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"Design And Characterization Of Mosquito Repellent Emulgel Formulations For Circumventing Infectious Diseases"

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Abstract:

Background: Herbal based essential oil (EO) containing catnip & citronella has been reported as an excellent mosquito repellent. But mild sensitive and volatile nature limit its topical application and hence it was intended to formulate a non-irritant, safe, and consistent product emulgel with sustained abode time on skin.

Results: The emulgel was made using solvent dispersion technique. Sensory evaluation, accuracy pH, softness, greasiness, and stickiness were all measured. The optimised batch (F4) was showing the viscosity (90249.67±139.95 cP), profile of texture (70.33±0.88 mg) as well as extrudability (639.62± 8.09 mg). In contrast

to the other mosquito repellents, F4 was found to have been secure for more than 90 days, as well as having a longer period in relation to repellency against mosquitoes.

Conclusions: Citronella and catnip oil in F4 were found to be intact using HS-GC. The primary annoyance index (PII 0.45) assigned F4 to the irritation barely perceptible category. Formulation F4 with distinct stability, texture profile, extended residence time on skin and lower the promise of PII have been observed. Industrial application and provided a promising substitute for synthetically produced products.

Keywords: Citronella oil, Catnip oil, Malaria, Mosquito repellent, Emulgel, Membrane permeation

Background

The dengue fever, malaria, zika, chikungunya, and other mosquito-borne illnesses are spreading all around the world [1]. The mosquitoes are the deadliest of all human disease vectors [2]. They are believed to be responsible for hundreds of millions of deaths per year [3, 4].There are few effective treatments available for those living in mosquito-infested areas [5].Half of the world's population living in mosquito-endemic areas; these infectious illnesses are considered a serious public health problem, resulting in significant disease burden [6]. Despite all of the existing measures in poor nations to minimize mosquito breeding and biting, disease transmission remains a challenge [7, 8]. The malaria, dengue fever, and Japanese encephalitis are all of major concerns in India's north-eastern regions. There have been several instances of mosquito-borne illnesses affecting large populations in a number of Indian towns [9, 10]. Several studies on laboratory-reared Anopheles stephensi have recently been conducted to assess the efficiency of mosquito repellent compositions [11]. In India, the 'type' form of Anopheles stephensi is a capable vector of malaria in urban settings, but the type 'mysorensis' plays a minor role in malaria transmission, possibly due to its zoophilic character [12, 13]. Anopheles mosquitoes of the 'intermediate' type are abundant in rural and peri-urban regions, but their function in malaria transmission is uncertain [14, 15, 16]. The mosquito species Aedes albopictus and Aedes albopictus are approved by the World Health Organization (WHO) for laboratory repellency testing. (Preferably Aedes aegypti), Anopheles (preferably Anopheles gambiae, Anopheles stephensi, or Anophelesalbimanus) and Culex (preferably Culex quinquefasciatus) species [17].

To avoid mosquito bites, people in tropical nations with low per capita income rely on smoke and plant materials. To combat mosquito-borne illnesses, prevent mosquito bites by using personal protective measures such as insecticide-treated bed nets and repellents [18]. Scientists in high-income nations are researching on mosquito repellent compositions that will protect military personnel operating in disease-prone areas [19, 20].

However, mosquitoes developed resistance to at least one insecticide in 80% of malaria endemic countries, resistance may build to artemisinin monotherapy by Plasmodium falciparum protozoa similar to how widespread chloroquine administration led to the re-emergence of malaria in Peru following those 30 years of low incidence [21]. Artemisinin resistance is marked by mutations in the K13 gene, but tetraoxane-based compounds have inhibitory properties against various protozoa strains [22]. Mosquito resistance is reducing the effectiveness of insecticides [23]. Mosquitoes are also adapting their feeding habits in response to mosquito nets. Mosquitoes have adapted to insecticide-treated nets by modifying their biting habits, according to Moiroux et al.[24]. The percentage of people biting outside increased from 45 to 68 % [25].

As a result, nets would be less successful for children under the age of five and pregnant women, who are the most vulnerable to the disease. Mosquito repellents applied to the skin (MR) are one way to avoid mosquito bites. N, N-diethyl-meta-toluamide (DEET) is an effective mosquito repellent, but it is prohibitively expensive for rural Sub-Saharan Africans [26–29]. Using native plants as a source of mosquito repellents can boost local economies while also protecting the population. Odalo et al.,extracted and tested the topical repellency of essential oils from Kenyan plants, Conyza newii (Compositae), Plectranthusmarrubioides (Lamiaceae), Lippiajavanica (Verbenaceae), Tetradenia riparia (Lamiaceae), and Tarchonanthuscamphoratus (Asteraceae) [30].Under the same experimental conditions (3 min, forearm exposure), the oils were more effective than DEET at repelling mosquitoes. The family of Nepeta cataria L. is Lamiaceae. The genus Nepeta is commonly cultivated and is native to temperate and tropical zones in Asia and Europe [31]. The conditions like chills, colds, constipation, headaches, infections, inflammations, rheumatism, sore throats, spasms, and stomach aches are treated with Nepeta catariain traditional medicine [32]. The essential oil of N. cataria has antibacterial and antimicrobial properties [33, 34]. The main constituent of this oil is Nepetalactone (NPL) [35–38]. The oil of catnip is a better spatial repellent than DEET has shown efficient topical repellency [39]. When formulated with isopropyl alcohol (1 % w/v), NPL is as active as DEET. The hydrogenated form of nepetalactone is dihydronepetalactone. It is twice active than DEET [40]. Catnip's essential oil, when applied to the skin, provided 480 min of protection [41]. Furthermore, N. cataria has a better safety profile than DEET [42]. Citronella oil, lemon oil, and eucalyptus oil are currently registered as insect repellents by the United States Environmental Protection Agency (USEPA) due to their low toxicity, high effectiveness, and high consumer satisfaction. These are effective alone or in combination with other natural or commercial insect repellents at concentrations ranging from 0.05 to 15% w/v [43–45]. Citronella oil does repel mosquitoes, the major components are citronellol, citronellal, and geraniol. Because of rapid volatility and irritant nature limits use in the said concentration range [46]. An emulgel can reduce volatile nature of oils and prevent direct contact with the skin which facilitates safe and efficient delivery of the oil for targeted time.

An essential part of the mosquito repellent manufacturing process is in-process quality control (IPQC). The specific tests are performed at various stages to validate the production process for the consistent quality of finished goods amongst the batches, dependable over time, and safe to use. The raw materials need to be tested for prerequisites as per specified specifications. Furthermore, as with interested formulation the pH, desired moisture content and gravity should be determined [46]. Other unambiguous quality control parameters including texture profile must be addressed properly in the production of emulgel (a semisolid formulation) in order to increase the durability, elegancy, and therefore consumer acceptance more deliberately.

The present emulgel of citronella oil and catnip oil mosquito repellent preparation using dispersion technique for the topical application. Thequality control testing of emulgel to achieve desired characteristics and to enhancepatient acceptance.

Methods

Batch steam distillation was used to make Citronella oil and Catnip oil. Tween20, Span 20, Carbopol 934P, and Propylene glycol were purchased from SD Fine Chemicals Ltd., Mumbai, MS, India. All other chemicals used were of laboratory grade.

Essential oil extraction

Batch steam distillation is the most common method for extracting oil (Fig. 1). However, practical yield of oil is less than 0.1%. It is an energy-consuming process and therefore identifying cost effective energy sources was a major hurdle [47, 48].

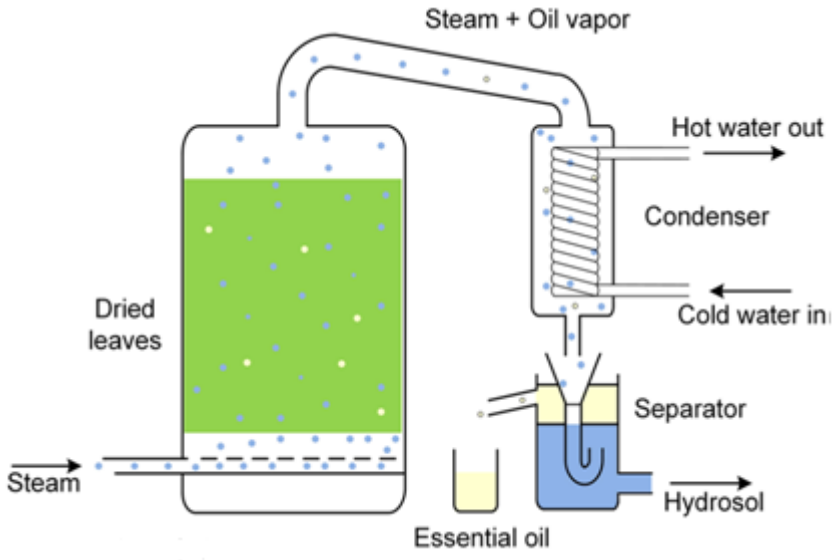


Fig.1 Citronella and catnip oils steam distillation

Preparation of emulgel

The general heuristics of mixing sequence and methodology used to prepare emulgel are summarized in Table 1. Although most of the heuristics are based on traditional methods, they can all be derived from a simple understanding of the emulgel preparation phenomenon. By soaking a weighed amount of carbopol 934P (1 %w/w) in 100ml of solvent-blend for 2 h, gels with 0.5–2% w/w catnip oil and 1-3 % w/w citronella oil were made. The weighed amount of drug emulgel was dispersed in the above dispersion with continuous stirring, then the necessary amount was added to the soaked carbopol dispersion to neutralize the polymer with continuous stirring, and finally a specific amount of viscosity modifier and permeation enhancer were added and stirring continued for about half an hour to obtain a sparkly clear gel [49, 50].

Table 1 Preparation of mosquito repellent emulgel

Sr. No.	Ingredients (%)	F1	F2	F3	F4	F5
1	Catnip Oil	0.5	1	1.5	1.8	2
2	Citronella Oil	3	1	2	1.8	1
3	Carbopol 934P	1	1	1	1	1.5
4	Liquid Paraffin	1	1	1	-	-
5	Propylene Glycol	5	5	5	5	5
6	Methyl Paraben	0.03	0.03	0.03	0.03	0.06
7	Propyl Paraben	0.01	0.01	0.01	0.01	0.06

8	Tween 20	0.6	0.6	0.6	1	0.6
9	Span 20	0.9	0.6	0.9	1.5	0.6
10	Ethanol	2.5	2.5	2.5	2.5	2.5
11	Distilled Water up to	100	100	100	100	100
Where, a and b are the weight in g and volume in mL respectively.						

Preparation of calibration curve

The absorbance of a series of working solutions containing 30g/mL of Catnip oil/Citronella oil were measured at 282nm and 277nm against a reagent blank[51].

Evaluation of emulgel

Spreadability

The spreadability of the emulgel formulations was measured using an apparatus suggested by Mutimer et al., which was restructured in the laboratory and used in the analysis[52,53].The specialized apparatus was used to assess the spreadability, which was then measured using a formula-

S = ml/t

Where, S = spreadability, m = weight tied to the upper slide, l = length of the glass slide and t = time in sec.

Viscosity

A Fungi Lab viscometer was used to calculate the viscosity of the gels that were prepared. The sample was filled in sample holder and the spindle no. 03 was submerged in it. After 2 min, the viscosity of the formulation was recorded [54].

Extrudability

The extrudability apparatus was properly fabricated in the laboratory. It consists of a wooden block angled at 45º with one end fitted with a small, long metal strip (tin) and the other end left open. On the inclined surface of a wooden block, an aluminum tube containing 20 g of gel was mounted for 30 sec; a 1 kg weight was put on the free end of the aluminum strip and just touched[55, 56].

Drug content uniformity

Using a Shimadzu Ultraviolet- visible (UV) spectrophotometer at 282 nm and 277 nm against a blank, the content of catnip oil and citronella oil was determined for triplicate determination [59]. The blank solution was made in the same way as the drug solution (EO).

In-vitro Diffusion study

A cylindrical glass tube (with a 22mm internal diameter and a height of 76 mm) was used as the apparatus, which was opened at both ends. Accurately weighed 1gm of emulgel containing 0.018 % w/w catnip oil and 0.018w/w citronella oil was spread evenly on the surface of the gelatin membrane and tied to one end of the tube, allowing the emulgel spread in inner surface of tube circumference [57, 58].

Skin irritation study

Experimental animals

The adult New Zealand white rabbits of either sex with a body weight of 2.5 kg were selected. The rabbits were acclimatized to the experimental environment for seven days (at 22°C with humidity regulation and a 12h dark/light cycle) before start of experiment. They were access to ample commercial rabbit diet and drinking water. Rehabilitation treatment following experimentation to the point of returning to a normal existence, as well as the care of such an animal, has been taken precedence over its intended statistical significance. The experiment was carried out according to the Organization for Economic Co-operation and Development (OECD) guideline number 404, which was last revised in 2002 [60], and the protocol for this research (registration number 1336/AC/10/CPCSEA) was approved by the Institutional Animal Ethics Committee (IAEC) in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals(CPCSEA),Government of India, guidelines.

Experimental protocol

Before 24 h of applying the sample, the back of each rabbit (n = 6) was clipped free of fur with a curved scissor. The skin clipped area was divided into two 1 × 1 inch test sites. Normal saline was used as the control, and lactic acid (98% in distilled water) was used as the standard irritant [61].Single test materials, namely, citronella oil and catnip oil 500 mg, cream base, F4 (corresponding to 500mg of the citronella oil and catnip oil) were applied on one test site of the animal at a time against vehicle control gauze was used to cover all of the wounds, and the rabbit's back was wrapped in a non-occlusive bandage. The bandage was removed after 4 h, the sites were macropathologically examined for skin irritation, and the observation was repeated after 24, 48, and 72 h [62]. Skin reactions are graded separately on a 0–4 scale for erythema and edoema. For erythema, a score of ‘0’ indicates ‘no erythema’, a score of ‘1’ indicates ‘very slight erythema/barely perceptible erythema’, a score of ‘2’ indicates ‘well-defined erythema’, a score of ‘3’ indicates ‘moderate to severe erythema’, and a score of ‘4’ indicates ‘severe erythema (beet redness) to slight Eschar formation’ (injuries in depth).For edoema, a score of ‘0’ indicates ‘no edoema’, a score of ‘1’ indicates ‘very slight edema/barely perceptible edoema’, a score of ‘2’ indicates ‘slight edoema’ (edges of area well defined by rising), a score of ‘3’ indicates ‘moderate edoema’ (raised approximately 1 mm), and a score of ‘4’ indicates ‘severe edoema’ (raised more than 1mm and extending beyond the area of exposure).The primary irritation index (PII) was calculated as the arithmetic mean of the values from the six animals, i.e., the six patches with the same test material. According to OECD test guideline number 404, test materials produced PII values of 0 as non-irritant, 0.04 to 0.99 as barely perceptible irritation, 1.00 to 1.99 as slightly irritant, 2.00 to 2.99 as mildly irritant, 3.00 to 5.99 as moderately irritant, and 6.00 to 8.00 as severely irritant [63].One-way analysis of variance (ANOVA) was used to compare the mean PII values of the experimental groups, followed by Tukey's post hoc test.

Mosquito repellent activity

Mosquito repellency was tested using a modified version of the designer equipment described by Tripathi et al. The apparatus consists of a chamber A that contains a smaller chamber B with a copper wire mesh sheet on top and is connected to chamber C outside of chamber A via a side tunnel The test material was placed in chamber A, and the mosquitoes were placed in chamber B. Mosquito repellent activity of citronella oil & catnip oil and F4 formulation of various groups such as G0, Gel base; citronella oil & catnip oil; F4 (1%), F4 (5%), and F4 (10%) are optimised prototypes with 1, 5, and 10% citronella oil and catnip oil, respectively. The key criterion for this investigation was mosquito movement across a defined distance (from chamber B to C) after 2 h of sample

application. On the copper wire mesh surface of chamber B of said designer apparatus, an anesthetized rabbit was positioned. In compartment B, sixty mature female mosquitoes were fed. About 100mg of each emulgel and 10L of citronella and catnip oils were poured individually on a cardboard sheet with dimensions (22 × 35 mm) and thickness (2.5 mm) comparable to commercially available mosquito mats. The processed cardboard sheet was positioned in the designer apparatus's large chamber A for 2 h. The number of mosquitoes maintained in both chambers (B and C) was recorded every 0, 15, 30, 45, 60, 90, and 120 min of experimental length [64].The percentage of repellency was determined using the following equation-

$$\% \text{ Repellency} = \frac{\text{Total no. of mosquitoes placed initially in chamber B} - \text{Total no. of mosquitoes present in chamber B}}{\text{Total no. of mosquitoes placed initially in chamber B}} \times 100$$

Head space gas chromatography (HS-GC)

Varian CP-3800 GC (Varian Associates, USA) with Combi Pal Head Space Gas Chromatography using DB-5 fused silica capillary column (30m length, 0.25mm internal diameter, and 0.25m film thickness) was fitted with Flame Ionization Detector was used to analyses Citronella oil and Catnip oil (FID). The oven temperature (60– 240°C) was set at 30°C per minute, with a final hold of 2 min. The injector and detector were both set to 280°C. The carrier gas was hydrogen at a rate of 1 mL/min, with a split ratio of 1: 40. The head space incubator was held at 110°C and 300 rpm for 15 min of incubation time. With a plunger speed of 250 L/sec and a flush time of 8.5 sec, the temperature of the head space syringe was held at 110°C. With a plunger injector speed of 500 µL/sec and the head space volume was 900 µL [65].

Statistical analysis

Data are presented as mean ± standard error mean (SEM). Data for different groups were compared using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. All statistical analyses were performed using GraphPad Prism, Version 5.01 (GraphPad software. Inc., USA).

Stability studies

The stability study of optimised formulation (F4) was carried out in compliance with the International Conference on Harmonization (ICH) guidelines. In the humidity chamber, the optimised cream formulation (F4) was stored in tightly sealed glass containers for 90 days at 25°C and 60% relative humidity. Samples were collected and physicochemical evaluation parameters such as colour, consistency, phase separation, texture analysis, and pH were evaluated at fixed intervals of 0, 30, 60, and 90 days [66].

Results

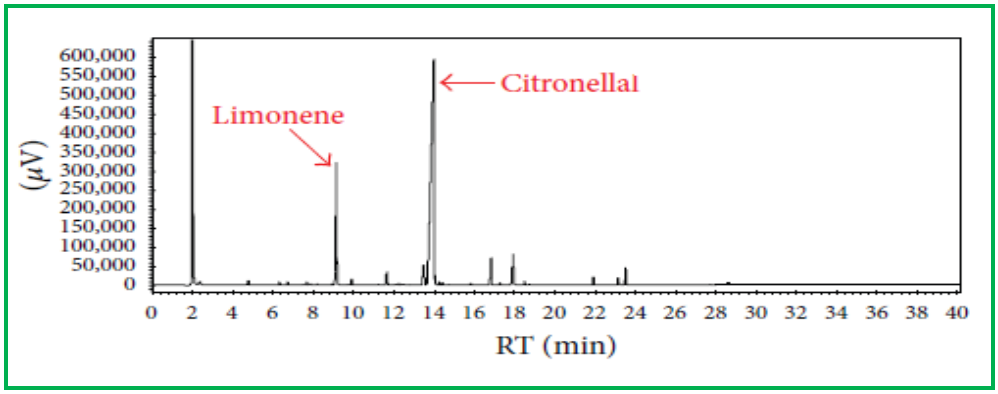
Evaluation of Emulgel

Sensorial assessment was used to assess the elegancy, emulsification, and quality of the first batches of formulated Emulgel. To produce emulgel, researchers measured different concentrations of the

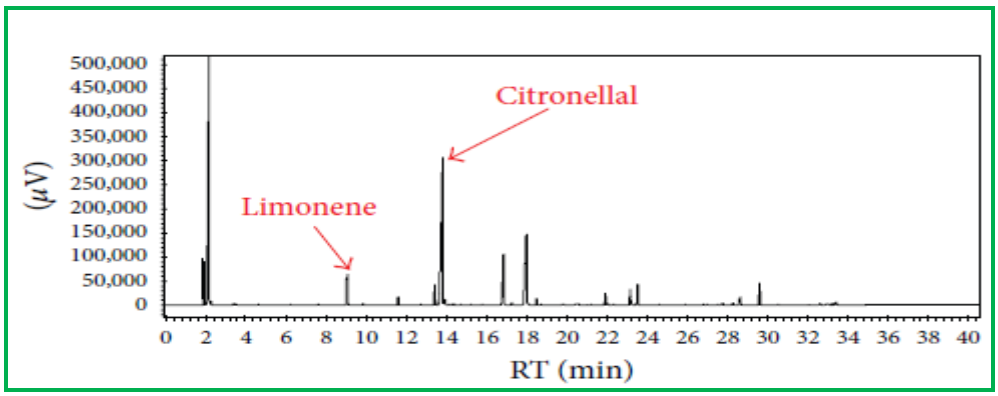
ingredients in the oil and aqueous phases to achieve the desired emulsification, viscosity, consistency, spreadability, and stickiness. The emulgel was white in colour, opaque, and uniform in appearance.

HS-GC

The GC chromatogram of citronella and catnip oilwas shown in Fig. 2. For the development of emulgel, sufficient concentrations of the ingredients in the oil and aqueous phases were tested to achieve the desired emulsification, viscosity, consistency, spreadability, and stickiness. The emulgel was white, opaque, and homogeneous.



(a)



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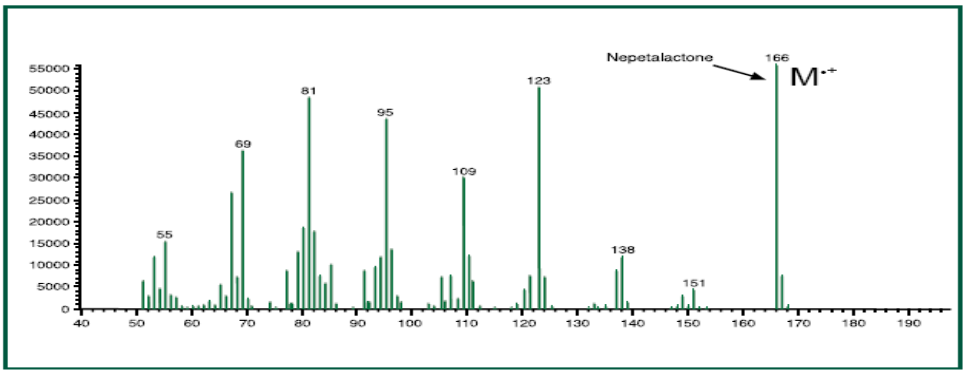

(c)

Fig.2 Analysis of Citronella and catnip oil from optimised batch using head space gas chromatography (HS-GC) (F4)Citronella oil and F4 (optimised batch) GC chromatograms, and catnip oil GC chromatogram.

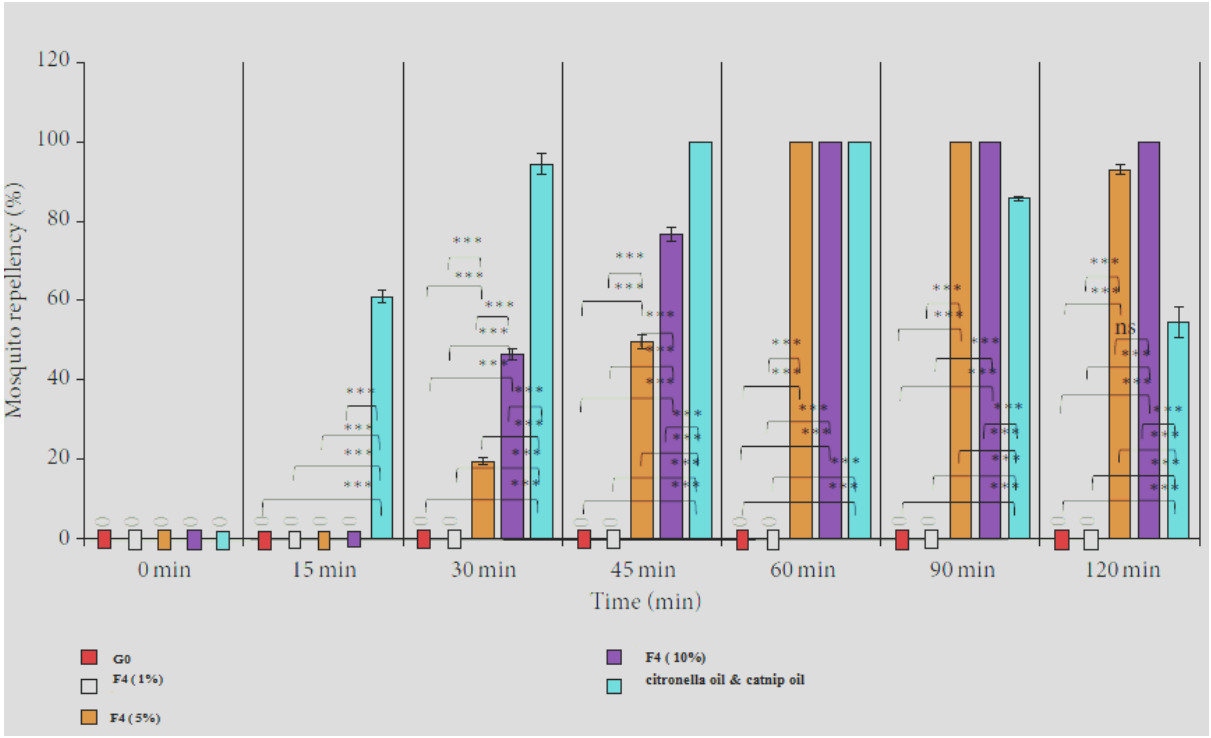

Fig. 3:Mosquito repellent activity of citronella oil & catnip oil and F4. Graphical representation of mosquito repellent activity of various groups such as G0, Gel base; citronella oil & catnip oil; F4 (1%), F4 (5%), and F4 (10%) are optimised prototypes with 1%, 5%, and 10% citronella oil & catnip oil, respectively. The data was presented as a mean SEM (n = 3). One-way analysis of variance (ANOVA) was wont to analyze the info, followed by Tukey's post hoc ergo propter hoc test. ***P 0.001 was considered statistically significant. Observations were representing batches that are not significantly different from one another.

Mosquito repellent activity

Figure 3 depicts a graphical representation of the percentage of mosquito repellency by different groups. At any time, the gel base (G0) and F4 with 1% citronella and catnip oil were unable to repel mosquitoes. Only citronella and catnip oil showed a significant difference (*** P 0.001) in the percentage of mosquito repellency (61 %) after 15 min compared to the other groups. At 30 min, F4 (5%), F4 (10%), and citronella & catnip oil demonstrated significant mosquito repellency (*** P 0.001); however, an intergroup variation study revealed that F4 (5%), F4 (10%), and citronella & catnip oil were also significantly different from each other. F4 (5%), F4 (10%), citronella & catnip oil were found to have higher percentage repellency values at 45 min than at 30 min, with citronella &catnip oil showing 100 percent repellency. At 60 min, F4 (5%), F4 (10%), citronella, and catnip oil exhibited 100 percent repellency. At 90 min, F4 (5 %) and F4 (10 %) showed 100 percent repellency again; however, the percentage repellency of citronella and catnip oil was observed to be declining significantly (***P 0.001).Citronella and catnip oil showed a further decrease in percentage repellency values after 120 min.F4 (5%) also showed a decrease in percentage repellency but was found to be non-significantly different from the value of F4 (10%), i.e., 100 %.This discovery revealed that the F4 (10%) increased the residence time of citronella and catnip oil on the surface. More than 50% mortality was also observed after 1 h in the case of pure citronella & catnip oil, while it was seen after 2 h and 1.5 h in the case of F4 (5%) and F4 (10%), respectively.

Table 2 Texture profile and viscosity of marketed formulation and prepared emulgel

Parameter	F1	F2	F3	F4	Marketed product (MP)
Viscosity (cP)	93160.67 ± 89.04	93161.67 ± 88.04	92160.37 ± 99.02	90249.67 ± 139.95	82959.33 ±35.83
Spreadability(mJ)	80.67 ± 1.43	81.47 ± 1.46	79.61 ± 1.48	70.33 ± 0.88	84.0± 1.00^{ns}
Extrudability (mJ)	740.67 ± 9.18	637.67 ± 8.06	632.61± 8.09	639.62± 8.09	567.64± 9.64

F4 was the optimised formulation, and MP was the marketed formulation, among the F1, F2, F3, and F4 formulations. Data was presented as mean ± SEM (n = 3). One-way analysis of variance (ANOVA) was wont to analyze the info, followed by Tukey's post hoc ergo propter hoc test. All of the groups are substantially different from one another (***P 0.001).

Viscosity

The optimised batch's viscosity was determined to be 90249.67 ±139.95 cP, while the viscosities of F1, F2, and F3 were 93160.67 89.037, 100030.67 36.70, and 82959.33 35.83 cP, respectively (Table 2). Spreadability data supports the prepared emulgel improved acceptability in terms of viscosity, on the skin.

Table 3: Erythema and edema scores for skin irritation index

		04 h						**24 h**						**48 h**										**72 h**	
Sample	**Reaction**	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	**PII**	**Results**
Saline	Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	Nonirritant
-	Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Base																										0.0416^{ns}	Irritation barely perceptible
Test site	Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0		
	Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0		
Control site	Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Standard irritant																										3.833 ^{ns}	Moderately irritant
Test site	Erythema	2	2	2	3	2	2	2	2	2	2	2	3	2	2	2	1	2	1	1	1	1	2	1	1		
	Edema	4	4	4	4	4	3	4	4	3	2	2	3	3	2	1	2	1	1	1	1	2	2	1	1		
Control site	Erythema	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0		
	Edema	0	1	0	1	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0		
F4(optimized batch)																										0.45 $^{ns/a}$	Irritation barely perceptible
Test site	Erythema	0	0	2	1	1	1	1	1	1	1	0	2	0	1	0	1	0	1	0	0	0	0	0	0		
	Edema	0	0	2	0	0	0	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0		

Control site	Erythema	0	1	0	1	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0		
	Edema	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		

For erythema, a score of 0 indicates no erythema, 1 indicates very mild erythema (barely perceptible), 2 indicates well-defined erythema, 3 indicates moderate to extreme erythema, and 4 indicates severe erythema (beet redness) with minor Escher formations0 indicates no edoema, 1 indicates very slight edoema (barely noticeable), 2 indicates slight edoema (edges of area well marked by definite rising), 3 indicates moderate edoema (raised approximately 1 millimeter), and 4 indicates extreme edoema. Evaluation of primary irritation index (PII): 0.00 indicates no annoyance, 0.04–0.99 indicates scarcely perceptible irritation, 1.00–1.99 indicates minor irritation, 2.00–2.99 indicates mild irritation, 3.00–5.99 indicates moderate irritation, and 6.00–8.00 indicates extreme irritation. One-way analysis of variance (ANOVA) was wont to analyze the info, followed by Tukey's post hoc ergo propter hoc test. When normal irritant and test control (emulgel formulation, Standard, and F4) were compared to vehicle control, ***P 0.001 was found to be non-significantly different from saline control. When F4 was compared to a marketed irritant, P 0.001 was found.

Skin irritation study

Table 3 summarizes the findings from the main skin irritation research. The primary annoyance index (PII) of the vehicle control and regular irritant groups differed significantly (P 0.001), indicating that lactic acid has irritation potential in the animals. According to OECD guidelines (OECD, 2002), the PII of an emulgel containing citronella oil and catnip oil (F4) was 0.45, which was found to be non-significantly different from the vehicle or placebo control groups, placing the prepared formulation in the category of discomfort barely perceptible and rendering it appropriate for topical application. Furthermore, when compared to the PII of the regular irritant community, F4 had a substantially lower PII. The erythema and edoema that are characteristic of the F4 formulation limit its usefulness and acceptability for topical application. According to our results, the optimised batch (F4) based on citronella oil and catnip oil outperformed the regular irritant in terms of skin tolerability, suggesting that it has the potential to increase patient acceptance and topical distribution as a mosquito repellent emulgel [49, 50].

Table 4: Stability study of optimized batch (F4)

Parameter	**On day**			
	0	**30**	**60**	**90**
Color[@]	Acceptable	Acceptable	Acceptable	Acceptable
Spreadability (mJ)	70.33 ± 0.88	70.33 ± 1.20	71 ± 1.53	70 ± 1.73
Extrudability (mJ)	639.67 ± 8.09	635.00 ± 13.89	646.67 ± 4.18	635.00 ± 8.14
Viscosity (cP)[*]	30150.7 ± 81.5	90249.67 ± 139.95	90149.67 ± 229.90	90583.00 ± 58.39
pH[*]	6.63 ± 0.02	6.79 ± 0.02	6.73 ± 0.01	6.77 ± 0.02

*Stability tolerance after one freeze/thaw period, based on sensorial assessment. Stable: a change of less than 10%, acceptable: a change of 10% to 20%, unstable: a change of 20% to 40%, and unacceptable: a change of more than 40%.The data was viewed and analysed as mean standard deviation (n = 3).

Stability Studies

Over the course of 90days analysis, the colour, consistency, viscosity, texture profile, and pH of F4 were found to be consistent, and no distinction was shown in Table 4.It demonstrates the consistency of the established emulgel formulation's quality and demonstrating the reproducibility of physical and chemical parameters

Discussion

Sensorial assessment was used to assess the elegancy, emulsification, and quality of the first batches of formulated emulgel. The GC chromatogram of citronella and catnip oil (Fig. 2) for the development of emulgel, sufficient concentrations of the ingredients in the oil and aqueous phases were tested to achieve the desired emulsification, viscosity, consistency, spreadability, and stickiness. The emulgel was white, opaque, and homogeneous.

The viscosity of marketed formulations and F4 Emulgel, the optimised batch's viscosity was determined to be 90249.67 ±139.95 cP, moreover spreadability data supports the established emulgels improved acceptability in terms of viscosity, which falls within the range of the given marketed formulation's viscosity, facilitating F4 application on the skin surface. Mosquito repellent discovery revealed that the F4 (10%) increased the residence time of citronella and catnip oil on the surface (Fig. 3).More than 50% mortality was also observed after 1 h in the case of pure citronella & catnip oil, while it was seen after 2 h and 1.5 h in the case of F4 (5%) and F4 (10%), respectively.

The erythema and edema scores for skin irritation index summarized in Table 3, the findings from the main skin irritation research. The primary annoyance index (PII) of the vehicle control and regular irritant groups differed significantly (P 0.001), indicating that lactic acid has irritation potential in the animals. According to OECD guidelines (OECD, 2002), the PII of an emulgel containing citronella oil and catnip oil (F4) was 0.45, which was found to be non-significantly different from the vehicle or placebo control groups, placing the prepared formulation in the category of discomfort barely perceptible and rendering it appropriate for topical application. In Stability Studies**,** over the course of a 90-day analysis, the colour, consistency, viscosity, texture profile, and pH of F4 were found to be consistent, and no distinction was observed (Table 4).

Conclusions

A mosquito repellent emulgel formulation that safe, stable and effective has been developed. Citronella oil and catnip oils being irritant to skin were successfully transformed into low irritancy and volatility emulgel to provide strong mosquito repellent effect. Promising effectively was preliminary confirmed by texture analyzer by estimating spreadability and extrudability of the emulgel formulation. The consistency and stability of the emulgel (F4) were found to be as per the marketed formulation. Although the safety and efficacy were appraised on animal model, still the clinical trials using human

volunteers are need to be conducted so as to meet regulatory requirements before bringing the product into the market.

List of abbreviations

WHO- World Health Organization

DEET- N, N-diethyl-meta-toluamide

EO- Essential oils

N. cataria- Nepeta cataria

NPL- Nepetalactone

USEPA- US Environmental Protection Agency

IPQC- In-process quality control

IAEC- Institutional Animal Ethics Committee

CPCSEA-Committee for the Purpose of Control and Supervision of Experiments on Animals

OECD -Organization for Economic Co-operation and Development

HS-GC- Head Space-Gas Chromatography

FID- Flame Ionization Detector

ICH- International Conference on Harmonization

GC-Gas Chromatography

MP-Marketed Product

cP-centipoises

ANOVA -Analysis of variance

MJ–mega Joule

PII- Primary irritation index

SEM-Standard Error of Mean

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