smooth smartweed. It is from

Polygonaceae family plant. It has red small flowers. A pure anthelmintic and molluscicidal terpenoid substance (PGA) has been derived by Eneh, 2012 from the methanolic extract of the leaves.

1.4.1 Habitat and Ecology:

This is an entirely glabrous plant, except the leaves which are often red-gland dotted, ochrea is completely eciliate. Grows mostly from plains to 1,000 m in or near water, in ditches, river banks, margins of lakes, pools and wadis, where it often forms dense, monospecific stands.

1.4.2 Distribution:

Fujian, Guangdong, Guangxi, Hainan, Hubei, Hunan, Taiwan, Bangladesh, Bhutan, India, Myanmar, Philippines, Sri Lanka, Thailand, Vietnam; Africa, Australia, North and South America, Pacific Islands

1.4.3 Taxonomic hierarchy:

According to the Integrated Taxonomic Information System (ITIS) Data Portal Classification:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Caryophyllales

Family: Polygonaceae

Genus: *Persicaria*

Species: *Persicaria glabra*

Bengali Name: Biskatali, Bihagni, Sada Kukri

Tribal Name: Ejadar (Chakma)

Common Name: Denseflower knotweed, smooth smartweed

1.4.4 Chemistry of *Persicaria glabra:*

1.4.4.1 Chemical constituents:

a) Flowers:

- Pigments
- Delphinidin-3,5-diglucoside
- Cyanidin-3,5-diglucoside
- Quercetin

b) Stem and seeds:

- Vanillic acid
- Syringic
- p-hydroxybenzoic
- Protocatechuic
- Gallic acid
- Coumaric acids
- Kaempferol
- Quercetin
- Hyperin.

c) Leaves:

- Flavonoids,
- Quercetin,
- Rhamnetin,
- Quercitrin,
- Avicularin

d) Aerial parts:

- Terpenoids
- Flavones.

1.4.5 Medicinal use:

Persicaria glabra is one of the traditional plants that is used as an analgesic agent in Sylhet and anti-cancer agent in the Chittagong Hill Tracts. Generally, juice of this herbs is used as painkiller and leaf paste is used as an anti-cancer drug.

Chapter 2: Methodology

2.1 Collection and identification of plant:

After extensive literature study about this plant and its availability, it was decided as the suitable candidate of phytopharmacological investigation. In this project work, the whole plant of *Persicaria glabra* was collected in the month of October 2016 from Khagrachari, Bangladesh. After that, its verification (Verification code number: 45023) was done by the National Herbarium of Bangladesh (NHB), Mirpur, and Dhaka by submitting plant sample.

2.2 Extraction procedure:

The extraction of medicinal plant:

The whole extraction procedures are divided into 2 parts:

a. Plant material preparation and drying (2-steps)

b. Extraction process (5-step)

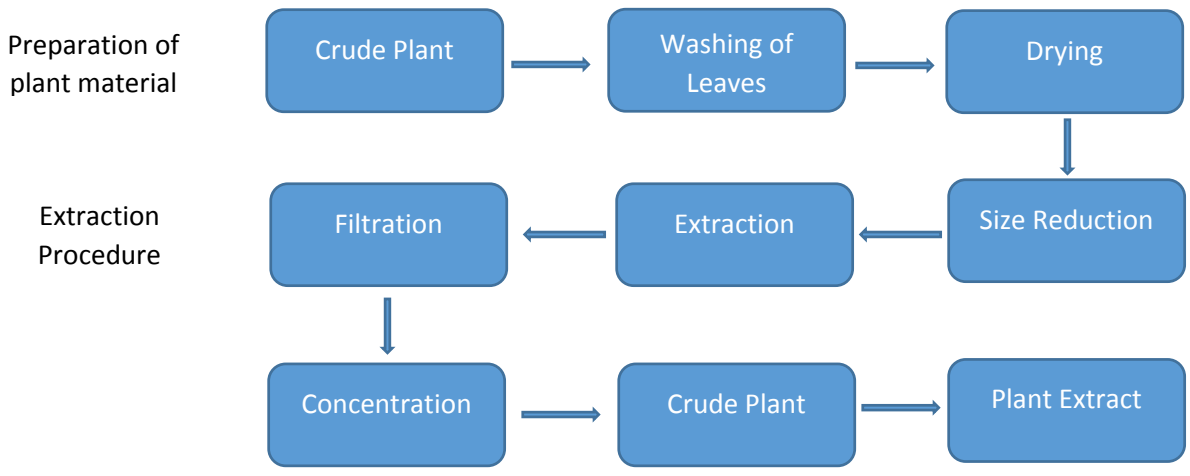


Figure2.1: Flow chart showing a step-by-step procedure for the extraction of ingredients from the crude medicinal plant

2.2.1 Preparation of plant material and drying:

Laves of *Persicaria glabra* were plucked off from plant stem and cleaned properly with fresh water to remove unnecessary dust particles and plant debris. After that clean leaves were shade dried for several days and dried leaves were then prepared for next procedure.

2.2.2 Extraction process:

2.2.2.1 Reduction in size and weighing:

The dried out and crunchy leaves were grounded finely as a granular particle with a high power grinding machine. After that, it was packed into an air-tight plastic container and proper labeling on the container was done. After that, labeled container was kept in a dark, cool and dry place till it required for next process. During the crushing procedure was continuing, essential measurement was taken to prevent cross-contamination.

2.2.2.2 Extraction:

To extract the chemical constituents from plant parts by maceration process, methanol was used as the organic solvent. Amber glass bottle was used for this process. About 400gm of grounded leave powder of *Persicaria glabra* which was drenched in 2L of methanol for 14 days period in a room temperature (22-25^{o}C) with random agitation.

The result of maceration process was a 2-layer phase. The lower-most phase is the sediment and the upper-most is a methanolic solution of the extract which was separated by a dirty suspension of plant parts.

2.2.2.3 Filtration process:

After 14 days of soaking, the substances of the bottle were emptied out first to filter them by using Whatman filter paper (pore size 100nm).

2.2.2.4 Drying:

After the collection of the filtrate, it was concentrated by using rotary evaporator (Heidolph) at 30^{o}C temperature with a rotation speed of 100rpm up to form the concentrated methanolic extract. After that, that concentrated methanolic extract was shifted into a glass jar wrapping

with foil paper and kept it in dark and dry place. After 3 days semi-solid extract of *Persicaria glabra* was formed.

2.3. Phytochemical screening:

The crude extract of *Persicaria glabra* was used for phytochemical screening to identify its chemical compound present in its leaves.

2.3.1 Procedure of extract preparation:

2-3 grams of dried methanol extract was mixed with 50ml methanol in a 100ml of conical flask. After that, that flask was labeled properly closing with cotton plugs and kept still for 1 to 2 hours. Later, the mixture was filtered through Whatman filter paper. Collected filtrates were used for phytochemical screening by following the standard process (Kokate *et al*., 2009; Evans and Trease 2002). The following qualitative tests were performed sequentially:

2.3.1.1 Tests for Alkaloids:

2.3.1.1.1 Mayer's test: A few drops of Mayer's reagent (Potassium mercuric iodide solution) was added in 1ml of plant extract. If cream color precipitation form then it will contain the presence of alkaloids.

2.3.1.1.2 Wagner's test: In 1ml of plant extract was added with the same amount of Wagner's reagent (Iodine in potassium iodide). If reddish brown color precipitation form then it will point to the presence of alkaloids.

2.3.1.1.3 Dragendorff's reagent test: 2ml of Dragendorff's reagent was added in 1ml of plant extract and later dilute HCL of 2ml was added in that solution. If orange color precipitation forms then it will confirm the presence of alkaloids.

2.3.1.1.4 Hager's test: A few drops of Hager's reagent (Saturated picric acid solution) was added in 2ml of plant extract. If bright yellow color precipitation form then it will point to the presence of alkaloids.

2.3.1.1.5 Tannic acid test: A few drops of tannic acids was added in 1ml of plant extract. If yellow-brown colored precipitation form then it will point to the presence of alkaloids.

2.3.1.1.6 $FeCl_3$ test: About 1-2 ml extract was mixed with a little amount of neutral ferric chloride solution in dropwise. If cream yellow precipitation forms then it will point to the presence of alkaloids.

2.3.1.2 Tests for glycosides:

2.3.1.2.1 Legal's Test: Addition of alkaline sodium nitroprusside and pyridine in extract solution results in the formation of cherry red color then it will confirm to the presence of glycosides.

2.3.1.2.2 Keller Killiani test: At first 1ml of glacial acetic acid was mixed-up with 1 ml of extract and cooled. After that 2-3 drops of ferric chloride was mixed and 2ml of concentrated H_2SO_4 was added carefully in sideways of test tube walls. If reddish brown colored ring at the junction of two layers form then it will point to the presence of glycosides.

2.3.1.2.3 Concentrated H_2SO_4 test: 1ml of Concentrated H_2SO_4 was added in 1ml of plant extract and kept still for 2 minutes. If reddish color precipitate form then it will point to the presence of glycosides.

2.3.1.2.4 Molish's test: In plant extract around 2-3 drops of Molish's reagents was added. Later, a few drops of concentrated H_2SO_4 was added properly. If reddish purple colored ring at the junction of two layers form then it will point to the presence of glycosides.

2.3.1.3 Test for phlobatannins: At first 2-3ml of 10% HCl was added in 10ml of plant extract in a boiling test tube which was boiled for 5-6 minutes. If red color precipitate occurs then it will point to the presence of phlobatannins.

2.3.1.4 Test for resins: 3-4ml of the $CuSO_4$ solution was mixed-up with plant extract which was shaken vigorously for 1-2 minutes and allowed to discrete. If green color precipitate occurs then it will point to the presence of resins.

2.3.1.5 Test for quinones: Alcoholic KOH solution was added in plant extract. If color ranging from red to blue occur then it will point to the presence of quinones.

2.3.1.6 Test for Saponins: In the test tube 5ml of the extract was taken and shaked vigorously to get a stable froth. 5-6 drops of olive oil were added into frothing solution. If the emulsion is formed then it will point to the presence of saponins.

2.3.1.7 Tests for phenols:

2.3.1.7.1 Ellagic acid test: A few drops of 5% (w/v) glacial acetic acid was added in plant extract. After that 5% (w/v) $NaNO_2$ solution was added. If muddy brown color form then it will point to the presence of phenols.

2.3.1.7.2 Phenol test: 1ml of the $FeCl_3$ solution was added in 2ml of plant extract. If the development of intense color form then it will point to the presence of phenols.

2.3.1.8 Tests for Tannins:

2.3.1.8.1. Ferric chloride test: A few drops of $FeCl_3$ was added in plant extract. If blackish color precipitate form then it will point to the presence of tannins.

2.3.1.8.2 Lead acetate test: A few drops of basic lead acetate was added in 1-2ml of plant extract. If bulky red color precipitate form then it will point to the presence of tannins.

2.3.1.8.3 Alkaline reagent test: A few drops of sodium hydroxide solution was added in plant extract. If red color form then it will point to the presence of tannins.

2.3.1.9 Tests for flavonoids:

2.3.1.9.1 Lead-acetate test: A few drops of basic lead acetate solution was added in 1-2ml of plant extract. If reddish brown color precipitate form then it will point to the presence of flavonoids.

2.3.1.9.2 $FeCl_3$ test: A few drops of neutral ferric chloride solution was added in 1-2ml of plant extract. If the deposition of blackish red color precipitate form then it will point to the presence of flavonoids.

2.3.1.9.3 Alkaline reagent test: A few drops of sodium hydroxide was added in 1-2ml of plant extract. If yellowish red color occurs then it will point to the presence of flavonoids.

2.3.1.10 Test for sterols:

2.3.1.10.1 Libermann-Burchard test: A few drops of acetic anhydride solution was mixed with 1-2ml of plant extract. After that, a few drops of concentrated H_2SO_4 was given beside the test tube walls in the mixture. If reddish brown color ring at the junction of two layers occur then it will point to the presence of sterols.

2.3.1.10.2 Salkowski test: 5ml of chloroform was added in 1-2ml of plant extract. After that, 1ml of concentrated H_2SO_4 was put beside the test tube walls. If the reddish color in the lower layer occurs then it will point to the presence of sterols.

2.4 Evaluation of Antioxidant activity:

In the modern scientific advancement, the therapeutic activates of plants have been extended all over the world because of antioxidant activities, absences of side effects and economic feasibility.

The antioxidant activity assay can be done by following methods:

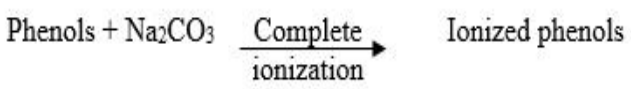
- Determination of total phenolic content
- DPPH assay

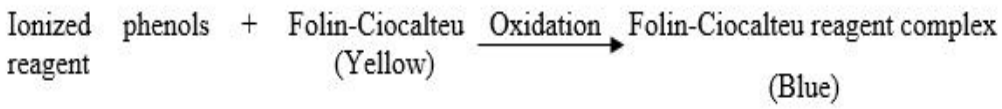
2.4.1 Determination of total phenolic content:

Neutralization and absorption of free radicals, reduction of triplet as well as singlet compounds and lastly, decomposition of peroxides of the human body are vital roles played by antioxidants (Osawa, 1994). Generally, the antioxidative action is shown by phenolics, phenolic acid, phenolic diterpenes, and flavonoids. Chemical properties of the phenolic compounds show that they exert their antioxidative properties by redox reaction (Pietta, 1998; Shahidi, Janitha and Wanasundara, 1992). Researches show that various amount of the phytochemicals retain antioxidant capacities which might be related to lower mortality rate and lower incidence human cancer (Velioglu *et al*, 1998).

2.4.1.1 Principle:

Phenols get ionized in an alkaline condition which is why the Folin-Ciocalteu reagent is used which readily gets ionized in phenolic solution. Oxidized reagent turns blue from yellow. Color change intensity is measured as absorbance at 760 nm by UV spectrophotometer. Absorbance indicates the TPC (Total Phenolic Content) of particular test compound (Harbertson and Spayed, 2006).





2.4.1.2 Materials and Methods:

Total phenolic content of leaves of the Total phenolic content of leaves of *Persicaria glabra* extract was measured by using the method which was designed by Skerget *et al*.,(2005) involving Folin-Ciocalteu reagent as an oxidizing agent and gallic acid as standard (Majhenic *et al*., 2007).

2.4.1.3 Materials:

- Folin-Ciocalteu reagent (10 fold diluted)
- Na_2CO_3 solution (7.5 %)
- Distilled water
- Methanol
- Beaker (100 and 200ml)
- Test tube
- Pipette (1ml)
- Pipette (5ml)

2.4.1.4 Composition of Folin-Ciocalteu reagent:

SL. No.	Component	Percent
1	Water	57.5
2	Lithium Sulfate	15.0
3	Sodium Tungstate Dihydrate	10.0
4	Hydrochloric Acid>=25%	10.0
5	Phosphoric Acid 85% solution in water	5.0
6	Molybdic Acid Sodium Dihydrate	2.5

2.4.1.5 Standard curve preparation:

As a standard for this procedure, we used gallic acid for our experimental purpose. Various concentration of gallic acid was prepared which was ranged from 0µg/ml TO 100µg/ml. 2.5ml Folin-Ciocalteu reagent (10 times diluted with water) and 2ml Na_2CO_3 (7.5% w/v) solution was taken where both of them were added in 0.5ml of gallic acid solution. 20 minutes incubation was done at room temperature for that mixture. At 760nm absorbance of that incubated mixture was taken by UV-spectrophotometer.

After that, absorbance was plotted in Y axis and concentration was plotted in X axis which gave a linear relationship in the graph. That graph was used as a standard curve to measure the total phenolic content of test sample.

2.4.1.6 Sample preparation:

To make a 2mg/ml concentrated sample solution, 2mg of extract was dissolved in required amount of distilled water.

2.4.1.7 Total phenolic content analysis:

The sample solution, 0.5ml of extract solution and 2.5ml of Folin-Ciocalteu reagent solution (10 times diluted) was taken. At room temperature the mixture was incubated for 20 minutes. Later, absorbance of incubated mixture was taken at a 760nm by UV-spectrophotometer. After that total phenolic content of the plant, extract was measured from the standard curve. This phenolic content of test sample was denoted as gallic acid equivalent/gm of the extract or GAE.

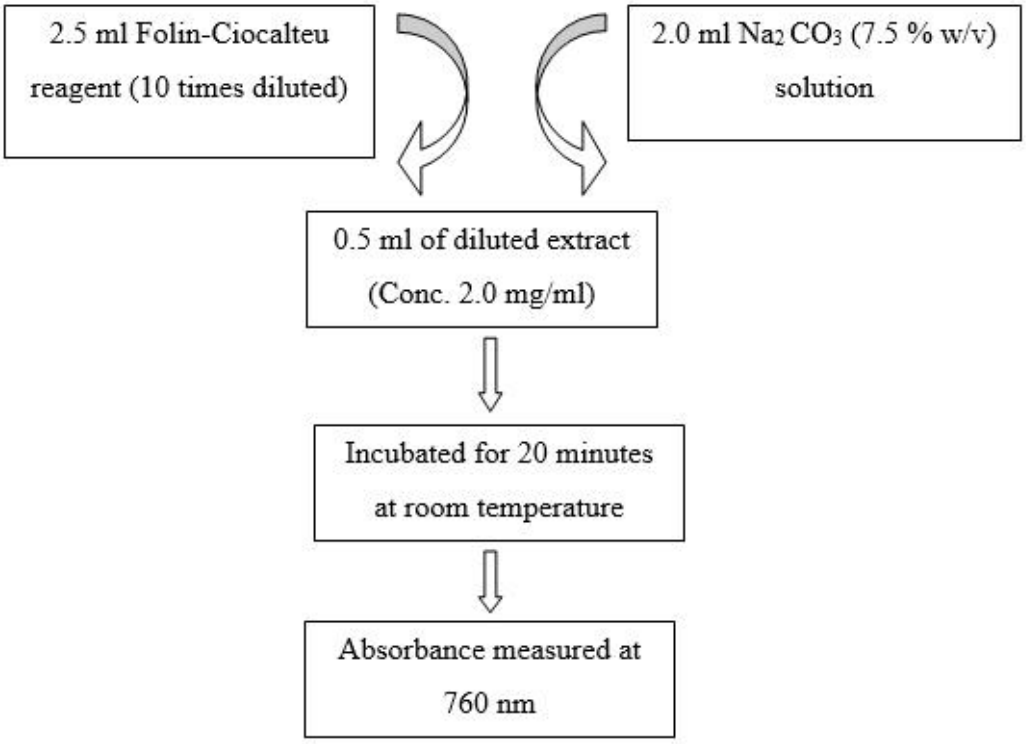


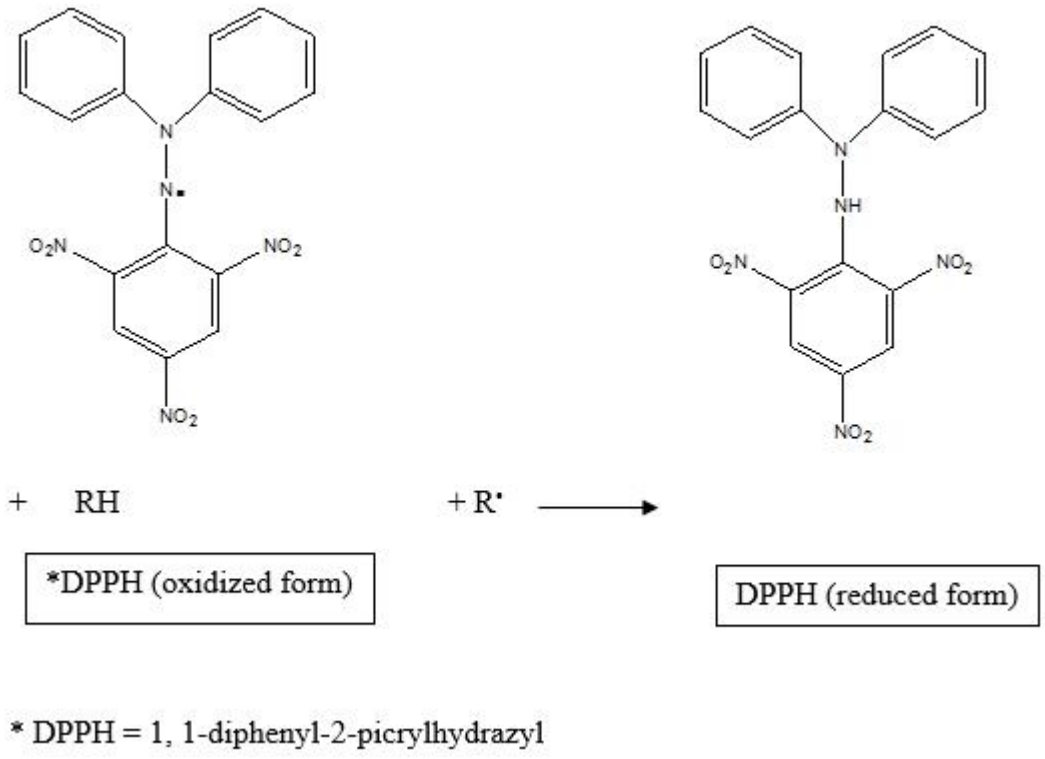
Figure 2.2: Schematic representation of the total phenolic content determination

2.4.2 DPPH assay:

2.4.2.1 Principle:

DPPH assay is simple and fast procedure to evaluate antioxidant activity of extract sample where its stability in the radical form is good (Bozin *et al.*, 2008). This method give reliable information regarding to antioxidant ability of test sample (Huang *et al*., 2005). Basic law of this assay is color change of DPPH solution from purple to yellow as the radical is quenched by antioxidant (Karagözler *et al*., 2008). Based on the method described by Brand-Williams *et al*., 1995 the free radical scavenging activity or antioxidant property of plant extracts was measured using DPPH (1, 1-diphenyl-2-picrylhydrazyl) reagent. Above mentioned procedure follows the addition of extract's methanol solution (2 ml) with DPPH methanol solution (3 ml, conc. 20µg/ml).

Decoloration of purple colored DPPH methanol solution by the test plant extract is compared with a standard ascorbic acid.



* DPPH = 1, 1-diphenyl-2-picrylhydrazyl

2.4.2.2 Materials and Methods:

DPPH was used to evaluate the free radical scavenging activity (antioxidant potential) of various compounds and medicinal plants (Choi *et al*., 2000; Desmarchelier *et al*., 1997).

1,1-diphenyl-2-picrylhydrazyl	UV-spectrophotometer
Pipette (5ml)	Beaker (100 and 200ml)
Ascorbic acid	Pipette (5ml)
Distilled water	Test tube
Methanol	Light-proof box

2.4.2.3 Preparation of control sample for measurement of antioxidant activity:

In positive control, we used Ascorbic acid (ASA). To get a 1000µg/ml concentrated mother solution of ASA, we dissolved the premeditated amount of ASA in methanol properly. After that, different concentrated solution ranging from 500 to 0.977µg/ml was made by serial dilution from mother solution.

2.4.2.4 Preparation of test sample:

The accurate amount of extract was measured to dissolve in methanol solvent to make a mother solution of 1000µg/ml concentration. Later, different concentrated solution ranging from 500 to 0.977µg/ml was made by serial dilution from mother solution which was kept in test tubes and marked properly.

2.4.2.5 Preparation of DPPH solution:

To get a 20µg/ml concentrated DPPH solution, we weighed 20mg powder of DPPH and dissolved in methanol solvent. DPPH solution was made in an amber glass bottle and kept in dark place because of its light sensitivity.

2.4.2.6 Assay of free radical scavenging activity:

3ml of methanolic solution of DPPH was mixed with 2ml of methanolic sample (extract or control) solution at a various concentration which was ranging from 500 to 0.977µg/ml. The prepared solution was kept in dark for 30 minutes at room temperature for reaction. After that, prepared solution absorbance was measured against methanol as blank by UV spectrometer at 517nm.

Percent of inhibition of free radical DPPH (I%) was calculated by given equation:

$$(I\%) = (1 - A_{sample}/B_{blank}) \text{ X } 100$$

In the given equation, B_{lank} is denoted as absorbance of the control reaction (contain all chemical reagents except the test material).

The concentration of extract provided 50% inhibition *(IC_{50})* which was calculated by using the graph of inhibition percentage vs. extract concentration. In this graph we will use logartmic trendline to get an equation by which we will determine the IC_{50} value for our extract sample.

General equation for calculating IC_{50} is given below –

$$y = mln(x) + c$$

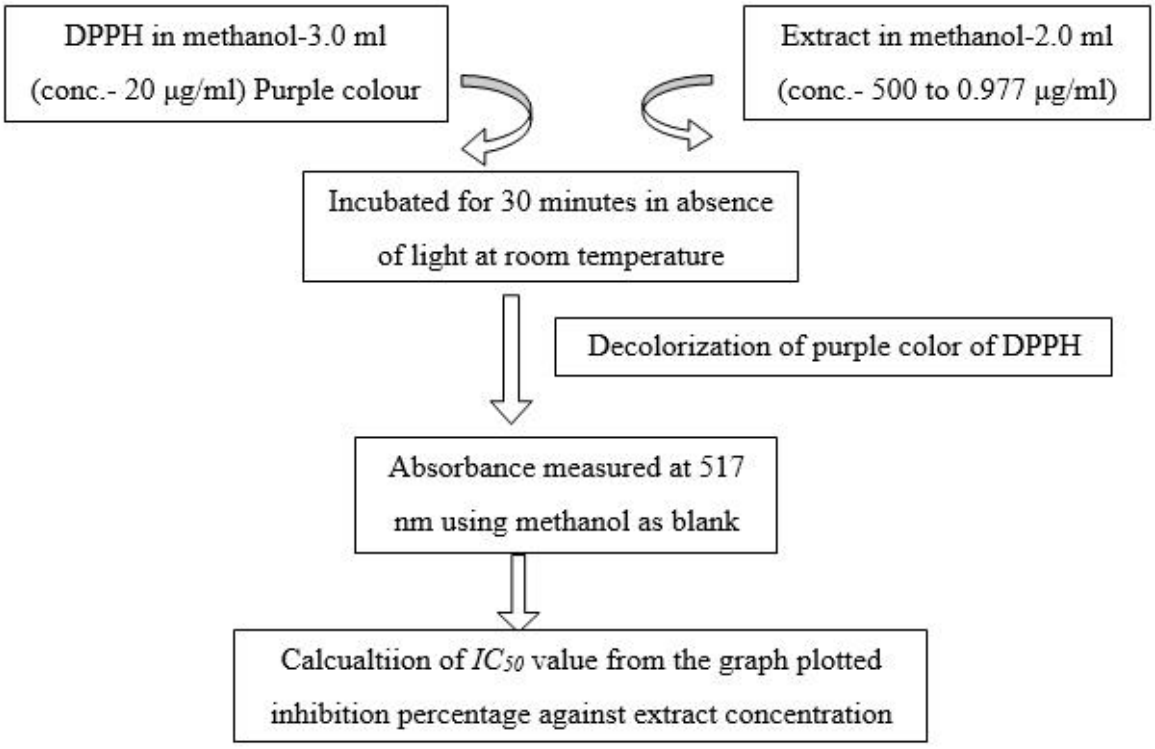


Figure 2.3: Schematic representation of the method of assaying free radical scavenging activity

2.4.3 Brine Shrimp Lethality Bioassay:

In a living entity, toxicity is shown is at higher doses by bioactive compounds. For compounds from both natural and synthetic sources brine shrimp lethality bioassay method is an effective and trustworthy one through which pure compounds, natural product extracts, as well as different solvent partitions, can be tasted. In vivo lethality investigation of a simple zoological organism (Brine shrimp nauplii) allows a close screening and fractionation. Results obtained from this experiment can play an important role in finding out certain pharmacological activities like antimicrobial, antiviral, pesticidal, anti-tumor activities etc which makes this

method a superior one than other cytotoxicity testing procedures. Rapid, inexpensive and use of less equipment or aseptic technique makes a good method to assay cytotoxicity.

2.4.3.1 Principle:

Inside the simulated sea water brine, shrimp eggs are hatched to get nauplii. The required amount of dimethylsulphoxide (DMSO) is weighed to prepare desire concentrated test sample. Visual inspection is done to count nauplii which were taken in test tubes. Those test tubes conatined simulated sea water of 5ml. After that, the variuos concentrated sample is added into test tubes by micropipette. Those test tubes are pre marked to avoid accidental mixup of chemical reagents. After that, test tubes are kept in dark place for 24 hours and counting of survivors is done after 24 hours.

2.4.3.2 Materials:

- Artemia salina leach (brine shrimp egg)
- Sea salt (NaCl)
- Small tank with perforated dividing dam to hatch the shrimp
- Electric Lamp
- Pipettes
- Micropipette
- Glass vials
- Magnifying glass
- Test tubes

2.4.3.3 Experimental Procedure:

2.4.3.3.1 Preparation of seawater:

In 1 liter of distilled water was taken and 38gm sea salt which was pure sodium chloride dissolved in distilled water. That solution was filtered to acquire clear solution.

2.4.3.3.2 Hatching of brine shrimps:

Our test organism *Artemia salina* leach (brine shrimp eggs) were collected from a pet shop. Inside the small tank, shrimp eggs were added from one side of the tank to cover that side. To

hatch the shrimp and get naupill we incubate those eggs for one day. During the hatching time, continuous oxygen supply was carried out. An electric lamp was used through the perforated tank to attract the hatched shrimp. Later, 10 living shrimps from that tank were taken for the experiment by using pasteur pipette where each test tube conatined5ml of simulated sea water.

2.4.3.3.3 Preparation of test samples of the experimental plant:

Inside the test tube, test samples were taken and 100µl of pure dimethyl sulfoxide (DMSO) was dissolved to make stock solutions. After that, inside the first test tube which contains 5ml of simulated sea water and 10 shrimp naupill was mixed with a 50µl stock solution (DMSO). So, the concentration of that prepared solution was 400µg/ml. By using serial dilution method, we made different concentration of stock solution. In every test tube, 5µl samples were added and fresh 50µl of DMSO was mixed in a stock solution of DMSO. Those various concentrations were made for different test tubes (Table 2.4).

Table 2.1: 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

2.4.3.3.4 Preparation of control group:

In this cytotoxic test, control groups were used to authenticate the test method and make sure that the acquired results are obtained only from the activity of test agent where other effects were not interrupted in this test. Basically, control groups are diveded into two types:

a) Positive control
b) Negative control

2.4.3.3.5 Preparation of the positive control group:

In the study of cytotoxicity, positive control is broadly accepted as a cytotoxic agent where test results are contrasted with result that obtains from positive control. In current study vincristine sulfate was used as a positive control. In DMSO, a specific amount of vincristine sulfate was dissolved to get desired concentration of 20µg/ml. After that, using this concentrated solution I had done serial dilution by using DMSO to prepare 10µg/ml, 5µg/ml, 2.5µg/ml, 1.25µg/ml, 0.625 µg/ml, 0.3125µg/ml, 0.15625µg/ml, 0.078125µg/ml and 0.0390µg/ml concentrated solution. After that, those diluted solution were added inside the premarked test tube which contains 10 living shrimp nauplii with 5ml of simulated sea water to get a positive control group.

2.4.3.3.6 Preparation of the negative control group:

Inside of three pre-marked test tubes which contain 5ml of simulated sea water and 10 living shrimp nauplii were mixed up with 100µl of DMSO in each test tube. In the case of rapid mortality of brine shrimps inside of those three test tubes, then it will be considered as an invalid test as the death of nauplii due to other reason than the effect cytotoxicity of those compounds.

2.4.3.3.7 Counting of nauplii:

Test tubes were inspected by using magnifying glass after 24 hours to count survivors number. Calculation of mortality percentage was done for every dilution. Data of concentration-mortality were studied statistically by using linear regression of Microsoft excel program. Median lethal concentration (LC_{50}) value is another way to express effectiveness or the concentration-mortality connection of plant constituent. It typifies the chemical concentration which can cause death of 50% test subjects after a definite peroid of time.

2.4.4 Antimicrobial screening:

Infectious diseases are one of the significant causes of deaths (approximately 50% cause of deaths) in a tropical region, which is not an astonishing scenario in case of lower developed

and developing countries. Moreover, the mortality rate due to infectious diseases is rising alarmingly in developed countries. In 1981, death caused by infectious disease placed 5th which increased rate became 58% by the time of 1992 and become the 3rd leading cause of human death. In the United State, the infectious disease causes approximately 8% death of their people (Pinner *et al*., 1996). On the other hand, increasing rate of antibiotics resistance in community-acquired and nosocomial infections are also shaking the health condition in the current world. Moreover, the rate of suffering from infectious diseases among people aged between 25 to 44 years has been increased (Pinner *et al*., 1996).

Those undesirable health tendencies creating an improvement action against infectious diseases in the medical and public health communities by developing approaches to management of infectious diseases and its preventive actions. This is the last key which can incorporate the progress of new antimicrobial agents (Fauci, 1998).

The first step is testing the vulnerability of different fungi and bacteria to any compound by antimicrobial screening. By this procedure, we can determine the capability of each test sample to inhibit the in vitro growth of fungus and bacteria. That capability can be determined by any of the following three techniques which are given below (Ayafor, 1972).

- Disc diffusion method
- Serial dilution method
- Bioautographic method

Between given techniques, disc diffusion method (Bayer *et al*., 1966) is broadly known for in vitro study for initial screening of test agents which may have antimicrobial activity. This is fundamentally a qualitative and quantitative test to identify the resistance or sensitivity of the microorganisms into the testing compound. However, no distinctive characteristics between bactericidal and bacteriostatic activity can be determined by this technique (Roland R, 1982).

2.4.4.1 Principle of disc diffusion method:

By this conventional method, antibiotics drawn-out from a restricted source through nutrient agar gel and generate a concentration gradient. In the dried out and sterilized filter paper discs (6mm in diameter) contained the test sample in a specific amount which is positioned on nutrient agar medium with uniformly seeded microorganisms. As a standard of antibiotic,

kanamycin discs and blank discs are used as a positive and negative control. Those plates are kept at 4°C temperature for 24 hours to allow maximum diffusion of the test materials to the surrounding media (Barry, 1976). After 24 hours those plates are inverted and incubated at 37°C for 24 hours for optimal growth of microorganisms. If the test materials have antimicrobial property, it will inhibit the growth of microorganisms in the near to the discs of that media and produce a clear distinct area which defined as a zone of inhibition. After that, the antimicrobial activity of test agent is then measured by determining the diameter of the zone of inhibition (in millimeter) (Barry, 1976; Bayer *et al*., 1966.)

In the present study, the crude extracts, as well as fractions, were tested for antimicrobial activity by disc diffusion method. The experiment was carried out more than once and the mean of the readings was taken.

2.4.4.2 Apparatus and reagents:

Filter paper discs	Autoclave
Nutrient Agar Medium	Laminar air flow hood
Petridishes	Spirit burner
Sterile cotton	Refrigerator
Micropipette	Incubator
Inoculating loop	Chloroform
Sterile forceps	Ethanol
Screw cap test tubes	Nosemask and Hand gloves

2.4.4.3 Test organisms:

The bacterial and fungal strains used in the experiment were collected from pure cultures from the department of Pharmacy, University of Dhaka. Both gram positive and gram negative organisms were taken for the test.

Table 2.2 Different strains used in antimicrobial screening

Gram positive Bacteria	*Gram negative Bacteria*	*Fungi*
Bacillus cereus	*Escherichia coli*	*Aspergillus niger*
Bacillus megaterium	*Salmonella paratyphi*	*Candida albicans*
Bacillus subtilis	*Salmonella typhi*	*Sacharomyces cerevacae*
Sarcina lutea	*Shigella boydii*	
Staphylococcus aureus	*Shigella dysenteriae*	
	Pseudomonas aeruginosa	
	Vibrio mimicus	
	Vibrio parahemolyticus	

2.4.4.4 Composition of culture medium:

To prepare subculture of test microorganisms, following culture media are generally used

a) Nutrient agar medium

Ingredients	Amount
Bacto peptone	0.5 gm
Sodium chloride	0.5 gm
Bacto agar	2.0 gm
Bacto yeast extract	1.0 gm
Distilled water q.s.	100 ml
pH	7.2 + 0.1 at 25^0C

b) Nutrient broth medium

Ingredients	Amount
Bacto peptone	0.5 gm
Bacto beef extract	0.3 gm
Distilled water q.s.	100 ml
pH	7.2 + 0.1 at 25^0C

c) Muller – Hunton medium

Ingredients	Amount
Starch	0.15 gm
Bacto agar	1.70 gm
Beef infusion	30 gm
Casamino acid	1.75 gm
Distilled water q.s.	100 ml
pH	7.3 + 0.2 at 25^0C

d) Tryptic soya broth medium (TSB)

Ingredients	Amount
Bacto tryptone	1.70 gm
Bacto dextrose	0.25 gm
Bacto soytone	0.30 gm
Di potassium hydrogen Phosphate	0.25 gm
Sodium chloride	0.50 gm
Distilled water q.s	100 ml
pH	7.3 + 0.2 at 25^0C

Among those culture medium, nutrient agar medium is used broadly. In the current study, nutrient agar medium is chosen for sensitivity test of microorganism for test material and preparation of fresh cultures.

2.4.4.5 Preparation of the medium:

A predetermined amount of each material were taken in the conical flask with the required amount of distilled water to make the necessary amount of those mediums. After that, those mixtures were heated inside the water bath to get a clear solution. At 25^{o}C temperature, pH of mediums was adjusted between 7.2-7.6 by using NaOH or HCl. 5ml and 10ml amount of medium were taken in screw cap test tubes to prepare plates and slant. After that, test tube caps were closed and sterilized by autoclave at a 121^{o}C temperature and 15lbs pressure for 20 minutes. Slant was prepared to make the fresh culture of fungi and bacteria for the sensitivity test.

2.4.4.6 Sterilization procedure:

The antimicrobial screening process was run under the laminar hood to avoid any type of cross contamination by test organism and contamination. Moreover, other precautions were maintained properly. The autoclaving was done at a 121^{o}C temperature and maintaining 15lbs/square pressure to sterile the petri dishes and glass instruments. Laminar hood, cotton, blank discs, micropipette tips and forceps were sterilized by keeping under UV light for one hour.

2.4.4.7 Preparation of subculture:

Under the laminar air cabinet that creates an aseptic condition to transfer test organisms from pure culture to agar slants in an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with aid of transfer loop to acquire fresh pure culture. After that those inoculated strains were incubated at 34^{o}C temperature for 24 hours to gain optimum growth of those microorganisms. In sensitivity test, those fresh cultures were used.

2.4.4.8 Preparation of the test plate:

Under the aseptic area, a subculture of test organisms was moved into the test tube which

contains around 10ml of sterilized and melted agar medium with the support of a sterilized transfer loop. Those test tubes were shaken with rotation to achieve an even distribution of microorganisms suspension. After that, suspension of microorganisms (bacteria and fungus) were transferred in the sterile Petri dish. Several times rotation of Petri dishes as clockwise and anticlockwise was done so that uniform distribution of test microorganisms occur in media.

2.4.8.9 Preparation of discs:

The calculated amount of test sample was dissolved in Chloroform or methanol to get the desired concentrations in a sterilized condition. Filter paper discs were reserved in a blank petridish under the laminar hood. After that, the discs were soaked with solutions of test samples and dried.

Table 2.4: Preparation of sample Discs

Plant part	**Test Sample**	**Dose** (μg/disc)
Leaf of *Persicaria glabra*	Methanolic extract of leaves	400

As a positive control standard Kanamycin (30 μg /disc) plates were used to assure the activity of standard antibiotic against the test organisms as well as compare the antimicrobial capacity between standard and test sample. Blank discs were used as negative controls to confirm that the filter paper and the residual solvents were not active themselves.

2.4.4.10 Diffusion and incubation:

Our sample discs, control discs and standard antibiotic disc were placed smoothly on the marked zone of agar plates which were pre-inoculated with test bacteria and fungus. After that, those plates were put inside the incubator for 24 hours at a 40^{o}C temperature in upside down position to let adequate diffusion of constituents from discs to encircle agar medium. After 24 hours, those plates were put in inverted position for another 24 hours at 37^{o}C temperature.

2.4.4.11 Determination of the zone of inhibition:

To determine the potency of test material as an antimicrobial agent, the size of the clear zone

of inhibition was measured by a transparent scale. The diameter of the zone of inhibition in millimeter unit indicates the prevention of growth of microorganisms by test material.

Chapter Three: Result and Discussion

3.1 Phytochemical screening:

Table3.1: Phytochemical screening of *Persicaria glabra*

Serial Number	Class of compound	Result
1	Alkaloid	++++++
2	Glycoside	+
3	Phlobatannin	+
4	Resin	-
5	Quinone	-
6	Phenol	++
7	Tannnin	-
8	Flavonoids	+
9	Sterol	++

Note: (+) means presence in a single method test, (++) means presence experimented in two methods,(+++) means presence experimented in three methods, (++++) means presence experimented in four methods, ,(+++++) means presence experimented in five methods and (-) means absence.

Interpretation: The phytochemical screening of *Persicaria glabra* showed the presence of alkaloids, flavonoids, glycosides, phenol, phlobatannin, resins, sterol and tannins whereas showing the absence of tannin, resin and quinone.

3.2 Determination of Total Phenolic Content:

The methanolic extract (ME) of the leaves of *Persicaria glabra* was subjected for total phenolic content determination. Folin-Ciocalteu reagent was used for this test. Depending on the absorbance values of the extract solutions, the investigation of the total phenolic content of various extracts was determined and differentiated with the standard solutions of gallic acid (table 3.1) equivalents. The total phenolic content of the samples are actually expressed as mg of GAE (gallic acid equivalent)/ gm of extractives and are given in table 3.2.

The total phenolic content was found to be 106.877 mg of GAE / gm of extract.

Table 3.2: Standard curve preparation by using gallic acid

Sl. No.	Concentration of the Standard (µg / ml) (X)	Absorbance (Y)	Regression line	R^2
1	100	0.800	y = 0.0081x - 0.0007	0.9975
2	50	0.423		
3	25	0.215		
4	12.5	0.123		
5	6.25	0.047		
6	3.125	0.007		
7	1.5625	0.003		
8	0.78125	0.000		
9	0.3906	0.000		
10	0	0.000		

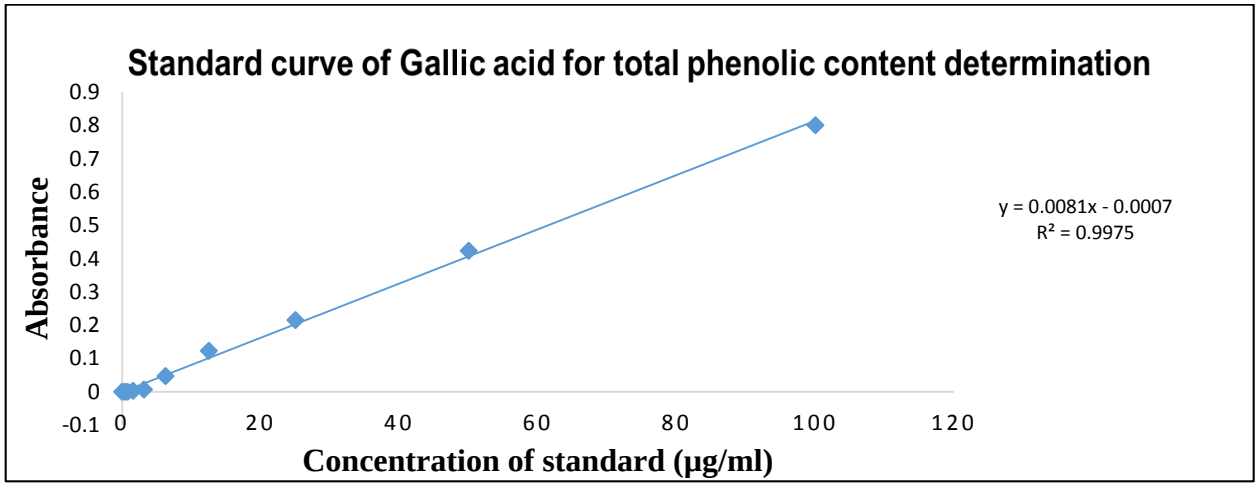


Figure 3.1: Standard curve of Gallic acid for total phenolic content determination

Table 3.3: Test samples for total phenolic content determination

Plant part	Sample code	Test Sample	Absorbance (Y)	Total phenolic content (mg of GAE / gm of extractives) (X)
Leaves of *Persicaria glabra*	ME	Methanolic extract	0.865	106.877

3.3 DPPH Assay:

The Methanolic extract of the leaves of *Persicaria glabra* (ME) was tested to free radical scavenging activity by using the method suggested byBrand-Williams *et al*., 1995. Reference standards were Ascorbic acid (ASA) and *tert*-butyl-1-hydroxytoluene (BHT).

In this study, Methanolic extract solution (ME) presented the notable free radical scavenging activity with an IC_{50} value of 5.524µg/ml for leaves of *Persicaria glabra*. The free radical scavenging activity of methanolic extract of leaves of *Persicaria glabra* is near to the standards. If compare between IC_{50} value of ME (5.524µg/ml) and ASA (3.01µg/ml), we can say that reducing power activity of ascorbic acid is slightly better than methanolic extract. On the other hand IC_{50} value of ME is better than BHT (21.17µg/ml).

Table 3.4: IC_{50} values of the standards and methanolic extract of leaves of *Persicaria glabra*

Plant part	Sample code	Test Sample	IC_{50} (µg/ml)
Leaves of *Persicaria glabra*	ME	Methanolic extract	5.524
ASA (Ascorbic acid) (standard)			3.01
BHT (*tert*-butyl-1-hydroxytoluene) (standard)			21.17

Table 3.5: IC_{50} value of Ascorbic acid (ASA)

Absorbance of the blank	Concentration (µg/ml) (X)	Absorbance of the extract	% inhibition (Y)	IC_{50} (µg/ml)
0.325	500	0.005	98.46	3.01
	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	

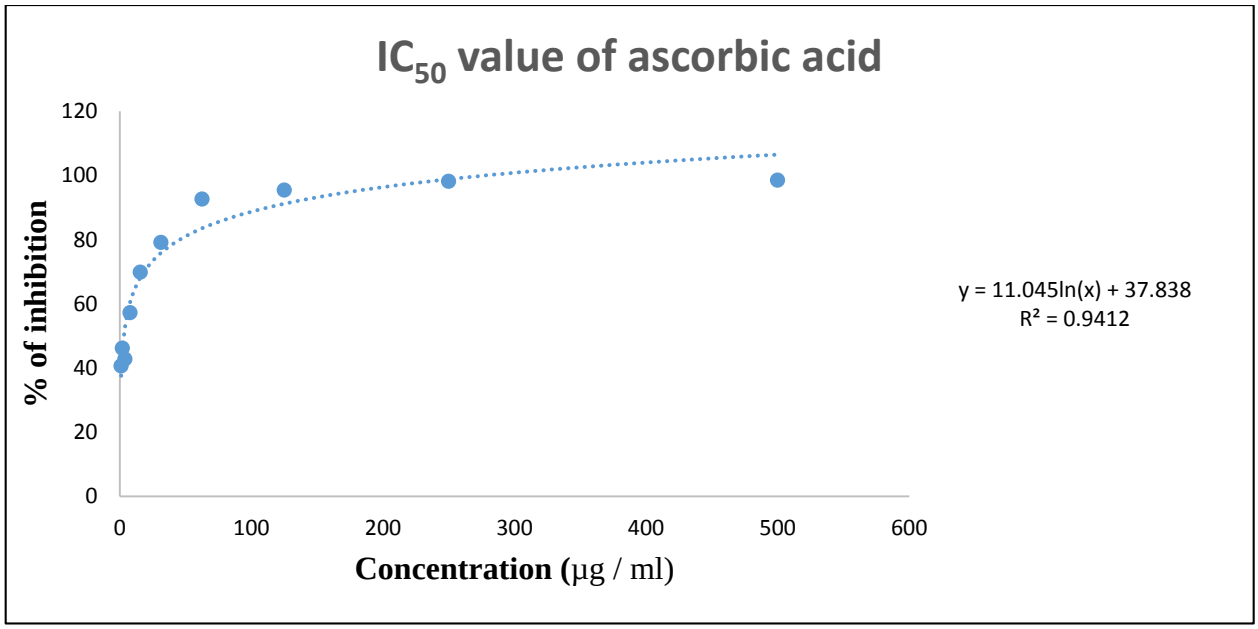


Figure 3.2: IC_{50} value of ascorbic acid

Table 3.6: IC_{50} value of *tert*-butyl-1-hydroxytoluene (BHT)

Absorbance of the blank	Concentration (µg/ml)	Absorbance of the extract	% inhibition (Y)	IC_{50} (µg/ml)
0.325	500	0.018	94.46	21.17
	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	

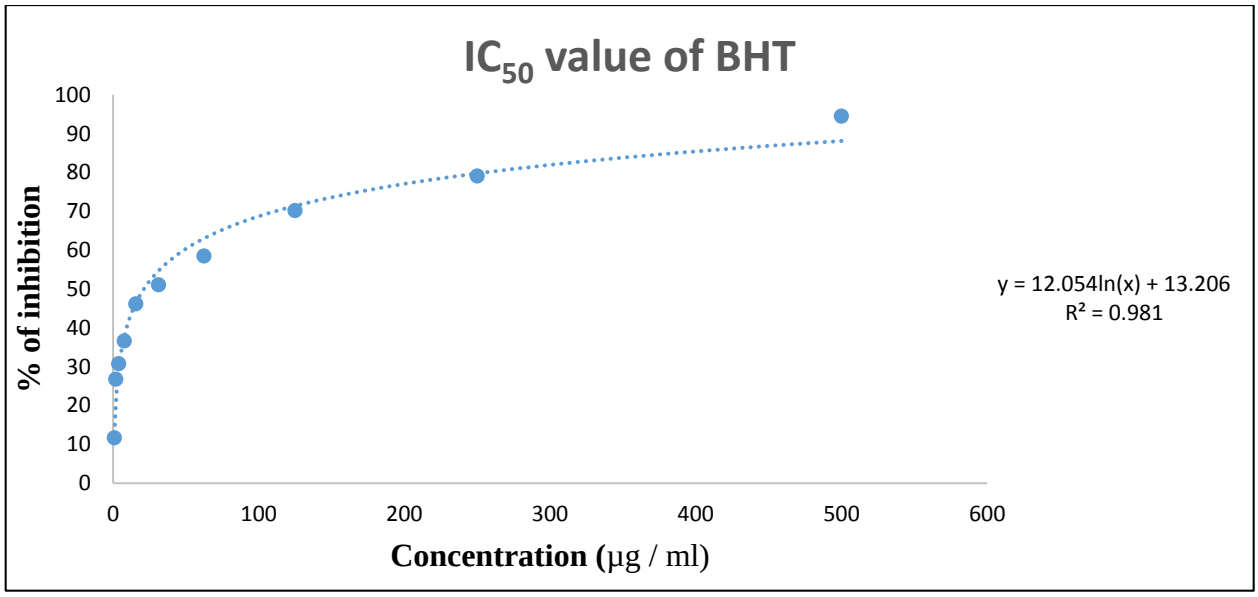


Figure 3.3: IC_{50} value of tert-butyl-1-hydroxytoluene (BHT)

Table 3.7: IC_{50} value of Methanolic extracts (ME)

Absorbance of the blank	Concentration (µg/ml) (X)	Absorbance of the extract	% Inhibition (Y)	IC_{50}
0.325	500	0.021	93.54	5.524
	250	0.028	91.38	
	125	0.042	87.08	
	62.5	0.066	79.69	
	31.25	0.083	74.46	
	15.625	0.116	64.31	
	7.813	0.144	55.69	
	3.906	0.193	40.62	
	1.953	0.209	35.69	
	0.977	0.224	31.08	

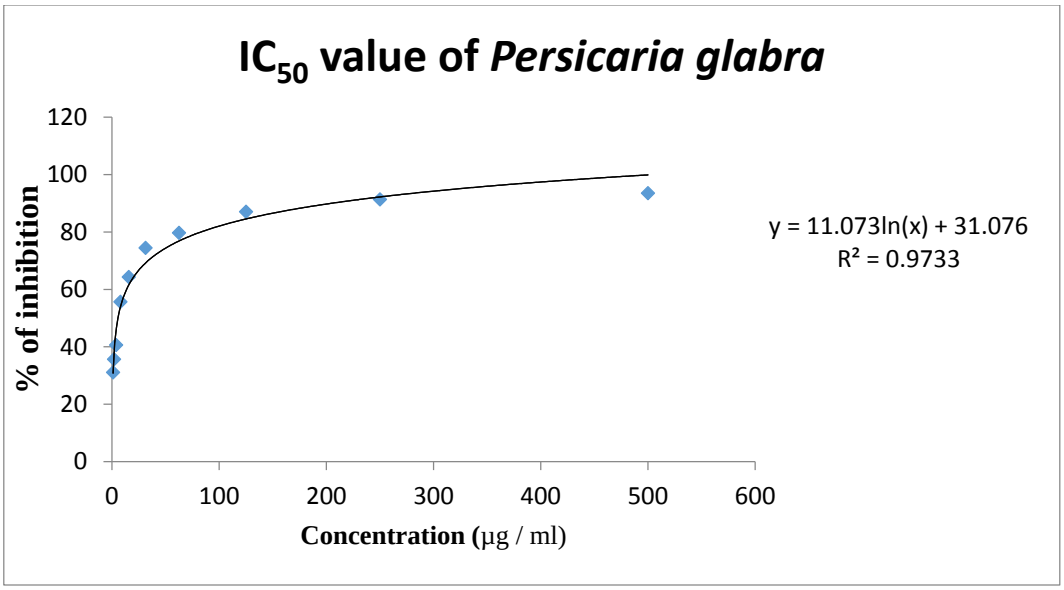


Figure 3.4: IC_{50} value of ME of leaves of *Persicaria glabra*

3.4 Brine Shrimp Lethality Bioassay:

The methanolic extract (ME) of leaves of *Persicaria glabra* was examined for brine shrimp lethality bioassay. The cytotoxicity of the extract to brine shrimp was observed and the results are given in Table 3.10.

The lethal concentration (LC_{50}) of the test sample was determined by plotting the percentage of the mortality rate of shrimps against the logarithm of concentration. The curve of regression analysis helps in gaining the best-fit line. Vincristine sulfate (VS) was used as positive control and the LC_{50} was found to be 0.35 µg/ml. The LC_{50} of the methanolic extract of leaves of *Persicaria glabra* was 0.516 µg/ml which is much higher than vincristine sulfate.

Table 3.8: LC_{50} values of the test samples of leaves of *Persicaria glabra*

Test samples	Regression line	R^2	LC_{50} (µg/ml)
ME	y = 25.97x + 36.606	0.828	0.516

Table 3.9: Effect of Vincristine sulfate (positive control) on shrimp nauplii

Conc. (µg/mL)	Log_{10} Conc.	% Mortality	LC_{50} (µg/mL)
0	-	0	0.35
0.039	-1.4089	20	
0.078125	-1.1072	30	
0.15625	-0.8061	30	
0.3125	-0.5051	40	
0.625	-0.2014	50	
1.25	0.09691	70	
2.5	0.39794	80	
5	0.6989	80	
10	1.00	90	
20	1.30102	100	

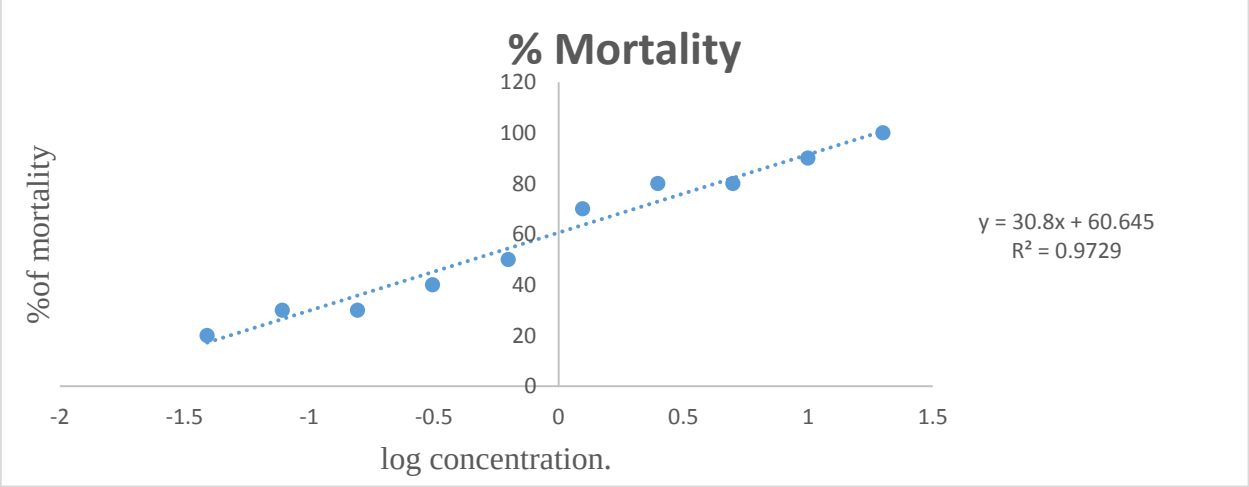


Figure 3.5: Plot of % mortality and predicted regression line of VS

Table 3.10: Effect of the methanolic extract (ME) of leaves of *Persicaria glabra* shrimp nauplii

Conc. (µg/mL)	$Log_{10\ conc.}$	% of mortality	LC_{50}
0	-	0	0.516
0.78125	-0.1071	20	
1.5625	0.1932	40	
3.125	0.4949	50	
6.25	0.7959	60	
12.5	1.0969	90	
25	1.3979	70	
50	1.69897	90	
100	2	80	
200	2.301	90	
400	2.6021	100	

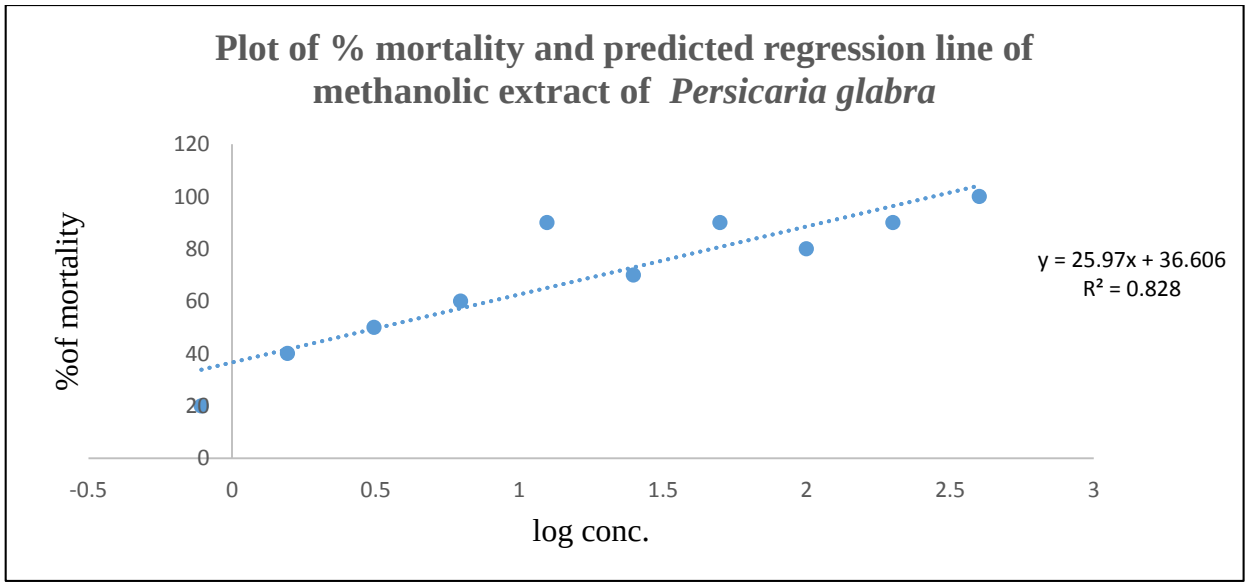


Figure 3.6: Plot of % mortality and predicted regression line of ME

3.5 Antimicrobial Screening:

The methanolic extract (ME) of leaves of *Persicaria glabra* was investigated to find out the antimicrobial activity with a concentration of 400 µg/disc in every case. The methanolic extracts of the leaves of *Persicaria glabra* showed some moderate antimicrobial activity against some different tested microorganisms.

The maximum zone of inhibition exhibited by ME was found to be 11mm against *Aspergillus niger*. The mild antimicrobial activities were tested against *Sacharomyces cerevacae* (having zone of inhibition of 10 mm), *Escherichia coli* (having zone of inhibition of 9 mm), *Sarcina lutea* (having zone of inhibition of 9 mm), *Bacillus subtilis* (having zone of inhibition of 8 mm), *Vibrio parahemolyticus* (having zone of inhibition of 18 mm), *Bacillus cereus* (having zone of inhibition of 7 mm), *Vibrio mimicus* (having zone of inhibition of 5 mm), *Bacillus megaterium* (having zone of inhibition of 4 mm), *Staphylococcus aureus* (having zone of inhibition of 4 mm), *Shigella boydii* (having zone of inhibition of 3 mm). Among all of the test microorganisms, the lowest activity was exhibited against *Pseudomonas aeruginosa* (having a zone of inhibition of 2 mm).

Table 3.11: Antimicrobial activity of test samples of bark of *Persicaria glabra*

Test microorganisms	**Diameter of zone of inhibition (mm)**	
	Extract	**Kanamycin**
Gram Positive Bacteria		
Bacillus cereus	7	37.6
Bacillus megaterium	4	38.3
Bacillus subtilis	8	35.0
Staphylococcus aureus	4	35.0
Sarcina lutea	9	37.3
Gram-negative bacteria		
Escherichia coli	9	37.0
Pseudomonas aeruginosa	2	35.6
Shigella boydii	3	35.1
Vibrio mimicus	5	37.3
Vibrio parahemolyticus	8	38.0
Fungi		
Aspergillus niger	11	37.0
Sacharomyces cerevacae	10	38.6

Chapter 4: Conclusion

The crude extracts of the leaves of *Persicaria glabra* can be a source of herbal medicine to efficiently treat some specific human diseases. The biological investigation of *Persicaria glabra* shows that it has high antioxidant properties that can treat oxidative stress. A high LC_{50} value of methanolic extracts of leaves of *Persicaria glabra* was also determined and compared with the standards. On the other hand, the leaf extract showed low antimicrobial activity against gram-positive and gram-negative bacteria and fungi.

Therefore, the study shows that the extracts of the leaves of *Persicaria glabra* might be a significant contributor to drug development with potential cytotoxic and antioxidant activity. However, extensive in-vivo investigations should be carried out to validate the existing herbal and traditional uses and to explore any other therapeutic activity along with their possible side effects. These will ultimately lead to the gathering of substantive understanding about the safety and efficacy of *Persicaria glabra*. Also, further phytochemical analysis of different extracts will be helpful in characterization and isolation of its active compounds. Sooner rather than later, anticancer activity on cell line for squamous cell carcinoma will be investigated by using A431 human cell line to decide the use of leaves extract of *Persicaria glabra* as an anticancer agent.

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