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Article *in* Entomological Research · May 2024

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RESEARCH PAPER

Marching towards bioeconomy: Larvicidal effect of three indigenous plant extracts from Sri Lanka—*Garcinia quaesita*, *Garcinia zeylanica*, and *Coleus hadiensis* on dengue vector *Aedes aegypti*

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Received 15 June 2023;
accepted 29 March 2024.

doi: 10.1111/1748-5967.12732

Abstract

Bioeconomy would be a beneficial solution for funding through the rational use of finances and human resources in order to achieve better productivity and fruitfulness of mosquito control programs. Synthetic insecticides are widely used to repress mosquitoes. However, it instigates problems such as insecticide resistance among mosquitoes such as developing resistance, bio-accumulation, and ecosystem destabilization. Natural plant-based products are a healthy alternative to use as mosquito larvicides. The country has to spend a considerable cost for buying synthetic insecticides too. Therefore, the present study was undertaken to evaluate larvicidal efficacy of *Garcinia zeylanica*, *Garcinia quaesita*, and *Coleus hadiensis* against *Aedes aegypti* mosquito larvae. Fresh leaves of plants were collected, and the aqueous crude extract was prepared. Phytochemicals were extracted using refluxing technique. A concentration series of crude extract of leaves were prepared separately from 20 to 100 mg/L. Batches of each containing 100 third instar larvae of *Ae. aegypti* were used for larval bioassays. *Ae. aegypti* mosquito larvae were evaluated in accordance with the guidelines of World Health Organization. The experimental setup was repeated four times at each concentration. Probit analysis was used to evaluate the relationship of mortality with the concentration of aqueous crude extract. The *G. zeylanica* and *G. quaesita* leaf extracts showed a dose-dependent effect against *Ae. aegypti* larvae after the 24 and 48 h exposure period. Interestingly, *C. hadiensis* did not show any dose-dependent effect against *A. aegypti* mosquito larvae. The percentage mortality rates have shown a significant variance among different concentrations ($P = 0.000$). The recorded LC_{50} and LC_{90} for aqueous crude extract of *G. zeylanica* were 27.167 and 52.861 g/L, respectively, and LC_{50} and LC_{90} for aqueous crude extract of *G. quaesita* were 36.841 and 76.036 g/L, respectively, after 24 h of exposure period. The *G. zeylanica* and *G. quaesita* plant's high larvicidal activity is supported by the presence of phytochemicals such as saponins, steroids, flavonoids, and phenol, which showed combination effects in terms of larvicidal action to mosquito larvae. Hence, there is a potential of *G. zeylanica* and *G. quaesita* aqueous leaf extracts as a key source for the development of an environment-friendly plant-based larvicide against *Ae. aegypti*. Hence, this warrants vector control entities to re-think "biological wealth for economic prosperity" with environmentally friendly country-wide control approaches for medically important disease vectors.

Key words: bioassay, exposure, phytochemical, potential

Introduction

Mosquitoes are the most medically important arthropod vectors of diseases because they can transmit a higher numbers of diseases than any other groups of arthropods. They transmit many medically important pathogens and parasites such as viruses, bacteria, protozoans and nematodes (Kettle 1995; Govindarajulu *et al*. 2015; Şengül Demirak & Canpolat 2022).

In terms of public health importance, mosquitoes are the most significant category of insects because they spread numerous diseases. They transmit most of the life-threatening diseases such as malaria, filariasis, Japanese encephalitis, dengue fever, chikungunya fever, yellow fever, West Nile virus infection, ZIKA fever, and so forth. Thus, it can affect the socio economic status of many nations, causing destructive effects to humans. Also, mosquitoes can act as pests for humans because it can cause allergic responses including local skin reactions and systemic reactions such as angioedema and urticarial (Maia & Moore 2011).

Vector borne diseases have become a major problem in the world, particularly in both tropical and subtropical regions. But these vectors occur mainly in tropical countries with about one million deaths been claimed yearly from malaria and filariasis (Southgate 1984). These illnesses not only have high rates of morbidity and mortality but also severely disrupt society and entail significant economic losses in underdeveloped nations. Many of the predominant mosquito species that spread diseases like malaria and dengue are anthropophagous, meaning they have physiological traits that enhance their capacity to feed on human blood (Ramasamy & Surendran 2016).

Sri Lanka is a tropical country which is suffering from high abundance of dengue cases annually and the primary vector of the disease; *Aedes aegypti* (Hari & Mathew 2018). Dengue epidemics are emerging according to statistics. Around 99,120 cases of dengue fever have been reported by the Ministry of Health's epidemiology unit in 2019, which is nearly twice as many as the 51,659 cases documented in 2018. More than 21,000 instances were reported in the month of November, 2019. Additionally, in 2020 across the nation, there have been at least 90 clinically verified dengue deaths reported. Only 58 dengue deaths were documented in 2019, according to Epidemiology Unit sources. Nearly 20,000 cases were reported only in the Colombo area in 2019, which was followed by the Gampaha district and the Kandy district (Epidemiology Unit Ministry of Health).

The Epidemiology Unit has selected five high-risk districts of Dengue in Sri Lanka, due to the persistent rainy weather: Colombo, Gampaha, Kalutara, Ratnapura, and Galle (Epidemiology Unit Ministry of Health). Health experts have urged the public to be watchful for mosquito breeding grounds and regularly eliminate them. A total of 31,162 suspected dengue cases were documented for the year 2020, and for January 2023, 914 suspected dengue cases from the entire island were reported to the epidemiology unit (Epidemiology Unit Ministry of Health).

Mosquito control is essential to prevent proliferation of mosquito borne diseases and to improve the quality of environment and public health. In the absence of an effective preventive measures or vaccine, the best approach is either killing, preventing mosquitoes from biting people or by killing the larvae at breeding sites of vectors, in order to interrupt the dengue transmission (Joseph *et al*. 2009; Şengül Demirak & Canpolat 2022).

Mosquito control includes the spraying chemical insecticides to the adult mosquitoes or by killing the mosquito larvae before emerging into adults. Application of synthetic insecticides such as organochlorine and organophosphate compounds is the major control in mosquito control (Ghosh *et al*. 2012). Application of synthetic insecticides has not been very successful due to human, technical, operational, ecological and many economic factors apart from developing resistant strains. Among them the increasing cost of new insecticides and annual exportation expenditures, effect on non-target populations especially on mammals, high toxicity to mammals, entering to food chains, bioaccumulation in non-target organisms' bodies, ecological imbalance, non-biodegradable nature, environmental pollution, and the emergence of refractory vector behavior is occurred. Use of many former synthetic insecticides in mosquito control programs has limited in recent years (Ghosh *et al*. 2012).

One approach to these arising problems has been to search for new and effective compounds that are easily degradable, do not have any ill effect on non-target populations, environmentally friendly, safe and easily available products at low cost. Also, to face the increasing emergence of mosquito resistance to chemical insecticides, a sound option lays on the use on new natural products, as alternative technique for using synthetic insecticides. These has triggered and urged the development of alternative techniques using natural products, as a mosquito larvicides and adulticides for killing mosquito larvae and adults respectively (Joseph *et al*. 2009; Şengül Demirak & Canpolat 2022).

Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Botanicals are basically secondary metabolites that serve as a means of defense mechanisms of the plants. Applications of phytochemicals as mosquito controlling agents were used since 1920s until the discovery of synthetic insecticides such as DDT in 1939 which sidetracked the application of phytochemicals in mosquito control programs. However, refocus on phytochemicals was occurred after facing several problems due to injudicious and over-application of synthetic insecticides (Ghosh *et al*. 2012).

Botanicals have widespread insecticidal properties and can obviously work as a new weapon in the arsenal of synthetic insecticides. Plants can be used as an alternative source of mosquito control agents because they constitute a rich source of bioactive chemicals (Ruchi & Pankaj 2015). Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities (Ghosh *et al*. 2012). Development of plant-based insecticides and repellents has been focused by many recent studies (Maia & Moore 2011; Şengül Demirak & Canpolat 2022; Deng *et al*. 2023; Priya *et al*. 2023).

While being effective, the phytochemicals reduce the risk of potentially adverse ecological effects. Also, they may prevent the possibility of the resistance that synthetic chemical insecticides typically bring about after prolonged use (Monzon *et al*. 1994; Priya *et al*. 2023). Unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise botanical blends of chemical compounds which act concertedly on both behavioral and physiological processes. Thus, there is a very less chance of pests developing resistance to such substances (Ghosh *et al*. 2012). Phytochemicals are easily biodegradable and have no ill effects on non-target organisms. They are potentially economical safe while being practical for control measures "being environmentally friendly" it become more practical and safer for the environment to use botanical insecticides to control mosquitoes also (Nayak 2015).

Sri Lanka as a tropical country, suffering from high abundance of dengue cases annually, has to spend a considerable cost for buying synthetic insecticides. But, it has instigated problems such as insecticide resistance among mosquitoes, bio-accumulation and ecosystem destabilization. Since natural plant-based products are a healthy alternative to use as mosquito larvicides, the present study was conducted to evaluate the efficacy of leaf extracts of three medicinal and endemic plants in Sri Lanka; *Garcinia quaesita*, *Garcinia zeylanica*, and *Coleus hadiensis* as a natural insecticide to control *Ae. aegypti* mosquito larvae. Mosquito larvicidal activity of *G. quaesita*, *G. zeylanica*, and *C. hadiensis* are not available to date.

Methodology

Test plant material collection and preparation

Fresh and healthy leaves from the *G. quaesita* were collected from Wadduwa, Sri Lanka (6°40′32.7″N 79°55′59.6″E). *G. zeylanica* plants were collected from Godigamuwa, Sri Lanka (6°45′10.6″N 79°59′50.4″E). *C. hadiensis* were collected from Boralesgamuwa, Sri Lanka (6°50′31.9″N 79°54′31.4″E). Plants were confirmed to the species level from Fruit and Research Institute Sri Lanka and from Henarathgoda Botanical Garden, Gampaha, Sri Lanka.

G. quaesita which belongs to the family of Clusiaceae and endemic to Sri Lanka. *G. quaesita* is commonly called "Goraka" but locally referred as "Rath Goraka" and Red mango and Brindle berry in English. This plant is up to 20 m tall. It produces flowers and fruits. The color of the fruit is red to orange-red (Fig. 1). It is one of the important medicinal plants, and has been used in herbal medicine to treat various diseases like Fevers, Heart diseases, Wounds, Fractures, Hemorrhoids, Abdominal pains, Nausea, Asthma, Vomiting, Swellings, Nervous system diseases, Warm infestations, Hypertensions and Hyperlipidemia.

G. zeylanica same as *G. quaesita*, belongs to Clusiaceae family. This plant is locally referred as "Ela Goroka" and "Kaha Goroka" (Fig. 1). This plant is up to 20 m tall. It has Fruits with a diameter of up to 8 cm and a glossy pale-yellow color. *G. zeylanica* is used to treat Ulcers, Rheumatism, Bowel problems, Anorexia, Chronic dyspepsia, for fractures and it has medical properties like Antiseptic (Kokilananthan *et al*. 2022). *Garcinia* is grown specifically to harvest its fruits, which are then used in food as a spice or condiment and in various forms of ayurvedic or traditional medicine (Kokilananthan *et al*. 2022).

C. hadiensis, also endemic to Sri Lanka, belongs to Lamiaceae family (Fig. 1). It is commonly called "Iriveriya" in Sinhala and Tulsi in English. This plant's maximum height is 1.5 meters and width is 1 m. The flowers are either white or violet. *C. hadiensis* has a Least Concern conservation classification and typically grows in grassland and forests. It is indigenous to Asia (Sri Lanka, the Maldives, and Yemen) and Africa (Rajesh *et al*. 2021). The plant is active in the treatment of Fevers, Dysentery, Diarrhea, Vomiting, Excessive thirst, Congestion of the liver Tarantula bites, Abscesses, and heart diseases and it has diuretic, cholagogue, and diaphoretic activities or properties.

All the leaves sample from each plant were washed well with tap water and then rinsed with distilled water. All three samples were air dried for 1 week at room temperature (28°C ± 2°C). Then they were cut into pieces and pulverized using an electrical blender separately.

Field collection of *Ae. aegypti* mosquito larvae

Third instar larvae of *Ae. aegypti* were collected from Kotikawaththa, Sri Lanka (6°55′50.1″N 79°54′50.6″E) and Boralesgamuwa, Sri Lanka (6°50′31.9″N 79°54′31.4″E) from different container-type breeding habitats. Sampling was performed using dipping and pipetting techniques according to the nature of the breeding habitat. Larvae were identified to the species level using standard identification keys (Chelliah 1984; Amerasinghe 1995; Rueda 2004;

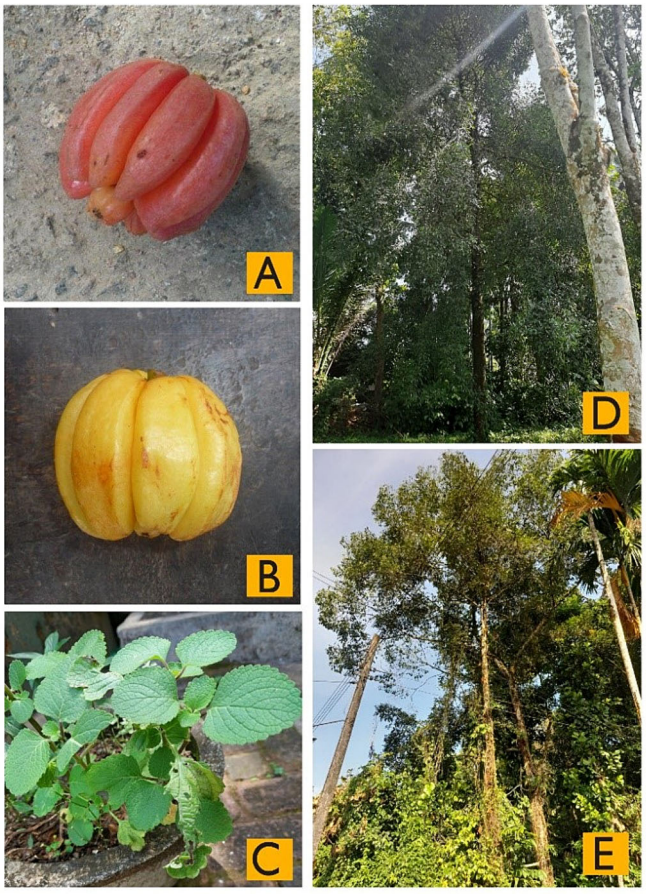

Figure 1 Medicinal end indigenous plants used for the study; (A) *Garcinia quaesita* fruit, (B) *Garcinia zeylanica* fruit, (C) *Coleus hadiensis* plant, (D) *Garcinia quaesita* tree, (E) *Garcinia zeylanica* tree.

Rattanarithikul *et al*. 2005). Identified third instar *Ae. aegypti* larvae were used for bioassays.

Aqueous crude extraction of plant materials

Two hundred grams each of pulverized leaves samples was placed in three bottom-rounded flasks (1000 mL). Distilled water was added to each bottom-rounded flask until pulverized leaves were covered. Then the soaked pulverized samples were refluxed separately in distilled water for 40 min at 40°C. Then the resulting solutions were filtered using a muslin cloth. Each refluxed solution was used for bioassay.

Larvicidal activity of aqueous crude extracts

Ae. aegypti mosquito larvae were evaluated with the accordance with guidelines of World Health Organization (WHO 2005).

From the stock solution (100 g/L); a concentration series of crude extract of leaves (40%, 60%, 80%, 100%) were prepared separately from refluxed solutions in separate glass beakers of 100 mL. This was done by initially adding the exact volume of the extract that is 40, 60, 80 and 100 mL (to each beaker having a concentration of 40%, 60%, 80%, 100% respectively) required to prepare the final concentration (based on $C_1V_1 = C_2V_2$ equation) by adding distilled water (except for the concentration of 100%).

Later, 20 mosquito larvae were added to each container. Five replicates were done for each of the test concentration with 20 mosquito larvae per each replicate. A control was run without any plant extract added. The number of dead mosquito larvae were counted after 24 and 48 h of exposure, and the percentage mortality was calculated.

Phytochemical screening

The phytochemical tests were performed for each plant extract for the identification of flavonoids, alkaloids, steroids, saponins, phenol, and tannins.

The 100% of the crude extract was put into six test tubes (six test tubes were taken for each crude extract of *G. quaesita*, *G. zeylanica*, and *C. hadiensis*). Then the chemical tests were carried out to identify each phytochemical as follows:

Test for flavonoids

Magnesium turnings (20 mg) were added to the crude extract. Then conc. Sulfuric acid was dropped through the side of the test tube. The formation of scarlet color indicates the presence of flavonoids.

Test for alkaloids

Mayer's test was carried out for this. Mayer's reagent (0.2 mL or 4 drops) were added to crude extract. A yellowish or white precipitate indicated the presence of alkaloids.

Test for steroids

Lieberman-Burchard's test was performed here. Few drops of acetic anhydride were added to the crude extract and mixed well. One milliliter of sulfuric acid was added from the side of the tube. The formation of red ring at the junction of the two layers indicated the presence of the steroids.

Test for saponin

A drop of Na_2CO_3 solution was added to 5 mL of plant extract in the test tube. After shaking vigorously, it was left to rest for 5 min. The foam formation indicated the presence of saponins.

Test for phenol

Phenol test was performed. To the extract, 0.5 mL of ferric chloride was added. Formation of intense blue green color indicated the presence of phenolics.

Test for tannins

Ferric chloride test was performed. One milliliter of 5% ferric chloride solution was added to the extract. The formation of greenish black revealed the presence of tannins.

The test for the phenol and tannins (performed for both phytochemicals at the same time)

Ferric chloride test was performed here. Two milliliters of 5% neutral ferric chloride solution was added to 1 mL of the crude extract. The dark blue color indicated the presence of phenol and tannins.

Data analysis

Data analysis was performed SPSS Software package. Linear regression analysis was carried out to test whether there is a significant difference between the mean percentage mortality of *Ae. aegypti* mosquito larvae, and *G. quaesita*, *G. zeylanica* and *C. hadiensis*, aqueous leaf extracts after 24 and 48 h of exposure. Probit analysis was carried out to find the LC_{50} of *G. quaesita*, *G. zeylanica* and *C. hadiensis*, aqueous leaf extracts on *Ae. aegypti* after 24 and 48 h of exposure. One-way ANOVA was carried out to test whether there is a significant difference between the mean percentage mortality percentage of *Ae. aegypti* in *G. quaesita*, *G. zeylanica* and *C. hadiensis*, aqueous leaf extracts.

Results

Larvicidal activity of *G. zeylanica, G. quaesita,* and *C. hadiensis* aqueous plant extract

The mean percentage mortality of *Ae. aegypti* mosquito larvae has increased with the increasing concentration of for *G. zeylanica* aqueous leaf extract (Fig. 2). Also mean percentage mortality of mosquito larvae has increased from 24 to 48 h of exposure (Figs. 2,3).

The mean percentage mortality of *Ae. aegypti* mosquito larvae has increased with the increasing concentration of *G. quaesita* aqueous leaf extract (Fig. 2). Also mean percentage mortality of mosquito larvae has increased from 24 to 48 h of exposure (Figs. 2,3).

Interestingly no mortality was observed for *C. hadiensis* aqueous leaf extract for any concentration of aqueous leaf extract used for the test (Figs. 2,3).

There is a significant positive relationship between the mean % mortality of mosquito larvae on increasing concentration of *G. zeylanica* aqueous leaf extract tested, for *Ae. aegypti* after 24 h exposure period ($P < 0.05$; $R^2 = 85\%$) and after 48 h exposure period ($P < 0.05$; $R^2 = 75\%$).

Moreover, there is a significant positive relationship between the mean % mortality of mosquito larvae on increasing concentration of *G. quaesita* aqueous leaf extract tested, for *Ae. aegypti* after 24 h exposure period ($P < 0.05$; $R^2 = 77\%$) and, anyhow after 48 h exposure period there was a comparatively weaker significant positive relation ($P < 0.05$; $R^2 = 56.8\%$).

Interestingly, there is no significant relationship between the mean % mortality of mosquito larvae on increasing concentration of *C. hadiensis* aqueous leaf extract tested, for *Ae. aegypti* after 24 and 48 h exposure periods ($P > 0.05$; $R^2 = 0\%$).

Therefore, the regression analysis results summarizes that only *G. quaesita* and *G. zeylanica* plant extracts has a significant relationship between the mean % mortality of mosquito larvae but not *C. hadiensis*.

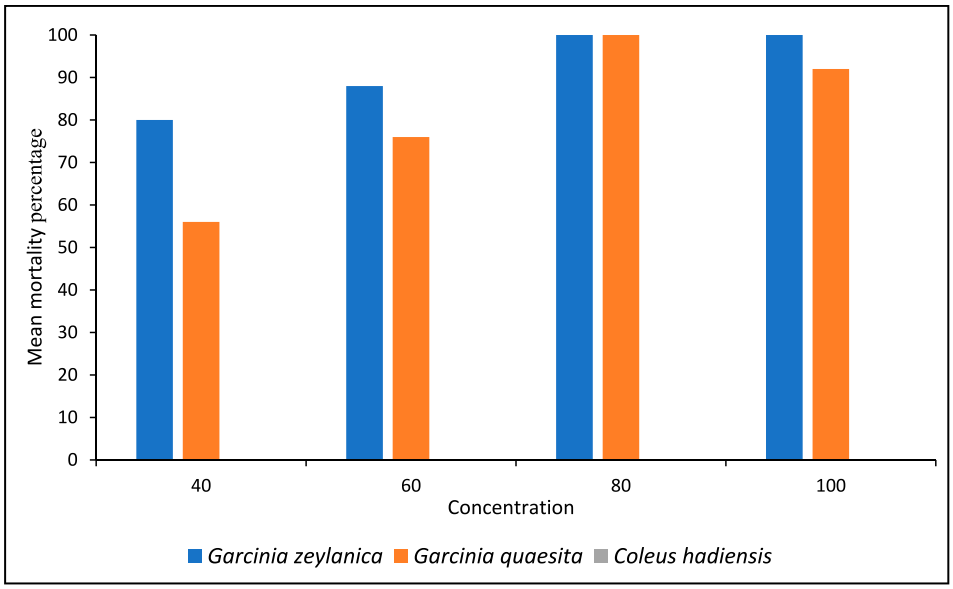

Figure 2 Relationship between the *Garcinia zeylanica*, *Garcinia quaesita*, and *Coleus hadiensis* aqueous leaf extracts' concentration and the % mortality of *Aedes aegypti* mosquito larvae (exposure period = 24 h).

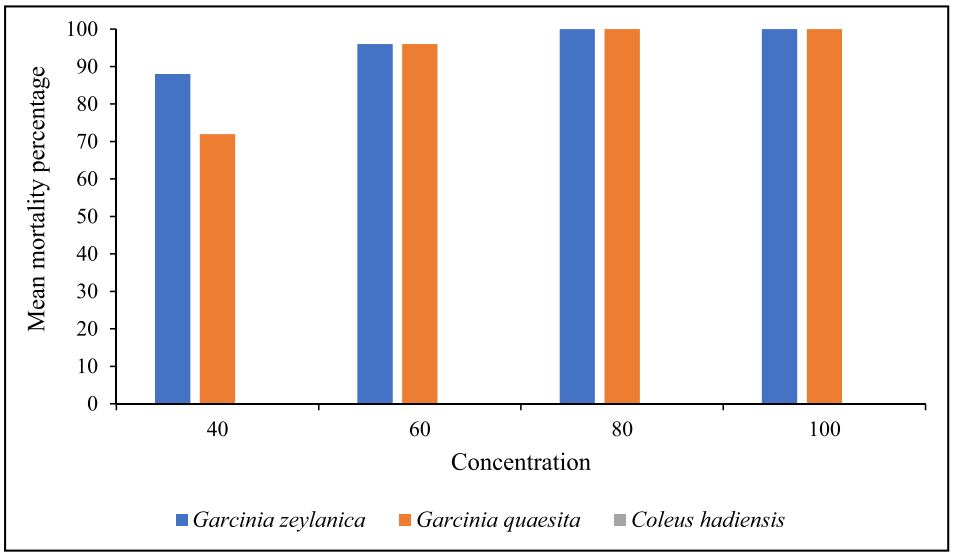

Figure 3 Relationship between the *Garcinia zeylanica*, *Garcinia quaesita*, and *Coleus hadiensis* aqueous leaf extracts' concentration and the % mortality of *Aedes aegypti* mosquito larvae (exposure period = 48 h).

Determination of the 24 and 48 h LC_{50} and LC_{90} for *G. zeylanica* and *G. quaesita* aqueous extracts

As regression analysis revealed that only *G. quaesita* and *G. zeylanica* plant extracts has a significant relationship between the mean % mortality of mosquito larvae but not *C. hadiensis*; LC_{50} and LC_{90} values were determined only for *G. quaesita* and *G. zeylanica*.

Kolmogorov–Smirnov test revealed that mean percentage mortality data for *G. zeylanica* and *G. quaesita* at 24 and 48 h are normally distributed. $P > 0.05$ was obtained for all tests of bioassay, revealed that data are normally distributed.

LC_{50} and LC_{90} for *G. zeylanica* aqueous extract

The LC_{50} for the 24 h exposure period was 27.167 g/L, for the *G. zeylanica* aqueous leaf extract on *Ae. aegypti* larvae, while its SE and the 95% confidence limits were 1.651 and 4.753–37.098 g/L of respectively. Meanwhile, the LC_{50} for the 48 h exposure period was 16.781 g/L, while its SE was 1.875 (Table 1).

The LC_{90} for the 24 h exposure period was 52.861 g/L, while its SE and the 95% confidence limits were 1.651 and 40.412–75.302 g/L of respectively. Further, the LC_{90} for the 48 h exposure period was 41.293 g/L, for the *G. zeylanica*, while its SE was 1.875 (Table 1).

Table 1 LC_{50} and LC_{90} statistical analysis of *Garcinia zeylanica* and *Garcinia quaesita* at 24 and 48 h exposure

Plant extract	Exposure period	LC_{50} (g/L)	LC_{90} (g/L)	Confidence interval for LC_{50} (g/L)	Confidence interval for LC_{90} (g/L)	Standard error (SE)
Garcinia zeylanica	24 h	27.167	52.861	17.753–37.098	40.412–75.302	1.651
Garcinia quaesita		36.841	76.036	27.304–45.570	63.392–112.575	1.087
Garcinia zeylanica	48 h	16.781	41.293	-	-	1.875
Garcinia quaesita		33.428	50.132	63.392–112.575	43.415–70.298	2.557

LC_{50} and LC_{90} for *G. quaesita* aqueous extracts

The LC_{50} for the 24 h exposure period was 36.841 g/L, for the *G. quaesita* aqueous leaf extract on *Ae. aegypti* larvae, while its SE and the 95% confidence limits were 1.087 and 21.304–45.570 g/L, respectively. Further, the LC_{50} for the 48 h exposure period was 33.428 g/L, while its SE and the 95% confidence limits were 2.557 and 16.55–39.525 g/L, respectively. And also, the LC_{90} for the 24 h exposure period was 76.036 g/L, while its SE and the 95% confidence limits were 1.087 and 63.392–112.575 g/L, respectively (Table 1).

The LC_{90} for the 48 h exposure period was 50.132 g/L, for the *G. quaesita* aqueous leaf extract on *Ae. aegypti* larvae, while its SE and the 95% confidence limits were 2.557 and 43.415–70.298 g/L, respectively (Table 1).

Comparison of the efficiencies of *G. zeylanica, G. quaesita,* and *C. hadiensis* plant extracts on *Ae. aegypti* third instar larvae

The percentage mortality rates have shown a significant variance among different concentrations of three plant varieties studied according to ONE-WAY ANOVA ($P = 0.000$).

Further, according to Post-Hoc Comparisons, at 95% confidence level there is a significant difference between *C. hadiensis* with *G. zeylanica* and *G. quaesita* ($P = 0.00$).

At 95% confidence level there is a significant difference between the 2 plant species; *G. zeylanica* and *C. hadiensis* ($P < 0.05$; $P = 0.00$); followed by *G. quaesita* and *C. hadiensis* ($P = 0.00$).

Interestingly, there is no significant difference between the larvicidal effect of 2 plant species; *G. zeylanica* and *G. quaesita* ($P = 0.462$).

Phytochemical screening of *G. zeylanica, G. quaesita,* and *C. hadiensis* plant extracts

Alkaloids, Saponins, Steroids, Flavonoids, Phenol were identified as phytochemicals present in both *G. zeylanica* and *G. quaesita* leaf extracts. But alkaloids were absent in *G. quaesita* which was found in *G. zeylanica* (Fig. 4). Only tannins were identified as a phytochemical present in *C. hadiensis* leaf extract (Table 2).

Discussion

The present study was conducted to find an environmentally safe and eco-friendly solution for the control of *Ae. aegypti* arbovirus vector. The controlling agent being a plant world comprises a rich untapped pool of phytochemicals that may be widely used in place of synthetic insecticides in mosquito control programs. Considering the phytochemical analysis done for alkaloids, saponins, steroids, flavonoids, tannins and phenols, *C. hadiensis* only had tannins, whereas *G. zeylanica* had all the aforementioned phytochemicals (alkaloids, saponins, steroids, flavonoids, phenol) except for tannins. Interestingly, *G. quaesita* had all the phytochemicals tested for, except alkaloids and tannins. Having only tannins may be the reason for not showing any larvicidal activity *C. hadiensis* on *Ae. aegypti* larvae. Plant based natural insecticides shown to have oviposition inhibiting, repellent or insect growth regulatory effects, and may help us to find chemicals that are safe, biodegradable, and target specific (Shaalan *et al*. 2005; Ghosh *et al*. 2012).

According to the analyzed data for 24 h exposure time of larvae, the lowest value of LC_{50} was recorded for *G. zeylanica* which was 27.167 g/L and the lowest value of LC_{50} at 48 h was recorded for *G. zeylanica*, which was 16.781 g/L. Similarly, the lowest LC_{90} values at 24 and 48 h were reported for *G. zeylanica* (Kaha Goraka) compared to *G. quaesita*. LC50 and LC90 represents the lethal concentration of the extract required to kill 50% and 90% of the larvae sample. Therefore, lower LC_{50} and LC_{90} values give better mortality than higher LC_{50} and LC_{90} values which is for *G. zeylanica*. Finally, considering the LC_{50} and LC_{90} values, the extract of the *G. zeylanica* is the most appropriate for potential controlling programs of mosquito larvae.

Kishore *et al*. (2011) reviewed the efficacy of phytochemicals against mosquito larvae according to their chemical nature and have described the mosquito larvicidal potentiality of several plant derived secondary materials such

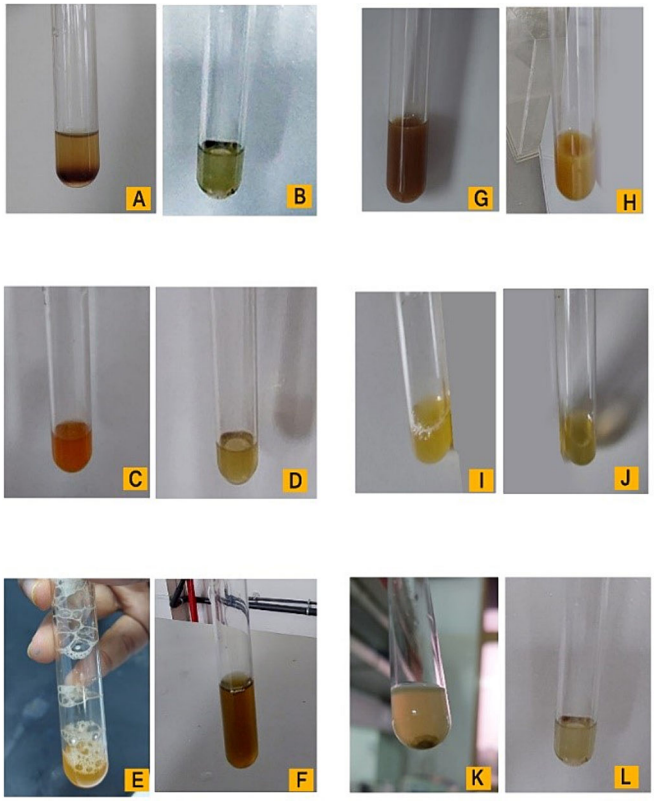

Figure 4 Steroid test [(A) positive, (B) negative], flavonoid test [(C) positive, (D) negative], saponin test [(E) positive, (F) negative], phenol test [(G) positive, (H) negative], tannin test [(I) negative, (J) positive], alkaloid test [(K) positive, (L) negative].

Table 2 Phytochemicals analyzed from leaf extracts of *Coleus hadiensis*, *Garcinia qauesita*, and *Garcinia zeylanica*

Plant species	Plant family	Plant part used	Target mosquito	Phytochemical tested	Result
Coleus hadiensis	Lamiaceae	Leaf	*Ae. aegypti*	Alkaloids	–
				Saponins	–
				Steroids	–
				Flavonoids	–
				Tannins	+
				Phenol	–
Garcinia zeylanica	Clusiaceae	Leaf	*Ae. aegypti*	Alkaloids	+
				Saponins	+
				Steroids	+
				Flavonoids	+
				Tannins	–
				Phenol	+
Garcinia quaesita	Clusiaceae	Leaf	*Ae. aegypti*	Alkaloids	–
				Saponins	+
				Steroids	+
				Flavonoids	+
				Tannins	–
				Phenol	+

as alkanes, alkenes, alkynes and simple aromatics, essential oils and fatty acids, lactones, terpenes alkaloids, steroids, isoflavonoids, pterocarpans and lignans (Son & Tram 2013).

Saponins are plant's bioactive compounds with significant insecticidal action and other biological properties. As well, saponins are responsible for amplifying pest insects' death

intensity, lowering food intake, retardation in development instability in development and declining reproduction (Gubitz *et al*. 1999). In addition, saponins are freely soluble and can be extracted in both aqueous and organic solvents and perform their action by attacking with cuticle membrane of the larvae, eventually disturbing the membrane, which is the main cause of larval death (Hostettmann & Marston 2002). On the other hand, flavonoids revealed an extensive scope of bio control potential of insecticidal activities. Tannins are also known to possess insecticidal properties (Azmathullah *et al*. 2011). Steroids derived from plants shows considerable larvicidal activity against mosquitoes (WHO 2005). Important phenolic in terms of insecticidal, repellent and feeding deterrent functions are flavonoids in plants. The *Annona glabra* plant's high larvicidal activity is supported by the presence of phytochemicals such as saponins, flavonoids, steroids and tannins which showed combination effects in terms of larvicidal action to mosquito larvae. The activity may not due to a single ingredient, but to a mixture of compounds, which may act synergistically.

Generally, the active toxic ingredients of plant extracts are secondary metabolites that are evolved to protect from herbivores. The mode of action of phytochemicals in target insect body could be several types. The insects that feed on these secondary metabolites potentially encountering toxic substances with relatively non-specific effects on a wide range of molecular targets. The targets are range from proteins (receptors, enzymes, signaling molecules, ion-channels and structural proteins), nucleic acids, bio membranes and other cellular components (Rattan 2010). This in turn, affects the insect physiology in many different ways and at various receptor sites, the principal of which is abnormality in the nervous system (such as, in neurotransmitter synthesis, storage release, binding and re uptake, receptor activation and function, enzymes involved in signal transduction pathway). However, Rattan (2010) reviewed the mechanism of action of plant secondary metabolites on insect body and documented several physiological disruptions, such as inhibition of acetylcholine-esterase (by essential oils), gamma aminobutyric acid-gated chlorine channel, sodium and potassium ion exchange disruption, and inhibition of cellular respiration. Such disruption also includes the calcium channel blockage of nerve cell membrane action, mitotic poisoning, disruption of molecular events of morphogenesis and alteration in the behavior and memory of cholinergic system (essential oils), hormonal balance disruption, and so forth. However, the most important activity is the inhibition of acetylcholine-esterase (AChE) activity is a key enzyme responsible for terminating the nerve impulse transmission through synaptic pathway. In addition, numerous toxicity to insect larvae result from physical properties of fatty acids; toxicity by inhalation due to aggregation and formation of thin film at the surface of water which does not allow respiration, and by penetration due to the amphibolic property of certain molecules, or these toxic compounds may enhance the toxicity of other toxic compounds (Rattan 2010).

These medicinally bioactive components exert antimicrobial action through different mechanism. Tannins cause inhibition in the cell wall synthesis by forming irreversible complexes with prolene rich protein (Mamtha *et al*. 2004). Saponins have the ability to cause leakage of proteins and certain enzymes from the cell (Zablotowicz *et al*. 1996). Terpenoids are responsible for dissolution of the cell wall of microorganism by weakening the membranous tissue (Hernández *et al*. 2000). Flavonoids which have been found to be effective antimicrobial substances against a wide array of microorganisms *in vitro* are known to be synthesized in response to microbial infection by plants. They have the ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Cowan 1999). Furthermore, steroids are known for their antibacterial activity specifically associated with membrane lipids and cause leakage from liposomes (Epand *et al*. 2007).

Although several studies have documented the efficacy of plant extracts as the reservoir pool of bioactive toxic agents against mosquito larvae, only a few have been commercially produced and extensively used in vector control programs. The main reason behind the failure in laboratory to land movements of bioactive toxic phytochemicals are poor characterization and inefficiency in determination of structure of exact active toxic ingredients responsible for larvicidal activity, which are essential for the development of specifications. In addition, the active ingredients can vary both in concentration and composition in the same plant species, in different clones, at different stages of plant growth and under different climatic and soil conditions. Adequate toxicological and eco-toxicological data are not available for many plant-based pesticides. So, scope for future research should me more focused on isolation of toxic larvicidal active ingredients. However, the plant extracts may be more effective than the individual active compounds due to a natural synergism that discourages the development resistance in vectors (Rattan 2010).

Being economically friendly" it becomes more practical and safer for the environment to use such botanical insecticides to control mosquitoes. On the other hand, this approach would be a beneficial solution for funding through the rational use of finances and human resources in order to achieve better productivity and fruitfulness of mosquito control programs. Hence, this warrants vector control entities to re-think "biological wealth for economic prosperity" with environmentally-friendly country-wide control approaches for medically important disease vectors.

Conclusions and recommendations

The *G. zeylanica* and *G. quaesita* leaf extracts showed a dose-dependent effect against *Ae. aegypti* larvae after the 24 and 48 h exposure period. LC_{50} values for *G. zeylanica* and *G. quaesita* leaf extracts on *Ae. aegypti* larvae was observed as 27.167 g/Land 36.841 g/L respectively. But, *C. hadiensis* did not show any dose-dependent effect against *A. aegypti* mosquito larvae. The percentage mortality rates have shown a significant variance among different concentrations and among of three plant varieties studied. There is a significant difference of larvicidal activity between *C. hadiensis* with *G. zeylanica* and *G. quaesita*. But, interestingly there is no significant difference between the larvicidal effect of 2 plant species; *G. zeylanica* and *G. quaesita*.

Saponins, steroids, flavonoids, and phenol were identified as phytochemicals present in both *G. zeylanica* and *G. quaesita* leaf extracts. But, alkaloids was absent in *G. quaesita* which was found in *G. zeylanica*. Only tannins were identified as the phytochemical present in *C. hadiensis* leaf extract. The efficacy of larvicidal activity may differ in *C. hadiensis*, *G. zeylanica* and *G. quaesita* due to the presence of varying phytochemicals in the extract.

In conclusion, aqueous extracts of *G. zeylanica* and *G. quaesita* may be bioactive against *A. aegypti* larvae. Thereby, aqueous leaf extracts of *G. zeylanica* and *G. quaesita* can serve as alternative form of mosquito control which is eco-friendly. Since these two plants used in the study are available in large quantities in terrestrial habitats of Sri Lanka, acquiring these plants for potential controlling measures is easy.

Field trials using aqueous leaf extracts of *G. zeylanica* and *G. quaesita* as a as potential natural larvicides for dengue controlling programs is recommended.

Author contributions

Research design, writing the manuscript, and data analysis: H.A.K. Ranasinghe and E.H. L. Perera. *Field collection of samples, bioassays and laboratory experimentation, writing the manuscript, and data analysis*: G.C.A. Perera, J.D.A.S. M. Jayakodi, J.A.H. Madhumika, and M.S. Ishara. *Overall supervision of the research work editing and reviewing the manuscript*: H.A.K. Ranasinghe, E.H. L. Perera, and M. Hettihewa. All authors read and approved the final manuscript.

Acknowledgments

This work was supported by the Faculty of Health Sciences, CINEC Campus, Malabe.

Conflicts of interest statement

There are no conflicts of interest.

Data availability statement

The datasets supporting the conclusions of this article are included in the article.

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