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FTIR ANALYSIS AND THE EFFECT OF LEAF EXTRACT OF ANTHOCEPHALUS CADAMBA AND BIOSYNTHESISZED GOLD NANOPARTICLES OF CYMBOPOGAN CITRATUS ON THE GROWTH AND DEVELOPMENT OF CULEX QUINQUIFASCIATUS (DIPTERA: CULICIDAE)

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ABSTRACT

The aim of the study was to find out the phytochemical constituents and to evaluate the functional group of methanolic leaf extract of Anthocephalus cadamba and the biosynthesized gold nanoparticles of Cymbopogan citratus. The individual and combined effect of both were studied for growth and developmental activity against Culexquinquifasciatus. The adult development activity against freshly emerged 1st instar larvae of Culex quinquifasciatus was tested using WHO protocol. The secondary metaboliteswas analyzed using FTIR.

Hence the present study clearly showed the adult emergence was greatly inhibited by the present active compounds in the methanolic leaf extract of Anthocephalus cadamba and the biosynthesized gold nanoparticles of Cymbopogan citratus.

INTRODUCTION

Culex is a genus of mosquito, and is important in that several species serve as vectors of important diseases, such as West Nile virus, filariasis, Japanese encephalitis, St. Louis encephalitis and avian malaria. Currently, worldwide there are approximately 120 million cases of lymphatic filariasis (WHO, 2000). Many approaches have been developed to control mosquito menace. One such approach to prevent mosquito borne disease is by killing mosquito at larval stage. The current mosquito control approach is based on synthetic insecticides and many herbal products have been used as natural insecticides before the discovery of synthetic organic insecticides (ICMR Bulletin, 2003; Iqbal Ahamad *et al*, 2006; Ramamoorthi and Kannan, 2007; Kareru *et al*, 2008; Muruganantham *et al*, 2009). screened the functional groups of bioactive compounds in the plant extract using different solvents. Anthocephalus cadamba (Roxb.) Rubiaceae, is widely distributed throughout Bangladesh, Nepal, India, Myanmar, Sri Lanka, Philippines, Indonesia, and Papua New Guinea (Banerji, 1977; 1978; Sahu *et al*, 2000; Niranjan *et al*, 2000; GRIN Databases, 2010). Various parts of this plant have traditionally been used as an antidiuretic, in the treatment of fever, anemia and tumor, and for the improvement of semen quality (Umachigi *et al*, 2007; Dr. Duke's Phytochemical and Ethnobotanical Databases, 2010). The leaves are recommended as a gargle to treat stomatitis (Slkar *et al*, 1992).

While previous bioactivity studies on this plant revealed its analgesic, anti-inflammatory, antimicrobial, anti-oxidant, antimalarial, antihepatotoxic activities, and antidiarrheal and wound-healing properties (Umachigi *et al*, 2007; Alam *et al*, 2008,), the phytochemical investigations resulted in the isolation of indole alkaloids, steroids, terpenoids, triterpenes and saponins from this plant (Banerji, 1977a; 1978b; Brown & Chapple, 1976; Kitagawa *et al*, 1996; Sahu *et al*, 1999a; 2000b). Along with botanical pesticides we also evaluated the toxicity effects of nanoparticles on mosquitoes. Nanoparticles are viewed as the fundamental building blocks of nanotechnology (Mansoori *et al*, 2005). Nanoparticles were synthesized from three different leaf conditions such as fresh leaves, sun-dried leaves and hot-air oven dried leaves.

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(Torresdey *et al*, 2003) demonstrated gold nanoparticle synthesis within live alfalfa plants from solid media There have been recent reports on phytosynthesis of gold nanoparticles by employing coriander leaves. (Narayana *et al*, 2008) sundried Cinnamomum camphora leaves (Huang *et al*, 2008), phyllanthin extract (Kasthuri *et al*, 2009a) and purified compound extracted from henna leaves (Kasthuri *et al*, 2009b). Research on biosynthesized nanoparticles and insect control should be geared towards introduction of faster and ecofriendly pesticides in future (Bhattacharyya *et al*, 2007)

Hence, an attempt is made in the present study to analyse the functional groups of phytoactive compounds present in the leaf extracts of Anthocephalus cadamba and biosynthesized Gold nanoparticles of Cymbopogan citratus. They were subjected to dose response bioassay for growth and developmental activity. Against culexquinquefasciatus.

MATERIALS AND METHOD

Collection &maintainance 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.

Collection of plant materials

The plant materials of Anthocephalus cadamba (Rubiaceae) and Cymbopogan citratus (for synthesis of Gold nanoparticles) were collected from in and around Palani hills, Palani, Tamilnadu, India. The plants were authentified at BSI (Botanical Survey of India) and the specimens were deposited at Zoology Department, Bharathiar University, and Coimbatore, India.

Preparation of plant extracts

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. 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.

Synthesis of gold nanoparticles

The collected fresh leaves of plant Cymbopogan citratus was washed three or four times with distilled water. 25 gm of leaves were chopped into fine small pieces and added to 100 ml of deionized water and boiled for 5 minutes and at known temperature (55^{o}C) using a water bath. This was filtered to get a clear aqueous extract.

Chloroauric acid ($HAuCl_4$), were purchased from Sigma Aldrich Chemcals was used in typical synthesis for gold nanoparticles using Cymbopogan Citratus and the leaf extract was added to lmM concentration of aqueous Chloroauric acid($HAuCl_4$),solution in 100 ml of conical flask stored at room temperature under dark condition.

Larval and pupal duration Assay

To determine the effect of Anthocephalus cadamba and Gold Nanoparticles on the growth and development of culexquinquefasciatus, different concentrations of both the extract were prepared in an enamel tray of 30cm x 25cm x 5 cm dimensions. Fifty eggs were released in treated water and allowed to hatch. The number of dead larvae and deformities, and percentage adult reaching adulthood was reported from the average of 5 replicates.

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, 1972).

RESULTS AND DISCUSSION

Insecticidal activity of plant extract, even from the same source, can be inherently variable for many readers. The chemical composition and broad spectrum of biological activity for plant extract can vary with plant age, the plant tisssues, geographical origin of plant, organ used in the distillation process, the type of distillation and the species and the age of a targeted post organism (chiasson *et al*, 2001). The presence of saponin, steroids, terpenoids, phenols, proteins and sugars in seaweeds of Enteromorpho intestinalis, Dictyota dichotoma, Acanthopora spicifera possess active compounds for development of larvicidal activity. (Ravikumar, *et al*, 2011).The extract of different parts of Withaniasomnifera on Triboliumcastenum causes morphological abnormalities and significant mortality.(MonaArora *et al*, 2011). It was suggested that nanoparticles, because of their small sizes, could act like haptens to modify protein structures, either by altering their function or rendering them antigenic, thus raising their potential for autoimmune effects (Klimuk *et al*, 2000). Effect of therapeutically used nanoparticles on cell membrane, mitochondrial function, prooxidant/antioxidant status, enzyme leakage, DNA, and other biochemical endpoints was elucidated.

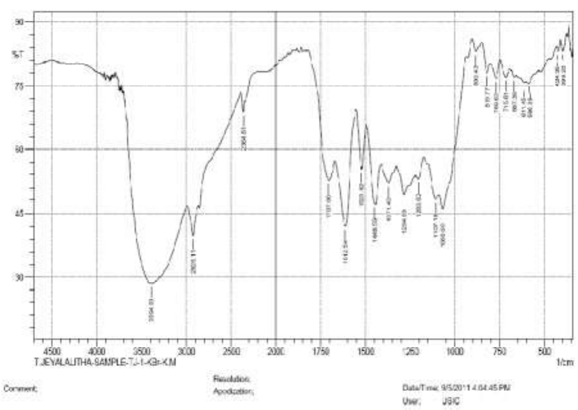
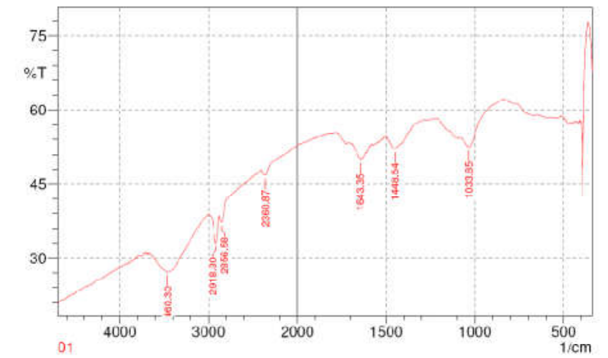


Fig. 1 FT-IR spectrum of crude extract of Anthocephalus cadamba

The FT-IR analysis showed that Anthocephalus cadamba leaf extract and biosynthesized gold nanoparticles of Cymbopogan citratus had many functional groups. These compounds they act on growth and development of culexquinquefasciatus.

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	Amine
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
		C-Cl stretch alkyl halides
18	611.45	C-Br stretch alkyl halides
		C-Cl stretch acid chlorides
		C-Cl stretch alkyl halides
19	586.38	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



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1033.85	52.5	3.42	1085.92	827.11	59.69	0.95
2	1448.54	52.09	0.55	1463.97	1419.61	12.42	0.09
3	1643.35	50	0.45	1691.57	1637.56	15.63	0.12
4	2360.87	46.73	0.77	2384.02	1955.82	126.93	-1.12
5	2856.58	37.2	2.48	2881.65	2810.28	29.53	1.23
6	2918.3	33.07	5.58	2995.45	2881.65	50.16	3.26
7	3460.3	27.17	0.04	3583.74	3458.37	69.36	0.57

Fig. 2 FT-IR spectrum of Biosynthesized Gold nanoparticles of Cymbopogan citratus

Table 2 FTIR analysis of synthesized gold nanoparticles synthesised with the help of *Cymbopogan citrates*

S.No.	Wave number	Functional groups
1.	3460.3 Alcohol/phenol O-H stretch	Amines N-H stretch (1 per N-H bond)
2.	O-H stretch 2918.30 Carboxylic Acid	Alranes C-H stretch
3.	2856.58 Carboxylic Acid O-H stretch	Alkyl/OH stretch
4.	2360.87	Phosphines P-H stretch
5.	1643.35 amides C=O stretch	R_2 C=N-R stretch
6.	1448.54 C= stretch aromatics	Nitro groups
7.	1033.85 alkyl halide	Amine

FTIR analysis was used for the characterization of the extract and the synthesized Gold nanoparticles and shown in fig.2and Tab.2. The FTIR spectra of (Lemon grass) Cymbopogan citratus extract before and after bioreduction did not show any significant changes. The FTIR spectrum of the leaf extract showed bands at 3460.3 and 1033.85 cm-1 is the characteristic of the hydroxyl functional group in alcohols and phenolic compounds. The band at 1033.85 cm-1 can be assigned to the amide 1 band of the proteins released by the Cymbopogan citratus or to alkyl halide C-f stretch. The FTIR spectrum of gold Nanoparticles showed bands at 1448.54, 1643.35, 2360.87, 2856.58 cm-1. The band at 1448.54 cm-1 corresponds to the c=c stretching vibration of Nitro groups of aromatics. The bands at 1643.35 and 2360.87 cm-1 corresponds to the amides of c=o stretching and P-H stretch of phosphines. The bands at 2856.58 and 2918.30 cm-1 can be assigned to the carloxylic acids. The observed peaks are more characteristic of flavonoids and terpenoids that are very abundant in lemon grass leaves extract. IGRs prevent an insect from reaching maturity by interfering with the molting process (greenhouse magazine, 2012).

This in turn curbs infestations because immature insects cannot reproduce (Wikipedia, 2010.) Because IGRs work by interfering with an insect's molting process, they take longer to kill than traditional insecticides. Death typically occurs within 3 to 10 days, depending on the product, the insect's life stage when the product is applied and how quickly the insect develops. Some IGRs cause insects to stop feeding long before they die. (greenhouse magazine, 2012) Hormonal IGRs typically work by mimicking or inhibiting the juvenile hormone (JH), one of the two major hormones involved in insect molting. IGRs can also inhibit the other hormone, ecdysone, large peaks of which trigger the insect to molt. If JH is present at the time of molting, the insect molts into a larger larval form; if absent, it molts into a pupa or adult (greenhousemagazine, 2012). IGRs that mimic JH can produce premature molting of young immature stages, disrupting larval development (greenhousemagazine, 2012). They can also act on eggs, causing sterility, disrupting behavior or disrupting diapause, the process that causes an insect to become dormant before winter.(James *et al*,1993) IGRs that inhibit JH production can cause insects to prematurely molt into a nonfunctional adult(James *et al*,1993). IGRs that inhibit ecdysone can cause pupal mortality by interrupting the transformation of larval tissues into adult tissues during the pupal stage (greenhousemagazine, 2012).

Table 3 Effect of methanolic extracts of of Anthocephalus cadamba on growth and development 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

Effect of extracts of *Anthocephalus cadamba* on **growth and development** 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.

Table 4 Effect of gold nanoparticles of *Cymbopogon citratus* on growth and development 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
0.2	75	16	71	70
0.4	71	22	66	63
0.8	64	30	58	58
1.6	51	49	44	46
3.2	25	69	35	30

** Significant at 1% level

Effect of biosynthesized gold nanoparticles of *Cymbopogan citratus* on **growth and development** of filarial vector *Culex quinquefasciatus* is given in table 20. For treatment the concentration taken were 0.2, 0.4, 0.8, 1.6 and 3.2 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 0.2, 0.4, 0.8, 1.6 and 3.2 the number of Adult reaching adulthood were 70, 63, 58, 46 and 30 respectively.

Table 5 Effect of gold nanoparticles of cymbopogan citratus and methanolic extracts of Anthocephalus cadamba on growth and development 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
0.01+1	73	23	64	61
0.05+5	69	25	60	59
0.1+10	56	34	52	55
0.5+15	39	50	40	34
1+20	18	66	24	19

** Significant at 1% level

Effect of biosynthesized gold nanoparticles of Cymbopogan citratus and extracts of Anthocephalus cadamba on growth and development of filarial vector Culex quinquefasciatus is given in table 22. For treatment the concentration taken were 0.1+1, 0.05+5, 0.1+10, 0.5+15 and 1+20 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 0.1+1, 0.05+5, 0.1+10, 0.5+15 and 1+20 ppm the numbers of adult reaching adulthood were 61, 59, 55, 34 and 19 respectively. The present results revealed that the individual (Arjunan *et al*,2013; Dhanasekaran *et al* , 201;.Banu *et al*, 2014; Marcard *et al*,1986) and the combined effect (Pushphalatha and muthukrishnan , 1999; shaalan *et al*, 2005) of green, biological synthesis of gold nano particles from cymbopogan citratus and the plant extract Anthocephalus cadamba have the potential to be utilized as a good, rapid, eco-friendly approach for the control of mosquito population. It is totally a new pathway but, can be effectively utilized for the efficient killing of mosquitoes. Therefore, biological control can thus provide an effective and environmental friendly approach, which can be used as an alternative to minimize the mosquito population.

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