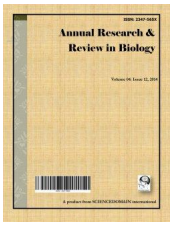

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Formulation and Evaluation of Novel and Natural Mosquito Repellent Liquid to Prevent Dengue Mosquitoes

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Authors' contributions

This work was carried out in collaboration between both authors. Author MS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author FNAR managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Syzygium aromaticum, *Cymbopogon citratus* and *Cinnamon cassia* are plants that grow wildly in many tropical countries of Asia such as Malaysia, China and Indonesia. They belong to the family of Myrtaceae, Graminae, and Lauraceae respectively. Commonly, *S. aromaticum* and *C. cassia* are used as spices in Malay cuisine. Their essential oils have a great smelling which able to repel the mosquitoes effectively with less harmful side effects to the users and the environment. The present study was to formulate and evaluate the mosquito repellent liquid against dengue mosquitos, which containing the combination of natural herbs. The plants materials were extracted with methanol using maceration method. The methanolic extract of the plant materials were formulated with the concentration of 10% extracts and 1% essential oils. The formulated liquid mosquito repellent was evaluated using standard procedure stated in SIRIM MS 1497:2007. Ten female mosquitoes were acclimatized and exposed to the odour of the formulated liquid mosquito repellent in the Y-Tube

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Olfactometer. The formulation showed a significant repellent activity against the dengue mosquitoes with mean of 66.67%, after three consecutive tests. In conclusion, we recommend this product to convert into market standards and need to be commercialized with low cost.

Keywords: Syzygium aromaticum; Cymbopogon citratus; Cinnamon cassia; mosquito repellent liquid; dengue mosquitos.

1. INTRODUCTION

Vectors are living organisms that can transmit infectious diseases between humans or from animals to humans. There are two types of vectors; mechanical vector in which it transmit pathogens by transporting them on their feet or mouthparts and the other one is biological vector that involved in the life-cycle of parasite or arbovirus which must pass through the vector in order to mature to an effective stage capable of being transmitted to human or animal host when a vector takes a blood meal. Mosquitoes are the best known disease vector majority of transmissions of the most important vector-borne diseases. Mosquitoes transmit most of the important vector-borne diseases such as encephalitis, malaria, dengue, chikungunya, Zika virus, and yellow fever.

Dengue is the most rapidly spreading insect-borne diseases with 50,000 dengue fever cases in five first months of 2016. In Malaysia, dengue fever seem to be the most concern vector-borne disease among all. This is due to alarming number of cases reported annually with statistic released by Ministry of Health (MOH) shown that Malaysia reaches 20,000 dengue cases in 2016 and consistent with 2015. Till now, there is no straightforward method of treatment available and although there is a discovery on dengue vaccine, namely Dengvaxia, however this vaccine status is still unknown and yet get approval by WHO [1].

A number of interventions to control mosquito do exist and the most convenient and easiest way is by the application of repellents. Mosquito repellents may effectively protect humans from vector-borne diseases as well as other problems caused by mosquitoes. N,N-Diethyl-meta toluamide, also called DEET is the active ingredient in the chemical-based mosquito repellent. It is readily available and frequently used mosquito repellent. However, several drawbacks of DEET have been reported, which it cause local skin irritation, including erythema and pruritis, at the site of application. Furthermore, it is also severe enough to cause sensory disturbance and affect motor capacity, memory and learning ability [2].

Cinnamon bark (*C. cassia*), clove buds (*S. aromaticum*), and lemon grass (*C. citratus*) are the potential natural mosquito repellent [3]. On the basis of chemical nature, these medicinal plants contain mixture of many compounds including isoprenoids mono and sesquiterpenes [4]. These chemicals act as carriers of the smell which found chiefly in the aromatic plants. Previous studies have shown that they possess repellent activity against mosquitoes. However, so far the effectiveness of the combination of those plants extracts or essential oils have not been studied and yet explored as a natural mosquito repellent [5-11]. This is supported by recent research that reported that different combinations of repellents appear to have synergistic effects and, therefore, better efficiency when compared to an isolated repellent. Therefore, the present study intends to formulate a poly herbal derivative which are crude extracts and essential oil into a formulation that specifically may prevent the mosquitoes bite as well as with the aim of reducing or eliminating chemical usage.

2. MATERIALS AND METHODS

2.1 Plant Collection

Fresh cinnamon bark (*C. cassia*), clove buds (*S. aromaticum*), and lemon grass (*C. citratus*) were collected from the local market, Ipoh district, Malaysia and authenticated. Essential oil of all these plants were purchased from the local market.

2.2 Plant Extraction

All the plant materials were washed thoroughly with distilled water and air-dried. Later, they were grinded into powder using an electric grinder. The extraction of the plants were carried out separately using maceration method. Weighed accurately 200 g of each cinnamon powder, clove bud powder and lemongrass powder were extracted for 2 weeks using 1000 mL of

methanol. The extracts were filtered and concentrated for a few days and then stored at room temperature till further use.

2.3 Preparation of Test Solution

All the three extracts and essential oil solutions were mixed on a volume-volume basis and diluted with absolute ethanol as diluents. Test solution containing mixture of plant extracts was prepared by taking 1 mL of each plant extract and mixed them together. Same procedures were followed for plant essential oils.

2.4 Formulation of the Mosquito Repellent Liquid

The mosquito repellent liquid was formulated by mixing methanolic extracts and essential oils of cinnamon, clove, and lemongrass along with ethanol, hexane and distilled water in the order mentioned in Table 1. Distilled water were added to the mixture until the final volume is 100 mL. All the constituents were mixed using mechanical stirrer for one hour. Finally, the mixture was poured into an empty container of mosquito repellent plug-in device and labelled as well (Fig. 1).

2.5 Evaluation of Formulated Mosquito Repellent Liquid

The repellent activity of the formulated mosquito repellent liquid is tested against the mosquitoes according to SIRIM MS 1497:2007 using Y-tube olfactometer. Cotton wool impregnated with test liquid was put at the treatment tube of the olfactometer while cotton wool without the test liquid is put at the control tube. Ten female's mosquitoes were introduced and allowed to acclimatize to the test environment with clean air for 15 minutes with no treatment. Odours in air was allowed to migrate down the branches, through the base leg and the holding port, from which test mosquitoes are released at the start of the test. After acclimatization, the doors was opened for exposure period. Number of mosquitoes were observed and recorded. The experiment was repeated three times in order to get the mean of percentage repellent activity and it was calculated using the following formula:

> % Repellent activity = Number of mosquitoes in control tube – Number of Mosquitoes in treatment / Number of mosquitoes in control tube X 100.

3. RESULTS AND DISCUSSION

The results obtained from behavioral response of dengue mosquitoes against the formulation using Y-maze olfactometer are shown in Table 2. At 10% concentration, the formulation which consist of extracts and essential oils of cinnamon, lemon grass and clove exhibited significantly higher repellent activity towards the mosquitoes. In first test, out of 10 mosquitoes inserted, only one mosquito present in the treatment tube with percentage repellent activity of 66.67%. Similarly, in the second test, only one mosquito present in the treatment tube. However, the percentage repellent activity was quite different of 33.33%. The highest percentage repellent activity was shown during the third trial with no mosquito present in the treatment tube. Overall, based on the percentage repellent activity obtained, the formulation shown a greater repellent activity with mean of 66.67%.

A plant extract is a substance or an active with desirable properties that is removed from the tissue of a plant, usually by treating it with a solvent. The ideal solvent used should be highly selective for the compound to be extracted, not react with the extracted compound or with other compound in the plant material, have low price, harmless to human and environment and be completely volatile. The common solvents used for extraction includes water, alcohol, ether and chloroform. The plants were extracted using various methods such as maceration, decoction, infusion, percolation, hot continuous extraction (soxhlet), counter-current extraction and etc.

Table 1. Composition of formulated mosquito repellent liquid

Components	Amount
Methanolic extract (Cinnamon, Clove, Lemongrass)	10% each plant extract
Essential oil (Cinnamon, Clove, Lemongrass)	0.1% each essential oil
Hexane	1 mL
Absolute ethanol	25 mL
Distilled water	Up to 100 mL

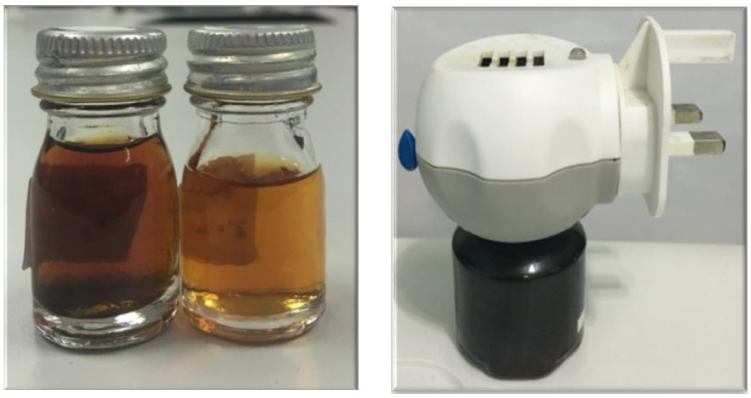

Fig. 1. Formulated mosquito repellent liquid

Table 2. Repellent activity of formulated mosquito repellent liquid

S. no	Number of mosquito in control tube (Tube A)	Number of mosquito in treatment tube (Tube B)	% Repellency	Mean % repellency
1	5	1	66.67	
2	2	1	33.33	66.67
3	4	0	100	

Various methods of extraction employed have their own degrees of limitations and advantages. On the other hand, essential oils are highly concentrated substances extracted from parts of the plants. These oils are often used for their flavour and their therapeutic or odoriferous properties. Essential oil composed of volatile components having minor constituents contain pleasant fragrance which are responsible for mosquito repellency and inhibit the orientation of blood sucking insects [12-15].

Many findings found that the plant essential oil have better repellent efficacy as compared to plant extracts. This is due to stronger scent by the essential oils compared to the plant extracts. Essential oils are so called because they can be thought of as the "essence" of the aromatic plant. While, plant extract can be figure out as essential oils dissolved in an appropriate solvents. They are therefore less strongly flavoured than the undiluted oil itself. Nevertheless, all the essential oils are claimed to be highly volatile and thereby contributes to their poor longevity as mosquito repellents [16-21]. Thus, essential oils are not suitable to be used as sole mosquito repellents.

This problem is overcome in the current study by preparing the herbal mosquito repellent formulation using highly volatile essential oils in combination with plant extracts. During the experiment, 10 mosquitoes were inserted into the Y-tube olfactometer. However, not all mosquitoes took part or response to the sample. In first trial, only 6 mosquitoes response, while in second trial 3 mosquitoes response and in last trial only 4 mosquitoes response. This might due to some factors that influence the response of the mosquitoes. This probably due to the satisfaction of the mosquitoes being sufficiently fed before the experiment started, means the mosquitoes were not in their fasting state. Moreover, due to time constraint the experiment was carried out at time between 4 pm till 5 pm which not an optimum biting time for the mosquitoes.

In addition, environment factors such as darkness and temperature of the place also contribute to that condition. The number of mosquitoes which do not response during the experiment were not taken into account. In Tube A, a control was used. The tube was inserted with wire gauze soaked with distilled water. Previously, the experiment was tested by using formulation which does not contain any essential oil and plant extracts as control. However, under this condition, the mosquitoes seem to repel to go to both tube (Tube A and Tube B) which reduce the participation of the mosquitoes towards the sample. Thus, it marked that the blank formulation also have the repellent properties but in a poor degree of repellent activity.

4. CONCLUSION

In this study, natural base mosquito repellent was formulated successfully. The repellent properties of the liquid containing essential oil and plant extract was evaluated and determined. The test is carried out thrice in order to get the mean percentage. The mean was found to be 66.67% which is more than half. Thereby, it shows that the formulation is very good. No any harmful effects reported. Thus, it is totally a safe product. The formulation was also ecological and economical.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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