

# Study of Antimicrobial Activity of Plant Extracts

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**Abstract:** *The present study entitled “Study of Antimicrobial Activity of Plant Extracts” was undertaken to evaluate the antimicrobial potential of selected medicinal plant extracts against various pathogenic microorganisms. Medicinal plants have been used traditionally for the treatment of infectious diseases due to the presence of bioactive phytoconstituents such as alkaloids, flavonoids, tannins, terpenoids, glycosides, and phenolic compounds. Increasing resistance of microorganisms towards synthetic antibiotics has created a need for the discovery of alternative antimicrobial agents from natural sources. In this study, selected plant materials were collected, authenticated, shade dried, and powdered. Extraction was carried out using suitable solvents such as ethanol, methanol, or aqueous media by Soxhlet extraction method. The obtained extracts were concentrated and subjected to preliminary phytochemical screening. Antimicrobial activity was evaluated against selected bacterial strains including Staphylococcus aureus, Escherichia coli, Bacillus subtilis, and fungal strains such as Candida albicans using agar well diffusion method and determination of zone of inhibition. The results demonstrated that plant extracts exhibited significant antimicrobial activity against both Gram-positive and Gram-negative microorganisms. Ethanolic extracts showed higher activity compared to aqueous extracts due to better extraction of phytoconstituents. The antimicrobial activity was concentration dependent. Presence of flavonoids, tannins, and alkaloids may contribute to the antimicrobial effect. The study concludes that medicinal plant extracts possess promising antimicrobial properties and may serve as potential sources for the development of safer and effective herbal antimicrobial agents.*

**Keywords:** Medicinal Plants, Antimicrobial Activity, Plant Extracts, Agar Well Diffusion, Phytochemical Screening, Herbal Medicine, Zone of Inhibition, Soxhlet Extraction

## I. INTRODUCTION

### 1.1 Herbal Medicine and Medicinal Plants

Medicinal plants have played a significant role in healthcare systems since ancient times. Plants contain numerous bioactive compounds that possess therapeutic activities useful in the prevention and treatment of diseases. Herbal medicine is considered one of the oldest systems of medicine practiced by humans and remains an important source of primary healthcare in many developing countries.

According to the World Health Organization (WHO), nearly 80% of the global population relies on traditional herbal medicines for their primary healthcare needs. Herbal medicines are preferred due to their natural origin, fewer side effects, affordability, and easy availability.

Medicinal plants produce secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, glycosides, phenolic compounds, and essential oils. These phytoconstituents exhibit various pharmacological properties including:

- Antimicrobial activity
- Antioxidant activity
- Anti-inflammatory activity
- Anticancer activity
- Antidiabetic activity
- Antiviral activity



Plant extracts are increasingly being explored as potential alternatives to synthetic antimicrobial agents because of rising microbial resistance.

### **1.2 Microorganisms**

Microorganisms are microscopic living organisms that include bacteria, fungi, viruses, and protozoa. Some microorganisms are beneficial, while others are pathogenic and cause diseases in humans, animals, and plants. Microbial infections are one of the leading causes of morbidity and mortality worldwide. Common infectious diseases include:

- Tuberculosis
- Pneumonia
- Urinary tract infections
- Gastrointestinal infections
- Skin infections
- Fungal infections

Pathogenic microorganisms invade host tissues, multiply rapidly, and produce toxins leading to disease conditions.

### **1.3 Classification of Microorganisms**

#### **1.3.1 Bacteria**

Bacteria are unicellular prokaryotic organisms. They may be classified based on shape as: Cocci

Spherical bacteria such as *Staphylococcus aureus*. Bacilli

Rod-shaped bacteria such as *Escherichia coli*. Spirilla

Spiral-shaped bacteria.

Bacteria are further classified into:

- Gram-positive bacteria
- Gram-negative bacteria Gram-positive Bacteria

These bacteria possess thick peptidoglycan cell walls and retain crystal violet stain. Examples:

- *Staphylococcus aureus*
- *Bacillus subtilis*

Gram-negative Bacteria

These bacteria possess thin peptidoglycan layers and an outer membrane. Examples:

- *Escherichia coli*
- *Pseudomonas aeruginosa*

#### **1.3.2 Fungi**

Fungi are eukaryotic organisms that include yeasts and molds. Some fungi cause infections such as candidiasis and ringworm.

Example:

- *Candida albicans*

### **1.4 Infectious Diseases**

Infectious diseases occur due to invasion of pathogenic microorganisms into the body. These diseases may spread through:

- Air
- Water
- Food
- Direct contact



- Vectors

Symptoms of infections include:

- Fever
- Inflammation
- Pain
- Weakness
- Tissue damage

Antimicrobial therapy is essential for controlling infectious diseases.

### **1.5 Antimicrobial Agents**

Antimicrobial agents are substances that destroy or inhibit the growth of microorganisms.

Types of Antimicrobial Agents

- Antibiotics
  - o Used against bacterial infections.
- Antifungal Agents
  - o Used against fungal infections.
- Antiviral Agents
  - o Used against viral infections.
- Antiseptics and Disinfectants
  - o Used for sterilization and prevention of infections.

### **1.6 Antibiotic Resistance**

Antibiotic resistance occurs when microorganisms become resistant to antimicrobial drugs. Major causes include:

- Overuse of antibiotics
- Incomplete treatment
- Self-medication
- Misuse of antibiotics

Drug-resistant microorganisms are difficult to treat and pose serious health threats. Examples:

- MRSA (Methicillin Resistant Staphylococcus aureus)
- Multidrug resistant E. coli

The emergence of resistant strains has increased the need for discovering new antimicrobial agents from natural sources.

### **1.7 Importance of Plant Extracts in Antimicrobial Therapy**

Medicinal plants contain numerous compounds capable of inhibiting microbial growth. Plant extracts are safer and less toxic compared to synthetic antibiotics.

Advantages include:

- Natural origin
- Reduced side effects
- Cost effectiveness
- Biodegradability
- Easy availability

Plant-derived antimicrobial agents are increasingly studied in pharmaceutical research.

### **1.8 Phytochemicals Responsible for Antimicrobial Activity Alkaloids**

Nitrogen-containing compounds exhibiting antibacterial activity.



Flavonoids  
Possess antioxidant and antimicrobial effects.  
Tannins  
Precipitate microbial proteins and inhibit enzymes.  
Terpenoids  
Disrupt microbial membranes.  
Glycosides  
Exhibit antimicrobial and anti-inflammatory activities. Phenolic Compounds  
Strong antioxidants with antimicrobial potential.

## **II. REVIEW OF LITERATURE**

### **2.1 Overview**

Medicinal plants have been widely investigated for their antimicrobial properties due to the increasing prevalence of microbial resistance toward synthetic antibiotics. Since ancient times, plants have served as important therapeutic agents because they contain bioactive compounds capable of inhibiting or destroying pathogenic microorganisms.

Researchers across the world have studied the antimicrobial activity of various plant extracts against bacteria, fungi, and other microorganisms. The antimicrobial effects are mainly attributed to secondary metabolites such as alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, glycosides, and essential oils.

The present chapter summarizes previous research work related to antimicrobial activity of medicinal plants, extraction methods, phytochemical constituents, and screening techniques.

### **2.2 Historical Background of Herbal Antimicrobials**

The use of medicinal plants for treatment of infections dates back thousands of years. Ancient systems of medicine such as Ayurveda, Siddha, Unani, and Traditional Chinese Medicine extensively utilized herbs for wound healing, fever, respiratory infections, and gastrointestinal disorders.

Before the discovery of antibiotics, plant-based remedies were the primary source of antimicrobial therapy. Even after the development of synthetic antibiotics, medicinal plants continue to be important because of their safety profile and effectiveness against resistant microorganisms. The World Health Organization (WHO) has emphasized the importance of traditional medicine and encouraged scientific evaluation of medicinal plants for antimicrobial applications.

### **2.3 Medicinal Plants as Sources of Antimicrobial Agents**

Plants produce secondary metabolites as defense mechanisms against microbial attack. These metabolites possess antimicrobial properties and protect plants from pathogens.

Major antimicrobial phytoconstituents include:

- Alkaloids
- Flavonoids
- Tannins
- Phenolic compounds
- Terpenoids
- Glycosides
- Essential oils

These compounds act through different mechanisms such as:

- Cell wall disruption
- Membrane damage
- Protein precipitation
- Enzyme inhibition



- DNA synthesis inhibition

Medicinal plants are considered promising alternatives to synthetic antibiotics due to:

- Lower toxicity
- Fewer side effects
- Cost effectiveness
- Reduced microbial resistance

## **2.4 Review of Previous Research Work**

### **2.4.1 Cowan MM (1999)**

Cowan extensively reviewed the antimicrobial properties of plant secondary metabolites. The study reported that flavonoids, tannins, terpenoids, and alkaloids possess significant antibacterial and antifungal activity.

The researcher suggested that medicinal plants may serve as important sources for new antimicrobial drugs.

#### **Findings**

- Flavonoids inhibit nucleic acid synthesis.
- Tannins precipitate microbial proteins.
- Alkaloids interfere with DNA replication.

### **2.4.2 Rios JL and Recio MC (2005)**

Rios and Recio discussed various techniques used for antimicrobial screening of medicinal plants. The researchers emphasized the importance of standardization in extraction procedures and antimicrobial assays.

#### **Findings**

- Agar diffusion method is commonly used for screening.
- Solvent selection significantly influences antimicrobial activity.
- Ethanolic extracts generally show higher activity than aqueous extracts.

### **2.4.3 Parekh and Chanda (2007)**

The researchers evaluated antibacterial activity of several Indian medicinal plants against pathogenic bacteria including *Escherichia coli* and *Staphylococcus aureus*.

#### **Method**

- Agar well diffusion method used.
- Ethanolic extracts prepared.

#### **Findings**

- Significant antibacterial activity observed.
- Gram-positive bacteria were more susceptible than Gram-negative bacteria.

### **2.4.4 Nostro A et al. (2000)**

Nostro and co-workers investigated antifungal activity of medicinal plant extracts against *Candida albicans*.

#### **Findings**

- Plant extracts inhibited fungal growth.
- Antifungal activity depended on extract concentration.
- Phenolic compounds contributed to activity.

### **2.4.5 Gupta VK et al. (2015)**

Gupta and colleagues studied antimicrobial activity of ethanolic extracts from medicinal plants against pathogenic bacteria.



### **Findings**

- Ethanolic extracts showed broad-spectrum antibacterial activity.
- Strong activity observed against *Staphylococcus aureus*.
- Presence of flavonoids and tannins confirmed.

## **III. AIM AND OBJECTIVES**

### **3.1 Aim**

To study the antimicrobial activity of selected medicinal plant extracts against pathogenic microorganisms.

### **3.2 Objectives**

- To collect and authenticate medicinal plants.
- To prepare plant extracts using suitable solvents.
- To perform phytochemical screening.
- To evaluate antimicrobial activity using agar well diffusion method.
- To determine zone of inhibition.
- To compare antimicrobial activity of different extracts.
- To analyze effectiveness against Gram-positive and Gram-negative bacteria.

### **3.3 Significance of the Study**

The present study is significant because:

- It promotes the use of natural antimicrobial agents.
- It helps in combating antibiotic resistance.
- It supports herbal medicine research.
- It may contribute to development of safer antimicrobial formulations.
- It provides scientific information regarding medicinal plants.

### **3.4 Expected Outcome of the Study**

The expected outcomes of the study were:

- Successful extraction of phytoconstituents.
- Detection of bioactive compounds.
- Significant antimicrobial activity against pathogens.
- Identification of most effective plant extract.
- Scientific support for herbal antimicrobial therapy.

## **IV. PLANT PROFILE**

### **4.1 Overview**

Medicinal plants are rich sources of bioactive phytoconstituents that possess various pharmacological activities including antimicrobial, antioxidant, anti-inflammatory, anticancer, and antiviral properties. The antimicrobial activity of medicinal plants is mainly attributed to secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, glycosides, and phenolic compounds. In the present study, selected medicinal plants known for their antimicrobial properties were chosen for extraction and evaluation against pathogenic microorganisms. The selected plants include:

- Neem (*Azadirachta indica*)
- Tulsi (*Ocimum sanctum*)
- Turmeric (*Curcuma longa*)
- Garlic (*Allium sativum*)
- Aloe vera (*Aloe barbadensis*)



These plants have been widely used in traditional systems of medicine such as Ayurveda, Siddha, and Unani for the treatment of infectious diseases.

#### 4.2 Neem (*Azadirachta indica*)

Biological Source:

Neem consists of fresh and dried leaves obtained from *Azadirachta indica*.

Scientific Classification

Category	Classification
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Sapindales
Family	Meliaceae
Genus	<i>Azadirachta</i>
Species	<i>indica</i>



Fig. 1: Neem (*Azadirachta indica*)

#### Geographical Source

Neem is widely distributed throughout India, Bangladesh, Sri Lanka, and tropical regions of Africa.

#### Morphological Characteristics

- Medium to large evergreen tree
- Height: 12–20 meters
- Leaves are pinnate and green
- Flowers are white and fragrant
- Fruits are oval-shaped drupes



**Chemical Constituents**

Neem contains several biologically active compounds including:

- Azadirachtin
- Nimbin
- Nimbidin
- Quercetin
- Flavonoids
- Tannins
- Alkaloids

**Antimicrobial Activity**

Neem extracts are effective against:

- Staphylococcus aureus
- Escherichia coli
- Pseudomonas aeruginosa
- Candida albicans

The antimicrobial activity is mainly due to flavonoids and limonoids.

**Uses**

- Skin infections
- Wound healing
- Dental infections
- Fever
- Fungal infections

**4.3 Tulsi (Ocimum sanctum)**

**Biological Source**

Tulsi consists of dried leaves and flowering tops of *Ocimum sanctum*.

**Scientific Classification**

Category	Classification
Kingdom	Plantae
Family	Lamiaceae
Genus	Ocimum
Species	sanctum





Fig. 2: Tulsi (*Ocimum sanctum*)

### **Geographical Source**

Tulsi is cultivated throughout India and Southeast Asian countries.

### **Morphological Characteristics**

- Aromatic herb
- Height: 30–60 cm
- Leaves are green or purple
- Strong pleasant odor
- Small purple flowers

### **Chemical Constituents**

- Eugenol
- Ursolic acid
- Rosmarinic acid
- Carvacrol
- Linalool
- Flavonoids Antimicrobial Activity Tulsi extracts inhibit:
- *E. coli*
- *S. aureus*
- *Bacillus subtilis*
- *Candida albicans*

Essential oils present in tulsi contribute to antimicrobial effects.

### **Uses**

- Respiratory infections
- Cough and cold
- Fever
- Skin infections

### **4.4 Turmeric (*Curcuma longa*)**

#### **Biological Source**

Turmeric consists of dried rhizomes of *Curcuma longa*.



**Scientific Classification**

Category	Classification
Kingdom	Plantae
Family	Zingiberaceae
Genus	Curcuma
Species	longa



Fig. 3: Turmeric (*Curcuma longa*)

**Morphological Characteristics**

- Perennial herb
- Yellow-orange rhizomes
- Broad leaves
- Aromatic odor

**Chemical Constituents**

- Curcumin
- Turmerone
- Demethoxycurcumin
- Essential oils

**Antimicrobial Activity**

Curcumin inhibits microbial growth by:

- Damaging cell membranes
- Inhibiting enzymes
- Preventing protein synthesis

**Uses**

- Wound healing
- Skin infections



- Gastrointestinal disorders
- Inflammatory diseases

#### 4.5 Garlic (*Allium sativum*)

##### Biological Source

Garlic consists of bulbs of *Allium sativum*.

##### Scientific Classification

Category	Classification
Kingdom	Plantae
Family	Amaryllidaceae
Genus	<i>Allium</i>
Species	<i>sativum</i>



Fig. 4: Garlic (*Allium sativum*)

##### Morphological Characteristics

- Bulbous herb
- Strong characteristic odor
- Narrow flat leaves
- White bulb cloves

##### Chemical Constituents

- Allicin
- Alliin
- Sulfur compounds
- Flavonoids

##### Antimicrobial Activity

Garlic exhibits broad-spectrum antimicrobial activity against:



- S. aureus
- E. coli
- Salmonella
- Candida albicans

Allicin is responsible for antimicrobial activity.

**Uses**

- Respiratory infections
- Gastrointestinal infections
- Cardiovascular disorders
- Fungal infections

**4.6 Aloe vera (Aloe barbadensis)**

**Biological Source**

Aloe vera consists of fresh leaves of Aloe barbadensis.

Scientific Classification

Category	Classification
Kingdom	Plantae
Family	Asphodelaceae
Genus	Aloe
Species	barbadensis

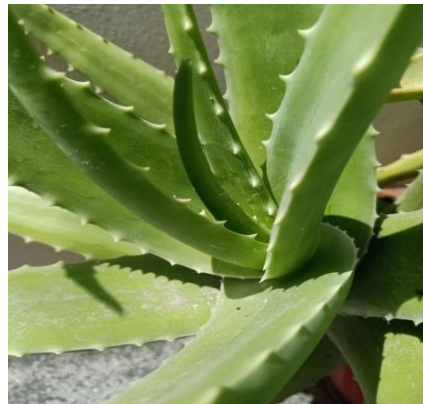


Fig. 5: Aloe vera (Aloe barbadensis)

**Category Classification**

Kingdom Plantae

Family Asphodelaceae

Genus Aloe

Species barbadensis

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**Morphological Characteristics**

- Succulent perennial herb
- Thick fleshy leaves
- Gel-filled leaf interior
- Green spiny leaves

**Chemical Constituents**

- Aloin
- Anthraquinones
- Saponins
- Vitamins
- Minerals

**Antimicrobial Activity**

Aloe vera gel inhibits:

- E. coli
- S. aureus
- Candida albicans

Anthraquinones and saponins contribute to antimicrobial activity.

**Uses**

- Burns
- Wounds
- Skin infections
- Cosmetic preparations

**4.7 Comparison of Selected Medicinal Plants**

Plant	Major Constituents	Main Activity
Neem	Azadirachtin, Nimbin	Antibacterial
Tulsi	Eugenol	Antifungal
Turmeric	Curcumin	Anti-inflammatory
Garlic	Allicin	Broad-spectrum antimicrobial
Aloe vera	Anthraquinones	Wound healing

**4.8 Importance of Selected Plants in Antimicrobial Research**

The selected medicinal plants are widely used in traditional medicine and possess scientifically proven antimicrobial properties. These plants are economical, easily available, and rich in bioactive compounds capable of inhibiting pathogenic microorganisms.



## V. MATERIALS AND METHODS

### 5.1 Introduction

Materials and methods constitute one of the most important sections of a research project because it provides detailed information regarding the experimental procedures used during the study. The present investigation was carried out to evaluate the antimicrobial activity of selected medicinal plant extracts against pathogenic microorganisms using standard microbiological techniques.

### 5.2 Materials Required

#### 5.2.1 Plant Materials

The following medicinal plants were selected for the study:

Plant Name	Part Used
Neem ( <i>Azadirachta indica</i> )	Leaves
Tulsi ( <i>Ocimum sanctum</i> )	Leaves
Turmeric ( <i>Curcuma longa</i> )	Rhizome
Garlic ( <i>Allium sativum</i> )	Bulb
Aloe vera ( <i>Aloe barbadensis</i> )	Leaves

#### 5.2.2 Chemicals and Reagents

The chemicals used in the study were of analytical grade.

Chemical	Purpose
Ethanol	Extraction solvent
Methanol	Extraction solvent
Distilled water	Preparation of solutions
Nutrient agar	Bacterial culture media
Sabouraud dextrose agar	Fungal culture media
Ferric chloride	Tannin test
Dragendorff's reagent	Alkaloid test
Mayer's reagent	Alkaloid test
Concentrated sulfuric acid	Glycoside test
Hydrochloric acid	Phytochemical tests

#### 5.2.3 Instruments and Apparatus

Instrument	Use
Soxhlet apparatus	Extraction
Hot air oven	Drying
Incubator	Incubation of cultures



Autoclave	Sterilization
Laminar airflow chamber	Aseptic handling
Electronic balance	Weighing
Water bath	Concentration of extracts
Petri dishes	Culture plates
Micropipettes	Sample transfer
Cork borer	Well preparation

### 5.3 Collection of Plant Materials

Fresh medicinal plants were collected from nearby local areas and herbal gardens. The collected plant materials were authenticated by a botanist.

The plants were washed thoroughly using distilled water to remove dust and foreign particles.

### 5.4 Drying of Plant Materials

The collected plant materials were shade dried at room temperature for approximately 10–15 days.

Importance of Shade Drying

- Prevents decomposition of active constituents
- Preserves phytochemicals
- Prevents microbial contamination
- Maintains therapeutic activity

After complete drying, the plant materials became brittle and suitable for grinding.

### 5.5 Powdering of Plant Materials

The dried plant materials were powdered separately using a mechanical grinder. The powdered materials were:

- Passed through sieve
- Stored in airtight containers
- Protected from moisture and sunlight

Fine powder increases extraction efficiency by increasing surface area.

### 5.6 Extraction Procedure

#### 5.6.1 Soxhlet Extraction Method

Soxhlet extraction was used for preparation of plant extracts.

#### Procedure

1. About 50 g of powdered plant material was placed in a thimble.
2. The thimble was inserted into Soxhlet apparatus.
3. Ethanol or methanol was added into round bottom flask.
4. Heating was continued for 6–8 hours.
5. Solvent repeatedly siphoned through plant powder.
6. Extract was collected and concentrated using water bath.
7. Dried extract was stored in airtight container.



### Flowchart of Extraction Process

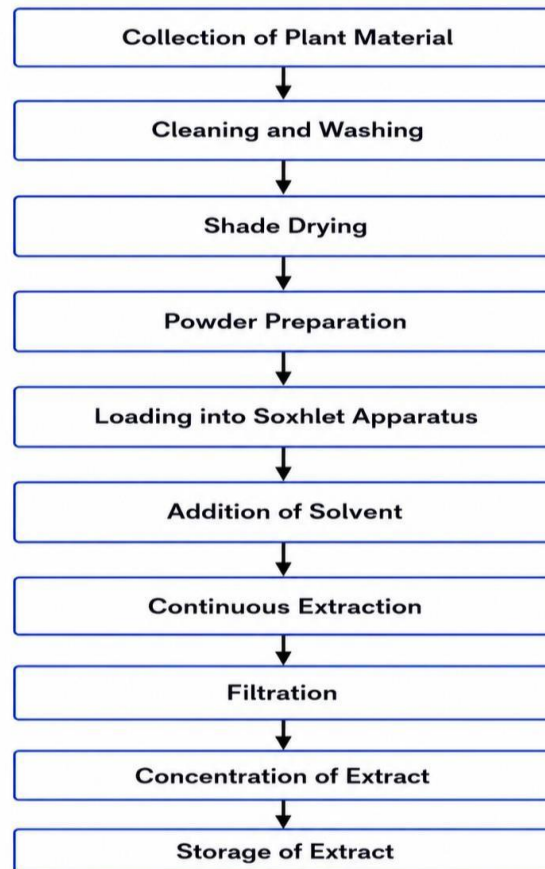


Fig. 6: Flowchart of Extract Preparation

#### 5.6.2 Advantages of Soxhlet Extraction

- Efficient extraction
- Continuous extraction process
- Requires less solvent

#### 5.7 Preparation of Extract Solutions

Different concentrations of extracts were prepared using distilled water or dimethyl sulfoxide (DMSO).

Example concentrations:

- 25 mg/ml
- 50 mg/ml
- 100 mg/ml

The prepared solutions were stored under refrigerated conditions.

#### 5.8 Microorganisms Used

The antimicrobial activity was evaluated against selected bacterial and fungal strains.



### 5.8.1 Bacterial Strains

Microorganism	Type
Staphylococcus aureus	Gram-positive
Bacillus subtilis	Gram-positive
Escherichia coli	Gram-negative
Pseudomonas aeruginosa	Gram-negative

### 5.8.2 Fungal Strain

Microorganism	Type
Candida albicans	Fungus

## 5.9 Preparation of Culture Media

### 5.9.1 Nutrient Agar Medium

Composition

Ingredient	Quantity
Peptone	5 g
Beef extract	3 g
Sodium chloride	5 g
Agar	15 g
Distilled water	1000 ml

#### Procedure

1. Ingredients dissolved in distilled water.
2. pH adjusted to 7.2.
3. Medium sterilized by autoclaving at 121°C for 15 minutes.
4. Sterile medium poured into Petri dishes.

### 5.9.2 Sabouraud Dextrose Agar

Used for fungal culture.

Composition

Ingredient	Quantity
Dextrose	40 g
Peptone	10 g
Agar	15 g
Distilled water	1000 ml



### 5.10 Sterilization

Sterilization is essential to prevent contamination during microbiological experiments. Methods Used

- Autoclaving
  - o Used for culture media and glassware.
- Dry Heat Sterilization
  - o Used for glass instruments.
- UV Sterilization
  - o Used in laminar airflow chamber.

### 5.11 Phytochemical Screening

Preliminary phytochemical tests were performed to identify bioactive constituents present in extracts.

#### 5.11.1 Test for Alkaloids Procedure

- Extract treated with Dragendorff's reagent.  
Observation
- Orange precipitate indicates presence of alkaloids.

#### 5.11.2 Test for Flavonoids Procedure

Extract treated with magnesium turnings and concentrated HCl.

Observation

Pink coloration indicates flavonoids.

#### 5.11.3 Test for Tannins Procedure

Ferric chloride solution added to extract.

Observation

Blue-black or green color indicates tannins.

#### 5.11.4 Test for Glycosides

Keller-Killiani Test Observation

Brown ring formation indicates glycosides.

#### 5.11.5 Test for Saponins Procedure

Extract shaken with water.

Observation

Persistent foam indicates saponins.

### 5.12 Evaluation of Antimicrobial Activity

#### 5.12.1 Agar Well Diffusion Method

Agar well diffusion method was used to determine antimicrobial activity.

#### Principle

Plant extract diffuses through agar medium and inhibits microbial growth around the well forming clear zones called zones of inhibition.



**Procedure**

1. Sterile nutrient agar plates prepared.
2. Microbial suspension spread uniformly using sterile swab.
3. Wells prepared using cork borer.
4. Plant extracts introduced into wells.
5. Standard antibiotic used as control.
6. Plates incubated at 37°C for 24 hours.
7. Zone of inhibition measured in millimeters.

**Advantages**

- Simple method
- Economical
- Rapid screening
- Suitable for multiple samples

**5.13 Determination of Minimum Inhibitory Concentration (MIC)**

MIC is the lowest concentration of extract preventing visible microbial growth.

**Procedure**

1. Serial dilution of extracts prepared.
2. Microbial inoculum added.
3. Tubes incubated for 24 hours.
4. Tubes observed for turbidity.

**Importance of MIC**

- Determines potency of antimicrobial agent
- Helps compare effectiveness of extracts

**5.14 Recording of Results**

Results were recorded by measuring:

- Zone of inhibition
- Presence or absence of microbial growth
- MIC values

Observations were tabulated for analysis.

**5.15 Statistical Analysis**

All experiments were performed in triplicate and results expressed as: Mean  $\pm$  Standard Deviation (SD)

Statistical analysis helps ensure reliability and reproducibility of results.

**5.16 Precautions**

- All glassware sterilized before use.
- Aseptic conditions maintained.
- Fresh microbial cultures used.
- Extracts stored properly.
- Cross contamination avoided.



## **VI. EXPERIMENTAL WORK**

### **6.1 Experimental**

Experimental work is the practical section of the project in which all procedures are carried out systematically for evaluation of antimicrobial activity of medicinal plant extracts. This chapter includes extraction of plant materials, preparation of microbial cultures, phytochemical screening, antimicrobial testing, and determination of minimum inhibitory concentration (MIC).

All experiments were performed under sterile and controlled laboratory conditions to ensure accuracy and reproducibility of results.

### **6.2 Experimental Design**

The study was designed to evaluate antimicrobial activity of selected medicinal plant extracts against pathogenic microorganisms using agar well diffusion method.

#### **Steps Involved**

1. Collection of plant materials
2. Drying and powdering
3. Soxhlet extraction
4. Preparation of microbial cultures
5. Phytochemical screening
6. Antimicrobial evaluation
7. MIC determination
8. Statistical analysis

### **6.3 Collection and Authentication of Plant Materials**

Fresh medicinal plants including neem, tulsi, turmeric, garlic, and aloe vera were collected from nearby herbal gardens and local areas.

The plants were authenticated by a qualified botanist to confirm their identity.

### **6.4 Preparation of Plant Materials**

#### **6.4.1 Washing**

The collected plant materials were washed thoroughly with distilled water to remove:

- Dust particles
- Soil
- Foreign matter

#### **6.4.2 Shade Drying**

The cleaned plant materials were shade dried at room temperature for 10–15 days. Importance of Shade Drying

- Prevents degradation of phytoconstituents
- Preserves medicinal properties
- Prevents fungal contamination

#### **6.4.3 Powdering**

The dried plant materials were powdered separately using a mechanical grinder. The powders were:

- Passed through sieve
- Stored in airtight containers



## 6.5 Preparation of Plant Extracts

### 6.5.1 Soxhlet Extraction Method

#### Principle

Continuous extraction of active constituents from powdered plant materials using suitable solvents.

#### Procedure

1. About 50 g of powdered plant material was accurately weighed.
2. Powder transferred into extraction thimble.
3. Thimble placed inside Soxhlet apparatus.
4. 250 ml ethanol added to round bottom flask.
5. Extraction carried out for 6–8 hours.
6. Solvent heated continuously.
7. Extract obtained after repeated siphoning.
8. Extract filtered using Whatman filter paper.
9. Filtrate concentrated on water bath.
10. Semi-solid extract collected and stored.

### 6.5.2 Percentage Yield of Extract

The percentage yield was calculated using the formula:

Percentage Yield =  $\frac{\text{Weight of Extract}}{\text{Weight of Powdered Drug}} \times 100$  Example Calculation

Weight of powdered drug = 50 g Weight of extract obtained = 8 g

Percentage Yield =  $\frac{8}{50} \times 100 = 16\%$

## 6.6 Preparation of Extract Solutions

Different concentrations of extracts were prepared for antimicrobial testing.

Concentrations Used

Concentration	Purpose
25 mg/ml	Low concentration
50 mg/ml	Moderate concentration
100 mg/ml	High concentration

## 6.7 Preparation of Culture Media

### 6.7.1 Nutrient Agar Medium

Composition

Ingredient	Quantity
Peptone	5 g
Beef extract	3 g
Sodium chloride	5 g
Agar	15 g
Distilled water	1000 ml



Procedure

1. Ingredients dissolved in distilled water.
2. pH adjusted to 7.2.
3. Medium sterilized by autoclaving.
4. Sterile medium poured into Petri dishes.

**6.7.2 Sabouraud Dextrose Agar**

Used for cultivation of fungal strains.

**6.8 Sterilization Procedure**

Sterilization was performed to avoid contamination.

Methods Used

Autoclaving

- Temperature: 121°C
- Pressure: 15 psi
- Time: 15 minutes Dry Heat Sterilization Used for glassware.

UV Sterilization

Laminar airflow chamber sterilized before use.

**6.9 Microorganisms Used**

Bacterial Strains

Microorganism	Type
<i>Staphylococcus aureus</i>	Gram-positive
<i>Bacillus subtilis</i>	Gram-positive
<i>Escherichia coli</i>	Gram-negative
<i>Pseudomonas aeruginosa</i>	Gram-negative

Fungal Strain

Microorganism	Type
<i>Candida albicans</i>	Fungus

**6.10 Preparation of Inoculum**

Microbial cultures were transferred into sterile saline solution.

The turbidity was adjusted to match standard microbial suspension.

**6.11 Evaluation of Antimicrobial Activity**

**6.11.1 Agar Well Diffusion Method**

Agar well diffusion method was used to determine antimicrobial activity.

**Principle**

The plant extract diffuses through agar medium and inhibits microbial growth around the well resulting in formation of clear zones.



**Procedure**

1. Sterile agar medium poured into Petri dishes.
2. Plates allowed to solidify.
3. Microbial suspension spread uniformly.
4. Wells prepared using sterile cork borer.
5. Different concentrations of extracts introduced into wells.
6. Standard antibiotic used as control.
7. Plates incubated at 37°C for 24 hours.
8. Zones of inhibition measured.

**Flowchart of Antimicrobial Testing**

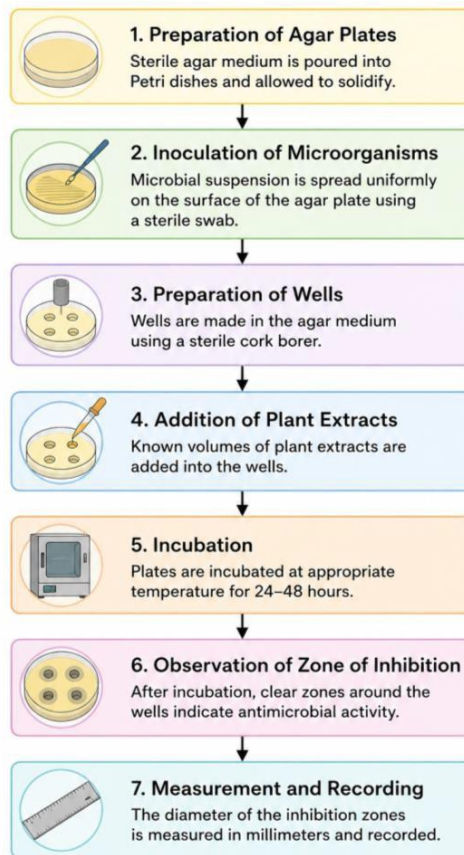


Fig. 7: Flowchart of Agar Well Diffusion Method

**6.12 Determination of Minimum Inhibitory Concentration (MIC)**

**Principle**

MIC is the minimum concentration preventing visible microbial growth.

**Procedure**

1. Serial dilutions of extracts prepared.
2. Microbial inoculum added into tubes.

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3. Tubes incubated for 24 hours.
4. Tubes observed for turbidity.

#### Interpretation

- Clear solution → No microbial growth
- Turbid solution → Microbial growth present

### 6.13 Experimental Observations

#### Phytochemical Screening Results

Test	Observation	Result
Alkaloids	Orange precipitate	Present
Flavonoids	Pink color	Present
Tannins	Blue-black color	Present
Glycosides	Brown ring	Present
Saponins	Foam formation	Present

### 6.16 Antimicrobial Activity Observations

Organism	Zone of Inhibition (mm)
<i>S. aureus</i>	18 mm
<i>E. coli</i>	15 mm
<i>B. subtilis</i>	17 mm
<i>C. albicans</i>	14 mm

### 6.17 Precautions

- Sterile conditions maintained throughout experiment.
- Fresh microbial cultures used.
- Contamination avoided.
- Glassware sterilized properly.
- Extracts stored in airtight containers.

### 6.18 Advantages of Experimental Method

- Simple and economical
- Reliable antimicrobial screening
- Suitable for herbal extracts
- Easy comparison of antimicrobial activity



## VII. RESULTS AND DISCUSSION

### 7.1 Overview

The present study was carried out to evaluate the antimicrobial activity of selected medicinal plant extracts against pathogenic microorganisms. Ethanolic extracts of neem, tulsi, turmeric, garlic, and aloe vera were prepared and subjected to phytochemical screening and antimicrobial evaluation using agar well diffusion method.

### 7.2 Percentage Yield of Plant Extracts

The percentage yield of extracts obtained after Soxhlet extraction was calculated. Formula Used

Percentage Yield =  $\text{Weight of Extract} / \text{Weight of Powdered Drug} \times 100$

Table 7.1: Percentage Yield of Plant Extracts

Plant Name	Weight of Powder (g)	Weight of Extract (g)	Percentage Yield (%)
Neem	50	8.0	
Tulsi	50	7.5	15%
Turmeric	50	9.0	18%
Garlic	50	6.5	13%
Aloe vera	50	7.0	14%

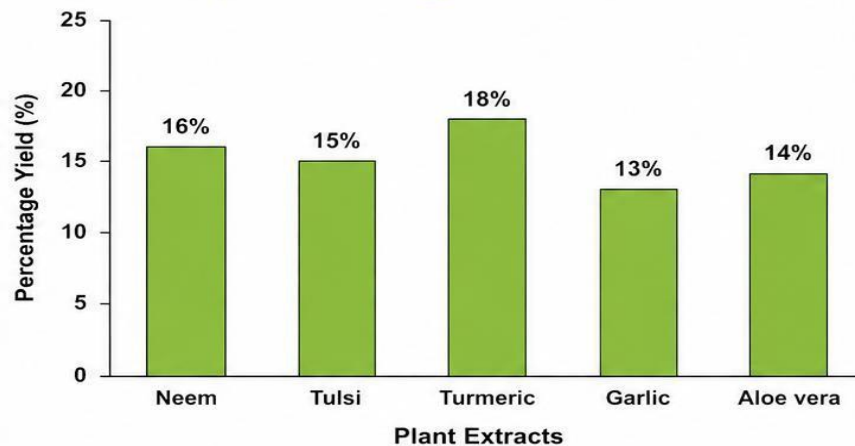


Fig. 8: Percentage Yield of Plant Extracts

### Discussion

Among all extracts, turmeric showed the highest percentage yield due to the presence of large amounts of extractable phytoconstituents. Garlic showed the lowest yield comparatively.

The extraction efficiency depends on:

- Solvent used
- Extraction time
- Nature of phytochemicals
- Moisture content



### 7.3 Results of Phytochemical Screening

Preliminary phytochemical analysis was performed to identify bioactive compounds present in plant extracts.

Table 7.2: Phytochemical Screening Results

Phytochemical	Neem	Tulsi	Turmeric	Garlic	Aloe vera
Alkaloids	Present	Present	Absent	Present	Present
Flavonoids	Present	Present	Present	Present	Present
Tannins	Present	Present	Present	Absent	Present
Glycosides	Present	Present	Present	Present	Present
Saponins	Present	Absent	Present	Present	Present
Phenolics	Present	Present	Present	Present	Present

### Discussion

The phytochemical screening confirmed the presence of various secondary metabolites responsible for antimicrobial activity.

- Alkaloids
  - o Known to inhibit microbial DNA replication.
- Flavonoids
  - o Possess antioxidant and antibacterial activities.
- Tannins
  - o Cause protein precipitation and membrane disruption.
- Phenolic Compounds
  - o Act by damaging microbial cell membranes.

The presence of these phytoconstituents supports the antimicrobial potential of medicinal plants.

### 7.4 Antimicrobial Activity Results

The antimicrobial activity was evaluated using agar well diffusion method. Zones of inhibition were measured in millimeters.

#### 7.4.1 Activity against *Staphylococcus aureus*

Table 7.3: Antibacterial Activity Against *S. aureus*

Plant Extract	Zone of Inhibition (mm)
Neem	18 ± 0.5
Tulsi	17 ± 0.4
Turmeric	16 ± 0.3
Garlic	19 ± 0.5
Aloe vera	14 ± 0.2
Standard Antibiotic	24 ± 0.4



**Discussion**

Garlic extract showed maximum activity against *S. aureus* due to the presence of allicin. Neem and tulsi also exhibited significant antibacterial effects.

**7.4.2 Activity against Escherichia coli**

Table 7.4: Antibacterial Activity Against *E. coli*

Plant Extract	
Neem	15 ± 0.4
Tulsi	14 ± 0.3
Turmeric	13 ± 0.2
Garlic	16 ± 0.4
Aloe vera	12 ± 0.2
Standard Antibiotic	22 ± 0.5

**Discussion**

The activity against *E. coli* was comparatively lower than Gram-positive bacteria because Gram-negative bacteria possess an outer membrane that restricts penetration of phytochemicals.

**7.4.3 Activity against Bacillus subtilis**

Table 7.5: Antibacterial Activity Against *B. subtilis*

Plant Extract	Zone of Inhibition (mm)
Neem	17 ± 0.3
Tulsi	16 ± 0.3
Turmeric	15 ± 0.2
Garlic	18 ± 0.4
Aloe vera	13 ± 0.2
Standard Antibiotic	23 ± 0.4

**Discussion**

Garlic and neem extracts showed strong antibacterial activity against *B. subtilis*. The activity may be attributed to sulfur compounds and flavonoids.

**7.4.4 Antifungal Activity Against Candida albicans**



Table 7.6: Antifungal Activity against *Candida albicans*

Plant Extract	Zone of Inhibition (mm)
Neem	14 ± 0.2
Tulsi	15 ± 0.3
Turmeric	13 ± 0.2
Garlic	16 ± 0.3
Aloe vera	12 ± 0.2
Standard Antifungal	21 ± 0.4

### Discussion

Garlic extract exhibited maximum antifungal activity against *Candida albicans*. Tulsi also demonstrated significant inhibition due to presence of essential oils and phenolic compounds.

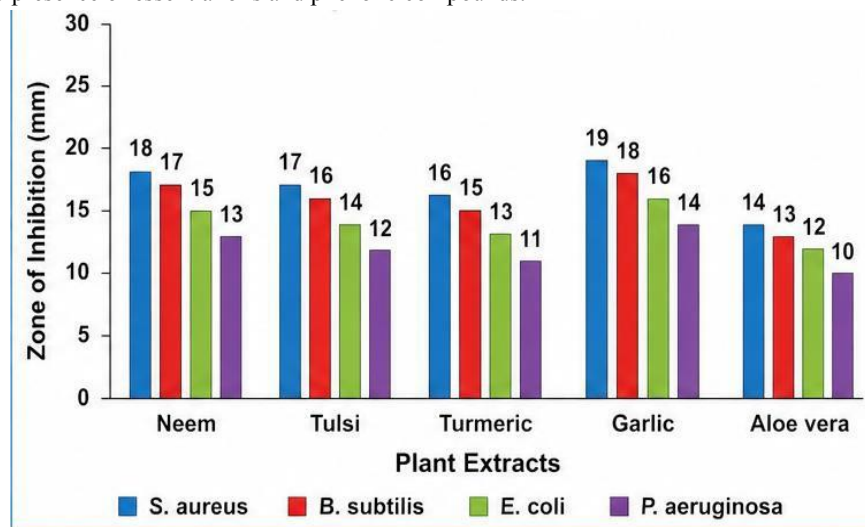


Fig. 9: Antibacterial Activity of Plant Extracts against different Bacteria

### 7.5 Comparative Antimicrobial Activity

Plant	Average Zone of Inhibition (mm)
Neem	16
Tulsi	15.5
Turmeric	14.25
Garlic	17.25
Aloe vera	12.75

Table 7.7: Comparative Activity of Plant Extracts



### Discussion

Garlic showed the highest overall antimicrobial activity followed by neem and tulsi. The superior activity of garlic may be due to:

- Presence of allicin
- Sulfur compounds
- Broad-spectrum antimicrobial action Aloe vera exhibited comparatively lower activity.

### 7.6 Minimum Inhibitory Concentration (MIC)

MIC values were determined using serial dilution method.

Plant Extract	MIC (mg/ml)
Neem	25
Tulsi	25
Turmeric	50
Garlic	12.5
Aloe vera	50

Table 7.8: MIC Values of Plant Extracts

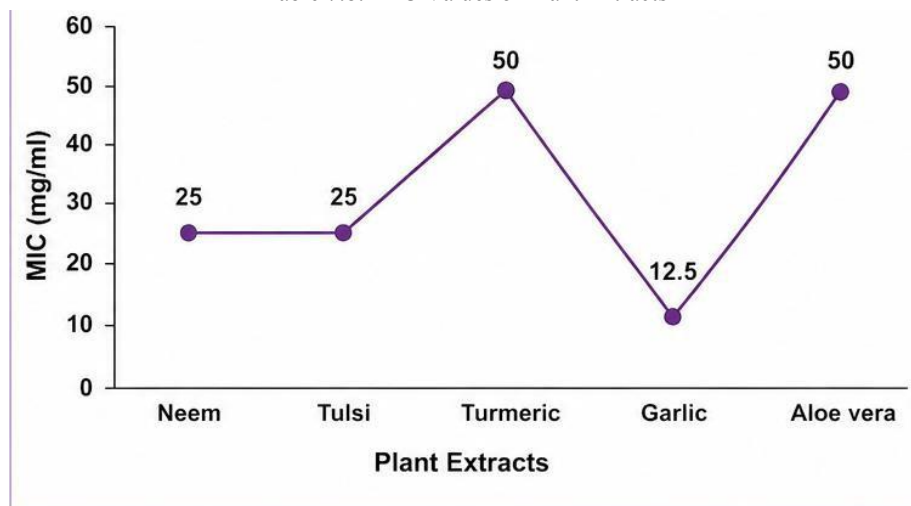


Fig. 10: Minimum Inhibitory Concentration (MIC) of Plant Extracts Discussion

Lower MIC values indicate stronger antimicrobial activity.

Garlic extract showed the lowest MIC value, indicating highest potency among all extracts.

## VIII. SUMMARY AND CONCLUSION

### 8.1 Summary

The present study entitled “Study of Antimicrobial Activity of Plant Extracts” was carried out to evaluate the antimicrobial potential of selected medicinal plant extracts against pathogenic microorganisms. Medicinal plants have long been used in traditional systems of medicine due to the presence of various bioactive phytoconstituents possessing therapeutic properties.

The study was designed to investigate the antimicrobial activity of selected medicinal plants including neem (*Azadirachta indica*), tulsi (*Ocimum sanctum*), turmeric (*Curcuma longa*), garlic (*Allium sativum*), and aloe vera (*Aloe*



barbadensis). These plants were selected based on their traditional medicinal importance and reported antimicrobial properties.

Fresh plant materials were collected, authenticated, washed, shade dried, and powdered. The powdered materials were subjected to Soxhlet extraction using ethanol as solvent. The extracts obtained were concentrated and stored for further evaluation.

Preliminary phytochemical screening was performed to identify the presence of active phytoconstituents such as:

- Alkaloids
- Flavonoids
- Tannins
- Glycosides
- Saponins
- Phenolic compounds

The phytochemical investigation confirmed that most extracts contained important secondary metabolites responsible for antimicrobial activity.

The antimicrobial activity of the extracts was evaluated against selected microorganisms including:

#### **Bacterial Strains**

- Staphylococcus aureus
- Bacillus subtilis
- Escherichia coli
- Pseudomonas aeruginosa

#### **Fungal Strain**

- Candida albicans

The agar well diffusion method was used for antimicrobial evaluation. Zones of inhibition were measured in millimeters and compared with standard antimicrobial agents. The results demonstrated that all plant extracts exhibited varying degrees of antimicrobial activity against tested microorganisms. Among the extracts evaluated, garlic showed the highest antimicrobial activity followed by neem and tulsi.

The study also demonstrated that Gram-positive bacteria were more susceptible to plant extracts than Gram-negative bacteria. This difference may be attributed to the structural differences in bacterial cell walls. Minimum inhibitory concentration (MIC) studies indicated that lower concentrations of garlic extract effectively inhibited microbial growth, suggesting greater antimicrobial potency.

The antimicrobial activity observed in the study may be attributed to the presence of phytoconstituents such as flavonoids, tannins, alkaloids, phenolic compounds, and essential oils. The results obtained from the present investigation support the traditional use of medicinal plants in the treatment of infectious diseases.

#### **8.2 Conclusion**

The present investigation confirmed that medicinal plant extracts possess significant antimicrobial activity against pathogenic microorganisms. Ethanolic extracts of neem, tulsi, turmeric, garlic, and aloe vera effectively inhibited bacterial and fungal growth.

Among all the tested extracts, garlic exhibited maximum antimicrobial activity, whereas aloe vera showed comparatively lower activity. The strong antimicrobial potential of garlic may be due to the presence of sulfur-containing compounds such as allicin.

Phytochemical screening confirmed the presence of various bioactive compounds including alkaloids, flavonoids, tannins, glycosides, saponins, and phenolic compounds, which are responsible for antimicrobial effects.



The study also revealed that:

- Ethanolic extracts were more effective than aqueous extracts.
- Gram-positive bacteria were more susceptible than Gram-negative bacteria.
- Antimicrobial activity increased with increase in extract concentration.

The findings of this study indicate that medicinal plants can serve as promising natural alternatives to synthetic antimicrobial agents. Herbal antimicrobial agents may help reduce the problems associated with antibiotic resistance, toxicity, and side effects of conventional drugs.

Therefore, medicinal plants possess immense potential for development of safe, economical, and effective antimicrobial formulations.

Further studies are recommended for:

- Isolation and purification of active constituents
- Toxicological studies
- Mechanism of action studies
- Clinical evaluation
- Development of herbal dosage forms

In conclusion, medicinal plants represent valuable sources of antimicrobial compounds and may contribute significantly toward future drug discovery and development of herbal medicines for management of infectious diseases.

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