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Larvicidal potential of rhizome extracts of *Elettaria cardamomum* (L.) Maton against filarial vector, *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae)

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ABSTRACT: Investigation on the larvicidal potential of *Elettaria cardamomum* (L.) Maton rhizome extracts against filarial vector, *Culex quinquefasciatus* Say, was undertaken with crude concentrations 0.1-0.5 per cent and 40, 50, and 60 ppm of each of petroleum ether, hexane and ethyl acetate rhizome extracts revealed that first instar larvae were most susceptible to crude rhizome extract with 100 per cent mortality at 0.5% after 24 hrs of exposure. Among three solvent extracts, ethyl acetate extract showed maximum mortality (96.66±3.33%) at 60 ppm after 72 hrs of exposure. LC_{50} values of larvicidal bioassays by crude rhizome extract were 0.1002, 0.0794, 0.1275 and 0.6334 ppm for 1st, 2nd, 3rd and 4th instars larvae after 72 hrs of exposure, respectively and LC_{50} values for larvicidal bioassays by petroleum ether, hexane, and ethyl acetate rhizome extracts were 48.3629, 40.9613 and 37.0282 ppm against 3rd instar larvae after 72 hrs of exposure, respectively. Preliminary phytochemical analyses of the rhizome extracts showed presence of secondary metabolites. Non target organisms, tadpoles of frog and 4th instar larvae of *Chironomus circumdatus,* were not affected by the crude as well as ethyl acetate rhizome extracts. Larvicidal efficacy of the rhizome extracts of *E. Cardamomum* against *Cx. quinquefasciatus* mosquito species has been reported first time.

KEY WORDS: Crude extract, solvent extracts, LC_{50} values, phytochemicals, regression

INTRODUCTION

Out of 300 different species of mosquitoes, 100 species act as vectors of several diseases (Rozendaal, 1997; Abou-Enaga, 2014). Mosquitoes transmit of many diseases, viz. dengue, dengue hemorrhagic fever, filariasis, malaria, yellow fever, chikungunia and Japanese Encephalitis (Ghosh *et al*., 2012; Sogan *et al*., 2018). *Culex quinquefasciatus* Say1823 (Diptera: Culicidae) is the vector of lymphatic filariasis disease (Mallick and Chandra, 2015a). In 50 countries of the world, 859 million people become threatened by lymphatic filariasis (WHO, 2021). Synthetic chemical insecticides are used to control mosquito and these insecticides cause the development of resistance in mosquitoes. Natural products of botanical origin have insecticidal efficacy and are bio degradable, safe to the environment, less harm to beneficial insects and are effective to control targeted species (Maharaj *et al*., 2011; Hwang *et al*., 2017; Mdoe *et al*., 2014; Shoukat *et al*., 2016). *Elettaria cardamomum* (L.) Maton, also known as true or green cardamom, is a perennial herbaceous plant.

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It belongs to Zingiberaceae family and is a commercially significant spice. It has anticancer, antioxidant, anti-inflammatory and antimicrobial properties (Alam *et al.*, 2021; Cárdenas Garza *et al.*, 2021). Many researchers experimented on different plants against several species of mosquitoes and unfolded their larvicidal potential (Rawani *et al*., 2010; Hossain *et al*., 2011; Mallick *et al*., 2014; Mallick *et al*., 2015a; Mallick *et al* 2015b; Mallick and Chandra, 2015b; Mallick and Chandra, 2016; Mallick, 2021). The study aims to investigate the larvicidal potential of rhizome extracts of *E. cardamomum* against the larvae of *Cx quinquefasciatus*.

MATERIALS AND METHODS

Rhizomes of *E. cardamomum* were collected from the Medicinal Plant Garden of M.U.C. Women's College, Purba Bardhaman, West Bengal, India ($23^0 16'$ N, $87^0 54'$ E) during the month of August (the monsoon season) and cleaned and subsequently dried on paper towel. Fresh cleaned rhizomes were cut into very small pieces, crushed by the electrical grinder and the juice of rhizomes was filtered through muslin cloth and the filtrate was used as stock test solution (filtrate of rhizome served as 100% concentrated solution). Extracts @ 0.1, 0.2, 0.3, 0.4, and 0.5 per cent concentrations were prepared for bioassays.

For the preparation of different solvent extracts of rhizome, maceration procedure was used (Sharma *et al.,* 2016). Collected cleaned rhizomes were cut into small bits and dried in shade for 14-15 days. Dried small pieces of rhizomes were ground in electric blender and thereafter sieved for obtaining powder material. Petroleum ether, hexane and ethyl acetate, were used to obtain solvent extracts. 50 g of powder material of rhizomes was socked first in 500 ml of petroleum ether, kept in a bottle and thereafter closed the mouth of the bottle tightly for a period of near about 15 days with frequent agitation daily. Final petroleum ether extract of rhizomes was filtered by What Man 42 no. filter paper and the filtrate extract was concentrated by evaporation, to obtain the semi-solid extract and kept in a refrigerator at 4^0 C for further bioassays. After obtaining petroleum ether extractive of rhizome, the same plant material of rhizomes was soaked successively in hexane and thereafter in ethyl acetate for a period of near about 15 days each, with frequent agitation daily. After filtering each of the hexane and ethyl acetate rhizome extracts was concentrated by evaporation and the semi-solid extract of hexane and ethyl acetate kept in a refrigerator at 4^0 C for bioassay experiments.

Preparation of graded concentrations of different semi-solid solvent extracts: After preliminary trialing, 40, 50, and 60 ppm graded concentrations of each of petroleum ether, hexane and ethyl acetate extract of rhizome, were prepared for larvicidal bioassay experiments. From each of semi-solid aforesaid solvent extracts of rhizomes, stock solutions were prepared on 5 per cent ethanol, separately. Petroleum ether, hexane and ethyl acetate rhizome extracts @ 0.1 g of each dissolved separately in 1 ml of ethanol and thereafter added 19 ml distilled water to get stock test solutions (5000 ppm) of the said different solvent extracts. From stock test solutions, graded concentrations of each of different solvent extracts of rhizome were prepared, by taking required volume of stock test solution of each solvent extract and mixing by required volume of water, to get different concentrations test solutions for larvicidal bioassays.

Test mosquito species: Larvae of *Cx. quinquefasciatus* mosquito were collected. Larvae of different instars were kept in a plastic tray with water. Larvae were provided powdered mixture of dog biscuits and dried yeast powder (ratio 3:1). Larval colonies were maintained at 27 ± 2^0 C temperature and 80-85 per cent relative humidity. Larvae transformed into pupae, and near about 200 pupae were transferred to two separate plastic bowls (225 ml capacity) containing water, and thereafter two plastic bowls with pupae were kept in a mosquito cage (30×30×30 cm) where adult mosquitoes emerged. Ten per cent glucose solution in a plastic bowl with a cotton wick was kept in the cage for adult mosquitoes feeding. On day five adults were provided with blood meal from restrained pigeon. Two plastic bowls with 100 ml water were kept in the cage for oviposition. F1 generation larvae were used for larvicidal bioassays.

Larvicidal bioassays were conducted according to standard protocol of WHO with suitable modifications (WHO, 2005). All instars larvae were used during bioassays with crude extract of rhizomes. Twenty larvae were put in different plastic bowls (225 ml capacity), each containing 100 ml of test solution of different doses of crude extract (0.1-0.5%), to investigate the percent mortalities of larvae. Negative control experiments were set on 100 ml of tap water only. Only 3rd instar larvae of *Cx. quinquefasciatus* were used for larvicidal bioassays with the different solvent extracts. Twenty larvae were put in plastic bowls, each containing 100 ml of test solution of different doses (viz., 40, 50, and 60 ppm) of each solvent extract. Ethanol treated control experiments (ethanol treated) were set on 100 ml of water with 0.5 ml of ethanol. Each set of experiment for crude as well as different solvent extracts was replicated three times, including three replicates of control experiments (for crude as well as solvent extracts) on separate three days. The percent mortalities were noted after 24, 48 and 72 hrs of post exposure cumulatively. Larvae were detected dead when the larvae were unable to move after touching their body with a fine brush.

Phytochemicals: Ethanol and water extracts of dried rhizomes were used for phytochemical analyses through standard protocols (Trease and Evans, 1989; Sofowara, 1993; Harborne, 1984) with modifications.

a) Alkaloids test (Mayer's test) **-** Five ml of ethanol extract was taken in a test tube to which 2 drops of 2N HCL and thereafter 2 drops of Mayer's reagent were added. The resultant pale yellow color precipitation indicated the presence of alkaloids.

b) Terpenoids test (Salkowski test) - 5 ml of ethanol extract was taken in a test tube and thereafter 5 ml of chloroform and 1 ml of concentrated H_2SO_4 were added carefully to it. The reddish brown coloration at the interface indicated the terpenoids.

c) Steroids test - 5 ml of ethanol extract was taken in a test tube and thereafter 2 ml of concentrated H_2SO_4 was gently added to it. The resultant brown color ring indicated steroids.

d) Flavonoids test **-** 5 ml of water extract was taken in a test tube and treated with 1 ml of NaOH solution. Intense color was observed and it became colorless when dilute HCL to it added, indicating the presence of flavonoids.

e) Tannins and phenol compounds test **-** 5 ml of water extract was taken in a test tube and 4-5 drops of ferric chloride were added to it. The occurrence of blue green coloration indicated tannins and phenol compounds.

f) Test of saponins (frothing test) - 8 ml of aqueous extract was taken in a test tube and was shaking vigorously. The persistence of frothing indicated the presence of saponin.

Effect of crude and ethyl acetate rhizomes extracts on non-target organisms: Tadpoles of frog and 4th instar larvae of *Chironomus circumdatus* were chosen as they live in the same habitat of mosquito larvae and were tested with appropriate lethal concentrations i.e. LC_{50} value (dose) of crude as well as ethyl acetate rhizome extracts, as per protocol, described by Mallick *et al.* (2016a) with modification. Twenty tadpoles of frog and twenty 4th instar *C. circumdatus* larvae were released in each of two beakers (each beaker of 500 ml contained 200 ml of pond water of aforesaid doses of crude rhizome extract), and the similar experiments for ethyl acetate rhizome extract was conducted. Each experiment for crude as well as ethyl acetate extracts was folded thrice on separate three days. Concurrently, negative control experiments with 200 ml of pond water only and ethanol treated control experiments with 199.5 ml of pond water with 0.5 ml of ethanol were run parallel. Mortality of non-target creatures was noted for a period of 24, 48, and 72 hrs of exposures, cumulatively.

Computer software, 'STAT PLUS-2009' – trial version and MS EXCEL-2007, was used to calculate the LC_{50} and LC_{90} values through Log probit analyses, as well as for R^2 (coefficient of determination), regression equation, mean per cent mortality, standard error, and ANOVA analysis.

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

Instars	Conc. (%)	Mortality % at different exposure periods (Mean mortality % ± Standard Error		
		24 h	48 h	72 h
1st	0.1	36.66±3.33	43.33±3.33	53.33±3.33
	0.2	60.00±5.77	70.00±5.77	76.66±3.33
	0.3	73.33±6.67	86.66±3.33	96.66±3.33
	0.4	86.66±6.67	93.33±3.33	96.66±3.33
	0.5	100.00±0.0	100.00±0.0	100.00±0.0
2nd	0.1	26.66±3.33	46.66±3.33	56.67±3.33
	0.2	40.00±5.77	50.00±0.00	63.33±3.33
	0.3	50.00±5.77	63.66±3.33	73.33±3.33
	0.4	56.66±3.33	66.67±3.33	76.66±8.82
	0.5	70.00±5.77	76.66±3.33	83.33±3.33
3rd	0.1	26.66±8.82	43.33±3.33	50.00±5.77
	0.2	30.00±5.77	46.67±3.33	53.33±3.33
	0.3	33.33±6.67	50.00±5.77	56.66±3.33
	0.4	43.33±8.82	56.67±3.33	63.33±3.33
	0.5	46.66±8.82	60.00±5.77	73.33±3.33
4th	0.1	10.00±0.00	16.66±3.33	23.33±3.33
	0.2	13.33±3.33	23.33±3.33	33.33±3.33
	0.3	16.66±3.33	30.00±0.00	36.66±3.33
	0.4	23.33±3.33	36.66±3.33	43.33±3.33
	0.5	26.66±3.33	40.00±5.77	46.66±3.33

No mortality in control (for all instars)

RESULTS AND DISCUSSION

In the larvicidal activity of crude rhizome extract, mortality increased with the increase in concentration and time of exposure in all the instars. First instar larvae were most susceptible to crude rhizome extract and showed cent percent mortality at 0.5 per cent after 24 hrs of exposure. No larval mortality was observed on the negative control experiments (Table 1). Crude rhizome extract experiments showed, gradually decreasing LC_{50} and LC_{90} values in time for different larval instars. R^2 values in almost all cases showed close to one which denotes that mortality percent was strongly correlated with the dose of the crude extract (Table 2). In the larvicidal efficacy experiments of petroleum ether, hexane and ethyl acetate rhizome extracts, the mortality increased with the increase in concentration and time of exposure. Among the three solvent rhizome extracts, ethyl acetate extract showed maximum mortality after 72 hrs of exposure. Ethanol treated control experiments did not show any mortality (Table 3). LC_{50} and LC_{90} values gradually decreased in time against 3rd instar larvae in the different solvent extracts. R^2 values are close to one in almost all cases. Among three solvent extracts, ethyl acetate extract showed low LC_{50} and LC_{90} values after 24, 48 and 72 hrs of exposure. Among three solvent extracts, the ethyl acetate extract showed as the most potent larvicide (Table 4). Crude rhizome extract of *E. cardamomum* showed great efficacy in larval mortality against filarial vector, *Cx. quinquefasciatus*. First instar

Table 2. Log probit and regression analyses of larvicidal activity of crude rhizome extract of *Elettaria cardamomum* against different larval instars of *Culex quinquefasciatus*

Instars	Periods(h)	LC_{50}(%)	LC_{90}(%)	Regression equations	R^2- values
1st	24	0.1483	0.4265	Y = 25.3280+153.3400 X	0.9916
	48	0.1215	0.3204	Y =37.6630+136.6700X	0.9527
	72	0.1002	0.2483	Y = 50.6600+113.3400X	0.9049
2nd	24	0.2767	1.8879	Y =17.6620+103.3400X	0.9943
	48	0.1428	2.2635	Y =36.9930+76.6700X	0.9237
	72	0.0794	1.1905	Y =50.6610+66.6700X	0.9901
3rd	24	0.7531	31.0841	Y =19.9970+53.3300X	0.9774
	48	0.2289	31.7545	Y =37.9940+46.6800X	0.9615
	72	0.1275	5.7803	Y = 42.3320+56.6600X	0.9686
4th	24	2.3693	47.7411	Y =5.0000+43.3200X	0.9912
	48	1.0482	28.9825	Y = 11.3270+60.0100X	0.9939
	72	0.6334	16.3633	Y =19.6640+56.6600X	0.9815

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

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

Solvent extracts	Conc. (ppm)	Mortality % at different exposure periods (Mean mortality % ± Standard Error		
		24 h	48 h	72 h
Petroleum ether	40	23.33 ±3.33	33.33±3.33	40.00±5.77
	50	33.33±3.33	43.33±3.33	50.00±5.77
	60	46.66±.6.67	56.66±6.67	63.33±8.82
Hexane	40	30.00±0.00	40.00±.5.77	50.00±5.77
	50	43.33±3.33	53.33±3.33	60.00±5.77
	60	66.66±3.33	70.00±5.77	76.66±8.28
Ethyl acetate	40	46.66±3.33	53.33±3.33	63.33±3.33
	50	60.00±3.33	70.00±0.00	73.33±3.33
	60	83.33±3.33	90.00±0.00	96.66±3.33

No mortality in control

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

Solvent extracts	Periods (h)	LC_{50}(ppm)	LC_{90}(ppm)	Regression equations	R^2- values
Petroleum ether	24	64.0288	143.4722	Y=23.8850+1.1665X	0.9966
	48	54.4058	130.2267	Y= 13.8850+1.1665X	0.9966
	72	48.3629	116.9358	Y=7.2150+1.1665X	0.9966
Hexane	24	51.2659	88.8038	Y=44.9867+1.8330X	0.9878
	48	46.3743	91.1377	Y=20.5567+1.5000X	0.9979
	72	40.9613	85.3110	Y=4.4300+1.3330X	0.9898
Ethyl acetate	24	42.4847	71.2085	Y=28.3450+1.8335X	0.9879
	48	39.5602	62.5172	Y= 20.565+1.8335X	0.9986
	72	37.0282	56.6899	Y=5.5517+1.6665 X	0.9744

R^2= Coefficient of determination; LC= Lethal Concentration

Y= Mortality; X= Concentration

larvae were most susceptible to crude rhizome extract of the plant. First instar larvae showed cent per cent mortality only at 0.5 per cent dose after 24 hrs of exposure. Second, third, and fourth instars larvae showed maximum mortality, 83.33±3.33, 73.33±3.33, and 46.66±3.33 per cent respectively after 72 hrs of exposure. First, second, third, and fourth instars larvae showed LC_{90} values 0.4265, 1.8879, 31.0841and 47.7411 per cent respectively after 24 hrs of exposure.

Patel *et al.* (2018) worked with crude leaf as well as berry extracts of *Solanum nigrum* to investigate the larvicidal activity against dengue vector, *Aedes aegypti* and observed that fourth instar larvae showed 100 per cent mortality at 5 per cent dose after 48 hrs of exposure and fourth instar larvae showed maximum percent mortality (52.33 and 99.33% at 5 % dose by the effect of crude green berry and crude black berry extracts, respectively, after 72 hrs of exposure). Rawani *et al.* (2013) reported larvicidal activity of crude *S. niagram* crude berry extract against *Cx. quinquefasciatus* at 3 per cent dose. Mallick Halder *et al.* (2011) reported cent per cent mortality of first instar larvae of *Cx. quinquefasciatus* with crude and methanol leaf extracts of *Typhonium trilobatum* at 0.4 per cent after 72 hrs of exposure.

In the present study, ethyl acetate extract showed the highest percent mortality among three solvent rhizomes extracts having LC_{50} value 37.0282 ppm against 3rd instar larvae after 72 hrs of exposure followed by petroleum ether (LC_{50} value 40.9613 ppm) and hexane (LC_{50} value 48.3629 ppm) rhizome extracts of the plant. Many researchers experimented with ethyl acetate extract of the different plants against larvae of *Cx. quinquefasciatus* mosquito species to observe their larvicidal activity. Kamaraj *et al.* (2010) worked with hexane, ethyl acetate and methanol extracts of *Zingiber zerumbet* L., *Gymnema sylvestre* (Retz) Schult, *Cassia angustifolia* Vahl, *Mimosa pudica* L., *Aristolochia indica* L., *Diospyros melanoxylon* Roxb., *Dolichos biflorus* L., and *Justicia procumbens* L. against early 4th instar larvae of *Cx. quinquefasciatus* Say and *Cx. gelidus* Theobald and reported highest larval mortality in ethyl acetate extract of *D. biflorus* against *Cx. quinquefasciatus* Say having LC_{50} and LC_{90} values 34.76 and 172.78 ppm, respectively, after 24 hrs of exposure. Kumar *et al.* (2012) observed cent per cent mortality of late third or early fourth instar of *Cx. quinquefasiciatus* at 250 ppm and 300 ppm doses of petroleum ether and ethyl acetate extracts of dried whole plant, *Tephrosia purpurea* (L) Pers. Mallick and Chandra

(2015c) reported 100 per cent mortality at 5 ppm with ethyl acetate leaf extract of *Annona reticulata* L. against 3rd instar larvae of *Cx. quinquefasciatus*, having LC_{50} and LC_{90} values 1.4556 and 6.6383 ppm after 48 hrs of exposure. Jayaraman *et al.* (2015) investigated hexane, choloroform, ethyl acetate, acetone and methanol extracts of seven aromatic plants against *Cx. quinquefasciatus, Ae. aegypti, and Anopheles stephensi* for 12 and 24 hrs of exposure periods and showed various levels of larvicidal activity but ethyl acetate extract of *Chloroxylon swietenia* showed the remarkable larvicidal activity; Larvae of *Cx. quinquefasciatus* showed LC_{50} and LC_{90} values 194.22 and 458.83 ppm, respectively, after 12 hrs of exposure. Bagavan *et al.* (2018) examined on hexane, ethyl acetate, chloroform, methanol and acetone extracts of leaves of the medicinal plants viz. *Leucas aspera*, *Acalypha indica*, *Ocimum sanctum, Achyranthes aspera*, and *Morinda tinctoria* against the early fourth instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti* for a period of 24 hrs of exposure, and they observed that ethyl acetate leaf extract of *Achyranthes aspera*s showed the highest larvicidal potential against both species. Other extracts showed moderate larvicidal efficacy. Saponin compound was isolated from ethyl acetate leaf extract of *A. aspera*s which showed LC_{50} values 18.20 and 27.24 ppm against *Ae. aegypti* and *Cx. quinquefasciatus* respectively.

In the completely randomized three way ANOVA analyses, using instars, hours and concentrations as independent variables and mortality percentage as dependent variable, showed statistical significance in larval mortality (F value = 6.1551 $p<0.05$) in terms of doses of crude rhizome extract, instars of *Cx. quinquefasciatus* and of time of exposure.

Preliminary phytochemical analyses of rhizome extracts of *E. cardamomum* revealed the presence of alkaloids, terpenoids, steroids, flavonoids, tannins, phenols and saponins in the of rhizome extracts. Among three solvent extracts, ethyl acetate extract was most potent in larval mortality. Petroleum ether and hexane are highly non-polar solvents and only non-polar phytocompounds may be present in petroleum ether and hexane rhizome extracts which may cause larval mortality, but the polarity of ethyl acetate is medium. Due to its medium polarity nature both polar and non-polar phytocompounds may be present in ethyl acetate rhizome extract which may cause larval mortality. Due to this reason, ethyl acetate rhizome extract showed larval mortality among the solvent extracts.

The test on the effect of crude and ethyl acetate rhizome extracts on non-target organisms indicated that there were no mortality and abnormal behaviour on frog tad poles and 4th instar larvae of *C. circumdatus* up to 72 hrs of exposure. Non-target organisms were non responsive to crude as well as ethyl acetate rhizome extracts, so their uses for controlling mosquito population will be safer.

There are preliminary phytochemical investigation of several plant extracts and mosquito larvicidal activity along with the observation of the effects on non-target organisms (Singha *et al*., 2011; Singha *et al*., 2012; Mallick *et al*., 2016a; Mallick et al., 2016b). In recent times, the insecticides of plant origin have been given more importance for their bio-degradable, nontoxic, and ecofriendly nature. Mosquito larvicidal property of *E. cardamomum* rhizome (ethyl acetate and crude extracts) has been unfolded for the first time and the findings of the present study revealed that crude as well as ethyl acetate rhizome extracts of *E. cardamomum* showed larvicidal potential against *Cx. quinquefasciatus*.

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