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(DIPHTERA)

JeyalalithaThirupathi., K.Murugan., Umayavalli M
and Sivapriya V



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Research Article

PHYTOCHEMICAL SCREENING OF LEAF EXTRACT OF ANTHOCEPHALUS CADAMBA AND ITS LARVICIDAL, PUPICIDAL, IGR ACTIVITY ON CULEXQUINQUEFASCIATUS (DIPTHERA)

JeyalalithaThirupathi¹, K.Murugan², Umayavalli M³ and Sivapriya V⁴

¹Department of Zoology, Arulmigu Palaniandavar College of Arts & Culture, Palani

²Department of Zoology, School of Life sciences, Bharathiar University,
Coimbatore 641046, Tamil Nadu, India

³Department of Chemistry, Arulmigupalaniandavar college of Arts and Culture, Palani

⁴Banari Amman Institute of Technology, Sathyamangalam

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ABSTRACT

Methanolic leaf extract of Anthocephalus cadamba was evaluated for phytoconstitutes present in them by UV/Vis absorption spectra, FT-IR Analysis. These studies indicated the possible information for correct identification and standardization of the plant materials.

The larvicidal activity of methanolic leaf extract of Anthocephalus cadamba was obtained by solvent extraction and then bioassayed following protocols showed LC50 values from 5 to 80ppm after exposure. The mortality was high as 100% in the 1st instars larva. Minimum mortality was 31% in IV Instar. The LC50 values and LC90 values were represented as follows. LC50 values and LC90 values for 1st instar was 0.612, 10.125, II instar was 1.291, 15.064 III rd instar was 3.526, 19.979 and IV instar was 5.631, 24.958 respectively.

The LC50 and LC90 value of pupal stage after the treatment of plant extract Anthocephalus cadamba was 10.521 and LC90 value was 35.227 respectively.

IGR activity on filarial vector culex quinquefasciatus after the treatment of plant extract Anthocephaluscadamba is given. The concentrations used for the treatment were 5, 10, 20, 40, 80ppm. Percentage inhibitions were 39, 54, 62, 69, and 75 respectively. EI50 and EI90 were 6.781 and 125.575. The results suggest that the investigated plant extracts are promising larvicides against filarial vector culex quinquefasciatus. This leads in the search for new and biodegradable plant derived larvicidal products.

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INTRODUCTION

Phytochemical are bioactive chemicals of plant origin. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components (Tiwari *et al.*, 2011). Phytochemical have been recognized as the basis for traditional herbal medicine practiced in the past and currently en vogue in parts of the world (Lalitha *et al.*, 2012). In the search for phytochemical that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region (Das *et al.*, 2010). Following such leads, plant parts are usually screened for phytochemicals that may be present. Then it can be used as the basis for a new pharmaceutical product. Mosquito larvicides from plant origin have been reported to overcome the environmental hazards associated with using synthetic chemicals in mosquito control programme (Kalyanasundram and Babu, 1982). The effects of

orange peel ethanol extract of Citrus sinensis had showed larvicidal, pupicidal, repellent and adulticidal activity against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus (Murugan *et al.*, 2012). Extracts from the medicinal plants are used to control mosquito vectors (Kovendan and Murugan, 2011). Limonoids such as azadiractin and gedunin, present in species from the meliaceae and rutaceae are recognized for their toxic effects on insects and are used in several insecticide formulations (Dua *et al.*, 1995). Aliphatic Amide from Seeds of Carica papaya are used as Mosquito Larvicide, Pupicide, Adulticide, Repellent and Smoke Toxicant (Anjali Rawani *et al.*, 2012). Anthocephalus cadamba (Rubiaceae) is widely distributed throughout India and is used as a folk medicine in the treatment of fever, anemia, uterine complaints, blood diseases, skin diseases, leprosy, dysentery, and for improvement of semen quality and also cure diabetes mellitus, diarrhoea, inflammation, haemoptysis, cough, vomiting, wounds, ulcers, debility and antimicrobial

*Corresponding author: JeyalalithaThirupathi

Department of Zoology, Arulmigu Palaniandavar College of Arts & Culture, Palani

activity. The leaves are recommended as a gargle in cases of stomatitis (Silkar et al, 1996). Some scientific studies have been carried out to reveal its antimalarial (Sianne and Fanie, 2002) and antihepatotoxic activities (Kapil, et al., 1995).

The main objective of the present research work was to check the presence or absence of the phytochemical constituents in the methanolic leaf extracts of Anthocephalus cadamba and their activity against the larvae of mosquito Culex quinquefasciatus the vector of bancroftian filariasis in India.

MATERIALS AND METHODS

Collection of plant and 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 each 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. Plant extracts were subjected to various qualitative chemical tests to screen for phytochemical constituents.

UV-VIS and FTIR Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis. For UV-VIS and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the UV-VIS and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

Larval and pupal toxicity test of plant extracts Anthocephalus cadamba

Laboratory colonies of mosquito and pupae were used for the larvicidal/ pupicidal activity. Twenty-five numbers of first, second, third and fourth instar larvae and pupae will be introduced into the 500 ml glass beaker containing 249 ml of de-chlorinated water and 1ml of desired concentrations of plant extracts Anthocephalus cadamba was added separately. Larval food was given for the test larvae. At each tested concentration 2 to 5 trials will be made and each trial consisted of three to four replicates. Mixing 1ml of acetone with 249 ml of de-chlorinated water set up the control. The control mortalities will be corrected by using (Abbott's formula 1925)

$$\text{Percentage mortality} = \frac{\text{number of dead larvae/pupae} \times 100}{\text{number of larvae/pupae introduced}}$$

LC₅₀, LC₉₀, regression equation and 95% confidence Limit of Lower Confidence of Limit (LCL) and Upper Confidence

Limit (UCL) were calculated from toxicity data by using probit analysis (6).

IGR Bioassay- (WHO, 2005)

IGR activity was assessed using 3rd instars. An accurate initial count of the larvae was made to avoid recording-missing (as dead) larvae as a result of cannibalistic or scavenging behaviour during long exposure period, larvae provided with a small amount of food (finely ground dog biscuits and Yeast) at a concentration of 10 mg/l at 2 day intervals. The food powder should be suspended in water and one or two drops added per cup until mortality counts were made. Mortality or survival is counted every day until the complete emergence of adults. At the end of the observation periods, the number of larvae that don't develop successfully into viable adults or larvae and pupae were dead as well as adult mosquitoes not completely separated from pupal cases were recorded and expressed as percent of inhibition emergence (EI). The experiment was terminated when all larvae or pupae in the controls had died or emerged as adults. The total mean emergence inhibitions were calculated on the basis of the number of third stage larvae exposed and EI is calculated using the formula.

$$\text{EI (\%)} = \frac{100 - [T \times 100]}{C}$$

Where T=% of emergence in treated cohorts and
C=% of emergence in the control

Statistical Analysis

The data gets from the bioassay subject to statistical analysis. The SPSS software package was computing all the data including profit analysis. (Finney, 1971)

RESULTS AND DISCUSSION

Mosquitoes are the most deadly vectors for several of these diseases causing organisms.

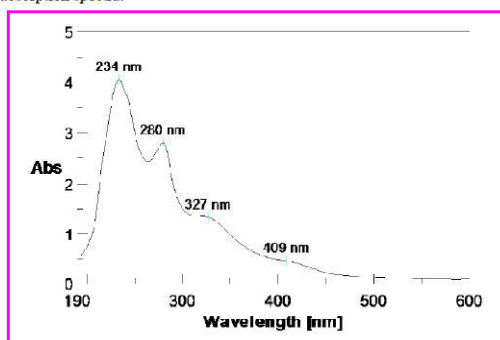
Lymphatic filariasis caused by Wuchereria bancrofti is an important public health problem in India. Both parasites produce essentially similar clinical presentations in man, related mainly to the pathology of the lymphatic system. Filariasis is endemic in 17 States and six Union Territories, with about 553 million people at risk of infection. Recently, the filarial control programme focused more on the elimination of mosquitoes in larval stage with plant extract. The advantage of targeting larvae is that they cannot escape from their breeding sites until the adult stage and also reduce overall pesticide use in control of adult mosquitoes. The tested solvent plant extracts have exerted a promising activity.

Different types of biological activities are played by the wide variety of secondary metabolites of plants. Most studies reported active compounds like Steroidal saponins, Alkaloid, Phenolic are responsible for mosquitocidal property.

In the present study the crude methanol extract of Anthocephalus cadamba is further analyzed by the UV/Vis. absorption spectra. Crude extracts shows the three absorption bands are 234 nm, 280 nm, 327 nm and 409 nm (Fig. 1). The absorption range between 200-400 nm indicates the presence of the flavonoids as a major constituent in the crude methanol

extract of *Anthocephalus cadamba*. In the previous studies it has been proved that the flavonoids are used as anti-inflammatory, anticancer, antiviral agents. (Umachigi *et al*, 2007) 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 *et al*, 2013) In previous research Bioactivity of four flavonoid compounds from *Poncirus trifoliata* L. was tested against the dengue fever vector (Rajkumar & jebanesan, 2008).

FIG 1: UV/Vis. absorption spectra.



The crude methanol extract of *Anthocephalus cadamba* is further analyzed by the FT-IR spectra to find out the functional groups. Fig. 2 shows the FT-IR spectra of crude methanol extract of *Anthocephalus cadamba*. Table 1 shows the observed stretching frequencies of crude methanol extract of *Anthocephalus cadamba* show bands are 3394.83, 2926.11, 2364.81, 1707.06, 1612.54, 1523.82, 1371.43, 1203.62, 1448.59, 1371.43, 1203.62, 1107.18, 1068.60, 819.77, 769.62, 715.61, 667.39, 611.45, 586.38, and 424.35. The bands around 3394.83, 2926.11, 2364.81, 1707.06, 1612.54 manifest major IR bands, While the minor bands at 1523.82, 1371.43, 1203.62, 1448.59, 1371.43, 1203.62, 1107.18 and 1068.60.

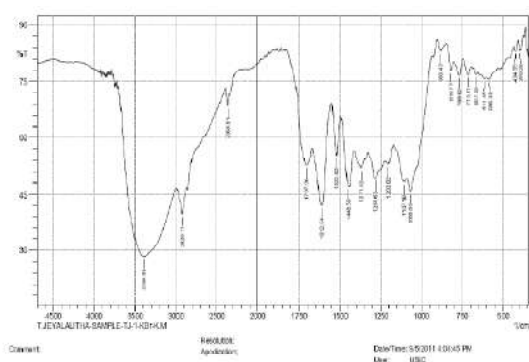
The band around 1371 cm^{-1} can be assigned to methyl groups. To a large extent, the band at 1068 cm^{-1} might be contributed by the -C=O groups of the polyphenols such as flavones, terpenoids and the polysaccharides present in the crude methanol extract of *Anthocephalus cadamba*. The bands around 1734 cm^{-1} can be assigned to C=O stretching vibrations of the carbonyl functional groups. The various functional groups observed in the different extracts probably indicate the presence of carbohydrates, carotenoid, Glycogen, aminoacids, amides, starch, caltropin, calotropogenin, phosphates, lipids, glycogen and cellulose. (Ragavendran *et al*, 2011, Thangarajan starlin *et al*, 2012., Kareru *et al*, 2013, Ramamurthy and Kennan 2007, Mueen Ahmed *et al*, 2005, Muruganatham *et al*, 2009), Presence of OH group has got the ability of forming hydrogen bounding capacity. So this extract has the higher potential towards inhibitory activity against microorganism. (Ashok kumar and Ramaswamy, 2013). Presence of peptide bounds also inhibits the growth of bacteria (KimA. Brogden, 2014).

Larval and pupal mortality of *Culex quinquefasciatus* after the treatment of methanolic extract of *anthocephalus cadamba* leaves was observed. In the present research Larval and pupal toxicity effect of leaves extract *Anthocephalus cadamba* on *Culex quinquefasciatus* is shown in the table 2. The percentage mortality of *Culex quinquefasciatus* after the treatment of leaves extract *Anthocephalus cadamba* on I to IV instars larvae and pupal from 5, 10, 20, 40 and 80 ppm were carried out. Higher mortality rate was 100 at 80 ppm concentration in the I instars larvae and the minimal mortality rate was 31 ± 0.5 at 5 ppm concentration in the IV instars larvae. At 5ppm concentration mortality was 46 ± 0.7 in the I instars larva. The LC_{50} values for the I instars was 12.152, II instars was 15.148, III instars was 21.817, and IV instars was 31.292 respectively.

Table 1: List of phytochemicals shown in FT-IR spectrum of crude extract of *Anthocephalus cadamba*

S.NO.	Wave Number	Functional Groups
1	3394.83	Phenols & Alcohols
2	2926.11	Carboxylic Acid
3	2364.81	Nitriles
4	1707.06	Aldehyde
5	1612.54	Amide
6	1523.82	Nitro Groups
7	1448.59	Secondary Amine
8	1371.43	Nitro Groups
9	1284.63	Aromatic amines
10	1203.62	Aliphatic Amines
11	1107.18	Ketones
12	1068.60	Amides
13	883.43	N-H Primary Secondary Amines
14	819.77	N-H Primary Secondary Amines
15	769.62	N-H Primary Secondary Amines
16	715.61	N-H Primary Secondary Amines
17	667.39	S-O stretch - Sulfonates
18	611.45	C-Cl stretch alkyl halides C-Br stretch alkyl halides C-Cl stretch acid chlorides
19	586.38	C-Cl stretch alkyl halides C-Br stretch alkyl halides C-Cl stretch acid chlorides
20	424.35	C-Cl stretch alkyl halides
21	399.28	C-Cl stretch alkyl halides

FIG 2: FT-IR spectra



against Culex (Kannathasan et al, 2007). It had been reported that four species of Vitex against Cx. quinquefasciatus showed differential larvicidal efficacy. (Kannathasan et al, 2008). The LC₅₀ value of pupal stage after the treatment of plant extract Anthocephalus cadamba, was 46.482 and LC₉₀ value was 149.970 respectively. Thus toxicological study showed the Plant extract Anthocephalus cadamba had larvicidal and pupicidal activity against Culex quinquefasciatus. The percentage mortality increases as the concentration increases. Thus this work proves that green plants represent a reservoir of effective chemicals, therapeutans and can provide valuable sources of natural pesticides (Kamalakkanan et al, 2009.)

Table 2: Larvicidal and pupicidal activity of filarial vector, Culex quinquefasciatus after the treatment of methanolic extracts of Anthocephalus cadamba

Larval and pupal stages	% of larval and pupal mortality (Mean ± SD)					Standard Error	LC ₅₀ (I.C ₉₀) (ppm)	95% Confidence limit		Chi square value
	Concentration (ppm)							LC ₅₀	LC ₉₀	
	5	10	20	40	80			LCL-UCL (ppm)	LCL - UCL (ppm)	
I	46±0.7 ^{cd}	49±0.5 ^{cd}	57±1.5 ^c	72±0.7 ^b	100 ^a	.003	12.152 (56.619)	5.58-22.30	41.39-103.29	6.399
II	43±1.6 ^{cd}	47±0.9 ^{cd}	53±2.1 ^c	67±0.9 ^b	98±1.1 ^a	.003	15.148 (64.717)	9.62-19.85	55.88-78.10	4.977
III	37±1.2 ^{cd}	41±1.1 ^{cd}	48±1.3 ^c	61±1.2 ^b	92±0.5 ^a	.003	21.817 (79.516)	16.10-27.09	68.54-96.25	1.534
IV	31±0.5 ^{cd}	35±0.9 ^{cd}	43±0.7 ^c	56±2.1 ^b	81±2.1 ^a	.002	31.292 (107.130)	24.87-38.00	86.58-127.18	.076
PUPAE	26±1.2 ^{cd}	29±1.6 ^{cd}	35±1.5 ^c	47±1.1 ^b	66±0.5 ^a	.002	46.482 (149.970)	37.15-59.69	119.05-211.22	2.986

Table 3: IGR activity of filarial vector, Culex quinquefasciatus after the treatment of methanolic extracts of Anthocephalus cadamba

Treatment	% inhibition	EI ₅₀ (ppm)	EI ₉₀ (ppm)
Control	00	00	00
5	39	6.781	125.575
10	54		
20	62		
40	69		
80	75		

** Significant at 1% level

LC₉₀ value of I instars was 56.619, II instars was 64.717, III instar was 79.516, and IV instars was 102.130 respectively. At 5 ppm concentration of leaves extract Anthocephalus cadamba the pupal mortality rate was 26 ± 1.2 where as it has been increased to 66 ± 0.5 at 80 ppm. The methanol extract of A. alnifolia leaf against C. quinquefasciatus had values of LC₅₀ for I, II, III & IV instar and pupae were 198.79, 172.48, 151.06, 140.69 and 127.98 ppm and LC(90) = 458.73, 430.66, 418.78, 408.83 and 386.26 ppm, respectively. The leaf extract of A. alnifolia also shows similar results as good larvicide. (koventhan, 2012) 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 have Larvicidal activity of on different instars of Culexquinquefasciatus and An. Stephensi (Pushpalatha E, Muthukrishnan J.1995). It have been reviewed that Larvicidal activity of fatty acid methyl esters of different species of Vitex

IGR activity if filarial, Culex quinquefasciatus after the treatment of extracts of Anthocephalus cadamba is given in table 2. The IGR activity of the extract of A. monophylla tested against the larvae of Culex quinquefasciatus showed EI₅₀ as 0.004 and EI₉₀ as 0.047 respectively (Sivagnaname and Kalyanasundaram, 2004). In the present work the concentrations used for the treatment were 5,10,20,40 and 80 ppm. Percentage inhibitions were 39, 54, 62, 69 and 75 respectively. EI₅₀ and EI₉₀ were 6.781 and 125.575. Interaction of acalypha alnifolia and vitex negundo showed the growth regulatory effect against Aedes aegypti (sivakamalakkanan et al, 2015) Growth regulatory effect is due to the presence of bioactive compounds, with many of these containing phytoecdysones, phytojuvenoids and anti-juvenile hormones, which act IGRs (Varma J, dubey NK, 1998).

CONCLUSION

Phytochemicals present in leaves of Anthocephalus cadamba indicates their potential as a sources of mosquitocides. Further more, isolation, purification and characterization of the phytochemicals present in the methanolic leaf extract of Anthocephalus cadamba will make studies interesting.

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- of Acalypha alnifolia (Euphorbiaceae) and (Verbenaceae) leaf extract of vitex negundo against Aedes aegypti (Diptera:Culicidae) *International journal of mosquito Research* 2015;2(1):47-52 .
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