Development And Evaluation 0f Herbal Mosquito Repellent Cream Containing Cymbopogon Flexuosus and Biophytum Sensitivum

Krishnendhu K[1], Praveena P[2], Sumayya K[3], Vishnupriya AV[4],
Shahal A M[5], Aishwarya Jith[6], Aleena K A[7], Raslamol.K[8]
[1,2,3,4,5]Students, Department of Pharmacognosy, Holy Grace Academy of Pharmacy, Affliated to Kerala University of Health Sciences, Mala, Thrissur-680732 Kerala
[6]Assistant Professor, Department of Pharmaceutics, Holy Grace Academy of Pharmacy, Affliated to Kerala University of Health Sciences, Mala, Thrissur-680732 Kerala
[7]Associate Professor, Department of Pharmacognosy, Holy Grace Academy of Pharmacy, Affliated to Kerala University of Health Sciences, Mala, Thrissur-680732 Kerala
[8]Dean-Academics & Professor, Department of Pharmaceutics, Holy Grace Academy of Pharmacy, Affliated to Kerala University of Health Sciences, Mala, Thrissur-680732 Kerala

Abstract—**Mosquito-borne diseases such as malaria, dengue, chikungunya, and Zika continue to pose significant public health challenges, particularly in tropical and subtropical regions. The limitations associated with synthetic mosquito repellents, including toxicity, environmental hazards, and the development of resistance, have driven the search for safer and eco-friendly alternatives. In the present study, a herbal mosquito repellent cream was developed and evaluated using Cymbopogon flexuous (lemongrass) and Biophytum sensitivum, both known for their larvicidal and repellent properties. Plant extracts were prepared and incorporated into an oil-in-water emulsion-based cream formulation. The formulated creams were evaluated for physicochemical parameters including pH, viscosity, spread ability, stability, and skin compatibility. The results of phytochemical screening confirmed the presence of bioactive constituents such as flavonoids, tannins, alkaloids, and saponins, which contribute to insecticidal activity. Among the formulations developed, F4 demonstrated optimal performance with suitable pH, good spread ability, acceptable viscosity, and non-greasy texture. The formulation exhibited good physical stability without phase separation and showed effective mosquito repellent activity, primarily attributed to the bioactive compounds present in Cymbopogon flexuous. Skin irritation studies indicated that the formulation is safe for topical application. In conclusion, the developed herbal mosquito repellent cream represents a safe, effective, and environmentally friendly alternative to conventional synthetic repellents, with significant potential for further development and large-scale application.**

Index Terms—**Herbal mosquito repellent, Cymbopogon flexuous, Biophytum sensitivum, Cream formulation, Phytochemical screening, Larvicidal activity, Natural repellent.**

I. INTRODUCTION

Mosquito-borne diseases continue to pose a serious global public health challenge, particularly in tropical and subtropical regions where environmental conditions such as high temperature, humidity, and seasonal rainfall create ideal habitats for mosquito breeding and survival. These diseases account for millions of infections and several hundred thousand deaths annually, significantly impacting public health systems and socio-economic development. In countries like India, the burden is especially high due to rapid population growth, unplanned urbanization, poor sanitation, and ineffective vector control measures.

Mosquitoes act as vectors for various pathogens, including protozoa, viruses, and helminths, causing diseases such as malaria, dengue, chikungunya, lymphatic filariasis, Zika virus infection, and Japanese encephalitis. Malaria, transmitted by Anopheles mosquitoes, remains a major cause of mortality in developing countries, while dengue and chikungunya, spread by Aedes aegypti, are rapidly increasing in urban areas. Culex species are responsible for diseases such as lymphatic filariasis and Japanese encephalitis, highlighting the significant impact of mosquito vectors on public health.

The mosquito life cycle consists of four stages: egg, larva, pupa, and adult. The larval stage is a key target for control strategies, as larvae are confined to aquatic habitats and have limited mobility. Targeting this stage is an effective preventive approach to interrupt the life cycle and reduce the emergence of adult disease-transmitting mosquitoes.

Conventional mosquito control strategies predominantly rely on synthetic chemical insecticides, including organophosphates, carbamates, and synthetic pyrethroids. These compounds exert their effects by disrupting the insect nervous system, leading to rapid mortality. However, their prolonged and indiscriminate use has led to several significant challenges. One of the major concerns is the development of insecticide resistance among mosquito populations, which reduces the long-term effectiveness of these agents. Additionally, chemical insecticides contribute to environmental pollution, including contamination of water bodies, toxicity to non-target organisms such as fish and beneficial insects, and disruption of ecological balance. Human exposure to these chemicals has also been associated with adverse health effects, including skin irritation, respiratory problems, and potential long-term toxicity. These limitations underscore the urgent need for safer, sustainable, and environmentally friendly alternatives.

In recent years, increasing attention has been directed toward plant-based products as natural mosquito control agents. Medicinal plants are rich sources of bioactive compounds such as alkaloids, flavonoids, terpenoids, phenolic compounds, and essential oils, which exhibit a wide spectrum of biological activities, including larvicidal, insecticidal, repellent, antifeedant, and growth-regulating effects. Plant-derived products are generally biodegradable, exhibit lower toxicity to non-target organisms, and are less likely to induce resistance in insect populations, making them attractive alternatives to synthetic insecticides.

Among the various medicinal plants, Cymbopogon flexuosus (lemongrass) has gained considerable attention due to its essential oil rich in citral (geranial and neral), along with other constituents such as geraniol, limonene, and myrcene. These compounds are known to exhibit strong mosquito-repellent and larvicidal activities. The mechanism of action involves interference with the mosquito's olfactory receptors, thereby preventing host detection, as well as disruption of the nervous system and metabolic processes. In addition to its insecticidal properties, Cymbopogon flexuous possesses antimicrobial, antifungal, antioxidant, and anti-inflammatory activities, which enhance its suitability for topical applications.

Another promising medicinal plant is Biophytum sensitivum, a small herb belonging to the Oxalidaceae family, traditionally known as "Mukkutti" in South India. The plant is rich in flavonoids, bioflavonoids (such as amentoflavone), phenolic compounds, tannins, and saponins. These phytoconstituents contribute to a wide range of pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, and analgesic effects. Importantly, extracts of Biophytum sensitivum have demonstrated significant larvicidal activity against mosquito species such as Aedes aegypti. The larvicidal mechanism is believed to involve disruption of respiratory and enzymatic processes, inhibition of larval growth, and interference with metabolic pathways, ultimately leading to larval mortality.

Despite the promising biological activities of these plant extracts, their direct application in crude form presents several limitations. These include poor physicochemical stability, rapid degradation of active constituents, volatility of essential oils, and reduced persistence under environmental conditions. Such limitations can significantly reduce their effectiveness in practical applications. Therefore, there is a need to develop suitable formulation strategies that can enhance the stability, bioavailability, and efficacy of these plant-based agents.

Although extensive research has been conducted on plant-based mosquito repellents and larvicides, several gaps remain unaddressed. Most studies have focused on the individual evaluation of plant extracts or essential oils, with limited exploration of their combined or synergistic effects. Specifically, there is a lack of research investigating the combined formulation of Cymbopogon flexuous and Biophytum sensitivum in a single topical delivery system.

Furthermore, while the larvicidal and repellent activities of these plants have been reported, insufficient attention has been given to formulation development, particularly in terms of optimizing cream-based systems for enhanced stability, controlled release, and user acceptability. There is also a notable scarcity of studies that simultaneously evaluate physicochemical parameters (such as pH, viscosity, and spreadability), skin safety, and biological efficacy (larvicidal activity) of such formulations.

In view of the above considerations, the present study is designed to develop and evaluate a herbal mosquito repellent cream incorporating extracts of Cymbopogon flexuosus and Biophytum sensitivum. The combination of these two plants is expected to produce a synergistic effect, enhancing both mosquito-repellent and larvicidal activities while also providing additional benefits such as antimicrobial and antioxidant protection for the skin.

The study aims to formulate a stable and effective cream using suitable excipients and to evaluate its physicochemical properties, including pH, viscosity, spreadability, and stability. Furthermore, the formulation will be assessed for skin safety and larvicidal efficacy to ensure its suitability for regular topical use.

By developing an eco-friendly, plant-based formulation, this research contributes to the advancement of sustainable mosquito control strategies and offers a safer alternative to conventional chemical insecticides.

II. MATERIALS AND METHODS

Figure 1: Photo of Biophytum sensitivum

Figure 2: Photo of Biophytum sensitivum

2.1 Plant Collection and Authentication

The whole plant of Biophytum sensitivum and leaves of Cymbopogon flexuosus were collected from local areas of Thrissur, Kerala. The collected plant materials were washed thoroughly with water to remove adhering dirt and foreign particles. The plants were authenticated by Aneesh K.S., College of Forestry, Kerala Agricultural University. The authentication certificate was obtained and preserved for reference.

Figure 3: Photo of collected and Dried Biophytum Sensitivum

Figure 4: Photo of the collected and cutted fresh Cymbopogon flexuous

2.2 Preparation of Plant Extract

The collected Biophytum sensitivum was shade-dried at room temperature and coarsely powdered. The powdered material was subjected to Soxhlet extraction using a suitable solvent (ethanol/methanol). The extract obtained was treated with chloroform and water to remove chlorophyll. The solvent was then evaporated using a water bath to obtain a concentrated and dried extract, which was stored in an airtight container until further use.

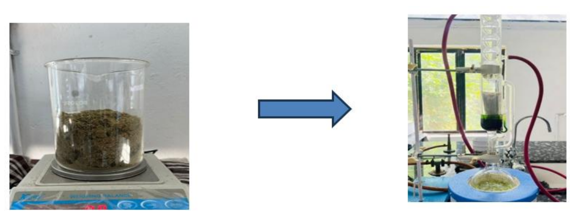

Figure 5 & 6: Dried Biophytum sensitivum powder & Soxhlet extraction process

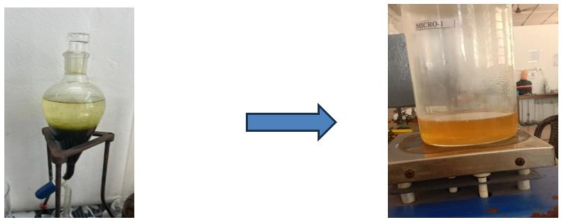

Figure 7 & 8: Removal of chlorophyll & Removal of solvent

2.3 Extraction of Essential Oil

Fresh leaves of Cymbopogon flexuosus were cut into small pieces (~2 cm) and subjected to steam distillation using a Clevenger apparatus. The essential oil obtained was separated, collected, and stored in a cool, dry place in an airtight container for further use.

Figure 9 & 10: Photo of cut leaves & Steam Distillation

2.4 Preliminary Phytochemical Investigation

The plant extract was subjected to qualitative phytochemical screening to identify the presence of various bioactive constituents.

Table 1: Preliminary Phytochemical Tests

Test	Procedure	Observation
Alkaloids (Dragendorff's Test)	Extract + Dragendorff's reagent	Orange/reddish-brown ppt
Flavonoids (Shinoda Test)	Extract + Mg ribbon + HCl	Pink/red/orange color
Tannins (Ferric Chloride Test)	Extract + $FeCl_3$	Blue-black/green color
Saponins (Frothing Test)	Extract + water, shaken	Stable foam formation
Glycosides (Keller–Killiani Test)	Extract + acetic acid + $FeCl_3$ + H_2SO_4	Brown ring at interface
Phenols	Extract + $FeCl_3$	Deep blue/black color
Terpenoids (Salkowski Test)	Extract + chloroform + H_2SO_4	Reddish-brown color
Steroids (Liebermann–Burchard Test)	Extract + chloroform + acetic anhydride + H_2SO_4	Green/bluish color

2.5 Preparation of Herbal Mosquito Repellent Cream

The cream was formulated as an oil-in-water (O/W) emulsion. Stearic acid reacts with potassium hydroxide to form potassium stearate, which acts as an emulsifying agent. Other excipients were used to improve consistency, stability, and skin compatibility.

Table 2: Ingredients and Their Functions

Ingredient	Function
Biophytum sensitivum extract	Active ingredient
Cymbopogon flexuosus oil	Active ingredient
Lanolin	Emollient
Cetyl alcohol	Thickening agent
Liquid paraffin	Emollient
Stearic acid	Emulsifier (with KOH)
Glycerin	Humectant
Potassium hydroxide	Alkali/emulsifier
Methylparaben	Preservative
Citric acid	pH adjuster
Vitamin E	Antioxidant
Distilled water	Vehicle

Table 3: Formulation of Cream (for 50 g)

Ingredient (g)	F1	F2	F3	F4
Biophytum extract	0.5	0.6	0.8	0.1
Cymbopogon oil	2.0	2.5	2.5	2.5
Lanolin	0.9	0.4	0.5	0.5
Cetyl alcohol	1.5	0.9	1.0	1.0
Liquid paraffin	1.0	2.5	1.5	1.0
Stearic acid	9.0	7.5	8.0	8.0
Glycerin	5.0	6.0	5.5	6.0
Potassium hydroxide	0.5	0.5	0.5	0.5
Methylparaben	0.05	0.05	0.05	0.05
Citric acid	—	—	0.05	0.05
Vitamin E	—	0.07	0.05	0.05
Distilled water	QS to 50	QS to 50	QS to 50	QS to 50

Procedure for Cream Preparation

1. Preparation of Oil Phase: Stearic acid, cetyl alcohol, lanolin, and liquid paraffin were melted together at 70–75°C.

2. Preparation of Aqueous Phase: Distilled water was heated to the same temperature. Glycerin, potassium hydroxide, and methylparaben were added and mixed thoroughly.
3. Emulsification: The aqueous phase was slowly added to the oil phase with continuous stirring to form an emulsion.
4. Incorporation of Active Ingredients: After cooling below 40°C, Biophytum sensitivum extract and Cymbopogon flexuosus oil were added.
5. Mixing and Cooling: The mixture was stirred continuously until a smooth, homogeneous cream was formed and then transferred into suitable containers.

Figure 11: Herbal mosquito repellent cream

2.6 Evaluation of Formulations

A. Physical Evaluation

The prepared cream formulations were evaluated for the following parameters:

- Color: Observed visually
- Odor: Characteristic odor
- Texture: Smooth, non-gritty
- Consistency: Firm and easily spreadable
- State: Semi-solid

B. Homogeneity

Homogeneity was determined by visual inspection for the presence of lumps, aggregates, or phase separation.

C. pH Determination

The pH of the cream was measured using a digital pH meter. One gram of cream was dispersed in 100 mL of distilled water and kept for 2 hours. The pH was measured in triplicate and the average value was recorded.

D. Spreadability

Spreadability was determined using a slide apparatus. About 2 g of cream was placed between two glass slides, and a weight was applied. The time required for the upper slide to move a fixed distance was noted.

Formula:

$$S = \frac{M \times L}{T}$$

Where:

S = Spread ability

M = Weight applied (g)

L = Length moved (cm)

T = Time (sec)

E. Viscosity

Viscosity was measured using a Brookfield viscometer at 12 rpm. The spindle was immersed in the cream sample, and readings were recorded after stabilization.

III. RESULT AND DISCUSSION

3.1 Phytochemical Analysis of Extract

Table 4: Phytochemical Screening of Extract

Phytochemical	Test	Observation	Result
Alkaloids	Mayer's Test	Cream precipitate	++
Alkaloids	Wagner's Test	Reddish-brown precipitate	++
Tannins	Ferric Chloride Test	Blue-black colour	+++
Flavonoids	Shinoda Test	Pink/red colour	++
Saponins	Foam Test	Persistent froth	+++
Glycosides	Keller–Killiani Test	Brown ring at junction	+

The preliminary phytochemical screening confirmed the presence of important bioactive constituents such as alkaloids, flavonoids, tannins, saponins, and glycosides. Tannins and saponins showed strong presence (+++), indicating their significant contribution to biological activity. These compounds are known for their larvicidal, antimicrobial, and antioxidant properties, which support the effectiveness of the formulation. Flavonoids and alkaloids also contribute to insecticidal and repellent activity, while glycosides, though present in lower amounts, may enhance overall pharmacological effects. The presence of these phytoconstituents justifies the selection of the plant extracts for mosquito repellent formulation.

3.2 Physical Evaluation of Cream

Table 5: Physical Evaluation

Parameter	F1	F2	F3	F4
Colour	Pale green	Pale yellow	Pale yellow	Pale yellow
Odour	Pleasant	Pleasant	Pleasant	Pleasant
State	Semisolid	Semisolid	Semisolid	Semisolid
Consistency	Thick cream	Soft cream	Thick cream	Creamy
Texture	Non-greasy	Non-greasy	Non-greasy	Non-greasy

All formulations exhibited acceptable physical characteristics such as pleasant odor, semisolid state, and non-greasy texture, indicating good patient acceptability. F2 and F4 showed better consistency and creaminess compared to F1 and F3, suggesting improved spreadability and ease of application.

3.3 pH of Herbal Mosquito Repellent Cream

Table 6: pH Values

Formulation	pH
F1	6.8
F2	7.32
F3	6.23
F4	5.36

The pH of all formulations was found to be within the acceptable range for topical application (approximately 5–7). F4 showed a slightly acidic pH (5.36), which is closer to normal skin pH and may be more suitable for minimizing skin irritation. F2 showed a slightly higher pH but still within acceptable limits.

3.4 Spreadability Test

Table 7: Spreadability

Formulation	Observation
F1	Not good
F2	Good
F3	Good
F4	Good

Spreadability is an important parameter for topical formulations. F2, F3, and F4 showed good spreadability, indicating ease of application and uniform distribution on the skin. F1 showed poor spreadability, likely due to its higher viscosity and thicker consistency.

3.5 Washability

Table 8: Washability

Formulation	Observation
F1	Not good
F2	Good
F3	Easily washable
F4	Easily washable

F3 and F4 exhibited excellent washability, indicating that the formulations can be easily removed from the skin without leaving residue. This enhances user convenience and acceptability. F1 showed poor washability, possibly due to higher oil content.

3.6 Viscosity

Table 9: Viscosity (cp)

Formulation	Viscosity (cp)
F1	4987
F2	2400
F3	6098
F4	5171

Viscosity plays a crucial role in determining the consistency and spreadability of creams. F3 exhibited the highest viscosity, which may contribute to thicker consistency but slightly reduced spreadability. F2 showed the lowest viscosity, correlating with better spreadability and smoother application. F4 maintained a balanced viscosity, providing both good consistency and ease of application.

IV. CONCLUSION

The present study successfully formulated and evaluated an herbal mosquito repellent cream using Biophytum sensitivum extract and Cymbopogon flexuosus essential oil. The formulation was developed as an oil-in-water (O/W) emulsion system to ensure better skin compatibility, spreadability, and user acceptability.

Preliminary phytochemical screening confirmed the presence of important bioactive constituents such as alkaloids, flavonoids, tannins, saponins, and glycosides, which are known for their insect-repellent, antimicrobial, and antioxidant properties. These phytoconstituents play a significant role in enhancing the therapeutic efficacy of the formulation.

Four different formulations (F1–F4) were prepared and evaluated for various physicochemical parameters including physical appearance, homogeneity, pH, spreadability, washability, and viscosity. All formulations showed acceptable characteristics; however, significant variation was observed among them:

- The pH values (5.36–7.32) were within the acceptable range for topical application, indicating safety and minimal risk of skin irritation.
- Spreadability and washability were found to be satisfactory for F2, F3, and F4, indicating ease of application and removal.
- Viscosity results revealed that F4 possessed balanced consistency, ensuring both stability and ease of spreading.
- Physical evaluation confirmed non-greasy texture, pleasant odor, and good consistency, enhancing patient compliance.

Among all formulations, F4 was identified as the optimized formulation due to its balanced physicochemical properties, suitable pH close to skin pH, good spreadability, easy washability, and appropriate viscosity.

The mosquito repellent activity of the cream can be attributed primarily to the presence of Cymbopogon flexuosus oil, which is rich in citronella compounds known for their effective insect-repellent action, along with the supportive bioactivity of Biophytum sensitivum.

Overall, the developed herbal cream is safe, effective, stable, and eco-friendly, offering a promising alternative to synthetic chemical repellents. The formulation demonstrates strong potential for commercial development and wider application in mosquito bite prevention with minimal side effects.

Further studies such as long-term stability testing, clinical evaluation, and large-scale production optimization are recommended to enhance its applicability and market potential.

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