

Phytochemical And Biological Evaluation of Three Indian Traditional Medicinal Plant

Mr. Jadhav Akash Anant, Prof. Komal A. Dongare Dr. Surwase K. P

Aditya Institute of Pharmaceutical, Beed

Abstract: Medicinal plants have been widely used in traditional systems of medicine for the treatment and prevention of various diseases. The therapeutic potential of medicinal plants is mainly due to the presence of biologically active phytochemical constituents such as alkaloids, flavonoids, tannins, glycosides, saponins, and phenolic compounds. India possesses a rich diversity of medicinal plants that play an important role in healthcare and pharmaceutical industries.

The present study was carried out to evaluate the phytochemical constituents and biological activities of three important Indian medicinal plants namely Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), and Amla (*Emblica officinalis*). The selected plant materials were collected, authenticated, shade dried, powdered, and extracted using ethanol by Soxhlet extraction method. Preliminary phytochemical screening of the extracts was performed for identification of major bioactive constituents. The study confirmed the presence of alkaloids, flavonoids, tannins, glycosides, saponins, carbohydrates, and proteins in the selected medicinal plants.

Biological evaluation of the extracts was carried out by antioxidant and antimicrobial studies. Antioxidant activity was evaluated using DPPH free radical scavenging assay, while antimicrobial activity was determined using agar well diffusion method against selected microorganisms including *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The results demonstrated that Amla exhibited highest antioxidant activity due to the presence of vitamin C and polyphenolic compounds. Neem showed strongest antimicrobial activity against tested microorganisms because of the presence of limonoids, alkaloids, and flavonoids. Tulsi also demonstrated significant antioxidant and antimicrobial activities due to essential oils and phenolic constituents.

Keywords: Medicinal plants, Phytochemical screening, Antioxidant activity, Antimicrobial activity, Neem, Tulsi, Amla, Herbal medicine, DPPH assay, Agar well diffusion method

I. INTRODUCTION

1.1 Introduction to Medicinal Plants

Medicinal plants are plants that contain naturally occurring chemical compounds capable of producing therapeutic effects in humans and animals. Since ancient times, plants have been used as an important source of medicine for the treatment and prevention of diseases. Traditional systems of medicine such as Ayurveda, Siddha, Unani, and Homeopathy mainly depend on herbal remedies prepared from medicinal plants[1].

India is known as the “Botanical Garden of the World” because of its rich biodiversity and wide variety of medicinal plants. More than 6,000 medicinal plant species are used in traditional healthcare systems in India. Herbal medicines are gaining worldwide popularity due to their safety, low cost, easy availability, and fewer side effects compared to synthetic medicines. Medicinal plants contain biologically active compounds known as phytochemicals. These compounds are produced naturally in plants and are responsible for their therapeutic activities. Phytochemicals include alkaloids, flavonoids, tannins, glycosides, phenolic compounds, terpenoids, steroids, and saponins. Such constituents exhibit various pharmacological activities including antioxidant, antimicrobial, anti-inflammatory, anticancer, antidiabetic, hepatoprotective, and immunomodulatory effects.



Scientific evaluation of medicinal plants through phytochemical and biological studies helps in identifying active constituents and validating traditional claims. This also contributes to the development of new herbal drugs and pharmaceutical products[2].

1.2 Importance of Medicinal Plants

Medicinal plants play a vital role in healthcare systems throughout the world. They are used for curing diseases, maintaining health, and improving immunity. Herbal medicines are widely accepted because they are considered natural and safer alternatives to synthetic drugs[3].

Importance of medicinal plants includes:

1. Source of therapeutic agents.
2. Used in traditional and modern medicine.
3. Economical and easily available.
4. Fewer side effects compared to synthetic drugs.
5. Useful in chronic disease management.
6. Rich source of antioxidants and bioactive compounds.
7. Used in pharmaceutical, cosmetic, and nutraceutical industries.
8. Support primary healthcare in rural populations.

According to the World Health Organization (WHO), nearly 80% of the world's population depends on herbal medicines for primary healthcare needs[4,6].

1.3 Phytochemicals

Phytochemicals are naturally occurring chemical compounds present in medicinal plants. They protect plants from microbial infections, insects, and environmental stress. These compounds also provide therapeutic benefits to humans[6].

Major Classes of Phytochemicals

1. Alkaloids

Alkaloids are nitrogen-containing compounds with strong physiological activities.

Examples:

- Morphine
- Quinine
- Caffeine

Biological activities:

- Analgesic activity
- Antimalarial activity
- Antibacterial activity

2. Flavonoids

Flavonoids are polyphenolic compounds known for antioxidant properties.

Biological activities:

- Antioxidant activity
- Anti-inflammatory activity
- Cardioprotective activity

3. Tannins

Tannins are phenolic compounds capable of precipitating proteins.



Biological activities:

- Antimicrobial activity
- Astringent action
- Wound healing activity

4. Saponins

Saponins produce foam in aqueous solutions.

Biological activities:

- Antifungal activity
- Cholesterol-lowering effect
- Immune stimulation

5. Glycosides

Glycosides contain sugar and non-sugar components.

Biological activities:

- Cardiotonic activity
- Laxative activity
- Anti-inflammatory activity

6. Terpenoids

Terpenoids are aromatic compounds responsible for fragrance and medicinal value.

Biological activities:

- Anticancer activity
- Antiviral activity
- Antimicrobial activity

1.4 Biological Activities of Medicinal Plants

Medicinal plants exhibit several biological activities due to the presence of phytochemicals[5].

1. Antioxidant Activity

Antioxidants neutralize harmful free radicals and prevent oxidative stress.

Benefits:

- Prevention of aging
- Protection against cancer
- Cardiovascular protection

2. Antimicrobial Activity

Medicinal plants inhibit the growth of microorganisms including bacteria and fungi[4].

Applications:

- Treatment of infections
- Preservation of food products
- Development of herbal antiseptics

3. Anti-inflammatory Activity

Plant extracts reduce inflammation and swelling.

Uses:

- Arthritis treatment



- Pain relief
- Skin disorders

4. Antidiabetic Activity

Some medicinal plants reduce blood glucose levels and improve insulin sensitivity.

Benefits:

- Diabetes management
- Prevention of diabetic complications

5. Hepatoprotective Activity

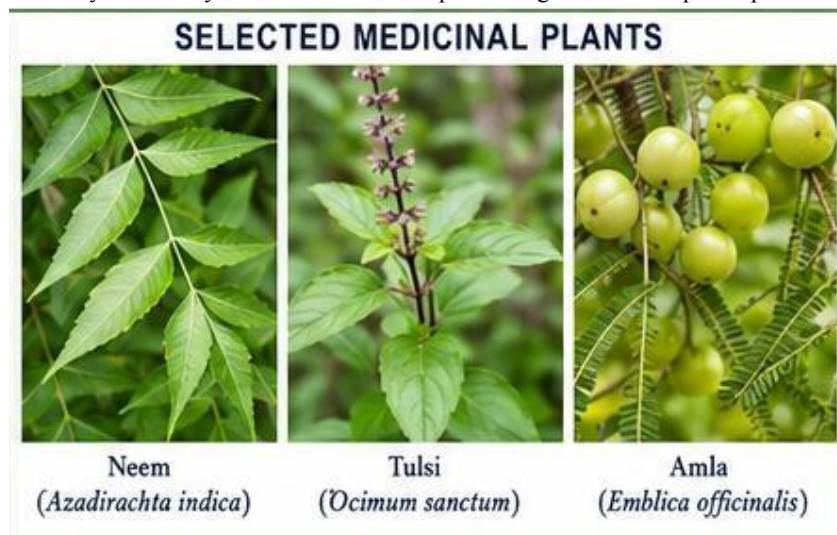
Certain phytochemicals protect liver cells from damage caused by toxins and drugs.

1.5 Selected Medicinal Plants

The present study focuses on the phytochemical and biological evaluation of three Indian medicinal plants:

1. Neem (*Azadirachta indica*)
2. Tulsi (*Ocimum sanctum*)
3. Amla (*Emblica officinalis*)

These plants are commonly used in Ayurvedic medicine and possess significant therapeutic potential.



1.6 Neem (*Azadirachta indica*)

Neem belongs to the family Meliaceae and is widely distributed in India. It is considered one of the most important medicinal trees [7,8,10].

Chemical Constituents

Neem contains:

- Azadirachtin
- Nimbin
- Nimbidin
- Quercetin
- Limonoids

Therapeutic Uses

Copyright to IJARSCT
www.ijarsct.co.in



DOI: 10.48175/IJARSCT-36268



1. Antibacterial activity
2. Antifungal activity
3. Antiviral activity
4. Antidiabetic activity
5. Skin disease treatment
6. Dental care applications

Neem leaves and bark are commonly used in herbal formulations.

1.7 Tulsi (*Ocimum sanctum*)

Tulsi is known as the “Queen of Herbs” due to its medicinal importance[3,6].

Chemical Constituents

Tulsi contains:

- Eugenol
- Ursolic acid
- Rosmarinic acid
- Flavonoids
- Tannins

Therapeutic Uses

1. Antioxidant activity
2. Antimicrobial activity
3. Immunomodulatory effect
4. Stress reduction
5. Respiratory disorder treatment

Tulsi is commonly used in herbal teas and Ayurvedic preparations.

1.8 Amla (*Emblica officinalis*)

Amla is a highly nutritious fruit rich in vitamin C and antioxidants[18].

Chemical Constituents

Amla contains:

- Ascorbic acid
- Gallic acid
- Ellagic acid
- Tannins
- Polyphenols

Therapeutic Uses

1. Antioxidant activity
2. Hepatoprotective activity
3. Anti-aging effect
4. Antidiabetic activity
5. Immunity enhancement

Amla is an important ingredient in many Ayurvedic formulations such as Chyawanprash.



1.9 Need for the Study

Although medicinal plants are widely used traditionally, scientific validation is necessary to confirm their therapeutic efficacy and safety. Phytochemical screening helps identify active constituents, while biological evaluation determines pharmacological activities.

The study is important because:

1. It validates traditional medicinal claims.
2. It identifies bioactive compounds.
3. It supports herbal drug development.
4. It promotes safe use of medicinal plants.
5. It contributes to pharmaceutical research.

II. REVIEW OF LITERATURE

2.1 Introduction

Medicinal plants have been extensively studied for their phytochemical constituents and therapeutic properties. Several researchers have reported that medicinal plants contain bioactive compounds responsible for various pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer effects.

The present chapter summarizes previous research work related to the phytochemical and biological evaluation of Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), and Amla (*Emblica officinalis*) [4,9].

2.2 Review of Literature on Neem (*Azadirachta indica*)

Neem is one of the most important medicinal plants in India and has been widely investigated for its therapeutic activities.

Phytochemical Studies on Neem

Researchers reported that Neem contains numerous bioactive compounds including:

1. Azadirachtin
2. Nimbin
3. Nimbidin
4. Gedunin
5. Quercetin
6. Limonoids
7. Flavonoids
8. Tannins

These compounds are responsible for the medicinal properties of the plant.

Several studies revealed that Neem leaves contain high concentrations of flavonoids and phenolic compounds which contribute to antioxidant activity.

Antimicrobial Activity of Neem

Various investigators studied the antimicrobial activity of Neem leaf extracts against bacterial and fungal strains [6,9].

Reported findings:

1. Ethanolic extract of Neem showed inhibitory activity against:
 - o *Escherichia coli*
 - o *Staphylococcus aureus*
 - o *Pseudomonas aeruginosa*
2. Neem extracts demonstrated antifungal activity against:
 - o *Candida albicans*



o *Aspergillus niger*

3. Methanolic extracts showed larger zones of inhibition compared to aqueous extracts.

The antimicrobial activity is mainly due to the presence of nimbidin and quercetin.

Antioxidant Activity of Neem

Several studies confirmed that Neem possesses strong antioxidant activity because of phenolic compounds and flavonoids[12,14].

Researchers observed that Neem extracts:

1. Scavenge free radicals effectively.
2. Reduce oxidative stress.
3. Protect cells from damage caused by reactive oxygen species.

Neem extracts showed significant DPPH radical scavenging activity in laboratory studies.

Anti-inflammatory Activity of Neem

Studies indicated that Neem inhibits inflammatory mediators and reduces swelling and pain.

Mechanism:

- Inhibition of prostaglandin synthesis
- Reduction of inflammatory cytokines

Neem bark and leaf extracts are commonly used in traditional medicine for inflammatory disorders.

Antidiabetic Activity of Neem

Research demonstrated that Neem extracts reduce blood glucose levels in diabetic experimental models.

Proposed mechanisms:

1. Enhancement of insulin secretion
2. Improvement of glucose uptake
3. Reduction of oxidative stress

Neem has shown potential in managing diabetes and diabetic complications.

2.3 Review of Literature on Tulsi (*Ocimum sanctum*)

Tulsi is regarded as one of the most sacred medicinal plants in India and has been widely studied for its medicinal properties[15].

Phytochemical Studies on Tulsi

Tulsi contains several phytochemical constituents including:

1. Eugenol
2. Ursolic acid
3. Rosmarinic acid
4. Flavonoids
5. Tannins
6. Saponins
7. Alkaloids

These constituents are responsible for the therapeutic activities of Tulsi.

Researchers reported that essential oil obtained from Tulsi leaves contains high levels of eugenol, which contributes significantly to antimicrobial and anti-inflammatory activities.

Antioxidant Activity of Tulsi

Many studies revealed that Tulsi possesses potent antioxidant properties.



Observed activities:

1. Free radical scavenging activity
2. Prevention of lipid peroxidation
3. Reduction of oxidative stress

The antioxidant activity is mainly due to:

- Flavonoids
- Phenolic compounds
- Eugenol

Tulsi extracts demonstrated significant DPPH and hydrogen peroxide scavenging activities.

Antimicrobial Activity of Tulsi

Researchers evaluated Tulsi extracts against several pathogenic microorganisms.

Findings:

1. Tulsi extract inhibited:
 - o Staphylococcus aureus
 - o Escherichia coli
 - o Salmonella typhi
2. Essential oil of Tulsi showed strong antifungal activity against:
 - o Candida albicans

The antimicrobial action is attributed mainly to eugenol and other volatile oils.

Immunomodulatory Activity of Tulsi

Studies suggested that Tulsi improves immune response by stimulating immune cells[13].

Benefits include:

1. Increased resistance to infections
2. Enhanced immune function
3. Stress reduction

Tulsi is therefore considered an adaptogenic herb.

Anti-stress and Neuroprotective Activity

Research showed that Tulsi reduces stress and anxiety by regulating cortisol levels and improving neurotransmitter balance.

Animal studies indicated that Tulsi protects brain cells from oxidative damage.

2.4 Review of Literature on Amla (*Emblica officinalis*)

Amla is an important medicinal fruit used extensively in Ayurvedic medicine.

Phytochemical Studies on Amla

Studies reported that Amla contains several important phytochemicals such as:

1. Vitamin C
2. Gallic acid
3. Ellagic acid
4. Polyphenols
5. Tannins
6. Flavonoids

These constituents are responsible for its strong antioxidant properties.

Amla fruit contains high amounts of natural antioxidants compared to many other fruits.

Antioxidant Activity of Amla

Numerous researchers demonstrated that Amla possesses excellent antioxidant activity.



Mechanism:

1. Neutralization of free radicals
2. Reduction of oxidative stress
3. Protection against cellular damage The antioxidant activity is mainly due to:

- Vitamin C
- Polyphenolic compounds
- Tannins

Amla extracts exhibited strong DPPH scavenging activity in various studies.

Antidiabetic Activity of Amla

Several studies reported that Amla reduces blood glucose levels and improves insulin sensitivity.

Proposed mechanisms:

1. Delayed glucose absorption
2. Improvement of pancreatic function
3. Reduction of oxidative stress

Amla is widely used in herbal antidiabetic formulations.

Hepatoprotective Activity of Amla

Researchers demonstrated that Amla protects the liver against toxic chemicals and drug- induced damage.

Effects observed:

1. Reduction in liver enzyme levels
2. Prevention of hepatic cell damage
3. Improvement of liver function

The hepatoprotective activity is due to antioxidant compounds present in Amla.

Anti-aging and Immunomodulatory Activity

Studies showed that Amla enhances immunity and delays aging due to its antioxidant potential. Regular consumption of Amla improves:

1. Skin health
2. Hair growth
3. Immune function
4. Overall vitality

2.5 Comparative Review of Selected Medicinal Plants

Activity	Neem	Tulsi	Amla
Antioxidant	Good	Excellent	Excellent
Antimicrobial	Excellent	Good	Moderate
Anti-inflammatory	Excellent	Good	Moderate
Antidiabetic	Good	Moderate	Excellent
Immunomodulatory	Moderate	Excellent	Excellent

2.6 Research Gap

Although several studies have been conducted on individual medicinal plants, comparative phytochemical and biological evaluation of Neem, Tulsi, and Amla using similar experimental conditions is limited.



There is a need for:

1. Comparative phytochemical screening.
2. Standardized extraction methods.
3. Comparative antioxidant studies.
4. Comparative antimicrobial evaluation.

Such studies help identify the most effective medicinal plant based on biological activities.

III. AIM, OBJECTIVES AND PLAN OF WORK

3.1 Aim of the Study

The aim of the present study is to perform phytochemical and biological evaluation of three selected Indian medicinal plants namely Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), and Amla (*Emblica officinalis*).

3.2 Objectives of the Study

The main objectives of the study are as follows:

- To collect and authenticate selected medicinal plant materials.
- To prepare extracts using suitable extraction methods.
- To perform preliminary phytochemical screening of plant extracts.
- To identify the presence of alkaloids, flavonoids, tannins, glycosides, saponins, and other phytochemicals.
- To evaluate antioxidant activity of the extracts.
- To study antimicrobial activity against selected microorganisms.
- To compare the biological activities of Neem, Tulsi, and Amla.
- To scientifically validate the medicinal importance of selected plants.

3.3 Need for the Study

Medicinal plants are widely used in traditional systems of medicine due to their therapeutic properties. However, scientific validation of these plants is essential to confirm their efficacy and safety.

Neem, Tulsi, and Amla are commonly used medicinal plants in Ayurveda and folk medicine. These plants contain several bioactive compounds responsible for different pharmacological activities.

The present study is therefore undertaken to:

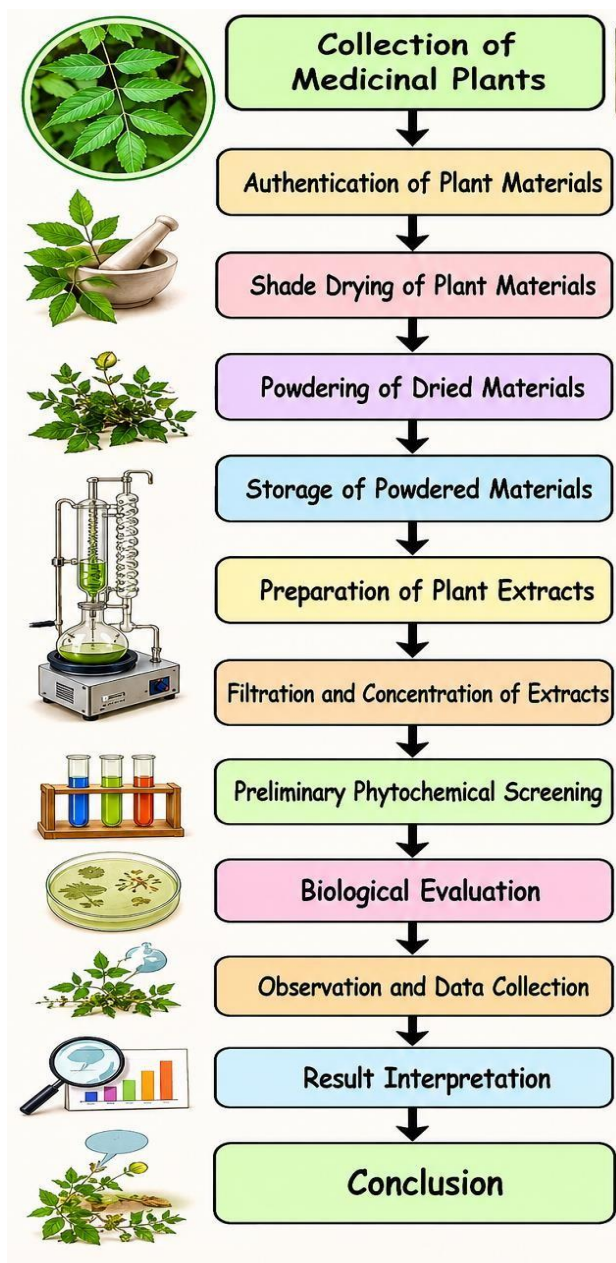
- Evaluate the phytochemical constituents of selected plants.
- Determine their biological activities.
- Support traditional medicinal claims through scientific evidence.
- Promote the development of herbal medicines.

3.4 Plan of Work

The present research work was carried out according to the following experimental plan.

Stepwise Plan of Work





IV. DRUG PROFILE

4.1 Introduction

Medicinal plants contain various bioactive compounds responsible for their therapeutic properties. In the present study, three important Indian medicinal plants namely Neem, Tulsi, and Amla were selected for phytochemical and biological evaluation[19].



4.2 Neem (*Azadirachta indica*) Synonyms

- Indian Lilac
- Margosa Tree
- Neem Tree

Biological Source

Neem consists of fresh and dried leaves, bark, seeds, and other parts of *Azadirachta indica*.

Family

Meliaceae

Taxonomical Classification

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

Geographical Source

Neem is widely distributed throughout India, especially in tropical and subtropical regions. It is also found in Sri Lanka, Bangladesh, Pakistan, and African countries [16,18].

Morphological Characteristics

Neem is a fast-growing evergreen tree that reaches a height of about 15–20 meters.

Leaves

- Compound and pinnate
- Dark green in color
- Bitter taste

Flowers

- Small and white
- Aromatic in nature

Fruits

- Smooth and oval-shaped
- Yellowish-green when ripe



Chemical Constituents

Neem contains several important phytochemicals including:

- Azadirachtin
- Nimbin
- Nimbidin
- Gedunin
- Salannin
- Quercetin
- Limonoids
- Tannins
- Flavonoids

Medicinal Uses

Neem is widely used in traditional medicine due to its therapeutic activities.

Uses include:

- Antibacterial agent
- Antifungal agent
- Antiviral agent
- Anti-inflammatory agent
- Antidiabetic agent
- Skin disease treatment
- Dental care preparations

4.3 Tulsi (*Ocimum sanctum*)

Synonyms

- Holy Basil
- Sacred Basil

Biological Source

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

Family

Lamiaceae

Taxonomical Classification

Category	Classification
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae



Genus	Ocimum
Species	sanctum

Geographical Source

Tulsi is cultivated throughout India and is commonly grown in household gardens and temples[20].

Morphological Characteristics

Tulsi is an aromatic erect herb reaching a height of about 30–60 cm.

Leaves

- Opposite arrangement
- Green or purple in color
- Aromatic odor

Flowers

- Small and purplish
- Arranged in racemes

Stem

- Hairy and branched

Chemical Constituents

Major phytochemicals present in Tulsi include:

- Eugenol
- Ursolic acid
- Rosmarinic acid
- Linalool
- Flavonoids
- Tannins
- Saponins

Medicinal Uses

Tulsi is widely used in Ayurveda for treatment of various disorders.

Uses include:

- Cough and cold treatment
- Fever management
- Respiratory disorders
- Stress reduction
- Antioxidant therapy
- Immunity enhancement



Biological Activities

Tulsi exhibits:

- Antioxidant activity
- Antimicrobial activity
- Anti-inflammatory activity
- Adaptogenic activity
- Antidiabetic activity

4.4 Amla (*Emblica officinalis*) Synonyms

- Indian Gooseberry
- Amlaki

Biological Source

Amla consists of fresh and dried fruits of *Emblica officinalis*.

Family

Phyllanthaceae

Taxonomical Classification

Category	Classification
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Malpighiales
Family	Phyllanthaceae
Genus	<i>Emblica</i>
Species	<i>officinalis</i>

Geographical Source

Amla is widely distributed throughout tropical and subtropical regions of India [18].

Morphological Characteristics

Amla is a medium-sized deciduous tree.

Fruits

- Round and light green
- Sour and astringent taste
- Rich in vitamin C

Leaves

- Small and simple



- Light green in color

Flowers

- Greenish-yellow in appearance

Chemical Constituents

Amla contains several important bioactive compounds including:

- Ascorbic acid
- Gallic acid
- Ellagic acid
- Emblicanin
- Tannins
- Flavonoids
- Polyphenols

Medicinal Uses

Amla is widely used as a rejuvenating agent in Ayurveda.

Uses include:

- Antioxidant therapy
- Liver protection
- Hair care preparations
- Digestive disorders
- Diabetes management
- Immunity enhancement

Biological Activities

Amla possesses:

- Antioxidant activity
- Hepatoprotective activity
- Anti-aging activity
- Antidiabetic activity
- Gastroprotective activity

4.5 Comparative Profile of Selected Medicinal Plants

Parameter	Neem	Tulsi	Amla
Family	Meliaceae	Lamiaceae	Phyllanthaceae
Part Used	Leaves	Leaves	Fruits
Major Constituents	Azadirachtin	Eugenol	Vitamin C
Major Activity	Antimicrobial	Antioxidant	Antioxidant
Traditional Use	Skin disorders	Respiratory disorders	Rejuvenator



4.6 Advantages of Selected Medicinal Plants

The selected medicinal plants possess several advantages:

- Natural source of therapeutic agents
- Easily available in India
- Cost-effective
- Fewer side effects
- Rich in phytochemical constituents
- Multiple pharmacological activities

V. MATERIALS AND METHODS

5.1 Plant Materials

Sr. No.	Plant Material	Biological Name	Part Used
1	Neem	<i>Azadirachta indica</i>	Leaves
2	Tulsi	<i>Ocimum sanctum</i>	Leaves
3	Amla	<i>Emblica officinalis</i>	Fruits

The plant materials were collected from local areas and authenticated by a botanist before experimental work.

5.2 Chemicals and Reagents

Chemicals/Reagents	Use
Ethanol	Solvent for extraction
Methanol	Preparation of DPPH solution
Distilled Water	Preparation of solutions
Hydrochloric Acid	Phytochemical testing
Sulfuric Acid	Glycoside test
Ferric Chloride	Test for tannins
Mayer's Reagent	Test for alkaloids
Dragendorff's Reagent	Confirmation test for alkaloids
Benedict's Reagent	Test for carbohydrates
Sodium Hydroxide	Test for flavonoids
DPPH Reagent	Antioxidant activity study
Glacial Acetic Acid	Glycoside test
Copper Sulfate	Protein test
Nutrient Agar	Culture medium for bacteria



Mueller Hinton Agar	Antimicrobial study
Sodium Chloride	Preparation of microbial suspension

5.3 Instruments and Equipment

Instrument/Equipment	Use
Soxhlet Apparatus	Extraction of phytochemicals
Hot Air Oven	Drying of plant materials
Electronic Balance	Accurate weighing
UV-Visible Spectrophotometer	Measurement of absorbance
Rotary Evaporator	Concentration of extracts
Incubator	Microbial culture incubation
Autoclave	Sterilization of media and glassware
Water Bath	Heating samples
pH Meter	Measurement of pH
Mechanical Grinder	Powdering of plant materials
Micropipettes	Transfer of small volumes
Petri Plates	Antimicrobial activity testing
Glassware	Laboratory experimental work
Mortar and Pestle	Grinding of samples
Cork Borer	Preparation of agar wells

5.4 Collection and Authentication of Plant Materials

Fresh Neem leaves, Tulsi leaves, and Amla fruits were collected from nearby herbal gardens and local areas. The collected plant materials were authenticated by a qualified botanist. The materials were washed thoroughly with water to remove dirt and foreign particles.

5.5 Drying and Powdering

The collected plant materials were shade dried at room temperature for 7–10 days. After complete drying, the materials were powdered separately using a mechanical grinder. The powdered materials were sieved to obtain uniform particle size and stored in airtight containers for further use.





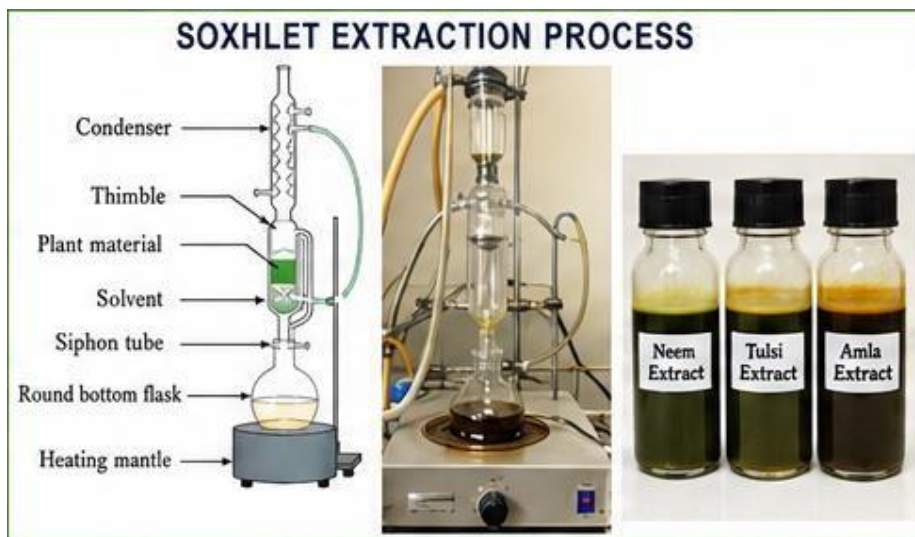
5.6 Preparation of Plant Extracts

Soxhlet Extraction Method

Procedure

1. About 50 g of powdered plant material was weighed accurately and placed inside a thimble made of filter paper.
2. The thimble was inserted into the Soxhlet apparatus. Approximately 300 ml of ethanol was added into the round bottom flask.
3. The apparatus was heated gently using a heating mantle. The solvent evaporated and condensed into the extraction chamber containing the plant powder.
4. Continuous extraction was carried out for 6–8 hours until the solvent in the siphon tube became colorless.
5. The obtained extract was filtered and concentrated using a rotary evaporator to remove excess solvent.
6. The concentrated extract was transferred into clean containers and stored under refrigerated conditions for further studies.





5.7 Preliminary Phytochemical Screening

The prepared extracts were subjected to qualitative phytochemical tests for identification of different phytochemical constituents.

Test for Alkaloids

Mayer's Test

Procedure

About 2 ml of plant extract was taken in a test tube and few drops of Mayer's reagent were added.

Observation

Formation of cream-colored precipitate indicated the presence of alkaloids.

Dragendorff's Test

Procedure

To 2 ml of plant extract, few drops of Dragendorff's reagent were added.

Observation

Formation of reddish-brown precipitate confirmed the presence of alkaloids.

Test for Flavonoids

Alkaline Reagent Test Procedure

A small quantity of extract was treated with sodium hydroxide solution.

Observation

Formation of intense yellow color that disappeared after addition of dilute acid indicated the presence of flavonoids.

Test for Tannins

Ferric Chloride Test

Procedure

Few drops of ferric chloride solution were added to the extract.

Observation

Formation of dark blue or greenish-black color indicated the presence of tannins.



Test for Saponins Foam

Test Procedure

About 5 ml of distilled water was added to the extract and shaken vigorously for several minutes.

Observation

Persistent foam formation indicated the presence of saponins.

Test for Glycosides Keller-Killiani Test Procedure

The extract was treated with glacial acetic acid containing ferric chloride solution followed by concentrated sulfuric acid along the side of the test tube.

Observation

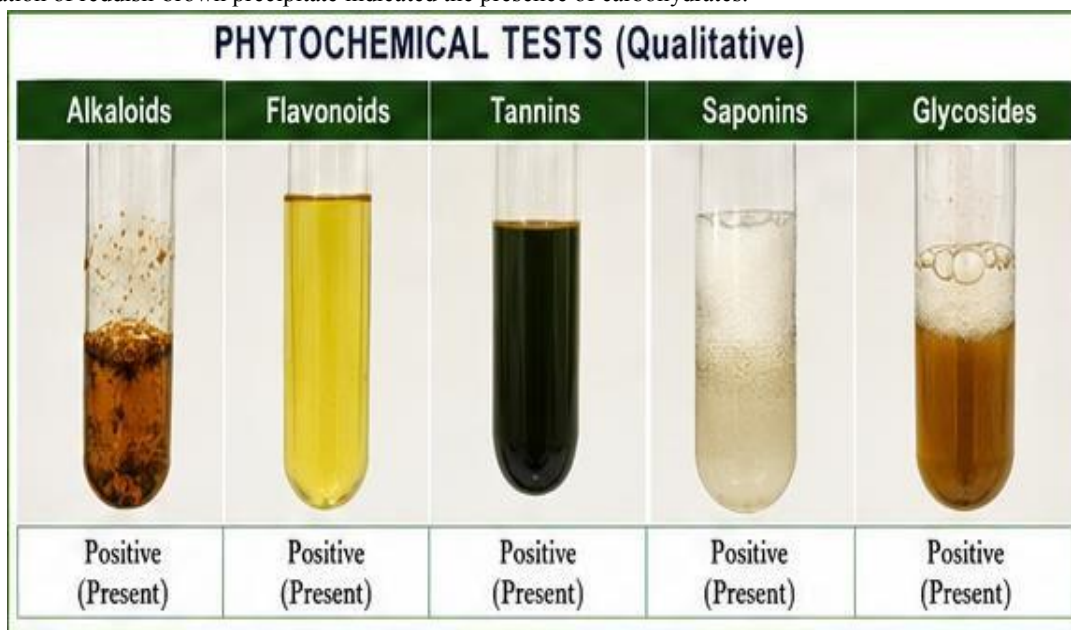
Formation of brown ring at the junction indicated the presence of glycosides.

Test for Carbohydrates Benedict's Test Procedure

The extract was mixed with Benedict's reagent and heated in a water bath for few minutes.

Observation

Formation of reddish-brown precipitate indicated the presence of carbohydrates.



Test for Proteins Biuret Test Procedure

The extract was treated with sodium hydroxide solution and few drops of copper sulfate solution.

Observation

Formation of violet color indicated the presence of proteins.



5.8 Evaluation of Antioxidant Activity

DPPH Radical Scavenging Method

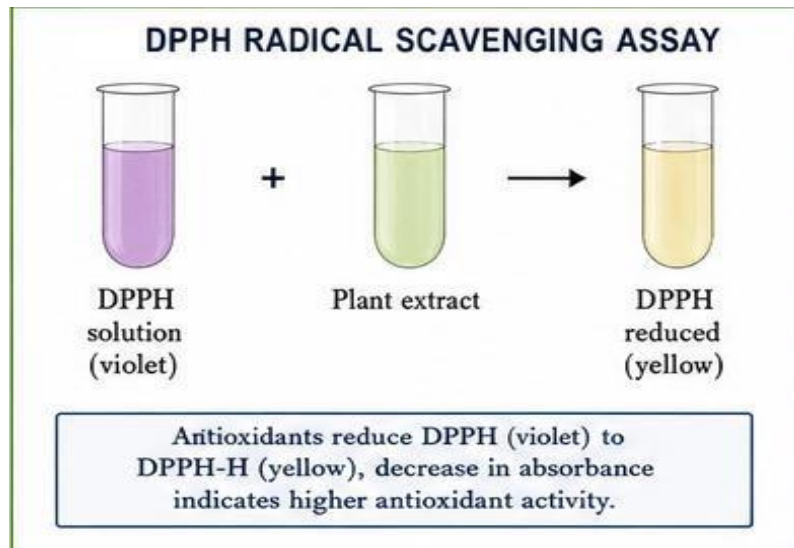
Principle

DPPH is a stable free radical having deep violet color. Antioxidants present in plant extracts reduce DPPH into yellow-colored diphenylpicrylhydrazine.

Reduction in color intensity indicates antioxidant activity.

Procedure

1. DPPH solution was prepared by dissolving DPPH reagent in methanol.
2. Different concentrations of plant extracts were prepared separately.
3. About 1 ml of plant extract solution was mixed with 3 ml of DPPH solution.
4. The mixtures were incubated in dark for 30 minutes at room temperature.
5. Absorbance was measured at 517 nm using UV-visible spectrophotometer.
6. Ascorbic acid was used as standard antioxidant.



Calculation

Percentage inhibition was calculated using the formula:

% Inhibition = $\frac{A_0 - A_1}{A_0} \times 100$ Where:

- A_0 = Absorbance of control
- A_1 = Absorbance of sample

5.9 Evaluation of Antimicrobial Activity

Agar Well Diffusion Method

Test Organisms

- Escherichia coli
- Staphylococcus aureus
- Candida albicans

Procedure

1. Sterile Mueller Hinton agar medium was prepared and poured into sterile petri plates.
2. After solidification, microbial cultures were spread uniformly over the agar surface using sterile cotton swab.



3. Wells of approximately 6 mm diameter were prepared using sterile cork borer.
4. Different concentrations of plant extracts were introduced into the wells using micropipettes.
5. The plates were incubated at 37°C for 24 hours.
6. After incubation, zones of inhibition around the wells were measured using a ruler.
7. Larger zone of inhibition indicated higher antimicrobial activity.

5.10 Statistical Analysis

All experiments were carried out in triplicate and the obtained results were expressed as mean values. The results of antioxidant and antimicrobial studies were compared among Neem, Tulsi, and Amla extracts.

5.11 Precautions

- Plant materials were dried properly before extraction.
- Glassware and culture media were sterilized before use.
- Solvents were handled carefully.
- Extraction temperature was maintained properly.
- Microbial studies were performed under aseptic conditions.
- Accurate measurements were taken during experimental work.

VI. EVALUATION PARAMETERS

6.1 Evaluation of Phytochemical Parameters

The prepared plant extracts of Neem, Tulsi, and Amla were evaluated for the presence of various phytochemical constituents responsible for biological activities.

The phytochemical screening included identification of alkaloids, flavonoids, tannins, glycosides, saponins, carbohydrates, and proteins.

6.2 Organoleptic Evaluation

Organoleptic evaluation was carried out using sensory characteristics of the plant materials.

Parameter	Evaluation
Color	Observed visually
Odor	Determined by smell
Taste	Determined carefully
Texture	Examined manually
Appearance	Evaluated visually

Importance

Organoleptic evaluation helps in identification and quality assessment of medicinal plant materials.

6.3 Determination of Extractive Value

Extractive value indicates the amount of active constituents extracted using a suitable solvent.

Procedure

A known quantity of powdered plant material was extracted with ethanol using Soxhlet apparatus.

The extract was filtered and evaporated to dryness.

The dried extract was weighed and percentage extractive value was calculated.



Calculation

$\% \text{ Extractive Value} = \frac{\text{Weight of Extract}}{\text{Weight of Plant Material}} \times 100$ Importance

- Determines extraction efficiency
- Indicates presence of soluble phytochemicals
- Useful for standardization of herbal drugs

6.4 Determination of Moisture Content

Moisture content was determined to evaluate stability and storage quality of plant materials.

Procedure

The powdered plant material was weighed and dried in a hot air oven at 105°C until constant weight was obtained.

Loss in weight was calculated as moisture content.

Calculation

$\% \text{ Moisture Content} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$ Importance

- Prevents microbial growth
- Improves stability of crude drugs
- Essential for storage conditions

6.5 Evaluation of Phytochemical Constituents Alkaloids

Observation

Formation of cream or reddish-brown precipitate indicated the presence of alkaloids.

Importance

Alkaloids possess:

- Antibacterial activity
- Analgesic activity
- Antimalarial activity

Flavonoids Observation

Formation of yellow color confirmed presence of flavonoids.

Importance

Flavonoids exhibit:

- Antioxidant activity
- Anti-inflammatory activity
- Cardioprotective effect

Tannins Observation

Formation of blue-black or green color indicated tannins.

Importance

Tannins possess:

- Antimicrobial activity
- Wound healing activity
- Astringent property

Saponins Observation

Persistent foam formation confirmed presence of saponins.



Importance

Saponins exhibit:

- Antifungal activity
- Immune stimulation
- Cholesterol lowering effect

Glycosides Observation

Formation of brown ring indicated glycosides.

Importance

Glycosides possess:

- Cardiotonic activity
- Anti-inflammatory activity
- Therapeutic importance

6.6 Evaluation of Antioxidant Activity

DPPH Radical Scavenging Assay

Antioxidant activity of plant extracts was evaluated using DPPH free radical scavenging method.

Principle

DPPH is a stable free radical having violet color. Antioxidants reduce DPPH into a yellow- colored compound resulting in decrease in absorbance.

Greater reduction in absorbance indicates higher antioxidant activity.

Parameters Evaluated

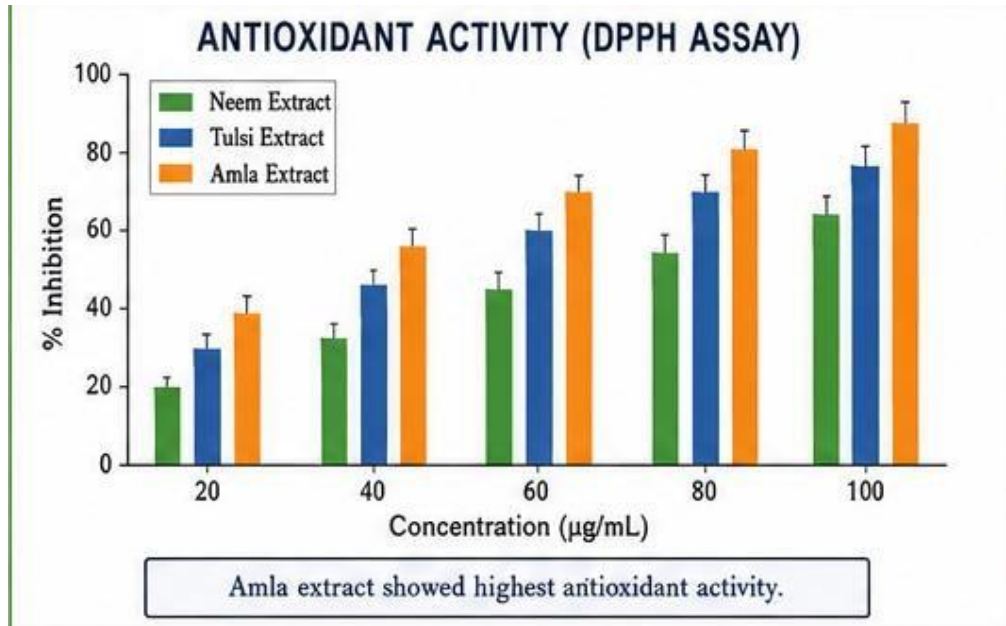
Parameter	Significance
Absorbance	Indicates free radical reduction
Percentage inhibition	Measures antioxidant activity
Concentration dependence	Evaluates effectiveness of extract

Calculation

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100 \text{ Where:}$$

- A₀ = Control absorbance
- A₁ = Sample absorbance





Interpretation

- Higher percentage inhibition indicates stronger antioxidant activity.
- Amla extract is expected to show maximum antioxidant activity due to high vitamin C content.

6.7 Evaluation of Antimicrobial Activity

Agar Well Diffusion Method

Antimicrobial activity was evaluated by measuring the zone of inhibition produced by plant extracts against microorganisms.

Test Organisms

Microorganism	Type
Escherichia coli	Gram-negative bacteria
Staphylococcus aureus	Gram-positive bacteria
Candida albicans	Fungus

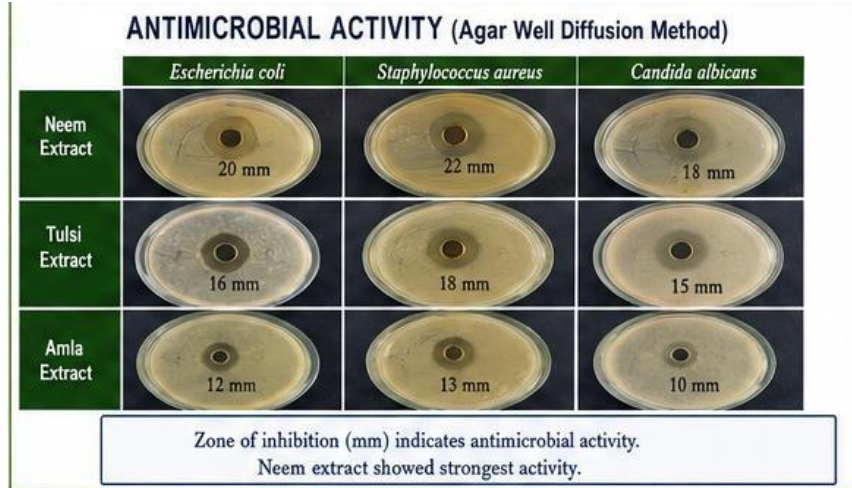
Parameters Evaluated

Parameter	Significance
Zone of inhibition	Indicates antimicrobial activity
Diameter of inhibition zone	Measures effectiveness of extract
Microbial sensitivity	Determines susceptibility



Procedure Summary

Plant extracts were introduced into wells prepared in agar plates inoculated with microbial cultures. After incubation, the diameter of clear zone around the wells was measured.



Interpretation

- Larger zone of inhibition indicates stronger antimicrobial activity.
- Neem extract is expected to exhibit maximum antimicrobial effect.

6.8 Comparative Evaluation of Selected Plants

The biological activities of Neem, Tulsi, and Amla were compared based on antioxidant and antimicrobial activities.

Plant	Major Activity
Neem	Antimicrobial activity
Tulsi	Antioxidant and antimicrobial activity
Amla	Strong antioxidant activity

6.9 Stability Evaluation

The prepared extracts were observed for stability during storage.

Parameters Observed

- Change in color
- Change in odor
- Precipitation
- Microbial contamination

Importance

Stability studies help determine storage conditions and shelf life of plant extracts.

6.10 Statistical Evaluation

The obtained experimental results were expressed as mean values from triplicate experiments. Comparative analysis was carried out among the selected medicinal plants.



VII. RESULTS AND DISCUSSION

7.1 Phytochemical Screening Results

The ethanolic extracts of Neem, Tulsi, and Amla were subjected to preliminary phytochemical screening for identification of important bioactive constituents.

The results obtained confirmed the presence of several phytochemicals responsible for biological activities.

Table 7.1 Preliminary Phytochemical Screening of Plant Extracts

Phytochemical Test	Neem	Tulsi	Amla
Alkaloids	Present	Present	Absent
Flavonoids	Present	Present	Present
Tannins	Present	Present	Present
Saponins	Present	Present	Absent
Glycosides	Present	Moderate	Present
Carbohydrates	Present	Present	Present
Proteins	Moderate	Present	Moderate

Discussion

- The phytochemical screening results indicated that all selected medicinal plants contain important bioactive compounds.
- Neem extract showed the presence of alkaloids, tannins, flavonoids, glycosides, and saponins which are responsible for antimicrobial and anti-inflammatory activities.
- Tulsi extract exhibited the presence of flavonoids, tannins, saponins, and essential phytoconstituents responsible for antioxidant and immunomodulatory activities.
- Amla extract showed high presence of flavonoids and tannins due to its rich polyphenolic content and vitamin C concentration.

The obtained results support the traditional medicinal uses of these plants.

7.2 Organoleptic Evaluation Results

The collected plant materials were evaluated for organoleptic properties.

Table 7.2 Organoleptic Characteristics of Selected Medicinal Plants

Plant Material	Color	Odor	Taste	Appearance
Neem Leaves	Dark Green	Characteristic	Bitter	Dried leaves
Tulsi Leaves	Green	Aromatic	Slightly Pungent	Dried leaves
Amla Fruits	Light Green	Characteristic	Sour	Dried fruit powder

Discussion

- Organoleptic evaluation helps in identification and quality assessment of medicinal plant materials.
- Neem leaves showed characteristic bitter taste due to the presence of limonoids and alkaloids.
- Tulsi possessed aromatic odor because of volatile oils such as eugenol.



- Amla exhibited sour taste due to high vitamin C content.

7.3 Extractive Value Results

Extractive values of selected medicinal plants were determined using ethanol as extraction solvent.

Table 7.3 Extractive Value of Plant Extracts

Plant Extract	Extractive Value (%)
Neem	12.4%
Tulsi	14.1%
Amla	16.8%

Discussion

Amla showed highest extractive value indicating greater amount of soluble phytochemicals.

Higher extractive value suggests better extraction efficiency and presence of more bioactive compounds.

Tulsi also showed appreciable extractive value due to essential oils and phenolic compounds. Neem extract exhibited comparatively lower extractive value.

7.4 Moisture Content Results

Table 7.4 Moisture Content of Plant Materials

Plant Material	Moisture Content (%)
Neem Leaves	6.2%
Tulsi Leaves	5.8%
Amla Fruits	7.1%

Discussion

- The moisture content of all plant materials was within acceptable limits.
- Lower moisture content prevents microbial contamination and improves stability during storage.
- Proper drying of plant materials contributed to better preservation of phytochemical constituents.

7.5 Antioxidant Activity Results

Antioxidant activity of the extracts was evaluated using DPPH radical scavenging assay.

Table 7.5 DPPH Radical Scavenging Activity

Plant Extract	Percentage Inhibition (%)
Neem	68.4%
Tulsi	74.2%
Amla	86.7%

- Among the selected medicinal plants, Amla exhibited highest antioxidant activity due to the presence of vitamin C, flavonoids, and polyphenolic compounds.
- Tulsi also demonstrated strong antioxidant activity because of eugenol and phenolic constituents.



- Neem extract showed moderate antioxidant activity compared to Amla and Tulsi.
- The results indicate that all selected plants possess free radical scavenging ability and can reduce oxidative stress.

7.6 Antimicrobial Activity Results

Antimicrobial activity was evaluated using agar well diffusion method.

Table 7.6 Antimicrobial Activity Against *Escherichia coli*

Plant Extract	Zone of Inhibition (mm)
Neem	20 mm
Tulsi	16 mm
Amla	12 mm

Table 7.7 Antimicrobial Activity Against *Staphylococcus aureus*

Plant Extract	Zone of Inhibition (mm)
Neem	22 mm
Tulsi	18 mm
Amla	13 mm

Table 7.8 Antifungal Activity Against *Candida albicans*

Plant Extract	Zone of Inhibition (mm)
Neem	18 mm
Tulsi	15 mm
Amla	10 mm

- Neem extract exhibited highest antimicrobial activity against bacterial and fungal strains.
- The antimicrobial effect of Neem may be attributed to the presence of nimbidin, quercetin, and limonoids.
- Tulsi also showed good antimicrobial activity due to essential oils and eugenol content.
- Amla demonstrated comparatively lower antimicrobial activity but showed better antioxidant potential.
- The obtained results confirmed that the selected medicinal plants possess broad- spectrum antimicrobial properties.

7.7 Comparative Discussion of Selected Medicinal Plants Neem

Neem demonstrated:

- Strong antimicrobial activity
- Moderate antioxidant activity
- Presence of alkaloids and tannins

Neem is highly effective against bacterial and fungal infections.

Tulsi

Tulsi showed:

- Good antioxidant activity
- Significant antimicrobial effect
- Presence of flavonoids and volatile oils



Tulsi acts as an effective immunomodulatory and protective agent.

Amla

Amla exhibited:

- Highest antioxidant activity
- Rich polyphenolic content
- High vitamin C concentration

Amla is highly beneficial in reducing oxidative stress and improving immunity.

7.8 Overall Interpretation of Results

The present study confirmed that Neem, Tulsi, and Amla contain important phytochemical constituents responsible for various biological activities.

The results demonstrated:

- Presence of alkaloids, flavonoids, tannins, and glycosides
- Significant antioxidant activity
- Effective antimicrobial activity Among the selected plants:
- Neem showed highest antimicrobial activity.
- Amla showed strongest antioxidant activity.
- Tulsi exhibited balanced biological activities.

VIII. SUMMARY AND CONCLUSION

8.1 Summary

Medicinal plants have been used since ancient times for the treatment and prevention of various diseases. Herbal medicines are gaining worldwide importance because of their therapeutic effectiveness, natural origin, low cost, and fewer side effects compared to synthetic drugs.

The present study was carried out to evaluate the phytochemical constituents and biological activities of three important Indian medicinal plants namely Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), and Amla (*Emblia officinalis*). The medicinal plants were collected, authenticated, shade dried, powdered, and extracted using ethanol by Soxhlet extraction method. The obtained extracts were subjected to preliminary phytochemical screening and biological evaluation.

Phytochemical studies confirmed the presence of important bioactive compounds such as:

- Alkaloids
- Flavonoids
- Tannins
- Glycosides
- Saponins
- Carbohydrates
- Proteins

These phytochemicals are responsible for various pharmacological and therapeutic activities.

The antioxidant activity of plant extracts was evaluated using DPPH radical scavenging assay. Among the selected plants, Amla showed highest antioxidant activity due to its rich vitamin C and polyphenolic content.

Antimicrobial activity was evaluated using agar well diffusion method against selected microorganisms including:

- *Escherichia coli*
- *Staphylococcus aureus*
- *Candida albicans*

Neem extract exhibited strongest antimicrobial activity among all selected medicinal plants.



The obtained results scientifically validated the traditional medicinal uses of Neem, Tulsi, and Amla.

8.2 Major Findings of the Study

The important findings observed during the study are summarized below:

- All selected medicinal plants contained important phytochemical constituents.
- Neem extract showed excellent antimicrobial activity.
- Tulsi extract exhibited good antioxidant and antimicrobial activities.
- Amla extract demonstrated highest antioxidant activity.
- Flavonoids and tannins were present in all extracts.
- The selected medicinal plants showed significant therapeutic potential.

8.3 Conclusion

The present study confirmed that Neem, Tulsi, and Amla are rich sources of phytochemical constituents possessing significant biological activities.

The phytochemical screening revealed the presence of bioactive compounds responsible for antioxidant and antimicrobial properties. Biological evaluation demonstrated that the selected medicinal plants possess considerable therapeutic potential.

Neem showed superior antimicrobial activity due to the presence of alkaloids, limonoids, and phenolic compounds.

Tulsi demonstrated balanced biological activities because of eugenol, flavonoids, and essential oils.

Amla exhibited excellent antioxidant activity mainly due to high vitamin C and polyphenolic content.

The results obtained from the present study support the traditional medicinal applications of these plants and indicate their potential use in development of herbal medicines and pharmaceutical preparations.

8.4 Future Scope

The present study provides useful information regarding phytochemical and biological properties of selected medicinal plants. Further studies may be carried out for:

- Isolation and purification of active constituents
- Identification of individual bioactive compounds
- In-vivo pharmacological studies
- Toxicological evaluation
- Development of herbal formulations
- Clinical evaluation of herbal products
- Standardization of plant extracts

Advanced research on these medicinal plants may contribute to the development of safer and effective natural therapeutic agents.

REFERENCES

1. Verma OP, Poonam S, Kamin A. Phytochemical Screening of *Ocimum sanctum* (Tulsi), *Azadirachta indica* (Neem) and *Phyllanthus emblica* (Amla). *International Journal of Current Microbiology and Applied Sciences*. 2019;8(4):682-686.
2. Islas JF, Acosta E, Delgado-Gallegos JL, et al. An overview of Neem (*Azadirachta indica*) and its potential impact on health. *Journal of Functional Foods*. 2020;74:104171.
3. Dhama K, et al. *Emblica officinalis* (Amla) with a particular focus on its antimicrobial potentials: A review. *Microbiology Research Journal International*.
4. Rao DMS, et al. Uncovering the role of Indian medicinal botanicals in COVID-19 prevention and management: A review. *Cureus*. 2024.



5. Tiwari A, Pandey A, Verma OP. Phytochemical screening of *Ocimum sanctum* (Tulsi), *Azadirachta indica* (Neem) and *Phyllanthus emblica* (Amla). *Asian Journal of Bio Science*. 2016;11(1):28-31.
6. Bole SB, et al. Comparative study of antibacterial and antioxidant activity of plant extract Amla, Tulsi and Neem. *Pharmacophore*. 2010;1(3):178-183.
7. Kumar R, et al. *Azadirachta indica*: A herbal panacea in dentistry. *International Journal of Life Science and Scientific Research*. 2018.
8. Andersa KN, et al. Proximate composition, phytochemical constituents and antioxidant activity of Neem leaf extracts. *Scientific African*. 2024.
9. Sireesha G, et al. Phytochemical composition and evaluation of herbal formulations containing Neem, Tulsi and Amla extracts. *World Journal of Pharmaceutical Research*. 2024.
10. Yamani HA, Pang EC, Mantri N, Deighton MA. Antimicrobial activity of Tulsi (*Ocimum tenuiflorum*) essential oil against bacterial species. *Frontiers in Microbiology*. 2016.
11. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Chapman and Hall; 1998.
12. Kokate CK. *Practical Pharmacognosy*. Vallabh Prakashan; 2010.
13. Khandelwal KR. *Practical Pharmacognosy Techniques and Experiments*. Nirali Prakashan; 2008.
14. Trease GE, Evans WC. *Trease and Evans Pharmacognosy*. Saunders Elsevier; 2009.
15. Sofowora A. *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books; 2006.
16. Evans WC. *Pharmacognosy*. WB Saunders Company; 2002.
17. *Indian Herbal Pharmacopoeia*. Indian Drug Manufacturers Association; 2002.
18. World Health Organization. *WHO Guidelines on Good Agricultural and Collection Practices for Medicinal Plants*. WHO; 2003.
19. Chopra RN, Nayar SL, Chopra IC. *Glossary of Indian Medicinal Plants*. CSIR Publications; 2006.
20. Sharma PV. *Dravyaguna Vijnana*. Chaukhamba Bharati Academy; 2005.
21. Anonymous. *The Ayurvedic Pharmacopoeia of India*. Government of India, Ministry of Health and Family Welfare; 2001.
22. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and antioxidant activity. *Methods in Enzymology*. 1999;299:152-178.
23. Brinda P, Sasikala P, Purushothaman KK. Pharmacognostic studies on medicinal plants. *Bulletin of Medicobotanical Research*. 1981;3:84-96.
24. Singh VK, Govil JN, Hashmi S, Singh G. *Recent Progress in Medicinal Plants*. Studium Press; 2005.
25. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and their antioxidant activity. *Food Chemistry*. 2006;99(1):191-203.
26. Calixto JB. Efficacy, safety and quality control of herbal medicines. *Brazilian Journal of Medical and Biological Research*. 2000;33:179-189.
27. Gupta RK, Sharma A. Antioxidant and antimicrobial activity of medicinal plants. *International Journal of Pharmaceutical Sciences*. 2011;3(2):45-50.
28. Prakash P, Gupta N. Therapeutic uses of *Ocimum sanctum* Linn (Tulsi). *Indian Journal of Physiology and Pharmacology*. 2005;49(2):125-131.
29. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of Neem (*Azadirachta indica*). *Current Science*. 2002;82(11):1336-1345.
30. Scartezzini P, Speroni E. Review on medicinal plants with antioxidant activity. *Journal of Ethnopharmacology*. 2000;71:23-43.
31. Govindarajan R, Vijayakumar M, Pushpangadan P. Antioxidant approach to disease management and role of medicinal plants. *Journal of Ethnopharmacology*. 2005;99:165-178.
32. Bhattacharya A, Chatterjee A, Ghosal S, Bhattacharya SK. Antioxidant activity of active tannoid principles of Amla. *Indian Journal of Experimental Biology*. 1999;37:676-680.



33. Kaur C, Kapoor HC. Antioxidant activity and phenolic content of medicinal plants. *International Journal of Food Science and Technology*. 2001;36:703-725.
34. Mandal S, et al. Antioxidant and antimicrobial activities of medicinal plants. *Indian Journal of Natural Products and Resources*. 2010;1(1):102-108.
35. Chatterjee A, Pakrashi SC. *The Treatise on Indian Medicinal Plants*. National Institute of Science Communication; 2003.
36. Ali A. *Textbook of Pharmacognosy*. CBS Publishers and Distributors; 2008.
37. De Pasquale A. Pharmacognosy: oldest modern science. *Journal of Ethnopharmacology*. 1984;11:1-16.
38. Cowan MM. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*. 1999;12(4):564-582.
39. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. *Indian Journal of Pharmacology*. 2000;32:S81-S118.
40. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*. 2001;109:69-75.

