

# A Novel Approach to Antifungal Therapy : Formulation and Evaluation of Jamun, Jaifal, Neem Oil Combination

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**Abstract:** *Fungal infections, both superficial and systemic, have become a significant public health concern, especially with the rise of antibiotic-resistant strains and increased use of synthetic antifungal agents. Many of these agents come with side effects, including skin irritation and other toxicities, prompting the need for alternative and natural treatments. Essential oils derived from plants have shown promising antifungal properties due to their bioactive compounds, making them an attractive alternative.*

**Keywords:** Fungal infections

## I. INTRODUCTION

Fungal infections, both superficial and systemic, have become a significant public health concern, especially with the rise of antibiotic-resistant strains and increased use of synthetic antifungal agents. Many of these agents come with side effects, including skin irritation and other toxicities, prompting the need for alternative and natural treatments. Essential oils derived from plants have shown promising antifungal properties due to their bioactive compounds, making them an attractive alternative.

Among various plant oils, Neem oil (*Azadirachta indica*), Jamun oil (*Syzygium cumini*), and Jaifal oil (*Myristica fragrans*) have been traditionally used in various cultures for their medicinal properties. Neem oil is well-known for its antifungal, antibacterial, and anti-inflammatory properties. Jamun oil is recognized for its antioxidant and antimicrobial activities, while Jaifal oil has antifungal and antioxidant effects. Combining these oils may create a synergistic effect, enhancing their therapeutic efficacy, particularly in the treatment of fungal infections.

This combination aims to create a natural, effective, and safe alternative to synthetic antifungal treatments. The oils' combined properties can provide not only antifungal action but also help with skin care, promoting healing and reducing irritation, making them ideal for topical formulations.

Ideal Property for oil Combination

### Antifungal Efficacy

At the core of the ideal formulation lies its potent antifungal activity. The combination of Jamun, Jaifal, and Neem oils must be effective against a broad spectrum of fungal pathogens, such as dermatophytes (*Trichophyton rubrum*), yeasts (*Candida albicans*), and molds (*Aspergillus niger*). The oil must not only be effective at low concentrations but also demonstrate rapid action upon application. This means that the formulation should show visible effects within 24-48 hours of use, ideally achieving full fungal inhibition in a short period. Additionally, the Minimum Inhibitory Concentration (MIC) should be low, ensuring that the oil is potent even when diluted, making it cost-effective for users. A combination of these oils offers a synergistic effect, enhancing the overall antifungal potential.

### Physical and Sensory Properties

The physical properties of the oil blend are crucial for ensuring ease of use, stability, and consumer acceptance. The oil should possess the following characteristics:

- **Clarity and Homogeneity:** The oil must be clear and free from any visible particulates or sedimentation, ensuring a smooth, uniform consistency. Phase separation should be absent, confirming the blend's chemical stability.
- **Consistency and Viscosity:** The ideal oil should have a medium viscosity, allowing it to spread evenly and comfortably on the skin without being excessively thick or overly runny. The right balance will ensure easy application, whether using it on the face, body, or scalp.
- **Non-Greasy and Fast Absorption:** It is essential that the oil is non-greasy and does not leave an oily residue after application. The formulation should absorb quickly into the skin, leaving it soft, moisturized, and free from excess oil, which is crucial for consumers who seek non-disruptive skincare routines.
- **Mild Odor:** Given that all three oils—Neem, Jamun, and Jaifal—have distinct natural scents, the formulation should possess a pleasant and mild aroma. The oil should not have a pungent or overpowering smell, but rather a natural, herbal scent that is soothing to the user.

### Skin Compatibility and Safety

One of the most important properties of any topical product is its skin compatibility, and for an antifungal oil, this is particularly crucial. The formulation should be safe for all skin types, including sensitive skin. The following aspects are essential:

- **Hypoallergenic and Non-Irritating:** The oil should be tested to ensure that it does not cause any skin irritation, redness, or allergic reactions upon application. A patch test can ensure that the product is hypoallergenic and safe for repeated use on different skin types.
- **Non-Drying:** In addition to its antifungal properties, the oil should also have moisturizing effects, preventing the skin from becoming dry, flaky, or irritated due to the antifungal treatment. This is especially important since fungal infections often lead to skin dryness and damage.
- **Healing and Calming:** The formulation should have anti-inflammatory properties, which would aid in reducing any inflammation caused by the fungal infection. Additionally, the oil should help in wound healing, as fungal infections can cause skin lesions or sores.

### Stability and Shelf-Life

For a product to be commercially viable, it must demonstrate good stability over time. This includes:

- **Oxidative Stability:** The oil blend must be resistant to oxidation, which could lead to the degradation of its active ingredients and unpleasant changes in smell or appearance. The presence of antioxidants (such as Vitamin E) in the formulation can help prevent rancidity and prolong shelf-life.
- **Physical Stability:** The oil should maintain its viscosity, color, and homogeneity over time, even under different storage conditions. It should not undergo any phase separation or significant changes when stored at room temperature or higher temperatures. The ideal formulation should be stable for at least 6 months to 1 year.
- **No Contaminants:** The formulation must remain free from microbial contamination, ensuring that the oil is safe for use even after prolonged storage. Antimicrobial preservatives can be added, but they should not compromise the natural integrity of the oils.

### Absorption and Bioavailability

The ideal oil blend should offer effective absorption into the skin to deliver its antifungal agents at the site of infection. The oils should have high bioavailability, allowing their active ingredients (such as nimbin from Neem, myristicin from Jaifal, and anthocyanins from Jamun) to penetrate the skin and act on the fungal pathogens efficiently. This ensures that the oil can be effective even with a small amount applied to the skin.

### **Chemical Composition and Active Ingredients**

The chemical composition of the oil blend is fundamental to its efficacy as an antifungal treatment. The ideal oil should contain high concentrations of active compounds that have well-documented antifungal, antimicrobial, and anti-inflammatory effects. The primary components include:

- **Neem oil:** Rich in compounds like nimbin and azadirachtin, which are known for their potent antifungal, antibacterial, and anti-inflammatory properties.
- **Jamun oil:** Contains anthocyanins and flavonoids, known for their antioxidant, antibacterial, and antifungal properties.
- **Jaifal oil:** Contains myristicin, a compound that provides antifungal, anti-inflammatory, and antioxidant benefits. These components should be present in optimal concentrations to provide maximum antifungal effectiveness while maintaining the safety of the formulation for long-term use.

### **User-Friendly Application**

- **Proper Dispensing:** The oil should be packaged in a dark-colored bottle (to protect it from light) with a dropper or nozzle for precise dispensing, preventing wastage and ensuring users can apply the right amount.
- **Compact and Travel-Friendly:** The packaging should be sturdy, easy to carry, and appropriate for travel or daily use, ensuring users can take the product with them without concern for leaks or spills.
- **Cost-Effectiveness and Market Competitiveness**

For this combination oil to reach a wide market, it must also be affordable. The production cost should be optimized to ensure that the product is competitively priced compared to synthetic antifungal treatments. At the same time, it should provide consumers with a natural, safer alternative that offers equal or superior efficacy, making it an attractive option for health-conscious individuals.

The ideal antifungal oil preparation of Jamun, Jaifal, and Neem oil should have the following characteristics:

- Strong and broad-spectrum antifungal activity.
- Non-irritating and skin-compatible for long-term use.
- Stable with a reasonable shelf-life and resistance to oxidation.
- Easy to apply, pleasant-smelling, and non-greasy.
- Safe for skin with no adverse reactions.
- Chemical integrity and active ingredients to maintain effectiveness.
- Affordable and convenient for users.

### **Ideal Property in short**

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- Affordable and convenient for users.

## **II. AIM AND OBJECTIVE**

**Aim:-** To study will be to investigate the antifungal effects of a combination of Jamun seed oil, Jaifal oil, and Neem oil against a variety of fungal pathogens. The specific objectives are as follows:

**Objectives:**

- To evaluate the antifungal efficacy of the combination of Jamun seed oil, Jaifal oil, and Neem oil against common fungal species such as *Candida albicans*, *Aspergillus niger*, and dermatophytes in both in vitro and in vivo models
- To determine the minimum inhibitory concentration (MIC) of each oil individually and in combination to establish the most effective concentrations for antifungal action.
- To assess the synergistic effects of the oil combination by calculating the fractional inhibitory concentration index (FICI) to confirm whether the combination offers enhanced efficacy compared to individual oils.
- To investigate the mechanism of action of the oil combination through biochemical and molecular assays that evaluate the disruption of fungal cell walls, interference in cell membrane integrity, and modulation of immune response.
- To evaluate the safety profile of the oil combination through cytotoxicity tests on human cell lines and animal models to determine the potential for topical and systemic application without causing harmful side effects.
- To compare the efficacy of the oil combination with conventional antifungal agents such as fluconazole and itraconazole, focusing on their ability to inhibit fungal growth and prevent recurrence

**PLAN OF WORK**

**LITERATURE REVIEW**

**Khan, M. et al. (2010).**

"Neem (*Azadirachta indica*) oil: A potent natural product with multiple therapeutic benefits." *International Journal of Phytomedicine*, 2(1), 15-21. • This study highlights the antimicrobial and antifungal effects of Neem oil, particularly its efficacy against dermatophytes and other fungal pathogens.

**Sreenivasan, R. et al. (2018).**

"Antioxidant and antimicrobial properties of Jamun (*Syzygium cumini*) seeds and oil." *International Journal of Pharmacy and Pharmaceutical Sciences*, 10(12), 58-64. Explores the antioxidant and antifungal activities of Jamun seed extract and oil, emphasizing their potential in treating fungal infections.

**Ali, S. et al. (2005).**

"The antifungal activity of *Azadirachta indica* (Neem) extracts against dermatophytes and other human pathogenic fungi." *International Journal of Dermatology*, 44(11), 1010-1015.

Reviews the antifungal properties of Neem oil against various fungal pathogens, including dermatophytes, highlighting its therapeutic use in skin infections.

**Rahman, M. et al. (2009).**

"Antifungal activity of *Myristica fragrans* (Nutmeg) essential oil against *Candida* species." *Asian Pacific Journal of Tropical Medicine*, 2(3), 218-220.

This study evaluates the antifungal effects of Nutmeg oil, particularly its activity against *Candida albicans* and other fungal pathogens.

**Rathi, N. et al. (2014).**

"*Syzygium cumini* (Jamun): A review on phytochemistry and pharmacological aspects." *Journal of Pharmacognosy and Phytochemistry*, 3(3), 49-56.

A comprehensive review of Jamun's medicinal properties, including its antimicrobial and antifungal activities, which support its potential for treating fungal infections.

**Satyavati, G.V. et al. (1976).**

"Medicinal plants of India: Essential oils of spices." Indian Journal of Medical Research, 64(1), 42-51.

This article reviews the medicinal properties of various essential oils, including those from Myristica fragrains(Jaifal), and discusses their antifungal activities

**Sivapragasam, V. et al. (2015).**

"Antifungal and antimicrobial activities ofNeem oil (Azadirachta indica): A review." Asian Journal of Pharmaceutical and Clinical Research, 8(4), 58-61.

Reviews the broad-spectrum antimicrobial and antifungal activities of Neem oil, including its potential as a treatment for skin and fungal infections.

**Boreddy, S. R. et al. (2012).**

"Antioxidant and antimicrobial activities of Mylistica fragrans (Nutmeg) essential oil." Journal of Medicinal Plants Studies, 1(4), 50-56. • Evaluates the antioxidant and antimicrobial properties of Nutmeg essential oil, including its role in fighting fungal infections.

**Jalali, M. et al. (2011).**

"Synergistic antifungal activity of essential oils: A review." Journal of Medicinal Plants Research, 5(17), 4023-4031. • Discusses the synergistic effects of combining various essential oils, including Neem, Jamun, and Jaifal oils, in enhancing their antifungal properties

**DRUG PROFILE:-**

Neem Seeds Oil:-

Biological Source - Seeds of neem trees Azadirachta Indica

Family-Meliaceae

Chemical Con. - Azadirachtin, nimbin and nimbidin, gedunin, salanin, margosin acid, terpenoids, triterpenoids.

Uses:-

Disrupting cell membrane integrity Inhibiting spore germination Inhibiting fungal enzymes Inducing oxidative stress



Jaifal Seeds Oil:-

Biological Source - seeds of nutmeg tree myristica fragrans

Family- Myristicaceae

Chemical Con. - Myristicin, Eugenol, Safrole, Terpenoids, Linalool, Camphene, phenolic Compound, Fatty Acid.

Uses:-

Disrupting cell membrane integrity Inhibiting spore germination Inhibiting fungal enzymes Inducing oxidative stress



**Jamun Seeds Oil:-**

Biological Source - Seeds of *Syzygium cumini*

Family - Myrtaceae

Chemical Con. - Phenolic Acids, Terpenoids, Flavonoids Alkaloids, Fatty Acid, Saponins, Essential Oil (Volatile Comp.) Eg. Eugenol.

Uses-

Disrupts fungal cell wall and membrane integrity Inhibits spore germination and mycelial growth Generates oxidative stress within fungal cell Suppresses enzymatic activities



**III. MATERIAL AND METHODS**

Ingredients and Their Roles

Active Ingredients:

Jamun Oil (*Syzygium cumini*): Antioxidant, antimicrobial, and anti-inflammatory properties.

Jaifal Oil (*Myristica fragrans*): Contains eugenol and myristicin, which have antifungal and analgesic effects.

Neem Oil (*Azadirachta indica*): Broad-spectrum antifungal activity due to azadirachtin, nimbin, and other bioactive compounds.

Carrier Oil (Base):

Coconut Oil or Sweet Almond Oil: Used as a base oil to dilute the active oils and improve skin penetration.

Stabilizers and Preservatives:

Vitamin E (Tocopherol): Acts as an antioxidant to prevent rancidity of oils.

Natural Preservative: (Optional) A small amount of benzyl alcohol or grapefruit seed extract can be used.

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Essential Oil (Optional):

Tea Tree Oil or Lavender Oil: Added for additional antifungal and soothing properties.

Formulation Ratio

Jamun Oil: 20%

Jaifal Oil: 20%

Neem Oil: 30%

Carrier Oil: 30%

The ratios can be adjusted based on specific antifungal requirements or skin sensitivity.

Detailed Step-by-Step Preparation Process for Jamun, Jaifal, and Neem Oil Combination

This process ensures the proper formulation of a stable and effective oil blend for antifungal use, incorporating Jamun, Jaifal, and Neem oils with a carrier and stabilizers. Follow the steps below for precise preparation.

Preparation of Workspace and Equipment

Sanitization: Clean and sterilize all equipment, including beakers, glass rods, funnels, and bottles, using ethanol or isopropyl alcohol. Allow them to air-dry.

Environment: Perform the preparation in a clean, dry, and well-ventilated area, free from dust and moisture

**Materials and Proportions**

Ingredient	Proportion(%)	Role
Jamun Oil	20%	Antioxidant, Antimicrobial
Jaifal Oil	20%	Proper Antifungal and anti-inflammatory
Neem Oil	30%	Broad-Spectrum antifungal action
Carrier Oil (Eg. Coconut or Sweet Almond Oil)	30%	Improves Skin Penetration and dilution
Vitamin E	0.5-1%	Antioxidant to Stabilize the oil
Optional Essential Oil (Eg. Tea Tree Oil)	1%	Enhances antifungal efficacy fragrance

**Preparation Process**

**Step 1: Weigh The Ingredient**

Use a digital weighing scale to precisely measure each ingredient. For a 100 mL batch:

Jamun Oil: 20 mL

Jaifal Oil: 20 mL

Neem Oil: 30 mL

Carrier Oil: 30 mL

Vitamin E: 0.5-1 mL (approximately 10-20 drops).

Essential Oil (if used): 1 mL (approximately 20 drops)

**Step 2: Base Oil Preparation**

1. In a clean, dry glass beaker, pour the carrier oil (30 mL). This dilutes the concentrated active oils, reducing potential irritation and improving skin absorption. 2. Add Vitamin E (0.5-1 mL) to the carrier oil and stir gently with a glass rod until well mixed. Vitamin E stabilizes the formulation and prevents oxidation of the oils.

**Step 3: Add Active Oils**

Add Jamun Oil: Slowly pour the measured Jamun Oil (20 mL) into the carrier oil mixture while stirring continuously. Ensure the blend is uniform before adding the next oil.

Add Jaifal Oil: Add Jaifal Oil (20 mL) gradually, stining gently to incorporate it fully into the mixture  
Add Neem Oil: Add Neem Oil (30 mL) last, as it has the highest propmption. Stir the mixture thoroughly to achieve a homogenous blend.

**Step 4: Incorporate Optional Additives**

If desired, add 1% of an essential oil like Tea Tree Oil (known for additional antifungal properties) or Lavender Oil (for fragrance and cahning effects).  
Stir the mixture for 5-10 minutes using a magnetic stiner (or glass rod) to ensure unifonn distribution of all components.

**Step 5: Filtration (Optional)**

If any oils contain impurities or sediments, filter the blend using a fine muslin cloth or filter paper. This step ensures clarity and smoothness in the final product.

**Step 6: Packaging**

Select Packaging: Use amber or dark glass bottles to protect the oils from light, which can degrade their active components.  
Transfer: Use a clean funnel to pour the prepared oil blend into the bottles, filling each to about 90% capacity to leave room for expansion.  
Seal the Bottles: Tightly close the bottles with ailtight caps to prevent contamination or oxidation.

**Evaluation Method Of Jamun, Jaifal, and Neem Oil Combination**

The evaluation of this oil involves assessing its physical, chemical, biological, and functional properties to ensure its stability, safety, and efficacy as an antifungal agent.

**Physical Evaluation**

**Appearance**

Objective: Assess the homogeneity and clarity of the oil.

**Method:**

Pour a small amount of the oil into a transparent container.

Observe for color consistency, phase separation, or visible paiticles under n01 mal and bright light conditions.

Criteria: The oil should be clear, uniform, and free from sediments.

**Odor**

Objective: Evaluate the natural smell of the oil blend.

**Method:**

Smell the oil directly after opening the container.

Check for any signs of rancidity or unpleasant odors.

Criteria: The oil should have a mild, natural smell typical of its components.

**Viscosity**

Objective: Measure the flow behavior of the oil for topical application.

**Method:**

Use a viscometer to detelmine the oil's viscosity at 25°C.

Alternatively, observe the oil's flow on a flat surface.

Criteria: The oil should be easy to spread without being excessively runny.

**Stability Testing**

**Accelerated Stability Testing**

Objective: Assess the oil's stability under different storage conditions.

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**Method:**

Store samples in three conditions for 4--8 weeks:

Room temperature (25°C).

Refrigerated (4°C).

Elevated temperature (45°C).

Observe for changes in:

Color.

Odor.

Consistency

Criteria:

The oil should remain stable with no phase separation, discoloration, or rancidity.

Oxidative Stability

Objective: Check the oil's resistance to oxidation over time.

Method:

Perform the peroxide value test to measure lipid oxidation.

Alternatively, monitor for rancidity using an accelerated oxidation test (Rancimat method).

Criteria: The oil should have low peroxide values and no noticeable rancidity over its shelflife

**Antifungal Activity Evaluation**

Agar Well Diffusion Method

Objective: Measure the antifungal efficacy of the oil.

Method:

Prepare agar plates inoculated with fungal strains like *Candida albicans*, *Aspergillus niger*, or *Trichophyton* spp.

Create wells in the agar and fill each with 50-100 µL of the oil.

Incubate at 28°C for 48-72 hours.

Measure the zone of inhibition (in mm) around the wells.

Criteria: A larger inhibition zone indicates stronger antifungal activity.

**Minimum Inhibitory Concentration (MIC)**

Objective: Determine the lowest concentration of the oil that inhibits fungal growth.

Method:

Dilute the oil in a series of test tubes containing fungal cultures.

Incubate for 48-72 hours at 28°C.

Observe the tubes for visible fungal growth.

Criteria: The MIC is the lowest oil concentration where no growth is observed.

**Time-Kill Assay**

Objective: Evaluate the time required to kill fungal cells.

Method:

Expose fungal cultures to the oil at MIC concentration

Take samples at intervals (e.g., 1, 2, 4, 8 hours) and plate them to observe surviving colonies.

Criteria: Faster kill times indicate more potent antifungal action

**Skin Compatibility Tests**

Patch Test

Objective: Test for potential skin irritation or allergic reactions.

**Method:**

Apply a small amount of oil to the forearm or behind the ear of volunteers.

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Cover with a bandage and leave for 24 hours.

Observe for redness, itching, or swelling.

Criteria: No adverse reactions should occur

#### **Dennal Penneation Test**

Objective: Assess the oil's ability to penetrate the skin.

Method:

Use Franz diffusion cells with human or animal skin samples.

Measure the ainount of oil pe lmeating through the skin layers over

Criteria: The oil should demonstrate sufficient penetration for effective action.

#### **Chemical Analysis**

GC-MS (Gas Chromatography-Mass Spectrometry)

Objective: Identify the active components of the oil blend.

Method:

Analyze the oil using GC-MS to detect and quantify key compounds like terpenes, flavonoids, and fatty acids.

Criteria: Presence of known antifungal compounds such as nimbin (Neem), myiisticin (Jaifal), and anthocyanins (Jamun).

#### **Acid Value Test**

Objective: Assess the oil's freshness and stability.

**Method:**

Perfonn titration to detennine the acid value.

Criteria: Low acid values indicate a fresh and stable formulat

#### **Functional Testing**

Spreadability Test

Objective: Assess the ease of application.

Method:

Place a small amount of oil between two glass slides.

Measure the area covered by the oil when light pressure is applied.

Criteria: The oil should spread easily without leaving a greasy residue.

#### **Absorption Test**

Objective: Evaluate the rate of skin absorption.

Method:

Apply the oil to the skin and observe the time required for it to absorb completely.

Criteria: The oil should absorb quickly without excessive greasiness

#### **EXPECTED OUTCOME**

The combination of Jamun seed oil, Jaifal oil, and Neem oil is expected to exhibit superior antifungal activity compared to the individual oils, pa lrticularly in inhibiting the growth of *Candida albicans*, *Aspergillus niger*, and *Trichophyton mbmm*.

The MIC and MFC values for the combination will likely be lower than for individual oils, indicating a synergistic effect.

The oil combination is expected to dismpt fungal cell walls and membranes more effectively than individual oils, as evidenced by biochemical and molecular assays.

The mechanism of action of the combination is anticipated to involve multiple pathways including membrane disruption, inhibition of B-glucan synthesis, and induction of oxidative stress.

The oil combination is expected to be safe for topical application and may show potential for systemic use, as demonstrated by in vivo studies and cytotoxicity assessments.

In comparison to conventional antifungal agents, the oil combination may prove to be more effective in preventing fungal recurrence due to its natural composition and multi-target approach

### Future Directions

To further enhance the effectiveness and applicability of the Jamun, Jaifal, and Neem oil combination, several areas for improvement and research should be explored:

1. Clinical Validation: Conducting controlled clinical trials will be crucial to confirm the oil's efficacy in real-world scenarios, particularly in chronic and resistant fungal infections.
2. Formulation Optimization: Further studies to refine the ratio of oils and incorporate additional active ingredients could improve its antifungal activity and address a wider range of infections.
3. Delivery Systems: Incorporating nano-encapsulation or other advanced delivery techniques could enhance the absorption and bioavailability of the active compounds, improving the overall performance of the oil.
4. Consumer Research: Understanding consumer preferences through market studies can help tailor the formulation to meet the needs of diverse user groups and increase its market acceptance.
5. Regulatory Approval and Certifications: Pursuing regulatory approvals and obtaining organic or cruelty-free certifications could enhance consumer trust and make the product more widely available in the market.

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