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RESEARCH ARTICLE

MOSQUITOCIDAL EFFICACIES OF MEDICINAL PLANT OF *COLEUS AROMATICUS* BENTH (LAMIACEAE) LEAF EXTRACTS CHIKUNGUNYA VECTOR, *AEDES AEGYPTI* (LINN.) (DIPTERA:CULICIDAE)

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Abstract

The mosquitocidal activity of *Coleus aromaticus* (*C. aromaticus*) leaf extracts against larvae of *Aedes aegypti* was investigated in the present study. The larvicidal activity were carried out as said by the recommendations of the WHO 2005; the 24 hrs LC_{50} values of the *C. aromaticus* leaf extract was determined by Probit analysis and ovicidal activity, to some extent customized method of Su and Mulla was performed. The ovicidal activity was resolute against *Ae*. *aegypti* to a variety of concentrations ranging from 40 - 200 ppm lower than the laboratory conditions. The egg hatch rates were assessed 24 hrs placement treatment. For repellency activity was definite against *Ae*. *aegypti* mosquito species at three concentrations *viz*., 1.0, 2.0 and 3.0 mg/cm^2 beneath the laboratory conditions and carried out according to the recommendations of the WHO 2009. The diethyl ether extract of *C. aromaticus* establish to more repellency than the additional extracts. A better-quality concentration of 3.0 mg/cm^2 provided 100% defense up to 120 minutes, the lower concentrations of 2.0 and 1.0 mg/cm^2 provided 100% defense up to 80 and 40 minutes, respectively. The domino effect clearly shows that larvicidal, ovicidal and repellent activity was dose reliant. From the results, it can be concluded the diethyl ether extract of *C. aromaticus* was an outstanding potential for controlling the dengue vector mosquito *Ae*. *aegypti*.

Keywords: *Coleus aromaticus,* Larvicidal, Ovicidal and Repellent acitivity, *Aedes aegypti.*

Introduction

Mosquito borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases (De Omena, 2007). A recent estimate shows that more than 50 million people are at risk of dengue virus exposure worldwide. Annually, there are 2 million infections, 5, 00, 000 cases of hemorrhagic fever, and 12, 000 deaths (Guha-sapir and Schimme, 2005). Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, *etc*., causing millions of deaths every year. *Aedes aegypti* (*Ae. aegypti*), a vector of dengue is widely distributed in the tropical and subtropical zones. Dengue fever incidence has increased fourfold since 1970 and nearly half the world's population is now at risk. *Ae. aegypti* is also the vector of dengue hemorrhagic fever, which is endemic to South East Asia, the Pacific islands area, Africa and the America (Senthilkumar *et al.,* 2007). Indeed, the present recrudescence of these diseases is due to the higher number of breeding place in today's throwaway the public and also increasing resistance of mosquitoes to current commercial insecticides. Although yellow fever has been reasonably brought under control with its vaccine, no vaccine is available for dengue. The only way of decreasing the incidence of this disease is thus the eradication of *Ae. aegypti* (Ravikumar *et al.,* 2011).

Currently, most insecticides are non-selective and can be harmful to other organisms and to the environment. There is an urgent need to develop new materials for controlling mosquitoes in an environmentally safer way, using biodegradable and target-specific insecticides against them (Isman, 2006; Pavela, 2007; Jawale *et al.,* 2010). Experience has shown that, aerial toxicants for the eradication of this mosquito are not effective, since it is highly domesticated and many adults rest indoors in hidden places such as closets. The only successful way of reducing mosquito densities to a level where dengue fever epidemics do not occur is by attacking the larval breeding places (Knio *et al.,* 2008). Bioactive organic compounds produced by plants can act as repellent, oviposition or food deterrents, growth inhibitors and toxins (Ezeonu *et al.,* 2001; Carlini and Grossi-de-Sa, 2002). *Coleus* species are found as herbs, sub shrubs or shrubs. They are often succulent with opposite leaves. Inflorescence is terminal or in the upper leaf axils and flowers are in compact *Cymose clusters* (Khare, 2007). It is known to possess antimicrobial (Murthy *et al.,* 2009; Ragasa, *et al.,* 1999; Pritima, *et al.,* 2008) antiepileptic, leishmanial and antioxidant activities (Gurgel, *et al.,* 2009; Buznego and Perez-saad 1999). Therefore, this study provides fist report on the mosquito larvicidal activity effect of *C. aromaticus* leaf extract against *Ae. aegypti* as target species.

Material and Methods

Collection and Identification of Plant material

Plant sampling was carried out during the growing season (October - November) of 2013 from different places of Karaikal region, Puducherry (UT). Bulk samples were air-dried in the shade and after drying each sample was ground to a fine powder. At the time of collection, two pressed voucher herbarium specimens were prepared per species and identified with the help of Plant Taxonomist, Department of Botany, Annamalai University, whenever possible, flowering or fruiting specimens were collected to facilitate taxonomic identification.

Extraction method

The dried leaves (100 g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with diethyl ether, hexane, benzene and acetone (500 ml, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure of 22-26 mm Hg at 45^{o}C by 'Rotavapour' and the residue obtained was stored at 4^{o}C by "Rotavapour' and the residue obtained was stored at 4^{o}C.

Mosquito rearing

Eggs of *Ae. aegypti* were collected from ICMR center, Virudachalam. The egg rafts were then brought to the laboratory. The eggs were placed in enamel trays (30 cm × 24 × cm × 5 cm) each containing 2 L of tap water and kept at room temperature (28 ± 2^{o}C) with a photoperiod of 16:8 hrs (L:D) for larval hatching. The larvae of each mosquito species were maintained in separate trays under the same laboratory conditions and fed with a powdered feed containing a mixture of dog biscuit and baker's yeast (3:1 ratio). The trays with pupae of each mosquito species were maintained in separate mosquito cages at (26 ± 2) ^{o}C and relative humidity of (85 ± 3)% under a photoperiod of 16:8 h (L:D) for adult emergence. Cotton soaked in 10% aqueous sucrose solution in a Petri dish to feed adult mosquitoes was also placed in each mosquito cage. An immobilized young chick was placed for 3 h inside the cage in order to provide blood meal especially for female mosquitoes. A plastic tray (11 cm×10 cm ×4 cm) filled with tap water with a lining of partially immersed filter paper was then placed inside each cage to enable the female mosquitoes to lay their eggs. The eggs obtained from the laboratory-reared mosquitoes were immediately used for toxicity assays or allowed to hatch out under the controlled laboratory conditions described above. Only the newly hatched larvae / pupae of *Ae. aegypti* were used in all bioassays.

Larvicidal activity

The larvicidal activity of crude extract was evaluated as per the protocol previously described by WHO (2005). From the stock solution, six different test concentrations (40, 80, 120, 160, and 200 mg/L) were prepared and tested against the freshly moulted (0–6 h) III instar larvae of *Ae. aegypti*. The test medium (500 ml plastic cups) was prepared by adding 1 ml of appropriate dilution of test concentrations and mixed with 249 ml of dechlorinated water to make up 250 ml of test solution. The larvae were fed with dry yeast powder on the water surface (50 mg/l). The control (without plant extracts) experiments were also run parallel with each replicate. For each experiment, six

replicates were maintained at a time. A minimum of 25 larvae per concentration was used for all the experiments. The larval mortality was observed and recorded after 24 h post-treatment. Percent mortality was corrected for control mortality using probit analysis (Abbot 1925).

Ovicidal activity

The method of Su and Mulla (1998) was slightly modified and used to test the ovicidal activity. The various concentrations as stated in the previous experiments were prepared from the stock solution. Before treatment, the eggs/eggs raft of *Ae. aegypti* were counted individually with the help of hand lens. Freshly hatched eggs (100) were exposed to DMSO in water served as control. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under a microscope. Each test was replicated five times. The hatchability was assessed 24 h post treatmment.

$$\% \text{ Mortality} = \frac{\text{Mortality at treatment-Mortality at control}}{\text{Mortality at control}} \text{ X } 100$$

Repellent activity

The repellent study was following the methods of world health organization (2009). 3-4 days old blood - starved female, *Ae. aegypti* mosquito (100) was kept in a net cage (45×45×40cm). The volunteer had no contact with lotion, perfumes or perfumed soaps on the day of the assay. The arms of the test person were cleaned with isopropanol. After air drying the arm only 25cm^2 of the dorsal side of the skin on each arm was exposed, the remaining area being covered by rubber gloves. The selected medicinal plant leaf extract at 1.0 to 3.0 mg/cm^2 concentration was applied. The control and treated arms were introduced simultaneously into the cage. The numbers of bitted were counted over 5 min every 30 min and the experiment were conducted five times. It was observed that there was no skin irritation from the plant extract. The percentage of repellency was calculated by the following formula.

$$\% \text{ Repellency} = [(T_a\text{-}T_b)/T_a] \times 100$$

Where T_a is the number of mosquitoes in the control group and T_b is the number of mosquitoes in the treated group.

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL), regression, chi-square, mean and standard deviation values were calculated using the SPSS (Statistical Package of Social Sciences) 12.0 software. The LC_{50} and LC_{90} value were calculated by using Probit Analysis (Finney 1971). Results with $p<0.05$ were considered to be statistically significant.

Results

In the preliminary screening of botanical extracts, result reveals that the plant extract of *C. aromaticus* was effective compared with the other solvent extracts. Therefore our present study was aimed to evaluate the efficacy of *C. aromaticus* leaf extract against the selected vector mosquito *Ae. aegypti*. The effect of leaf diethyl ether, hexane, benzene and acetone extracts *C. aromaticus* are tested at 40-200 ppm and showed larvicidal activity against the larvae of *Ae. aegypti* are presented in Table 1. The plant extracts showed moderate larvicidal effects after 24 h. The experiments conducted for evaluating larvicidal efficacy of leaf of *C. aromaticus.* Among the extracts tested, the highest larvicidal activity was observed in diethyl ether extract of *C. aromaticus* against *Ae. aegypti* with the LC_{50} and LC_{90} values being 73.49 and 80.16 ppm, respectively. The data is statistically significant at $P < 0.05$. The mean percent of egg hatchability of *Ae. aegypti* are tested with four different solvents at different concentrations of *C. aromaticus* leaves extracts, and the results are listed in Table 2. Among the extracts tested for ovicidal activity against *Ae. aegypti,* the diethyl ether extract of *C. aromaticus* exerted 100% mortality (*i.e.,* no hatchability was recorded; Table 2) at 120, 160 and 200, respectively. Control eggs showed the 100% hatchability. The repellent activity of the leaf extracts *C. aromaticus* showed repellent against *Ae. aegypti,* in Table 3. A higher concentration of 3.0 mg/cm^2 provided 100% upto 120, 160 and 200 min against *Ae. aegypti,* respectively.

Table 1. Larvicidal activity of the *C. aromaticus* extracts against *Ae. aegypti*

Name of extract	Concentration (mg/L)	%mortality ± SD	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	Regression	*Chi*-square
	40	33.6±4.15				
	80	60.8±2.94	73.49	80.16		
Diethyl ether	120	82.4±3.28	(71.78-75.24)	(78.45-81.89)	Y=34.00x-58.45	6008.2
	160	98.2±1.78				
	200	100.0±0.0				
	40	13.2±2.58				
	80	15.4±3.57	85.93	99.25		
Hexane	120	28.4±2.50	(83.12-88.83)	(96.38-102.20)	Y=20.47x-34.60	1884.2
	160	41.6±3.18				
	200	56.4±2.50				
	40	24.2±2.68				
	80	51.8±2.96	76.03	82.68		
Benzene	120	71.4±2.60	(74.38-77.72)	(81.04-84.36)	Y=35.17x-61.15	6012.3
	160	92.8±3.42				
	200	98.6±1.94				
	40	16.4±2.70				
	80	27.6±2.30	80.56	89.82		
Acetone	120	40.8±2.94	(78.44-82.75)	(87.69-92.01)	Y=27.12x-46.69	3533.8
	160	61.2±2.77				
	200	76.2±2.28				

Significant at P<0.05 level

Table 2. Ovicidal activity of the *C. aromaticus* extracts against *Ae. Aegypti*

Plant extract	Percentage of egg hatchability					
	Concentration used (ppm)					
	Control	40	80	120	160	200
Diethyl ether	100±0.0	55.8±3.4	29.4±2.6	NH	NH	NH
Hexane	100±0.0	88.6±2.5	67.6±1.9	53.6±2.1	36.8±1.6	16.4±1.8
Benzene	100±0.0	59.8±2.2	41.6±2.6	19.6±2.3	NH	NH
Acetone	100±0.0	78.4±2.3	59.4±1.8	41.2±2.7	19.8±2.2	NH

Values represents mean of five replications.

Table 3. Repellent activity of the *C. aromaticus* extracts against *Ae. aegypti*

Plant extract	Concentration (mg/cm^2)	% of repellency					
		30	40	80	120	160	200
	1.0	100±0.0	100±0.0	95.4±3.5	86.8±1.6	71.6±2.3	61.4±1.1
Diethyl ether	2.0	100±0.0	100±0.0	100±0.0	90.4±1.1	81.6±1.5	73.2±1.7
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	93.4±2.6	81.8±2.2
	1.0	90.6±1.9	82.4±2.6	65.4±1.6	46.8±1.4	32.6±1.8	20.2±1.3
Hexane	2.0	100±0.0	90.8±2.9	70.2±2.7	56.8±2.3	41.2±2.8	20.8±2.5
	3.0	100±0.0	98.4±0.8	87.8±2.2	67.8±2.1	50.4±1.8	38.6±1.9
	1.0	100±0.0	95.6±1.8	82.6±1.9	70.4±1.5	63.8±2.1	51.6±1.5
Benzene	2.0	100±0.0	100±0.0	93.2±1.4	83.4±2.1	72.8±1.6	64.2±2.5
	3.0	100±0.0	100±0.0	100±0.0	97.6±1.9	84.4±3.1	71.2±2.6
	1.0	100±0.0	86.8±1.3	66.2±2.2	50.8±1.6	39.2±1.4	23.4±1.8
Acetone	2.0	100±0.0	97.2±1.0	83.2±1.7	71.4±1.5	59.8±2.6	49.4±2.5
	3.0	100±0.0	98.4±0.8	87.8±2.2	67.8±2.1	50.4±1.8	38.6±1.9

Mean ± SD value of the five replications.

Discussion

The mosquitocidal activities of the leaf extract domino effect are also comparable with former reports. Rahuman and Venkatesan (2008) reported that the petroleum ether extract of *Citrullus colocynthis* (*C. colocynthis*), methanol extracts of *Cannabis indica*, *Cannabis sativus*, *Momordica charantia* and acetone extract of *Trichosanthes anguina* against the larvae of *Ae*. *aegypti* (LC_{50}=74.57, 309.46, 492.73, 199.14 and 554.20 ppm) and against *Cx*. *quinquefasciatus* (LC_{50}=88.24, 377.69, 623.80, 207.61 and 842.34 ppm), respectively. Mullai and Jebanesan (2007) have reported that ethyl acetate, petroleum ether and methanol leaf extracts of *C. colocynthis* and *Cucurbita maxima* showed LC_{50}= values od 47.58, 66.92 and 118.74 ppm and 75.91, 117.73 and 171.64 ppm, respectively, against *Cx*. *quinquefasciatus* larvae. Baranitharan and Dhanasekaran (2014) reported that the ethyl acetate, hexane, chloroform and acetone extracts of *C. caudata* showed LC_{50} values of *Ae. aegypti* are 97.19, 112.85, 99.17 and 109.67 mg/L; *An*. *stephensi* are 96.04, 104.16, 97.13 and 106.53 mg/L; *Cx*. *quinquefasciatus* are 94.76, 102.95, 95.98 and 105.09 mg/L, respectively. Krishnappa and Elumalai (2014) reported that the hexane, diethyl ether, dichloromethane, acetone and methanol extracts of *Achras sapota* and *Cassia auriculata* against *An*. *stephensi.* The LC_{50} values of hexane, diethyl ether, dichloromethane, acetone and methanol extracts of *Achras sapota* and *Cassia auriculata* against *An*. *stephensi* larvae in 24 h were 54.82, 48.85, 45.37, 39.82 and 39.54 mg/L; 97.44, 89.47, 86.33, 78.44 and 74.82 mg/L, respectively. Gokulakrishnan *et al.* (2012) reported that the larvicidal and ovicidal efficacy of different solvent leaf extract of *Ariitolochia indica* against *Anopheles stephensi*. The hatch rates were assessed 48 h after treatment. The LC_{50} and LC_{90} values of acetone, benzene, chloroform, hexane and methanol extracts of *A. indica* against *An*. *stephensi* larvae in 24 h were 76.29, 58.82, 53.59, 65.84, 51.78 and 205.85, 193.23, 185.16, 196.72 and 181.00 ppm, respectively. Baranitharan and Dhanasekaran (2014) investigated that the LC_{50} and LC_{90} values of ethyl acetate followed by hexane, chloroform and acetone of *Ageratina adenophora* against *Cx*. *quinquefasciatus* larvae I-instar in 24 h were 136.75, 145.69, 139.49 and 143.64 mg/L; 149.07, 158.24, 151.95 and 156.14 mg/L,

respectively. Dhanasekaran *et al.,* (2013) have reported that the LC_{50} value of ethanol crude extracts of selected indigenous medicinal plants, particularly with C. argentea was 134.4 for dengue vector *Ae. aegypti.* Rajkumar and Jebanesan (2002) who observed that increase in the concentration of leaf extract of *S. aerianthum* induced the oviposition attractant activity in *Cx*. *quinquefasciatus.* Chowdhury *et al.,* (2009) have reported that the chloroform and methanol extracts of mature leaves of *Solonum villosum* (*S. villosum*) showed the LC_{50} value for all instars between 24.20 and 33.73 ppm after 24 h and between 23.47 and 30.63 ppm after 48 h of exposure period against *An*. *subpictus*. The larvicidal activity of *Croton sparciflrorus* leaf extracts with four different solvents like ethyl acetate, hexane, dichloromethane and diethyl ether was tested against the three vector mosquitoes. Among the four solvents the maximum larvicidal activity was observed in ethyl acetate. The LC_{50} values of *Croton sparciflorus* against three vector mosquitoes of *Ae. aegypti, An*. *stephensi* and *Cx*. *quinquefasciatus* were 34.02, 28.88 and 36.22 ppm, 74.57, 65.35 and 79.89 ppm, respectively (Baranitharan *et al.,* 2014). The isolated compound saponin from ethyl acetate extract of *Achyranthes aspera* was effective against the larvae of *Ae. aegypti* and *Cx*. *quinquefasciatus* with LC_{50} value of 18.20 and 27.24 ppm, respectively (Bagavan *et al.,* 2008).

Conclusion

In the present study, it could be concluded that diethyl ether extracts of *C. aromaticus* used as larvicidal, ovicidal and repellent activity against the mosquito vector, *Aedes aegypti.* Furthermore, the results of the present study may donate to a diminution in the relevance of synthetic insecticides, which in turn increases the opening for nature control of various medically significant pests by botanical pesticides. Further studies on the tested plant including mode of action, synergism with the biocides under field condition are needed.

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