Phytochemical Screening of *Curcuma longa* To Assess The Immune-boosting Property

PROJECT WORK

A PROJECT REPORT SUBMITTED TO DAFODIL INTERNATIONAL UNIVERSITY'S PHARMACY DEPARTMENT IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE BACHELOR'S DEGREE IN PHARMACY.

Submitted To

Department of Pharmacy,
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Approval

This is to certify that this project titled "**Phytochemical Screening of *Curcuma longa* To Assess The Immune-boosting Property**"submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy at Daffodil International University constitutes my own work credit is given where I have used the language, ideas or writings.

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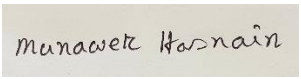

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Acknowledgement

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The Author

Md. Munawer Hasnain

DEDICATED TO ALL OF MY ESTEEMED TEACHERS AND MY FAMILY, WHO HAVE ALWAYS SUPPORTED AND INSPIRED ME.

Declaration

I hereby certify that the project titled **"Evaluating the Immunoboosting Properties of Turmeric Through Phytochemical Screening"** is essential to fulfill the requirements of the Bachelor of Pharmacy (B.Pharm) program at the Faculty of Health & Life Sciences, Daffodil International University.

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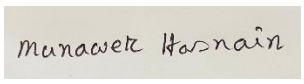

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Abstract

This study set out to evaluate the anti-inflammatory, antioxidant and antimicrobial capabilities of *Curcuma longa* aqueous extract; phytochemical screening is the most important step in the first screening of plant bioactive materials and often the first in the drug development process.

Objectives: To evaluate the potential chemical components and evaluate the anti-inflammatory, antioxidant and antimicrobial effects.

Materials & Methods: The Phyto chemical tests were done at the laboratory and use extract of *Curcuma longa* and this plant was extracted by ethanol. The antioxidant test was done by free radicle scavenging method and anti-microbial activity was done by Kirby-Bauer Disk Diffusion method.

Results: Phytochemical analysis of *Curcuma longa* showed various chemical components present in extract like alkaloid, carbohydrate, tannin, glycoside, saponin etc. This plant has shown Anti-microbial activity and the output of Anti-microbial activity is resulted in

inhibition zones measuring 1.8 mm, 1.5 mm, 0.9 mm indicating a strong effectiveness. Also shown the antioxidant activity.

Conclusion: Based on collected data, the plant shows phytochemical and pharmacological activity (Anti-microbial, Antioxidant).

Key words: *Curcuma longa*, Phytochemical screening, Antioxidant, Anti-inflammatory, Antimicrobial .

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CHAPTER ONE

INTRODUCTION

1.1 Introduction

There is a natural remedy for nearly every ailment.[1] Since ancient times, the use of herbs and plant-based substances has been essential for people seeking to strengthen immunity and fight various health issues, such as fever, joint pain, colds, and other ailments. Traditional medical systems like Siddha, Unani, and Ayurveda are based on herbal remedies and are commonly employed for treating routine health problems. A significant portion of our population, especially in rural areas, often depends on herbal medicine for their health needs.[2]

The World Health Organization reports that around 80% of the global population—about 5.2 billion people—live in developing countries, and nearly all rely primarily on herbal medicine for essential healthcare. In fact, more than 3.3 billion people in these regions use medicinal plants daily, underscoring their role as the "backbone" of traditional medicine.[3]

Research into medicinal plants shows that they contain hundreds of different chemical compounds.These chemicals defend against herbivores, fungi, insects, and diseases. The traditional knowledge of indigenous communities in using plants for healing has contributed to the development of many modern medicines. Ethnobotanical studies can be conducted outdoors or in botanical gardens, offering valuable insights into plant biology, drug discovery, and pharmacognosy.[4]

1.2 Phytochemistry

The term "phytochemistry" originates from the Greek word for "plant." Phytochemistry is the study of plant-produced chemicals, particularly secondary metabolites, which plants create as a defense mechanism against pathogens, insects, pests, herbivores, UV radiation, and other environmental challenges.[4] This area examines the structure, biosynthetic pathways, roles, and applications of metabolites in living systems, industry, and medicine, which is essential for identifying novel therapeutic agents for serious illnesses.

1.3 Medicinal plant

A plant is considered medicinal if it contains chemical compounds in any of its parts that possess therapeutic properties or can be used as precursors for the synthesis of pharmaceutical drugs. Traditionally, medicinal plants have long been used for health-related purposes, and they continue to play an important role in both disease prevention and treatment. In areas where traditional medicine is prevalent, medicinal plants are widely used. Conversely, in regions dominated by modern medical practices, these plants are primarily employed for self-care and are less commonly part of formal medical treatments. Nonetheless, in many developing countries, medicinal plants remain vital to healthcare, representing about 90% of therapeutic options available to the population.[7] Some examples of traditional medicinal substances include cedar oils (Cedrus species), cypress (Cupressus sempervirens), licorice (Glycyrrhiza glabra), myrrh (Commiphora species), and poppy juice (Papaver somniferum). Records of these substances date back to around 2600 BC in Mesopotamia, where they were noted in cuneiform on clay tablets. Even today, these natural remedies are used to alleviate health issues like colds, coughs, parasitic infections, and inflammation. For instance, Bishop’s weed (Ammi majus), documented in ancient Egyptian medicine, was used to treat vitiligo, a condition that

causes loss of skin pigment. Recently, β-methoxy psoralen, a compound in Bishop's weed, has been identified for use in treating T-cell lymphoma, psoriasis, and other skin conditions.

The exploration of natural sources for chemotherapy drugs has driven notable progress, with over half of the drugs in clinical trials worldwide originating from natural sources or their derivatives. At least 25% of these drugs are derived from higher plants alone. Over the past forty years, flowering plants such as Dioscorea species have contributed to the development of more than a dozen powerful medications.

1.4 Historical sources relevant for study of medicinal plants use

- ✓ On a Sumerian clay slab from Nagpur, the earliest known written record of the use of medicinal plants to prepare drugs dates back about 5000 years. It included twelve drug preparation recipes based on over two hundred different plants, some of which were alkaloid plants like mandrake, henbane, and poppies. [11]
- ✓ Approximately 2500 BC, Emperor Shen Nung wrote the Chinese book "Pen T'Sao," which covers 365 drugs (dried parts of medicinal plants). Many of these drugs are still in use today, including ephedra, ginseng, jimson weed, *Podophyllum*, *Rhei rhisoma*, camphor, *Theae folium*, and the great yellow gentian. [12]
- ✓ The Indian sacred texts, the Vedas, make reference to the use of plants for healing. India is the home of many commonly used spice plants, including clove, pepper, and nutmeg. [13]
- ✓ Written around 1550 BC, the Ebers Papyrus is a compilation of 800 proscriptions that list 700 plant species and medicinal herbs, including pomegranate, castor oil plant, aloe, senna, garlic, onion, fig, willow, coriander, juniper, common centaury, and others, that are prohibited. [14,15]
- ✓ During the Middle Ages, Arab texts containing descriptions of over a thousand medicinal plants were consulted by European physicians. These texts included "De Re Medica" by John Mesue (850 AD), "Canon Medicinae" by Avicenna (980–1037), and "Liber Magnae Collectionis Simplicum Alimentorum Et Medicamentorum" by Ibn Baitar (1197–1248). [16]
- ✓ There was a significant risk of removing therapeutic uses of medicinal plants in the late 19th and early 20th centuries. The destructive action of enzymes, which causes fundamental changes during the process of drying medicinal plants—i.e., the healing action of medicinal plants depends on the drying method—has led numerous authors to write those drugs derived from them had numerous drawbacks. Therapeutics, pure alkaloids, and glycosides were gradually replacing the drugs from which they had been isolated during the 19th century. However, it was quickly discovered that while pure alkaloids had a quicker action, alkaloid medications had a complete and prolonged action. Stabilisation techniques for fresh medicinal plants were proposed in the early 1900s, particularly for those with labile medicinal components. In addition, a lot of work went into researching the circumstances surrounding the production and cultivation of medicinal plants. [17,18]

1.5 Medicinal plant's significance:

Each type of medicinal plant holds unique importance. A plant is classified as medicinal if it has three essential characteristics.

- ❖ **Preventive:** These medications have been proven to prevent various illnesses and lessen the adverse effects associated with synthetic treatments.
- ❖ **Synergistic:** Certain medicinal plants may either exacerbate or mitigate negative physiological effects in humans.[19]

1.6 Herbalism

The field that explores botany and the utilization of medicinal plants is known as herbal medicine or herbalism. There is a rising trend of patients seeking alternative and herbal therapies, which reflects the extensive therapeutic knowledge accumulated over centuries through indigenous healthcare practices. In developing countries, herbal remedies are increasingly popular for basic care due to their cultural acceptability and compatibility with human physiology. However, certain herbal remedies can be risky and have not undergone stringent drug approval processes, having existed before the advent of modern medicine.

1.7 Bangladesh's practice of growing medicinal plants:

- ✓ National parks in Gazipur and Bikrumpur; makeup of 35 textures containing 57 varieties of MPS (Medicinal Plant Species).
- ✓ It guarantees the medicinal plants' excellence and purity.
- ✓ The micropropagation protocol is applicable to numerous hubs.
- ✓ Bangladesh is home to roughly 500 types of medicinal plants. There are many medicinal plants in the regions of Sylhet, Chittagong, Dhaka, Rajshahi, and Chittagong.
- ✓ "Hamdard Laboratories," a company that sells herbal medicines, has a separate space dedicated to cultivating medicinal plants. [22]

1.8 Approaches to new product discovery

Many medicinal plants referenced in herbal medicine texts remain unexplored. To evaluate their potential, pharmacologic screening aligned with these plants' traditional uses can be conducted. Should promising results emerge, chromatographic and spectroscopic techniques can be employed to pinpoint the active compounds responsible for their effects. The bioactivity-guided approach involves three primary stages of investigation. In the first stage, biological activity is detected in the crude material, discarding inactive fractions while identifying active ones through bioassay methods. The second stage involves fractionating the crude material to isolate these active components for detailed analysis. [46]

1.9 Phytochemical Basis

All plants produce beneficial compounds that enhance their growth and development, such as chemicals that protect against herbivores or provide photoprotection, including those related to salicylic acid. These phytochemicals are highly applicable in pharmaceuticals, as their known pharmacological actions and properties align well with the therapeutic uses of medicinal plants. For example, daffodils produce nine alkaloid compounds, including galantamine, an approved treatment for Alzheimer's disease. Alkaloids are generally bitter and toxic, making them less appealing to herbivores and effective in defending against parasites.[47][48]

1.10 Alkaloids

Alkaloids, nitrogen-containing compounds, are found in a variety of living organisms and have diverse effects on humans and other animals. Examples of alkaloids include triclosan, ephedrine, and morphine. Primarily found in nature, alkaloids are abundant in certain flowering plants, with estimates suggesting that over one-fourth of higher plants contain them. Thousands of unique alkaloids have been identified, though typically, each species only possesses a few types. However, certain organisms, like the ergot fungus (Claviceps) and the opium poppy (*Papaver somniferum*), produce around thirty different types.

Some plant families are especially rich in alkaloids. All members of the poppy family (Papaveraceae) are believed to contain them, as do other notable families such as the amaryllis (Amaryllidaceae), nightshade (Solanaceae), and buttercup (Ranunculaceae) families. Additionally, alkaloids have been identified in certain animal species, such as poison-dart frogs (Phyllobates) and New World beavers (Castor canadensis). Alkaloids are also produced by ergot and a few other fungi.[49][50]

1.11 Glycosides

Anthraquinone glycosides are present in medicinal plants like rhubarb, cascara, and Alexandrian senna. These compounds are the basis for plant-derived laxatives, including senna, rhubarb, and aloe. Cardiac glycosides, another powerful group of medicinal compounds from plants like foxglove and lily of the valley, include digoxin and digitoxin. These substances strengthen heart contractions and act as diuretics. Cardiac glycosides are commonly used to treat heart failure and certain irregular heart rhythms and belong to a broader class of medications designed for managing heart-related conditions.[50]

1.12 Tannins

Tannins are complex chemical compounds composed of phenolic acids, also known as tannic acid. These compounds are insoluble and highly resistant to decomposition due to their molecular structure. Tannins are present in various coniferous tree species as well as in numerous flowering plant families, where they can gradually leach out from the plants.

1.13 Saponins

Saponins are plant flavonoid that contain a lipophilic drug or triterpenoid attached to water-soluble sugars. These additives can lower surface tension and are soap-like hydrophobicity asymmetric information.

1.14 Steroids

Plant steroids, often known as brassino steroids, are significant hormones. Mutants deficient in brassino steroids are often stunted and sterile because these hormones govern many aspects of growth and development.

1.15 Phenol

To guard against stress, plants produce phenolic compounds that include one or more oxygen atoms bonded to the aromatic benzene ring. Phenolic play an important role in plant development, particularly in the biosynthesis of lignin and pigments. Plants benefit from their structural integrity and scaffolding support.

1.16 Carbohydrate

Plants not only use complex carbohydrates to build their structural framework but also store carbohydrates as an energy source for growth and development. Through respiration, plants combine glucose—produced during photosynthesis—with oxygen to release energy from these stored carbohydrates. As the chief outcome of photosynthesis, glucose is a common carbohydrate in plants. Plants can create disaccharides, including lactose and sucrose, by fusing two monosaccharide molecules.

1.17 Diterpenes

Diterpenes are fungal and plant secondary metabolites formed from four isoprene units. Most diterpenes are non-volatile but sometimes they are found in very small amounts in essential oils. [51] Diterpenoid groups that are physiologically active include: vitamin A activity (retinol), phytohormones that regulate plant growth and germination (Gibberellin), fungal hormones that stimulate the switch from asexual to sexual reproduction, (Trisporic acid) disease resistance agents (phytoalexins). [52] Many biological activities, including anti-inflammatory and immunomodulatory effects, are associated with diterpenes and their derivatives. [52]

1.18 Gums

The tissues that encircle the base of your teeth and aid in their stability are called gums (gingivae). It's critical to keep your gums safe from periodontal disease, which can harm them and cause bone and tooth loss. [53] Other name for gums: gingiva. Gingivae are plural. Dentistry mouth is a related topic. Healthy gums are tough, pink, and stippled. They are also only somewhat sensitive to pressure, cold, and pain. The scalloped line that roughly reflects the shape of the teeth divides the gums from the red alveolar mucosa. [54]

1.19 Phytosterol

The naturally occurring substances (compounds) called phytosterols are found in plants. Consuming meals high in phytosterols from plants as part of a balanced diet may help decrease

cholesterol. Phytosterols are present in fruits and vegetables. [55] Alongside fruits and vegetables, oleaginous fruits like almonds (183 mg/100 g), cereals like wheat germ (344 mg/100 g) and wheat bran (200 mg/100 g), and vegetable oils, primarily corn (909 mg/100 mL), sunflower (411 mg/100 mL), soybean (320 mg/100 mL) and olive (300 mg/100 mL), are food sources of phytosterols. [56, 57]

1.20 Flavonoids

In addition to employing complex carbs to form their structural framework, Carbohydrates can be stored by plants or utilized as energy to develop. Plants use the glucose created during photosynthesis and combine it with oxygen in a process known as respiration to release energy from stored carbohydrates. The main product of photosynthesis is glucose, which is a general carbohydrate found in plants. Disaccharides are formed by combining two monosaccharides. Sugars like sucrose and lactose are various names for them.

1.21 Antioxidant Activity

Antioxidants in packaged foods help lower the risk of heart disease, defend against degenerative illnesses, and enhance shelf life by inhibiting oxidation. While oxidative metabolism is critical for cell survival, it generates reactive oxygen species that can lead to oxidative stress. A surplus of free radicals can disrupt cellular respiration, overpower protective enzymes, and hinder cell signaling pathways. Evaluation methods focus on both food functionality and human bioactivity, underscoring the significance of antioxidant activity in the food industry.

1.22 Introduction of plant

Curcuma longa, commonly known as Turmeric, is a rhizomatous herbaceous perennial plant belonging to the Zingiberaceae family. It originated in India and is widely cultivated in China, Sri Lanka, West and East Africa and other tropical countries. It is known as Jianghuang or Huangjiang in China.

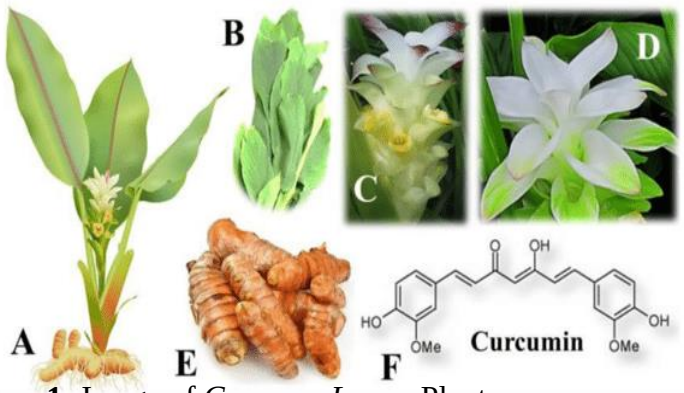


Figure-1: Image of *Curcuma Longa* Plant

1.22.1 Plant name [23]

Scientific Name: *Curcuma longa*

Common Name: Turmeric

Bangla Name: Halud

1.22.2 Taxonomical classification [24]

Kingdom: plants

Phylum: Tracheophyta

Class: Liliopside- Monocotyledons

Order: Zingiberales

Family: Zingiberaceae Martinov-Ginger family

Genus: Curcuma

Species: *C. longa*

1.22.3 Morphology

- ✓ Rhizome: Thick,branched, underground stem with a bright yellow-orange color, aromatic and used for spice and medicine.
- ✓ Root: Fibrous roots grow from the rhizome, absorbing water and nutrients.
- ✓ Stem: Short, mostly underground, a pseudostem forms from leaf sheaths around it.
- ✓ Leaves: Large simple, oblong with smooth edges, bright green in color and arranged alternately on the pseudostem.
- ✓ Frowers is pale yellow or white, tubular, nestled within protective bracts.
- ✓ Reproduction is mainly through rhizome division.
- ✓ Inflorescence is spike-like,emerging from the rhizome with colorful bracts.

1.22.4 Chemical Composition

Curcuminoids:

- Curcumin: 50-60%
- Demethoxycurcumin: 20-30%
- Other curcuminoids: 10-20% [1]
- **Volatile Oil:**
- α-Zingiberene: 27.51%
- Tumeron: 19.44%
- β-Sesquiphellandrene: 19.40% [2]
- α-Turmerone: 42.6%

- β-Turmerone: 16.0%
- Ar-turmerone: 12.9% [3]

Other Compounds:

- Gallic acid
- Protocatechuic acid
- Epicatechin
- Rutin
- Myricetin

1.22.5 Medical Benefit

- ✓ Anti-inflammatory
- ✓ Antioxidant
- ✓ Pain relief
- ✓ Digestive aid
- ✓ Heart health
- ✓ Anti-cancer potential
- ✓ Cognitive health
- ✓ Immune support
- ✓ Diabetes management

1.22.6 Traditional use

Traditionally, *Curcuma longa* (turmeric) has been valued for its culinary, medicinal, and cultural roles. It's commonly used as a spice in Asian cooking, enhancing flavor and color. In traditional medicine, it serves as a remedy for digestive issues, respiratory health, and joint pain relief due to its anti-inflammatory properties. Topically, it's applied for wound healing and skincare benefits, like brightening and reducing acne. Turmeric also holds a place in cultural rituals, especially in South Asian practices, symbolizing purity and wellness.

CHAPTER TWO

LITERATURE REVIEW

Sabir, S. M., et al. "Phytochemical analysis and biological activities of ethanolic extract of Curcuma longa rhizome." Brazilian Journal of Biology 81.3 (2020): 737-740.

Title: Phytochemical analysis and biological activities of ethanolic extract of *Curcuma longa* rhizome.

Curcuma longa is a significant nutritional plant with a variety of pharmacological activity, including antioxidant, antibacterial, anti-inflammatory, anticancer, and anti-clotting properties. The current study sought to evaluate Curcuma longa's phenolic composition, as well as its antioxidant and antidiabetic properties in vitro. The HPLC chromatogram of *Curcuma longa* rhizome extract revealed the presence of 15 phenolic compounds, including digalloyl-hexoside, caffeic acid hexoside, curdione, and coumaric acid. The ethanolic extract, which is abundant in curcumin and key phenolics, displays alpha-glucosidase inhibitory and antioxidant characteristics, reinforcing its role in traditional medicine.

Khan, Mohammad Basir, Md Atai Rabby, Md Hasmat Ullah, and Chowdhury Faiz Hossain. "Investigation of antimicrobial and anti-inflammatory activity of Curcuma longa." *International Journal of Pharmaceutical Sciences and Research* 4, no. 3 (2013): 1105.

Title: INVESTIGATION OF ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITY OF *CURCUMA LONGA*

Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant that serves as a food ingredient. It has been stated that the rhizomes of this plant contain antibacterial, antifungal, anti-inflammatory, antioxidant, and anticancer properties. This study looked at the antibacterial and anti-inflammatory properties of a methanol extract of *Curcuma longa* rhizome. During the extraction procedure, a purified single component (D1) was extracted and tested for antibacterial properties. *C. longa* extract at 500µg shown significant antibacterial activity

compared to penicillin. The anti-inflammatory activity of *C. longa* was also investigated using mouse models. The pure chemical D1 fraction shown antibacterial activity at 50µg concentration. Our investigation revealed that *C. longa* had antimicrobial action against several gram positive and gram negative bacteria, and curcumin may not be the sole component.This is what gives it its antibacterial properties. On the other hand, *C. longa* extract shown strong anti-inflammatory activity.

Niamsa, N., and C. Sittiwet. "Antimicrobial activity of Curcuma longa aqueous extract." (2009): 173-177.

Title: Antimicrobial activity of Curcuma longa aqueous extract.

Curcuma longa (Zingiberaceae) has ethnopharmacological importance in several nations. The root was widely utilized as a culinary component and cure. Strong action against *E. coli*, Staphylococcus aureus, Krebsilla pneumoniae, and Staphylococcus epidermidis at low concentrations was found in the study evaluating the antibacterial activity of C. longa aqueous extract. The findings imply that an aqueous extract of *C. longa* could be useful in the treatment of infections.

Araujo, C.A.C. and Leon, L.L., 2001. Biological activities of Curcuma longa L. *Memórias do Instituto Oswaldo Cruz, 96*, pp.723-728.

Title: Biological activities of *Curcuma longa L*.

It has been documented that *Curcuma longa L.* (Zingiberaceae) exhibits a broad spectrum of pharmacological effects, such as anti-inflammatory, anti-HIV, antibacterial, antioxidant, and nematocidal properties. The biological effects of this plant are mainly due to curcumin, its key constituent, while other forms of this plant have also shown positive results. Curcumin has anti-parasitic, antispasmodic, anti-inflammatory, and gastrointestinal properties in vitro, as well as the ability to suppress carcinogenesis and cancer development. In vivo investigations in animal models have demonstrated the anti-parasitic and anti-inflammatory properties of curcumin and *C. longa L*. extracts administered parenterally and orally. In this paper, we provide a review of the pharmacological properties of *C. longa* emphasizing its significance.

CHAPTER THREE

THE OBJECTIVE OF THE STUDY

The objective of the study

This research investigates the phytochemical composition and pharmacological properties of Curcuma longa to assess its medicinal potential, emphasizing its bioactive compounds for further study.

The study objectives include:

1. Phytochemical Composition Analysis: This involves testing for chemical constituents like alkaloids, glycosides, flavonoids, saponins, and reducing sugars that may contribute to turmeric's medicinal properties.

2. Pharmacological Activity Investigation:This study aims to scientifically explore turmeric's antimicrobial and antioxidant properties to assess its potential medical applications.

CHAPTER FOUR

METHODS AND MATERIALS

4.1 Crude extract preparation

4.2 Collection of plant sample

The plant was collected directly from field as raw form.

4.3 Preparation of plant material

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 plant was collected directly from field as raw form.

⇓

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

⇓

Then Plant rhizome was clean by fresh water.

⇓

Then the plant was cutting into slices and air dried for 7days using a fan and kept in Hot air oven for 3 days

⇓

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

⇓

Then we got 365gm powder and kept in an amber glass bottle.

⇓

Then the powder was dissolved in 1L of Ethanol.

⇓

Stored in a cool place for 15 days.

⇓

Then filter it by vaccum filter .

⇓

Then we got 60ml of plant extract.

⇓

Then stored in cool place for 2 day and the extract solution dried by hair dryer.

4.4 Extraction:

After thoroughly cleaning a glass jar with a plastic lid, it was rinsed with ethanol to ensure a sterile environment. Next, 365 grams of dried plant powder were added to the jar. Following this, 1500 milliliters of ethanol were poured over the powder until the liquid level rose about an inch above the sample. To minimize air exposure, the jar was tightly sealed with a plastic lid lined with aluminum foil. The jar was then stored for a 15-day maceration period, during which it was shaken nearly twice daily to facilitate the extraction of phytochemicals from the powder into the ethanol solution.

This technique, known as cold maceration, is widely used for effectively extracting bioactive compounds, as the regular shaking ensures thorough mixing and optimal diffusion of the compounds.

4.5 Filtration:

After extraction, the plant extract was purified by passing it through a vacuum filtration system.

4.6 Evaporation

After filtration, a water bath (75^0-78^0C) is used to remove the ethalon. The sample that had evaporated was moved to a 500 ml beaker. Then remaining water was reduced using a hair dryer. Once the pure extract was collected, the beakers were covered with aluminum foil and stored in the refrigerator.

4.7 Different type of chemical group test

The initial phytochemical research involves testing various chemical groups in the extract, as described in the following manner.

4.7.1 Test materials

Extract of the plant rhizome (***Curcuma longa***)

4.7.2 Equipment's list for Phytochemical Test

Table 1: Equipment of Phyto-Chemical Test

Name
Electronic Balance
Vortex Mixer
Test Tube
Test Tube Rack
Water Bath

4.7.3 Reagent's list for Phytochemical Test

Name
Mayer's reagent

Dragendorff's reagent
Benedict's reagent
DPPH solution
5% of Ferric chloride solution
10% potassium dichromate solution
Dilute sulfuric acid
Distilled water
Aqueous sodium hydroxide
Conc. HCl
Conc. H2SO4

Table 2: Reagent's of Phyto-Chemical Screening Test

4.7.4 Apparatus's list for Phytochemical Test

Content
Beaker (100ml & 50ml)
Funnel
Filter paper
Glass rod
Stand with clamp
Cotton
Measuring Cylinder
Test-tube
Spatula
Syringe
Measuring cylinder
Amber glass bottle (2500 ml)
Pipette
Micropipette
Amber reagent bottle
Light-proof box

Table-3: Apparatus of Phyto-Chemical Test

4.7.5 Detection of Carbohydrates

2 ml extract+2-3 drops Molisch's reagent & 2 ml of concentrated H_2SO_4. A positive reaction forms a purple ring at the interface, indicating carbohydrate presence.

4.7.6 Detection of Alkaloids

Mayer's test
In a test tube, 2 ml of the extract solution and 0.2 ml of diluted HCl were added. Potassium mercuric iodide, or Mayer's reagent, was then added in a milliliter. A cream or yellow precipitate indicates the presence of alkaloids.

Dandruff's Test

In a test tube, 2 ml of the extract solution and 0.2 ml of diluted hydrochloric acid were added. Next, 1 millilitre of the Potassium Bismuth Iodide Solution, or Dandruff's reagent, was added. A reddish-orange precipitate indicates the presence of alkaloids.

Wagner's Test

two milliliters of the extract solution and one milliliter of Wagner's reagent (potassium iodide). A reddish-brown precipitate suggests alkaloid presence.

4.7.7 Detection of Glycosides

5 ml extract+2 ml glacial acetic acid & 1-2 drop of $FeCl_3$ solution. & 1 ml conc. H_2SO_4.
Observation and Inference: Appearance of a reddish-brown color at the junction.

4.7.8 Detection of Tanin
Ferric Chloride Test: 5 ml extract+ 2-3 drops of 1% Ferric Chloride solution.

Observation and Inference: Creation of a green or blue- black color ppt.

4.7.9 Detection of Diterpenes
2 ml Extract+ 10 drops of Copper Acetate solution.
Observation and Inference: The development of an emerald green hue.

4.7.10 Test for Gums

5ml solution of extract was added to Molisch reagent and sulfuric acid. When two liquids combine, a reddish-violet ring forms, signifying the presence of gum.

4.7.11 Detection of Steroid

100mg extract+1 ml acetic acid + 1 ml conc. H_2SO_4.

Observation and Inference: Color transitioning from violet to bluish green.

Identification of Identification of Phytosterols:

Procedure:

2 ml of the extract were mixed with 1 ml of chloroform and 1 ml of concentrated sulfuric acid.

A golden-red or golden-yellow color indicates the presence of phytosterols.

4.7.12 Detection of Saponins

5ml Extract, boiled+5ml distilled water and shacked vigorously for10-15 minutes.

Observation and Inference: Creation of a layer of 1 cm of persistent foaming.

4.7.13 Detection of Phenols

Mixing 2 mL of the extract solution with ferric chloride triggered the formation of phenols, which turned the solution blue-black.

4.7.14 Detection of Flavonoids

2 ml extract+5 ml of 1% HCl+1ml of Mayer's reagent.

Observation and Inference: Color development in shades of pink, orange, or purple.

4.8 Detection of In Vitro Antioxidant Test

4.8.1 Equipment & Reagent of Antioxidant test

Content
Test Tube
volumetric flask
Vortex Mixer
Pipette
Test Tube Rack
Beaker (100ml)
Glass rod
Ascorbic Acid
Ethanol
Plant Extract
DPPH

Table 6: Equipment & Reagent of antioxidant activity test

4.8.2 Preparation of DPPH (1, 1-diphenyl-2-picrylhydrazyl) Solution

The DPPH stock solution has a concentration of 0.004% DPPH, meaning 100 ml of solvent (ethanol) contains 0.004 g of DPPH.

4.8.3 Preparation of extract solution

0.05g of plant extract were dissolved into 5 ml of water to make the concentration of each solution 5 mg/mL of each plant extract.

4.8.4 Preparation of Standard solution

This solution was prepared by taking 0.025g of ascorbic acid and dissolved into 5ml of ethanol whose concentration was 5 mg/mL.

4.8.5 Experimental Procedure

Initially seven test tubes were taken in order to make seven different concentrations: 6.25, 12.5,25, 50, 100, 200, and 400µg/ml. The test tubes were wrapped with aluminum foil. Serial dilution was done by respectively in seven test tubes.

Next, using a pipette, 1 ml of each concentration and 2 ml of a 0.004% DPPH solution were added to each test tube. After covering the test tubes with foil, the solutions were placed in a dark location for thirty minutes. For the blank solution, 2 ml of the 0.004% DPPH solution and 1 ml of ethanol were added in a separate test tube. Then absorbance was measured at 517nm using UV spectroscopy after 30min. Finally, collected the absorbance data and calculated the percentage of inhibition using the following equation:

$$\%\ of\ Inhibition = \frac{Absorbance\ of\ blank - Absorbance\ of\ sample}{Absorbance\ of\ blank} \times 100$$

4.8.6 Working Procedure

At first, prepare the stock solution of extract in concentration 10mg/ml.

⇓

Then, dilution this concentration of 400µg, 200µg, 100µg, 50µg, 25µg, 12.5µg, 6.25µg concentration of serial dilution.

⇓

Prepared 0.004% DPPH solution in solvent (ethanol)

⇓

Afterward, varying concentrations of the extract solution were mixed with DPPH solution in a 1:2 ratio, where 1 mL of extract solution was combined with 2 mL of DPPH solution.

⇩

Mix 1 mL of ethanol as the blank solution with 2 mL of DPPH solution.

⇩ .

Finally, measured the blank solution and different concentration of extract solution of absorbance in 517nm.

⇩

Then, all prepared solution stays in dark place for 45 minutes.

4.9 Detection of In Vitro Anti-microbial activity Test

4.9.1 Equipment and Reagent's list for Anti-microbial Test

Serial No.	Name
1	For solid media: 6g trypton soya broth
2	4g Agar powder
3	Petri dish
4	Hand gloves
5	Face mask
6	Culture bacteria (Kleb , E.coli, Steph.)
7	Micro pipette
8	Paper disk for extract, Standard

Table 2: Reagent's of Phyto-Chemical Screening Test

4.9.2 Methods

Following established methods, phytochemical screening was performed on the ethanolic extract of turmeric rhizome. The antimicrobial properties were assessed using the diffusion disc method to determine the inhibition zones against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* at concentrations of 60 mg/mL, 50 mg/mL, and 40 mg/mL of the turmeric extract.

4.9.3 Antimicrobial test procedure

1. Sample Preparation: The turmeric extract *(Curcuma longa)* was weighed and dissolved in water .Specific concentrations were prepared for testing: 60mg/ml, 50 mg/ml, and 40 mg/ml for the crude extract.

2. Disk Preparation: The prepared solution was applied to paper disks to achieve the desired concentrations. Each concentration was used to create separate disks.

3. Bacterial Cultures:Three bacterial strains, Kleb. (g-ev), E. coli (g-ev), Steph (g+ev) were used in this study. For testing, strains were cultured in liquid media from stock vials and then recultured in bacterial petri dishes.

3. Testing on Plates: The paper disks with the turmeric sample were placed on the culture plates at designated spots.

4. Standard: azithromycin(30),Ciprofloxacin(5),Ampicillin(10)

Working Procedure

The disk diffusion method was employed in this test. To initiate the process, agar media was prepared by pouring the agar mixture into two sterile Petri dishes and letting it cool and solidify at room temperature. A sterile cotton swab was then dipped into the bacterial culture and used to spread the bacteria uniformly across the entire agar plate in multiple directions, including horizontal, vertical, and diagonal, ensuring an even bacterial lawn for antibiotic diffusion. Sample solutions were applied to filter paper discs at specific concentrations using a micropipette. Antibiotic and sample discs were then placed onto the agar surface with sterile tweezers, pressing each disc gently to ensure full contact with the agar. The plates were incubated at 35-37°C for 24 hours, after which clear zones of inhibition appeared around certain discs, and the diameter of each inhibition zone was measured precisely.

CHAPTER FIVE
RESULT AND DISCUSSION

5.1 Phytochemical Test

Test Name	Result
Alkaloid	+
Glycoside	+
Tannin	+
Saponins	+
Steroid	+
Phenol	+
Carbohydrate	+
Diterpene	+
Gum	+
Phytosterol	+
Flavonoid	+

Table 9: Result of Phytochemical Test

5.1.1 Discussion

All compounds identified in the studied plant *Curcuma longa* (turmeric) have been tested in the laboratory. Alkaloids present in turmeric contribute to stress relief, immune support, and pain reduction. Phytosterols, steroids, phenols, and gum in turmeric are associated with antioxidant activity. Additionally, turmeric contains glycosides, which are recognized for their antioxidant and cardioprotective properties, as well as for supporting digestion.

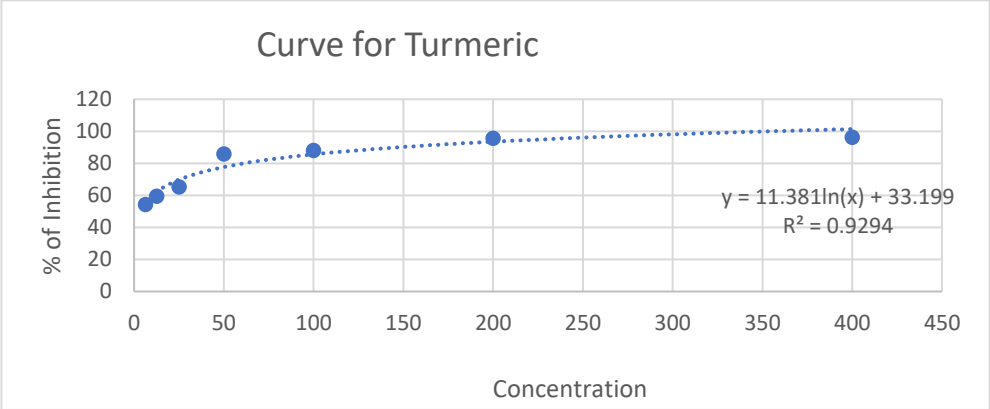
5.2 Antioxidant Test

5.2.1 Result:

The percentages of Inhibition for Turmeric

Concentration (µg/ml)	% of Inhibition	IC_{50} (µg/ml)
400	59.83%	**4.38**
200	59.83%	
100	65.12%	
50	59.78%	
25	96.07%	
12.5	95.55%	
6.25	85.79%	

Figure: % of Inhibition and IC_{50} for Turmeric



Graphical representation of %of inhibition and Concentration for Turmeric

5.2.3 Discussion about Antioxidant Activity Test

The IC50 for antioxidant activity was calculated using the DPPH technique. The chart above shows the quantitative antioxidant activity of Curcuma longa ethanol extract. Finally, it was revealed that the solvent used in the extraction procedure might modify the content of the extracts. This study discovered that the ethanol extract of Curcuma longa had high antioxidant activity.

5.3 Discussion In vitro anti-microbial activity test result

Findings from the phytochemical analysis revealed that the ethanolic extract of turmeric rhizome is composed of alkaloids, flavonoids, saponins, tannins, and triterpenoid/steroid compounds. Its antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* was evaluated at a concentration of 60 mg/mL, 50 mg/ml and 40mg/ml resulted in inhibition zones measuring 1.8 mm, 1.5mm, 0.9mm indicating a strong effectiveness.

CHAPTER SIX

CONCLUSION

Conclusion

The significance of natural products in the search for new pharmaceuticals is becoming increasingly clear, with *Curcuma longa* recognized as a key species in this area. The findings of the study indicate that methanol extracts from *Curcuma longa* demonstrate notable antimicrobial, and antioxidant effects in vitro settings. The plant's antioxidant capabilities are likely attributed to its phenolic compounds and other phytochemicals, which also enhance its antimicrobial and anti-inflammatory actions. Although many studies have aimed to validate the medicinal benefits of *Curcuma longa*, the phytochemical analysis identified a wide variety of compounds, such as alkaloids, carbohydrates, glycosides, and flavonoids. Further research is suggested to clarify the specific active phytochemical compounds responsible for these effects and to explore their mechanisms.

CHAPTER SEVEN
REFERENCE

References

1. Monkheang, P., Sudmoon, R., Tanee, T., Noikotr, K., Bletter, N., & Chaveerach, A. (2011). Species diversity, usages, molecular markers and barcode of medicinal Senna species (Fabaceae, Caesalpinioideae) in Thailand. *Journal of Medicinal Plants Research*, *5*(26), 6173-6181.
2. Gupta, S. S. (1994). Prospects and perspectives of natural plant products in medicine. Indian J Pharmacol, 26(1), 1-12.
3. Davidson-Hunt I. Ecological ethnobotany: stumbling toward new practices and paradigms. MASA J. 2000; 16:1–13.
4. Ahn, K. (2017). "The worldwide trend of using botanical drugs and strategies for developing global drugs". BMB Reports. 50 (3): 111–116. doi:10.5483/BMBRep.2017.50.3.221. PMC 5422022. PMID 27998396
5. Breslin, A. (2017). The chemical composition of green plants. Sciencing, Leaf Group Ltd, 76.
6. Egbuna, C., Mukherjee, M., Rao, G. N., Gido, L. J. F. J., & Tijjani, H. (2018). Introduction to phytochemistry. In Phytochemistry (pp. 3-36). Apple Academic Press.
7. Penso, G. (1980). The role of WHO in the selection and characterization of medicinal plants (vegetable drugs). Journal of ethnopharmacology, 2(2), 183-188.
8. Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. African journal of traditional, complementary and alternative medicines, 10(5), 210-229.
9. Ahmed, M., Kumari, N., Mirgani, Z., Saeed, A., Ramadan, A., Ahmed, M. H., & Almobarak, A. O. (2022). Metabolic syndrome; Definition, Pathogenesis, Elements, and the Effects of medicinal plants on its elements. Journal of Diabetes & Metabolic Disorders, 21(1), 1011-1022.

10. Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular aspects of Medicine, 27(1), 1-93.

11. Kelly K. History of medicine. New York: Facts on file; 2009. pp. 29–50.

12. Wiart C. Etnopharmacology of medicinal plants. New Jersey: Humana Press; 2006. pp. 1–50.

13. Tucakov J. Healing with plants – phytotherapy. Beograd: Culture; 1971. pp. 180–90.

14. Bojadzievski P. The health services in Bitola through the centuries. Bitola: Society of science and art; 1992. pp. 15–27.

15. Gorunovic M, Lukic P. Pharmacognosy. Beograd: Gorunovic M; 2001. pp. 1–5.

16. Tucakov J. Pharmacognosy. Beograd: Institute for text book issuing in SR. Srbije; 1964. pp. 11–30.

17. Lukic P. Pharmacognosy. Beograd: SSO Faculty of Pharmacy; 1985. pp. 8–22.

18. Kovacevic N. Fundamentals of pharmacognosy. Beograd: Personal edition; 2000. pp. 170–1.

19. Royal Botanic Gardens, Kew (continuously updated). Plants of the World Online Kew.

20. Hard to swallow". Nature. 448 (7150): 105–06. 2007. Bibcode:2007Natur.448S.105. doi:10.1038/448106a. PMID 17625521.

21.al, S. K., & Shukla, Y. (2003). Herbal medicine: current status and the future. Asian pacific journal of cancer prevention, 4(4), 281-288.

22. Kuhn, M. (1868). Filices Africanae: Revisio critica omnium hucusque cognitorum cormophytorum Africae indigenorum additamentis Braunianis novisque Africanis speciebus ex reliquiis Mettenianis adaucta. Accedunt Filices Deckenianae et Petersianae.

23.http://www.flowersofindia.net/catalog/slides/Small%20Knotweed.html

24.https://indiabiodiversity.org/species/show/23084526.

25.https://www.medicalnewstoday.com/articles/318522

26.H. Gibbs, J. Fletcher, P. Blombery et al., "Venous thromboembolism prophylaxis guideline implementation is improved by nurse directed feedback and audit," Thrombosis Journal, vol. 9, no. 1, p. 7, 2011.

27. National Health and Medical Research Council, Stop the Clot: Integrating VTE Prevention Guideline Recommendations into Routine Hospital Care, National Health and Medical Research Council, 3rd edition, 2011.

28. Naess, S. C. Christiansen, P. Romundstad et al., "Incidence and mortality of venous thrombosis: a population-based study," Journal of Thrombosis and Haemostasis, vol. 5, no. 4, pp. 692–699, 2007.

29. Rasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Effect of Fagoniaarabica (Dhamasa) on in vitro thrombolysis. BMC Complement Altern Med. 2007; 7:36.

30.V. R. Winrow, P. G. Winyard, C. J. Morris and D. R. Blake, Br. Med. Bull., 1993, 49, 506

31.V. Bauer, R. Sotnikova, J. Machova, S. Matyas, V. Pucovsky and M. Stefek, Life Sci., 1999, 65, 1909

32.G. U. Bae, D. W. Seo, H. K. Kwon, H. Y. Lee, S. Hong, Z. W. Lee, K. S. Ha, H. W. Lee and J. W. Han, J. Biol. Chem., 1999, 274, 32596

33.M. G. Lindley, Trends Food Sci. Technol., 1998, 9, 336.

34.CRC Handbook of Free Radicals and Antioxidants, 1989; 1: 209-221.

35. Antioxidant Vitamins Benefits Not Yet Proved (editorial) NEJM, 1994; 230(15): 1080-1081.

36. Antioxidants and Physical Performance, Critical Reviews in Food Science and Nutrition, 1995:35(1&2): 131-141.

37. Anti-inflammatory and antinociceptive activities of Lippia nodiflora Linn. Ahmed F, Selim MS, Das AK, Choudhuri MS. Pharmazie. 2004 Apr;59(4):329-30. PMID: 15125587

38. Yekkirala, A. S., Roberson, D. P., Bean, B. P., & Woolf, C. J. (2017). Breaking barriers to novel analgesic drug development. Nature reviews Drug discovery, 16(8), 545-564.

39. Papich, M. G. (1997, May). Principles of analgesic drug therapy. In Seminars in Veterinary Medicine and Surgery (Small Animal) (Vol. 12, No. 2, pp. 80-93).

40. Halpern, L. M. (1973). Analgesics and other drugs for relief of pain. Postgraduate Medicine, 53(6), 91-100.

41. Vane JR, Bakhle YS, Botting RM: Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol 1998; 38: 97–120.

42. Mitchell JA, Akarasereenont P, Themermann C, Flower RJ, Vane JR: Selectivity of non-steroidal antiinflammatory drugs are inhibitors of constitutive and inducible cyclogenase. Proc Natl Acad Sci U S A 1993; 90: 11693–7

43. Yekkirala, A. S., Roberson, D. P., Bean, B. P., & Woolf, C. J. (2017). Breaking barriers to novel analgesic drug development. Nature reviews Drug discovery, 16(8), 545-564.

44. Toma W, Gracioso JD, Hiruma-Lima CA, Andrade FD, Vilegas W, Brito AS. Evaluation of the analgesic and antiedematogenic activities of Quassia amara bark extract. J Ethnopharmacol, 2003; 85(1): 19-23.

45. Nahar, U. J., Akter, M., Bhuiyan, M. M. R., & Rahmatullah, M. (2017). Evaluation of analgesic activity of methanolic extract of Holarrhena antidysenterica leaves by tail immersion and hot plate assay methods. World J. Pharm. Res, 7, 172-178.

46. Laird, S. A. & ten Kate, K. 2002. Linking biodiversity prospecting and forest conservation. In selling forest environmental services (ed. S. Pagiola, J. Bishop and N. Landell-mills).

47. Bastida, Jaume, Rodolfo Lavilla, and Francesc Viladomat. "Chemical and biological aspects of Narcissus alkaloids."

48. Brewster, James T., et al. "Classics in chemical neuroscience: Donepezil." ACS chemical neuroscience 10.1 (2018): 155-167.

49. Birks, Jacqueline S. "Cholinesterase inhibitors for Alzheimer's disease." Cochrane database of systematic reviews 1 (2006).

50. Gurib-Fakim, Ameenah. "Medicinal plants: traditions of yesterday and drugs of tomorrow." Molecular aspects of Medicine 27.1 (2006): 1-93.

51.https://www.sciencedirect.com/topics/chemistry/diterpene#:~:text=Diterpenes%20are%20fungal%20and%20plant,amounts%20in%20essential%20oils%20

52.http://nou.edu.ng/coursewarecontent/CHM%20318%20MARCH%202021.pdf

53. Activities and Molecular Mechanisms of Diterpenes, Diterpenoids, and Their Derivatives in Rheumatoid Arthritis. Evid Based Complement Alternat Med. 2022 Mar 25; 2022:4787643. doi: 10.1155/2022/4787643. PMID: 35368757; PMCID: PMC8975657.

54.https://my.clevelandclinic.org/health/body/24972-gums

55. Britannica, T. Editors of Encyclopaedia (2019, August 16). gum. Encyclopedia Britannica. https://www.britannica.com/science/gum-anatomy

56.https://my.clevelandclinic.org/health/drugs/17368-phytosterols-sterols--stanols

57. AbuMweis, S. S., Marinangeli, C. P., Frohlich, J., & Jones, P. J. (2014). Implementing phytosterols into medical practice as a cholesterol-lowering strategy: overview of efficacy, effectiveness, and safety. Canadian Journal of Cardiology, 30(10), 1225-1232.

58. Gupta, A. K., Savopoulos, C. G., Ahuja, J., & Hatzitolios, A. I. (2011). Role of phytosterols in lipid-lowering: current perspectives. QJM: An International Journal of Medicine, 104(4), 301-308.

59. Pratt, Charlotte W.; Cornely, Kathleen (2013). Essential Biochemistry (Third ed.). Wiley. p. 626. ISBN 978-1118083505.

60. Meneveau, N., Schiele, F., Vuillemenot, A., Valette, B., Grollier, G., Bernard, Y., & Bassand, J. P. (1997). Streptokinase vs alteplase in massive pulmonary embolism: a randomized trial assessing right heart haemodynamics and pulmonary vascular obstruction. European heart journal, 18(7), 1141-1148.

61. Young, Kung-Chia, et al. "Plasminogen activation by streptokinase via a unique mechanism." Journal of Biological Chemistry 273.5 (1998): 3110-3116.

62. Wang, S., Reed, G. L., & Hedstrom, L. (1999). Deletion of Ile1 changes the mechanismof streptokinase: evidence for the molecular sexuality hypothesis. Biochemistry, 38(16), 5232- 5240.

63. Wang, Xiaoqiang, et al. "Crystal structure of the catalytic domain of human plasmin complexed with streptokinase." Science 281.5383 (1998): 1662-1665.

64. Hansen, Carlo. "The genus Campimia (Melastomataceae)." Willdenowia (1988): 147-151.

65. Tambaro S, Reali R, Volonterio A, Zanda M, Olimpieri F, Pinna GA, Lazzari P [2013] NESS002 ie: A new fluorinated thiolendopeptidase inhibitor with antinociceptive activity in an animal model of persistent pain, Pharmacology, Biochemistry and Behavior, pp. 1-7

66. Harper D [2001]. Online Etymology Dictionary: Analgesia. Retrieved December 3:2012.

67. Tripathi KD [2008]. Essentials of Medical Pharmacology, Sixth edition, Jaypee brothers' medical publishers [p] ltd. pp.453.

68. Kulkarni SK. Handbook of Experimental Pharmacology (2012).4th ed. Vallabh Publication, New Delhi.

69. Muhammad Naveed (2014). In-vitro models for management of pain. Pharmacology and Pharmacy: 5: 92-96.