Phytochemical Screening Of *Ocimum sanctum* To Assess The Immune-boosting Property

Project Report

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

Submitted To

Department of Pharmacy

Faculty of Health & Life Sciences

Daffodil International University

Submitted By

Student ID: 203-29-412

Department of Pharmacy

Faculty of Health & Life Sciences

Daffodil International University

……

October 2024

APPROVAL

This project paper, **"Phytochemical Screening of *Ocimum sanctum* To Assess The Immune-boosting Property",** submitted to the Department of Pharmacy, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Bachelor programmed and approved as to its style and contents.

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Dr. Muniruddin Ahmed
Professor and Head
Department of Pharmacy
Faculty of Health & Life Sciences
Daffodil International University

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DECLARATION

I hereby declare that this project report **"Phytochemical Screening Of *Ocimum sanctum* To Assess The Immune-boosting Property ",** is done by me under the supervision of Dr. Sharifa Sultana Associate Professor and Associate Head, Department of Pharmacy, Faculty of Health & Life Sciences, Daffodil International University. I am declaring that this project is my original work. I also declare that neither this project nor any part therefore has been submitted elsewhere for the award of Bachelor or any degree.

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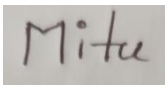

……

Amena Akter Mitu

Student ID: 203-29-412

Department of Pharmacy

Faculty of Health & Life Sciences

Daffodil International University

Submitted To

……

Dr. Sharifa Sultana

Associate Professor and Associate Head

Department of Pharmacy

Faculty of Health & Life Sciences

ACKNOWLEDGEMENT

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

I'm a lot of thankful to my honorable thesis supervisor **Dr. Sharifa Sultana** Associate Professor and Associate Head, Department of Pharmacy, Faculty of Health & Life Sciences, Daffodil International University for her brilliant direction and steady oversight just as for giving essential data in regards to the task and furthermore for her help in finishing the project.

I also wish to offer my respect to all of the teachers of Department of Pharmacy, Faculty of Health & Life Sciences, Daffodil International University and thankful to other members for their excellent cooperation with us.

Finally, I would like to express my gratitude towards my parents and other family members for their kind cooperation and encouragement which helped me in completion of this project.

My Parents,

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

My teacher,

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

Abstract

The study investigates the phytochemical composition, antioxidant capacity, and antimicrobial activity of *Ocimum sanctum* (Tulsi), a medicinal herb traditionally known for its immunity-boosting properties. Phytochemical screening confirmed the presence of key bioactive compounds, including alkaloids, saponins, phenols, glycosides and flavonoids, which are associated with immunomodulatory, antioxidant, and antimicrobial effects. Antioxidant analysis demonstrated notable free radical scavenging activity of the ethanol extract with an IC50 value of 17.33 µg/ml, compared to ascorbic acid with an IC50 of 9.11 µg/ml, suggesting that *Ocimum sanctum* can effectively counteract oxidative stress, supporting immune function. However, antimicrobial testing revealed limited inhibition against bacterial strains like *E. coli* and no substantial inhibition for *Staphylococcus* and *Klebsiella*, highlighting the need for further investigation to enhance its antibacterial efficacy. Overall, the findings support *Ocimum sanctum* as a valuable natural supplement for immunity enhancement, with its potent phytochemicals and antioxidant properties. Future research should focus on optimizing its formulation to enhance therapeutic potential.

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CHAPTER ONE

INTRODUCTION

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Introduction

1. Introduction

Herbal medicine has been a cornerstone of traditional healthcare systems for centuries, and in recent years, there has been growing scientific interest in the medicinal properties of various herbs. One such herb, *Ocimum sanctum*, commonly known as Tulsi or Holy Basil, has been highly revered in Ayurvedic and other traditional medicinal practices for its remarkable health benefits [1]. Tulsi is considered an "elixir of life," due to its potential to boost immunity, alleviate stress, and promote overall well-being. Phytochemicals are naturally occurring compounds found in plants that have been shown to possess therapeutic properties. Tulsi is rich in several bioactive compounds such as eugenol, ursolic acid, rosmarinic acid, and flavonoids, which contribute to its **antioxidant, antimicrobial,** and adaptogenic effects [2]. These properties make Tulsi a potent herb for enhancing the body's defense mechanisms against various diseases. This study aims to explore the phytochemical profile and pharmacological potential of Tulsi, focusing particularly on its role in immunity enhancement. By analyzing its chemical constituents and understanding its biological activities, this research seeks to validate the traditional uses of Tulsi and highlight its significance in modern therapeutic practices, especially in the context of boosting immunity [3].

Figure 1: *Ocimum sanctum [5]*

1.1 Medicinal plant in Bangladesh

Bangladesh, with its rich biodiversity and favorable climatic conditions, is home to a wide variety of medicinal plants that have been used for centuries in traditional healing practices. These plants play a significant role in the country's rural healthcare system, where traditional medicine continues to be a primary form of treatment for many communities [4]. The indigenous knowledge of medicinal plants has been passed down through generations, contributing to the extensive use of herbal remedies for treating various ailments. The country's medicinal flora includes more than 500 species of plants, many of which are recognized for their therapeutic properties. Notable medicinal plants of Bangladesh include Neem (Azadirachta indica), Tulsi (Ocimum sanctum), Turmeric (Curcuma longa), Aloe Vera (Aloe barbadensis), Ashwagandha (Withania somnifera), and Basak (Justicia adhatoda). These plants are widely used in both traditional and modern medicine for their anti-inflammatory, antimicrobial, antioxidant, and immune-boosting properties. In Bangladesh, the use of medicinal plants is not limited to traditional healers; it is also gaining increasing recognition in the pharmaceutical industry. With growing global demand for natural and plant-based medicines, these medicinal plants offer immense potential for drug development [6]. Various government and private sector initiatives have been launched to promote the sustainable cultivation, research, and commercialization of medicinal plants, making them an important resource for the healthcare and economic development of the country. Despite their importance, medicinal plants in Bangladesh face challenges such as habitat loss, over-harvesting, and lack of standardized cultivation practices. Efforts are needed to conserve this valuable natural resource and ensure its sustainable use for future generations [7].

1.2 Plants in traditional medicine

For centuries, plants have been a foundational element of traditional medicine systems around the world. They are the primary source of natural remedies used in healing practices such as Ayurveda, Traditional Chinese Medicine (TCM), Unani, and indigenous medical systems. These healing traditions have been based on the knowledge of plants' medicinal properties, passed down through generations, and have played a vital role in treating various diseases and maintaining overall health. Medicinal plants possess a wide array of

bioactive compounds like alkaloids, flavonoids, tannins, glycosides, terpenoids, and saponins, which have been studied for their therapeutic effects [8]. For instance, herbs like Neem (Azadirachta indica) are renowned for their antiseptic and anti-inflammatory properties, Tulsi (Ocimum sanctum) is known for boosting immunity and combating respiratory infections, while Ginger (Zingiber officinale) is widely used for its anti-nausea and digestive benefits. Plants in traditional medicine are used to treat a variety of conditions, including digestive disorders, infections, inflammation, pain, skin diseases, and even chronic diseases like diabetes and hypertension [9]. They offer holistic healing by balancing the body's systems and often have fewer side effects compared to synthetic drugs. Furthermore, herbal medicines are often more affordable and accessible, especially in rural or underdeveloped regions, making them a critical component of primary healthcare. In modern times, scientific research has validated many of the uses of medicinal plants, leading to the development of plant-based pharmaceuticals. The integration of traditional plant knowledge into modern medicine has led to the discovery of key drugs like aspirin from willow bark (Salix spp.) and the anti-cancer compound paclitaxel from the Pacific yew tree (Taxus brevifolia). However, with increasing demand for herbal remedies and plant-based medicines, there is a growing need to ensure the sustainability of medicinal plant resources. Conservation, sustainable harvesting practices, and proper documentation of traditional knowledge are essential to preserve these valuable resources for future generations. The resurgence of interest in plant-based therapies reflects a global shift towards natural, holistic healing and the enduring importance of plants in human health [10].

1.3 Chemical Composition of *Ocimum sanctum*

Ocimum sanctum, commonly known as Tulsi or Holy Basil, is a medicinal herb renowned for its wide array of therapeutic properties. The plant contains a rich profile of bioactive compounds that contribute to its pharmacological effects, such as antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory activities. The chemical composition of Tulsi includes various phytochemicals, which can be categorized into essential oils, flavonoids, phenolic compounds, and other secondary metabolites [11].

1. **Essential Oils**

   Ocimum sanctum is known for its aromatic essential oils, which are responsible for many of its medicinal properties. Key components of the essential oils include:

   - **Eugenol** (up to 70%): A phenolic compound with strong antiseptic, antioxidant, and anti-inflammatory properties.
   - **Methyl eugenol**: Known for its antibacterial and insecticidal activity.
   - **Carvacrol**: Exhibits antimicrobial and anti-inflammatory actions.
   - **Linalool**: A terpene with anti-anxiety and antimicrobial effects.
   - **Caryophyllene**: Known for its analgesic and anti-inflammatory effects.
   - **α-Pinene**: Contributes to its antimicrobial and bronchodilatory properties [12].

2. **Flavonoids**

   Tulsi is a rich source of flavonoids, which are powerful antioxidants and anti-inflammatory agents. Some important flavonoids found in *Ocimum sanctum* include:

   - **Apigenin**: Known for its anti-inflammatory, anti-cancer, and antioxidant properties.
   - **Luteolin**: Acts as an antioxidant, and has potential anti-cancer and anti-inflammatory effects.
   - **Quercetin**: Exhibits antioxidant, antihistamine, and antiviral activities [13].

3. **Phenolic Compounds**

   Phenolic acids in Tulsi contribute to its potent antioxidant activity. These include:

   - **Rosmarinic acid**: Known for its anti-inflammatory and antioxidant effects, which help in protecting cells from oxidative stress.
   - **Caffeic acid**: Exhibits antioxidant, anti-inflammatory, and antimicrobial properties.

4. **Triterpenoids**

   Tulsi contains several triterpenoids, which are known for their anti-inflammatory, analgesic, and immune-modulating properties:

- **Ursolic acid**: A bioactive compound with anti-inflammatory, anticancer, and antimicrobial properties.
- **Oleanolic acid**: Known for its hepatoprotective, anti-inflammatory, and antioxidant activities [14].

5. **Other Constituents**

   In addition to the major categories of compounds, Tulsi contains several other biologically active constituents such as:

   - **Saponins**: Known for their antimicrobial and immune-boosting effects.
   - **Tannins**: Provide astringent and antioxidant properties.
   - **Vitamins and Minerals**: Tulsi is also rich in vitamins A and C, calcium, zinc, and iron, which contribute to its role in boosting immunity and overall health [15].

1.4 Therapeutic use and pharmacological activity of *Ocimum sanctum*

Ocimum sanctum, commonly known as Tulsi or Holy Basil, is one of the most important medicinal plants in traditional Ayurvedic and other ancient medical systems. Its broad spectrum of therapeutic uses is attributed to its rich chemical composition, which includes essential oils, flavonoids, triterpenoids, and phenolic compounds. These bioactive components confer various pharmacological properties that have been scientifically studied and validated. Below are the major therapeutic uses and pharmacological activities of *Ocimum sanctum*:

1. **Immunomodulatory Activity**

Tulsi is widely recognized for its ability to boost the immune system. It enhances both cellular and humoral immune responses, making the body more resistant to infections. Its immunomodulatory effects are primarily attributed to compounds like ursolic acid, rosmarinic acid, and eugenol, which help in modulating immune responses and stimulating the production of antibodies [16].

- **Therapeutic Use**: Tulsi is used to improve immune function, making it helpful in preventing colds, flu, and other infectious diseases.

2. **Antioxidant Activity**

Tulsi has strong antioxidant properties, which help in neutralizing free radicals that cause oxidative stress. This action is largely due to phenolic compounds such as rosmarinic acid and flavonoids like luteolin and apigenin.

- **Therapeutic Use**: It is used in the management of conditions related to oxidative stress, including premature aging, cardiovascular diseases, and neurodegenerative disorders.

3. **Anti-inflammatory Activity**

The anti-inflammatory effects of *Ocimum sanctum* are attributed to bioactive compounds like eugenol, ursolic acid, and caryophyllene. These compounds inhibit the production of inflammatory mediators like prostaglandins and cytokines.

- **Therapeutic Use**: Tulsi is commonly used to treat inflammatory conditions such as arthritis, bronchitis, and inflammatory bowel disease (IBD) [17].

4. **Antimicrobial and Antiviral Activity**

Tulsi exhibits broad-spectrum antimicrobial properties against bacteria, viruses, fungi, and protozoa. Compounds like eugenol, carvacrol, and linalool contribute to its antibacterial, antiviral, and antifungal activities. It is especially effective against *Staphylococcus aureus*, *Escherichia coli,* and *Candida albicans*.

- **Therapeutic Use**: Tulsi is used to treat skin infections, respiratory infections, and digestive disorders caused by harmful microorganisms [18].

5. **Adaptogenic and Anti-stress Activity**

Tulsi is considered an adaptogen, a substance that helps the body adapt to stress and restore physiological balance. Its anti-stress effects are due to its ability to reduce cortisol levels and enhance the body's resilience to physical and emotional stressors.

- **Therapeutic Use**: Tulsi is widely used for reducing stress, anxiety, and depression. It helps in improving mental clarity and endurance under stressful conditions.

6. **Antidiabetic Activity**

Studies have shown that *Ocimum sanctum* helps in regulating blood glucose levels by enhancing insulin secretion and improving insulin sensitivity. It also reduces oxidative stress, which plays a key role in the development of diabetes complications [18].

- **Therapeutic Use**: Tulsi is used as a natural remedy for controlling diabetes and preventing complications associated with high blood sugar, such as nephropathy and neuropathy [19].

7. **Cardioprotective Activity**

The cardioprotective effects of Tulsi are attributed to its antioxidant and lipid-lowering properties. It helps in reducing cholesterol and triglyceride levels while improving blood circulation and protecting against ischemia-induced heart damage.

- **Therapeutic Use**: It is used to promote cardiovascular health, reduce high blood pressure, and prevent heart diseases like atherosclerosis and myocardial infarction.

8. **Hepatoprotective Activity**

Tulsi has been shown to protect the liver from damage caused by toxins, drugs, and oxidative stress. Compounds like ursolic acid and rosmarinic acid contribute to its hepatoprotective effects by enhancing the liver’s detoxification pathways and reducing oxidative damage.

- **Therapeutic Use**: It is used in the treatment of liver disorders, including fatty liver disease, hepatitis, and drug-induced liver toxicity [20].

9. **Anticancer Activity**

Several studies have indicated that *Ocimum sanctum* possesses anticancer properties, which are primarily linked to its ability to induce apoptosis (programmed cell death) and inhibit cancer cell proliferation. Compounds like eugenol and ursolic acid are believed to interfere with cancer cell signaling pathways.

- **Therapeutic Use**: Tulsi has been studied for its potential role in cancer prevention and as an adjunct in cancer therapy, particularly for skin, lung, liver, and breast cancers.

10. **Antiulcer Activity**

Tulsi has gastroprotective properties, which help in preventing and treating gastric ulcers. Its antiulcer effects are attributed to its ability to reduce gastric acid secretion, increase mucus production, and enhance the healing of ulcerated tissues.

- **Therapeutic Use**: It is used in the treatment of peptic ulcers and other gastrointestinal disorders, such as acid reflux and gastritis [21].

11. **Antipyretic and Analgesic Activity**

Ocimum sanctum has been traditionally used to reduce fever and relieve pain. Its antipyretic and analgesic activities are linked to the presence of compounds like eugenol and linalool, which act on the central nervous system to reduce pain and fever.

- **Therapeutic Use**: Tulsi is commonly used as a remedy for fever, headaches, and body aches.

12. **Respiratory Benefits**

Tulsi is particularly beneficial for respiratory health. It helps to relieve symptoms of asthma, bronchitis, and other respiratory disorders due to its bronchodilatory, expectorant properties. It is also effective in treating coughs, colds, and sinusitis.

- **Therapeutic Use**: Tulsi is widely used in treating respiratory conditions, improving lung function, and alleviating symptoms of allergies and asthma [22].

1.5 Evaluation of Anti-oxidant activity

Oxidative stress, induced by the imbalance between free radicals and antioxidants, plays a crucial role in the pathogenesis of various diseases, including cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders. Antioxidants are molecules that inhibit the oxidation of other molecules, thereby preventing cell damage. They work by neutralizing free radicals or reactive oxygen species (ROS), which are highly reactive and unstable molecules that can damage lipids, proteins, and DNA. The evaluation of antioxidant activity is essential for understanding the protective mechanisms of natural and synthetic compounds against oxidative stress. Natural antioxidants, commonly found in fruits, vegetables, and medicinal plants, have garnered significant attention due to their potential health benefits. These include phenolic compounds, flavonoids, vitamins, and enzymes that act as potent scavengers of free radicals. The assessment of antioxidant activity involves various in vitro and in vivo methods to quantify the ability of a compound or extract to neutralize free radicals. Common in vitro assays include DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP (Ferric Reducing Antioxidant Power). Each method provides unique insights into the efficacy of antioxidants under different conditions. In this study, we aim to evaluate the antioxidant potential of selected compounds or plant extracts using standardized methods, which will contribute to the understanding of their role in mitigating oxidative damage and promoting health [23].

1.6 Evaluation of Anti-microbial activity

The rise of microbial resistance to conventional antibiotics has become a critical global health issue, prompting the search for new antimicrobial agents from natural, synthetic,

and semi-synthetic sources. Antimicrobial agents play a vital role in controlling the growth of microorganisms, including bacteria, fungi, and viruses, which are responsible for numerous infectious diseases in humans, animals, and plants. Evaluating the antimicrobial activity of substances is an essential step in discovering new therapeutic agents that can effectively inhibit or kill pathogenic microorganisms. Many natural compounds, especially those derived from plants, are known for their bioactive properties, including antimicrobial effects. Such compounds have been used traditionally in medicine and have gained renewed interest due to their potential in addressing antibiotic resistance. The antimicrobial activity of a compound or extract is typically evaluated using various in vitro techniques, such as the disk diffusion method, broth or agar dilution methods, and minimum inhibitory concentration (MIC) determination. These assays help determine the spectrum of activity (broad-spectrum or narrow-spectrum) and the effectiveness of a substance against different microbial strains. In this study, we aim to assess the antimicrobial activity of selected agents by using standardized methods. The results of this evaluation will provide valuable insights into the potential use of these agents as alternative treatments for microbial infections, contributing to the development of new antimicrobial strategies [24].

CHAPTER TWO

LITERATURE REVIEW

Chapter TWO
Literature Review

Phytochemical and Pharmacological Insights into *Ocimum sanctum* as an Immunity Booster

Ocimum sanctum (Tulsi), commonly known as Holy Basil, has been extensively studied for its wide range of therapeutic applications. This review synthesizes existing literature on the phytochemical constituents and pharmacological activities of *Ocimum sanctum*, focusing on its role as an immunity booster. The review highlights key compounds, including eugenol, ursolic acid, and apigenin, which contribute to its antioxidant, antimicrobial properties. These bioactive compounds demonstrate the ability to scavenge free radicals, modulate inflammatory responses, and inhibit the growth of pathogenic microorganisms, providing a holistic approach to immune enhancement. Additionally, the therapeutic applications of *Ocimum sanctum* in the management of various diseases are discussed, supporting its potential as a natural immunomodulator [25].
This review seeks to present a comprehensive analysis of the phytochemical composition and pharmacological properties of Ocimum sanctum, with a focus on its antioxidant and antimicrobial benefits. By examining numerous studies, the paper highlights the primary phytochemicals—such as flavonoids, tannins, and phenolic acids—that contribute to its therapeutic effects. These compounds show strong antioxidant capabilities, helping to counter oxidative stress, which plays a role in chronic inflammation and infections. Additionally, the review investigates how Ocimum sanctum influences immune responses, underscoring its potential as a natural agent for enhancing immunity and preventing infectious diseases [22].

CHAPTER THREE

PURPOSE OF THE STUDY

Chapter THREE
Purpose of the study

3. Objective of the study

The purpose of this study is to perform a detailed phytochemical and pharmacological analysis of Ocimum sanctum (Tulsi), focusing on its **antioxidant and antimicrobial** properties. By investigating its bioactive compounds and their effects on boosting immunity, the research aims to scientifically validate Tulsi's traditional medicinal uses and explore its potential for developing natural therapeutic agents for immune system support.

CHAPTER FOUR

MATERIALS & METHODS

4. Materials & methodology

Analyzing phytochemicals entails studying the bioactive substances found in plants. These chemicals are extracted, isolated, and characterized using a variety of techniques and equipment.

4.1 Description of plant extraction is given below:

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

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

⇓

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

⇓

Then whole plant was clean by fresh water.

⇓

Then the plant leaves was dried under sun two days and air-dried for 15 days by fan.

⇓

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

⇓

Then we got **800 gm powder** and kept in an amber glass bottle.

⇓

Then the powder was dissolved in **1.5L of ethanol**.

⇓

Stored in cool place for 15 days.

⇓

Then filter it by vacuum filtration.

⇓

Finally the extract solution was dried by hair dryer.

4.2 Extraction:

A glass jar with a plastic top was thoroughly cleaned before being given an ethanol rinse. Subsequently, 1500 milliliters of ethanol were poured to the jar containing 800 grams of the dry powder, before the surface of the sample was sufficiently coated, reaching a height of one inch above the jar. The jar was appropriately airtight, with a plastic cover that was coated with aluminum foil. The procedure described above was done for twelve days. To enhance the extraction procedure, the jar was shook nearly twice a day.

4.2.1 Filtration

Vacuum filtration is a technique used in laboratories to separate solids from liquids quickly and efficiently. This method involves using a vacuum to draw the liquid through a filter, leaving the solid behind on the filter surface.

Figure 2: Filtration of extract Vacuum Filtration Apparatus

4.3 My investigation

In my investigation I have been gadget

- Phytochemical Screening test
- Anti-oxidant activity
- Anti-microbial activity

4.4 Phytochemical Assessment

As part of the phytochemical evaluation process, plant phytochemicals are examined and assessed. Plants naturally contain bioactive molecules called phytochemicals, which may have positive effects on human health. These compounds help the plant prevent disease, maintain links with its environment, and strengthen its defense mechanisms. Phytochemicals encompass a wide range of compounds, including as phenolic acids, alkaloids, flavonoids, terpenoids, saponins, and more.

4.4.1 Reagents applied for the diverse chemical group analysis

Stock Solution Preparation:

At first, mix 1 gm extract(Mixture of Ocimum sanctum with ethanol) with 40 ml ethanol.

4.4.1 Assessment for alkaloids

Mayer's evaluation

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

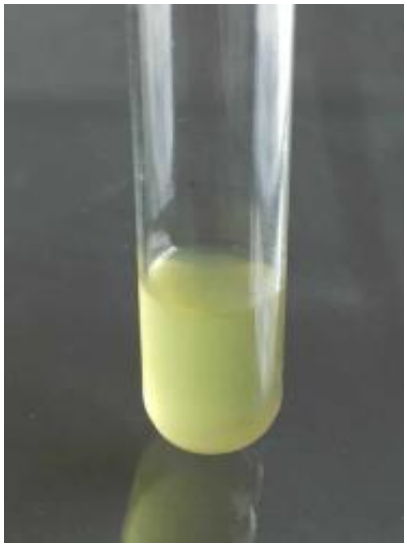


Figure 3: Mayer's evaluation

Dragendorff's examination

2 ml of stock solution and 0.2 ml of diluted hydrochloric acid were put into a test tube. Dragendorff's reagent (1 ml) was then added. An orange-brown precipitated was observed, indicating the possible involvement of alkaloids.

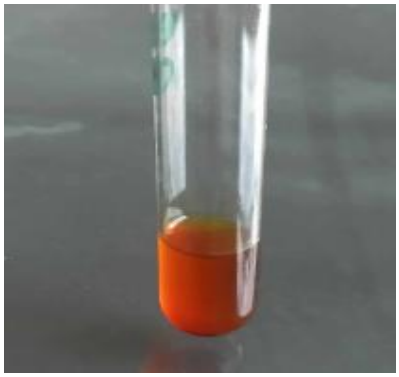


Figure 4: Dragendorff's examination

Wagner's Test

2 ml of stock solution and 1 ml of Wagner's reagent was applied. The appearance of a precipitate with a reddish indicated the existence of alkaloids.

Figure 5: Wagner's Test

4.4.2 Assessment for Flavonoids

1 ml of the stock solution is mixed with a few drops of ethanol and diluted H_2SO_4, then boiled. A color appears, and upon adding NaOH, the color vanishes, indicating the presence of flavonoids.

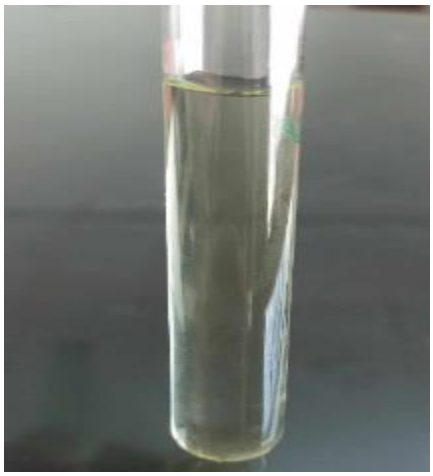


Figure 6: Flavonoids analysis of extract

4.4.3 Assessment for Saponins

After diluting 1 milliliter of the aqueous solution with 20 milliliters of distilled water, it was agitated in a glutted container for fifteen minutes. A foam layer just one centimeter thick indicates the presence of saponins.

Figure 7: Saponins analysis of extract

4.4.4 Test for Phenol

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

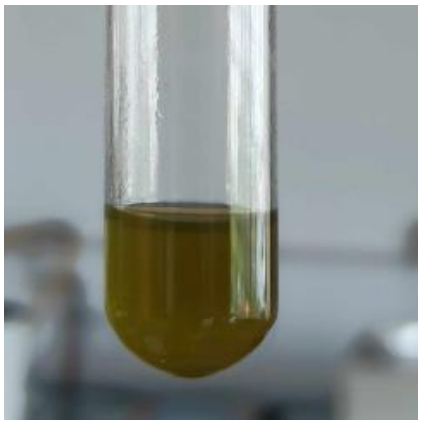


Figure 8: Test for Phenol

4.4.5 Acid Hydrolysis Test

The extract, when mixed with water and boiled with diluted H_2SO_4, and then neutralized with NaOH, showed positive results for the presence of glycosides.

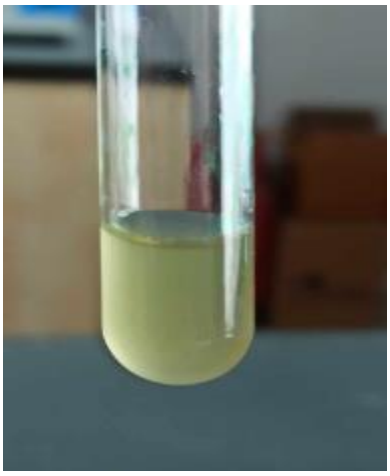


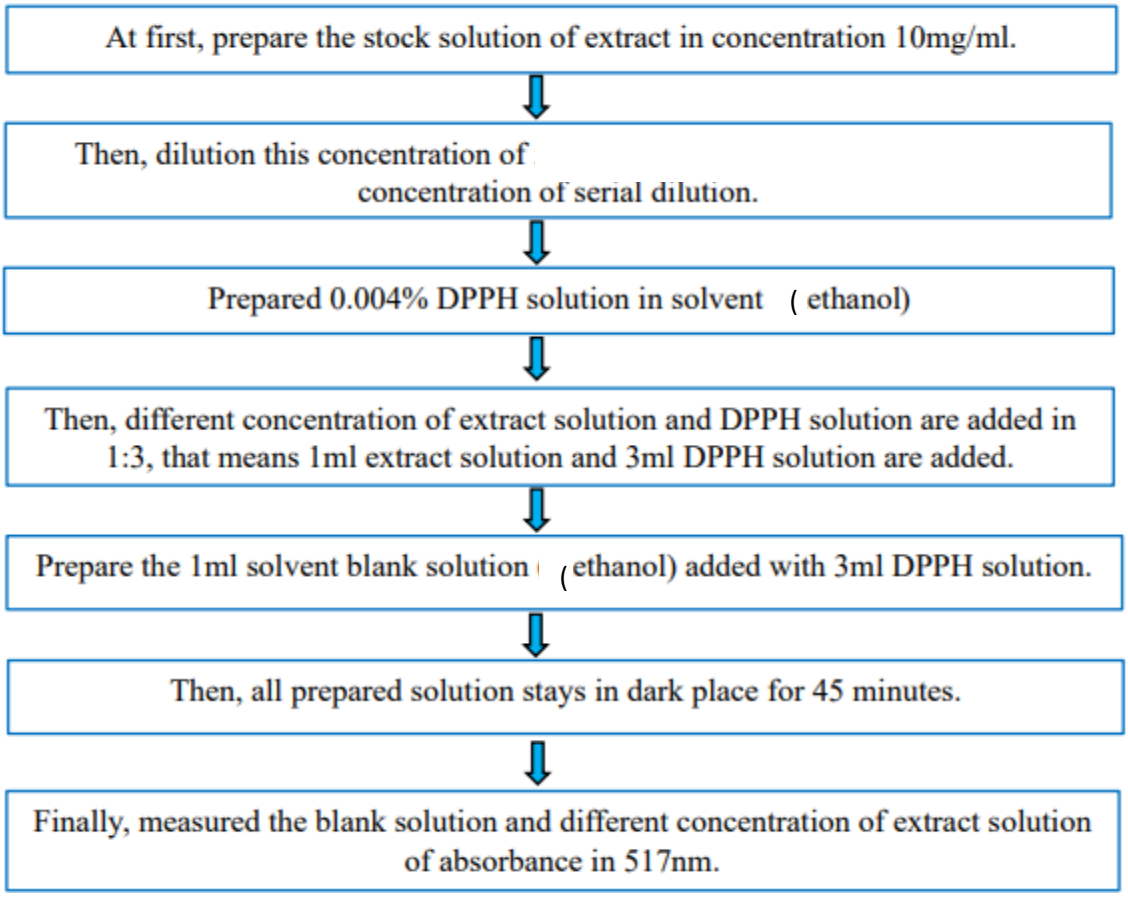
Figure 9: Acid Hydrolysis Test

4.5 Antioxidant Test

The plant extraction stock solution (10 mg/ml) was prepared in ethanol and used in a serial dilution. The first six concentration kinds are prepared using six volumetric flasks: **6.25, 12.5, 25, 50, 100, 200 and 400** ug/mL. We encircle volumetric flasks and test tubes with foil paper. Six volumetric flasks are prepared with varying extract dilutions and labeled appropriately.

Two milliliters of the sample for every concentration level and two milliliters of the 0.004% DPPH solution are pipetted into each of the six test tubes. Each test tube is wrapped in foil paper, and the solution is then left in a dark room for half an hour. In another tube for testing, combine two milliliters of 0.004% DPPH and two milliliters of methanol to create the blank solution. Next, UV Spectroscopy is used to measure absorption.

4.5.1 Working Procedure of Antioxidant Test



4.6 Test of antimicrobial activity by disc diffusion method

4.6.1 Principle of disc diffusion method

In this method, a measured amount of each test sample was dissolved in a specific volume of solvent to prepare solutions of known concentrations (µg/ml). Sterile Matricel (BBL, Cocksville, USA) filter paper discs were then impregnated with known quantities of the test substances using a micropipette and subsequently dried. Standard antibiotic and antifungal discs, which were treated with the same solvent used for dissolving the samples and then dried, served as positive and negative controls, respectively. These discs were placed on petri dishes containing an appropriate agar medium inoculated with the test organisms using a sterile transfer loop for antimicrobial screening. The plates were maintained at 40°C for 12 hours to promote optimal diffusion. During this time, several processes occur simultaneously: The test material is released from the dried disc as it absorbs water from the agar. The test material diffuses from the disc into the surrounding medium, following the physical laws governing molecular diffusion through agar gel.

This process leads to a gradual concentration gradient of the test material in the agar surrounding each disc. The plates are then incubated at 37°C for 12-18 hours to allow microbial growth. If the test material has antimicrobial activity, it will inhibit microorganism growth, producing a clear "zone of inhibition." The antibacterial effect is evaluated by measuring the diameter of this inhibition zone in millimeters. Each experiment was performed more than twice, and the average measurement was recorded.

4.6.2 Apparatus

- Filter paper discs
- Petri dishes
- Sterile napkin tissue
- Micropipette
- Electronic balance
- Hot plate
- Sprit lamp
- Test tubes
- Beaker
- 10 ml volumetric flask
- Nose masks and hand gloves
- Inoculating loop

- ❖ Sterile forceps
- ❖ Laminar air flow
- ❖ Autoclave
- ❖ Incubator
- ❖ Indelible Marker

4.6.3 Reagents

- ➢ Standard antibiotic discs (Kanamycin 30 µg/disc and Fluconazole 50 µg/disc)
- ➢ 70 % Ethanol
- ➢ Nutrient agar medium
- ➢ MacConkey agar (MAC) medium

4.6.4 Microorganisms used for the activity test

Both gram positive and gram-negative bacterial strains and antifungal stains were taken for the test. These organisms were collected from the Microbiology Lab. of Pharmacy Discipline, Daffodil international university.

Gram negative	Gram Positive
1. *Escherichia coli* (MacConkey agar)	1. *Staphylococcus aureus* 2. *Klebsiella* (Nutrient agar medium)

Table 1: List of microbes used for screening of antimicrobial activity.

4.6.5 Culture media

A nutrient-rich mixture used in laboratories to support the growth and proliferation of a culture (a population of microorganisms) is known as a culture medium. Microorganisms have diverse nutritional needs, leading to significant variations in the chemical makeup of culture media. Some microbes can grow in media containing only inorganic compounds, while others require organic components like amino acids, sugars, purines or pyrimidines, vitamins, or coenzymes. In addition to specific nutrients, each microorganism type has unique physical requirements for growth. For instance, some bacteria cannot grow at temperatures below 40°C, while others are unable to thrive above 20°C, and some require temperatures near human body temperature (around 37°C).

4.6.6 Preparation of test sample

3 mg and 1.5 mg of the each crude extract of endophytic fungi were accurately measured by the electronic balance and taken in two small volumetric flasks. Then 600µl ethanol was added and triturated in unidirectional manner using a vortex mixer, the concentration of these solutions became 250 µg/100µl and 500 µg/ 100µl.

4.6.7 Preparation of Standard Sample

We used standard kanamycin of 30µg/disc against bacteria and as standard fluconazole 50µg/disc, we dissolved 5 mg fluconazole powder in 1 ml methanol to make 50 µg/10 µl solution and soaked every disc.

4.6.8 Application of discs

Three types of discs were used for antibacterial screening:

a) Sample discs
b) Standard discs and
c) Blank discs

Each broth plates were divided into four portions i.e. two for samples (250 µg/100 µl and 500 µg/ 100 µl.), one for standard and one for blank.

4.6.9 Sample disc application

Sterile filter paper discs, each 5 mm in diameter, were used as sample discs. Two sterile discs were carefully placed onto the designated areas using sterile forceps to ensure full contact with the medium surface. In an aseptic environment under laminar airflow, 10 µl of test samples from solutions with concentrations of 25 µg/10 µl and 50 µg/10 µl were applied to the discs using a micropipette, resulting in final concentrations of 250 µg and 500 µg per disc, respectively.

4.7 Blank disc application

25 sterile filter paper discs (5 mm in diameter) were taken as blank discs. 100µl ethanol was applied on the blank discs as negative control. They ensure that the residual solvent's activity and the filter paper were not active themselves.

4.8 Standard disc application

Kanamycin (30 µg/disc) was used as a positive control to verify the effectiveness of the standard antibiotic against the test organisms and to compare its antimicrobial response with that of the test samples. The plates were then incubated upside down at 37°C for 16-18 hours for antibacterial testing and 48-72 hours for antifungal testing.

4.9 Determination of zone of inhibition

After proper incubation, the antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition in term of millimeter with a calibrated scale.

CHAPTER FIVE
RESULT & DISCUSSION

Chapter five
Results and Discussion

5.1 Result of phytochemical evaluation of *Ocimum sanctum* (Tulsi)

Test Name	Result
Alkaloid	+
Saponin	+
Phenol	+
Flavonoid	+
Glycoside	+

Table 2: Result of phytochemical evaluation of *Ocimum sanctum* (Tulsi)

5.2 Discussion on phytochemical evaluation of *Ocimum sanctum* (Tulsi)

The phytochemical evaluation of *Ocimum sanctum* (Tulsi) reveals the presence of various bioactive compounds. Based on the test results provided, here's a detailed discussion of each compound type identified:

1. **Alkaloids**

- **Tests Conducted**: Mayer's, Dragendorff's, and Wagner's tests, all of which were positive.
- **Interpretation**: The positive results for alkaloid detection tests suggest that *Ocimum sanctum* contains significant alkaloid compounds. Alkaloids are known for their wide range of pharmacological activities, including antimicrobial, and analgesic effects. This presence supports Tulsi's use in traditional medicine for its therapeutic effects.

2. **Saponins**

- **Test Conducted**: Saponin test, which yielded a positive result.

- **Interpretation**: Saponins are glycosidic compounds known for their ability to form foams in aqueous solutions and are linked to benefits such as lowering cholesterol levels, boosting the immune response, and exhibiting anti-cancer properties. The presence of saponins in Tulsi may enhance its health benefits, supporting its role in cardioprotective and immune-boosting applications.

3. **Phenols**

- **Test Conducted**: General phenol test, which was positive.
- **Interpretation**: Phenolic compounds are known antioxidants, contributing to the plant's free radical scavenging activity. This antioxidant property is important for reducing oxidative stress, potentially lowering the risk of chronic diseases, including heart disease and certain cancers. Phenols also possess anti-inflammatory and antimicrobial properties, which may contribute to Tulsi's therapeutic effects.

4. **Flavonoids**

- **Test Conducted**: General flavonoid test, with a positive result.
- **Interpretation**: Flavonoids are important plant metabolites with antioxidant, anti-inflammatory, and antimicrobial activities. Their presence in *Ocimum sanctum* could be a key contributor to its therapeutic potential, especially for cardiovascular and immune health. This further validates Tulsi's reputation as a medicinal herb in traditional practices.

5. **Glycoside**

- **Test Conducted**: Acid hydrolysis test, which was positive.
- **Interpretation**: Acid hydrolysis is often used to detect glycosidic linkages in complex phytochemicals like saponins and flavonoid glycosides. A positive result indicates the presence of glycosides, compounds that can yield active aglycones when metabolized. These aglycones may have various bioactivities, reinforcing the potential therapeutic profile of *Ocimum sanctum*.

The phytochemical profile of *Ocimum sanctum*, as evaluated through these tests, supports its use in traditional medicine and its role as a multi-functional therapeutic agent. This combination of alkaloids, saponins, phenols, and flavonoids underscores its antioxidant, anti-inflammatory, antimicrobial, and cardioprotective properties, aligning with the reputed health benefits of Tulsi.

5.3 Result of Antioxidant evaluation

Table 3: *IC50 for ascorbic acid (standard):*

Concentration (µg/ml)	% of Inhibition	IC_{50} (µg/ml)
400	92.48%	**9.11**
200	89.35%	
100	87.78%	
50	85.79%	
25	78.57%	
12.5	69.33%	
6.25	63.74%	

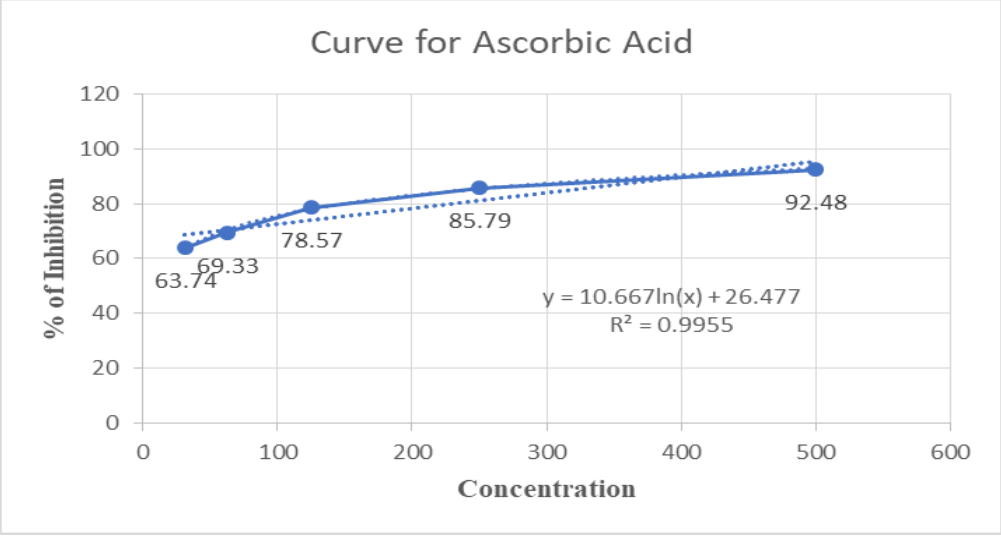


Figure 10: Graphical representation of % of Inhibition and concentration for Standard

Table 4: IC50 for ethanol extract of *Ocimum sanctum*

Concentration (µg/ml)	% of Inhibition	IC_{50} (µg/ml)
400	82.77%	**17.33**
200	80.88%	
100	78.93%	
50	74.34%	
25	66.82%	
12.5	59.78%	
6.25	55.67%	

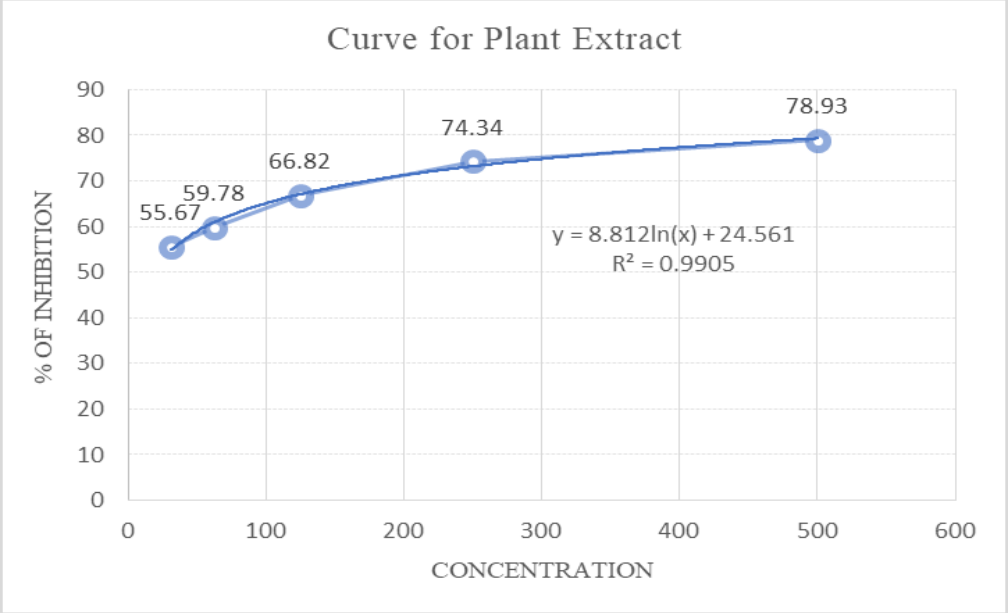


Figure 11: Graphical representation of % of Inhibition and concentration for Sample

5.3.1 Discussion on the antioxidant activity of ethanol extract of *Ocimum sanctum*

The antioxidant activity of the ethanol extract of *Ocimum sanctum* (Tulsi) can be discussed by comparing its IC50 value with that of the standard.

IC50 Value Comparison:

- The IC50 value for the *Ocimum sanctum* ethanol extract is **17.33 µg/ml**, while the standard antioxidant (likely a known antioxidant like ascorbic acid) has an IC50 of **9.11 µg/ml**.
- The IC50 value represents the concentration at which the extract or compound inhibits 50% of the free radicals. A lower IC50 indicates stronger antioxidant activity, as less sample is needed to achieve this inhibition level.

Antioxidant Potency:

- Although the extract has a higher IC50 than the standard, indicating it is less potent, an IC50 of 17.33 µg/ml still suggests good antioxidant activity. In pharmacological contexts, extracts with IC50 values below 20 µg/ml are often considered effective antioxidants.
- *Ocimum sanctum* has various phenolic and flavonoid compounds, which contribute significantly to its antioxidant activity. These phytochemicals are known for their ability to scavenge free radicals, reducing oxidative stress in cells.

Potential Applications:

- While the extract is not as potent as the standard antioxidant, it still has valuable antioxidant properties that could be beneficial in formulations where natural antioxidants are preferred.
- Its effectiveness suggests it could be useful in nutraceuticals or herbal supplements aimed at managing oxidative stress-related health conditions,

such as inflammation, cardiovascular diseases, or even as a protective agent against aging.

The ethanol extract of *Ocimum sanctum*, with an IC50 of 17.33 µg/ml, demonstrates strong antioxidant activity, though it is slightly less potent than the standard (IC50 = 9.11 µg/ml). This aligns with the traditional use of Tulsi in various health-promoting applications, supported by its phenolic and flavonoid content.

5.4 Anti-microbial activity of ethanol extract of *Ocimum sanctum*

Table 5: Anti-microbial activity of ethanol extract of *Ocimum sanctum*

Bacteria Name	**Antibiotic Name**	**Zone of Inhibition (Standard)**	**Zone of Inhibition (Sample)**	
E. coli	Ciprofloxacin	19 mm	1000 µg/µL	Not found
			1500 µg/µL	9 mm
			2000 µg/µL	11 mm
Staphylo	Azithromycin	Not grown	40 µg/µL	Not found
			50 µg/µL	Not found
			60 µg/µL	Not found
E. coli	Ampicillin	Not grown	100 µg/µL	Not found
			250 µg/µL	Not found
			500 µg/µL	Not found
Klebsiella	Ciprofloxacin	30 mm	60 µg/µL.	Not found
			100 µg/µL	Not found
			250 µg/µL	Not found

5.4.1 Discussion on Anti-microbial activity of ethanol extract of *Ocimum sanctum*

The antimicrobial activity of ethanol extract of *Ocimum sanctum* (Tulsi) was assessed against different bacteria, including *Escherichia coli*, *Staphylococcus* species, and

Klebsiella, using a range of concentrations. Here is a breakdown based on the results presented in the table:

1. **E. coli and Ciprofloxacin Comparison**

- **Ciprofloxacin** (Standard Antibiotic): Produced a 19 mm zone of inhibition, indicating its effective bactericidal activity against *E. coli*.
- **Ocimum sanctum Ethanol Extract**:
    - At **1000 µg/µL concentration**: No inhibition zone was observed, suggesting insufficient antibacterial efficacy at this level.
    - At **1500 µg/µL concentration**: A 9 mm zone of inhibition was observed, indicating some activity against *E. coli* but notably weaker than the standard ciprofloxacin.
    - At **2000 µg/µL concentration**: The inhibition zone increased to 11 mm, demonstrating that higher concentrations of the extract have slightly better efficacy but still fall short of ciprofloxacin's effect.

This indicates that *Ocimum sanctum* ethanol extract has moderate inhibitory effects on *E. coli* at higher concentrations, though it is less effective compared to ciprofloxacin.

2. **Staphylococcus and Azithromycin Comparison**

- **Azithromycin** (Standard Antibiotic): The *Staphylococcus* cultures did not grow, implying the bacterium is very sensitive to azithromycin.
- **Ocimum sanctum Ethanol Extract**:
    - No inhibition was observed at concentrations of **40 µg/µL, 50 µg/µL, or 60 µg/µL**.

The absence of inhibition suggests that *Ocimum sanctum* ethanol extract at these tested concentrations does not possess significant antibacterial properties against *Staphylococcus* species.

3. **E. coli and Ampicillin Comparison**

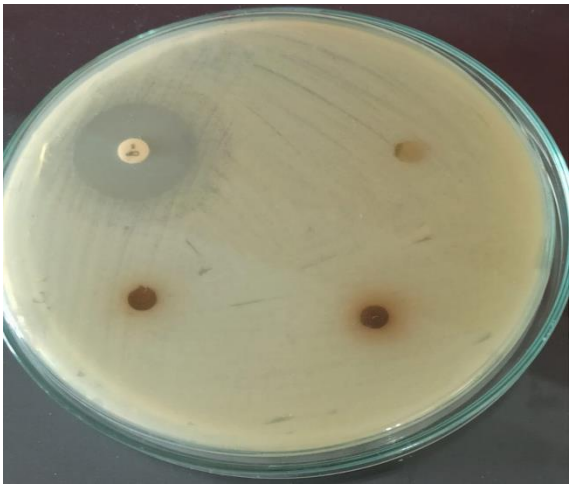
- **Ampicillin** (Standard Antibiotic): *E. coli* was not able to grow, showing its high susceptibility to ampicillin.
- **Ocimum sanctum Ethanol Extract**:
    - Tested at **100 µg/µL, 250 µg/µL, and 500 µg/µL concentrations**, the extract did not show any inhibitory effect.

This result further supports that *Ocimum sanctum* extract does not significantly impact *E. coli* at these concentrations, particularly when compared to ampicillin.

4. **Klebsiella and Ciprofloxacin Comparison**

- **Ciprofloxacin** (Standard Antibiotic): Exhibited a 30 mm inhibition zone, indicating strong antibacterial efficacy against *Klebsiella*.
- **Ocimum sanctum Ethanol Extract**:
    - Tested at **60 µg/µL, 100 µg/µL, and 250 µg/µL concentrations**, the extract failed to exhibit any inhibition.

The lack of inhibition zones for *Klebsiella* implies that, at the tested concentrations, *Ocimum sanctum* does not have a substantial antimicrobial effect against this bacterium. The ethanol extract of *Ocimum sanctum* exhibited mild antimicrobial activity against *E. coli* at higher concentrations (1500 µg/µL and 2000 µg/µL) but was generally ineffective against *Staphylococcus* and *Klebsiella* under the conditions tested. This suggests that while *Ocimum sanctum* may have some potential as an antimicrobial agent, especially against specific bacteria at higher concentrations, it is much less potent compared to standard antibiotics like ciprofloxacin, azithromycin, and ampicillin. Further studies may focus on increasing extract concentrations or exploring other methods of extraction to potentially enhance its efficacy.



CHAPTER SIX

CONCLUSION

Chapter six
Conclusion

6.1 Conclusion

The study on Ocimum sanctum (Tulsi) provides significant insights into its phytochemical properties, antioxidant capacity, and antimicrobial potential, aligning with its traditional use as an immunity-boosting herb. Phytochemical screening confirms the presence of bioactive compounds such as alkaloids, saponins, phenols, and flavonoids, which are known to contribute to various therapeutic activities, including immune modulation, antioxidant defense, and antimicrobial effects. The antioxidant evaluation of Ocimum sanctum extract revealed an IC50 value of 17.33 µg/ml, indicating substantial free radical scavenging activity, though slightly less potent than ascorbic acid, which had an IC50 of 9.11 µg/ml. This antioxidant property suggests that Ocimum sanctum may play a protective role against oxidative stress-related cellular damage, potentially strengthening the immune system. However, the antimicrobial results indicate limited effectiveness of Ocimum sanctum ethanol extract against certain bacterial strains at tested concentrations, as evidenced by the minimal inhibition zones. While there was some inhibition against E. coli at higher concentrations, no significant antibacterial effect was observed against other tested bacteria like Staphylococcus and Klebsiella, even at higher doses. In conclusion, Ocimum sanctum demonstrates valuable phytochemical and antioxidant properties, confirming its potential as an immunity-boosting agent. Although the antimicrobial activity was limited, its phytoconstituents still make it a promising natural supplement to support immune health. Future studies could explore enhanced formulations or combinations with other antimicrobial agents to broaden its therapeutic applications. By defending against infections and shielding cells from oxidative stress, substances with antibacterial and antioxidant qualities can improve immunological function. Antioxidants protect the immune system, while antimicrobials lessen the strain on it by preventing pathogenic microbes. Herbs, spices, fruits, and vegetables are examples of natural sources that frequently include these beneficial chemicals. To support overall immunological health, Tulsi is high in flavonoids, which have antibacterial and antioxidant properties. These compounds also reduce inflammation, increase pathogen resistance, and protect cells.

CHAPTER SEVEN

REFERENCE

Chapter seven
Reference

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