

Laboratory Evaluation of the Efficacy of Insect Repellent Products Extracted from *Melaleuca Cajuputi* Powell against Mosquito

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Abstract

Melaleuca cajuputi Powell or known as cajuput tree is a native tree to Peninsular Malaysia where it easily grows near to the swampy forest. The essential oil extracted from its leaf have many benefits due to its chemical compound. One of its benefits is to work as an insect repellent. Hence, the main objective of this study is to evaluate the biological efficacy of the *M. cajuputi* Powell based product against the mosquito. The essential oil extracted from the fresh leaves through steam distillation was formulated into two products which are Mosquito repellent roll-on and mosquito repellent diffuser. Mosquito repellent roll on undergo skin repellent analysis using Bioassay Method for Mosquito Repellent on Human Skin followed Malaysian Standard Procedure while mosquito repellent diffuser was analyzed for spatial repellent using choice test method developed by Vector Control Research Unit (VCRU). Both samples with 5% concentration were tested against the dengue vector (*Aedes aegypti*) in the laboratory. The skin repellent results were compared with Malaysian Standard repellent which is 10% DEET. Mosquito Repellent Roll On has shown complete protection against mosquito landing/biting up to 1 hour and start to decrease from 1'30'' hours to 4'00'' until 31.37% which are lower than Malaysia Standard. Besides that, Mosquito Repellent Diffuser showed an excellent repellency from 30 minutes to 3 hours from 83.33% to 100% of repellency. Thus, the conclusion from this study, cajuput oil could be used as a botanical insecticide since the plant is widely available in Malaysia especially in Selangor, Terengganu, Pahang, and Kelantan. This study is very crucial to the botanical insecticide development in Malaysia to sustain our environment by reducing harmful residue to nature due to conventional insecticides uses.

Keywords: *Cajuput oil, Melaleuca cajuputi Powell, skin repellent, spatial repellent*

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1. Introduction

In 2017, Dengue cases are still number one among

infectious disease in Malaysia [1]. This trend continued until 2019 when the sudden increase of

dengue cases is worrisome [2] and urge Malaysian to take responsibilities in controlling the disease together. Surprisingly, the Health Department Malaysia also mentioned that fogging is not the solution but only the immediate response to kill the mosquito [2]. Based on the articles from the newspaper above, dengue cases as one of the mosquito-borne disease are still unsolved and difficult to avoid from be the outbreak cases. Every year, there are millions of deaths and hundreds of illness cases have been distinguished worldwide due to mosquito-borne disease because of the mosquito is the prominent vector of all disease-transmitting arthropod [3]. Thus, the alarming condition of the mosquito-borne disease has affected all countries and become major health concern either in developing or developed countries [4]. Even though human nowadays becoming more modern and many technologies have been developed, people still depending on the synthetic or chemical insecticides in controlling vector-borne disease [5] and until now, there is no effective vaccine that have been proven to prevent human from affected the mosquito-borne disease [6].

Chemical or synthetic insecticides have succeeded to control the vector especially mosquito but continuously used of this alternative have led to several complications. The complications that have been discussing for many years by scientist are development resistance of insecticides towards the vectors particularly from endemic countries and the most affected are the environment where it causes environmental pollution on a living organism [7]. Currently, the chemical insecticides that have shown excellence protection against mosquitoes are DEET and permethrin, but the adverse effect such as skin eruption and toxic encephalopathy have been reported [6]. Besides the health effect, the progressive change of the current situation has led to economic demands or increase in cost. Therefore, another alternative that less in cost, environmentally friendly and effective are needed to overcome the challenge and difficulties.

Due to these problems and concern, application of plant extracts as an organic or botanical insecticide has become the research interest worldwide [4]. Although the application of natural products has been known since ancient times [8], this alternative just increases its demands presently. Tropical Asia and Southeast Asia are the locations that have a high concentration of botanical insect repellent with more than 2300 plant species [9]. There are many plants extracted that have been studied and have the efficacy to become insect repellent

specifically in repelling *Aedes aegypti*, *Aedes albopictus*, *Anopheles dirus*, and *Culex quinquefasciatus* because of all the mention species are the major vector cause the mosquito-borne disease. Example of the plants that have been studied previously and prove in becoming mosquito repellent was lemongrass, aromatic turmeric, citronella, eucalyptus, cajaput tree and others [16]. Most of the botanical extracts have been formulated becoming repellent products such as spray, lotion, stick, roll on and patches.

In this study, *M. cajuputi* Powell has been chosen as the main ingredient in the roll on and mist diffuser repellent due to some reasons. Firstly, based on the previous study *M. cajuputi* Powell has an effect on the mosquitoes either, larvicidal and adulticides. Before this, the repellent spray and lotion have been that have *M. cajuputi* Powell extraction have been studied but lack study of roll-on repellent and mist diffuser repellent.

Hence, the purpose of this paper is to evaluate the biological efficacy of the repellent products formulated by *M. cajuputi* Powell extraction through bioassay test. The significant of this study is to extend the knowledge of the *M. cajuputi* Powell extraction as the insecticide properties.



Figure 1: Extraction of essential oil through steam distillation

2. Methodology

Collection and Preparation of Specimen

Plant specimen of *Melaleuca cajuputi* Powell were obtained from the mini garden park at the Universiti

Tenaga Nasional (UNITEN). Collection of plant specimens were identified by the researchers itself based on the morphological characteristics. The guidance of identification was taken from the Forest Research Institute of Malaysia (FRIM). Collected fresh leaves were extracted to produce the essential oil through steam distillation. All the extraction process was done by the researcher at the laboratory with some modification of the method. Firstly, the apparatus was set up (Fig. I) with the distillation flask, boiling flask, distillation chamber, condensation tube, collection tube, and water flow container. Then, the leaves were separated from the stem or stick and weighed equal with distilled water volume before put into the distillation flask. Then, the leaves were left to be extracted with controlling the water temperature in the water container not exceed 10- degree Celsius. This step is crucial to get pure and clear essential oil. The essential oil was collected after three hours extraction and the oil was slowly drawn off from the collecting tube. This method was repeated until getting enough essential oil for product formulation.

There were two samples or product that have been used to test the biological efficacy of the repellent product with the *M. cajuputi* Powell essential oil as the active compound. The products are roll-on repellent and mist diffuser repellent. The products were formulated using essential oil, carrier oil and purified water which each sample has 5% essential oil. Then, all the samples were sent to the Vector Control Research Unit, Universiti Sains Malaysia to perform two bioassay tests which is human skin test and choice test method.

Human Skin Repellent

Test Chamber Method (Fig. II) was used to test the effectiveness of Roll-On Repellent when applying to the human skin. The method was conducted essentially following the Malaysian Standard (MS 1497:2007). Experiments were conducted in screened, wooden cages measuring 60 x 60 x 60 cm. The cage was divided into two compartments with 15 cm diameter circular openings fitted with cloth sleeves for each compartment. A fresh batch of 25 female sucrose-fed *Aedes aegypti* mosquitoes (aged 5 to 7 days old) were introduced into each compartment through the circular opening. The right arms of human volunteers are left untreated whilst the left arms are treated with the test sample. Roll On Repellent will be applied on the left arm. Application is left to dry before covering it with a rubber sleeve within 30 minutes after treatment. Both hands (up

to the wrists) are covered with thick rubber gloves to prevent unwanted bites. For each arm, a surface area of 24 cm² (3 cm x 8 cm) is exposed to mosquito landing/biting activities through an opening on the rubber sleeves. The arms are exposed simultaneously for 5 minutes every 30 minutes and the number of mosquitoes landing/biting is recorded. The assessment period shall continue up to 4 hours post-application of the test sample. A fresh batch of mosquitoes is introduced to replace the exposed mosquitoes at each assessment period. The experiment is done in triplicates. All test to be conducted at a temperature of 26 °C ± 2 °C and relative humidity of 65-85%. The effectiveness of a repellent product is assessed by determining the percentage reduction of mosquito biting/landing on the treated arm when compared with the untreated arm. The formulation as stated below: % Reduction

$$= \frac{\text{No. mosquito on untreated arm} - \text{no of mosquito on treated arm}}{\text{No mosquito on untreated arm}} \times 100\%$$



Figure 2: Test Chamber Method

Choice Test Method Repellent

The test was conducted in two connected chambers (Fig. III). The size of each chamber was 70 cm x 70 cm x 70 cm. The two chambers were connected with the tunnel size 15 cm x 15 cm with the window in the middle of the tunnel. The function of the window (A) was for releasing the mosquito. The repellent sample Mosquito repellent Diffuser attached with the heating device was placed in the chamber (B). The Chamber (C) was left untreated. The repellency was evaluated at the interval of 30 minutes, 1 hour and every 30 minutes until 3 hours. At

each interval, 50 mosquitoes were released into the tunnel, and a number of mosquitoes in each chamber was recorded after 30 minutes. Each interval, the fresh mosquitoes were used. The calculation of the repellency stated below:

$$\text{Repellency (\%)} = ((A+C) / 50) 100$$

A = Window where mosquitoes were released

C = Untreated Chamber



Figure 3: Choice Test Method

3. Results

The efficacy of Roll-On Repellent that contains *M. cajuputi* Powell essential oil as the main ingredient was tested against mosquito, *Aedes aegypti*. According to the Malaysian Standard procedure, the sample should be compared with the 10% DEET (control from Malaysian Standard). Table 1 compares the percentage reduction of mosquito landing/biting on the treated arm between the Roll-on Repellent and MS Standard Repellent. During 4 hours of observation, roll on repellent have shown complete protection against mosquito landing or biting up to 1 hour. Mosquitoes start to landing or biting the treated arm after a 1- hour exposure. Compared to the MS Repellent, mosquitoes start to landing / biting after 2 hours and 30 minutes. Based on the results, Malaysian Standard Repellent shown more protection compared to the Roll-on repellent with the average percentage reduction or protection were 93.05 ± 0.78 and 66.58 ± 3.06 respectively. Although the Roll-on repellent could not be achieved or below than MS Repellent total reduction, Roll-on repellent have shown the protection against mosquito more than 50%. These results suggest that the Roll-On Repellent have the capability to completely keep away mosquito when reapplication after 1 hour.

Mist Diffuser Repellent has tested its repellency towards mosquito using Choice Test Method. Table 2 shows the effectiveness of Mist Diffuser Repellent to keep away the mosquitoes. During three hours of observation, Mist Diffuser Repellent has provide repellency against *Aedes aegypti* from 30 minutes to 3 hours from 83.33% to 100%. Start at 2 hours 30 minutes, it shows complete protection against mosquitoes. Hence, Mist Diffuser Repellent that contains 5% of *M. cajuputi* Powell has proven that could provide good landing or biting protection against mosquitoes within 3 hours of application.

4. Discussion

An initial objective of this present study was to evaluate the efficiency of the natural household insecticides product formulated from the extracted essential oil of *M. cajuputi* Powell against mosquitoes. It is interesting to note that both of the product has the capabilities to be mosquito repellent products. These present findings seem to be consistent with other research which found *M. cajuputi* Powell extract effective toward larvicidal and adulticidal of *Aedes aegypti* and *Aedes albopictus* [11], [12]. While the study done by [6] stated that lotion repellent formulated from 10% *M. cajuputi* Powell essential oil, absolute ethanol, and additives have 87.9% repellency against *Aedes aegypti*. In addition, this study also stated that the repellent lotion could give the protection against *Aedes albopictus* and *Anopheles dirus* up to 8 hours and agree by the [10]. On top of that, [13] also agree that *M. cajuputi* Powell extracts have the repellent effect against *Aedes aegypti*, *Aedes albopictus*, *Anopheles dirus* and *Culex quinquefasciatus*. Meanwhile, extraction from *M. cajuputi* Powell has shown the highest mortality against *Aedes aegypti* and *Aedes albopictus* [4].

There are several possible clarifications that might affect this result. Firstly, the effectiveness of a botanical household insecticide depends on many factors such as the type of active ingredient, formulation, temperature, and humidity [12]. Then, the synergistic effects of a combination of phytochemicals from each plant extract are the reason for the repellent activities against certain mosquito species [6]. Furthermore, there are several phytochemicals of essential oil extracted from *M. cajuputi* Powell that have insecticidal activities.

Table 1: The Repellency Effects Of Roll On Repellent Tested Against *AedesAegypti*

	Roll On Repellent (% unknown active ingredient – ISO/013/2018-a)		Malaysian Standard Repellent (10% DEET)	
	No of mosquito landed on the untreated arm	No of mosquito landed on the treated arm	No of mosquito landed on the untreated arm	No of mosquito landed on the treated arm
0'30"	10.67±0.67	0.00±0.00	4.00±1.00	0.00±0.00
Reduction		100.00±0.00	100.00±0.00	
1'00"hours	10.33±0.88	0.00±0.00	10.67±2.19	0.00±0.00
% Reduction		100.00±0.00	100.00±0.00	
1'30" hours	10.33±1.76	2.67±0.33	11.00±1.53	0.00±0.00
% Reduction		71.50±7.95	100.00±0.00	
2'00" hours	8.33±0.67	2.00±0.00	11.33±0.88	0.00±0.00
% Reduction		75.66±2.12	100.00±0.00	
2'30" hours	8.33±0.88	3.00±0.58	11.67±0.67	0.00±0.00
% Reduction		63.81±6.92	100.00±0.00	
3'00" hours	9.00±1.00	4.00±0.58	11.33±0.67	1.00±0.00
% Reduction		55.71±2.97	91.11±0.56	
3'30" hours	8.67±0.88	5.67±0.67	10.00±0.58	2.00±0.00
% Reduction		34.34±5.07	79.87±1.17	
4'00" hours	13.33±1.76	9.00±1.00	7.67±0.33	2.33±0.33
% Reduction		31.37±6.60	69.64±3.72	
TOTAL	79.00±1.15	26.33±2.03	77.67±4.41	5.33±0.33
% Reduction		66.58±3.06	93.05±0.78	

Table 2: Mean Percentage of Repellency of Mist Diffuser Repellent against *AedesAegypti*

Duration	Time expose the sample	Mean % Repellency ± SD
Fresh	0'30"	83.33±1.15
	1'00"	89.33±1.15
	1'30"	91.33±2.31
	2'00"	94.67±5.03
	2'30"	100.00±0.00
	3'00"	100.00±0.00

The phytochemicals are caryophyllene, β -caryophyllene, 1,8-cineole, chavicol, citral, p-cymene, limonene, linalool, myrcene, α -pinene, α -terpineol, γ -terpinene and terpinene-4-ol [13]. Many plant extracts and essential oils that are highly volatile could work in giving protection against mosquitoes in the vapor phase [12], [14]. Then, the quality of essential oil also plays a crucial role in extracting the important chemical compound that might use in repelling insect. The quality of essential oil affecting by the species, cultivating conditions, maturation of harvested plant, plant storage, plant preparation, and extraction method [6].

Even though these current findings could not reveal the effectiveness of all the repellent products formulated by *M. cajuputi* Powell worldwide, it could be used as the information and guidance for the next research. It is because the chemical compounds extracted are different depends on the chemical surrounding, soil nutrition, and others. While the formulation of the products also acts as the main reason in insecticidal activities. Thus, the variety of chemical compounds produce and the difference of insecticidal activities are important issues for upcoming research.

5. Conclusion

The main goal of this study was to evaluate the effectiveness of two products formulated by *M. cajuputi* Powell essential oil through bioassay test. The result of this present study reveals that both of the products which are Roll-On Repellent and Mist Diffuser could repel the adult mosquitoes with the average protection almost 70% and 100% repellency respectively. In conclusion, this finding indicated that both of the products have a good potential to be a household insecticide where it could be used when applying to the human skin and room environment. The findings from this study have extended the knowledge between the *M. cajuputi* Powell essential oil and biological efficacy test. The several information contributions of this study are the relationship between active ingredient percentage with protection percentage, the sample used with mosquito species and phase targeted and interaction between phytochemical involve in give protection against the mosquito. Besides the contribution from this study, there are several limitations such as the collection of specimen from the wild plant means the plant did not control the nutrient intake, local condition, and harvesting process. Additionally, this study examined the effectiveness of the product with one concentration of the active ingredient only. Hence, future research should obtain the specimen from the well-treated plant to control

the phytochemicals found. Next, this study will be more valuable if could formulate the product with various concentration of active ingredients. This is essential to trace the correct concentration that could give 100% protection for up to 4 hours and above.

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