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Development of mosquito repellant gel formulations from various natural volatile oils: comparative study with the marketed formulation odomos®

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ABSTRACT

DEET based mosquito repellents were found to be comparatively harmful to the person suffering from urea cycle disorders such as ornithine transcarbamylase (OTD) deficiency and, are therefore, contraindicated in individuals. These situations lead to the budding necessity of natural mosquito repellents which will have inexpensive, effectual, non-toxic, environment-friendly, and biodegradable attributes. Inspired from the upcoming global need, a carbopol 940 based mosquito repellant gel formulation was prepared from the essential oils of *Cymbopogon nardus*, *Murraya Koenigii*, *Cymbopogon citratus*, *Tridax procumbens*, *Eucalyptus globules*, and *Azadirachta indica*, and further evaluating them for their appearance, pH, viscosity, spreadability, extrudability, swelling index, and accelerated studies. The mosquito repellent potential was evaluated and simultaneously compared with the positive control (Odomos®). In several developing nations, where the majority of the people do not have access to mosquito net, high-cost mosquito repellant creams, and miscellaneous physical methods, this gel formulation may be an effective, inexpensive, and easily accessible way to prevent mosquito-borne diseases, like malaria, dengue, etc. in the lower sections of the society.

Keywords: Mosquito, Repellent, Malaria, Gel, Formulation, DEET.

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INTRODUCTION

Globally, the tropical and sub-tropical regions are primarily affected by vector-borne diseases[1]. Mosquito is the sole vector for the transmission of malaria, dengue, and chikungunya which transmits more than 750 million people every year. Nearly 3 million lost their life annually, of which nearly 90% of the mortality lies in infants[2]. Currently, for the prevention of mosquito-borne diseases, the control of mosquito larval growth and personal protection from mosquito bites by the use of mosquito nets and mosquito repellant remained the chief methods[3]. Scientific innovations include mosquito vaccines, but it is still at a nascent level and is not yet recommended for human use[4].

For the complete elimination of adult mosquito and mosquito larva across the planet, dichloro diphenyl trichloroethane (DDT) was sprayed a few decades back, however mosquitoes developed rapid resistance and over the years, this became a rising problem for all individuals and there is an unmet need for the solution[5]. Many commercial brands have flooded the market with N, N-diethyl-meta-toluamide (DEET) based mosquito repellent. For meeting the challenges, a large population across the globe started purchasing these DEET based mosquito repellents[6]. Everything was going well until it was noticed that the chemical component causes toxicity with hyperammonemia and encephalopathy in children after ingestions and applications[7]. DEET was found to be comparatively harmful to the person suffering from urea cycle disorders such as ornithine transcarbamylase (OTC) deficiency and is therefore, contraindicated in individuals[8].

These situations lead to the budding necessity of natural mosquito repellents which will have inexpensive, effectual, non-toxic, environment-friendly, and biodegradable attributes. Inspired from the upcoming global need, a carbopol 940 based mosquito repellant gel formulation was

prepared from the essential oils of *Cymbopogon nardus*, *Murraya Koenigii*, *Cymbopogon citratus*, *Tridax procumbens*, *Eucalyptus globules*, and *Azadirachta indica*, and further evaluating them for their appearance, pH, viscosity, spreadability, extrudability, swelling index, and accelerated studies.

MATERIALS AND METHODS

Instrumentation

The spectroscopic analysis was performed on a double-beam Ultraviolet-Visible Spectrophotometer (Model: Shimadzu® UV-1800, Japan) having a spectral bandwidth 1 mm and connected to a computer. The weighing function was carried out by Shimadzu balance (Kyoto, Japan) model AUW220D. The pH was measured using VSI® digital pH meter of model VSI-1B. The viscosity was estimated by Brookfield Digital Viscometer (using spindle 6). The stability chamber of Bio-Technics, India was used for the accelerated stability studies.

Chemicals

Oils of citronella (*Cymbopogon nardus*), eucalyptus (*Eucalyptus globules*), lemongrass (*Cymbopogon citratus*), and neem (*Azadirachta indica*) were commercially procured. All chemicals and solvents used for the study were of analytical grade and purchased from Sigma-Aldrich (Germany) through a local vendor at Nagpur. Double distilled water apparatus (Borosil®, India) was used for the study.

Collection and Authentication of plant material

The leaves of *Tridax procumbens* and *Murraya Koenigii* were purchased from a local vendor at Nagpur and were further authenticated by Dr. Dongarwar, Department of Botany, Nagpur University, Nagpur, Maharashtra. The authenticated sample was then submitted to the library museum with sample number 4798 and 4799, respectively.

Extraction of essential oils

The leaves of *Tridax procumbens* and *Murraya Koenigii* were suitably powdered (50 g) and were subjected to hydrodistillation. Water (containing a little amount of glycerin) was added to the weighed quantity of plant material in a round bottom flask which was placed on a heating mantle. The flask was connected with the Clevenger-arm apparatus, containing a few silica beads to prevent bumping. The flow of water was allowed to run in the condenser. While boiling, the volatile oils were carried along with the steam into the graduated distillate receiving tube and excess water returned to the flask. A layer of solvent, a mixture of dichloromethane and diethyl ether (1:1 ratio), was added to the distillation arm. The essential oils dissolved in the organic solvent mixture present in the graduated distillate receiving arm. Heating was continued for about 5 hrs duration and the assembly was allowed to cool. Finally, the aqueous layer and the organic layer were collected separately. Afterward, the organic layer was allowed to dry over anhydrous sodium sulfate and the aqueous layer was extracted twice with dichloromethane. At last, the combined solvents were evaporated and essential oil was obtained. The essential oils were weighed and stored in the refrigerator at 4ºC temperature until it was used for the experiment[9].

Preparation of gel formulation

A gel formulation containing 12.5% v/v of active ingredients was prepared by using conventional method[10] **(Table 1)**.

Table 1: Formulation chart of mosquito repellant gel

INGREDIENTS	F1	F2	F3
Cymbopogon nardus oil	2 mL	2 mL	2 mL
Eucalyptus globules oil	2 mL	2 mL	2 mL
Murraya koenigii oil	2 mL	1.5 mL	1.5 mL
Cymbopogon citratus oil	0.5 mL	0.25 mL	0.25 mL
Tridex procumbens oil	2 mL	1.5 mL	1 mL
Azadirachta indica oil	3 mL	2.5 mL	2.5 mL
Ethanol	1 mL	1 mL	1 mL
Carbopol 940	1.3 g	2 g	2.5 g
Propylene glycol	5 mL	5 mL	5 mL
Methyl paraban	0.3 g	0.3 g	0.3 g
Tween 80	3 mL	3 mL	3 mL
Triethanolamine	qs	qs	qs
Distilled water	qs	qs	qs

Evaluation of gel formulation

Physical evaluation

The formulated polyherbal gel was visually evaluated for color, appearance, and transparency. The smoothness of the gel was estimated by rubbing the formulation between the fingers to observe the smoothness, clumps, roughness, and homogeneity[11].

Washability

The washability of formulations was examined by applying the gel on the skin and then evaluating the ease and the extent of washing it with distilled water and manually observing the effect[12].

Skin irritation test

The formulated gel in the quantity of 0.5 g was applied to the normal hairless skin at an area of 6 cm^2 and then covered with a semi-occlusive bandage for the duration of 1 hr. After the application time, the bandage was removed, the applied gel was scrapped off completely, and the area was visually inspected for any rashes or similar symptoms. The test was done for a period of 7 days. The results were expressed in terms of grades[13].

Spreadability

Based on the principle of the slip-drag feature of the polyherbal dermal gel, the spreadability was determined. The protocol involved taking 2 g of formulation and placing it on a ground slide and sandwiching it by an analogous

glide slide, having a hook attached. A heavy mass was applied to the slides to remove the entrapped air so as to form a uniform film between the slides. The excess gel content was scrapped off from the edges. Following it, the top slide was made to drag 50 g intensity[14]. The time needed by the top slide to cover a distance of 6 cm was determined from the formula:

$$\textbf{Spreadability (S)} = \frac{M \times L}{T}$$

where, M = weight tied to the upper slide (20 g); L = length of glass slide (6 cm); T = time taken (sec) to separate the glide slides from each other.

The pH of the dermal gel was evaluated with the digital calibrated pH meter. 1 g of the formulation was dissolved in 25 mL of distilled water and the glass electrode was dipped into it until constant reading obtained. The pH measurement was performed thrice for each formulation and the average reading was noted[15].

Viscosity

The viscosity of the formulation was determined by using the Digital Brookfield Viscometer using spindle no. 6 at 10 rpm and temperature of 25±1°C. A sufficient quantity of gel was filled in appropriate wide mouth container n such way that it should sufficiently allow to dipped the spindle and allowed to settle over 30 min before the measurements[16].

Extrudability

The extrudability of the prepared formulation was determined by first filling the gels (100 g) into a capped collapsible aluminum tubes and sealed by using manual ointment sealing machine. The tubes (containing different formulations) were placed in between two slides and properly clamped. It was followed by placing 500 g weight over the slides and ultimately opening the cap where the extruded ribbon length was noted after 10 min[17].

Swelling index

The swelling index of the prepared dermal polyherbal gel was determined by taking 2 g of gel in a beaker containing 10 mL of distilled water. After 1 hr, the swelled formulation was removed from the beaker and was put on a petridish[18]. The content was re-weighed and the swelling index was estimated from the formula:

$$\textbf{Swelling index (Si)} = \frac{Wt - Wo}{Wo} \times 100$$

where, Wt = weight of swollen at t time; Wo = original weight of gel at zero time.

Accelerated stability studies

The optimized formulation (G4) was subjected to accelerated stability study (40°C±2°C temperature; 75%±5%, relative humidity) for the duration of 90 days. The prepared gel formulation was kept in a PVC container and covered with a black foil. The critical parameters such as physical appearance, pH, viscosity, spreadibility, and extrudability were evaluated[19].

Mosquito repellant activity

Initially, the forearm of volunteers was thoroughly washed with soap and completely dried. The left arm served as the control that was kept inside the mosquito cage. The frequency of the vector landed on the forearm in the duration of 30 seconds. If the mosquitoes landed >10 then the study was commenced. After 30 seconds of time duration, the arm was carefully removed from the mosquito cage. Afterward, the right arm smudged with mosquito repellent gel formulation was entered and analogously the study was performed at 30, 60, 120, 240, and 480 minutes. The number of mosquitoes that landed was determined and compared with the positive control (Odomos®)[20]. The study was performed in triplicate manner.

RESULT AND DISCUSSION

All the fabricated three gel formulations (F1-F3) displayed a brown, less to more translucent, homogenous smooth textured appearance with no solid particles or grittiness found on touching between the fingers. The translucency is largely influenced by the concentration of carbopol 940 in the gel formulations. A lower % of carbopol 940 leads to an enhancement in the clarity of the gel formulations. On the application of the gel formulations for 7 days, no skin irritation, edema, rashes, erythema, or any dermatological reaction or specific inflammation. Furthermore, a brilliant washability attribute has been observed for all the developed formulations (**Table 2**).

The pH of the gel formulations was found to be in the range of 7.1-7.3 which lies in the normal pH range of the skin. Viscosity is an imperative factor which influences pharmaceutical properties such as spreadability, extrudability, pourability attribute from the container, etc. The viscosity of the formulations lies in the range of 44600-52900 cps. The rheological study indicated that with an increase in the torque, the shear stress extensively increases which results in a decrease in the formulation viscosity. The formulations presented the spreadability in the range of 14.21-16.83 g.cm/sec which reflected that the gel formulation can be easily spread by a small amount of shear. A relative study of spreadability and viscosity revealed that with an enhancement of formulation viscosity, the spreadability reduces significantly. The formulated gel preparations displayed a notable extrudability with a large volume of extrudes (++ to +++). With an increase in the viscosity of the formulation, the extrudability decreases alongside and prevents easy extrusion from the collapsible tube. The swelling index was observed in the range of 109-122%. The swelling index signified the matrix nature of the gel formulation which facilitates a controlled release of the drug (**Table 3**).

Table 2: Physical evaluation of the formulated gel formulations.

Formulation	Color	Transparency	Smoothness	Washability	Irritability
F1	Brown	Less Translucent	Smooth	Good	No irritation
F2	Brown	Translucent	Smooth	Good	No irritation
F3	Brown	More Translucent	Smooth	Good	No irritation

Table 3: Evaluation parameters of fabricated gel formulations.

Formulation	pH	Spreadability (g.cm/sec)	Viscosity (cp)	Extrudability	Swelling index (%)
F1	7.3	16.83	52900	++	109
F2	7.2	15.44	49900	++	114
F3	7.1	14.21	44600	+++	122

On subjecting the optimized formulation (F3) at accelerated conditions (40±2°C and 75±5% RH) for 90 days, no substantial disparity in the pH, viscosity, spreadability, swelling index, extrudability, and physical appearance were detected. A change in pH by 0.1 unit, viscosity by 600 cps, swelling index by 9%, spreadability by 0.79 g.cm/sec have been noticed considerably (**Table 4**). However, no changes in the physical appearance, translucency, and smoothness have been seen after the study. In overall, the formulation remained stable for the 3 months duration and is expected to remain in his original form for a longer duration in tropical and sub-tropical regions.

The formulation F3 expressed the highest mosquito repellent activity of 87.37% in the 0th hr and continued to perform up to 85.16% in the 3rd hr. The other formulations F1 and F2 displayed less activity 79% to 80% after 3 hrs, while the standard product (Odomos®) presented nearly 99% efficacy after the lapse of 3rd hr (**Table 5**).

Table 4: Accelerated study of optimized gel formulation (F3).

Duration	pH	Spreadability (g.cm/sec)	Viscosity (cp)	Extrudability	Swelling index (%)
0th Day	7.1	16.83	44600	**+++**	122
90th Day	7.0	17.62	44000	**+++**	113

Table 5: Mosquito screening results of the formulated gel.

Treatment	Repellency (%)			
Stability	**0**	**1 hr**	**2 hr**	**3 hr**
Positive control	100±1.66	99.25±1.27	98.56±1.76	98.11±1.54
F1	84.29±1.86	82.38±1.13	81.55±1.21	79.43±1.33
F2	86.39±1.46	83.49±1.96	81.17±1.59	80.79±1.44
F3	87.37±1.15	86.13±1.55	85.94±1.88	85.16±1.99

CONCLUSION

The prepared six different volatile oil based mosquito repellent gel formulation was proved to be an effective measure in preventing mosquito-borne diseases owing to its useful application in the human body. In several developing nations, where the majority of the people do not have access to mosquito net, high-cost mosquito repellant creams, and miscellaneous physical methods, this gel formulation may be an effective, inexpensive, and easily accessible way to prevent mosquito-borne diseases, like malaria, dengue, etc. in the lower sections of the society.

CONFLICT OF INTEREST

None declared.

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