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Biological Activity of Root Extract *Decalepis hamiltonii* (Wight & Arn) against Three Mosquito Vectors and Their Non-Toxicity against the Mosquito Predators

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Abstract: Bioactive molecules of plant origin play a significant role as defensive agents in different insect species. Chemical compounds in medicinal plants have been an exciting alternative to standard methods of controlling mosquito larvae. The present study evaluates the different solvent extracts of *D. hamiltonii* for toxicity against three different mosquito larvae. Bioassay revealed that the effect of the methanol extracts increased the larval mortality with increasing concentration. The highest larval mortality was observed in Culex quinquefasciatus with 98.33%, followed by 95 and 90% mortality in Aedes aegypti and Anopheles stephensi, at 24 h exposure. GC-MS analysis of methanol extract of *D. hamiltonii* showed six major peak compounds. They are benzaldehyde, 2-hydroxy-4-methoxy-(10.35%), dodecanoic acid (11.02%), n-hexadecanoic acid (21.05%), linoleic acid methyl ester (14.20%), oleic acid (21.04%), octadecanoic acid (22.21%). The level of α and β Carboxylesterases gets significantly decreased post-treatment with the methanol extract of *D. hamiltonii* in a dose-dependent manner.In contrast, glutathione S-transferase (GST) and cytochrome-P450 (CYP450) levels get uplifted steadily when the dosage gets increased. The ratio of GST level has drastically proclaimed to in *Ae. aegypti* 0.702 mg/m Lin parallel to *Cx. quiquefasciatus* (0.656 mg/mL) and *An. stephensi* (0.812 mg/mL). Cytochrome P450 (CYP450) activity was observed to increase significantly post-treatment with the sub-lethal dosage of methanol extract of *D. hamiltonii*. Correspondingly, the non-target screening against the aquatic predators reveals that the crude root extracts and their derivatives are ecologically safe and less toxic. Overall, the present research highlights the chemical characterization of crude methanol extracts of *D. hamiltonii*, their insecticidal activity against the medically challenging pests, and their non-target activity delivers an ecologically safe, and target specific bio-active agents and suitable substitute for chemical pesticides.

Keywords: *D. hamiltonii*; methanol; Carboxylesterases; CYP450; mosquito predators

1. Introduction

Mosquitoes act as a principal vector for many diseases that affect humans and other beneficial animals. It is estimated that the >10,000 death rate was recorded due to arthropods, especially medically challenging mosquito vectors [1]. The key diseases spread by mosquito vectors include Malaria, Dengue, Yellow Fever, Filariasis, Chikungunya, Schistosomiasis, and Japanese encephalitis [2]. In addition, mosquitoes are responsible for causing

allergic reactions in humans, including local skin allergy and systematic reactions like angioedema [3].

The dengue vector *Aedes aegypti* (L.) (Diptera: Culicidae) is widespread in the areas of tropical and subtropical nations. The incidence of Dengue fever has risen quadruple in 1970, with an estimate of over 50 percent of 1.5 billion people living in regions with a risk of dengue transmission, which is almost half of the world's population [4]. Dengue Fever is endemic in Asian countries, chiefly spread by *Ae. aegypti* [5]. It is estimated that dengue transmission is exposed to over 50 million people per year across the nations [6]. Generally, Societies with dengue are identified with hemorrhagic diseases. Similarly, *Culex quinquefasciatus* Say (Diptera: Culicidae) mosquitoes are considered painful and persistent biters responsible for spreading a neglected tropical disease named filariasis. Lymphatic filariasis is commonly known as elephantiasis affecting more than 1.3 billion people across 72 countries [7].

In general, managing mosquito vectors highly relies on spraying commercial insecticides, and due to higher insecticide resistance controlling arthropods faces more significant challenges [8,9]. Mosquitocidal agents derived from the botanical source are considered essential and natural alternative tools, especially from plant crude extracts, essential oils, and, more importantly, the bioactive compounds isolated from the green extracts biodegradable and non-toxic to other beneficial species [10]. Naturally, phytochemicals are derived from the extracts or essential oils extracted from the plants' different parts [11]. As compared to the commercial pesticide impacts towards non-targets, phytochemicals were recognized as a readily biodegradable agent and notably less toxic to the non-targets than chemical pesticides [12].

The bioactive compounds struggle with the continuous selection pressures on the herbivores and other ecological factors as a mechanism in the plants. Different phytochemical classes, including alkaloids, steroids, terpenoids, essential oils, and phenolics of different species, have been documented for their insecticide activity [13]. Plant-based insecticides were having a wide range of impacts concerning the plant species, mosquito species, geographical location, the parts used and the technique of extraction, and the polarity of solvents used during removal.

Traditionally plant species are having diversified medicinal and insecticidal properties with a wide range of chemical metabolites existed. Over two thousand plant species have different modes of action against other pests with a wide range of chemical derivatives. Previous research focused on the larvicidal, adulticidal, and repellent activity has been proved on different species of plant members such as Solanacéae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae, and Rutaceae [14].

There is plenty of research previously focused on plant extracts and their chief derivatives with profound insecticidal, repellent, and adulticidal activity [15,16]. Similarly, several studies have displayed steady larvicidal activity against the malarial vector [17]. There are plenty of bio-active compounds that showed a unique mode of physiological and biochemical actions against different mosquito vectors. Previous research of [18] synthesized a new novel bio-based extract named "PONNEEM" (based on *Azadirachtaindica* and *Pongamia glabra*) commercially synthesized to control *An. stephensi* and *Cx. quinquefasciatus*. Correspondingly, methanol leaf extracts of *Justicia adhatoda* commonly known as Malabar nut displayed >95% of larval mortality the lepidopteran pest [19]. In addition, previous research of Selin-Rani et al. (2016) [20] illustrates that the methanolic leaf extracts of *Alingium salvifolium* showed 85% mortality rate against the different instars of *Spodoptera litura*. The methanolic leaf extracts of *Cassia fistula* L. commonly referred as golden shower established 98% mortality rate against *Ae. aegypti* larvae. Moreover, nineteen plant derived extracts from six different plant species from northeastern Brazil (*Clitoriopsis mollis*, *Philodendron grazielae*, *Merremia aegyptia*, *Roureadoniana*, *Setariaverticillata*, and *Tilia americana*) displayed >75% larval mortality on the fourth instar larvae of dengue vector [21]. It is evident that the selection of plant extracts for mosquito larvicidal actions could enable the discovery of

new dynamic agents for effective arthropod management [22]. The above statement was well interrelated with our current research on managing mosquito vector.

Efforts are required for integrated pest management using the broad pharmacological knowledge on the phytochemicals against medically challenging pests. It is apparent to analyze the past review stating that the *Dendrocalamus hamiltonii*(Nees and Arn. ex Munro), commonly known as Hamilton's bamboo and their chief Phyto-compounds, defends them against herbivores and toxic chemical pesticides. The insecticidal activity of methanolic root extracts of *D. hamiltonii* against five stored grain pests displayed that they are the potential to control stored product pests and delivered biologically safe natural grain protectant [23]. Similarly, a major bio-active compound Decalesides I and II derived from root extracts of *D. hamiltonii* showed selective toxicity on the targeting tarsal chemosensory/gustatory region of the stored grain pests [24]. Correspondingly, n-hexane extracts of *Epaltes pygmaea* D.C. showed intense insecticidal activity against the dengue and filarial vector [25,26].

Thus, the present study investigates the multipotent activity of methanol extracts from root *D. hamiltonii* with their chief derivatives against the significant arthropods *Ae. aegypti*, *An, stephensi*, and *Cx. quinquefasciatus* and its non-target activity against the selected mosquito predators *Anisops bouvieri*, *Deinococcus indicus* and *Toxorhynchites splendens* which shares same ecological niche of the selected arthropod vectors.

2. Materials and Methods

2.1. Collection of Plant Material

Decalepis hamiltonii (Nees and Arn. ex Munro) root samples were collected from the Kolli hills (11°24′85′′ N, 78°33′87′′ E,) Namakkal District, Tamil Nadu, India. Healthy fresh roots were collected in the early morning from in and around Kolli Hills Station, Southern India. The plant taxonomy was authenticated with the botanical scientist, and its voucher number—AU/SRC/1/18/2014/PP/134 was recorded. The collected roots were washed and shadow dried at room temperature for seven days until they become brittle, then pulverized to powder.

2.2. Preparation of Extraction

A soxhlet extraction method (boiling point ranging from 45–80 °C for 8 h) was carried out with 100 g of powdered plant material using serious of 500 mL organic solvents of increasing polarity viz, hexane, chloroform, ethyl acetate, methanol and distilled water until exhaustion.

The powdered material (100 g) was packed and extracted with 500 mL of methanol solvent using soxhlet extraction apparatus (Borosil, Mumbai, India) for 48 h until the solvent extracted no more color. The extract was concentrated under reduced pressure using a rotary vacuum evaporator (Heidolph, Schwabach, Germany) under reduced pressure at 40 °C to yield the crude extract. The viscous solution of extract was obtained from rotary vacuum evaporator.

The residue remaining after the methanol extraction was dried and after the complete solvent removal again subjected into chloroform solvent extraction as per the method described above. The methanol soluble portion was concentrated using a rotary evaporator followed by water bath drying. The yield of serious of extraction are 1.13 g/mL, 1.23 g/mL, 1.65 g/mL, 1.95 g/mL and 2.13 g/mL respectively in hexane, chloroform, ethyl acetate, methanol and distilled water. The crude extracts were kept at 4 °C in a refrigerator for further analysis.

2.3. GC-MSAnalysis

The toxicity of larval mosquito vectors has been tested on various solvent extracts (Chloroform, Ethyl acetate, and methanol). The toxicity of methanol extracts is highest, based on the preliminary examination of *D. hamiltonii*. Therefore, for further tests, methanol extract was used. The methanolic crude extracts derived from *D. hamiltonii* were identified based on spectroscopic analysis, 2 µL of crude and fractions were dissolved in HPLC

grade methanol and subjected to GC and MS JEOL GC mate equipped with secondary electron multiplier. JEOL GCMATE II GC-MS (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was fused silica 50 m × 0.25 mm I.D. Analysis conditions were 20 min at 100 °C, 3 min at 235 °C for column temperature, 240 °C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1 µL) was evaporated in a split less injector at 300 °C. The run time was 22 min. The compounds were identified by gas chromatography coupled with mass spectrometry. The molecular weight, molecular formula and structure of the compounds of test materials were ascertained by interpretation on mass spectrum of GC-MS using the database of National Institute Standard and Technology (NIST).

2.4. Mosquito Culture

The larvae of selected mosquito species were taken from Salem District, Tamil Nadu, India. The collected larvae were placed in sterile plastic containers covering tap water and were kept under in-vitro condition. Complete tests were performed at 27 ± 2 °C and 70–80% relative humidity (RH) under a 14:10 light/dark phase. For feeding larvae yeast and dog biscuits were mixed and given under laboratory conditions.

2.5. Larvicidal Bioassay

The larvicidal assays were executed based on the adapted methodology of WHO [27]. Bioassay was carried out using WHO (2005) procedure with slight modifications. The larvae were placed into 200 mL disposable plastic cups containing 25 mL of the test solution with different concentrations (1.0, 1.5, 2.0, 2.5, and 3.0 mg/L) (24.5 mL dechlorinated tap water and 0.5 mL of methanol dissolved test extract) and incubated at 27 °C. During the treatment period, larval food was added to each test cup, particularly if high mortality is noted in control.

Larvae were considered dead when they were unable to reach the surface of the solution when the cups were disturbed. The larvae were considered moribund if, at the end of 24 h, they showed no sign of swimming movements even after gentle touching with a glass rod. The dead and moribund larvae were recorded after 24 h as larval mortality. The whole set up was kept undisturbed for another 24 h and mortality counts were recorded again after 48 h. The number of dead larvae was determined at the start of the experiment (0, 24 and 48 h). The larvicidal tests with more than 20% mortality in controls and if pupae formed were discarded and repeated again.

The treatments were replicated five times, and each replicated set contained one control. An aqueous solution of methanol (0.5%) was employed as the negative control. Treatments that showed at least 50% mortality within 48 hr was followed-up by further bioassays of the same sample at different concentrations in order to determine the concentration required to kill 50% (LC_{50}) and 90% (LC_{90}) of the larvae present. The analyses of the follow-up assay were carried out according to the Probit analysis. Percentage mortality in the treatments was corrected when necessary for mortality in the controls using Abbott [28] formula.

2.6. Chemicals

Individual chemicals octadecanoic acid and oleic acid displayed higher peak area in the GC-MS analysis were purchased commercially from Sigma-Aldrich (≥98.5% Grade I, (capillary G.C., Mumbai, India).

2.7. Enzyme Assay

The treated and control fourth instar larvae were washed with double distilled water, and the adhering water was totally removed from the surface by blotting with tissue paper. The larvae were separately homogenized in Eppendorf tubes using a hand homogenizer in 500 µL of ice-cold sodium phosphate buffer (20 mM, pH 7.0) to enzyme activity. The homogenates were centrifuged (8000× *g* at 4 °C) for 20 min and supernatants were used

for the further analyses. The final homogenates were held on ice until used in assays. The complete enzyme assay experiments were performed based on the adapted methodology of [29].

2.7.1. Carboxylesterase Assays

The larval extracts in 0.1 M phosphate potassium pH 7.2 were prepared to (20 µL; 84 µg of protein) which was mixed with 500 µL of a solution containing 0.3 mM α- or β-naphthyl acetate in 0.1 M phosphate potassium at pH 7.2 containing 1% acetone. The reaction mixture was incubated for 20 min at 30 °C. Then, 0.1 mL of a mixture containing 0.3% Fast Blue B and 3.3% sodium dodecyl sulfate (SDS) was added. After centrifugation (3000× *g*, 28 °C), the supernatant absorbance at 590 nm was recorded. One unit of enzyme activity was defined as the amount of enzyme required to generate 1 µmol of α- or β-naphthol per minute.

2.7.2. Glutathione S-Transferase Assay

Fourth instar larvae were homogenized in 250 µL of 50 mM sodium phosphate buffer (pH 7.2) before being centrifuged at 15,000× *g* at 4 °C for 20 min (Eltek-RC4100F). The Sigma-Aldrich (Catalog 0410, Bangalore, India) GST assay kit was used to evaluate the conjugation of the thiol group of glutathione to the 1-chloro-2, 4-dinotrobenzene (CDNB) substrate. A total of 20 µL of homogenate was added to each well before 200 µL of solution containing Dulbecco's phosphate buffer (Sigma-Aldrich, Bangalore, IN), glutathione reduced (4 mM), and CDNB (2 mM). Rodriguez et al. (2001) determined the saturation concentration of CDNB to be 50 mM and the optimum time for reading to be 3 min. The 96-well flat-bottom UV microplate (Catalog 3635, Corning, Haryana, India) was immediately loaded onto a Synergy HT microplate reader (BioTek, Mumbai, India). After a 1-min lag time, the absorbance was read at 340 nm and the samples were read each 60 s for 3 min. Protein concentrations were verified using an albumin standard and bicinchoninic acid protein assay kit (Catalog TP0l00, Sigma Aldrich, Bangalore, India). The activity of GST was expressed as µmol/mg protein/min substrate conjugated.

2.7.3. Cytochrome P450 Assay

Larvae were placed in 40 mM sodium phosphate buffer (pH 7.2) and chilled before dissection. The heads, last abdominal segment, and digestive system were removed from the larvae. Carcasses were then used for determining the cytochrome P450 activity. The assays were based on the measurement of ethoxycoumarin-O-de-ethylase activity in the body walls. The methods of Boyer et al. (2005) were modified to determine the effect of plant extracts on the cytochrome P450 activity in the larvae. Black, round-bottom 96-well microplates were filled with 100 µL of a 0.4 mM 7-ethoxycoumarin solution containing 50 mM sodium phosphate buffer (pH 7.2). Individual larval carcasses were placed in each well and incubated for 4 h at 30 °C. The reaction was stopped with the addition of 50 µL of glycine buffer (1 mM, pH 10.4) and 50 µL of ethanol. Larval carcasses remained at the bottom of the well and were not detached before reading. Six wells containing 100 µL of phosphate buffer, 50 µL of glycine buffer, and 50 µL of ethanol served as control. The fluorescence of the reaction medium was measured from the top of the wells using a Synergy HT Microplate Reader (Bio-Tek Instruments, Mumbai, India) with 400 nm excitation and 480 nm emission filters. The production of 7-hydroxycoumarin (7-OH) was expressed as µmol 7-OH/mg larvae/min.

2.8. Non-Target Toxicity against Mosquito Predaotrs

The non-target screening of methanolic extracts of *D. hamiltonii* and their chief derivatives octadecanoic acid and oleic acid was investigated and related with the market available pesticide Temephos (Sigma-Aldrich, analytical standard, PESTANAL®, Mumbai, India). *Deinococcus indicus*, *Anisops bouvieri*, and *Toxorhynchites splendens* (third instar larvae) were taken from the similar biological niche of malarial and dengue vector maintained in

the discrete bowls casing water (45 cm diameter and 25 cm depth) at 26 ± 3 °C and 80% Relative humidity (RH), and second instars mosquito larvae of *Ae. aegypti*, *An. stephensi and Cx. quinquefasciatus* were given as feed. The selected non-targets were open to different dosages (*D. hamiltonii* methanol extracts-5.0, 10.0, 15.0 and 20.0 mg/L and octadecanoic acid and oleic acid- 1.0 mg/L), and the results were compared with Temephos at the different dosages (0.2, 0.4, 0.8 and 1.0 mg/L). For each treatment 10 replication was performed and five replications were utilized as control (without any exposure to chemicals) and the death rate was experiential post 24 h of treatment.

2.9. Data Analysis

The larvicidal experimental data were exposed to the analysis of variance (ANOVA, and square root transformed percentages) and the obtained statistical results were considered as five replicates means. Statistical significance within each larvicidal groups were analyzed using Tukey's multiple range test (significance at $p < 0.05$) and the lethal concentrations of larvae in 24 h were calculated by the Probit analysis with a dependability interval of 95% using the Minitab® 17 program. For determining enzyme inhibition level, Microcal Software (Sigma plot 11) was used to plotting the graphs.

3. Results

The total yield of different solvent crude extracts of *D. hamiltonii* displayed 1.13 g/mL, 1.23 g/mL, 1.65 g/mL, 1.95 g/mL and 2.13 g/mL respectively in Hexane, Chloroform, Ethyl acetate, Methanol and distilled water.

3.1. GC-MS Analysis

GC-MS analysis of methanol crude extract of *D. hamiltonii* showed six major peak compounds benzaldehyde, 2-hydroxy-4-methoxy (10.35%), dodecanoic acid (11.02%), n-hexadecanoic acid (21.05%), linoleic acid methyl ester (14.20%), oleic acid (21.04%) and octadecanoic acid (22.10%) displayed in Table 1.

Table 1. Chemical characterization of crude root methanol extracts of *D. hamiltonii*.

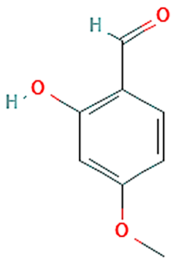
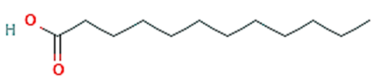
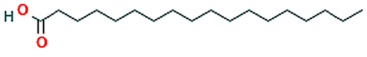
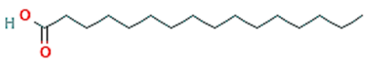
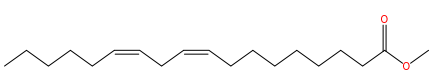
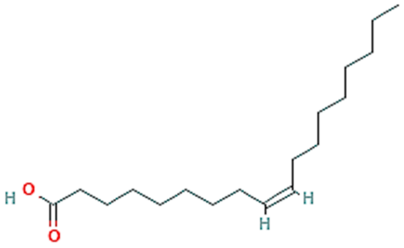
S. No	Name of the Compounds	Structure	R. Time	Peak Area (%)	M. Formula	M. Weight
1	Benzaldehyde, 2-hydroxy-4-methoxy-		11.868	10.35	$C_8H_8O_3$	152.15
2	Dodecanoic acid		15.658	11.02	$C_{12}H_{24}O_2$	200.32
3	Octadecanoic acid		18.437	22.10	$C_{18}H_{36}O_2$	284.5
4	n-Hexadecanoic acid		21.058	21.05	$C_{16}H_{32}O_2$	256.42
5	Linoleic acid methyl ester		22.506	14.20	$C_{19}H_{34}O_2$	294.4721

Table 1. *Cont.*

S. No	Name of the Compounds	Structure	R. Time	Peak Area (%)	M. Formula	M. Weight
6	Oleic acid		23.240	21.04	$C_{18}H_{34}O_2$	282.5
		Total Peak Area (%)		**99.76**		

3.2. Larvicidal Activity

The larvicidal activity of methanolic root extract of *D. hamiltonii* against the fourth instar larvae of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* was displayed (Figure 1). The larval mortality is profound in the maximum dosage of 3.0 mg/mL against all the three mosquito larvae. Despite the larvicidal activity was profound in the *Cx. quinquefasciatus* (95.5%- $F_{4,20}$ = 14.43, $p \leq 0.001$), followed by *Ae. aegypti* (92.56%- $F_{4,20}$ = 13.73, $p \leq 0.001$) and *An. stephensi* (90.18%- $F_{4,20}$ = 12.48, $p \leq 0.001$) respectively at the maximum dosage of 3.0 mg/mL. Though there is significance between the 1.0 and 1.5 mg/mL in *Ae. aegypti* (18.20%- $F_{4,20}$ = 13.73, $p \leq 0.001$) and *An. stephensi* (14.18%- $F_{4,20}$ = 12.48, $p \leq 0.001$) respectively.

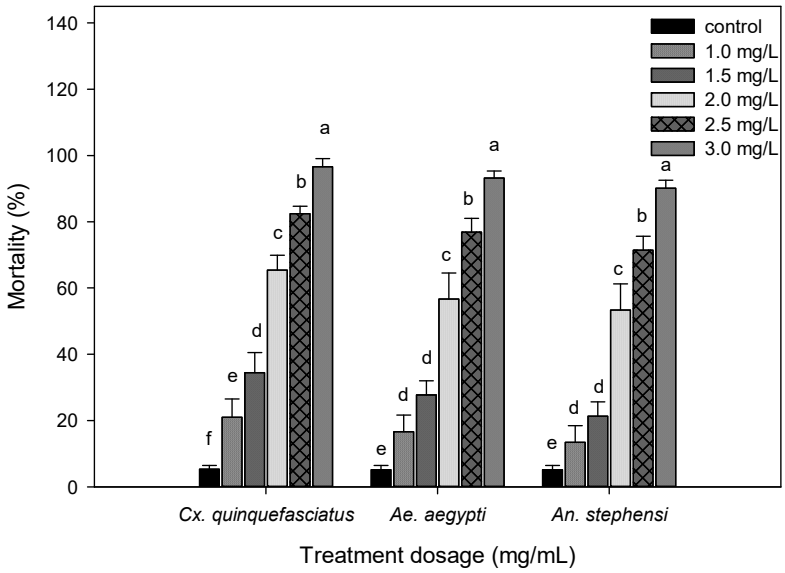


Figure 1. Larvicidal activity of root extracts of *D. hamiltonii* against fourth instar larvae of *Ae. aegypti, An. stephensi*, and *Cx. quinquefasciatus.* According to Tukey's test, the means (SEM±) followed by the same letters above bars indicate no significant difference ($p \leq 0.05$).

Correspondingly, the larvicidal activity against *Ae. aegypti* treated with highest peak area compound showed significant larval mortality rate in the octadecanoic acid (0.1 mg/mL) (98.35%- $F_{4,20}$ = 18.23, $p \leq 0.001$) followed by oleic acid (0.1 mg/mL) (96.77%- $F_{4,20}$ = 18.23, $p \leq 0.001$) respectively. Similarly, the larvicidal activity of *An. stephensi* also showed prominent mortality rate post treatment with octadecanoic acid (0.1 mg/mL) (95.55%- $F_{4,20}$ = 11.23, $p \leq 0.001$) followed by oleic acid (0.1 mg/mL) (93.17%- $F_{4,20}$ = 16.23, $p \leq 0.001$) respectively. Similar trends were observed in the *Cx. quinquefascia-*

tus with octadecanoic acid (0.1 mg/mL) (94.11%- $F_{4,20}$ = 11.23, $p \leq 0.001$) followed by oleic acid (0.1 mg/mL) (92.18%- $F_{4,20}$ = 16.23, $p \leq 0.001$), respectively (Figure 2).

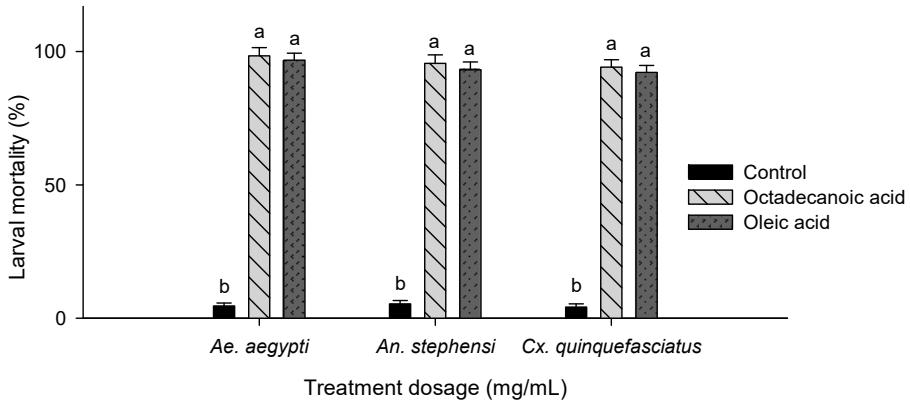


Figure 2. Larval mortality of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* treatment with 0.1 mg/mL of octadecanoic acid and oleic acid. According to Tukey's test, the means (SEM±) followed by the same letters above bars indicate no significant difference ($p \leq 0.05$).

3.3. Enzyme Activity

The enzyme association degeneration completed period and then tenable with reduction in the protein displayed at the sub-lethal concentration (0.172 mg/mL). In all the three mosquito larvae inhibition of α-carboxylesterase was observed to be significant in *Ae. aegypti* (0.171 mg/mL) as connected to *Cx. quinquefasciatus* (0.195 mg/mL), and *An. stephensi* (0.183 mg/mL) correspondingly (Figures 3 and 4). Likewise, β-carboxylesterase level gets reduced to 0.461, 0.410 and 0.373 µmol/mg/min at the highest treatment concentration of 0.197 mg/mL against *Culex*, *Anopheles* and *Aedes* mosquitoes. In difference, the level of GST enzyme improved in a concentration reliant manner. The GST level gets inclined significantly in *Ae. aegypti* (0.201 mg/mL) in parallel to *An. stephensi* (0.810 mg/mL) and *Cx. quinquefasciatus* (0.653 mg/mL), respectively (Figure 5). Likewise, Cytochrome P450 (CYP450) activity improved knowingly post-treatment with the sub-lethal concentration against all the three arthropods (0.201 mg/mL) and it was significant compared to control (Figure 6).

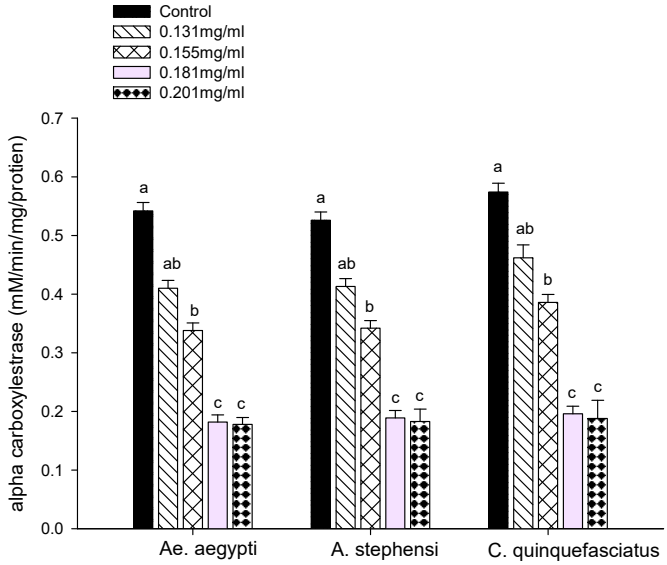


Figure 3. α-Carboxylesterasesenzyme activity of fourth instar larvae of three mosquito vectors after treatment with a sub-lethal dosage of methanol extract of *D. hamiltonii.* The data were fitted on a polynomial (regression) model, whereas vertical bars indicate standard error (±SEM). Means (SEM±) followed by the same letters above bars indicate no significant difference ($p \leq 0.05$).

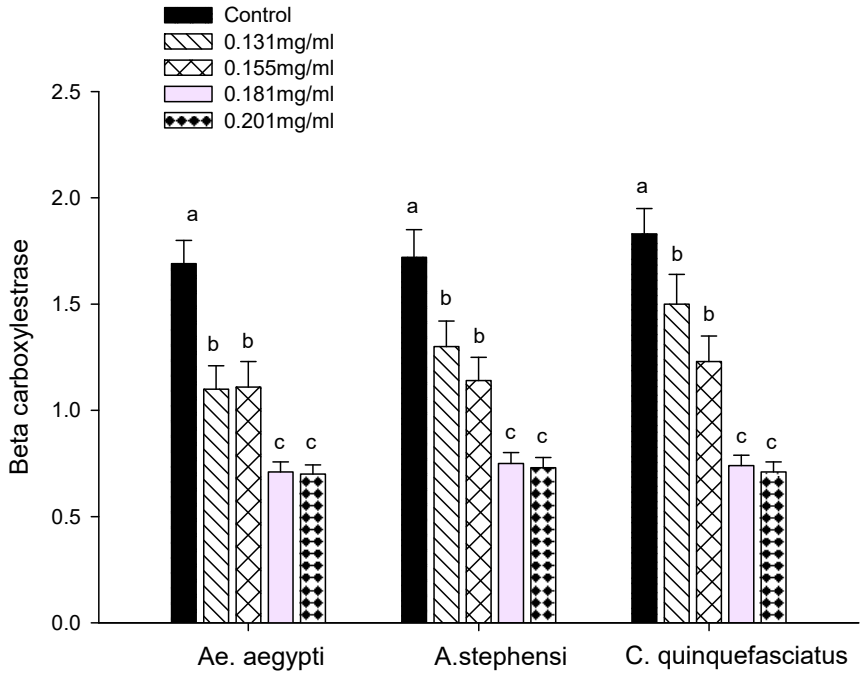


Figure 4. β-Carboxylesterasesenzyme activity of fourth instar larvae of three mosquito vectors after treatment with the sub-lethal dosage of methanol extract of *D. hamiltonii.* The data were fitted on a polynomial (regression) model, whereas vertical bars indicate standard error (±SEM). Means (SEM±) followed by the same letters above bars indicate no significant difference ($p \leq 0.05$).

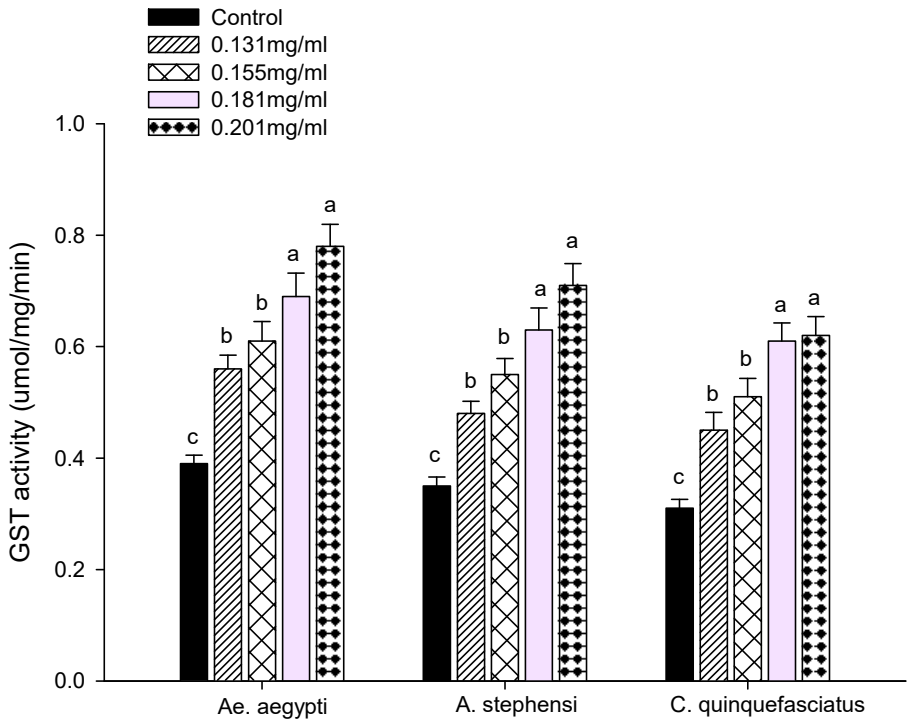


Figure 5. GST activity enzyme activity of fourth instar larvae of three mosquito vectors after treatment with a sub-lethal dosage of methanol root extract of *D. hamiltonii.* Means (SEM±) followed by the same letters above bars indicate no significant difference ($p \leq 0.05$).

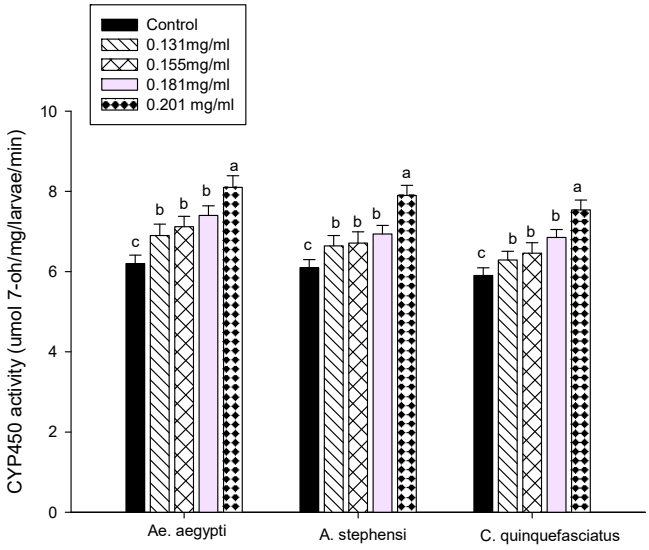


Figure 6. CYP450 enzyme activity of fourth instar larvae of three mosquito vectors after treatment with a sub-lethal dosage of methanol root extracts of *D. hamiltonii.* Means (SEM±) followed by the same letters above bars indicate no significant difference ($p \leq 0.05$).

3.4. Non-Target Activity of Mosquito Predators

The non-target toxicity of methanol root extracts of *D. hamiltonii* and their chief derivatives octadecanoic acid and oleic acid compared with Temephos on the mosquito predators *Tx. splendens*, *A. bouvieri*, and *D.indicus* displayed considerable mortality rate against the crude root extracts (5, 10, 15 and 20 mg/L) and its major derivatives (1.0 mg/L) signifies reduced toxic effect against all the three mosquito predators as of compare to the chemical pesticide Temephos (Figure 7). The mortality rate of *A. bouvieri*, *Tx. splendens* and *D.indicus* was maximum with 27.89% ($F_{4,20}$ = 14.22, $p \leq 0.0001$), 30.88% ($F_{4,20}$ = 19.77, $p \leq 0.0001$) and 34.22% ($F_{4,20}$ = 11.79, $p \leq 0.0001$) against the methanolic crude extracts of *D. hamiltonii* 20 mg/L respectively. Similarly, the mortality rate of octadecanoic acid and oleic acid (1 mg/L) was found to be less than 15% mortality rate against all the three aquatic predators and there is no significance difference with control in *A. bouveri* ($F_{4,20}$ = 9.52, $p \leq 0.0001$) and Tx. splendens ($F_{4,20}$ = 11.26, $p \leq 0.0001$). However, significance difference was displayed between octadecanoic acid and oleic acid and control in *D. indicus* ($F_{4,20}$ = 18.88, $p \leq 0.0001$). Despite the rate mortality rate against the Temephos (1.0 mg/L) showed 94.21% ($F_{4,20}$ = 20.95, $p \leq 0.0001$), 93.13% ($F_{4,20}$ = 13.39, $p \leq 0.0001$) and 96.63% ($F_{4,20}$ = 17.84, $p \leq 0.0001$) against the three mosquito predators, respectively.

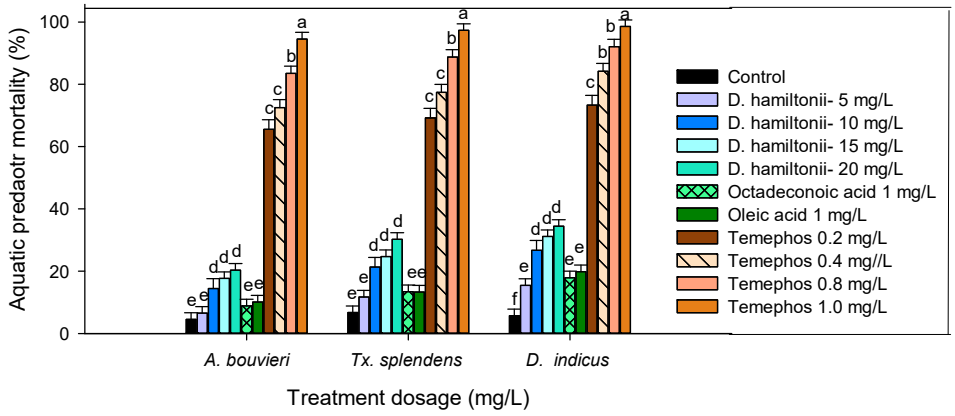


Figure 7. Effects of methanol root extracts of *D. hamiltoni* and their chief derivatives octadecanoic acid and oleic acid against non-target organisms *A. bouvieri*, *Tx. splendens* and *D. indicus* compared with commercial pesticide, Temephos. Means (SEM±) followed by the same letters above bars indicate no significant difference ($p \leq 0.05$) using Probit analysis.

4. Discussion

The present research on ecotoxicology has drifted towards botanical extract warehouse to explore the novel bio-pesticides. Moreover, plant derivatives have displayed potential efficacy as a mosquitocidal agent as an alternative to commercial pesticides [30,31]. Since then, plant extracts enriched with novel phytochemicals, many of them displayed targeted action against mosquito larvae [32–34]. Botanical extracts have significant attention as potential insect control agents against agriculture and medically challenging pests [35–37]. From the past decade, global researchers have proved that the derivatives of plant extracts, essential oils and their derivatives had substantial agent in treating mosquito larvae [29,38–40]. As the plant extracts are considered a low-cost agent, easily degradable and more importantly they are harmless against the non-target organisms and delivers different mode of actions against the targeted pests which decline the chances of getting resistance [41,42]. Remarkably, major plant compounds are target specific against the mosquito larvae and placed a vital position in the integrated pest management, IPM programs in the past three decades [43].

Chemical characterization of methanol extract of *D. hamiltonii* displayed six major compounds with higher peak area percentage observed in octadecanoic acid followed by oleic acid. Previously, [44] also investigated that the crude extract of *Thalictrum javanicum* and their chief derivatives oleic acid showed strong mosquitocidal activity against the dengue filarial vector. Similarly, octadecanoic acid-3, 4-tetrahydrofuran diester displayed significant alteration in the oxidative phosphorylation pathway in the Itch mite *Sarcoptesscabiei* [45,46]. In support of the above findings, all these compounds might play a major role in delivering larvicidal, enzyme inhibition, and antibacterial activity against the mosquito vectors and microbes. Generally, plant derived chemicals are having unique mode of actions against different insect pests [47]. Our finding states that the *D. hamiltonii* methanol extract acts as an essential representative in contradiction of larvae *Ae. aegypti*, *An.stephensi* and *Cx. quinquefasciatus*. Earlier, 90% larval mortality was exhibited at 4% concentration of *Leucas aspera* leaf extract against fourth instar larvae of *An. stephensi* [48]. The ethanol extract of *Leucas aspera* (commonly known as Thumba) treatment displayed significant LC_{50} range of 9.70-I instar, 10.27-II instar, 10.82-III instar, 11.30-IV instar, and 12.73-pupae mg/mL, respectively against *An. stephensi* [49]. Similarly, it has been reported that *Leucas aspera* methanol leaf extract having LC_{50} values ranging from 148.93 to 417.07 mg/L and the LC_{90} values ranging from 449.72 to 912.94 mg/L against *An. stephensi* first to fourth instar larvae and pupae, respectively [50]. Methanol leaf extract of *Vitex negundo* (horseshoe vitex), *V. trifolia* (simple leaf chaste-tree), *V. peduncularis* (white chaste-tree), and *V. altissima* (peacock chaste tree) showed LC_{50} dosage of 212.57-*Cx. quinquefasciatus*, 41.41, 76.28 of *An. stephensi* [51]. Global researchers illustrated thatgreen extracts and their derivatives are usually harmless to human and other well-beings. Indeed, their bio-active molecules displayed significant mortality rate against insect pests [52]. Many plant arrangements manifest toxicity to different mosquito type larvae [53]. *D. hamiltonii* reported that root extract functions against insecticidal activity [54].

The enzyme contributes to converting oxygen into a substrate from molecular oxygen and minimizing the other oxygen atoms into water. Oxidase was involved in oxidizing and reducing enzymes. The GSTs are a set of different enzymes which, for detoxification and oxidative harm defense, come in combination with glutathione and xenobiotic substances [55]. The relation of the electrophilic compound with reduced glutathione (GSH) would be catalyzed. In many insects, there have been high levels of GST activity associated with insecticide resilience. By combining a decreased GSH to insecticides and primary toxic metabolic products, GSTs can generate resistance to various insecticides. The most significant report is DDT and resistance pyrethroids. Plants produce chemicals, mainly bioactive compounds that misunderstand the incessant selection pressure from predators and other biological factors through their defensive action [56].P450 enzymes are extensive in the sustentacular vertebrate cell, presumably for olfactory receptor persistence. If that was the case, an attraction from this complex formation could be expected through the

ionotropic pathogen [16]. Stimulation of metabolic detoxifying mechanism has an essential role in adapting host insects to plants. As a function of esterase activity, carboxyl was considered exposure of a wide variety of target insects to various plant derivatives. In the larvae, α and β-to-lower concentrations of α and esterase activity were controlled. *Aedes aegypti* selectable plant species and the addition of secondary metabolites previously described [57] can prevent a protein enzyme's actions by binding. In contrast, a stimulating effect can occur in enzyme activities by increasing enzyme affinity to its substrate. Many previous research findings have shown that the use of essential detoxifying enzymes in plant chemicals is altered or inhibiting [58]. Itshowed an increase in the rate of GST activity in IV instar larvae of dengue mosquito exposed to methanolic leaf extract of *J. adhatoda* with their major derivative 3-hydroxy-2,3-dihydropyrrolo(2,1-b)quinazolin-9 (1 h) one (26.37%). Correspondingly, the activity of essential enzymes (esterases, GST, and CYP450) of dengue mosquito severely affected post-treated with dynamic compound andrographolide derived from *Andrographis paniculata* (Acanthaceae) at the maximum dosage of 12 ppm [55].

The methanol root extract from *D. hamiltonii* was published and displayed broad-spectrum of biological activity [58]. A primary mode of action is cell wall interference, protein synthesis inhibition, nucleic acid interference, and metabolic pathway inhibition [36]. Different root absorptions will be joint, homogenized, and used for further analysis with powder or oil. In terms of changes in the physical and chemical analysis that can affect the stability and acceptability of formulations, the strength of prepared formulations will be assessed. Thus, in this research, the methanol root extracts of *D. hammiltoni* and their major bio-active compounds delivered profound larvicidal and enzymatic properties against different mosquito vectors. Moreover, mosquitocidal agents were measured to be operative only if it is target-specific and needs to non-toxic or less toxic against non-targets which shares the same biological niche [41,59,60]. As similar to the above statement, the crude root extracts along with their derivatives are harmless/less toxic against the aquatic predators and considered to be a safe green based mosquitocides.

5. Conclusions

The present investigation delivers a baseline toxicity of plant derived methanol root extracts of *D. hammiltoni* against the medically challenging pests of dengue, malaria and filarial vector. Thus, the present research reveals the following outcomes. (i) The larvicidal activity of crude extracts of *D. hammiltoni* along with their chief chemicals displayed profound mortality rate against all the three mosquito larvae. (ii) The sub-lethal dosage of crude extract showed significant changes in the major biomarker enzymes of all the three arthropod vectors. (iii) The non-target toxicity screening against three beneficial species delivers that the crude extracts of *D. hammiltoni* and their derivatives are ecologically safe. The future perspective of the research will be focused on exploring the chemical characterization of *D. hammiltoni* root extracts and detecting the mosquitocidal potential of their individual chemistry.

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