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Design and evaluation of herbal mosquito repellent and air cleansing dhoop sticks: *In vitro* evaluation

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Abstract
The growing concerns over the adverse effects of synthetic chemicals in commercial mosquito repellents and air purifiers have led to an increasing demand for natural alternatives. This study aims to design and evaluate herbal mosquito repellent and air cleansing dhoop sticks by assessing their efficacy through *in vitro* methods. Various plant-based ingredients, including essential oils and herbal extracts known for their repellent and purifying properties, were selected and incorporated into the formulation of dhoop sticks. The prepared sticks were evaluated with respect to mosquito repellant activity using mosquito net method and air purifying potential using agar plate method. The formulated sticks showed promising mosquito repellant activity and air cleansing potential. Thus, preparation of dhoop sticks using natural ingredients could be promising alternative.

Keywords: Herbal dhoop sticks, mosquito repellant potential, natural mosquito repellant

Introduction

Mosquitoes are blood sucking creature which annoy humans by causing buzzing sound. There are number of diseases such as malaria and dengue which are caused due to mosquito bite. There are ample of mosquito repellent available in the market but most of them are from synthetic origin. DDT (Dichloro-diphenyl-trichloroethane), BHT (Butylated Hydroxy Toluene) and Deodorised kerosene are few of the synthetic mosquito repellents with extreme harmful side effects on humans as well as on the environment. Most of them are carcinogenic, mutagenic and some of them even cause renal and hepatic toxicity if inhaled or ingested [1]. Beside these terrible drawbacks on human health and on the environment, they are also expensive which maked it uneconomical. Herbal dhoop stick was prepared to provide better alternative. As it contains eco-friendly and easily available natural ingredients such as cowdung, neem, camphor, bay leaf, clove oil and dhoop powder. Neem (*Azadirachta indica*) is a well-known herb, which shows potent anti-microbial property. Azadirachtin is the phytoconstituent present in neem and it is responsible for showing mosquito repellent activity. Camphor is widely used due to its strong characteristic odour. It is isolated from *Cinnamomum camphora.* The active constituent present in the camphor is borneol and has strong characteristic odour. The mosquitoes find this odour miserable. Bay leaf (*Laurus nobilis*) is widely used as an important condiment in the Indian cuisine. Terpenoids are the secondary metabolite present in the bay leaf which acts against mosquitoes. Clove oil is extracted from flower buds of *Eugenia caryophyllus*. Eugenol is known for its Analgesic property in the medical field. It has characteristic odour which is responsible for the mosquito repellent activity. Cow dung is easily available and is used for the ignition purpose. Dried cow dung cakesare used in every house hold in remote areas, as the smoke it cause makes mosquitoes feel unpleasant. Dhoop powder is used for the aromatic purpose. Ingredients used in the Herbal Dhoop stick are natural and environment friendly, which makes it cost effective alternative [2].

There are number of chemical receptors which are present in the mosquitoes. Female *Anopheles* mosquitoes are more attracted towards Lactic acid and Carbon dioxide which is present in the sweat of the humans. Chemical receptors of mosquito get activated by lactic acid. The mosquito repellent destroys the lactic acid receptor. As the lactic acid receptors are destroyed the mosquitoes shows knock down effect [3].

Materials and Methods

Fresh Neem leaf *(Azadirachta indica)* and Bay leaf *(Laurus nobilis)* were collected from the medicinal garden of the institution and it was allowed to shade dry at temperature not exceeding 25℃ until it loses all its moisture. Cow dung was collected from nearby cow farm and subjected to dry in open sun till it loses all its moisture. Clove *(Eugenia caryophyllus)* and camphor *(Cinnamomum camphora)* was bought from the market. Clove oil was extracted from clove by using the method of steam distillation. All dry ingredients were subjected to size reduction by using electric grinder separately. After reducing the size, individual samples were passed through the sieve (mesh no 66). Above process was repeated until fine powder was achieved.

Preparation of mosquito repellant dhoop sticks

Previously weighed dry ingredients were taken (As represented in Table 1) in mortar and pestle and mixed properly to get a uniform mixture. Then required quantity of clove oil was added, triturated and water was added gradually to get dough like consistency. The dough was filled in plastic syringe to get cylindrical shape and dried in tray dryer at temperature not exceeding 30℃ [4, 5, 6].

Table 1: Formulation of one dhoop stick

Ingredients	Quantity
Cow dung	5 gm
Neem powder *(Azadirachta indica)*	2 gm
Bayleaf powder *(Laurus nobilis)*	1 gm
Camphor *(Cinnamomum camphora)*	1 gm
Dhoop powder	1 gm
Clove oil *(Eugenia caryophyllus)*	0.5 ml
Water	Q.S.

Evaluation of mosquito repellent activity of Dhoop sticks using mosquito cage method

Mosquitoes required for the evaluation activity of dhoop stick were collected by the help of polyester mosquito net. In the evening time when the mosquitoes are highly active, the net was waved slowly around the damp areas and grass field to trap the mosquitoes. Then it was transferred to a case of size 70 cm X 70 cm. Around 50 mosquitoes were taken in the case at 6 - 9 PM. Two sticks of size 3 cm each were burned at two corners of room diagonally with sufficient ventilation. Case containing mosquitoes was kept at the centre of the room. The activity of mosquitoes was observed and noted for next three hours [7, 8, 9].

Evaluation of air cleansing potential of Dhoop sticks

The air cleansing potential of prepared dhoop sticks was assessed agar petri plate method. The areas selected for assessment of air cleansing potential was bathroom. Briefly, one open presterilized agar petri plate was kept at each area for 30 minutes and incubated to judge the contamination of air each area before exposing to dhoop stick. The one dhoop stick was completely burned at each area at next step. After complete burning of stick, one more open presterilized agar petri plate was kept at each area for 30 minutes and incubated to judge the contamination of air after exposing to stick. The microbial colonies on petri plates were compared [10].

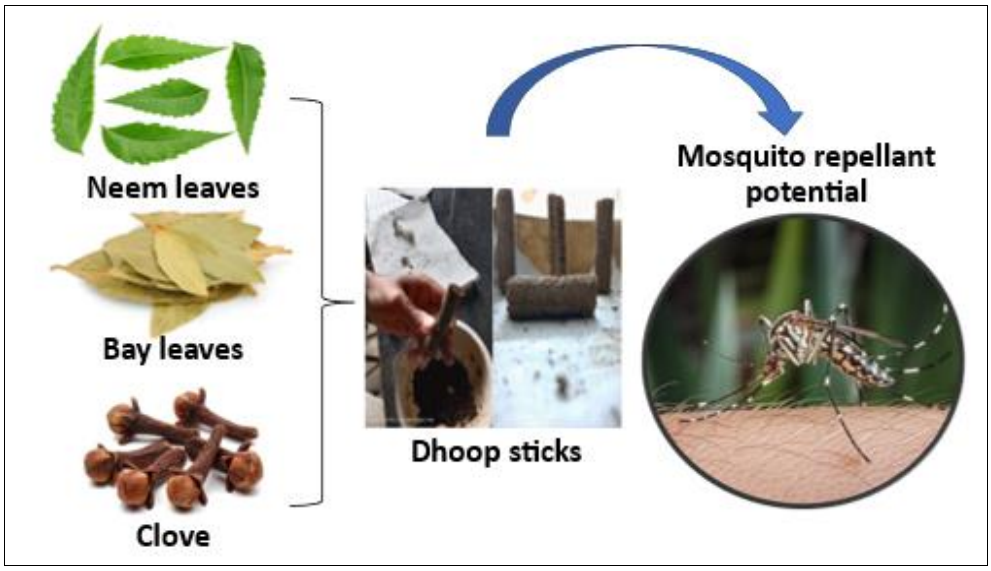

Fig 1: Mosquito repellant dhoop sticks

Distribution and feedback of Dhoop sticks at rural area

To judge the elegance and consumer acceptance, the prepared sticks were distributed to local villagers of Morvande village, Tal. Khed, Dist. Ratnagiri and few questions were asked related to elegance as well as acceptance and feedbacks of the villagers were recorded using google form.

Fig 2: Distribution of dhoop sticks to villagers for feedback purpose

Results and discussion

Preparation of mosquito repellant dhoop sticks

The mosquito repellant dhoop sticks were prepared by using all herbal ingredients which are easily available at rural area. The prepared dhoop sticks were found to be intact. Figure 3 represents prepared dhoop sticks.

Fig 3: Prepared dhoop sticks

Evaluation of mosquito repellent activity

Mosquito net method was used to evaluate the efficacy of dhoop stick. Two dhoop stick were burned completely in the room and behavior of mosquito was observed for three hours. In between 7 to 8 pm 30 mosquitoes were aligned on the net and 9 mosquitoes were observed with knock down effect. In next hour maximum number of mosquitoes (30) were observed with knock down effect. Whereas, in next one hour the number of mosquitoes observed with knock down effect were increased from 30 to 41. Thus, formulated dhoop sticks have showed promising mosquito repellent potential.

Table 2: Evaluation of mosquito repellant activity of prepared dhoop sticks

Behaviour of the mosquitoes	7 to 8 pm	8 to 9 pm	9 to 10 pm
Free movement of the mosquitoes	6	0	0
Number of mosquitoes aligned on the net	30	17	7
Mosquitoes struggling for life	5	3	2
Mosquito with knock down effect	9	30	41

Evaluation of air cleansing potential of Dhoop sticks

The air cleansing potential of formulated dhoop sticks was assessed using agar petri plates. As highlighted in figure 4. The microbial colonies on petri plate before exposure with dhoop stick were found to be more than microbial colonies after exposure with dhoop stick. Thus, formulated dhoop sticks could be promising in reduction of microbial contamination of room.

Fig 4: Microbial colonies on agar petri plate before and after exposure with dhoop stick

Distribution and feedback of Dhoop sticks at rural area

The formulated dhoop sticks were distributed to local villagers and few questions were asked to them in order to collect the feedback. It was found that, 100% dhoop sticks were completely burned. The 15.4% villagers showed dhoop sticks were quite irritant to eyes. The 100 % villagers were ready to accept the formulated dhoop sticks.

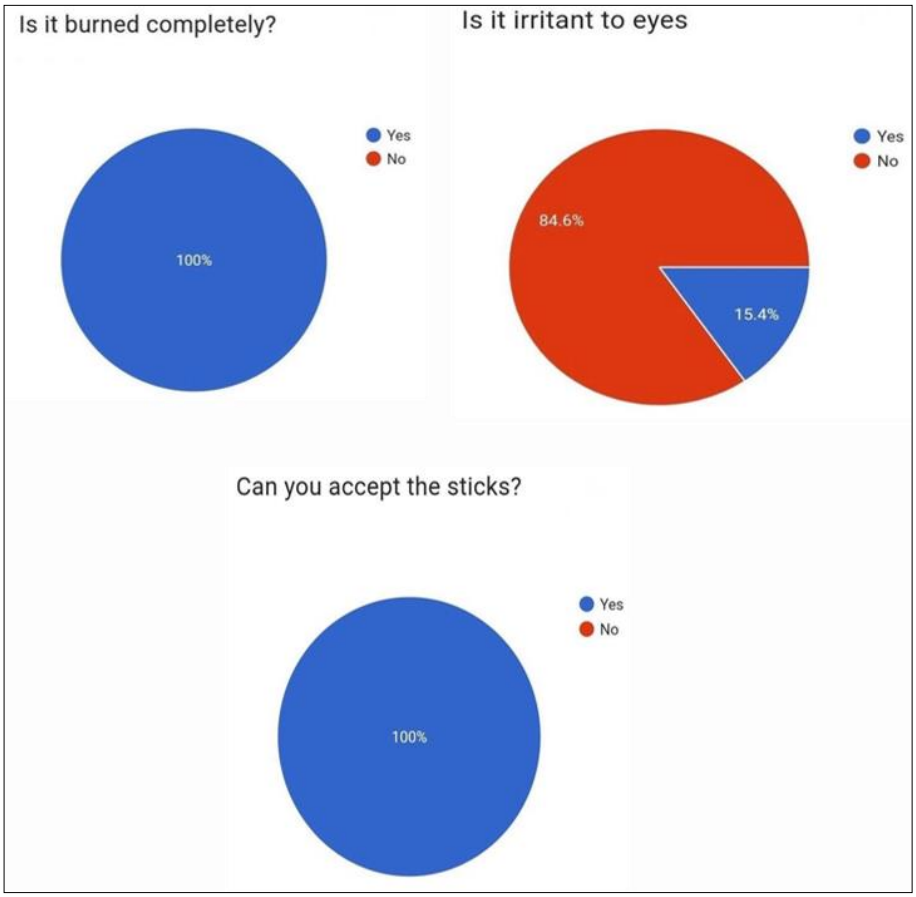

Fig 5: Feedback of formulated dhoop sticks from local villagers

Conclusion

A survey was conducted in nearby rural area for their traditional technic for getting rid of mosquitoes. Neem, Camphor, Bayleaf, Cow dung, Camphor, Clove oil and Dhoop powder were selected for the purpose. Then a through literature search was done on these herbal materials for their effects. The prepared Dhoop stick shows long lasting effect against mosquito. The prepared Dhoop stick

was subjected for evaluation by using Mosquito cage method and result was found to be adequate for protection against mosquito.

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