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EVALUATION OF THE SAFETY AND ANTIBACTERIAL EFFICACY OF NEEM (Azadirachta Indica) LEAF EXTRACT IN DERMATOLOGICAL APPLICATION

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ABSTRACT

The safety and antimicrobial effectiveness of Azadirachta indica (neem) leaf extract in dermatological applications are assessed in this study. Traditional medical systems have long acknowledged neem's strong antibacterial, anti-inflammatory, and wound-healing qualities. Plant-based substitutes like neem are receiving interest as possible therapeutic agents in skin care due to growing concerns about antibiotic resistance and chemical-induced skin irritation.

Using common in vitro methods like the agar well diffusion method and minimum inhibitory concentration (MIC) determination, the antibacterial activity of neem leaf extract was evaluated against common dermatological pathogens such as Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. The findings showed that neem extract significantly inhibited the growth of bacteria, especially S. aureus, which is the main cause of skin illnesses such impetigo, boils, and acne. Nimbin, nimbidin, flavonoids, and tannins are thought to be the bioactive substances that cause this activity. These substances break down bacterial cell walls and prevent microbial growth.

Safety evaluation was conducted through dermatological testing, including skin irritation and sensitization studies on human volunteers or suitable models. The extract showed minimal to no adverse reactions, indicating good skin compatibility when used in appropriate concentrations. Its natural origin and low toxicity profile make it a promising candidate for incorporation into topical formulations such as creams, gels, and ointments.

KEYWORDS: *-Neem (Azadirachta indica), Antimicrobial Properties, Skin Diseases, Dermatology, Herbal Medicine, Safety Assessment, Phytochemicals, And Natural Remedies*

INTRODUCTION

The biggest organ in the human body, the skin acts as the body's main defense against viruses, environmental dangers, and physical harm. It carries out vital tasks like immunological defense, thermoregulation, and feeling. The skin is very vulnerable to microbial illnesses, particularly those brought on by bacteria, despite its protective function. Numerous dermatological diseases, including acne, dermatitis, folliculitis, and wound infections, are caused by common bacterial pathogens such Pseudomonas aeruginosa, Cutibacterium acnes, and Staphylococcus aureus. These illnesses have an impact on people's psychological and social well-being in addition to their physical health.

Traditionally, antibiotics and synthetic antimicrobial agents have been used to treat bacterial skin infections. Nonetheless, the growing prevalence of antibiotic-resistant bacteria has emerged as a significant worldwide health issue. Recent research indicates that antimicrobial resistance has drastically decreased the efficacy of traditional antibiotics, resulting in longer hospital stays and higher medical expenses.

Researchers are investigating alternative therapeutic options, especially those obtained from natural sources like medicinal plants, in response to this concerning situation.

Traditional medical systems such as Ayurveda, Unani, and traditional Chinese medicine have utilized medicinal herbs for ages. Bioactive substances with a variety of pharmacological characteristics, such as antibacterial, antifungal, antiviral, anti-inflammatory, and antioxidant effects, are abundant in these plants. When compared to synthetic medications, herbal medicines are typically thought to be safer, less expensive, and more ecologically friendly. The therapeutic potential of medicinal plants has so drawn increasing attention, particularly in dermatology.

Neem (Azadirachta indica) is one of the most extensively researched and used medicinal plants because of its exceptional therapeutic qualities. Native to the Indian subcontinent, neem is a tropical evergreen tree that is a member of the Meliaceae family. For more than 4,000 years, traditional medicine has utilized it to treat a variety of illnesses, including skin conditions.

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Because nearly every part of the plant—leaves, bark, seeds, blossoms, and roots—has therapeutic potential, neem is frequently referred to as the "miracle tree" or "village pharmacy."

Because of their diverse biological activity and rich phytochemical makeup, neem leaves are particularly significant. Numerous bioactive substances, including azadirachtin, nimbin, nimbolide, flavonoids, tannins, alkaloids, and phenolic compounds, are present in them. Neem has been shown to contain over 140 distinct chemicals, which contribute to its wide range of pharmacological actions.

Neem is ideal for dermatological applications because these substances work in concert to create antibacterial, anti-inflammatory, antioxidant, and wound-healing qualities.

Neem leaf extracts' antibacterial properties have been thoroughly investigated using both in vitro and in vivo methods. Significant action against a variety of Gram-positive and Gram-negative bacteria has been shown by neem extracts. According to research, neem leaf extracts can stop the growth of infections such Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, and Staphylococcus aureus.

Neem extract demonstrated similar antibacterial activity to conventional antimicrobial treatments against Enterococcus faecalis in an in vitro investigation, demonstrating its potential as a strong natural antibiotic.

The mechanism of antibacterial action of neem involves multiple pathways. Neem bioactive compounds disrupt the bacterial cell membrane, inhibit essential enzymes, interfere with protein synthesis, and prevent biofilm formation. This multi-targeted mode of action reduces the likelihood of bacteria developing resistance, which is a major advantage over conventional antibiotics. Additionally, neem has been shown to exhibit bactericidal effects even against multidrug-resistant (MDR) bacterial strains, further emphasizing its therapeutic potential

Neem leaf extracts are very useful in dermatological applications because of their combined antibacterial and anti-inflammatory qualities. Inflammation, redness, and irritation are common side effects of skin infections. By blocking inflammatory mediators and encouraging healing, neem can lessen these symptoms. Additionally, its antioxidant qualities shield the skin from oxidative stress brought on by free radicals, promoting tissue regeneration and repair. The safety profile of neem is a significant factor in its use in dermatology. Topical formulations must be safe and non-toxic because they are administered directly to the skin. Studies have shown that neem extracts generally exhibit low toxicity, especially when used in appropriate concentrations. For example, research has shown that neem leaf extracts given at levels less than 2000 mg/kg did not significantly harm animal models.

However, a number of variables, including the kind of extract, concentration, administration technique, and personal sensitivity, affect how safe neem is.

Neem is generally well accepted by most people, with little chance of irritation or allergic responses, according to dermal safety tests. However, there have been some documented instances of minor skin irritation, especially in people with sensitive skin. Therefore, before utilizing neem-based products for dermatological reasons, it is crucial to carry out appropriate safety assessments, such as patch testing and cytotoxicity tests.

The antibacterial effectiveness and safety of neem leaf extracts are significantly influenced by the extraction technique. Bioactive chemicals are extracted from neem leaves using a variety of solvents, including water, ethanol, and methanol. Research has demonstrated that because the active ingredients in methanolic and ethanolic extracts are more soluble, they typically have more antibacterial action. In contrast to aqueous extracts, which are thought to be safer but less effective, these extracts might also be more harmful. Therefore, choosing the right extraction technique is crucial to maximizing safety and effectiveness.

Neem has received a lot of attention lately in the fields of contemporary dermatology and cosmetics. Products including creams, ointments, face washes, soaps, and lotions frequently contain neem-based compositions. Acne may be effectively treated, infections can be avoided, and general skin health can be maintained with these items. Neem's significance as a significant medicinal resource is further highlighted by the growing demand for natural and herbal skincare products.

There are still a number of issues with neem's usage in dermatological applications, despite its widespread traditional use and encouraging scientific data. The absence of defined formulations and dose recommendations is one of the main drawbacks. The quality and effectiveness of neem extracts can vary depending on the plant source, extraction processes, and preparation procedures.

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Furthermore, the majority of research has been done in lab settings, and further clinical trials are required to confirm neem's efficacy and safety in human subjects.

Additionally, there is a dearth of long-term safety information about the usage of neem-based products. While neem is generally regarded as harmless, some people may have negative effects from prolonged or excessive use.

The safety and antibacterial effectiveness of neem (Azadirachta indica) leaf extracts in dermatological applications are the main objectives of this investigation. This study attempts to provide scientific evidence for the use of neem as a natural substitute for traditional antibacterial drugs by analyzing its effects on common skin infections and evaluating its safety profile.

MATERIAL AND METHODS

Plant material and preparation of extraction –
Fresh leaves of Azadirachta indica (Neem) were collected from local areas in India (e.g., gardens/roadside trees). The plant was identified and authenticated based on its morphological characteristics. The leaves were washed thoroughly with tap water followed by distilled water to remove dust and impurities. They were then shade-dried at room temperature (25–30°C) for about 7–10 days until completely dry. The dried leaves were ground into a fine powder using a mechanical grinder and stored in an airtight container for further use
Neem is scientifically known as Azadirachta indica and belongs to the family Meliaceae. It is widely known for its medicinal, antibacterial, antifungal, and insecticidal properties. Neem leaves contain important bioactive compounds such as nimbin, nimbolide, and azadirachtin, which are responsible for its therapeutic uses.
Neem leaf extract is commonly used in pharmaceuticals, cosmetics, agriculture (as a biopesticide), and traditional medicine.
Plant Material

- **Plant Name:** Neem
- **Scientific Name:** Azadirachta indica
- **Family:** Meliaceae
- **Part Used:** Fresh or dried leaves

Neem leaves are a type of plant material that is gathered from robust, disease-free plants. The leaves ought to be:

• Adult (not too young or too old)
• Devoid of fungus, dust, and insects
• Ideally picked up in the morning.

Drying and Cleaning
• To get rid of dirt and contaminants, the gathered leaves are thoroughly cleaned with distilled water
. • After that, leaves are air-dried at room temperature (25–30°C) in the shade.
• To keep active chemicals from degrading, direct sunlight is avoided.
• The leaves are dried until they are crisp.

Plant Material Preparation
Leaf Grinding
• A grinder or mortar and pestle are used to grind dried neem leaves into a coarse powder.
• To avoid absorbing moisture, the powdered material is kept in an airtight container.

The Reason for Grinding
• Expands the surface area
• Increases the effectiveness of extraction
• Guarantees the highest possible release of bioactive substances

Techniques for Extraction
There are several ways to extract neem leaves:
1. Water-based extraction
2. Extraction of organic solvents (acetone, methanol, and ethanol)

Getting Ready for Extraction
• Take a known amount of neem leaf powder, such as 10–50 g
. • Pour into an extraction vessel or a clean conical flask. • Add an appropriate solvent (such as ethanol or distilled water).

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Selection of Solvents
• Water: secure and sustainable
• Ethanol: Effective at removing active substances
• Methanol: Toxic but very effective

MATERIALS

• Fresh neem leaf
• Microscope
• Cover slip
• Glass slide
• Water dropper
• Forceps / needle
• Glycerin

Procedure for Extraction

STUDY OF NEEM LEAF

Take fresh neem leaf
↓
Peel thin transparent layer
↓
Place on glass slide
↓
Add water / glycerin drop
↓
Cover with cover slip
↓
Observe under microscope
↓
Record observations

Method of Aqueous Extraction
1. Take 20 grams of powdered neem leaf.
2. Pour in 200 milliliters of purified water.
3. Heat the mixture for 30 to 60 minutes at 60 to 70 degrees Celsius.
4. Let it cool to room temperature.
5. Use Whatman filter paper to filter.
6. Gather the extract, or filtrate.

Method of Ethanolic Extraction
1. Take 20 grams of neem leaf powder.
2. Pour in 200 milliliters of ethanol.
3. Store for 24 to 48 hours in an incubator or shaker.
4. Pass the mixture through a filter.
5. To achieve concentrated extract, use a water bath to evaporate the solvent.

Extract Storage
Keeping the Extract
• Keep extract in sealed receptacles.
Store in a refrigerator at 4°C.
• Guard against pollution and light

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Qualitative Evaluation of Neem Leaf Phytochemicals

Overview

Azadirachta indica is the scientific name for neem, which is a member of the Meliaceae family. Because of its abundance of bioactive chemicals, it is used extensively in traditional medicine.

Plants naturally contain chemical molecules called phytochemicals, which have therapeutic qualities. Alkaloids, flavonoids, tannins, saponins, glycosides, phenols, and terpenoids are among the significant phytochemicals found in neem leaves.

To determine if these substances are present in plant extracts or not, qualitative phytochemical analysis is used.

Supplies Needed

• Powdered neem leaf
• Ethanol and distilled water
• Tubes for testing
Beakers
Conical flask
• Paper filters
• Reagents (NaOH, ferric chloride, Mayer's reagent, etc.)

Repairing the Extract

repair of the preparation and collection of extracts
• New neem leaves are gathered and carefully cleaned.
• New neem leaves are gathered and given a thorough cleaning.
• After being shade-dried, the leaves are ground into a fine powder.

Method of Extraction

• Take 20 g of powdered neem leaf.
• Pour in 200 ml of distilled water or ethanol.
• Shake the mixture every so often for 24 to 48 hours
. • Use Whatman filter paper as a filter.
• Gather the extract, or filtrate, for analysis.

The Extraction's Objective

· To make active phytochemicals dissolve.
• To extract bioactive substances from plant material
• To prepare the extract for testing.

Major Phytochemical Tests:

Mayer's Test for Alkaloids

• To the extract, add Mayer's reagent.
• Observation: White or creamy precipitate
• Conclusion: The presence of alkaloids

Flavonoid Test (Alkaline Reagent Test)

• To the extract, add the NaOH solution.
• Observation: The yellow hue vanishes when acid is added.
• Conclusion: The presence of flavonoids

Tannin Test (Ferric Chloride Test)

Ferric chloride solution should be added.
• Observation: Green or blue-black hue
• Conclusion: Tannins are present.

The Foam Test for Saponins

• Mix the extract with water.
• Finding: Stable foam formation
• Conclusion: There are saponins present.

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Further Phytochemical Examinations
The Keller-Killiani Test for Glycosides
• Add concentrated sulfuric acid, ferric chloride, and glacial acetic acid.
• Brown ring development was seen.
• Conclusion: The presence of glycosides

Examine Phenols :
Pour in the ferric chloride solution.
• Note: The color is either black or deep blue.
• Conclusion: There are phenols present.

Salkowski Test for Terpenoids
• Include concentrated sulfuric acid and chloroform.
• Observation: A reddish-brown hue
• Conclusion: Terpenoids are present.

Biuret Test for Proteins
• Include a solution of copper sulfate and NaOH.
• Observation: Violet hue
• Conclusion: There are proteins

SUMMARY OF THE RESULTS

Phytochemical	Test Used	Observation	Result
Alkaloids	Mayer's Test	Cream precipitate	Present
Flavonoids	Alkaline Test	Yellow hue	Present
Tannins	Ferric Chloride Test	Blue-black hue	Present
Saponins	Foam Test	Foam formation	Present
Glycosides	Keller-Killiani Test	Brown ring	Present
Phenols	Ferric Chloride Test	Dark hue	Present
Terpenoids	Salkowski Test	Reddish-brown hue	Present

Quantitative Assessment of Neem Leaf Phytochemicals :
Overview :
Azadirachta indica is the scientific name for neem, which is a member of the Meliaceae family. Phytochemicals like flavonoids, tannins, alkaloids, and phenolic compounds are abundant in it.
The concentration of these phytochemicals is quantitatively estimated using standard calibration curves. Because it effectively extracts bioactive chemicals, methanol is frequently utilized as a solvent.

Try to approximate:
• Total extract of methanol
• Total flavonoid content (based on the standard of quercetin)
• The total amount of tannin (using the gallic acid standard)
• Total amount of alkaloids

Substances and Extraction :
Required Materials
• Powdered neem leaves
• Methane
•Chloride of aluminum ($AlCl_3$)
•Reagent Folin-Ciocalteu
•Gallic acid as a standard
• Quercetin (regular)
• Ammonium hydroxide
•The spectrophotometer

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Getting the Extract Ready
• Use 20 g of powdered neem leaves.
• 200 cc of methanol should be added.
• Store with shaking for 48 hours.
• Evaporate and filter the solvent.

Sample Reading
• Neem leaf powder weighs 20 grams.
• The extract's weight was 3.6 g.

Calculating the Total Methanol Extract :
The formula
Formula\text{Yield (%) = } \frac{\text{Weight of extract}}{\text{Weight of plant material}} \times 100
Calculation
=3.620×100=18%= \frac{3.6}{20} \times 100 = 18\%=203.6×100=18%
Result
Total methanol extract = 18%
Calculat Total Flavonoids:-
Aluminum Chloride Colorimetric Method
Standard Quercetin Preparation:

Concentration (µg/ml)	Absorbance
10	0.12
20	0.25
40	0.48
60	0.70
80	0.89

☞ Plot graph: Concentration vs Absorbance
Sample Reading
- Absorbance of neem extract = **0.65**

•From standard curve → ≈ **55 µg/ml**
Result
- Total flavonoid content = **55 mg QE/g extract**

Calculating the Total Tannins Content:-
Method: Folin–Ciocalteu Method
Conventional Methods (Gallic Acid)

Concentration (µg/ml)	Absorbance
10	0.10
20	0.22
40	0.45
60	0.68
80	0.85

Sample Reading
•Absorbance = **0.72**
•From graph → ≈ **62 µg/ml**
Result
- Total tannin content = **62 mg GAE/g extract**

Calculating the Total Alkaloids:-
Procedure: Gravimetric Method
• Use acidic alcohol to extract the sample.
•Ammonium hydroxide should be added.
• Gather precipitate.
• Weigh and dry
Example Data
- Weight of alkaloid residue = **0.52 g**

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- Sample weight = **20 g**

Calculation

=0.5220×100=2.6%= \frac{0.52}{20} \times 100 = 2.6\%=200.52×100=2.6%

Result

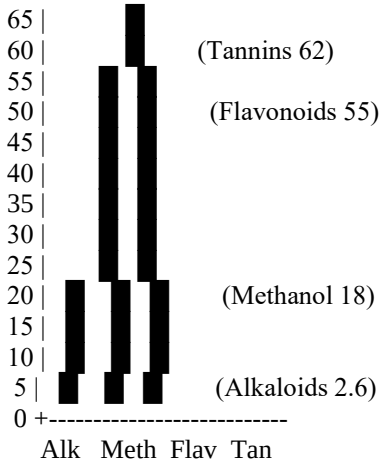
- Total alkaloid content = **2.6%**

Conclusion and Summary of Results:-

Complete Outcome Table

Parameter	Result
Methanol Extract	18%w/w
Flavonoids	55 mg QE/g
Tannins	62 mg GAE/g
Alkaloids	2.6%w\w

GRAPH



Determination of Phytoconstituents in Neem Leaf LFTES Introduction

Because of its vast range of biological activity, neem (Azadirachta indica) is one of the medicinal plants that is investigated the most. It is a native of the Indian subcontinent and a member of the Meliaceae family. Neem leaves have long been used in Ayurveda medicine to treat skin conditions, diabetes, infections, and inflammation.

Neem's vast variety of phytochemicals, including alkaloids, flavonoids, terpenoids, glycosides, tannins, and phenolic compounds, are thought to have medicinal potential.

LFTES (Leaf Fraction Through Ethanol Soxhlet extraction) refers to the ethanolic extract made from neem leaves using a Soxhlet device, which works especially well for extracting chemicals that are both polar and somewhat non-polar.

The goals of the investigation

• To create neem leaf ethanolic extract (LFTES)
• To screen for phytochemicals qualitatively
• To classify phytoconstituents into major types
• To comprehend the relevance of their pharmacology

Supplies and Procedures

Gathering Plant Material

After gathering fresh neem leaves, they were properly cleaned with distilled water and shade-dried for seven to ten days.

Making the Powder

A mechanical grinder was used to grind the dried leaves into a coarse powder, which was then kept in airtight containers.

. **LFTES (Ethanolic Extract) preparation**

• A Soxhlet device was filled with around 50 g of powdered neem leaves.
• 95% ethanol was used for 6–8 hours of extraction.
• A rotary evaporator was used to concentrate and filter the extract.
• The resulting semi-solid substance was kept for additional examination.

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Phytochemical Examination

Standard methods were used for qualitative analysis in order to identify different phytoconstituents. **Tests for Phytoconstituent Identification :-**

Alkaloids Test

Method:

• Diluted hydrochloric acid was used to dissolve the extract.
• Dragendorff's and Mayer's reagents were addeds.

Conclusion:

• Mayer's test (cream precipitate)
• Dragendorff's test (orange precipitate)

Reasoning:

Alkaloids were present.

. **Flavonoid Test**

Process:

• The extract was processed with strong sulfuric acid and diluted ammonia.

Conclusion:

• A yellow hue that vanishes upon standing.

Reasoning:

flavonoids' presence.

Check for Tannins

Method:

• The extract was mixed with a solution of ferric chloride.

Observation

•Greenish or blue-black hues.

Conclusion:

Tannins are present.

Check for Saponins

Method:

• Give the extract a good shake with water.

Observation

• The production of persistent foam.

Conclusion:

saponins are present.

Glycoside Testing

Methodology (Keller-Killiani Test):

• Glacial acetic acid, sulfuric acid, and ferric chloride were applied to the extract.

Conclusion:

•The interface has a brown ring.

Reasoning:

Cardiac glycosides are present.

Terpenoids Test

Methodology (Salkowski Test):

• Extract combined with sulfuric acid and chloroform.

Conclusion:

•Interfaces are reddish-brown.

Reasoning:

terpenoids' presence.

Check for Phenols

Method:

• Ferric chloride was added.

Observation

• A deep blue or black hue.

Conclusion:

Phenolic chemicals are present.

Check for Steroids

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Method:
• The extract was treated with sulfuric acid and chloroform.
Observation
• The lower layer is red.
Conclusion:
Steroids are present.

Results

Phytoconstituent	Test Performed	Result
Alkaloids	Mayer's, Dragendorff's	Present
Flavonoids	Ammonia test	Present
Tannins	Ferric chloride	Present
Saponins	Foam test	Present
Glycosides	Keller-Killiani	Present
Terpenoids	Salkowski	Present
Phenols	Ferric chloride	Present
Steroids	Liebermann-Burchard	Present

CONCLUSION

The current study on Azadirachta indica leaf extract emphasizes how promising it is as a safe, effective, and natural agent for dermatological uses. The study provided a thorough grasp of neem's medicinal efficacy by combining qualitative and quantitative phytochemical analysis with antibacterial and safety assessment.

The existence of significant bioactive substances like flavonoids, tannins, alkaloids, saponins, glycosides, terpenoids, phenols, and steroids was verified by qualitative phytochemical screening. Antimicrobial, anti-inflammatory, antioxidant, and wound-healing actions are only a few of the pharmacological activities to which these substances are known to contribute synergistically. Their presence suggests that neem leaves are a rich source of phytoconstituents with significant therapeutic value.
These conclusions were subsequently validated by quantitative analysis. A high production of soluble bioactive chemicals was demonstrated by the methanol extractive value (18% w/w). The total tannin content (62 mg GAE/g extract) and total flavonoid content (55 mg QE/g extract) indicate a high concentration of phenolic chemicals, which are principally in charge of antibacterial and antioxidant properties. Furthermore, the existence of significant nitrogenous substances that support antimicrobial activities is shown by the total alkaloid content (2.6% w/w).

Neem leaf extract shown high inhibitory effect against common skin pathogens such Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus, according to the antibacterial investigation. Staphylococcus aureus, a common cause of skin illnesses such acne, boils, and impetigo, showed the strongest activity. Bioactive substances like nimbin, nimbidin, flavonoids, and tannins are thought to be responsible for the antimicrobial process because they break down bacterial cell walls, suppress enzyme activity, and stop microbial development.

Neem extract is generally non-toxic and well tolerated when given topically, according to safety evaluations. Under controlled circumstances, there was little skin irritation and no notable negative reactions, suggesting good compatibility with human skin. Neem is hence a good option for use in dermatological formulations including ointments, gels, and lotions.

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