"Exploring the Phytochemical Profile and Therapeutic Potential of the Methanolic Extracts of *Xantolis assamica* leaves: An In-vivo & In-vitro Approaches"

A dissertation submitted to Daffodil International University's Department of Pharmacy, Faculty of Allied Health Sciences, partially meets the requirements for a Bachelor's Degree in Pharmacy.

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APPROVAL

This is to ensure that the project **"Exploring the Phytochemical Profile and Therapeutic Potential of the Methanolic Extracts of *Xantolis assamica* leaves,"** which was turned in for credit towards the Bachelor of Pharmacy degree from Daffodil International University's Department of Pharmacy, is entirely original work of mine. I have been given credit for any original ideas, writings, or language used in the project.

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Md. Atikur Rahman

The Author,

DEDICATION

In great appreciation, I devote this endeavor to the Almighty Allah as well as my ever-supportive family, instructors, and friends, whose encouragement propelled me forward.

DECLARATION

I declare that this project, titled **"Exploring the Phytochemical Profile and Therapeutic Potential of the Methanolic Extracts of Xantolis assamica Leaves,"** is my own original work, carried out under the supervision of **Md. Mizanur Rahman**, an assistant professor in the Department of Pharmacy at Daffodil International University Faculty of Health and Life Sciences, and in partial fulfilment of the Bachelor of Pharmacy degree requirements. All sources have been properly acknowledged and listed in the bibliography. This project had not previously been submitted for any other degree or qualification.

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CERTIFICATE

This is to certify that the project titled **"Exploring the Phytochemical Profile and Therapeutic Potential of the Methanolic Extracts of Xantolis assamica Leaves,"** submitted by Md. Atikur Rahman is the outcome of his independent and original work. All sources from which ideas and extracts were obtained are recognized and mentioned in the project. The project is free of plagiarism and has not been submitted elsewhere for a degree or qualification.

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Abstract

Background: Modern scientific inquiry consistently explores the medicinal potential of plants by analyzing their chemical composition and therapeutic effects. This study focuses on investigating the phytochemicals and healing properties of methanolic extracts from the leaves of *Xantolis assamica*, a plant native to Bangladesh and Assam.

Objectives: The primary goals of this research were to analyze the phytochemical constituents of methanolic extracts using gas chromatography-mass spectrometry (GC-MS), assess thrombolytic activity, and evaluate hypoglycemic effects through an oral glucose tolerance test (OGTT) on Swiss Albino mice.

Methods: After drying, *X. assamica* leaves underwent methanol extraction. GC-MS analysis identified various phytochemicals present. Thrombolytic activity was evaluated in vitro, and OGTT was conducted on Swiss Albino mice.

Results: GC-MS analysis revealed diverse phytochemicals in the methanolic extract, including fatty acid esters, triterpenes, diterpenes, and steroids. The extract exhibited significant thrombolytic activity, with a clot dissolution rate of 32%. Additionally, OGTT demonstrated reduced blood glucose levels in mice treated with the extract compared to control and standard groups at 120 minutes. The plant extract, administered at a dosage of 250 mg/kg and 500 mg/kg body weight, resulted in blood glucose levels of 6.2±1.27 and 5.33±0.78 mmol/L, respectively. In comparison, the standard and control groups exhibited blood glucose values of 6.27±0.88 and 5.36±2.12 mmol/L, respectively.

Conclusion: This study underscores the therapeutic potential of *X. assamica* leaves, particularly their thrombolytic and hypoglycemic effects. Further research is warranted to elucidate underlying mechanisms and explore the development of natural remedies.

Title	Page No
Abstract	vii
Content	vii-vii
List of Figure	vii
List of Table	vii

Table of Contents

Chapter One
Introduction

List of Tables

List of Figures

CHAPTER ONE

INTRODUCTION

1.1 General Introduction

The various roles that plants play have contributed to our understanding of their significance in treating human ailments and have made them an essential source of both traditional and innovative medicines (1). Since the dawn of time, people have used herbs and plant-based products as medicine to increase immunity or resistance against a variety of ailments, including fever, joint pain, colds, and coryza. The foundation of the ancient Siddha, Unani, and Ayurvedic medical systems, which are used to treat common ailments, is plant-based medicine. The bulk of individuals in our nation, particularly those who reside in rural areas, often employ herbal medicines (2). The World Health Organization estimates that 5.2 billion people on the planet live in less developed countries, and nearly all of them only utilize herbal medicine to meet their basic medical needs. Every day, 3.3 billion individuals in underdeveloped nations use medicinal herbs, which are the mainstay of traditional medicine (3). Plants are important medical subjects because their structures are safe for use in developing new pharmaceuticals. There are recognized therapies for about 33% of human ailments, and demand for botanical prescriptions is considerable (4).

Plants are an excellent source of new chemicals with medicinal potential for medication development (5). The process of producing medicinal plants involves hundreds of chemical components. These substances are toxic to plants, animals that consume herbivorous foods, insects, and illnesses (6). Many novel chemicals derived from plants are now commonly utilized as medicines in one or more countries across the globe. These days, a lot of medications are just replications or synthetic versions of substances that are found naturally. There has been a lot of interest in secondary metabolism recently because to the growing importance of secondary metabolites in the marketplace, especially in relation to the potential use of tissue culture technologies to modify the production of bioactive plant metabolites (5).

Research on phytochemical pharmacology and conventional medicine have produced chemically powerful medications that can treat a variety of human illnesses, including infections. Geologists created organic medications, which are now a major player in the pharmaceutical sector.

1.2 Phytochemistry

The Greek term meaning "plant" is the name's etymological origins (7). The scientific study of plants' secondary metabolites, which serve as a defense mechanism against illnesses, pests, and environmental dangers, is known as phytochemistry (8). Another name for phytochemicals is "man-friendly medicines" since they treat ailments without putting people in danger (9). The scientific study of the secondary metabolites that plants make to defend themselves against illnesses, pests, and environmental dangers is known as phytochemistry. It looks at the parts, synthesis, uses, and workings of biological systems. Understanding phytochemicals is critical to the creation of novel therapeutic agents and the search for new treatments. This information is essential for researching new medications and treating severe ailments (8).

1.3 Herbal Medicine

The practice of herbal medicine, also known as herbalism, involves the exploration of plant species and their medicinal properties (10). Patients are increasingly looking into complementary and alternative treatments. Generations of traditional healers have gathered centuries of valuable knowledge to create natural herbal remedies. These remedies are gaining popularity in developing countries for essential medical care due to their cultural acceptance, better compatibility with the human body, and lower risk of adverse effects. However, a new study casts question on the safety of several herbal medications because they have been linked to harmful side effects. A drug approval process that verifies safety and efficacy has not been applied to most herbal medications that are currently on the market. The World Health Organization (WHO) characterizes traditional medicine as any healing method that has been practiced for centuries and predates the emergence and spread of modern medicine. This encompasses the utilization of natural remedies (11).

1.4 Medicinal Plant

The earliest kind of medication comes from medicinal plants, which have been used in traditional medicine in many countries for thousands of years. Over the millennia, human groups have shared actual information regarding their beneficial impacts (12). Natural products are an important source of pharmacological compounds, and many contemporary medications utilized in modern pharmacotherapy are originated from traditional herbal medicine (13).

Compared to nations that only employ scientific medicine, those that practice traditional medicine tend to use medicinal herbs more frequently. In contrast, in the latter, the use of medicinal plants has largely been limited to self-treatment and has virtually disappeared from official therapy. Approximately 90% of individuals in various developing countries rely on medicinal plants as a key component of their medical treatment (14).

The natural substances consisted of oils derived from various plants, including different species of Commiphora (known as myrrh), Cupressus sempervire (cypress), Glycyrrhizaabra (licorice), and different species of Cedrus (cedar). The earliest known writings were crafted in cuneiform on clay tablets in Mesopotamia around 2600 BC. Currently, a range of conditions such as inflammation, parasitic infections, common colds, and coughs are managed using these medicinal solutions Ammi majus, sometimes called bishop's weeds, is a skin disorder that causes pigment loss. Egyptian medicines is the source of this information. β-methoxy psoralen, discovered in a plant known for treating psoriasis, T-cell lymphoma, and other skin disorders, has sparked interest in the potential of plants as sources for medicinal treatments. Over half of pharmaceuticals explored in global clinical trials consist of natural chemicals or their derivatives, with at least a quarter originating from higher plants. In the past forty years, plants like Dioscorea spp. and other flowering plants have given rise to approximately twelve potent drugs (15).

1.5 Significance of Medicinal Plants

Each type of medicinal plant serves a certain function. A plant is considered medicinal if it satisfies these three requirements.

Preventive: According to research, these drugs can prevent a number of illnesses. It decreases the side effects of synthetic medications.

Synergistic: Certain medicinal herbs may cause negative physiological effects in humans to worsen or to reduce.

Official: Certain plants are used as "formula medicines," often used to treat incurable diseases like cancer (16).

1.6 Status of Medicinal Plants in Bangladesh

This study looks at the current state and market environment for medicinal plants in Bangladesh, a nation in the Indus valley that is home to 2,000 different types of medicinal plants, 449 of which are officially registered. The historic use of therapeutic herbs by healers, known as Kavirajs, is uncertain. Many cultures, such the Chakma, Marma, Rakhine, Tripura, Garo, and Khashia each with its own oral tradition have advanced the use of therapeutic herbs. Traditional medical practices have persisted because people still have faith in nature, even in the face of the widespread use of modern healthcare systems. Generations have passed along their knowledge of how to use therapeutic flora. There are no laws, rules, or government limitations pertaining to the production, sale, or preservation of therapeutic plants. Roughly 422 businesses produce goods with botanical medicines (17).

1.7 Opportunities in Drug Discovery from Medicinal Plants

Plant drug development necessitates a multidisciplinary strategy integrating biological, phytochemical, ethnobotanical, and botany methods. New chemical compounds, or lead molecules, are still being found in plants that can be used to generate drugs for a variety of pharmacological targets, including pain, cancer, HIV/AIDS, malaria, and Alzheimer's disease. Numerous naturally occurring plant-based products, including as tiotropium analogues, arteether, galantamine, paclitaxel, and camptothecin, are being used in clinical settings and some are even going through phase II and III trials.

In addition to sourcing and certifying plant materials, high-throughput screening bioassay techniques, and scaling up of bioactive lead compounds, plant-based drug development initiatives remain a significant source of new drug discovery leads. It's up to you to solve the issue. Current research on the identification of novel medications from medicinal plants employs a multidisciplinary approach combining botanical, phytochemical, biological, and molecular techniques. The identification of therapeutic plants continues to yield novel and significant insights into a range of pharmacological targets, such as pain, cancer, AIDS, Alzheimer's disease, and malaria. Drug discovery from medicinal plants is still a significant source of novel drugs, but there are still many obstacles to overcome, such as finding plant materials, choosing and using the right high-throughput screening bioassays, and scaling up active molecules (7).

1.8 Basics of GC–MS Analysis.

The mass spectral fragmentation patterns, which are indicative of a compound's chemical structures, are combined with the relative gas chromatographic retention times and elution patterns of mixture constituents to identify compounds using gas chromatography–mass spectrometry (GC–MS) (18). Typical GC-MS system operations include the following: 1) gas chromatography is used to separate specific compounds from mixtures; 2) components that have been separated are moved into an ionizing chamber; 3) ionization occurs; 4) mass analysis is performed; 5) an electron multiplier is used to detect ions; and 6) a computer system is used for data gathering, processing, and display. The distinct organic molecules enter the MS as they elute from the GC column. They are subjected to an electron stream during ionizations, which causes them to fracture into ions. By dividing the mass of the fragment by its charge, one may determine the mass to charge ratio (M/Z). The M/Z ratio indicates the fragment's molecular weight, while the charge is nearly always +1. Mass analyzers of the quadrupole or magnetic sector types are typically used in MS configurations. Four electromagnets in a group focus each fragment via a slit in the detector of a quadrupole GC-MS. These aim to selectively guide certain pieces, one by one (scan), until the whole M/Z range is obtained. As a result, a mass spectrum is produced, which is a graph that displays the molecular weight (or relative abundance, or signal strength) versus the M/Z ratios (19). Every chemical has a distinct fingerprint, and software comes with a library of spectra for unidentified substances (18) (19).

1.9 Thrombolytic Activity

A thrombus is a form of blood clot that forms within the body's circulatory system. It clings to the site of creation and stops the flow of blood. 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 (20). Venous thromboembolism (VTE) has a major detrimental influence on community economics as well as health (21). Annually in Australia, around 30,000 hospital admissions are attributed to VTE, resulting in approximately 5,000 patient fatalities. Among vascular illnesses in Caucasian populations, this illness ranks third after myocardial infarction and stroke (22). Estimates indicate that two to three hospital admissions per 1000 involve VTE, an acute event that is followed by a main diagnosis (20). Both pulmonary embolism

(PE) and deep vein thrombosis (DVT) represent key manifestations of VTE that can be life-threatening for individuals of any gender (22).

Thrombolytic treatment increases blood flow, breaks up harmful clotting of blood, and shields vital organs and tissues from harm. Thrombolysis is the process of injecting medications that break up clots into the circulation through an IV line or a long catheter to the location of the blockage. An further alternative is to manually break up or remove the material clot using a long catheter with a piece of equipment at the tip (23). Acute pulmonary embolism and ischemic strokes are mostly caused by blood clots in the arteries feeding the heart, which are broken up by thrombolysis therapy. It also disintegrates thrombi in the heart's expanding chambers. The legs, pelvis, and upper limbs contain veins that have the potential to burst and cause deep vein thrombosis (DVT). If the clot fractures and travels to a lung artery, this might cause a sudden pulmonary embolism. If the occurrence of a blood clot is considered life-threatening, thrombolysis may be an option immediately when symptoms of a heart attack, stroke, or pulmonary embolism manifest, preferably within one to two hours of diagnosis (22).

1.9.1 Mechanisms of Thrombolysis

Thrombolytic drugs function by prompting plasminogen to split, resulting in the production of plasmin, which aids in breaking up blood clots. Blood clots are able to maintain their structure intact because of the fibrin crosslinks, which are broken down by the proteolytic enzyme known as plasmin. Thrombolytic medications are sometimes referred to as "fibrinolytic drugs" and "plasminogen activators" based on how they work. The three primary classes of fibrinolytic medications are SK (UK), tPA (tissue plasminogen activator), and urokinase. Nonetheless, the effects of the three medication classes are identical. They all break up blood clots using different techniques. methods to change their fibrin clot preference. The graphic on the right shows the fibrinolytic processes of SK and tPA. Derivatives of tPA are the most widely used thrombolytic medications due to their greater selectivity in activating fibrin-bound plasminogen, particularly for cerebral and coronary vascular clots. Tissue plasminogen activator causes clot lysis in the following order:

- Fibrin on the surface of the clot is bound by tPA.
- Initiates the activation of plasminogen that is bound to fibrin.
- Plasmin is released from the plasminogen attached to fibrin.

- Pleurosmin breaks down fibrin molecules, dissolving blood clots. Plasmin, a protease, can also disintegrate fibrin molecules to make clots disappear. It's essential to note that plasmin doesn't only break down fibrin but also other proteins.

Circulatory proteins such as fibrinogen exhibit relative specificity, leading to less breakdown of circulating fibrinogen during clot dissolution with tPA in the United Kingdom and the Republic of Korea. While tPA primarily targets clot-bound plasminogen, it also activates circulating plasminogen, prompting the release and synthesis of plasmin. The breakdown of circulating fibrinogen can result in an unintended systemic fibrinolytic state. Therapeutic antiplasmin acts by deactivating plasmin, similar to naturally occurring antiplasmin in circulation. Unlike tPA, which has a preference for binding to fibrin linked with clots, SK, although lacking enzymatic activity, binds to plasminogen and releases plasmin with equal affinity to both circulating and non-circulating plasminogen. Therefore, SK not only leads to substantial breakdown of blood clots but also causes significant degradation of fibrinogen. As a result, tPA is commonly preferred over SK as a thrombolytic agent, particularly for dissolving blood clots in the coronary and cerebral arteries. Patients who have recently become infected with streptococci may need much higher dosages of SK in order to achieve thrombolysis because SK is derived from streptococci. It's critical to keep in mind that the age of the clot affects how well thrombolytic medications work. Breaking down as a result of increased fibrin cross-linking and compactness in older clots. Administration of thrombolytic drugs is crucial within the first two hours following the onset of an acute myocardial infarction. Subsequently, higher doses may be necessary to achieve the intended clot breakdown as the drug's effectiveness diminishes over time (24).

1.9.2 Streptokinase

A metabolic byproduct of beta-hemolytic streptococci, streptokinase interacts with plasminogen to produce an active complex with protease activity, which converts plasminogen to plasmin. Streptokinase is an indirect fibrinolytic drug (25). Extensive, placebo-controlled trials have demonstrated the mortality benefit of streptokinase in patients suffering from acute myocardial infarction (AMI, 25). Hydrolyzing fibrinolytic is possible with streptokinase complexes containing human mast cells. The production of fibrin occurs when bond cleavage activates additional

unbound plasminogen. The three domains that comprise streptokinase are amino acid residues 1–150, residues 1–150, and residues 1–150 together. 288–414, 288–414, and 151–287, respectively, are the residues. No single individual can activate plasminogen on their own; however, each domain binds plasminogen (26). Proteins other than human called streptokinase can cause life-threatening allergic reactions when they enter the bloodstream. Anti-streptokinase antibodies in circulation have an effect on the risk of this immunological response. This immunogenicity limits the number of streptokinase treatments that can be administered (25).

1.10 Oral Glucose Tolerance Test (OGTT)

In order to diagnose type 2 diabetes and glucose intolerance, the oral glucose tolerance test, or OGTT, is a simple diagnostic that is widely used in clinical settings. Blood is drawn for testing insulin and glucose levels at -30, 60, and 120 minutes after an overnight fast and either a standard meal or a standard oral glucose load (75 g of anhydrous glucose). Insulin resistance is not as well-measured by this test as glucose tolerance is. Moreover, insulin secretion, the actions of incretins, and the body's capacity to metabolize glucose load all influence glucose tolerance.

After two hours of a 75 gm glucose load, those with plasma glucose levels of ≥200 mg/dL are diagnosed with diabetes mellitus. If the plasma glucose level is between 140 and 190 mg/dL two hours after the glucose load, impaired glucose tolerance is diagnosed.
Glucose clearance can be calculated by using a glucose tracer in conjunction with insulin, C-peptide, and glucose levels. But there are several drawbacks to OGTT. First, even within the same individual, there might be variations in the absorption of glucose and the emptying of the stomach. Furthermore, it has been noted that glucose measurements are insufficient in providing information on the interactions between glucose and insulin (27).

CHAPTER TWO

PLANT PROFILE

2.1 Introduction

The genus Xantolis comprises trees and shrubs of the Sapotaceae family, and it was first recognized as a separate entity in 1838 (28). A genus of blooming plants is called Xantolis assamica. Charles Baron Clarke provided the initial description of it, and Pieter van Royen gave it the precise name (29). This species is native to Bangladesh and Assam. It is a tree that thrives mostly in the tropical wet biome (30).

2.1.1 Plant name

Scientific Name: *Xantolis assamica*

Common Name: Assam ironwood

Vernacular Name: Assamixantol

2.1.2 Synonym

- ✓ *Planchonella assamica*
- ✓ *Planchonella assamica*
- ✓ *Pouteria assamica*
- ✓ *Sideroxylon assamicum*

2.2 Taxonomy classification (30).

Kingdom: Plante

Phylum: Streptophyta

Class: Equisetopsida

Subclass: Magnoliidae

Order: Ericales

Family: Sapotaceae

Genus: Xantolis

Species: *Xantolis assamica*

2.3 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 ferruginously villous surface. As they grow, they become glabrescent, and when they dry, they turn dark brown or blackish.

Figure -1: Xantolis assamica.

2.4 Traditional use

- ❖ Leaf paste is applied to treat swelling.

Chapter Three

Objectives of the Study

3.1 Objectives of the Study

This research investigation used a two-pronged approach to look into the methanolic extracts of *Xantolis assamica* leaves. While the second goal looks into the plant's possible health advantages, the first goal is to determine the chemical composition of the plant. Below is a summary of these goals:

1. **Unveiling the Phytochemical Profile:**

   - To identify and measure the phytochemical components found in the methanolic extracts of *Xantolis assamica* leaves, use GC-MS methods.
   - Identify the main substances and their concentrations, paying particular attention to those that make up more than 1% of the ionic chromatogram. This will give a clear image of the chemical makeup of the plant.

2. **Exploring Therapeutic Potential:**

   - **Thrombolytic Activity:** In order to determine whether *Xantolis assamica* leaf extract has thrombolytic capability, determine how well it dissolves blood clots. Its effectiveness will be evaluated by contrasting it with that of the common thrombolytic medication streptokinase.
   - **Blood Sugar Regulation:** To find out how the extract affects blood sugar levels, use the Oral Glucose Tolerance Test (OGTT). This will be contrasted with the results of a common antidiabetic drug called glimepiride. The objective is to ascertain whether the extract has antidiabetic qualities by examining how it affects blood sugar regulation at different intervals following administration.

By fulfilling these goals, the research will offer important new understandings of the chemical makeup of *Xantolis assamica* leaves as well as their potential for medicinal uses, especially in the fields of blood sugar regulation and blood clot management.

Chapter Four

Literature Review

1. Richert JC, Masuram S, Sneddon J. Fundamental concepts, apparatus, and specific uses of gas chromatography and mass spectrometry for the identification of organic substances. Letters Analytical. 2007 May 1;40(6):1003–12.

The basic ideas and tools of the analytical technique known as gas chromatography mass spectrometry (GC-MS) are covered in this succinct review. The demonstration of GC-MS's suitability for a range of inquiries intended to identify organic compounds from throughout the world highlights how widely accepted and used the technology is. A few examples demonstrate how GC-MS is a crucial and useful component of several field investigations involving the measurement and identification of organic compounds.

2. **Mohammedberhan A, Zewdu B, Zewdneh S. Otostegia integrifolia leaf extract in methanol In rats with diabetes, high glucose, and healthy rodents, beth lowers blood glucose levels. 2015;15(19) BMC Complementary & Alternative Medicine.**

The antidiabetic properties of Otostegia integrifolia in rodents were examined in this study. For the tests of glucose tolerance, hypoglycemia, and diabetes, male rats or mice were split into five groups. Different times were used to measure the blood glucose levels. The fourth-hour fasting blood BGL was considerably lower in the O. integrifolia 100 mg/kg and 200 mg/kg group as compared to the control group. An intra-group analysis revealed that BGL was significantly lower at the 1st, 2nd, 3rd, and 4th hours post-treatment at 200 mg/kg of O. integrifolia than it was at baseline. O. integrifolia extract at 200 mg/kg demonstrated a substantial reduction in blood glucose levels in the hypoglycemic and OGTT models when compared to negative controls and at all time periods.

3. **Gupta SS: Opportunities and viewpoints for natural plant products in healthcare. Indian Journal of Pharmacology, 26(1), 1-2, Jan. 1, 1994.**

The vast majority of individuals generally use herbal treatments, particularly those who reside in rural areas. For many of the medicinal plants that have been researched, there is an abundance of published scientific data. Very few plant-based drugs have reached the clinical stage, and not even

twelve plant-based treatments could be found in the National Formulary. For this reason, significant work goes into producing herbal medicines that have medicinal potential. From a broader perspective, this article looks at several plants that have been connected to antiviral, anti-allergic, anti-inflammatory, anti-atherosclerotic, anti-fertility, and antidiabetic qualities.

CHAPTER FIVE

METHODS AND MATERIALS

5.1.1 Plant Collection

Several writers have recently focused on the flora of Bangladesh, identifying many species as new to the nation—*Xantolis assamica* being one among them (31). Leaves of xoantolis assamica were gathered for this study from the Hazarikhil Wildlife Sanctuary in Fatikchari, Chattogram.

Figure-2: Xantolis assamica leaves collection.

5.1.2 Preparation of Methanolic Extraction

Unrefined medicine is kept separate from the plant and animal and used without modifying its chemical makeup. I organized raw concentrate in several steps.

Firstly, the leaves of the plant were collected from Hazarikhil Wildlife Sanctuary, Fatikchari, Chattogram.

↓

The plant was air-dried with a fan for seven days after being sun-dried for two days.

↓

I used a blender to grind the plant's leaves into fine powder after thoroughly drying them.

↓

I got 400g powder and kept it in an amber glass bottle

↓

After that, the powder and 2.5 liters of methanol were added to the Soxhlet apparatus.

↓

In Soxhlet apparatus, extraction presses were operating for four days.

The fluid concentration was evaporated with a rotary evaporator.

After being kept in a cold location for two days, the extract was split into methanol and the extract solution was dried with a hair drier.

I took 98.5g of crude extract at last.

5.2 Phytochemical Profiling by GC-MS

Gas chromatography mass spectrometry plays a pivotal role in identifying and measuring volatile and semi-volatile organic compounds within intricate combinations. In various scenarios, the utilization of this method proves highly beneficial as it can determine the molecular weights and, in occasional instances, the elemental compositions of unfamiliar organic compounds found in intricate mixtures. GC-MS is commonly employed for quantifying pollutants present in both drinking water and wastewater, playing a fundamental role in EPA-endorsed protocols. Furthermore, the measurement of drugs and their byproducts in biological fluids like blood and urine is achievable through this approach. Applications in forensics and pharmacology are both important. By comparing spectra with reference spectra or by interpreting spectra a priori, GC-MS can be utilized to identify unknown organic substances. Analyzing industrial products to ensure their quality control and the identification of reaction products by synthetic organic chemists are other common uses. The organic compounds need to be in solution in order to be injected into the gas chromatograph in order to perform GC-MS. Hexane or dichloromethane are two examples of volatile, organic solvents. Typical analytical sensitivities for each component vary from 1 to 100 pg, contingent upon the ionization method. Sample preparation can be as easy as partially dissolving the sample in an appropriate solvent or as involved as performing intensive cleanup techniques with several types of liquid chromatography. The duration of the gas chromatographic run, which normally lasts between 20 and 100 minutes, dictates the instrumental analysis time in addition to the time needed for sample preparation. A further hour to twenty hours (or longer) may be needed for data processing, depending on the level of information required. GC-MS has certain drawbacks. GC-MS can only examine substances whose vapor pressures are greater than 10^10 torr. Analyzing many substances with lower pressures is possible if they are chemically derivatized (trimethylsilyl ethers, for instance). On aromatic rings, positional substitution is frequently hard to

determine. Certain isomeric chemicals, such as naphthalene and azulene, can frequently be separated chromatographically but cannot be differentiated by mass spectrometry. Overall analytical process calibration controls quantitative correctness. An accuracy of ±20% relative standard deviation is normal when using isotope internal standards (32).

The plant extract was analyzed at the Bangladesh Reference Institution of Chemical Management (BRICM) using gas chromatography in conjunction with electron ionization quadrupole mass spectrometry (GC-MS).

5.3 Thrombolytic activity test of sample

5.3.1 Principle

Once blood artery damage has caused bleeding, thrombocytes, also referred to as platelets, aggregate with coagulation factors to create a blood clot. A thrombus, or blood clot, forming inside a blood vessel, is called thrombosis. Blood cannot flow through the circulatory system properly as a result of it. As blood clots form in veins, venous thromboembolism follows. Potential outcomes include pulmonary embolism and deep vein thrombosis. Heart attacks and strokes can result from atherothrombosis, which is the buildup of clots in the arteries.

5.3.2 Test apparatus for thrombolytic activity

Serial No.	Apparatus
1	Test Tube
2	Glass Rod
3	Vortex Mixture
4	Eppendorf Tube
5	Eppendorf Tube Rack
6	Beaker (50ml)
7	Incubator
8	Cotton
9	Syringe
10	Syringe Filter
11	Micropipette

Table -1: Test apparatus for thrombolytic activity

5.3.3 Reagent of thrombolytic activity test

Serial No.	Content
1	Blood (Human)
2	Streptokinase
3	Distilled Water
4	Sample Extraction

Table -2: Reagent used in thrombolytic activity test

5.3.4 Procedure

5.3.4.1 Preparing an extract solution for thrombolytic action

100 milligrams of the plant extract were mixed in 10 milliliters of distilled water and thoroughly agitated on a vortex mixer. The mixture was left undisturbed overnight, after which the clear liquid portion was separated by decanting and subsequently filtered through Whatman No. 1 filter sheets. This method was employed to assess the effectiveness of the mixture in dissolving blood clots in a controlled laboratory environment. (33).

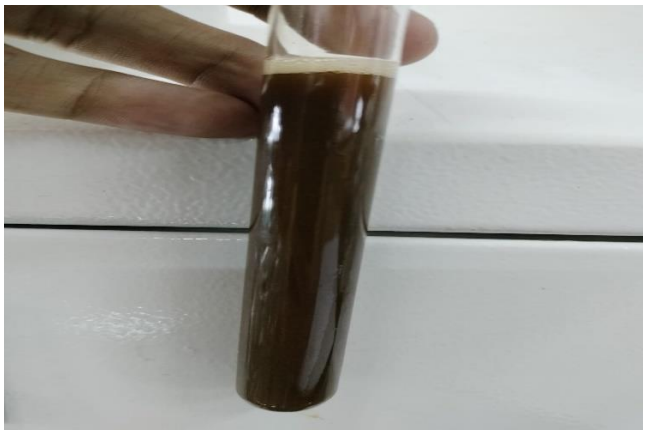

Figure-3: Preparing extract solutions for thrombolytic action.

5.3.4.2 Preparation of Streptokinase (SK) Solution as a Standard

The standard solution used for the 15,000,000 I.U. lyophilized ATPase (Streptokinase) alpine tube was commercially available from Beacon Pharmaceutical Ltd. Following this, five parts of water were mixed with the streptokinase in the vial, which was then thoroughly stirred. This resulting suspension of 100µl (30,000 I.U.) was utilized for in vitro thrombolysis (33).

5.3.4.3 Preparation of Eppendorf Tube

Three Eppendorf tubes underwent sanitization, weighing, and cleaning, followed by disinfection with distilled water. Subsequently, each tube was distinguished by marking them with a permanent marker to differentiate them from one another.

5.3.4.4 Collection of blood sample

A healthy male volunteer between the ages of 20 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.

5.3.4.5 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.

↓

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

↓

The tube was utilized to weigh the original blood clot following the extraction of the serum.

↓

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.

↓

* Administer the typical dosage of 100µl or 30000 IU and withdraw 100µl or 1000µg/100µl while utilizing 100µl of distilled water for comparison.

↓

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

↓

"The tube containing the blood fluid used for lysis was extracted.".

↓

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

↓

Weight of the second clot is equivalent to the weight of the second clot with the tube subtracted by the weight of the blank tube.

↓

Finally, weight of Lysis clot = first clot weight – second clot weight

↓

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

Figure-4: First and Final Clot of Blood (DIU Lab)

5.4 Experimental Animals

From Jahangirnagar University in Dhaka, Bangladesh, 30-35 gramme Swiss Albino mice were procured. At five to six weeks of age, the mice were housed in standard settings, which included a 12-hour light-dark cycle, a temperature range of 22 to 25 °C, and a humidity level of 60 to 70%. The animals spent a set amount of time enclosed in cages. Twelve hours of light and twelve hours of darkness make up the light cycle. The mice were fed traditional pelleted diet obtained from Dhaka's Jahangirnagar University. The animals used in this study were carefully bred in accordance with the guidelines our lab uses when doing research on animals.

Figure-5: Swiss Albino Mice.

5.5 Oral Glucose Tolerance Test (OGTT)

Overnight fasting Swiss Albino mice were employed for the Oral Glucose Tolerance Test (OGTT). The study involved four groups of mice receiving distinct treatments: 250 mg/kg of leaf extract, 500 mg/kg of leaf extract, 2 mg/kg of glimepiride, and a control group administered with distilled water. Following pretreatment with either distilled water, glimepiride, or leaf extract, a glucose solution was administered to each mouse 30 minutes later at a dose of 2 grams per kilogram. Blood glucose levels of the mice subjected to the glucose load were subsequently measured at baseline (-30 minutes), as well as at 60 and 120 minute post-administration. Blood glucose levels were assessed using a glucometer, specifically the UNICHECK (EBCARE) Diabetes Machine manufactured by Visgeneer, along with compatible blood glucose test strips (34).

Figure-6: Oral Glucose Tolerance Test on Swiss Albino Mice.

CHAPTER SIX

RESULT AND DISCUSSION

6.1 Result of GC-MS-Based Phytochemical Analysis of Plant Extracts at Bangladesh reference Institution for Chemical Measurements (BRICM)

Upon analysis, 61 distinct compounds were identified. However, only20 of these compounds had an area exceeding 1% in the overall ionic chromatogram, indicating their prominence in the sample. Figure 6 presents the Total Ionic Chromatogram, providing an overview of the compound distribution. The key compounds identified, their retention times (RT), area percentages, compound names, and chemical classifications are detailed in Table 3

Library Search Report

Sample Information

Sample ID : MSD24032501
User ID : NA
Method File : G:\GC-MSMS\2023\plant extract\METHOD/Plant Extract_CutT3.5_V 0.5_11.09.2023
Data Path : G:\GC-MSMS\2024\plant extract\DATA\2024.03.28
Acq on : 28.03.2024
Solvent used : Methanol

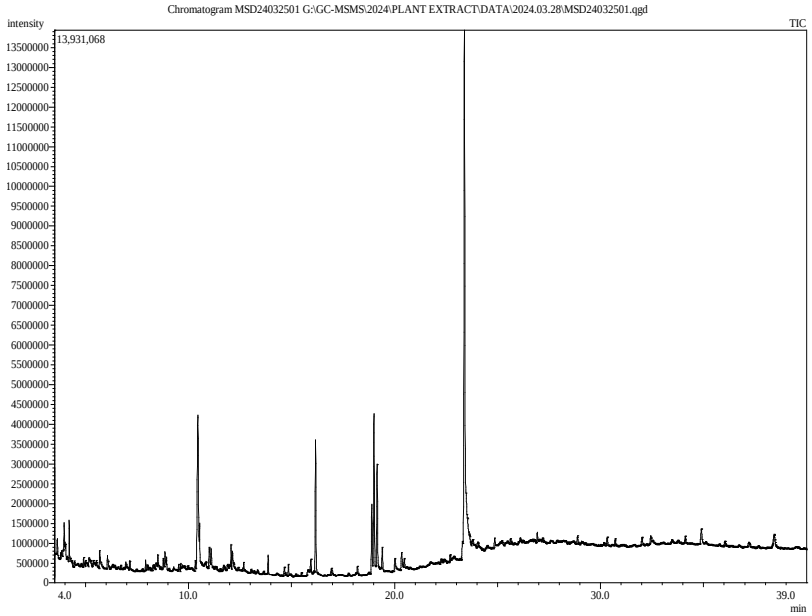

Figure-7: Total Ionic Chromatogram

6.1.1 Most Probable Compounds

SL.NO.	R. TIME	AREA %	COMPOUND	Group
1	3.529	1.42	N-Methyl- I -adamantaneacetamide	Amide
2	23.39	26.03	9-Octadecen amide, (Z)	Amide
3	3.609	1.35	1, 2-B is(trimethylsilyl)benzene	Aromatic hydrogen
4	24.037	1.33	Triacontane, 1-bromo-	Alkane
5	24.519	1.22	7-Hexadecenal, (Z)	Aldehyde
6	24.87	1.24	1 8-Methyl-nonadecane- 1,2-dio, trimethylsilyl ether	Alcohol Derivative
7	19.159	3.86	Phytol	Diterpene
8	10.413	4.18	2-(Isobutoxymethyl) oxirane	Epoxid
9	16.173	2.59	Hexadecanoic acid, methyl ester	Fatty Acid Ester
10	19.001	3.09	9,12,1 5-Octadecatrienoicacid, methyl ester, (Z,Z,Z)	Fatty Acid Ester
11	19.412	1.35	Methyl stearate	Fatty Acid Ester
12	24.925	1.29	Pentalene, octahydro-2- [(2-octyl)decyl]	Hydrocarbon
13	3.99	2.32	3, 3 -Dimethoxy-2-butanone	Ketone
14	11.096	1.02	Phenol, 3,5-bis(1, 1-dimethylethyl)	Phenol
15	32.542	1.04	2,2' -Methylenebis-(6-tert-buty1)-4-ethylphenol, O Pentafluoropropionyl-	Phenol Derivative
16	24.786	1.84	C(1 4a)-Hom o -27 -nor gammacer- 13 -en -21 -one, 3 -methoxy-, (3. alpha.)	Steroid
17	28.226	13.35	Lupeol	Triterpene
18	30.331	1.84	Squalene	Triterpene
19	34.901	4.73	Onocerin	Triterpenoid
20	39.266	4.69	2-(3-Acetyloxy -4,4,10,13, I 4-pentamethyl-Z,3,5,6,7,1 7,12,1 5,16,17 decahydro- 1	Triterpenoid

Table-3: Probable compound and their classes of the Plant Extract of *Xantolis assamica*.

Discussion

The GC-MS analysis of the methanolic extracts of *Xantolis assamica* leaves revealed the presence of various phytochemical compounds belonging to different chemical groups. Notable compounds include fatty acid esters such as hexadecanoic acid methyl ester and methyl stearate, as well

triterpenes like lupeol, squalene, and onocerin. Additionally, diterpenes like phytol and steroids like C(14a)-Hom o-27-nor gammacer-13-en-21-one were identified. The leaves of *Xantolis assamica* exhibit a variety of medicinal properties, such as antimicrobial, antioxidant, and anti-inflammatory effects, indicating their potential therapeutic benefits. Further investigations are warranted to elucidate the specific biological activities and mechanisms of action associated with these compounds, contributing to the development of novel natural remedies.

6.2 Result of Thrombolytic Activity Test

Sample	**Blank tube weight (gm.)**	**1st clot + tube weight (gm.)**	**1st clot weight (gm.)**	**2nd clot + tube weight (gm.)**	**2nd clot weight (gm.)**	**Lysis weight (gm.)**	**% of lysis**
Control (Distilled water)	0.80	1.679	0.89	1.46	0.67	0.22	5%
Standard (Streptokinase)	0.860	1.870	1.01	1.10	0.24	0.77	76%
Xantolis assamica Leaf Extract	0.82	1.671	0.851	1.391	0.571	0.28	32%

Table-4: Result of Thrombolytic Activity Test.

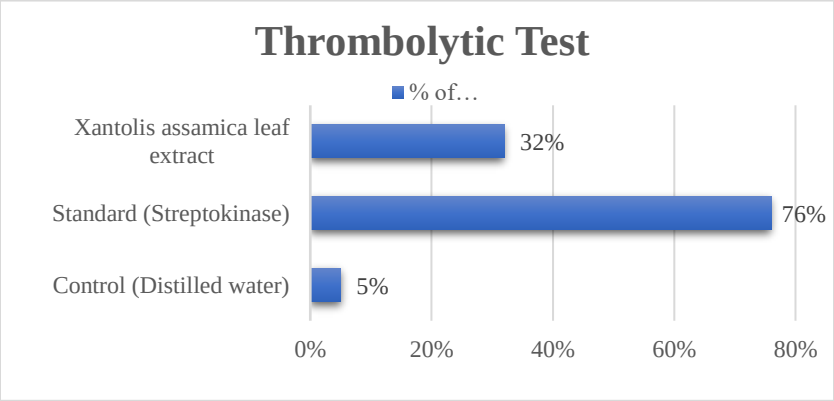

Figure-8: Graphical representation of Thrombolytic Activity Test.

Discussion

Streptokinase used as the standard which was previously reported 76% clot lysis. The clot lysis rate in distilled water (Control) was 5%. Methanol extract of Polygonum Xantolis assamica plants showed 32% clot lysis in research. The result of thrombolytic activity of the methanol extract of Xantolis shown excellent antioxidant activity when compared with standard (Streptokinase).

6.3 Oral Glucose Tolarance Test (OGTT)

Treatment	Blood glucose levels (mmol/L)		
	-30 min	60 min	120 min
Control (D.W)	9.63±0.15	9.16±1.30	5.36±2.12
Standard (Glimepiride)	8.93±1.21	7.63±1.01	6.27±0.88
Plant Extract-250	9.1±0.56	7.9±0.52	6.2±1.27
Plant Extract-500	8.7±0.17	7.47±0.33	5.33±0.78

Table -5: The oral glucose tolerance test (OGTT) result.

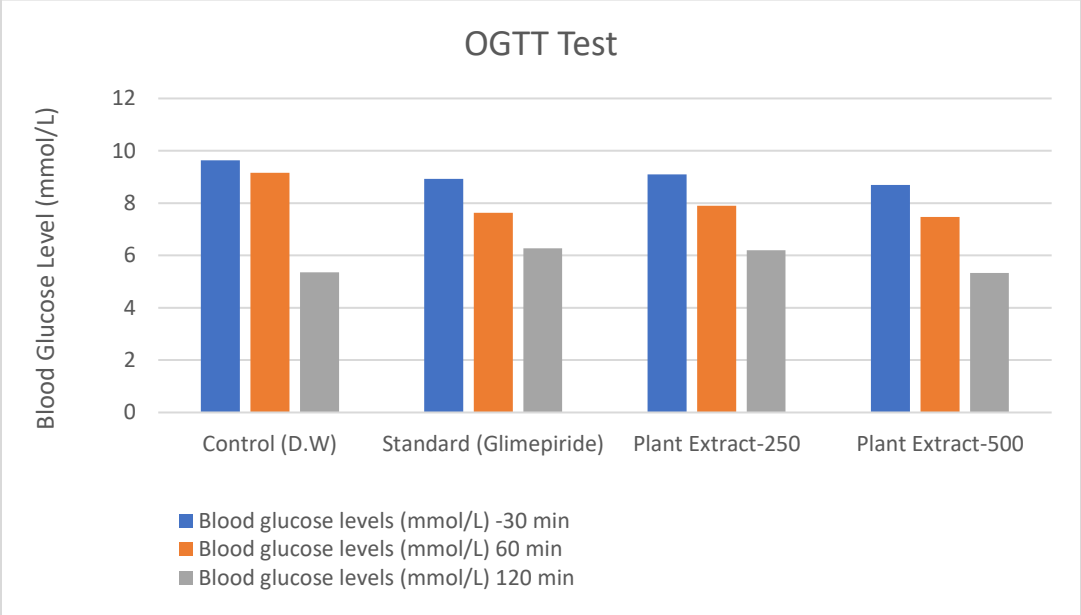

Figure-9: Graphical Presentation of OGTT Test.

Discussion:

Using the oral glucose tolerance test, the glucose content of the blood samples was measured at -30, 60, and 120 minutes, respectively. The groups treated with 500 mg/kg of plant extract had significantly lower blood glucose levels than the control and standard treatment groups (Glimepiride) at -30, 60, and 120 minutes. After 60 minutes, the group treated with plant extract (250 mg/kg) had higher blood glucose levels than the standard group (glimepiride).

Chapter Seven

Conclusion

7.0 Conclusion

The methanolic extract from *Xantolis assamica* leaves contains a diverse range of phytochemicals, including fatty acid esters, triterpenes, diterpenes, and steroids, suggesting potential therapeutic benefits such as antibacterial and antioxidant activities. The extract exhibited significant thrombolytic activity with a clot dissolution rate of 32%, indicating its potential as a natural alternative to conventional thrombolytic drugs. Furthermore, the extract demonstrated antidiabetic potential as evidenced by the oral glucose tolerance test (OGTT), where mice treated with the extract showed reduced blood glucose levels at 120 minutes compared to control and standard groups. Specifically, administration of the extract at dosages of 250 mg/kg and 500 mg/kg body weight resulted in blood glucose levels of 6.2±1.27 and 5.33±0.78 mmol/L, respectively, compared to 6.27±0.88 mmol/L in the standard group and 5.36±2.12 mmol/L in the control group. These findings validate the traditional medicinal uses of *Xantolis assamica* and highlight its promising therapeutic potential, warranting further research for the development of novel treatments.

Chapter Eight

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