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RESEARCH

Larvicidal Efficacy of Different Plant Parts of Railway Creeper, *Ipomoea cairica* Extract Against Dengue Vector Mosquitoes, *Aedes albopictus* (Diptera: Culicidae) and *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT. Natural insecticides from plant origin against mosquito vectors have been the main concern for research due to their high level of eco-safety. Control of mosquitoes in their larval stages are an ideal method since *Aedes* larvae are aquatic, thus it is easier to deal with them in this habitat. The present study was specifically conducted to explore the larvicidal efficacy of different plant parts of *Ipomoea cairica* (L.) or railway creeper crude extract obtained using two different solvents; methanol and acetone against late third-stage larvae of *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.) (Diptera: Culicidae). Plant materials of *I. cairica* leaf, flower, and stem were segregated, airdried, powdered, and extracted using Soxhlet apparatus. Larvicidal bioassays were performed by using World Health Organization standard larval susceptibility test method for each species which were conducted separately for different concentration ranging from 10 to 450 ppm. Both acetone and methanol extracts showed 100% mortality at highest concentration tested (450 ppm) after 24 h of exposure. Results from factorial ANOVA indicated that there were significant differences in larvicidal effects between mosquito species, solvent used and plant parts ($F = 5.71$, df $= 2$, $P < 0.05$). The acetone extract of *I. cairica* leaf showed the most effective larvicidal action in *Ae. aegypti* with LC_{50} of 101.94 ppm followed by *Ae. albopictus* with LC_{50} of 105.59 ppm compared with other fractions of *I. cairica* extract obtained from flower, stem, and when methanol are used as solvent. The larvae of *Ae. aegypti* appeared to be more susceptible to *I. cairica* extract with lower LC_{50} value compared with *Ae. albopictus* ($F = 8.83$, df $= 1$, $P < 0.05$). Therefore, this study suggests that the acetone extract of *I. cairica* leaf can be considered as plant-derived insecticide for the control of *Aedes* mosquitoes. This study quantified the larvicidal property of *I. cairica* extract, providing information on lethal concentration that may have potential for a more eco-friendly *Aedes* mosquito control program.

Key Words: *Aedes*, biological control, *Ipomoea cairica*, mosquito, plant extracts

Dengue, the most important viral infection for humans transmitted by bites of infected *Aedes* mosquitoes, is the major public health problem in tropical and subtropical regions (WHO 2012). Today, the geographic distribution of dengue epidemics includes 124 countries worldwide whereby 3.61 billion people are at risk of being infected and every year 500 million of them contract infection effectively (Beatty et al. 2010). Malaysia, with a population at ~27.7 million and a population density of 84 per sq. km, has consecutively recorded rising cases of dengue outbreaks since 1980 (Lam 1993). Dengue fever (DF) and dengue hemorrhagic fever (DHF) are caused by dengue virus transmitted to susceptible humans mainly by *Aedes aegypti* and *Aedes albopictus* (Guha-Sapir and Schimmer 2005). Clinical manifestation of dengue infection ranges from classical DF to much more severe or fatal DHF and dengue shock syndrome. The increase in dengue cases is considered to be a reflection of the development towards massive infrastructure as well as urbanization, which is a favorable factor for breeding of *Ae. aegypti* (Muhammad Azami et al. 2011, Gubler 2002).

In the absence of effective dengue vaccine and antiviral treatment, the only strategy to prevent disease transmission and disease is by controlling the principle vectors such as eliminating larval habitats, reducing abundance and lifespan of adult mosquitoes, and preventing human–mosquito contact (Eisen et al. 2009, Rohani et al. 2011). The ideal method for controlling mosquito infestation is by preventing mosquito breeding places. Because mosquito larvae are aquatic, it is much easier to control the mosquito population in the larval stage as compared with adult. Public health policies have adopted various control of Culicidae by synthetic insecticides such as organochlorines and organophosphates compounds. However, it has not been very successful due to human, environment, economical, technical, and operational factors (Ghosh et al. 2012). In recent years, the use of many former insecticides in mosquito control operations has been limited due to high cost of synthetic insecticides, concern for environment sustainability, their nonbiodegradable nature, harmful effect on human health, and increasing resistant rates (Ghosh et al. 2012).

One of the possible ways to overcome these problems is by the use of plant-derived insecticides as alternatives for synthetic insecticides or for use in integrated management programmes (Shaalan et al. 2005, Waliwitiya et al. 2009, Ghosh et al. 2012). Plants produce a broad range of bioactive chemical compounds (Bernhoft 2010) and are source of substitute agents for the control of insect vectors (Rahuman et al. 2009). Phytochemical insecticides are more prominent than synthetic insecticides due to their rapid environmental biodegradation, specific to target organisms (Cetin et al. 2004, Mann and Kaufman 2012), and cause fewer or lesser threat to human health and environment (Isman 2006). Natural products of plant origin with insecticidal properties have been tried in the past for control of variety of insect pests and vectors (Das et al. 2007). Approximately, more than 2,000 plant species have been reported to show insecticidal properties. Ghosh et al. (2012) reviewed the mosquitocidal activities of various herbal products from edible crops, ornamental plants, trees, shrubs, herbs, grasses, and marine plants against different vector species. Azadirachtin, the active compound of neem has long been recognized for its mosquitocidal properties (Isman 2006). Several other plant species such as *Piper nigrum* (Park et al. 2002), *Piper longum* (Lee 2000), *Tagetes minuta*

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(Perich et al. 1994), and *Curcuma domestica* (Ranaweera 1996) has been proven to show excellent larvicidal properties against mosquitoes.

Ipomoea cairica (L.) (Family: Convolvulaceae) is an indigenous plant, which is commonly known as "Railway creeper." This plant is a climbing herbaceous perennial and widely distributed in almost all tropical regions (Liu et al. 2011). It is normally used for fencing of domestic and peri-domestic situations and also has some medicinal properties (Ferreira et al. 2006, Thomas et al. 2004). Drink made from crushed leaf of *I. cairica* is used for treatment of body rashes, especially the one accompanied by fever (Thomas et al. 2004). The essential oil of *I. cairica* leaf by steam distillation showed excellent repellency effects against *Anopheles stephensi* (Rajkumar and Jebanesan 2007) and showed remarkable larvicidal activity against *Culex tritaeniorhynchus, Ae. aegypti*, *An. stephensi,* and *Culex quinquefasciatus* (Thomas et al. 2004). As this plant grow abundantly in the wild, it is worthwhile to identify its active components that cause larval mortality and explore its remarkable larvicidal properties as an alternative to chemical larvicides. Thus, this study was carried out to determine larvicidal efficacy of *I. cairica* extract against F1 late third-stage larvae from wild-strain mosquitoes of *Ae. albopictus* and *Ae. aegypti* using two different solvents; methanol and acetone. Three different parts of *I. cairica* leaf, flower, and stem were tested. The study postulated that all plant parts of *I. cairica* will possess larvicidal activity and toxicity against larvae of *Ae. albopictus* and *Ae. aegypti*.

Materials and Methods

Plant Materials. The plant materials of *I. cairica* were collected from Bayan Baru, Penang, Malaysia (5° 25′ N, 100° 19′ E) by using random sampling method. The plant was identified by using the key to the taxa of *Ipomoea* based on cotyledon characteristics (Ogunwenmo 2003).

Preparation of Extracts. *I. cairica* plant was segregated as leaf, flower, and stem and air-dried for 7–10 d under shade. Dried materials were powdered mechanically by using commercial electrical stainless steel blender (Panasonic: MX-899TM). The finely ground plant parts were extracted using Soxhlet apparatus (Favorit, Malaysia) separately using two different solvents; methanol (2,000 ml, Qualigens) and acetone (2,000 ml, Qualigens). In total, 40 g ground-plant material were placed in paper thimble (Favorit cellulose extraction thimbles: size 43 × 123 mm), together with some clean pebbles to ensure optimum solvent flow through the plant powder. The apparatus was set to the boiling point of solvents; methanol (65°C) and acetone (56°C) (Amer and Paxton 1956). The apparatus was run for 3 h, until the solvent color in the siphon side arm turns almost clear. The procedure was repeated twice by replacing the plant powder, 40 g of powder for each round, in the paper thimble.

The solvents from collected plant extracts were removed by using rotary evaporator. The speed was set up to 100 rpm and the temperature of water bath was fixed to boiling point of methanol at 65°C and acetone at 55°C. The solvent from the concentrated crude extract were further removed by placing it in an electrical oven at 37°C. The crude residue of these plants varies with the solvents used (Govindarajan 2010). The collected crude extract was weighed and stored in the petri dish at 4°C until later use (Thomas et al. 2004).

Stock solutions at 10,000 ppm were prepared by dissolving 1 g of extract in 100 ml of respective solvent. From the stock solution, 1,000 ppm was prepared by dissolving 50 ml of the solution in 450 ml of distilled water and subsequent dilutions were made as per requirements to prepare different concentrations ranging from 10 to 450 ppm.

Mosquito Colonies. F1 larvae of wild-strain mosquitoes were used in this study. Eggs of *Ae. aegypti* were obtained by placing ovitraps in Bagan Dalam, Butterworth, Penang (5° 24' N, 100° 23' E); while *Ae. albopictus* was obtained from Durian Valley, Universiti Sains Malaysia, Penang, (5° 26' N, 100° 49' E). Collected larvae were brought back to laboratory and were reared in enamel trays containing dechlorinated tap water. The culture was maintained at (28 ± 2)°C, 70–85% relative humidity, with a photo period of 14-h light and 10-h dark. Larvae were fed with a mixture of dog biscuit, beef liver, yeast, and milk powder with ratio of 2:1:1:1 by weight and prepared as a fine powder.

Larvicidal Bioassay. Larvicidal bioassays were performed as per standard World Health Organization (2005) larval susceptibility test method in 350-ml paper cups containing 250 ml of test medium (distilled water and plant extracts solution) and 25 early fourth-stage larvae of *Ae. albopictus* and *Ae. aegypti*. A homogenous population of late third-stage larvae (5 d old and 4–5 mm in length) were chosen for this study (WHO 2005). Initially, the mosquito larvae were exposed to a wide range of test concentrations and a control to find out the activity range of the extract solution. After determining the mortality of larvae in this wide range of concentrations, a narrower range (eight concentrations ranging from 10 to 450 ppm, yielding between 0 and 100% mortality in 24 h of exposure) was selected as test concentrations for larvicidal bioassays. In total, four replicates were set for each concentration. One control of distilled water with 1 ml of 10% of respective solvent was kept with each set of experiment. Solvent was added into the control containers to ensure it was identical to the test solutions which may have also contained the solvent. Experiments were conducted at room temperature (28 ± 2)°C. After 24 h of exposure, the number of dead larvae was recorded and the percentage of mortality values was calculated. Larvae with total absence of movement, even after touch, were considered as dead (Oliveira et al. 2010).

Statistical Analysis. The data were subjected to log-probit analysis for calculating LC_{50} and LC_{95} with 95% confidence limit using the SPSS 20.0 (Statistical Package of Social Sciences) software. A factorial analysis of variance (ANOVA) was performed using concentration, species, solvents, and plant parts as variables to find the significant differences between the above parameters and on larval mortality. Mortality data were tested for normality (Shapiro-Wilk) prior to analysis. The data were natural-log transformed ($\ln[y+1]$) due to violations of homogeneity of variance (Blaustein et al. 2005). Larval mortality was served as dependent variable and concentration, species, solvents, and plant parts were treated as fixed factors.

Results

The crude extract obtained from different parts of *I. cairica* plant produced impressive results on larvicidal activity against late third-stage larvae of *Ae. albopictus* and *Ae. aegypti* under laboratory conditions. It is evident from the study that the rise in the concentrations of the crude extracts caused an increase in percentage mean mortality of larvae (Fig. 1). No mortality was recorded at concentration of 10 ppm. At the highest concentration tested (450 ppm) which induced 100% mortality, the larvae reacted in terms of behavioral aspects such as restless movement for some time, followed by sluggishness, convulsions, and subsequently paralysis at the bottom of test cups and died slowly. In comparison to normal larvae (Fig. 2A), the larvae exposed to high concentrations showed severe effects such as darkening or blackening of abdomen (Fig. 2B) and larvae with twisted abdomen (Fig. 2C). As in most of the cases, *Ae. aegypti* larvae were found to be more susceptible than *Ae. albopictus,* and acetone extract of *I. cairica* leaf produced highest mortality. Therefore, the morphological deformities caused by acetone extract of *I. cairica* leaf on *Ae. aegypti* larvae were selected as examples as shown in Figure 2. The morphogenetic anomalies suggested a general toxic effect of the extract, which was found to be dose dependent.

The highest mortality was observed in acetone extracts of all the three plant parts tested and it was statistically significant than methanol extracts ($F = 127.32$, df = 1, $P = 0.000$; Table 2). Different extracts of leaf, flower, and stem also revealed significant difference between each other ($F = 83.38$, df = 2, $P = 0.000$; Table 2) whereby the leaf produced the most effective larvicidal activity (Table 1). The highest larval mortality was recorded in the acetone extract of *I. cairica* leaf with LC_{50} of 101.94 and 105.59 ppm and LC_{95} of 447.78 and 321.56 ppm against

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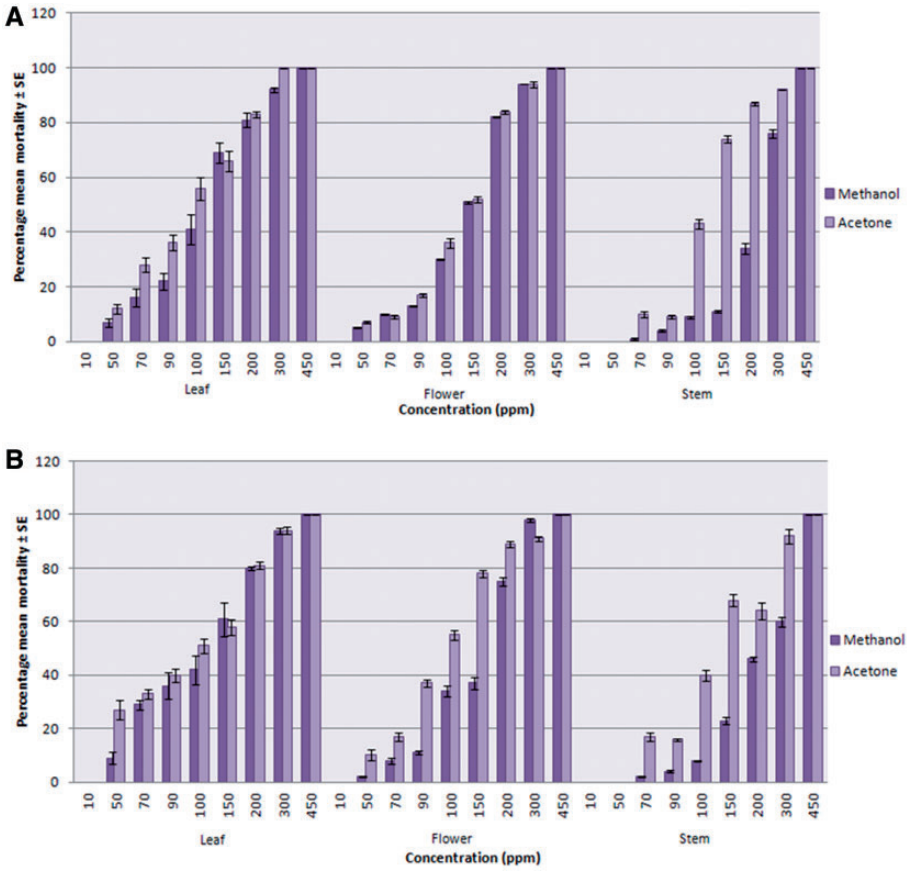

Fig. 1. Percentage mean mortality of late third-stage larvae of *Ae. albopictus* (A) and *Ae. aegypti* (B) in response to different parts of *I. cairica* extract.

Ae. aegypti and *Ae. albopictus* larvae, respectively (Table 1). This was followed by acetone extract of *I. cairica* flower with LC_{50} of 105.53 and 132.47 ppm and LC_{95} of 278.67 and 319.46 ppm against *Ae. aegypti* and *Ae. albopictus* larvae, respectively. Crude extracts from *I. cairica* stem showed significantly lower larval mortality but 100% mortality was reached at concentration of 450 ppm for both solvents and species tested. The effect of *I. cairica* extract on *Ae aegypti* was significantly differed from *Ae. albopictus* larvae ($F = 8.83$, df = 1, $P = 0.003$; Table 2). As shown in Table 2, results from factorial ANOVA indicated that there were significant differences in larvicidal effects between mosquito species, solvent used, and plant parts ($F = 5.71$, df = 2, $P < 0.05$). The result indicated that the acetone extract of *I. cairica* leaf showed the best larvicidal efficacy towards *Ae. aegypti* larvae. No mortality was recorded in the control treatment.

Discussion

We demonstrated that the biocontrol potentiality of crude solvent extract of *I. cairica* against late third-stage larvae of *Ae. albopictus* and *Ae. aegypti* has been well established in laboratory conditions. Previous studies of *Ipomoea* have been observed earlier that essential oil of *I. cairica* can possess remarkable larvicidal property as it caused 100% mortality in larvae of *Cx. tritaeniorhynchus*, *Ae. aegypti*, *Ae. stephensi,* and *Cx. quinquefasciatus* at concentrations of 100, 120, 120, and 170 ppm, respectively (Thomas et al. 2004). Rahuman et al. (2009) reported that the acetone, hot water, methanol, and petroleum ether extracts of *I. carnea* leaf had showed different larvicidal activity against fourth-stage larvae of *Cx. quinquefasciatus*. The variation in the larvicidal potentiality of crude extract against different mosquito species is something common as the toxicity and active compounds of *I. cairica* vary according to the geographical origin of the plant, solvent used and methods of extraction, plant parts from which they are extracted and developmental stage of the plant (Sukumar et al. 1991).

The larvae exposed to high concentrations of extract showed severe morphological deformities. Several other authors reported morphological aberrations induced by plant extracts on mosquito larvae. Saranya et al. (2013) observed that aqueous leaf extract of *Spathodea campanulata* affect *Ae. aegypti* larval morphology such as dechitinized larva with damaged digestive tract and exuvia of the proceeding instar attached to the dead. Similarly, Arivoli and Tennyson (2011) found that after treated with crude leaf extracts of *Abutilon indicum*, larvae of *Ae. aegypti*, *Ae. Stephensi,* and *Cx. quinquefasciatus* had strated sclerotization, which appeared to be a feature of pupal cuticle. In present study, larvae exposed to *I. cairica* extract showed darkening or blackening of abdomen and twisted abdomen. Comparable abnormalities were reported by Khater and Shalaby (2008) when larvae of *Culex pipiens* showed abnormalities such as pigmented and twisted larvae after exposure to some commercially available plant oils.

The bioactivity of plant-based insecticides against mosquito larvae vary significantly according to solvent used in extraction and the mosquito species tested (Shaalan et al. 2005). This is an agreement with the present study whereby acetone extract of *I. cairica* was found to show better larvicidal activity against larvae of *Ae. aegypti* and *Ae. albopictus* compared with methanol extracts of *I. cairica*. In the present study, acetone and methanol were used since they have different polarities with polarity index of 5.1 and 6.1, respectively. It has been reported that a converse relationship exist between extract efficacy and solvent polarity where efficacy increase with decreasing polarity (Mulla and Su 1999) which lays in line with the present findings. Moderately polar solvents such as acetone and methanol were selected in this study since they mainly extract steroids and alkaloids. Ghosh et al. (2012) reported

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Fig. 2. Morphological deformities induced by acetone extract of *I. cairica* leaf in larvae of *Ae. aegypti*: (A) normal larvae, (B) larvae with blackened abdomen after treatment, (C) larvae with twisted abdomen after treatment.

Table 1. Larvicidal activity of different solvents crude extracts of *Ipomoea carica* plant parts against late third-stage larvae of *Ae. albopictus* and *Ae. aegypti*

Mosquito species	Solvent	Parts used	LC50 (ppm)	LC95 (ppm)	Regression equation
Ae. albopictus	Methanol	Leaf	122.12	315.73	$Y = 3.99X - 8.32$
		Flower	138.45	306.94	$Y = 4.76X - 10.19$
		Stem	231.3	512.07	$Y = 4.77X - 11.27$
	Acetone	Leaf	105.59	321.56	$Y = 3.40X - 6.88$
		Flower	132.47	319.46	$Y = 4.33X - 9.13$
		Stem	145.79	356.87	$Y = 4.23X - 9.16$
Ae. aegypti	Methanol	Leaf	114.78	319.53	$Y = 3.70X - 7.62$
		Flower	152.00	375.32	$Y = 4.19X - 9.14$
		Stem	238.37	704.38	$Y = 3.50X - 8.31$
	Acetone	Leaf	101.94	447.78	$Y = 2.56X - 5.14$
		Flower	105.53	278.67	$Y = 3.90X - 7.89$
		Stem	132.94	375.99	$Y = 3.64X - 7.74$

ppm, parts per million; LC_{50}, Lethal concentration required to kill 50% of the population exposed; LC_{95}, Lethal concentration required to kill 95% of the population exposed.

that biochemicals extracted using moderately polar solvents produced good larvicidal bioassay results.

Generally, plant phytochemicals are secondary metabolites that are developed to protect them from insects that feed on them. The insects that feed on secondary metabolites encounter toxic effects that in turn affect insect physiology in different means and at various receptor sites (Ghosh et al. 2012). It has been reported that *I. cairica* contains alkaloid (Lin et al. 2008), which is primarily toxin to insects (Kennedy and Wightman 2011) and potentially serves as natural mosquito larvicides (Talontsi et al. 2011). Mann and Kaufman (2012) reviewed that nicotine, anabasine, and ryanodine are common alkaloids used as pesticides. The mode of action of alkaloids on insect vectors varies by the structure of the molecules but they are mainly reported to inhibit acetylcholinestrase (AChE) and sodium channels. Ghosh et al. (2012) stated that inhibition of AChE is most important physical disruption of plant secondary metabolites on insects, as it functions as key enzyme responsible for termination of nerve impulse transmission through synaptic pathway. Correspondingly, the insects exposed to substances containing AChE will be paralyzed and eventually die, as observed in mosquito larvae in recent study.

It is evident from our study that rise in the concentrations of the crude extracts was the main cause of the mortality in both of the mosquito species tested. Rawani et al. (2010) also stated that the results of regression analysis of crude extract of *Solanum nigrum* (L.) leaves

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Table 2. Analysis of variance on larval mortality comparing concentrations, species, solvents, and plant parts used

Source of variation	df	MS	*F*-value	*P*-value
Concentration	7	36.96	244.93	**0.000***
Species	1	1.33	8.83	**0.003***
Solvent	1	19.21	127.32	**0.000***
Parts	2	12.58	83.38	**0.000***
Concentration * Species	7	0.27	1.80	0.088
Concentration * Solvent	7	1.11	7.38	**0.000***
Concentration * Parts	13	1.08	7.14	**0.000***
Species * Solvent	1	0.25	1.65	0.200
Species * Parts	2	0.06	0.42	0.658
Solvent * Parts	2	3.56	23.59	**0.000***
Concentration * Species * Solvent	7	0.16	1.08	0.374
Concentration * Species * Parts	13	0.15	0.97	0.480
Concentration * Solvent * Parts	13	0.39	2.59	**0.002***
Species * Solvent * Parts	2	0.86	5.71	**0.004***
Concentration * Species * Solvent * Parts	13	0.23	1.53	0.107

df, degree of freedom; MS, mean-squared value.
Significant values are given in bold.

against *Cx. quinquefasciatus* was positively correlated with the concentrations of the extracts. A similar trend was observed by Dharmagadda et al. (2005) when the percentage mortality of *Ae. aegypti*, *Cx. quinquefasciatus*, and *Ae. stephensi* larvae increase with an increasing concentration of *Targetes patula* essential oil from 6.25 to 150 ppm.

Variation of larvicidal potential of *I. cairica* changed with the different plant parts used for extraction. Similar finding was reported by Bansal et al. (2012) when seed, leaf, and flower extract from *Calotropis procera*, *Tephrosia purpurea*, and *Prosopis juliflora* produced variable larvicidal efficacy against *Ae. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. Anees (2008) reported that different solvents extracts of leaf and flower of *Ocimum sanctum* produced different larvicidal activity against larvae of *Ae. aegypti* and *Cx. quinquefasciatus* where crude extract of leaf showed higher larvicidal activity on both species tested. This finding is in line with the present study, whereby the crude extracts from *I. cairica* leaf produced the highest larvicidal activity as compared with flower and stem of the plant. In our study, more bioactive compound was located in the leaf when compared with the flower and stem. This is due to the highest larval mortality with the lowest lethal concentration was reported when tested with leaf extract compared with other plant part. Phytochemicals can be extracted from specific parts of a plant such as leaf, stem, flowers, seeds, barks, and roots which is known to contain an accumulated concentration of bioactive chemicals which is responsible for varying toxicity towards test organisms (Shaalan et al. 2005).

In the present study, both of the tested mosquito species exhibited varying susceptibility levels which may be due to differences in physiological characteristic of the two species. *Ae. aegypti* appeared to be more susceptible with most fractions of the extract compared with *Ae. albopictus*. Similarly, Sulaiman et al. (2009) have reported that fourth-stage larvae of *Ae. aegypti* was more susceptible than *Ae. albopictus* when tested with *Acorus calamus* (L.) extract for larvicidal activity. Shaalan et al. (2005) reported that the difference in lethal concentration value has been observed when tested upon mosquito larvae of different species as they exhibit contrasting susceptibilities to the same phytochemicals due to variations that exist in responses between genera and species.

In conclusion, the study showed that *I. cairica* has produced good larvicidal activity against late third-stage larvae of *Ae. albopictus* and *Ae. aegypti*. However, the leaf produced the most effective larvicidal effect, followed by the flower and stem. Because the plant of the present study is widely distributed in Malaysia, mostly as invasive plant, the commercial exploitation could provide an important step in the development of new novel plant-based insecticide as one of the alternatives to expensive and environmentally harmful chemical insecticides. Finding an environmentally friendly insecticide for control of mosquito vectors is considered to be of paramount importance to reduce the negative impacts caused by chemical insecticides to the environment. Further studies are necessary to elucidate the active compounds that are responsible for the larvicidal property of the plant, determination sublethal effects and other possible biological activities of the plant for greener approach in *Aedes* mosquito control programmes.

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