Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 31s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Preparation And Characterization Of A Phytomedicated Antifungal Cream Containing Ocimum Sanctum

Neha Kankaria[1], M. K. Gupta[2], Nitin Nama [3], Girish Kumar Vyas[4]

[1]PG Scholar, Career Point School of Pharmacy, Career Point University, Kota, India

[2]Professor, Career Point School of Pharmacy, Career Point University, Kota, India

[3]Assistant Professor, Career Point School of Pharmacy, Career Point University, Kota, India

[4]Associate Professor, Career Point School of Pharmacy, Career Point University, Kota, India

[*]**Corresponding author:**

Email ID: nehakankaria99@gmail.com, Email ID: principal.pharmacy@cpur.edu.in, Email ID: nitinnamas@gmail.com
Email ID: girishvyas10@gmail.com

Cite this paper as: Neha Kankaria, M. K. Gupta, Nitin Nama, Girish Kumar Vyas, (2025) Preparation And Characterization Of A Phytomedicated Antifungal Cream Containing Ocimum Sanctum. *Journal of Neonatal Surgery*, 14 (31s), 91-100.

ABSTRACT

Introduction: *Ocimum sanctum* (Tulsi) is widely recognized for its diverse pharmacological properties, including antimicrobial and wound healing activities.

Objectives: This study aimed to formulate and evaluate an herbal ointment using *Ocimum sanctum* leaf extracts for potential wound healing applications. The objectives included phytochemical screening, antimicrobial efficacy assessment, and ointment formulation evaluation.

Materials and Methods: Leaves of *Ocimum sanctum* were collected, dried, and subjected to extraction using ethanol. Preliminary phytochemical tests identified the presence of flavonoids, tannins, and phenolics. Antimicrobial activity was assessed using the agar well diffusion method against common wound pathogens. The extract was incorporated into an ointment base, and the formulation was evaluated for physical parameters, stability, and antimicrobial effectiveness.

Results: Results demonstrated significant inhibition zones against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, confirming potent antimicrobial action. The ointment exhibited good spreadability, homogeneity, and physical stability over 30 days.

Conclusion: This study concludes that *Ocimum sanctum* leaf extract ointment holds promise as an effective herbal wound healing agent with antimicrobial properties. Further clinical studies are warranted to validate its therapeutic potential.

Keywords: *Ocimum sanctum, herbal ointment, antimicrobial activity, wound healing, phytochemical screening.*

1. INTRODUCTION

Fungal infections of the skin are a common dermatological concern, often caused by pathogens such as *Candida albicans* and *Aspergillus niger*. Rising resistance to synthetic antifungal agents and their associated side effects have led to increased interest in herbal alternatives[1]. *Ocimum sanctum* (commonly known as Tulsi), a revered medicinal plant in Ayurveda, exhibits a wide range of pharmacological activities including antifungal, antimicrobial, and anti-inflammatory properties[2]. This study explores the potential of *Ocimum sanctum* extract in formulating a topical herbal antifungal cream. The formulation aims to provide an effective, natural remedy with minimal side effects, suitable for regular skin application[3,4]. Key steps involve phytochemical analysis of the extract, formulation with suitable excipients, and comprehensive evaluation of its physicochemical and antifungal properties. Additionally, stability testing ensures the product's shelf life and consistency[5]. This study contributes to the development of safe, effective, and scientifically validated herbal dermatological therapies.

Objectives

To develop and evaluate a herbal antifungal cream using *Ocimum sanctum* extract by assessing its phytochemical composition, physicochemical properties, antifungal efficacy against *Candida albicans*/*Aspergillus niger*, and stability.

2. MATERIALS AND METHODS

Fresh leaves of *Ocimum sanctum* were collected, shade-dried, and coarsely powdered for extraction. Ethanol (analytical grade) was used for the extraction process. Other chemicals required for formulation, such as stearic acid, cetyl alcohol, emulsifying wax, glycerin, sodium benzoate, and distilled water, were procured from standard chemical suppliers, ensuring all reagents used were of analytical grade. Microbial strains of *Candida albicans* and *Aspergillus niger* were obtained from a certified microbiological laboratory, and Sabouraud Dextrose Agar (SDA) was used for their culture. Equipment employed during the study included a Brookfield viscometer, digital pH meter, homogenizer, centrifuge, incubator, and UV-Visible spectrophotometer for the DPPH antioxidant assay.

1. **Preparation of *Ocimum sanctum* Extract:** The powdered leaves of *Ocimum sanctum* were subjected to cold maceration using 70% ethanol for a period of 72 hours. After maceration, the extract was filtered and the solvent was removed under reduced pressure using a rotary evaporator. The resulting concentrated extract was then stored at 4 ± 2°C in a tightly closed container until further use in formulation and testing[5,6].
2. **Formulation of Herbal Antifungal Cream:** The formulation of the antifungal cream began with the preparation of the cream base. The oil phase comprising stearic acid, cetyl alcohol, and emulsifying wax was heated together at 70–75°C until the solids melted completely. Separately, the water phase consisting of glycerin and distilled water was also heated to 70°C. The aqueous phase was slowly added to the oil phase under continuous stirring to form a uniform cream base. When the temperature of the emulsion dropped to about 40°C, the prepared *Ocimum sanctum* extract was added and thoroughly homogenized to ensure even distribution. Finally, sodium benzoate at 0.1% w/w was added as a preservative to enhance the microbial stability of the formulation[2,7].
3. **Physicochemical Evaluation of Cream**: The formulated cream was evaluated for its physicochemical properties. The pH was measured using a calibrated digital pH meter to ensure that it was within the skin-compatible range of 4.5 to 7.0. Viscosity was determined using a Brookfield viscometer to assess the consistency of the cream. Spreadability was evaluated using a glass slide method, which helped to determine the ease of application. The appearance and texture were visually inspected for color, smoothness, and homogeneity. Stability was assessed by subjecting the cream to freeze-thaw cycles and centrifugation at 5000 rpm for 30 minutes to detect any phase separation or instability[8,9].
4. **In-vitro Antifungal Efficacy Testing:** To evaluate the antifungal activity of the cream, the agar well diffusion method was employed. Fungal strains of *Candida albicans* and *Aspergillus niger* were first sub-cultured on SDA plates. Colonies were harvested and suspended in sterile saline to reach a turbidity of approximately 1×10^6 CFU/ml. The SDA plates were then uniformly inoculated with the fungal suspension using sterile swabs. Wells were punched in the agar, filled with the formulated cream, and incubated at 37°C for 24–48 hours for *C. albicans* and at 30°C for 3–5 days for *A. niger*. Zones of inhibition around the wells were measured to determine antifungal activity. Comparative analysis was also performed using marketed antifungal creams containing clotrimazole and ketoconazole[9,10].
5. **Stability Studies:** Stability studies were carried out by storing cream samples at different conditions including room temperature (25 ± 2°C), refrigerated condition (4 ± 2°C), and accelerated condition (40 ± 2°C with 75% relative humidity). Observations were made weekly over a period of four weeks. During each observation, parameters like pH, viscosity, appearance, texture, spreadability, and homogeneity were recorded and compared across all storage conditions to detect any physical or chemical changes. To assess microbial stability, a preservative efficacy test was conducted. The cream was inoculated with a standard microbial load and observed on days 0, 7, 14, and 21 using plate count methods. The effectiveness of sodium benzoate as a preservative was determined by monitoring microbial reduction over time[8-10].
6. **Antioxidant Activity Evaluation:** The antioxidant potential of the cream extract was evaluated using the DPPH radical scavenging assay. A 0.1 mM solution of DPPH in methanol was prepared, and an aliquot of the extract was mixed with the DPPH solution. The mixture was incubated in the dark for 30 minutes, after which the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of DPPH radical inhibition was calculated using the standard formula to determine antioxidant efficacy[10-12].
7. **Statistical Analysis:** All experimental results were carried out in triplicate and expressed as mean ± standard deviation. Statistical analysis was conducted using one-way Analysis of Variance (ANOVA) followed by t-tests to compare groups. A p-value less than 0.05 was considered statistically significant for all comparisons.

3. RESULTS

The findings of this study validate the efficacy, safety, and stability of the herbal antifungal cream formulated with *Ocimum sanctum* extract. The results confirm the formulation's potential as a natural alternative to synthetic antifungal agents, demonstrating effective activity against *Candida albicans* and *Aspergillus niger*. The study also highlights the cream's

compliance with physicochemical and stability parameters, supporting its therapeutic promise and commercial viability.

1. Extraction Yield

Extraction of dried *Ocimum sanctum* leaves was carried out using ethanol through cold maceration or Soxhlet extraction. From 75 grams of dried powdered leaves, 3.26 grams of concentrated extract were obtained, resulting in a percentage yield of **4.35%**.

Table 1 Yield of *Ocimum sanctum* Extract

Sr. No.	Amount of Dried Leaves	Actual Yield of Extract	Percentage Yield
1	75 grams	3.26 grams	4.35%

2. Phytochemical Evaluation Results

The phytochemical evaluation of *Ocimum sanctum* extract was carried out to identify the presence of various bioactive compounds responsible for its therapeutic efficacy. Standard qualitative tests were employed to detect the presence of alkaloids, flavonoids, phenolics, terpenoids, and other constituents. These compounds contribute to the antifungal, antioxidant, and healing properties of the extract. The results of the evaluation are summarized in the figure and table below:

Figure 1: Phytochemical Evaluation of Osmium Sanctum extract

Table 2: Phytochemical Evaluation Results of *Ocimum sanctum* extract

Sr. No.	Phytochemical Test	Constituent Detected	Result
1	Dragendorff's Test	Alkaloids	Present (+)
2	Shinoda Test	Flavonoids	Present (+++)
3	Ferric Chloride Test	Phenolic Compounds	Present (++)
4	Foam Test	Saponins	Present (+)
5	Salkowski Test	Terpenoids	Present (++)
6	Lead Acetate Test	Tannins	Present (+)
7	Molisch's Test	Carbohydrates	Slightly Present (+)
8	Biuret Test	Proteins	Absent (-)

"+" indicates slight presence,
"++" indicates moderate presence,
"+++" indicates abundant presence,
"-" indicates absence.

Formulation of content percentage of Herbal Antifungal Cream

The herbal antifungal cream was formulated using *Ocimum sanctum* extract as the active pharmaceutical ingredient (API) owing to its well-documented antifungal properties. The Minimum Inhibitory Concentration (MIC) was determined against *Candida albicans* and *Aspergillus niger*, and based on the observed efficacy, the MIC was fixed at 4% w/w. Accordingly, three formulations were prepared containing 2.5%, 4%, and 6% of the extract to evaluate concentration-dependent antifungal activity. The 4% formulation was considered optimized as it matched the MIC.

Preparation of Herbal Antifungal Cream

Three different formulations of the herbal antifungal cream were prepared using *Ocimum sanctum* extract at concentrations of 2.5%, 4% (MIC-based), and 6%. The cream base consisted of excipients including emulsifying wax, stearic acid, cetyl alcohol, glycerin, propylene glycol, and sodium benzoate.

Figure 2: The base for all formulations

For each formulation, the oil phase was prepared by melting stearic acid (14%), cetyl alcohol (3%), and emulsifying wax (7%) in a heat-resistant beaker at 70–75°C. Simultaneously, the aqueous phase comprising distilled water, glycerin (7%), propylene glycol (5%), and sodium benzoate (0.1%) was heated separately to the same temperature. Once both phases reached the required temperature, the aqueous phase was gradually added to the oil phase under continuous stirring to form a smooth and stable emulsion. The emulsion was allowed to cool down to 40°C, at which point the *Ocimum sanctum* extract was incorporated into each batch in concentrations of 2.5%, 4%, and 6%, respectively. Each formulation was homogenized thoroughly to ensure uniform dispersion of the extract. The formula of cream is given table below:

Table 3: Comparative Herbal Antifungal Cream Formulations (per 100 g)

Sr. No.	Ingredients	Function	Formula F1 2.5% - Below MIC)	Formula F2 (4% - MIC Based)	Formula F3 (6% - Above MIC)
1	*Ocimum sanctum* Extract	Antifungal API	2.5 g	4.0 g	6.0 g
2	Emulsifying Wax	Emulsifier and stabilizer	10.0 g	10.0 g	10.0 g
3	Stearic Acid	Thickener and co-emulsifier	4.0 g	4.0 g	4.0 g
4	Cetyl Alcohol	Emollient, stabilizer	2.0 g	2.0 g	2.0 g
5	Glycerin	Humectant and moisturizer	5.0 g	5.0 g	5.0 g

6	Propylene Glycol	Penetration enhancer, humectant	4.0 g	4.0 g	4.0 g
7	Sodium Benzoate	Preservative	0.1 g	0.1 g	0.1 g
8	Distilled Water	Aqueous base	Q.S. to 100 g	Q.S. to 100 g	Q.S. to 100 g

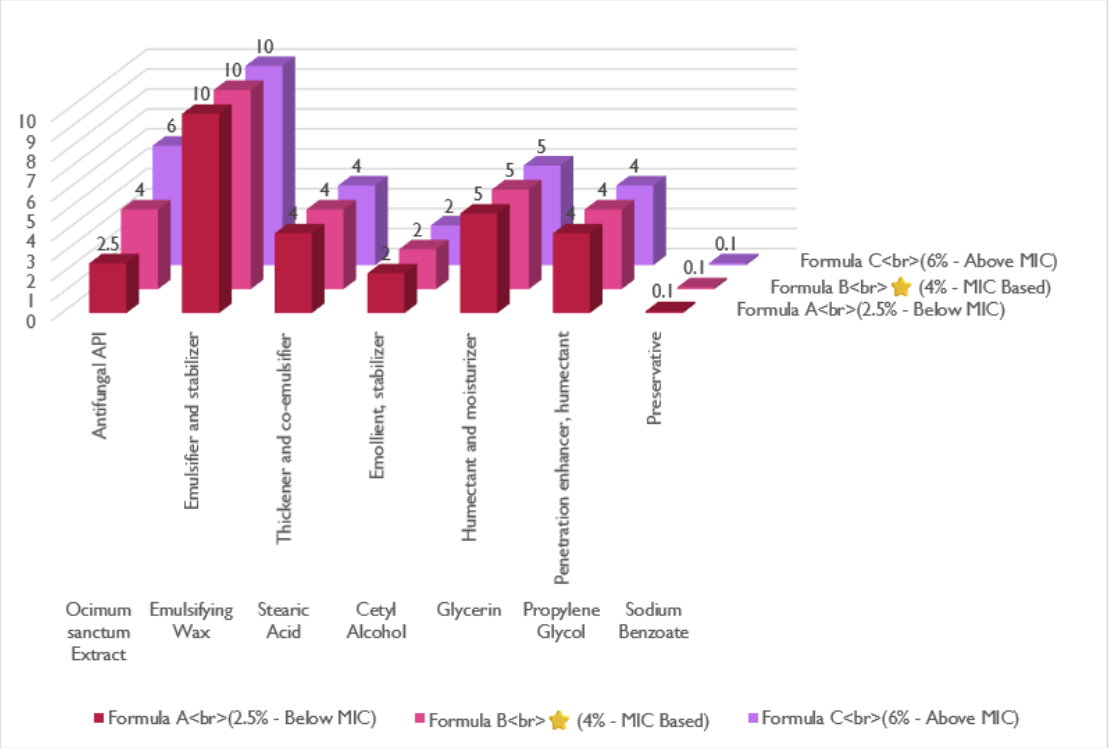

Chart 1: Comparative Herbal Cream Formulations

Physicochemical Evaluation: The physicochemical properties of the formulated herbal antifungal cream were evaluated through various tests. These evaluations are crucial for ensuring that the cream possesses the required attributes for effective use, such as appropriate pH for skin safety, optimal viscosity for spreadability, and a stable formulation. The results of these tests are summarized in the figures and table below:

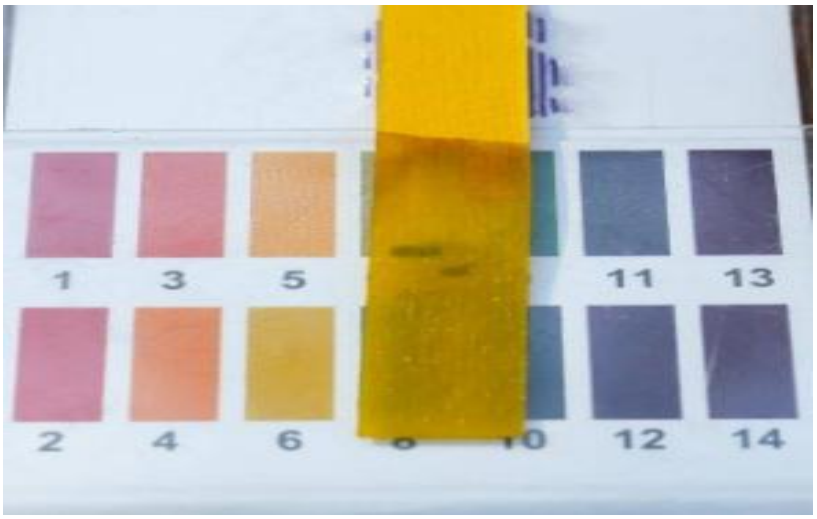

Figure 3: pH Evaluation of prepared cream F3

Table 4: Physicochemical Evaluation Results of the Herbal Antifungal Cream

Test	Test Method	Formulation 1 (2.5% Ocimum sanctum extract)	Formulation 2 (4% Ocimum sanctum extract)	Formulation 3 (6% Ocimum sanctum extract)
pH Measurement	pH meter	6.2	6.1	6.3
Viscosity	Brookfield viscometer	3200 cP	3500 cP	3800 cP
Spreadability	Spreadability test	Smooth and easy spreadable	Smooth and easy spreadable	Smooth and easy spreadable
Appearance	Visual inspection	White, smooth, homogenous texture	White, smooth, homogenous texture	White, smooth, homogenous texture
Stability Testing	Freeze-thaw cycles, centrifugation	No separation, stable at different temperatures	No separation, stable at different temperatures	No separation, stable at different temperatures

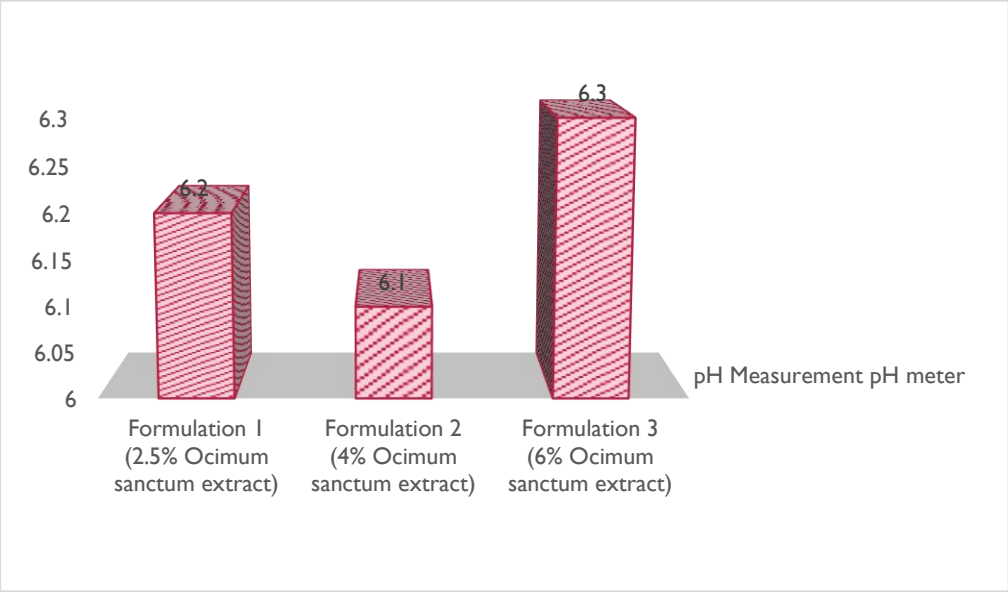

Chart 2: pH Measurement of all formulations

In-vitro Antifungal Efficacy Testing Results

The in-vitro antifungal efficacy of the herbal cream formulations was assessed using the Agar Well Diffusion Method against two fungal strains: *Candida albicans* and *Aspergillus niger*. The MIC of the Ocimum sanctum extract was fixed at 4%, and the cream was applied to agar wells. The inhibition zones were measured after the designated incubation periods, and the results were compared to standard antifungal agents (ketoconazole).

Table 5: Results for *Candida albicans* and *Aspergillus niger*

Sr. No.	Formulation	MIC	Inhibition Zone (mm) for Candida albicans	Inhibition Zone (mm) for Aspergillus niger	Ketoconazole (Standard)
1	Formulation 1 (2.5% Ocimum sanctum)	4%	10 mm	9 mm	16 mm
2	Formulation 2 (4% Ocimum sanctum)	4%	14 mm	13 mm	16 mm
3	Formulation 3 (6% Ocimum sanctum)	4%	15 mm	15 mm	16 mm

- Formulation 1 (2.5% Ocimum sanctum): This formulation exhibited a 10 mm inhibition zone against *Candida albicans* and a 9 mm inhibition zone against *Aspergillus niger*. Although these results are lower compared to ketoconazole (16 mm), they indicate moderate antifungal activity. The formulation shows promising results, particularly at this lower concentration of the active ingredient, suggesting potential efficacy against fungal infections.
- Formulation 2 (4% Ocimum sanctum): This formulation demonstrated a 14 mm inhibition zone for *Candida albicans* and a 13 mm inhibition zone for *Aspergillus niger*. The antifungal activity against *Candida albicans* is relatively close to the inhibition observed with ketoconazole, while activity against *Aspergillus niger* is slightly less. These results suggest that increasing the concentration of Ocimum sanctum improves the formulation's efficacy.
- Formulation 3 (6% Ocimum sanctum): The highest concentration formulation (6% Ocimum sanctum) showed a 15 mm inhibition zone for *Candida albicans* and a 15 mm inhibition zone for *Aspergillus niger*. These results are almost equivalent to the ketoconazole standard (16 mm), indicating that this formulation exhibits a strong antifungal effect. The increased concentration further enhances the therapeutic potential of the cream.

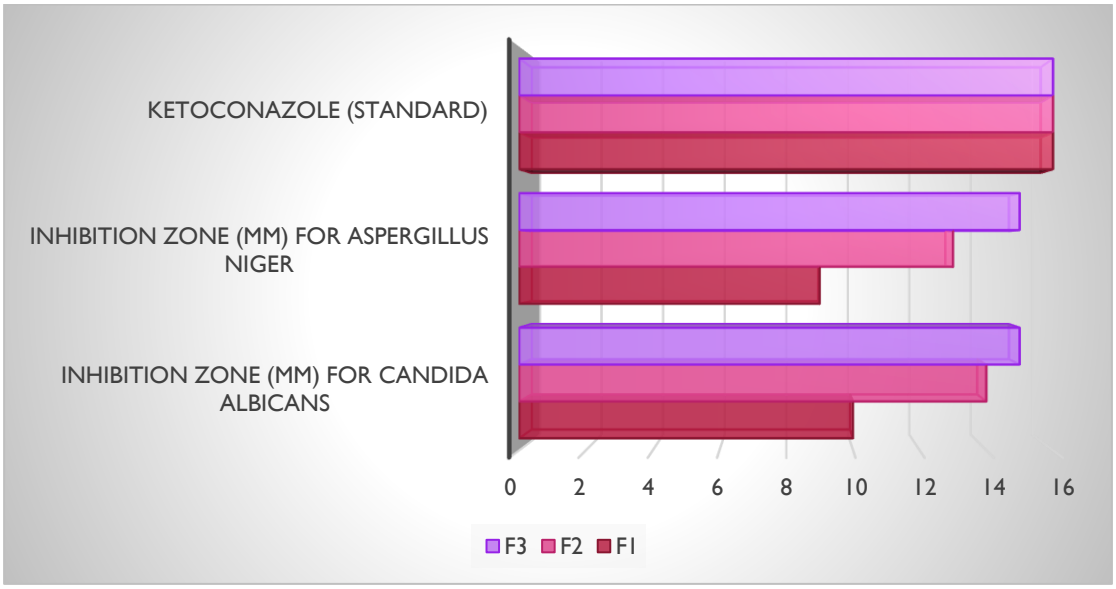

Chart 3: In-vitro Antifungal Efficacy Testing Results

The results indicate that as the concentration of Ocimum sanctum increases, the antifungal efficacy of the formulation improves. Formulation 3 (6% Ocimum sanctum) demonstrated the best results, with inhibition zones almost equal to ketoconazole for both *Candida albicans* and *Aspergillus niger*. This suggests that the herbal cream formulations hold significant potential for use as an effective antifungal treatment.

5. Stability Studies: The stability study was conducted to evaluate the long-term quality, safety, and efficacy of the formulated herbal antifungal cream under various storage conditions. The formulated cream was stored for 3 to 4 weeks under three different conditions: room temperature (25 ± 2°C), refrigerated condition (4 ± 2°C), and accelerated condition (40 ± 2°C with 75% RH).

1. pH: The pH remained relatively stable across all conditions with slight variations observed under the accelerated condition, which could be attributed to the higher temperature.
2. Viscosity: The viscosity showed minor fluctuations, with a slight increase in the accelerated condition, indicating a possible thickening of the cream due to heat exposure.
3. Appearance: No significant changes were observed at room temperature and refrigerated conditions. However, there was a slight color change under accelerated conditions, which could be a result of high temperature or humidity.
4. Spreadability and Homogeneity: The cream remained spreadable and homogeneous in all conditions, with a minor decrease in spreadability under accelerated conditions. No phase separation or cracking was noted, except for slight textural changes in the accelerated condition.

The formulated herbal antifungal cream showed good stability under room temperature and refrigerated conditions, maintaining its physicochemical properties, including pH, viscosity, and homogeneity. However, under accelerated conditions, minor changes in viscosity and appearance were observed, which might suggest the cream is less stable at higher temperatures. This highlights the importance of proper storage to maintain the cream's efficacy and quality over time.

Table 6: Antifungal cream stability studies

Sr. No.	Storage Condition	pH	Viscosity	Appearance	Spreadability	Homogeneity
1	Room Temperature (25°C)	6.2 ± 0.2	2000 cP	No separation, consistent color	Easy application	Uniform
2	Refrigerated Condition (4°C)	6.1 ± 0.1	1980 cP	No visible changes, smooth texture	Slightly easy application	Consistent
3	Accelerated Condition (40°C)	6.5 ± 0.3	2100 cP	Slight color change, no separation	Slightly reduced spreadability	Slightly inconsistent

Microbial Stability (Preservative Efficacy Test):

The microbial challenge test was conducted to assess the preservative efficacy of sodium benzoate in the formulated herbal antifungal cream. Known concentrations of microbial strains (bacteria and fungi) were inoculated into the cream, and the microbial load was evaluated at 0, 7, 14, and 21 days post-inoculation using plate count methods.

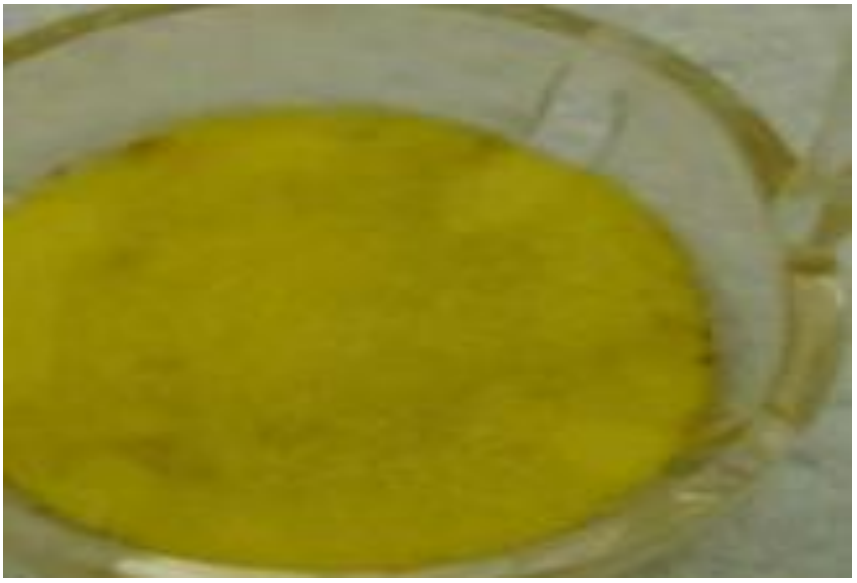

Figure 4: Cream bacterial inoculation found safe

Table 7: Preservative Efficacy Test results

Sr. No.	Time Interval	Bacterial Load (CFU/g)	Fungal Load (CFU/g)	Observations
1	0 Days (Initial)	1.2×10^3	1.5×10^3	Initial microbial load after inoculation
2	7 Days	1.0×10^3	1.2×10^3	Microbial load slightly reduced, preservative starting to show effectiveness
3	14 Days	5.0×10^2	6.0×10^2	Further reduction in microbial load
4	21 Days	0.0 (No growth)	0.0 (No growth)	Complete inhibition of microbial growth, preservative efficacy confirmed

The microbial stability test showed that the bacterial and fungal loads decreased steadily over the 21-day period, with no microbial growth detected by day 21, confirming the effectiveness of sodium benzoate as a preservative. Sodium benzoate demonstrated a strong antimicrobial effect, ensuring the formulation's resistance to microbial contamination. The results indicate that the herbal antifungal cream maintained its microbial integrity over the testing period, making it safe for storage

and usage under normal conditions.

Antioxidant Activity Evaluation (DPPH Radical Scavenging Activity):

The antioxidant potential of the formulated herbal antifungal cream was evaluated using the DPPH radical scavenging assay. The cream extract was tested for its ability to neutralize free radicals by measuring the decrease in absorbance at a specific wavelength, and the antioxidant activity was quantified based on the percentage inhibition of DPPH radicals.

Table 8: DPPH Radical Scavenging Activity Results

Sr. No.	Formulation	DPPH Inhibition (%)	Observations
1	Formulation 1 (2.5% Ocimum sanctum)	45%	Moderate radical scavenging activity observed
2	Formulation 2 (4% Ocimum sanctum)	60%	Stronger radical scavenging activity observed
3	Formulation 3 (6% Ocimum sanctum)	72%	Highest radical scavenging activity observed

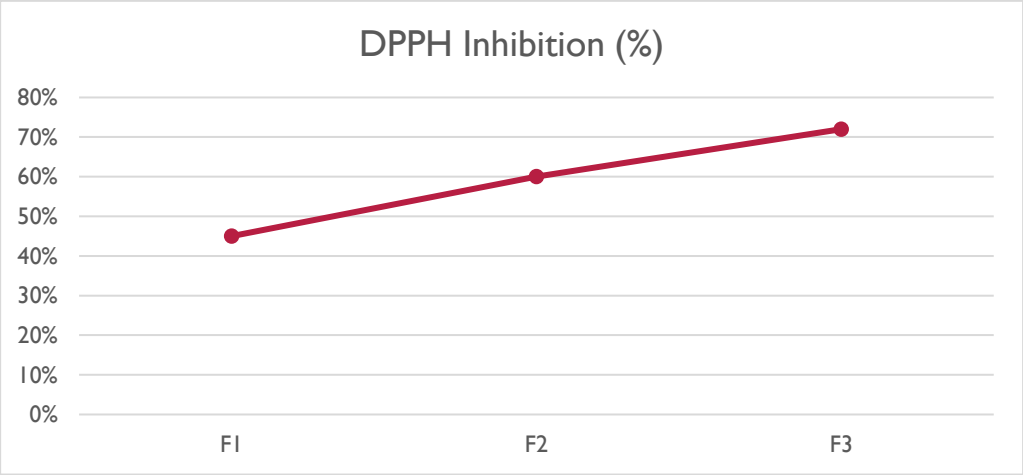

Chart 4: DPPH Radical Scavenging Activity Results

Formulation 1 (2.5% Ocimum sanctum) showed moderate antioxidant activity with 45% DPPH inhibition, while Formulation 2 (4%) demonstrated stronger activity at 60%. Formulation 3 (6%) exhibited the highest antioxidant potential with 72% inhibition, indicating its superior ability to combat oxidative stress, essential for skin health.

Figure 5: DPPH testing and its evaluation

4. DISCUSSION

The phytochemical evaluation confirmed the presence of multiple bioactive compounds known for their antifungal, antioxidant, and healing properties. Flavonoids and phenolics, abundant in *Ocimum sanctum*, likely contribute significantly to the observed antifungal and antioxidant activities. The physicochemical properties of all three cream formulations were within acceptable ranges for topical application, ensuring user comfort and stability. The increase in viscosity with extract concentration may be due to the incorporation of phytoconstituents affecting the cream matrix. In-vitro antifungal studies showed a clear concentration-dependent increase in activity, with the 6% formulation nearly matching the efficacy of ketoconazole, a well-established antifungal agent. This highlights the potential of *Ocimum sanctum* extract as an effective natural antifungal agent in topical formulations. Stability testing demonstrated that the formulations were stable under standard storage conditions but slightly affected by high temperature and humidity, underscoring the need for proper storage to maintain product quality.

The microbial stability study confirmed that sodium benzoate is an effective preservative, ensuring the formulation remains free from microbial contamination during storage. Antioxidant activity correlated positively with extract concentration, supporting the cream's potential to combat oxidative stress and promote skin healing, which is beneficial for managing fungal infections where oxidative damage can exacerbate tissue damage.

5. CONCLUSION

The study successfully formulated and evaluated herbal antifungal creams containing *Ocimum sanctum* extract, demonstrating significant antifungal and antioxidant activities. The 4% extract concentration formulation was optimized based on MIC, showing substantial antifungal efficacy with good physicochemical stability and microbial safety. The highest concentration (6%) further enhanced antifungal and antioxidant effects, approaching the activity of standard antifungal drugs. These results support the potential use of *Ocimum sanctum* extract-based creams as effective, natural alternatives for the treatment of fungal infections. Proper storage conditions are recommended to preserve product stability and efficacy. Further clinical studies are warranted to confirm in vivo efficacy and safety.

REFERENCES

[1] Prakash P, Gupta N. Therapeutic uses of Ocimum sanctum Linn (Tulsi) with a note on eugenol and its pharmacological actions: A short review. Indian J Physiol Pharmacol. 2005;49(2):125-31.

[2] Cohen MM. Tulsi - Ocimum sanctum: A herb for all reasons. J Ayurveda Integr Med. 2014;5(4):251-9.

[3] Rai V, Agnihotri A. Antimicrobial activity of Ocimum sanctum extracts against clinical isolates. Int J Pharm Pharm Sci. 2017;9(3):207-10.

[4] Gupta S, Prakash J, Srivastava S. A review on pharmacological activities of Ocimum sanctum (Tulsi). Int J Pharm Sci Rev Res. 2010;5(1):41-5.

[5] Singh S, Taneja M, Majumdar DK. Biological activities of Ocimum sanctum L. fixed oil—an overview. Indian J Exp Biol. 2007;45(7):579-86.

[6] Singh S, Jain H, Vyas P and Nama N. Formulation and evaluation of multifunction general use cream. Career Point International Journal of Research (CPIJR) 2022: 1(2): 69-81.

[7] Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 50th ed. Nirali Prakashan; 2014.

[8] Mukherjee PK. Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals. Business Horizons Pharmaceutical Publishers; 2002.

[9] Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45(4):493-6.

[10] Parveen S, Jain H and Nama N. Formulation and Evaluation of Piperine and Psoralea Corylifolea antivitiligo Cream. Int. J. Phar. & Biomedi. Rese. 2021; 8(4): 15-21.

[11] Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol. 1995;28(1):25-30.

[12] Indian Pharmacopoeia Commission. Indian Pharmacopoeia. Vol. II. Government of India, Ministry of Health and Family Welfare; 2018.