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PHYTOCHEMICAL SCREENING AND MOSQUITO LARVICIDAL EFFICACY OF ROOT EXTRACTS OF *Elettaria cardamomum* (L.) Maton AGAINST *Culex quinquefasciatus* Say

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AUTHOR'S CONTRIBUTION

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Larvicidal efficacy of crude root extracts (0.1 to 0.5% concentrations) of *Elettaria cardamomum* (L.) Maton (*E. cardamomum* (L.) Maton) were tested against 1st to 4th instar larvae of *Culex quinquefasciatus* Say (*Cx. quinquefasciatus* Say) for a period of 24, 48, and 72 hrs of exposure. Petroleum ether, hexane, and ethyl acetate root extracts with 30, 40, and 50 ppm concentrations each were tested against only 3rd instar larvae of *Cx. quinquefasciatus* up to 24, 48, and 72 hrs of exposure. Crude root extract showed great efficacy in larvicidal activity. First instar larvae were most susceptible to crude root extract. Only at 0.3% concentration of crude root extract, first instar larvae showed cent percent mortality after 24 hrs of exposure. Second, third, and fourth instar larvae showed 96.66±3.33%, 96.66±3.33% and 60.00±5.77% mortality respectively at 0.5% concentration of crude root extract. Petroleum ether, hexane, and ethyl acetate root extracts showed LC_{50} values = 30.47, 42.22, and 58.81 ppm respectively after 72 hrs of exposure. Among tested three solvent root extracts, petroleum ether root extract showed the best result in larvicidal efficacy followed by hexane and ethyl acetate root extracts. Phytochemical analyses of root extracts revealed the presence of several secondary metabolites. Negative control and ethanol treated control experiments did not show any larval mortality. Statistical significance was determined through three ways ANOVA analyses for larvicidal bioassays by crude root extract. Mortality percent of larvae showed significant values in terms of instar, hour, and concentration of crude root extract. In conclusion, crude and petroleum ether root extracts of the plant may be used to control of *Cx. quinquefasciatus* mosquito population. Experimented non-target creatures were non responsive to crude as well as petroleum ether root extracts, so its uses will be also eco friendly.

Keywords: *Elettaria cardamomum*; *Culex quinquefasciatus*; mortality; bioassay; significant.

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1. INTRODUCTION

Mosquitoes transmit parasites and pathogens and cause many vector borne diseases, such as malaria, Japanese encephalitis (JE), yellow fever, filariasis, dengue which are major human health issues in many countries and ultimately affect the socioeconomically status of several nations. It also causes allergy and local skin irritation to human when it bites [1, 2]. Filariasis also called elephantiasis is a tropical disease caused by parasitic nematodes viz. *Wuchereria bancrofti* (90% cases), *Brugia malayi* (9% cases) and *Brugia timori* (1% case) which harbor in the lymph vessels and lymph glands of human and leads to tremendous swellings of different parts of the body. *Culex quinquefasciatus* (*Cx. quinquefasciatus*) is the principal vector of bancroftian filariasis. Fifty one million people were infected globally due to filariasis in 2018. [3]. To stop the spread of mosquito borne diseases, mosquito control is essentially done through using insecticides of synthetic chemical compounds such as, organochlorine and organophosphate. These synthetic chemical insecticides cause severe human health problems, harmful effects to environment, toxicity to non-targeted creatures and growing insect resistance varieties [4, 5]. Plants produce many bio active chemicals (secondary metabolites) which destroy a limited number of species including targeted insects. So, plants are a source of alternative agents for controlling insect vectors and these naturally occurring botanical insecticides are easily biodegradable, suitable alternative agents to synthetic chemical insecticides and may be used to control strategies of integrated pest management [6]. Many researchers have done their experiments on plant extracts in recent and past to find out their larvicidal efficacy against different species of mosquitoes [7-19]. *Elettaria cardamomum* (L.) Maton (*E. cardamomum* (L.) Maton) (Family: Zingiberaceae) are designated as 'queen of spices' and are very ancient, and expensive spices [20, 21]. In Ayurveda, *E. cardamomum* were used in treatment in ulcers, hypertension, anorexia, constipation, cardiovascular diseases, asthma, bronchitis, and gastrointestinal disorders [22]. It has many pharmacological properties like, antimicrobial, gastro-protective, anti-oxidant, immune-stimulant, anti-hypertensive, anticancer, anti-inflammatory [23]. The present research work aims mainly to unfold the larvicidal efficacy of root extracts of *E. cardamomum* (L.) Maton against *Cx. quinquefasciatus* mosquito species.

2. MATERIALS AND METHODS

2.1 Collection of Roots of the Plant

Elettaria cardamomum plants were collected with rhizomes and roots by me from the medicinal plant garden of Maharajadhiraj Uday Chand Women's College, Purba Bardhaman, West Bengal, India (23 ^{0}16' N, 87^{0} 54' E) during the rainy season (month of August). After collection of some whole plants, roots were separated from rhizomes of plants. Roots were cleaned properly by running tap water and thereafter rinsed with distilled water and subsequently dried on paper towel. The voucher specimen (voucher no. MUCZH-02) was deposited in the laboratory, Department of Zoology, Maharajadhiraj Uday Chand Women's College Purba Bardhaman, West Bengal, India. Voucher specimen was identified by professor Dr. A Mukhopadhyay, Department of Botany, The University of Burdwan, Burdwan, West Bengal, India.

2.2 Preparations of Crude Root Extract and Different Doses

Cleaned roots of *E. cardamomum* were cut into very small pieces and kept in a cleaned glass beaker (250 ml), then crushed in an electrical grinding machine, the juice of roots was filtered through muslin cloth and filtrate of the crude extract was used as a stock test solution (100%). From stock test solution 0.1% to 0.5% doses were prepared for larvicidal bio assays. Different mentioned doses of crude root extract solution (100 ml) were made taking required volume of stock crude extract test solution of roots and mixing required volume of tap water.

2.3 Preparation of Solvent Root Extracts

Maceration procedure was used for the preparation of different solvent root extracts of the plant as per protocol of Sharma et al. [24]. Collected cleaned roots of *E. cardamomum* were cut into very small pieces and dried in shade at the environmental temperature for a period of 14-15 days. The dried small pieces of roots were ground using electrical stainless steel grinding machine and sieved to get fine powdered materials. 50 g ground fine powdered materials of roots of the plant were dipped in petroleum ether (500 ml) kept in a brown color glass bottle and thereafter closed the mouth of the bottle tightly for a period of 10 days with frequent agitation daily (at least one hour per day). After 10 days the petroleum ether extract of roots of the plant were collected and filtered through What Man No. 42 filter paper and the filtrated extract was concentrated by direct evaporation and semi solid extract was obtained after evaporation of total petroleum ether. Thereafter same plant material was socked in hexane (500 ml) and ethyl acetate (500 ml) successively for a period of 10 days each, maintaining the same procedure as described as before to obtain petroleum ether semi solid extract. All the semi solid extracts of foresaid different

solvents were stored in a refrigerator at 4^0 C for further bioassays.

2.4 Preparation of Different Doses of Different Solvent Root Extracts

Graded concentrations viz., 30, 40, and 50 ppm of each of petroleum ether, hexane, and ethyl acetate root extracts were used for larvicidal bioassays. Stock solution was prepared on 5% absolute alcohol for each aforesaid solvent root extracts. 0.1 g of each of petroleum ether, hexane, and ethyl acetate root extracts of the plant dissolved separately in 1 ml of ethanol and thereafter added 19 ml distilled water to get stock test solutions (5000 ppm) of different solvent root extracts. From stock test solution required graded concentrations (30, 40, and 50 ppm) of different solvent root extracts were prepared through dilution method and ultimately to obtain 100 ml of mentioned different doses of each of petroleum ether, hexane, and ethyl acetate root extracts of the plant.

2.5 Test Mosquito Species

Larvae of *Cx. quinquefasciatus* were collected from cemented drains of Burdwan town. Larvae of different instars were kept in a big plastic tray with tap water. Larvae were provided powdered mixture of dog biscuits and dried yeast powder in 3:1 ratio. Larval colonies were maintained at 27 ± 2^0 C temperature and 80-85% relative humidity and kept free from exposure to pathogens, or insecticides [12]. Bioassay experiments were conducted in the laboratory, Department of Zoology, Maharajadhiraj Uday Chand Women's College, Purba Bardhaman, West Bengal, India.

2.6 Larvicidal Bioassay Experiment

Larvicidal bioassays were performed as per standard protocol of WHO, 2005 with some suitable modifications [25]. All larval instars (first to fourth) were used for larvicidal bioassays by crude root extract of the plant. Twenty larvae (20) were released in different plastic bowls (225 ml capacity), contained each with 100 ml of test solution of different doses of crude root extract (0.1-0.5%) to examine the per cent mortality of larvae. Negative control experiments were arranged on 100 ml of tap water only. Only third instar larvae of *Cx. quinquefasciatus* mosquito were used for bioassays (larvicidal) with different solvent (petroleum ether, hexane, and ethyl acetate) root extracts. Twenty (20) larvae were released in plastic bowls of 225 ml capacity, each containing 100 ml of test solution of different doses like 30, 40, and 50 ppm of each of aforesaid different solvent root extracts. Control experiments (ethanol treated) were set on 100 ml of tap water with 0.5 ml of ethanol. Each set of experiment for crude as well as different solvent root extracts were folded thrice, including control experiments on different three days. The per cent mortalities were recorded after 24, 48 and 72 hrs of post exposure cumulatively. Larvae were detected dead when they were unable to move after touching in their body or siphon with a fine brush.

2.7 Phytochemical Screening

Preliminary phytochemical analyses were performed as per protocol of Trease and Evans [26], Harborne [27], and Sofowara [28] with suitable modifications. Ethanol and water root extracts of *E. cardamomum* were used for phytochemical analyses. For the preparation of water root extract, 500 mg of dried root powdered material was taken in a conical flask and added 100 ml of distilled water and boiled in a hot water bath at 100 ° C for 30 minutes and cooled. The root tissues were homogenized and shaken well. The extract was charcoal filtered to remove pigments and then filtered through Whatman No. 1 filter paper. The clear supernatant was used as water root extract. For the preparation of ethanol root extract, 500 mg of dried root powdered material was taken in a conical flask and added 100 ml of 80% ethanol and boiled in a hot water bath at 100 ° C for 30 minutes. The root tissues were homogenized and shaken well. The extract was charcoal filtered to remove pigments and then filtered through Whatman No. 1 filter paper. The clear supernatant was used as ethanol root extract.

2.7.1 Test for steroids

4 ml of extract (ethanol) was taken in a cleaned test tube and then 2 ml of concentrated H_2SO_4 was added through the internal wall of the test tube. Brown color ring developed which indicated the presence of steroids in the sample.

2.7.2 Test for alkaloids (Mayer's test)

4 ml of ethanol extract was taken in a cleaned test tube and then added first 2N HCL (2 drops) and thereafter added Mayer's reagent (3 drops). Precipitation of pale yellow color indicated the presence of alkaloids in the sample.

2.7.3 Test for terpenoids (Salkowski test)

4 ml of sample (ethanol extract) was taken in a test tube and then 5 ml of chloroform and 1 ml of concentrated H_2SO_4 was added one after another through the internal wall of the test tube very carefully. At the interface reddish brown color was developed which indicated the presence of terpenoids in the sample.

2.7.4 Test for flavonoids

4 ml of sample (water extract) was taken in a test tube and then added 1 ml of sodium hydroxide solution. Intense color was developed but became colorless after adding dilute HCL which indicated the presence of flavonoids in the sample.

2.7.5 Test for tannins and phenolic compounds (Ferric chloride test)

4 ml of sample (water extract) was taken in a cleaned test tube and therefore added 3-4 drops of ferric chloride solution. Development of blue green coloration indicated that the sample contained tannins and phenolic compounds.

2.7.6 Test for saponins (frothing test)

8 ml of sample (water extract) was taken in a cleaned test tube and was shaken vigorously. Formation of many small bubbles (frothing) in the sample indicated of the presence of saponines.

2.8 Effect of Crude and Petroleum Ether Root Extracts of *Elettaria cardamomum* on Non-Targeted Creatures

Non-targeted creatures dwell in the same habitat of the mosquito larvae. One vertebrate (Tadpol of toad) and one invertebrate (4^{th} instar *Chironomus circumdatus* larvae) non-targeted creatures have been tested with doses LC_{50} value of crude as well as petroleum ether root extracts against 3^{rd} instar larvae of *Cx. quinquefasciatus* after 24 hrs of exposure respectively as per protocol adopted by Suwannee et al. [29]. Twenty (20) tadpoles of toad and twenty (20) 4th instar of *Chironomus circumdatus* larvae were released in two separate 500 ml glass beaker, each contained 200 ml of solution with 0.16 % concentration (i.e. LC_{50} value of crude root extract against 3^{rd} instar larvae of *Cx. quinquefasciatus* after 24 hrs of exposure) of crude root extract of the plant. Another two beaker (500 ml) were taken, each contained 200 ml of solution of petroleum ether root extracts of the plant with 44.14 ppm concentration (*i.e.* LC_{50} value of petroleum ether root extract against 3^{rd} instar larvae of *Cx. quinquefasciatus* after 24 hrs of exposure) and Twenty (20) tadpoles of toad and twenty (20) fourth instar of *Chironomus circumdatus* larvae were released in two separate 500 ml glass beaker to observe the effect of petroleum ether root extracts. Negative control experiments were set on 200 ml of tap water only. Data of % mortality were noted up to 24, 48, and 72 hrs of exposure cumulatively. Each experiment was conducted three times including negative control (only 200 ml tap water only) and ethanol treated control experiments (200 ml tap water with 1 ml of ethanol) on separate three days.

2.9 Statistical Analyses

Computer software 'STAT PLUS-2009' – Trial version and MS Excel- 2007 were used to calculate the mean per cent mortality, standard error, LC_{50} and LC_{90} values (Log probit analyses), regression equations, coefficient of determination (R^2) and ANOVA analyses.

3. RESULTS

Table 1 demonstrates the larvicidal efficacy of crude root extract of *Elettaria cardamomum* against *Culex quinquefasciatus* mosquito species. Percent mortality of larvae increased with increase in concentration and time of exposure. Highest per cent mortality for all instar larvae was observed at 0.5 % concentration. First instar larvae were most susceptible and they showed cent percent mortality only at 0.3% concentration of crude root extract after 24 hrs of exposure. No larval mortality was observed on negative control experiments.

Table 2 depicted that $LC_{50,}$ LC_{90}, regression equations and R^2 values of larvicidal activity by the application of crude root extract of *Elettaria cardamomum* against different larval instar of *Culex quinquefasciatus* mosquito. LC_{50} and LC_{50} values gradually decreased in time for different larval instars. Co-efficient of determination (R^2) values close to one (1) in all most all cases which indicated that per cent mortality is strongly co-related with concentration of the crude root extract.

Fig. 1 depicted the graphical representation of LC_{50} and LC_{90} values of larvicidal activity by crude root extract of *Elettaria cardamomum.* From Fig. 1 it was clear that LC_{50} and LC_{90} values gradually decreased in time for all instar larvae.

Table 3 depicted the larvicidal efficacy of petroleum ether, hexane, and ethyl acetate root extract of *Elettaria cardamomum* against *Culex quinquefasciatus*. Percent mortality of larvae increased with increase in concentration and time of exposure for different solvent root extracts. Highest per cent mortality of third instar larvae of *Culex quinquefasciatus* was observed at 50 ppm concentration. Among three solvent root extracts, petroleum ether root extract showed highest % mortality. Highest % mortality (i.e. 96.67±3.33 %) was observed at 50 ppm concentration after 72 hrs of

exposure. Ethanol treated control experiments did not show any mortality.

Table 4 depicted that $LC_{50,}$ LC_{90}, regression equations and R^2 values of larvicidal activity of petroleum ether, hexane and ethyl acetate root extract of *Elettaria cardamomum* against third instar larvae of *Culex quinquefasciatus* respectively. LC_{50} and LC_{50} values were calculated, LC_{50} and LC_{50} values gradually decreased in time for larvicidal activity (third instar) by petroleum ether, hexane and ethyl acetate root extract. Co-efficient of determination (R^2) values close to one (1) in all most all cases for larvicidal activity (third instar larvae) of said different solvent root extract which indicated that per cent mortality is strongly co-related with the concentration of the different solvent root extract of the plant.

Table 1. Mortality percent of different instars of *Culex quinquefasciatus* exposed to different concentrations of crude root extract of *Elettaria cardamomum*

Instar	Concentration (%)	Mortality % at different exposure periods (Mean mortality % ± Standard Error		
		24 hrs	48 hrs	72 hrs
1st	0.1	70.00±5.77	80.00±5.77	86.66±6.67
	0.2	93.33±3.33	100.00±0.00	100.00±0.00
	0.3	100.00±0.00	100.00±0.00	100.00±0.00
	0.4	100.00±0.00	100.00±0.00	100,00±0.00
	0.5	100.00±0.00	100.00±0.00	100.00±0.00
2nd	0.1	53.33±3.33	66.66±3.33	76.66±3.33
	0.2	63.33±3.33	73.33±3.33	83.33±3.33
	0.3	66.66±3.33	76.66±3.33	86.66±3.33
	0.4	70.00±5.77	73.33±3.33	90,00±5.77
	0.5	80.00±.5.77	83.33±3.33	96.66±3.33
3rd	0.1	40.00±5.77	50.00±5.77	63.33±3.33
	0.2	53.33±3.33	60.00±5.77	70.00±5.77
	0.3	63.33±3.33	73.33±3.33	76.66±3.33
	0.4	73.33±3.33	83.33±3.33	86.66±3.33
	0.5	76.66±3.33	93.33±3.33	96.66±3.33
4th	0.1	13.33±3.33	20.00±5.77	26.66±3.33
	0.2	16.66±3.33	23.33±3.33	30.00±5.77
	0.3	23.33±3.33	30.00±0.00	36.66±3.33
	0.4	26.66±.6.7	36.66±.6.67	43.33±3.33
	0.5	40.00±5.77	50.00±.5.67	60.00±5.77

Control: *No mortality (For all instars)*

Table 2. Log probit analyses and regression analysis of larvicidal activity of crude root extract of *Elettaria cardamomum* against different larval instar forms of *Culex quinquefasciatus*

Instars	Periods (hrs)	LC_{50} (ppm)	LC_{90}(ppm)	Regression equations	R^2 values
1st	24	0.0757	0.1572	Y =72.6650+66.6700X	0.8112
	48	0.0842	0.1094	Y = 84.0000+40.0000X	0.7071
	72	0.0783	0.1038	Y = 89.3280+26.6800X	0.7071
2nd	24	0.0870	2.1016	Y = 48.6610+60.0100X	0.9762
	48	0.0187	2.8739	Y = 64.6600+33.3400X	0.8704
	72	0.0276	0.3304	Y = 72.6610 +46.6700X	0.9900
3rd	24	0.1603	1.2542	Y = 33.3340+93.3200X	0.9826
	48	0.1179	0.5747	Y = 39.0010+109.9900X	0.9986
	72	0.0744	0.4879	Y = 53.6660+83.3200X	0.9944
4th	24	1.1044	13.6446	Y = 4.9940+63.3400X	0.9645
	48	0.6924	9.0580	Y = 9.9990+73.3300X	0.9722
	72	0.4620	6.2351	Y = 15.3270+80.0100X	0.9577

R^2= Coefficient of determination; LC= Lethal Concentration; Y= Mortality; X= Concentration

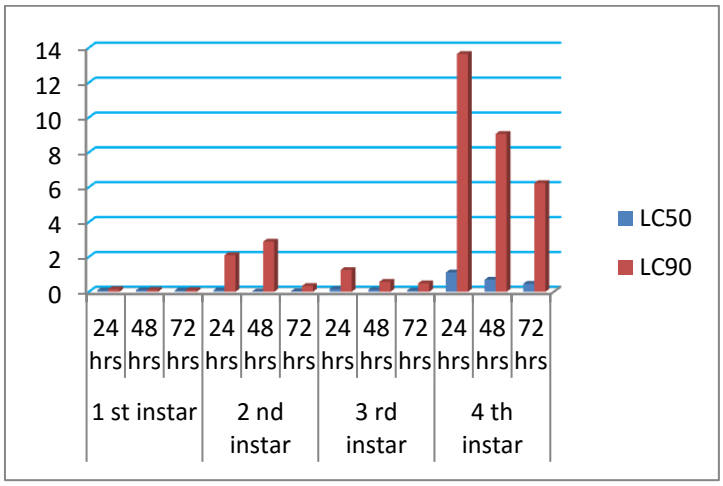

Fig. 1. Graphical representation of LC_{50} and LC_{90} values (for crude root extract)

Table 3. Mortality percent of third instars larvae of *Culex quinquefasciatus* exposed to different concentrations of different solvent extracts of roots of *Elettaria cardamomum*

Solvent extracts	Concentration (ppm)	Mortality % at different exposure periods (Mean mortality % ± Standard Error		
		24 hrs	48 hrs	72 hrs
	30	30.00±5.77	46.67±3.33	53.33±3.33
Petroleum ether	40	50.00±5.77	56.67±3.33	66.67±3.33
	50	50.00±5.77	73.33±8.82	96.67±3.33
Hexane	30	16.67±3.33	23.33±3.33	36.67±3.33
	40	26.67±3.33	30.33±0.00	43.33±3.33
	50	36.67±3.33	53.33±3.33	60.00±0.00
Ethyl acetate	30	6.67±3.33	16.67±3.33	23.33±3.33
	40	23.33±3.33	26.67±3.33	33.33±3.33
	50	33.33±3.33	33.33±3.33	46.67±3.33

Control: *No mortality*

Table 4. Log probit and regression analyses of different solvent extracts of roots of *Elettaria cardamomum* on third instar larvae of *Culex quinquefasciatus*

Solvent extracts	Periods (hrs)	LC_{50}(ppm)	LC_{90}(ppm)	Regression equations	R^2- values
Petroleum ether	24	44.1366	127.2691	Y =-0.2222+0.1167X	0.5034
	48	32.9095	85.3049	Y =0.5556+0.1333X	0.6316
	72	30.4721	48.4337	Y =1.4444+0.2167X	0.8926
Hexane	24	66.1520	187.9920	Y =1.3333+0.1000X	0.7500
	48	50.1160	112.7218	Y =2.4444+0.1500X	0.8322
	72	42.2191	130.1251	Y =-0.0000+0.1167X	0.8167
Ethyl acetate	24	60.2940	113.3823	Y =-3.2222+0.1333X	0.8276
	48	67.9528	192.4406	Y =-1.4444+0.1000X	0.5870
	72	58.8050	163.7768	Y =-1.5556+0.1167X	0.6336

R^2= Coefficient of determination; LC= Lethal Concentration; Y= Mortality; X= Concentration

Fig 2 depicted the graphical representation of LC_{50} and LC_{90} values of larvicidal activity (third instar larvae) by different solvents root extracts of *E. cardamomum*. LC_{50} and LC_{90} values gradually decreased in time.

Table 5 showed completely randomized three ways ANOVA analyses using instars, hours, and Concentrations (crude root extract of the plant) as independent variable and per cent mortalities as dependent variable. Larval mortality ($p<0.05$) showed

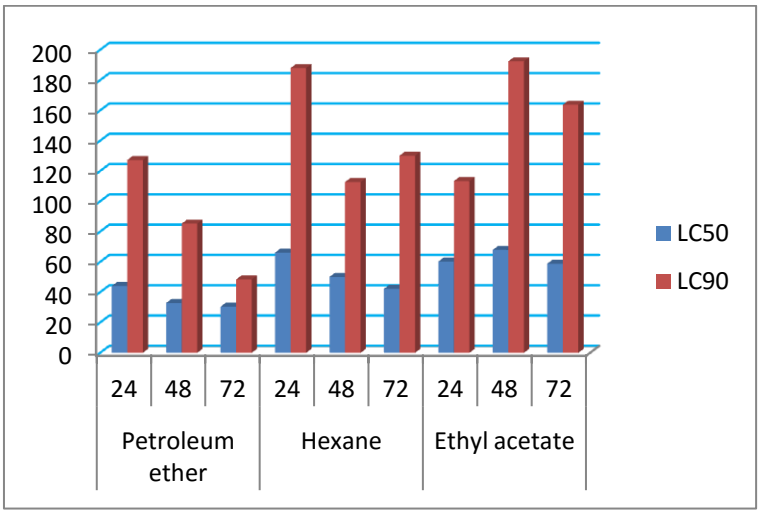

Fig. 2. Graphical representation of LC_{50} and LC_{90} values (for different solvents root extracts)

Table 5. Completely randomized three way ANOVA analyses using instars (I) of *Cx. quinquefasciatus*, hour (H), and Concentration of crude root extract of *Elettaria cardamomum* (C) as three independent parameter

Source of variation	Sum of squares(SS)	Degree of freedom(df)	Mean of squares(MS)	F value	p-level
Instars(I)	968.9056	3	322.9685	747.4414	0*
Time(H)	54.7444	2	27.3722	63.3471	0*
Conc.(C)	145.8556	5	29.1711	67.5103	0*
I×H	10.4111	6	1.7352	4.0157	0.0011*
I×C	31.4556	15	2.097	4.8531	0*
H×C	3.7278	10	0.3728	0.8627	0.5702(N.S)
I×H×C	6.5611	30	0.2187	0.5061	0.9828(N.S)
Within groups	46.6667	108	0.4321		
Total	1268.3278	179	7.0856		

** = Significant at p<0.05; N.S. = Not Significant*

statistical significance in terms of concentration (crude root extract), instar, and time of exposure. But larval mortality showed insignificance value in terms of interaction of hour and concentration and also showed insignificance value in terms of interaction of instar, hour and concentration.

Table 6. Result of preliminary phytochemical analyses of root extracts of *Eleteria cardamomum*

Phytochemicals	Presence(+)/Absence(-)
Alkaloids	+
Terpenoids	+
Steroids	+
flavonoids	+
Tannins	+
phenols	+
Saponins	+

Table 6 represented the result of preliminary phytochemical analyses of root extracts of *Eleteria cardamomum*. Phytochemical analyses revealed the presence of several secondary metabolites.

Table 7 and Table 8 represented the effect of crude and petroleum ether root extracts on tested non targeted creatures respectively. Non target creatures showed no mortality up to 24, 48, and 72 hrs of exposure with the application of crude as well as petroleum ether root extracts of the plant.

4. DISCUSSION

Crude and petroleum ether root extracts of *E. cardamomum* showed effective larvicidal activity against larvae of *Culex quinquefasciatus* mosquito species. First instar larvae showed 100.00 % mortality only at 0.3% concentration of crude root extract. Second, third, and fourth instar larvae showed highest per cent mortality i.e. 96.66±3.33, 96.66±3.33, and 60.00±5.77% only at 0.5% concentration after 72 hrs of exposure respectively. Among petroleum ether,

Table 7. Effect of crude root extract of *Eletteria cardamomum* on non-targeted creatures

Non target organisms	Exposure time periods		
	24 hrs (M%±SE)	48 hrs (M%±SE)	72 hrs (M%±SE)
Chironomus circumdatus larvae (4th instar)	0.00±0.00	0.00±0.00	0.00±0.00
Tadpoles of Toad	0.00±0.00	0.00±0.00	0.00±0.00

Control: *No mortality; M=mortality; SE= Standard Error*

Table 8. Effect of petroleum ether root extract of *Eletteria cardamomum* on non-targeted organisms

Non target organisms	Exposure time periods		
	24 hrs (M%±SE)	48hrs (M%±SE)	72hrs (M%±SE)
Chironomus circumdatus larvae (4th instar)	0.00±0.00	0.00±0.00	0.00±0.00
Tadpoles of Toad	0.00±0.00	0.00±0.00	0.00±0.00

Control: *No mortality; M=mortality; SE= Standard Error*

hexane, and ethyl acetate solvent root extract, petroleum ether extract showed the best result. LC_{50} values of petroleum ether, hexane, and ethyl acetate solvent root extracts were 44.14, 66.15, and 60.29 ppm respectively after 24 hrs of exposure. Plants produce several secondary metabolites. These secondary metabolites may be responsible for this larvicidal activity. Petroleum ether is completely a non polar solvent and this solvent root extract contained several non polar compounds which may be the cause for this larvicidal activity. Crude root extract also contained several secondary metabolites which are mainly polar in nature and these polar chemical compounds may cause this larvicidal activity. Many researchers worked with petroleum ether and crude extracts of roots and other plant parts against *Cx. quinquefasciatus* and other species of mosquitoes. Mallick and Chandra [30] worked with crude and petroleum ether, hexane, and ethyl acetate solvent root extracts of *Annona reticulata* L. plant against larvave of *Cx. quinquefasciatus* mosquito up to 24, 48, and 72 hrs of exposure. They observed that first, second, third, and fourth instar larvae showed 100.00, 83.33, 83.33 and 60.00 % mortality by crude root extract only at 0.05% concentration after 24 hrs of exposure and petroleum ether, hexane, and ethyl acetate root extracts showed LC_{50} values 10.5, 9.7, 0.9 ppm concentrations after 24 hrs of exposure against third instar larvae. Kumar et al. [31] worked with petroleum ether and ethyl acetate extract of dried whole plant, *Tephrosia purpurea* (L) Pers. and examine the larvicidal potential against late third or early fourth instar larvae of *Cx. quinquefasiciatus* mosquito. They observed that larvae showed 100 per cent mortality at 250 ppm dose of petroleum ether extract of the plant. Rahuman and Venkateson (2008) [32] experimented on fourth instar larvae (early) of *Cx. quinquefasciatus* and *Aedes aegypti* (*Ae. aegypti*) by the application of petroleum ether, hexane, ethyl acetate, methanol and acetone leaf extracts of five cucurbitaceous plant species viz. *Momordica charantia*, *Coccinia indica*, *Citrullus colocynthis*, *Cucumis sativus* and observed that all extracts showed moderate efficacy in larvicidal activity but petroleum ether leaf extract of *Citrullus colocynthis* showed highest larvicidal efficacy against *Cx. quinquefasciatus* with LC_{50} value 88.24 ppm after 24 hrs of exposure. Petroleum ether, acetone and ethanol leaf extracts of *Tribulus terrestris* were tested against third instar larvae and adult mosquito of *Ae. aegypti*. The petroleum ether extract showed more efficacies with LC_{50} value 64.4 ppm followed by acetone and ethanol leaf extracts having LC_{50} values 173.2 and 376.4 ppm respectively. Ilahi and Ullah [33] worked with different solvent extracts of different parts of *Artemisia vulgaris* L. against third and fourth instar larvae of *Cx. quinquefasciatus* up to 24 hrs of exposure and they observed that LC_{50} values of methanol root extract of the plant was 9141.0 ppm and methanol leaf and stem extracts were 803.2 and 2224.2 ppm respectively. Adhikari et al. [34] worked with crude and petroleum ether leaf extract of *Swietenia mahagoni* to observe its larvicidal activity against *Cx. quinquefasciatus*. They observed that first, second third and fourth instar larvae showed 96.66%, 100%, a 90.00% and 60.00% mortality at 50 ppm dose respectively after 72 hrs of exposure. Nganjiwa et al. [35] worked with ethanol root and leaf extracts of *Eucalyptus globules*, *Balanites aegyptica*, and *Balanites aegyptica* against fourth instar larvae of mosquito species up to 24 hrs of exposure. They recorded that at 10 ppm dose, ethanol root extracts of *Eucalyptus globulus*, *Balanites aegyptica*, *Calotropis procera* showed LC_{50} values 6.92, 7.24, and 6.61 ppm respectively. Sheikh et al. [36] worked with petroleum ether, acetone and ethanol leaf extracts of *Tribulus terrestris* to observe the larvicidal activity against third instar larvae and adult mosquito of *Ae. aegypti*.

The petroleum ether extract showed more efficacies with LC_{50} value 64.4 ppm followed by acetone and ethanol leaf extracts having LC_{50} values 173.2 and 376.4 ppm respectively. Further, tested non target creaturs were non responsive to crude as well as petroleum ether root extracts of *E. cardamomum* which indicated that the tested root extracts are eco-friendly larvicidal agent.

5. CONCLUSION

From the above discussion it may conclude that crude as well as petroleum ether root extracts of *E. cardamomum* (L.) Maton can be used effectively to destroy the different instar larvae of *Cx. quinquefasciatus* Say mosquito species and its uses will be very eco-friendly as experimented non-target organisms did not response to crude as well as petroleum ether root extracts of the plant. Further study is needed to observe its larvicidal activity against other species of mosquitoes. Larvicidal activity of root extract of *E. cardamomum* has been reported first time.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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