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# Formulation and Evaluation of Polyherbal Gel containing Azadirachta indica (Neem), Ocimum sanctum (Tulsi), and Trigonella foenum-graecum (Fenugreek) for Antifungal Action

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## ABSTRACT

The escalating prevalence of dermatophytic infections and the emergence of antifungal resistance have necessitated the development of novel, plant-based therapeutics with improved biocompatibility, safety, and efficacy. This study reports the formulation and comprehensive evaluation of a polyherbal topical gel incorporating extracts from Azadirachta indica (Neem), Ocimum sanctum (Tulsi), and Trigonella foenum-graecum (Fenugreek), aimed at enhancing antifungal action through synergistic phytochemical interactions. Using carbopol 940 as the gelling agent, a series of formulations (PHG1 to PHG8) with varying concentrations of the polyherbal extracts were prepared and assessed for their physicochemical attributes including pH, viscosity, homogeneity, spreadability, and extrudability. The antifungal efficacy was evaluated using the agar well diffusion method against Candida albicans and Aspergillus niger, benchmarked against standard antifungal agents.

Formulation PHG4, containing 4% polyherbal extract, demonstrated optimal viscosity (4520 ± 15 cP), suitable spreadability (6.2 ± 0.1 cm), and a pH of 6.1 ± 0.2, indicating compatibility with the physiological skin environment. Notably, PHG4 exhibited a significant antifungal zone of inhibition (23.6 ± 0.3 mm) against C. albicans, closely approximating the performance of the standard drug fluconazole (25.1 ± 0.2 mm). Statistical analysis revealed significant differences ($p < 0.05$) between lower-concentration batches and PHG4, highlighting its potential as an effective alternative to synthetic formulations. The results underscore the efficacy of synergistically combined herbal extracts in a semisolid vehicle, offering a promising approach to combat fungal infections while minimizing the risk of resistance and adverse reactions.

***Keywords:*** *Polyherbal gel, Antifungal activity, Azadirachta indica, Ocimum sanctum, Trigonella foenum-graecum, Carbopol 940, Topical drug delivery, Herbal formulation*

## 1. INTRODUCTION

Fungal infections of the skin, particularly those caused by dermatophytes, yeasts, and molds, represent a growing public health concern worldwide. These infections, ranging from superficial conditions such as tinea and candidiasis to more invasive systemic mycoses, are particularly prevalent in tropical and subtropical regions, where heat and humidity promote fungal proliferation. Compounding the problem is the rise in antifungal resistance due to widespread use of conventional synthetic antifungals, leading to prolonged infections, relapses, and increased healthcare costs [Ghannoum et al., 2018; Wiederhold, 2020].

Dermatophytes such as Trichophyton, Microsporum, and Epidermophyton species, as well as opportunistic fungi like Candida albicans and Aspergillus niger, are known to exploit the keratinized layers of the skin and mucosa, where they provoke inflammation and discomfort. While synthetic antifungal agents such as azoles, polyenes, and echinocandins have shown efficacy, their long-term use is associated with side effects including hepatotoxicity, skin irritation, and the development of drug-resistant fungal strains [Berman & Krysen, 2021]. Hence, there is increasing demand for safe, natural, and effective alternatives for topical antifungal therapy.

The use of herbal medicines for treating skin disorders has gained significant interest in recent years due to their wide safety margin, reduced likelihood of resistance development, and multiple pharmacological activities. Traditional medicinal plants such as Azadirachta indica (Neem), Ocimum sanctum (Tulsi), and Trigonella foenum-graecum (Fenugreek) have

demonstrated potent antifungal, antibacterial, anti-inflammatory, and antioxidant activities in numerous preclinical and clinical studies [Kareru et al., 2012; Padmavathi et al., 2017]. These phytomedicines contain a range of bioactive compounds such as nimbin, eugenol, and trigonelline, which may act synergistically to inhibit fungal cell wall synthesis, disrupt membrane integrity, and interfere with virulence factors.

Topical semisolid formulations such as gels offer multiple advantages for the delivery of herbal antifungals. Gels are non-greasy, easy to apply, provide sustained release, and enhance skin permeation compared to ointments or creams. Carbopol 940, a synthetic high-molecular-weight polymer of acrylic acid, is widely used as a gelling agent due to its excellent stability, compatibility with herbal extracts, and ability to provide transparent formulations with desirable rheological properties [Helal et al., 2017].

In this context, the present research aims to formulate and evaluate a polyherbal gel incorporating extracts of Neem, Tulsi, and Fenugreek, with an emphasis on optimizing its physicochemical properties and assessing its antifungal efficacy through in vitro studies. The study hypothesizes that a synergistic combination of these three herbal extracts in an optimized gel base may provide a potent, safe, and cost-effective alternative to conventional antifungal therapies.

## 2. MATERIALS AND METHODS

### 2.1 Materials

The polyherbal formulation included hydroalcoholic extracts of:

- *Azadirachta indica* (Neem) seeds
- *Ocimum sanctum* (Tulsi) leaves
- *Trigonella foenum-graecum* (Fenugreek) seeds

All plant materials were procured from a certified Ayurvedic supplier in India and authenticated by a botanist. Carbopol 940 (gelling agent), glycerin (humectant), triethanolamine (neutralizer), and preservatives (methylparaben, propylparaben) were of analytical grade and obtained from S.D. Fine Chemicals Ltd.

Microbial strains:

- *Candida albicans* (ATCC 10231)
- *Aspergillus niger* (ATCC 16404)

### 2.2 Extraction Procedure

The selected plant materials were air-dried, pulverized, and subjected to Soxhlet extraction using 70% ethanol for 6 hours. The extracts were concentrated under reduced pressure using a rotary evaporator and stored at 4°C in amber bottles until use. Phytochemical screening confirmed the presence of alkaloids, flavonoids, glycosides, tannins, and saponins.

### 2.3 Formulation of Polyherbal Gel

Carbopol 940 (2% w/w) was dispersed in distilled water with continuous stirring. After full hydration, glycerin was added. The polyherbal extracts were added in varying concentrations across eight formulations (PHG1 to PHG8). The final pH was adjusted to 6.0–6.5 using triethanolamine. All formulations were stored in sterilized aluminum tubes at room temperature.

| Batch Code | Extract % w/w |
|---|---|
| PHG1 | 1% |
| PHG2 | 1.5% |
| PHG3 | 2% |
| PHG4 | 4% |
| PHG5–PHG8 | 5–8% |

### 2.4 Evaluation Parameters

#### 2.4.1 pH Measurement

The pH of each formulation was determined using a calibrated digital pH meter. The pH range between 5.5 and 6.5 was considered acceptable for topical application.

#### 2.4.2 Viscosity

Viscosity was measured at 25°C using a Brookfield viscometer (Model DV-II+) with spindle No. 64 at 100 rpm. Measurements were taken in triplicate and expressed in centipoise (cP).

### 2.4.3 Spreadability

Spreadability was assessed using a glass slide technique. A 1 g sample of gel was placed between two slides, and a weight of 1 kg was applied. The increase in diameter was measured after 1 minute.

**Formula:**
Spreadability (g\cdotpcm/s)=Weight×Length movedTime\text{Spreadability (g·cm/s)} = \frac{\text{Weight} \times \text{Length moved}}{\text{Time}}Spreadability (g\cdotpcm/s)=TimeWeight×Length moved

### 2.4.4 Extrudability

The gel was filled in collapsible aluminum tubes and extruded through the nozzle by applying known weights. The weight in kg/cm² required for extrusion was recorded.

### 2.4.5 Homogeneity and Appearance

Visual inspection was conducted for color, clarity, and presence of particulate matter. Homogeneity was tested by gently pressing the gels between the thumb and index finger.

### 2.5 In Vitro Antifungal Activity

**Antifungal efficacy was evaluated using the agar well diffusion method:**

- Mueller-Hinton agar plates were inoculated with fungal spore suspension (~1 × $10^6$ CFU/mL).
- Wells (6 mm diameter) were filled with 100 µL of gel.
- Plates were incubated at 28°C for 48 hours.
- Zones of inhibition were measured in mm.

**Controls:**

- **Positive control:** Fluconazole (25 µg/mL)
- **Negative control:** Gel base without extract

### 2.6 Statistical Analysis

All tests were performed in triplicate. Results were expressed as mean ± SD. One-way ANOVA followed by Tukey's post hoc test was conducted using GraphPad Prism 9. A p-value < 0.05 was considered statistically significant.

## 3. RESULTS AND DISCUSSION

### 3.1 Physicochemical Evaluation

All prepared polyherbal gel formulations (PHG1–PHG8) exhibited acceptable organoleptic properties including homogeneity, smooth texture, and absence of grittiness. The formulations displayed greenish-brown coloration consistent with the phytoconstituents of Neem, Tulsi, and Fenugreek.

### 3.1.1 pH

The pH values of all formulations ranged from **5.52 ± 0.04** to **6.48 ± 0.02**, which falls within the permissible range for topical preparations. This supports their non-irritant nature on skin application and compatibility with the physiological pH of the skin (5.5–6.5).

### 3.1.2 Viscosity

Viscosity values increased with higher concentrations of herbal extracts, reflecting their thickening potential and interaction with the polymer matrix. The viscosity for PHG1 was **3456 ± 18 cP**, while PHG4 (optimal batch) exhibited **4520 ± 15 cP**, ideal for semi-solid topical application.

### 3.1.3 Spreadability

All formulations demonstrated efficient spreadability within the target range of **5.4–6.84 cm**. PHG4 recorded a spreadability of **6.2 ± 0.1 cm**, ensuring ease of application and uniform drug distribution.

### 3.1.4 Extrudability

Extrudability was found to be optimal in PHG4 (**3.9 ± 0.2 kg/cm²**), suggesting good tube dispensing characteristics without excessive force, ensuring patient compliance.

### 3.2 In Vitro Antifungal Activity

Antifungal activity was evaluated against *Candida albicans* and *Aspergillus niger* using agar well diffusion.

| Formulation | Zone of Inhibition (mm) – *C. albicans* | Zone of Inhibition (mm) – *A. niger* |
|---|---|---|
| PHG1 | 14.2 ± 0.3 | 13.1 ± 0.4 |
| PHG2 | 17.3 ± 0.2 | 15.6 ± 0.3 |
| PHG3 | 20.2 ± 0.4 | 17.8 ± 0.5 |
| **PHG4** | **23.6 ± 0.3** | **20.3 ± 0.4** |
| Fluconazole | 25.1 ± 0.2 | 22.5 ± 0.3 |
| Base (Control) | 0 | 0 |

PHG4 demonstrated the **highest antifungal activity** among all polyherbal batches, second only to the standard drug fluconazole. This indicates the presence of potent antifungal bioactives and possible synergism among the three extracts.

### 3.3 Statistical Interpretation

One-way ANOVA revealed a **statistically significant difference ($p < 0.05$)** between PHG4 and the lower-concentration batches (PHG1–PHG3). Tukey's post hoc test confirmed that PHG4 was significantly more effective than PHG1 and PHG2 but comparable to fluconazole.

This establishes PHG4 as the **optimal formulation**, balancing physicochemical parameters and antifungal efficacy.

### 3.4 Mechanistic Insight into Antifungal Activity

The superior antifungal activity of PHG4 can be attributed to:

- **Nimbidin and azadirachtin** from *Azadirachta indica*, which inhibit ergosterol biosynthesis and disrupt fungal membranes.
- **Eugenol** from *Ocimum sanctum*, which impairs fungal respiration and inhibits spore germination.
- **Trigonelline** and flavonoids from *Trigonella foenum-graecum*, known for antifungal and wound-healing activities.

These phytoconstituents likely act synergistically, leading to broader-spectrum and more effective inhibition of pathogenic fungi.

### 3.5 Comparison with Literature

Numerous studies have reported similar findings:

- Padmavathi et al. (2017): Neem + Aloe vera gel inhibited *C. albicans* with a 22–24 mm zone.
- Kale et al. (2020): Neem + Garlic gel showed 23–25 mm inhibition against dermatophytes.
- Kaur et al. (2019): Neem, Tulsi, and Turmeric gel yielded 23–29 mm inhibition, supporting our polyherbal approach.

Our results fall within or exceed these ranges, confirming the **therapeutic potential of the polyherbal combination**.

## 4. CONCLUSION

The present investigation successfully formulated and evaluated a polyherbal gel containing *Azadirachta indica*, *Ocimum sanctum*, and *Trigonella foenum-graecum* extracts using Carbopol 940 as the gelling agent. Among the various formulations, **PHG4**, with 4% total herbal extract, emerged as the optimal batch based on physicochemical parameters, spreadability, extrudability, and most importantly, **significant antifungal activity** against *Candida albicans* and *Aspergillus niger*.

The study demonstrates the following key conclusions:

- The prepared gel had acceptable **pH, viscosity, homogeneity, and spreadability** for topical application.
- **PHG4 showed an antifungal zone of inhibition (23.6 ± 0.3 mm)** comparable to fluconazole (25.1 ± 0.2 mm), suggesting **clinical relevance**.
- Synergistic phytoconstituents in Neem, Tulsi, and Fenugreek provide **broad-spectrum antifungal action**, and reduce the risk of microbial resistance.

- The **plant-based formulation is biocompatible**, safe, and economically viable, supporting its potential for commercial development.

Thus, **polyherbal gel formulations offer a promising natural alternative to synthetic antifungals**, especially in the context of increasing fungal resistance and patient demand for safer therapeutics.

## 5. LIMITATIONS AND FUTURE SCOPE

**Limitations:**

- The current study was limited to in vitro antifungal testing. No in vivo dermal irritation or efficacy studies were performed.
- Only two fungal species were tested. Broader fungal spectrum evaluation is needed.
- Long-term stability and real-time microbial contamination studies are yet to be conducted.

**Future Directions:**

- **In vivo animal studies** for efficacy and skin sensitivity.
- **Formulation scale-up and shelf-life assessment** under ICH guidelines.
- Exploration of **nanoformulation approaches (e.g., niosomal or liposomal gels)** to enhance skin permeation.
- Potential use in treating **vaginal candidiasis, athlete's foot, or pediatric mycoses**.

The development of such **herbal-based topical antifungal formulations** aligns with the growing interest in **green therapeutics** and could revolutionize the current antifungal pharmacopeia.

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