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Research Article

REPELLENT ACTIVITY AND GCMS ANALYSIS OF ANTHOCEPHALUS CADAMBA LEAF EXTRACT AGAINST CULEXQUINQUEFASCIATUS

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ABSTRACT

Bioactive compounds were extracted from the leaves of Anthocephalus cadamba using standard method**.** Different compounds of the most active extract were identified by the gas chromotography-massspectrometry (GC-MS) analysis.

All plant extracts showed significant larvicidal activity against A. aegypti mosquito larvae at 0.05 level of significance. Tinospora rumphii leaf extract is the most effective mosquito larvicide which is manifested by the highest percentage mortality on the larvae of 90% and 93% after 24 and 48 hours respectively; with an LC50 and LC90 values of 10 mg/mL and 46 mg/mL respectively after 48 hours of exposure. Citrus grandis bark and Tinospora rumphii stem extracts showed a significant difference on the increased of the mortality of mosquito larvae with increasing concentrations of the plant extracts at 0.05 level of significance. The high larvicidal activity of Tinospora rumphii leaf is supported by the abundance of phytochemicals which show synergestic effects in terms of larvicidal action to mosquito larvae. The larvicidal activities of the three plants differ according to the plant species and part used which is supported by the presence of several bioactive chemicals.

INTRODUCTION

Mosquito is one of the notorious creations in the animal kingdom. They are vectors of several disease causing pathogens having the potentiality to kill more than a million victims annually around the world (Vatandoost *et al*., 2001). In tropical developing countries human filariasis is a socioeconomic problems and the pathogens of filariasis is transmitted by culex quinquifasciatus mosquito. Lymphatic filariasis is a tropical disease infecting about 120 million people worldwide within which 44 million have chronic manifestation (Bernhard *et al*., 2003). In Indian subcontinent the pathogen of lymphatic filariasis is Wucheriabancrofit, which is transmitted by female Culex Quinquifasciatus mosquitoes (Rajkumar *et al*., 2005). Therefore, the only efficacious approach of minimizing the incidence of these diseases is to control mosquito population by application of insecticides at larval habitats. Mosquito in the larval stage are attractive target for control operation due to their low mobility in the breeding habitats (Howard *et al*., 2007). One of the methods available for the control of mosquito population is over and the injuidicious application of persistent synthetic insecticides resulting undesirable effect including biomagnifications through food chain, development of insecticides resistance, toxic effect on human and other non target organisms. More detailed studies naturally occurring insecticides are needed to avoid the adverse effect of synthetic insecticides.

In recent years, research proved effectiveness of plant derived secondary componds such as saponins (Wiseman *et al*., 2005)], steroid (Chowdhury *et al*, 2008 & Ghosh *et al*, 2008), Isoflavonoid (Joseph *et al*, 2004) essential oils (Cavalcanti *et al*, 2004), alkaloids and tannins (Khanna *et al*., 2007) as mosquito larvicides. Plant compounds and the synthetic derivatives i.e., essential oil alternative source of mosquito repellent agent (Yang *et al*., 2004).

The objective of the present study was to observe the larvicidal activity and repellent activity of leaf extract of Anthocephalus cadamba on Culex quinquifasciatus mosquitoes.

MATERIALS AND METHOD

Collection & Maintenance of egg, larva and adult mosquitoes

The eggs of *Culex quinquefasciatus* were collected. The mosquito larval, pupal culture was maintained in our laboratory. The adult female mosquitoes were reared.

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Collection of plant & Preparation of plant extracts

The plant materials of *Anthocephalus cadamba* (Rubiaceae) were **collected from in and around Palani hills, Palani, Tamilnadu, India. The plant materials of** *Anthocephalus cadamba* leaves washed with tap water, shade dried at room temperature and powdered by an electrical blender. From sample, 100g of the plant materials were extracted with 300ml of organic solvent methanol for 8hrs in a soxhlet apparatus (Vogel, 1978) the crude plant extracts were evaporated to dryness in rotary vacuum evaporator. The extract were subjected to various qualitative chemical tests to screen for phytochemical constituents. One gram of the plant residue was dissolved in 100 ml of Acetone (stock solution) and considered as 1% stock solution. From this stock solution, different concentrations were prepared.

Larvicidal Bioassay

Different concentrations ranging from 5ppm to 80 ppm were prepared for larvicidal activity. The number of dead larvae were counted after exposure and the percentage of mortality was reported from the average of 5 replicates. However at the end, the selected test samples turned out to be equal in their toxic potential.

The efficacy of the plant extracts as larvicide against the dengue-vector Aedes aegypti mosquito was evaluated in accordance with the guidelines of World Health Organization13. Batches of 20 third-instars larvae of Aedes aegypti were placed in a small plastic container with 50 ml dechlorinated water and lay in the netted area in the Laboratory room at 30-320C. For the control group, the mosquito larvae were exposed to 60 mg/mL methanol since it is the solvent used in the extraction of different plant samples. The experimental group is the methanolic extracts of the stem/bark and leaf of Jatropha curcas, Tinospora rumphii and Citrus grandis with 20 mg/mL, 40 mg/mL, 60 mg/mL concentrations. These concentrations were chosen after the pre-test//pretreatment conducted. Identification of the mosquito larvae were done by tapping it with a needle in the siphon or cervical area. Each treatment was conducted in three replicates. The effects of the plant extracts were monitored through carefully counting the number of dead larvae after 24 and 48 hours of treatment, and the percentage mortality was computed. Percentage mortality = Number of dead larvae x 100 Number of larvae introduced Statistical Analysis: The statistical tools that were used in this

GC-MS analysis

GC-MS analysis of active fractions of *Anthocephalus cadamba* were analysed individually using Agilent GC_MS 5975 Inert XL MSD (United States) gas chromatography equipped with J&W 122 – 5532G DB-5ms 30 x 0.25mm x 0.25µm and mass detector (EM with replaceable horn) was operated in EMV mode. Helium was used as carrier gas with the flow rate of 1.0 ml min^{-1}. The injection port temperature was operated at 250°C. The column oven temperature was held at 80°C for 2min then programmed at 10° min^{-1} to 250°C, which was held for 0 min, and then at 5°C min^{-1} to 280°C which was held for 9 mins. Electron impact spectra in positive ionization mode were acquired between m/z 40 and 450.

Repellent Activity of plant extracts *Anthocephalus cadamba*

Repellent activity of plant compounds tested with human volunteers. For the repellent activity of **plant extracts *Anthocephalus cadamba*** percentage protection in relation to dose method was adopted (W.H.O, 1996, Murugan *et al*., 2006). Three to four days old blood starved female adult mosquitoes (100) will be kept in a net cage. The arms of the test person cleaned with isopropanol. After air-drying the arm only 25 cm^2 of the dorsal side of the skin on each arm will be exposed, the remaining area being covered by rubber gloves.

The **plant extracts *Anthocephalus cadamba*** was dissolved in isopropanol and this alcohol served as control. The **plant extracts *Anthocephalus cadamba*** at 0.5, 1.0 and 2.0 mg/cm^2 concentrations will be applied. The control and treated arms introduced simultaneously into the cage. The number of bites counted over 5 min every 60 min, from 20.00 to 6.00 hrs. The experiment was conducted five times. The percentage protection calculated by using the following formula.

$$\% \text{ Protection} = \frac{\text{(Number of bites received by control arm) - (Number of bites received by treated arm)}}{\text{Number of bites received by control arm}} \times 100$$

T = The number of mosquitoes collected from treated areas.

Statistical Analysis

The data gets from the bioassay subject to statistical analysis. The SPSS software package was computing, all the data including profit analysis, correlation co-efficient and mean of the sample (Finney, 1971)

RESULTS AND DISCUSSION

Today, the environmental safety is considered to be of paramount importance. Hence an insecticide should be eco-friendly, which is generally not observed in chemical or synthetic pesticides. This safety could only be ascertained through plant-based insecticides. Phytochemicals may serve as suitable alternatives to synthetic insecticides. In future as these are relatively safe, inexpensive and readily available in most parts of the world. In the present study, bioactive compounds from leaf extract of Anthocephalus cadamba particularly was found to have higher rate of larvicidal and pupicidal activity against Culex quinquifasciatus. Effect of extracts of *Anthocephalus cadamba* on various stages of filarial vector *Culex quinquefasciatus* is given in table 1. For treatment the concentration taken were 5,10,20,40 and 80 ppm. In control the percentage larvae moulted was 100 percent, deformities of larval pupae intermediate was 100%, percentage of normal pupae reaching adulthood was 100 percent, Similar trend has been followed for different concentration and values had been recorded. For 5, 10, 20, 40 and 80 ppm the number of Adult reaching adulthood were 81, 74, 64, 49 and 35 respectively.

Many reported that Flavonoid extract from flower-buds of *Vitex* particularly was found to have higher rate of larvicidal activity against *An. Stephensi* and *Ae. aegypti*, whereas in the case of other extracts (obtained from different parts), the concentrations had to be increased for better larvicidal effect (Keerthi Gautam *et al*, 2013). In fact many researchers have

reported that Larvicidal activity of partially purified extracts of leaves of *V. negundo, Nerrium oleander* and seeds of *Syzygium jambolanum* on different instars of *Culexquinquefasciatus* and *An. Stephensi* (Pushphalatha *et al*., 1995). It have been reviewed that Larvicidal activity of fatty acid methyl esters of different species of *Vitex* against *Culex* (Kannathasan *et al*., 2008). Differential larvicidal efficacy of four species of *Vitex*against *Culex quinquefasciatus* had been also reported (Kannathasan *et al*., 2007). Whole plant ethanolic extract of *A.paniculata* had been studied for larvicidal, pupicidal, adulticidal and ovicidal properties against the malaria vector (Kuppusamy *et al*., 2011).

Synergistic activity of A. paniculata with Bacillus thuringiensis against malaria vector An.stephensi was also tested (Kuppusamy *et al*., 2011). Larvicidal and ovicidal efficacy of different extracts of A. paniculata was tested against Culex quinquefasciatus and Ae. Aegypti (Govindarajan *et al*., 2011). Bioactivity of four flavonoid compounds from Poncirus trifoliate L. was tested against the dengue fever vector (Rajkumar *et al*., 2008). Many plant extracts and essential oils manifest repellent activity against different mosquito species (Marimuthu., 2010)

The biological activity of the plant extracts might be due to a variety of compounds in *Solanum tribolium* plant, including phenolics, terpenoids and alkaloids. These compounds may jointly or independently contribute to causing oviposition deterrent and skin repellent activity against *An.Stephensi (Jed., 2005)*.

Table 1 Effect of methanolic extracts of Anthocephalus cadamba on various stages of filarial vector, *Culex quinquefasciatus*.

Treatment	% of larval which moulted	% deformities of larval pupae intermediate	% of normal pupae	Number of adult reaching adulthood (%)
Control	100	100	100	100
5	89	7	84	81
10	84	12	78	74
20	75	19	67	64
40	55	40	51	49
80	38	55	42	35

** Significant at 1% level

Table 1 shows the effect of anthocephalus cadamba on various stages of filarial vector, Culex quinquefasciatus. In control the percentage of larvae moulted was highly reduced when treated with Anthocephalus cadamba. At 5 ppm, 10 ppm, 20 ppm, 40 ppm and 80 ppm. The percentage of larvae moulted was 81, 74, 64, 49 and 35. In control the % deformities of larval pupal intermediate are 100. After the treatment % deformities of larval pupal intermediate are more. In 5 ppm it was 7%. In 10ppm, 20 ppm, 40 ppm it is 12%, 19%, 40%.

Maximum deformities have been observed in 80 ppm as 55%. The percentage of normal pupae in control is 100. When treated with anthocephalus extract at 5 ppm was 84%, at 10 ppm was 78%, at 20 ppm was 67% at 40 ppm was 51%, at 80 ppm was 42%. The percentage of adult reaching adulthood in control is 100 whereas treated shows minimum mosquito larvae in 5 ppm as 81% maximum reduction in 80 ppm as 35%. In 10 ppm, 20ppm, 40ppm% of adult reaching adulthood was 74%, 64% and 49%.

Table 2 Repellent activity of plant extracts *Anthocephalus cadamba* on adult mosquitoes, *Culex quinquefasciatus*

Repellent activity observation time (hour)	Number of mosquitoes fed Plant extract Anthocephalus cadamba Concentration in ppm			
	0.5	1.0	2.0	Control
18.00 - 19.00	*0*	*0*	*0*	*0*
19.00 – 20.00	*6*	*10*	*0*	*0*
20.00 – 21.00	*10*	*8*	*1*	*4*
21.00 – 22.00	*8*	*8*	*1*	*12*
22.00 – 23.00	*12*	*2*	*3*	*14*
24.00 – 01.00	*5*	*2*	*2*	*10*
01.00 – 02.00	*4*	*2*	*1*	*8*
02.00 – 03.00	*8*	*1*	*3*	*18*
Fed Mosquito	*53*	*33*	*11*	*66*
Unfed Mosquito	*47*	*67*	*89*	*34*
% of mosquito	*19.6*	*50.0*	*78.7*	-

The repellent activity of Anthocephalus cadamba on Culex quinquefasciatus is given in the Table 2. The repellent activity was carried out in the evening from18.00-03.00 am. The repellency was minimum at 0.5 ppm showed 19.6%, protection. The repellency was maximum at 1.0 ppm, which showed 50.00% protection. An average protection was at 2.0 ppm which showed 78.7% protection against Culex quinquefasciatus.

Repellent activity of Ageratum houstonianumMill. (Asteraceae) leaf extracts against Anophelestephensi, Aedesaegypti and Culexquinquefasciatus (Diptera:Culicidae was determined for hexane, ethyl acetate and methanol crude extracts of Ageratum houstonianum (A.houstonianum) leaves against adult Anopheles stephensi, Aedes:aegypti (Ae.aegypti) and Culexquinquefasciatus (Cx.quinquefasciatus) of fixed concentration of Bioassay on laboratory reared Swiss albino mice by topical application at a 0.01 per cent with coconut oil as a base. Crude leaf extracts of A.houstonianumin combination with coconut oil repelled vector mosquitoes. Amongst the three extracts, methanol extract gave the maximum protection of 95.0% against Culex quinquefasciatus. Hexane and ethyl acetate extracts gave a maximum of 93.4% protection against An.stephensi.

The crude extracts of A. houstonianum leaves in combination with coconut oil showed repellent activity with repellent quotient ranging from 0.6 to 0.9 (SamuvelTennyson *et al*., 2012) Phytochemicals such as alkaloids, flavonoids and monoterpenes are known for their mosquito repellent activity. The phytochemical study for Tagetes erecta L. showed positive results for tannins, saponins test, alkaloids test, reducing compound test and cardioglycosides test and showed negative result on anthraquinones. Callistemon brachyandrus Lindl. showed positive result on tannins test, saponins test, alkaloids test, reducing compound test and cardioglycosides test and showed negative result on anthraquinones. Tagetes erecta L. showed 40% of mortality rate against Anopheles stephensi and Culex infulus. So, this plant source has maximum larvicidal activity against Anopheles stephensi and Culex infulus. Callistemon brachyandrus Lindl showed maximum mortality rate 13.33% against Culexinfulus. So, this plant source has maximum larvicidal activity against Culex infulus (Pavitha *et al*., 2014)

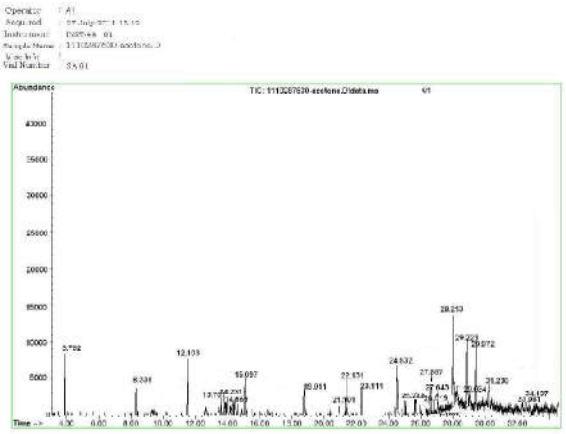

Fig.1 GCMS spectra for Crude extract of *Anthocephalus Cadamba*

GC-MS spectrum of crude methanol extract of Anthocephalus cadamba is shown in fig. 1. GC-MS is used to analysis the mass value of the compound present in the crude methanol extract of Anthocephalus cadamba. In this analysis 35 compounds have been identified. The retention time, name, molecular weight and the structure of some of the components were ascertained.

The compounds identified in GC-MS are mostly hydrocarbons, carboxylic acids, olefinic compounds, esters, sulphur, amine, amides, etc. which have been reported to have bioactivity. The bioactive compounds which have been screened in the present study can be assigned to the synergistic effects.

Table 3 GC-MS analysis of *Anthocephalus cadamba* dry leaves extract

S.No	R.T	NAME OF THE COMPOUND	PEAK OF AREA %
1	3-737	3-Butyn-1-01 2-Propenamide	1-90
2	4-405	1,2-Propanediol, 3-Chloro-1,2,3,4-Butanetetrol, Urethane	2.27
3	6.048	4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-1-Pentanamine, N-methyl-	1.01
4	7.257	Ethan amine, 2-propoxy-L-Alanine, N-glycyl- 2-Isopropoxyethylamine	1.12
5	7.373	1,2-Benzenediol Phenol, 2-ethoxy-Phenol, 4-butoxy-	4.29
6	8.146	Phenylethanolamine Ethanamine, 2,2' –dithiobis- Carbondithioic acid, O,S-diethyl ester	0.49
7	8.649	Propane	0.95
8	11.820	D-Allose 1,6-Anhydro-.beta.-D glucopyranose(Levoglucosan) Phosphoric triamide,N,N',N"-tris (hydrazinocarbonyl)	1.69
9	12.023	1,6-Anhydro-.beta.-D-glucopyranose(Levoglucosan) Nonanoic acid L-Mannose, 6-deoxy-	0.48
10	14.006	Pyrazine, methoxy-, 1-oxide 2-Ethyl-4-hexene-1-ol,7-(1-Hydroxy-ethyl) -3,3-dimethyl-bicyclo(4.1.0) heptan-2-one	0.57
11	14.412	Propanedioic acid, Propyl-Hexanoic acid, 6-bromo-Heptane	11.21
12	15.620	Myo-Inositol, 4-C-methyl- Methyl (methyl 4-O-methyl-.alpha.-d-mannopyranoside) urinate Silane, ethyltrimethyl- 3-cis-Methoxy-5-cis-methyl-1R-cyclohexanol	0.90
13	16.587	Enolide, 3-(2,6-dideoxy-4-O-.beta.-D-glucopyranosyl-3-O-methyl-.beta.-D-ribo-hexopyranosyl)oxyl)-5,14-dihydroxy-19-oxo-,(3.beta.,5.beta.)-Decanoic acid, 3-methyl-	8.65
14	16.800	2-O-Methyl-D-mannopyranosa Thiophene,tetrahydro-2-methyl-D-Fructose, 3-O-methyl-	1.71
15	16.858	Enolide, 3-(2,6-dideoxy-4-O-.beta. –D-glucopyranosyl)oxy) -5,14-dihydroxy-19-oxo-(3.beta.,5.beta.)-Thiophene, tetrahydro-2-methyl- Thiophene, tetrahydro-2-methyl	1.12
16	17.061	Thiophene, tetrahydro-2-methyl- Thiophene, tetrahydro-2-methyl-2,2,4-Trimethyl-3-Pentanol	5.68
17	17.177	Thiophene, tetrahydro-2-methyl-Thiophene, tetrahydro-2-methyl 2-Trimethylsilyl-1,3-dithiane	2.39
18	17.264	Thiophene, tetrahydro-2-methyl-(Methylthio)-acetonitrile 3-Methylmannoside	2.74
19	17.419	Enolide, 3-[(2,6-dideoxy-4-O-.beta.-D-glucopyranosyl-3-O-methyl-.beta.-D-ribo-hexopyranosyl)oxyl]-5,14-dihydroxy-19-oxo-,(3.beta.,5.beta.)- D-Fructose, 3-O-methyl-3-Hexanol, 2,4-dimethyl-	6.23
20	17.515	Thiophene, tetrahydro-2-methyl-Thiophene, tetrahydro-2-mwthyl-.alpha.-D-Xylofuranoside, methyl 3 –O-methyl-	2.64
21	17.583	-enolide, 3-[(2,6-dideoxy-4-O-.beta.-D-glucopyranosyl-3-O-methyl-.beta.-D-ribo-hexopyranosyl)oxyl]-5,14-dihydroxy-19-oxo-, (3.beta.,5.beta.)-Thiophene, tetrahydro-2-methyl-Thiophene,tetrahydro-2-methyl-	2.92
22	17.709	.alpha. –D-Xylofuranoside, methyl 2-O-methyl- .alpha. –D-Xylofuranoside, methyl 3 –O- methyl- Thiophene, tetrahydro-2-methyl-	4.32
23	17.776	Thiophene, tetrahydro-2-methyl-.alpha.-D-Xylofuranoside,methyl-O-methyl- 2-Trimethylsilyl-1,3-dithiane	4.23
24	17.873	Thiophene, tetrahydro-2-methyl-.alpha. –D-Xylofuranoside, methyl 3 –O-methyl- 2,2-Diethoxytetrahydrofuran	3.20
25	18.037	Thiophene, tetrahydro-2-methyl-Thiophene, tetrahydro-2-methyl-Hydrazine, 1,1-dipropyl-	8.05
26	18.076	Thiophene,tetrahydro-2-methyl-.alpha.-Methyl mannofuranoside 2-Butenoic acid, 4-hydroxy-, methyl ester	4.50
27	18.327	Hexadecanoic acid, methyl ester Pentadecanoic acid, 14-methyl-,thyl ester Hexadecanoic acid, 15-methyl-,thyl ester	0.84
28	19.362	n-Hexadecanoic acid	5.93
29	21.750	Phytol	0.42
30	22.398	9,12-Octadecadienoic acid(Z,Z)-9,12-Octadecadienoic acid, methyl ester, (E,E)- 11,14-Eicosadienoicacid,methyl ester	0.28
31	22.524	13-oxabicyclotridecane 1,15-hexadecadiene 9,12-octadecadienoyl chloride	1.85
32	22.891	Tetracosenoic acid, methylester,(Z)-Oleic acid 9-Tetradecenal, (Z)	0.56
33	28.383	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester 9-(2',2'- Dimethypropanoilhydrazon)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxyl] fluorine 2,4,7,14-Tetramethyl-4-Vinyl-tricy clo[5.4.3.0(1,8] tetradecan-6-ol	0.01
34	30.200	Cyclopropane carboxamide,2-cyclopropyl-2-N-(1-cyclopropylethyl) 1,2-15,16-diepoxyhexadecane 1-propanamine,N,2-dimethyl-,N-nitroso	0.15
35	31.225	2,6,10,14,18,22-tetracosahexaene 2,6,10,15,19,23-hexamethyl-9all-E)-Squalene	4.69

CONCLUSIONS

The findings of the present investigation revealed the Anthocephalus cadamba leaf extract have good larvicidal pupicidal and repellent activity against culex quinquefasciatus. The bioactive compounds identified in GC-MS study can be assigned to the synergistic effects. This plant extracts showed that has good effective mosquito control properties and also can act as an ecofriendly, bio-pesticide for further vector control programmes.

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