Formulation of Herbal Antifungal Ointment A Review

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Abstract: Fungal infections, also known as mycoses, range from superficial skin issues to severe systemic diseases. Due to the rising resistance to synthetic antifungal agents and their potential side effects, herbal alternatives have gained increased interest. This study aims to formulate and evaluate a herbal antifungal ointment using Melia azedarach (China Berry) and Hygrophila auriculata (Marsh Barbel), both of which are known for their traditional medicinal properties. The selected plants were collected locally, authenticated botanically, and subjected to methanol and ethanol extraction. The phytochemical constituents of the plants—such as limonoids, flavonoids, alkaloids, and triterpenes—contribute to their antifungal efficacy. The extracts were evaluated for their minimum inhibitory concentration (MIC) against *Aspergillus niger*, a common causative agent of fungal infections. Following extraction and MIC evaluation, the herbal ointment was formulated using standard excipients including stearic acid, glycerin, paraffin, and preservatives. The final formulation was tested for its antifungal activity and stability. The results demonstrated promising antifungal potential with minimal risk of side effects, supporting the efficacy of herbal combinations as a cost-effective and safe alternative for treating fungal skin infections.

Key Words: Herbal antifungal ointment, *Melia azedarach*, *Hygrophila auriculata*, Minimum Inhibitory Concentration (MIC), *Aspergillus Niger.*

INTRODUCTION

Fungal infections, or mycoses, are diseases caused by the overgrowth or invasion of fungi into human tissues. These infections may affect the outermost layers of the skin or mucosal surfaces, or progress deeper into underlying tissues, bones, and even systemic circulation, depending on the type and virulence of the fungus as well as the host's immune response. Mycoses are increasingly recognized as significant causes of morbidity and mortality, particularly in patients with weakened immune systems, such as those with HIV/AIDS, cancer, organ transplants, or chronic illnesses.

Fungal infections are broadly classified into four categories based on the depth and location of infection:

1. Superficial Mycoses – Affect the outermost layer of skin or hair shafts. These are usually non-invasive and include both inflammatory (e.g., tinea) and non-inflammatory types (e.g., pityriasis versicolor).
2. Cutaneous Mycoses – Involve keratinized tissues like nails and hair, often caused by dermatophytes such as *Trichophyton* species.
3. Subcutaneous Mycoses – Penetrate deeper into the skin and subcutaneous tissue, often following trauma (e.g., sporotrichosis).
4. Systemic Mycoses – Can disseminate to internal organs and are typically life-threatening. Examples include infections caused by *Histoplasma*, *Cryptococcus neoformans*, and *Aspergillus* species.

Fig: Types of Fungal Infections

Causative Fungal Agents

The most common fungal pathogens include:

- *Candida albicans*: a commensal organism that can cause opportunistic infections like oral thrush and vaginal candidiasis.
- *Aspergillus* species: especially *A. fumigatus* and *A. niger*, which are associated with respiratory and systemic infections.
- *Cryptococcus neoformans*: often associated with severe meningoencephalitis in immunocompromised patients.

These fungi thrive in warm, moist environments, making body folds, mucosal membranes, and skin a favorable site for colonization. Environmental factors (humidity, poor hygiene), prolonged use of antibiotics, immunosuppressants, or corticosteroids, and underlying chronic diseases further increase susceptibility.

Challenges with Conventional Antifungal Therapy

Conventional antifungal agents such as azoles (e.g., fluconazole), polyenes (e.g., amphotericin B), and allylamines (e.g., terbinafine) have been the mainstay of treatment. However, they present several challenges:

- Drug resistance due to prolonged or inappropriate use
- Toxicity and side effects, especially hepatotoxicity and nephrotoxicity
- High cost and limited accessibility in rural or low-resource areas
- Recurrent infections, especially in chronic cases

These limitations have shifted scientific interest toward natural and plant-based antifungal alternatives with fewer adverse effects, better tolerability, and sustainable sourcing.

Herbal Approaches to Antifungal Therapy

Traditional medicine and ethnobotanical knowledge have long used plants with antifungal properties, such as:

- Turmeric (Curcuma longa) – Contains curcumin, a potent antifungal and anti-inflammatory compound
- Garlic (Allium sativum) – Rich in allicin, effective against *Candida* and *Aspergillus*
- Black walnut (Juglans nigra) – Contains juglone with antifungal potential Aloe vera, Calendula, Echinacea, Cinnamon, and others

Among these, China Berry (*Melia azedarach*) and Marsh Barbel (*Hygrophila auriculata*) have demonstrated notable antifungal activity in various studies, making them strong candidates for herbal formulation development.

Plant Profile: Melia azedarach (China Berry)

- Common Names: Bakayan, Ghora Neem
- Family: Meliaceae
- Active Phytochemicals:
  - Limonoids – Azadirachtin, Meliatoxin (known for insecticidal and antifungal action)
  - Triterpenoids – Anti-inflammatory properties
  - Flavonoids – Rutin, Wogonin (antioxidant and antifungal effects)
  - Alkaloids – Quinine, Furoquinoline alkaloids (broad-spectrum antimicrobial activity)
- Uses: Widely used for treating skin infections, expelling intestinal worms, and acting as an insecticide

Plant Profile: Hygrophila auriculata (Marsh Barbel)

Common Names: Gokul Kanta, Talimkhana

- Family: Acanthaceae
- Active Phytochemicals:
  - Flavonoids – Apigenin 7-O-glucuronide (anti-inflammatory, antifungal)
  - Alkaloids – Asteracanthine (antibacterial and hepatoprotective)
  - Triterpenes – Lupeol, Betulin (healing, antimicrobial)
  - Steroids – Stigmasterol (enhances skin barrier function)
- Uses: Traditionally used in Ayurveda for treating liver disorders, urinary tract infections, and skin diseases including fungal infections.

Active Ingredients in Herbal Antifungal Ointment

1. Chinaberry Extract (*Melia azedarach*) – Active Pharmaceutical Ingredient (API)

- Role: Primary antifungal agent
- Action: Contains limonoids (azadirachtin, meliatoxin), flavonoids, alkaloids, and terpenoids that:
  - Disrupt fungal cell membrane integrity
  - Inhibit spore germination
  - Interfere with ergosterol biosynthesis
  - Show fungistatic and fungicidal effects against *Candida*, *Aspergillus*, and dermatophytes

2. Marsh Barbel Extract (*Hygrophila auriculata*) – API

- Role: Secondary antifungal and healing agent
- Action: Rich in flavonoids (apigenin), alkaloids, and triterpenoids like lupeol and betulin that:
  - Inhibit fungal enzymes and mitochondrial functions
  - Induce oxidative stress leading to fungal apoptosis
  - Support tissue repair and reduce inflammation

3. Stearic Acid – Thickening Agent

- Role: Provides cream consistency and structure
- Action: Helps in forming an occlusive base, which:
  - Aids in retaining moisture
  - Enhances skin penetration of the active ingredients

4. Potassium Hydroxide & Sodium Carbonate – pH Adjusters / Mild Alkalizers

- Role: Maintain the pH of the formulation
- Action
  - Create a slightly alkaline environment unfavorable for fungal growth
  - Assist in emulsification and cream stability

5. White Soft Paraffin – Emollient/Base

- Role: Skin softener and ointment base
- Action
  - Forms a protective barrier to lock in moisture
  - Aids in prolonged contact of actives with the infected area
  - Soothes dry, cracked, or itchy skin

6. Methanol – Extraction Solvent (for plants)

- Role: Used only in the preparation phase (evaporated before final formulation)
- Action
  - Extracts phytochemicals efficiently
  - Does not remain in the final product after evaporation

7. Glycerin – Humectant/Moisturizer

- Role: Moisturizes and maintains skin hydration
- Action
  - Keeps the skin soft, helping reduce irritation from fungal infection
  - Enhances dermal absorption of herbal extracts

8. Methyl Paraben – Preservative

- Role: Prevents microbial contamination
- Action
  - Maintains the shelf-life and microbial safety of the cream
  - Prevents secondary bacterial infections in treated areas

9. Rose Oil – Fragrance and Functional Excipient

- Role: Provides fragrance and additional antifungal/anti-inflammatory benefits
- Action
  - Contains geraniol, citronellol, and eugenol that have mild antifungal and antiseptic effects
  - Offers cooling and soothing effect to irritated or inflamed skin

10. Distilled Water – Solvent

- Role: Used to dissolve other ingredients and form the emulsion
- Action
  - Provides the aqueous phase for the cream
  - Helps in proper mixing and consistence

Summary Table:

Sr.no.	Ingredient	Role	Antifungal/Supportive Action
1.			
2.	Chinaberry Extract	API	Cell membrane disruption, enzyme inhibition
3.	Marsh Barbel Extract	API	Induces oxidative stress, promotes healing
4.	Stearic Acid	Thickening agent	Improves consistency, supports penetration
5.	Potassium Hydroxide	pH adjuster	Maintains alkaline environment
6.	Sodium Carbonate	pH adjuster	Enhances emulsification, antifungal support
7.	White Soft Paraffin	Emollient	Barrier formation, skin protection
8.	Methanol	Extraction solvent	Extracts actives (evaporated before use)
9.	Glycerin	Humectant	Moisturizes skin, improves absorption
10.	Methyl Paraben	Preservative	Prevents contamination
11.	Rose Oil	Soothing agent/fragrance	Mild antiseptic, cooling effect
12.	Distilled Water	Solvent	Base for emulsion

Types of Fungal Infections Treated

Based on the nature and bioactivity of the ingredients, the ointment is best suited for:

- Superficial Mycoses: Including tinea corporis (ringworm), tinea pedis (athlete's foot), tinea cruris (jock itch), and candidiasis of skin folds.
- Cutaneous Mycoses: Fungal infections involving keratinized tissues such as the epidermis, hair, and nails.
- Mild Secondary Skin Fungal Infections: Due to its anti-inflammatory and soothing properties.

Mechanism of Action

The antifungal effect of the formulation results from the synergistic activity of bioactive phytochemicals in both herbs, working through the following mechanisms:

1. Disruption of Fungal Cell Membranes

Limonoids (from Chinaberry) and flavonoids (from both herbs) disrupt fungal cell membrane integrity by interacting with ergosterol or membrane lipids, leading to leakage of intracellular contents.

2. Inhibition of Spore Germination

The extract components inhibit fungal spore germination, preventing colonization and spread.

3. Oxidative Stress Induction

Some phytochemicals increase reactive oxygen species (ROS) within fungal cells, damaging DNA and proteins.

4. Protein Synthesis Inhibition

Alkaloids and triterpenoids interfere with fungal protein synthesis, thereby halting growth and reproduction.

5. Anti-inflammatory Action

Flavonoids like rutin, apigenin, and wogonin reduce local inflammation and redness, aiding healing and symptom relief.

6. Immune-Modulatory Effect

Certain plant compounds may boost local immune response, enhancing clearance of infection.

Advantages of Herbal Ointment

1. Natural Antifungal Properties: Rich in limonoids, triterpenoids, flavonoids, and alkaloids which are known to effectively inhibit fungal growth.
2. Multi-Target Mechanism: Acts on multiple fungal pathways (cell membrane disruption, spore inhibition, ROS generation), reducing the risk of resistance.
3. Anti-inflammatory & Healing: Compounds like apigenin and rutin help reduce inflammation and promote skin healing.
4. Antibacterial Action: Also effective against certain bacteria, offering protection in mixed infections or contaminated wounds.
5. Moisturizing & Skin-Friendly: Formulated with glycerin and paraffin, improving skin texture and barrier function.
6. Low Side Effects: Minimal risk of irritation or toxicity compared to synthetic antifungals.
7. Eco-Friendly & Biodegradable: Plant-based formulation is safer for the environment during production and disposal.
8. Traditional Usage Validation: Both plants have a history in Ayurveda and traditional medicine for skin disorders and infections.

9. Local & Economical Sourcing: Plants are commonly available and affordable, especially in tropical regions like India.
10. Immune-Boosting Potential: Some phytochemicals may support local immune responses in fungal-infected skin areas.

Disadvantages of Herbal Ointment

1. Variability in Potency: Active compound concentration can vary depending on plant maturity, harvesting season, and extraction method.
2. Slower Action: May take longer to show effects compared to synthetic antifungals like clotrimazole or terbinafine.
3. Shorter Shelf Life: Without advanced preservatives, herbal products can degrade faster over time.
4. Lack of Standardization: Difficult to ensure consistent formulation unless standardized extract ratios are used.
5. Potential for Mild Allergic Reactions: Although rare, some individuals may react to plant compounds like alkaloids or essential oils.
6. Limited Penetration in Severe Infections: May not effectively treat deep or systemic fungal infections.
7. Poor Regulatory Control: Herbal formulations may lack stringent quality checks if not GMP-certified.
8. Need for Refrigeration: Some formulations may need cold storage to maintain efficacy.
9. Research Gaps: Limited large-scale clinical studies to fully validate safety, efficacy, and long-term use.
10. Extraction & Formulation Time: Preparation involves multiple steps (drying, extraction, filtration, formulation), which can be labor-intensive.

Evaluation of herbal ointment

A visual examination was used to check physical characteristics, including color and smell.

Consistency: Smooth consistency and no indications of greed are present. Solubility Ether, alcohol, and chloroform are all soluble in and miscible with boiling water.

Washability: After the combination had been applied to the skin, the ease of water washing was evaluated.

PH: A digital pH metre was used to determine the herbal ointment's pH. One hundred milliliters of distilled water was used to make the ointment solution, which was then left to settle for two hours. The solution's pH was measured three times, and the average value was determined was 6.93 pH.

Spread ability: To test the spread ability, we sandwiched extra sample between two slides that had been uniformly crushed using a predetermined weight for a specific amount of time. To determine spread ability, the time needed to separate the two slides was utilised. Improved spread ability is the end consequence of speedier slide separation. Spread ability was determined using the formula below.

$S=M\times L/T$ Where, S = Spread ability M denotes the weight of the upper slide. L = Glass slide length T is the duration needed to separate the slides.

Extrudability: A tube-shaped container was used to store the mixture. Extrudability was calculated as the weight of cream needed to extrude 0.5 cm of cream ribbon in 10 seconds.

Diffusion analysis: The diffusion research was carried out using the agar nutritional medium. A board with a hole in the middle was filled with ointment. It was apparent how long the ointment took to diffuse. (After 60 seconds)

Stability study: A four-week physical stability test on the herbal cream was conducted at different temperatures, including 2°C, 25°C, and 37°C. It was found that the herbal ointment was physical steadiness across a range of temperatures, including 2°C, 25°C, and 37°C, four weeks soon.

Stability studies data of formulated ointment

No. Of days	Physical appearance	Ph evaluation
Initial	++ No change in color	6.8
30	++	6.8
60	+ Slight change in color	6.5
90	+	6.4

CONCLUSION

The developed herbal antifungal ointment, utilizing the bioactive extracts of *Melia azedarach* and *Hygrophila auriculata*, demonstrated excellent formulation characteristics, therapeutic promise, and

shelf stability. The product exhibited a visually acceptable appearance, retaining a pleasant color and odor throughout the study period, with no signs of phase separation, rancidity, or microbial contamination, confirming its physical integrity. The smooth consistency ensured ease of application and comfort during topical use. The ointment's solubility in organic solvents such as ether, alcohol, and chloroform, as well as miscibility with boiling water, suggested broad compatibility for pharmaceutical processing and effective dispersion of active phytoconstituents. Washability testing revealed that the formulation could be easily rinsed off with water after skin application, an essential attribute for patient compliance and hygiene. The pH value of approximately 6.93 aligns closely with human skin's natural pH (5.5–7), indicating that the ointment is non-irritating and dermatologically safe for long-term use. Evaluation of spread ability demonstrated efficient distribution of the ointment across the skin surface, a critical factor in ensuring uniform application and absorption of the active ingredients. Similarly, extrudability measurements confirmed the product's suitability for dispensing from standard ointment tubes, enhancing user convenience. Diffusion analysis, performed using an agar nutrient medium, indicated effective and rapid release of active components, with diffusion evident within 60 seconds. This suggests prompt onset of antifungal activity, which is crucial in managing infections early and effectively. The stability study, carried out over a period of 90 days at temperatures of 2°C, 25°C, and 37°C, demonstrated that the ointment maintained its physical and chemical properties over time. Only a slight change in color and a minor reduction in pH (from 6.8 to 6.4) were observed by the end of the test period. These changes remained within pharmaceutically acceptable limits, supporting the formulation's robustness and storage compatibility under various environmental conditions.

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