Thesis On

Phytochemical Screening and In Vitro Thrombolytic Potential of the Methanolic Extract of Persicaria maculosa

Submitted To

The Department of Pharmacy,

Faculty of Allied Health Sciences,

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In the partial fulfillment of the requirements for the degree of Masters of Pharmacy

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APPROVAL

This Thesis, Phytochemical Screening and In Vitro Thrombolytic Potential of the Methanolic Extract of Persicaria maculosa, submitted to the Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Masters of Pharmacy and approved as to its style and contents.

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DECLARATION

I, at this moment, announce that I am carrying out this project study under the supervision of "Mr. Md. Mominur Rahman, Lecturer, Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, Impartial Compliance with the Masters of Pharmacy Degree Requirement (M. Pharm). This thesis, I declare, is my original work. I also state that neither this project nor any part thereof has been submitted for the Bachelor's award or any degree elsewhere.

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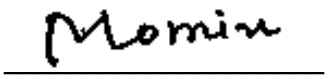

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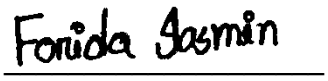

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DECLARATION

I dedicate this work to my parents and my teachers and my friends.

Abstract

Traditional medicine uses persicaria maculosa to cure a variety of ailments, including headaches, gout, rhinitis, hemorrhoids, the common cold, and venereal diseases. Traditional medicine recommends it to prevent stomach cancer and uses it topically to heal burns and skin sores. The National Botanical Garden of Bangladesh provided the Persicaria maculosa, and 2500 ml of methanol were used to extract its 500 g of dry plant powder. Alkaloids, glycosides, saponins, and flavonoids all showed good findings in a number of phytochemical studies that were carried out. The results of the thrombolytics activation include Blank 9.84%, Streptokinase 68.81%, and 12.33% for the Persicaria Maculosa extract in methanol. My research's results suggest that further study is needed to determine if the plant may treat headaches, gout, and rhinitis.

Keywords. Cancer, Drug, Antiviral, Treatment, Persicaria, Neuroprotective.

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Chapter One: Introduction

1.1.Plant

The photosynthetic eukaryotes that make up the kingdom Plantae are plants. Fungus and certain algae were originally included in the plant kingdom, but they are now uniformly omitted, along with prokaryotes, from contemporary definitions of the word "Plantae" (the archaea and bacteria). The plant group Viridiplantae (Latin for "green plants"), which is sister to the Glaucophyta, is composed of green algae and the clade Embryophyta (land plants). The second group of plants consists of flowers, trees, ferns, hornworts, liverworts, and mosses. Most plants have many cells in their bodies. Green plants get the largest bulk of their energy requirements from photosynthesis, which is carried out by primary chloroplasts created through endosymbiosis with cyanobacteria. The chlorophylls a and b found in their chloroplasts give them their green color. Parasitic or mycotrophic plants may nevertheless produce flowers, fruit, and seeds while lacking the photosynthesis and chlorophyll required for normal development. Plants are known for their sexual reproduction, generational alternation, and extensive asexual reproduction. Between 260 and 290 thousand of the approximately 320,000 plant species are capable of reproducing by methods other than seed production. [1] Green plants create the majority of the molecular oxygen that is needed by Earth's ecosystems, and they also produce a significant portion of the world's food. For ages, mankind has been cultivating grain, fruit, and vegetable plants to provide them with a basic diet. For millennia, people have utilized plants as a source of food, fiber, paper, medicine, and a variety of psychoactive drugs. [2] The branch of biology that focuses on plants is called botany. Before, there were two groups for anything that could breathe air or eat: plants and animals. Some assert that Aristotle (384–322 BC) was the first to compare animals and plants, which ordinarily do not move (which are often mobile to capture their food). These two classifications evolved into the kingdoms Vegetabilia (later Metaphyta or Plantae) and Animalia when Linnaeus (1707-1778) laid the groundwork for the modern system of scientific taxonomy (also called Metazoa). Once it became clear that the original concept of the plant kingdom comprised numerous unrelated taxa, fungi, and a few other types of algae were divided into their own kingdoms. Nevertheless, these species are nevertheless referred to as plants in certain informal contexts. In this situation, a citation is necessary. A multicellular creature with cell walls rich in cellulose and primary chloroplasts that perform photosynthesis is often referred to as a "plant." [3-4]

1.2.Medicinal plants

The traditional medical practices of many cultures have long recognized and used therapeutic plants, sometimes known as medicinal herbs. Plants produce a wide array of chemicals for a number of reasons, including protection against herbivorous animals, insects, fungi, and illnesses. Numerous phytochemicals with biological actions that are either confirmed or highly suspected have been found. The benefits of consuming a complete plant as medicine are uncertain, however, since a single plant includes so many different phytochemicals. Numerous plants with potential medicinal lack thorough scientific studies proving their effectiveness and safety, as well as describing their phytochemical composition and potential pharmacological effects. [5] Between around 3000 BC and 3000 AD, the Sumerian civilization developed clay tablets that mention hundreds of medicinal plants, including opium. One such ancient Egyptian manuscript is the Ebers Papyrus. [6] There are several applications for medicinal plants, and they are also essential commercially. They are composed of active components (Stary) that are used to treat a variety of human ailments. Plant extracts have been researched, created, and put up as potential medicinal agents. Medicinal plants are now the subject of a lot of study due to their particular properties, such as being a rich source of phytochemicals with potential therapeutic uses that might result in the creation of innovative medications. According to studies, most phytochemicals derived from plants, such as phenolics and flavonoids, are beneficial to your health and aid in the prevention of cancer. [7]. Because it has been shown that a plant-based diet increases life expectancy among Okinawan people, who have the highest percentage of centenarians, the contemporary Mediterranean diet and the DASH (Dietary Approaches to Stop Hypertension) diet both contain a meal that is rich in phytochemicals [8–9]. There are several fruits and vegetables that contain these chemicals. The drive to find and create skin products from natural sources as alternatives to synthetic and conventional pharmaceuticals has increased interest in the research and commercial usage of medicinal plants [10]. Among the intriguing natural sources are plant extracts, essential oils, and essential oils. The presence of large amounts of phenolic and flavonoid compounds in medicinal plants has been linked to their antioxidant qualities, which are thought to contribute to the prevention of age-related illnesses, especially those brought on by oxidative stress. Research on medicinal plants is extremely significant and should be addressed on par with research on conventional therapies because of the valuable phytochemicals present in medicinal plants and the

trend toward natural products in the pharmaceutical and cosmeceutical industries. Pre-extraction and extraction procedures are the first steps in the research of medicinal plants, and they are crucial steps in the processing of the bioactive chemicals generated from plant materials. Maceration and Soxhlet extraction are conventional methods that are often used in the context of small-scale research or small industrial businesses (SMEs). Processing medicinal plants have advanced significantly, using modern extraction methods, including supercritical fluid extraction, microwave-assisted extraction, and ultrasound-assisted extraction, among others (SFE). These developments aim to increase yield while reducing costs. The methods are also continuously changed and enhanced. The optimum extraction process must be carefully chosen since there are so many different ones accessible. To aid in the choice of acceptable techniques, this overview contains a review of the guiding principles, benefits, and drawbacks of the frequently used methods, as well as instances from recent years.

1.3.Medicinal Plants (Importance and Uses)

Various plants utilized in herbalism, some of which have therapeutic characteristics, are referred to as medicinal plants. These healing herbs are recognized as a plentiful supply of ingredients for drug manufacturing and research. These plants are also essential to the development of human civilizations all around the world. Numerous plants are also recommended for their therapeutic properties since they are thought to be important providers of nutrition. Ginger, green tea, walnuts, and a few other plants are among them. The active ingredients in aspirin and toothpaste are thought to come from other plants and their derivatives.

Alternative Medicine

The idea of using plants as medicine is now at the core of what is now referred to as "Alternative Medicine," a cultural phrase. However, conventional wisdom holds that only pills should be used for medicine. Even most of the pills and capsules we use every day are made from plants. Many different medicines are created using plant extracts. Many pharmaceuticals, such as laxatives, blood thinners, antibiotics, and antimalarials, include compounds derived from plants. Additionally, morphine, foxglove, periwinkle, and yew were used to extract taxol, vincristine, and morphine, respectively. [11]

1.4.Common medicinal plants names

Tulsi

The fragrant perennial plant Ocimum tenuiflorum [or Ocimum sanctum L.] belongs to the Lamiaceae family and is often known as holy basil, tulsi, or Tulasi. It originated on the Indian subcontinent and is currently planted across Southeast Asia's tropical areas.

Ginger

The ginger flowering plant's rhizome, sometimes called ginger root or ginger is used as a spice and in traditional medicine. It is a perennial plant with thin leaf blades growing on annual pseudostems that are about a meter tall.

Indian bael

The Aegle marmelos tree is a rare species found in India and Southeast Asia. It is also known as bael, Bengal quince, golden apple, bitter Japanese orange, stone apple, and wood apple. As a naturalized species, it has established itself in those regions as well as Bangladesh, Sri Lanka, and Nepal.

Sweet flag

Psychoactive compounds can be found in the flowers of the Acorus calamus plant. It belongs to the genus Acorus in the family Acoraceae and grows to a height of several feet in wetland areas.

Coriander

The Apiaceae family includes the annual herb coriander. Some other names for it are Chinese parsley, dhania, and cilantro. Although the entire plant can be eaten, it is the fresh leaves and the dried seeds that are most commonly utilized in recipes.

Basil

Basil, sometimes known as big basil, is a herb from the Lamiaceae family that is commonly used in cooking. It's a delicate plant that's cooked in dishes all across the world. The type of basil commonly known as "basil" in Western cuisine is actually sweet basil, also called Genovese basil. The basil plant originates from the tropics, from Central Africa to Southeast Asia.

Hidden-lilies

Some members of the Zingiberaceae family, such as turmeric and the Siam tulip, belong to the genus Curcuma. Southeast Asia, southern China, the Indian subcontinent, New Guinea, and northern Australia are all places where you can find them in their natural habitat.

Lemon grass

The grass family member Cymbopogon has many common names, including lemongrass, barbed wire grass, silky heads, Cochin grass, Malabar grass, oily heads, citronella grass, and fever grass.

Lavender

Flowering plants in the genus Lavandula are members of the mint family, Lamiaceae. Originating in the Old World, you can spot it everywhere, from northern and eastern Africa, the Mediterranean, southwest Asia, and India to Cape Verde and the Canary Islands.

Ashwagandha

As a member of the nightshade (Solanaceae) family, the evergreen shrub Withania somnifera, sometimes called ashwagandha or winter cherry, is native to India, the Middle East, and some areas of Africa. Similar in appearance are several other species in the genus Withania.

German chamomile

Chamomile, often known as blue chamomile, wild chamomile, fragrant mayweed, or Matricaria chamomilla, is an annual plant in the daisy family Asteraceae.

Centella Asiatica

Centella Asiatica is a perennial herb of the family Apiaceae. It is also known as Gotu kola, Kodava, Indian pennywort, and Asiatic pennywort. Its natural habitats include the tropical zones of Africa, Asia, and Australia, as well as the islands of the western Pacific.

Peppermint

Peppermint is a mix of watermint and spearmint, two different types of mint. Although it is said to have originated in Europe or the Middle East, today, you can find this plant being grown in many different parts of the world. It occasionally appears in the wild alongside its parent species.

Calendula

The pot marigold, also known as Calendula officinalis, is a member of the daisy family, Asteraceae. Other common names for this flower are common marigold, ruddles, Mary's gold, and Scotch marigold. Since it has been cultivated for so long, its exact origin is unclear; nonetheless, it was likely domesticated from a garden, and its likely native range includes southern Europe.

Garlic

The genus Allium includes the bulbous blooming plant commonly known as garlic. The onion, shallot, leek, chive, Welsh onion, and Chinese onion are all near relatives.

Heart-leaved moonseed

Originally found in the tropical parts of the Indian subcontinent, Tinospora cordifolia is a herbaceous vine belonging to the family Menispermaceae. Although Ayurvedic practitioners have used it to treat a wide range of conditions, scientific studies have yet to confirm the medicine's supposed therapeutic benefits.

1.5.Ethnobotany

The scientific study of plants and their uses as discovered by customs and folklore associated with a particular people's native flora and fauna is known as ethnobotany. An ethnobotanist uses this method to document the various applications of natural flora in daily life, including food, medicine, wine, and even clothing. Richard Evans Schultes frequently referred to as the "father of ethnobotany," defined the discipline as "the scientific study of plants from various civilizations." Since Schultes's time, the goal of ethnobotany has evolved from merely studying plants to applying that information to modern civilization, particularly in the pharmaceutical sector. Fundamental issues in ethnobotany include the protection of intellectual property and the equal distribution of financial gains. [12-13]

1.6.Ethnopharmacology

The link between various cultures and the therapeutic qualities of plants is investigated in ethnopharmacology. This has connections to ethnobotany and the use of medicinal plants since it is a source of lead compounds for medication development. [14] Traditional medicine has traditionally been the main focus, but this approach has been very successfully used to examine novel treatments as well. [15-16] It entails research on the following topics: identification, ethnotaxonomy (cognitive categorization), traditional pharmaceutical form preparation, bio-evaluation of the potential pharmacological action of such preparations (ethnopharmacology), the potential for clinical effectiveness, and socio-medical aspects implied in the uses of these compounds (medical anthropology).

1.7.Introduction of *Persicaria maculosa*

Buckwheat is the common name for members of the Polygonaceae family of plants, which includes the annual Persicaria maculosa (also known as Polygonum persicaria). This plant goes by the names lady's thumb, spotted lady's thumb, Jesusplant, and redshank. [18-19] Its distribution extends from Iceland to Portugal and even as far east as Japan, covering a large portion of Eurasia. [20-21] Subsequently, it was first seen in the Great Lakes region in 1843 and has since expanded throughout much of the continent; it is also common in North America as an imported and invasive species. [22]

Description

Fig 01: *Persicaria maculosa*

Persicaria maculosa is an annual plant with a stiff, drooping stem and swollen joints that may reach a height of 1 meter (3 feet 3 inches)[23]. A different leaf is fastened to each one, forming an alternate pattern. The leaf blades have a narrowly oval form and entire edges and often feature a mark in the center that is either brown or black. Each leaf has stipules that are fused together to produce a loose, sheath-like covering for the stem that is fringed with long hairs at the top. These stipules are located at the base of each leaf. The bloom spike is extremely thick. Each little pink flower has a perianth that consists of four or five connected lobes at the base. There are two styles and six stamens that have merged into two carpels. The achene-shaped fruit is glossy and dark black on all three sides. The months of July and September in the Northern Hemisphere are when this plant blooms. [24]

1.8.Scientific classification

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Order: Caryophyllales

Family: Polygonaceae

Genus: Persicaria

Species: *P. maculosa*

Fig 02: Polygonaceae

1.9.Phytochemical chemicals

Established methods were utilized to detect secondary metabolites in the extract of Persicaria maculosa, including alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, proteins, amino acids, glycosides, and anthraquinones. In the extract, several chemicals were discovered. [25]

1.10.Medicinal uses

It is used to cure a variety of ailments, including hemorrhoids, the common cold, venereal infections, headaches, gout, and rhinitis. It may be used topically to treat wounds and other skin damage, and conventional medicine advises taking it orally to lower the chance of getting stomach cancer.

1.11.Botanical Aspects

An annual herb, Persicaria maculosa, has a stem that is erect, highly floppy and has swollen joints. It may reach a height of up to one meter (3 feet 3 inches). The almost stalkless leaves are arranged in an alternating pattern. The leaf blades often have a narrow oval form, and all of their edges are present. Additionally, they typically have a mark in the center that is either brown or black.

Persicaria maculosa common name

The annual plant Persicaria maculosa, commonly known as Polygonum persicaria, is a member of the buckwheat family Polygonaceae. Some of the popular names for this plant are redshank, Jesusplant, lady's thumb, and spotted lady's thumb.

1.10.Pharmacological activities

Antioxidant activity

Persicaria and Polygonum species have an extraordinarily high amount of antioxidant activity. This is so that the bodies of these species can get rid of excess free radicals, maintaining regular metabolic functions in the process. ABTS (2,20-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), NBT (Nitroblue tetrazolium), FRAP (Ferric Reducing Antioxidant Power), TEAC (Trolox Equivalent Antioxidant Capacity), CUPRAC (Cupric Reducing Antioxidant Capacity), and DPPH (2,2-diphenyl-2-picryl hydroxy (Copper Chelating Activity). [26]

Antinociceptive Activity

The antinociceptive properties of the n-Hex, EtOAc, and MeOH extract from P. hydropiper were examined in Swiss albino mice of either sex using the acetic acid-induced writhing method. Ethyl acetate extract showed a moderately dose-dependent effect, with writhing inhibition of 54.95% at a dose of 500 mg/kg, compared to the conventional aminopyrine, which suppressed writhing by 73.62% at a dosage of 50 mg/kg. A 400 mg/kg b.w. dosage of a crude EtOH extract of P. hydropiper leaves demonstrated strong antinociceptive action when compared to the aspirin-based medication, resulting in reductions in the number of the abdominal writhing of 41.02% and 69.23%, respectively. This was also observed by Many et al. [27]. At 400 mg/kg b.w., P. barbata extracts in petroleum ether, and chloroform (Chl) inhibited the writhing response by 46.8% and 44.8%, respectively, compared to 62.2% for aminopyrine, the positive control. The scientists concluded that this behavior was being caused by apolar molecules, most likely sterols or terpenoids. With a percentage of writhing inhibition of 53.57% and 50%, respectively, at a dosage of 500 mg/kg for P. acuminata, EtOH extracts of the leaves and stems showed antinociceptive effects [28]. These results are equivalent to those for conventional diclofenac (57.7% at a dose of 25 mg/kg). The antinociceptive qualities of the crude MeOH extract of P. verticillatum rhizomes were assessed by Khan et al. They found that dose-dependently and via an opioid pathway, the extract may significantly lower (72%) the number of writhes caused by acetic acid. There are several alkaloids and saponins in the extract.

Antitumoral, Cytotoxic, and Anticancer Activity

The antitumoral and anticancer effects of Persicaria and Polygonum species have been the subject of an investigation on a global scale. These species are widespread and inhibit a variety of cancer-causing cell lines. [29] Hepatocellular carcinoma cell lines include HepG2, Huh-7, SMMC-7721, HCCLM3, and Hep3B; human leukemia cell lines include Jurkat, HL60, THP-1, CCRF-CEM, K562, U- 937, K562, and P338; colon cancer cell lines include CaCo-2, HCT116, HT-29, CT-26, RKO, Colo320, and SW620; and breast cancer cell lines include M (fibroblast).

Antiviral Activity

Several species of Persicaria and Polygonum have shown antiviral activity against a range of viruses, including HHV-1 and HSV-1 (human herpes virus 1); EBV-EA (Epstein-Barr virus);

H1N1 A/PR/8/34, H1N2 A/HK/8/68, and B/Lee/40 (influenza A and B); HIV- 1VB59 and HIV-1UG070 (immunodeficient It could be highlighting the inhibitory effects of viscoazulone from P. viscosum on HIV-1 reverse transcriptase and the inhibition of HIV-1 protease (56%) by polygonumins A from P. minor. [30]

Anti-Depressant and Sedative

P. glabra's aqueous extract was examined by Nizar et al. for its potential as an antidepressant. The results showed that the extract (50, 100, and 200 mg/kg) significantly decreased the immobility time of mice during the behavioral despair test (BDT) and tail suspension test and increased the hyperactivity scores in an L-dopa-induced hyperactivity test with values comparable to the reference imipramine (15 mg/kg) (TST). An open-field experiment was conducted to examine the depressive effects of four sesquiterpenes (viscosumic acid, viscozulenic acid, viscoazucine, and viscoazulone), as well as the flavonoid glycoside quercetin-3-O-(6-feruloyl)- -D-galactopyranoside that was isolated from the aerial parts of P. viscosum [31]. Viscoazucine and viscoazulone were the most efficient depressants, causing consistent decreases in mouse movement (number of movements at 0 min = 143.97 and 137.95 and at 240 min = 23.92 and 27.93, respectively). The other drugs are just slightly depressed people.

Neuroprotective Activity

Won and Ma [32] evaluated the neuroprotective efficacy of the aqueous-MeOH extract of P. aviculare by conducting an experiment in which glutamate was used to induce neurotoxicity in primary cultures of rat cortical cells. This experiment was carried out in order to determine whether or not the extract was effective. In contrast to the standards CNQX (59.2%) and MK-801 (70.8%), the data indicate that a significant neuroprotective potency of 50.1% was discovered at a concentration of 100 g/mL. In addition, the juglandin that was extracted from crude P. aviculare showed good neuroprotective activity in mice that had LPS-induced Parkinson's disease. This activity included attenuating memory deficits, promoting the expression of synaptic markers (SYP, PSD-95, and SNAP-25), reducing the production of pro-inflammatory cytokines (IL-1, TNF-, IL-18, and COX-2), and blocking the TLR4/NF-B The neuro-inflammatory effects of P. aviculare extract were also studied, and it was discovered that the extract had the ability to reduce lethargy-like behavior, the brain chemicals corticosterone, serotonin, and catecholamines (all of which are

related to fatigue), and it had the ability to inhibit the production of the protein TNF- (tumor necrosis factor) [33]. Orientin, a pyrone glucoside that was isolated from P. Orientalis, was studied for its potential neuroprotective effects on the PC12 pheochromocytoma cell line of mice, which had been stimulated by H2O2. According to the findings, orientin did not have a negative effect on PC12 cells, but it did have the potential to reduce the damage to PC12 cell viability that was produced by H2O2 at concentrations higher than 40 g/mL. Additionally, orientin decreased the number of reactive oxygen species (ROS) that formed in cells as a result of H2O2-induced activation of signaling proteins (such as MAPKs, AKT, and Src) [34]. ROS are responsible for neurodegenerative diseases.

Chapter Two:
Materials and Method

2.1 Plant Materials

Persicaria maculosa leaves that were completely matured were taken from the National Botanical Garden in Dhaka, Bangladesh. The plant leaves were divided, properly cleansed with tap water, dried in the shade, homogenized to a fine powder, and then kept in an airtight container.

2.2 Extraction of Plant Material

In accordance with Table 1, the powder was steeped in 2300 ml of methanol to create extracts. For fourteen days, the jars were kept sealed with foil paper, sometimes being stirred. Then, Whatman No. 1 filter paper, cotton, and cloth were used to filter the soaking leaves. The filtrate was dried for 40 minutes in a rotary evaporator at 50 °C. It was afterward put into a beaker and held in the fume hood to allow the solvent to evaporate further. After a week, a sticky extract was produced, which was stored at room temperature in a dry area. For pharmacological and phytochemical analysis, the crude extract was employed.

Table 1: Amount of plant material soaked in selected solvents

No. of Container	**Amount of plant materials**	**Amount of Solvent**
Container 1	500gm	Total 2300ml of methanol

Fig 03 : Extraction of Plant Material

2.3 Collection of human blood

7 ml of venous blood was drawn from healthy volunteers (n = 5) who had no history of smoking or using lipid-lowering medications, oral contraceptives, or anticoagulant therapy. The blood was then transferred to various pre-weighed(W1) sterile micro-centrifuge tubes (1 ml/tube) while maintaining aseptic precautions.

2.4. Phytochemical screening

Standard phytochemical tests were performed on the newly produced extracts to identify several compounds, including alkaloids, glycosides, saponins, resins, tannins, flavonoids, and reducing sugar.

2.4.1. Detection of alkaloids

(Hager's test)

In a few milliliters of diluted hydrochloric acid, methanol extract was dissolved, then filtered. To 2 ml of filtrate, a few drops of Hager's reagent (picric acid-saturated aqueous solution) were added. The test results are positive when there is a large yellow precipitate.

2.4.2. Detection of glycosides

(Kellar – Kiliani test)

Two ml of the filtrate were combined with one ml of glacial acetic acid, one ml of FeCl3, and one ml of H2SO4 (methanol extract). Glycosides are indicated by a green-blue hue.

2.4.3. Detection of saponins

(Foam test)

With vigorous shaking, five milliliters of distilled water were added to five milliliters of filtrate (aqueous extract). Saponins are an indication of a stable foam.

2.4.4. Detection of saponins

(Salkowski's Test)

Chloroform was used to treat the extracts before filtering. A few drops of strong sulfuric acid were added to the filtrates, which were then agitated and left to stand. The presence of steroids is indicated by a golden-yellow appearance.

2.4.5. Detection of resins

(Precipitate test)

15 ml of filtrate was combined with 20 ml of distilled water (methanol extract). A precipitate that shows resins are present.

2.4.6. Detection of resins

(Acetone-water Test)

Acetone was used to treat the extracts. Water was added in a small quantity, then shaken. Turbidity's appearance suggests the existence of resins.

2.4.7. Detection of tannins

(Braymer's test)

Two to three ml of (methanol extract) filtrate were added to a few drops of FeCl3 (10%) solution. The presence of tannins points to a dark blue or greenish-grey solution.

2.4.8. Detection of flavonoids

(Alkaline Reagent test)

A few drops of NaOH solution were added to a small amount of (methanol extract) filtrate, followed by the addition of a small amount of diluted HCl. Flavonoid presence implies a NaOH-yellow solution that becomes colorless when diluted HCl is added.

2.4.9. Detection of reducing sugar

(Benedict's Test)

In a test tube, 5ml of Benedict's solution was added to 5ml of the plant material's aqueous extract filtrate. It was then boiled for 5 minutes, after which time it was let to cool naturally. The presence of reducing sugar is confirmed by a cuprous oxide precipitate that is red in color.

2.5. Thrombolytic assay

The leaves in vitro clot lysis activity were tested using the Prasad et al. method[10] .'s with a few minor adjustments. The micro-centrifuged tubes underwent a 45-minute incubation period at 37°C. The serum was entirely withdrawn from the tubes after the clot had formed (without disturbing the clot), and each tube that had a clot was weighed again to calculate its weight (clot weight = weight of the tube containing the clot minus the weight of the tube alone).

A 100 l solution of various extracts (aqueous, ethyl acetate, and methanolic extract), with a concentration of 1 mg/mL, was applied in accordance with each micro-centrifuge tube containing a pre-weighed clot. The numbered control tubes received 100 l of Streptokinase as a positive control and 100 l of sterilized distilled water as a negative non-thrombolytic control, respectively. All of the tubes were then re-incubated for 90 minutes at 37°C, and clot lysis was checked. The collected fluid was taken from the tubes after incubation, and they were weighed once again to determine the weight difference after clot breakup. Finally, the weight difference was computed, and the result was reported as a percentage of clot lysis using the equation below.

% clot lysis= (Weight of the lysis clot, W5/ Weight of clot before lysis, W3)*100

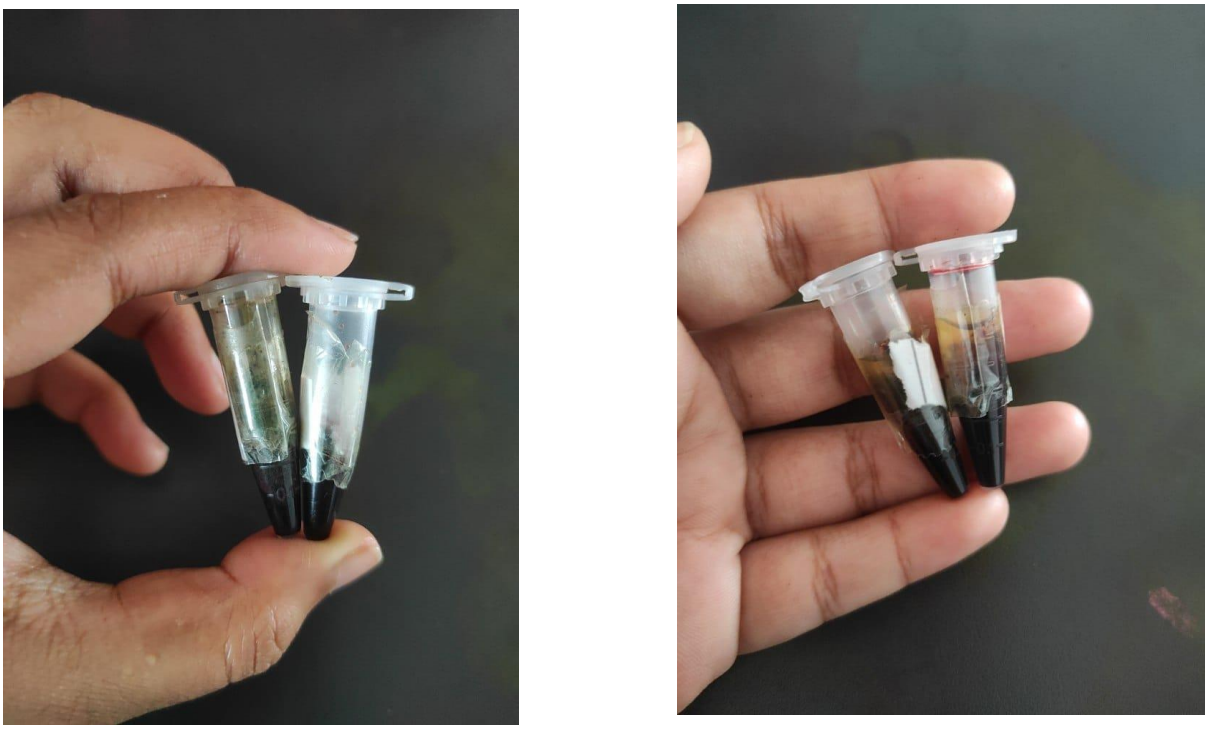

Fig 04 : Thrombolytic assay

Chapter Three:
Result and Discussion

3.1 Results of phytochemicals and pharmacological Evaluation of plant extract

3.1.1 Result of phytochemical screening

Phytochemical screening of the *Persicaria maculosa* extract showed the presence of alkaloids, glycosides, saponins, and flavonoids. Results of the preliminary phytochemical screening of *Persicaria maculosa* are presented in Table 2.

Table 2. Phytochemical analysis of *Persicaria maculosa* in Methanol.

	Extracts
Phytochemical	**Methanol**
Alkaloids	+
Glycosides	+
Saponins	+
Resins	-
Tannins	-
Flavonoids	+
Reducing Sugar	-

Key: (+) Present, (-) Absent

3.1.2 Result of thrombolytic Evaluation

In the event of in-vitro thrombolytic activity, adding 100 l Streptokinase (30,000 I.U.) to the clots and incubating for 90 minutes at 37° C resulted in 68.81% clot lysis. When clots were treated with 100 µl sterile distilled water (negative control), clot lysis was minimal (3.44%). The Methanolic extracts of *Persicaria maculosa* showed 12.33% clot lysis in an in-vitro thrombolytic activity investigation. The effective clot lysis % was calculated using a statistical model that included a negative control (sterile distilled water), a positive control (Streptokinase), and *Persicaria Maculosa* herbal medicines, which are shown in Table 3:

Table 3: Effect of *Persicaria maculosa* extract on in-vitro clot lysis.

Fraction	Weight of empty vial, W1	Weight of clot-containing vial before blood clot disruption, W2	Weight of clot-containing vial after clot disruption, W3	Weight of clot before clot disruption, W4	Weight of clot after clot disruption, W5	% of clot lysis
Blank	0.83	1.44	0.61	1.38	0.06	9.84%
Streptokinase	0.83	1.75	0.93	1.15	0.64	68.81%
***Persicaria maculosa* extract in Methanol**	0.84	1.57	0.73	1.66	0.09	12.33%

Chapter Four: Conclusion

4. Conclusion

A member of the family Polygonaceae, sometimes known as the buckwheat family, the annual plant known as *Persicaria maculosa* (also known as Polygonum persicaria). A number of names are used to refer to this plant, including lady's thumb, spotted lady's thumb, Jesusplant, and redshank. It is used in the treatment of conditions such as rhinitis, gout, headaches, the common cold, and venereal infections. Wounds and other skin injuries may be healed by applying it topically to the affected area, while traditional medicine recommends taking it to ward off stomach cancer. Following the collection of this plant from the National Botanical Garden of Bangladesh, a total of 500 grams of plant powder was extracted using 2500 milliliters of Methanol. A number of phytochemical tests were performed, the results of which are presented in table 2, and positive results were discovered for alkaloids, glycosides, saponins, and flavonoids. Finally, thrombolytic activities were able to be identified, as demonstrated in (table 3). The findings for the activation of thrombolytics are as follows: Blank 9.84%, Streptokinase 68.81%, and the *Persicaria maculosa* extract in Methanol reveals 12.33%.

Chapter Five: Reference

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