JAZ INDIA

# Journal of Advanced Zoology

*ISSN: 0253-7214*

*Volume **45** Issue **01 Year 2024** Page **21:32***

# Toxicity of *Elytraria acaulis* (L. F.) Lindau (Acanthaceae) to the Larvae of Vector Mosquitoes

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***Article History***

Received: 17 May 2023
Revised: 25 Sept 2023
Accepted: 12 Oct 2023



***Abstract***

*Synthetic pesticides, which are non-biodegradable and have detrimental effects on the environment, non-targeted organisms, and human health, are often used to control mosquitoes. This situation fostered and prompted the creation of substitutes utilizing natural products like phytoextracts and phytochemicals. The current study was set out to determine the toxicity of leaf extracts from Elytraria acaulis on the early third instar larvae of Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus at doses of 31.5, 62.5, 125, 250, 500 and 1000mg/L at 24 and 48 hours of exposure. All extracts, with the exception of aqueous, demonstrated potent larvicidal effectiveness with 100% larval death in all the three studied vector mosquitoes after 48 hours. The ethanol extract showed the maximum larvicidal activity and 100% larval mortality in Aedes aegypti after 24 hours, and its respective $LC_{50}$ values against Aedes aegypti, Anopheles stephensi, and Culex quinquefasciatus were 31.98, 560.29, 603.81mg/L and 20.43, 46.13 and 60.08mg/L after 24 and 48 hours. The treated larvae exhibited extremely restless behaviour, including wiggling, sinking, floating, slowness, paralysis, sinking to the bottom of the glass beaker, and ultimately death. Qualitative phytochemical study of Elytraria acaulis leaves revealed the presence of alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, tannins, terpenes, and terpenoids. The ethanolic extract GC-MS examination identified main phytocompounds, including imidazole, imidazolidinone, phytol, phytol acetate, octacosane, thymol 1-thiocarbonylimidazolide and methoxyacetic acid to determine the larvicidal mechanism of action and the cause of larval death. It is quite exciting to note, based on the results of the current investigation, that Elytraria acaulis leaf extracts, particularly ethanol extract, demonstrated good larvicidal efficacy. The present study documents the first report on the effectiveness of Elytraria acaulis ethanolic leaf extract against the larvae of Aedes aegypti, Anopheles stephensi, and Culex quinquefasciatus.*

**Keywords:** *Elytraria acaulis, leaf extracts, phytochemical constituents, larvicidal, Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus*

## 1. Introduction

Man could travel to Mars, but it would take years to defeat the mosquito, a tiny buzzing vampire creature. Mosquitoes, the 'flying syringes' and 'public enemy number one' have been man's biggest enemy since the dawn of time[1-3]. Dengue, malaria, and lymphatic filariasis are mosquito/vector-borne diseases that are carried by the bite of vector mosquitoes, *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*[4-8]. Vector control, which employs a variety of conventional and synthetic insecticides, is the primary strategy for preventing mosquito/vector-borne illnesses[9], but have adverse effects on the environment, non-target creatures, and human health[10]. As a result, there is a backlash against the use of chemical pesticides, and there is an urgent need for insecticidal agents of natural origin that are extremely effective, target-specific, and safe for both human health and the environment. Phytoextracts and phytocompounds have become increasingly popular as phytoinsecticides/pesticides against

mosquitoes as they are eco-friendly, quickly biodegradable, and non-toxic to humans and other living things, and have the potential to reduce the environmental impact of traditional pesticides. Since then, reliable reviews of botanical insecticides with mosquito-killing properties have been widely documented[11–23].

*Elytraria acaulis*, a little shrub distributed throughout South Africa and India grows in sandy or rocky soils, shady dry regions, and is commonly termed Asian scaly stem, Bull foot herb, Nilakadambu or Pumikatambu in Tamil, and Patharchatta in Hindi. It has been traditionally used for wound healing, venereal diseases, abscesses, pneumonia, boils, burns, tonsillitis, stomachaches, toothaches, as well as skin infections brought on by ringworm[24], leucorrhoea[25,26,] arthritis, body aches, and fits[27]. The plant's infusion is also recommended as a treatment for cough[28]. Antihyperglycemic[29], antidiabetic[30], antidiarrheal[31], antihelmintic[32,] antiseptic and anti-inflammatory[33], hepatoprotective[34], antioxidant[32,35-38,] antimicrobial[36,39,40,] antifungal[41,] antibacterial [38,42-44,] and anticancer [45] are some of its pharmacological properties. With regard to its insecticidal, only two studies conducted by Munusamy et al.[46] and Sukumaran and Maheswaran[47,] have evaluated its mosquito larvicidal activities against *Aedes aegypti* and *Culex quinquefasciatus*, respectively. Hence, a paucity of knowledge still lies on the larvicidal effectiveness of its leaf extracts against vector mosquitoes. Henceforth, the current investigation was the first to document the larvicidal toxicity of various solvent extracts of *Elytraria acaulis* leaves against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*.

## 2. Materials and Methods

### *Plant material collection and extract preparation*

*Elytraria acaulis* found in the Western Ghats, Tamil Nadu, India (10.938011°N 76.687177°E), was collected and brought to the laboratory. Using morphological key characteristics and an identification guide, the Department of Botany, Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu, India, verified and confirmed the taxonomy identification of the obtained plant. The fresh and mature leaves of this plant were cleaned in dechlorinated water and allowed to air dry at room temperature in the shade. The dried leaf was then sieved after being pounded into a coarse powder with an electric blender. Thereafter, three litres of butanol, ethyl acetate, acetone, ethanol, and distilled water, each were used to soak one kilogram of finely powdered leaves for 72 hours. The solvent-extracted material was then transferred to a soxhlet extractor after filtering[48.] Soxhlet extraction was carried out in the increasing order of solvent polarity. The extracted material was then centrifuged for 10 minutes at 4°C at 5000 rpm, filtered using Whatman No. 1 filter paper, and the supernatant was collected in a separate flask. The filtered material was then condensed to obtain the solidified crude phytoextracts, which were then air dried to completely evaporate the solvents. Each crude solvent extract was then concentrated using a rotary vacuum evaporator. The resultant crude solvent extracts were then stored at 4°C in amber-colored sterile vials for bioassay.

### *Gas Chromatography-Mass Spectrometry (GC-MS) analysis*

This analysis was done on the larvicidal extract that was the most effective. The Elite-5MS (5% biphenyl, 95% dimethylpolysiloxane, 30m×0.25mm ID× 250µmdf) packed fused silica column was utilized, and the components were separated using helium as the carrier gas at a constant flow of 1mL/min. During chromatographic run, the injector temperature was set to 260 °C. The extract sample (1µL) was injected into the device with 60°C oven temperature for two minutes, 300°C at 10 °C per minute, and 300 °C, where it was kept for six minutes. Mass detector was operated at 240 °C for transfer line and ion source each, 70 eV for the electron impact in the ionisation mode, 0.2s for the scan period, and 0.1s for the scan interval. Fragments ranged in from 40 to 600Da. The component spectra were compared to the database of component spectra stored in National Institute for Standards and Technology's GC-MS library. Prior to GC-MS analysis, *Elytraria acaulis*, butanol, ethyl acetate, acetone, ethanol, and aqueous leaf extracts were qualitatively screened for alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, tannins, terpenes, and terpenoids in accordance with standard procedures[49].

### *Culture of test vector mosquitoes*

Immatures of *Aedes* and *Culex* obtained from cisterns using a dipper, and from open drains using a ladle, respectively, were transported in plastic containers to the laboratory, and placed in enamel larval

salvers until adult emergence. With the help of an aspirator, *Anopheles* adults were collected from cattle sheds, and transferred to laboratory in a one-foot mosquito cage. Using the mosquito identification key, the adults of each vector mosquito species were verified and confirmed before rearing[50,51]. After receiving a blood meal, cyclical generations of each vector mosquito were housed apart in two-foot mosquito cages in insectary (27±2°C, 70-80% RH). The oviposited eggs were removed from the mosquito cages using ovitraps, transferred to the larval rearing chamber in enamel trays, and given larval food (yeast and dog biscuits in a 1:3) when hatched. The larvae on becoming pupae were moved to a different mosquito cage in enamel bowls, for adult emergence.

***Larvicidal bioassay***

With minor alterations, World Health Organization[52] protocol was adopted for this bioassay. The required test concentrations (31.5, 62.5, 125, 250, 500 and 1000mg/L) and quantity of test solution were prepared using serial dilution of 1.0% stock solutions of each crude solvent leaf extract. For the bioassays, healthy early third instar larvae from the laboratory-colonized $F_1$ generation were chosen as the test instar because they had a bigger body length than first and second instar, and because the fourth instar develops into a pupa in around 48 hours. Twenty numbers of each vector mosquito for each replication of each trial were put separately to 250mL glass beakers containing distilled water and the desired test concentration. For positive and negative controls, distilled water (250mL), and Tween 80 (1.0mL) dissolved in distilled water (249mL), were kept separate and run simultaneously. Larvae were provided larval diet during the experiment. When a needle was inserted into a respiratory siphon of larva, no sign of movement by the larvae was considered moribund, and was scored dead. Three replicates in each trial and a total of three trials were performed. Larval mortality was calculated after 24 and 48 hours, and additionally, every two hours from the time of treatment exposure until 48 hours, the behavior of treated larvae was observed and recorded.

***Statistical analysis of data***

Percentage of larval mortality was calculated, and Abbott's formula[53] was used to rectify control mortality when it varied between 5% and 20%. IBM SPSS statistics version 27 was used for statistical analysis of data[54]. Regression, chi-square and probit analysis were performed on the mortality data. One-way analysis of variance with Duncan's multiple comparison difference post-hoc tests were performed to determine whether and at what concentrations precisely, the mortality in treated bioassays significantly differed from that of the controls, as well as whether there were notable differences in response between the solvent extracts, and the differences were deemed significant at $P \leq 0.05$ level.

## 3. Results and Discussion

Leaf extracts of *Elytraria acaulis* tested effective against the larvae of tested vector mosquito species. No larval death in either positive or negative controls were reported. After 24 hours of exposure, ethanol and ethyl acetate extracts caused 100% larval mortality in *Aedes aegypti* at 250 and 1000mg/L, respectively (Table 1; Figure 1). The ethanol extract showed the highest levels of larval mortality in *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* with respective $LC_{50}$ values of 31.98, 560.29, and 603.81mg/L at the lowest dosage after 24 hours (Table 3). After 48 hours, every extract aside from aqueous exhibited 100% larval death in every tested vector mosquito species (Table 2; Figure 1). In *Aedes aegypti*, ethanol and ethyl acetate extracts caused 100% larval mortality at 31.5 and 125 mg/L, respectively; in *Anopheles stephensi*, it was ethanol, ethyl acetate, and acetone extracts at 500 mg/L; and in *Culex quinquefasciatus*, only the ethanol extract showed 100% mortality at 500 mg/L. The $LC_{50}$ values of ethanol extract against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* at the lowest concentration after 48 hours of exposure were 20.43, 46.13 and 60.08 mg/L, respectively (Table 4). Overall assessment from this study portrayed the ethanolic extract to have had the highest impact on the larvae of the three vector mosquito species. Regarding behaviour, all treated larvae showed signs of unusual agitation, writhing, sinking, floating, sluggishness, paralysis, sinking to the bottom of the glass beaker, and ultimately death. Regarding the phytochemical analysis, *Elytraria acaulis* leaf extracts revealed the presence of alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, tannins, terpenes and terpenoids. The GC-MS analysis of its ethanolic extract revealed notable phytocompounds such as imidazole, imidazolidinone, phytol, phytol acetate, octacosane, thymol 1-thiocarbonylimidazolide, and methoxyacetic acid.

**Table 1.** Larvicidal activity of *Elytraria acaulis* leaf extracts against vector mosquitoes at 24 hours

| Solvent extracts | Control | | Treated concentrations (mg/L) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Positive | Negative | 31.5 | 62.5 | 125 | 250 | 500 | 1000 |
| ***Aedes aegypti*** | | | | | | | | |
| **Butanol** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 14.00 ±0.00$^{b23}$ | 14.66 ±0.57$^{bc3}$ | 15.00 ±0.00$^{bc2}$ | 15.66 ±0.57$^{cd2}$ | 16.33 ±0.57$^{d2}$ | 17.66 ±0.57$^{e2}$ |
| **Ethyl acetate** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 11.33 ±0.57$^{b12}$ | 14.66 ±0.57$^{c2}$ | 16.33 ±0.57$^{d2}$ | 17.66 ±0.57$^{e2}$ | 19.33 ±0.57$^{f2}$ | 20.00 ±0.00$^{f2}$ |
| **Acetone** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 9.66 ±0.57$^{b1}$ | 12.33 ±0.57$^{c1}$ | 13.33 ±0.57$^{d2}$ | 14.66 ±0.57$^{e2}$ | 16.33 ±0.00$^{f2}$ | 17.66 ±0.00$^{g2}$ |
| **Ethanol** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 15.33 ±0.57$^{b}$ | 17.66 ±0.57$^{c3}$ | 19.66 ±0.57$^{d2}$ | 20.00 ±0.00$^{d2}$ | 20.00 ±0.00$^{d2}$ | 20.00 ±0.00$^{d2}$ |
| **Aqueous** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 5.66 ±0.57$^{b1}$ | 6.00 ±0.00$^{bc1}$ | 6.33 ±0.57$^{bc1}$ | 6.66 ±0.57$^{bc1}$ | 7.33 ±0.57$^{c1}$ | 7.66 ±0.57$^{c1}$ |
| ***Anopheles stephensi*** | | | | | | | | |
| **Butanol** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 7.66 ±0.57$^{b12}$ | 8.66 ±0.57$^{c3}$ | 9.00 ±0.00$^{cd3}$ | 9.66 ±0.57$^{d3}$ | 10.66 ±0.57$^{ce3}$ | 12.00 ±0.00$^{f2}$ |
| **Ethyl acetate** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 7.66 ±0.57$^{b12}$ | 8.00 ±0.00$^{cd2}$ | 8.66 ±0.57$^{de12}$ | 9.00 ±0.00$^{e2}$ | 10.33 ±0.57$^{f23}$ | 11.00 ±0.00$^{f2}$ |
| **Acetone** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 5.00 ±0.00$^{b2}$ | 6.66 ±0.57$^{c3}$ | 7.66 ±0.57$^{d23}$ | 8.66 ±0.57$^{e3}$ | 11.00 ±0.00$^{f3}$ | 11.66 ±0.57$^{g2}$ |
| **Ethanol** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 5.66 ±0.57$^{b1}$ | 7.00 ±0.00$^{c1}$ | 8.66 ±0.57$^{d12}$ | 9.66 ±0.57$^{e3}$ | 11.33 ±0.57$^{f3}$ | 12.33 ±0.57$^{g2}$ |
| **Aqueous** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 2.66 ±0.57$^{b1}$ | 2.66 ±0.57$^{b1}$ | 3.00 ±0.00$^{b1}$ | 3.00 ±0.00$^{b1}$ | 3.00 ±0.00$^{b1}$ | 3.00 ±0.00$^{b1}$ |
| ***Culex quinquefasciatus*** | | | | | | | | |
| **Butanol** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 5.66 ±0.57$^{b12}$ | 6.00 ±0.00$^{bc3}$ | 6.66 ±0.57$^{cd2}$ | 7.66 ±0.57$^{e2}$ | 9.33 ±0.57$^{f2}$ | 11.00 ±0.00$^{g2}$ |
| **Ethyl acetate** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 5.00 ±0.00$^{b1}$ | 5.66 ±0.57$^{c}$ | 6.66 ±0.57$^{d1}$ | 7.66 ±0.57$^{e1}$ | 10.00 ±0.57$^{f1}$ | 11.66 ±0.57$^{g12}$ |
| **Acetone** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 5.66 ±0.57$^{b1}$ | 6.00 ±0.00$^{bc1}$ | 6.66 ±0.57$^{c1}$ | 7.66 ±0.57$^{d2}$ | 8.66 ±0.57$^{e2}$ | 10.66 ±0.57$^{f2}$ |
| **Ethanol** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 5.00 ±0.00$^{b2}$ | 6.00 ±0.00$^{c34}$ | 6.66 ±0.57$^{c2}$ | 8.66 ±0.57$^{d2}$ | 11.00 ±0.00$^{e2}$ | 12.33 ±0.57$^{f2}$ |
| **Aqueous** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 1.66 ±0.57$^{ab1}$ | 2.00 ±0.00$^{ab1}$ | 2.00 ±0.00$^{ab1}$ | 2.33 ±0.57$^{b1}$ | 3.00 ±0.00$^{c1}$ | 3.00 ±0.00$^{c1}$ |

Values are mean±standard deviation of larval mortality of three replicates of three trials; Different superscript alphabets in rows indicate values significant than respective controls, and different superscript numerical in columns indicate values significant between the extracts at $P<0.05$ level by one way ANOVA followed by Duncan's multiple comparison post-hoc test performed; Similar superscript alphabets and numerical in rows and columns indicate no significant variation

Acanthaceae family members have reportedly shown to display mosquitocidal properties[55-65]. The current study's findings were found to be superior to those of earlier studies on *Elytraria acaulis*, where the hexane, chloroform and methanol extracts of its root displayed $LC_{50}$ values of 207.39, 230.05 and 268.83; 219.98, 261.73 and 316.23mg/L against the larvae of *Culex quinquefasciatus* and *Aedes aegypti*, respectively[46]; and the powder of this plant had $LC_{50}$ values of 116.07 and 124.25mg/100mL against larvae of *Culex quinquefasciatus* and *Aedes aegypti*, respectively[47]. Additionally, in this study, the treated larvae lengthened and turned black. The same information was provided by Sukumaran and Maheswaran[47] in their investigation on the effects of exposing larvae of *Aedes aegypti* and *Culex quinquefasciatus* to *Elytraria acaulis* in powder form. The present study's behavioural analysis also identified a relationship between the effects of *Elytraria acaulis* leaf extracts on the nervous system and motor coordination of treated larvae, where the symptoms (excitation, convulsions, paralysis, and larval death) were suggestive of nerve poisons. The susceptibility of several mosquito larval genera to the same phytoextracts/phytochemicals varies. *Anopheles* larvae can be more or less susceptible to botanical compounds than *Aedes* and *Culex* because their susceptibility can fluctuate, while *Aedes* larvae are more durable and resistant to botanical extracts than *Culex*[12]. Based on this study's findings, *Aedes aegypti* larvae were found to be more susceptible, followed by *Anopheles stephensi*, when compared to *Culex quinquefasciatus* on the basis of low $LC_{50}$ values displayed by the solvent leaf extracts of *Elytraria acaulis*.

**Table 2.** Larvicidal activity of *Elytraria acaulis* leaf extracts against vector mosquitoes at 48 hours

| Solvent extracts | Control | | Treated concentrations (mg/L) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Positive | Negative | 31.5 | 62.5 | 125 | 250 | 500 | 1000 |
| ***Aedes aegypti*** | | | | | | | | |
| **Butanol** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 16.66 ±0.57$^{b12}$ | 17.66 ±0.57$^{c3}$ | 18.00 ±0.00$^{d2}$ | 19.33 ±0.57$^{e2}$ | 20.00 ±0.00$^{e2}$ | 20.00 ±0.00$^{e2}$ |
| **Ethyl acetate** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 15.66 ±0.57$^{b1}$ | 18.00 ±0.00$^{c}$ | 20.00 ±0.00$^{d1}$ | 20.00 ±0.00$^{d1}$ | 20.00 ±0.00$^{d1}$ | 20.00 ±0.00$^{d12}$ |
| **Acetone** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 15.66 ±0.57$^{b1}$ | 16.66 ±0.00$^{bc1}$ | 17.66 ±0.57$^{bc}$ | 19.00 ±0.00$^{c2}$ | 19.33 ±0.57$^{c2}$ | 20.00 ±0.00$^{c2}$ |
| **Ethanol** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 19.66 ±0.57$^{b2}$ | 20.00 ±0.00$^{b34}$ | 20.00 ±0.00$^{b2}$ | 20.00 ±0.00$^{b2}$ | 20.00 ±0.00$^{b2}$ | 20.00 ±0.00$^{b2}$ |
| **Aqueous** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 6.66 ±0.57$^{ab1}$ | 7.33 ±0.57$^{bc1}$ | 8.00 ±0.00$^{c1}$ | 8.33 ±0.57$^{d1}$ | 8.66 ±0.57$^{d1}$ | 9.00 ±0.00$^{d1}$ |
| ***Anopheles stephensi*** | | | | | | | | |
| **Butanol** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 9.66 ±0.57$^{b12}$ | 12.00 ±0.00$^{c3}$ | 14.66 ±0.57$^{d2}$ | 15.66 ±0.57$^{e2}$ | 16.66 ±0.57$^{f2}$ | 17.66 ±0.57$^{g2}$ |
| **Ethyl acetate** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 11.33 ±0.00$^{b1}$ | 13.66 ±0.57$^{c}$ | 15.66 ±0.57$^{d1}$ | 18.33 ±0.57$^{e1}$ | 20.00 ±0.00$^{f1}$ | 20.00 ±0.00$^{f12}$ |
| **Acetone** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 13.33 ±0.57$^{b2}$ | 15.66 ±0.57$^{c34}$ | 16.66 ±0.57$^{d2}$ | 19.33 ±0.57$^{e2}$ | 20.00 ±0.00$^{e2}$ | 20.00 ±0.00$^{e2}$ |
| **Ethanol** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 15.00 ±0.00$^{b1}$ | 16.66 ±0.57$^{c1}$ | 17.66 ±0.57$^{c1}$ | 19.33 ±0.57$^{d2}$ | 20.00 ±0.00$^{d2}$ | 20.00 ±0.00$^{d2}$ |
| **Aqueous** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 5.66 ±0.57$^{b1}$ | 6.00 ±0.00$^{b1}$ | 6.00 ±0.00$^{b1}$ | 6.00 ±0.00$^{b1}$ | 6.66 ±0.57$^{bc1}$ | 6.66 ±0.57$^{bc1}$ |
| ***Culex quinquefasciatus*** | | | | | | | | |
| **Butanol** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 9.33 ±0.57$^{b12}$ | 12.00 ±0.00$^{c3}$ | 14.33 ±0.57$^{d2}$ | 17.00 ±0.00$^{e2}$ | 18.66 ±0.57$^{f2}$ | 20.00 ±0.00$^{g2}$ |
| **Ethyl acetate** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 10.66 ±0.57$^{b1}$ | 12.33 ±0.57$^{c}$ | 14.33 ±0.57$^{d1}$ | 17.33 ±0.57$^{e1}$ | 18.33 ±0.57$^{f1}$ | 19.00 ±0.00$^{f12}$ |
| **Acetone** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 15.00 ±0.00$^{b2}$ | 15.66 ±0.57$^{c34}$ | 16.66 ±0.57$^{d2}$ | 17.66 ±0.57$^{e2}$ | 19.66 ±0.57$^{f2}$ | 20.00 ±0.00$^{f2}$ |
| **Ethanol** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 14.00 ±1.00$^{b1}$ | 16.00 ±0.00$^{c1}$ | 16.66 ±0.57$^{cd1}$ | 17.66 ±0.57$^{d2}$ | 20.00 ±0.00$^{e2}$ | 20.00 ±0.00$^{e2}$ |
| **Aqueous** | 0.00 ±0.00$^{a1}$ | 0.00 ±0.00$^{a1}$ | 4.00 ±0.00$^{ab1}$ | 4.00 ±0.00$^{ab1}$ | 4.33 ±0.47$^{ab1}$ | 4.33 ±0.57$^{ab1}$ | 4.66 ±0.57$^{ab1}$ | 5.00 ±0.00$^{b1}$ |

Values are mean±standard deviation of larval mortality of three replicates of three trials; Different superscript alphabets in rows indicate values significant than respective controls, and different superscript numerical in columns indicate values significant between the extracts at $P<0.05$ level by one way ANOVA followed by Duncan's multiple comparison post-hoc test performed; Similar superscript alphabets and numerical in rows and columns indicate no significant variation

The larvicidal activity of *Elytraria acaulis* leaf extracts in this study may be attributable to a number of bioactive phytocompounds, such as alkaloids, flavonoids, saponins, steroids, tannins, terpenes, and terpenoids, which may act synergistically or separately to kill mosquito larvae because they are toxic to immature mosquitoes. Sukumaran and Maheswaran[47] reported that the larvicidal activity of *Elytraria acaulis* was caused by the presence of alkaloids, flavonoids, proteins, amino acids, glycosides, carbohydrates, phenols, steroids, saponins, and tannins, and the same is corroborated to the current study. Alkaloids, amino acids, carbohydrates, flavonoids, glycosides, phenols, phenolics, phytosterols, proteins, saponins, steroids, tannins, and terpenoids are among the major groups of phytochemicals found in *Elytraria acaulis*[33,35,66-68], besides ethers, esters, carboxylic acids and amides[69]. The present study's findings supported the existence of these phytochemical subgroups, and the ethanolic extract of this plant also demonstrated the presence of terpenes, terpenoids, alkaloids, flavonoids, saponins, and tannins. The phytocomponents, imidazole, imidazolidinone (alkaloids), phytol, phytol acetate, octacosane (terpenes), thymol 1-thiocarbonylimidazolide (terpenoid), and methoxyacetic acid might have interacted with the cuticle membrane of the larvae, disarranged the membrane, acted as mitochondrial poison, which is most likely the cause of larval mortality. Additionally, they can also attack and damage the nervous system, midgut epithelium, gastric caeca and malpighian tubules[70,71].

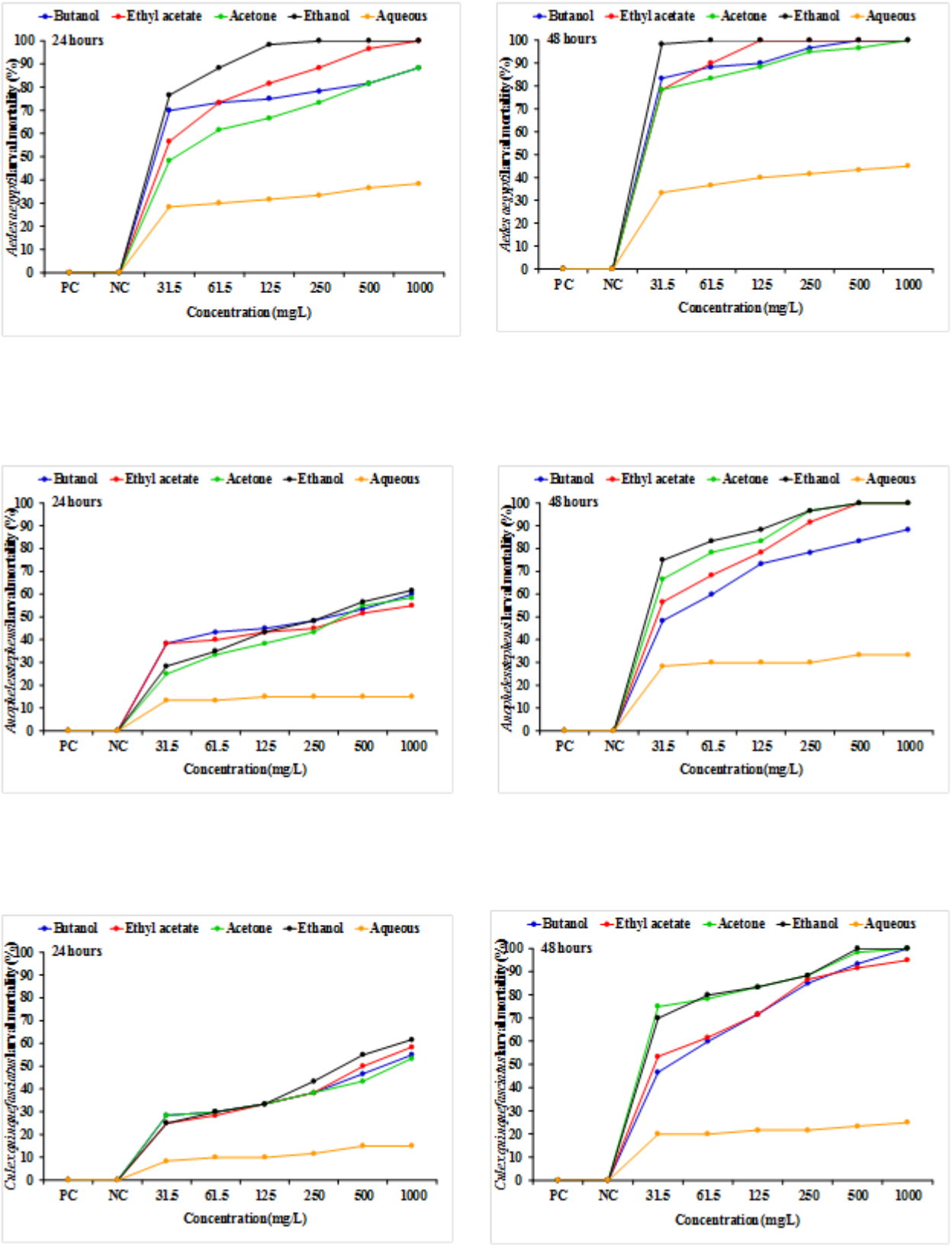


**Figure 1.** Percent larval mortality of vector mosquitoes on exposure to *Elytraria acaulis* leaf extracts

**Table 3** Statistical inference of *Elytraria acaulis* leaf extracts against larvae of vector mosquitoes at 24 hours

| Solvent extracts | $LC_{50}$ (mg/L) | $LC_{90}$ (mg/L) | Intercept±S.E. | Slope±S.E. | $\chi^2$ | Regression equation | $R^2$ | *P* value |
|---|---|---|---|---|---|---|---|---|
| | | | | ***Aedes aegypti*** | | | | |
| **Butanol** | 102.10 | 746.93 | 8.76±1.50 | 0.012±0.004 | 151.93* | Y=8.762+0.012x | 0.563 | 0.001* |
| **Ethyl acetate** | 78.30 | 240.06 | 8.66±1.54 | 0.015±0.004 | 319.88* | Y=8.660+0.015x | 0.653 | 0.004* |
| **Acetone** | 186.49 | 754.87 | 7.14±1.26 | 0.014±0.003 | 113.00* | Y=7.144+0.014x | 0.684 | 0.001* |
| **Ethanol** | 31.98 | 59.92 | 10.80±1.88 | 0.013±0.005 | 507.05* | Y=10.801+0.013x | 0.525 | 0.001* |
| **Aqueous** | 1118.50 | 2708.44 | 3.62±0.63 | 0.005±0.002 | 44.35* | Y=3.414+0.007x | 0.759 | 0.003* |
| | | | | ***Anopheles stephensi*** | | | | |
| **Butanol** | 566.06 | 1658.74 | 5.04±0.87 | 0.009±0.002 | 66.90* | Y=5.043+0.009x | 0.659 | 0.001* |
| **Ethyl acetate** | 644.62 | 1840.39 | 4.86±0.84 | 0.008±0.002 | 63.63* | Y=4.864+0.008x | 0.637 | 0.001* |
| **Acetone** | 629.79 | 1561.62 | 3.89±0.71 | 0.010±0.002 | 52.46* | Y=3.898+0.010x | 0.769 | 0.001* |
| **Ethanol** | 560.29 | 1482.85 | 4.32±0.78 | 0.010±0.002 | 58.98* | Y=4.321+0.010x | 0.749 | 0.001* |
| **Aqueous** | 2953.68 | 5729.93 | 1.70±0.30 | 0.002±0.001 | 19.11† | Y=3.414+0.007x | 0.473 | 0.638† |
| | | | | ***Culex quinquefasciatus*** | | | | |
| **Butanol** | 719.27 | 1744.23 | 3.59±0.63 | 0.009±0.002 | 44.27* | Y=3.597+0.009x | 0.776 | 0.003* |
| **Ethyl acetate** | 661.58 | 1659.36 | 3.40±0.61 | 0.010±0.002 | 43.45* | Y=3.401+0.010x | 0.818 | 0.004* |
| **Acetone** | 758.60 | 1838.33 | 3.59±0.62 | 0.008±0.002 | 44.30* | Y=3.594+0.008x | 0.759 | 0.003* |
| **Ethanol** | 603.81 | 1457.27 | 3.55±0.65 | 0.011±0.002 | 46.43* | Y=3.556+0.011x | 0.821 | 0.002* |
| **Aqueous** | 2314.02 | 4219.63 | 1.15±0.21 | 0.002±0.001 | 13.51† | Y=3.414+0.007x | 0.703 | 0.918† |

$LC_{50}$ & $LC_{90}$: Lethal concentration that kills 50% and 90% of the treated larvae respectively; $\chi^2$: Chi-square value; $R^2$: Coefficient of determination; *Values significant at $P \leq 0.05$ level; †Values not significant at $P \leq 0.05$ level

**Table 4** Statistical inference of *Elytraria acaulis* leaf extracts against larvae of vector mosquitoes at 48 hours

| Solvent extracts | $LC_{50}$ (mg/L) | $LC_{90}$ (mg/L) | Intercept±S.E. | Slope±S.E. | $\chi^2$ | Regression equation | $R^2$ | *P* value |
|---|---|---|---|---|---|---|---|---|
| | | | | ***Aedes aegypti*** | | | | |
| **Butanol** | 40.38 | 111.18 | 10.70±1.84 | 0.013±0.005 | 143.81* | Y=10.701+0.013x | 0.530 | 0.001* |
| **Ethyl acetate** | 29.22 | 50.69 | 10.97±1.91 | 0.013±0.005 | 37.59* | Y=10.976+0.013x | 0.513 | 0.020* |
| **Acetone** | 49.63 | 195.83 | 10.20±1.76 | 0.014±0.004 | 264.32* | Y=10.206+0.014x | 0.556 | 0.001* |
| **Ethanol** | 20.43 | 27.06 | 12.08±2.07 | 0.012±0.005 | 2.09† | Y=12.082+0.012x | 0.441 | 1.000† |
| **Aqueous** | 876.00 | 2363.02 | 4.47±0.77 | 0.006±0.002 | 57.54* | Y=3.414+0.007x | 0.570 | 0.001* |
| | | | | ***Anopheles stephensi*** | | | | |
| **Butanol** | 167.46 | 724.19 | 7.43±1.33 | 0.014±0.003 | 126.10* | Y=7.439+0.014x | 0.664 | 0.001* |
| **Ethyl acetate** | 71.51 | 176.26 | 8.49±1.52 | 0.016±0.004 | 82.07* | Y=8.492+0.016x | 0.669 | 0.001* |
| **Acetone** | 53.85 | 132.51 | 9.50±1.67 | 0.015±0.004 | 206.75* | Y=9.507+0.015x | 0.607 | 0.001* |
| **Ethanol** | 46.13 | 119.23 | 10.15±1.76 | 0.014±0.004 | 642.85* | Y=10.157+0.014x | 0.566 | 0.001* |
| **Aqueous** | 1358.20 | 3253.38 | 3.55±0.61 | 0.004±0.002 | 42.72† | Y=3.414+0.007x | 0.525 | 0.006† |
| | | | | ***Culex quinquefasciatus*** | | | | |
| **Butanol** | 111.39 | 312.98 | 7.37±1.34 | 0.016±0.003 | 104.48* | Y=7.373+0.016x | 0.728 | 0.001* |
| **Ethyl acetate** | 122.59 | 492.02 | 7.80±1.40 | 0.015±0.003 | 221.66* | Y=7.800+0.015x | 0.682 | 0.001* |
| **Acetone** | 60.54 | 207.57 | 9.59±1.65 | 0.014±0.004 | 888.71* | Y=9.595+0.014x | 0.597 | 0.001* |
| **Ethanol** | 60.08 | 177.36 | 9.48±1.65 | 0.014±0.004 | 122.80* | Y=9.484+0.014x | 0.606 | 0.001* |
| **Aqueous** | 1855.00 | 3925.76 | 2.47±0.43 | 0.003±0.001 | 29.23† | Y=3.414+0.007x | 0.550 | 0.138† |

$LC_{50}$ & $LC_{90}$: Lethal concentration that kills 50% and 90% of the treated larvae respectively; $\chi^2$: Chi-square value; $R^2$: Coefficient of determination; *Values significant at $P \leq 0.05$ level; †Values not significant at $P \leq 0.05$ level

The efficiency of the larvicidal agent is significantly influenced by the extraction solvent choice[13]. The solvents should be chosen with great care and skill based on the phytochemical profile of the plant/plant part employed in order to achieve a potent extract[72] because there is a correlation between the efficiency of the extract and solvent polarity. The primary factors determining the choice of solvent are the quantity of phytochemicals to be extracted, the pace of extraction, and the variety of different compounds extracted.[73]. The solvent selected will depend on the intended purpose of the extract as well as the specific chemicals to be extracted. Ethanol can be used to extract alkaloids, flavonoids, sterols, tannins and terpenoids[74]. Every ethanolic plant extract that has been linked to mosquito larvicidal activity has been identified by the present authors[75]. The ethanolic extract was discovered to be the most effective among the other solvent extracts in the current study. Ethanol could extract the bioactive phytocompounds responsible for immature mosquitocidal activity as they had exhibited $LC_{50}$ values of 20.43, 46.13, and 60.08mg/L against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*, respectively in this study. Komalamisra et al.[57] tested 96 ethanolic extracts from different parts of 84 Thai plant species for larvicidal activity against *Aedes aegypti*, and extracts from six of them showed strong larvicidal activity, with $LC_{50}$ values ranging between 16.0 and 48.2mg/L. When tested against *Aedes albopictus* and *Culex quinquefasciatus* larvae, ethanolic extracts from a few plants exhibited $LC_{50}$ values that ranged from 31.8 to 155.0ppm[76]. These results validated the findings of the present investigation.

The behaviour displayed by the treated mosquito larvae in the current study can be related to the effects of phytocompounds on the larval nervous system and motor coordination. Due to the effects of the bioactive phytochemical compounds in *Elytraria acaulis* leaf extracts on the larval death of the studied vector mosquitoes, the following can be deduced as a possible explanation for their larvicidal properties. Alkaloids have an adverse effect on mosquito larvae, causing them to move slowly, become translucent, and change colour. The present investigation made a note of this. They cause acetylcholinesterase or sodium channel disruption, which stops the transmission of nerve impulses through synaptic pathways. Additionally, they tighten blood vessels and lessen the activity of the autonomic nervous system, all of which aid in the death of mosquito larvae [77]. Flavonoids attacks the central nerve ganglia, submerges the nerves, paralyses the nerve cells and kills mosquito larvae[78]. They further obstruct the function of the larvae's respiratory system, obstruct electron transfer, cause denaturation and protein coagulation, and decrease the permeability of the digestive tract's cell walls, which obstructs the flow of nutrients and kills the larvae. Samuel et al.[79] reported that *Aedes aegypti* larvae metabolic processes stopped by the flavonoids in *Citrus limon* leaf extracts, altered its skin appearance, disrupted body metabolism, drained the larvae energy, and caused it to spasm before it died. This study also turned out something similar. Rey et al.[70] found that dipteran larvae treated with phenolic compounds developed lesions on their midgut. As stomach poison, saponins can kill larvae after they enter their bodies through the digestive system, and interferes with physiological functions, including ion transport, osmoregulation, nutrition, absorption, and digestion[80,81]. Tannins bind proteins in the digestive tract, acting as a stomach toxin that hinders the larvae digestion and stops the larvae from absorbing proteins [82]. Terpenoids may also denaturize the mosquito larvae digestive system because they interfere with the stability of the midgut cell membrane, stop the larvae from feeding, and ultimately result in their demise[80,81]. If any of these may have contributed to the larval deaths in the current study, further investigation is necessary on the same.

## 4. Conclusion

The bioactive phytochemicals in phytoextracts, which, when isolated in pure form, will undoubtedly have excellent mosquitocidal capabilities, are being studied further as a result of successful exploratory studies on the ability of potential mosquitocidal property. The results of the present study will serve as the foundation for further investigation into the active phytoconstituents that have a harmful effect on mosquito larvae. To better understand the potential mechanisms of action of the biologically active phytocomponents present in the ethanolic leaf extract of *Elytraria acaulis*, a study on larvicidal phytochemicals should be carried out.

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