Daffodil International University

A dissertation submitted to the Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University in the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm)

Project On

Phytochemical Screening, Determination of Total Tannin Content & Evaluation of Antioxidant & Thrombolytic Activities of Leaves Extract of *Symplocos macrophylla*.

Submitted To

The Department of Pharmacy

Faculty of Allied Health Sciences

Daffodil International University

Submitted By

Student ID: 171-29-1029

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APPROVAL

This is to certify that the investigation that are figured in this project are original and have not been submitted before in substance for any degree or diploma of this university. The entire present work submitted to the Department of Pharmacy, Daffodil International University, as a project work for the partial fulfillment of the degree of bachelor of pharmacy; is based on the result of authors (ID: 171-29-1029) own investigation.

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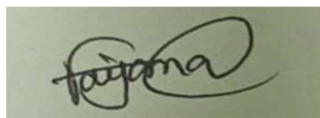

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Declaration

I'm declaring that this project work 'Phytochemical Screening, Determination of Total Tannin Content and Evaluation of Antioxidant & Thrombolytic Activities of Leaves Extract of *Symplocos macrophylla*' submitted to the Daffodil International University, is done by me under the supervision of Farjana Islam Aovi, Lecturer (Senior Scale), Department of Pharmacy, Daffodil International University. I'm also declaring that the investigation that are figured in this project are original and have not been submitted anywhere for any degree of bachelor or diploma.

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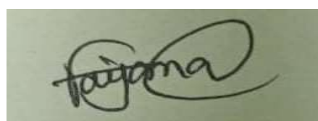

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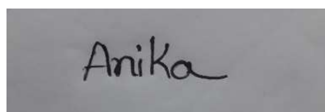

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// Acknowledgement

At the beginning I would like to say ‘Alhamdulillah’ for expressing my gratitude to Almighty Allah to enable me to complete my project work.

The success and final outcome of this project required a lot of guidance, assistance and support from many people and I am lucky enough to have got these all along for the finishing of my entire project work. I am very grateful & would like to express my deep sense of gratitude to them.

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Anika Tahsin

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Dedication

Dedicated to my beloved Parents & Teachers.

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Abstract

In this present study, the leaves extract of *Symplocos macrophylla* Wall. were subjected to a phytochemical screening, determination of total Tannin content and evaluation of Antioxidant and Thrombolytic Activity. Preliminary phytochemical screening shows the presence of alkaloids, glycosides, tannins, saponins, gums and phenols. Total tannin contents in methanol extract of *Symplocos macrophylla* Wall. leaves were found 0.6367 mg of QE/gm. The % of inhibition of leaves extract for DPPH test of antioxidant activity is 78.06%. and it shows that *Symplocos macrophylla* Wall. has some antioxidant activity. In the evaluation of thrombolytic activity, leaves extract showed 55.37% of clot lysis.

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CHAPTER

ONE

INTRODUCTION

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1.1 GENERAL INTRODUCTION

Natural substances that found from plants and uses for the treatment of various types of diseases have been proven that nature stands a golden mark to show a relationship between human and the environment. Plant plays a key role in medicine and human health. [1] Plants have been used for medicinal purpose from the period before written records and no concept of surgery existed. The role of plants in traditional medicine cannot be described in words. The use of plant parts to treat diseases is all-embracing among countries not having become industrial. Plants are used in medicine to maintain and improve health conditions physically, mentally and spiritually as well as to treat diseases. [2] Even today, around 1.4 billion people in South Asia alone have no idea about modern health care and depends on traditional medicine to treat various diseases. On a universal basis, nearby 50,000 to 80,000 plant species are used as natively and pharmaceutical derivatives for life-threatening conditions including diabetes, severe asthma, hypertension, epilepsy and cancer. Nowadays, the demand for plant-based medicine is increasing, for this reason it is necessary to test and determine the quality, safety and ability of these plant-based medicines by scientific methodologies like botanical, phytochemical, analytical, and molecular evaluations. [3] 'Crude drugs of natural or biological origin' this term is used by pharmacists and pharmacologists to describe the parts of plants or whole plant which has medicinal properties.[4] These medicinal plants with chemically active compounds show's different therapeutic properties of plant and animal drugs.[5] The WHO supports and promotes to the addition of herbal medicines in national health care programs because it is accessible at a law price within the reach of a common people. Herbal medicines are considered to be much safer than the synthetic drugs. [4]

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1.2 PHYTOCHEMISTRY

The study of phytochemicals or chemicals that derived from plants is called phytochemistry. The compounds found in plants are of many kinds, but major biosynthetic classes are: alkaloids, flavonoids, tannins, saponins, phenols, glycosides, carbohydrates, steroids etc. [6]

1.3 MEDICINAL PLANTS

Recovery with medicinal plants is an old treatment process from prehistoric period. A medicinal plant is any plant that contains substances which can be used for therapeutic purposes. When a plant is called as medicinal, it is understood that the plant is useful in drug, has therapeutic purpose or has an active ingredient of a medicinal preparation. [7] Herbal medicines are in great demand in the developed and developing countries for preliminary health care because of their various biological and medicinal properties, higher safety, minimal side effects and inexpensiveness. [8] Medicinal plants are rich sources of ingredients that can be used in drug development. Treatment with medicinal plants is known as very safe because of their minimal side effects. The biggest advantage of the herbal remedies is that any gender and ages of people can use it. [9]

1.3.1 IMPORTANCE OF MEDICINAL PLANTS

Medicinal plant plays an important role in the prevention and treatment of disease. The researches and uses of herbal medicine in the treatment of diseases are increasing day by day. Due to the presence of natural compounds, medicinal plants contains major source of molecules

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with medicinal properties. Medicinal plants are useful for relieving and healing human diseases due to presence of phytochemical constituents. Medicinal plants are important source to treat the serious diseases in all over the world. Their properties of healing have been transmitted from centuries to centuries among human communities. Active compounds that are formed in secondary metabolism, normally responsible for the phytochemical constituents of plant species used in the whole world for the prevention of diseases. [10]

1.4 HARBAL MEDICINE

The use of plants to prevent disease, maintain human health and decrease the rate of side effects is known as herbal medicine. An herb is a plant or plant part used for its scent, flavor, therapeutic properties, active constituents and biological uses. Herbal medicines are one kind of dietary supplement. They are sold in the market as many forms like tablets, capsules, powders, suspension, extracts, fresh and dried plants. People use herbal medicines to maintain and improve their health conditions and to avoid side effects of synthetic drugs. In this 20th century there are many people can be found who believes that products labeled as natural are always safe and better for their health. This is not necessary to be true in case of all herbal medicines. [11,12]

1.5 PHYTOCHEMICAL SCREENING

Phytochemical screening of any plant means the extraction, screening, determination and identification of the medicinally active or bioactive constituents found in that plants. Some of the medicinally active components are alkaloids, glycosides, flavonoids, tannins, saponins, gums, steroids, phenols and they can be found from plant. [26]

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1.5.1 ALKALOIDS

Alkaloids are a large group of chemical constituents that made by plants and have nitrogen. There are many alkaloids that contain mighty pharmacologic effects. The alkaloids contain cocaine, nicotine, strychnine, caffeine, pilocarpine, morphine, atropine, ephedrine, mescaline, tryptamine etc. [13,14]

1.5.2 GLYCOSIDES

Glycosides are a naturally occurring components in which a carbohydrates portion which contains one or more sugars, is combined with a hydroxy compound. The hydroxy is a derivative of phenol or an alcohol, which consist of many glucose units. A large number of glycosides found in plants, flower and fruits. [15]

1.5.3 TANNINS

The word tannin comes from the German word ‘tanna’, which means oak. Tannin is a complex chemical substance that derived from phenolic acids. Sometimes tannin is called tannic acid. Tannins are classified as phenolic compounds and found in many plant species. They contain a large number of molecules that bind easily with cellulose, proteins, starches, and minerals. These chemical substances are resistant also insoluble to decomposition. Tannins found in many plant species and a number of flowering plant families. [16]

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1.5.4 SAPONINS

The name saponin derived from their ability of forming stable and soap like foams in aqueous solutions. Chemically, saponin contains a carbohydrate moiety which is attached to a triterpenoid or a steroid. Saponins are a group of compounds that distributed in the worldwide plant kingdom. they are characterized by a skeleton derived of the 30-carbon precursor oxidosqualene and glycosyl residues are attached with it. The most common skeleton was oleanane and present in most of the plant in the world. Saponins are bitter in taste. Generally, they are subdivided into triterpenoid, spirostanol, and furostanol saponins. [17]

1.5.5 GUMS/ CARBOHYDRATES

Gum is a mixture of chemical constituents with the ability to form gels that improves the viscosity of solutions and stabilize the foams and emulsions. The plant gums are complex compounds that executed from carbohydrates. Generally, they are salts potassium, magnesium, calcium of acidic polysaccharides. Gum is generally associated with water soluble and modified polysaccharides. Plant gums are naturally occurring from various plant barks, seeds, leaves and seaweed extracts. [18]

1.5.6 PHENOLS

Phenols are organic compounds that characterized by a hydroxyl group (-OH) which is attached to a carbon atom which is a part of an aromatic ring. Phenols and alcohols are similar, but phenols build stronger hydrogen bonds. Plants forming compounds that contains phenol are

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called antioxidants. That means phenols can stop the reaction of free radicals in the body with other molecules and can prevent the damage of the DNA and long-term health diseases. [19]

1.6 ANTIOXIDANTS

Antioxidants are the substances that can stop the reaction of free radicals with other molecules in the body to slow down or prevent the damage of DNA and cells. Free radicals are reactive oxygen and nitrogen species that produced in the body by various endogenous systems and exposure to different pathological states. The balance between free radicals and antioxidants is very important for body to maintain healthy physiological function. The cell damage that causes because of the reaction of free radicals is known as oxidative stress. Antioxidants are also called 'free-radical scavengers.' Antioxidants can be found both naturally and artificially. Antioxidants that are formed naturally or plant-based are a type of phytonutrient. [20,21]

1.7 THROMBOLYTIC ACTIVITY

Thrombosis is the formation of a deadly blood clot inside a blood vessel artery which is called arterial thrombosis or inside a vein which is called venous thrombosis, opposing the blood flow via circulatory system. If thrombosis is formed it can slowdown or decrease the rate of normal blood flow. This can cause serious injury like heart attack, stroke and venous thromboembolism. Thrombolytic therapy is a treatment of dissolving dangerous clots in blood vessels to increase blood flow also preventing damage of tissues, cells and organs. Synthetic drugs that used in

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the treatment of thrombolysis may causes adverse effects like major bleeding, cardiac arrhythmias, cerebrovascular hemorrhage and anaphylactic reaction. To avoid and decrease the adverse effects it is a necessary to research naturally forming safe thrombolytic agents. [22]

1.7.1 STREPTOKINASE

Streptokinase is a protein, which is produced by various strains of h-hemolytic streptococci with a molar mass of 47 kDa. H-hemolytic streptococci is made of 414 amino acid residues. SK is not an enzyme itself. At pH, approximately 7.5 the protein shows its highest activity. The isoelectric pH of the protein is 4.7. This protein contains a single chain polypeptide which is connected with the pro-activator plasminogen. This enzymatic complex activated the alteration of inactive plasminogen and shows fibrinolytic activity. [23,24]

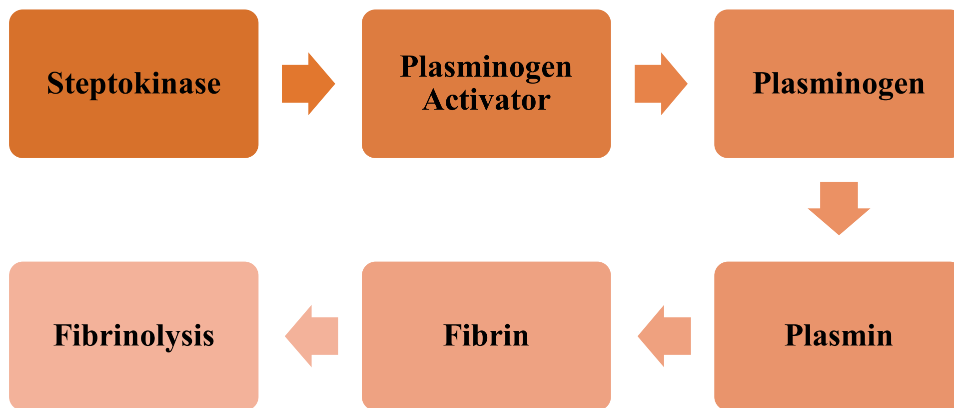

Figure-1: Mechanism of Streptokinase

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1.8 OBJECTIVES OF THE PROJECT WORK

The research work of *Symplocos macrophylla* Wall. was chosen:

- ✓ To determine the presence of phytochemical constituents.
- ✓ To determine total tannin content.
- ✓ To evaluate the antioxidant and thrombolytic activities.

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CHAPTER

TWO

PLANT PROFILE

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2.1 PLANT REVIEW

2.1.1 INTRODUCTION OF PLANT

Scientific Name: *Symplocos macrophylla* Wall.

Common Name: Sankan, Malankuruvi, Malankurvi

Locality: Lawachara National Park, Kamalgonj, Moulovibazar

Synonym: *Symplocos gardneriana Wight, Symplocos barberi Gamble*

2.1.2 SCIENTIFIC CLASSIFICATION

Kingdom: Plantae

Family: Symplocaceae

Genus: Symplocos

Species: Symplocos mscrophylla Wall.

Symplocos macrophylla Wall. is an unresolved name.

2.1.3 DESCRIPTION

Habit: Trees up to 10 m tall.

Habitat: Very rare; Evergreen forests; Found growing in forests as undergrowth.

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Leaves: Leaves are simple, alternate, crowded at apex and spiral.

Flowers: White in spike.

Fruit and Seed: Drupe, cylindrical up to 2 cm long, greenish-white in color.

2.1.4 PLANT PART USED IN THE PROJECT WORK

- Leaves

2.1.5 IMAGE OF THE PLANT

Figure-2: Image of *Symplocos macrophylla* Wall.

2.2 LITERATURE REVIEW

No literature about this plant has been found.

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CHAPTER

THREE

MATERIALS & METHODS

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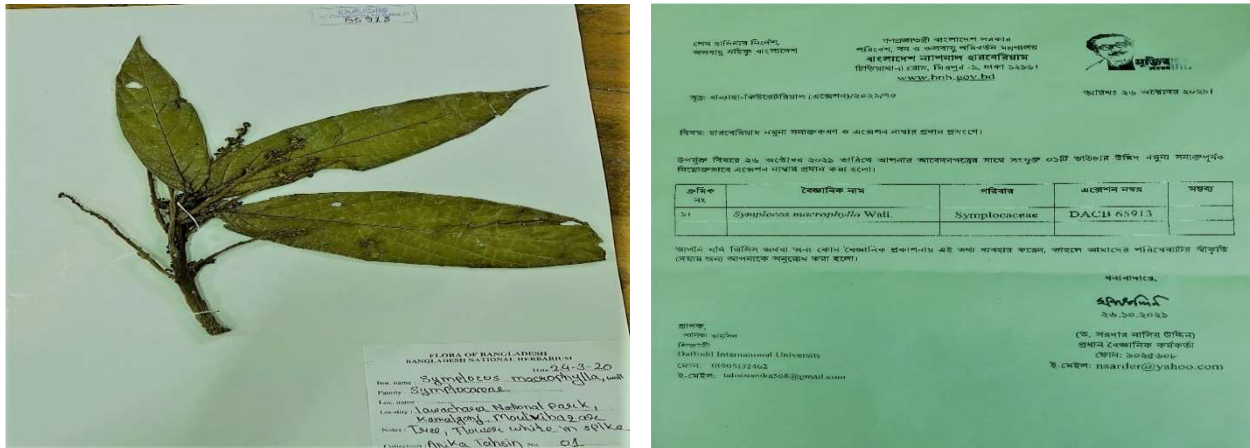
3.1 EXPERIMENTAL PLANT

Symplocos macrophylla Wall. included in family Symplocaceae, inspected in this study.

Plant Name	Family	Part of the plant used
Symplocos macrophylla Wall.	Symplocaceae	Leaves

3.1.1 PLANT COLLECTION AND IDENTIFICATION

The plant was collected in fresh condition from Lawachara National Park, Kamalgonj, Moulovibazar. Expert of National Herbarium (Mirpur, Dhaka) identified as ***Symplocos macrophylla* Wall.** (DACB Accession No: 65913).



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১।	*Symplocos macrophylla* Wall.	Symplocaceae	DACB 65913	

স্বাক্ষরিত
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Figure-3: Identification of *Symplocos macrophylla* Wall.

3.2 PREPARATION OF CRUDE EXTRACT

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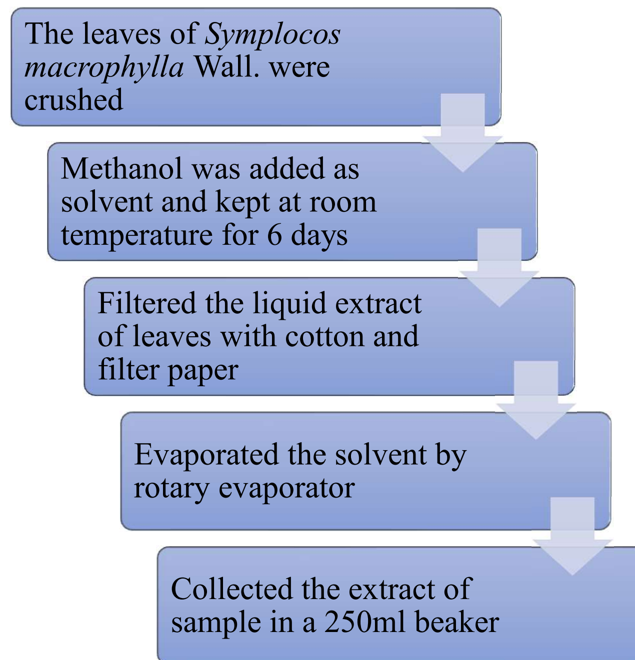

Figure-4: Preparation of Crude Extract

3.2.1 PLANT MATERIAL PREPARATION

Leaves were separated from the collected plant and washed them with water to remove all the debris. After that, the leaves were dried in the room temperature for few days until proper drying. The dried leaves were crushed into powder with the help of grinder and then that, the powder was stored into an air tight container for further use. [25]

3.2.2 EXTRACTION PROCESS

- ✓ A big glass jar was taken, washed it with water and then rinse out with methanol.
- ✓ Then 93.3461gm crushed leaves powder was taken in the jar and 400ml methanol (up to 2 inch above of the powder surface) was added to it.
- ✓ The jar was closed properly with plastic cover so that air cannot enter into the jar.

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- ✓ After that the jar was kept in a dry dark place for 6 days and shaken it 2 times a day regularly. [25]

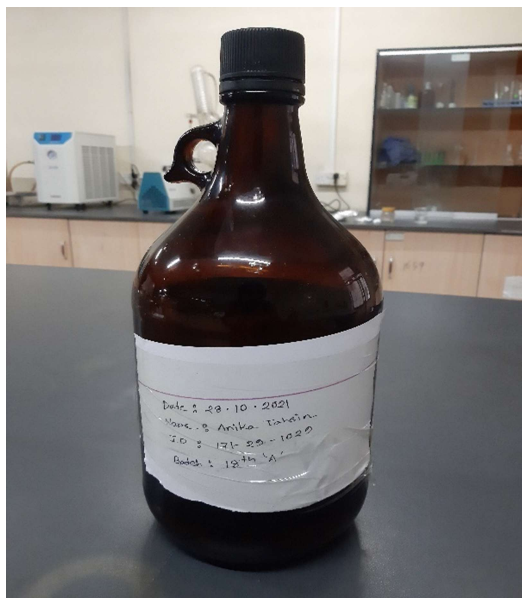

Figure-5: Extraction of the leaves powder

3.2.3 FILTRATION PROCESS

- ✓ After 6 days the liquid extract of leaves was filtered with cotton and filter paper.
- ✓ The extract was filtered and re-filtered with cotton for 2 times and then filter paper for 2 times with the help of a funnel.
- ✓ The filtrate was 250ml and collected in a 500ml beaker.
- ✓ Then the beaker was covered with aluminum foil paper to close it and make a hole in the middle of the foil paper so that, the methanol can evaporate.
- ✓ The beaker with the extract was kept in a dry place for 3 days. [25]

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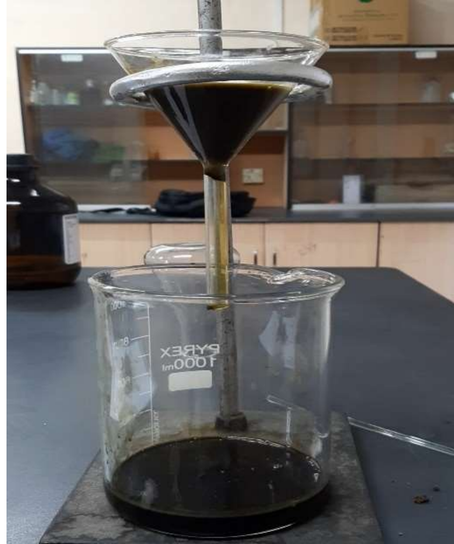

Figure-6: Filtration of the leaves powder extract

3.2.4 EVAPORATION PROCESS

- ✓ After 3 days the liquid extract was evaporated with the help of rotary evaporator.
- ✓ The rotary evaporator was run and evaporation was done under 64°C temperature.
- ✓ Then the sample extract was collected in a 250 ml beaker and covered it with an aluminum foil paper and make a hole in the middle of the foil paper.
- ✓ The beaker was kept for 4 days in a dry place at room temperature to evaporate the remaining methanol. [25]

Figure-7: Evaporation of methanol from the liquid extract

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3.3 PHYTOCHEMICAL SCREENING METHOD

3.3.1 REAGENTS PREPARATION

Mayer's reagent:

- ✓ At first, mercuric chloride (1.36 gm) was dissolved in distilled water (60 ml).
- ✓ Separately, potassium iodide (5 gm) was dissolved in distilled water (20 ml).
- ✓ Then both solutions were mixed.

Fehling's reagent A:

- ✓ Copper sulphate (34.64 gm) and concentrated sulfuric acid (0.50 ml) were dissolved in distilled water and adjusted the volume up to 500 ml.

Fehling's reagent B:

- ✓ Sodium potassium tartrate (176 gm) and sodium hydroxide (77 gm) were dissolved distilled water and adjusted the volume up to 500 ml.

Dragendroff's Reagent:

- ✓ Basic bismuth nitrate (1.7 gm) and tartaric acid (20 gm) were dissolved in 80 ml distilled water.
- ✓ Potassium iodide (16 gm) was dissolved in 40 ml distilled water.
- ✓ Then both solutions were mixed.

Molish Reagent:

- ✓ Pure α-naphtha (2.5 gm) was dissolved in 25 ml ethanol.

Benedict's Reagent:

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- ✓ Cupric sulphate (1.73 gm), sodium citrate (1.73 gm) and anhydrous sodium carbonate (10 gm) were dissolved in distilled water and adjusted the volume up to 100 ml.

3.3.2 TEST PROCEDURES FOR PHYTOCHEMICAL SCREEING

- **Alkaloids Test**

  Mayer's Test

  - ✓ Solution of the sample extract (2 ml) and dilute hydrochloric acid (0.2 ml) were taken in a test tube.
  - ✓ Then mayer's reagent (1 ml) was added in the test tube.

Note: Formation of yellow color indicates the presence of alkaloids. [26]

  Dragendroff's Test

  - ✓ Solution of the sample extract (2 ml) and dilute hydrochloric acid (0.2 ml) were taken in a test tube.
  - ✓ Then dragendroff's reagent (1 ml) was added in the test tube.

Note: Precipitation of orange brown color indicates the presence of alkaloids. [26]

- **Flavonoids Test**

  - ✓ Alcoholic solution of the sample extract (0.5 ml) was taken in a test tube.
  - ✓ A small piece of zinc dust was added in the test tube.
  - ✓ Then a few drops of concentrated hydrochloric acid were added to it and boiled the mixture for few minutes.

Note: Formation of red color indicates the presence of flavonoids. [26]

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- **Glycosides Test**
  - ✓ A small amount of alcoholic extract solution of the sample was taken in a test tube with distilled water (1 ml).
  - ✓ Then 4-5 drops of aqueous sodium hydroxide were added to the test tube.

Note: Formation of yellow color indicates the presence of glycosides. [26]

- **Tannins Test**
  - ✓ Solution of the sample extract (6 ml) was taken in a test tube.
  - ✓ Then 5% ferric chloride solution (1 ml) was added in the test tube.

Note: Formation of greenish black color indicates the presence of tannins. [26]

- **Saponins Test**
  - ✓ Solution of the extract (1 ml) was taken in a graduated cylinder.
  - ✓ Then diluted it with distilled water (20 ml).
  - ✓ Graduated cylinder was shaken for 10-15 minutes.

Note: Formation of a layer of foam up to 1 cm. indicates the presence of saponins. [26]

- **Steroids Test**
  - ✓ Solution of chloroform extract (1 ml) was taken in a test tube.
  - ✓ Then sulfuric acid (1 ml) was added in the test tube.

Note: Formation of red color indicates the presence of steroids. [26]

- **Gums and Carbohydrates Test**
  - ✓ Solution of the extract (5 ml) was taken in a test tube.
  - ✓ Then molish reagent and sulfuric acid were added to it.

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Note: Formation of red-violet color ring in the joint of two solutions indicates the presence of gums and carbohydrates. [25]

- **Phenols Test**
  - ✓ Small amount of the sample extract was taken and 2% $FeCl_3$ (2 ml) was mixed with it.

Note: Formation of black or blue-green color indicates the presence of phenols. [26]

3.4 DETERMINATION OF TOTAL TANNIN CONTENT

3.4.1 REAGENTS

- ✓ Folin-Ciocalteu Reagent (FCR)
- ✓ Sodium Carbonate

3.4.2 APPARATUS

- ✓ Beaker
- ✓ Test Tube
- ✓ Pipette
- ✓ Volumetric flask

3.4.3 INSTRUMENT

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- ✓ UV/ Visible spectrophotometer

3.4.4 SOLUTION PREPARATION

- ✓ Extract of the sample (5mg) was mixed with methanol (99-110%, 10ml)

3.4.5 35% Na_2CO_3 PREPARATION

- ✓ Na_2CO_3 (35 gm) was dissolved in distilled water and adjusted the volume up to 100 ml.

3.4.6 PROCEDURE

The total tannin content was determined by Folin-Ciocalteu method.

- ✓ At first solution of sample extract (0.1 ml) was taken a volumetric flask containing 7.5 ml of distilled water.
- ✓ Then Folin-Ciocalteu phenol reagent (0.5 ml) and 35% Na_2CO_3 solution (1 ml) was added to the volumetric flask and adjust the volume up to 10 ml with distilled water.
- ✓ After that, the mixture was shaken and kept in a dark place for 30 min.
- ✓ Standard solutions of Quercetin were prepared in 5 concentrations (0.1 – 0.5 mg/ml).
- ✓ The absorbances were measured against the blank at 725 nm with an UV visible spectrophotometer of sample and standard.
- ✓ The tannin content was expressed in terms of mg of QE/g of extract. [27]

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3.5 EVALUATION OF ANTIOXIDANT ACTIVITY

3.5.1 REAGENTS

- ✓ Methanol (99-100%)
- ✓ DPPH
- ✓ Ascorbic acid

3.5.2 APPARATUS

- ✓ Beaker
- ✓ Test Tube
- ✓ Measuring Cylinder

3.5.3 INSTRUMENT

- ✓ UV/ Visible spectrophotometer

3.5.4 SOLUTION PREPARATION

- ✓ At first, prepared 500 µg/ml solution of extract as stock solution by dissolving extract (10 mg) in methanol (99-100%, 20 ml).
- ✓ Then prepared 6 concentrations of solution (200,100, 50, 10, 5 and 1 µg/ml) by serial dilution.

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- 6 concentrations of ascorbic acid solution (200, 100, 50, 10, 5 and 1 μg/ml) was prepared in the same way.
- After that, DPPH powder (4 mg) was mixed with methanol (99-100%, 100ml) to prepare DPPH solution (0.004%).

3.5.5 PROCEDURE

- 7 test tubes were taken and labeled them as 500, 200, 100, 50, 10, 5 and 1 μg/ml.
- Extract solution of each concentration (1 ml) was taken in the 7 labeled test tubes for each concentration.
- Then DPPH solution (3 ml) was added in 7 test tubes and kept them in a dark place for 30 minutes.
- After that, solution of ascorbic acid was also taken in another 7 test tubes and DPPH solution was added to it in the same way.
- Kept them in a dark place for 30 minutes also.
- Only methanol was taken in a test tube as blank and DPPH was also added to it.
- After 30 minutes, the absorbances were measured against the blank at 517 nm with an UV visible spectrophotometer of each test tube and noted them.
- At last, % of inhibition was calculated.

% Of inhibition= {(OD of Blank - OD of Sample) / OD of Blank}× 100 [28]

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3.6 EVALUATION OF THROMBOLYTIC ACTIVITY

3.6.1 REAGENTS

- ✓ Streptokinase
- ✓ Blood
- ✓ Distilled Water

3.6.2 APPARATUS

- ✓ Micro Centrifuge Tubes
- ✓ Micropipette
- ✓ Incubator
- ✓ Electric Balance
- ✓ Tissue paper

3.6.3 PREPARATION OF SAMPLE

- ✓ Extract (0.5 g) was dissolved in distilled water (10 ml) and shaken very well.

3.6.4 PREPARATION OF STREPTOKINASE (SK)

- ✓ Distilled water (5 ml) was mixed with streptokinase vial of 15,00,000 I.U. and 30,000 I.U (100 µl) was used for the experiment.

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3.6.5 BLOOD COLLECTION

- ✓ Blood (3 ml) was collected from a healthy volunteer without a medical history of anticoagulant therapy.

3.6.6 PROCEDURE

- ✓ At first 3 micro centrifuge tubes were taken, labeled and weighted the empty tubes.
- ✓ Blood (100 μl) was taken to each of the 3 micro centrifuge tubes and incubated them for 45 minutes at 37° C.
- ✓ Carefully serum was removed completely without disturbing the clot.
- ✓ Then weighted again the centrifuge tubes having clot for calculating the weight of the clot by using this formula:

Weight of the clot = Weight of the tube containing clot – Weight of the empty tube

- ✓ Sample (100 μl) was added to a clot containing micro centrifuge tube.
- ✓ SK (100 μl) was added in another clot containing micro centrifuge tube, as a positive control.
- ✓ Distilled water (100 μl) was added to the clot containing last micro centrifuge tube as a negative control.
- ✓ Then the 3 tubes were incubated again for 90 minutes at 37° C for clot lysis.
- ✓ After 90 minutes, the fluid that released was removed and tubes were weighted again for the difference of weight between them after clot lysis.

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- ✓ Difference between the weight of tubes before clot lysis and weight of tubes after clot lysis was exposed as % of clot lysis. [29,30]

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CHAPTER

FOUR

RESULT

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4.1 YIELD VALUE OF EXTRACT

The weight of the leaves powder was = 94.3461 gm

The weight of the leaves extract was= 14.49 gm

% yield = (14.49 gm/93.3461 gm) ×100

= 15. 52%

4.2 PHYTOCHEMICAL SCREEING

4.2.1 RESULT

Phytochemical screening was done to determine the presence of phytochemical constituents or active constituents in the leaves extract of *Symplocos macrophylla* Wall. The result is shown in the table 1:

Table-1

Tested Groups	Result
Alkaloids	+
Glycosides	+
Flavonoids	-
Tannins	+
Saponins	+
Gums	-

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Steroids	-
Phenols	+

Note: + = Presence of the tested group; - = Absence of the tested group.

Figure-8: Presence of the tested group

4.3 DETERMINATION OF TOTAL TANNIN CONTENT

4.3.1 RESULT

Total tannin contents in the leaves extract exposed in terms of Quercetin equivalent (mg of QE/gm of the extract). Absorbance of leaves extract was 0.8232 nm.

Table-2

Methanol Extract of Leaves	mg of QE/gm of the extract
	0.6367

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Total tannin content was determined in the leaves extract of *Symplocos macrophylla* Wall. by using Folin-Ciocalteu's phenol Reagent is expressed in the terms of Quercetin equivalent (standard or calibration curve equation of quercetin: y = 1.181x + 0.0713; R^2 = 0.988). The total tannin content in the leaves extract of *Symplocos macrophylla* Wall. was 0.6367 mg QE/gm.

Table-3

Concentration (mg/ml)	Absorbance of Quercetin (nm)
0.1	0.205
0.2	0.307
0.3	0.405
0.4	0.524
0.5	0.687

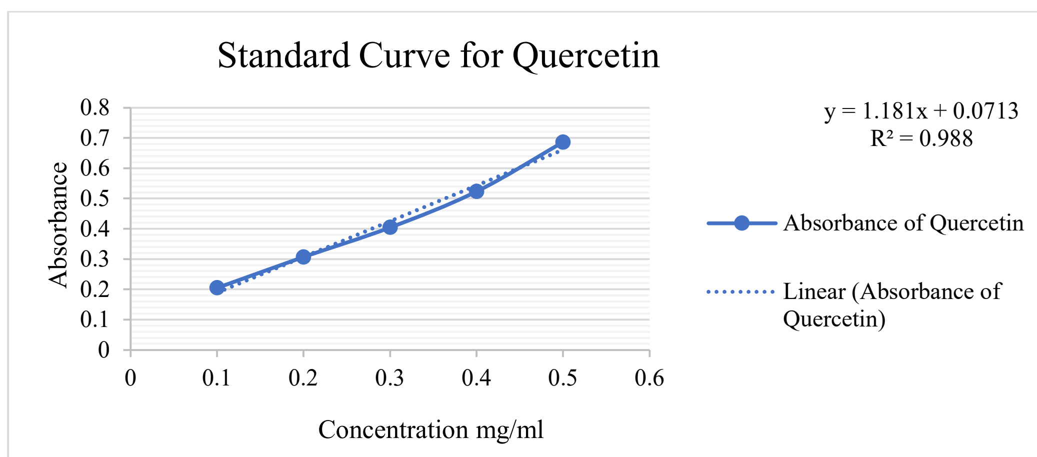

Figure-9: Calibration curve Quercetin for determining total Tannin content

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4.4 EVALUATION OF ANTIOXIDANT ACTIVITY

4.4.1 RESULT

Table-4

Concentration (µg/ml)	Blank Absorbance (nm)	Standard (Ascorbic Acid)		Extract of Leaves	
		Absorbance (nm)	% Of Inhibition	Absorbance (nm)	% Of Inhibition
1	0.903	0.228	82.83%	0.298	67.00%
5		0.216	80.62%	0.287	68.21%
10		0.209	78.74%	0.252	72.10%
50		0.201	77.74%	0.192	78.74%
100		0.192	76.85%	0.176	80.51%
200		0.175	76.08%	0.105	88.37%
500		0.155	74.75%	0.077	91.47%

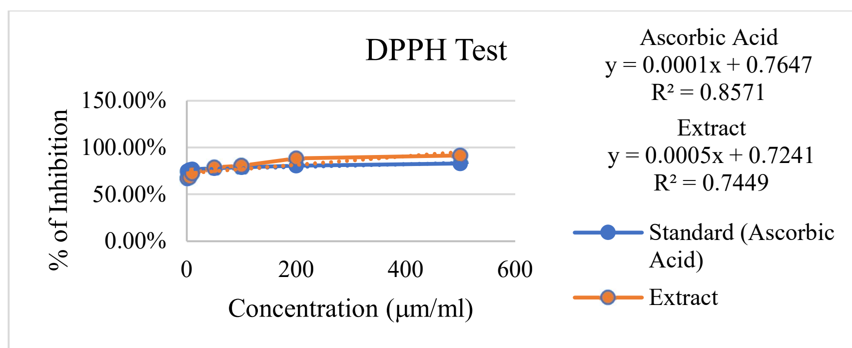

Figure-10: Calibration curve for DPPH test of antioxidant activity

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Here,

Concentration is in X axis and % of Inhibition is in Y axis.

% Of inhibition of ascorbic acid for DPPH test of antioxidant activity is 78.23% and the % of inhibition of leaves extract for DPPH test of antioxidant activity is 78.06%.

Calibration curve equation for Ascorbic Acid is, $y = 0.0001x + 0.7647$; $R^2 = 0.8571$

Calibration curve equation for Sample Extract is, $y = 0.0005x + 0.7241$; $R^2 = 0.7449$

4.5 EVALUATION OF THROMBOLYTIC ACTIVITY

4.5.1 RESULT

Table-5

Sample	Weight of Blank Tube (gm) A	Weight of clot with tube (gm) B	Weight of clot (gm) C = B-A	Weight of Tube with clot after lysis (gm) D	Weight of lysis (gm) E = B-D	% Of clot lysis =(E/C)×100%	Mean ± SEM % of clot lysis
Streptokinase (Standard)	0.83	2.11	1.28	1.37	0.74	57.81%	47.51

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Distilled Water (Control)	0.86	1.95	1.09	1.63	0.32	29.36%	±
Symplocos macrophylla Wall. leaves extract	0.87	2.08	1.21	1.41	0.67	55.37%	0.0743

Streptokinase used as a positive control.

Distilled water used as a negative control.

Figure-11: Evaluation of thrombolytic activity

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CHAPTER

FIVE

DISCUSSION

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5.1 DISCUSSION

5.1.1 YIELD VALUE

The cleaned leaves were dried in the room temperature for extraction. After proper drying, grinding and cold extraction with methanol the yield value was calculated 15.52%.

5.1.2 PHYTOCHEMICAL SCREENING

Phytochemical screening shows that the leaves extract of *Symplocos macrophylla* Wall. contains alkaloid, glycoside, tannin, saponin and phenol.

5.1.3 TOTAL TANNIN CONTENT

The calibration curve or standard curve represent strong positive linear correlation (R^2 = 0.988) because it is very close to the value 1. It represents that the absorbance increases respectively with the increase of concentration. The total tannin content was 0.6367 mg of QE/gm.

5.1.4 ANTIOXIDANT ACTIVITY

% Of inhibition of positive control ascorbic acid for DPPH test of antioxidant activity is 78.23% and the % of inhibition of leaves extract for DPPH test of antioxidant activity is 78.06%. It represents that the *Symplocos macrophylla* Wall. contains some antioxidant activity.

5.1.5 THROMBOLYTIC ACTIVITY

Addition of Streptokinase (100 µl) as a positive control (30,000 IU) in the clots and 90 minutes0f incubation at 37° C, showed 57.81% of clot lysis. Addition of distilled water (100 µl) in the clots and 90 minutes of incubation showed 29.36% of clot lysis. On the other hand, *Symplocos*

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macrophylla Wall. leaves extract showed 55.37% of clot lysis. The Mean ± SEM difference found 47.51 ± 0.0743.

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CHAPTER

SIX

CONCLUSION

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5.1 CONCLUSION

In Bangladesh plant have been used form many years ago like thousand years as medicine for many diseases. There are many researches can be found that are done scientifically to evaluate the therapeutic activity, active compounds of these medicinal plants. This research also has been done to determine the phytochemical screening, total tannin content and evaluate the antioxidant and thrombolytic activity of *Symplocos macrophylla* Wall. leaves. The phytochemical screening showed that the leaf extract of *Symplocos macrophylla* Wall. contains phytochemical constituents like alkaloids, glycosides, tannins, saponins and phenols. Total tannin content in methanol extract of *Symplocos macrophylla* Wall. leaves were found 0.6367 mg of QE/gm. The % of inhibition of leaves extract for DPPH test of antioxidant activity is 78.06%. It represents that the Symplocos macrophylla Wall. contains some antioxidant activity. In the evaluation of thrombolytic activity of leaves extract showed 55.37% of clot lysis. Many more pharmacological activities of this plant are yet to be discovered or studied.

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CHAPTER

SEVEN

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