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Screening of selected local plant extracts for their repellent activity against *Aedes albopictus* mosquitoes

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Abstract: This preliminary study selected the extracts of 33 Malaysian plants to screen for repellent activity against *Aedes albopictus* (*Ae. albopictus*) mosquitoes, a mosquito that is native to Southeast Asia and is one of the vectors for the Dengue virus. The repellent potential of the plant extracts was studied under a controlled environment, at 27-28°C and a humidity of 75 to 80%. A set concentration of plant extract was applied on the skin of hairless rats which act as the blood meal host for the *Ae. albopictus* mosquitoes. Starved mature female *Ae. albopictus* mosquitoes were used and observation was done by observational parameters such as the time took to land and begin blood feed. Results indicated that out of the 33 plant extract used, seven showed potential as a viable mosquito repellent, which is *Muntingia calabura* fruit extract, *Ipomoea aquatica* leaf extract*, Glycine max* polysaccharide extract*, Annona muricata* fruit and polysaccharide extract*, Mangifera indica* leaf extract, and *Citrus hystrix* peel extract. These local plants have the potential as a viable option as a commercial mosquito repellent, but further testing is needed.

Keywords: Repellent test, *Aedes albopictus,* plant extract

INTRODUCTION

Malaysia has a large population of mosquitoes, which thrive in the hot and humid climate. Mosquitoes act as a vector for the spread of several dangerous diseases such as Dengue fever, Malaria and Chikungunya, among others. In Malaysia, the diseases are endemic to the area, with cases of Dengue fever, in particular, is exhibiting exponential growth in number, with the years between 2014 to 2017 recording a historical amount of cases (Suppiah et al., 2018). One of the species of mosquito common in Malaysia is *Ae. albopictus. Ae. albopictus* or more commonly referred to as the Asian tiger mosquito is a species native to the area between India, Southeast Asia and Japan, thus, is native in Malaysia that falls in the area (Schmidt et al., 2020).

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One method of reducing the number of Dengue cases is by using mosquito repellent products. Chemical mosquito repellents are widely available for the average consumer and have been proven effective for repelling mosquitoes. However, the average chemical mosquito repellent can cause undesirable side effects, and they tend to harm the environment. It is common for chemical mosquito repellent to cause skin irritation and eye irritation among other side effects (Trivedi et al., 2018). As such, there is a growing interest among the modern consumer in natural alternative in mosquito repellent product. Natural chemical mosquito repellent products are safer for the consumer and are generally better for the environment. On the other hand, natural mosquito repellent products that are available on the market are not as effective as their chemical counterpart, thus, making the use void for this purpose. As such, we need to find other alternative sources that can function as a natural mosquito repellent to produce a more effective natural mosquito repellent.

MATERIAL AND METHODS

Plant material: The plant specimens (Table 1) was collected from various locations in Malaysia. The plant's sample was stored at the herbarium of the Forest Research Institute Malaysia (FRIM), Kepong. The plants were then separated by the different plant parts. Plants parts are dried in a hot air oven at 45 °C until totally dried (Thongwat et al., 2017). Polysaccharides are extracted using ethanol extraction (Ma et al., 2017). Protein extraction required the use of the Acetone/TCA precipitation method as protein is difficult to extract using common methods (Niu et al., 2018). The resulting extract was then used for the screening.

Mosquito rearing: Mosquito larvae were collected from the area of Klang, and raised in the laboratory on a diet of liver broth powder. Once the larvae emerge, the adult mosquitoes were collected into cups, anaesthetized with a small amount of acetone to allow sorting of the mosquito according to species. The *Ae. albopictus* mosquito was then allowed to mature for 5 days, fed with a solution of 10% sugar solution with added vitamin B12.

Test mosquitoes: Ae. albopictus mosquito was starved for 4 hours. Females were then aspirated and separated into mosquito cups, with 3 adult female mosquitoes per cup. The cups were labelled according to the intended sample to be tested.
Test hairless rats: The hairless rats were laboratory-reared and fed a steady diet of rat pellet, and ad libitum access to fresh water. A total of 9 female hairless rats was used, all aged 12 weeks old.

Test plant extract solution: Plants extracts were measured for a precisely 5mg amount in separate test tubes. 1 ml of filtered tap water was each added to the extracts. The mixture was vortexed for 5 minutes each to ensure the solutions will be well mixed. The resulting solutions resulted in a concentration of 5mg/ml.

Repellency evaluation: The mosquito repellence screening method is adapted from a study done by Kiplang'at and Mwangi (2013). Based on the method used in their study, changes were made to better accommodate the materials and test subjects available. Laboratory raised mature female mosquitoes was starved before the screening test for 4

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Table 1. The plant specimens were collected, processed into different plant parts, and underwent different extraction methods.

Common name	Scientific name	Part
Karas	*Aquilaria malaccensis*	Bark
Jamaican Cherry	*Muntingia calabura*	Fruit
God's Crown	*Phaleria macrocarpa*	Fruit
Melinjo	*Gnetum gnemon*	Fruit
Noni	*Morinda citrifolia*	Fruit
Kaffir Lime	*Citrus hystrix*	Fruit
River Tamarind	*Leucaena leucocephala*	Fruit
Tamarind	*Tamarindus indica*	Fruit
Pepper	*Piper nigrum*	Fruit
Okra	*Abelmoschus esculentus*	Fruit
Soursop	*Annona muricata*	Fruit
Kaffir Lime	*Citrus hystrix*	Fruit peel
Water Spinach	*Ipomoea aquatica*	Leaf
Pandan	*Pandanus amaryllifolius*	Leaf
Kratom	*Mitragyna speciose*	Leaf
Patawali	*Tinospora crispa (Miers)*	Leaf
Josephine Pineapple	*Ananas cosmosus*	-
Soursop	*Annona muricata*	-
Roselle	*Hibiscus sabdariffa*	-
Noni	*Morinda citrifolia*	-
Soybean	*Glycine max*	-
Roselle	*Hibiscus sabdariffa*	-
Longjack	*Eurycoma longifolia*	-
Fenugreek	*Trigonella foenum-graecum*	-
Longjack	*Eurycoma longifolia*	Root
Ginger	*Zingiber officinale Roscoe*	Root
Greater Galangal	*Alpinia galanga*	Root
African Oil Palm	*Elaeis guineensis*	Sap
Water Spinach	*Ipomoea aquatica*	Stem
Longjack	*Eurycoma longifolia*	Whole plant
Sarang Semut	*Myrmecodia pendans*	Whole plant
Indian Pennywort	*Centella asiatica*	Whole plant
Mango	*Mangifera indica*	Young leaf

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hours. 102 mature female mosquitoes were collected using a mechanical aspirator to be counted and verified. The male mosquitoes are not used. The mosquitoes were then transferred to mosquito cups, with 3 mature female mosquitoes for each mosquito cups. The mosquito cup was modified from a plastic test tube with a 30 mm inner diameter. The end of the plastic tubes was cut off and closed with a net. The other end of the test tube came with screw caps with was left as is, allowing the top to be open and closed securely.

The mosquitoes were carefully introduced to each cup. They were then left for 1 hour to acclimatize themselves. The cups were labelled according to the test sample to be used to prevent inaccuracies in the results. The control for the screening is filtered tap water, to observe the mosquitos' natural behaviour. Test for the repellency of the extract solution was commenced by first laying the hairless rat on one side of their body and cleaning the surface with filtered tap water. The surface was allowed to air dry and the extract solution was applied. The solution will then allowed to air dry, and the mosquito cups will be held onto the skin of the hairless rat with applied plant extract solution. The mosquitoes were observed for landing and blood-feeding behaviours. The cups were withdrawn as soon as the mosquito attempted to feed. The process will be repeated on the other side of the hairless rat body, to minimize the number of rats required. All tests were done during the day, between 0900 to 1800 hrs. The results were recorded as the time taken to begin feeding on the host. The results are then sorted into three categories which are under three minutes, under 5 minutes and 10 minutes.

RESULTS AND DISCUSSION

From the test done, out of 33 plant extract, seven plant extract worked as mosquito repellent using a 5mg ml^{-1} extract solution. Samples *Muntingia calabura* fruit extract, *Ipomoea aquatica* leaf extract*, Glycine max* polysaccharide extract*, Annona muricata* fruit and polysaccharide extract*, Mangifera indica* leaf extract, and *Citrus hystrix* peel extract deterred the mosquito from feeding for at least 10 minutes (Table 2) in the test done. While several plant samples deterred the mosquitoes from biting in the first 3 minutes, that is the *Gnetum gnemon* extract, *Elaeis guineensis* sap extract, and *Mitragyna speciose* extract, these plant extracts did not deter the mosquitoes from biting at a longer period. The method used in this study was adapted from a previous study by Kiplang'at and Mwangi (2013) gives the advantage of using adult parental generation mosquitoes for a repellency test. This is in contrast to the standard method for mosquito repellent test from WHO, which is the Arm-in-Cage test. The Arm-in-Cage test requires human test subject to hold their arm in a cage full of starved female mosquitoes (Pushpalatha & Athira, 2021). The test thus disqualifies parental generation mosquito from use. The method used also allows rapid disqualification of samples, as the mosquito cups are easier to remove from the host compared to when the host is placed in a cage. The extracts of 33 local plants were tested for any repellency effect against the local strain of *Ae. albopictus* mosquitoes. Of the 33 samples, seven showed potential as a repellent agent. The seven extracts are *Muntingia calabura* fruit extract, *Ipomoea aquatica* leaf extract*, Glycine max* polysaccharide extract*, Annona muricata* fruit and polysaccharide extract*, Mangifera indica* leaf extract, and *Citrus hystrix* peel extract. The *Gnetum gnemon* extract, *Elaeis guineensis* sap extract, and *Mitragyna speciosa* extract have prevented the mosquitoes from feeding in the first three minutes but failed to do so by the five-minute mark, thus, the plant extract has a weaker potential as a mosquito repellent.

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Table 2. Time is taken for the mature female *Ae. albopictus* mosquitoes to begin feeding on the host

SAMPLE	<3 minutes	<5 minutes	<10 minutes
CONTROL	Yes	-	-
Aquilaria malaccensis	Yes	-	-
Muntingia calabura	No	No	No
Phaleria macrocarpa	Yes	-	-
Gnetum gnemon	No	Yes	-
Morinda citrifolia	Yes	-	-
Citrus hystrix	Yes	-	-
Leucaena leucocephala	Yes	-	-
Tamarindus indica	Yes	-	-
Piper nigrum	Yes	-	-
Abelmoschus esculentus	Yes	-	-
Annona muricata	No	No	No
Citrus hystrix	No	No	No
Ipomoea aquatica	No	No	No
Pandanus amaryllifolius	Yes	-	-
Mitragyna speciose	No	Yes	-
Tinospora crispa	Yes	-	-
Ananas cosmosus	Yes	-	-
Annona muricata	No	No	No
Hibiscus sabdariffa	Yes	-	-
Morinda citrifolia	Yes	-	-
Glycine max	No	No	No
Hibiscus sabdariffa	Yes	-	-
Eurycoma longifolia	Yes	-	-
Trigonella foenum-graecum	Yes	-	-
Eurycoma longifolia	Yes	-	-
Zingiber officinale	Yes	-	-
Alpinia galanga	Yes	-	-
Elaeis guineensis	No	Yes	-
Ipomoea aquatica	Yes	Yes	-
Eurycoma longifolia	Yes	-	-
Myrmecodia pendans	Yes	-	-
Centella asiatica	Yes	-	-
Mangifera indica	No	No	No

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In this screening test, *Muntingia calabura* succeeded in repelling the starved female mosquitoes from feeding on the host for the full duration of the test. *Muntingia calabura* is more known by its common name Jamaican cherry, or its Malay name "Kerukup Siam" or "Ceri Kampung". *Muntingia calabura* is a fast-growing shrub, reaching an average of 40 ft or around 12.2 m in height. *Muntingia calabura* have to gain more attention in recent years with the rising popularity of natural remedies, as studies have shown the plant exhibit antibacterial, anti-inflammatory, and antidiabetic activities among other potential benefits (Sarojini & Mounika, 2018). Studies have shown the plant also exhibits insecticidal properties, as studies show that the plant's flower and fruit extract is toxic to *Plutella xylostella* larvae and pupae (Sarojini and Mounika, 2018) (Bandeira et al, 2012). No studies have been done on the effects of *Muntingia calabura* extract on mosquitoes, but further study could potentially be beneficial to understand the effect of Muntingia *calabura* fruit extract on mosquitoes.

Ipomoea aquatica leaves were dried, ground finely and vortexed together with filtered tap water for five minutes**.** The resulting solution successfully deterred mosquito from feeding on the host, showing repellency potential. *Ipomoea aquatica* or water spinach is a vegetable common in tropical countries and is generally considered a water weed. Common is wetland environment, this plant is commonly eaten by locals, but not widely cultivated (Ali & Kaviraj, 2018). The plant is more widely studied for its potential in agriculture, such as a cheap nutritional additive to farm fish feed (Ali & Kaviraj, 2018). No studies have been conducted on the insecticidal effect of the plants of mosquitoes, a study done in 2019 tested *Ipomeae aquatica* for antifeedant activity against Pomacea canaliculata Lamarck, or golden apple snail. However, the study concluded the *Ipomeae aquatica* does not have high antifeedant activity. As there is a lack of information on the topic, further testings should be done.

Glycine max polysaccharide extract worked to deter the mosquitoes from biting the host, as the mosquito refrained from biting throughout the time given**.** Glycine max or soybean is a legume plant that originates from Southeast Asia, though they were domesticated from wild soybean around 5000 years ago (Mammadov et al., 2018). Studies done on soybean are focused more on their nutritional value. There are no studies that research the effect of the *Glycine max* extract on mosquitoes.

Both the fruit extract and the polysaccharide extract of *Annona muricata* plant prevent the *Ae. albopictus* mosquito from landing. *Annona muricata* or soursop plant is an evergreen tree that thrives in the tropical and subtropical regions. The plant is widely studied, and results indicate that the plant has anti-stress, immunomodulatory, anti-inflammatory, antidepressant, and anticancer activities, among other beneficial activities (Gavamukulya et al., 2017). *Annona muricata* was also studied for its insecticidal activities, and have shown to exhibit a larvicidal effect on *Aedes aegypti* (*Ae. aegypti*) mosquitoes (Parthiban et al., 2020).

The young leaf extract of the *Mangifera indica* plant has exhibited repellent activity in this study. *Mangifera indica* or mango is an evergreen tropical tree that fruits were cultivated in the Indian subcontinent for centuries. The fruit is rich in nutrient and the plant itself is commonly used in traditional medicine, the plant was studied to have antioxidant,

anti-inflammatory, antidiabetic and anticancer activities (Mirza et al., 2020). The plant has not been studied for any repellent effect, but the fruit of *Mangifera indica* have commonly used as bait for adult mosquitoes (Meza et al., 2020).

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The rind of the *Citrus hystrix* fruit was effective as a repellent in the test conducted. *Citrus hystrix* or kaffir lime is a species of citrus that is native to the tropical region of Southeast Asia and southern China. Thus, the plant is also native in Malaysia (Agouillal et al., 2017). The plant leaves and fruit is very aromatic with a unique flavour, making them a staple in local cuisines. Studies on the plant proved that the plant has many beneficial properties, and the plant has traditionally used as a digestive reliever and to manage high blood pressure (Agouillal et al, 2017). The *Citrus hystrix* have also shown repellent activity against mosquitoes and is proven effective against *Ae. aegypti* (Rosanty et al., 2019). Another study on the essential oil of the lime concluded that the oil has ovicidal, larvicidal and pupicidal on *Ae. aegypti* (Subekti et al., 2017).

CONCLUSION

To conclude, from the 33 different plant extracts used as a preliminary screening for repellent activities, seven of the plant extracts exhibits potential as a repellent against *Ae. albopictus.* From the result, some of the extracts have the previous studies that finding corroborates with the result of this study, while some plant extract has no previous study done on their repellent effect on mosquitoes. This highlight the importance of further research on the potential extracts, and further data is needed to have a more firm reason for their repellent effect on the *Ae. albopictus* mosquito in this screening test.

DECLARATION OF CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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