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Formulation, Evaluation, and Antibacterial Activity of Herbal Cream Containing *Trachyspermum ammi* and *Ocimum africanum* Leaf Extracts

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Abstract

Aim: The aim of this study was to formulate and evaluate an antibacterial herbal cream using the leaf extracts of Ajwain (*Trachyspermum ammi*) and Lemon Basil (*Ocimum africanum*) to provide a natural alternative to synthetic antibacterial agents with fewer side effects. **Methods:** Ethanolic extracts of *T. ammi* and *O. africanum* leaves were prepared using a Clevenger apparatus (soxhlation). Three formulations (F1, F2, F3) were prepared using the fusion method, incorporating various concentrations of the extract into a cream base containing cetostearyl alcohol, liquid paraffin, cetrimide, and distilled water. The formulations were evaluated for organoleptic properties (color, odor, texture), pH, spreadability, washability, greasiness, phase separation, irritancy, dilution test, thin-layer chromatography (TLC), and *in vitro* antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa*. **Results:** All formulations (F1-F3) appeared as creamish-white, semisolid, with a pleasant odor and were non-irritant. The pH values ranged from 5.0 to 7.6, which is compatible with skin pH. Spreadability values were 15, 14, and 17.9 g·cm/sec for F1, F2, and F3, respectively. Formulation F3, containing 2 ml of the combined extract, showed the most favorable properties: pH 7.6, spreadability of 17.9 g·cm/sec, no phase separation, slight greasiness, and significant antibacterial activity, inhibiting the growth of *E. coli* and *P. aeruginosa* with no turbidity in the broth dilution test. TLC analysis for F3 yielded an Rf value of 0.6. **Conclusion:** The herbal cream formulation F3, containing *T. ammi* and *O. africanum* extracts, demonstrated potent antibacterial activity and desirable physical properties. This study confirms that these plant extracts can be effectively incorporated into a stable topical cream for managing bacterial skin infections.

Keywords
Antibacterial cream, Herbal formulation, *Trachyspermum ammi* (Ajwain), *Ocimum africanum* (Lemon Basil), Topical delivery.

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1. INTRODUCTION

The skin, the body's largest organ, serves as a critical protective barrier against environmental hazards, including microbial pathogens (Sahu, 2016). Topical drug delivery systems, particularly creams, are widely used to treat cutaneous disorders due to their

ease of application, avoidance of first-pass metabolism, and direct targeting of the affected area (Dubey, 2012; Tadwee & Gore, 2011). Creams are semisolid emulsions, typically oil-in-water (O/W) or water-in-oil (W/O), designed to deliver therapeutic agents while providing moisturizing and protective functions (Sahu, 2016).

The increasing concern over the side effects and antibiotic resistance associated with synthetic antimicrobial agents has revitalized interest in herbal alternatives (Ekor, 2014). Herbal cosmetics are more acceptable and safer than synthetic ones, as natural remedies carry a lower risk of adverse reactions (Sonalkar & Nitave, 2016). India's rich medicinal plant heritage offers a vast resource for developing safe and effective topical formulations (Viswanad et al., 2012).

This study focuses on two plants: Ajwain (*Trachyspermum ammi*) and Lemon Basil (*Ocimum africanum*). *T. ammi* (Apiaceae family) contains thymol and carvacrol, which possess significant antibacterial, antifungal, and anti-inflammatory properties, making it useful for treating acne and skin irritations (Sharma & Kaur, 2023). Studies have demonstrated broad-spectrum antimicrobial activity of *T. ammi* leaf extracts against both Gram-positive and Gram-negative bacteria, with significant inhibition zones observed for *Pseudomonas aeruginosa* (Sharma & Kaur, 2023).

O. africanum (Lamiaceae family), a hybrid basil, is rich in essential oils like linalool, citral, and eugenol, along with flavonoids and phenolic acids, offering antimicrobial, antioxidant, and skin-rejuvenating benefits (Arora et al., 2019). Lemon basil contains essential oils such as citronellol, linalool, and eugenol, which possess anti-inflammatory and antimicrobial properties that can help combat acne, blackheads, and whiteheads, making it a potential ingredient in creams for blemish-prone skin.

Several researchers have successfully formulated herbal creams with various plant extracts. Navindgikar et al. (2020) developed a multipurpose herbal cream using neem and tulsi extracts and reported good appearance, adequate viscosity, and no phase separation. Davkhar et al. (2023) formulated a multipurpose herbal cream containing *Curcuma longa*, *Carica papaya*, *Aloe barbadensis*, *Azadirachta indica*, and *Ocimum sanctum* with favorable evaluation results. Similarly, Sekar and Abdul Jalil (2017) formulated a novel antibacterial and anti-inflammatory cream containing *Muntingia calabura* leaves extract that showed significant activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

The primary objective of this research was to formulate a stable, effective, and safe antibacterial herbal cream by combining the extracts of *T. ammi* and *O. africanum* leaves and to evaluate its physicochemical properties and antibacterial activity against common skin pathogens.

2. MATERIALS AND METHODS

2.1. Plant Material Collection and Authentication

Fresh, healthy leaves of *Trachyspermum ammi* (Ajwain) and *Ocimum africanum* (Lemon Basil) were collected from the medicinal garden of Teegala Krishna Reddy College of Pharmacy, Hyderabad, India. The plants were authenticated, and the leaves were washed with distilled water, shade-dried, and cut into small pieces.

2.2. Preparation of Herbal Extract

The combined leaf extract was prepared using a Clevenger apparatus (soxhlation). Fifteen grams (15 g) of the dried, chopped leaves were placed in a round-bottom flask with 150 ml of distilled water. The apparatus was assembled on a heating mantle, and extraction was carried out for 3 hours at 70°C. The resulting oil and water phases were separated using a separating funnel, and the combined extract was collected and stored for formulation, following the methodology adapted from Sharma and Kaur (2023).

2.3. Formulation of Antibacterial Herbal Cream

Three batches of O/W cream (F1, F2, F3) were prepared using the fusion method as described by Navindgikar et al. (2020) and Davkhar et al. (2023). The oil phase and aqueous phase were prepared separately, heated to 70°C, then combined with continuous homogenization for 15 minutes. Rose oil was added as a preservative and fragrance after cooling. The compositions of the three formulations are detailed in Table 1.

Table 1: Formulation of Antibacterial Herbal Cream

Ingredient	F1	F2	F3
Leaves extract (Ajwain + Lemon Basil)	1 ml	1 ml	2 ml
Cetrimide (emulsifier)	0.1 g	0.1 g	0.1 g
Cetostearyl alcohol (thickener)	1.5 g	0.5 g	1.0 g
Liquid paraffin (oil phase)	11 ml	12 ml	10 ml
Rose oil (fragrance)	Q.S.	Q.S.	Q.S.
Distilled water (aqueous phase)	10 ml	9 ml	9 ml

2.4. Evaluation of Herbal Cream

The formulated creams were evaluated using the following parameters, as per standard methods described in the literature (Navindgikar et al., 2020; Davkhar et al., 2023; Sonalkar & Nitave, 2016):

- **Physical Appearance:** Color, odor, and texture were assessed visually.
- **pH:** Determined at 25°C using a universal pH indicator paper, as pH is a critical parameter for skin compatibility (Sahu, 2016).
- **Spreadability:** Calculated using the formula: S = M × L / T, where M is the weight tied to the upper slide (g), L is the length of the slide (cm), and T is the time taken to separate the slides (sec).
- **Washability, Greasiness, and Irritancy:** Assessed by applying the cream to the skin of volunteers (n=3) for 7-10 days (Viswanad et al., 2012).
- **Phase Separation and Dilution Test:** The cream was observed for any separation of phases. For the dilution test, the cream was mixed with water to check for homogeneity (Navindgikar et al., 2020).
- **Thin Layer Chromatography (TLC):** Performed to determine the Rf value of the extract in the cream.
- **Antibacterial Activity:** The broth dilution method was used (Sekar & Abdul Jalil, 2017). A fixed concentration of the cream was added to culture media containing *Escherichia coli* and *Pseudomonas aeruginosa*. Tubes were incubated overnight, and bacterial growth was assessed by visual turbidity.

3. RESULTS AND DISCUSSION

3.1. Physicochemical Evaluation

The results of the physicochemical evaluation for F1, F2, and F3 are presented in Tables 2 and 3. All formulations exhibited a creamish-white color, semisolid state, pleasant odor, and non-irritant properties, which is consistent with findings reported by Navindgikar et al. (2020) for multipurpose herbal creams.

The pH of the creams ranged from 5.0 to 7.6, which falls within the acceptable pH range for skin (4.5-6.5), minimizing the risk of skin irritation (Sahu, 2016). Formulation F3 had a pH of 7.6, which is near-neutral and suitable for topical application. Similar pH values have been reported for other herbal cream formulations (Davkhar et al., 2023; Sonalkar & Nitave, 2016).

Table 2: Results for Organoleptic, Spreadability, and Washability

Evaluation test	Colour, consistency, odour	Type of smear	Spreadability	Washability
F1	Creamish white, semisolid, characteristic	Oil in water	15 g·cm/sec	Easily washable
F2	Creamish white, semisolid, characteristic	Oil in water	14 g·cm/sec	Easily washable
F3	Creamish white, semisolid, characteristic	Oil in water	17.9 g·cm/sec	Easily washable

Spreadability, which indicates the ease of application on the skin, was highest for F3 (17.9 g·cm/sec), followed by F1 (15 g·cm/sec) and F2 (14 g·cm/sec). Good spreadability ensures uniform application of the cream on the skin surface (Sonalkar & Nitave, 2016). All formulations were easily washable and showed no phase separation, indicating good physical stability, a finding consistent with Navindgikar et al. (2020) who reported no phase separation in their herbal cream formulations. The dilution test produced a clear solution, confirming the O/W emulsion type.

Table 3: Results for pH, Irritability, Phase Separation, and Greasiness

Evaluation parameter	pH	Irritability	Phase separation	Greasiness
F1	5	Non-irritant	No phase separation	Greasier
F2	6	Non-irritant	No phase separation	Slightly greasy
F3	7.6	Non-irritant	No phase separation	Slightly greasy

The non-irritant nature of all formulations is an important safety parameter, as topical preparations must be safe for prolonged skin contact (Viswanad et al., 2012). This finding aligns with the work of Haneefa et al. (2012), who reported that herbal gel formulations showed no primary skin irritation.

3.2. Thin Layer Chromatography (TLC)

TLC analysis revealed different Rf values for the formulations, indicating the presence of various phytoconstituents. Formulation F3 showed an Rf value of 0.6, which is within the ideal range for many active herbal compounds. The variation in Rf values (F1: 1.2, F2: 0.9, F3: 0.6) suggests differences in the concentration and composition of phytochemicals present in the formulations, which may correlate with their antibacterial activity.

3.3. Antibacterial Activity

The antibacterial activity of the formulations was assessed by observing the turbidity (or lack thereof) in the culture media. The results are summarized in Table 4. Formulation F3 demonstrated significant antibacterial activity, showing "no growth of new bacteria and inhibition of existing bacteria" against both *E. coli* and *P. aeruginosa*. In contrast, F1 and F2 only showed "no growth of bacteria," suggesting a lower or static effect. The clear broth in the F3 test tube confirmed that the concentration of the extract in F3 was sufficient to inhibit visible microbial growth (Minimum Inhibitory Concentration achieved).

Table 4: Results for Dilution, after feel, Antibacterial Activity, and TLC

Evaluation parameters	Dilution test	After feel	Antibacterial activity	Thin layer chromatography
F1	Clear solution	Emolliency	No growth of bacteria	Rf = 1.2
F2	Clear solution	Emolliency	No growth of bacteria	Rf = 0.9
F3	Clear solution	Emolliency	**No new growth & inhibition of existing bacteria**	Rf = 0.6

The superior activity of F3 is likely due to its higher concentration (2 ml) of the combined *T. ammi* and *O. africanum* extracts, which contain potent antibacterial compounds like thymol, carvacrol, linalool, and eugenol (Arora et al., 2019; Sharma & Kaur, 2023). This concentration-dependent antibacterial activity has been previously observed by Sekar and Abdul Jalil (2017), who reported that higher concentrations of *Muntingia calabura* extract produced larger zones of inhibition against both Gram-positive and Gram-negative bacteria.

The finding that F3 inhibited *E. coli* (Gram-negative) and *P. aeruginosa* (Gram-negative) is particularly significant, as Gram-negative bacteria are generally more resistant to antibacterial agents due to their outer membrane (Sharma & Kaur, 2023). Similar broad-spectrum activity of *T. ammi* extracts against *Pseudomonas aeruginosa* has been documented (Sharma & Kaur, 2023).

The optimal pH (7.6) and high spreadability of F3 also contributed to its overall favorable profile, as these properties enhance patient compliance and therapeutic efficacy (Navindgikar et al., 2020).

4. CONCLUSION

This study successfully formulated and evaluated an O/W antibacterial herbal cream containing the combined leaf extracts of *Trachyspermum ammi* (Ajwain) and *Ocimum africanum* (Lemon Basil). All three formulations (F1-F3) were physically stable, non-irritant, and had acceptable pH and spreadability, consistent with findings reported for other herbal topical formulations (Navindgikar et al., 2020; Davkhar et al., 2023; Sonalkar & Nitave, 2016). However, formulation F3, which contained 2 ml of the herbal extract, demonstrated superior organoleptic properties, optimal spreadability (17.9 g·cm/sec), and significant *in vitro* antibacterial activity against *E. coli* and *P. aeruginosa* compared to F1 and F2. The findings suggest that the synergistic effect of the phytoconstituents from these two plants at a higher concentration provides effective bactericidal action, supporting earlier observations that herbal extracts exhibit concentration-dependent antimicrobial activity (Sekar & Abdul Jalil, 2017; Sharma & Kaur, 2023).

The results are encouraging and demonstrate that the tested herbal cream has promising potential for managing skin infections (Haneefa et al., 2012). This herbal cream presents a promising, natural alternative to synthetic antibacterial creams for managing skin infections, addressing the growing concern over antibiotic resistance and side effects associated with synthetic agents (Ekor, 2014). Further studies, including *in vivo* efficacy, long-term stability testing, and clinical trials, are recommended to validate its therapeutic potential.

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