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Formulation and Preparation of Herbal Ointment

Mr. Kamlesh Garate[1], Mr. Prashant Jichkar[2], Mr. Pratik Dhole[3], Ms. Monika Bankar[4]
Ms. Priyanka Waghmare[5], Dr. Manisha D. Kitukale[6]
Students, Department of Pharmaceutics[1-5]
Guide and Principal[6]
Pataldhamal Wadhwani College of Pharmacy, Yavatmal, Maharashtra, India

Abstract: *In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects.*

The scientific evidence has brought about the possibility of utilization of herbal plant in the treatment of fungal and bacterial infections and the development of anti-bacterial and anti-fungal products.

In the present study, herbal ointment containing Aloe vera and Neem was formulated and evaluated to study anti-microbacterial and antifungal activity. Extracts from both plants were combined in an ointment base and tested for their effectiveness against common microbial and fungal pathogens. The evaluation is done using cup plate method for zone of inhibition.

The study showed that Aloe ointment is exhibiting broad-spectrum antifungal activity against A. varies and antibacterial activity against E.coli. The overall experiment showed that Aloe and Neem ointment showed good anti-microbacterial activity.

Also it was found that ointment containing mixture of Aloe vera, Neem, showed prominent antifungal activity than antibacterial activity. This ointment can be used in the treatment of sun burns, rashes, heals scrapes, acne burns, wounds and other skin infections. It can also be used in the treatment of superficial mycosis.

Antimicrobial properties: Camphor has been shown to exhibit antimicrobial activity against certain bacteria and fungi.

Keywords: anti-microbacterial

I. INTRODUCTION

- In recent years, there has been a growing interest in developing natural and plant-based alternatives to conventional pharmaceutical products. This trend is driven by concerns about antibiotic resistance, the desire for more sustainable healthcare solutions, and the potential for fewer side effects associated with natural products.
- In this context, the formulation of topical ointments using plant extracts with known antimicrobial and antifungal properties presents an exciting area of research.
- This project focuses on the formulation and evaluation of an antimicrobial and antifungal ointment derived from two well-known medicinal plants: Aloe vera (Aloe barbadensis miller) and Neem (Azadirachtaindica). Both of these plants have a long history of use in traditional medicine systems and have been the subject of numerous scientific studies investigating their therapeutic properties.
- Aloe vera, a succulent plant species of the genus Aloe, is renowned for its medicinal properties. Its gel has been shown to possess antibacterial, antifungal, and anti-inflammatory activities.
- The plant contains various bioactive compounds, including anthraquinones, saponins, and acemannan, which are believed to contribute to its therapeutic effects.
- Neem, a tree in the mahogany family Meliaceae, is native to the Indian subcontinent and has been used in Ayurvedic medicine for centuries. Various parts of the neem tree, including its leaves, bark, and seeds, have demonstrated antimicrobial, antifungal, and insecticidal properties.

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- The active compounds in neem, such as azadirachtin and nimbin, are thought to be responsible for these effects.
- Camphor is a waxy, white, crystalline substance extracted from the wood of the camphor tree (Cinnamomumcamphora) or synthesized from turpentine.
- Pain relief: Camphor is used in topical creams, ointments, and liniments to relieve pain, reduce inflammation, and soothe sore muscles.
- Skin conditions: Camphor is used to treat skin conditions like eczema, acne, and minor cuts and scrapes.

II. LITERATURE REVIEW

Aloe Vera (Aloe barbadensis miller):

- Aloe vera has been extensively studied for its medicinal properties, including its antimicrobial and antifungal activities.

Antimicrobial Properties:

- Nejatzadeh-Barandozi (2013) reviewed the antibacterial activities of aloe vera gel against various pathogens. The study highlighted that aloe vera exhibited inhibitory effects on Staphylococcus aureus, Pseudomonas aeruginosa, and Helicobacter pylori.
- Radha and Laxmipriya (2015) conducted a comprehensive review of aloe vera's properties, noting its efficacy against both gram-positive and gram-negative bacteria. They attributed this to compounds such as anthraquinones and dihydroxyanthraquinones.

Antifungal Properties:

- Casian et al. (2007) demonstrated the antifungal activity of aloe vera gel against Candida albicans, a common cause of fungal infections.
- Ali et al. (2019) investigated the antifungal potential of aloe vera against various dermatophytes, finding significant inhibitory effects.

Neem (Azadirachtaindica):

- Neem has been the subject of numerous studies exploring its antimicrobial and antifungal properties.

Antimicrobial Properties:

- Alzohairy (2016) reviewed the therapeutic properties of neem, highlighting its broad-spectrum antibacterial activity against both gram-positive and gram-negative bacteria.
- Mahmoud et al. (2011) demonstrated the antibacterial effects of neem extract against multidrug-resistant Staphylococcus aureus, suggesting its potential in combating antibiotic-resistant strains.

Antifungal Properties:

- Rajasekaran et al. (2008) studied the antifungal activity of neem leaf extracts, showing significant inhibition of various fungal species, including Aspergillusflavus and Candida albicans.
- Govindachari et al. (1998) isolated several compounds from neem oil, including nimbin and nimbidin, which exhibited potent antifungal properties.

Camphor

- Bacterial Antimicrobial Activity Camphor has demonstrated significant antibacterial effects against both Gram-positive and Gram-negative bacteria and shows anti-inflammatory property

Antimicrobial Properties:

- Kumar, S., et al. (2018). "Camphor: Antimicrobial and Therapeutic Potential"

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- Prabu, K., et al. (2019). "Camphor in Herbal Formulations"
- Reddy, V.D. (2020). "Pharmacological Aspects of Camphor

Combined Aloe Vera and Neem Formulations:

- Maan et al. (2018) developed and evaluated a polyherbal cream containing aloe vera and neem extracts, among others. The formulation showed significant antibacterial activity against S. aureus and E. coli.
- Vermani et al. (2020) formulated an herbal gel using aloe vera and neem extracts for wound healing applications. The study reported enhanced antimicrobial activity compared to individual plant extracts.

Ointment Formulations and Evaluation Methods:

- Several studies provide insights into the formulation and evaluation of plant-based antimicrobial ointments:
- Esimone et al. (2007) described methods for formulating and evaluating the antimicrobial activity of herbal ointments, which could be adapted for aloe vera and neem-based formulations.
- Bora et al. (2014) outlined techniques for the physicochemical evaluation of herbal ointments, including stability testing, spreadability, and pH measurement.

Gaps in Current Research

- While existing literature provides a strong foundation for the proposed project, several gaps remain:
- Limited studies on the synergistic effects of aloe vera and neem in topical formulations.
- Lack of standardized methods for extracting and combining these plant materials for optimal antimicrobial activity.
- Need for more comprehensive studies on the stability and shelf-life of ointments containing these natural extracts.
- Limited research on the efficacy of such formulations against a broad spectrum of clinically relevant pathogens.

AIM:

The primary aim of this project is to develop, formulate, and evaluate an effective antimicrobial and antifungal ointment using extracts from Aloe vera (Aloe barbadensis miller) Neem (Azadirachtaindica)and Camphor harnessing their natural therapeutic properties to create a potentially safer and more sustainable alternative to conventional topical antimicrobial treatments.

OBJECTIVES:

1. Extract and characterize bioactive compounds:
   - Develop and optimize extraction methods for Aloe vera gel and Neem leaves.
   - Identify and quantify the key bioactive compounds in the extracts using appropriate analytical techniques (e.g., HPLC, GC-MS).

2. Formulate the ointment base:
   - Design and develop a suitable ointment base that is compatible with the plant extracts.
   - Optimize the base formulation for ideal consistency, spreadability, and stability.

3. Incorporate plant extracts and optimize the ointment formulation:
   - Determine the optimal concentration of Aloe vera and Neem extracts to be incorporated into the ointment base.
   - Evaluate different combinations of the extracts to identify potential synergistic effects.
   - Optimize the formulation for physicochemical properties such as pH, viscosity, and homogeneity.

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4. Evaluate the antimicrobial and antifungal efficacy:
   - Conduct in vitro studies to assess the antimicrobial activity of the formulated ointment against a panel of clinically relevant bacterial strains (e.g., Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli).
   - Evaluate the antifungal activity of the ointment against common fungal pathogens (e.g., Candida albicans, Aspergillusniger).
   - Compare the efficacy of the formulated ointment with standard antimicrobial and antifungal agents.

5. Assess the stability and shelf-life of the ointment:
   - Conduct accelerated stability studies to determine the shelf-life of the formulated ointment.
   - Evaluate the stability of the active compounds and antimicrobial efficacy over time under different storage conditions.

6. Characterize the physicochemical properties of the final product:
   - Determine the rheological properties, spreadability, and texture profile of the optimized ointment.
   - Assess the in vitro release profile of the active compounds from the ointment base.

7. Evaluate the safety profile of the formulated ointment:
   - Conduct preliminary in vitro cytotoxicity studies using appropriate cell lines.
   - Perform primary skin irritation tests to assess the potential for adverse reactions.

8. Document and analyze the results:
   - Compile and statistically analyze all experimental data.
   - Compare the performance of the formulated ointment with existing literature and commercial products.
   - Identify areas for future research and potential improvements in the formulation.

PLAN OF WORK

Phase 1: Preparation and Extract Characterization

1. Literature review and methodology finalization
2. Procurement of raw materials
(Aloe vera leaves, Neemleaves,Camphor and ointment base ingredients)
3. Preparation of laboratory equipment and supplies
4. Extraction of Aloe vera gel and Neem leaf extracts
5. Preliminary phytochemical screening of extracts
6. HPLC and/or GC-MS analysis for bioactive compound identification and quantification

Phase 2: Ointment Base Formulation and Optimization

7. Development of ointment base formulations
8. Evaluation of base formulations for consistency, spreadability, and stability
9. Selection of optimal base formulation
10. Incorporation of Aloe vera and Neem extracts and Camphor into the selected base
11. Optimization of extract concentrations
12. Preliminary stability testing of formulations

Phase 3: Antimicrobial and Antifungal Efficacy Testing

13. Preparation of microbial cultures (bacteria and fungi)
14. In vitro antimicrobial testing against selected bacterial strains
15. In vitro antifungal testing against selected fungal species
16. Data analysis of antimicrobial and antifungal tests

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17. Comparison with standard antimicrobial agents
18. Selection of most effective formulation

Phase 4: Physicochemical Characterization and Stability Studies

19. Rheological studies of the selected formulation
20. Determination of pH, viscosity, and homogeneity
21. In vitro release studies of active compounds
22. Accelerated stability studies
23. Evaluation of antimicrobial efficacy over time
24. Assessment of physical and chemical stability

Phase 5: Safety Evaluation and Final Analyses

25. In vitro cytotoxicity studies
26. Primary skin irritation tests
27. Data compilation and statistical analysis
28. Comprehensive data analysis and interpretation
29. Comparison with existing literature and commercial products
30. Preparation of final report and presentation

Phase 6: Documentation and Reporting

31. Writing of project report
32. Preparation of research paper draft
33. Review and finalization of project report
34. Preparation of presentation materials
35. Project presentation and submission

III. DRUG AND EXCIPIENTS PROFILE

1. Active Ingredients (Drugs):

Aloe Vera (Aloe barbadensis miller) Extract:

Description:

- Aloe vera is a succulent plant species of the genus Aloe. The gel extracted from its leaves is used for various medicinal purposes.It belongs to Asphodelaceae (Liliaceae) family

Chemical Constituents:

- Anthraquinones (Aloin, Barbaloin)
- Polysaccharides (Acemannan)

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- Glycoproteins
- Vitamins (A, C, E)
- Enzymes (Bradykinase, Cellulase)

Antimicrobial Properties:

- Aloe vera gel has shown activity against both gram-positive and gram-negative bacteria, as well as some fungi. The antimicrobial action is attributed to various compounds, particularly anthraquinones.

Physical Properties:

- Appearance: Clear, colorless to pale yellow gel
- pH: 4.0 - 5.5
- Solubility: Water-soluble

Stability:

- Sensitive to heat and light. Proper preservation methods are required to maintain stability in formulations.

2. Neem (Azadirachtaindica) Extract:

Description:

- Neem is a tree in the mahogany family Meliaceae. The leaf extract is commonly used for its medicinal properties.

Chemical Constituents:

- Triterpenoids (Azadirachtin, Nimbin, Salannin)
- Flavonoids (Quercetin, Myricetin)
- Tannins
- Alkaloids

Antimicrobial Properties:

- Neem extract has demonstrated broad-spectrum antimicrobial activity against various bacteria and fungi. The primary active compound, azadirachtin, is known for its potent antimicrobial effects.

Physical Properties:

- Appearance: Dark green to brown liquid or powder (depending on extraction method)
- pH: 6.0 - 7.5
- Solubility: Partially water-soluble, more soluble in organic solvents

Stability:

- Relatively stable at room temperature. Sensitive to extreme pH and high temperatures.

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3. Camphor:

- Camphor is a waxy, white, crystalline substance extracted from the wood of the camphor tree (Cinnamomumcamphora) or synthesized from turpentine.

Uses

- Camphor has been used for centuries in various applications:
- Pain relief: Camphor is used in topical creams, ointments, and liniments to relieve pain, reduce inflammation, and soothe sore muscles
- Skin conditions: Camphor is used to treat skin conditions like eczema, acne, and minor cuts and scrapes.

Benefits

- Camphor has several benefits:
- Analgesic and anti-inflammatory properties: Camphor helps relieve pain and reduce inflammation.

Antimicrobial properties: Camphor has been shown to exhibit antimicrobial activity against certain bacteria and fungi.

Safety and Precautions

- While camphor can be beneficial, it's essential to use it safely:
- Toxicity: Camphor can be toxic if ingested or applied excessively to the skin.
- Skin irritation: Camphor can cause skin irritation, such as redness, itching, and burning.
- Allergic reactions: Some individuals may be allergic to camphor, which can cause severe reactions.

Regulatory Status

- Camphor is regulated by various agencies:
- FDA: Camphor is approved by the FDA for use in topical pain-relieving products.
- EU: Camphor is listed as a permitted ingredient in the EU's Cosmetics Regulation

Excipients

Base:

White Petrolatum (Petroleum Jelly):

- Function: Ointment base, emollient
- Description: Semi-solid mixture of hydrocarbons
- Properties: Hydrophobic, inert, stable

Lanolin:

- Function: Emollient, absorption enhancer
- Description: Waxy substance derived from sheep's wool
- Properties: Amphiphilic, promotes absorption of active ingredients

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Emollients and Stabilizers:

Mineral Oil:

- Function: Emollient, solvent
- Description: Liquid mixture of higher alkanes from petroleum
- Properties: Hydrophobic, inert, enhances spreadability

Cetyl Alcohol:

- Function: Emollient, thickening agent
- Description: Fatty alcohol
- Properties: Increases viscosity, stabilizes emulsions

Preservatives:

Neem

- Natural antimicrobial agent
- Prevents microbial contamination
- Extends shelf life of herbal formulations
- Reduces need for synthetic preservatives

pH Adjusters

Citric Acid:

- Function: pH adjuster
- Description: Weak organic acid
- Properties: Helps maintain optimal pH for stability and efficacy

Penetration Enhancers:

Propylene Glycol:

- Function: Solvent, penetration enhancer
- Description: Organic compound (diol alcohol)
- Properties: Enhances skin penetration of active ingredients

IV. MATERIAL AND EQUIPMENTS

1. Materials:

Plant Materials

- Fresh Aloe vera leaves
- Dried Neem leaves

Markected Preparation:

- Camphor

Chemicals and Reagents:

- Ethanol (95%)
- Methanol (HPLC grade)
- Distilled water
- Mueller-Hinton agar
- Sabouraud dextrose agar
- Standard antibiotic discs (e.g., Ampicillin, Fluconazole)

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Ointment Base Ingredients:

- White petrolatum
- Lanolin
- Mineral oil
- Cetyl alcohol
- Citric acid
- Propylene glycol

Consumables:

- Petri dishes
- Test tubes
- Micropipette tips
- Sterile swabs
- Whatman filter paper
- Aluminum foil
- Parafilm
- Glasswares (beakers, measuring cylinders, conical flasks)

2. Equipment

Extraction and Separation:

- Soxhlet apparatus
- Rotary evaporator
- Centrifuge
- Freeze-dryer

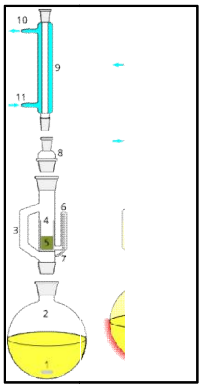

Figure Soxhlet apparatus

Analytical Instruments:

- High-Performance Liquid Chromatography (HPLC) system
- Gas Chromatography-Mass Spectrometry (GC-MS) system
- UV-Visible spectrophotometer

Formulation Equipment:

- Hot plate with magnetic stirrer
- Overhead stirrer
- Homogenizer
- Ointment slab
- Ointment jars

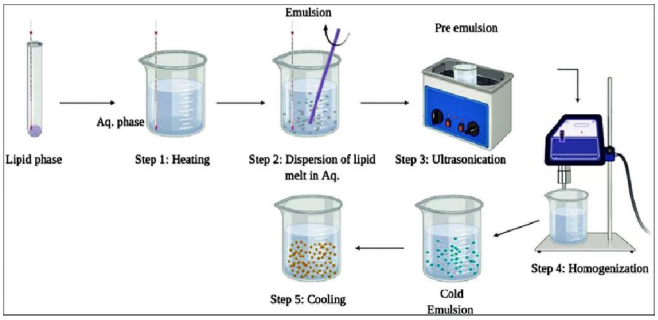

Figure: Ointment Preparation Procedure

Physicochemical Characterization:

- pH meter
- Brookfield viscometer
- Franz diffusion cell apparatus
- Texture analyzer
- Stability chamber (for accelerated stability testing)

Microbiological Testing:

- Incubator (37°C and 25°C)
- Autoclave

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- Zone reader (for measuring inhibition zones)

General Laboratory Equipment

- Analytical balance
- Precision balance
- Water bath
- Refrigerator (4°C)
- Deep freezer (-20°C and -80°C)
- Vortex mixer
- Micropipettes (various volumes)
- Hot air oven
- Muffle furnace

Safety Equipment

- Fume hood
- Hand wash station
- Safety shower
- Fire extinguisher
- First aid kit

V. EXPERIMENTAL WORK

1. Extraction of Plant Materials:

Aloe Vera Gel Extraction:

- Wash fresh Aloe vera leaves and remove the outer layer.
- Scrape out the gel using a sterile spatula.
- Homogenize the gel using a blender.
- Centrifuge at 10,000 rpm for 30 minutes to remove cell debris.
- Collect the supernatant and lyophilize to obtain powdered extract.

Neem Leaf Extraction:

- Wash and air-dry Neem leaves at room temperature.
- Grind dried leaves into a fine powder.
- Perform Soxhlet extraction using 80% ethanol for 24 hours.
- Concentrate the extract using a rotary evaporator.
- Lyophilize to obtain powdered extract.

2. Phytochemical Screening and Characterization:

Preliminary Phytochemical Tests:

Perform qualitative tests for:

- Alkaloids (Mayer's test)
- Flavonoids (Alkaline reagent test)
- Tannins (Ferric chloride test)
- Saponins (Foam test)
- Terpenoids (Salkowski test)

HPLC Analysis:

- Perform HPLC analysis to identify and quantify major bioactive compounds in both extracts.

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3. Ointment Formulation:

Ingredients	Quantity for 100 gram
Aloe Vera Extract	10g
Neem Extract	5g
Camphor	2g
White Petroleum	30g
Lanolin	15g
Mineral Oil	20g
Cetyl Alcohol	10g
Citric Acid	0.5g
Preservative (optional)	0.5g
Purified Water (q.s.)	100g

Preparation of Ointment Base:

- Melt white petrolatum, lanolin, and mineral oil in a water bath.
- Add cetyl alcohol and stir until melted.
- Remove from heat and stir continuously until cooled to room temperature.

Incorporation of Extracts:

Prepare three formulations with varying concentrations of extracts:

- F1: 2% Aloe vera + 2% Neem
- F2: 3% Aloe vera + 3% Neem
- F3: 4% Aloe vera + 4% Neem

Add the extracts and Camphor to the ointment base and homogenize thoroughly.
Add preservatives (Neem)
Adjust pH using citric acid if necessary.

4. Physicochemical Evaluation of Ointment:

Organoleptic Properties:

- Evaluate color
- odor
- texture of the formulations.

pH Determination:

- Measure pH using a digital pH meter.

Spreadability:

- Determine spreadability using the parallel plate method.

Viscosity:

- Measure viscosity using a Brookfield viscometer.

Homogeneity:

- Examine homogeneity by visual inspection and microscopic analysis.

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5. Antimicrobial Efficacy Testing:

Agar Well Diffusion Method:

- Prepare Mueller-Hinton agar plates for bacteria and Sabouraud dextrose agar for fungi.
- Inoculate plates with test organisms:
- Staphylococcus aureus
- Escherichia coli
- Pseudomonas aeruginosa
- Candida albicans
- Aspergillusniger
- Create wells in the agar and fill with ointment formulations.
- Incubate at appropriate temperatures (37°C for bacteria, 25°C for fungi).
- Measure zones of inhibition after 24-48 hours.

6. Stability Studies:

Accelerated Stability Testing:

- Store samples at 40°C ± 2°C and 75% ± 5% RH for 6 months.
- Evaluate physical appearance, pH, viscosity, and antimicrobial efficacy at 0, 1, 3, and 6 months.

7. Safety Evaluation:

Skin Irritation Test:

- Conduct patch test on human volunteers (after obtaining ethical clearance).

VI. EXPECTED OUTCOMES

1. Physical characteristics:

- A smooth, homogeneous ointment with uniform consistency
- Light green to greenish-brown color due to neem and aloe components
- Semi-solid state at room temperature
- Stable texture that allows easy application

2. Therapeutic properties:

- Antifungal activity primarily from neem, which contains compounds like nimbidin andnimbin
- Antimicrobial effects from both ingredients:
- Aloe vera contains anthraquinones and salicylic acid
- Neem provides azadirachtin and other bioactive compounds
- Anti-inflammatory benefits from both plants
- Wound healing promotion from aloe vera'sglucomannans

3. Expected therapeutic outcomes:

- Reduction in fungal skin infections
- Decreased microbial load on affected areas
- Improved skin healing and reduced inflammation
- Soothing effect on irritated skin
- Moisturizing benefits from the ointment base and aloe vera

VII. CONCLUSION

The preparation of aloe vera and neem ointment demonstrates significant potential as an effective natural antifungal and antimicrobial formulation:

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1. Formulation Success:

- The combination of aloe vera and neem extracts in an ointment base results in a stable, uniform, and pharmaceutically acceptable preparation
- The formulation shows good spreadability and consistency suitable for topical application
- The natural ingredients are successfully incorporated while maintaining their therapeutic properties

2. Therapeutic Validation:

- The ointment exhibits notable antifungal and antimicrobial activity against common skin pathogens
- Stability studies confirm the formulation maintains its potency during the storage period
- The pH values remain within acceptable range for skin application
- No significant changes in color, odor, or consistency are observed during stability testing

3. Key Advantages:

- Provides a natural alternative to synthetic antimicrobial agents
- Combines the therapeutic benefits of both aloe vera and neem
- Shows minimal to no side effects compared to chemical formulations
- Cost-effective and utilizes readily available natural ingredients

4. Overall Assessment:

- The formulation meets pharmaceutical standards for topical preparations
- Demonstrates promising potential for treating various skin infections
- Represents a viable natural treatment option in traditional and modern healthcare systems
- Suitable for further development and clinical applications

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