Phytochemical investigation & Evaluation of Anti-helminthic and Thrombolytic Activity of Methanolic Extract of Tagetes (Marigold Flower)

Project Report

[In the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy]

Submitted To

Department of Pharmacy

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APPROVAL

This project paper, **"Phytochemical investigation & Evaluation of Anti-helminthic and Thrombolytic Activity of Methanolic Extract of *Tagetes* (Marigold Flower)",** submitted to the Department of Pharmacy, Faculty of Health & Life Sciences Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Bachelor programmed and approved as to its style and contents.

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DECLARATION

I hereby declare that this project report **"Phytochemical investigation & Evaluation of Anti-helminthic and Thrombolytic Activity of Methanolic Extract of *Tagetes* (Marigold Flower)",** is done by me under the supervision of Ms. Tahmina Afroz Assistant Professor Department of Pharmacy, Faculty of Health & Life Sciences, Daffodil International University. I am declaring that this project is my original work. I also declare that neither this project nor any part therefore has been submitted elsewhere for the award of Bachelor or any degree.

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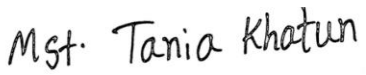

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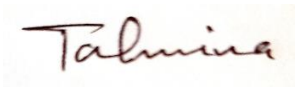

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I might want to communicate my profound applause to the All-powerful Allah who has given me the capacity to finish my undertaking work and the chance to concentrate in this subject.

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My Parents,

The persons who always encourage me in every sphere of my life.

My teacher,

The persons who guided me in this process and the faculty who kept me on track.

Abstract

The study aimed to evaluate the anti-helminthic and thrombolytic activity of the methanolic extract of Marigold flower through a series of in vitro assays. Phytochemical screening of the extract revealed the presence of various bioactive compounds, including alkaloids, glycosides, tannins, steroids, phenols, carbohydrates, gums, and reducing sugars, while saponins were absent. The thrombolytic activity was assessed by measuring the percentage of clot lysis, where the Marigold flower extract demonstrated a clot lysis percentage of 80.50%, compared to 9.90% for the control and 91.03% for the standard streptokinase. The anti-helminthic activity was evaluated using different concentrations (5, 10, and 20 mg/mL) of the methanolic extract. The extract at 20 mg/mL concentration induced paralysis in 18 minutes and death in 46 minutes, closely resembling the standard drug Albendazole, which showed paralysis and death at 16 minutes 52 seconds and 44 minutes, respectively. These findings indicate that the methanolic extract of Marigold flower possesses notable thrombolytic and anti-helminthic properties, making it a promising candidate for further pharmacological studies.

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CHAPTER ONE
INTRODUCTION

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1.1 Introduction

Medicinal plants have long played a significant role in traditional healthcare systems, providing potential sources of therapeutic agents. One such plant is the marigold (Tagetes spp.), which is well-known for its ornamental value but also recognized for its various pharmacological properties [1]. Among the active compounds found in marigold flowers are flavonoids, terpenoids, and essential oils, which are believed to contribute to the plant's medicinal benefits. Anthelmintic drugs are widely used to treat parasitic worm infections, a common health problem, particularly in developing countries. Resistance to current anthelmintic agents, however, is increasing, leading to a need for new, effective alternatives. Similarly, thrombolytic agents, which help in dissolving blood clots, are critical for managing cardiovascular diseases such as heart attacks and strokes [2]. The search for natural thrombolytic agents is of growing interest as they offer potential benefits with fewer side effects compared to synthetic drugs. In this study, we aim to evaluate the anthelmintic and thrombolytic activity of the methanolic extract of marigold flowers. The choice of methanolic extract is based on the solvent's ability to extract a wide range of bioactive compounds. By assessing both the anthelmintic and thrombolytic properties, this research seeks to explore the therapeutic potential of marigold, contributing to the development of plant-based treatments for parasitic infections and clot-related disorders [3].

Figure 1: *Tagetes* (Marigold Flower)

1.2 Role of Phytochemicals in Marigold flower Plant Defense and Human Health

Phytochemicals are bioactive compounds produced by plants that play crucial roles in both plant defense mechanisms and human health. These natural substances, which include alkaloids, flavonoids, terpenoids, and phenolic acids, have been widely studied for their potential benefits. Here's how they contribute to both plant defense and human health:

Role of Phytochemicals in Plant Defense

1. **Physical Defense**: Phytochemicals can form protective barriers, such as waxes and lignins that make plants more resistant to physical damage or pest invasion.
2. **Chemical Defense**: Many phytochemicals act as deterrents against herbivores, insects, and pathogens:
    - **Alkaloids** (e.g., caffeine, nicotine): Toxic too many pests and insects.
    - **Phenolic Compounds** (e.g., tannins): Bind to proteins in herbivore saliva, reducing digestibility.
    - **Terpenoids** (e.g., essential oils): Emit strong odors that repel insects and microbes.
    - **Glucosinolates** (in cruciferous plants): Produce toxic compounds when plants are damaged, deterring herbivores and pathogens [4].
3. **Antimicrobial Properties**: Some phytochemicals inhibit the growth of fungi, bacteria, and viruses that could damage plants. For example, saponins and phytoalexins prevent infection by creating a toxic environment for pathogens.
4. **Signal Molecules**: Phytochemicals like salicylic acid (a precursor to aspirin) help trigger plant defense responses, such as the activation of systemic acquired resistance (SAR), which protects the plant from further attacks [5].

Role of Phytochemicals of Marigold flower in Human Health

1. **Antioxidant Properties**: Phytochemicals like flavonoids, carotenoids, and phenolic acids neutralize free radicals in the human body, reducing oxidative stress

and lowering the risk of chronic diseases such as cancer and cardiovascular diseases [6].

2. **Anti-inflammatory Effects**: Certain phytochemicals like curcumin (from turmeric) and resveratrol (from grapes) have potent anti-inflammatory effects. They help reduce inflammation linked to conditions like arthritis, diabetes, and Alzheimer's disease.
3. **Cancer Prevention**: Many phytochemicals, including sulforaphane (from broccoli) and lycopene (from tomatoes), have been shown to inhibit cancer cell growth, induce apoptosis (programmed cell death), and prevent the formation of carcinogens in the body [7].
4. **Cardiovascular Health**: Phytochemicals like polyphenols found in tea, grapes, and cocoa improve heart health by lowering blood pressure, reducing LDL cholesterol, and improving endothelial function.
5. **Antimicrobial and Antiviral Properties**: Some phytochemicals, such as allicin (from garlic) and epigallocatechin gallate (EGCG, found in green tea), have antimicrobial effects that help fight infections and boost the immune system.
6. **Hormonal Balance**: Phytochemicals like phytoestrogens (e.g., in soy) mimic estrogen, which can help alleviate menopausal symptoms and reduce the risk of hormone-related cancers like breast and prostate cancer [8].

Phytochemicals are essential for both plant defense and human health. In plants, they protect against environmental stresses, pests, and pathogens, while in humans, they contribute to disease prevention, immune support, and overall health promotion. Their multifaceted roles make them valuable both in agriculture and nutrition [9].

1.3 Bioactive Components in Edible and Medicinal Plants

Edible and medicinal plants have been revered for centuries across diverse cultures for their therapeutic and nutritional benefits. The health-promoting properties of these plants are largely attributed to the presence of bioactive components-naturally occurring compounds that offer physiological benefits beyond basic nutrition. These compounds, which include alkaloids, flavonoids, polyphenols, terpenoids, and glycosides, play crucial roles in promoting wellness, preventing diseases, and supporting overall health [10].

Bioactive components exhibit a range of biological activities, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. As scientific research has progressed, there has been a growing interest in understanding how these compounds interact with the human body at molecular levels, providing evidence-based support for their use in both traditional medicine and modern therapeutic applications [11]. In addition to their health benefits, bioactive components found in edible plants enhance food preservation and flavor, making them valuable not only in health care but also in the food and pharmaceutical industries. This exploration into bioactive compounds opens new avenues for developing functional foods, dietary supplements, and plant-based medicines aimed at promoting health and preventing chronic diseases [12].

1.4 Chemical Composition of marigold flowers

Marigold flowers (Tagetes species), widely known for their vibrant colors and distinctive fragrance, are not only ornamental but also possess a rich and diverse chemical composition. These compounds contribute to the flower's medicinal, cosmetic, and agricultural applications. Key chemical constituents of marigold flowers include flavonoids, carotenoids, essential oils, saponins, phenolic acids, sterols, and tannins [13]. The bright pigments, such as lutein and β-carotene, are responsible for the flowers' vivid hues and have antioxidant properties, while flavonoids like quercetin and luteolin provide anti-inflammatory and antimicrobial benefits. Marigold's essential oils, rich in limonene and ocimene, give the flowers their distinctive aroma and offer additional uses in perfumery and pest control. These bioactive compounds make marigolds a valuable resource in herbal medicine, cosmetics, and sustainable agriculture [14].

1.5 Therapeutic use and pharmacological activity of marigold flowers

Marigold flowers (*Tagetes* species) have long been valued in traditional medicine for their therapeutic uses and pharmacological activities. Their rich chemical composition, including flavonoids, carotenoids, essential oils, and other bioactive compounds, contributes to a wide range of health benefits [15]. Here are some key therapeutic uses and pharmacological activities of marigold flowers:

1. Anti-inflammatory Activity

Marigold flowers, particularly due to their flavonoid and phenolic content, exhibit significant anti-inflammatory properties. These compounds inhibit inflammation pathways, making marigold useful for soothing skin irritations, wounds, and inflammatory conditions like arthritis [16].

2. Antioxidant Properties

The presence of carotenoids (such as lutein and β-carotene) and flavonoids in marigold flowers gives them potent antioxidant activity. These compounds neutralize free radicals, reduce oxidative stress, and protect cells from damage, potentially lowering the risk of chronic diseases, including cancer and heart disease.

3. **Antimicrobial and Antifungal Activity**

Marigold essential oils (such as limonene, ocimene, and tagetone) possess antimicrobial and antifungal properties, making the flower effective against bacterial and fungal infections. This activity has been traditionally utilized to treat skin infections and promote wound healing.

4. Wound Healing

Marigold extracts have been widely used in topical formulations for wound healing. Their anti-inflammatory, antimicrobial, and astringent properties accelerate the healing process and reduce scarring, which is why marigolds are often used in ointments and skin creams [17].

5. Eye Health

The high lutein content in marigold flowers supports eye health by protecting against macular degeneration and cataracts. Lutein is known for filtering harmful blue light and improving visual function.

6. Gastroprotective Activity

Marigold extracts are known to have protective effects on the stomach lining, reducing gastric inflammation and preventing ulcers. This activity is attributed to the flower's antioxidant and anti-inflammatory compounds.

7. Antispasmodic and Analgesic Effects

Marigold flowers have antispasmodic properties, helping to alleviate muscle spasms, cramps, and gastrointestinal discomfort. They may also act as a mild analgesic, reducing pain in various conditions [18].

8. Anticancer Potential

The antioxidant and anti-inflammatory properties of marigold compounds are being studied for their anticancer potential. The bioactive constituents may help inhibit the growth of cancer cells and prevent tumor formation.

9. Insecticidal and Repellent Properties

Marigold essential oils are used as natural insecticides and repellents. Their compounds, particularly tagetone and limonene, are effective against various pests and are often utilized in eco-friendly pest control solutions.

10. Skin Care and Cosmetic Use

Marigold flowers are widely used in skincare products for their soothing, anti-aging, and hydrating properties. Their ability to promote wound healing, reduce inflammation, and protect against oxidative stress makes them popular in creams, lotions, and serums.

The therapeutic uses and pharmacological activities of marigold flowers are diverse, ranging from anti-inflammatory and antimicrobial effects to antioxidant and wound-healing benefits. These properties have made marigold a valuable plant in both traditional and modern medicine, as well as in cosmetic formulations and sustainable agriculture [19].

1.6 Anti-helminthic evaluation

Anthelmintics are drugs used to treat infections caused by parasitic worms (helminthes) in both humans and animals. These drugs are primarily used to target and eliminate worms such as roundworms, tapeworms, and flukes. The evaluation of Anthelmintics involves several steps to ensure their effectiveness, safety, and optimal use.

1. **Efficacy Testing:**
    - **In Vitro Testing:** Anthelmintic activity is first tested in controlled laboratory environments on worm larvae or adult parasites to assess the drug's direct effect.
    - **In Vivo Testing:** After in vitro studies, the drug is tested on live animals or humans infected with helminths. The reduction in worm load, fecal egg count, and symptom alleviation are evaluated [20].
2. **Mechanism of Action:**
    - Understanding how the drug works is crucial. Common mechanisms include paralysis of the parasite (e.g., via muscle depolarization) or disruption of nutrient uptake, leading to starvation of the parasite.
3. **Safety and Toxicity:**
    - **Acute and Chronic Toxicity:** The potential toxic effects of the drug on the host organism (humans or animals) are evaluated. This includes checking for side effects and long-term impacts.
    - **Therapeutic Index:** The ratio of the effective dose to the toxic dose is determined to ensure that the drug is safe at therapeutic levels [21].
4. **Pharmacokinetics and Pharmacodynamics:**

- **Absorption, Distribution, Metabolism, and Excretion (ADME):** How the drug is absorbed into the bloodstream, distributed in tissues, metabolized by the liver, and excreted (usually in urine or feces) is studied.
- **Drug Bioavailability:** The concentration of the drug in the body and how long it remains effective is evaluated.

5. **Resistance Monitoring:**
    - Over time, parasitic worms can develop resistance to anthelmintic drugs. Monitoring for signs of resistance, especially in livestock, is important. This often involves repeated treatments and assessing the worm load reduction over time.
6. **Clinical Trials:**
    - **Phase I, II, and III Trials:** New Anthelmintics are subjected to clinical trials to assess safety in healthy individuals (Phase I), efficacy in infected individuals (Phase II), and large-scale efficacy and safety testing (Phase III) [22].
7. **Field Trials:**
    - In the case of veterinary Anthelmintics, field trials are conducted in livestock or wild animals to assess the practical application, effectiveness, and economic benefits of the treatment in real-world conditions.
8. **Formulation Development:**
    - Development of an appropriate formulation (tablets, capsules, suspensions, etc.) that ensures stability, ease of administration, and good bioavailability is also critical.
9. **Regulatory Approval:**
    - Before an anthelmintic can be marketed, it must be approved by regulatory authorities like the FDA (Food and Drug Administration) or EMA (European Medicines Agency), based on efficacy, safety, and quality control data [23].

1.7 Thrombolytic evaluation

Thrombolytic evaluation is essential for determining the effectiveness and safety of treatments designed to dissolve blood clots, a process known as thrombolysis. Blood clots, or thrombi, can block blood flow in vessels, causing serious conditions such as heart attacks, strokes, and pulmonary embolism. Thrombolytic therapy works to restore blood flow by breaking down these clots, preventing tissue damage and improving patient health. In vivo studies are crucial for assessing the thrombolytic potential of various drugs and treatments in living organisms, often using animal models that closely resemble human biology. These studies provide important insights into the effectiveness, mechanisms, and potential side effects of thrombolytic agents under realistic physiological conditions [24].

Several techniques are commonly used to evaluate thrombolytic efficacy:

- ❖ Animal models:

Rodents (like mice and rats) and larger animals (such as rabbits or pigs) are used to replicate human thrombotic conditions. Blood clots are induced in certain vessels, like the coronary or cerebral arteries, and treated with thrombolytic agents to study clot breakdown and the restoration of blood flow.

- ❖ Blood flow measurements:

Tools like Doppler ultrasound or laser Doppler flowmetry are used to monitor blood flow before and after treatment, helping to measure the extent of clot dissolution and the restoration of vessel function.

- ❖ Histological analysis:

Examining the clots and surrounding tissues reveals the structural changes caused by thrombolytic treatments, including clot breakdown, tissue reperfusion, and potential adverse effects like bleeding or tissue damage [25].

- ❖ Coagulation parameters:

Besides monitoring clot dissolution, these evaluations also check coagulation markers like activated partial thromboplastin time (aPTT) and prothrombin time (PT) to assess the broader effects of thrombolytic agents on blood clotting and hemostasis.

Results from these in vivo studies help refine thrombolytic treatments, guiding the development of candidates for clinical trials. They also highlight potential risks and inform strategies to enhance the safety and effectiveness of thrombolytic therapies in practice. Ultimately, these evaluations play a vital role in understanding how blood clots dissolve and in translating innovative treatments from the lab to clinical use, with the goal of reducing thrombotic disease complications and improving patient outcomes [26].

CHAPTER TWO

PURPOSE OF THE STUDY

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2.1 Purpose of the study

The primary purpose of this study is to evaluate the anthelmintic and thrombolytic potential of the methanolic extract of **marigold (Tagetes spp.) flowers**. Marigold has been traditionally used in various medicinal practices, yet its efficacy in treating parasitic infections and clot-dissolving properties remains underexplored. This study aims to investigate the extract's effectiveness as an anthelmintic agent by assessing its activity against parasitic worms and to determine its thrombolytic activity by examining its ability to break down blood clots. By exploring these therapeutic potentials, the study seeks to contribute to the growing body of knowledge on natural remedies and potentially identify new, effective bioactive compounds for pharmaceutical applications.

CHAPTER THREE

LITERATURE REVIEW

CHAPTER THREE
LITERATURE REVIEW

Analysis 1:

Throughout history, medicinal plants have been vital to human healthcare, offering a wide range of phytochemicals. These phytochemicals primarily consist of complex compounds known as secondary metabolites. The secondary metabolites produced by plants have numerous therapeutic applications in both traditional and modern medicine. This growing interest is largely due to the high costs, reduced efficacy, and significant side effects of many synthetic drugs, along with the emergence of drug-resistant pathogens. Consequently, there has been a push to find safer, low-toxicity treatments derived from medicinal plants. Various secondary metabolites from different species in the Asteraceae family (Compositae) are significant in biomedicine, pest control, and other fields because they contain alkaloids, flavonoids, quinones, lignans, steroids, and terpenoids. Tagetes patula L. is a key species within Asteraceae, widely utilized in folk and modern medicine as well as in the cosmetic and pharmaceutical industries. This chapter discusses the phytochemical composition of various extracts of T. patula L. and reviews the current literature on its traditional medicinal uses, pharmacological effects, and biocidal properties [27].

Analysis 2:

The use of medicinal plant extracts and secondary metabolites is gaining popularity worldwide as a natural alternative to synthetic chemicals in both traditional and allopathic medicine. This article explores the medicinal properties of Tagetes erecta, commonly known as Genda Phul (marigold), based on research conducted between 2006 and 2014. Various parts of the plant are beneficial for conditions such as fevers, astringency, digestive issues, scabies, liver problems, eye diseases, blood purification, bleeding hemorrhoids, rheumatism, colds, and bronchitis. Tagetes erecta is rich in important phytochemicals and exhibits a range of pharmacological activities, including anti-nociceptive, anti-inflammatory, antioxidant, insecticidal, larvicidal, hepatoprotective, antipyretic, wound healing, antibacterial, antimicrobial, antiepileptic, and antifungal effects. This review summarizes the research conducted on the plant's chemical constituents, pharmacological

actions, toxicological studies, and its traditional and non-pharmacological applications over the years [28].

Analysis 3:

The flower extract of commercially available Aztec marigold (Tagetes erecta) was assessed for its in vitro antioxidant properties and in vivo analgesic effects on abdominal writhing induced by acetic acid. The findings demonstrated significant antioxidant potential in the Aztec marigold flowers, along with a dose-dependent analgesic effect at doses of 100 and 300 mg/kg. These antioxidant and analgesic activities align well with the traditional medicinal applications of Aztec marigold as an anti-inflammatory and pain-relieving agent. Copyright © 2008 John Wiley & Sons, Ltd [29].

CHAPTER FOUR

MATERIALS & METHODS

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MATERIALS & METHODS

4. Materials & methods

Examining the bioactive components present in plants is necessary for the analysis of phytochemicals. A range of methods and tools are used to extract, isolate, and characterize these compounds.

4.1 Description of plant extraction is given below:

Unprocessed medicine is extracted from the plant and animal and used without modifying its chemical composition. I organized raw concentration in several steps. Here's a general description of the process:

Firstly, the plant leaves was collected from my native area.

⇓

Undesirable material and the plant parts were removed from the experimental.

⇓

Then whole plant was clean by fresh water.

⇓

Then the plant leaves was dried under sun two days and air-dried for 14 days by fan.

⇓

After drying the plant leaves well, I have been crushed the plant into fine powders by blender.

⇓

Then we got **250 gm powder** and kept in five bags in an extraction chamber.

⇓

Then **2.5L of methanol** were taken in boiling flasks.

⇓

Started extraction process for 5 days by using Soxhlet apparatus.

⇓

Then collected the methanol extract.

⇓

Then evaporated by rotary machine.

⇓

Finally separated extract from methanol.

4.2 Extraction:

A glass jar with a plastic top was thoroughly cleaned before being given a methanol rinse. Subsequently, 1500 milliliters of methanol were poured to the jar containing 52 grams of the dry powder, before the surface of the sample was sufficiently coated, reaching a height of one inch above the jar. The jar was appropriately airtight, with a plastic cover that was coated with aluminum foil. The procedure described above was done for twelve days. To enhance the extraction procedure, the jar was shook nearly twice a day.

4.2.1 Filtration

After the process of extraction, sterile cotton and filter paper were used to filter the plant's extract. Both items were subsequently immersed in the required solvents and put within a funnel. The filtered material was stored in a glass jar.

Figure 2: Filtration of extract via funnel with filter paper

4.2.3 Evaporation

The liquid extraction was evaporated using an evaporator that rotates. At a temperature of 65 to 670 degrees Celsius and a spinning speed of 34 to 38 rotations per minute, methanol was evaporated. The liquefied material was transferred to a 50 milliliter beaker. After the pure material was gathered, the resultant solution was put just beneath the fan in beakers wrapped with aluminum foil. A cold hair drier was then used to completely eliminate the leftover water after the rest of the component in the resulting extract had begun to evaporate.

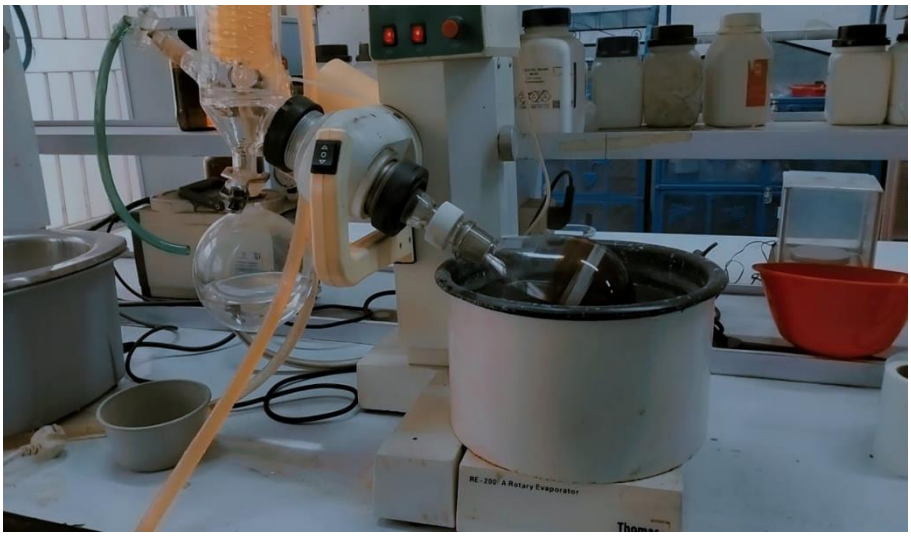


Figure 3: Evaporation of extract via Evaporator

4.3 My investigation

In my investigation I have been gadget

- Phytochemical Screening test
- Thrombolytic activity
- Anti-helminthic activity

4.4 Phytochemical Assessment

As part of the phytochemical evaluation process, plant phytochemicals are examined and assessed. Plants naturally contain bioactive molecules called phytochemicals, which may have positive effects on human health. These compounds help the plant prevent disease,

maintain links with its environment, and strengthen its defense mechanisms. Phytochemicals encompass a wide range of compounds, including as phenolic acids, alkaloids, flavonoids, terpenoids, saponins, and more.

4.4.1 Reagents applied for the diverse chemical group analysis

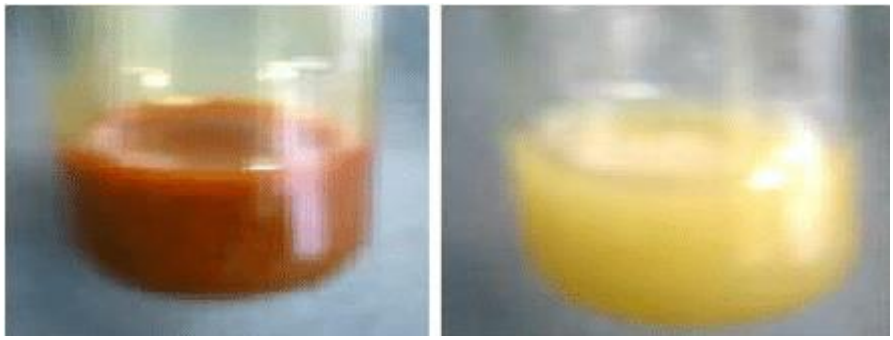
4.4.1 Assessment for alkaloids

Mayer's evaluation

Two milliliters of extort solution and 0.2 milliliters of diluted hydrochloric acid were put into a test tube. Then, 1 milliliter of Mayer's reagent was applied. The appearance of a precipitate with a yellow tint indicated the existence of alkaloids.

Dragendroff's examination

Two milliliters of extort solution and two milliliters of diluted hydrochloric acid were put into a test tube. Dragendroff's reagent (1 mL) was then added. An orange-brown precipitated was observed, indicating the possible involvement of alkaloids.



[a] **[b]**

A=Mayer's analysis

B= Dragendroff's analysis of extract

4.4.2 Assessment for Flavonoids

The presence of flavonoids is shown by the red color, which was obtained by adding 2 mL of droplets of strong hydrochloric acid to a 5 mL amount of an alcoholic solution of the plant component.

Figure 4: Flavonoids analysis of extract

4.4.3 Assessment for Saponins

After diluting 5 milliliter of the aqueous solution with 5 milliliters of distilled water, it was agitated in a glutted container for fifteen minutes. A foam layer just one centimeter thick indicates the presence of saponins.

Figure 5: Saponins analysis of extract

4.4.4 Assessment for Tannins

10 ml of purified water will be used to absorb 5g of extraction (Equivalent 2 mL). The mixture will then be filtrated, and the resultant filtrate will be treated with 2-3 drops of ferric chloride reagents. The formation of a blue-black, green, or blue-green precipitation suggests the presence of tannins.

Figure 6: Tannins analysis of extract

4.4.5 Assessment for Glycosides

0.2 mL of concentrated HCL was added after 2 mL of the sample was initially taken. The evaluation of yellow color indicated the existence of glycosides. Fehling liquid contains a tiny amount of a heated alcohol extract. The amount of red brick that precipitated was measured to ascertain the presence of glycosides. After the substance was combined with alcohol and water, a little portion was heated with a few drops of diluted sulfuric acid. After neutralizing Fehling solution with sodium hydroxide water, bring it to a boil. The red brick precipitated was assessed as a glycoside indication.

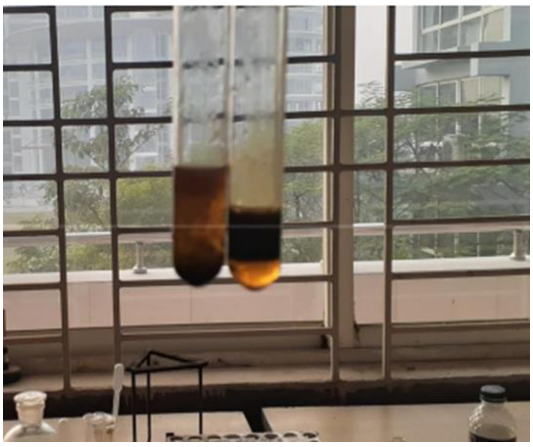


Figure 7: Glycosides analysis of extract

4.4.6 Test for Phenol

A 2-3 drops neutral ferric chloride solution was diluted with 2 milliliters of the extract; the dark blue coloring indicated the presence of phenolic chemicals.

4.4.7 Test for Steroid

One milliliter of concentrated H2SO4 was added to two milliliters of extract in a test tube. Bright yellow or red coloration.

4.4.8 Test for gums

Sulfuric acid was added after the resultant extract and Molish reagent were combined in five milliliters. A reddish-violet ring formed when two liquids were combined, indicating the existence of gums and carbs.

4.4.9 Test for Reducing Sugar (Benedict's test)

After mixing Benedict's reagent with one milliliter of the analyte sample, place the mixture in a boiling water bath and heat it for three to five minutes. The development of a brick-red-colored cuprous oxide precipitate indicates the presence of reducing sugars in the analyte.

4.4.10 Test for Carbohydrates

2 ml of concentrated H2SO4 plus 2-3 drops of Molisch's reagent and 2 ml of extract. The existence of carbohydrates was indicated by a red or purple ring.

4.5 Thrombolytic Activity Test

4.5.1 Equipment of thrombolytic activity test

Equipment	Description/Use
Centrifuge	Used to separate plasma or serum from blood samples.
Water Bath	Maintains samples at a constant temperature, usually required for incubation steps during thrombolytic assays.
Spectrophotometer/Plate Reader	Measures the absorbance of the sample, used to quantify clot lysis based on optical density.
Pipettes (Automatic/Micropipettes)	Used for precise measurement and transfer of reagents and samples during the assay.
Glass/Plastic Tubes	Holds blood samples or reaction mixtures.
Test Plates (96-well or others)	Microplates used to perform the assay in a high-throughput manner, typically for fibrinolysis detection.
Incubator	Maintains the optimal conditions (temperature, CO_2, humidity) required for cell or sample incubation.
Coagulation Analyzer	Specialized equipment for measuring coagulation parameters such as fibrinolytics activity.
Reagent Reservoirs	Holds reagents for easy pipetting.
Thrombin/Plasminogen Activator Kit	Specific kits for inducing and measuring thrombolysis.
Stirrer or Vortex Mixer	Used to mix samples or reagents evenly.
Fibrinogen Solution	A key reagent used to create a clot for assessing thrombolytic activity.
Clotting Activator (Calcium Chloride)	Used to trigger the clotting process in samples.
Timer	For precise measurement of incubation times during the assay.

Table 1: Equipment of thrombolytic activity test

4.5.2 Reagent of thrombolytic activity test

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

Table 2: Reagent of thrombolytic activity test

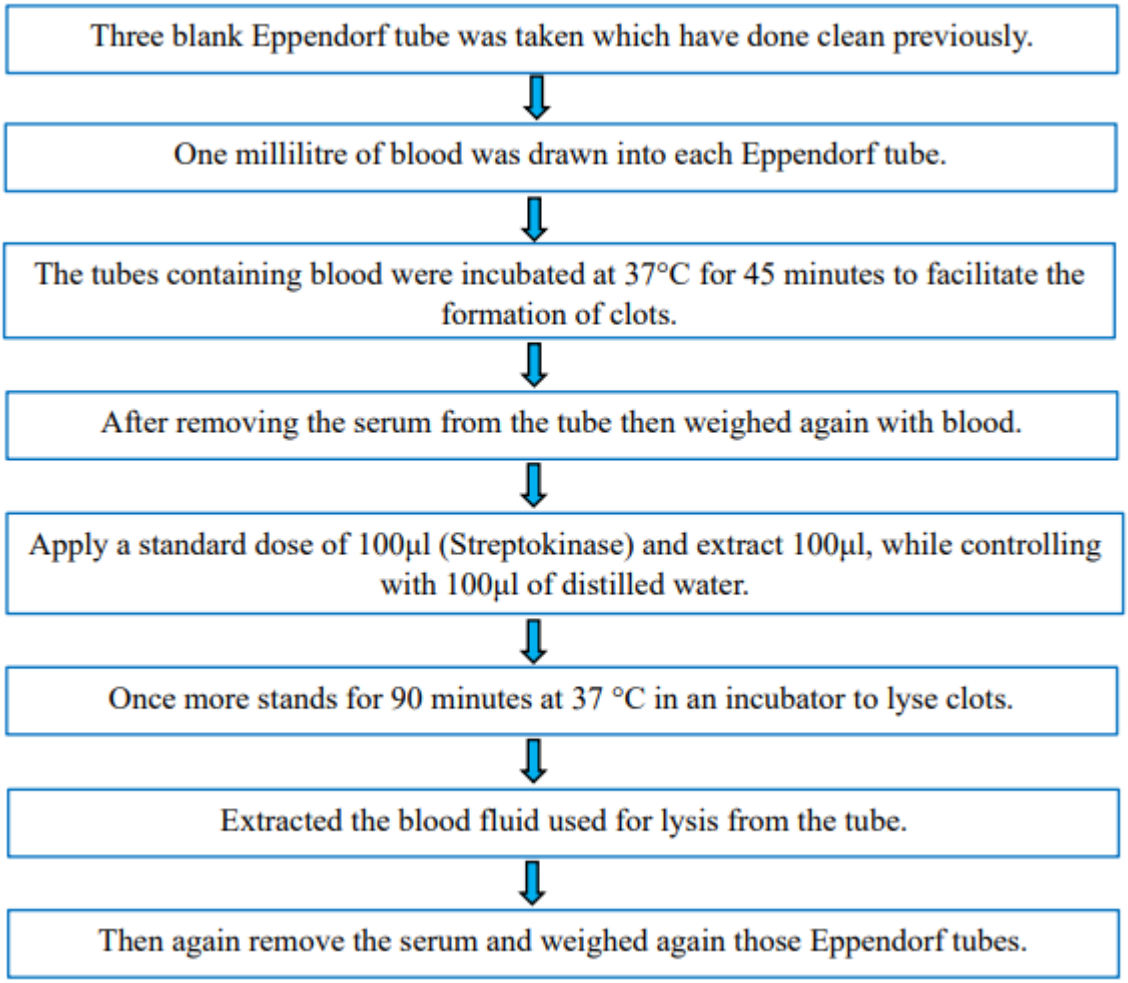
4.5.3 Preparation of test sample

In the beginning, 25 mg of crude extract suspended in 2.5 ml of distilled water were stirred with a vortex mixer. The suspension was allowed to settle overnight before being filtered using syringe filter paper to collect the soluble supernatant. With the solution, ex-vivo cardioprotective action could now be accomplished.

4.5.4 Preparation of Streptokinase Solution as Standard

The readily accessible 15, 00,000 I.U. lyophilized ATPase (Streptokinase) alpine tube (Beacon Pharmaceutical Ltd.) served as the standard solution. After that, five water was added to the streptokinase vial and carefully stirred. This suspension was used in 100µl (30,000 I.U.) for in vitro thrombolysis.

4.5.5 Working Procedure of Thrombolytic Test



4.6 Anti-helminthic activity test

4.6.1 Materials and Reagents Required:

- ✓ Methanolic extract of Marigold flower (prepared by standard extraction methods).
- ✓ Phosphate-buffered saline (PBS).
- ✓ Petri dishes.
- ✓ Beakers.
- ✓ Measuring cylinders.
- ✓ Test worms (e.g., *Pheretima posthuma* or *Ascaris lumbricoides*).
- ✓ Standard anthelmintic drug (e.g., Albendazole, Piperazine citrate).
- ✓ Stopwatch.
- ✓ Distilled water.

- ✓ Syringe/filter paper (for filtration, if needed).

4.6.2 Preparation of Worms:

- Collect adult earthworms (*Pheretima posthuma*) or helminths such as *Ascaris lumbricoides* from fresh soil or a verified source.
- Wash the worms with normal saline to remove any dirt or debris.

4.6.3 Test Sample Preparation:

- Prepare different concentrations of the **methanolic extract** of **Marigold flower** (e.g., 5 mg/mL, 10 mg/mL & 20 mg/mL) in distilled water.
- Use **Albendazole or Piperazine citrate** as the standard drug at the same concentrations (positive control).
- Use **distilled water** as a negative control.

4.6.4. Experimental Procedure:

1. **Grouping of Worms:**
    - Divide the worms into groups (6–8 worms per group).
    - Assign groups for each concentration of methanolic extract, positive control (Albendazole/Piperazine citrate), and negative control (distilled water).
2. **Incubation with Extract:**
    - Place the worms in petri dishes containing 25 mL of the respective test solutions (methanolic extract or control solutions).
    - Ensure that the worms are fully immersed in the solution.

4.6.5 Observations and Data Collection:

- Record the **time taken for paralysis** of the worms (time when no movement occurs, except when shaken).
- Record the **time of death** (time when no movement occurs even after exposure to external stimuli like shaking).
- Use a stopwatch for accurate measurement.

4.6.6 Interpretation of Results:

- Compare the anthelmintic activity of the **methanolic extract of Marigold flower** with the standard drug and the control.
- Evaluate the efficacy by comparing the **paralysis time** and **time of death** for the worms in each group.
- Shorter times for paralysis and death indicate stronger anthelmintic activity.

CHAPTER FIVE
RESULT & DISCUSSION

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RESULT & DISCUSSION

5.1 Result and Discussion of phytochemical evaluation

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

Table 3: Result of phytochemical evaluation of *Tagetes* (Marigold Flower)

The following table lists the several **Marigold Flower** extracts together with their matching dry extract amounts, physical attributes, and qualitative chemical analyses.

5.2 Thrombolytic Activity Test

Solution	Blank Tube Weight gm	1st clot+ Tube Weight gm	1st clot Weight gm	2nd clot+ Tube Weight gm	2nd clot Weight gm	% Of lysis
Standard	0.786	1.681	0.892	1.598	0.812	**91.03%**
Control	0.779	1.385	0.606	1.325	0.546	**9.90%**
Marigold Flower	0.761	1.664	0.903	1.488	0.727	**80.50%**

Figure 8: Thrombolytic Activity Test of ***Tagetes*** **(Marigold Flower)**

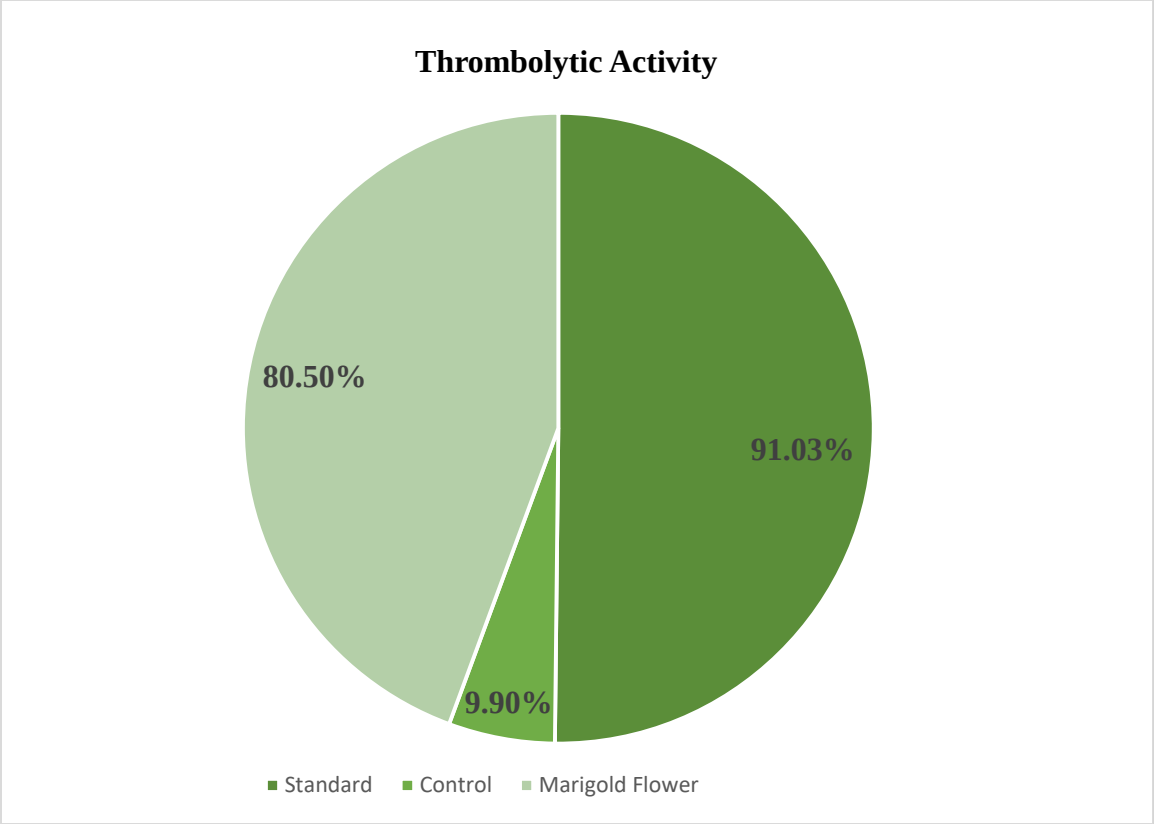

Figure 9: Graphical representation of Thrombolytic Activity Test of *Tagetes* (Marigold Flower)

5.2.1 Discussion about Thrombolytic Activity Test of *Tagetes* (Marigold Flower)

Thrombolytic activity refers to the ability of a substance to break down blood clots (thrombi) and restore normal blood flow. This test compares the thrombolytic potential of marigold flower extract against a standard thrombolytic agent and a control. The data provided helps to evaluate the percentage of clot lysis, which is a critical measure in assessing thrombolytic efficacy.

The results presented show three groups: a standard thrombolytic agent, a control (which typically involves no treatment or an inactive substance), and the marigold flower extract. Here's a breakdown:

- **Standard (91.03% lysis)**

The standard thrombolytic agent shows a high percentage of clot lysis (91.03%), as expected. This serves as a positive control for the experiment, demonstrating that the

experimental setup effectively evaluates clot breakdown. The tube weight differences before and after the clot formation, as well as clot weight before and after lysis, clearly indicate the effectiveness of the standard agent in dissolving clots.

- **Control (9.90% lysis)**

The control group exhibits a clot lysis of 9.90%, which is comparable to the standard agent. This could indicate that the control used may have some inherent activity in clot dissolution or there might be an external factor influencing this group, causing an unexpected high lysis. Usually, in such experiments, the control is expected to show minimal lysis, especially if it represents no treatment.

- **Marigold Flower Extract (80.50% lysis)**

The marigold flower extract shows a clot lysis percentage of 80.50%, which is lower than both the standard and control groups. This suggests that the marigold extract has some thrombolytic activity but is less potent than the standard thrombolytic agent. However, the relatively high percentage of lysis indicates that the extract does have a significant effect on clot dissolution.

The difference between the clot weights before and after lysis in the marigold group reflects its ability to break down clots, but the lower percentage of lysis suggests that it may require further refinement or higher doses for it to be as effective as the standard. Additionally, the bioactive compounds present in marigold flowers, such as flavonoids and carotenoids, may contribute to this thrombolytic effect, and their mechanism of action could be explored further.

5.3 Result of Anti-helminthic activity

Group	Concentration (mg/mL)	Paralysis Time (min)	Death Time (min)
Methanolic Extract (Marigold Flower)	5	1 hours 28 minutes	3 hours 12 minutes
Methanolic Extract (Marigold Flower)	10	31 minutes	54 minutes
Methanolic Extract (Marigold Flower)	20	18 minutes	46 minutes
Standard (Albendazole)	20	16 minutes 52 second	44 minutes
Control (Distilled water)	---	No paralysis observed	No death observed

Table 4: Evaluation outcomes of Anti-helminthic activity of *Tagetes* (Marigold Flower)

5.3.1 Discussion on Anti-helminthic Activity

The results of the study evaluating the anti-helminthic activity of the methanolic extract of marigold flower at different concentrations, compared to the standard drug Albendazole, and the control (distilled water) provide valuable insights into the effectiveness of this plant extract.

1. **Effect of Methanolic Extract of Marigold Flower**:
    - The methanolic extract of marigold flower exhibited significant anti-helminthic activity in a concentration-dependent manner. At a concentration of **5 mg/mL**, paralysis was observed after **1 hour and 28 minutes**, with the death of the worms occurring after **3 hours and 12 minutes**. This indicates moderate activity at lower concentrations.
    - Increasing the concentration of the extract to **10 mg/mL** led to a substantial decrease in both the time to paralysis and the time to death. Paralysis occurred after **31 minutes**, and death followed after **54 minutes**, suggesting improved potency with a higher concentration.
    - At the highest tested concentration, **20 mg/mL**, the methanolic extract showed even stronger activity, with paralysis observed in just **18 minutes**

and death occurring in **46 minutes**. This demonstrates that the extract's efficacy increases as its concentration rises.

2. **Comparison with Standard (Albendazole)**:
    - The standard drug, **Albendazole** (at 20 mg/mL), caused paralysis after **16 minutes and 52 seconds**, followed by the death of the worms in **44 minutes**. These times are only slightly faster than the methanolic extract at the same concentration (**20 mg/mL**). The closeness of these values suggests that at higher concentrations, the methanolic extract of marigold flower approaches the anti-helminthic efficacy of Albendazole.
    - This comparison highlights the potential of marigold flower extract as a natural anti-helminthic agent, especially at higher concentrations. However, Albendazole remains slightly more potent and faster-acting overall.
3. **Control Group**:
    - In the control group, which used **distilled water**, no paralysis or death was observed in the worms, indicating that the observed effects in the experimental groups were due to the action of the methanolic extract and Albendazole, and not due to any external factors.

CHAPTER SIX

CONCLUSION

CHAPTER SIX
CONCLUSION

6.1 Conclusion

The methanolic extract of marigold flower demonstrated significant phytochemical properties, containing a variety of bioactive compounds, such as alkaloids, glycosides, tannins, steroids, phenols, carbohydrates, gums, and reducing sugars, while lacking saponins. These phytochemicals suggest the potential for diverse biological activities. In terms of thrombolytic activity, the marigold extract exhibited notable clot lysis of 80.50%, though slightly less effective than the standard streptokinase (91.03%) and control (9.90%). This result indicates that the marigold extract holds promise as a natural thrombolytic agent, though further refinement could enhance its efficacy. The anti-helminthic activity of the marigold flower extract was dose-dependent, with higher concentrations (20 mg/mL) showing potent activity in inducing paralysis and death of worms, close to the efficacy of the standard drug Albendazole. The results suggest that the marigold flower extract could be an effective natural anti-helminthic agent, particularly at higher concentrations, making it a potential alternative to synthetic drugs. In conclusion, the methanolic extract of marigold flower possesses considerable anti-helminthic and thrombolytic activities, and further research may optimize its use in therapeutic applications.

CHAPTER SEVEN

REFERENCE

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REFERENCES

7.1 References

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