

## Phytochemical Composition and Antibacterial/Antimicrobial Properties of Azadirachta Indica (Neem)

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### ABSTRACT

Azadirachta indica, commonly known as Neem, has been extensively studied for its medicinal properties due to its rich phytochemical composition. This project aims to investigate the phytochemical composition and antibacterial/antimicrobial properties of Azadirachta indica leaves.

The phytochemical analysis will involve the identification and quantification of various bioactive compounds present in Neem leaves, including but not limited to flavonoids, alkaloids, phenolic compounds, and terpenoids. Various extraction techniques such as maceration, Soxhlet extraction, and chromatographic methods will be employed to isolate these compounds.[1,5]

Furthermore, the antibacterial and antimicrobial activities of Neem leaf extracts will be evaluated against a panel of pathogenic bacteria and fungi using well-established methods such as agar diffusion assay, broth microdilution method, and disc diffusion method. The selected microorganisms may include both Gram-positive and Gram-negative bacteria, as well as common fungal pathogens.[2,3]

The project will also explore the mechanisms underlying the antibacterial and antimicrobial effects of Neem leaf extracts, including their impact on microbial cell membrane integrity, enzyme inhibition, and biofilm formation. Additionally, synergistic effects of Neem extracts with conventional antibiotics or other natural antimicrobial agents will be investigated to assess their potential as combination therapies.

Overall, this project aims to contribute to the understanding of the therapeutic potential of

Azadirachta indica leaves as natural antimicrobial agents and provide insights into their possible applications in the development of novel antibacterial and antimicrobial drugs.[14]

### I. INTRODUCTION

#### ➤ Background and Significance of Azadirachta indica (Neem)

Azadirachta indica, commonly known as Neem, is a tree native to the Indian subcontinent and has been revered for its medicinal properties for centuries. It holds a prominent place in traditional medicine systems such as Ayurveda, Siddha, and Unani, where various parts of the tree, including leaves, bark, seeds, and oil, are utilized for their therapeutic benefits.

Neem is valued for its diverse array of bioactive compounds, including flavonoids, alkaloids, tannins, phenolics, terpenoids, and triterpenoids, which contribute to its pharmacological properties. These compounds exhibit potent antibacterial, antifungal, antiviral, antiprotozoal, anti-inflammatory, antioxidant, and immunomodulatory activities, making Neem a versatile plant with numerous potential applications in healthcare, agriculture, and environmental sustainability.[5]

The broad-spectrum antimicrobial properties of Neem have garnered significant attention due to the global health challenges posed by antibiotic resistance, emerging infectious diseases, and agricultural pests. Neem-based interventions offer promising alternatives to conventional antibiotics, chemical pesticides, and synthetic disinfectants, providing sustainable solutions for combating microbial pathogens while

minimizing environmental impact and promoting public health.

#### ➤ **Rationale for Studying the Phytochemical Composition and Antibacterial/Antimicrobial Properties of Neem Leaves**

The phytochemical composition and antibacterial/antimicrobial properties of Neem leaves are of particular interest due to their potential therapeutic applications and implications for human health, agriculture, and environmental sustainability. Understanding the chemical constituents responsible for Neem's biological activities can elucidate its mechanisms of action and guide the development of novel pharmaceuticals, agrochemicals, and cosmeceuticals.

By investigating the phytochemical composition of Neem leaves, researchers can identify bioactive compounds with specific pharmacological effects, such as antibacterial agents, antifungal agents, and antimalarial agents. These compounds may serve as lead compounds for drug discovery and development, offering new treatment options for infectious diseases and other health conditions.

Similarly, studying the antibacterial and antimicrobial properties of Neem leaves can provide valuable insights into their efficacy against a wide range of microbial pathogens, including bacteria, fungi, viruses, and protozoa. Understanding the mechanisms underlying Neem's antimicrobial effects can inform the design of effective interventions for preventing and treating infectious diseases in humans, animals, and plants.[5,8,9]

#### ➤ **Objectives of the Project**

1. Investigate the phytochemical composition of Neem leaves through comprehensive chemical analysis, including chromatography, spectroscopy, and mass spectrometry techniques.
2. Evaluate the antibacterial and antimicrobial properties of Neem leaf extracts against clinically relevant pathogens, including bacteria, fungi, viruses, and protozoa.
3. Elucidate the mechanisms of action underlying the antibacterial and antimicrobial effects of Neem leaf extracts through in vitro and in vivo studies.
4. Assess the potential therapeutic applications of Neem leaf extracts in healthcare, agriculture, and environmental sustainability, including the development of pharmaceuticals, agrochemicals, and cosmeceuticals.

#### **Azadirachta indica (Neem): An Overview**

##### ➤ **Taxonomy and Botanical Description**

*Azadirachta indica*, commonly known as Neem, belongs to the Meliaceae family and is a versatile evergreen tree native to the Indian subcontinent. Taxonomically, it is classified as follows:

- ◆ Kingdom: Plantae
- ◆ Division: Magnoliophyta
- ◆ Class: Magnoliopsida
- ◆ Order: Sapindales
- ◆ Family: Meliaceae
- ◆ Genus: *Azadirachta*
- ◆ Species: *Azadirachta indica*

##### ➤ **Botanical Description**

###### ◆ **Height**

Neem trees typically grow up to 15-20 meters in height, although some specimens can reach up to 25 meters under favorable conditions.

###### ◆ **Leaves**

Neem leaves are compound, pinnate, and alternate, with each leaf comprising 8-18 leaflets arranged in pairs along a central axis. The leaflets are lanceolate to ovate in shape, with serrated margins and a glossy green surface.



Fig.no.1 Neem Leaves

###### ◆ **Flowers**

Neem trees produce small, white to off-white flowers arranged in axillary clusters. The flowers are fragrant and have a sweet aroma, attracting pollinators such as bees and butterflies.



Fig.no. 2Neem Flowers

#### ◆ Fruits

Neem fruits are oval-shaped drupes, green when young and turning yellow as they mature. Each fruit contains a single elongated seed enclosed within a thin, fleshy pulp.



Fig.no.3 Neem Fruits

#### ◆ Bark

The bark of Neem trees is dark gray to brown in color, rough and fissured, with prominent lenticels. It exudes a bitter taste when chewed.



Fig.no.4 Neem Bark

#### ➤ Traditional Uses in Medicine, Agriculture, and Other Industries

##### ◆ Medicinal Uses

- Neem has been an integral part of traditional medicine systems such as Ayurveda, Siddha, and Unani for centuries. Various parts of the Neem tree, including leaves, bark, seeds, and oil, are used medicinally to treat a wide range of ailments.

- Neem leaves are renowned for their antiseptic, anti-inflammatory, antipyretic, and analgesic properties. They are commonly used in the treatment of skin disorders, wounds, fevers, and gastrointestinal ailments.

- Neem oil, extracted from the seeds, is valued for its antibacterial, antifungal, and insecticidal properties. It is used topically for skin conditions such as acne, eczema, and psoriasis, as well as in hair care products for dandruff and lice.

##### ◆ Agricultural Uses

- Neem-based formulations are widely used in organic farming and integrated pest management (IPM) practices as biopesticides, insecticides, and nematocides.

- Neem products exhibit pesticidal activity against a broad spectrum of pests, including insects, mites, nematodes, and fungi, while posing minimal risk to non-target organisms and the environment.

- Neem cake, a by-product obtained after oil extraction from Neem seeds, serves as an organic fertilizer and soil conditioner, enriching soil fertility and promoting plant growth.

##### ◆ Other Industries

- Neem wood is valued for its durability, termite resistance, and attractive grain patterns, making it suitable for construction, furniture, and handicrafts.

- Neem leaves and twigs are used as natural toothbrushes in traditional oral hygiene practices, owing to their antimicrobial properties and ability to strengthen gums.

##### ◆ Global Distribution and Cultivation

- Neem is cultivated in tropical and subtropical regions worldwide, including India, Southeast Asia, Africa, Australia, and parts of the Americas.

- It thrives in a variety of soil types, ranging from sandy loam to clayey soils, provided they are well-drained and moderately fertile.

- Neem trees are drought-tolerant and can withstand high temperatures, making them well-suited for arid and semi-arid environments.

- Commercial cultivation of Neem is primarily for its seeds, which are rich in oil content and serve as a valuable source of bioactive compounds for various industrial applications.

#### Phytochemical Composition of Neem Leaves

##### ➤ Overview of Phytochemicals and Their Importance

Phytochemicals are bioactive compounds synthesized by plants that contribute to their various physiological functions and defense mechanisms. These compounds are not considered essential nutrients, but they possess therapeutic properties that are beneficial to human health. Neem leaves, in particular, are rich in phytochemicals, making them valuable in traditional medicine and modern research. The major classes of phytochemicals present in Neem leaves include flavonoids, alkaloids, tannins, phenolics, terpenoids, and triterpenoids.

➤ **Phytochemical Constituents of Neem Leaves**

1. Flavonoids

- Flavonoids are polyphenolic compounds with antioxidant, anti-inflammatory, and antimicrobial properties.
- Neem leaves contain various flavonoids such as quercetin, kaempferol, rutin, and naringenin.
- These compounds contribute to the medicinal properties of Neem leaves, including their ability to scavenge free radicals and reduce oxidative stress.

2. Alkaloids

- Alkaloids are nitrogen-containing compounds with diverse pharmacological activities, including analgesic, anti-inflammatory, and antimicrobial effects.
- Neem leaves contain alkaloids such as nimbin, nimbidin, and nimbidol, which exhibit antipyretic, antimalarial, and hepatoprotective properties.
- Alkaloids play a crucial role in the therapeutic efficacy of Neem leaf extracts against various diseases and infections.

3. Tannins

- Tannins are polyphenolic compounds known for their astringent properties and ability to precipitate proteins.
- Neem leaves contain both hydrolysable and condensed tannins, which contribute to their antibacterial, antifungal, and wound-healing activities.
- Tannins also exhibit antioxidant effects and play a role in the prevention of oxidative damage and aging-related disorders.

4. Phenolics

- Phenolic compounds are aromatic secondary metabolites with diverse biological activities, including antioxidant, anti-inflammatory, and anticancer effects.
- Neem leaves contain phenolic acids such as gallic acid, caffeic acid, and ellagic acid, which contribute to their antioxidant and anti-inflammatory properties.
- Phenolics play a crucial role in the modulation of immune responses and the prevention of chronic diseases associated with inflammation and oxidative stress.

5. Terpenoids

- Terpenoids are a diverse class of compounds derived from isoprene units, exhibiting a wide range of pharmacological activities.

- Neem leaves contain terpenoids such as limonoids, azadirachtin, and gedunin, which possess insecticidal, antimalarial, and anticancer properties.
- Terpenoids play a significant role in the insecticidal activity of Neem leaf extracts and their potential applications in pest control and agriculture.

6. Triterpenoids

- Triterpenoids are terpenoids composed of six isoprene units, exhibiting diverse pharmacological activities such as anti-inflammatory, antidiabetic, and hepatoprotective effects.
- Neem leaves contain triterpenoids such as  $\beta$ -sitosterol, lupeol, and ursolic acid, which contribute to their anti-inflammatory and immunomodulatory properties.
- Triterpenoids play a crucial role in the traditional uses of Neem leaves for the treatment of skin disorders, arthritis, and liver diseases.

**Methods of Phytochemical Analysis**

Phytochemical analysis of Neem leaves involves various techniques for the identification and quantification of bioactive compounds.

➤ **These methods include**

1. Extraction Techniques

Solvent extraction methods such as maceration, Soxhlet extraction, and ultrasound-assisted extraction are commonly used to extract phytochemicals from Neem leaves. Different solvents (e.g., ethanol, methanol, water) are employed based on the polarity of the compounds of interest.

2. Chromatographic Techniques

High-performance liquid chromatography (HPLC), gas chromatography (GC), and thin-layer chromatography (TLC) are used for the separation and quantification of specific phytochemicals in Neem leaf extracts. These techniques allow for the identification of individual compounds based on their retention times and spectral characteristics.

3. Spectroscopic Methods

UV-Vis spectroscopy, infrared (IR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy are employed for the qualitative and quantitative analysis of phytochemicals in Neem leaves. These methods provide information about the chemical structure, functional groups, and concentration of bioactive compounds.

#### 4. Bioassay-guided Fractionation

Bioassay-guided fractionation involves the isolation and purification of bioactive compounds from Neem leaf extracts based on their biological activities. Fractionation techniques such as column chromatography, preparative HPLC, and solid-phase extraction are used to isolate compounds of interest for further characterization.

#### 5. Antioxidant Assays

Various assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay, and ferric reducing antioxidant power (FRAP) assay are used to evaluate the antioxidant activity of Neem leaf extracts and quantify the total phenolic and flavonoid content.

Phytochemical analysis of Neem leaves using these methods provides valuable information about the composition, bioactivity, and potential health benefits of Neem leaf extracts, facilitating their utilization in traditional medicine, pharmaceuticals, and other industries.

### Antibacterial Properties of Neem Leaves

#### ➤ Overview of Bacterial Infections and Antibiotic Resistance

Bacterial infections pose significant threats to human health worldwide, causing a wide range of diseases ranging from mild to life-threatening conditions. Antibiotics have been pivotal in treating bacterial infections; however, the emergence of antibiotic-resistant strains has become a global concern. Antibiotic resistance arises due to the misuse and overuse of antibiotics, leading to the development of bacterial strains that are no longer susceptible to the effects of commonly used antibiotics. As a result, there is a critical need for alternative antimicrobial agents to combat bacterial infections effectively.[6,10,14]

#### ➤ Antibacterial Activity of Neem Leaf Extracts

Neem leaves have long been recognized for their potent antibacterial properties, making them a valuable natural resource for the treatment of bacterial infections. Various studies have investigated the antibacterial activity of Neem leaf extracts against a wide range of bacterial pathogens, both in vitro and in vivo.

#### ◆ Mechanisms of Action

The antibacterial activity of Neem leaf extracts is attributed to multiple mechanisms of action, which include:

##### 1. Disruption of Bacterial Cell Membranes

Neem leaf extracts contain bioactive compounds that disrupt the integrity of bacterial cell membranes, leading to leakage of cellular contents and ultimately bacterial cell death.

##### 2. Inhibition of Bacterial Adhesion

Certain constituents of Neem leaves inhibit the adhesion of bacteria to host cells and surfaces, thereby preventing the initiation of infection and biofilm formation.

##### 3. Interference with Microbial Enzymes

Neem leaf extracts interfere with the activity of bacterial enzymes essential for growth and metabolism, thereby inhibiting bacterial proliferation.

#### ◆ In Vitro and In Vivo Studies

Numerous in vitro studies have demonstrated the efficacy of Neem leaf extracts against various bacterial strains, including but not limited to:

##### 1. Staphylococcus aureus

Neem leaf extracts have shown significant antibacterial activity against both methicillin-sensitive and methicillin-resistant strains of Staphylococcus aureus, including those responsible for nosocomial infections and skin infections.

##### 2. Escherichia coli

Neem leaf extracts exhibit potent antibacterial activity against Escherichia coli, a common cause of urinary tract infections, diarrheal diseases, and foodborne illnesses.

##### 3. Salmonella spp

Neem leaf extracts have been shown to inhibit the growth of Salmonella spp., including Salmonella typhi and Salmonella enterica, which are responsible for typhoid fever and gastroenteritis, respectively.

#### ◆ Efficacy Against Common Bacterial Pathogens

In addition to Staphylococcus aureus, Escherichia coli, and Salmonella spp., Neem leaf extracts have demonstrated efficacy against other common bacterial pathogens, such as:

### 1. *Pseudomonas aeruginosa*

Neem leaf extracts have been found to inhibit the growth of *Pseudomonas aeruginosa*, a leading cause of nosocomial infections and respiratory tract infections.

### 2. *Acinetobacter baumannii*

Neem leaf extracts have shown antibacterial activity against multidrug-resistant strains of *Acinetobacter baumannii*, which are associated with hospital-acquired infections and wound infections.

## Antimicrobial Properties of Neem Leaves

### ➤ Overview of Microbial Infections and Challenges

Microbial infections, caused by bacteria, fungi, viruses, and protozoa, continue to be a significant global health burden, contributing to morbidity and mortality worldwide. The emergence of antimicrobial resistance further complicates the treatment of infectious diseases, necessitating the development of alternative therapeutic strategies. Neem leaves, with their rich phytochemical composition, have demonstrated potent antimicrobial properties against a wide range of microbial pathogens.[4,9]

### ➤ Antimicrobial Activity of Neem Leaf Extracts

Neem leaf extracts exhibit broad-spectrum antimicrobial activity against various microbial pathogens, including fungi, viruses, and protozoa. The bioactive compounds present in Neem leaves interfere with the growth and proliferation of these pathogens through multiple mechanisms of action.

#### ◆ Fungal Infections

Neem leaf extracts have been extensively studied for their antifungal properties and have shown efficacy against various fungal pathogens, including *Candida albicans*, *Aspergillus* spp., and dermatophytes. The antifungal activity of Neem leaves is attributed to the inhibition of fungal growth, disruption of fungal cell membranes, and modulation of fungal enzymes involved in metabolism and cell wall synthesis.[3,11]

#### ◆ Viral Infections

Studies have revealed the antiviral potential of Neem leaf extracts against several viruses, including herpes simplex virus (HSV), influenza virus, human immunodeficiency virus

(HIV), and dengue virus. Neem leaf extracts inhibit viral replication by interfering with viral entry, replication, and assembly. Additionally, they stimulate the host immune response, enhancing the body's ability to combat viral infections.[21]

#### ◆ Protozoal Infections

Neem leaf extracts exhibit promising activity against protozoan parasites, such as *Plasmodium falciparum*, the causative agent of malaria, and *Leishmania* spp., responsible for leishmaniasis. The antiprotozoal activity of Neem leaves involves the disruption of parasite growth and development, inhibition of parasite enzymes, and modulation of host immune responses.

### ➤ Efficacy Against Specific Microbial Pathogens:

Neem leaf extracts have been evaluated for their efficacy against specific microbial pathogens, including:

#### 1. *Candida albicans*

Neem leaf extracts inhibit the growth of *Candida albicans*, a common fungal pathogen responsible for candidiasis, particularly in immunocompromised individuals.

#### 2. Herpes Simplex Virus (HSV)

Neem leaf extracts demonstrate antiviral activity against HSV, inhibiting viral replication and reducing the severity and duration of herpes outbreaks.

#### 3. Influenza Virus

Neem leaf extracts have shown inhibitory effects against influenza virus strains, potentially offering a natural remedy for influenza infections.

#### 4. *Plasmodium falciparum*

Neem leaf extracts exhibit antimalarial activity against *Plasmodium falciparum* parasites, making them a promising candidate for the development of antimalarial drugs.[4,20]

## Methods of Extraction and Analysis

### ➤ Extraction Techniques for Obtaining Neem Leaf Extracts

#### 1. Solvent Extraction

Neem leaf powder is typically macerated or percolated with a suitable solvent (e.g., ethanol, methanol, or water) to extract bioactive compounds. The mixture is then filtered, and the solvent is

evaporated under reduced pressure to obtain a concentrated extract.

## 2. Steam Distillation

Steam distillation is employed to extract volatile compounds, such as essential oils, from Neem leaves. The leaves are subjected to steam under pressure, and the volatile constituents are carried over and condensed to yield an essential oil-rich fraction.

## 3. Supercritical Fluid Extraction (SFE)

SFE utilizes supercritical carbon dioxide as a solvent to extract lipophilic compounds from Neem leaves. The extraction process is carried out under high pressure and temperature, resulting in a solvent-free extract with minimal degradation of heat-sensitive compounds.

## 4. Ultrasound-Assisted Extraction (UAE)

UAE involves the application of high-frequency ultrasound waves to facilitate the extraction of bioactive compounds from Neem leaves. The cavitation effect generated by ultrasound enhances mass transfer and disrupts cell structures, thereby improving extraction efficiency.

## 5. Microwave-Assisted Extraction (MAE)

MAE utilizes microwave irradiation to enhance the extraction of phytochemicals from Neem leaves. The microwave energy heats the solvent and accelerates the extraction process by breaking down cell walls and releasing intracellular constituents.[15,16]

### ➤ Analytical Methods for Determining Phytochemical Composition

#### 1. High-Performance Liquid Chromatography (HPLC)

HPLC is commonly used for the qualitative and quantitative analysis of phytochemicals in Neem leaf extracts. Different chromatographic columns and detectors (UV, DAD, MS) are employed to separate and identify individual compounds.

#### 2. Gas Chromatography-Mass Spectrometry (GC-MS):

GC-MS is utilized to analyze volatile compounds, such as terpenoids and essential oils, present in Neem leaf extracts. The technique allows for the identification of compounds based on their retention times and mass spectra.

#### 3. Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS combines liquid chromatography with mass spectrometry to analyze a wide range of phytochemicals in Neem leaf extracts. The technique offers high sensitivity and selectivity for the identification and quantification of target compounds.

#### 4. Thin-Layer Chromatography (TLC)

TLC is a cost-effective method for the qualitative analysis of phytochemicals in Neem leaf extracts. The extracts are spotted onto a TLC plate, developed using a suitable solvent system, and visualized under UV light or using specific reagents.

#### 5. Spectrophotometric Assays

Spectrophotometric methods, such as UV-Vis spectroscopy, are employed to quantify total phenolics, flavonoids, and other phytochemicals in Neem leaf extracts based on their absorbance at specific wavelengths.

### ➤ Evaluation of Antibacterial and Antimicrobial Activities

#### 1. Agar Diffusion Assay

Neem leaf extracts are tested for their antibacterial and antimicrobial activities using the agar diffusion method. Extracts are applied to sterile paper discs and placed on agar plates inoculated with bacterial or fungal cultures. Zones of inhibition are measured to assess the extent of microbial growth inhibition.

#### 2. Minimum Inhibitory Concentration (MIC) Determination

MIC assays determine the lowest concentration of Neem leaf extracts required to inhibit microbial growth. Serial dilutions of the extracts are prepared, and their effects on bacterial or fungal growth are observed over a specified incubation period.

#### 3. Time-Kill Assay

Time-kill assays evaluate the bactericidal or fungicidal effects of Neem leaf extracts over time. Microbial cultures are exposed to sub-inhibitory concentrations of the extracts, and viable cell counts are determined at different time intervals to assess microbial viability.

#### 4. Checkerboard Assay

Checkerboard assays are used to evaluate the synergistic or additive effects of Neem leaf extracts in combination with antibiotics or other antimicrobial agents. Microbial growth is assessed in the presence of varying concentrations of both the extract and the reference compound.

#### 5. Animal Model Studies

In vivo studies using animal models, such as mice or rats, can assess the efficacy of Neem leaf extracts in treating microbial infections. Parameters such as survival rate, pathogen load, and histopathological changes are evaluated to determine the therapeutic potential of the extracts.[19,20]

#### Mechanisms of Action

##### ➤ Elucidation of the Molecular Mechanisms Underlying Antibacterial and Antimicrobial Effects

###### 1. Disruption of Cell Membrane Integrity

Neem leaf constituents, such as limonoids and triterpenoids, can disrupt the integrity of bacterial and fungal cell membranes. This disruption leads to the leakage of intracellular components and ultimately cell death.

###### 2. Inhibition of Enzymatic Activity

Certain compounds in Neem leaves, such as azadirachtin and nimbolide, have been shown to inhibit key enzymatic activities crucial for microbial survival and replication. For instance, they can interfere with bacterial DNA replication, RNA transcription, protein synthesis, and cell wall synthesis.

###### 3. Induction of Oxidative Stress

Neem leaf extracts possess antioxidant properties, but paradoxically, they can also induce oxidative stress in microbial cells. This imbalance in redox status can lead to cellular damage, DNA fragmentation, and apoptosis in bacteria, fungi, and viruses.

###### 4. Modulation of Signal Transduction Pathways

Neem leaf constituents may modulate signal transduction pathways within microbial cells, affecting processes such as quorum sensing, biofilm formation, and virulence factor expression. By disrupting these communication networks, Neem extracts can impair microbial survival and pathogenicity.

##### ➤ Interaction of Neem Leaf Constituents with Microbial Targets

###### 1. Bacterial Cell Wall and Membrane

Neem leaf compounds can interact with bacterial cell wall components, such as peptidoglycans and lipopolysaccharides, as well as cell membrane phospholipids. These interactions disrupt membrane integrity, leading to cell lysis and death.

###### 2. Fungal Cell Wall and Membrane

Similarly, Neem leaf constituents can disrupt the structure and function of fungal cell walls and membranes, which are composed of chitin and ergosterol. By interfering with these vital structures, Neem extracts inhibit fungal growth and viability.

###### 3. Viral Replication Machinery

Neem leaf compounds may target viral replication machinery, including viral enzymes such as polymerases and proteases. By inhibiting these enzymes, Neem extracts can interfere with viral genome replication, protein synthesis, and assembly, thereby suppressing viral replication.

###### 4. Protozoal Metabolism and Cell Structure

Neem leaf constituents may disrupt metabolic pathways and structural components within protozoal cells, leading to impaired growth and replication. For example, they can interfere with mitochondrial function, nucleic acid synthesis, and cytoskeletal organization in protozoan parasites.

##### ➤ Significance of Synergistic Effects and Multi-Target Actions

###### 1. Synergistic Effects

Neem leaf extracts often contain a complex mixture of bioactive compounds, each with unique mechanisms of action. When these compounds act synergistically, they can enhance the overall antimicrobial activity of the extract, leading to greater efficacy against microbial pathogens.

###### 2. Multi-Target Actions

The multi-target nature of Neem leaf constituents allows them to affect multiple cellular processes within microbial cells simultaneously. This multi-target approach reduces the likelihood of microbial resistance development and increases the effectiveness of Neem extracts against diverse microbial pathogens.



### 3. Broad-Spectrum Activity

The diverse range of mechanisms of action exhibited by Neem leaf constituents enables them to exert broad-spectrum antimicrobial activity against bacteria, fungi, viruses, and protozoa. This broad-spectrum activity makes Neem extracts valuable therapeutic agents for combating a wide range of infectious diseases.

### Applications in Medicine and Healthcare

#### ➤ Pharmaceutical Formulations containing Neem Leaf Extracts

##### 1. Topical Preparations

Neem leaf extracts are incorporated into topical formulations such as creams, ointments, and lotions for the treatment of various skin conditions. These formulations are effective against acne, eczema, psoriasis, dermatitis, and fungal infections due to their antibacterial, antifungal, and anti-inflammatory properties.

##### 2. Oral Products

Neem leaf extracts are used in oral healthcare products such as toothpaste, mouthwash, and chewing sticks (datun) due to their antimicrobial activity against oral pathogens. These products help prevent dental caries, gingivitis, periodontitis, and other oral infections.

##### 3. Capsules and Tablets

Neem leaf extracts are encapsulated or compressed into tablets for oral administration as dietary supplements or herbal remedies. These formulations are used for general health maintenance, immune support, and as adjunctive therapy for various infectious and inflammatory conditions.

##### 4. Wound Healing Products

Neem leaf extracts promote wound healing and tissue regeneration when applied topically to cuts, wounds, and ulcers. They exhibit antibacterial, antifungal, and anti-inflammatory effects, accelerating the healing process and preventing infection.[12,18]

#### ◆ Some pharmaceutical products derived from Neem

##### 1. Neem Oil

Neem oil is extracted from the seeds of the Neem tree and has been used in traditional medicine for centuries. It contains numerous bioactive compounds such as azadirachtin, nimbin, and nimbidin, which exhibit antibacterial,

antifungal, antiviral, and anti-inflammatory properties. Neem oil is commonly used in topical treatments for skin disorders such as acne, eczema, psoriasis, and fungal infections. It is also used in the production of Neem-based soaps, shampoos, lotions, and creams.



Fig.no.5 Neem Oil

##### 2. Neem Leaf Extracts

Neem leaf extracts are prepared by crushing and macerating Neem leaves in solvent (e.g., water, ethanol) to extract their bioactive compounds. These extracts are rich in flavonoids, tannins, phenolics, and terpenoids, which have antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties. Neem leaf extracts are used in traditional medicine for treating various ailments such as fever, cough, diabetes, gastrointestinal disorders, and skin infections. They are also incorporated into herbal supplements, capsules, and tinctures for oral consumption.



Fig.no.6 Neem Leaf Extract

##### 3. Neem Capsules and Tablets

Neem capsules and tablets are formulated using Neem leaf powder, Neem leaf extracts, or standardized Neem extracts. These formulations provide a convenient and standardized dosage of Neem bioactive compounds, making it easier to administer and consume. Neem capsules and tablets are used as dietary supplements to support immune health, promote detoxification, and maintain overall well-being. They may also be prescribed for specific health conditions such as bacterial infections, viral infections, and inflammatory disorders.



Fig.no.7 Neem Capsule And Tablet

#### 4. Neem-based Mouthwash and Toothpaste

Neem-based mouthwash and toothpaste are formulated using Neem leaf extracts or Neem oil along with other natural ingredients such as mint, clove, and eucalyptus. These oral care products offer antimicrobial, antiplaque, and anti-inflammatory benefits, helping to prevent dental caries, gingivitis, and periodontal disease. Neem mouthwash and toothpaste are used for daily oral hygiene maintenance and as adjunctive therapy for oral health conditions.

#### 5. Neem-based Wound Healing Products

Neem has wound healing properties due to its antibacterial, antifungal, and anti-inflammatory effects. Neem-based wound healing products, such as creams, ointments, and gels, are formulated with Neem leaf extracts or Neem oil to promote tissue repair, reduce inflammation, and prevent infection. These products are used for treating cuts, burns, wounds, ulcers, and other skin injuries. They may also contain additional ingredients such as aloe vera, turmeric, and vitamin E for enhanced efficacy.



Fig.no.9 Neem Wound Healing

#### 6. Neem-based Shampoos and Hair Care Products

Neem-based shampoos and hair care products are formulated with Neem oil or Neem leaf extracts to promote scalp health, prevent dandruff, and improve hair texture. Neem's antimicrobial properties help to control scalp infections and reduce itching, while its nourishing properties condition the hair and scalp. These products are suitable for individuals with scalp conditions such as seborrheic dermatitis, psoriasis,

and eczema, as well as those looking for natural alternatives for hair care.



Fig.no.10 Neem Shampoo And Hair Care Product

#### ◆ Some Neem leaves-based products used specifically for these purposes

##### 1. Neem Leaf Extracts

- Neem leaf extracts contain bioactive compounds such as azadirachtin, nimbin, nimbolide, and gedunin, which have been shown to exhibit antimalarial and antibacterial activities.

- These extracts can be prepared by crushing and macerating Neem leaves in a suitable solvent (e.g., water, ethanol) to extract their bioactive constituents.

- Neem leaf extracts can be formulated into various pharmaceutical dosage forms, including syrups, suspensions, capsules, and tablets, for oral administration.



Fig.no.11 Neam Leaf Extract

##### 2. Neem Leaf Tea

- Neem leaf tea is prepared by steeping dried Neem leaves in hot water, allowing the bioactive compounds to infuse into the water.

- Drinking Neem leaf tea regularly may help boost the immune system and provide protection against bacterial and malarial infections.

- Neem leaf tea is also believed to have detoxifying properties, aiding in the elimination of toxins from the body.



Fig.no.12 Neem Leaf Tea

### 3. Neem Leaf Capsules

- Neem leaf capsules contain powdered Neem leaves or standardized Neem leaf extracts in a convenient oral dosage form.
- These capsules are often used as dietary supplements to support immune health and provide natural protection against bacterial and malarial infections.
- Neem leaf capsules may be taken daily as a preventive measure or used in conjunction with other antimalarial or antibacterial treatments.



Fig.no.13 Neem Leaf capsule

### 4. Neem Leaf Paste

- Neem leaf paste is prepared by grinding fresh Neem leaves into a fine paste using water or another suitable liquid as a binder.
- This paste can be applied topically to the skin to treat bacterial skin infections such as acne, boils, and wounds.
- Neem leaf paste may also be applied to mosquito bites and other insect bites to alleviate itching and inflammation.



Fig.no.14 Neem Leaf Paste

### 5. Neem Leaf Extract Mouthwash

- Neem leaf extract mouthwash is formulated with Neem leaf extracts, along with other natural ingredients such as mint and clove, to provide antibacterial and antimalarial benefits for oral hygiene.
- Rinsing with Neem leaf extract mouthwash may help reduce oral bacteria and prevent dental caries and gum disease.
- Neem leaf extract mouthwash can be used as part of a daily oral care routine or as needed for oral infections or inflammation.



Fig.no.15 Neem Leaf Extract Mouthwash

These Neem leaves-based products offer natural and effective alternatives for combating bacterial and malarial infections, providing both preventive and therapeutic benefits. However, it's essential to consult with a healthcare professional before using these products, especially if you have any underlying health conditions or are taking medications.

## ◆ Clinical Studies and Evidence-Based Medicine

### 1. Antibacterial Efficacy

Clinical studies have demonstrated the antibacterial efficacy of Neem leaf extracts in the treatment of bacterial infections, such as acne vulgaris, bacterial vaginosis, and periodontal disease. These studies have shown comparable or superior efficacy to conventional antibiotics, with fewer side effects and lower risk of antibiotic resistance.

### 2. Antifungal Activity

Clinical trials have evaluated the antifungal activity of Neem leaf extracts in the treatment of fungal infections, including candidiasis, dermatophytosis, and onychomycosis. Neem-based formulations have shown promising results in reducing fungal colonization and improving clinical outcomes.

### 3. Antiviral Effects

Preliminary clinical evidence suggests that Neem leaf extracts may have antiviral activity

against herpes simplex virus (HSV), human papillomavirus (HPV), and influenza virus. Further research is needed to elucidate the efficacy and safety of Neem-based interventions for viral infections.

#### 4. Anti-inflammatory Properties

Neem leaf extracts exhibit anti-inflammatory effects by inhibiting pro-inflammatory mediators such as cytokines, prostaglandins, and leukotrienes. Clinical studies have investigated the use of Neem-based formulations in inflammatory skin disorders, allergic rhinitis, and inflammatory bowel disease.

### ➤ Therapeutic Potential in Treating Infectious Diseases, Skin Disorders, and Oral Health Problems

#### 1. Infectious Diseases

Neem leaf extracts have broad-spectrum antimicrobial activity against bacteria, fungi, viruses, and protozoa, making them effective in the treatment of various infectious diseases. These include respiratory infections, urinary tract infections, gastrointestinal infections, sexually transmitted infections, and vector-borne diseases such as malaria and dengue fever.

#### 2. Skin Disorders

Neem leaf extracts are beneficial in the management of numerous skin disorders, including acne, eczema, psoriasis, dermatitis, scabies, and fungal infections. Their antibacterial, antifungal, and anti-inflammatory properties help alleviate symptoms, reduce inflammation, and promote skin healing.

#### 3. Oral Health Problems

Neem leaf extracts are used in oral healthcare to maintain oral hygiene, prevent dental caries, and treat oral infections. They inhibit the growth of oral pathogens such as *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Candida albicans*, thereby reducing the risk of dental decay, periodontal disease, and oral candidiasis.[12,14]

### Applications in Agriculture and Pest Management

#### ➤ Neem-based Pesticides and Insecticides

##### 1. Botanical Insecticides

Neem-based pesticides and insecticides are derived from Neem seed extracts, particularly neem oil and neem cake. These formulations contain bioactive compounds such as azadirachtin,

salannin, and nimbin, which exhibit insecticidal properties against a wide range of agricultural pests.

##### 2. Mode of Action

Neem-based pesticides disrupt the growth and development of insect pests by interfering with their feeding, molting, reproduction, and metamorphosis. Azadirachtin, the primary active ingredient, acts as a feeding deterrent, growth regulator, and oviposition deterrent, leading to reduced pest populations and crop damage.

##### 3. Target Pests

Neem-based pesticides are effective against a variety of agricultural pests, including aphids, caterpillars, beetles, mites, thrips, whiteflies, and leafhoppers. They are particularly useful in controlling pests that have developed resistance to synthetic chemical pesticides

#### ➤ Biofertilizers and Soil Amendments

##### 1. Neem Cake

Neem cake, a by-product obtained after extracting oil from Neem seeds, is used as a biofertilizer and soil conditioner. It contains residual nutrients such as nitrogen, phosphorus, potassium, and micronutrients, along with bioactive compounds that benefit soil health and plant growth.

##### 2. Nutrient Cycling

Neem cake enhances soil fertility and nutrient cycling by providing organic matter, improving soil structure, and promoting microbial activity. It helps maintain soil moisture, reduce soil erosion, and increase nutrient availability to plants, resulting in improved crop yields and quality.

##### 3. Pest and Disease Suppression

Neem cake has been shown to suppress soil-borne pathogens, nematodes, and weed seeds, reducing the incidence of soil-borne diseases and weeds. Its allelopathic effects inhibit the germination and growth of weed seeds, contributing to weed control in agricultural fields.[13]

#### ➤ Eco-friendly Alternatives for Crop Protection and Pest Control

##### 1. Integrated Pest Management (IPM)

Neem-based products play a key role in integrated pest management (IPM) strategies, which emphasize the use of multiple tactics to manage pests while minimizing environmental

impact. Neem-based pesticides are compatible with biological control agents, cultural practices, and resistant crop varieties, offering sustainable solutions for pest management.

### 2. Reduced Chemical Dependency

Neem-based pesticides provide eco-friendly alternatives to synthetic chemical pesticides, reducing the reliance on conventional pest control methods that may have adverse effects on human health, beneficial organisms, and the environment. They offer safer alternatives for farmers, consumers, and ecosystems.

### 3. Organic Agriculture

Neem-based products are widely used in organic agriculture and sustainable farming systems due to their natural origin, low toxicity, and minimal environmental impact. They help farmers comply with organic certification standards and meet consumer demand for pesticide-free produce.

## Challenges and Limitations

### ➤ Issues Related to Standardization and Quality Control

#### 1. Variability in Composition

Neem leaf extracts contain a complex mixture of bioactive compounds whose composition can vary depending on factors such as plant genetics, growing conditions, and extraction methods. Standardizing Neem-based products to ensure consistent quality and efficacy poses a challenge for manufacturers and regulators.

#### 2. Analytical Techniques

Analyzing the phytochemical composition of Neem extracts requires sophisticated analytical techniques such as HPLC, GC-MS, and LC-MS. However, the availability of standardized reference materials and validated analytical methods for quantifying specific compounds remains limited, hindering accurate quality control and regulatory compliance.

#### 3. Authentication and Adulteration

Authenticating Neem-based products and detecting potential adulterants or contaminants is essential for ensuring product safety and efficacy. However, the lack of reliable authentication methods and quality control standards increases the risk of product adulteration, substitution, or contamination.

### ➤ Safety Concerns and Potential Adverse Effects

#### 1. Toxicity Risk

While Neem leaf extracts are generally considered safe for human and environmental health, certain bioactive compounds such as azadirachtin and limonoids may exhibit toxicity at high concentrations. Accidental ingestion or improper use of Neem-based products can lead to adverse effects such as nausea, vomiting, diarrhea, and liver damage.

#### 2. Allergic Reactions

Individuals with allergies to Neem or related plants may experience allergic reactions, dermatitis, or respiratory symptoms upon exposure to Neem leaf extracts. Sensitivity to Neem-based products should be considered, especially in topical applications or agricultural settings where direct contact is likely.

#### 3. Ecotoxicity

While Neem-based pesticides are considered environmentally friendly alternatives to synthetic chemical pesticides, excessive or indiscriminate use may have unintended ecological consequences. Non-target organisms, including beneficial insects, pollinators, and aquatic organisms, may be adversely affected by Neem residues in the environment.

### ➤ Regulatory Hurdles and Commercialization Challenges

#### 1. Regulatory Approval

Obtaining regulatory approval for Neem-based products can be challenging due to the lack of standardized guidelines and regulatory frameworks governing their registration and marketing. Regulatory agencies may require extensive safety and efficacy data to assess product safety and compliance with existing regulations.

#### 2. Market Acceptance

Despite the potential benefits of Neem-based products, market acceptance and consumer awareness remain key challenges. Limited awareness of Neem's therapeutic properties, perceived efficacy compared to conventional treatments, and pricing considerations may affect market uptake and commercialization efforts.

#### 3. Intellectual Property Rights

Protecting intellectual property rights associated with Neem-based innovations,

formulations, and technologies presents challenges due to the widespread traditional knowledge and prior art surrounding Neem's medicinal and agricultural uses. Securing patents, trademarks, or other forms of intellectual property protection may require navigating complex legal and cultural landscapes.

### Future Directions and Research Opportunities

#### ➤ Emerging Trends in Neem Leaf Research

##### 1. Biotechnological Approaches

Advances in biotechnology, including genetic engineering and bioprospecting, offer opportunities to enhance Neem leaf extracts' efficacy, specificity, and safety. Biotechnological interventions can facilitate the production of novel Neem-based formulations with improved therapeutic and agricultural properties.

##### 2. Omics Technologies

Integration of omics technologies such as genomics, transcriptomics, proteomics, and metabolomics can provide comprehensive insights into Neem's molecular mechanisms of action, phytochemical composition, and biological activities. High-throughput omics approaches enable systematic exploration of Neem's therapeutic potential and identification of novel bioactive compounds.

##### 3. Nanoformulations

Nanotechnology-based approaches, such as nanoemulsions, nanoparticles, and nanosuspensions, offer innovative strategies for enhancing Neem leaf extracts' bioavailability, stability, and targeting efficiency. Nanoformulations can improve Neem-based product delivery systems for pharmaceutical, agricultural, and cosmetic applications.

#### ➤ Innovative Approaches for Enhancing Efficacy and Bioavailability

##### 1. Synergistic Formulations

Combining Neem leaf extracts with other natural products, phytochemicals, or synthetic compounds can enhance synergistic effects and therapeutic outcomes. Formulating Neem-based combinations with complementary mechanisms of action may improve efficacy against multidrug-resistant pathogens and agricultural pests.

##### 2. Drug Delivery Systems

Designing novel drug delivery systems, such as liposomes, microspheres, and hydrogels, can improve Neem leaf extracts' solubility, stability,

and release kinetics. Targeted delivery systems enable controlled release and site-specific accumulation of bioactive compounds, minimizing systemic side effects and maximizing therapeutic efficacy.

##### 3. Biocompatible Carriers

Utilizing biocompatible carriers, such as nanoparticles, liposomes, and cyclodextrins, can enhance Neem leaf extracts' cellular uptake, intracellular delivery, and pharmacokinetic profiles. Carrier-mediated delivery systems protect bioactive compounds from degradation and facilitate their transport across biological barriers.

#### ➤ Collaborative Efforts in Interdisciplinary Research and Development

##### 1. Academic-Industry Partnerships

Collaboration between academic institutions and industry stakeholders fosters translational research, technology transfer, and commercialization of Neem-based innovations. Academic expertise in basic science and clinical research complements industry capabilities in product development, manufacturing, and marketing.

##### 2. Global Collaborations

International collaborations promote knowledge exchange, capacity building, and cross-cultural understanding in Neem leaf research and development. Collaborative initiatives involving researchers, policymakers, and community stakeholders facilitate the adoption of Neem-based interventions for global health, agriculture, and environmental sustainability.

##### 3. Interdisciplinary Consortia

Interdisciplinary consortia bring together experts from diverse disciplines, including biology, chemistry, pharmacology, agriculture, and engineering, to address complex challenges in Neem leaf research.

## II. CONCLUSION

In conclusion, the exploration of the phytochemical composition and antibacterial/antimicrobial properties of *Azadirachta indica* (Neem) leaves represents a significant endeavor with multifaceted implications for various fields. Through this project, we have delved into the rich diversity of bioactive compounds present in Neem leaves, including flavonoids, alkaloids, tannins, phenolics, terpenoids,

and triterpenoids. These compounds contribute to the remarkable therapeutic and agricultural potential of Neem, offering a natural arsenal against microbial pathogens.

By investigating the mechanisms underlying the antibacterial and antimicrobial effects of Neem leaf extracts, we have gained valuable insights into their mode of action, including the disruption of cell membranes, inhibition of enzymatic activity, induction of oxidative stress, and modulation of signal transduction pathways. These findings not only deepen our understanding of Neem's pharmacological properties but also pave the way for the development of novel therapeutic interventions and agricultural solutions.

The implications of this research extend far beyond the laboratory, with profound implications for public health, agriculture, and environmental sustainability. Neem-based products offer promising avenues for the treatment and prevention of infectious diseases, skin disorders, and oral health problems, while also providing eco-friendly alternatives for pest management, soil fertility enhancement, and crop protection. By embracing Neem's natural virtues, we can foster a healthier, more resilient world while preserving the delicate balance of our ecosystems.

As we conclude this project, it is evident that the exploration of Neem's phytochemical composition and antibacterial/antimicrobial properties is not merely an academic pursuit but a journey towards transformative change. By continuing to unravel the mysteries of this remarkable plant and harnessing its potential for the betterment of humanity and the planet, we can forge a path towards a greener, more sustainable future. In doing so, we honor the legacy of Neem as a beacon of hope and healing, inspiring generations to come.

#### ➤ Summary of Key Findings and Insights

- Neem leaves contain a diverse array of phytochemicals, including flavonoids, alkaloids, tannins, phenolics, terpenoids, and triterpenoids, which contribute to their medicinal and agricultural properties.

- Neem leaf extracts exhibit potent antibacterial, antifungal, antiviral, and antiprotozoal activities, making them effective against a wide range of microbial pathogens

- The mechanisms of action underlying the antibacterial and antimicrobial effects of Neem leaf extracts involve disruption of cell membranes, inhibition of enzymatic activity, induction of

oxidative stress, and modulation of signal transduction pathways.

- Neem-based products, including pharmaceutical formulations, pesticides, biofertilizers, and oral health products, offer sustainable solutions for human health, agriculture, and environmental sustainability.<sup>284</sup>

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