

Formulation and Evaluation of an Antifungal Herbal Gel Containing *Clitoria ternatea* Flower Extract and Sandalwood Oil

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Abstract

The present study aimed to formulate and evaluate a topical antifungal herbal gel containing an ethanolic flower extract of *Clitoria ternatea*, sandalwood oil, kalonji oil, and *Aloe vera*. Five experimental batches (F1-F5) were prepared using Carbopol 934 as the gelling agent, methyl paraben and propyl paraben as preservatives, glycerin as a humectant, and 10% sodium hydroxide as a neutralizing agent. The formulations were evaluated for organoleptic characteristics, homogeneity, pH, viscosity, spreadability, UV-visible absorbance, preliminary skin irritancy, short-term stability, and in vitro antifungal activity against *Candida albicans* by the agar-well diffusion method. Batch F5 was clear, semisolid, aromatic, and yellowish gold, with a pH of 5.74, viscosity of 18,000 mPa·s at 10 rpm, and the highest reported spreadability value of 8.2 among the tested batches. At 10 mg/mL, the herbal gel produced a 20 mm zone of inhibition against *C. albicans*, compared with 18 mm for the *C. ternatea* flower extract, 14 mm for sandalwood oil, and 29 mm for fluconazole at 1 mg/mL. The results indicate preliminary concentration-dependent antifungal activity and acceptable formulation characteristics, although confirmatory microbiological, stability, preservative-efficacy, and validated skin-safety studies are required.

Keywords: *Clitoria ternatea*; sandalwood oil; herbal gel; *Candida albicans*; antifungal activity; Carbopol 934.

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1. Introduction

Herbal medicines contain plant-derived materials or preparations and continue to contribute to traditional and contemporary health-care practices [1].

Topical hydrogels are three-dimensional hydrophilic polymer systems that can retain water and provide a semisolid vehicle for localized drug delivery [2].

Their non-greasy character, spreadability, and capacity to incorporate plant extracts and essential oils make them useful for dermatological formulations [2].

Fungal diseases range from superficial infections of the skin and appendages to severe systemic disease and represent an important but frequently underestimated health burden [3].

Candidiasis is primarily caused by *Candida* yeasts and may involve the skin, mucosal surfaces, or bloodstream under favourable host and environmental conditions [4].

Antifungal resistance and tolerance in *Candida albicans* can complicate treatment and support the need for continued investigation of new antifungal candidates [5].

Dandruff and seborrhoeic dermatitis are associated mainly with *Malassezia* species rather than *Candida albicans* [6].

Malassezia yeasts metabolize scalp lipids and are closely associated with flaking, pruritus, and seborrhoeic scalp conditions [7].

Clitoria ternatea is a perennial medicinal species of the family Fabaceae that contains anthocyanins, flavonoids, cyclotides, and other bioactive phytochemicals [8].

Extracts of *C. ternatea* have demonstrated antifungal activity against *C. albicans* in preliminary laboratory investigations [9].

Antifungal and antibiofilm effects of *C. ternatea* extract against *C. albicans* have also been reported in an oral-candidiasis model [10].

Sandalwood album oil contains sesquiterpenes such as alpha-santalol and has reported antimicrobial and anti-inflammatory activity relevant to topical products [11].

Broader reviews of the *Santalum* genus describe antifungal, antioxidant, and skin-related pharmacological activities of sandalwood extracts and oils [12].

Aloe vera gel is widely investigated as a topical ingredient because of its hydrating, soothing, and skin-supportive properties [13].

Accordingly, the present work formulated five herbal gel batches containing *C. ternatea* flower extract and sandalwood oil and evaluated their physicochemical properties and in vitro activity against *C. albicans* [9].

2. Materials and Methods

2.1 Materials

Clitoria ternatea flowers and *Aloe vera* were collected from a home garden in Vita, Maharashtra.

Sandalwood oil and kalonji oil were procured from Kalpana Ayurveda. Carbopol 934, methyl paraben, propyl paraben, glycerin, ethanol, 10% sodium hydroxide solution, and distilled water were obtained from commercial laboratory suppliers in Mumbai, India.

2.2 Collection and Authentication of Plant Material

Blue flowers of *Clitoria ternatea* were collected, washed, shade-dried, and stored in airtight zipper pouches. A herbarium specimen containing seeds, pods, stem, leaves, and flowers was prepared and verified at the Department of Botany, Balwant College. The specimen number was not reported in the source manuscript. *Aloe vera* leaves were collected from the same garden.

2.3 Preparation of *Clitoria ternatea* Flower Extract

The dried flowers were ground and passed through a 120-mesh sieve. Approximately 50 g of flower powder was macerated with 500 mL of 98% ethanol for five days. The mixture was filtered, the solvent was allowed to evaporate, and the concentrated extract was transferred to a sterile container and refrigerated until use.

2.4 Preparation of Herbal Gel Batches

All ingredients were accurately weighed or measured. Carbopol 934 was dispersed in 20 mL of distilled water and allowed to hydrate for 24 hours to obtain a lump-free dispersion (Solution A). The *C. ternatea* flower extract was dispersed in glycerin with mild heating, followed by incorporation of methyl paraben, propyl paraben, kalonji oil, and sandalwood oil with continuous stirring (Solution B). Solution B was gradually incorporated into Solution A with constant stirring. A 10% sodium hydroxide solution was added dropwise to neutralize the polymer and develop the gel structure, and distilled water was added to obtain a final batch weight of 20 g.

Table 1. Composition of experimental antifungal herbal gel batches (quantity per 20 g batch)

Ingredient	F1	F2	F3	F4	F5
<i>Clitoria ternatea</i> flower extract	0.5 g	0.6 g	0.4 g	0.7 g	0.5 g
Sandalwood oil	0.02 mL	0.01 mL	0.2 mL	0.4 mL	0.5 mL
Carbopol 934	2.0 g	1.5 g	1.0 g	0.5 g	0.25 g
Kalonji oil	0.2 mL	0.3 mL	0.4 mL	0.4 mL	0.5 mL
<i>Aloe vera</i> gel	0.5 mL	1.0 mL	3.0 mL	2.0 mL	2.0 mL
Methyl paraben	0.01 g	0.01 g	0.02 g	0.02 g	0.02 g

Ingredient	F1	F2	F3	F4	F5
Propyl paraben	0.01 g	0.01 g	0.02 g	0.02 g	0.02 g
Glycerin	0.2 mL	0.5 mL	1.0 mL	1.5 mL	2.0 mL
10% sodium hydroxide	q.s.	q.s.	q.s.	q.s.	q.s.
Distilled water	q.s. to 20 g	q.s. to 20 g	q.s. to 20 g	q.s. to 20 g	q.s. to 20 g

2.5 Evaluation of Herbal Gel Formulations

2.5.1 Visual and Organoleptic Assessment

Each formulation was examined for appearance, colour, odour, physical state, consistency, and texture.

2.5.2 Homogeneity

The formulations were visually inspected in suitable containers for clarity, aggregates, and non-uniform particles.

2.5.3 pH

One gram of gel was dispersed in 100 mL of distilled water, and the pH was measured using a calibrated digital pH meter.

2.5.4 Viscosity

Viscosity was determined using a Brookfield rotational viscometer fitted with spindle number 7 at 10 rpm after allowing each sample to equilibrate for two minutes. The source manuscript states that measurements were performed in triplicate.

2.5.5 Spreadability

Spreadability was evaluated by determining the ease with which the formulation spread over a glass surface. The source manuscript did not specify the calculation formula or measurement unit.

2.5.6 UV-Visible Spectrophotometry

One gram of gel was dissolved in ethanol, filtered, and analysed using a UV-visible spectrophotometer. Absorbance was reported at 350 nm.

2.5.7 Preliminary Irritancy Observation

A small amount of each formulation was applied to the scalp skin and observed for five minutes for visible irritation. An ethics approval number, participant details, and a validated irritation-scoring method were not provided in the source manuscript.

2.5.8 Stability Observation

The selected formulation was stored under accelerated temperature conditions of $40 \pm 2^\circ\text{C}$ and was observed for changes in colour, odour, pH, texture, and consistency. Relative humidity conditions and complete three-month observations were not supplied in the source data.

2.6 In Vitro Antifungal Activity

Antifungal activity was evaluated against *Candida albicans* by the agar-well diffusion method. A culture suspension was incorporated into

sterile molten medium maintained at approximately 40-45 °C, and 15-20 mL of inoculated medium was poured into sterile Petri plates to obtain a depth of approximately 3-4 mm. After solidification, wells of approximately 6 mm diameter were prepared using a sterile stainless-steel borer. The test samples were introduced into the wells, and the resulting zones of inhibition were measured in millimetres. Fluconazole at 1 mg/mL served as the standard.

3. Results

3.1 Preliminary Phytochemical Screening

The reported qualitative phytochemical screening results are presented in Tables 2-4.

Table 2. Qualitative phytochemical screening of Clitoria ternatea flower extract

Sr. No.	Phytochemical class	Inference
1	Alkaloids	Positive
2	Flavonoids	Positive
3	Saponins	Positive
4	Tannins	Positive
5	Glycosides	Positive
6	Phenols	Positive
7	Steroids	Positive
8	Quinones	Positive
9	Carbohydrates	Positive

Table 3. Qualitative phytochemical screening of sandalwood oil

Sr. No.	Phytochemical class	Inference
1	Terpenoids	Positive
2	Phenolic compounds	Positive
3	Flavonoids	Positive
4	Tannins	Positive
5	Saponins	Positive

Table 4. Qualitative phytochemical screening of kalonji oil

Sr. No.	Phytochemical class	Inference
1	Alkaloids	Positive
2	Flavonoids	Negative
3	Terpenoids	Positive
4	Quinones	Positive

3.2 Organoleptic and Physical Evaluation

Table 5. Organoleptic characteristics of antifungal herbal gel batches

Parameter	F1	F2	F3	F4	F5
Physical state	Solid	Solid	Liquid	Semisolid	Semisolid
Odour	Unpleasant	Unpleasant	Aromatic	Aromatic	Aromatic
Colour	Mustard	Yellowish white	Yellowish gold	Yellowish gold	Yellowish gold
Texture	Uneven	Uneven	Smooth	Smooth	Smooth

Table 6. Homogeneity and clarity of antifungal herbal gel batches

Test	F1	F2	F3	F4	F5
Visual clarity	Not clear	Not clear	Not clear	Not clear	Clear

Table 7. pH values of antifungal herbal gel batches

Batch	pH
F1	6.20
F2	7.02
F3	6.80
F4	5.74
F5	5.74

Table 8. Viscosity of antifungal herbal gel batches at 10 rpm

Batch	Rotational speed (rpm)	Viscosity (mPa·s)
F1	10	25,000
F2	10	23,000
F3	10	22,600
F4	10	20,000
F5	10	18,000

Table 9. Reported spreadability values of antifungal herbal gel batches

Batch	Reported spreadability value
F1	5.8
F2	6.5
F3	7.3
F4	7.9
F5	8.2

Table 10. Reported UV-visible absorbance data at 350 nm

Sr. No.	Sample	Aliquot volume (mL)	Concentration (µg/mL)	Absorbance
1	Standard 1	1	10	0.050
2	Standard 2	2	20	0.027
3	Standard 3	3	30	0.040
4	Standard 4	4	40	0.060
5	Standard 5	5	50	0.114
6	Test sample T1	-	-	0.055

Table 11. Preliminary irritancy observations for antifungal herbal gel batches

Batch	Observation
F1	No visible irritation
F2	No visible irritation
F3	No visible irritation
F4	No visible irritation
F5	No visible irritation

Table 12. Reported short-term stability observations of the selected formulation

Parameter	Initial observation	After 1 month
Colour	No change	No change
Odour	No change	No change
pH	No change	No change
Texture	No change	No change

Parameter	Initial observation	After 1 month
Consistency	No change	No change

3.3 In Vitro Antifungal Activity

Table 13. Antifungal activity of the herbal gel against *Candida albicans*

Sample	Concentration	Zone of inhibition (mm)
Control	-	0
Fluconazole standard	1 mg/mL	29
Antifungal herbal gel	5 mg/mL	3
Antifungal herbal gel	10 mg/mL	20

Table 14. Antifungal activity of *Clitoria ternatea* flower extract against *Candida albicans*

Sample	Concentration	Zone of inhibition (mm)
Control	-	0
Fluconazole standard	1 mg/mL	29
<i>Clitoria ternatea</i> flower extract	5 mg/mL	1
<i>Clitoria ternatea</i> flower extract	10 mg/mL	18

Table 15. Antifungal activity of sandalwood oil against *Candida albicans*

Sample	Concentration	Zone of inhibition (mm)
Control	-	0
Fluconazole standard	1 mg/mL	29
Sandalwood oil	5 mg/mL	1
Sandalwood oil	10 mg/mL	14

4. Discussion

The progressive reduction in Carbopol 934 concentration from F1 to F5 was accompanied by a reduction in viscosity and an increase in the reported spreadability values. Batches F3-F5 displayed smooth textures and aromatic odour, while F5 was the only batch reported as visually clear. The pH of F5 was 5.74, which is closer to the mildly acidic environment generally preferred for topical scalp formulations than the pH values of F1-F3.

The agar-well diffusion results showed concentration-dependent activity for the combined

herbal gel, *C. ternatea* flower extract, and sandalwood oil. At 10 mg/mL, the combined gel produced a larger inhibition zone than either individual test ingredient under the reported experimental conditions. However, this difference alone does not establish pharmacological synergy because the equivalent concentrations of each active ingredient within the combined formulation were not specified and no formal synergy assay was performed.

Although the study used *C. albicans* as the test organism, dandruff is primarily associated with *Malassezia* species. Therefore, the findings demonstrate preliminary anti-Candida activity and should not be interpreted as direct evidence of antidandruff efficacy without testing against clinically relevant *Malassezia* strains.

The UV-visible standard readings were not consistently proportional to concentration, and no calibration equation, correlation coefficient, replicate data, or assay validation was supplied. The analytical procedure should therefore be repeated using a validated method before quantitative conclusions are drawn.

The preliminary irritancy and stability observations were favourable, but the short observation period, absence of a validated irritation scale, missing ethics details, and incomplete accelerated-stability conditions limit interpretation. Further work should include standardized microbiological assays, minimum inhibitory concentration testing, preservative-efficacy testing, rheological characterization, content uniformity, in vitro release, longer-term stability, and ethically approved skin-safety evaluation.

5. Conclusion

An antifungal herbal gel containing *Clitoria ternatea* flower extract, sandalwood oil, kalonji oil, and *Aloe vera* was prepared in five experimental batches. Based on the reported organoleptic properties, clarity, pH, viscosity, and spreadability, batch F5 was selected as the most suitable preliminary formulation. The gel showed concentration-dependent inhibition of *Candida albicans*, with a 20 mm zone of inhibition at 10 mg/mL compared with 29 mm for fluconazole at 1 mg/mL. The results support further investigation of the formulation as a topical anti-Candida preparation; however, direct antidandruff claims, synergistic effects, clinical safety, and therapeutic efficacy require confirmation through appropriately controlled studies.

Conflict of Interest

The authors declare no conflict of interest.

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