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Original Research Article

FORMULATION, PHYSICOCHEMICAL EVALUATION, AND COMPARATIVE INSECT REPELLENT EFFICACY OF A POLYHERBAL TOPICAL CREAM USING COLD MIXING AND ULTRASONICATION TECHNIQUES

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Abstract: Insect-borne diseases continue to pose major public health challenges, particularly in tropical regions, necessitating effective personal protective measures such as topical insect repellents. The present study aimed to formulate and evaluate a polyherbal insect repellent cream containing eucalyptus oil, neem oil, marigold extract, and camphor using cold mixing and ultrasonication techniques. Three formulations (F1–F3) were developed with varying concentrations of active ingredients and evaluated for physicochemical properties, stability, spreadability, viscosity, drying time, washability, and insect repellency using the filter paper method. Among the formulations, F1 showed excellent spreadability, quick drying, easy washability, and high user acceptability with a repellency duration of approximately 4 hours. F3 exhibited the longest repellency (about 7 hours), and its physical stability and texture were significantly improved after ultrasonication. Comparative evaluation with a marketed product demonstrated that the developed formulations were competitive in terms of stability, safety, and repellency performance. The study confirms that optimized polyherbal topical creams can serve as effective, safe, and user-friendly alternatives to synthetic insect repellents.

Keywords: Insect repellent cream, Polyherbal formulation, Neem oil, Ultrasonication technique, Physicochemical evaluation

INTRODUCTION

Insect-borne diseases remain a major public health concern, particularly in tropical and subtropical regions. Mosquitoes and other biting insects act as vectors for several life-threatening diseases such as malaria, dengue, chikungunya, Zika virus infection, filariasis, and Japanese encephalitis. In the absence of effective vaccines for many of these conditions, personal protective measures such as topical insect repellents are considered the first line of defense[1-3]. Insect repellents are chemical or natural substances that deter insects by creating a volatile vapor barrier on the skin, thereby masking human odor or producing an aversive stimulus to insects. Synthetic repellents like DEET, picaridin, and IR3535 are widely used because of their long-lasting protection. However, concerns regarding skin irritation, toxicity, and environmental safety have increased interest in herbal and plant-based alternatives[4].

Herbal insect repellents derived from essential oils such as neem, citronella, eucalyptus, and lemongrass offer advantages of better skin compatibility, biodegradability, and lower toxicity. However, these natural agents often exhibit limitations such as poor stability and shorter duration of action. Therefore, there is a need to develop optimized topical formulations that can enhance the stability, efficacy,

and safety of herbal or combined insect repellent systems[4,5].

Topical creams are among the most preferred dosage forms due to their ease of application, non-greasy nature, controlled drug release, and high patient compliance. A well-designed insect repellent cream can provide prolonged protection, skin moisturization, and minimal irritation. Hence, the development and scientific evaluation of an effective topical insect repellent cream is of significant pharmaceutical and public health relevance. The present study focuses on the development, physicochemical characterization, and comparative evaluation of a topical insect repellent cream with a marketed formulation to establish its quality, stability, and insect-repelling potential[5,6].

2. Objectives

- To formulate a stable and skin-compatible insect repellent cream using selected active ingredients.
- To evaluate the physicochemical properties of the developed cream, including pH, viscosity, spreadability, and stability.
- To compare the insect-repellent effectiveness of the formulated cream with a marketed insect repellent product.
- To evaluate the synergistic insect-repelling effect of a polyherbal blend using advanced bioassay techniques.

MATERIAL AND METHODS

3.1 **Active Herbal Ingredients**: The insect repellent creams were formulated using three primary herbal actives: eucalyptus oil, neem oil, and marigold extract. Eucalyptus oil (from *Eucalyptus globulus* leaves) and neem oil (from *Azadirachta indica* seeds) were used as natural volatile repellents with additional antimicrobial and skin-soothing properties. Marigold extract (*Tagetes erecta* flowers, in-house prepared) was incorporated for its mild insect-repellent, anti-inflammatory, and wound-healing effects. Camphor was included as a solid aromatic agent providing an additional repellent effect, cooling sensation, and masking of body odour.

3.2 **Excipients**: Cetyl alcohol and stearic acid were used as consistency builders and co-emulsifiers to obtain a stable oil-in-water cream with acceptable viscosity and structure. Lanolin, mineral oil, and glycerine were incorporated as emollient and humectant bases to improve moisturization, film formation, and spreadability. Potassium hydroxide (KOH) was employed in small quantity for partial neutralization of stearic acid, thereby forming in-situ soap and stabilizing the emulsion. Benzyl alcohol was used as a preservative (q.s.) and purified water served as the aqueous continuous phase and solvent.

3.3 **Sources of Materials**: All ingredients were procured from reliable commercial or in-house sources given below.

Source of materials used in insect repellent cream are

- Eucalyptus oil: natural essential oil obtained from *Eucalyptus globulus* leaves via local supplier
- Neem oil: cold-pressed oil from *Azadirachta indica* seeds via local supplier
- Marigold extract: prepared in-house from *Tagetes erecta* flowers
- Camphor: natural/synthetic grade from *Cinnamomum camphora* or turpentine-derived camphor
- Cetyl alcohol, mineral oil, stearic acid, glycerine, potassium hydroxide, and preservative: analytical/pharmaceutical grade from standard chemical suppliers
- Lanolin: commercial grade from sheep wool
- Purified water: laboratory prepared

3.4 **Equipment**: The following equipment were used in the experimental work: Ultrasonicator (probe or bath type), beakers, magnetic stirrer, weighing balance, pH meter, and suitable cream containers for filling and storage.

3. **Methods and Procedure**

4.1 **Formulation Design**

4.1.1 **Composition of Cream Formulations**: Three formulations (F1, F2, and F3) of herbal insect repellent cream were prepared by varying the concentration of active oils while keeping the base components constant. The composition was as follows:

- **Eucalyptus oil:** 1.5%, 3.5%, and 5.5% in F1, F2, and F3, respectively
- **Neem oil:** 4%, 6%, and 4% in F1, F2, and F3
- **Marigold extract:** 1.5%, 3.5%, and 5.5% in F1, F2, and F3
- **Camphor:** 0.5 g, 1 g, and 1.5 g in F1, F2, and F3
- **Cetyl alcohol:** 2% in all formulations
- **Lanolin:** 1% in all formulations
- **Mineral oil:** 2% in all formulations
- **Stearic acid:** 15% in all formulations
- **Glycerine:** 10% in all formulations
- **Potassium hydroxide:** 1% in all formulations
- **Preservative (benzyl alcohol):** q.s.
- **Purified water:** q.s. to 100%

Figure 1: Eucalyptus oil Neem oil, Camphor and Cetyl alcohol used for formulation

4.1.2 **Cold Mixing Technique (F1 as Optimized Cold-Mix Formula):** Cold mixing was used to prepare creams containing volatile and heat-sensitive herbal components. All ingredients were processed at room temperature (not exceeding ~40 °C).

- The required quantities of glycerine, KOH, and preservative were dissolved in a portion of purified water to form the **aqueous phase**.
- In a separate beaker, stearic acid, cetyl alcohol, lanolin, mineral oil, and camphor were blended to form the **oil phase**. Any mild warming necessary to soften waxy materials was kept minimal and the mixture was allowed to cool back to near room temperature.
- The aqueous phase was slowly added to the oil phase under continuous stirring on a magnetic stirrer to form a preliminary emulsion.
- After obtaining a uniform cream base, eucalyptus oil, neem oil, and marigold extract were added gradually with gentle stirring to avoid excessive volatilization of the essential oils.
- The final weight was adjusted with purified water, and the cream was mixed until a homogeneous, smooth product (F1) was obtained and then filled into clean, labeled containers.
- The same procedure was followed to prepare F2 and F3 with their respective concentrations of herbal actives, initially by cold mixing[7,8].

4.1.3 **Ultrasonication Technique (for F3 Optimization):** To improve the homogeneity and dispersion of the higher oil-loaded formulation (F3), ultrasonication was employed.

- The pre-emulsified cream (prepared as described above) was transferred to a suitable vessel compatible with the ultrasonicator.
- The probe of the ultrasonicator was immersed into the cream, and sonication was performed at an appropriate amplitude and frequency (within the typical range of ~20 kHz) for a predetermined duration, with intermittent cooling to avoid excessive temperature rise.
- Ultrasonication induced localized shear and cavitation, reducing droplet size and improving uniformity of the oil globules and herbal actives within the cream matrix.
- The ultrasonicated F3 cream was allowed to equilibrate to room temperature and then stored in suitable containers for further evaluation [9,10].

4.2 **Preformulation Studies**

4.2.1 **Solubility Studies:** Solubility of the herbal actives (eucalyptus oil, neem oil, marigold extract, and camphor) was evaluated in various solvents such as water, ethanol, glycerine, and oils using standard equilibrium solubility methods. Excess drug was added to the solvent, shaken, equilibrated, filtered, and visually inspected to guide phase selection and base composition.

4.2.2 **pH Compatibility:** Aqueous dispersions (1–10% w/v) of the active ingredients were prepared with distilled water, and pH was measured using a calibrated digital pH meter to ensure compatibility with skin pH (4.5–6.5) [11,12].

4.2.3 **Partition Coefficient:** The partition behaviour of the actives between n-octanol and water was determined using a separating funnel. After shaking and phase separation, the concentration in each phase was quantified by UV spectrophotometry, and log P was interpreted to estimate affinity for lipid versus aqueous phases.

4.2.4 **Melting/Boiling Point Determination:** Melting points (camphor, stearic acid) and boiling-related behaviour (for volatile oils) were assessed using a melting point apparatus or suitable heating setup. These data assisted in selecting processing temperatures and storage conditions[12].

4.2.5 **Stability of Active Ingredients:** Samples of the individual actives and selected blends were subjected

to stress conditions (e.g., higher temperature, light exposure, and ambient humidity) in a stability chamber or UV cabinet. Changes in colour, odour, pH, and UV assay of active content were monitored to evaluate inherent stability prior to formulation [13].

4.3 **Evaluation of Formulated Creams [14-18]**

4.3.1 **Physical Appearance and Organoleptic Properties:** The formulated creams (F1–F3) were visually examined for colour, texture, homogeneity, phase separation, and odour under normal light conditions.

4.3.2 **pH Measurement:** A 1% w/w dispersion of each formulation in distilled water was prepared, and pH was determined using a calibrated pH meter to ensure skin compatibility.

4.3.3 **Viscosity:** Viscosity of the creams was measured using a suitable viscometer and expressed in centipoise (cP), which reflects spreadability and consistency.

4.3.4 **Spreadability:** Spreadability was assessed by placing a fixed amount of cream between two glass slides and measuring the area or time required for spreading under a known load. The formulations were qualitatively graded as excellent, moderate, or poor based on their spreading characteristics [14-15].

4.3.5 **Washing-Off Ability:** A defined quantity of cream was applied onto glass slides and washed under running tap water. The ease of removal was graded as easy, slight greasy, or greasy.

4.3.6 **Drying Time:** Drying time was determined using a moisture analyzer by monitoring the time (up to 3 minutes) required for the cream film to attain a steady weight, and was categorized as quick, moderate, or slow[16].

4.3.7 **Short-Term Stability and Phase Separation Study:** Filled cream containers were stored at 4 °C, room temperature (~25 °C ± 2 °C), and 40 °C ± 2 °C for 15 days. Samples were periodically checked for phase separation, changes in appearance, odour, and consistency to assess physical stability [17].

4.3.8 **UV Absorbance of Cream Extracts:** Cream samples were suitably diluted to obtain concentrations between 10 and 50 µg/mL, and absorbance was recorded using a UV spectrophotometer. The increase in absorbance with concentration (0.121–0.611 from 10–50 µg/mL) was used to confirm linearity for further quantitative estimations.

4.3.9 **Insect Repellency (Filter Paper Technique):** Insect repellent efficacy was evaluated using the filter paper method. A known quantity of cream was applied onto half of a filter paper, leaving the other half untreated as control. The paper was placed in a petri dish or chamber containing insects (e.g., mosquitoes), and the number of insects landing or remaining on treated versus untreated areas was monitored over time. The duration for which insects avoided the treated surface was recorded as repellency time (hours) [18].

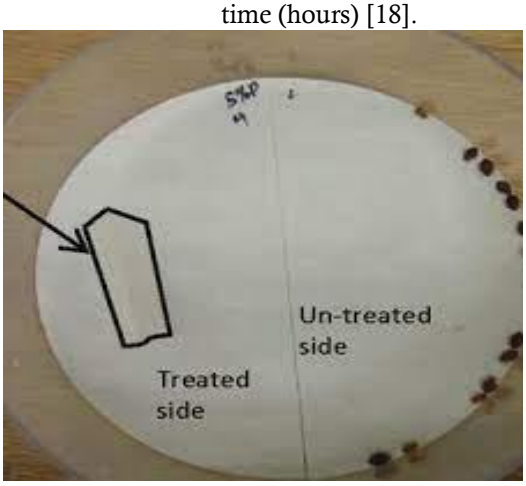

Figure 2: Insect Repellency filter paper treated

4.3.10 **Comparative Study with Marketed Product:** The optimized formulations (F1 by cold mixing and F3 after ultrasonication) were compared with a marketed insect repellent cream (Oudomass). Parameters such as texture, appearance, colour, odour, spreadability (g·cm/sec), pH, viscosity, drying time, washability, short-term stability, phase separation, skin irritation, insect repellency duration, user acceptance, and presence of preservative were evaluated under identical test conditions.

RESULTS AND DISCUSSION

6.1 **Physicochemical Characteristics of Formulations F1–F3**: All formulations produced smooth oil-in-water creams with distinct visual and sensory differences. F1 appeared as a white, smooth cream with mild herbal odour, whereas F2 and F3 were slightly yellowish to yellowish thick creams with progressively stronger odour due to higher oil content.

Table 6.1: Physicochemical characteristics of insect repellent cream formulations (F1–F3)

Sr No.	Parameter	F1	F2	F3
1	Appearance	White smooth cream	Slightly yellowish cream	Yellowish thick cream
2	Odour	Mild herbal	Strong herbal (higher oil level)	Strongest herbal (highest oil content)
3	pH	6.4	6.1	5.8
4	Viscosity	Moderate	High	Very high
5	Spreadability	Excellent	Moderate	Poor
6	Phase Separation	No	No	Slight after 7 days
7	Stability (15 days)	Stable at all test conditions	Stable at all test conditions	Slight oil separation at elevated temp
8	Washing off ability	Easy	Slight greasy	Greasy
9	Drying time	Quick	Moderate	Slow
10	User acceptability	High	Medium	Medium

The pH values of F1, F2, and F3 were 6.4, 6.1, and 5.8, respectively, indicating that all formulations fell within or close to the desirable skin-compatible range. The slight decrease in pH with increasing oil and acidic components in F3 remained acceptable and did not produce irritation in the skin test.

Viscosity increased with oil load: F1 exhibited moderate viscosity, F2 high viscosity, and F3 very high viscosity. Correspondingly, spreadability decreased from excellent (F1) to moderate (F2) and poor (F3), demonstrating the expected inverse relationship between viscosity and ease of spreading.

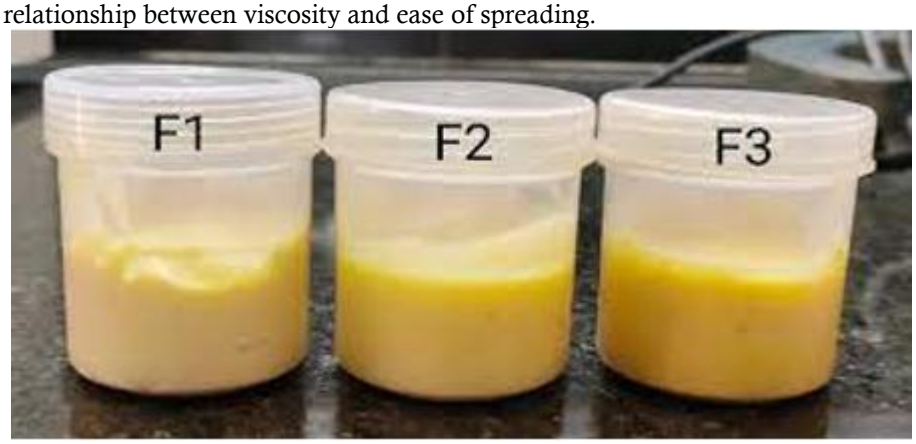

Figure 3: Formulated repellents

Short-term stability studies over 15 days at 4 °C, room temperature, and 40 °C showed that F1 and F2 remained physically stable without phase separation. F3 exhibited slight oil separation after 7 days and minor instability at elevated temperature, consistent with its higher oil content and thicker texture.

Washing-off ability correlated with the richness of the base: F1 was easily washable, F2 showed slight greasiness, and F3 was distinctly greasy and more resistant to washing. Drying time also varied, with F1 drying quickly, F2 at a moderate rate, and F3 drying slowly, reflecting its higher occlusive and oily nature.

The UV absorbance data for cream extracts (0.121–0.611 between 10–50 µg/mL) showed a proportional increase with concentration, supporting the suitability of UV spectrophotometry for further quantitative analyses of active content and stability.

6.2 **Insect Repellency Performance**: Insect repellency tests using the filter paper technique demonstrated that all formulations provided measurable protection, but efficacy increased with active oil concentration. F1, F2, and F3 showed repellency durations of approximately 4, 5.5, and 7 hours, respectively, with F3 exhibiting the longest protection time owing to its highest combined level of eucalyptus, neem, marigold, and camphor.

Table 6.2: Insect repellency duration of formulations (F1–F3)

Sr No.	Formulation	Insect Repellency Duration (hours)	Qualitative Performance
1	F1	4.0 ± 0.5	Good protection
2	F2	5.5 ± 0.5	Better protection

3	F3	7.0 ± 0.5	Highest and prolonged protection

However, the superior repellency of F3 was offset by its relatively poor spreadability, slow drying, and greasy feel, which reduced overall user acceptability to a moderate level. In contrast, F1 offered a balanced profile, combining good repellency (4 ± 0.5 hours) with excellent spreadability, quick drying, easy washability, and high user acceptance, making it more suitable for routine use, especially in warm climates where non-greasy products are preferred.

6.3 **Effect of Ultrasonication on F3**: Application of ultrasonication to F3 improved the overall uniformity and texture of the high oil-loaded cream. The process enhanced dispersion of the herbal oils, reduced droplet size, and minimized early phase separation, resulting in better physical stability and more acceptable consistency without compromising repellency (7 ± 0.5 hours). Despite these improvements, F3 remained thicker and slightly greasy compared to F1, and user acceptance was moderate rather than high.

Table 6.3: Comparative evaluation of F3 before and after ultrasonication

Sr No.	Parameter	F3 (Before Ultrasonication)	F3 (After Ultrasonication)
1	Appearance & texture	Yellowish, very thick, slightly coarse	Yellowish, smoother, more uniform cream
2	Odour	Strongest herbal, intense	Strong herbal, more acceptable
3	Viscosity	Very high	High but more manageable
4	Spreadability	Poor	Improved to moderate
5	Phase separation	Slight oil separation after 7 days	Markedly reduced; no visible separation in 15 days
6	Stability (15 days)	Stable with minor oil separation	Improved physical stability at all test conditions
7	Drying time	Slow	Slightly improved but still slower than F1
8	Insect repellency	~7.0 hours	~7.0 hours (maintained highest repellency)
9	User acceptability	Moderate (greasy, very thick)	Moderate–good (better texture, still rich/greasy)

6.4 **Comparison with Marketed Product (Oudomass)**: When compared with the marketed formulation Oudomass, F1 and ultrasonicated F3 demonstrated competitive performance.

Table 6.4: Comparison of optimized formulations (F1 and F3) with marketed product (Oudomass)

Sr No.	Parameter	F1 (Cold Mixing)	F3 (Ultrasonication)	Oudomass (Marketed Product)
1	Texture & appearance	Smooth, creamy	Thick, slightly oily	Smooth, semi-thick
2	Colour	White	Yellowish	Pale yellow
3	Odour	Mild herbal	Strong herbal (camphor, neem)	Pleasant floral–herbal
4	Spreadability (g·cm/sec)	18.2 ± 0.3	13.6 ± 0.4	17.8 ± 0.5
5	pH	6.4 ± 0.05	5.8 ± 0.1	6.2 ± 0.1
6	Viscosity (cP)	6200 ± 150	9800 ± 250	~7000 ± 200
7	Drying time (min)	4–5	8–9	5–6
8	Washability	Easy	Moderate	Easy
9	Stability (15 days at 40 °C)	Stable	Slight odour change, acceptable stability	Stable
10	Phase separation	No	No	No
11	Skin irritation test	No irritation	No irritation	No irritation
12	Insect repellency duration	4.0 ± 0.5 hours	7.0 ± 0.5 hours	6.0 ± 0.5 hours
13	Active ingredients	Neem, Eucalyptus, Marigold	Neem, Eucalyptus, Marigold	Neem, Citronella, Camphor
14	User acceptance	High	Moderate	High
15	Preservative	Added (benzyl alcohol)	Added (benzyl alcohol)	Present

Oudomass exhibited a smooth, semi-thick pale-yellow cream with pleasant floral-herbal odour, spreadability of 17.8 ± 0.5 g·cm/sec, pH 6.2 ± 0.1, viscosity around 7000 ± 200 cP, drying time of 5–6 minutes, easy washability, and stable behaviour at 40 °C, without skin irritation. Its insect repellency duration was approximately 6 ± 0.5 hours.

F1 showed comparable texture, pH (6.4 ± 0.05), and spreadability (18.2 ± 0.3 g·cm/sec), with easy washability,

quick drying (4–5 minutes), and high user acceptance, though its repellency duration (4 ± 0.5 hours) was slightly lower than that of Oudomass. F3, on the other hand, surpassed Oudomass in repellency time (7 ± 0.5 hours) but had higher viscosity (~9800 ± 250 cP), slower drying (8–9 minutes), and only moderate user acceptance due to its strong odour and greasier feel. Both F1 and F3 remained non-irritant in skin irritation tests and contained suitable preservatives.

6.5 **Optimization and Selection**: Based on the integrated evaluation of physicochemical properties, stability, repellency, and user perception:

- **F1 (Cold Mixing)** was identified as the best formulation for daily use and cold-mix processing. It presented a light, stable emulsion with excellent spreadability, quick drying, easy washability, mild herbal odour, and high user acceptance, while still providing reasonable repellency (~4 hours).
- **F3 (Ultrasonication)** was identified as the ideal choice when maximum repellency is desired. After ultrasonication, it delivered the longest protection (~7 hours) with acceptable stability and texture, although at the cost of increased viscosity, greasiness, and stronger odour.

The study demonstrates that a rational combination of herbal insect-repellent oils with appropriate cosmetic excipients, processed by either cold mixing (for lighter daily creams) or ultrasonication (for high-oil, long-acting preparations), can yield safe, stable, and effective herbal insect repellent creams that are competitive with marketed products.

CONCLUSION

The present investigation successfully demonstrated the formulation and evaluation of stable, safe, and effective polyherbal insect repellent creams using cold mixing and ultrasonication techniques. Among the developed formulations, F1 emerged as the most suitable for regular daily use due to its excellent spreadability, quick drying, easy washability, mild odour, and high user acceptance, while still providing effective repellency. F3, particularly after ultrasonication, showed the highest repellent efficacy with prolonged protection of up to 7 hours, making it ideal for situations requiring extended insect protection. Comparative evaluation with a marketed formulation confirmed the competitive performance of the developed creams in terms of stability, safety, and repellency. Overall, the study establishes that rational polyherbal combinations and appropriate processing techniques can produce efficient, eco-friendly, and consumer-acceptable insect repellent topical formulations.

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