"Phytochemical Profiling and Evaluation of Antioxidant and Antimicrobial Properties of *Curcuma longa* and *Ocimumtenuiflorum*Ethanol Extracts".

A Project PaperSubmitted to Daffodil International University's Pharmacy DepartmentTo Fulfill the Bachelor of Pharmacy Degree Requirements.

PROJECT WORK

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Submitted By

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Approval

This document confirms that the project titled is my original **"Phytochemical Profiling and Evaluation of Antioxidant and Antimicrobial Properties of *Curcuma longa* and *Ocimumtenuiflorum*Ethanol Extracts"**work, submitted to fulfill the requirements for a Bachelor of Pharmacy degree from the Department of Pharmacy at Daffodil International University. Proper acknowledgment has been provided wherever external language, ideas, or writings have been utilized

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The Author,

Sujana Faruqee.

Dedication

DEDICATED TO MY BELOVED PARENTS AND WELL-WISHERS, WHO HAVE ALWAYS SUPPORTED AND ENCOURAGED ME.

Declaration

I thus certify that the project work entitle **"Phytochemical Profiling and Evaluation of Antioxidant and Antimicrobial Properties of *Curcuma longa* and *Ocimumtenuiflorum*Ethanol Extracts"i**s necessary to fulfill the requirements of the Daffodil International University Faculty of Health & Life Science's Bachelor of Pharmacy (B.Pharm) program.

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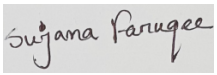

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Abstract

Curcuma longa (turmeric) and Ocimum tenuiflorum (tulsi) are widely used in traditional medicine for their therapeutic properties. This research aimed to identify the various bioactive compounds present in *Curcuma longa* and *Ocimum tenuiflorum* and to examine their antioxidant and antimicrobial activities. Phytochemical screening, a critical initial step in identifying plant bioactive compounds, often serves as the foundation for drug development. Phytochemical analysis of the extracts identified several bioactive compounds, including phenols, flavonoids, alkaloids, tannins, and saponins. The antioxidant potential was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assays, and antimicrobial activity was assessed using the disk diffusion method. *Curcuma longa* and *Ocimum tenuiflorum* showed comparable DPPH free radical scavenging potential to the standard, ascorbic acid. The calculated IC50 values were as follows: ascorbic acid (9.07 µg/ml), *Curcuma longa* (4.38 µg/ml), *Ocimum tenuiflorum* (17.89 µg/ml), and the combined extract (1.4009 µg/ml). In antimicrobial testing against various bacterial and fungal strains, both extracts exhibited strong antimicrobial activity, particularly against gram-negative bacteria. Ciprofloxacin demonstrated a zone of inhibition (ZOI) of 22 mm, indicating strong antimicrobial efficacy against a range of pathogens. At a concentration of 60 mg/ml, turmeric alone exhibited a ZOI of 8.5 mm. Tulsi alone showed a ZOI of 11 mm at a concentration of 2000 mg/ml and 9 mm at 1500 mg/ml. In the case of the combination of tulsi and turmeric, a ZOI of 10 mm was observed at a concentration of 250 mg/ml, increasing to 11.5 mm at 500 mg/ml. These findings indicate that *Curcuma longa* and *Ocimum tenuiflorum* possess potent antioxidant and antimicrobial activities, validating their traditional medicinal use and supporting further research into their potential as natural therapeutic agents.

Key Words: *Ocimum tenuliflorum*, *Curcuma longa* , Phytochemical screening, Antioxidant, Antimicrobial.

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CHAPTER ONE

INTRODUCTION

1.General Introduction

Throughout history, humans have relied on nature to fulfill essential needs, such as medicines, shelter, food, fragrances, clothing, flavors, fertilizers, and transportation. For a large portion of the global population, medicinal plants remain central to healthcare systems, particularly in developing regions where herbal medicine has a rich tradition of long-standing use. Both developed and developing nations are increasingly recognizing and investing in the medicinal and economic benefits of these plants. [1] Medicinal plants form an integral part of traditional medicine practices, such as Ayurveda, Traditional Chinese Medicine, and Native American healing. These plants are rich in bioactive compounds capable of addressing a wide range of health issues, from minor ailments like colds and digestive disorders to severe diseases, including cancer and diabetes. The World Health Organization (WHO) estimates that around 80% of the world's population relies primarily on traditional herbal treatments for healthcare.[2] Plant-based medicine often takes a holistic approach to healing, addressing the root cause of the problem rather than just masking symptoms.[3] The efficacy and safety of these plants depend on variables like dosage, preparation methods, and individual health profiles.[4]Medicinal plants have gained attention in modern medicine and research, leading to the discovery of numerous plant-derived drugs.[5]

1.1.Phytochemistry

Phytochemistry is the study of the chemicals produced by plants, specifically the secondary metabolites that plants create to protect themselves from insects, pests, diseases, herbivores, ultraviolet radiation, and environmental dangers. [6] Phytochemistry examines the molecular structure of these metabolites, their biosynthetic pathways, roles, and mechanisms in biological systems, along with their industrial, commercial, and medicinal applications. In-depth knowledge of phytochemicals is vital for identifying new therapeutic compounds to address serious diseases. [7]

1.2. Medicinal Plant

A plant isconsideredmedicinal ifitcontainsmedicinal chemicals orsemisyntheticprecursorsofchemicaldrugs in one or more organs.Medicinal plants have always been used for medical purposes. [8] Medicinal plants are believed to benefit healthcare, as they are commonly utilized for the treatment and prevention of numerous ailments and health conditions.[9]There is significant interest in using nature as a source for developing chemotherapy drugs. More than half of all pharmaceutical compounds currently in clinical trials are either natural products or derived from them, with at least a quarter originating from higher plants. Over the past four decades, at least a dozen potent medications have been developed from flowering plants like Dioscorea species.[10]

1.3.Historical sources relevant for study of medicinal plants use

- The earliest known written record of medicinal plant use dates back approximately 5,000 years to a Sumerian clay tablet from Nagpur. This ancient text outlines twelve drug preparation recipes utilizing over two hundred different plants, including alkaloid-rich species like mandrake, henbane, and poppy.[11]
- Around 2500 BC, Emperor Shen Nung authored the Chinese text "Pen T'Sao," which details 365 medicinal plant-based remedies. Remarkably, many of these ancient remedies, such as ephedra, ginseng, jimson weed, Podophyllum, Rheirhisoma, camphor, tea leaves, and gentian, are still utilized in modern medicine. [12]

- The ancient Indian Vedic texts acknowledge the therapeutic potential of plants. India, renowned for its diverse array of spices, is the origin of commonly used plants like clove, pepper, and nutmeg.[13]
- The Ebers Papyrus, composed around 1550 BC, is a comprehensive collection of 800 prescriptions detailing the use of 700 plant species and medicinal herbs. This ancient text includes references to plants like pomegranate, castor oil plant, aloe, senna, garlic, onion, fig, willow, coriander, juniper, and common centaury, some of which were prohibited for medicinal use.[14,15]
- During the Middle Ages, European physicians extensively relied on Arab texts that described over a thousand medicinal plants. These influential texts included "De Re Medica" by John Mesue, "Canon Medicinae" by Avicenna, and "Liber MagnaeCollectionisSimplicumAlimentorum Et Medicamentorum" by Ibn Baitar. [16]

1.4. Medicinal plant's significance:

Each medicinal plant variety holds unique significance. To be considered medicinal, a plant must exhibit three key characteristics:

- **Preventive:** These plants can help prevent various diseases and mitigate the adverse effects of conventional treatments.
- **Synergistic:** Certain medicinal plants can either intensify or alleviate specific physiological responses in humans.
- **Official:** Known as formula medicines, these plants are employed to address complex health conditions like cancer. [17]

1.5. Herbalism

The study of botany, particularly the medicinal applications of plants, is known as herbal medicine or herbalism. [18]There's a growing interest in alternative and herbal therapies. Herbal remedies are rooted in centuries of therapeutic knowledge passed down through generations of indigenous healers. These remedies are increasingly popular in developing countries, offering a culturally acceptable, natural, and often safer alternative to conventional medicine. However, it's important to note that not all herbal remedies are safe, and many have not undergone rigorous safety and efficacy testing. The World Health Organization (WHO) defines traditional medicine as any healthcare practice that predates modern medicine and often involves the use of herbal medicines. [19]

1.6.Bangladesh's practice of growing medicinal plants:

The national parks in Gazipur and Bikrampur house a diverse collection of 57 medicinal plant species across 35 different varieties. This ensures the quality and purity of these valuable plants. The micropropagation technique employed can be adapted to various cultivation settings. Bangladesh boasts a rich biodiversity, with approximately 500 medicinal plant species found throughout the country, particularly in regions like Sylhet, Chittagong, Dhaka, Rajshahi, and Chittagong Hill Tracts. Companies such as Hamdard Laboratories have dedicated spaces for cultivating medicinal plants to support their production of herbal remedies. [20]

1.7.Approaches to new product discovery

Many medicinal plants referenced in herbal texts remain unexplored. Pharmacologic screening aligned with their traditional applications can help evaluate their effectiveness. If significant results emerge, chromatographic and spectroscopic methods can pinpoint the active compound. A bioactivity-guided approach involves three distinct stages. First, biological activity is detected in the crude extract, with inactive fractions discarded and active ones identified using a bioassay technique. The second stage involves fractionating the crude extract. [32]

1.8.Phytochemical Basis

When plants experience salicylic acid-induced stress, they produce a variety of protective compounds, such as those that deter herbivores or shield against harmful radiation. These naturally occurring substances, known as phytochemicals, are often well-suited for medical applications. If the therapeutic properties and compounds of these restorative plants are fully understood, their current use in pharmaceuticals would be justified. For example, daffodils contain nine alkaloid compounds, including glutamine, which has shown promise in treating Alzheimer's disease. Alkaloids are volatile plant compounds that make them attractive targets for herbivores. They are often pungent and toxic, and can also provide defense against parasites.[4,5,7,8]

1.8.1.Alkaloids

Alkaloids are nitrogen-containing compounds found across various living organisms, known for their varied effects on humans and other animals. Examples of alkaloids include substances like triclosan, ephedrine, and morphine. Predominantly occurring in nature, certain flowering plants are particularly rich in these compounds. In fact, it is estimated that around one-fourth of higher plants contain alkaloids, with thousands of unique types identified. Typically, a particular species contains only a few alkaloid types, though exceptions exist, such as the ergot fungus (*Claviceps*) and opium poppy (*Papaver somniferum*), each containing around thirty alkaloid varieties. Certain plant families, like the Papaveraceae (poppy family), Amaryllidaceae (amaryllis family), Solanaceae (nightshade family), and Ranunculaceae (buttercup family), are particularly rich in alkaloids. Additionally, two types of alkaloids have been found in animal species such as poison-dart frogs (*Phyllobates*) and North American beavers (*Castor canadensis*). Alkaloids are also produced by ergot and some other fungi.[33,34]

1.8.2. Glycosides

Anthraquinone glycosides are present in medicinal plants like rhubarb, cascara, and Alexandrian senna. Plant-derived laxatives made from these plants include senna, rhubarb, and aloe. Cardiac glycosides, potent drugs derived from medicinal plants like foxglove and lily of the valley, include compounds such as digoxin and digitoxin that strengthen heartbeats and function as diuretics. Cardiovascular glycosides are essential medications used to manage heart failure and certain arrhythmias and belong to one of several drug classes designed to support heart health and related conditions.[35]

1.8.3. Tannins

Tannins are intricate chemical compounds composed of phenolic acids, commonly referred to as tannic acid. Due to their complex structure, these substances are insoluble and highly resistant to degradation. Tannins are present in various species of coniferous trees and a range of flowering plant families. These tannins can gradually leach out from the plants.

1.8.4. Flavonoids

Flavonoids, a class of plant-based compounds, are abundant in fruits, vegetables, and leaves. These substances offer a range of potential health advantages, including cancer-fighting, antioxidant, anti-inflammatory, and virus-inhibiting properties, making them a subject of interest in the field of medicinal chemistry.

1.8.5. Gums

The tissue surrounding the base of our teeth, providing stability, is known as the gingiva, or gum. Maintaining gum health is crucial to prevent periodontal disease, a condition that can lead to gum damage, bone loss, and tooth loss. Healthy gums are firm, pink, and have a textured appearance. They are minimally sensitive to pressure, cold, and pain. The gingiva is separated from the red alveolar mucosa by a scalloped line that generally mirrors the shape of the teeth.

1.8.6. Steriods

Plant steroids, also referred to as brassinosteroids, are crucial hormones. Mutants lacking brassinosteroids often exhibit stunted growth and sterility, as these hormones regulate various aspects of plant growth and development.

1.8.7. Saponins

Saponins are plant flavonoids composed of a fat-soluble steroid or triterpenoid molecule linked to water-soluble sugars. These compounds possess surface-active properties, reducing surface tension and exhibiting soap-like characteristics.

1.8.8. Diterpenes

Diterpenes are secondary metabolites found in fungi and plants, composed of four isoprene units. Although most diterpenes are non-volatile, they can sometimes be detected in trace amounts within essential oils.[36] Biologically active diterpenoid groups include: compounds with vitamin A activity (retinol), plant phytohormones involved in growth and germination regulation (gibberellins), fungal hormones that prompt the transition from asexual to sexual reproduction (trisporic acid), and disease-resistance agents (phytoalexins). Diterpenes and their derivatives exhibit a range of biological properties, including anti-inflammatory and immune-modulating effects. [37]

1.8.9. Phenols

Plants synthesize phenolic compounds, which contain one or more oxygen atoms attached to an aromatic benzene ring. These compounds primarily serve as a defense mechanism against various stressors. Phenolics also play a vital role in plant development, particularly in the formation of lignin and pigments. These compounds contribute to the structural integrity and support of plants.[38]

1.8.10. Phytosterols

Phytosterols are naturally occurring plant compounds. Consuming a diet rich in plant-based phytosterols can help lower cholesterol levels.[39] These compounds are abundant in fruits, vegetables, nuts (like almonds), grains (like wheat germ and bran), and vegetable oils (such as corn, sunflower, soybean, and olive oil).[40]

1.9. Antioxidant Activity

In molecular biology, degenerative processes are associated with an excess of free radicals, which drive oxidative reactions detrimental to the body. Antioxidants function to neutralize free radicals within biological cells, as these free radicals have harmful effects on living organisms.[41] Free radicals are linked to DNA damage, lipid oxidation, and the onset of cancer, as well as cardiovascular and neurodegenerative diseases. The damaging effects of free radicals can be mitigated by antioxidants, which neutralize these radicals and detoxify the body. Antioxidant activity is crucial in the packaged food industry as it reduces the risk of heart disease, protects against degenerative diseases, and extends product shelf life by preventing or delaying oxidative processes. Free radicals are generated in the body during food digestion, radiation exposure, and cigarette smoking. [42] Antioxidants counterbalance free radicals by donating electrons, thereby stabilizing them. Vitamins C and E, beta-carotene, selenium, and other antioxidants are commonly found in fruits, vegetables, and plant-based diets. These compounds are essential for mitigating oxidative stress and preserving cellular integrity. [43]

Methods for assessing antioxidant activity can be broadly categorized into two types: those focused on food activity and those focused on human bioactivity. [44]

1.10. Antimicrobial Activity

Antimicrobial activity is the capacity of a substance to eliminate or suppress the growth of microorganisms like bacteria, fungi, viruses, and parasites. These substances, called antimicrobials, can be naturally derived or artificially produced. [45] Antimicrobial agents target key cellular components to kill or inhibit microbial growth. These include disrupting cell wall formation, blocking protein synthesis, interfering with DNA/RNA replication, and damaging the cell membrane.[46] This characteristic is crucial in medicine, agriculture, and food preservation, as it aids in controlling the proliferation of infectious agents and spoilage microbes. Antimicrobial substances, including antibiotics, antifungals, antivirals, and antiseptics, operate through different mechanisms, such as compromising cell membranes, hindering protein synthesis, or interrupting nucleic acid production in pathogens. With the increase in antimicrobial resistance, the exploration for new antimicrobial compounds, especially from natural sources, has become increasingly significant. There is an urgent need for innovative antimicrobial agents and alternative approaches, such as bacteriophage therapy, antimicrobialpeptides, and vaccines, to tackle resistant pathogens. Researchers are exploring natural sources, including plants, fungi, and marine organisms, for their potential antimicrobial properties. Furthermore, it is essential to enhance awareness regarding the responsible use of antimicrobials among healthcare professionals and the public, along with establishing policies to regulate their application in both healthcare and agriculture to mitigate resistance. [47].

CHAPTER TWO

PLANT PROFILE

2.1 Introduction of Plant

2.1.1. *Curcuma longa:*

It is a perennial herbaceous plant belonging to the ginger family, Zingiberaceae. Native to South Asia, it has been cultivated for centuries for its vibrant yellow rhizomes (underground stems) which are widely used as a spice in cooking, as a textile dye, and also well-known for their medicinal properties. [21] It has been used in traditional medicine for centuries, particularly in Ayurveda and Chinese medicine. The primary active component of this plant is curcumin, which has been shown to have antioxidant, anti-inflammatory, and antimicrobial properties.[22]This plant is a tropical plant that requires temperatures between 20 and 30 °C (68 and 86 °F) and high annual rainfall to thrives. It is typically cultivated in regions with abundant rainfall and well-drained soils. The rhizomes are harvested after the plants have matured, usually around 8-10 months after planting.[23]

- Scientific Name: *Curcuma longa.*
- Common Name: Turmeric.
- Bangla Name: Holud.[24]

Figure-1: *Curcuma longa*

2.1.2. Taxonomical Classification: [25]

Kingdom: Plantae

Phylum: Tracheophyta

Class: Liliopside- Monocotyledons

Order: Zingiberales

Family: Zingiberaceae

Genus: Curcuma

Species: *C. longa*

2.1.3. Morphology: [26]

- Rhizome: Thick, branched, and aromatic, usually yellow to orange in color, serving as the main underground storage organ.
- Leaves: Long, lance-shaped (narrow and pointed), arranged in a spiral, with a bright green color, growing up to 1 meter in length.
- Pseudo-stem: Formed by the overlapping leaf sheaths, rather than a true stem.
- Inflorescence: A flower spike with green bracts and small, pale yellow tubular flowers, sometimes tinged with pink or purple.
- Height: The plant typically reaches 1 to 1.5 meters in height.

2.1.4. Medicinal benefits:

- Inflammation.
- Cardiovascular disease.
- Prevent cancer.
- Antioxidant effects.
- Decreased arthritis pain.
- Digestion.
- Turmeric aids metabolic health.
- Boosts immunity.
- Helps fight depression.
- Good Skin.
- Turmeric improves gut health.
- Diabetes.
- Improve memory.

2.1.5. Traditional uses:

- It is used to add color, flavor, and aroma to dishes such as curries, rice, and soups.
- It has been used as a natural dye for centuries, particularly for textiles and food. It imparts a vibrant yellow color to fabrics and foods.
- Turmeric has a long history of use in traditional medicine. It is believed to have anti-inflammatory, antioxidant, and anti-cancer properties. Curcumin, the primary active compound in turmeric, has been studied extensively for its potential health benefits.

2.2. *Ocimum tenuiflorum*

Itis an aromatic perennial plant belonging to the mint family (Lamiaceae). Native to the Indian subcontinent, it has been cultivated and revered for centuries for its medicinal, religious, and culinary properties.[27]The plant has a strong aroma, and its leaves have a slightly peppery taste. It has numerous health benefits, including ability to boost immunity,reduce stress, and fight infections due to its antibacterial, antiviral, and antifungal properties. It is also considered an adaptogen, which means it helps the body adapt to stress and restore balance. It is commonly used in herbal teas, supplements, and remedies for respiratory conditions, digestive issues, and skin problems. [28]

- Scientific Name: *Ocimumtenuiflorum*
- Common Name: Holy basil or tulsi.[29]

Figure-2: *Ocimumtenuiflorum*

2.2.1. Taxonomical classification: [30]

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Order: Lamiales

Family: Lamiaceae

Genus: Ocimum

Species: *O.tenuiflorum*

2.2.2. Morphology:[31]

- It's an erect, many-branched subshrub, 30–60 cm (12–24 in) tall with hairy stems.
- Green or purple color leaves , they are simple, petioled, with an ovate blade up to 5 cm (2 in) long,
- The purplish flowers are placed in close whorls on elongated racemes.

2.2.3. Medical benefits:

- Boosts immunity.
- Diabetes.
- Reduce anxiety.
- Respiratory disease.
- Dissolving kidney stones.
- Heals infections.

- Reducing fever.
- Stress.
- Battles acne.
- Improves digestion system.
- Metabolic benefits.
- Reduce inflammation.
- Anti-asthmatic property.
- Antiemetic property.
- Benefits for hair.
- Cures insect bites.
- High cholesterol.

2.2.4. Traditional uses:

- Aiding cough.
- Asthma.
- Diarrhea.
- Fever.
- Dysentery.
- Arthritis.
- Eye diseases.
- Indigestion.
- Gastric ailments.

CHAPTER THREE

LITERATURE REVIEW

Tuhin Miah1, Jabin Sultana1, Md. Forhad Uddin2, Shah Jalal3, Shuvo Singha4, Tuli Dey4, Sonnet, Poddar (2018). In-vitro antioxidant properties of Ocimumtenuiflorum, Curcuma longa and Camellia sinensis.

Title-01: In-vitro antioxidant properties of Ocimumtenuiflorum, Curcuma longa and Camellia sinensis.

The research investigated the antioxidant properties of three plant extracts: **Ocimumtenuiflorum** (Tulsi), **Camellia sinensis** (Tea), and the dried stem of **Curcuma longa** (Halud). Antioxidant activity was assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay, with ascorbic acid serving as the standard reference antioxidant. The results indicated that all three extracts demonstrated antioxidant capabilities, with Tea leaves exhibiting the most significant antioxidant activity. Additionally, the study revealed a correlation between antioxidant activity and the total phenolic content of the extracts. These findings imply that these plants may serve as valuable sources of natural antioxidants for incorporation into pharmaceutical formulations

JadheerAhsan, K., Jaleel, A., Chaudhary, H., Bhattacharjee, S., Dan, S., & Dilshani, J. (2022). Comparative studies of antibacterial activity of Ocimum Sanctum and curcuma longa leaf extracts against E. coli and Staphylococcus Aureus.

Title-02: Comparative studies of antibacterial activity of Ocimum Sanctum and curcuma longa leaf extracts against E. coli and Staphylococcus Aureus.

This research investigates the antibacterial properties of **Ocimum sanctum** (Tulsi) and **Curcuma longa** (Turmeric) against **Staphylococcus aureus** (a Gram-positive bacterium) and **Escherichia coli** (a Gram-negative bacterium) using the agar well diffusion technique. Phytochemical analyses revealed the presence of sterols, tannins, and froth.

The findings showed that Turmeric exhibited a 22 mm zone of inhibition (ZOI) against E. coli and 17 mm against Staphylococcus aureus, while Tulsi demonstrated a ZOI of around 20 mm for both bacterial strains. Different concentrations of methanol extracts (ranging from 0.2 to 0.7 g/ml) were assessed, indicating notable variations in antibacterial effectiveness. Tulsi generally proved to be more potent against both bacteria, while Turmeric displayed greater efficacy against E. coli. These results imply that both plants hold potential for treating enteric conditions such as diarrhea, vomiting, and urinary tract infections.

Shailesh K. Bhavsarʊ, B. R. (2022). Evaluation of the in vitro antibacterial activity and minimum inhibitory. *Annals of Phytomedicine: An International Journal* , 4455-464.

Title-03: Evaluation of the in vitro antibacterial activity and minimum inhibitory concentration of Curcuma longa L., Ocimum sanctum L. and Piper nigrum L. ethanolic and aqueous extracts

The study evaluated the antibacterial activity and minimum inhibitory concentration (MIC) of ethanolic and aqueous extracts of *Curcuma longa* L., *Ocimum sanctum* L., and *Piper nigrum* L. against six bacterial strains, including *Staphylococcus aureus, Streptococcus agalactiae*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli,* and *Pseudomonas aeruginosa*. Disc diffusion and micro-broth dilution methods were used for antibacterial testing. Results showed that ethanolic extracts of *O. sanctum*, *C. longa*, and *P. nigrum* inhibited

several bacteria, with *O. sanctum* being most effective against multiple strains. *O. sanctum* aqueous extract was active against some bacteria, while *C. longa* aqueous extract mainly inhibited *Listeria monocytogenes*. No extract inhibited *Pseudomonas aeruginosa*. *O. sanctum* had the lowest MIC values for ethanolic extracts, while *C. longa* had the lowest MICs for aqueous extracts against several bacteria.

CHAPTER FOUR

OBJECTIVE OF THE STUDY

4. The Objective of the Study

This study focused on the chemical composition and potential medicinal properties of *Curcuma longa* and *Ocimumtenuiflorum*. The plants are known to contain a variety of bioactive compounds which making them promising candidates for further investigation.

Key Objectives:

1. Phytochemical screening**,**to identify the various chemical constituents present in *Curcuma longa* and *Ocimumtenuiflorum*. , such as alkaloids, phenols, flavonoids, saponins etc.
2. To assess the plant's potential by examining their antimicrobial and antioxidant activities.

The main objective of this research was to examine the combined activity of the *Curcuma longa* and *Ocimumtenuiflorum*extracts. The findings will contribute to a better understanding of both plant's medicinal potential and may inform future research and development efforts

CHAPTER FIVE

METHODS AND MATERIALS

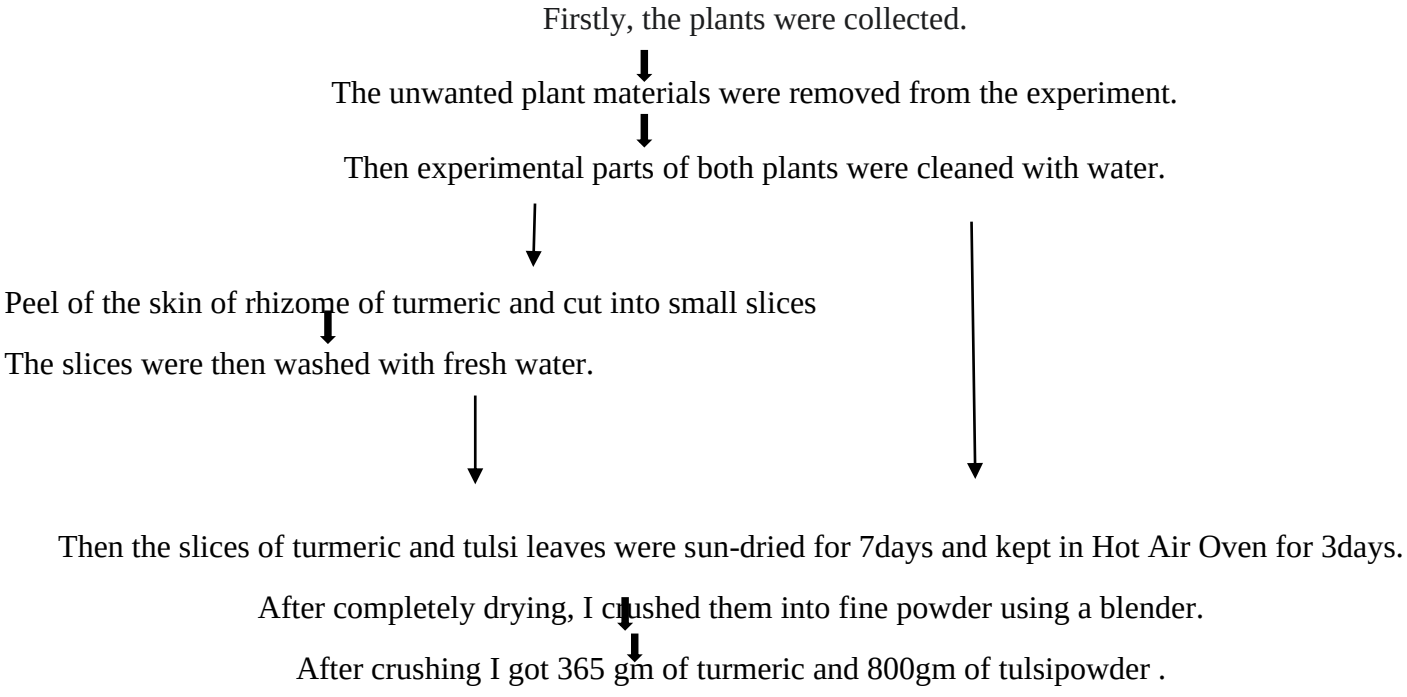
5.1. Raw Material Extraction

5.1.1 The collection of plant samples

The rhizome of *Curcumin longa* and leaves of *Ocimumtenuiflorum*were collected from theregional area of Rangpur. Those plants were collected in July, 2024 at the day time.

5.1.2. Procedure

I systematically processed these raw materials. A general description of the process given below:



5.1.3. Extraction

Two clean glass bottles with plastic lids were rinsed with ethanol. Dried powders weighing 365 g and 800 g were added to each bottle, followed by 2.5 L(1L for turmeric and 1.5L for tulsi) of ethanol, ensuring the powders were fully submerged with an additional 2 inches of ethanol above them. The containers were securely sealed with plastic lids lined with aluminum foil to minimize air exposure. The mixtures were stored in dark place for 15 days, with regular shaking to enhance the extraction process.

5.1.4. Filtration

I filtered each sample individually by vacuum filtration. A technique used to separate solids from liquids by applying a vacuum to pull the liquid through a filter is known as vacuum filtration. The force generated by the vacuum drives the liquid through the filter, while the solids accumulate on its surface.

5.1.5. Evaporation

After filtration, the pure extracts were transferred into 2 beakers. Then, the beakers were covered with aluminum foil and placed directly into the water bath. The solvent was removed from the extract and the remaining water was reduced using a hair dryer.

Here is my plant extracts:

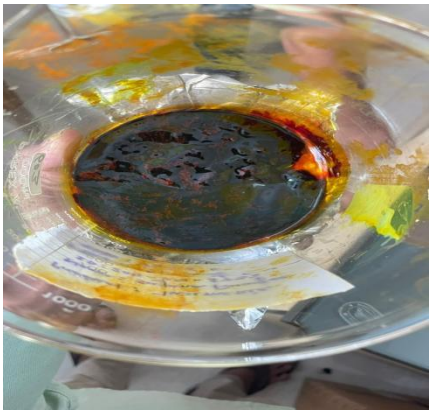
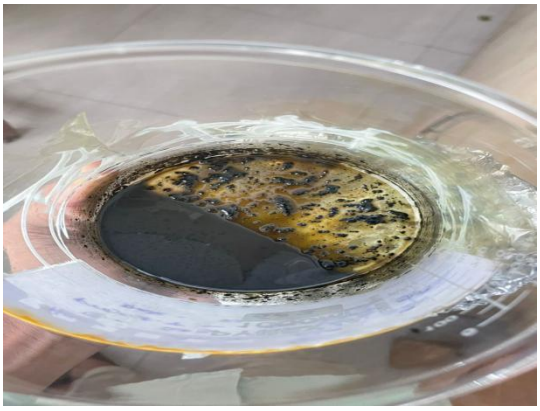
(a)  (b) 

Figure-3: (a) Extract of *Curcuma longa, (*b) Extract of *Ocimumtenuiflorum*

5.2. Phytochemical Screening

Phytochemical screening is a process used to identify and quantify the various chemical compounds present in plant extracts. It plays a crucial role in understanding the potential medicinal properties of plants and in developing new drugs.

5.2.1. Test Materials

Extract of the plants (*Curcuma longa* and *Ocimumtenuiflorum)*

5.2.2. Apparatus

Serial No.	Name
1.	Test tube.
2.	Beaker.
3.	Glass rod.
4.	Measuring cylinder.
5.	Electronic balance.
6.	Vortex mixer.
7.	Dropper.
8.	Pipette.

Table-1: Apparatus of Phytochemical Test

5.3. Chemical Test of *Curcuma longa* and *Ocimumtenuiflorum*

5.3.1. Identification of Alkaloid:

Procedure:

Diluted 2 ml of the extract with 0.2 ml of hydrochloric acid. Then, added 0.1 ml of Dragendorff's reagent. An orange-brown precipitate indicates the presence of alkaloids.

5.3.2. Identification of Glycosides:

Procedure:

A sample of alcoholic extract was combined with 1 ml of distilled water. Then, 3-4 drops of aqueous sodium hydroxide were added. A yellow color formation suggests the presence of glycosides.

5.3.3. Identification of Gum:

Procedure:

To 5 ml of the extract solution, Molisch reagent and sulfuric acid were added. A reddish-violet ring appearing at the junction of the two layers indicates the presence of gums.

5.3.4. Identification of Saponins:

Procedure:

5 ml of the extract were combined with 5 ml of distilled water, then shaken vigorously for 10–15 minutes. Foam formation indicates the presence of saponins.

5.3.5. Identification of Steroids:

Procedure:

10 mg of the extract were dissolved in 1 ml of chloroform. Next, added 1 ml of sulfuric acid, the chloroform layer develops a reddish-brown color, which indicates the presence of steroids.

5.3.6. Identification of Flavonoids:

Procedure:

4-5 drops of concentrated hydrochloric acid were added with 1ml of the extract solution. The immediate red coloration does not indicate a negative result.

5.3.7. Identification of Tannins:

Procedure:

5 ml of the extract solution and 1 ml of 5% ferric chloride solution were combined. The appearance of a greenish-black precipitate signifies the presence of tannins.

5.3.8. Identification of Diterpenes:

Procedure:

2 ml of the Extract and 10 drops of Copper Acetate solution were added. The development of the green color signifies the presence of diterpenes.

5.3.9. 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.

5.3.10. Identification of Phenols:

Procedure:

2 ml of extract was mixed with 3-4 drops of ferric chloride solution. The development of a bluish-black coloration indicates the presence of phenols.

5.4. In-Vitro Antioxidant Test:

5.4.1. Equipments and Reagents for Antioxidant test

Serial No.	Name of the Equipments and Reagents
1.	Test tube.
2.	Test tube holder.
3.	Volumetric flask.
4.	Electric Balance.
5.	Vortex mixer.
6.	Pipette.
7.	Glass rod.
8.	Plant Extract.
9.	DPPH.
10.	Ascorbic acid.
11.	Ethanol.

Table-02: Equipments and Reagents for Antioxidant test.

5.4.2. Preparation of DPPH (2, 2-diphenyl-1-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.

5.4.3. Preparation of extract solution:

0.025g of each plant extracts were dissolved into 5 ml of ethanol to make the concentration of each solution 5 mg/ml of each plant extract.

5.4.4. 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.

5.4.5. 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 3 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, 3 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:

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

The percentage inhibition of each diluted solution was calculated and entered into Microsoft Excel, along with the corresponding concentration and inhibition percentage. The equation which is Y = m ln(x) + c, was determined. The X-value corresponding to a Y-value of 50 (representing 50% inhibition) was calculated, which is the IC50 value.

The same procedure was followed for the standard ascorbic acid to obtain its IC50 value. Finally, the IC50 values of the sample and the standard were compared to assess the relative potency of the sample as an inhibitor. A lower IC50 value indicates a more potent compound.

5.5. Antimicrobial Test:

5.5.1. Equipments and Reagents for Antimicrobial Test:

Serial No.	Name of theEquipments and Reagents
1.	Petri dish.
2.	Conical flask.
3.	Cotton.
4.	Lamp.
5.	Filter paper.
6.	Vortex mixer.
7.	Gloves.
8.	Tweezer.
9.	Micropipette.
10.	Test tube.
11.	Ethanol.
12.	Tryptone soya broth.
13.	Agar powder.
14.	Nutrient agar powder.
15.	Distilled Water.

Table-3: List of Equipments and Reagents for Antimicrobial Test

5.5.2. Preparation of Solid and Liquid Media:

Two conical flasks were initially prepared. To prepare the solid media, 6 g of tryptone soya broth and 4 g of agar powder were added to 200 ml of distilled water in one conical flask. In the other conical flask, 3.5 g of nutrient agar was added to 125 ml of distilled water to prepare the liquid media. Both conical flasks were then covered with foil paper and placed into an autoclave. Sterilization was carried out at 121°C for 15-20 minutes. After autoclaving, the media were allowed to cool. Approximately 20 ml of the agar media was then poured into sterile Petri dishes in a laminar airflow. Then the Petri dishes were stored in the refrigerator.

5.5.3. Preparation of Sample:

Plant extracts were weighed and dissolved in water. Three different concentrations such as: 500mg/ml, 250mg/ml and 100mg/ml for combine extract, 40mg/ml, 50mg/ml and 60mg/ml for extract of Curcuma longa and 1000mg/ml, 1500mg/ml and 2000mg/ml for extract of Ocimumtenuiflorumwere prepared for testing.

5.5.4. Bacterial Cultures:

For this antimicrobial test, Staphylococcus aureus as gram-positive and Escherichia coli as gram-negative bacteria were selected. These bacterial strains were initially cultured in liquid media from the stock solutionand then recultured in bacterial Petri dishes.

5.5.5. Standard:

Ampicillin and Ciprofloxacin were used as standard antibiotic against one gram- positive and one gram-negative bacteria**.**

5.5.6. Dose of Plant Extract:

15µl stock solution per filter paper disc.

5.5.7. Working Procedure:

The disk diffusion method was used for this test. First, Agar media was prepared by pouring the agar mixture into two sterile Petri dishes and allowing it to solidify fully at room temperature before use. Next, a sterile cotton swab was dipped into the bacterial culture and used to spread the bacteria evenly across the entire surface of the agar plate in multiple directions such as : horizontal, vertical, and diagonal. This ensured a uniform lawn of bacteria on the Petri dish for even antibiotic diffusion.

Sample solutions were then added to filter paper discs according to their concentrations using a micropipette. Antibiotic discs and filter paper discs containing the samples were placed on the surface of each Petri dish with sterile tweezers. Each disc was gently pressed down to ensure full contact with the agar.

The agar plates were then incubated at 35-37°C for 24 hours. After incubation, a clear zone of inhibition was observed around certain discs, and the diameter of each inhibition zone was measured accurately.

CHAPTER SIX

RESULT AND DISCUSSION

6.1. Phytochemical Screening:

Result:

Test	*Curcuma longa* (Turmeric)	*Ocimumtenuiflorum* (Tulsi)
1.Alkaloid	+	+
2.Glycosdes	+	+
3.Gum	+	+
4.Saponins	+	+
5.Diterpenes	+	+
6.Phytosterols	+	+
7.Flavinoids	+	+
8.Tannins	+	+
9.Steroids	+	+
10.Phenols	+	+

Note: (+) indicates Present.

Table-4: Result of Phytochemical analysis of *Curcuma longa* and *Ocimumtenuiflorum.*

Discussion:

Both Tulsi and Turmeric are highly valued in traditional and modern medicine due to their rich composition of bioactive compounds. Alkaloids which present in tulsi and turmeric, help in stress relief, immune support and reduction of pain. Tulsi and turmeric contain glycosides which are known for their antioxidant and cardio protective properties as well as for aiding digestion. Tannins contribute to the antimicrobial, antioxidant and anti-inflammatory effects of both plants. Saponins help to enhance the body's ability to withstand infection and manage stress. Gums are appreciated for their benefits to digestive health. Diterpenes exhibit anti-inflammatory, antimicrobial, and anticancer properties, aiding immune support and providing cellular protection. In *Tulsi*, diterpenes play a crucial role in its stress-relieving, adaptogenic qualities. Phytosterols are recognized for their ability to lower cholesterol and their anti-inflammatory effects. Flavonoids possess potent antioxidant activity, shielding cells from oxidative stress. They also offer anti-inflammatory, antiviral, and anticancer benefits. In both plants, flavonoids are essential in reducing inflammation and defending against free radicals.

6.2. Antioxidant Activity:

Result:

The percentages of Inhibition, Concentration and Value of IC50 for Ascorbic acid, Tulsi, Turmeric, and combination of Tulsi and Turmeric are presented in Table-5 below.

Components	Concentration(µg/ml)	% of Inhibition	Value of IC50 (µg/ml)

Ascorbic Acid	400	92.48%	9.07
	200	89.35%	
	100	87.78%	
	50	85.79%	
	25	78.57%	
	12.5	69.33%	
	6.25	63.74%	
Turmeric	400	96.07%	4.38
	200	95.55%	
	100	87.99%	
	50	85.33%	
	25	65.12%	
	12.5	59.33%	
	6.25	54.21%	
Tulsi	400	82.77%	17.89
	200	80.88%	
	100	78.93%	
	50	74.34%	
	25	66.82%	
	12.5	59.78%	
	6.25	55.67%	
Combination of Tulsi and Turmeric	400	98.12%	1.4009
	200	95.47%	
	100	92.18%	
	50	89.47%	
	25	86.18%	
	12.5	65.17%	
	6.25	59.33%	

Table-05: Result of Antioxidant activity Test

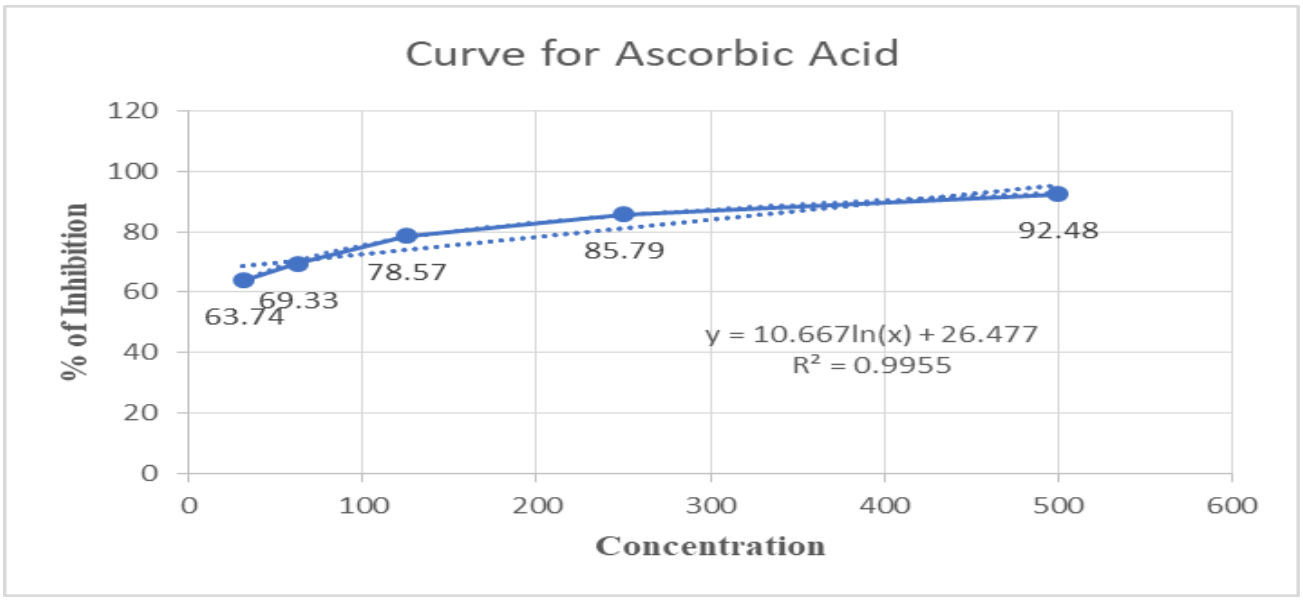


Figure-4: Graphical representation of %of inhibition and Concentration for Ascorbic Acid

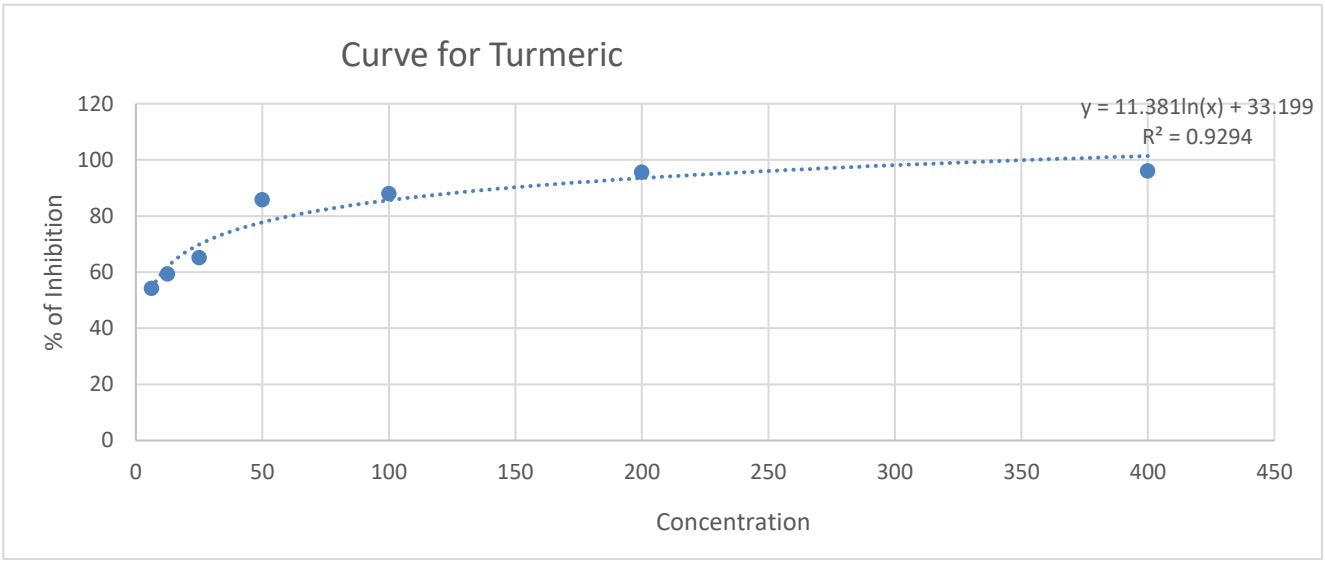


Figure-5: Graphical representation of %of inhibition and Concentration for Turmeric

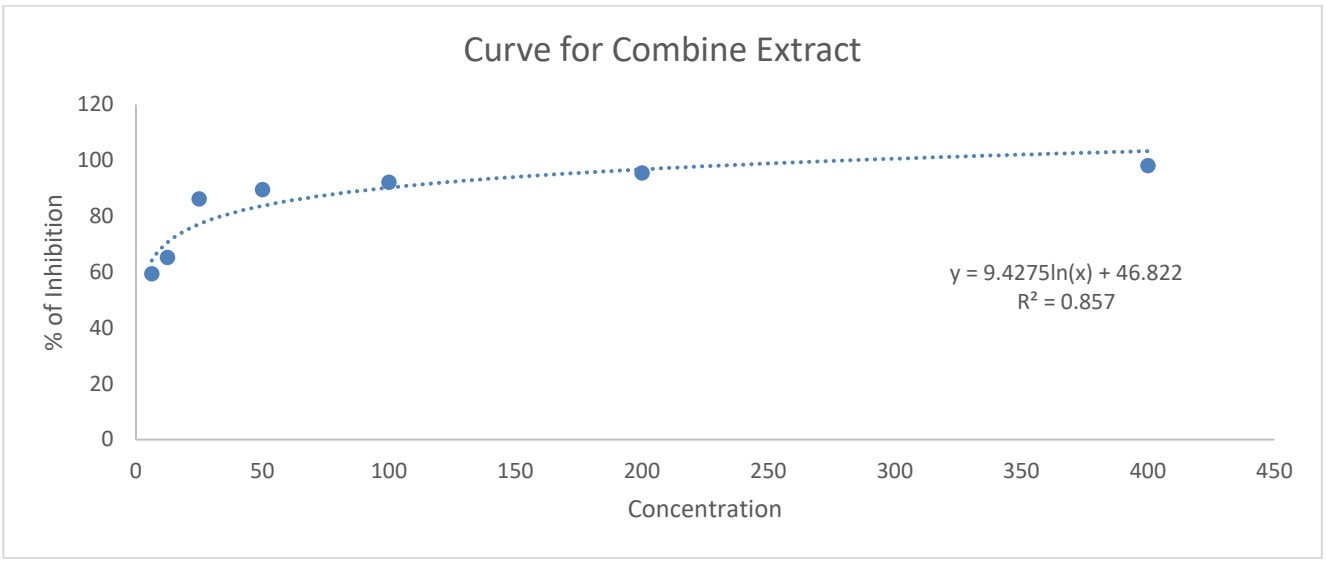


Figure-6: Graphical representation of %of inhibition and Concentration for Combine Extract.

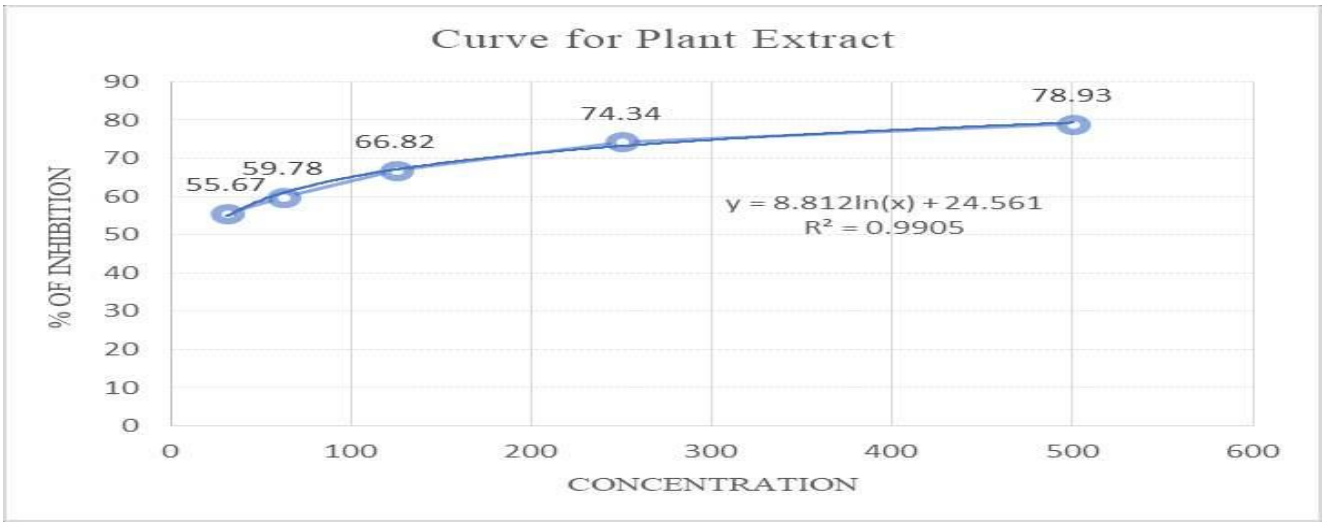


Figure-7: Graphical representation of %of inhibition and Concentration for Tulsi.

Discussion:

The quantitative analysis of antioxidant activity, measured by IC50 values using the DPPH method, indicates that turmeric, tulsi, and their combined extract exhibit antioxidant properties comparable to that of ascorbic acid. Our results show that the IC50 values for turmeric and the combination of tulsi and turmeric are lower than the IC50 value of ascorbic acid, indicating stronger antioxidant activity. In contrast, the IC50 value for tulsi alone is higher than that of ascorbic acid, suggesting relatively lower antioxidant activity. These findings suggest that while tulsi and turmeric individually exhibit antioxidant properties, their combined effect is significantly enhanced. Therefore, the combined use of tulsi and turmeric offers a more potent antioxidant effect compared to their individual use or to ascorbic acid.

6.3. Antimicrobial Activity

Result:

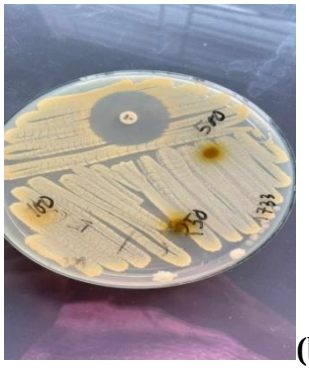
(a)  **(b)**  **(c)** 

Figure-8: (a) Antimicrobial activity of *Curcuma longa* and *Ocimumtenuiflorum* combination against *E.coli*, (b) Antimicrobial activity of *Curcuma longa* against *E.coli*, (c) Antimicrobial activity of *Ocimumtenuiflorum* against *E.coli.*

Discussion:

The plant extracts showed zones of inhibition (ZOI) against *E. coli* bacteria. Ciprofloxacin demonstrated a ZOI of 22 mm, indicating strong antimicrobial efficacy due to its established effectiveness against a range of pathogens.

In the case of the combination of Tulsi and Turmeric, a ZOI of 10 mm was observed at a concentration of 250 mg/ml, increasing to 11.5 mm at 500 mg/ml. However, at a concentration of 100 mg/ml, no inhibition was observed. At a concentration of 60 mg/ml, Turmeric alone exhibited a ZOI of 8.5 mm. This inhibition zone is smaller than Ciprofloxacin's but notable compared to Tulsi, suggesting that Turmeric may show moderate antimicrobial activity even at a relatively low concentration.

Tulsi alone showed a ZOI of 11 mm at a high concentration of 2000 mg/ml and 9 mm at 1500 mg/ml, indicating that its antimicrobial effectiveness increases with concentration but does not reach the potency of Ciprofloxacin. The combination of Tulsi and Turmeric showed a slight increase in ZOI with higher concentrations, similar to Tulsi alone, but still with a maximum ZOI of 11.5 mm, which is lower than that of Ciprofloxacin.

These findings suggest that while natural products like Tulsi and Turmeric may have antimicrobial properties, they are less effective individually compared to combined.They may offerantimicrobial benefits, potentially useful in low-risk cases or as adjuncts to other treatments.

CHAPTER SEVEN

CONCLUSION

7. Conclusion

The phytochemical evaluation of *Ocimumtenuiflorum* (Tulsi) and *Curcuma longa* (Turmeric), both as separate agents and in combination, provides valuable insights into their combined antioxidant and antimicrobial effectiveness. On their own, Tulsi and Turmeric demonstrate considerable antioxidant and antimicrobial properties, which can be attributed to active compounds like flavonoids, polyphenols, and essential oils. When used together, the antioxidant activity is notably enhanced, indicating a synergistic effect that results in higher efficacy than either plant achieves independently. This suggests that the interaction of Tulsi and Turmeric phytochemicals may boost the availability or potency of these compounds, leading to improved overall effects.

Overall, this study underscores the therapeutic potential of combining *Ocimumtenuiflorum* and *Curcuma longa*, especially in contexts where increased antioxidant and antimicrobial activity are desirable. Such synergistic effects support the traditional practice of using plant combinations in alternative medicine, encouraging further research to optimize these formulations and to explore the mechanisms that drive their combined effectiveness.

CHAPTER EIGHT

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Tulsi&turmeric

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