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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

process was repeated for three cycles which took about 3 h until the color of the solvent became semitransparent as a result of complete extraction of all plant contents. The whole process was recurred for three times to have enough crude extracts for the larval bioassay study. To remove excess solvent, the crude extracts were subjected to evaporation process using rotary vacuum evaporator machine under a reduced pressure for about 25–30 min at 66 °C with speed of 100 r/min. Then the remaining excess solvent was removed by placing the crude extract in oven at 40 °C for 24 h. The crude extract obtained after the removal of the excess solvent was stored in a refrigerator at 4 °C until further use. This procedure was done separately for each plant part and plant species.

2.4. Preparation of different concentrations

To obtain 10 000 mg/L of stock solution, 1 g paste from the crude extract was weighed and dissolved in 100 mL methanol. Sequential serial concentrations were made from the stock solution using distilled water. Concentrations ranging between 50 and 1 300 mg/L were used in this study.

2.5. Larvicidal bioassays

Late 3rd instar and early 4th instar larvae of *Ae. aegypti* and *Ae. albopictus* were used in this experiment according to the World Health Organization standard larvicidal bioassay [23]. A total of 20 larvae were placed in a 250 mL paper cup containing 200 mL of the different serial concentrations of extracts. A range of concentrations between 50 and 1 300 mg/L were prepared and each concentration was replicated three times for all the treatments. Control comprised of 1 mL of 10% of methanol in 199 mL of distilled water. Larvae were not given any food during the experiment. Mortality observations were made after 24 h exposure for all the treatments. Larvae were counted as dead when they were motionless after probing with a needle. All the experiments were conducted under laboratory conditions at a temperature of (28 ± 3) °C and a relative humidity of (70 ± 10)%.

2.6. Statistical analysis

The LC_{50} and LC_{95} values were calculated using probit analysis. A univariate analysis of variance was conducted using SPSS version 20 to examine the effects on the different mosquito strain, and the effects of concentration and plant part of crude extracts on *Aedes* larval mortality. The percentage of larval mortality was considered as the dependent variable whereas concentration, *Aedes* species and plant part were considered as the fixed factors. Larval mortality was expressed in percentage and was log-transformed to fulfill the ANOVA assumptions. The level of significance for the statistical analysis was set at $P < 0.05$.

3. Results

All the plant parts tested in this experiment for the larvicidal activities confirmed their larvicidal activities against *Aedes* spp. *G. renghas* demonstrated the strongest larvicidal activities as it caused 100% mortality by its bark extract at 600 mg/L against the *Ae. albopictus* laboratory strain (Figure 1A) followed by *M. indica*, *M. fasciculiflora* and *A. occidentale*. Whereas, to kill 100% of *Ae. aegypti* laboratory strain, more *G. renghas* bark extract was needed with 700 mg/L (Figure 1B). This indicated that laboratory strain of *Ae. albopictus* is more susceptible to *G. renghas* bark extract compared to *Ae. aegypti*. Whereas, field strain for both of the *Ae. aegypti* and *Ae. albopictus* requires higher plant concentration for 100% mortality as compared to the laboratory strain (Figure 2A and 2B). In this study, laboratory strain for both *Aedes* species is more susceptible as compared to field strain (Table 1). Similarly, on the other hand *Ae. albopictus* was found to be very susceptible to all the plants crude extracts in both laboratory and field strains as compared to the *Ae. aegypti* (Table 1). The difference of response between the species in both the strains towards the plant extracts is also significant (Tables 2 and 3).

The best LC_{50} and LC_{95} values were given by *G. renghas* as compared to the other three plants for both the leaf and bark extracts and against laboratory and field strains (Table 1). The lowest LC_{50} and LC_{95} values of 240.17 and 607.64 mg/L respectively were demonstrated by *G. renghas* bark extract against *Ae. albopictus* laboratory strain which caused mosquito larvae mortality at low dosage (Table 1), making it the most efficient plant extract in this study for mosquito larvae killing. While, the highest LC_{50} and LC_{95} values of 804.21 and 1 473.49 mg/L respectively were demonstrated by *A. occidentale* leaves extract against *Ae. aegypti* field strain (Table 1) which concluded that this plant extract is less efficient in killing mosquito larvae. Significant differences were observed between the mortalities caused by the crude extracts of the four plants against

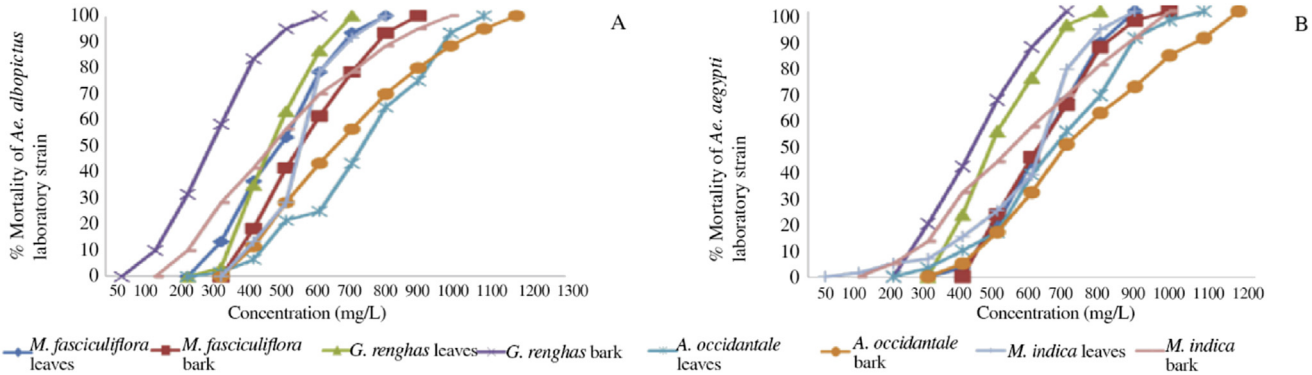

Figure 1. Mortality of mosquito larvae laboratory strains due to different concentration of methanolic crude extracts of 8 plant parts from family Anacardiaceae.
A: *Ae. albopictus*; B: *Ae. aegypti*.

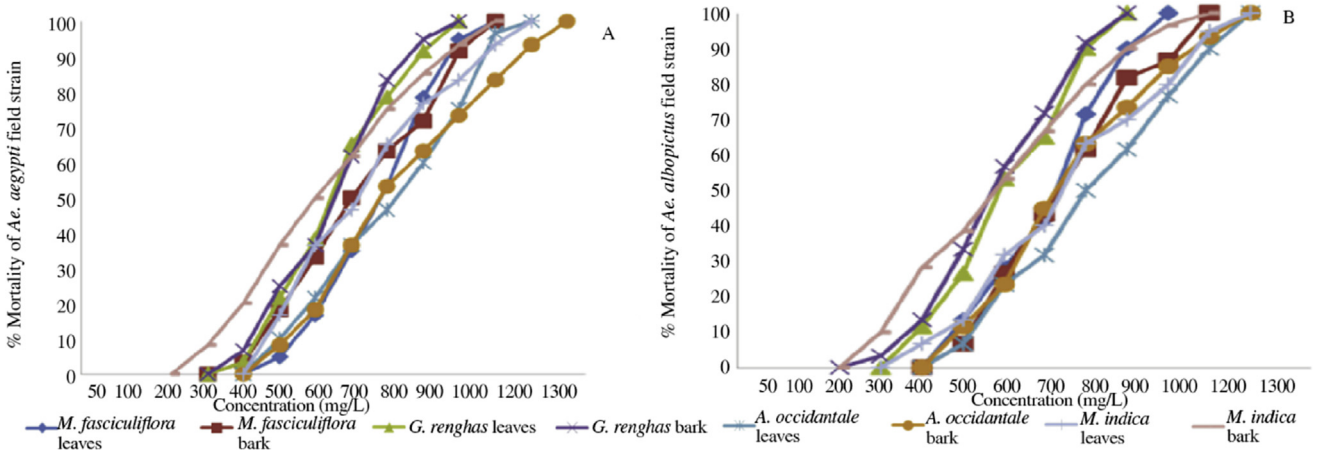

Figure 2. Mortality of mosquito larvae field strains due to different concentration of methanolic crude extracts of 8 plant parts from family Anacardiaceae. A: *Ae. aegypti*; B: *Ae. albopictus*.

Table 1

Mean LC_{50} and LC_{95} (mg/L) values of *M. fasciculiflora*, *G. renghas*, *A. occidentale* and *M. indica* leaf and bark extracts with 95% confidence limit of larval efficacy on *Ae. aegypti* and *Ae. albopictus* from laboratory and field strains.

Plant extract	*Ae. aegypti* (laboratory)		*Ae. albopictus* (laboratory)		*Ae. aegypti* (field)		*Ae. albopictus* (field)	
	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}
M. fasciculiflora leaves	619.22	915.68	454.75	801.75	753.01	1085.45	692.73	1048.83
M. fasciculiflora bark	615.40	951.79	535.59	888.68	696.35	1222.25	727.79	1131.74
G. renghas leaves	485.83	739.85	447.99	704.21	634.09	996.28	589.60	960.98
G. renghas bark	418.84	758.45	240.17	607.64	623.27	975.84	566.61	922.69
A. occidentale leaves	656.42	1121.03	698.85	1214.32	804.21	1473.49	791.16	1318.46
A. occidentale bark	711.99	1282.09	638.47	1199.78	791.67	1347.37	729.70	1190.72
M. indica leaves	570.36	1113.93	528.12	743.21	697.36	1229.40	722.28	1278.10
M. indica bark	523.91	1234.79	431.19	1076.30	582.06	1197.51	550.59	1171.95

Table 2

One-way ANOVA measuring the effects of different plant species and concentrations on laboratory strains of *Ae. aegypti* and *Ae. albopictus* larvae mortality after 24 h of exposure.

Source	*df*	MS	*F*-ratio	*P*
Aedes laboratory strains	1	259.44	83.25	0.000*
Plant parts	7	359.61	115.40	0.000*
Concentration	12	1524.71	489.27	0.000*
Aedes laboratory strains × plant parts × concentration	39	4.27	1.37	0.080*

df: Degree of freedom; MS: Mean square values. *: Significant values at 5% significant level.

Table 3

One-way ANOVA measuring the effects of different plant species and concentrations on field strains of *Ae. aegypti* and *Ae. albopictus* larvae mortality after 24 h of exposure.

Source	*df*	MS	*F*-ratio	*P*
Aedes field strains	1	32.75	12.75	0.000*
Plants parts	7	175.76	68.43	0.000*
Concentration	11	1774.75	691.01	0.000*
Aedes field strains × plant parts × concentration	48	1.11	0.43	1.000*

df: Degree of freedom; MS: Mean square values. *: Significant values at 5% significant level.

both *Aedes* species of laboratory strains (MS = 259.44, *df* = 1, *P* = 0.000) (Table 2) as well as field strains (MS = 32.75, *df* = 1, *P* = 0.000) (Table 3).

4. Discussion

The current study discovered the toxic nature of all the 4 plants from family Anacardiaceae that demonstrated the larvicidal activities against the dengue vectors *Ae. aegypti* and *Ae. albopictus*. *G. renghas* was proved to be the most toxic with the lowest lethal concentration followed by *M. indica*, *M. fasciculiflora* and *A. occidentale*. It has been known that the compounds responsible for the toxic effect of family Anacardiaceae are anthocyanosides, flavonones, coumarins, alkaloids, saponins, polyphenols, phytosterols, fatty acids, hydrocarbons, tanins, steroids, triterpenoids, anacardic acid and reducing sugars [20,24]. Anacardiaceae has been known for its medicinal use for prevention, cure and treatment of the diseases as the oldest practice known [25]. These compounds are also reported for their antimicrobial activities against bacteria, fungi and viruses [26], anti-inflammatory effects [27], antifeedant activity against some lepidopteran pests [28], repellent and larvicidal effects against some of the vector mosquitoes [22].

Plant extracts of different parts of *A. occidentale* have been reported to have larvicidal properties against *Ae. aegypti*. Effectiveness of CNSL obtained from *Anacardium* fruit is reported for its larvicidal activities against *Ae. aegypti* [20,29].

Methanolic extracts of *A. occidentale* leaf were used along with *Lantana camara* root by Tripathy *et al.* against *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* and were found more effective against *Ae. aegypti* [30]. Similarly, comparative examination of the nutshell, bark and leaf of *A. occidentale* done in Nigeria, for the larvicidal properties using the aqueous extracts against *An. gambiae*, confirmed that the bark extracts were much better as compared to other parts extracts [31].

Similarly, mango plant is also reported several times for its activities against dengue vectors [6,22,32]. Alwala *et al.* identified the repellent properties of oils of *M. indica* leaves due to its hydrocarbon compounds against the *An. gambiae* [22]. Methanolic extracts of *M. indica* showed no toxicity while the aqueous and acetone extracts showed its bioactivity against the dengue vector *Ae. aegypti*, revealing that the plant has the toxic activities which may change with the selection of the solvent [32]. Whereas, Adebajo *et al.* analyzed the methanolic extracts of *M. indica* leaves against *Ae. aegypti* and found to be effective against the fourth instar larvae, and the efficacy increased with the extended exposure [33]. *M. indica* stem bark extracts were analyzed and presence of flavonoids, alkaloids, phytosterols, saponins, tannins and cardiac glycosides were revealed [34], which are also reported for their activities against different vector pests and pathogens [6,35].

Even though few reports are available on the larvicidal properties of Anacardiaceae family, no work has been done on the potential of *G. renghas* and *M. fasciculiflora*. *G. renghas* and *M. fasciculiflora* plant parts were used for the first time in this study to assess their larvicidal activities against the dengue vectors and they exhibited remarkable results. *G. renghas* leaves as well as bark extracts presented the lowest LC_{50} and LC_{95} values for both *Ae. aegypti* and *Ae. albopictus* species either laboratory or field strains followed by the *M. fasciculiflora*. The poisonous resins present in both plants from family Anacardiaceae may be the reason for their effectiveness as larvicide, which supports the search of active compounds used as biological pesticides to minimize the hazardous effects of synthetic insecticides.

The larvicidal potential of all the plant extracts tested were promising against *Aedes* species of both the laboratory strains as well as field strains, but the laboratory strains appeared to be more susceptible as compared to the field strains. Increase in the resistance level was found in field strain due to the pre-existing resistance level in field strain towards the insecticides, genes frequencies and resistance mechanism evolved in inheritance [36]. Chaiyasit *et al.* also reported the laboratory strain of *Ae. aegypti* as more susceptible than the field strains towards 5 essential oils containing pyrethroids because of the fact that the study area was introduced with synthetic organophosphates which lead to the increase in the tolerance level of the *Ae. aegypti* field strain [37].

The LC_{50} and LC_{95} values shown by the family Anacardiaceae in this study against dengue vectors have sketched the significant potential of larvicidal activities. The current study revealed that the *Ae. albopictus* is more susceptible than the *Ae. aegypti* in both laboratory strains as well as field strains. Previous studies also have showed that the *Ae. albopictus* was not resistant to the synthetic insecticides whereas the *Ae. aegypti*, collected from different locations in Thailand, showed promising resistance to different insecticides [38]. *Ae. albopictus* has also showed more susceptibility than *Ae. aegypti* to crude extracts of different plants [39]. *Ae. albopictus* was mentioned as more susceptible while *Ae. aegypti* showed some resistance when tested with insect growth regulator triflumuron, a chitin synthesis inhibitor [40]. *Ae. aegypti* was found having higher level of resistance as compared to *Ae. albopictus* due to its endophilic nature, in which it tends to inhabit inside human habitat and consequently experienced higher exposure to the insecticides specifically mosquito adulticides [41].

Polar solvents such as methanol, ethanol, ethyl acetate and acetone are mostly used for the extraction of phenolic and antioxidant compounds [42]. Methanol was selected in this study for the extraction of active compounds from plants of family Anacardiaceae because methanol was found to be a better extractant followed by ethanol and water [43]. Anacardiaceae family is reported for the presence of alkaloids, polyphenols and saponins [44], anacardic acid, cardanol and cardol, which are highly antioxidant and are having effective larvicidal activities against dengue vectors [19,29,45].

Different plant parts have different phytochemical compounds which have different toxicities to target species [46]. Therefore, in current study, both the plant parts have demonstrated different larvicidal activities. Stem bark extracts of the plants have displayed better results in this study than the leaf extracts. These results also illustrated that all the plant parts are having different active chemical compounds responsible for diverse activities against various organisms. The toxicity of phytochemical compounds depends on the factors including the age of plant parts, organ development, type of plant material, seasonal variation, chemical or mechanical injuries, pollution, pests and diseases which may be the reasons of different LC_{50} and LC_{95} values against different pest species [47].

To avoid the detrimental effects caused by the chemicals for the control of dengue vectors, natural and nontoxic bioactive compounds of plant origin can be used as an alternate control measure [48]. This study finally proposed new alternative potential biopesticides from local flora, which is easily available with low technology and can easily be integrated into the ongoing mosquito management programs. Hereby, it can reduce the cost of mosquito management rather than using conventional chemical control, which is more expensive than the biological control comprising of plant extracts and is more effective and target specific [49].

This study concluded that *G. renghas* and *M. fasciculiflora* can be one of new potential biopesticides. These results also emphasized the need of further research and investigation to find out the bioactive compounds of *G. renghas* and *M. fasciculiflora* and their activities against other vector pests. This may help in enhancement of the bioactivity of their phytochemicals and replacement of the synthetic insecticides in future.

Conflict of interest statement

We declare that we have no conflict of interest.

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