ORIGINAL ARTICLE

Formulation and Evaluation of Controlled Release Herbal Mosquito Repellent Gel Containing Encapsulated Essential Oils Obtained from Natural Sources Indigenous to Northeast India

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Abstract

Aim: To formulate and evaluate controlled release herbal mosquito repellent gel containing encapsulated essential oils (EO) obtained from natural sources indigenous to North East India. **Material & Methods:** Essential oils were extracted from the fruits of *Zanthoxylum acanthopodium* (ZA) and *Litsea cubeba* (LC). The essentials oils were analysed using Gas chromatography and Mass spectrometry. Essential oils loaded Chitosan-Sodium lauryl sulphate (Chitosan-SLS) microparticles were prepared using ionic cross-linking method. Essential oils loaded microparticles were characterized using Fourier transformed infrared spectroscopy, Differential scanning calorimetry, Particle size and Encapsulation efficiency determination. Essential oils containing microparticles were loaded into a gel base containing Carbopol 934 and Lutrol F 127. Formulated gels were characterized by organoleptic characteristics, homogeneity test, pH determination, Viscosity determination, *In vitro* membrane permeation and Acute dermal irritation study. Mosquito repellent study was carried out for the gels using non-blood-fed females *Aedes aegypti* mosquitoes. **Results & Discussion:** The percentage yield of essential oils from the fruits of *Zanthoxylum acanthopodium* and *Litsea cubeba* were 0.8647 % w/w and 2.485 % w/w respectively. Encapsulation efficiencies of ZA EO loaded Chitosan-SLS microparticles and LC EO loaded chitosan-SLS microparticles particles were 97.16 % w/w and 96.64 % w/w respectively. Organoleptic properties of EO containing chitosan-SLS microparticles loaded gels were white in colour, having characteristic odour, homogenous and washable. pH of the gels were found within the range of 5.35 to 5.85. The formulated gel containing 3 % w/w essential oil in the encapsulated form (G3) demonstrated 21.49 % w/w permeation of essential oil in the study of *in vitro* membrane permeation. Maximum mosquito repellent activity was observed with G3 gel. Complete protection time for G3 gel was 2 h against *Aedes aegypti*. Protection time of G3 gel was observed up to 3 h. Acute dermal irritation study confirmed that there was no irritation effect due to the gels. Retained sample stability testing demonstrated that there was no significant change in gels over three months. **Conclusion:** The study resulted in successful development of a novel herbal controlled release mosquito repellent gel using essential oils from North East India.

Key words: *Aedes aegypti*, dermal toxicity, essential oil, gel, mosquito

INTRODUCTION

Mosquitoes are the vectors responsible for spreading different types of fatal human diseases such as malaria, Japanese encephalitis, dengue, filariasis, and yellow fever. It has been reported that mosquitoes are the vectors which alone are responsible for transmission of diseases to >700 million people and cause over 1 million deaths annually across the globe[1,2] According to the American Mosquito Control Association, mosquito control is conventionally divided into two areas of responsibility: Public and individual. It is typically performed

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based on the Integrated Mosquito Management concept. It is based on social, ecological, and economic, a key criterion which typically integrates multidisciplinary methodologies into pest management strategies to effectively protect public health and the environment and improve the quality of life.[3] According to pesticide law, a pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest.[3,4]

Distinct types of insect repellents:

a. Product applied on skin (cream, gel, patch…etc.)
b. Clip-on products typically have a pad containing repellent and a fan or any other mechanism that disperses the repellent near the person.
c. Spatial repellents: They employ heating mechanism to disperse repellent. Typical examples of dispersal mechanisms for spatial repellents include lanterns, torches, table-top diffusers, candles, and coils.

Due to the lack of availability of vaccine, effective drugs as well as emergence of resistant virus or vector, mosquito repellents are precisely a proactive step in this field. Due to the low cost of mosquito repellent, they is an effective way to prevent this mosquito-borne disease. Extensive research on mosquito control is still going on due to probable risk of emergence of the highly resistant vectors. The major research programs focus on the development of mosquito repellent from natural sources because synthetic chemical compounds cause environmental hazards.[5] Essential oils (EOs) from plants are natural sources for most of the repellent. EO of plants contains active ingredients for mosquito repellent activity. Commonly used EOs in marketed mosquito repellent formulations are citronellol oil, eucalyptus oil, and catnip oil.[6,7] The present study was carried out to formulate a suitable controlled release topical formulation of two EOs extracted from *Zanthoxylum acanthopodium* (ZA) and *Litsea cubeba* (LC). Many local tribes of Northeast traditionally use the two plants as mosquito repellents. Previous study from our laboratory demonstrated ZA EO as a potential mosquito repellent using ointment formulation for dermal application.[8] Controlled release of EOs remains a challenging task. Due to the volatility of EOs at room temperature, the essential evaporates rapidly. To control the rate of evaporation of EOs, one commonly used method is encapsulation of EOs within polymeric membrane. Lai *et al.* reported EO-loaded beads. They used sodium alginate as a polymer. Two specific types of cross-linking agents were used. These were calcium chloride and glutaraldehyde. They observed a prolong and slow release oil from the beads. They reported drug release from the beads ranging from 9.45% ± 0.96% to 32.92% ± 3.24% after 24 h.[9] Morett *et al*. reported that encapsulation of oil in microcapsule reduces the loss of the active principles, leading to high-loaded microparticles that offer protection against environmental agents.[10] Natrajan *et al.* reported the encapsulation of EO in chitosan-alginate nanocapsules. A typical release of EO demonstrated a slow and sustained release at neutral pH up to 48 h. Percentage encapsulation efficiency of turmeric oil and lemongrass oil was found to be 71.1% and 86.9% of the total oil, respectively.[11] Voncina *et al.* reported encapsulation of rosemary oil in ethyl cellulose microcapsules. They found 30% w/w oil content in encapsulated microparticles, whereas average void space was 40% in prepared capsules. The particle size of microcapsules varied was reported in the range of 10–90 µm.[12] Wattanasatcha *et al*. reported the preparation of thymol polymeric nanosphere for antibacterial activity evaluation. They formulated thymol polymeric nanosphere with ethyl cellulose and methylcellulose in 1:1 ratio. Thymol loading was 43.53% w/w. Effective bacterial suppression was found with thymol-encapsulated nanosphere-incorporated aqueous gel and cream formulation.[13] Sansukcharearnpon *et al.* reported the preparation of self-assembled nanoparticles with a polymeric blend for encapsulation of fragrance. They prepared self-assembled nanoparticles of ethyl cellulose, hydroxypropyl methylcellulose, and polyvinyl alcohol by solvent displacement method. They used a dialysis process for solvent displacement. The process gave drug loading ≥40% with ≥80% encapsulation efficiency.[14]

MATERIALS AND METHODS

Materials

The fruits of ZA were collected from Meghalaya, Northeast India. The collected plant specimen was identified at the Botanical Survey of India, Eastern Regional Center, Shillong, India. The plant specimen was identified and authenticated as ZA DC, family Rutaceae, through letter no. BSI/ERC/2015/Plant identification/343. The fruits of LC were collected from Meghalaya, Northeast India. The collected plant specimen was identified at the Botanical Survey of India, Eastern Regional Center, Shillong, India. The plant specimen was identified and authenticated as LC (Lour.) Pers. (Lauraceae) through the letter no. BSI/ERC/Tech/2017/350. Chitosan from shrimp shells (Degree of deacetylation ≥75.0%) ($C_6H_{11}NO_4$) and lutrol F 127 was purchased from HiMedia Laboratories Pvt. Ltd. Carbopol 934, triethanolamine, sodium lauryl sulfate (SLS), methanol (HPLC grade), and ethanol (HPLC grade) were purchased from Research-Lab Fine Chem Industries.

Methods

Method of oil extraction

Fresh fruits were crushed using a mortar and pestle. The grinded material was subjected to hydrodistillation for 6 h using Clevenger-type apparatus. EO was collected in a sterilized amber colored glass vial. EO was stored at refrigerated condition until analysis and use.[15]

Identification of terpenoid compounds

Constituents of EO were separated and identified by gas chromatography–mass spectrometry (GC-MS) on an Agilent 5921A GC hooked to an Agilent 5975 mass selective detector. GC was equipped with HP-5MS (30 m × 250 µm × 0.25 µm).

The GC settings were as follows: The initial oven temperature was held at 50°C for 1 min and ramped at 20°C/min to 280°C for 1 min. The injector temperature was maintained at 250°C. The sample (10 µl, dilute to 1% with methanol) was injected in the splitless mode. The carrier gas was helium at a flow rate of 1 ml/min. Spectra were scanned from 50 m/z to 550 m/z at two scans/s. Identification of constituents was made by comparing their mass spectra with those stored in NIST08 (NIST, Gaithersburg, MD, USA) and W8N08 (John Wiley and Sons, Inc., USA). Relative percentages for the individual constituents of the oil were determined from the GC peak area % report.[16]

Preparation of EO loaded particles

About 500 mg of chitosan was dispersed in 50 ml of 1% v/v acetic acid and kept overnight at room temperature. A weighed amount of EO (750 mg) was added to 5 ml of 1% w/v SLS and mixed by vigorous shaking. The emulsion was injected dropwise using 0.287 mm nozzle into 10 ml of 1% w/v chitosan hydrogel (kept in an ice bath) in a magnetic stirrer at 300 rpm. When the emulsion came into contact with chitosan hydrogel, SLS-chitosan particles were formed by ionic cross-linking method. After 3 min of addition, 1 ml of 1M NaOH was added dropwise with continuous stirring for 10 min at 300 rpm. The prepared particles were collected by filtration and subjected to analysis.

Characterization of EO-Loaded Particles

Encapsulation efficiency

Method I (for ZA EO-loaded particles): The total amount of precipitate was collected in a test tube and 3 ml of 10% v/v methanolic acetic acid was added. The mixture was stirred to dissolve the precipitate, and volume was made up to 10 ml with 10% v/v methanolic acetic acid. Further, 0.1 ml was diluted up to 10 ml with methanol. A blank was prepared by the same method using precipitate without EO.

Method II (for LC EO-loaded particles): The total amount of precipitate was collected in a test tube and 3 ml of 10% v/v ethanolic acetic acid was added. The mixture was stirred to dissolve the precipitate, and volume was made up to 10 ml with 10% v/v ethanolic acetic acid. Further, 0.5 ml was diluted up to 10 ml with ethanol. A blank was prepared by the same method using precipitate without EO.

Fourier-transform infrared (FT-IR) analysis

FT-IR spectra were recorded for chitosan, chitosan-SLS physical mixture, chitosan-SLS complex, and EO-loaded chitosan microparticles using Bruker Alpha FT-IR Spectrometer in the range of 4000 cm^{-1}–600 cm^{-1} at room temperature.

Differential scanning calorimetry (DSC) analysis

DSC curves were recorded for each pure EO, blank chitosan particles, and EO-loaded chitosan-SLS microparticles. About 10 mg samples were placed in aluminum pans and then hermetically sealed with aluminum lids. Thermal analyses were performed from 40°C under a dry nitrogen atmosphere with a heating rate of 10°C min^{-1}.[17]

Particle size analysis

Particle size was measured using the method of optical microscopy. Edmundson's equation was used to calculate the average particle size.

$$d\,mean=\left(\frac{\sum nd^{p+f}}{\sum nd^{f}}\right)^{1/p} \quad (1)$$

n is the number of particles
d is an equivalent diameter

p is an index related to the size of an individual particle. p = 1, p = 2, or p = 3 is an expression of the particle length, surface, or volume, respectively. When the value of p is positive, the mean is arithmetic. The mean is geometric when the value of p is zero. For negative value of p, the mean is harmonic. The value of frequency index f varies from 0 to 3. When f has values of 0, 1, 2, or 3, the size frequency distribution is expressed in terms of the total number, length, surface, or volume of the particles, respectively.[18-20]

Method of Preparation of EO-Loaded Particle-Containing Gel

The formulations were prepared by adding a weighed amount of Carbopol 934 and Lutrol F 127 (Poloxamer 407) in distilled water. The polymer/water mixture was kept in the refrigerator for overnight to dissolve the solid particles. The gel system was processed in an overhead stirrer at 1000 rpm for 5 min. The required amount of wet chitosan-SLS microparticles was added to the gel base, and then, the formulations were processed in an overhead stirrer at 1000 rpm for 5 min. G1 gel, G2 gel, and G3 gel were prepared at 1% w/w EO, 2% w/w EO, and 3% w/w EO concentration, respectively, using an equivalent amount of EO-loaded chitosan-SLS microparticles. For N,N-diethyl-m-toluamide (DEET) containing gel (D1) and unencapsulated EO containing gel (G4) preparation, the required amount of DEET was directly added to the gel base without any encapsulation and mixed thoroughly as per the same method for EO-loaded particle-containing gel. All the samples were kept at room temperature for evaluation [Table 1].[21]

Characterization of Gel Formulation

Organoleptic characteristics

Formulations were tested for organoleptic properties such as odor, color, texture, phase separation, and greasiness.[22]

Homogeneity test

About 100 mg of gel was pressed between the thumb and the index finger to notice the consistency of gel that any particles being attached or detached on the finger.[22]

Table 1: Composition of gel formulations of EO-loaded particles

Formulation code	Ingredient (% w/w)		
	Active ingredient	Carbopol 934	Lutrol F 127
G1	Microparticles containing EO equivalent to 1% w/w EO	0.5	15
G2	Microparticles containing EO equivalent to 2% w/w EO	0.5	15
G3	Microparticles containing EO equivalent to 3% w/w EO	0.5	15
G4	3% w/w EO	0.5	15
D1	3% w/w DEET	0.5	15

EO: Essential oils, DEET: N, N-diethyl-m-toluamide

Washability

Gels were rubbed on backside skin of the hand. After drying, the layer was washed with tap water and observed whether it is washable or not.[22]

pH determination

Nearly 2 g of gel was dissolved in 20 ml distilled water, and pH was measured using calibrated pH meter.[23]

Spreadability

It was be measured by spreading of 0.5g of the gel on a circle of 2cm diameter premarked on a glass plate, and then, a second glass plate was placed over the first plate. 500 g of weight was placed over upper glass plate for 5min. The diameter of the circle after spreading of the gel was determined.[24,25]

$$\text{Percentage spread by area} = \frac{A_2}{A_1} \times 100 \quad (2)$$

Where A_1 = 2 cm and A_2 = Final area after spreading of gel.

Viscosity determination

Viscosity of the gels was determined by cone and plate geometry viscometer (Brookfield Viscometer). Typical run was comprised of 1 rpm at 25°C using spindle No. 60.[26]

EO Content in Encapsulated Chitosan-SLS Microparticles

The standard curve for mixed oil of ZA EO and LC EO was developed. A weighed amount of sample was taken in a semi-permeable membrane bag and placed in 25 ml of methanol for overnight at refrigerated condition. Exact amount of blank gel was taken in another semipermeable membrane bag and placed in 25 ml of methanol for overnight at refrigerated condition. Solutions were filtered separately, and absorbance for both solutions was measured. Difference in the absorbance was used for the calculation of oil content.

In vitro membrane permeation

Permeation studies were conducted using Franz diffusion cells. Dialysis membrane was used for the permeation study.

Table 2: Erythema and eschar formation

Skin reaction	Grading
No erythema	0
Very slight erythema	1
Well-defined erythema	2
Moderate-to-severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4

Maximum possible: 4

A weighed amount of test sample was carefully placed in the donor cell, maintaining a complete and intimate contact with the membrane surface. The receptor compartment contained a water-ethanol solution (50:50), to allow "sink" condition and to sustain EO solubilization. These compartments were constantly stirred and thermostated at 32°C with a water jacket. The system was allowed to equilibrate for 30 min before the collection of first sample. An aliquot of receptor medium was withdrawn at predetermined intervals and replaced with the fresh medium until approximately constant absorbance was obtained.[27]

Acute dermal irritation study

Acute dermal irritation study was carried with prior approval from the Institutional Animal Ethical Committee of Dibrugarh University vide Registration no. 1576/GO/E Re/S/11/CPCSEA, Approval no. IAEC/DU/151. Fifteen female albino Wistar rats were taken and divided into five groups. Each group contains three rats. The five groups were control group, G1 gel group, G2 gel group, G3 gel group, and D1 gel-treated group. Approximately 24 h before the study, fur was carefully removed from the dorsal area of the trunk of the test animals by electrical clipping. Test substance was applied to a small area of 6 cm^2 and adequately covered with a gauze patch, which was held in place with a non-irritating tape. After 4 h, the test site was observed for any irritation or corrosion, after that residual test substance washed with water and observed for 14 days. Initially, one animal from each group was tested. After 14 days, two additional animals from each specific group were tested to confirm no irritation or corrosion effect. All animals were carefully examined for possible signs of erythema and edema, and the responses were scored at 60 min and then at 24, 48, and 72 h after removal of the patch [Tables 2 and 3].[25,28]

Mosquito repellent study

Mosquito repellent study was carried out as per the WHO protocol WHO/HTM/NTD/WHOPES/2009.4 with slight modification. Two mosquito cages (size: 35–40 cm per side) each containing 200–250 non-blood-fed female *Aedes aegypti* mosquitoes were used. Mosquitoes were reared, maintained, and tested (in a separate space or room) at 27 ± 2°C temperature, ≥80 ± 10% relative humidity, and a 12:12 (light: dark) photoperiod. Mosquitoes were host-seeking, of uniform age, preferably 5–7 days post-emergence. Complete protection time and percentage protection were determined for the gel formulations. Complete protection time is calculated as the number of minutes elapsed between the time of repellent application and the first mosquito landing and/or probing. 1 ml of the 20% ethanolic DEET solution was used as standard with the equivalent amount (weight/weight) of the candidate repellent on the other arm. In both cases, treatments were applied to ≈600 cm^2 area of the forearm skin between the wrist and elbow. 1 g of gels was applied to one arm, and 1 ml of the DEET standard solution is applied to the other arm. After 30 min, the repellent-treated arm was inserted into the appropriate cage and exposed for 3 min to determine landing and probing activity. Next, the DEET-applied arm was exposed to determine the landing and probing activity. This procedure was repeated at 30- or 60-min intervals and should be used consistently throughout the experiment until first mosquito landing and probing. After that further, readings were taken to find out percentage protection.[29]

$$\text{Percentage protection} = \frac{C - T}{C} \times 100\% \quad (3)$$

C - Average number of mosquito landing and probing in untreated arm

T - Number of mosquito landing and probing in treated arm.

Stability test

Stability tests were carried out as per retained sample stability testing. Samples were kept at room conditions and analyzed at pre-determined intervals of 1 month, 2 months, and 3 months.[30,31]

RESULTS AND DISCUSSION

Percentage Yield of ZA EO and LC EO

The percentage yield of ZA EO was 0.8647% w/w, and the percentage yield of LC EO was 2.485% w/w.

Composition of ZA EO and LC EO

D-limonene was the major compound having percentage content 24.0696% of total content in the composition of ZA EO. Other important essential constituents were present in low concentration. Citronellal and D-limonene were the major compounds having percentage content 14.35% and 5.3% of total content of LC EO, respectively. Other important EO constituents were present in low concentration. The composition of ZA EO and LC EO was found to be significantly different from literature data [Tables 4 and 5].[16,32-35]

FT-IR Analysis

From the interpretation of FT-IR spectra, it was found that there was no interaction between EO and polymer. FT-IR

Table 3: Edema formation

Skin reaction	Grading
No edema	0
Very slight edema	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised >1 mm and extending beyond area of exposure)	4

Maximum possible: 4

Table 4: Major constituents of ZA EO using GC-MS technique

Compounds	Percentage content
D-limonene	24.0696
Ocimene	6.1857
Eucalyptol	2.0292
2-Pyridinecarboxaldehyde	0.003
Limonene	0.9786
Terpinyl formate	0.4921
1S-α- Pinene	4.893
1R-α-Pinene	1.928
α-Pinene	1.416
α-Pinene	0.6454
Isobornyl methacrylate	0.475
Myrtenyl acetate	0.5017
(-)-trans-Myrtanyl acetate	0.0678
Caryophyllene oxide	0.13
Camphene	0.3895
1,7,7-trimethyl-Tricyclo[2.2.1.0 (2,6)]heptane	3.7135
1-Hepten-3-yne	2.7394
1-Pentanol	0.024
3,5-dimethyl-benzenemethanol	0.541
(E)-3 (10)-Caren-4-ol	0.5914
Caryophyllene	2.6209
α-Caryophyllene	0.7701

ZA EO: *Zanthoxylum acanthopodium* essential oils, GC-MS: Gas chromatography–mass spectrometry

Table 5: Major constituents of LC EO using GC-MS technique

Compounds	Percentage content
D-Limonene	5.3004
Limonene	2.7889
Ocimene	2.9161
1R-α-Pinene	2.4380
Citronellal	14.3514
1S-α-Pinene	0.6391
Myrcenyl acetate	1.0915
2,6-dimethyl-2,6-Octadiene	6.3514
Caryophyllene oxide	0.2917
(R)-(+)-Citronellic acid	0.4320
Copaene	0.1674
Caryophyllene	0.4239
α-Farnesene	0.4910
Camphene	0.4298
(E, Z)-α-Farnesene	0.0170
Caryophyllene oxide	2.3079
Longifolenaldehyde	0.3021
Geranylgeraniol	2.8633
Peucelinendiol	0.5704
(-)-α-Pinene	0.7290
(+)-(-)-3-carene	1.9753
Linalool	1.7601

GC MS: Gas chromatography–mass spectrometry, LC EO: *Litsea cubeba* essential oils

spectra confirmed the encapsulation EO in Chitosan-SLS microparticles. For ZA EO-loaded microparticles, prominent IR bands were observed at 2959 cm^{-1}, 2974 cm^{-1}, and 2874 cm^{-1} which occurred approximately at nearby positions for ZA EO IR bands at 2965 cm^{-1}, 2923 cm^{-1}, and 2880 cm^{-1}. For LC EO-loaded microparticles, prominent IR bands were observed at 2958 cm^{-1}, 2922 cm^{-1}, 1453 cm^{-1}, and 1376 cm^{-1} which occurred approximately at nearby positions for LC EO IR bands at 2962 cm^{-1}, 2914 cm^{-1}, 1453 cm^{-1}, and 1376 cm^{-1}. Therefore, it was confirmed that EOs were entrapped within chitosan-SLS microparticles [Figures 1 and 2].

DSC

DSC was carried to study the thermal transition behavior of the particles. As ZA EO and LC EO contain >100 individual compounds in each EO, so a complex pattern of curves was observed for the particles. ZA EO showed a relatively sharp peak at 179.25°C where LC EO showed a complex curve with a board peak at 202.67°C. For the blank Chitosan-SLS microparticles (Blank complex), a board peak was observed at 85.22°C which might be due to the evaporation of residual water molecules in the microparticles. A board peak for LC EO microparticles (LC complex) was observed at 196.22°C due to the oil. In case of ZA EO-loaded particles (ZA complex), a board peak was observed at 122.81°C. This shift in peak position may be due to the presence of moisture in the particle [Figure 3].

Particle Size

The average particle size for ZA EO-loaded microparticles was found within the range of 250.7 ± 0.94 μm. The average

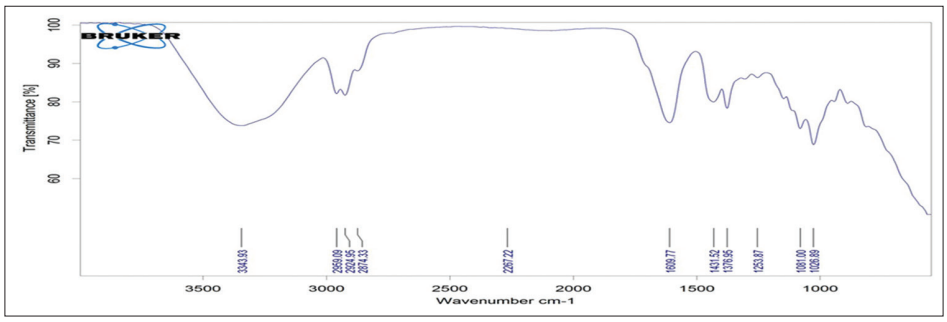

Figure 1: Fourier-transform infrared spectrum of *Zanthoxylum acanthopodium* essential oil-loaded chitosan-sodium lauryl sulfate microparticles

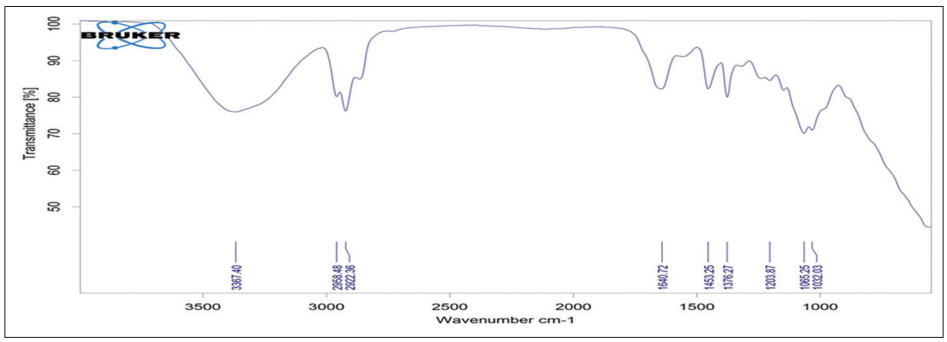

Figure 2: Fourier-transform infrared spectrum of *Litsea cubeba* essential oil-loaded chitosan-sodium lauryl sulfate microparticles

particle size for LC EO-loaded microparticles was found within the range of 233.5 ± 1.5 µm. It was observed that EO droplets entrapped inside the polymer microparticles [Figures 4 and 5].

Organoleptic Characteristics of Gel Formulation

Essential oil-based gels were white in color and possess characteristic odor. DEET gel was colorless and possesses characteristic odor.

Homogeneity and Washability

All the gels were homogeneous and washable.

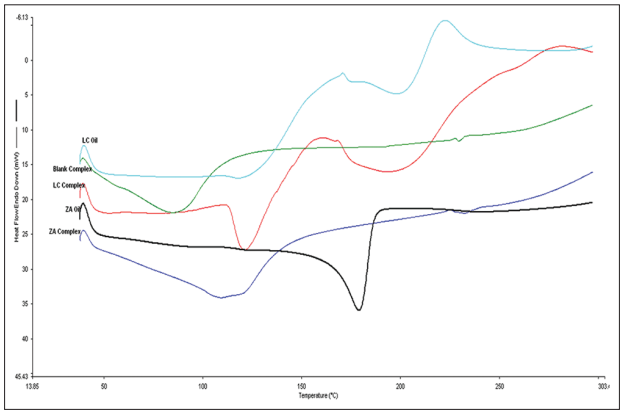

Figure 3: Comparison of differential scanning calorimetry thermograms

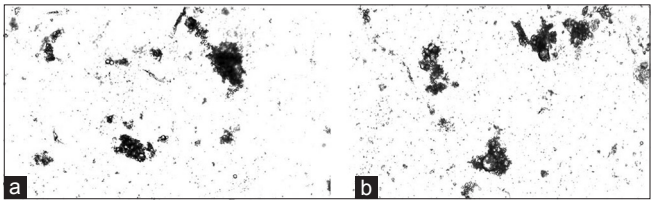

Figure 4: *Image of Zanthoxylum acanthopodium essential oils (ZA EO) and Litsea cubeba (LC) EO-loaded microparticles using optical microscope at ×100 magnification. (a) ZA EO-Loaded microparticles, (b) LC EO-loaded microparticles*

pH Determination

pH of the gels was found within 5.35–5.85 which in the range of skin pH.

Spreadability and Viscosity

Spreadability and viscosity for gels were found to be suitable for dermal application [Table 6].

EO content in the Gel Samples

Essential oil content in gel formulations was found to be 96.44% w/w, 95.6% w/w, and 97.81% w/w for G1, G2, and G3 gel, respectively [Table 7].

In vitro Membrane Permeation Study

This study confirmed that there was a minimal permeation of EO through the semipermeable membrane. *In vitro* EO permeation study demonstrated that the permeation of EO is significantly low from EO-loaded microparticles containing gel formulations. The gel containing EO directly

Table 6: pH, spreadability and viscosity of gel formulations

Formulation code	pH	Spreadability (%)	Viscosity (poise)
G1	5.37	190	4680
G2	5.61	180	5184
G3	5.84	165	5244

Table 7: Essential oil contents in gel formulations

Formulation code	EO content (mg/g gel)
G1	9.6449
G2	19.1264
G3	29.3440

EO: Essential oils

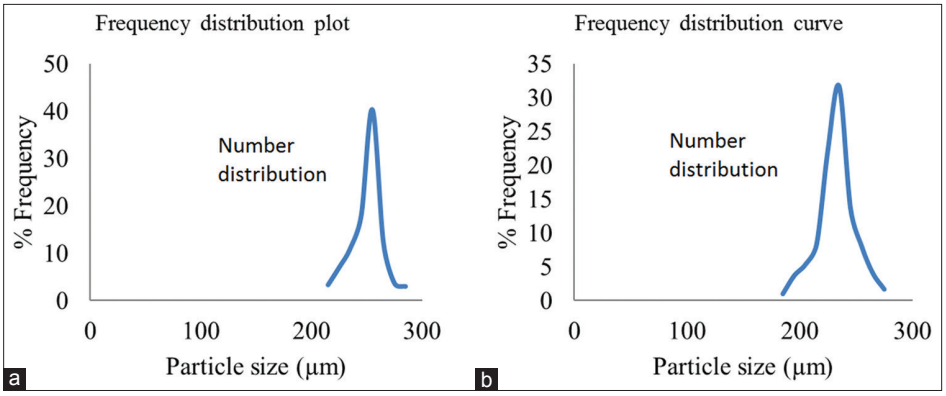

Figure 5: Frequency distribution curve for *Zanthoxylum acanthopodium* essential oil (ZA EO)-loaded and *Litsea cubeba* (LC) EO microparticles. (a) ZA EO-loaded microparticles, (b) LC EO-loaded microparticles

Table 8: Acute dermal irritation data for animals of different groups

Animal group	Effect	Grading of effect at different time intervals after application of gels						
		4 h	5 h	24 h	48 h	72 h	7th day	14th day
Control group	Erythema	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0
D1 group	Erythema	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0
G1 group	Erythema	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0
G2 group	Erythema	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0
G3 group	Erythema	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0

Table 9: Complete protection time and duration of total repellent activity for the gel formulations

Formulation code	Complete protection time (min)	Duration of total repellent activity (min)
G1	30	90
G2	90	150
G3	120	180
G4	60	120
D1	270	330

incorporated without encapsulation showed faster release than EO-encapsulated oil-loaded gel. This signifies that there is a slow release of EO from EO containing particle-loaded gel [Figure 6].

Acute Dermal Irritation Study

There were no irritation and corrosion of skin during the study period of 14 days of preliminary study and confirmatory study. No erythema and edema were observed. Therefore, the prepared formulations can be considered as safe for further studies [Table 8, Figures 7 and 8].

Mosquito Repellent Study

Mosquito repellent studies were carried out for gel formulations. The complete protection time was 2 h. This complete protection time was observed with G3 gel containing encapsulated oil. G3 gel demonstrated protection up to 3 h. The complete protection time for G4 gel was 1 h. It clearly indicates that EO-encapsulated particle-loaded gels are better than EO containing gel (unencapsulated) in mosquito repellent activity. Although the mosquito repellent activities of the gel formulations were found to be less than DEET at the same concentration, these gels can be the safer alternative to synthetic repellents. Marketed products containing oil of lemon eucalyptus provide only 6 h of mosquito repellent activity with 30% w/w EO.[36] In comparison to that, the prepared gel formulations can be considered as better

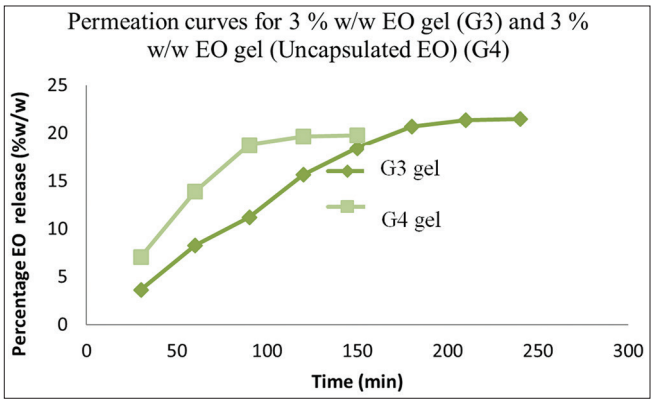

Figure 6: *In vitro* membrane permeation curve for G3 gel and G4 gel

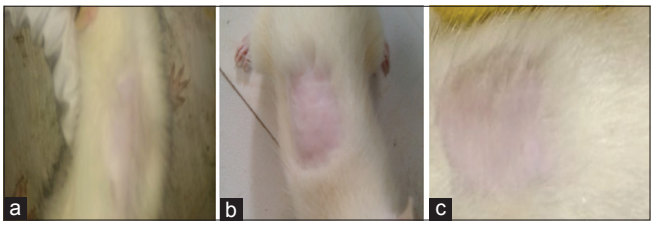

Figure 7: (a-c) Photograph of gel application sites of animals from groups G1, G2, and G3 (left to right) after 5 h of application of the essential oils containing gel during preliminary study

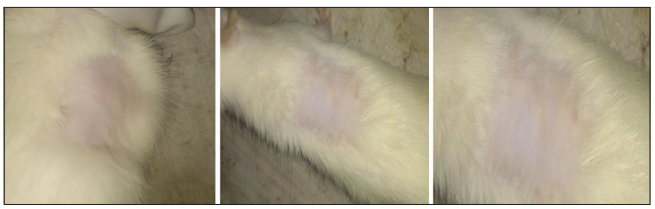

Figure 8: Photograph of gel application sites of animals from groups G1, G2, and G3 (left to right) before application of the essential oil containing gel during preliminary study

Table 10: Organoleptic properties and pH of gels during stability test period

Duration (month)	Formulation code	Color	Odor	Homogeneity	pH
1	G1	White	Characteristics	Homogeneous	5.42
	G2	White	Characteristics	Homogeneous	5.61
	G3	White	Characteristics	Homogeneous	5.85
2	G1	White	Characteristics	Homogeneous	5.44
	G2	White	Characteristics	Homogeneous	5.64
	G3	White	Characteristics	Homogeneous	5.87
3	G1	White	Characteristics	Homogeneous	5.44
	G2	White	Characteristics	Homogeneous	5.65
	G3	White	Characteristics	Homogeneous	5.86

Table 11: EO content in gels during stability test period

Duration (month)	Formulation code	EO content (mg/g gel)
1	G1	9.5573
	G2	18.8964
	G3	28.8372
2	G1	9.4716
	G2	18.7152
	G3	28.5987
3	G1	9.3529
	G2	18.4534
	G3	28.2450

EO: Essential oil

because the prepared gel demonstrated 3 h of mosquito repellent activity with only 3% w/w concentration of EO. EO incorporated nanoparticle formulation could be investigated to increase the duration of action of EO, but there is a risk of potential toxicity [Table 9].[37]

Stability Test

The formulations were found to be stable during the period of testing. A gradual decrease in EO content was observed during the testing period [Tables 10 and 11].

CONCLUSION

The development of a novel herbal controlled release mosquito repellent gel was successful. Organoleptic properties, spreadability, pH, and viscosity were found suitable for topical application of the formulation. The formulation can be further studied for optimization and scale-up activities. This study successfully established controlled release of EO that occurred in two ways; first, release of EO was controlled due to encapsulation and secondly, it was controlled due to Lutrol F 127 (Poloxamer 407) gel base. This formulation technique could be further investigated for other insect repellent volatile oils.

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