A dissertation submitted to Daffodil International University, Department of Pharmacy, Faculty of Allied Health Sciences, partially satisfies the requirements for a bachelor's degree in pharmacy.

PROJECT WORK

Evaluation of Phytochemical Screening and Investigation of Analgesic, CNS, Thrombolytic, and Antidiarrheal Activities of Methanolic Extracts from Xantolis Assamica Flowers in Experimental Mice.

SUBMITTED TO

Department of Pharmacy,
Faculty of Allied Health Sciences
Daffodil International University

SUBMITTED BY

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Date of Submission:
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APPROVAL

A project paper investigating the medicinal properties of the flower of Xantolis Assamica has been submitted and accepted by the Department of Pharmacy at Daffodil International University for partial fulfillment of a Bachelor of Pharmacy degree.

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DECLARATION

I'm declaring that this project work **"Evaluation of Phytochemical Screening and Investigation of Analgesic, CNS, Thrombolytic, and Antidiarrheal Activities of Methanolic Extracts from Xantolis Assamica Flowers in Experimental Mice."** submitted to the Daffodil International University, is done by me under the supervision of **Md. Mizanur Rahman, Assistant Professor, Department of Pharmacy**, Daffodil International University. I'm also declaring that the investigations that are figured in this project are original and have not been submitted anywhere for any degree of bachelor or others.

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- Charity Sutar

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Dedication

Dedicated to my beloved Parents & Teachers.

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ABSTRACT

The main object of evaluation of phytochemical Screening and Investigation of Analgesic, CNS, Thrombolytic, and Antidiarrheal Activities of Methanolic Extracts from Xantolis Assamica Flowers in Experimental Mice. Phytochemical screening such as alkaloids, glycosides, steroids, flavonoids, tannins, saponins, and gum tests the therapeutic value determination of the plant is done and the presence of alkaloids, glycosides, saponins, flavonoids, tannins is found in this plant. An acetic acid-induced writhing test was used to measure analgesic activity. CNS activities was evaluated by using hole board, hole cross and open field test. Thrombolytic activity of plant extract was done with the help of human blood. Anti diarrheal test was done by castor oil induced. The acetic acid-induced writhing test was used to examine the analgesic impact of the methanolic extract of Xantolis Assamica flowers. The writhing caused by acetic acid was significantly ($P<0.05$) reduced at doses of 250 mg/kg and 500 mg/kg. Using hole cross, hole board, and open field tests, the CNS depressant exertion was investigated by monitoring the decrease in locomotor and exploratory activities at doses of 250 mg/kg and 500 mg/kg body weight. The thrombolytic activities from flower extract of Xantolis Assamica was observed and the clot lysis effect was remarkable compared to control and blank. Overall, the study suggested that the methanolic extract of Xantolis Assamica flowers has remarkable analgesia, CNS depressant, thrombolytic and moderate effect against antidiarrheal effects.

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CHAPTER ONE INTRODUCTION

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1.1 General Introduction

Plants have a significant impact on global health and are a valuable source of medicine. It is well recognized that medicinal plants and herbs are a significant potential source of therapeutic or healing help. In health systems around the world, the utilization of therapeutic plants has become increasingly prevalent [1]. In medicine, plants are used to cure certain diseases and disorders as well as to preserve and improve physical, mental, and spiritual health. Many plants have been utilized in traditional Chinese, Indian, and African medical practices for the past 3,000 years the majority of these plants have medicinal benefit that is supported by Western standards [2]. The study of medicinal plants has attracted more attention recently as scientists try to use cutting-edge biotechnological techniques, like plant tissue culture and herbal biotechnology, to maximize their therapeutic potential. It is impossible to overestimate the significance of medicinal plants in the creation of medicines and herbal cures, as well as their role as a sustainable source of bioactive substances, which makes them a priceless and long-lasting resource in the field of healthcare [3]. Many ethnic groups employ a variety of plant species to treat a wide range of illnesses, from minor infections to skin conditions, asthma, malaria, and a host of other conditions. Based on their ethnomedical applications, we evaluated the potential of plants as sources of chemotherapeutic medicines and antibacterial activity [4]. Since herbal medicines are accessible and reasonably priced, the World Health Organization promotes and supports their use in public health campaigns. largely plant-based and utilized as a combination, oil, powder, decoction, extract, etc. Active chemical components discovered in crude extract or paste of natural products currently in use can now be isolated and identified thanks to advancements in separation, isolation, and characterization procedures and a rise in science-based knowledge [5].

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1.2 Phytochemistry

The study of phytochemicals, or compounds made from plants, is known as phytochemistry. The structure of the many secondary metabolites that are present in plants, their roles in plant and human biology, and their manufacture are all described by phytochemists. Among the many other compounds found in medical plants, the following are biosynthetic: alkaloids, flavonoids, tannins, phenols, glycosides, sugars, steroids, and saponins. Plants create bioactive substances called phytochemicals to defend themselves. Phytochemicals have antibacterial, antidiarrheal, anthelmintic, antiallergic, antispasmodic, and antiviral properties in addition to their potent antioxidant properties [6].

1.3 Medicinal of Plants

Since ancient times, medicinal plants have been utilized in both traditional and contemporary medical techniques. For a variety of purposes, such as protection and safety against insects, fungi, illnesses, etc., plants produce hundreds of different chemical components. Anything that can be utilized as a therapeutic object is considered a medicinal plant. When a plant is considered medicinal, it indicates that it has therapeutic potential, has been identified as the active ingredient in a medical treatment, or both. In both traditional and modern medicine, medicinal plants are used to treat specific conditions, preserve health, or both. Botanical medicine, often known as natural product medicine, is medicine made from natural materials that have therapeutic or health-promoting qualities [7].

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1.4 Importance of Medicinal Plants

Medicinal plants are a valuable gift of nature that have been used since ancient times to treat various ailments. The amount of research on using herbal remedies to treat ailments is increasing every day. Due to the presence of natural compounds, medicinal plants are the primary source of molecules with therapeutic properties. The evolution of human civilization has been significantly influenced by medicinal plants. New medications can be made from medicinal plants, and many contemporary medications are made indirectly from plants. Over 250,000 species of flowering plants are thought to exist. Understanding plant toxicity and safeguarding people and animals from natural toxins are two benefits of studying medicinal plants [8].

1.5 Herbal Medicine

Herbal products, botanical products, or phytomedicines are plant-based items used to treat illnesses or preserve health. The active compounds in herbal medications are derived from plant parts like leaves, roots, or flowers. The use of medicinal plants to prevent and treat illnesses is known as herbal medicine. This includes both standardized and processed herbal extracts as well as traditional and widely used medications in each nation. About 80% of the world's population, particularly millions of individuals living in large rural areas of poor nations, have their health needs met by herbal medicines, according to WHO confirmation [9].

1.6 Phytochemical Screening

Because of its possible pharmacological properties, which include antineoplastic, antimicrobial, antioxidant, anti-inflammatory, analgesic, CNS-depressant, anti-diabetic, anti-hypertensive, antidiarrheal, and other effects, medicinal plants have been utilized to treat a variety of illnesses.

A medicinal plant's therapeutic value is determined by its phytoconstituents, either separately or in

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combination. Steroids, glycosides, terpenes, gums, alkaloids, flavonoids, phenolics, tannins, and saponins, among others. By identifying the phytochemicals in a plant, one can anticipate its pharmacological activity. Although phytochemicals are now identified using a variety of contemporary methods, traditional qualitative assays are still widely used for plants' initial phytochemical screening. The phytochemicals found in medicinal plants are the source of medications used to treat a variety of illnesses and are also essential for the discovery of novel drugs [10].

1.6.1 Alkaloids

Alkaloids are primarily found in plants and are especially common in several families of flowering plants. Alkaloids are any of a class of naturally organic nitrogen-containing bases. Alkaloids have diverse and important physiological effects on humans and other animals disease. Numerous alkaloids have potent pharmacologic effects. The alkaloids include chemicals such as cocaine, nicotine, strychnine, caffeine, pilocarpine, morphine, atropine, ephedrine, and mescaline. High-performance thin-layer chromatography (HPTLC) and related techniques may be used for the efficient quantitative analysis of alkaloids [11].

1.6.2 Glycosides

Glycosides are naturally occurring compounds that include one or more sugars or uranic acid as part of their carbohydrate component. In living things, glycosides have significant quantitative functions. Passive glycosides are a common form of chemical storage in plants. These can be made active by hydrolyzing enzymes. Plants produce a lot of glycosides, frequently during flowering [12].

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1.6.3 Tannins

The complex chemical substance known as tannin is derived from tannin acids. Tannin is also known as tannic acid. Tannins are classified chemically into two primary groups condensed and hydrolyzable. Hydrolyzable tannins. Hydrolyzable tannins (decomposable in water, with which they form other substances) produce different water-soluble products like gallic acid and protocatechuic acid and sugars. They are commonly found in the roots, wood, bark, flower, and fruits of many plants [13].

1.6.4 Saponins

There are about 2600 species in the family Caryophyllaceae, and they are found on every continent. They mostly serve as decorative plants and agricultural weeds. Certain species have long been employed as emulsifiers in food preparation or in herbal medicine. The high concentration of triterpenoid saponins serves as the foundation for these uses. Ribosome-inactivating proteins (RIPs), which have the potential to be extremely hazardous, are also typical of this family. The most common was the olean skeleton, which is present in most important plants around the world. There are bitter saponins. In reality, they are divided into three categories of saponins: furostanol, spirostanol, and triterpenoid [14].

1.6.5 Gums

Carbohydrates, along with proteins and fats, are one of the three macronutrients in the human health. Atoms of carbon, hydrogen, and oxygen make up these molecules. In the human body, carbohydrates provide an effective function. They provide energy, aid in the regulation of insulin and blood glucose metabolism, take part in the metabolism of triglycerides and cholesterol, and support fermentation [15]. Glue is a chemical modification that can create gels that stabilize foams

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and emulsions and increase the viscosity of liquids. Plant gums are complex substances made from carbohydrates. These are often calcium, magnesium, and potassium salts of acidic polysaccharides. Gums are linked to modified polysaccharides and are soluble in water. Plant gums are naturally produced from extracts of several plant parts, including bark, seeds, leaves, and flowers [16].

1.6.6 Phenols

Phenols In organic chemistry, phenols called phenolics, are a class element conforming of one or further hydroxyl groups (− O H) clicked directly to a sweet hydrocarbon. The phenol C6H5OH. Phenolic composites are classified as simple phenols or polyphenols based on the number of phenol units in the patch. Phenol is both industrially synthesized and produced by organic [17].

1.6.6 Flavonoids

Plants contain a class of natural compounds called flavonoids, which act as signaling molecules, UV protectors, pollinator-attracting colorants, and antimicrobials. They are a vital component of the mortal diet and are potent antioxidants with a number of health-promoting properties that help avoid chronic illnesses including diabetes, obesity, and cancer. Flavonoids have gained widespread attention as potential therapeutic targets as a result of these products. As natural food coloring and ingredients and nutraceuticals, flavonoids are generally significant from a commercial standpoint.17]

1.6.7 Steroids

The functional groups affixed to these rings and the rings' oxidation state distinguish different steroids. Hundreds of different steroids are found in fungi, animals, and plants. Numerous further have been produced as synthetic medicines. Three steroid classes are set up in the mortal body, androgens and estrogens.[17]

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1.7 Analgesic Activity

Acetic Acid-Induced Writhing Test: This chemical technique involves injecting mice with irritants such as acetic acid and phenylquinone to cause discomfort of peripheral origin. The reduction in writing frequency suggests that the test substance has analgesic properties [18].

1.8 CNS-Depressant Activity

Pentobarbital sodium-induced sleep duration extension in rats was used to test the CNS depressing effect. Examine the potential mechanism of this effort by assessing its effects on monoamine neurotransmitters in the brain, such as serotonin and dopamine. Ethanol extracts of leaves, flowers, and seeds maintain their potent CNS depressive action, according to gross behavioral investigations. It has been demonstrated that leaves, flowers, seeds, and dinghy (600 mg/kg) significantly and cure-dependently extend the onset and duration of sleep, which lowers dopamine and raises serotonin levels. This suggests that the ethanol extracts of seeds, leaves, and flowers may have a CNS depressive effect because of a decrease in dopamine and an increase in serotonin [19].

CNS-Depressant Behavioral Pattern Test:

1. **Open Field Test.**
2. **Hole Board Test &**
3. **Hole Cross Test.**

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1.9 Antidiarrheal (Castor Oil-Induced)

Gastrointestinal illnesses brought on by various bacteria, viruses, and parasites are the most frequent causes of diarrhea. Food, drinking water, and unhygienic surroundings can all spread this virus. The pathophysiology of electrolyte and water transport, in addition to other pathological conditions, is typically caused by four main mechanisms: decreased electrolyte absorption, increased luminal osmolarity and electrolyte secretion, and accelerated intestinal motility that reduces transit time.

The prevalence of diarrhea remains high in spite of international organizations' attempts to control the disease. Although some antibiotics are used as antidiarrheal medications, they can occasionally have negative side effects and cause germs to become resistant to them. Thus, the pursuit of safer and more potent drugs derived from plants remains a crucial field of active research.[20]

1.10 Thrombolytic Activity

One type of blood clot that develops in the body's circulatory system is called a thrombus. It sticks to the site of creation and stops the blood flow. Thrombosis is the medical term for the onset of a thrombus. For people who are genetically predisposed to blood clotting and are sedentary, their risk of developing a thrombus is higher. Damage to an artery, vein, or surrounding tissue can potentially result in a thrombus. Thrombolytic treatment increases blood flow, breaks up harmful clotting of blood, and shields vital organs and tissues from harm. Thrombolysis is the process of administering medications that break up clots into the bloodstream to the blockage location through an IV line or a long catheter. Another choice is to physically break up or remove the material clot using a long catheter with a piece of equipment at the tip.[21]

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1.11 Plant Profile

The genus Xantolis includes trees and shrubs of the Sapotaceae family and was first recognized as a separate entity in 1838. A species of flowering plant is called Xantolis Assamica. Charles Barron Clarke first described it and Peter van Rooyen gave it the correct name. Bangladesh and Assam are the species' native habitats. This tree is primarily found in tropical wet biomes.[22]

1.11.1 Introduction of Plant

Scientifics Name: Xantolis Assamica

General Name: Assam ironwood

Vernacular Name: Assamixantol

Locality: Assam (India), Bangladesh.

1.11.2 Synonym

- ✓ Planchonella assamica,
- ✓ Pouteria assamica &
- ✓ Sideroxylon assamicum

1.11.3 Scientific Classification of Plant

Kingdom: Plante

Phylum: Streptophyta

Class: Equisetopsida

Subclass: Magnoliidae

Order: Ericales

Family: Sapotaceae

Genus: Xantolis

Species: Xantolis assamica [22]

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1.12 Plant Morphology

- Huge evergreen tree with a maximum height of 45 meters.
- Initially yellowish or ferruginously villous, branchlets are terete or angular, ranging in diameter from 2 to 5 mm, and eventually turn glabrescent.
- The leaves measure 6–16.5 by 2–7 cm and have an ovate, elliptic, or broadly lanceolate shape with a widely cuneate or rhomboid base and an acute or sharply acuminate apex.
- Flowers are dull white, pendulous, and arranged in few-flowered clusters.
- The fruits are ellipsoid or ovoid, measuring 2-3 by 0.8-1.5 cm, with one or two seeds, and a yellowish or ferruginous villous surface. As they grow, they become glabrescent, and when they dry, they turn dark brown or blackish.

1.13 Plant Part Used in the Project Work

- ✓ Flower

1.13.1 Image of the Plant

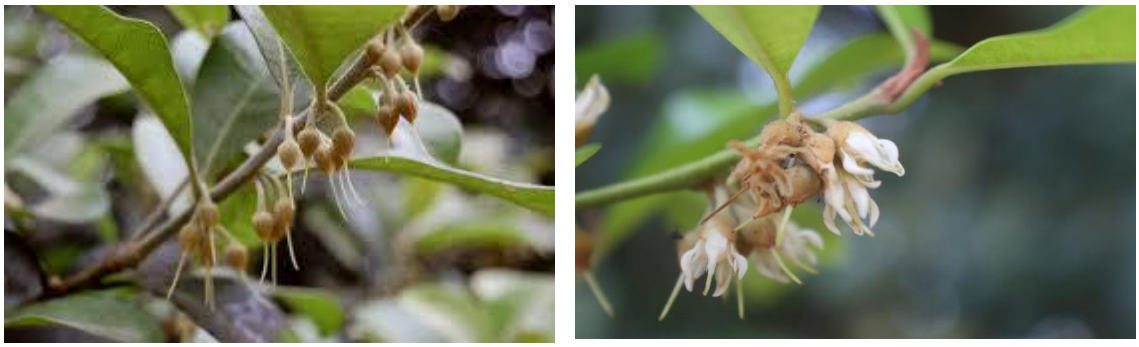

Figure-1: Xantolis Assamica.

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1.14 Literature Review

1.14.1

The analysis revealed that the plant is rich in essential minerals and nutrients like iron, copper, calcium, magnesium, and manganese, crucial for human health. The plant extract contained alkaloids, tannins, saponins, glycosides, phenols, and flavonoids, according to phytochemical extraction utilizing solvent extraction. Quantitative analysis showed that tannins were the most abundant phytochemical scanning test.

1.14.2

In accordance with OECD guideline version 18, 80% methanol was used to extract the air-dried leaves and roots of G. purpurascens, and an acute oral toxicity study was conducted for the 80% methanolic extract. To check for the existence of various ingredients, a preliminary phytochemical screening was conducted. Centrally mediated analgesic action was assessed using the hot plate method, while acetic acid-induced writhing was utilized to investigate peripheral analgesic activity.

1.14.3

The standard models of depression, including the open field, hole cross, hole bord forced, and sleeping period tests in mice, were used to assess the central nervous system (CNS) depressive action of C. diffusa. The animals were split up into three test groups, each with five mice, a control group, and a positive control group. The control group was given distilled water (0.1 mL/mouse, p.o.), while the test groups were given extract orally at doses of 50, 100, and 200 mg/kg body weight. The typical medication was diazepam (1 mg/kg, i.p.)

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CHAPTER TWO
OBJECTIVES OF THE STUDY

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2.1 Objectives of the Project Work

This research investigation used a two-pronged approach to look into the methanolic extracts of Xantolis Assamica flower. While the second goal investigates the plant's possible health advantages, the first goal is to determine the chemical composition of the plant.

Below is a summary of these object:

2.1.1 General Objective

- ✓ To evaluation of Phytochemical Screening and Investigation of Analgesic, CNS, Thrombolytic, and Antidiarrheal Activities of Methanolic Extracts from Xantolis Assamica Flowers in Experimental Mice.

2.1.2 Specific Objectives

- To determine the presence of phytochemical screening of Xantolis Assamica flowers.
- To assess the methanolic extract of Xantolis Assamica flowers' analgesic properties.
- To examine the effects of the methanolic extract of Xantolis Assamica flowers on the Central Nervous System (CNS).
- To assess the anti-diarrheal (caused by castor oil) properties of the Xantolis Assamica flower extract.
- To investigate the Thrombolytic activity of the methanolic extract by Xantolis Assamica.

By fulfilling these goals, the research will offer important new understandings of the chemical makeup of Xantolis Assamica flower as well as their potential for medicinal uses.

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CHAPTER THREE
MATERIALS & METHOD

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3.1 Experimental Plant

Xantolis Assamica. included in member Sapotaceae.

Plant Name	Family	Vernacular Name	Part of the plant used
Xantolis Assamica	Sapotaceae	Assamixantol	Flower

3.1.1 Plant Collection and Identification

For the investigation, the flower of Xantolis Assamica were collected by the authors from the Hazarikhil Wildlife Sanctuary in Fatikchari, Chattogram. An experienced botanist from the Bangladesh National Herbarium (DACB), Mirpur, Dhaka, Bangladesh, recognized and verified the plant.

Figure-2: Xantolis Assamica Flower Collection.

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3.2 Preparation of Crude Extract

Figure 3: Preparation of Crude Extract of Xantolis Assamica.

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3.2.1 Plant Material Preparation

During transit, the gathered plant material was wrapped in plastic sheets, carefully cleaned under running water to get rid of any dirt, let to dry at room temperature in the shade, and then reduced in size with a mill and pestle. The flower is then allowed to dry fully for a few days at room temperature. Using a grinder, the dried flowers are ground into a powder, which is then kept for later use in an airtight container [23].

3.2.2 Extraction Process

- After washing it with water, a large glass jar was rinsed with methanol.
- Next, 2 liters of methanol was added to a jar containing 350gm of crushed flower powder, which was placed 2 inches above the powder's surface.
- To stop air from getting inside, the jar was securely sealed with a plastic cover.
- Following that, the jar was shook twice a day on a regular basis and stored in a dry, dark spot for six days.

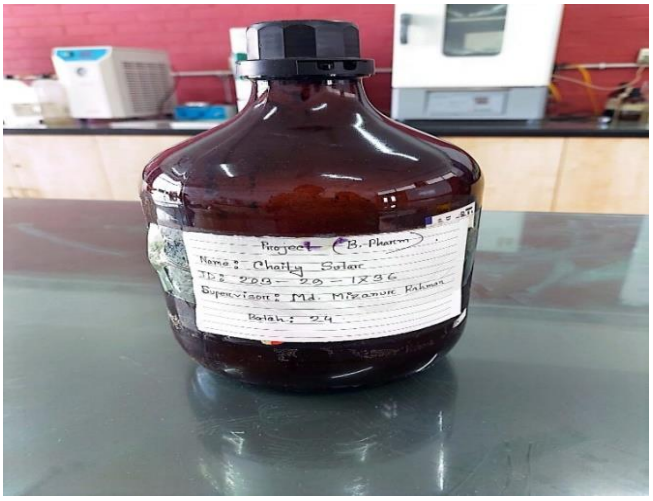

Figure-4: Extraction of the Flower Powder

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3.2.3 Filtration Process

- After six days, cotton and filter paper were used to filter the flower's liquid extract.
- With the aid of a channel, the passage was filtered and re-filtered twice using cotton and twice using filter paper.
- The 250 ml filtrate was gathered in a 500 ml beaker.
- Aluminum foil paper was placed over the beaker to seal it and make a hole in the middle of it, in addition to letting the methanol evaporate.
- The beaker containing the extract was kept in a dry place for three days.

Figure-5: Filtration of the Flower Powder Extract.

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3.2.4 Evaporation Process

- ✓ Within three days by using rotary the liquid was evaporated.
- ✓ Rotary evaporator was used to carry out the evaporation procedure at 50°C.
- ✓ A hole was then created in the middle of the aluminum foil after the sample extract had been collected in a 250 ml beaker and covered with it.
- ✓ The beaker was kept at room temperature in a dry place for four days in order to evaporate the leftover methanol.

Figure-6: Evaporation of methanol from the liquid extract

3.3 Materials and Instruments Used

Beaker, conical flask, measuring cylinder, funnel, glass stirrer, cotton, spatula, Bunsen burner, test tube, water bath, oven, aluminum foil paper, hand gloves, mortar and pestle, analytical weight balance, test-tube holder, refrigerator, bottle, capillary tube, filter paper.[23]

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3.4 Drugs and Chemicals Used

3.4.1 Phytochemical Screening

Chloroform, Methanol, Ferric Chloride, Hydrochloric Acid, NaOH, Sulfuric Acid and all other chemicals.

3.4.2 Analgesic Activities

Diclofenac Na. The suppliers of Diazepam and Ibuprofen were Square Pharmaceuticals Ltd. in Bangladesh. We imported acetic acid and regular saline water (0.9%) NaCl from Bangladesh's Beximco Infusion Ltd. every other compound was useful at the analytical level.

3.4.3 Castor oil-induced anti-diarrheal:

Castor oil, charcoal meal (10% activated charcoal in 5% gum acacia), loperamide (Square Pharmaceuticals Ltd.), dimethyl sulfoxide, and a 0.9% sodium chloride solution (Orion Infusions Ltd., Bangladesh) were used for the antidiarrheal activity test.

3.4.4 CNS Depressant Activity Test: Methanol, Diazepam (1 mg/kg), Thiopental sodium (Gonoshastha Pharmaceuticals Ltd., Bangladesh), and Diazepam (Square Pharmaceutical Ltd., Bangladesh) were employed in the open field, hole cross test. Fifteen minutes prior to the trial, the medications were injected intraperitoneally.

3.4.5 Thrombolytic Activity Test: A healthy male volunteer between the ages of 22 and 28 who has not recently used oral contraceptives, or anticoagulant medication had his venous blood drawn for the samples. About 5 mL of blood from each volunteer was placed into a pre-micro centrifuge tube, which was weighed. The volunteers then divided the clots into standard, sample, and blank tubes, which were then kept apart from one another.

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3.5 Experimental Animals

For the purpose of evaluating biological activity, healthy adult Swiss albino mice of both sexes, weighing between 20 and 40 grams and aged between 6 and 8 weeks, were acquired from the Department of Pharmacy at Jahangirnagar University in Sever, Dhaka. The animals were housed in cages with a 12-hour light and dark cycle and at room temperature. Food, water, and standard lab chow were all freely available to them. Prior to the experimental sessions, the animals were given seven days to adjust to their surroundings. Before the studies began, the animals were separated into groups of four and fasted for the whole night. The Institutional Animal Ethics Committee's rules were followed for conducting animal experiments [24].

3.6 Phytochemical Screening

The primary classes of chemicals (tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids) contained in plant extracts were identified using qualitative phytochemical screening in accordance with established techniques [25].

3.6.2 Materials of Phytochemical Screening

Chloroform, Methanol, Ferric Chloride, Hydrochloric Acid, NaOH, Sulfuric Acid and all other chemicals. [25].

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3.6.3 Chemicals and Reagents

- Dragendroff's Reagent
- Molish's Reagent
- Ferric Chloride
- Sodium Hydroxide
- Hydrochloric Acid (HCl)
- Chloroform

3.7 Test Procedures for Phytochemical Screening

3.7.1 Alkaloids Test

The plant 0.2 ml HCI and 0.1 ml Dragendroff's Reagent were added to 100 mL of water to dissolve 2 ml of extract. Alkaloids were present when a precipitate with an orange-brown hue appeared.

3.7.2 Tannin Testing

After adding 5% ferric chloride and boiling about 5 ml of the plant extract with 10 ml of distilled water, the mixture was examined for greenish-black coloration, which would suggest the presence of tannins.

3.7.3 Saponin Testing

20 ml of distilled water and 01 ml of the extract were mixed together and shaken. The existence of saponins was established by the froth that formed after 15 minutes of shaking.

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3.7.4 Flavonoid Testing

1ml extract mixed with 3-4 drops conc. Hydrochloric acid. Finally, identified the presence of Flavinoids by red colour.

3.7.5 Glycosides Test

1ml extract mixed with 2-3 drops NaOH. Finally, observed the Glycosides by yellow color formation.

3.7.6 Steroid Testing

Steroids prevented the formation of radish brown when 10 mg of the crude extract was combined with 1 mL of chloroform and 1 mL of H2SO4.

3.7.7 Gums

Add 1 mL of concentrated sulfuric acid down the side of the test tube, letting it create a layer without mixing, and then add 2 drops of Molisch's reagent (α-naphthol) to 5 ml of extract. No carbohydrates were found.

3.8 Evaluation of Analgesic Activities of the Extract

Acetic Acid-induced Writhing Test: A mouse model of acetic acid-induced writhing was used to examine the compounds' analgesic properties. There were four mice in each of the four groups into which the animals were split. Animals in Group I were given water as a vehicle, Group II were given 10 mg/kg body weight of Diclofenac Na, and animals in Groups III and IV were given 250 mg/kg and respectively 500 mg/kg body weight (per oral) of the methanolic extract of Xantolis Assamica flower. Diclofenac-Na was injected intraperitoneally 15 minutes before to the acetic acid injection, whereas test samples and the vehicle were given orally 30 minutes prior to the intraperitoneal administration of

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1.0% v/v acetic acid. The mice were watched for particular body spasms known as "writhing" for the next ten minutes after a 05 minute break [25].

3.9 Evaluation of CNS Depressant Activity

3.9.1 Hole Cross Test

The technique was carried out in accordance with Takagi et al. [26]. There were four animals in each of the three groups—control, standard, and test. The standard group got phenobarbital orally at a dose of 100 mg/kg body weight, the control group was given vehicle (water), whereas the test group was given a methanolic extract of Xantolis Assamica flower at doses of 250 mg/kg and 500 mg/kg p.o. Each animal was then placed on one side of the chamber, and every 30 minutes on 0, 30, 60, and 90 minutes during the study period, the number of times each animal went through the hole from one chamber to the other was recorded [26].

3.9.2 Open Field Test

This experiment was conducted in accordance with Gupta et al.'s description. [27] There were four animals in each of the three groups—control, standard, and test. The test group was given crude extract at 250 and 500 mg/kg p.o., the control group was given vehicle (water), and the standard group was given phenobarbita orally at a dose of 100 mg/kg body weight. The animals were set out in a series of squares on the floor of an open field that measured 100 cm by 100 cm by 40 cm. Throughout the study period, the number of squares each animal visited was tallied for three minutes on 0, 30, 60, and 90 minutes [27].

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3.9.3 Hole Board Test

Monitoring mouse activity in the experimental area and selecting the mice with the lowest and highest levels of behavior analysis were the goals of the test. The number of head-dipping incidents in the holes throughout the course of a three-minute session determined the score.

I utilized of a 600 x 600 x 450 mm square hole-board arena. To keep the animals from becoming distracted by visual signals, the translucent Plexiglas walls were covered. The arena's bottom included sixteen round holes, each 50 mm in diameter and uniformly spaced at equal intervals.[28]

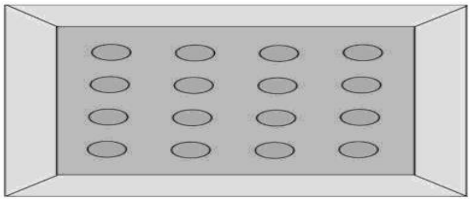

Figure 7. Hole-Board Apparatus

3.10 Anti-Diarrheal (Castor Oil-Induced)

Both male and female mice weighing 25–30 g were starved for eighteen hours. Four groups (n = 3) of the rats chosen for the castor oil-induced diarrheal test were created. Group II was administered loperamide (0.2 ml) as the standard group, whereas Group I was given 1% twin 80 orally as the control group. Doses of 250 mg/kg = 0.1 ml extract and 500 mg/kg = 0.2 ml extract were administered to groups III–IV. All groups were given 0.3 mL of castor oil orally after one hour. After that, they were put in cages with adsorbent paper lining, and for four hours, they were watched for the presence of distinctive diarrheal droppings. The total number of feces in the control group was taken to be 100%.

Percent inhibition of defecation in mice was calculated by using the following equation:

$$\% \text{ inhibition} = \{(Mo – M)/Mo\} \times 100$$

Where, Mo = Mean defecation of control & M = Mean defecation of test sample [29].

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3.11 Thrombolytic Activity Test

3.11.1 Working procedure and dose calculation of Thrombolytic activity

Standard:

Streptokinase =1500000 IU/5ml

Dose: 30000 IU in 100µl

Where,

1500000 IU streptokinase dissolved in 5 ml distilled water

= 100 µl distilled water contains 30000 IU streptokinase

3.11.2 Extract Dose:

1000 µg in100 µl

Extract Concentration:

Stock solution=100mg/10ml

Where,

10ml of distilled water with 100mg of extract Or, 100000 µg extract dissolved in 10000 µl distilled water 1000 µg extract dissolved in 100 µl distilled water = 100µl distilled water i.e. 1000µg/100 µl

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3.12 Thrombolytic Examine Operating Process

A blank tube was obtained and measured initially.

One milliliter of blood was contained in each tube, taken from a healthy individual.

The blood samples were placed in the incubator at a temperature of 37°C for 45 minutes to encourage the development of blood clots.

After the serum was extracted, the first clot was weighed using a tube.

The tube was used to weigh the initial blood clot after the serum was withdrawn.

The weight of the first clot is calculated by subtracting the weight of the blank tube from the weight of the tube containing the first clot.

Use 100µl of pure water for comparison, then administer the standard dosage of 100µl or 30000 IU and withdraw 100µl or 1000µg/100µl.

Incubate at 37°C for 90 minutes to break down clots.

The tube that held the lysis-related blood fluid was taken out.

The weight of the tube was used to evaluate the second clot.

The weight of the second clot is calculated by subtracting the weight of the blank tube from the weight of the second clot with the tube.

Next, the weight of Lysis clot = first clot weight – second clot weight

Finally, Clot lysis = (Weight of the lysis clot / Weight of clot before lysis) × 100

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Figure-8: Final Clot of Blood.

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CHAPTER FOUR RESULT

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4.1 Yield Value of Extract

The flower powder weighed 94.3461 grams. The flower extract weighed 14.49 grams, and its yield percentage was 14.49 grams divided by 93.3461 grams. × 100 = 15. 52%.

4.2 Phytochemical Screening Result

To find out whether the flower extract of Xantolis Assamica had phytochemical ingredients or active constituents, phytochemical screening was conducted.

Test of Group	Result
Alkaloids	+
Glycosides	+
Flavonoids	-
Tannins	+
Saponins	+
Gums	-
Steroids	-
Note that (+) denotes the presence of the tested group and (-) denotes its absence.	

Table 1: Determine The Presence of Phytochemical Screening.

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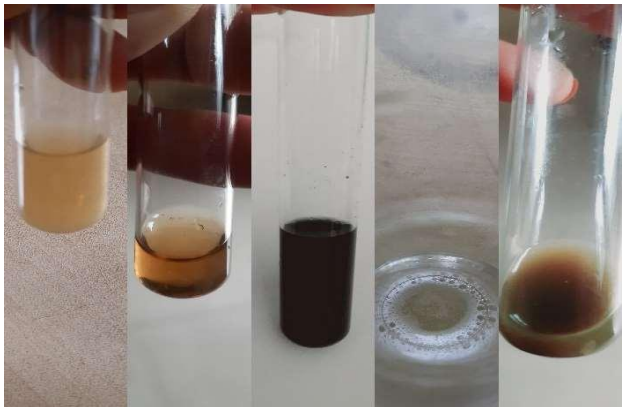

Figure-9: Presence of the Tested Group

4.3 Analgesic Activity Result

Acetic Acid-induced Writhing Test

Group	Number of Writhing (Mean±SEM)	% Inhibition of pain
Control	11.0±0.55	00
STD	6.60±1.03*	40.00
Test Drug Dose 1	4.80±1.24**	56.36
Test Drug Dose 2	8.0±0.71	27.27

Table 2: Effect of Test Drug on acetic acid induced abdominal writhing in mice

N.B: Data were analyzed by one way ANOVA following Dunnet's post hoc test. Values are expressed as Mean±SEM, n=6. *($p< 0.05$) = significant, ** ($p< 0.01$) = highly significant, *** ($p< 0.001$) = very highly significant compared to control group.

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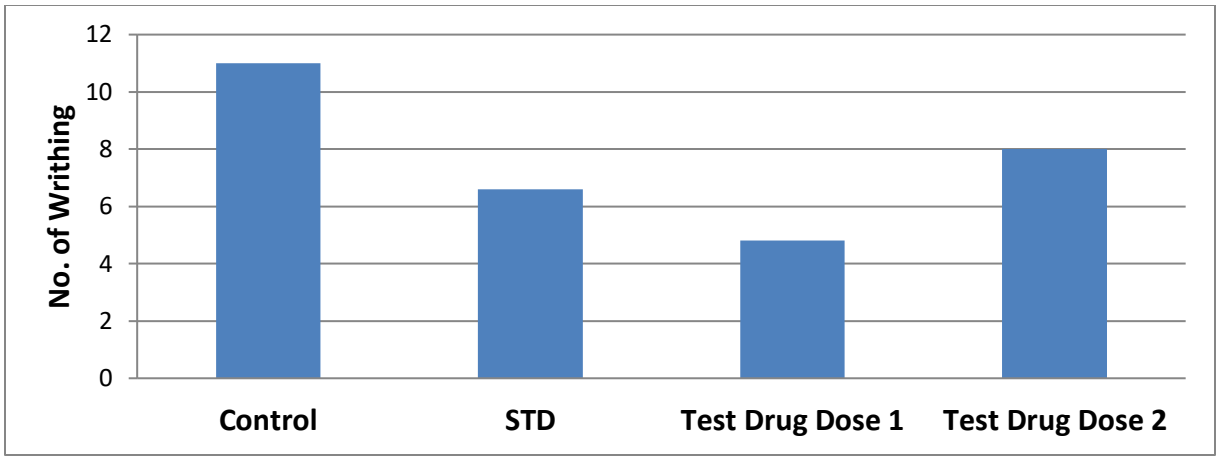

Graph-01: Impact of the Test Drug on Mice's Abdominal Writhing Caused by Acetic Acid.

4.4 Evaluation of CNS Depressant Activity Result

4.4.1 Open Field Test

Group	Area Travelled (Mean±SEM)			
	0 min	30 min	60 min	90 min
Control	3.80±0.58	3.60±0.25	2.80±0.20	2.60±0.25
STD	4.00±0.45	5.80±0.80*	8.40±2.94	6.80±0.86**
Test Drug	3.00±0.32	2.00±0.45	1.40±0.25	2.40±0.75

Table 3: Effect of Test Drug on area travelled in open field test in mice.

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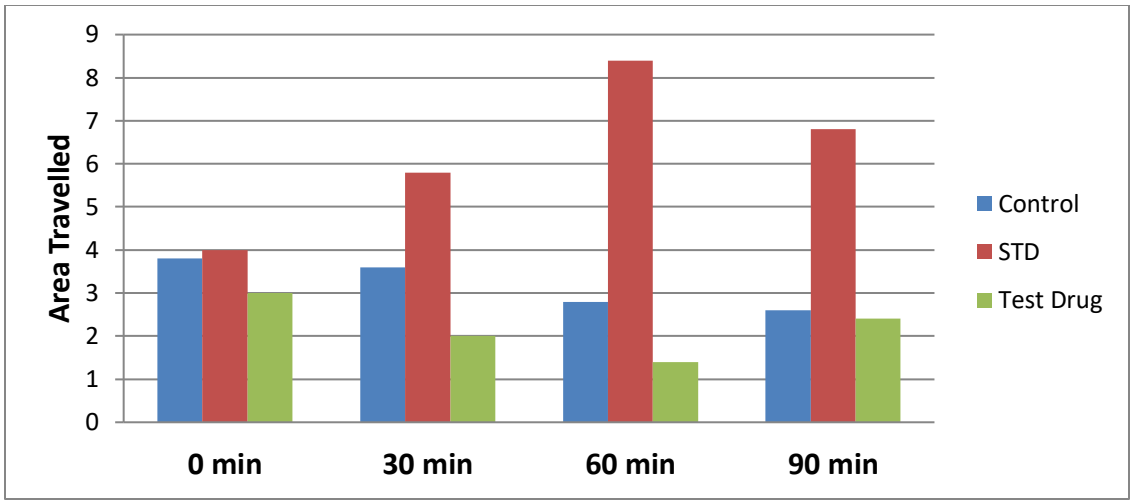
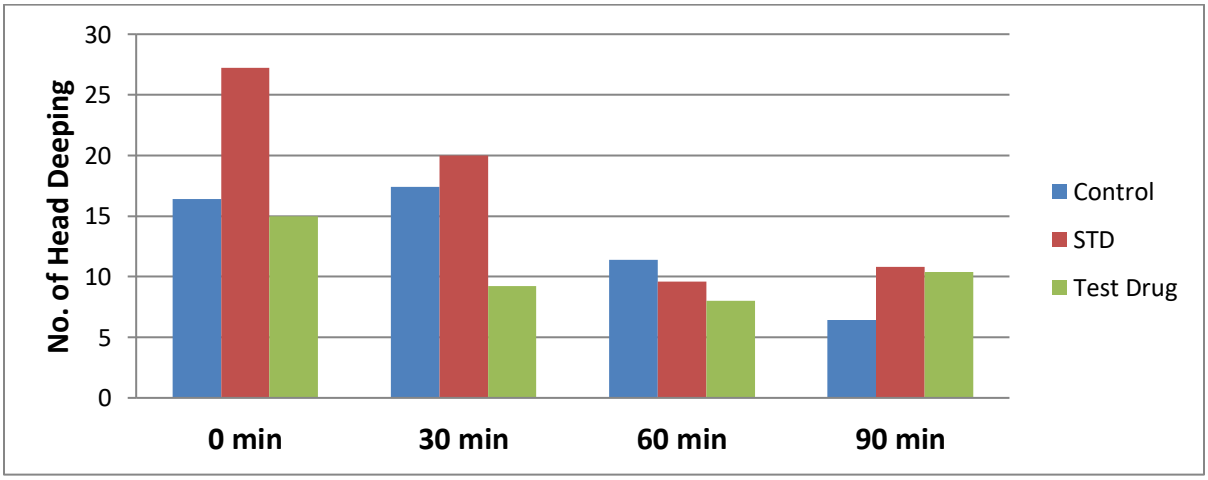


Graph-02: Effect of Test Drug on area travelled in open field test in mice.

4.4.2 Hole Board Test

Group	Number of Head Deeping (Mean±SEM)			
	0 min	30 min	60 min	90 min
Control	16.40±1.57	17.40±2.58	11.40±0.87	6.40±0.68
STD	27.20±3.34*	20.00±3.62	9.60±0.51	10.80±1.02
Test Drug	15.00±1.82	9.20±2.78	8.00±1.00*	10.40±2.42

Table 4: Effect of Test Drug on number of Head Deeping in Hole Board test in mice.



Graph-03: Effect of Test Drug on number of Head Deeping in Hole Board test in mice.

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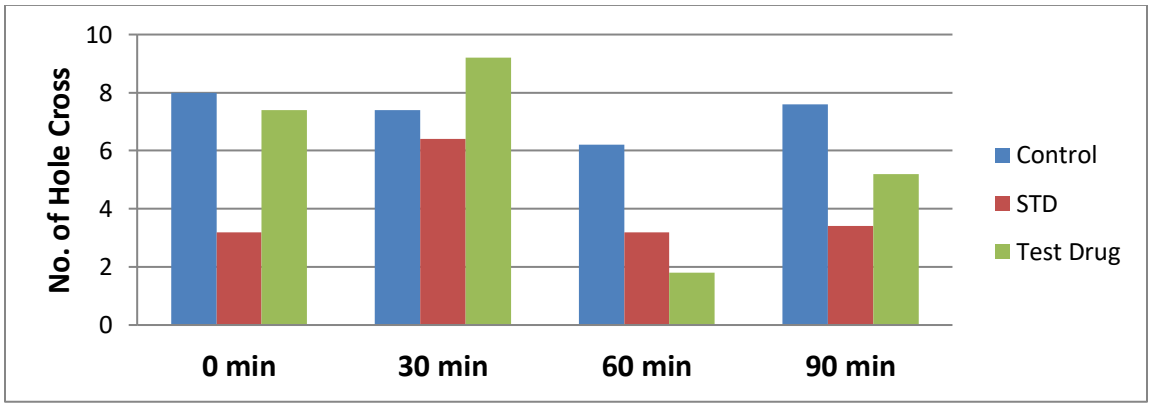
4.4.3 Hole Cross Test

Group	Number of Hole Cross (Mean±SEM)			
	0 min	30 min	60 min	90 min
Control	8.00±0.71	7.40±1.12	6.20±0.80	7.60±0.51
STD	3.20±0.38*	6.40±0.98	3.20±0.66*	3.40±0.51**
Test Drug	7.40±1.57	9.20±2.11	1.80±0.38**	5.20±1.02

Table 5: Effect of Test Drug Hole Cross in mice.



Graph 4: Effect of Test Drug Hole Cross in mice.

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4.5 Anti-Diarrheal (Castor Oil-Induced) Result

Code no.	Mice No.	Total no. of diarrheal feces				Total faces	Avg.	MEAN	SD
		1 hour	2 hour	3 hour	4 hour				
CTL (1% tween 80)	1	1	1	2	2	5	4.3	4.3	0.6
	2	0	1	1	1	4			
	3	0	0	2	1	4			
STD (Loperamide)	1	0	0	1	1	2	2.0	2.0	0.0
	2	0	0	1	1	2			
	3	0	0	0	2	2			
MXA (250)	1	0	1	1	1	3	3.3	3.3	0.6
	2	0	0	1	2	3			
	3	0	1	1	2	4			
MXA (500)	1	0	1	1	1	3	3.0	3.0	0.0
	2	0	1	1	1	3			
	3	0	1	1	1	3			

Table 6: Effect of Test Drug on area travelled Anti-Diarrheal in mice.

Group	Total Diarrhea	% of Reduction Diarrhea
Control	13	00%
Standard	06	53.85%
MXA 250	10	23.07%
MXA 500	9	30.77%

Table 6: Effect of Test Drug on area total Diarrhea in mice.

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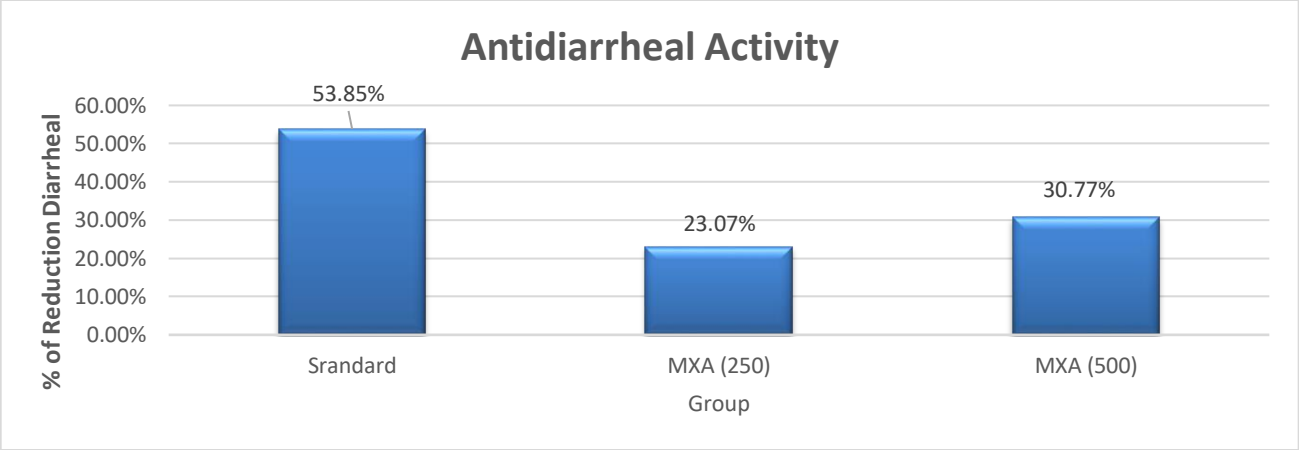

Graph 5: Effect of Test Drug on Anti-Diarrheal (Castor Oil-Induced).

4.6 Thrombolytic Activity Test result

Solution	Blank tube weight (gm.)	1st clot + tube weight (gm.)	1st clot weight (gm.)	2nd clot + tube weight (gm.)	2nd weight clot (gm.)	Weight of lysis (gm.)	% Of Lysis
Group - I [Standard]	0.83	1.85	1.02	1.16	0.33	0.69	69%
Group - II [Control]	0.77	1.58	0.81	1.47	0.7	0.1358	13.58%
Group - III [Sample]	0.76	1.48	0.72	1.20	0.44	0.3854	38.54%

Table 07: Effect of Test of Thrombolytic

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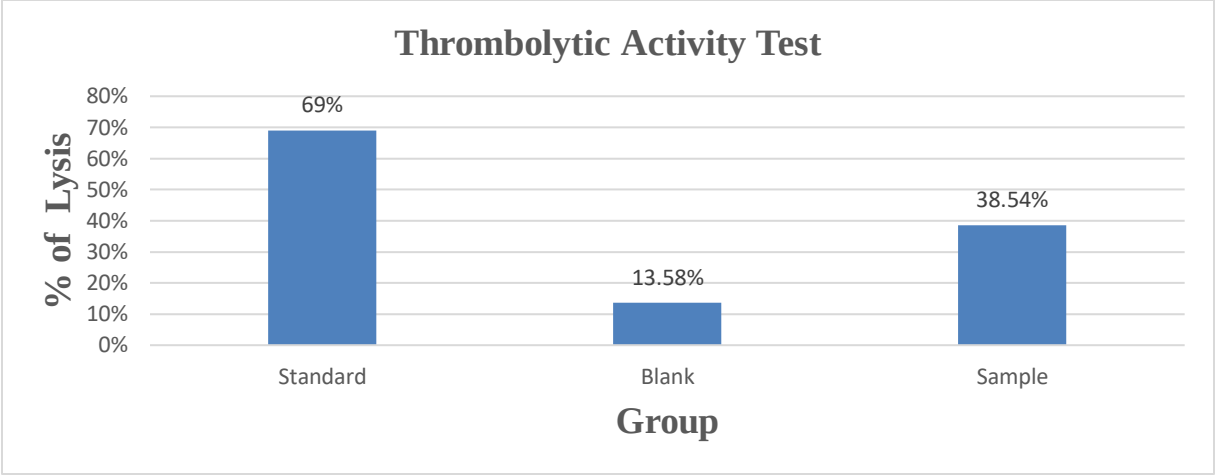

Graph 06: Effect of Test of Thrombolytic

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CHAPTER FIVE
DISCUSSION

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5.1 Yield Value

For extraction, the cleaned flowers were dried at room temperature. Finally, yield value was determined to be 15.85% following appropriate drying, grinding, and cold extraction with methanol.

5.2 Phytochemical Screening

Xantolis Assamica floral extract is shown by phytochemical screening. Alkaloids, glycosides, tannins, and saponins are present. Gum steroids and flavonoids are not present.

5.3 Analgesic Activities (Acetic Acid-induced Writhing Test)

Mice receiving 250 mg/kg and 500 mg/kg of Xantolis Assamica flower extract showed suppression of the acetic acid-induced writhing test, with respective values of 56.36% and 27.27%. had a strong analgesic effect ($p < 0.05$).

5.4 CNS depressant Activity

The CNS depressant activity was evaluated by observing the reduction of locomotor and exploratory activities in the open field test, hole board test and hole cross tests at a dose of 250 mg/kg and 500 mg/kg body weight. Find result show (Table-03,04,05).

5.5 Thrombolytic Activity

In this investigation, the methanol extract of Xantolis shown good thrombolytic activity when compared to the standard (Streptokinase). A higher percentage of lysis indicates that more of the clot was dissolved, while a lower percentage of lysis indicates that less of the clot was dissolved. The results suggest that the plant extract may be a potential anti-thrombotic agent. In further research is needed to confirm these findings and to determine the safety and accuracy of the extract for use.

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5.6 Anti-diarrheal (Castor Oil-Induced)

Following four hours, the administration of loperamide at a dosage of 500 mg resulted in a respective reduction of diarrhea by 53.83%. The values corresponding to the Minimum Effective mice Dose are 250 mg/kg each, with a respective 23.07%. Following of 500 mg/kg, the rate of inhibition of diarrhea is more significant than 250mg/kg. Overall, the potency of the observed order of inhibition of defecation and diarrhea is significant.

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CHAPTER SIX CONCLUSION

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6.1 Conclusion

The study of the phytochemical and therapeutic effect of methanolic extracts from Xantolis assamica flower reveals considerable pharmacological activity. The phytochemical screening revealed a diverse array of bioactive compounds, including alkaloid, glycoside, tannin and saponin. These compounds have been associated with various pharmacological properties, such as analgesic activities, CNS activities, thrombolytic activity and antidiarrheal. Furthermore, the plant extract exhibited significant thrombolytic activity, suggesting its potential for preventing or treating thrombotic disorders. Additionally, the extract demonstrated potent analgesic activity, indicating its ability to protect against diseases. The extract offers opportunities for future investigation into its potential as an antidiabetic drug.

According to the study's overall findings, Xantolis Assamica flowers may be effective natural remedies for conventional medical applications, and more investigation into their potential for therapeutic use is encouraged.

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CHAPTER SEVEN
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