Anti-allergic, analgesic activity, and phytochemical screening test of leaf extract Diospyros sylvatica on swiss-albino mice

PROJECT WORK

This is a project report that has been submitted to the Pharmacy Department of Daffodil International University in order to fulfill the requirements for a Bachelor's degree in Pharmacy.

Submitted To

Department of Pharmacy

Faculty of Health & Life Sciences

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Submitted By

ID: 201-29-346

Batch: 23

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APPROVAL

The project paper "Anti-allergic, analgesic activity, and phytochemical screening test of leaf extract Diospyros sylvatica on swiss-albino mice" was turned in to the Daffodil International University Department of Pharmacy in the Faculty of Health and Life Sciences. It was found to be satisfactory in meeting some of the requirements for completing the Bachelor of Pharmacy degree and was given the go-ahead for its format and content.

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Department of Pharmacy

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Daffodil International University

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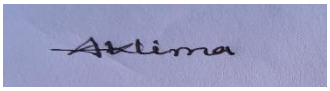
DECLARATION

I certify that the project work title is "Anti-allergic, analgesic activity, and phytochemical screening test of leaf extract Diospyros sylvatica on swiss-albino mice" to meet Daffodil International University Faculty of Health & Life Science's Bachelor of Pharmacy (B.Pharm) program requirements.

Supervised By



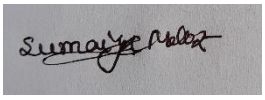
Aklima Akter

Assistant Professor

Department of Pharmacy

Daffodil International University

Submitted By



Sumaiya Meloz

ID: 201-29-346

Batch: 23rd

Department of Pharmacy

Daffodil International University

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I am grateful to Allah for granting me the opportunity to successfully complete my assignment and focus on this specific subject matter.

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We want to extend our profound gratitude to our cherished friends and other members of the Department of Pharmacy at Daffodil International University.

Ultimately, I express gratitude towards my parents and family members for consistently supporting and motivating my academic pursuits.

DEDICATION

I dedicate this work first to Allah Ta'ala and then to my family, especially my parents, teachers, and friends, who have always supported me.

ABSTRACT

Diospyros sylvatica, commonly referred to as Indian persimmon or wild ebony, has a rich history of traditional medicinal use owing to its diverse pharmacological properties.

Objective: This study aims to investigate the analgesic and anti-allergic potential of the methanol extract from Diospyros sylvatic leaves. A phytochemical screening was performed to identify the extract's bioactive components.

Materials and methods: The anti-allergic potential was assessed using a cow milk-induced allergic response model. The anti-allergic test was performed at Labtec Diagnostic Services under the supervision of Md Amirul Islam Amir, the lab in charge. To induce mouse allergies, Alcet (Levocetrizine Dihydrochloride 5mg tablet) was used as the standard, and cow's milk was used as the control group for Swiss albino mice. After seven days of experimental medicine and sample administration, mice were killed, and blood samples were taken. A blood sample of two to three millilitres was taken using the heart puncture procedure. The analgesic activity was investigated using the tail immersion method, which assesses the response to painful stimuli. The results showed that the time it took for the tail to be withdrawn in the tail immersion test increased in a manner that depended on the dose. This suggests that the extract from the Diospyros sylvatica plant may have pain-relieving benefits. Moreover, the extract exhibited a notable reduction in allergic reactions caused by cow milk, indicating its anti-allergic effects.

Results: The phytochemical examination of Diospyros sylvatica extract revealed flavonoids, alkaloids, carbohydrates, tannins, glycosides, saponins, and more. The control group had a serum IgE level of 97.03±3.03 IU/ml and an eosinophil count of 7±1.15 IU/ml. This implies that the allergy was observed in the animals of the control group when compared to the normal range. The IgE level and eosinophil count for the standard group of animals were measured to be 64.93±0.88 and 3.33±0.88, respectively. Respectively, this demonstrates the effectiveness of the treatments in lowering allergies, as the IgE levels and eosinophil counts of the control group animals are significantly higher than the standard. The serum IgE level and eosinophil count in the plant sample group were measured to be 77.60±5.44 IU/ml (400mg/kg) and 6±0.57 (400mg/kg) correspondingly. This finding is significant as it demonstrates that the plants have a beneficial effect in reducing animal allergies compared to the standard since we have obtained a standard value and a sample value that are very similar.

Diospyros sylvatica tail immersion test results and albino mice control group. The extract (100 and 200 mg/kg) reduced discomfort compared to the control group. The present extension showed checks at 30, 60, 120, and 180 minutes following extract loading in mice. Most elongations, up to 300%, occurred after 180 minutes. It performed 316.5% like the reference medication.

Conclusion: The study found anti-allergic and analgesic properties in Diospyros sylvatica methanol extracts.

Keywords: Sample (Diospyros Sylvatica), anti-allergic activity, analgesic activity, Levocetirizine, Cow’s Milk, phytochemical screening, Swiss albino mice

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List of Abbreviation	
LgE	Immunoglobulin
DPPH	2,2-Diphenyl-1-Picrylhydrazyl

Chapter-one
Introduction

1.1 General Introduction

Plants are the primary and indispensable constituent of the universe. Throughout history, humans have utilized plants as medicinal remedies. Plants were recognized as a significant reservoir of medicinal substances for therapeutic purposes through extensive observations and experimentation during the early phases of human civilization. [1]

Diospyros sylvatica, commonly known as the African ebony or Mozambique ebony, is a species of flowering tree belonging to the family Ebenaceae. D. sylvatica is highly esteemed for its dense and dark wood, as well as its decorative qualities. It plays a crucial role in the culture and economy of Sub-Saharan Africa, where it is naturally found. Diospyros sylvatica belongs to the genus Diospyros, which consists of more than 500 species of trees and shrubs found mainly in tropical and subtropical climates across the globe. D. sylvatica is distributed across many African countries, such as Mozambique, Tanzania, Zimbabwe, and Malawi. It is well-suited to a wide range of environments, from humid evergreen forests to arid woods. Diospyros melanoxylon, D. peregrina, D. sylvatica, and D. tomentosa are all plants that are used in Indian traditional medicine to treat a lot of different illnesses, like dysentery, diarrhea, cholera, and fevers that come and go. They are also used to treat bronchitis, carbuncles, coughs, cramps, pneumonia, syphilis, tumors, and more. [2]

People have used medicinal plants to treat illnesses for a long time because they contain ingredients that are good for you. Eighty percent of the people in the world still use them as traditional medicines. Chemicals made by plants help the body fight off many diseases, such as malaria, epilepsy, baby seizures, diarrhea, and infections caused by fungi and bacteria. Plants make many different kinds of bioactive molecules, which makes them a great place to get many different kinds of medicines. There are more than half of all modern drugs that come from natural products, and natural products are a big part of drug development efforts. In recent years, herbal drugs have become more popular because they work well and don't cost too much. [3]

Figure-1: Diospyros sylvatica (leaves)

1.2 Scientific classification of plant

Kingdom: Plantae

Phylum: Tracheophyta

Class: Equisetopsida C. Agardh

Order: Ericales Bercht.& J.Presl

Family: Ebenaceae

Genus: Diospyros

Species: Diospyros sylvatica Roxb**.**

1.3 Phytochemistry

The name comes from Greek "plant". Plants produce compounds, especially secondary metabolites, to defend against diseases, insects, pests, herbivores, UV radiation, and environmental dangers. [4] This is called phytochemistry. Phytochemistry considers these metabolites' structural makeup, biosynthetic processes, biological roles and effects, and industrial, commercial, and medical uses. Understanding phytochemicals is essential for developing new treatments for critical diseases.[5]

1.4 Part of the plant that was used in the study (Leaves)

The plant's leaves are simple, arranged in an alternating pattern, and positioned in two vertical rows. The petiole has a length of 0.3–1.2 cm and is smooth and flat on top. The lamina is 6–15 x 3-6 cm in size and has an elliptic to narrow elliptic shape. It has a pointed and twisted tip, a sharp or slightly tapered base, a curled edge when dry, and a smooth, drying black midrib. Tertiary nerves typically exhibit a reticulo-percurrent pattern, while secondary nerves are slender and appear in pairs. [6]

1.5 An Overview of Allergy

Allergies, or allergic disorders, are a range of conditions that occur when the immune system becomes overly sensitive to chemicals in the environment that are usually safe. The disorders encompassed in this category are hay fever, food allergies, atopic dermatitis, allergic asthma, and anaphylaxis. Possible symptoms encompass ocular redness, pruritic skin rash, sneezing, coughing, rhinorrhea, dyspnea, or edoema. It is important to understand that food intolerances and food poisoning are distinct disorders. [7] Typical allergies consist of pollen and specific types of food.Metals and other pollutants can potentially contribute to the occurrence of such issues. Severe reactions often occur as a result of food, bug bites, and drugs. Their development is influenced by

a combination of genetic and environmental factors. The fundamental process includes the binding of immunoglobulin E antibodies (IgE), which are a component of the body's immune system, to an allergen. This binding occurs on mast cells or basophils, leading to the activation of the release of inflammatory chemicals, such as histamine. The diagnosis is usually established by considering an individual's medical history. In specific instances, conducting additional examinations of the skin or blood could prove to be beneficial. Positive test results, however, may not always indicate a substantial allergy to the chemical being tested. [7, 8, 9]

Three Roman emperors from the Julio-Claudian dynasty, Augustus, Claudius, and Britannicus, are thought to have a background of atopy.The Viennese paediatrician Clemens von Pirquet first used the word "allergy" in 1906. He came up with it after noticing that people who had been injected with horse serum or the smallpox vaccine usually had stronger responses to second injections.The word "allergy" comes from the Ancient Greek words ἄλλος allos, which means "other," and ἦργον ergon, which means "work."[10, 11]

All kinds of sensitivities used to be called allergies, and they were all thought to be caused by the immune system not working right. Many years later, it became clear that different types of diseases were linked to immune systems that weren't working properly. Philip Gell and Robin Coombs came up with a new way to group things in 1963. [12, 13] They called the four types of hypersensitivity responses Type I through Type IV hypersensitivity. With this new grouping, the word "allergy," which was sometimes defined as a real allergy, was limited to type I hypersensitivities, which are also known as "immediate hypersensitivity" responses that happen very quickly and involve IgE antibodies. The finding of the antibody class called immunoglobulin E (IgE) was a big step forward in our understanding of how allergies work. In 1966 and 1967, IgE was found at the same time by two separate groups: Ishizaka's team at the Children's Asthma Research Institute and Hospital in Denver, USA and Gunnar Johansson and Hans Bennich in Uppsala, Sweden.In April 1969, they put out a paper together. [14]

Almost every system in the body is affected by allergies, which can show up in a lot of different ways and cause a lot of different symptoms. Research done in Europe has shown that up to 30% of people with allergic rhino conjunctivitis [7, 8] and 15% of people with allergic skin problems may also have asthma. That's the same number that was released for other parts of the world, like the USA and Australia. Food allergies are getting worse and more common. They can be made worse by medicines, work settings, and even insect stings that can be fatal. Contrary to what most people think, allergies are not harmless diseases. About 15% to 20% of allergy sufferers have a serious condition that makes them unable to do anything. They live in constant fear of having an asthma attack or anaphylactic shock that could kill them [9]. There is, however, a big difference in the number of people with allergies from one part of the EU to another, with a curve going from south to north and east to west [14,15]. It's scary that most allergy problems start in youth and get worse when a person is at their most productive. Up to 45% of Europeans between the ages of 20 and 40 have allergic rhinitis. The numbers may even be too low because a lot of people don't report their symptoms or get the wrong doctor's opinion. In fact, it is thought that at least 45% of people have never been diagnosed.[16]

Figure-2: Sign and symptoms of allergy

1.6 Basis in Phytochemistry

All pants create herbivore-resistance and photoprotective chemicals when salicylic acid corrosion begins. These phytochemicals are well-suited for use as medications, and their pharmacological properties and components in restorative plants support their medical use. Daffodils contain nine alkaloid components, including glutamine, and are approved for Alzheimer's treatment. Herbivores eat volatile plant stems called alkaloids. They stink and poison. Additionally, they can fight parasites. [17][18] A medicinal plant's therapeutically active or inert component molecule is identified using phytochemical screening. Thus, photochemical screening is increasingly important in physiology. The phytochemical screening process in this study includes a Diospyros sylvatica capacity bunch test. Flavonoids, alkaloids, tannins, gums, steroids, phenol, sugars, saponins, glycosides, etc. are useful. This material occurs mostly in plant stems, leaves, bark, and flowers. New technology separates these blends. It involves separating, screening, and publicizing medicinal plant-based compounds. Plant bioactives include flavonoids, alkaloids, steroids, and phytosterols. This is interesting because it can lead to the identification of beneficial experts and the discovery of new financial resources like oils, gums, tannins, and precursors for combining complex compounds. Understanding plant material components helps estimate value. [19]

1.7 Analgesic activity

Analgesics are drugs that relieve pain. This group of drugs is used to put someone to sleep. These drugs are also called painkillers or analgesics. Analgesics work on the central nervous system and the nerves around it in different ways. Usually, prostaglandins are caused by pain. Analgesics stop the production of prostaglandins and also block processes in the brain and the body. Cyclooxygenase is a special enzyme that makes prostaglandin. Pain killers stop cyclooxygenase from being made and ease pain. [20]

CHAPTER-TWO

THE AIM OF THE STUDY

2. Objective of the study

Almost every system in the body is affected by allergies, which can show up in many different ways and cause many other symptoms. Research done in Europe has shown that up to 30% of people with allergic rhinoconjunctivitis and 15% of people with allergic skin problems also have asthma. [7,8]

Therefore, The objective of this study is to analyze the phytochemical composition and potential biological effects of the methanol extract derived from Diospyros sylvatic Leaf. Here is a detailed outline of the investigation's specific objectives:

Chemical components analysis: The study aims to analyze the phytochemical extract of Diospyros sylvatica leaf methanolic extract phytochemically. This study will evaluate many phytochemical activities in plants, including the presence or absence of alkaloids, glycosides, flavonoids, saponin, reducing sugar, and more.

Pharmacological activity assessment: Another objective of this project is to investigate the scientific foundations of the entire plant's thrombolytic and antioxidant capabilities as prospective medical applications. The study tests the extract's efficacy in several biological systems using bioassays.

Discovery of Potential New Medicines: Finding bioactive molecules that have important pharmacological effects could lead to the creation of new drug candidates or natural product-based medicines. This part of the study is very important for finding new ways to do pharmaceutical research and development and making more drugs available.

CHAPTER-THREE
LITERATURE REVIEW

3. Literature Review

I conducted this literature review using PubMed, Google Scholar, and other credible journals. Many studies employing the plants I chose have been published, but they're unconnected to my research.

- **Title: Investigation of Indian Diospyros Species for Antiplasmodial Properties.**

T Malaria is still a deadly disease, even after decades of intensive research. We desperately need new antimalarials because Plasmodium falciparum is becoming increasingly resistant to existing drugs. This article talks about testing four types of Indian Diospyros plants—Diospyros melanoxylon, D. peregrina, D. sylvatica, and D. tomentosa—to see if they can kill chloroquine-sensitive (3D7) and chloroquine-resistant (K1) strains of Plasmodium falciparum. Six of the eight methanolic extracts showed potent activity against strain 3D7 (IC50 = 16.5–92.9 μg ml−1), and five of these also showed activity against strain K1 (IC50 = 20.5–121.6 μg ml−1). Diospyros sylvatica was the most active (IC50 = 16.5–29.4 μg ml−1) and should be investigated further. [21]

- **Antitermitic quinones from Diospyros sylvatica.**

A total of six quinones were extracted from the roots of Diospyros sylvatica using chloroform. These quinones were identified as 2-methyl-anthraquinone, plumbagin, diosindigo, diospyrin, isodiospyrin, and microphyllone. An experiment was conducted to assess the impact of the root extract on the alignment and viability of the subterranean termite, Odontotermes obesus. Furthermore, the survival of the subterranean termite was assessed by testing four of these quinones. During a direct-choice experiment, the presence of an extract-treated filter disc resulted in a noticeably stronger repellant effect compared to the solvent-treated filter disc. The no-choice experiment demonstrated the hazardous nature of the extract, as well as the tested quinones. It also exhibited a significant mortality rate among the O. obesus workers after 48 hours of forced exposure. The primary components shown to be effective in killing termites were plumbagin, isodiospyrin, and microphyllone. However, diospyrin did not exhibit toxicity towards termites at the measured dose. D. sylvatica is the first known source of all the quinones. [22]

- **Green synthesis, characterization and antimicrobial activity of silver nanoparticles using methanolic root extracts of Diospyros sylvatica**

The objective of the present investigation is to produce the initial biosynthesized silver nanoparticles using silver acetate and methanolic root extracts of the Ebenaceae plant, Diospyros sylvatica. The TEM investigation revealed that the average diameter of Ag NPs is around 8 nm, which closely matches the typical crystallite size of 10 nm measured by X-ray diffraction (XRD) research. In addition, the inquiry has been broadened to encompass the antibacterial effectiveness against pathogenic strains of bacteria and fungus, as well as Gram-positive and Gram-negative bacteria. The bioinspired Ag-NP demonstrated significant effectiveness against all tested bacterial strains, with the level of activity increasing as the dosage levels increased. [23]

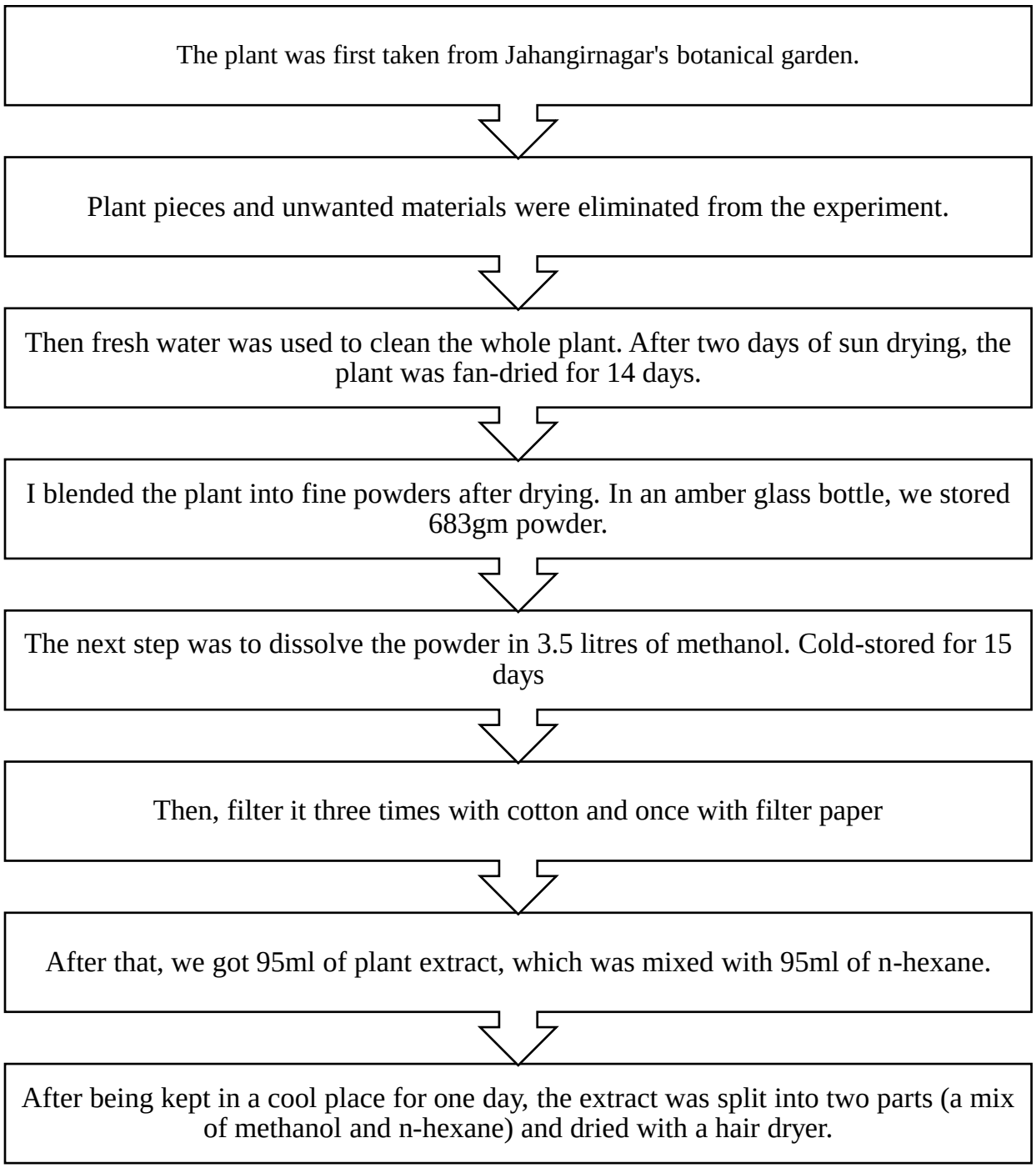
CHAPTER-FOUR
MATERIALS AND METHODS

4.1 Extraction Preparation

4.1.1 Plant Collection

The D. sylvatica plant used in the experiment was taken from the botanical area of Jahangirnagar University Savar in Dhaka, Bangladesh, in August 2023. The plant has medium-sized green leaves.

4.1.2 Prepare plant material



4.1.3 Extraction

A bottle jar with a glass was cleaned very well, and then it was rinsed with methanol. After adding 3.5L of methanol to the jar with 683g of dry materials, the sample was covered up to an inch above the top. The jar was properly sealed with a plastic lid wrapped in metal foil to keep air out. It looked like the surgery took 15 days. The jar was shaken approximately two to three times daily to increase extraction.

4.1.4 Filtration

The extracted plant extract was filtered using sterile cotton and filter paper. Cotton and paper were funneled after rinsing with the solvent. The collection filtrate was in a glass container revisited refiltration. After the plant extract was made, it was filtered through clean cotton and filter paper. The cotton and paper were rinsed with the suitable liquid and then put in a funnel. What had been filtered was put in a glass jar, and the process of refiltration was repeated.

Figure-3: Filtration process

4.1.5 Evaporation

A rotating evaporator evaporated the fluid concentrate, which was 64.7 degrees Celsius in temperature. The thick piece was in a 100 ml container. Once the pure concentration was gathered, the aluminum paper was put over the measuring glasses, and they were put right under the fan. I used a hair dryer to get rid of some of the water that was still in the concentrate so that the dissolvable would go away.

Figure-4: Rotary process

4.1.6 Different type of test

To determine the composition of synthetic compounds in Diospyros sylvatica plant and their qualities, I conducted various tests:

- ✓ Phytochemical Screening
- ✓ Anti-Allergic Test
- ✓ Analgesic Test

4.2 Phytochemical Screening

4.2.1 Test Materials

- Extract of the whole plant *(Diospyros sylvatica)*

4.2.2 Equipment's list for Phytochemical Screening

Serial No.	Name
1	Electronic Balance
2	Vortex Mixture
3	Test Tube
4	Test Tube Rack
5	Water Bath

Table 1: Equipments of Phyto-Chemical Screening Test

4.2.3 Reagent's list for Phytochemical Test

Serial No.	Name
1	Mayer's reagent
2	Benedict's reagent
3	Dragendorff's reagent
4	Dilute sulfuric acid
5	DPPH solution
6	10% of potassium dichromate solution
7	Aqueous sodium hydroxide
8	5% of Ferric chloride solution
9	Distilled water
10	Conc. H2SO4
11	Conc. HCL

Table 2: Equipment of Pyto-Chemical Screening Test

4.2.4 Apparatus's list for Phyto-chemical Test

Serial No.	Name
1	Beaker (100ml & 50ml)
2	Funnel
3	Filter paper
4	Glass rod
5	Stand with clamp
6	Cotton
7	Measuring Cylinder
8	Test-tube
9	Spatula
10	Syringe
11	Measuring cylinder
12	Amber glass bottle (2500 ml)
13	Pipette
14	Micropipette
15	Amber reagent bottle
16	Light-proof box

Table 3: Apparatus list for Phyto-chemical Test

4.2.5 Test for Alkaloids

Mayer's test: Two milliliters of extract solution and diluted hydrochloric acid were added to a test tube. Next, a milliliter of Mayer's potassium mercuric iodide reagent was added. Yellow precipitate implies alkaloids.

Dragendroff's Test: Two milliliters of extract solution and diluted hydrochloric acid were added to a test tube. Next, a milliliter of Dragendroff's reagent, Potassium Bismuth Iodide Solution, was added. An orange-brown precipitate implies alkaloids.

Wagner's Test: 2 ml extract solution and 1 ml Wagner's reagent (potassium iodide). Brown or red precipitation contains alkaloids.

4.2.6 Test for Carbohydrates

2 ml concentrated H2SO4, 2-3 drops Molisch's reagent, 2 ml extract. Red or purple rings signified carbs.

4.2.7 Test for Reducing Sugar

Benedict's test

Heat Benedict's reagent and one milliliter of the analyte sample in a boiling water bath for three to five minutes. A brick-red cuprous oxide precipitate confirms the reducing sugars in the analyte.

Fehling's test (Standard test)

Equal quantities of Fehling's solutions A and B were combined with two milliliters of plant material aqueous extract and one milliliter each. Briefly boiled. Reduced sugar formed a crimson or brick-red precipitate

4.2.8 Test for Flavonoids

One milliliter of crude extract was combined with a few drops of strong hydrochloric acid. Flavonoids provide instant red color.

4.2.9 Test for Glycosides

A few drops of liquid NaOH were added to a test tube containing a small amount of alcoholic extract and 1 mL of water. The yellow color shows that glycosides are present.

4.2.10 Test for Tanin

A few drops of a light yellow 10% ferric chloride solution were mixed with 2 mL of the extract's water-based solution. A greenish-black sediment showed that tannin was present.

4.2.11 Test for Diterpenes

The extract was mixed with water and put in ten drops of copper acetate solution. An emerald green color formed, which showed that diterpenes were present.

4.2.12 Test for Gums

Some extract (5 ml) was mixed with Molisch reagent and sulfuric acid. A reddish-violet ring forms when two liquids mix, which means gum is present.

4.2.13 Test for Phytosterol

2 ml of extract, 1 ml chloroform, and concentrated H2SO4 were added to a test tube until it. Golden red or golden yellow appearance.

4.2.14 Test for Steroid

100mg extract was added to 1 ml acetic acid and 1 ml conc. H2SO4 caused Violet to blue-green metamorphosis, which involved steroids.

4.2.15 Test for Saponin

Twenty milliliters of distilled water diluted with one milliliter of extract solution. Next, shake it hard for 15 minutes. Saponin will form translucent foam at this time.

4.2.16 Test for Phenol

One milliliter of the extract was combined with two milliliters of 5% neutral ferric chloride; the dark blue hue indicated phenolic chemicals.

4.3 Test for Anti-Allergic Activity (Cow Milk Induced Allergy Method) and Analgesic activity test

4.3.1 Choice of Drugs for anti-allergic and analgesic activity test

The trials used Healthcare Pharmaceutical Ltd., Dhaka's Alcet (Levocetirizine Dihydrochloride 5mg tablet), purchased from Super Shop of Daffodil Smart City, Bangladesh. This investigation used reagent-grade cow's milk. Experiments used analytical-grade chemicals.

The research used diclofenac, specifically diclofenac sodium 100 mg/kg tablet, manufactured by ARISTOPHARMA LTD. in Dhaka, Bangladesh. The Super Shop is the supplier of Daffodil Smart City in Bangladesh. This experiment used acetic acid of reagent grade. The research used chemicals of analytical grade.

4.3.2 Preparation of Samples

To make the suspension, one dose of Diospyros sylvatica extract (400 mg/kg) was mixed with distilled water.

4.2.3 Experimental Methodology

Swiss albino mice from Jahangirnagar University Lab were used for this experiment. The average mouse weight was 38-50g. Before the experiment, the mice spent five days in colony cages in the department's animal room, which is kept at 25–30 degrees Celsius. The bedding was changed every

day for hygiene. After seven days, controlled group rats were given 0.5ml/kg body weight of raw cow's milk to elicit allergy. The mice were divided into three groups of five rats for the investigation. Three groups were-

- Control Group
- Group-2 (Standard drug)
- Group-1 (Sample)

4.3.4 Medication and Sample

Group	Medication & Sample	Time Duration
Control	Cow's Milk	7 Days
Standard	Levocetirizine Dihydrochloride 5mg tablet	
Sample	One dose of sample 400mg/kg	

Table 4: Medication and sample combination performed throughout the study

4.3.5 Hematological Sampling

After seven days of experimental medicine and sample administration, mice were killed, and blood samples were taken. Two to three milliliters of blood were taken using cardiac puncture. After centrifuging the blood, serum was collected and kept for future research.

4.3.6 Biological test

Two biochemical tests were conducted as part of the research. They are:

- IgE Antibody
- Eosinophil

Labtec Diagnostic Services evaluates every test under the guidance of Md Amirul Islam Amir Sir, the Lab in Charge.

4.3.7 Analgesic activity in vivo

Analgesic activity can be assessed in many ways in pharmacological research. We used the "Tail immersion technique" to measure analgesia.

4.3.8 Experimental animals

The average mouse weight was 25–30 grams. Mice maintained their typical habitat. The circumstances were a 12-hour light/dark cycle, 25±2°C temperature, and 55-65% relative humidity.

4.3.9 Tail Immersion Test

The process entails immersing the tail in water that has been heated to a specified temperature. The presence of analgesic activity in the sample leads to an increase in the frequency of tail flicking. The screening and test had respective cutoff times of thirty-five seconds. The test animals were categorized into three groups, including three mice. The animals were administered medication 30 minutes before their distal tail (2-3 cm) was submerged in a water bath at 55 ± 0.5°C. The animals were administered diclofenac Na (10.0 mg/kg) as a recognized benchmark. The duration of tail flicking from the water was measured before, during, and after a medicine delivery lasting 30, 60, 120, and 180 minutes.

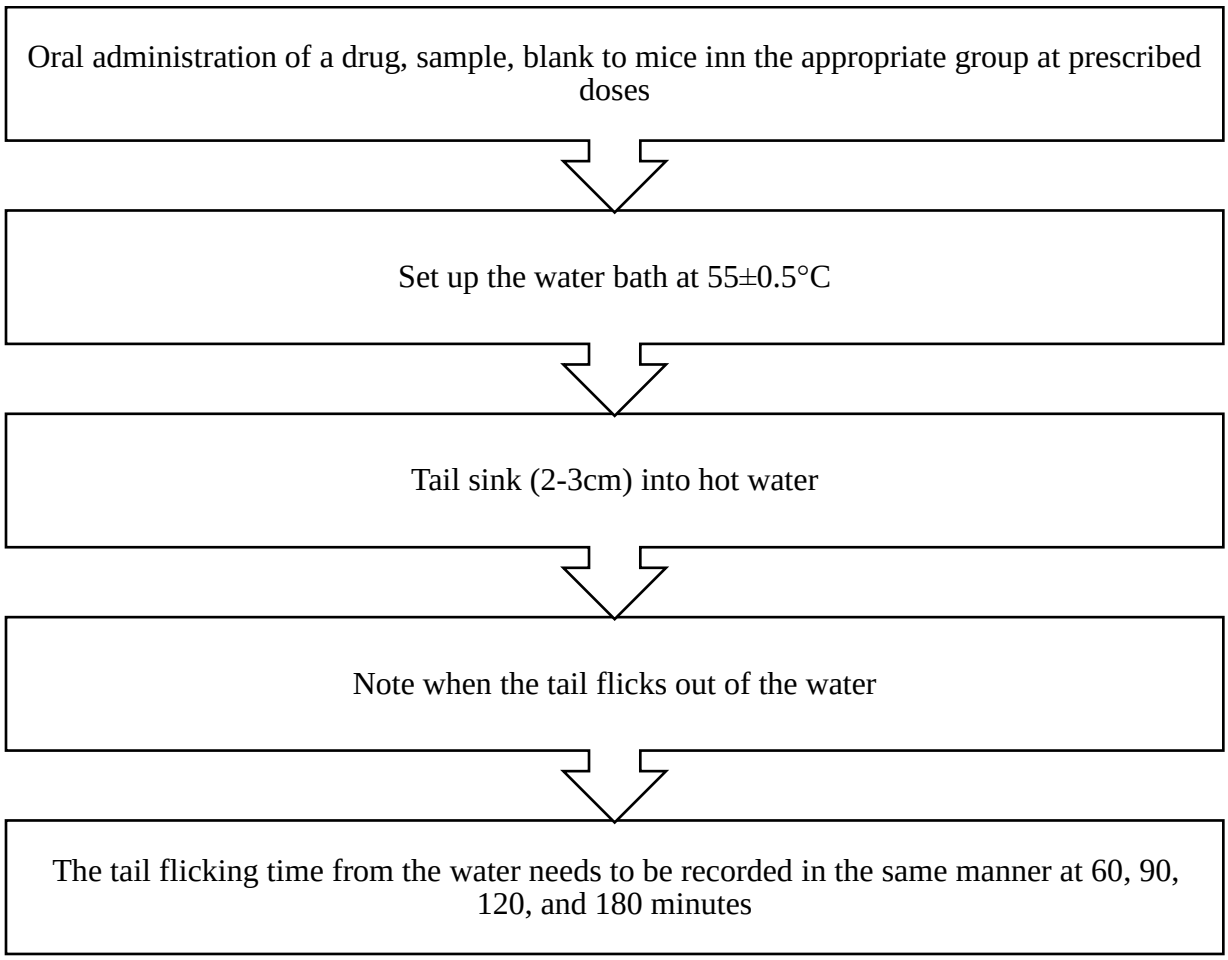
Figure 5: Tail immersion

4.3.10 Equipment and apparatus for analgesic test

Serial No.	Apparatus
1	Heating mantle
2	Disposable syringe
3	Test tube
4	Stop watch
5	Mice feeding needle
6	Thermometer
7	Vortex mixture
8	Glass rod

Table 5: Equipment and apparatus for analgesic test

4.3.11 Tail Immersion Method



CHAPTER-FIVE

RESULTS AND DISCUSSIONS

5.1 Phytochemical Test

Serial No.	Test Name	Result
1	Alkaloid (Mayer's & Draggendroff's test)	+
2	Glycoside	+
3	Tannin	+
4	Saponin	•
5	Steroid	+
6	Phenol	+
7	Carbohydrate	+
8	Diterpene	+
9	Gum	•
10	Phytosterol	+
11	Reducing Sugar (Benedict & Fehling Test)	+
12	Flavonoid	+

(+) indicates Positive & (-) indicates Negative

Table-6: Result of Phytochemical Test

Discussion

Two chemicals were absent from Diospyros sylvatica, while the rest were discovered and analyzed in the lab. Flavonoids and glycosides are the parts that cause allergy reactions. Phytosterol, steroids, and phenol also cause anti-allergic effects.

5.2 Anti-Allergic Test

5.2.1 Effects of Cow's Milk, Standard drug and Sample on Serum on IgE Antibody

Group Name	Compounds	Serum IgE (IU/ml)	Normal Range (IU/ml)
Control	Cow's milk	97.03 ± 3.03	5-10
Standard	Levoeetrizine Dihydrochloride 5mg tablet	64.93 ± 0.88	
Sample	400 mg/kg	77.60 ± 5.44	

Table-7: Effects of Cow's Milk, Standard drug and Sample on Serum on IgE Antibody

Discussion

Based on the provided table, it can be observed that the serum IgE level for the control group was 97.03±3.03 IU/ml. This implies that the allergy was observed in the animals of the control group when compared to the normal range. However, the IgE level in the standard group of animals was 64.97±0.88 IU/ml, indicating that they effectively reduced allergies compared to the control group, which had much higher IgE levels. The plant sample group exhibited a serum IgE level of 77.60±5.44 IU/ml (400mg/kg), indicating a remarkable finding demonstrating plants' ability to mitigate animal allergies. This is a great finding that shows plants can lower animal allergies compared to the standard, since the sample value and standard value are so close.

5.2.2 Effects of Cow's Milk, Standard drug and Sample on Eosinophil

Group Name	Compounds	Eosinophil (Mean+SEM)	Normal Range (%)
Control	Cow's milk	7 ± 1.15	~2
Standard	Levoeetrizine Dihydrochloride 5mg tablet	3.33 ± 0.88	
Sample	400 mg/kg	6 ± 0.57	

Table-08: Effects of Cow's Milk, Standard drug and Sample on Eosinophil

Discussion

From the above table, we see that the control group has an eosinophil count of 7±1.15, which means that the animals in this group were also subjected to an allergen-induction process. In contrast, the eosinophil count in the standard group was only 3.33±0.88 which demonstrating that these animals are excellent in alleviating allergy conditions. The eosinophil count in the plant sample group was 6±0.57 (400mg/kg). That means plants shows moderate anti-allergic activity when compared with standard.

5.2.3 Results of in vivo analgesic activity

Group Name	Compounds	30 min	60 min	120 min	180 min
Control	Saline	1.33 ± 0.33	1.66 ± 0.33	2 ± 0.57	2 ± 1
Standard	Diclofenac sodium	4.33 ± 0.33 (225.5%)	5.66 ± 0.33 (240.9%)	6.33 ± 0.66 (216.5%)	8.33 ± 0.33 (316.5%)
Sample	100 mg/kg	2.67 ± 0.58 (100.7%)	3.66 ± 0.66 (116.8%)	4.66 ± 0.33 (133%)	6.60 ± 0.33 (233%)
	200 mg/kg	3.33 ± 0.88 (200%)	4.33 ± 0.66 (160.8%)	6.33 ± 0.33 (216.5%)	8 ± 0.57 (300%)

Table-09: Results of in vivo analgesic activity

Discussion

Table 8 shows the results of the tail exposure test for Diospyros sylvatica and control group of albino mice. Compared to the control group, the extract (100 and 200 mg/kg) made the painful feelings much less intense. There were cheaks on the present extension at 30, 60, 120 and 180 minutes after the extract was loaded into mice. After 180 minutes, the extract had the most elongations, up to 300%. This was the same as the reference drug's performance (316.5%).

CHAPTER-SIX
CONCLUSION

7. Conclusion

Natural products are becoming essential in medication discovery. *Diospyros sylvatica* is vital for finding and using natural medications.

The study found anti-allergic and analgesic properties in *Diospyros sylvatica* methanol extracts.

Many studies have tried to prove these medicinal plants, but phytochemical analysis showed flavonoids, alkaloids, carbohydrates, glycosides, steroids, phenol, tannin, diterpenes, gum, phytosterol, reducing sugar, and no saponin or flavonoids. This test also showed anti-allergic properties.

CHAPTER-SEVEN
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