



Research Article

## Formulation and Evaluation of A Herbal Gel Containing Powdered Extract of Tulsi Leaves for the Treatment of Fungal Infections

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DOI: <https://doi.org/10.5281/zenodo.20958067>

### Abstract

Dermatophytosis, a fungal infection of the skin, is a widespread public health concern. The increasing resistance to antifungal drugs and the side effects associated with conventional chemical treatments highlight the need for new, plant-based therapeutic options. Tulsi (*Ocimum sanctum*), a well-known medicinal herb, has shown significant antifungal properties mainly due to its essential oil components such as eugenol and linalool. However, developing a stable, effective, and user-friendly topical delivery system for Tulsi extract remains a challenge. Gel-based formulations are considered a promising approach, as they are non-greasy, easy to apply, provide a cooling effect and allow controlled drug release. The present study aims to compile and critically analyse the existing literature on the formulation and evaluation of antifungal herbal gels containing Tulsi. The gel formulation was prepared using a standardised method. It was then evaluated for its *in vitro* antifungal activity against common fungal pathogens. In addition, key physicochemical properties such as pH, viscosity, spreadability, and drug content were assessed.

### Manuscript Information

- ISSN No: 2583-7397
- Received: 12-05-2026
- Accepted: 18-06-2026
- Published: 27-06-2026
- IJCRM:5(3); 2026: 1203-1211
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- Plagiarism Checked: Yes
- Peer Review Process: Yes

### How to Cite this Article

Kadam K, Salgar S, Shewale A. Formulation and Evaluation of A Herbal Gel Containing Powdered Extract of Tulsi Leaves for the Treatment of Fungal Infections. Int J Contemp Res Multidiscip. 2026;5(3):1203-1211.

### Access this Article Online



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**KEYWORDS:** Herbal gel, Tulsi leaf extract, Fungal infection, Antifungal activity, Formulation.

## 1. INTRODUCTION

Topical gel formulations are commonly used for application on the skin. Fungal infections, caused by different types of pathogenic fungi, are a major burden on global health care systems and can lead to chronic conditions if not treated properly. The increasing emergence of drug-resistant fungal strains has made treatment more difficult, highlighting the need for alternative therapies. Gels are semi-solid preparations in which a liquid phase is thickened using suitable agents [1]

*Ocimum sanctum*, often known as Tulsi, is a widely esteemed aromatic herb with both culinary and medicinal significance, classified under the family Lamiaceae. It has been employed in traditional Ayurvedic formulation for more than three millennia. The fungicidal activity of Tulsi is due to the action of secondary Metabolites that are present in Tulsi, including alkaloids, glycosides, saponins, tannins, ascorbic acids, eugenol and several other metabolites. [2]

Topical gel formulations are commonly used for application on the skin due to their ease of use, ability to deliver active compounds locally, and enhanced penetration compared to creams or ointments. Fungal infections, caused by various pathogenic fungi such as *Candida*, *Aspergillus*, and *Trichophyton species*, pose a significant burden on global health care systems. These infections can become chronic if not treated appropriately, and the emergence of drug-resistant fungal strains has complicated conventional therapy, highlighting the need for alternative antifungal strategies. Gel formulations are semi-solid preparations in which a liquid phase is thickened using suitable gelling agents, providing an effective medium for delivering antifungal agents.[3]

### Fungal Infection:

Fungal infections (mycoses) are diseases caused by fungi such as yeasts and moulds, affecting the skin, nails, hair, or internal organs.[4] They are classified into superficial, cutaneous, subcutaneous, and systemic infections based on the site of involvement.[5] Common causative organisms include *Candida*, *Aspergillus*, and *Cryptococcus*. These infections are more common in individuals with weakened immunity, poor hygiene, or underlying conditions like diabetes. Symptoms vary from mild irritation and itching to severe systemic complications. Diagnosis involves microscopy, culture, and molecular methods, while treatment includes antifungal drugs such as azoles and polyenes. Early diagnosis and proper management are important to prevent complications.[6][7]

## TYPES OF FUNGAL INFECTION:

### 1. SUPERFICIAL FUNGAL INFECTION:

Fungal infections that are superficial. Your skin, nails, and mucous membranes (such as your mouth, throat, or vagina) are all impacted by superficial fungal infections. The following are instances of superficial fungal infections:

**Ringworm:** Ringworm is caused by dermatophytes, a class of fungi that feed on the cells of the skin, hair, and nails. Dermatophytes are capable of infecting various keratinised tissues, including the hands (*tinea magnum*), scalp (*tinea*

*capitis*), groin and inner thighs (*tinea cruris*), and the facial region involving hair follicles (*tinea barbae*), among other sites.

**Candidiasis:** *Candida* (most often *Candida albicans*) is responsible for candidiasis, a mycotic infection involving the skin and mucous membranes (mucocutaneous). These include oesophageal candidiasis, vulvovaginal candidiasis, oral thrush, some forms of diaper rash, and candidal intertrigo.[8]

### 2. SUBCUTANEOUS FUNGAL INFECTION:

If a fungus enters a tissue injury, generally from an injury sustained while handling plants (such as a thorn scratch), you may develop a fungal infection beneath the epidermis (subcutaneous). They result in skin problems like rashes and ulceration. Tropical and subtropical geographical regions have higher rates of subcutaneous fungal infections.

#### Among the examples are:

##### Rosegardener's disease, or sporotrichosis:

The sporotrichosis is caused by the *Sporotrichosis* fungus. Sporotrichosis can also affect other regions of your body, such as your lungs.

**Mycosis chromoblastosis:** Chromato blastomycosis can be caused by a wide variety of fungi. Chronic (long-lasting) skin infections may result from it [9].

### 3. DEEP FUNGAL INFECTION

Deep fungal infections can occur in organs other than the cutaneous and systemic its such as the brain, blood, lungs, or urinary tract. Certain infections are opportunistic, which means that they often only infect those with compromised immune systems.

#### Invasive or deep fungal diseases include:

**Histoplasmosis:** The etiological agent of histoplasmosis, *Histoplasma*, can invade the central nervous system, lungs, or other bodily regions. It is frequently seen in the lowlands of the Mississippi and Ohio rivers.

**Aspergillosis:** The mould that causes aspergillosis, *Aspergillus*, is associated with several lung illnesses, such as chronic pulmonary aspergillosis and allergic bronchopulmonary aspergillosis (ABPA). It may potentially develop into a fungal ball (aspergilloma) or infect other areas of your body.[10]

#### **Ocimum Sanctum:**

*Ocimum sanctum* (Tulsi) is a well-known medicinal plant with significant antifungal activity. Its bioactive constituents, such as eugenol, ursolic acid, and linalool, contribute to its inhibitory effect against fungal species, including *Candida albicans*, *Aspergillus niger*, and *Trichophyton*. The antifungal mechanism involves disruption of fungal cell membranes and inhibition of growth. Due to its safety and natural origin, *Ocimum sanctum* is widely used in herbal formulations for the management of fungal infections.[11][12].

## Antifungal Mechanism Exhibited by *Ocimum Sanctum*:

### 1. Disruption of Cell Membrane Integrity:

The volatile oil of *O. sanctum* contains fat-soluble compounds such as eugenol and linalool that can enter fungal cell membranes. This disrupts the membrane, making it more permeable and causing leakage of essential cellular contents like ions and nucleotides, ultimately leading to cell death. This effect has been observed in *Candida* species, including *C. albicans* and *C. tropicalis*. [13]

### 2. Inhibition of Ergosterol Biosynthesis:

Eugenol, a major component of *O. sanctum* essential oil, interferes with the production of ergosterol, an important

component of fungal cell membranes. This disruption weakens the membrane, affecting its function and integrity, and inhibits fungal growth. Studies have reported this effect in *Candida* species. [14]

### 3. Production of Reactive Oxygen Species (ROS):

Bioactive compounds in *O. sanctum* essential oil can stimulate the production of reactive oxygen species (ROS) inside fungal cells. ROS cause oxidative damage to lipids, proteins, and DNA, which can lead to fungal cell death. This mechanism contributes significantly to the fungicidal efficacy of *O. sanctum*. [15]



Fig2. *Ocimum Sanctum*

## 4. MATERIAL AND METHOD

**Chemicals:** Carbopol 940, Propylene glycol, methyl paraben, Propyl paraben, Triethanolamine, Glycerin, Rose water, Distilled water.

**Equipments:** Digital balance, pH paper, Brookfield viscometer, stirrer, Rotary evaporator.

### Preparation of Plant Extract:

Maceration is a simple and widely used extraction technique for preparing tinctures and concentrated herbal extracts. In this

method, dried or finely powdered Tulsi plant material is placed in a closed container with a suitable solvent such as ethanol, and allowed to stand for an extended period ranging from a few days to several weeks with occasional shaking to enhance extraction. After the maceration process, the mixture is filtered using filter paper to separate the liquid extract from the solid residue. The obtained filtrate is then concentrated by removing the solvent, typically using a rotary evaporator under reduced temperature and pressure, resulting in a thick extract in solid form. [16][17][8]

### Ingredients Used in Formulation:

Sr. No.	Ingredients	Functional Category
1.	Tulsi Extract	Antifungal Agent
2.	Carbapol940	Gelling Agent
3.	Propylene Glycol	Solubilizing Agent
4.	Methyl paraben, propyl Paraben	Preservatives
5.	Triethanolamine	pH Adjuster
6.	Glycerin	Moisturising Agent
7.	Rosewater	Fragrance
8.	Distilled water	Vehicle

### Method of Preparation of gel:

Carbopol 0.2 g was dispersed in distilled water with continuous stirring and allowed to hydrate for 30 minutes. Methyl paraben 0.002 g and propyl paraben 0.008 g were dissolved in a small quantity of warm distilled water and cooled. Propylene glycol

1.6 mL of glycerine and 0.2 mL were added to the hydrated Carbopol dispersion, followed by the preservative solution. Tulsi extract 0.1 g was incorporated with continuous stirring. Triethanolamine was added dropwise to adjust the pH to 6.5–7.0 and to form the gel. Finally, rose water was added q.s. and

the volume was made up to 20 g with distilled water to obtain a homogeneous gel.[19][20]

**Formulation Table:** Formula for preparation of gel.

INGREDIENTS	B1(gm/ml)	B2(gm/ml)	B3(gm/ml)
Tulsi extract	0.1	0.16	0.2
Carbapol	0.2	0.3	0.36
Propylene glycol	1.6	1.8	2
Glycerine	0.2	0.24	0.28
Triethanolamine	q.s	q.s	q.s
Methyl paraben	0.002	0.02	0.02
Propyl paraben	0.008	0.008	0.08
Rosewater	q.s	q.s	q.s
Distilled water	Maketo20	Maketo20	Maketo20

#### Evaluation Parameter:

1. Physical evaluation
2. Clarity
3. Spreadability
4. Measurement of Ph
5. Viscosity
6. Skin irritation
7. Antifungal activity

**1. Physical Evaluation:** Visual inspection was conducted to evaluate the physical characteristics of the sample, including its colour, consistency, and odour [21].

- **Colour:** Visual inspection was performed to assess and confirm the colour of the formulations.
- **Consistency:** The consistency of the formulations was evaluated through topical application to the skin.
- **Odour:** The odour of the formulations was evaluated by dispersing water and subsequently assessing its olfactory characteristics.

**2. Clarity:** The clarity of all three batches was determined by visual inspection

**3. Spreadability:** Spreadability is defined as the time required for two glass slides to separate when a specified quantity of gel is placed between them under an applied load, and it is expressed in seconds. A lower separation time indicates better spreadability of the formulation. The spreadability was calculated using the following formula:

$$S=M \times L \div T$$

Were,

M=weight tied to upper slide, L = length of glass slide  
T=time taken to separate the slides [22][23]

**4. Measurement of pH:** The gel formulation's pH was measured with a pH paper.[24]

**5. Viscosity:** The viscosity of the gel formulation was measured using a Brookfield viscometer. An appropriate spindle was selected and immersed in the gel sample maintained at  $25 \pm 1^\circ\text{C}$ . The measurement was carried out at a

fixed rotational speed, and the reading was recorded once it became stable.[25][26]

**6. Skin irritation test:** The formulated herbal and fungal gel was applied topically to the skin and monitored for any signs of irritation, erythema, or rash development.[27]

**7. Anti-fungal activity:** The inocula of them microorganism was prepared from the fungal cultures.15ml of Sabouraud Dextrose agar (Himedia) medium was poured into clean, sterilised Petriplates and allowed to cool and solidify.100 µl of broth of fungal strain was pipette out and spread over heme dium evenly with a spreading rod till it dried properly. Wells of 6mm in diameter were bored using a sterile corn borer. Solutions of the compounds(100µl/ml) were prepared in water, and 100µl of the prepared test solutions and standards were added to the wells. The petri plates were incubated at  $37^\circ\text{C}$  for 24 h. Miconazole (1mg/ml) was prepared as a positive control, and DMSO was used as a negative control. Antifungal activity was evaluated by measuring the diameters of the zone of inhibition (ZI).[28][29][30]

#### 5. RESULTS AND DISCUSSIONS

1. Physical evaluation
2. Clarity
3. Spreadability
4. Measurement of Ph
5. Viscosity
6. Skin irritation
7. Antifungal activity

**1. Physical evaluation:** The herbal antifungal gel formulation was observed to be yellowish-green in colour, with a pleasant odour and a smooth, uniform consistency.

**2. Clarity:** Visual inspection was performed to assess the clarity of the gel formulation. The herbal antifungal gel was found to be clear

**3. Spreadability:** Spreadability is an important parameter influencing patient compliance and ensures uniform application of the gel. An ideal gel formulation should exhibit good spreadability, requiring less time for application. The herbal antifungal gel formulation showed a spreadability value of 9.48g·cm/sec.



Fig 3. Spreadability

**4. Measurement of pH:** The antifungal gel formulation exhibited a pH of 6, which falls within the acceptable range of 4–7 for topical herbal gel formulations. This pH range is

suitable for skin application, indicating that the formulation is compatible with the skin.



Fig 4. Measurement of pH

**Viscosity:** The viscosity of the gel formulation was determined using a Brookfield viscometer. Viscosity is an important parameter for characterising gels, as it influences spreadability,

extrudability, and drug release. The herbal antifungal gel exhibited a viscosity of 1628cps



Fig 5. Viscosity

1. **Skin irritation test:** The prepared herbal antifungal gel formulation was applied to the skin of the hand and exposed to sunlight for 4–5 minutes. The formulation

was found to be non-irritating and compatible with the skin.



Fig 6. Skin irritation test (Before)



Fig 7. Skin irritation test (After)

8. **Anti-fungal Activity:**

The antifungal profile of B1, B2, and B3 was evaluated by measuring the zone of inhibition against fungal strains C.

albicans (ATCC 10231) via the well diffusion method. The compounds B1, B2, and B3 exhibited good activity as compared to the standard Miconazole.

SR. NO	SAMPLES	ZONEINDIAMETER (mm)
1	Control	00
2	Standard (Miconazole)	27
3	B1	21
4	B2	24
5	B3	26

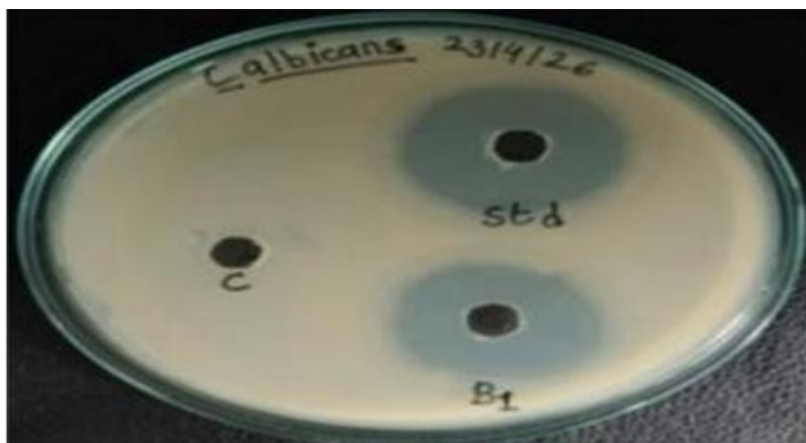


Fig 8. Anti-Fungal Activity of Sample B1



Fig 9. Anti-Fungal Activity of Sample B2



Fig 10. Anti-Fungal Activity of Sample B3

**6. RESULT AND DISCUSSION**

Following batch-wise evaluation of the formulations for parameters such as pH, viscosity, and spreadability, it was

observed that the formulation containing the tulsi leaf extract exhibited optimal performance. This formulation was therefore selected for further antifungal studies due to its desirable physicochemical characteristics.

**Various physical parameters such as colour, odour, pH, and viscosity**

1	B1	Yellowish green	Pleasant	6	1152cps
2	B2	Yellowish green	Pleasant	6	2244cps
3	B3	Yellowish green	Pleasant	6	1488cps

**Various parameters such as spreadability, clarity and skin irritation**

Sr. No	Formulation code	Spreadability	Clarity	SkinIrritation
1	B1	10	Quite Clear	No irritation
2	B2	9.3	Quite Clear	No irritation
3	B3	9.16	Quite Clear	No irritation

## 7. CONCLUSION

The present study focused on the formulation and evaluation of three batches (B1, B2, and B3) of herbal antifungal gel containing varying concentrations of *Ocimum sanctum* (Tulsi) extract. All formulations were evaluated for physicochemical parameters and antifungal activity. All three batches exhibited acceptable properties, including pH within the skin-compatible range (~6), good clarity, homogeneous consistency, and absence of skin irritation, indicating their suitability for topical application.

Comparative evaluation revealed that formulation variables, particularly the concentration of Tulsi extract, significantly influenced viscosity, spreadability, and antifungal activity. Batch B1 showed good spreadability with lower viscosity but comparatively lower antifungal activity. Batch B2 demonstrated higher viscosity and improved antifungal effect, though with slightly reduced spreadability. Batch B3 exhibited the highest antifungal activity, nearly comparable to the standard drug, along with acceptable viscosity and spreadability.

Overall, an increase in Tulsi extract concentration resulted in enhanced antifungal efficacy without adversely affecting the formulation characteristics. Among all batches, Batch B3 was identified as the optimised formulation due to its superior antifungal activity and satisfactory physicochemical properties. Thus, the developed herbal gel formulation shows promising potential as an effective and safe topical antifungal agent.

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