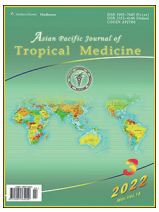

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Larvicidal activity of the pericarp extract of *Garcinia mangostana* against dengue vector *Aedes aegypti* in Sri Lanka

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ABSTRACT

Objective: To assess the larvicidal activity of mangosteen (*Garcinia mangostana*) against larval stages of *Aedes aegypti* mosquitoes.

Methods: A crude extract was prepared in ethanol from powdered mangosteen pericarps. A concentration gradient (0.01-4.92 g/L) was prepared from the stock solution. Seven batches of 25 third instar larvae of *Aedes aegypti* were used for larval bioassays. Larval mortality rates were observed after one and 24 hours. Cholesterol and total lipid contents in 20 randomly selected dead larvae at each trial were assessed by colorimetric method. The experimental setup was repeated five times. The General Linear Model and Probit analysis were used to evaluate the relationship of mortality with cholesterol level, total lipid level and cholesterol to total lipid ratio.

Results: The percentage mortalities significantly varied with different concentrations ($F_{7,32}$=385.737; P<0.001). The LC_{50} and LC_{99} values were (0.041 ± 0.006) g/L and (10.616 ± 1.758) g/L, respectively after 24 hours. There was no mortality recorded within the one-hour exposure time. Only the cholesterol content ($F_{5,24}$=173.245; P<0.001) in larvae exposed to different concentrations denoted a significantly decreasing trend within 24-hour exposure. Larvae that were exposed to the lowest concentration (0.55 g/L) showed a higher cholesterol level (22.67 ± 1.33) μg.

Conclusions: The *Garcinia mangostana* extract acts as an effective sterol carrier protein inhibitor that inhibits cholesterol uptake in *Aedes aegypti* mosquitoes. Hence, it could be explored for use as a key source for the development of an environment-friendly plant-based larvicide.

KEYWORDS: Mangosteen; Mosquito; Larvicide; *Garcinia mangostana*; *Aedes aegypti*

1. Introduction

The global incidence of dengue has dramatically increased over recent decades. It is estimated that approximately 390 million dengue cases are reported annually. Among them, 500 000 cases develop into severe hemorrhagic conditions and nearly 25 000 would end up in death[1]. At the global level, half of the global population is at risk of dengue infection, making a series of health concerns[2].

The history of dengue in Sri Lanka dates back to 1965, which recorded the first indigenous case[3]. After several decades, the first outbreak appeared in 1989[4]. Thereafter in 1996, dengue developed into an endemic status, especially in the Western Province of the country. At present, Sri Lanka is experiencing extreme outbreaks of dengue every year since 2002. The highest epidemic of dengue in Sri

Significance

Mangosteen (*Garcinia mangostana*) fruit has an xanthone rich pericarp. It has an inhibitory activity for sterol carrier protein-2 that regulates the cholesterol uptake in mosquitoes. However, in Sri Lanka, the larvicidal activity of this plant to control mosquito vectors has not been evaluated. Therefore, the present study evaluates the potential use of *Garcinia mangostana* pericarp extract as a larvicide to control dengue vector *Aedes aegypti*.

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Lanka was recorded in 2017, reporting a total of 186 101 cases with more than 390 deaths[5].

Since there is no specific medicine or effective vaccine available to treat dengue thus far, vector control is considered as the key approach in dengue control efforts[6]. Vector control strategies include environmental management, biological control, chemical control and use of personal protection methods to minimize human-mosquito contact[7]. Use of synthetic insecticides in controlling mosquitoes is widely used in vector control programmes. However, this has several limitations as mosquitoes tend to develop resistance to insecticides, high cost, harmful effects on human health or other non-target populations, non-biodegradable nature and a higher rate of biological magnification[8]. Therefore, scientists have diverted to explore chemical compounds in natural sources.

Many plants consist of insecticidal phytochemicals produced by plants to protect themselves against herbivorous insects as secondary compounds[9]. Application of botanical larvicides for the control of vector mosquitoes is considered as more protective, pro-environment, target-specific and safer[8]. *Garcinia (G.) mangostana* (mangosteen) is one of the plants that have a larvicidal activity on immature stages of insect vectors[10].

Mangosteen has received wider scientific attention due to its xanthone rich pericarp. Xanthones are heterocyclic compounds, which are biologically active in numerous pathways[10,11]. Xanthone derivatived from mangosteen pericarp consists of anti-plasmodial[12], anticancer[13], antimicrobial[12], and antioxidant[13] properties. Major xanthone taken from the pericarp of the mangosteen fruit is α-mangosteen[14]. It has been identified as a mosquito sterol carrier protein inhibitor that targets sterol carrier protein-2 (SCP-2), which is partially responsible for intracellular cholesterol transport in insects[10]. Other than transporting, SCP-2 involves cholesterol uptake and storage mechanisms, which is necessary for the life cycle of the insect[15].

Aedes (Ae.) aegypti has an independent gene that is similar to vertebrate SCP-2 known as AeSCP-2. This protein also has a high level of expression in the midgut of the mosquito larvae and shows a high binding affinity to cholesterol[16]. AeSCP-2 in mosquitoes has been identified as a vital protein for their larval development and fertility[15,16]. Cholesterol is necessary for growth, development and egg production in mosquitoes. It is important in the continuation of the mosquito life cycle as it is a precursor for molt-promoting hormones commonly known as ecdysteroids[15]. In the absence of essential cholesterol levels to maintain the required level of those hormones, the ecdysis may not happen and result in death during the larval stages of insects[17]. Therefore, disease-causing insect vector management can be done by targeting cholesterol metabolism, which is essential for the development of growth regulators insects.

Mangosteen is a well-growing plant in Sri Lanka. There can be changes in the chemical properties of the mangosteen grown in different regions of the world. Even though α-mangostin and its inhibitory activity of SCP-2 protein have been tested against mosquito larval populations in other countries, the larvicidal activity due to the inhibition of the SCP-2 protein-mediated cholesterol uptake in mosquitoes has not been evaluated in Sri Lanka. Therefore, the objective of the present study was to determine the mosquito larvicidal effect of the pericarp of mangosteen against the dengue vector *Ae. aegypti* in Sri Lanka.

2. Materials and methods

2.1. Rearing of Ae. aegypti larvae

Eggs of *Ae. aegypti* were obtained from the mosquito rearing facility at the Department of Parasitology, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka. Egg sheets were soaked in deoxygenated distilled water and kept for a few hours at room temperature (25-28 ℃) until the hatching of first instar (L1) larvae. The hatched larvae were transferred into larval rearing trays (25 cm × 25 cm × 7 cm) and reared with a daily dose of a larval diet comprising 12.5 g of tuna meal, 9.0 g of bovine liver powder, 3.5 g of brewer's yeast dissolved in 100 mL of distilled water[18]. The larvae were reared up to the third instar stage (L3) and used for the present experiment.

2.2. Preparation of mangosteen pericarp crude extraction

Kalutara is considered as the hometown of mangosteen in Sri Lanka and the fruit is abundantly available. The soil condition and weather in Kalutara is ideal during the months of the season for the fruit's growth[19]. Fresh fruits of mangosteen were obtained from naturally growing trees from Kaluthara, Sri Lanka (6°34'46.3" N; 79°58'08.8" E). The plant was authenticated from the Herbarium at the Deparment of Plant and Molecular Biology, Faculty of Science, University of Kelaniya, Sri Lanka. The fruits were cleaned with distilled water and wiped out with paper towels. The pericarp was removed and dried in an oven at 46 ℃ until the plant materials were completely dried.

Dried particles were ground at 48 700 × *g* using an electrical grinder (Singer, KA-ELITE, Sri Lanka) and 15.00 g of powdered product was added into four conical flasks (250 mL), separately. A volume of 125 mL of absolute ethanol was added into each conical flask and closed with a cotton plug. The flasks containing the pericarp powder of mangosteen with 100% ethanol were shaken for a 24-hour duration using an electric platform shaker (Innova 2000-200132350, Germany). The resultant suspensions were filtered by gravity

through a Whatman No. 1 filter paper. The four filtrates of ethanolic extracts were mixed and the solvent was kept for evaporation using a rotary evaporator (IKA, RV10, Germany). The concentrated sample (8.65 g) was re-dissolved in 25 mL of absolute ethanol to prepare the stock solution. Subsequent extracts were prepared similarly.

2.3. Larvicidal bioassay of mangosteen extract

A concentration series of mangosteen pericarp crude extract (0.003%, 0.006%, 0.2%, 0.5%, 1.2%, 1.5% and 1.8% w/v) which represent the concentrations of 0.01 g/L, 0.02 g/L, 0.55 g/L, 1.37 g/L, 3.28 g/L, 4.10 g/L and 4.92 g/L were prepared in plastic containers (250 mL) using the stock solution prepared. For this, volumes of 7 μL, 14.25 μL, 500 μL, 1 250 μL, 3 000 μL, 3 750 μL and 4 500 μL from the stock solution were added into each tray and top up to 250 mL, respectively. The larvicidal effect of the crude extract was assessed according to insecticide susceptibility guidelines described by the World Health Organization under laboratory conditions[20]. A blank control was prepared without adding the crude extract and positive controls were set up with 4 500 μL (2% v/v) of ethanol.

Batches of 25 third instar larvae of *Ae. aegypti* were transferred into each plastic container (25 cm × 25 cm × 7 cm). The number of dead mosquito larvae was counted at one hour and 24-hour intervals since the introduction of larvae. The percentage mortality of *Ae. aegypti* larvae at each concentration of the crude extract were calculated. Collected dead larvae from test trials at each concentration and live larvae from controls were preserved in microcentrifuge tubes containing distilled water and stored under -20 ℃. The entire experimental setup was repeated five times.

2.3. Extraction of lipids from preserved mosquito larvae

The extraction of lipids from preserved mosquito larvae was performed as described by Van Handel[21]. A total of 20 preserved larvae were randomly selected from each treatment group (concentration) for the extraction. Larvae were placed in a glass tube (7.2 cm height; 1.2 cm diameter). They were crushed within 1 mL of chloroform: methanol (1:1) mixture using a glass rod. Tubes were centrifuged using a micro-centrifuge (CliftonNE000GT/1, Korea) at 125 216 × *g* for 2 min and the supernatant was divided into two equal portions. Each portion (0.4 mL) was transferred into a separate clean glass tube. The solution of each tube was evaporated by nitrogen fluxing and each portion was used for total lipid and cholesterol level analysis, separately.

2.4. Determination of the total lipid level in Ae. aegypti larvae

Sulfuric acid (2 mL) was added to each glass tube with preserved mosquito larvae and tubes were heated for 10 min in a boiling water bath and kept in an ice bath for 10 min to cool down. Samples were transferred into the cuvettes. The absorbance of the solution was measured at 520 nm and 0.5 mL of the sample was transferred into a separate glass tube, as recommended by Van Handel[21].

A volume of 1 mL of Vanillin-phosphoric acid reagent (0.24 mg/mL) was added into the glass tube with the sample. This mixture was kept for 45 min for color development. The absorbance measurements were carried out using a colorimeter (Aimil, 018000, India) at 520 nm against a reagent blank. The total lipid mass was determined according to the standard curve prepared using linoleic acid standard (SRLchem, India). Five replicates were performed using treated groups from each concentration.

2.5. Detection of total cholesterol level in Ae. aegypti larvae

The method described by Burke *et al*. was used for the determination of total cholesterol levels[22]. Sulfuric acid (0.5 mL) was added to each tube containing processed mosquito samples. Ferric chloride ($FeCl_3$) (0.75 mL) was added and kept for 5 min for color development. Samples were transferred into the cuvettes and the absorbance was measured by a colorimetric method (Aimil, 018000, India) at 520 nm. The blank was prepared using 0.75 mL of $FeCl_3$ with 0.5 mL of sulfuric acid. Five replicates were performed for larvae treated with different concentrations. The total cholesterol mass was determined using the correlations of the standard curve prepared with known cholesterol masses.

2.6. Data analysis

All data were entered into Microsoft Excel worksheets. The effect of mangosteen crude extract concentration on the percentage mortality of third instar *Ae. aegypti* larvae was evaluated by the General Linear Model (GLM), followed by Turkey's mean separation test as *post–hoc* analysis. Subsequently, a Probit analysis was performed to determine the toxic effect of *G. mangostana* pericarp crude extract on the third instar *Ae. aegypti* larvae within 24 hours. Based on the Probit analysis, the lethal concentration 50 (LC_{50}) and lethal concentration 99 (LC_{99}) values were calculated along with 95% confidence intervals.

The effect of crude extract concentration on the total lipid level, cholesterol mass, and cholesterol to total lipid ratio of treated larvae was also analyzed using the GLM followed by Turkey's mean separation test at a 95% level of confidence. The normality of the percentage mortality of third instar *Ae. aegypti* larvae, total lipid levels at each treatment, cholesterol mass and cholesterol to total lipid ratio of treated larvae were evaluated based on the Anderson-Darling test. The variables were continuous and normally distributed without any significant outliers. A linear relationship was observed between mortality rate and other parameters. Therefore, Pearson correlation analysis was used to determine the degree of correlation among the percentage mortality of third instar *Ae. aegypti* larvae with the potential risk factors, namely total lipid level, cholesterol

mass and cholesterol to total lipid ratio of treated larvae. Finally, a linear step-wise regression model (with P_{α}=0.5 for entry and P_{α}=0.1 for removal of predictors) was used to identify the actual risk factor behind the mortality of third instar *Ae. aegypti* larvae after being exposed to *G. mangostana* pericarp crude extract. The SPSS (version 23; IBM Corporation) was used for statistical analysis.

3. Results

3.1. Larvicidal activity of mangosteen pericarp crude extract on Aedes larvae

No mortality of *Ae. aegypti* third instar larvae were observed within one hour of exposure time. The mean percentage mortality of *Ae. aegypti* exposed at different concentrations of mangosteen pericarp crude extract after a 24-hour exposure period is given in Table 1. The mean percentage mortality increased with higher concentrations of the crude extract. The percentage mortality rates of *Ae. aegypti* larvae denoted a significant variation among different concentrations of the crude extract at 95% level of confidence ($F_{7,32}$=385.737; P<0.001). The highest mortality (100%) was observed at the highest extract concentration of 4.92 g/L (Table 1).

Table 1. Mean mortality percentages of *Aedes aegypti* third instar larvae treated with different concentrations of mangosteen pericarp crude extract after 24-hour exposure.

The concentration of the crude extract (g/L)	Mean percentage mortality of *Aedes aegypti* larvae (%)	95% *CI*
4.92	100.0 ± 5.00[a]	95.00-105.00
4.10	97.3 ± 4.87[b]	92.43-102.17
3.28	95.1 ± 4.76[c]	90.34-99.86
1.37	92.9 ± 4.65[d]	88.25-97.55
0.55	86.3 ± 4.32[d]	81.99-90.62
0.02	33.3 ± 1.67[e]	31.63-34.97
0.01	24.0 ± 1.20[e]	22.80-25.20
Control	1.3 ± 0.07[f]	1.26-1.40

Note: Values are mean ± standard error (SE). Different superscript letters (a, b, c, d, e, and f) show significant difference among the groups based on the General Linear Model followed by the Tukey's pairwise comparison at 95% level of confidence.

3.2. Determination of 24-hour LC_{50} and LC_{99} for exposed Ae. aegypti larvae

The 24-hour percentage mortality curve of *Ae. aegypti* third instar larvae, after being exposed to different concentrations of mangosteen pericarp crude extract, retrieved from Probit analysis is illustrated in Figure 1. The LC_{50} of the *Ae. aegypti* larvae was (0.041 ± 0.006) g/L (0.031-0.053 g/L), while the LC_{99} remained as (10.616 ± 1.758) g/L (7.829-15.035 g/L).

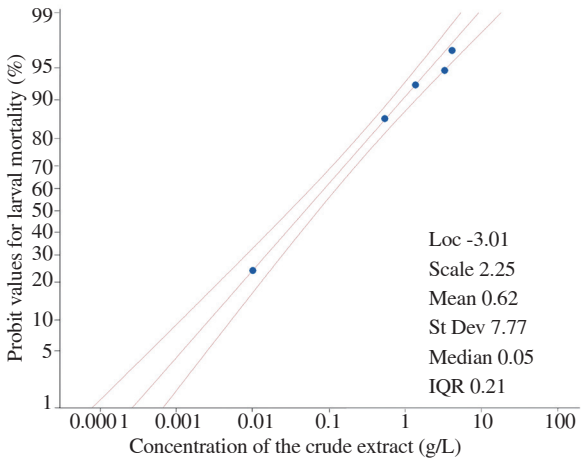

Figure 1. Probit analysis for the mortality of *Aedes aegypti* larvae treated with different concentrations of the mangosteen pericarp crude extract after 24-hour exposure. The middle line indicates the probit curve, while other two lines show the lower and and upper confidence levels.

3.3. Cholesterol level in the Ae. aegypti larvae treated at different concentrations

The highest mean cholesterol mass of (58.67 ± 3.77) μg was observed from the larvae exposed to the control treatment. The lowest cholesterol content [(6.00 ± 0.67) μg] was denoted by the larvae treated with the highest mangosteen pericarp extract concentration (Table 2). The statistics of GLM indicated that the crude extract concentration of mangosteen resulted in a significant decrement of the cholesterol content in the *Ae. aegypti* larvae ($F_{5,24}$ =173.245; P<0.001) at 95% level of confidence.

Table 2. Mean absorbance of cholesterol in *Aedes aegypti* larvae treated at each concentration (n=100) of mangosteen pericarp crude extract along with the cholesterol masses calculated using the cholesterol standard curve.

Concentration (g/L)	Mean cholesterol absorbance at 520 nm	Calculated mass of cholesterol (μg)
4.92	0.045 ± 0.005	6.00 ± 0.67[a]
4.10	0.063 ± 0.011	8.33 ± 1.45[a,b]
3.28	0.115 ± 0.005	15.33 ± 0.67[b,c]
1.37	0.123 ± 0.019	16.33 ± 2.56[c,d]
0.55	0.170 ± 0.010	22.67 ± 1.33[d]
Control	0.440 ± 0.028	58.67 ± 3.77[e]

Note: Values are mean ± standard error (SE). Different superscript letters (a, b, c, d, and e) show significant difference among the groups based on the General Linear Model followed by the Tukey's pairwise comparison at 95% level of confidence.

3.4. Total lipid level in the Ae. aegypti larvae treated at different concentrations

The highest mean lipid mass [(290.0 ± 70.00) μg] was observed from the larvae exposed to the control treatment, while the

lowest was observed at the highest mangosteen pericarp extract concentration (4.92 g/L) as (145.0 ± 5.00) µg (Table 3). The total lipid levels in the *Ae. aegypti* larvae indicated a declining trend with the increasing concentration of the crude extract (Table 3). However, the General Linear Model indicated that there was no significant effect on the total lipid content in *Ae. aegypti* larvae caused by the exposure to mangosteen pericarp extract with different concentrations ($F_{5,24}$=2.580; P=0.078) at 95% level of confidence.

Table 3. Mean absorbance of lipid in *Aedes aegypti* larvae treated at each concentration (n=100) of mangosteen pericarp crude extract along with the lipid masses calculated using the standard curve.

Concentration (g/L)	Mean absorbance of lipids at 520 nm	Calculated mass of lipid (µg)
4.92	0.145 ± 0.005	145.0 ± 5.00[a]
4.10	0.188 ± 0.008	187.5 ± 8.29[a]
3.28	0.210 ± 0.057	210.0 ± 56.57[a]
1.37	0.255 ± 0.036	255.0 ± 35.70[a]
0.55	0.273 ± 0.062	272.5 ± 61.79[a]
Control	0.290 ± 0.070	290.0 ± 70.00[a]

Note: Values are mean ± standard error (SE). The same superscript letter (a) shows no significant differences among the groups based on the General Linear Model followed by Tukey's pairwise comparison at 95% level of confidence.

3.5. Total cholesterol to total lipid ratio in treated Ae. aegypti third instar larvae

Variation of the mean cholesterol to total lipid ratio in *Ae. aegypti* after a 24-hour exposure period at the different concentrations of mangosteen pericarp crude extract is depicted in Figure 2. The lowest cholesterol to lipid ratio (0.04 ± 0.01) was observed from the larvae exposed to the highest concentration of the crude extract (4.92 g/L). The cholesterol mass to lipid mass ratio in the larvae also reported a declining trend in the increasing of the crude extract concentration (Figure 2). However, this trend remained non-significant based on the statistics of GLM ($F_{5,24}$=2.42; P=0.111) at a 95% level of confidence.

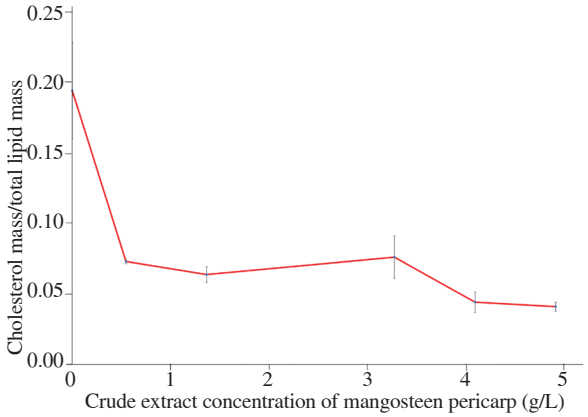

Figure 2. Variation of the mean cholesterol/total lipid ratio in *Aedes aegypti* larvae after 24-hour exposure at different concentrations of mangosteen pericarp crude extract.

3.6. Denominators of the larvicidal activity of mangosteen pericarp crude extract on Aedes larvae

It was noted that even though cholesterol level (Pearson's correlation coefficient r=-0.935), total lipid level (r=-0.887) and cholesterol/total lipid ratio (r=-0.865) in the exposed *Ae. aegypti* larvae denoted strong negative correlation coefficients with the percentage mortality at different concentrations, only the cholesterol mass and percentage mortality remained significant (P<0.05) at a 95% level of confidence (Table 4).

The best-fitting model converged by step-wise linear regression (Equation 1), included only the cholesterol mass as the predictor for the percentage mortality of *Ae. aegypti* larvae exposed to different crude extract concentrations as the significant predictor (P<0.05), while the other two predictors were excluded. The cholesterol mass was characterized by a variance inflation factor value of 1 suggesting a lower level of correlation among the predictors. With an R^2 value of 87.45% (Adjusted R^2=83.20%), the current model explained an adequate level of variance in the larvicidal activity of mangosteen pericarp crude extract against *Ae. aegypti* larvae.

Percentage mortality of *Ae. aegypti* larvae=101.375-11.249 cholestrol mass (Equation 1)

Therefore, based on the findings of GLM and step-wise linear regression, it is ostensible that the negative effect of mangosteen pericarp crude extract on the cholesterol uptake is a significant factor for the mortality of *Ae. aegypti* larvae.

4. Discussion

Despite vigorous surveillance activities and disease management practices, dengue continues a devastating impact on public health and livelihoods in Sri Lanka[23,24]. Various limitations in the existing methods of vector control including the adverse effects caused by the use of synthetic chemicals, signify the importance of assessing effective, environmentally friendly methods for vector control[25]. There are extracts in the plant material that inhibit SCP-2, which ultimately limits cholesterol transport in insects[10]. Therefore, the absence of essential cholesterol levels affects their molting process and causes death during the larval stages of insects[17]. Mangosteen is a plant that is enriched majorly with -mangosteen in the pericarp in its fruits[14] which is usually not edible and discard as waste material after consumption. This chemical constituent in the mangosteen plant has indicated the larvicidal effect against insects by inhibiting the SCP-2[10].

The larvicidal property in *G. mangostana* plant has been evaluated for six mosquito species namely; *Ae. aegypti*, *Anopheles (An.) stephensi, An. gambiae, Culex pipiens, An. quadrimaculatus*, and *Culex*

Table 4. Correlation matrix retrieved from the correlation analysis.

Parameter	Concentration of pericarp crude extract	Mortality percentage	Cholesterol mass	Total lipid mass	Cholesterol mass/total lipid mass
Concentration of pericarp crude extract		0.981*	-0.926*	-0.856	-0.832
Mortality percentage	0.981*		-0.935*	-0.887	-0.865
Cholesterol mass	-0.926*	-0.935*		0.981**	0.976**
Total lipid mass	-0.856	-0.887	0.981**		0.942*
Cholesterol mass/total lipid mass	-0.832	-0.865	0.976**	0.942*	

Note: *Correlation is significant at 0.05 level (2-tailed); **correlation is significant at 0.01 level (2-tailed).

quinquefasciatus[10] in other countries. However, the effective use of this plant species, as a larvicide for mosquito vector control has not been attempted in Sri Lanka. The current study indicates that the larvicidal activity of the mangosteen pericarp crude extract increased in a dose-dependent manner with an LC_{50} of 41 μg/mL. However, this value is higher compared to the values reported previously (LC_{50}=5.52 μg/mL)[26]. This may be due to the difference in the chemical properties of the variety used in the two studies, since the chemical properties of plants may vary with geographical location, environmental and climatic factors[27]. Previous studies carried out using mangosteen stem bark crude extracts prepared with different solvents such as hexane, ethyl acetate and ethanol have indicated an LC_{50} of 180.7 μg/mL, 30.1 μg/mL, and 188.1 μg/mL, respectively for *Ae. aegypti*[28]. In the same study, purified α-mangostin from the stem bark crude extract contributed to the lowest LC_{50} (19.4 μg/mL), which had given the highest bioactivity. The present study has reported a lower LC_{50} value for the ethanolic crude extract compared to previous studies indicating a high larvicidal effect. This may be mainly due to the higher α-mangostin concentration in the mangosteen pericarp used in the current study compared to the mangosteen stem bark used in previous investigations[29].

The assessment of cholesterol levels shows a dramatic reduction in the extract concentration of mosquito larvae[30]. From the larvae that were exposed to the concentration series of the crude extract, the lowest cholesterol mass [(6.00 ± 0.67) μg] was reported at the highest treatment dose. At the genetic level, overexpression or knockdown of *AeSCP-2* gene expression is shown to affect the cholesterol level in mosquitoes[30]. Therefore, the observed mortality in the present study may be due to the unavailability of free AeSCP-2 that leads to the inhibition of cholesterol uptake in the *Ae. aegypti* mosquitoes. This indicates that there is a potential to use mangosteen pericarp extraction to inhibit the uptake of cholesterol in the *Ae. aegypti* larvae. However, the particular mode of action of these compounds is yet to be implicit. Therefore, some investigations are needed to confirm the mechanism of these compounds to inhibit the action of AeSCP-2 more specifically on targeted insects[17].

The mortality of mosquito larvae occurs only after a considerable time from the moment of initial exposure since the toxicity mediated by SCP inhibitor is a slow-acting process[30]. In this study also, no larval mortality was recorded at a one-hour exposure period of the extract. Therefore, it is likely that the mortality of the larvae may not occur due to acute toxicity and it may be due to a change in the physiological activity within the cells subjected to the exposure time. The observation of long-term toxicity at the 24-hour exposure period confirmed its long-term physiological effect in terms of the toxicity mediated by the SCP inhibitors.

There are several ways to evaluate the effect of the extract on larval cholesterol uptake. Radiolabelled cholesterol analogs can be used to monitor the kinetics of cholesterol uptake or larval extracts can be analyzed by chromatographic methods to identify the lipid distribution[31]. Due to limitations in resources, cholesterol uptake was indirectly determined by quantifying the total cholesterol levels in the larvae using Lieberman-Burchard-Zack method. This method allows for convenient direct measurement of cholesterol with common laboratory chemicals while being sensitive enough to monitor small quantities of both esterified and unesterified cholesterol[32]. Therefore, it was used as the method of choice in the present evaluation.

In this study, the cholesterol measurement may depend on the number of mosquito larvae used for the assay and the body size of the larvae. Therefore, to compensate for the above limitation, a constant number of larvae with similar body sizes were used for comparative assays. Total cholesterol levels of the larvae treated with different concentrations of the extract were monitored after a 24-hour exposure period at different treatment doses. Since cholesterol depletion was observed significantly with the increase of extract concentration, total lipid assay was also performed in the same sample. The total lipid assay was used primarily as a method to compensate for variation between larval groups used for analysis and also to obtain secondary evidence for lipid depletion due to the uptake inhibition of cholesterol. Normalization with total body protein content was not included since the extraction of lipids with organic solvents may lead to precipitation of protein, which makes the colorimetry protein assays difficult to conduct.

The ratio of total lipid to cholesterol level was expected to decrease with the increasing levels of the extract concentration, based on the assumption that only cholesterol levels will change with the extract incubation. In the present study, a weak correlation was observed with the changes in the total lipid to cholesterol ratio according to the GLM. Besides, the total lipid assay includes cholesterol which

significantly decreased with the extract concentration. However, since an equal number of larvae with similar sizes were used for comparative essays, it can be assumed that the decrease in the cholesterol level in response to the extract concentration is due to the inhibition in the cholesterol uptake.

Similar to cholesterol, a dose-dependent declining trend in total lipid was also observed after a 24-hour exposure period. This may be due to depletion of both cholesterol and any other lipids, caused by either reduction of uptake or *de novo* biosynthesis. In this study, a detailed analysis of other lipids was not performed as the total lipid content does not directly reflect the cholesterol level. However, since the reduction of absolute total lipid mass was in response to the larvae treated at different concentrations of the extract, the reduction of the absolute total cholesterol mass indicates that the uptake or synthesis of other lipids could also be affected by the extract.

The relationship between mortality and lipid ratios was not statistically significant indicating that the normalization of cholesterol uptake with total lipids may create limitations. Normalization of data with total protein or DNA content could be a better indicator, but the extraction method did not facilitate due to precipitation. Despite the limitations in normalization, it can be concluded that mortality is primarily resulted due to the deprivation of cholesterol in treated samples. The statistical analysis of the factors that could affect the mortality rate (total cholesterol mass, total lipids, and cholesterol to lipid ratio) also confirmed that the mortality was primarily mediated by cholesterol deprivation.

The biggest drawback of the study is that all assumptions and interpretations were made assuming α-mangostin as the effective agent responsible for the larvicidal effect. Even though it has been proven from available literature, the present study was not able to quantify the chemical composition in the crude extracts due to limitations in funds. Therefore, since there are other bioactive molecules in mangosteen extracts, it is possible that some or all of those active substances, as well as differences in the solvent, have contributed to the overall larvicidal effect. Further, plant material was collected from Kaluthara District, Sri Lanka which is considered as the prominent city for mangosteen in the country. However, dose levels of chemical compositions in the plant material in some other part of Sri Lanka, or anywhere could be different. Therefore, the outcomes could be different because crude extracts will vary with locality, plant strain, and season of the year.

In conclusion, to the best of our knowledge, the present study documents the first study in Sri Lanka to assess the larvicidal effect of mangosteen crude extract agisnt *Ae. aegypti* mosquitoes. The percentage mortality of the *Ae. aegypti* larvae differed significantly with the concentration of the crude extract of *G. mangostana* pericarp. Cholesterol assay indicates that there is a dose-dependent depletion of cholesterol levels in the larvae with the increasing crude extract concentrations. The total lipid content of the treated larvae declined with the increase of the crude extract. The toxicity of larvae was primarily mediated by inhibition of the cholesterol uptake of *Ae. aegypti*. Therefore, *G. mangostana* crude extract appears to be with the potential as a mosquito larvicide. Further, it is recommended to conduct a detailed characterization of the chemical substances in the plant material to identify the active ingredient/s responsible for the larvicidal activity. It will be worthwhile to study and develop a slow-release formulation combined with UV-blocking additives, which will allow them to remain biologically active for a considerable time in the outdoor environment. Further, detailed investigations confirm the exact mode of actions at the cellular level and potential impact on non-target organisms needs to be performed.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Authors' contributions

NG Designed the research, overall supervised the research work, conducted larval bioassays and wrote the manuscript; YW conducted bioassays, biochemical analysis and writing of the manuscript; DA supervised the research work and reviewed the manuscript; LU did the experimental design, statistical analysis and writing of the manuscript; TW designed the biochemical assays, supervised and reviewed the manuscript. All authors read and approved the final manuscript.

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