

DEVELOPMENT OF MOSQUITO REPELLENT FORMULATIONS AND EVALUATION FOR ITS ACTIVITY

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ABSTRACT

Essential oils have been reported to have many pharmacological activities, one of which is their property to repel the mosquitoes and insects. The marigold infused oil is reported to be antibacterial, astringent, antifungal, used in insect bites etc. Mosquito repellent cream of different concentrations i.e. 5% and 7.5% , a combination of marigold oil with two essential oils i.e. clove oil and dill oil were formulated and evaluated as described in the American Society for Testing and Materials (ASTM) standard E951-83 Laboratory testing of non-commercial mosquito repellent formulation on the skin. All formulations were subjected to sensory evaluation with respect to color, fragrance, appearance, shine and ease of applications as well as constant pH, clarity, homogeneity and spreadability.

Keywords: Mosquito repellents, Marigold infused oil, Skin sensitivity, Tagetes sp.(Asteraceae), Anethum graveolens (Apiaceae), Eugenia Caryophyllata (Myrtaceae).

INTRODUCTION

With over many species of mosquitoes believed to be responsible for spreading diseases such as yellow fever, dengue hemorrhagic fever, epidemic polyarthritis, encephalitis and malaria.^{1,4} According to the World Health Organization (WHO) such diseases cause more than 3 million deaths annually.² There are many treatments for Malaria and other mosquito transmitted diseases but it is always better to prevent the disease. Hence, the term mosquito and other repellent came into existence. Topical or other application of mosquito repellent repels the mosquitoes from biting. Mosquito repellent can be prepared synthetically or naturally.

However, most synthetic chemical repellents, especially DEET, can be readily absorbed through the skin, causing many accidental poisonings, especially of children. They also can poison wildlife. DDT in particular has been shown to be very harmful to the environment, and DEET is suspected to be a carcinogen, teratogen and/or mutagen. Therefore, another, preferably non-toxic, means of repelling insects is desired. Many natural Substances are known to repel insects and mosquitoes.³

India represents a vast repository of diverse flora of considerable medicinal importance. Many researchers have reported the bioactivity of (Essential oils) *Eucalyptus* sp, *Cedrus deodara*, *Cymbopogon* sp. and *Tagetes minuta* against mosquitoes.^{4,5} Here the research work is available to determine the bioactivity of essential oils directly on the adult mortality. The present work evaluates bioactivity of the oils from three plants *Tagetes* sp. (Asteraceae), *Anethum graveolens* (Apiaceae) and *Eugenia Caryophyllata* (Myrtaceae). Essential oil is very important class of phytochemicals. We can see the use of essential oils in many herbal products like turmeric in cosmetic and clove in dental. They have been reported to have many pharmacological activities, one of which is their property to repel the mosquitoes and insects. It has been proved that essential oils like cinnamon oil, peppermint oil, etc are good mosquito repellents.^{6; 7.} The drug selected for study is dill oil and clove oil which are effectual oils.⁸ Along with the essential oils the marigold infused oil is incorporated since the marigold flowers are the good source of essential oil.⁹ The marigold infused oil is reported to be antibacterial, astringent, antifungal, used in insect bites etc^{10, 11, 12}.

MATERIAL AND METHOD

I) Preparation of plant material

The flowers and plants of marigold were collected from a nursery at Vangani village (Mumbai). The plants were identified and voucher specimens were deposited at the herbarium of Botany department of Nagpur University. All the chemicals were purchased from S.R. Traders, Ulhasnagar, and Mumbai. The mosquitoes were collected from Haffkine Institute for training, Research & Testing, Parel, and Mumbai.

II) Extraction of marigold oil

The collected marigold flowers were introduced into a glass jar. And the glass jar was then filled with castor oil. The marigold flowers were kept for extraction.

(Infusion) for about 3 weeks. After 3 weeks the Marigold oil was filter and collected into second jar which was incorporated in the formulation.

FORMULATION

Infusion of marigold oil was prepared first and was evaluated for repellent activity. The researchers found significant repellency activity in marigold oil which was compared with castor oil since the infusion was prepared in castor oil. With the data of marigold oil repellency activity it was decided to prepare a mosquito repellent cream of different concentrations i.e. 5% and 7.5% , a combination of marigold oil with two essential oils i.e. clove oil and dill oil.

Preparation of Mosquito repellent product

Mosquito repellent cream: - An aqueous cream (oil in water type) was prepared by emulsifying the essential oils in water with an emulsifying wax. The formula for the insect repellent cream is as follows:

Essential oil	5% or 7.5%
Cetyl alcohol	2%
Lanolin	1%
Mineral oil	2%
Stearic acid	15%
Glycerin	10%
Pot. Hydroxide	1%
Water Q.S.	100%

EVALUATION OF MOSQUITO REPELLENT ACTIVITY

Mosquito repellent activity was assessed by using the test cage as described in the American Society for Testing and Materials (ASTM) standard E951-83 Laboratory testing of non-commercial mosquito repellent formulation on the skin. The test procedure was similar to that describe by Buescher et al (1982) and Gupta et al (1989) .According to the method 20 mosquitoes were transferred into the cage with the help of aspirator tube. The formulations to be tested were applied to the forearms of the volunteers. The applied hand was introduced into the cage and the number of bites was recorded. After every 30 minutes the volunteer hands were subjected to the cage for 3 minutes and the numbers of mosquito bites were noted. The test procedure was replicated three times. Same procedure was implemented for control(blank formulation), marigold oil and castor oil. The Percentage protection provided by repellent cream can be expressed by the formula (Lillie et al., 1988):

$$\text{Percent Protection} = \frac{\text{Bites on control} - \text{Bites on treated}}{\text{Bites on check}} * 100$$

Primary skin irritation study¹³

Guinea pigs of either sex (300-350) were used for the study. The animals were housed in standard housing conditions of temperature $27 \pm 2^{\circ}$ C and humidity of 60 ± 5 % RH. The animals were fed with suitable diet and water ad-libidum. Two animals were used for study. The back of animals was shaven before the study. Five patches of equal area (1cm^2 each) were mark on shaven back. The following preparations were applied.

1. Clean with 5% marigold oil
2. Cream containing 7.5% marigold oil
3. Cream containing combination of volatile oil
4. Cream without active ingredient
5. Control site

Procedure: 0.5 gm cream was applied on marked area, spread uniformly and covered cotton gauze, which was secured by hypo-allergic adhesive tape. The entire trunk was wrap with an impervious material for 24 hrs period of exposure. This material aids in maintaining the test patch in position and retards evaporation of volatile substances. During the test period, guinea pigs were fed at regular interval. At the end of 24 hrs the patches were remove and the skin was observed for any visible changes such as erythema or edema. Evaluation was done by using scale given by Draize. Observation was repeated after 72 hrs.

Draize Scale for the evaluation of the skin reaction

A. Erythema and Eschar formation		
a.	No erythema	01
b.	Very slight erythema (barely perceptible)	1
c.	Well define erythema	2
d.	Moderate to severe erythema	3
e.	Severe erythema (beet redness) to Slight eschar formation, injuries in depth	4
Total possible erythema score		4

Oedema formation		
a.	No oedema formation	0
b.	Very slight oedema (barely perceptible)	1
c.	Slight oedema (edges of oedema well defined by definite raising)	2
d.	Moderate oedema (area raised approx 1 mm)	3
e.	Severe oedema (raised more than 1 mm and expanding behind the area of exposure)	4
Total possible oedema score		4
Total possible score of primary irritation		8

pH Determination: The pH of the various gel formulations was determined by using digital pH meter.

Spreadability: It was determined by wooden block and glass apparatus. Weights about 20gm were added to the pan and the time as noted for upper slide (movable) to separate completely from the fixed slide².

RESULTS AND DISCUSSION

For a repellent to be successful, it must first have to high % protection against mosquito bites. Second, it should be toxicologically safe at the rate of application for which it is intended. Third, it should be easy to apply and pleasant on skin.

Formulation parameters

All formulations were subjected to sensory evaluation with respect to color, fragrance, appearance, shine and ease of applications. All formulations had almost constant pH, homogeneous, emollient, non-greasy and easily removed after the application. The pH values of all developed formulations were found to be in the range of 6.7 to 7.

All developed formulations showed good clarity and homogeneity with absence of lumps or aggregates. Easy spreadability is one of the important characteristics of any topical preparations as far as patient compliance is concerned. Moreover if the formulation spreads easily, its application to the area of skin would be more comfortable.

Skin sensitivity study

The skin irritation study is very important as many cosmetics preparation have some inherent irritation levels. All formulations were safe in respect to skin irritation and allergic sensitization as the primary irritation index by Draize Patch technique was found to be zero, and there was no any report of any oedema or redness. Thus indicating skin acceptability of these formulations for topical application

Mosquito Repellency

Mosquitoes have very olfactory chemoreceptor on sensitive antennae which becomes stimulated by very distinct odors, it may either increase or decrease attractiveness to the host. This behavior of reducing the attraction of mosquitoes to their host had been observed during formulation containing combination of essential oils and marigold oil.

Mosquito repellent activity of extracted marigold oil was checked against *Aedes* mosquito.

Based on the results of Marigold oil (59.05% protection) against mosquito bites, it suggested that the oil has the potential to be developed into formulation for mosquito repellency. Hence, the extracted marigold oil was incorporated in cream base in different concentration and evaluated for its activity. The cream containing 7.5% marigold oil provided high level of protection (56.90%) against mosquito bites. Subjects treated with cream containing 7.5% marigold oil received only 8 mosquito bites in 90 min. The mean numbers of bits received for control volunteer were 18.67. Subjects treated with cream containing 5% marigold oil received only 10.67 mosquito bites in 90 min. The mean number of bits received for control volunteers were 19.33. This indicates that the cream containing 7.5% provided high effectiveness.

A cream base containing combination of volatile oil with 5% marigold oil as an active ingredient had been also tested for mosquito repellent activity. The significance repellency effect exhibited by the formulation containing combination of volatile oils with % protection indicated excellent protection.

Table 1 Values of evaluation parameters of developed formulations

Batch	Clarity	Homogeneity	pH	Spreadability g.cm/sec	Skin irritation
5 % MO Formulation	Clear	Good	6.8	6.0	Nil
7.5 % MO Formulation	Clear	Good	6.8	6.5	Nil
5 % MO + CO+ DO Formulation	Clear	Good	6.8	7.0	Nil

MO: Marigold oil; CO: Cinnamon oil; DO: Dill oil

Table 2 Evaluation of mosquito repellent activity of developed formulations

Batch	Mean Bites		% Mean protection
	Test	Control	
Marigold oil	3±0*(0.008899)	7.67±2.082	59.05±10.14
5 % MO Formulation	10.67±2.52* (0.003494)	19.33±1.53	44.12±15.83
7.5 % MO Formulation	8±1.73*(0.003591)	18.67±3.22	56.90±7.02
5 % MO + CO+ DO Formulation	3.33±2.52* (0.000425)	20±2	82.67±14.52

Values are represented as mean± S.D. of mosquito bites.*indicates the significantly (p <0.01) different compared to respective control values. (Students unpair T-test) [MO: Marigold oil; CO: Cinnamon oil; DO: Dill oil]

Based on the percentage of protection, the formulation containing combination of volatile oils was found to be the best followed by the formulation containing marigold oil. In case of marigold oil formulation, the % protection increased with increasing concentration of marigold oil so dose dependant response was observed.

CONCLUSION

Active ingredients are the focus and responsible factor of all mosquito repellent formulations. The cream containing 7.5% marigold oil provided high level of protection (56.90%) against mosquito bites. In case of marigold oil formulation, the % protection increased with increasing concentration of marigold oil so dose dependant response was observed. Natural repellents such as herbal essential oils have been employed as alternative compounds for repelling mosquitoes and other insects. There are reports on the insect repellency from mint, citronella, basil, thyme, neem, and lemongrass. Although the history of DEET and other prominent repellents such as dimethyl phthalate is proved, it is accurate to state that the combination of different essential oils influences repellent performance. At present study, it confirmed that natural, non-DEET formulation could be used as mosquito repellents.

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