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Research Article

DEVELOPMENT OF HERBAL MOSQUITO-REPELLENT FORMULATIONS AND THEIR COMPARATIVE EVALUATION

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ABSTRACT

Objective: The present research work aimed to develop herbal mosquito repellents as gels, incense sticks, and liquids for plug-in devices and also to assess their performance characteristics.

Methods: Herbal materials possessing mosquito-repellent activity were selected from the literature review based on percentage repellency and protection period. In this study, lavender, rosemary, lemongrass, and cedarwood oils were selected as actives. Span 20 and tween 80 were used to emulsify the volatile materials and then incorporated into Carbopol 934 base to form F1 and F2 gels which were evaluated for homogeneity, pH, spread ability, viscosity, and extrudability. Incense sticks were developed using charcoal and jigat as base materials. The prepared incense sticks F3 and F4 were evaluated for burning time, ash weight, and smoke. Solutions F5 and F6 were prepared using surfactants and cosolvents and were evaluated for phase separation. All formulations were screened for their mosquito repellency by screen cage method and their performance was compared to that of the marketed herbal repellents.

Results: Reared mosquitoes were identified as *Culex* species by microscopical observations of the antenna and maxillary palps. Mosquito repellency in 4 h is 93%, 87%, 89%, 79%, 89%, and 85% for F1, F2, F3, F4, F5 and F6, respectively. Among all, gel formulation F1 expressed the highest mosquito-repellent activity with 93% and is a better candidate.

Conclusions: Gel, incense sticks, and liquids prepared for use in plug-in devices exhibited reasonably good percentage repellency comparable to that of the standard. All the developed formulations are suitable for indoor protection from mosquito bites. However, safety, stability, and field studies need to be carried out to demonstrate suitability for regular use.

Keywords: Mosquito, Lavender, Rosemary, Lemongrass, Cedarwood, Emulgels, Incense Sticks, Liquids for plug-in device, Mosquito-repellent activity.

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INTRODUCTION

Vectors are the organisms that transmit a parasite or pathogens from one infected person to another causing serious infections. According to the WHO, every year deaths due to vector-borne diseases account for over 17% with more than one billion cases and over one million deaths. In the Indian scenario, malaria, dengue, chikungunya, filariasis, encephalitis, and kala-azar are of major concern [1]. National Vector-Borne Disease Control Program data of 2012 indicated that 50% of total malaria cases are due to *Plasmodium falciparum* [1,2]. Lancet's study claims that the malaria toll in India is 40 times the government count [2].

Malaria is caused by the genus *Plasmodium*, namely *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae.* Vectors for the genus *Plasmodium* are various mosquito species *Anopheles (A. arabiensis*, *A. gambias*, *A. funestus*, and *A. stephensi), Aedes (A. aegypti and A. albopictus), and Culex falciparum* [3]. The disease is transmitted through mosquito bites. Both humans and mosquitoes are considered to be parasite's hosts. *Anopheles* and *Culex* species are most active during the dawn and dusk and also at night whereas, *Aedes* species are active during the day time. Breeding sites for *Anopheles* and *Aedes* species are fresh water bodies and *Culex* species are polluted stagnant water bodies [4].

A few interventions to reduce the disease burden are the elimination of breeding sites, sanitation hygiene, and regular waste removal. For indoor protection, spraying insecticides, fitting doors and windows with wire mesh, appropriate clothing, mosquito nets, and use of mosquito-repellent creams, plug-in devices, coils, incense sticks, etc., [2,5,6]. Long-lasting insecticide nets (LLINs) are advocated by the WHO as they offer both physical and chemical protection. The use of LLINs reduced malaria incidence by 50% in sub-Saharan Africa which accounts for more than 90% of global cases. The durability and cost of nets are reasons for concern [7].

Commercially available mosquito repellents contain synthetic chemicals which are toxic and can cause skin/eye irritation, cough, and asthma and their long-term use is unwarranted in children, the elderly, and pregnant women [8,9]. The literature review indicated various herbs and essential oils, namely lavender, mint, sage, lemongrass, citronella, rosemary, basil, camphor, cedar, clove, eucalyptus, lemon eucalyptus, geranium, chamomile, peppermint, neem, pyrethrum to possess mosquito-repellent activity [8-12]. Researchers were successful in developing herbal formulations and many are available in the market for regular use.

The present research work aimed to develop a herbal formulation by designing it as a gel, incense stick, and solution for plug-in device and to evaluate and compare the repellent activity of the formulations with the marketed products.

METHODS

Materials

Essential oils of *Lavandula angustifolia* (lavender), *Rosmarinus officinalis* (rosemary), Cymbopogon citratus (lemongrass), and *Juniperus barbadensis* (cedarwood) were procured from Natural Biotech Solutions, New Delhi, India. Camphor and charcoal were purchased from the local market, in Hyderabad, India. Jigat and neem powder were procured from Shyam Sunder Ayurvedic, Hyderabad, India. Span 20, tween 80, Carbopol 934, triethanolamine, and ethanol were purchased from SD Fine Chem Limited, Mumbai, India. All chemicals and solvents used were of analytical grade.

Methods

Preparation of emulgel

Emulgels were prepared following the procedure mentioned by Kumar *et al*., 2022 [13], and a flow chart of the same is indicated in Fig. 1. The formulated gel contains total of 25% w/w of active ingredients.

Table 1: Composition of emulgels

Ingredients	F1 (%)	F2 (%)
Rosemary essential oil	10	10
Lavender essential oil	7.5	7.5
Lemongrass essential oil	7.5	-
Cedarwood essential oil	-	7.5
Span 20	0.5	0.5
Tween 80	0.5	0.5
Carbopol 934	0.5	0.5
Triethanolamine (drops)	3	3
Distilled water (gm^2)	50	50

Table 2: Composition of incense sticks

Ingredients	F1 (%)	F2 (%)
Rosemary essential oil	10	10
Lavender essential oil	7.5	7.5
Lemongrass essential oil	7.5	-
Cedarwood essential oil	-	7.5
Neem powder	10	10
Jigat powder	35	35
Charcoal powder	20	20
Camphor	5	5
Distilled water (gm^2)	50	50

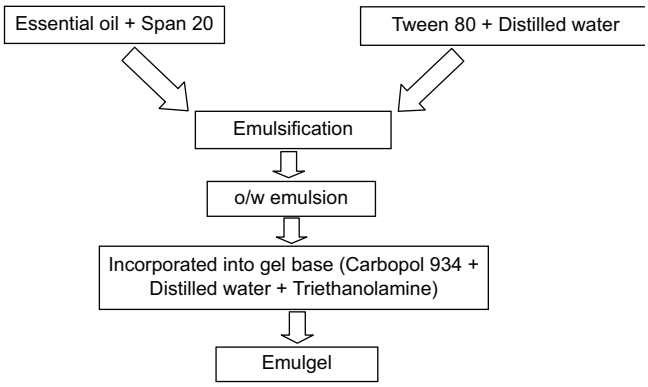

Fig. 1: Schematic representation for the procedure of emulgel

Preparation of incense sticks

Incense sticks were prepared following the procedure mentioned by Bahadur *et al*., 2020 [14]. The formulated incense sticks contain a total of 25% w/w of actives. All the base ingredients, neem, charcoal, jigat, and camphor, were finely powdered and passed through a sieve and mixed thoroughly. Oils were added and mixed to attain uniformity. Water Qs were used to attain a dough-like consistency. Small quantities were hand rolled and dried in shade for 24 h.

Preparation of liquid for plug-in device

The liquid for the plug-in device was prepared following the procedure mentioned by Sekar and Rahim., 2017 [15]. The formulated liquid contains a total of 25% w/w of active ingredients. Solution of oils in ethanol was added to the aqueous phase containing tween 80. Emulsification was achieved by magnetic stirring for 30 min.

Evaluation of emulgel formulation

Physical evaluation

The formulated gel was visually evaluated for color and transparency. The smoothness of the gel was evaluated by simply rubbing the formulation between fingers to feel for smoothness, clumps, roughness, and homogeneity [16].

pH

The pH of the gel was evaluated with the digitally calibrated pH meter. 1 g of the formulated gel was dissolved in 25 mL of distilled water. The measurement of pH was performed in triplicate and the average reading was calculated [16].

Spreadability

Two gram of formulated gel was sandwiched between two similar glass slides. 200 gm weight was applied on the slides to get rid of the entrapped air to form a uniform film between the slides. The top slide was dragged and the time taken to separate the glass slides from one another was determined from the formula:

$$\text{Spreadability (S)} = ML\backslash T$$

where M = weight placed on the upper slide (200 g); L = length of a glass slide (6 cm); T = time taken (sec) to separate the glass slides from one another [16].

Viscosity

The viscosity of the formulated gel was determined using the digital Brookfield viscometer using spindle no. 64 at 10 rpm and temperature of 25±1°C. The corresponding dial reading was noted [16].

Fig. 2: Collection and rearing of mosquitoes

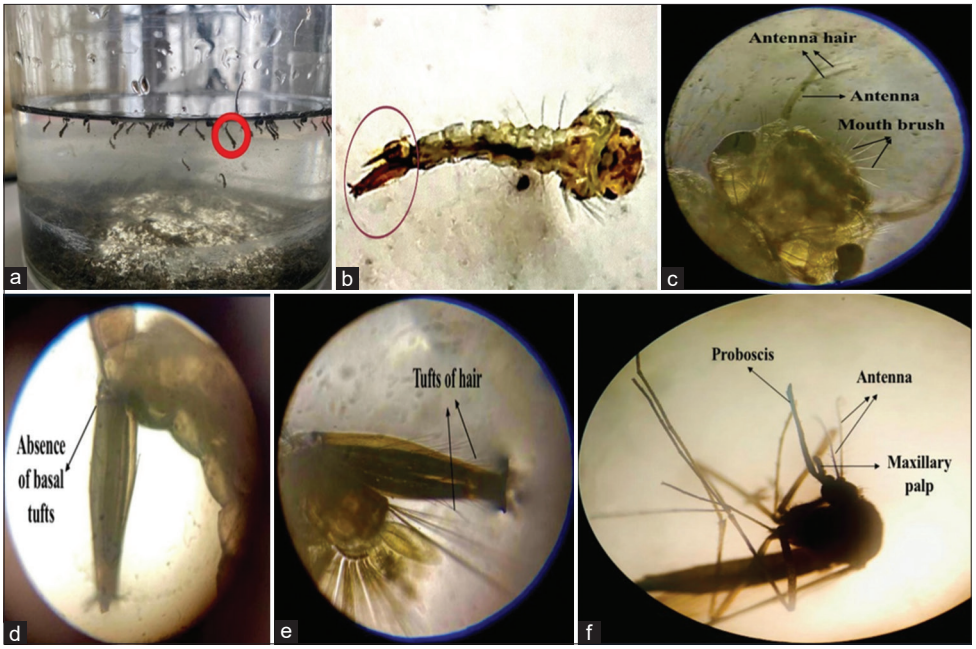

Fig. 3: Pictures indicating characters of reared mosquitoes. (a) Resting position of larvae, (b) Microscopic view of the presence of siphon, (c) Microscopic view of head, (d) Microscopic view of the absence of basal tufts, (e) Microscopic view of a row of tufts along siphon, (f) Microscopic view of a mosquito

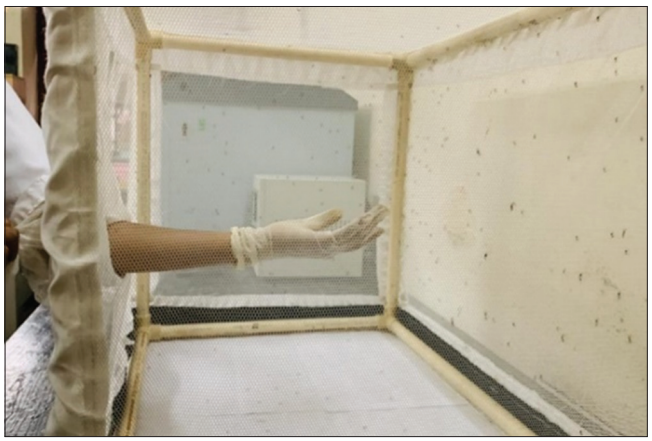

Fig. 4: Mosquito-repellent activity - Treated arm

Fig. 5: Mosquitoes with no movement and lying on the floor

Fig. 6: Mosquitoes with no movement and lying on the floor

Table 3: Composition of liquid for plug-in device

Ingredients	F1 (%)	F2 (%)
Rosemary essential oil	10	10
Lavender essential oil	7.5	7.5
Lemongrass essential oil	7.5	-
Cedarwood essential oil	-	7.5
Ethanol	22.5	22.5
Tween 80	7.5	7.5
Distilled water (gm^2)	50	50

QS: Quality standards

Table 4: Physical evaluations of emulgel

Formulation	Color	Transparency	Homogeneity	Washability
F1	Off-white	Not transparent	Good	Easily washable
F2	Off-white	Not transparent	Good	Easily washable

Table 5: Evaluation parameters of emulgel

Formulation	pH	Spreadability (g.cm/s)	Viscosity (cp)	Extrudability
F1	6.53	25.32	4766	++
F2	6.64	21.42	4730	++

Table 6: Evaluation of incense sticks

Formulation	Burning time (min)	Ash weight (g)	Odor
F3	80±5	0.165	Pleasant
F4	85±5	0.178	Pleasant

Extrudability

Fifteen grams of gel is transferred into a collapsible aluminum tube and sealed using a manual ointment machine. The tube was sandwiched

Table 7: Morphological and microscopical features of different mosquito species

Species	Resting angle to the surface	Siphon the tufts siphon	Basal tufts	Hair along at the end	Antennae with	Maxillary palps
Anopheles	Horizontal	Absent	Present	Absent	Absent	Long
Aedes	Hanging at an angle	Present	Present	Absent	Absent	Short
Culex	Hanging at an angle	Present	Absent	Present	Present	Short

Table 8: Behavior of mosquitoes on arms treated with F1 and F2

Duration of time positive control F1 F2 (h)	Repellency (%)	Repellency (%)	Repellency (%)
1	100	100	95
2	100	100	92
3	99	95	89
4	98	93	87

between two glass slides. A weight of 500 gm was placed on them and simultaneously opened the cap. The length of the extruded ribbon in 10 min was noted. The test was carried out in triplicate [16].

Washability

The ease of wash ability of gel with water was observed visually after application [16].

Evaluation of incense sticks formulation [17]

Burning time and ash

The burning time of incense sticks varies depending on thickness, length, and quality. The time taken for the incense sticks to burn down (in min) was recorded. The total ash obtained was weighed.

Odor and irritability

The incense sticks were observed for odor and irritation to the eyes or throat on burning.

Smoke visibility

The visibility of smoke was observed.

Evaluation of liquid for plug-in device formulation

Phase separation

The stability of the formulation was evaluated by visually observing the phase separation of the liquid.

Collection and rearing of mosquitoes

Mosquito larvae were collected from the stagnant waters of Hussain Sagar Lake, Hyderabad. They were fed using fish food and the pupae were allowed to emerge into adults. Sucrose solution was used as mosquito feed. Adult mosquitoes were transferred into another net cage to test mosquito-repellent activity [18].

Identification of mosquito

Larvae and adult species were identified by morphological and microscopical examination [19]. Fig. 3 depicts the features of larvae and adults.

Screening of emulgels for mosquito-repellent activity

100 mosquitoes were transferred carefully into the net cage (60 cm × 50 cm). Initially, the volunteer's forearm was thoroughly washed with soap and dried. The right forearm acts as control and was placed in the net cage for 30 s to assess their activity. A minimum of 10 landings is an indication of the suitability of commencement. If the mosquitoes were more than 10, the study was commenced. The left forearm was treated with the mosquito-repellent gel formulation and the untreated skin was covered with a glove. The forearm was placed inside the net cage for 3 min and the number of mosquitoes that landed and probed the skin was recorded. The observations were repeated every 30, 60, 120, and 240 min and compared with the positive control odomos. The experiment was performed in triplicate [16].

Screening of incense sticks for mosquito-repellent activity

100 mosquitoes were transferred carefully into the net cage (60 cm × 50 cm). The screen cage was placed in the center of the room and an incense stick was lit and placed at a distance adjacent to the cage. The duration of the study was 4 h. The behavior of the mosquitoes was compared with the positive control (black dragon). The experiment was performed in triplicate [14].

Screening of liquid for mosquito-repellent activity

One hundred mosquitoes were transferred carefully into the net cage (60 cm × 50 cm). The formulated liquid in plug-in device was placed at one corner of the net cage. The duration of the study was 4 h. The behavior of the mosquitoes was compared with the positive control (Good night). The experiment was performed in triplicate.

RESULTS AND DISCUSSION

Evaluation of emulgels

Gel formulations F1 and F2 were evaluated for appearance, homogeneity, and washability and the results are presented in Table 4.

The data obtained for spreadability, pH, extrudability, and viscosity are indicated in Table 5. The pH of the gel formulations lies in the normal pH range of the skin. Spreadability studies demonstrated that they spread uniformly with minimum shear. The extrudability is satisfactory indicating good flow.

Evaluation of incense sticks

Table 6 includes data for burning time, ash weight, odor, irritability, and smoke visibility. The odor was pleasant and the subject did not experience any irritation.

Evaluation of liquid for plug-in device

No phase separation was observed in 24 h.

Identification of mosquito species

The observations specified below indicated that the reared mosquitoes are Culex species.

Mosquito-repellent activity

The formulated emulgels F1 and F2 were evaluated for mosquito-repellent activity and compared with the positive control (odomos).

Mosquito repellency in 4 h was determined for F1 and F2 and is 93% and 87%, respectively. The standard product (odomos) demonstrated 98% repellency. F1 displayed better results than F2.

Mosquito-repellent activity

The formulated incense sticks were evaluated for mosquito-repellent activity and compared with the positive control (black dragon).

Mosquito repellency in 4 h was determined for F3 and F4 and is 89%, and 79%, respectively. The standard product (Black dragon) demonstrated 100% repellency. F3 showed better results than F4.

Mosquito-repellent activity

The formulated liquid for plug-in device was evaluated for mosquito-repellent activity and compared with the positive control (Good night).

Table 9: Behavior of mosquitoes when incense sticks are ignited

Duration of time (h)	Formulations	Number of mosquitoes freely moving in the net	Number of mosquitoes aligned to the net	Number of mosquitoes with no movement and lying on the floor	Repellency (%)
1	Positive control	45	37	18	55
	F3	48	43	9	52
	F4	51	43	6	49
2	Positive control	28	37	35	72
	F3	30	52	18	70
	F4	34	53	13	66
3	Positive control	10	34	56	90
	F3	22	52	27	78
	F4	28	51	27	72
4	Positive control	l0	29	71	100
	F3	11	54	35	89
	F4	21	52	27	79

Table 10: Behavior of mosquitoes for liquid in plug-in devices

Duration of time (h)	Formulations	Number of mosquitoes freely moving in the net	Number of mosquitoes aligned to the net	Number of mosquitoes with no movement and lying on the floor	Repellency (%)
1	Positive control	40	47	13	60
	F5	46	44	10	54
	F6	48	45	7	52
2	Positive control	25	51	24	75
	F5	32	47	21	68
	F6	40	45	15	60
3	Positive control	13	50	37	87
	F5	21	47	32	79
	F6	28	50	22	72
4	Positive control	44	9	47	96
	F5	11	45	44	89
	F6	15	54	31	85

Mosquito repellency in 4 h was determined for F5 and F6 to be 89%, and 85%, respectively, whereas the standard product (Good night) with 96% repellency. F5 showed better results than F6.

CONCLUSION

Selected volatile oils were formulated as gels, incense sticks, and liquids for in plug-in devices. The actives were selected based on protection time and % repellency. The formulations were studied for mosquito-repellent activity using net cages. Results obtained indicated formulations containing lemongrass oil possess better repellency. Those containing cedarwood are slightly inferior. Gel F1 exhibited 93% repellency in 4 h and is a better candidate. The performance of all formulations is comparable to the marketed formulations. All the developed formulations are suitable for indoor protection from mosquito bites.

Further studies to demonstrate safety, stability, and field experiences to demonstrate suitability for regular use need to be carried out.

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AUTHORS CONTRIBUTION

- All authors have contributed in the designing and executing the research work and preparation of the manuscript.

CONFLICT OF INTEREST

There are no conflicts of interest regarding the research project.

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