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# Lethal response of the dengue vectors to the plant extracts from family Anacardiaceae

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ABSTRACT

**Objective:** To explore the larvicidal activities of different plant parts of *Melanochyla fasciculiflora* (*M. fasciculiflora*), *Gluta renghas* (*G. renghas*), *Anacardium occidentale* and *Mangifera indica* from family Anacardiaceae against the laboratory and field strains of dengue vectors *Aedes aegypti* and *Aedes albopictus* (*Ae. albopictus*).

**Methods:** Leaves and bark parts of study plants were collected from Taman Nageri, Bukit Pancor and Teluk Bahang National Park, Penang, Malaysia. Leaves and stem barks were separated, air dried, ground and extracted with methanol by Soxhlet apparatus. Crude extract was obtained by evaporating the extra solvent in rotary evaporator. The 4th instar larvae from laboratory and field strains were exposed to 50–1 300 mg/L concentrations according to World Health Organization standard larval bioassay. Larval mortality was recorded after 24 h of exposure.

**Results:** Highest larvicidal activity was exhibited by *G. renghas* bark extract against *Ae. albopictus* laboratory strain at 600 mg/L. *G. renghas* also showed the highest larvicidal activities for other strains as compared to other plant extracts, followed by *Mangifera indica* and *M. fasciculiflora* and *Anacardium occidentale*.

**Conclusions:** *Ae. albopictus* has been found to be more susceptible as compare to *Aedes aegypti* in both laboratory and field strains in this study. *G. renghas* and *M. fasciculiflora* were tested for the first time and exhibited prompting larvicidal activities against dengue vectors. These results revealed that all the plants especially *G. renghas* and *M. fasciculiflora* have the higher larvicidal activities and can be used for the control of dengue vector as a new environment friendly, target specific and low cost phytochemical.

## 1. Introduction

Mosquitoes are very important insect due to their vital role as a vector in the diseases transmission [1]. They can spread diseases such as dengue, malaria, filariasis, yellow fever, and Japanese encephalitis; the dengue viruses which are transmitted by the infected females of the family Culicidae *i.e. Aedes aegypti* (*Ae. aegypti*) and *Aedes albopictus* (*Ae. albopictus*) have become a great distress for the international public health in recent years [2,3]. Gibbons declared *Ae. aegypti* as the main vector for the arboviral infections of dengue viruses in tropical and subtropical regions [4]. Worldwide, about 50–100 million people are infected yearly and almost 2.5% of those infected people died [5]. In Malaysia, dengue outbreak cases are reported rising every year since 1980 [6]. Mosquitoes exist all over the world except for the places which are frozen perpetually [7]. Among the 3 500 species of mosquito [8], most are native to the tropic and subtropic regions of the world [7].

Control strategies are more imperative nowadays as the increase in resistance towards the synthetic insecticides among mosquito populations, and it becomes more challenging to control the vector borne diseases [9]. *Ae. aegypti* has already showed its resistance towards dichloro-diphenyl-trichloroethane all over the world except in some African countries [10]. Resistance to organophosphate has been documented in Americas and Caribbean region, while Asian region reported pyrethroid resistance in *Aedes* mosquitoes [11]. In addition to resistance, insecticide applications are modeling a great risk to decrease of

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the biodiversity, bioaccumulation and eradication of non-target organisms and natural enemies of the insect pest [12].

The harmful impacts of insecticides on health and environment have driven the quest of alternative environment friendly pesticide. To minimize the threats offered by the synthetic insecticides, the concern in biological control of mosquitoes grew bigger in the early 20th century [13]. The global flora encompasses massive number of phytochemicals that may now replace the synthetic pesticides [14].

Phytochemicals are better alternatives for the synthetic insecticides and can be used in vector control programs with possible success that may equivalent to the synthetic insecticides [15]. Number of plant species have been tested for their activities against different vectors and found to be target specific, readily degradable and environmentally safe [16]. A few examples on the successful effects of phytochemical from plant include leaves of *Cassia fistula* which displayed ovicidal and larvicidal activities against *Anopheles stephensi* (*An. stephensi*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) [16]. The bioactive compounds found in other plants *e.g. Ervatamia coronaria* have completely exhibited ovicidal activities against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* [17], while *Cryptomeria japonica* leaf essential oil was reported for its toxic nature against *Ae. aegypti* larvae [18].

Cashew nut shell liquid (CNSL) obtained from the plants of *Anacardium* has demonstrated significant lethal activity against termites at very low concentrations [19]. CNSL was analyzed and anacardic acid, cardanol, cardol, carbachol, orcinol, butylated hydroxy toluene and quercetin were found as main constituents. Among these constituents, cardol, cardanol and anacardic acid were affirmed to have larvicidal activities against *Ae. aegypti* [20]. *Mangifera indica* L. (*M. indica*) leaves essential oils were reported to have hydrocarbons, triterpenes, phenolics, carotenoids, saponins, vitamins and fatty acid as their major constituents and these chemicals are held responsible for repellent effect on female African malarial vector, *Anopheles gambiae* (*An. gambiae*) [21,22].

The growing trend and positive response of the community towards the phytochemicals and its environment friendly behavior create an open ground for the research and innovation of the plant based insecticides. Keeping in view the toxic activities of family Anacardiaceae which were demonstrated against other mosquito species, this study was designed to test the lethal effect of plants from family Anacardiaceae on dengue vectors: *Ae. aegypti* and *Ae. albopictus*. The selected plants were *Anacardium occidentale* (*A. occidentale*), *M. indica*, *Melanochyla fasciculiflora* (*M. fasciculiflora*) and *Gluta renghas* (*G. renghas*). These plants were selected due to their poisonous resins and their easy availability in the urban and suburban areas of Malaysia.

## 2. Materials and methods

### 2.1. Mosquito cultures

Two species from two strains were used in this experiment: *Ae. aegypti* and *Ae. albopictus* of laboratory and field strains. Laboratory strains were obtained from the insectarium of Vector Control Research Unit, Universiti Sains Malaysia, where the mosquitoes have been cultured under laboratory conditions since 1960s for more than 600 generations. The eggs collected on Whatman No. 1 filter paper were immersed in a plastic tray containing 500 mL of seasoned water. The eggs hatched after soaking in seasoned water.

The field strain of *Ae. aegypti* and *Ae. albopictus* were obtained from two locations which located at Flat Hamna (5°20′53.9″ N, 100°18′02.8″ E) and Bukit Jambul (5°20′06.7″ N, 100°17′26.0″ E) residential apartments using ovitrap method. Locations were selected due to high population of *Aedes* which is associated with high number of dengue cases in Penang. Ovitraps were made of tin cans, painted in black and filled with 300 mL of seasoned water with wooden hardboard paddles. The hardboard paddle was used for the attachment of eggs during oviposition. A total of 10 ovitraps were placed at both locations to obtain wild field strain of *Aedes* eggs. Wooden paddles were collected weekly and replaced with new ones. This collection was carried out for a month to have enough number of field strain eggs of *Ae. aegypti* and *Ae. albopictus*. The paddles collected from the field were kept in laboratory, let to dry for 48 h, and eggs on the paddles were counted under microscope. Paddles were then soaked in seasoned water to let the eggs hatched. The eggs took about 24–48 h to hatch. Mosquito culture was maintained at a temperature of (28 ± 3) °C, relative humidity of (70 ± 10)% and a photoperiod of 12 h light and 12 h dark. The larvae were fed with fine powdered food, a mixture of dog biscuit, yeast, beef liver and powdered milk at a ratio of 2:1:1:1 by weight. The emerged larvae were reared under laboratory conditions till adult stage. During adult stage the mosquitoes were separated according to the species. *Ae. aegypti* and *Ae. albopictus* were selected for the study which were then kept in separate cages with 10% sugar solution on the cotton swab. Both the species were blood fed on rats. After 24 h of blood feeding, oviposition substrate made of Whatman No. 1 filter paper in cone shape was placed on Petri dish. A total of 5 mL of water was added to moisten the filter paper for the mosquito to lay eggs in the cage. The eggs laid on the filter paper were allowed to dry and after 3 days the collected eggs were immersed in seasoned water to obtain the F1 generation. This F1 generation was used for the bioassay study.

### 2.2. Plant species

Mature leaves and bark parts of *A. occidentale*, *M. indica*, *M. fasciculiflora* and *G. renghas* were selected for the study. These plant parts were collected from Teluk Bahang National Park, Penang (5°27′38.56″ N, 100°12′18.69″ E) and Taman Nageri, Bukit Pancor (5°10′10.607″ N, 100°32′37.291″ E), Malaysia. Plant samples were verified and confirmed for the species by the herbarium staff of School of Biological Sciences, Universiti Sains Malaysia.

### 2.3. Plant extract preparations

Bark and leaves were left in laboratory to dry under normal environment condition. Leaves took 10–14 days to be dried until the weight was constant. Bark took about 20–25 days to completely dry. Dried leaves were ground mechanically using Panasonic stainless steel blender while the dried bark was mashed by using a tabletop hammer mill. The powdered samples were then extracted using methanol solvent in Soxhlet apparatus. A total of 2000 mL of methanol and 50 g of powdered sample were used in this extraction. Powdered sample was placed in a cellulose thimble (Favorit cellulose extraction thimbles: 43 mm × 123 mm in size) and inserted in the extraction tube of Soxhlet apparatus. The solvent was boiled at methanol boiling point at 66 °C using heating mantle. The