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Development and Optimization of Herbal Cream for Ringworm: A Natural Approach to Antifungal Treatment

Kiran Dudhat[1*], *Krushil* Thummar[1], *Yuvrajsinh* Jadeja[1], *Kiran* Dekavadiya[1]

[1]School of Pharmacy, RK University, Kasturbadham, Rajkot, India

Abstract. The present study focuses on the formulation and evaluation of a herbal cream intended for the treatment of ringworm, a common fungal infection. With growing global interest in plant-based remedies, particularly those with antimicrobial properties, this research utilizes natural ingredients known for their antifungal efficacy. The cream was developed using ethanolic extracts of Aloe vera gel, Azadirachta indica (Neem), and Butea monosperma (Palash) prepared via maceration. The cream base comprised beeswax, liquid paraffin, borax, methylparaben, rose oil, and distilled water, and the formulation was completed using the slab method to ensure homogeneity. Five trial batches (F1H to F5H) were prepared and evaluated for physical appearance, pH, viscosity, phase separation, and skin irritancy. All formulations exhibited favorable visual properties, appropriate pH and viscosity, stability at room temperature, and no signs of skin irritation or erythema during application. Antifungal screening against Trichophyton, Microsporum, and Epidermophyton revealed that the herbal ingredients demonstrated notable antifungal activity. The overall results confirmed that all five formulations were physically stable, non-irritant, and suitable for topical application, highlighting the potential of herbal-based creams as effective, safe, and natural alternatives for managing fungal skin infections.

1. Introduction

Fungal skin infections, particularly ringworm (dermatophytosis), are a common dermatological concern due to their widespread occurrence, high recurrence rate, and limitations of conventional treatments. Caused by dermatophytes like Trichophyton, Microsporum, and Epidermophyton, ringworm affects the skin, hair, and nails, often requiring topical therapy. Although synthetic antifungal creams are readily available, their prolonged use can lead to adverse effects, skin sensitivity, and resistance. These challenges have shifted focus toward safer, plant-based alternatives [1]. Herbal medicines have gained attention for their therapeutic efficacy and minimal side effects [2]. Herbal creams, formulated using natural plant extracts in an emulsion base, offer multiple advantages: they are rich in bioactive compounds, remain on the skin for extended durations, and provide sustained therapeutic action [3, 4]. This study focuses on developing and evaluating an herbal cream incorporating Neem (Azadirachta indica), Palash (Butea monosperma), and Aloe Vera (Aloe barbadensis miller) three traditionally used medicinal plants known for their antifungal and skin-healing properties [5].

Neem is widely recognized for its antimicrobial, antifungal, anti-inflammatory, and regenerative actions. Its active constituents, such as azadirachtin, nimbidin, and nimbin, have demonstrated broad-spectrum antifungal effects. Neem also soothes irritated skin, reduces inflammation, and promotes healing beneficial for ringworm-infected areas. Palash, also known as flame of the forest, contains potent flavonoids like medicarpin and

isoformononetin, which exhibit significant antifungal activity. Its flower and gum extracts have shown efficacy comparable to some synthetic agents, making it an effective component in topical antifungal formulations [6]. Aloe Vera complements these effects by providing hydration, soothing inflammation, and aiding in skin repair. It contains polysaccharides and glycoproteins that support wound healing, relieve itching and burning, and restore the skin barrier. Aloe Vera also exhibits mild antimicrobial activity, further enhancing the cream's overall effectiveness [7].

The formulation of the herbal cream involved the maceration of plant materials in ethanol to extract key phytoconstituents, ensuring maximum bioavailability. The resulting product was a smooth, semi-solid emulsion suitable for topical application [8]. The cream was stable under ambient conditions, non-irritant, and easy to apply. In-vitro antifungal studies confirmed its efficacy, showing a clear zone of inhibition against ringworm-causing fungi. Unlike synthetic formulations, this herbal cream is free from harsh chemicals, parabens, and synthetic preservatives, reducing the risk of allergic reactions, especially in sensitive skin. It maintains the natural pH of the skin and supports overall skin health through its rich content of vitamins, minerals, and essential nutrients [9, 10]. This study aims to develop a safe and effective herbal cream incorporating Palash, Neem, and Aloe Vera for the treatment of ringworm. The cream was evaluated for its physicochemical properties, skin compatibility, and antifungal activity. The formulation offers a natural, non-irritant alternative to synthetic agents, promoting skin healing while minimizing side effects.

2. Material and Methods

All-natural crude drugs used in this formulation, including Palash (Butea monosperma), Neem (Azadirachta indica), and Aloe Vera (Aloe barbadensis), were procured from Dhanvantari Ayurvedic Store, Rajkot. These herbal ingredients were selected for their proven antifungal, antimicrobial, and skin-soothing properties. Synthetic excipients such as beeswax, liquid paraffin, borax, methyl paraben, and rose oil were sourced from Loba Chemie, Mumbai (India). All excipients used were of analytical grade to ensure consistency and reliability in the formulation.

2.1 Preformulation

2.1.1 Extraction processes of Palash

The ethyl acetate and petroleum extracts of palash show the antifungal activity against Cladosporium cladosporioide. The chemical constituent that was responsible for this antifungal activity was medicarpin. Its activity against fungus was found to be greater than the standard fungicide that is Benlate. Palash in a hot air oven, dried leaves were gathered, cleaned with distilled water, and stacked. Leaves were pulverized following adequate drying. Afterward, a volumetric flask containing 8 g of Palash leaf powder and 50 mL of ethyl acetate was shaken for three days on a REMI RSB-12 mechanical shaker. The solution was then boiled on a water bath at 80– 100 °C. Then contaminants were filtered using muslin cloth. The process was then completed using the filtrate, also known as the filter product, which is an extract or clear solution made from palash leaves [11].

2.1.2 Extraction processes of Neem

Gathered neem leaves were washed with distilled water, then dried in a hot air oven. The leaves were pulverized after thorough drying. After that, a volumetric flask containing 5g of powdered neem leaves and 50 mL of dimethyl sulfoxide was shaken for three days using a REMI RSB-12 mechanical shaker. The mixture was then concentrated to 20 mL using a water bath heated to 80–100 °C, contaminants were then removed by filtering through muslin fabric. The preparation process then employed the filtrate, or filter product, which is an extract or solution that is clearly made from neem leaves [12].

2.1.3 Extraction processes of Aloe vera gel

Picked and cleaned with distilled water Aloe Vera leaves that are ripe, strong, and new. Following thorough cleaning of the leaves, a sterile knife was used to cut the outer part of the leaf into two halves. The colorless parenchymatous tissue that makes up the aloe vera gel was then cut away using a sterile knife. The contaminants and fibers are then removed by filtering the liquid through muslin fabric. Following that, the preparation processes the filtrate, a clear aloe Vera gel that is the end result of the filter observe antifungal activity [13].

2.2 Formulation of herbal cream

Palash extract, Aloe Vera gel, and Neem extract are combined, then forcefully mixed to create a smooth cream. Add a few drops of rose fragrance oil after that. Beeswax and liquid paraffin are heated to 75 degrees in a borosilicate glass beaker. Place this cream on the slab, add a few drops of distilled water if the temperature is °C, and keep it at that heating level. (Oil phase). Borax and methylparaben must be dissolved in distilled water and heated to 75 °C in a separate beaker while the cream is being mixed geometrically on a slab to the beaker. This will give the cream a smooth texture [5]. The different five formulations were prepared (Table 1) with different ratio of herbal drug palash and neem.

Table 1. Trial batch Formulation of herbal cream

Ingredients	F1H	F2H	F3H	F4H	F5H
Palash extract (gm)	1.5	1.4	0.4	0.6	1
Neem extract (gm)	0.5	0.4	1.4	0.2	0.8
Aloe vera gel (ml)	1	1	1	1	1
Bees wax (gm)	3	3	3	3	3
Liquid paraffin(ml)	10	10	10	10	10
Borax (gm)	0.3	0.3	0.3	0.3	0.3
Methylparaben (gm)	0.03	0.03	0.03	0.03	0.03

Distilled water (ml)	2	2	2	2	2
Rose oil (ml)	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.

2.3 Evaluation of cream

All physical, chemical, and antifungal evaluations were performed in triplicate (n=3) to ensure statistical robustness.

2.3.1 Physical evaluation

In this experiment, the cream's colour, odor, condition, and texture were assessed.

2.3.2 pH

A digital PH meter was used to determine the PH after dissolving 0.5 g of cream in 50 mL of distilled water. The five formulations, F1H, F2H, F3H, F4H and F5H, all had pH values that were closer to skin pH than those of the control group, suggesting that they may be used on the skin without risk [5, 14].

2.3.3 Viscosity

Cream's viscosity was measured using a Brooke field viscometer at a temperature of 25 °C and spindle No. 63 at 25 RPM [5, 14].

2.3.4 Spreadability

The spread ability was measured in terms of how long it took two slides operating under a specific load to separate from the cream positioned in between the slides. The spread ability is improved by separating the two slides more quickly. Glass slides with uniform dimensions were selected from two sets. The cream formulation was then put on a slide that was cut to the proper size. The formulation was then covered by another slide. The cream between the two slides was then evenly compressed to produce a thin layer after which a weight or specific load was added to the top slide. The extra formulation that had adhered to the slides was then scraped off once the weight was removed. The weight that was linked to the upper slide gave it the freedom to fall off without resistance. It was recorded how long it took the upper slide to come off. Where 'm' represents a normal weight of 30 grams that is either fastened, while 'l' is the 5 cm length of a glass slide, and 't' signifies the duration of an action measured in seconds [14].

$$\textbf{Spread ability} = m \times l / t \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

2.3.5 Phase separation

At a temperature between 25 and 100 °C, prepared cream was kept in a closed container out of the light. Then, phase separation was observed throughout a 30-day, 24-hour period. The phase separation was examined, and any changes were noted.

2.3.6 Greasiness

In this instance, a cream smear was put to the skin's surface and its grease- or oil-like consistency was assessed. All five formulas were non-greasy, as shown by the findings.

2.3.7 Irritancy

On the left-hand dorsal surface, mark a one cm2 region. The cream was then administered to that region, and the duration was recorded. Following that, it is evaluated for irritation, erythema, and edema, if present, for a period of up to 24 hours, and reported. The findings indicated that none of the five formulations F1H, F2H, F3H, F4H and F5H exhibited signs of irritability, erythema, or edema [15].

2.3.8 Washability

A little quantity of cream was applied to a hand to assess washability, and the hand was subsequently washed under running water. It was simple to wash all five formulations.

2.3.9 In-Vitro Anti-Fungal study of cream

To evaluate the antifungal activity of the formulated cream, an in vitro study was conducted using the agar diffusion method. Initially, 100 mL of distilled water was taken in a conical flask, and 2 g of potato dextrose agar was added and dissolved thoroughly. The flask and sterile Petri plates were then autoclaved for 45 minutes. After sterilization, the PDA medium was poured into the Petri plates and allowed to cool and solidify. A clinical sample of ringworm was collected from an infected patient and evenly spread over the surface of the solidified agar under aseptic conditions, using two burners to maintain sterility. The formulated cream was then applied onto the inoculated plates, which were incubated at 37°C for 24 hours. After incubation, the plates were examined for zones of inhibition around the cream application site. The diameter of the zone of inhibition reflects the sensitivity of the fungal strain to the antifungal agent present in the cream [15]. A commercially available antifungal agent, such as (Specify Drug, e.g., Ketoconazole) cream, was utilized as the positive control for direct comparison of inhibitory zones.

2.4 Experimental Design and Optimization Using 3² Full Factorial Design

The formulation of herbal cream formulation was improved using a 3² Full Factorial Design, which assessed the impact of important independent parameters on its critical reaction characteristics. The Concentration of base (Bees wax) (X_1), the Concentration of Borex (X_2), were the independent variables chosen for optimization. The three levels of each factor designated as -1, 0, and +1 were examined in order to methodically evaluate how each affected the dependent variables [16, 17]. Response criteria that were taken into consideration for optimization included Viscosity (Y_1), Spread ability (Y_2), Zone of inhibition (Y_3). With the use of the experimental design, polynomial regression equations

were created to characterize the relationships between independent and dependent variables. The regression equation was displayed as follows:

$$Y = \beta^0 + \beta^1 X^1 + \beta^2 X^2 + \beta^{12} X^1 X^2 + \beta^{11} X^{12} + \beta^{22} X^{22}$$

Factors having a p-value of below 0.05 were deemed statistically significant in influencing the formulation outcomes after statistical analysis to assess the variables' relevance. In order to get the Viscosity, spread ability, and Zone of inhibition, the ideal formulation parameters were found through the optimization process. Bees wax ranged from 2.8 g to 3.5 g, influencing the cream's viscosity and texture, while Borax ranged from 0.2 g to 0.4 g, affecting emulsification and stability. This design enabled comprehensive optimization by evaluating both individual and interactive effects of the variables on the final cream properties.

3. Results and Discussion

3.1 Evaluation of trial batches of herbal cream

Table 2. Evaluation of trial batches of herbal cream

Batch	pH	Viscosity (Cps)	Spreadability (g.cm/sec)	Zone of inhibition
F1H	6.4±0.22	21020±5.28	12.67±1.83	Present
F2H	6.6±0.31	11810±4.82	12.75±1.97	Present
F3H	6.2±0.18	18820±7.68	13.45±1.33	Present
F4H	6.9±0.27	16640±4.56	14.10±1.73	Absent
F5H	6.7±0.14	18562±7.41	14.75±1.62	Absent

3.1.1 Appearance

All five formulations (F1H–F5H) of the herbal cream exhibited a uniform light green color with a pleasant, graceful odor. The creams were semisolid in state with a smooth texture, indicating good consistency and homogeneity suitable for topical application.

3.1.2 pH

The pH of the cream was found to be 6.2 to 6. 9, which is weakly acidic value as per table 2.

3.1.3 Viscosity

Viscosity A Brooke field viscometer was used to test the viscosity of cream at a temperature of 25 degrees and spindle number 63 at 2.5 RPM. The findings indicated that all five formulations had appropriate viscosity as per Table 2.

3.1.4 Spread ability

Spreadability is an important parameter for topical formulations as it determines the ease with which the cream can be applied to the skin. Among the six formulations tested, batch F5 exhibited the highest spread ability (14.75 g·cm/sec), indicating superior ease of application, while batch F1 showed the lowest value (12.67 g·cm/sec).

3.1.5 Phase separation

In a dark, enclosed space with a temperature range of 25 to 100 °C, prepared cream was maintained. After that, phase separation was tested for 30 days over 24 hours. Phase separation was examined, and no changes were noted. The findings show that none of the five formulations showed phase separation.

3.1.6 Greasiness

The cream was smeared onto the skin's surface, and the consistency of the smear oily or grease like was assessed. We may conclude from the findings that none of the five formulas were greasy.

3.1.7 Washability

A little quantity of cream was applied to a hand for the washability test, and the hand was subsequently washed under running water. It was simple to wash all five formulations.

3.1.8 In-vitro Anti-Fungal Study

The antifungal spot is surrounded by a zone of inhibition where fungal colonies cannot develop. The zone of inhibition can be used to gauge how susceptible a fungus is to an antifungal [15]. From the research, we found the zone of inhibition in the three formulations F1H, F2H, and F3H. It indicated that the prepared herbal cream has antifungal activity. Large "zones of inhibition" surrounding the discs, which show the antifungal is effective against the current fungus strain, are apparent as soon as the findings of the experiment are complete. The absence of a "zone of inhibition" is a sign that the antifungal is not working. The F2H batch has a large zone of inhibition as compared to F1H and F3H (Table 2).

3.2 Optimization of Herbal cream formulation

A number of evaluation criteria, such as spreadability, viscosity, pH, appearance, washability, greasiness, phase separation, and antifungal activity (zone of inhibition), were used to optimize the herbal cream formulation. Among the nine formulations (F1 to F9), Formulation F3 was identified as the optimized batch based on its superior physicochemical and pharmacological performance.

Table 3. Designed Batches for Herbal Cream Formulation (3^2 Factorial Design)

Run	Factor 1 Bees wax (gm)	Factor 2 Borax (gm)	Response 1: Viscosity (cP)	Response 2: Separability (g.cm/sec)	Response 3: Zone of Inhibition (mm)
F1	3.5	0.2	11210±8.23	5.5±0.02	12.5±1.11
F2	3.5	0.3	12020±9.10	6.2±0.08	15±0.68
F3	3.5	0.4	18520±10.22	7.5±0.09	18.5±1.49
F4	3	0.2	15210±12.11	5.8±0.05	11.8±1.29
F5	3	0.3	15020±13.17	6.5±0.02	14.2±1.11

F6	3	0.4	16562±18.23	7.8±0.08	17±1.93
F7	2.8	0.2	19502±14.75	6±0.05	10.5±1.56
F8	2.8	0.3	20120±15.7	6.8±0.02	13±1.20
F9	2.8	0.4	21020±12.84	8.2±0.09	15.8±0.89

3.2.1 Spreadability

Beeswax may also cause semi-crystalline domains that reduce flow resistance in the final formulation if not properly balanced with emulsifiers like borax. Beeswax also strongly affected spreadability. These findings align with the understanding that excess beeswax creates a thicker, less pliable base, making it harder to spread the formulation evenly on the skin. F3 showed excellent spreadability of 7.5 ± 0.09 g·cm/sec, allowing smooth and even application over the skin with minimal effort. It's ideal consistency avoided excessive runniness or resistance, thus enhancing patient compliance and facilitating efficient delivery of the active ingredients across the infected area [18]. Lower beeswax improves spreadability (e.g., F3: 7.5 g.cm/sec at 3.5 g beeswax vs. F6: 7.8 g.cm/sec at 3.0 g). Beeswax stiffens the base, so higher concentrations reduce spreadability due to increased consistency and decreased fluidity. However, optimal levels are needed for structural integrity. Generally, increasing borax concentration decreased spreadability due to enhanced emulsion stiffness, but smoother emulsions formed at optimal levels of borax may slightly improve glide. For instance, F2 (0.3 g borax) showed a spreadability of 6.2 g·cm/sec, which was more favorable than the lower borax formulation F1 (5.5 g·cm/sec), yet slightly less than F3 (7.5 g·cm/sec). This indicates that borax's effect on spreadability is closely tied to how well it interacts with beeswax to create a balanced cream structure [19, 20].

3.2.2 pH

The pH of formulation F3 was within the desirable range of 5.5–7.0, making it compatible with human skin and reducing the risk of irritation or allergic reactions. This also helps preserve the skin's acid mantle and enhances the tolerability of the cream for prolonged use [18].

3.2.3 Viscosity

The F3 had a high viscosity of 18,520±10.22 cP, ensuring a stable, semi-solid consistency that remained intact during application. The formulation was neither too thick to hinder spreadability nor too thin to cause dripping, striking an ideal balance for topical retention and ease of use. In the formulation of herbal creams, beeswax plays a pivotal role as an emollient, structuring agent, and thickener. It significantly influences the viscosity and texture of the cream by forming a semi-solid matrix that entraps the aqueous phase. Interestingly, while beeswax is generally known to increase viscosity, a decreasing trend in viscosity was observed with its higher concentrations [21, 22]. The observed inverse relationship (e.g., lower viscosity for F1 with 3.5 g beeswax vs. F7 with 2.8 g is likely due to insufficient emulsification at higher beeswax loads, leading to microstructural phase separation and compromised homogeneity, which reduces the apparent viscosity of the matrix. For instance, F7 with 2.8 g beeswax showed a viscosity of 19,502 cP, whereas F1

with 3.5 g showed only 11,210 cP. This inverse relationship may be due to poor emulsification or microstructural phase separation at higher beeswax concentrations, which reduces homogeneity and apparent viscosity [23]. The borax, served as a key emulsifying agent by reacting with the fatty acids in beeswax to form sodium soaps, which help stabilize oil-in-water emulsions. An increase in borax concentration showed a direct and expected increase in viscosity. The formation of a more structured emulsion matrix due to borax's emulsifying action likely contributed to this increased viscosity. A stronger internal network within the cream also prevents breakdown under stress, which is ideal for topical applications requiring extended residence time [14, 24].

3.2.4 Appearance and texture

The cream was light green in color, semi-solid, and exhibited a smooth texture with a pleasant odor. These aesthetic properties contribute to user acceptability, especially when treating chronic or visible skin conditions like fungal infections.

3.2.5 Washability

The F3 was easily washable under running water, improving patient convenience and ensuring hygienic use. Its non-greasy nature and residue-free rinsing made it suitable for repeated daily application without skin buildup.

3.2.6 Phase Separation

No phase separation was observed over a 30-day stability study, indicating good physical stability and efficient emulsification between aqueous and lipid phases [25].

3.2.7 In-Vitro Antifungal Activity

When considering antifungal activity, beeswax appeared to have a beneficial effect on enhancing the zone of inhibition. Higher beeswax levels, especially in F3 (3.5 g), correlated with better retention on the skin surface, potentially allowing for prolonged contact and improved penetration of active phytoconstituents like Palash and Neem extracts. The occlusive nature of beeswax likely helped in creating a barrier that sustained the delivery of antifungal agents to the target site. F3 demonstrated the largest zone of inhibition (18.5 ± 1.49 mm), reflecting the highest antifungal activity among all batches. This suggests effective inhibition of common dermatophytic fungi such as *Trichophyton, Microsporum, and Epidermophyton* species due to synergistic effects of Palash and Neem extracts. Borax also had a significant effect on antifungal activity, as seen by the increase in the zone of inhibition with higher borax concentrations. The zone expanded from 12.5 mm in F1 (0.2 g borax) to 18.5 mm in F3 (0.4 g borax). This enhancement is attributed to improved emulsification and better drug dispersion in the cream, allowing the active compounds to be released more effectively. Furthermore, borax slightly raises the pH, which may enhance the solubility and bioavailability of the herbal actives against fungal pathogens [26, 27]. The combined effect of beeswax and borax was particularly evident in the optimized batch F3 (3.5 g beeswax and 0.4 g borax), which demonstrated the highest viscosity, acceptable spreadability, and the largest zone of inhibition among all batches. This synergy suggests that the correct proportion of structuring agent (beeswax) and emulsifier (borax) is essential for achieving an ideal balance of physical and therapeutic properties. Conversely, suboptimal combinations, such as low beeswax with low borax (F7), led to poor antifungal activity and unstable cream characteristics [28].

3.3 Graphical presentation of 3^2 full Factorial Design

3.3.1 Viscosity

The impact of borax (Factor B) and beeswax (Factor A) on the viscosity of the herbal cream is depicted in the response surface plots (2D and 3D) in Figure 1. The 2D contour plot displays a gradient from low (blue) to high (yellow) viscosity, suggesting that higher viscosity is a result of increasing concentrations of borax and beeswax. This pattern is further supported by the 3D surface map, which shows a peak viscosity at larger concentrations of both variables. The non-linear response indicates that borax and beeswax have an interaction effect on viscosity. These results demonstrate that in order to optimize viscosity and attain the required uniformity for efficient application, the two components must be carefully balanced [29, 30]. The curved topography of the 3D plot indicates a non-linear synergistic influence, suggesting that the combination of borax and beeswax enhances rheological strength more than either factor alone. This highlights the importance of carefully tuning their proportions to obtain an optimal viscosity range necessary for uniform application, stability, and consumer acceptability [31].

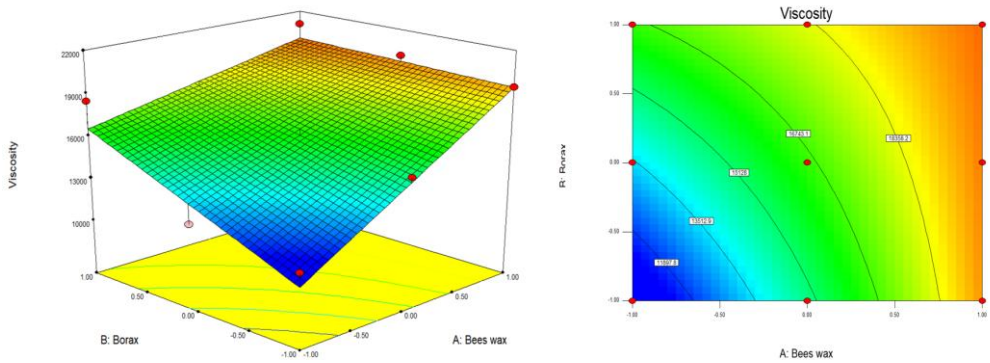

Figure 1: Response surface plots (2D and 3D) for Viscosity

3.3.2 Spreadability

Higher borax concentrations increase spread ability, but beeswax has the opposite effect, according to the 2D contour plot (Figure 2), which displays a gradient from low (blue) to high (yellow) spread ability. This pattern is supported by the 3D surface plot, which displays maximum spreadability at greater borax and lower beeswax levels. The almost linear response indicates that borax is a major factor in improving spreadability. These results demonstrate that in order to obtain the intended application qualities, the ideal ratio of borax to beeswax must be maintained [32]. Borax likely improves spreadability by reducing internal structural rigidity and modifying the cream's flow behavior, allowing easier deformation under minimal force. Conversely, beeswax contributes to stiffness and reduces mobility of the lipid matrix, thereby lowering spreadability. The nearly linear response pattern indicates that borax is the dominant factor governing this parameter. Scientifically, an optimal balance is crucial because excessive softness may compromise product retention on the skin, whereas excessive stiffness impairs patient compliance. This establishes borax–beeswax ratio as a critical determinant for achieving desirable sensorial and application properties [31, 33].

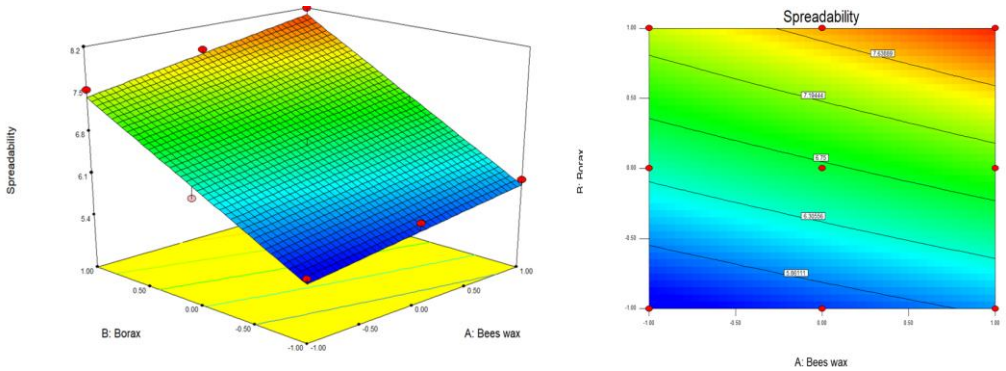

Figure 2: Response surface plots (2D and 3D) for Spreadability

3.3.3 In-Vitro Anti-Fungal study (Zone of Inhibition)

The impact of borax (Factor B) and beeswax (Factor A) on the antifungal activity of the herbal cream, as determined by the zone of inhibition, is depicted in the response surface plots (2D and 3D). A gradient from low (blue) to high (yellow/red) inhibitory zones can be shown in the 2D contour map, demonstrating how much the antifungal activity is enhanced by greater borax concentrations (Figure 3). This pattern is supported by the 3D surface map, which shows that the biggest zone of inhibition occurs at intermediate beeswax and high borax levels [29, 34]. According to the linear pattern, borax is more important in enhancing bioactivity. Beeswax at moderate concentrations may enhance occlusiveness, increasing localized retention of actives, while excessively high levels hinder release, reducing bioactivity. The predominantly linear trend underscores borax as the key enhancer of antifungal efficacy. These findings suggest that optimizing borax enhances both formulation performance and therapeutic potential, particularly for topical antifungal applications [35, 36].

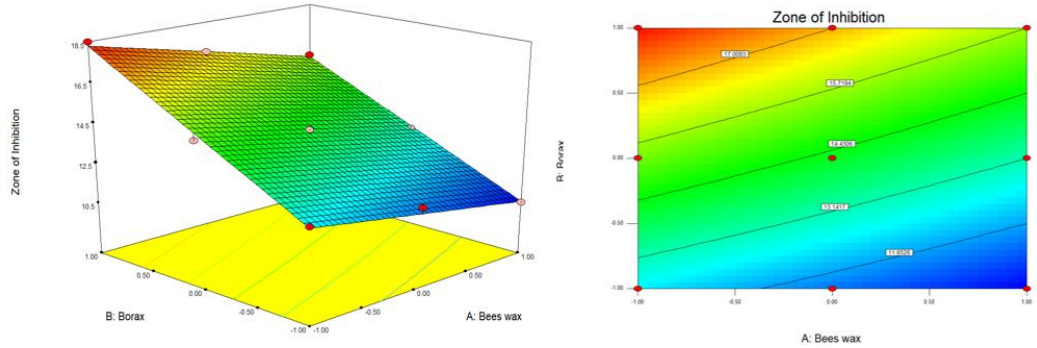

Figure 3: Response surface plots (2D and 3D) for Zone of Inhibition

3.4 Overlay Plot of optimized batch of herbal cream

The overlay plot (Figure 4) demonstrates the design space for the optimized herbal cream formulation, integrating key response parameters: viscosity, spreadability, and zone of inhibition. The yellow region within the plot indicates the area where all three formulation responses meet the desired criteria, signifying the ideal composition. The gray regions represent combinations of borax and beeswax that result in suboptimal formulation

characteristics. The inset values in the plot denote the optimized levels of responses: viscosity of 16,398.2 cps, spreadability of 7.5281 g·cm/sec, and a zone of inhibition of 16.5842 mm. The corresponding optimized formulation was obtained using beeswax concentration of 3.5 g and borax concentration of 0.4 g. The low % bias across all three responses confirms that the observed values are in close agreement with the predicted values from the design (Table 4). This validates the statistical model and confirms that the selected concentrations of borax and beeswax effectively control the critical quality attributes of the herbal cream. The formulation thus exhibits a stable and effective profile in terms of viscosity (ensuring ease of application and retention), spreadability (patient compliance), and antifungal efficacy (zone of inhibition).

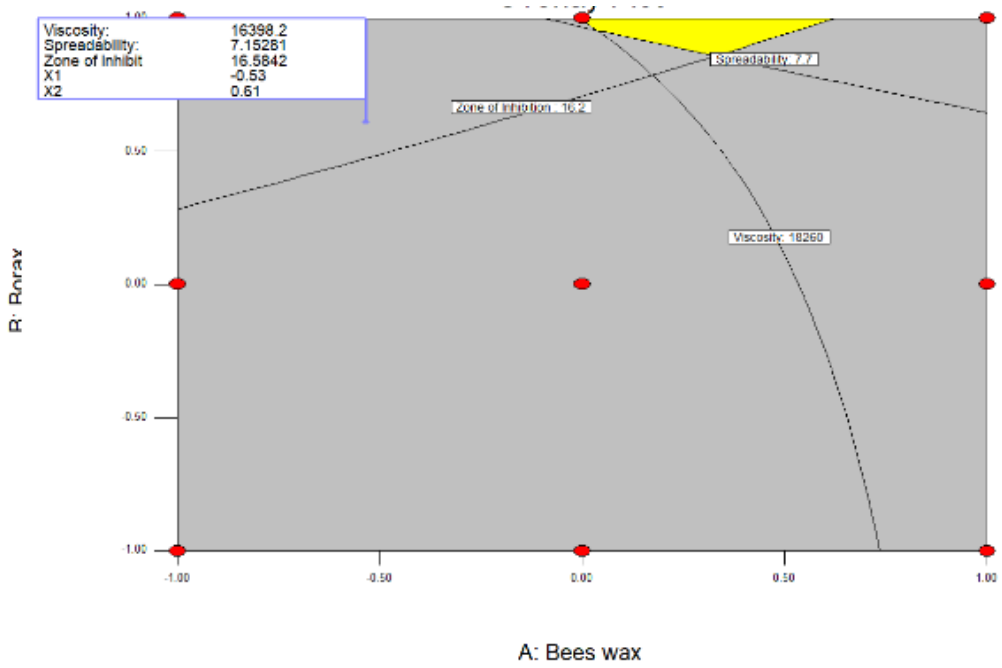

Figure 4: Overlay Plot of optimized herbal cream

Table 4. Predicted vs. Observed Values of Optimized Herbal Cream (Checkpoint Batch)

Parameters	Predicted Value	Observed Value	% Bias
Viscosity (cps)	16,398.2	16,450.3 ± 14.25	-0.31 %
Spreadability (g·cm/sec)	7.5281	7.50 ± 0.17	0.37 %
Zone of Inhibition (mm)	16.5842	16.42 ± 0.21	0.98 %

3.5 Stability Study of Optimized Herbal Cream

The optimized herbal cream formulation demonstrated excellent stability over the three-month study period (Table 5). Viscosity showed only a marginal decline, remaining well within the acceptable range to ensure sufficient product consistency and topical retention. The minor variations in spreadability indicate consistent ease of application, with no phase separation or significant textural changes over time. The zone of inhibition exhibited only slight reductions over time, maintaining its antifungal effectiveness, which signifies that the herbal actives remained stable and bioavailable in the cream matrix [35, 37]. The controlled

presence of beeswax and borax helped preserve the emulsion structure and sustained release properties.

Table 5. Stability Study of Optimized Herbal Cream Batch for 3 Months

Time (Months)	Viscosity (cps)	Spreadability (g·cm/sec)	Zone of Inhibition (mm)
0 (Initial)	16,450.3 ± 14.25	7.50 ± 0.17	16.42 ± 0.21
1 Month	16,398.7 ± 16.52	7.48 ± 0.19	16.35 ± 0.23
2 Months	16,320.6 ± 15.38	7.46 ± 0.21	16.30 ± 0.26
3 Months	16,278.2 ± 17.01	7.45 ± 0.22	16.28 ± 0.25

4. Conclusion

This study developed an optimized herbal cream using Butea monosperma and Azadirachta indica extracts for effective ringworm treatment. A statistical approach optimized beeswax (3.5 g) and borax (0.4 g) concentrations, achieving ideal viscosity (16,450.3 cps), spreadability (7.50 g·cm/sec), and antifungal efficacy (16.42 mm zone of inhibition). The formulation's reliability was validated by minimal bias between predicted and observed values. Stability tests over three months confirmed consistent physicochemical properties and antifungal activity. The cream's synergistic herbal actives and controlled-release matrix offer a promising natural alternative for ringworm management, with potential for clinical evaluation and commercial development. Future clinical investigation, including extensive patch testing and sensitization studies, is essential to definitively confirm the non-allergic and long-term safety profile of the high concentrations of herbal extracts in the optimized formulation.

Conflict of Interest

The authors report no financial or any other conflicts of interest in this work.

Ethical Approval

There is no participation of human and animals or their biological materials; therefore, no ethical approval is required.

Authors' Contribution

Kiran Dudhat conceptualized the study, supervised the overall work, manuscript drafting and finalized the manuscript. Krushil Thummar contributed to data collection, literature review, and experimental execution. Yuvrajsinh Jadeja and Kiran Dekavadiya assisted in data analysis and manuscript editing. All authors reviewed and approved the final version of the manuscript.

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Data Availability

All the data is available with the authors and shall be provided upon request.

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