Final Report of Major Research Project

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ISOLATION OF NOVEL PESTICIDAL COMPOUND(S) FROM SELECTED INDIGENOUS MEDICINAL PLANTS WITH SPECIAL REFERENCE TO MOSQUITOCIDAL ACTIVITIES AGAINST IMPORTANT VECTOR MOSQUITOES (DIPTERA: CULICIDAE).

Submitted To

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Submitted by

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Introduction

With respect to the human well-being, mosquitoes are of great economic impact because their bites are annoying and may cause skin allergies, and they are vectors for a number of diseases, such as malaria, yellow fever, dengue, filariasis, and certain types of encephalitis such as West Nile Fever (Service, 1993). Mosquito-borne diseases, such as filariasis, malaria, dengue, yellow fever, and Japanese encephalitis, contribute significantly to disease burden, death, poverty, and social debility in tropical countries (Jang *et al*., 2002). Lymphatic filariasis caused by *Wuchereria bancrofti* and transmitted by mosquito *C. quinquefasciatus* is found to be more endemic in the Indian subcontinent. It is reported that *C. quinquefasciatus* infects more than 100 million individuals worldwide annually. It is estimated that every year at least 500 million people in the world suffer from one or other tropical diseases that include malaria, lymphatic filariasis, Japanese encephalitis, schistosomiasis, dengue, trypanosomiasis and leishmaniasis. One to two million deaths are reported annually due to malaria worldwide. Lymphatic filariasis affects at least 120 million people in 73 countries in Africa, India, Southeast Asia and Pacific Islands. These diseases not only cause high levels of morbidity and mortality but also inflict great economic loss and social disruption on developing countries such as India, China, etc. India alone contributes around 40% of global filariasis burden and the estimated annual economic loss is about 720 (Hotez *et al*., 2004).

Control of the mosquito larvae is frequently dependent on continued applications of organophosphates and insect growth regulators. An obvious method for the control of mosquito-borne diseases is the use of insecticides, and many synthetic agents have been developed and employed in the field with considerable success. However, one major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment. It has also provoked undesirable effects, including toxicity to non target organisms, and fostered environmental and human health concerns (Yang *et al*., 2002).

Thus, the effort towards mosquito control continues to be an important strategy in preventing the mosquito-borne diseases (Billingsley *et al*., 2008). Use of synthetic chemicals with insecticidal properties such as organochlorines, organophosphates, carbamates, and pyrethroids has been proven to be the most important effective method to control mosquitoes and other insect pests all over the world. The toxicity problem, together with the growing incidence of insect resistance, has called attention to the need for novel insecticides and for more detailed studies of naturally occurring insecticides (Ansari *et al*., 2000).

The acquisition of new strategies or natural products for mosquito control has intensified over the past decades because of concerns over chemical contaminations to the environments. The use of plant active principles as mosquito control agents can be effective, and it has been shown to minimize the impact that most pesticide compounds impose on the environment.

Significance of the study

As the mosquitoes are menaces to human life in various ways from disturbing the physiology of human to challenging the immune system. The present line of research are definitely pave the way for the exploration of new, natural and human-ecofriendly mosquitocidal compound, in turn this made an imminent research for the society in the need of hour.

It is a fact that the compounds isolated from the plants are environmentally safer, non-toxic; yet the efficacy of these botanical pesticides is threatened by development of resistance in insect pest populations. The selected plants have given useful compounds and utilized to develop a new eco-friendly product, which is the current need for IPM program. On the other hand it is suggested that implementation of some management strategies such as use of botanical in combination with low toxic chemical pesticides, would combat development of resistance in insect pests.

Objectives

- To investigate the hexane, diethyl ether, dichloromethane, ethyl acetate and methanol crude extracts of selected plants for their larvicidal activity against the fourth instar larvae of *C. quinquefasciatus, A. aegypti* and *An. stephensi.*
- To investigate the hexane, diethyl ether, dichloromethane, ethyl acetate and methanol crude extracts of selected plants for their ovicidal activity against the freshly laid eggs of *C. quinquefasciatus, A. aegypti* and *An. stephensi.*
- To investigate the hexane, diethyl ether, dichloromethane, ethyl acetate and methanol crude extracts of selected plants for their repellent activity against the freshly emerged adults of *C. quinquefasciatus, A. aegypti* and *An. stephensi.*
- To investigate the fractions of promising crude extract(s) of selected plants for their larvicidal, ovicidal and repellent activity against the freshly emerged larvae, freshly laid eggs and adults of *C. quinquefasciatus, A. aegypti* and *An. stephensi.*
- To isolate and elucidate the structure of promising compound(s) / active principle(s) from effective fraction by using various spectral analysis *viz.,* TLC, CC, UV, IR, HPLC and NMR (^{C}NMR and ^{H}NMR) spectral data.

METHODOLOGY

Plant collection and processing

Plant sampling was carried out during the growing season (March– April). Fully developed leaves of the selected were be collected from in and around Yelagiri hills, Salem district, Tamil Nadu, India (Plate 1a - d). At the time of collection, two pressed voucher herbarium specimens were prepared per species for identification and confirmation with the help of plant taxonomist, Department of Botany, Annamalai University. Bulk samples were air-dried in the shade and after drying, each sample was ground to a fine powder.

List of plants selected for evaluation

S. No.	Plant Name	Family
1.	*Coleus aromaticus*	Lamiaceae
2.	*Ageratina adenophora*	Asteraceae

Extraction method

The dried leaves (500g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with hexane, diethyl ether, dichloromethane, ethyl acetate and methanol (2500ml, Ranchem), in a Soxhlet apparatus separately until exhaustion (Plate 2a - c). The extract was concentrated under reduced pressure 22–26 mmHg at 45°C by 'Rota-vapour' and the residue obtained will be stored at 4°C (Plate 3a & b).

Mosquito rearing

Eggs of *Ae. aegypti, An. stephensi* and *C. quinquefasciatus* were collected from ICMR centre, Virudachalam, Cuddalore District, Tamilnadu. The egg rafts were then brought to the laboratory (Plate 4a - d). The eggs were placed in enamel trays (30×24×5 cm) each containing 2 *l* of tap water and kept at room temperature (28 ± 2°C) with a photoperiod of 16:8 h (L:D) for larval hatching. The larvae of each mosquito species were individually maintained in trays under the same laboratory conditions and fed with a powder 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°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 solutions in a Petri dish to feed adult mosquitoes were 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× 10×4 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 subject to toxicity assays or allowed to hatch out under the controlled laboratory conditions described above. Only the newly hatched specific instars of larvae or the pupae of different mosquito species were used in all bioassays.

BIOASSAYS

Larvicidal activity

The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO (2005) (Plate 5a - c). From the stock solution different test concentrations were prepared and they were tested against the freshly moulted (0 – 6 hrs) third instar larvae of *An. stephensi*, *Ae. Aegypti* and *C. quinquefasciatus*. DMSO (emulsifier) in water was treated as control. The larvae of these mosquito species (25 larvae) were introduced in 500-ml plastic cups containing 250 ml of aqueous medium (249 ml of dechlorinated water + 1ml of emulsifier) and the required amount of plant extract was added. The larval mortality were observed and recorded after 24 h of post treatment. For each experiment, five replicates were maintained at a time. The percentage of mortality was calculated by using Abbott's formula (Abbott, 1925). The LC_{50}, LC_{90}, 95% confidence limit of Lower Confidence Limit (LCL) and Upper Confidence Limit (UCL), chi-square values and the degrees of freedom were calculated by using Probit analysis with Statistical Package for Social Sciences (SPSS) 17.0 Version in MS-Excel, 2007.

Plate 1(a & b): Medicinal plants

Coleus aromaticus *Ageratina adenophora*

Plate 1(c& d): Leaf Powder

Plate 2 (a & b): Extraction of Plant Material by Soxhlet apparatus

Plate 2 (c): Extraction of leaf powder of *Coleus aromaticus* & *Ageratina denophora*

Plate 4 (a & b): Laboratory culture of mosquito larvae: *Culex quinquefasciatus*

Plate 4 (c & d): Laboratory culture of mosquito larvae: *Aedes aegypti*

Plate 5 (a & c): Experimental set up

Ovicidal activity

The method of Su and Mulla (1998) were slightly modified to suit with the present experiment for testing the ovicidal activity of the plant extracts. The various concentrations as stated in the previous experiments were prepared from the stock solution. Before treatment, the eggs of *An. stephensi, Ae.aegypti* and *C. quinquefasciatus* were counted individually with the help of hand lens. Freshly laid eggs of these mosquito species (100) were exposed to each concentration of leaf extract until they hatched or died. Eggs 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 48 h post treatment by the following formula.

$$\%\text{Ovicidal Activity} = \frac{\text{No. of eggs hatched}}{\text{Total no. of eggs treated}} \text{ X } 100$$

Repellent Activity

The repellent study was following the method of WHO (2005). Three-day-old blood-starved female *Cx. quinquefasciatus, Ae.aegypti*and *An. stephensi*mosquitoes (100) were kept in a net cage (45 cm × 30 cm × 45 cm). The volunteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. The arms of volunteer, only 25 cm^2 dorsal side of the skin on each arm was exposed and the remaining area covered by rubber gloves. The crude extract was applied at 1.0, 2.5 and 5.0 mg/cm^2 separately in the exposed area of the fore arm. Only ethanol served as control. The time of the test dependent on whether the target mosquitoes day-or night biters. *Ae.aegypti* will be tested during the day time from 07.00 to 17.00h, while *Cx. quinquefasciatus* and *An. stephensi* were tested during the night from 19.00 to 05.00h. The control and treated arm were introduced simultaneously in to the mosquito cage, and gently tapping the sides on the experimental cages, the mosquitoes were activated. Each test concentration was repeated six times. The volunteer conducted their test of each concentration by inserting the treated and control arm in to the same cage for one full minute for every five minutes. The mosquitoes that landed on the hand were recorded and then shaken off before imbibing any blood; making out a 5 minutes protection. The percentage of repellency was calculated by the following formula.

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

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

Determination of lethal concentrations

Lethal concentration (LC_{50}) represents the concentration of the test material that caused 50% mortality of the test (target and non-target) organisms within the specified period of exposure, and it

was determined by exposing various developmental stages of the mosquitoes to different concentrations of the extract. Based on the mortality of the test organisms recorded in these bioassays, LC_{50} and LC_{90} were calculated along with their fiducial limits at 95% confidence level by probit analysis using SPSS software package 17.0 (Statistical Package of Social Sciences) software. Results with $p<0.05$ were considered to be statistically significant.

Hypothesis to be Tested: The selected plant crude extracts do possess the potentiality of mosquitocidal (=larvicidal/mosquito ovicidal) activity.

PHYTOCHEMICAL ANALYSIS

The above said bioassays were repeated with fractions at 5, 10, 15, 20 and 25ppm concentrations against selected mosquitoes.

Preliminary studies on phytochemical screening

The preliminary phytochemical screening was carried out for the quality of various organic compounds present in the effective crude extracts.

Steroid:Liebermann- Burchard test: A few mg of the substance in chloroform is treated with a few drops of acetic acid, acetic anhydride and two drops of concentrated H_2SO_4 the mixture was heated gently if necessary. Development of blue or green colour indicated the presence of steroid.

Triterpenoid: Noller's: A few mg of the substance in a dry test tube is treated with a bit of tin foil and 0.5 ml of thionyl chloride. Heated gently if required. Development of pink colour indicated the presence of triterpenoid.

Sugars/glycosides: A few mg of the substance is mixed with equal quantity of anthrone and treated with two drops of concentrated H_2SO_4. Heated gently on a water bath. Development of dark green colour indicated the presence of sugar/glycosides.

Acid: A few mg of the substance is treated with aqueous $NaHCO_3$. Effervescence shows the presence of acid, which is due to liberation of CO_2.

Quinone: A few mg of the substance in alcohol is treated with H_2SO_4 or aqueous NaOH. Coloration indicates the presence of quinoid compounds.

Coumarin: A few mg of substance in alcohol is treated with alcoholic NaOH. Development of yellow colour indicates the presence of coumarin.

Flavanoid:Shimoda test: A few mg of the substance in alcohol is treated with magnesium foils and a few drops of concentrated HCL. Development of red or pink colour indicates the presence of flavanoid.

Furanoid: Ehrlich test: A few mg of the substance in alcohol is treated with a pinch of paradimethyl amino benzaldehyde and a few drops of concentrated HCL. Development of red or pink colour indicates the presence of furanoid.

Tannin: A few mg of substance in alcohol is treated with a few drops of aqueous lead acetate. Precipitation indicates the presence of Tannin.

Alkaloid:Dragendorff's test: A few mg of substance in acetic acid (filtered if necessary) is treated with two drops of dragendorff reagent (potassium mercuric iodide). Development of red or orange precipitation indicates the presence of alkaloid. Excess reagent should be avoided.

Phenol: A few mg of the substance in alcohol is treated with alcoholic ferric chloride. Any coloration indicates the presence of phenolic compounds.

SECONTARY PHYTOCHEMICAL ANALYSIS

Thin layer and Column chromatography

The methanolic extract of three plants was analyzed by TLC with different solvent systems. Plants extract were analyzed by using Column Chromatography with different solvent system.

Infrared spectroscopy

IR is used to probe bond vibrations and bending in molecules and to reveal the types of functional groups present in compound. Functional group region is in the range from 4000-1600 cm-1 and finger print region is from 1550-660 cm-1.

Gas Chromatography-Mass Spectroscopy Analysis

Gas chromatography-mass spectroscopy (GC-MS) was performed using a mass detector Turbo mass gold-Perkin Elmer particular identifier and a Elite-5MS (5% Diphenyl/ 95% Dimethy poly siloxane) slender segment. The stove temperature was customized from 50 to 280ºC at the rate of 5ºC min-1 and stopped at this temperature for 36 min. The delta and interface temperatures were 250 and 280ºC, respectively. The transporter gas was heat a stream rate of 1.0 ml min-1 (consistent stream). The sample (2µl) was injected at a split of 10:1. Electron sway mass spectrometry was conveyed at 70eV. Particle source and fourfold temperature were kept up at 250 and 200ºC separately (Kumaravel *et al.,* 2010).

Mass spectrometry

Mass spectra were recorded at the Department of Instrumentation, Indian Institute of Technology using a Manchester using a Micromass PLATFORM II (ES) and Termo Finnigan MAT95XP (Accurate mass) instrument. Mass spectrometry provides both molecular weight and fragmentation pattern of the compound. It relies of production of ions from a parent compound and the subsequent characterization of the pattern that are produced.

Statistical analysis

The average adult mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and Chi-square values were calculated using the SPSS 12.0 version software. Results with $p \leq 0.05$ were considered to be statistically significant.

EXPERIMENTAL RESULTS

The bioactivity of two different medicinal plant extracts of *Coleus aromaticus* and *Ageratina adenophora* were evaluated against three vector mosquitoes, such as *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*. Five different solvents hexane, diethyl ether, dichloromethane, ethyl acetate and methanol have been used for the extraction of medicinal plants. The extract was concentrated by 'Rota-vapour' and the required concentrations were prepared further for the bioassay studies.

Larvicidal activity of *Coleus aromaticus*

Larvicidal activity of *C. aromaticus* with hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extracts have been tested against freshly moulted larvae of three mosquitoes, such as *A. aegypti, An. stephensi* and *C. quinquefasciatus*. Larvae of uniformed size, hale and healthy were subjected to different concentrations *viz.,* 50, 100, 150, 200, and 250 ppm. The data pertaining to the per cent larval mortality is presented in the table 1 and figure 1. Larvicidal activities of *C. aromaticus* with different solvent extracts are presented in the table 2 - 6 and figure 2. Among the five solvent extracts tested, the highest larvicidal activity was observed in methanol extract of *C.aromaticus* against *A. aegypti, An. stephensi* and *C. quinquefasciatus* with LC_{50} and LC_{90} value of 28.66 and 69.19; 22.20 and 58.80; 31.10ppm and 74.31 ppm, respectively. The recorded data were found statistically significant (Table 6; DMRT, $p<0.05$).

Larvicidal activity of *Ageratina adenophora*

Larvicidal activity of *A. adenophora* with hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extracts have been tested against freshly moulted larvae of three mosquitoes, such as *A. aegypti, An. stephensi* and *C. quinquefasciatus*. Larvae of uniformed size, hale and healthy were subjected to different concentrations *viz*., 60, 120, 180 240, 300 and 360 ppm. The per cent larval mortality of three mosquitoes exposed to five different solvent extracts of *A. Adenophora* is depicted in Table 7 and Figure 3. The calculated LC_{50} and LC_{90} value of *A. Adenophora* with hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extracts against three mosquites are presented in Table 8 to 12 and Figure 4. The methanol extract of *A. Adenophora* exhibited maximum larvicidal activity with LC_{50} and LC_{90} value of 137.02 and 243.99; 108.52 and 185.99; 161.22 and 280.47 ppm against *A. Aegypti, An. stephensi* and *C. quinquefasciatus*, respectively. The recorded data were found statistically significant (Table 12; DMRT, $p<0.05$).

Table 1: **Larvicidal activity of the *Coleus aromaticus* extract against *Ae.aegypti, An. Stephensi* and *Cx. quinquefasciatus***

Mosquitoes	Solvents	Per cent Larval Mortality (%) ± SD				
		50ppm	100ppm	150ppm	200ppm	250ppm
Ae. Aegypti	Hexane	20.2±1.30	36.6±1.41	56.8±2.91	72.6±2.38	84.2±1.92
	Dichloromethane	32.2±1.09	48.4±1.67	64.2±1.81	80.6±1.94	100.0±0.0
	Diethyl ether	28.4±1.64	44.2±1.48	60.2±1.14	76.4±1.34	88.6±2.07
	Ethyl acetate	36.2±2.38	56.2±1.51	76.4±2.28	92.8±2.34	100.0±0.0
	Methanol	44.2±1.81	64.2±1.92	84.4±1.94	100.0±0.0	100.0±0.0
An. Stephensi	Hexane	24.6±2.16	34.8±3.50	48.8±3.20	60.8±4.30	70.4±2.94
	Dichloromethane	40.6±2.86	56.4±2.30	76.6±2.68	92.8±2.88	100.0±0.0
	Diethyl ether	32.8±3.13	44.6±3.78	64.4±2.28	80.2±1.92	92.4±2.28
	Ethyl acetate	44.2±2.44	60.8±3.80	80.6±2.60	100.0±0.0	100.0±0.0
	Methanol	52.2±1.81	68.6±2.50	88.6±2.19	100.0±0.0	100.0±0.0
Cx. quinquefasciatus	Hexane	12.2±2.12	28.6±2.96	44.4±2.77	64.8±3.78	76.2±2.68
	Dichloromethane	24.6±2.50	40.8±3.20	52.8±3.03	68.6±3.28	84.8±3.01
	Diethyl ether	20.6±2.30	36.8±3.24	48.6±2.60	64.8±3.84	80.8±3.01
	Ethyl acetate	32.2±1.94	52.4±2.60	72.8±3.63	88.4±2.79	100.0±0.0
	Methanol	40.2±1.48	60.4±2.19	76.2±1.94	92.6±2.12	100.0±0.0

Value represents Mean ± S.D. of five replications.
*mortality of the larvae observed after 24h of exposure period WHO (2005).

Table 2. Larvicidal activity of *Coleus aromaticus* hexane extract tested against the freshly moulted (0-6h old) 3rd instar larvae of selected mosquitoes species.

Species	LC_{50} (ppm)	95%Confidence Limits (ppm)		LC_{90} (ppm)	95%Confidence Limits (ppm)		χ^2 (*df*)	Regression
		LCL	UCL		LCL	UCL		
A. aegypti	55.28	49.88	60.42	110.70	101.08	124.29	0.221	y=2.5943x+0.651
An. stephensi	63.47	56.23	71.14	143.91	125.56	174.35	0.130	y=1.8265x+1.786
C. quinquefasciatus	69.59	64.08	75.71	129.34	116.91	147.63	0.765	y=2.6682x+0.250

Table 3. Larvicidal activity of *Coleus aromaticus* dichloromethane extract tested against the freshly moulted (0-6h old) 3rd instar larvae of selected mosquitoes species.

Species	LC_{50} (ppm)	95%Confidence Limits (ppm)		LC_{90} (ppm)	95%Confidence Limits (ppm)		χ^2 (*df*)	Regression
		LCL	UCL		LCL	UCL		
A. aegypti	41.58	18.23	55.47	88.44	71.20	137.20	10.305	y=-3.4024x+9.978
An. stephensi	32.32	26.33	37.18	74.95	68.92	83.02	4.293	y=-3.3564x+10.164
C. quinquefasciatus	54.77	48.68	60.49	117.47	105.95	134.50	0.702	y=2.2794x+1.1971

LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality.
LCL = Lower Confidence Limit; UCL = Upper Confidence Limit;
Values in a column with a different superscript alphabet are significantly different at P <0.05 (MANOVA; LSD -Tukey's Test).

Table 4. Larvicidal activity of *Coleus aromaticus* diethyl ether extract tested against the freshly moulted (0-6h old) 3rd instar larvae of selected mosquitoes species.

Species	LC_{50} (ppm)	95%Confidence Limits (ppm)		LC_{90} (ppm)	95%Confidence Limits (ppm)		χ^2 (*df*)	Regression
		LCL	UCL		LCL	UCL		
A. aegypti	47.89	52.73	52.73	106.06	96.31	120.09	0.075	y =2.4268x+1.1137
An. stephensi	45.04	38.65	50.52	102.62	93.30	115.97	0.956	y =2.6258x+0.8686
C. quinquefasciatus	60.55	54.70	66.44	123.65	111.35	141.94	0.486	y=2.2747x+1.0846

Table 5. Larvicidal activity of *Coleus aromaticus* ethyl acetate extract tested against the freshly moulted (0-6h old) 3rd instar larvae of selected mosquitoes species.

Species	LC_{50} (ppm)	95%Confidence Limits (ppm)		LC_{90} (ppm)	95%Confidence Limits (ppm)		χ^2 (*df*)	Regression
		LCL	UCL		LCL	UCL		
A. aegypti	34.02	28.54	38.57	74.57	68.78	82.26	3.203	y=-3.213x+9.897
An. stephensi	28.88	11.23	41.35	65.35	51.79	103.66	7.658	y=-7.422x-15.932
C. quinquefasciatus	21.22	46.05	46.05	79.89	67.68	103.97	6.709	y=3.1914x+9.7472

LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality.
LCL = Lower Confidence Limit; UCL = Upper Confidence Limit;
Values in a column with a different superscript alphabet are significantly different at $P < 0.05$ (MANOVA; LSD -Tukey's Test).

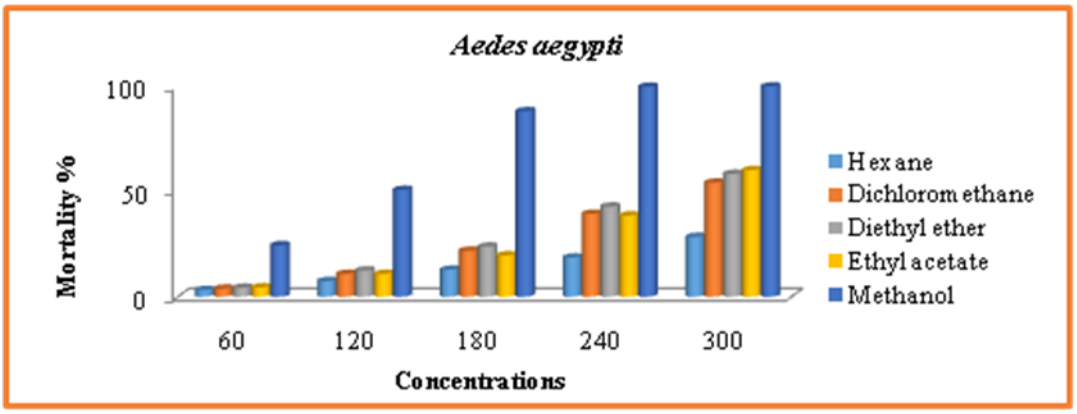
Table 6. Larvicidal activity of *Coleus aromaticus* methanol extract tested against the freshly moulted (0-6h old) 3rd instar larvae of selected mosquitoes species

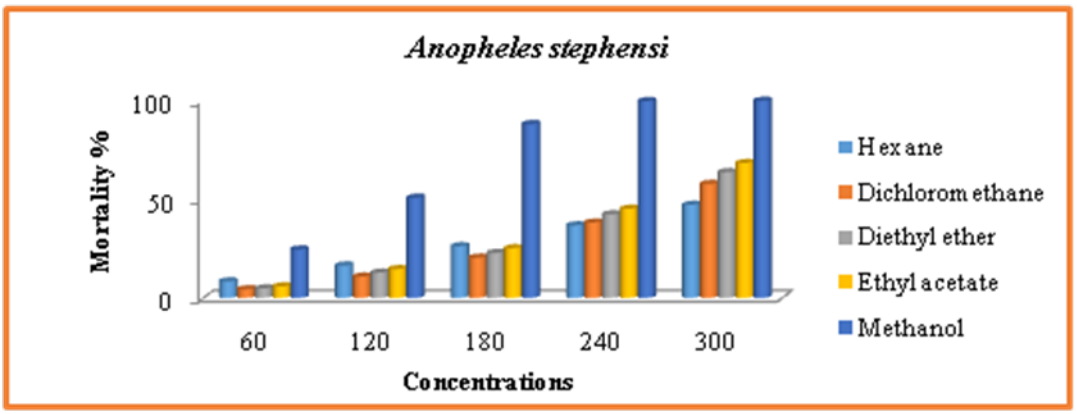
Species	LC_{50} (ppm)	95%Confidence Limits (ppm)		LC_{90} (ppm)	95%Confidence Limits (ppm)		χ^2 (*df*)	Regression
		LCL	UCL		LCL	UCL		
A. aegypti	28.66	22.44	33.59	69.19	63.49	76.82	6.679	y=-7.431x+15.998
An. stephensi	22.20	15.30	27.32	58.80	53.58	65.88	5.889	y=-7.7103x+16.576
C. quinquefasciatus	31.10	24.84	36.11	74.31	68.25	82.45	3.770	y=-3.395x+10.251

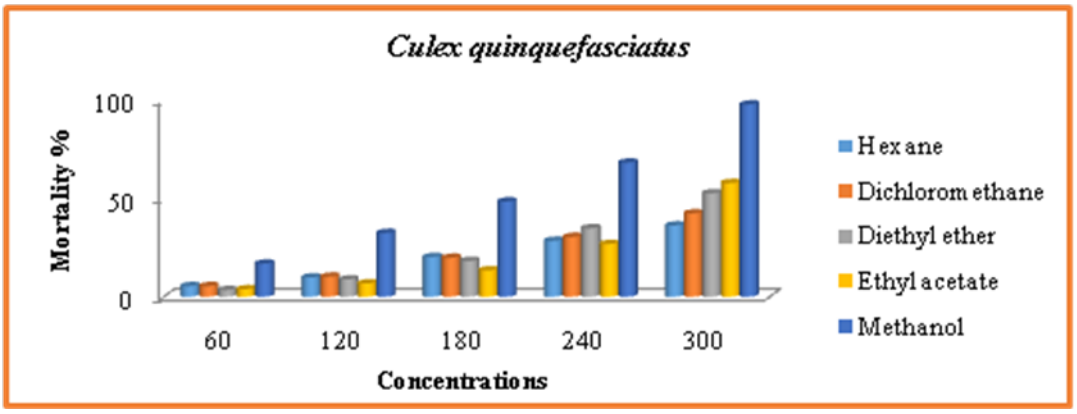
LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality.
LCL = Lower Confidence Limit; UCL = Upper Confidence Limit;

Values in a column with a different superscript alphabet are significantly different at P <0.05 (MANOVA; LSD -Tukey's Test).

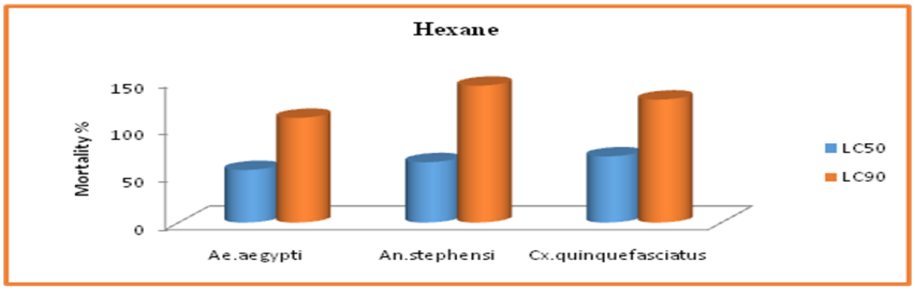
Graph 1: Percent mortality of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* tested with different solvent extract of *Coleus aromaticus*

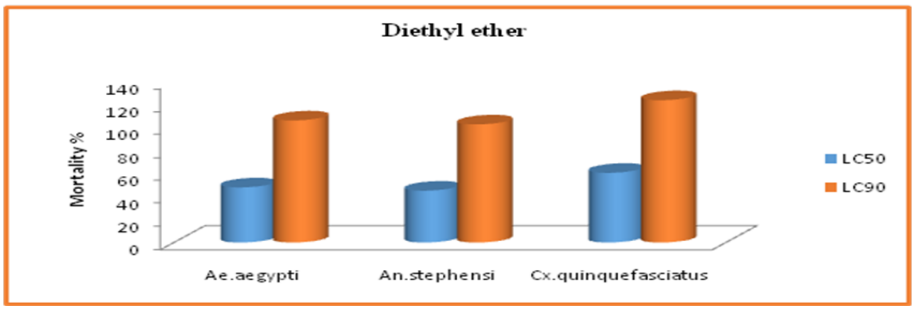




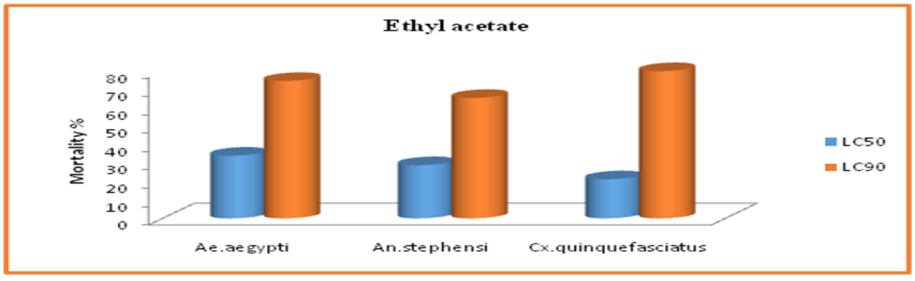


Graph 2: Larvicidal activity of *Coleus aromaticus* tested against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*





Graph 2a: Larvicidal activity of *Coleus aromaticus* tested against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*



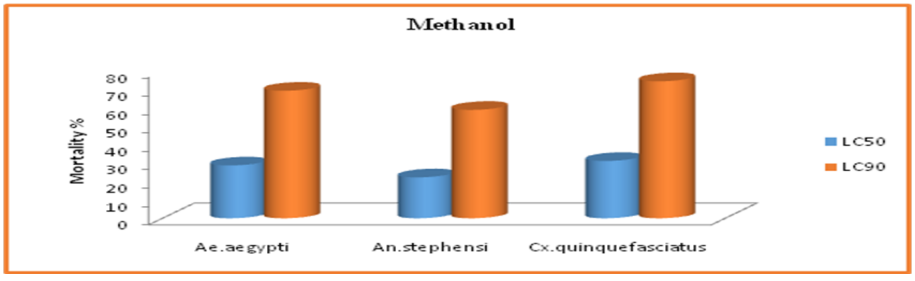


Table 7: Larvicidal activity of the *Ageratina adinophora* extract against *Ae. aegypti, An. stephensi and Cx. quinquefasciatus*

Mosquitoes	Solvents	Larval Mortality (%) ± SD					
		60ppm	120ppm	180ppm	240ppm	300ppm	360ppm
Ae. aegypti	Hexane	3.2±1.09	7.4±1.51	12.8±2.28	18.6±1.67	28.4±1.81	44.8±2.28
	Dichloromethane	3.8±3.01	10.8±2.94	21.8±1.78	39.4±1.51	54.2±2.58	65.8±1.78
	Diethyl ether	4.2±1.09	12.4±2.60	23.8±2.77	42.8±3.11	58.4±2.19	70.2±2.28
	Ethyl acetate	4.4±1.14	10.8±2.94	19.6±1.81	38.6±2.30	60.2±1.78	83.4±1.94
	Methanol	24.4±3.2	50.8±1.92	88.2±1.09	99.8±0.44	100.0±0.0	100.0±0.0
An. stephensi	Hexane	8.4±1.14	16.4±1.51	26.2±1.92	36.8±1.92	47.2±1.64	58.4±2.07
	Dichloromethane	4.4±1.16	10.8±2.28	20.4±2.70	38.2±2.16	57.8±2.16	76.8±2.58
	Diethyl ether	4.8±1.09	12.8±2.28	22.8±3.83	42.4±2.88	63.8±1.64	86.4±1.81
	Ethyl acetate	5.8±1.78	14.6±1.94	25.2±2.16	45.2±2.38	68.4±2.50	89.2±2.38
	Methanol	24.6±1.9	50.8±2.58	88.2±2.86	99.8±0.44	100.0±0.0	100.0±0.0
Cx. quinquefasciatus	Hexane	5.4±1.67	9.8±1.78	20.2±1.78	28.4±1.81	36.2±1.48	52.4±1.67
	Dichloromethane	5.4±1.14	10.2±1.92	19.8±11.64	30.4±2.07	42.4±2.50	54.2±2.58
	Diethyl ether	3.4±1.67	8.8±1.92	18.2±2.16	34.8±2.38	52.6±2.88	65.8±2.04
	Ethyl acetate	3.8±2.48	6.8±1.30	13.4±3.20	26.8±1.30	57.8±2.16	81.2±2.77
	Methanol	16.8±2.4	32.4±2.30	48.4±2.50	68.2±1.09	97.8±0.83	100.0±0.0

Value represents Mean ± S.D. of five replications.
*mortality of the larvae observed after 24h of exposure period WHO (2005).

Table 8. Larvicidal activity of *Ageratina adenophora* hexane extract tested against the freshly moulted (0-6h old) 3rd instar larvae of selected mosquitoes species.

Species	LC_{50} (ppm)	95%Confidence Limits (ppm)		LC_{90}(ppm)	95%Confidence Limits (ppm)		Slope	χ^2 (*df*)
		LCL	UCL		LCL	UCL		
A. aegypti	333.13	308.02	367.29	560.33	500.78	650.61	2.217445	0.893
An. stephensi	314.82	212.82	376.83	568.18	465.17	737.58	5.19284	0.453
C. quinquefasciatus	352.25	323.50	393.21	591.48	523.59	697.46	2.07405	0.910

Table 9. Larvicidal activity of *Ageratina adenophora* dichloromethane extract tested against the freshly moulted (0-6h old) 3rd instar larvae of selected mosquitoes species.

Species	LC_{50} (ppm)	95%Confidence Limits (ppm)		LC_{90} (ppm)	95%Confidence Limits (ppm)		Slope	χ^2 (*df*)
		LCL	UCL		LCL	UCL		
A. aegypti	290.48	273.21	311.04	471.26	433.49	523.72	2.78718	1.235
An. stephensi	274.83	260.16	291.38	431.20	401.70	470.56	3.03219	0.302
C. quinquefasciatus	335.58	310.41	369.87	560.31	501.13	649.90	2.20188	0.300

LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality.
LCL = Lower Confidence Limit; UCL = Upper Confidence Limit;
Values in a column with a different superscript alphabet are significantly different at P <0.05 (MANOVA; LSD -Tukey's Test).

Table 10. Larvicidal activity of *Ageratina adenophora* diethyl ether extract tested against the freshly moulted (0-6h old) 3rd instar larvae of selected mosquitoes species.

Species	LC_{50} (ppm)	95%Confidence Limits (ppm)		LC_{90} (ppm)	95%Confidence Limits (ppm)		Slope	χ^2 (*df*)
		LCL	UCL		LCL	UCL		
A. aegypti	276.25	260.30	294.56	449.29	415.49	495.36	2.91991	1.344
An. stephensi	254.46	241.38	268.53	395.99	371.75	427.40	3.32589	1.985
C. quinquefasciatus	281.00	310.41	318.04	468.35	432.33	518.08	2.88245	0.544

Table 11. Larvicidal activity of *Ageratina adenophora* ethyl acetate extract tested against the freshly moulted (0-6h old) 3rd instar larvae of selected mosquitoes species.

Species	LC_{50} (ppm)	95%Confidence Limits (ppm)		LC_{90} (ppm)	95%Confidence Limits (ppm)		Slope	χ^2 (*df*)
		LCL	UCL		LCL	UCL		
A. aegypti	269.52	256.48	283.87	405.39	381.07	436.96	3.211977	9.238
An. stephensi	241.57	138.73	324.61	472.20	370.55	668.14	4.23911	12.074
C. quinquefasciatus	282.85	269.64	297.65	415.95	391.01	448.60	3.232214	7.118

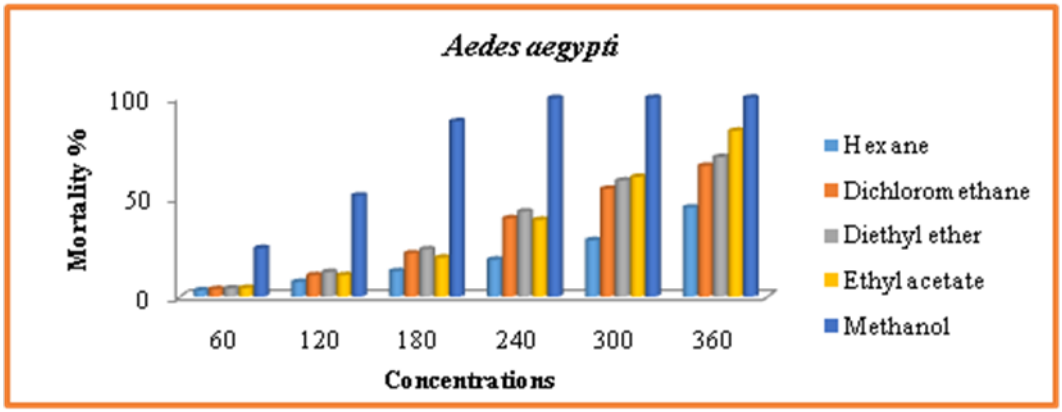
LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality.
LCL = Lower Confidence Limit; UCL = Upper Confidence Limit;
Values in a column with a different superscript alphabet are significantly different at P <0.05 (MANOVA; LSD -Tukey's Test).

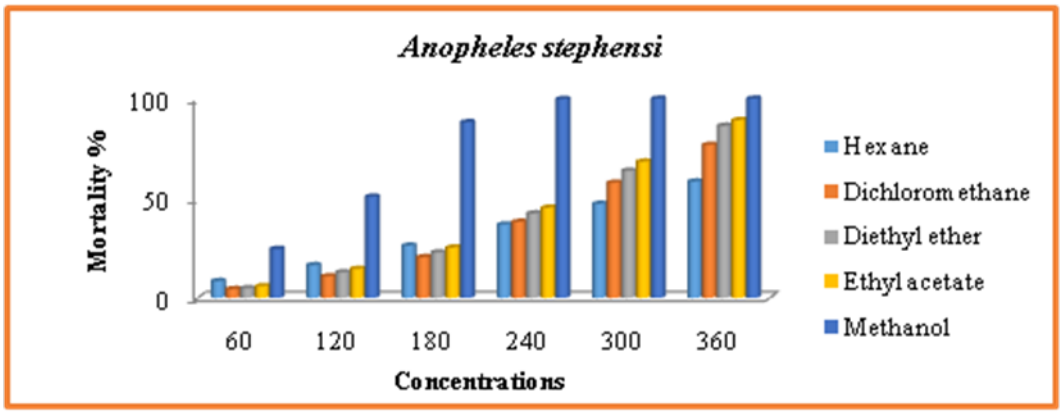
Table 12. Larvicidal activity of *Ageratina adenophora* methanol extract tested against the freshly moulted (0-6h old) 3rd instar larvae of selected mosquitoes species.

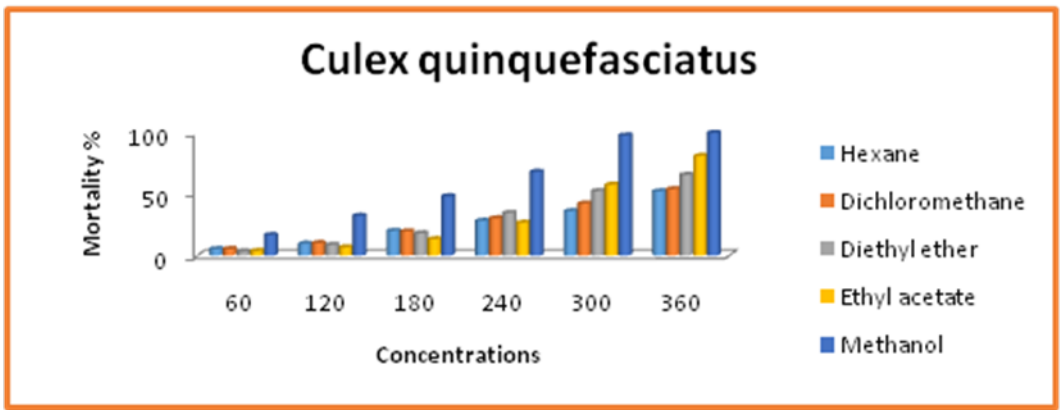
Species	LC_{50} (ppm)	95%Confidence Limits (ppm)		LC_{90} (ppm)	95%Confidence Limits (ppm)		Slope	χ^2 (*df*)
		LCL	UCL		LCL	UCL		
A. aegypti	137.02	125.17	148.02	243.34	227.94	262.76	3.06941	7.024
An. stephensi	108.52	96.37	130.25	185.91	164.25	208.28	4.01254	4.026
C. quinquefasciatus	161.22	149.04	172.76	280.47	263.63	301.53	3.44331	4.482

LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality.
LCL = Lower Confidence Limit; UCL = Upper Confidence Limit;
Values in a column with a different superscript alphabet are significantly different at P <0.05 (MANOVA; LSD -Tukey's Test).

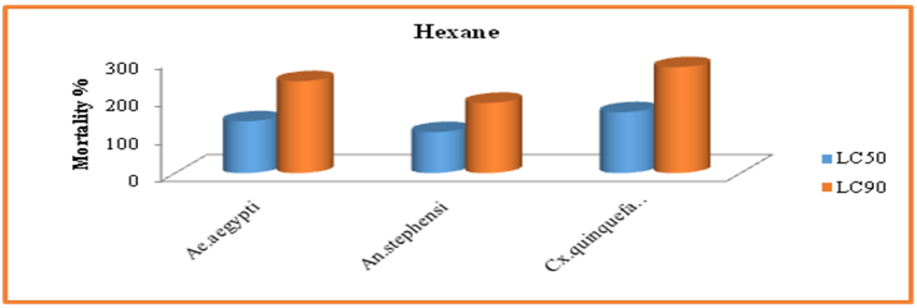
Graph 3: Percent mortality of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* tested with different solvent extract of *Ageratina adenophora*

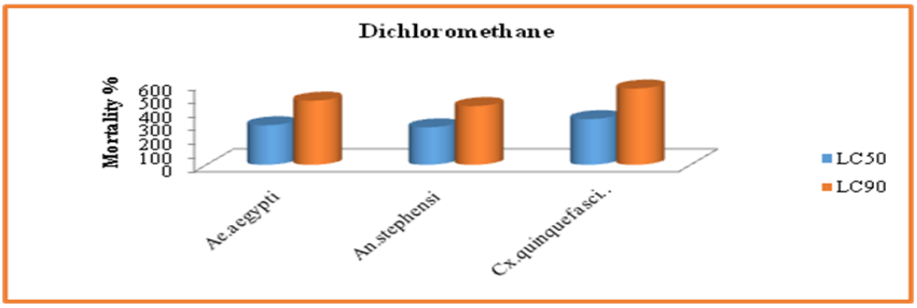


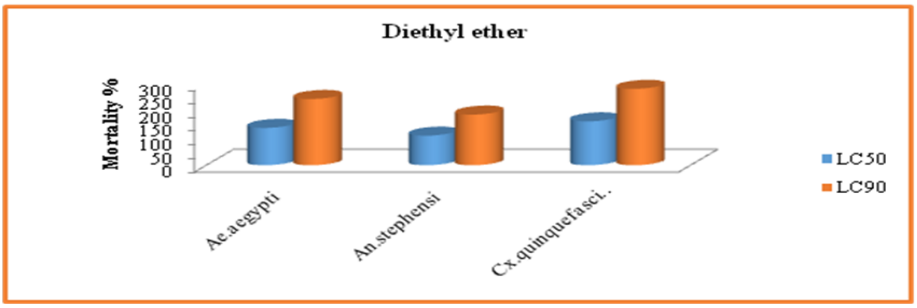




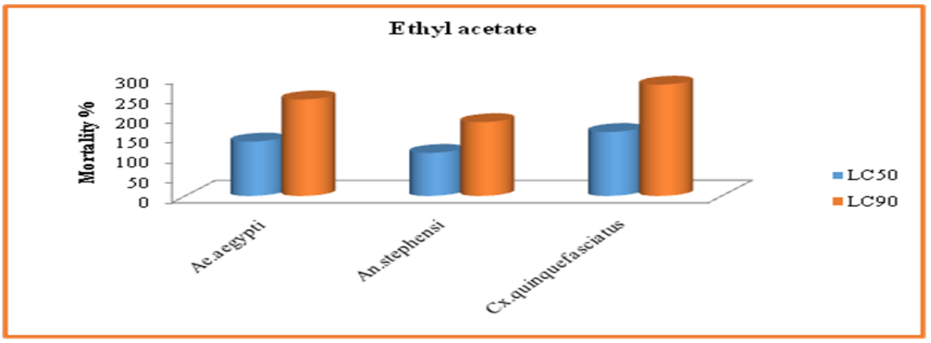
Graph 4: Larvicidal activity of *Ageratina adenophora* tested against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*

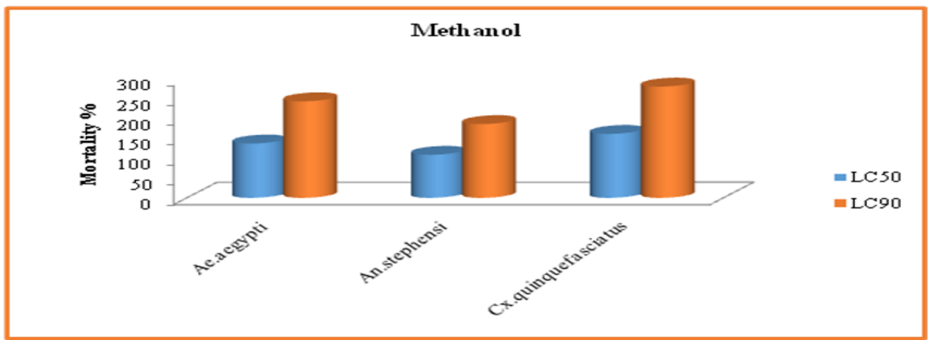






Graph 4a: Larvicidal activity of *Ageratina adenophora* tested against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*





Ovicidal activity of *Coleus aromaticus*

The mean percent of egg hatchability of *A. aegypti, An. stephensi* and *C. quinquefasciatus* were tested with five different solvents at different concentrations of *C. aromaticus* leaves extracts, and the results are listed in Table 13. Among the extracts tested for ovicidal activity against *A. aegypti, An. stephensi* and *C. quinquefasciatus*, maximum ovicidal activity with mathanol extract of *C.aromaticus* exerted 100% mortality (*i.e.,* no hatchability) was recorded (Table 13) at 250ppm, respectively. Control eggs showed the 100% hatchability. The data obtained in the experiments were statistically significant over the control.

Ovicidal activity of *Ageratina adenophora*

The mean percent of egg hatchability of *A. aegypti, An. stephensi* and *C. quinquefasciatus* were tested with five different solvents at different concentrations of *A. adenophora* leaves extracts, and the results are listed in Table 14. Among the extracts tested, maximum ovicidal activity with mathanol extract of *A. adenophora* exerted 100% mortality (*i.e.,* no hatchability) was recorded (Table 14) at 300 ppm against *An. stephensi* and *C. quinquefasciatus*. Control eggs showed the 100% hatchability. The data obtained in the experiments were statistically significant over the control.

Repellent activity of *Coleus aromaticus*

The repellent activity of the leaf extracts of *C. aromaticus* with hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extract has been tested against three to four days old, blood starved 100 adult female mosquitoes, *Ae. aegypti, An. stephensi* and *Cx. Quinquefasciatus*. Table (15 -19) showed repellent activity against three mosquitoes. A higher concentration of 5.0 mg/cm^2 provided 100% protection upto 240 min against *Ae. aegypti,* followed by 210 min. against *An. stephensi* and *Cx. quinquefasciatus*.

Repellent activity of *Ageratina adenophora*

The repellent activity of the leaf extracts of *A. Adenophora* with hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extract were tested against three to four old, blood starved 100 adult female mosquitoes *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus*. The data pertaining to the repellent activity is presented in the table 20 to 24. The repellent activity of methanol extract was found to be most effective and at higher concentration (3.0 & 4.5) provided 100% protection up to 320 min against *C. quinquefasciatus* and *Ae. Aegypti*, respectively and up tp 280 min against *Ae. Aegypti* (Table 24). In the above results it is evident that methanol and ethyl acetate extract of *A. adenophora* exhibited strong repellent activity against the selected mosquito species.

Table 13. Ovicidal activity of *Coleus aromaticus* different extracts tested against selected mosquitoes.

Name of the species	Percentage of egg hatch ability, 48hrs post treatment				
	Concentrations tested (ppm)				
	50	100	150	200	250
Hexane					
A. aegypti	96.8±1.64^{e}	80.6±1.81^{d}	64.2±2.16^{c}	42.6±2.30^{b}	24.2±1.48^{ab}
An. stephensi	89.4±2.30^{e}	75.6±1.51^{d}	59.4±1.81^{bc}	36.8±2.16^{b}	12.8±1.48^{a}
C. quinquefasciatus	87.6±1.81^{e}	71.2±2.16^{cd}	56.4±1.18^{bc}	33.2±1.30^{b}	10.8±1.48^{a}
Control	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
Dichloromethane					
A. aegypti	90.6±1.81^{e}	79.2±2.16^{d}	52.6±2.30^{bc}	24.2±2.16^{ab}	16.2±1.48^{a}
An. stephensi	86.6±1.51^{e}	71.2±1.48^{cd}	53.4±1.81^{bc}	31.6±1.94^{b}	9.2±1.48^{a}
C. quinquefasciatus	84.2±1.78^{de}	63.6±2.60^{c}	45.4±1.94^{bc}	20.8±2.16^{ab}	NH
Control	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
Diethyl ether					
A. aegypti	87.8±1.64^{e}	72.4±2.30^{cd}	44.2±2.16^{b}	19.2±1.48^{a}	6.4±1.51^{a}
An. stephensi	82.6±2.30^{de}	66.4±1.67^{c}	36.2±1.48^{b}	10.4±1.94^{a}	NH
C. quinquefasciatus	79.8±1.64^{d}	59.6±2.19^{bc}	29.4±2.30^{ab}	NH	NH
Control	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
Ethyl acetate					
A. aegypti	85.4±2.30^{de}	66.8±1.48^{c}	31.6±1.81^{b}	14.2±2.04^{a}	3.2±1.48^{a}
An. stephensi	79.4±2.50^{d}	50.8±2.16^{bc}	28.4±1.18^{ab}	NH	NH
C. quinquefasciatus	70.8±2.16^{cd}	31.8±2.58^{b}	NH	NH	NH
Control	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
Methanol					
A. aegypti	80.8±1.78^{de}	63.6±1.94^{c}	29.2±2.16^{ab}	7.4±1.81^{a}	NH
An. stephensi	75.4±1.51^{d}	45.8±2.28^{bc}	21.6±2.30^{ab}	NH	NH
C. quinquefasciatus	60.8±1.78^{bc}	20.6±2.19^{a}	NH	NH	NH
Control	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00

Values represent Mean ± S.D.of five replications.
Different alphabets in the column are statistically significant at $p<0.05$. (MANOVA; LSD -Tukey's Test).
Eggs in control groups were sprayed with no phytochemicals.

Table 14. Ovicidal activity of *Ageratina adenophora* different extracts tested against selected mosquitoes.

Name of the species	Percentage of egg hatch ability, 48hrs post treatment					
	Concentrations tested (ppm)					
	50	100	150	200	250	300
Hexane						
A. aegypti	70.8±2.28^{e}	65.6±1.94^{d}	57.8±3.03^{c}	53.2±2.58^{c}	48.6±2.19^{bc}	42.4±2.96^{ab}
An. stephensi	58.3±1.57^{cd}	46.5±1.42^{b}	37.8±1.81^{ab}	32.1±1.28^{a}	23.6±1.72^{a}	18.6±1.62^{a}
C. quinquefasciatus	64.2±2.16^{d}	58.8±1.48^{cd}	51.6±1.81^{c}	46.8±1.92^{b}	41.2±2.16^{a}	35.2±1.92^{a}
Control	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
Dichloromethane						
A. aegypti	65.6±1.94^{d}	51.4±1.81^{c}	47.2±1.48^{b}	41.6±2.30^{ab}	35.2±2.16^{a}	30.6±2.19^{a}
An. stephensi	54.8±1.64^{c}	40.2±2.16^{ab}	32.6±2.60^{a}	28.4±1.81^{a}	18.6±1.94^{a}	14.2±2.16^{a}
C. quinquefasciatus	60.4±1.81^{d}	45.2±1.78^{c}	37.2±1.48^{ab}	34.2±1.09^{a}	25.6±1.51^{a}	18.6±1.14^{a}
Control	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
Diethyl ether						
A. aegypti	59.4±1.81^{d}	46.2±1.09^{bc}	37.4±1.81^{ab}	31.2±2.16^{a}	26.4±1.81^{a}	20.2±2.16^{a}
An. stephensi	50.4±2.30^{c}	37.2±1.78^{ab}	27.4±1.51^{a}	24.2±1.64^{a}	13.4±1.14^{a}	9.6±0.54^{a}
C. quinquefasciatus	54.6±1.94^{c}	41.4±2.30^{ab}	32.6±1.51^{a}	27.6±2.19^{a}	22.2±2.38^{a}	16.6±1.94^{a}
Control	**0.00±0.00**	**0.00±0.00**	**0.00±0.00**	**0.00±0.00**	**0.00±0.00**	**0.00±0.00**
Ethyl acetate						
A. aegypti	57.8±1.92^{c}	48.2±2.58^{bc}	36.2±1.48^{a}	30.2±2.16^{a}	24.6±2.30^{a}	17.8±1.92^{a}
An. stephensi	46.15±1.38^{b}	35.47±1.88^{a}	24.93±1.64^{a}	19.39±1.75^{a}	12.82±1.69^{a}	NH
C. quinquefasciatus	51.4±2.07^{c}	42.2±2.16^{ab}	29.2±2.16^{a}	24.4±2.50^{a}	18.4±1.81^{a}	11.2±1.92^{a}
Control	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
Methanol						
A. aegypti	51.8±2.16^{c}	43.4±2.07^{ab}	31.2±2.16^{a}	22.6±0.89^{a}	16.2±1.09^{a}	12.2±2.16^{a}
An. stephensi	41.9±1.26^{ab}	31.72±1.65^{a}	21.44±1.43^{a}	12.25±2.74^{a}	NH	NH
C. quinquefasciatus	46.8±1.48^{b}	37.4±1.51^{ab}	25.4±1.81^{a}	16.8±1.64^{a}	9.8±1.78^{a}	NH
Control	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00

Values represent Mean ± S.D. of five replications. Different alphabets in the column are statistically significant at $p<0.05$. (MANOVA; LSD -Tukey's Test). Eggs in control groups were sprayed with no phytochemicals.

Table 15. Repellent activity of *Coleus aromaticus* hexane extract tested against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* at different concentrations.

Percent of repellency								
Concentration (mg/cm^2)	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**	**210 min**	**240 min**
Aedes aegypti								
1.0	96.6±2.19	87.4±1.81	75.4±1.81	66.6±1.67	54.6±2.60	43.8±2.28	33.4±2.40	20.6±1.51
2.0	100.0±0.0	95.2±2.16	85.4±1.81	76.6±1.94	65.8±2.28	55.2±2.16	44.4±2.50	34.2±2.16
3.0	100.0±0.0	100.0±0.0	96.2±2.16	87.4±2.70	77.4±2.60	69.2±1.92	58.8±2.16	50.4±1.81
4.0	100.0±0.0	100.0±0.0	100.0±0.0	98.8±1.30	88.4±1.81	78.4±1.81	70.6±2.60	62.2±2.68
5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	99.6±0.54	91.2±1.78	81.4±2.07	74.8±2.58
Anopheles stephensi								
1.0	92.6±2.07	81.4±1.81	67.2±1.78	56.6±1.51	46.8±2.16	36.2±1.92	25.4±1.81	13.2±1.92
2.0	94.2±2.16	84.6±2.30	74.2±2.16	62.4±2.30	51.2±2.04	41.4±2.40	29.6±1.51	16.4±2.07
3.0	98.2±1.48	89.2±1.78	78.8±2.58	67.2±1.58	56.4±1.81	46.2±1.48	36.2±1.78	25.6±1.51
4.0	100.0±0.0	92.8±2.16	83.2±2.68	72.2±2.16	61.6±2.30	49.2±2.28	38.2±2.16	29.6±1.81
5.0	100.0±0.0	100.0±0.0	96.8±1.92	87.6±1.94	78.2±2.04	67.6±1.67	56.6±1.81	47.2±1.48
Culex quinquefasciatus								
1.0	91.2±1.92	82.4±1.67	71.2±1.92	60.4±1.81	49.4±2.50	38.2±2.28	27.8±1.92	17.2±1.94
2.0	95.2±1.92	86.2±2.16	75.4±1.81	64.4±2.50	53.4±1.81	42.4±1.67	30.2±1.92	19.2±2.16
3.0	100.0±0.0	95.2±1.92	86.2±2.16	74.4±2.40	63.8±1.92	53.2±1.92	41.8±2.16	31.2±2.16
4.0	100.0±0.0	100.0±0.0	96.6±1.94	87.6±2.30	77.2±2.16	66.2±1.48	56.4±2.07	45.2±1.92
5.0	100.0±0.0	100.0±0.0	100.0±0.0	97.2±2.04	88.2±1.64	77.8±1.92	67.2±1.48	55.4±1.94

Table 16. Repellent activity of *Coleus aromaticus* dichloromethane extract tested against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* at different concentrations.

Percent of repellency								
Concentration (mg/cm^2)	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**	**210 min**	**240 min**
Aedes aegypti								
1.0	100.0±0.0	95.2±1.78	85.8±1.64	75.2±1.92	64.8±2.77	53.6±2.19	41.8±1.92	30.8±1.92
2.0	100.0±0.0	100.0±0.0	93.4±2.07	84.2±2.38	74.2±2.16	62.2±2.38	51.4±2.07	40.8±1.92
3.0	100.0±0.0	100.0±0.0	100.0±0.0	95.8±1.92	87.6±1.94	77.4±2.50	67.2±2.38	57.8±1.92
4.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.2±1.92	88.4±1.81	80.2±1.78	71.6±2.30
5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	98.2±1.78	90.4±1.81	82.4±1.94
Anopheles stephensi								
1.0	94.6±1.81	86.2±2.04	75.8±1.92	63.8±1.92	53.8±2.16	43.4±2.07	31.6±2.30	18.4±1.81
2.0	97.4±2.30	88.6±2.30	78.2±2.38	66.8±2.28	56.2±2.04	45.6±1.81	35.4±1.81	23.6±3.20
3.0	100.0±0.0	94.4±2.40	84.2±2.16	71.6±2.19	59.4±1.81	48.6±2.19	37.2±1.30	25.4±1.81
4.0	100.0±0.0	100.0±0.0	94.4±1.81	84.2±2.38	72.6±2.60	61.8±2.48	50.6±1.94	48.4±1.81
5.0	100.0±0.0	100.0±0.0	100.0±0.0	95.2±2.16	87.6±1.94	76.4±2.07	66.8±1.92	56.2±1.48
Culex quinquefasciatus								
1.0	97.8±1.92	87.6±1.51	78.4±2.50	67.8±2.58	56.6±1.94	45.8±1.92	36.2±1.48	25.8±1.64
2.0	100.0±0.0	94.8±1.64	82.2±1.64	71.2±2.28	59.8±2.38	49.6±2.40	38.4±1.81	29.2±2.38
3.0	100.0±0.0	100.0±0.0	95.2±1.92	84.6±2.30	71.6±1.81	60.6±1.94	48.4±2.50	36.2±1.92
4.0	100.0±0.0	100.0±0.0	100.0±0.0	96.8±1.30	86.2±3.27	75.2±1.64	64.4±2.40	53.8±1.92
5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.6±1.51	88.2±1.92	77.8±1.92	67.4±2.30

Value represents Mean ± S.D. of five replications.
Repellent activity assayed by the method of WHO, (1996).

Table 17. Repellent activity of *Coleus aromaticus* diethyl ether extract tested against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* at different concentrations.

Percent of repellency								
Concentration (mg/cm^2)	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**	**210 min**	**240 min**
Aedes aegypti								
1.0	100.0±0.0	100.0±0.0	94.6±1.67	85.8±1.92	76.6±1.94	56.2±2.04	45.2±2.04	34.4±2.30
2.0	100.0±0.0	100.0±0.0	100.0±0.0	93.6±1.67	85.4±1.81	73.6±1.94	61.2±2.04	50.8±2.16
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	95.8±1.92	88.2±2.94	78.4±2.60	67.6±2.70
4.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.4±1.14	89.6±2.19	80.4±1.81
5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	98.6±1.14	92.4±2.07
Anopheles stephensi								
1.0	98.4±1.14	90.4±2.30	83.8±2.16	71.2±2.16	58.6±2.60	46.4±2.30	35.2±2.77	22.2±2.30
2.0	100.0±0.0	96.2±1.78	88.6±2.50	77.4±2.40	66.8±1.92	55.8±1.92	45.2±1.48	32.2±2.68
3.0	100.0±0.0	100.0±0.0	95.6±1.94	86.4±1.81	75.6±1.94	64.4±2.40	52.2±2.68	40.6±1.94
4.0	100.0±0.0	100.0±0.0	100.0±0.0	97.6±1.67	88.4±2.50	78.6±2.88	67.4±2.07	56.4±2.70
5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	98.4±1.51	90.8±1.92	81.8±1.92	70.4±2.30
Culex quinquefasciatus								
1.0	100.0±0.0	97.6±1.94	87.6±2.60	75.2±2.16	62.6±2.30	50.8±2.28	39.4±2.50	26.4±1.81
2.0	100.0±0.0	100.0±0.0	95.2±2.16	83.8±2.38	71.2±2.16	58.4±2.50	47.4±2.88	35.8±1.48
3.0	100.0±0.0	100.0±0.0	100.0±0.0	96.6±1.94	88.2±2.77	78.4±2.50	67.4±2.96	55.8±1.48
4.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.8±1.64	89.6±2.19	78.4±2.50	66.6±2.30
5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.8±1.64	88.6±2.19	77.6±2.60

Table 18. Repellent activity of *Coleus aromaticus* ethyl acetate extract tested against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* at different concentrations.

Percent of repellency								
Concentration (mg/cm^2)	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**	**210 min**	**240 min**
Aedes aegypti								
1.0	100.0±0.0	100.0±0.0	100.0±0.0	91.6±2.60	84.2±2.38	72.6±2.60	60.8±2.28	49.8±2.77
2.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.6±2.40	83.8±2.38	70.8±2.58	62.2±2.68
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	95.8±1.48	87.6±1.94	75.8±2.58
4.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	98.2±1.48	89.4±2.50
5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	99.2±0.83
Anopheles stephensi								
1.0	100.0±0.0	100.0±0.0	91.4±2.70	79.8±2.77	68.8±2.58	55.4±2.70	40.8±2.58	29.4±2.50
2.0	100.0±0.0	100.0±0.0	100.0±0.0	92.6±2.30	81.8±1.92	69.4±2.50	56.6±2.30	42.8±2.77
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	88.8±2.58	77.4±2.40	64.6±2.30	49.4±3.20
4.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	92.8±2.38	79.6±2.50	69.6±2.70
5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	90.2±1.30	77.4±2.88
Culex quinquefasciatus								
1.0	100.0±0.0	100.0±0.0	95.8±1.92	83.4±1.94	71.6±2.60	58.4±3.20	44.8±2.86	32.4±2.30
2.0	100.0±0.0	100.0±0.0	100.0±0.0	95.6±2.30	83.6±2.19	71.8±2.68	59.4±2.60	46.8±2.38
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.6±2.50	82.8±1.92	69.4±3.20	52.4±2.96
4.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.8±2.48	83.6±2.70	72.2±2.77
5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	94.2±2.68	81.8±1.92

Value represents Mean ± S.D. of five replications.
Repellent activity assayed by the method of WHO, (1996).

Table 19. Repellent activity of *Coleus aromaticus* methanol extract tested against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* at different concentrations.

Percent of repellency								
Concentration (mg/cm^2)	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**	**210 min**	**240 min**
Aedes aegypti								
1.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	92.8±3.11	81.2±3.03	69.4±2.50	55.8±2.94
2.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.2±2.38	84.8±2.58	72.6±2.88
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	95.6±2.30	87.6±3.28
4.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.6±1.94
5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Anopheles stephensi								
1.0	100.0±0.0	100.0±0.0	100.0±0.0	88.2±3.27	75.8±2.16	63.6±3.13	46.4±2.30	31.2±2.77
2.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	91.2±2.86	78.6±3.20	68.2±2.86	49.4±3.20
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	89.6±3.13	76.4±3.20	63.8±2.77
4.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	90.8±2.77	79.6±2.60
5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.8±2.28
Culex quinquefasciatus								
1.0	100.0±0.0	100.0±0.0	100.0±0.0	92.2±2.16	80.4±1.51	67.6±1.81	50.2±1.64	34.2±1.64
2.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.8±1.92	83.4±1.94	72.2±1.48	54.6±1.14
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.2±1.48	80.4±1.94	69.2±2.16
4.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	94.8±1.48	84.4±2.30
5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	96.6±1.51

Table 20. Repellent activity of *Ageratina adenophora* hexane extract tested against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* at different concentrations.

Percent of repellency								
Concentration (mg/cm^2)	**40 min**	**80 min**	**120 min**	**160 min**	**200 min**	**240 min**	**280 min**	**320 min**
Aedes aegypti								
1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	87.8±2.28	79.2±2.48
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	92.4±1.81	85.2±1.48
4.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	95.4±2.07	90.8±2.77
Anopheles stephensi								
1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	84.6±2.7	76.6±2.6
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	89.8±2.5	81.8±2.3
4.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.4±2.9	88.4±1.7
Culex quinquefasciatus								
1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	91.2±2.04	84.2±1.78
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	95.2±1.48	87.6±1.67
4.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.6±1.51	93.2±1.92

Value represents (Mean ± S.D.) of five replications.
Repellent activity assayed by the method of WHO, (1996).

Table 21. Repellent activity of *Ageratina adenophora* dichloromethane extract tested against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* at different concentrations.

Concentration (mg/cm^2)	Percent of repellency							
	40 min	80 min	120 min	160 min	200 min	240 min	280 min	320 min
Aedes aegypti								
1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	78.8±2.16
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	87.2±2.28
4.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Anopheles stephensi								
1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	84.6±2.7	76.6±2.6
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	89.8±2.5	81.8±2.3
4.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.4±2.9	88.4±1.7
Culex quinquefasciatus								
1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	86.4±1.81
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
4.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0

Table 22. Repellent activity of *Ageratina adenophora* diethyl ether extract tested against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* at different concentrations

Concentration (mg/cm^2)	Percent of repellency							
	40 min	80 min	120 min	160 min	200 min	240 min	280 min	320 min
Aedes aegypti								
1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	82.6±1.51
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	90.4±1.67
4.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Anopheles stephensi								
1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	85.4±1.81
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	89.2±1.30
4.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.2±1.48
Culex quinquefasciatus								
1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	90.8±1.78
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
4.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0

Value represents Mean ± S.D.of five replications.
Repellent activity assayed by the method of WHO, (1996).

Table 23. Repellent activity of *Ageratina adenophora* ethyl acetate extract tested against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* at different concentrations.

Percent of repellency								
Concentration (mg/cm^2)	**40 min**	**80 min**	**120 min**	**160 min**	**200 min**	**240 min**	**280 min**	**320 min**
Aedes aegypti								
1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	91.6±1.14
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.6±2.07
4.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Anopheles stephensi								
1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	87.6±2.5
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	89.2±1.8
4.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	95.4±1.9
Culex quinquefasciatus								
1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	93.6±2.30
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
4.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0

Table 24. Repellent activity of *Ageratina adenophora* methanol extract tested against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* at different concentrations.

Percent of repellency								
Concentration (mg/cm^2)	**40 min**	**80 min**	**120 min**	**160 min**	**200 min**	**240 min**	**280 min**	**320 min**
Aedes aegypti								
1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	94.6±1.67
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
4.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Anopheles stephensi								
1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	89.6±2.8
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	94.4±2.2
4.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	98.7±2.6
Culex quinquefasciatus								
1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	97.6±1.51
3.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
4.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0

Value represents Mean ± S.D. of five replications. Repellent activity assayed by the method of WHO, (1996).

Phytochemical screening of different extract of *Coleus aromaticus and Ageratina adenophora*

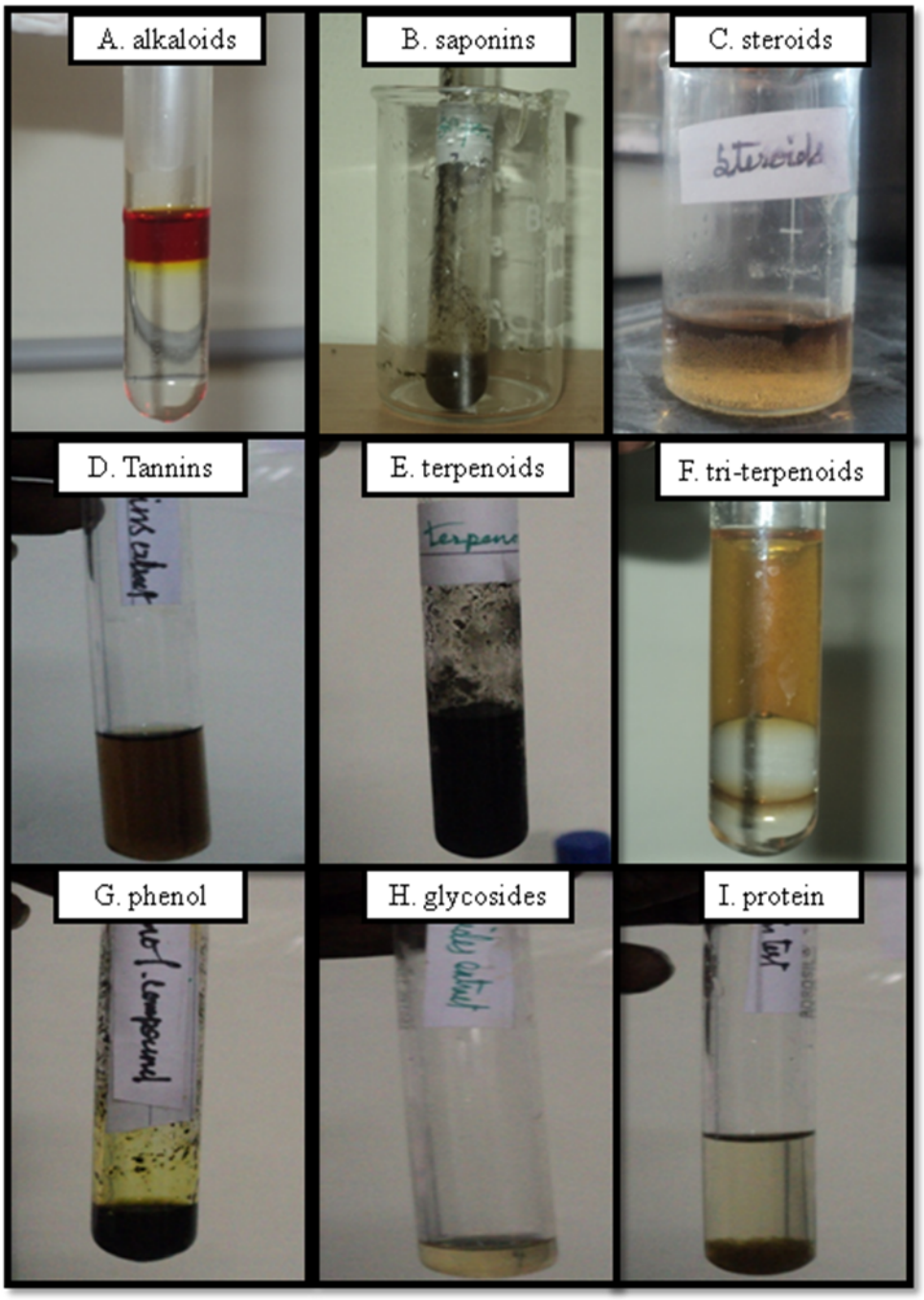
The leaf extracts of *C. aromaticus* and *A. adenophora* were screened for the presence of major phytochemical groups and mosquitocidal activity. Plant extract was analysed with thin layer chromatography with different solvents *viz.*, acetone, ethyl acetate, benzene and methanol revealed a wide number of bioactive compounds present in the plant (Table 25). The present results revealed that the methanol extracts *C. aromaticus* contains alkaloids, saponins, steroids, tannins, terpenoids, tri-terpenoides, phenols, glycosides and proteins (Plate 6 A-I). Hexane : ethyl acetate (9:1) gave 2 and 4 fractions,

Table 25. Phytochemical screening of *Coleus aromaticus* leaf extracts

S.No	Chemical constituents	Solvent			
		Methanol	Ethyl acetate	Acetone	Benzene
1.	Alkaloids	+++	+++	+++	++
2.	Flavonoids	--	+	--	--
3.	Saponins	+++	++	++	+
4.	Steroids	+++	++	+	+
5.	Tannins	++	+++	+	++
6.	Terpenoids	+++	+	++	++
7.	Tri-terpenoids	++	--	--	+
8.	Anthraquinones	--	--	--	--
9.	Amino acid	--	--	--	--
10.	Phenol	+++	+++	++	--
11.	Glycosides	++	+	--	+
12.	Carbohydrate	--	--	--	--
13.	Protein	+++	++	+++	+
14.	Phytosteroids	--	--	+	--

+++ : Abandance of the phytochemical group; ++: presence of the phytochemical group; + : trace of the phytochemical group; --: absence of the phytochemical group

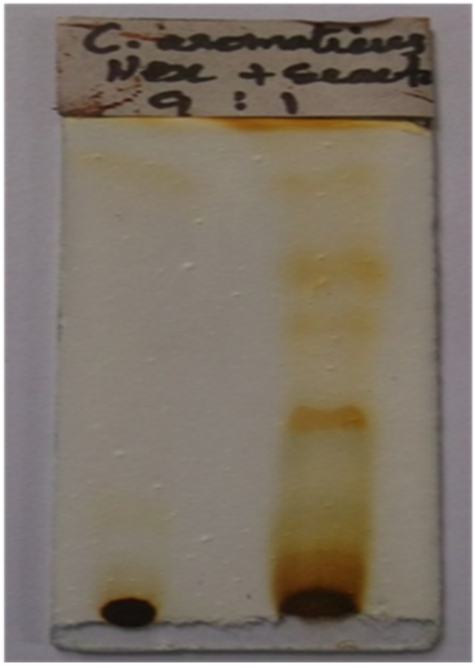
Plate 6: Phytochemical screening



Fourier transforms infrared spectroscopy analysis (FT-IR)

Plant extracts were analyzed by using Thin Layer Chromatography with different solvent systems (Plate 7). Hexane: ethyl acetate (9: 1) gave 2 and 4 fractions, two fractions have been obtained in hexane: ethyl acetate (1.5: 9.5), four fractions has been obtained in hexane: ethyl acetate (1:10) (Plate 8). FT-IR analysis was carried out, to identify the functional groups of the methanol extract, *Coleus aromaticus*. FT-IR spectrum indicated the clear peaks with (360, 3566, 3456, 2927, 2860, 2380, 1742, 1640, 1561, 1457, 1397, 1106, 704 and 662 cm^{-1}) different values (Figure 5a). Above the peak values they corresponded to functional groups like amide group (medium, N-H stretching 3650 cm^{-1}), alcohol groups (strong broad, OH stretching bended 3566 and 3456 cm^{-1}), alkenes groups (strong, C-H stretching 2927 and 2860 cm^{-1}), ester and saturated aliphatic groups (strong, C=O stretching 1742 cm^{-1}), alkenes groups (medium, -C=C- stretching 1640 cm^{-1}), alkenes groups (variable, -C-H bending 1457 and 1397 cm^{-1}), alcohol, carboxylic acid, ester, ethers groups (strong, C-O stretching 1106 cm^{-1}), aromatics groups (strong, C-H "oop" 704 cm^{2}), alkynes groups (broad and strong, -C≡C-H: C-H bend 662 cm^{2}). The functional groups such as alcohols, amide, alkenes, ester, aliphatic, carboxylic acid, ethers, aromatics and alkynes confirmed their presence in methanol extract.

Plate 7: Thin Layer Chromatography



Fraction

Plate 8: Column Chromatography
(Isolation of fractions)

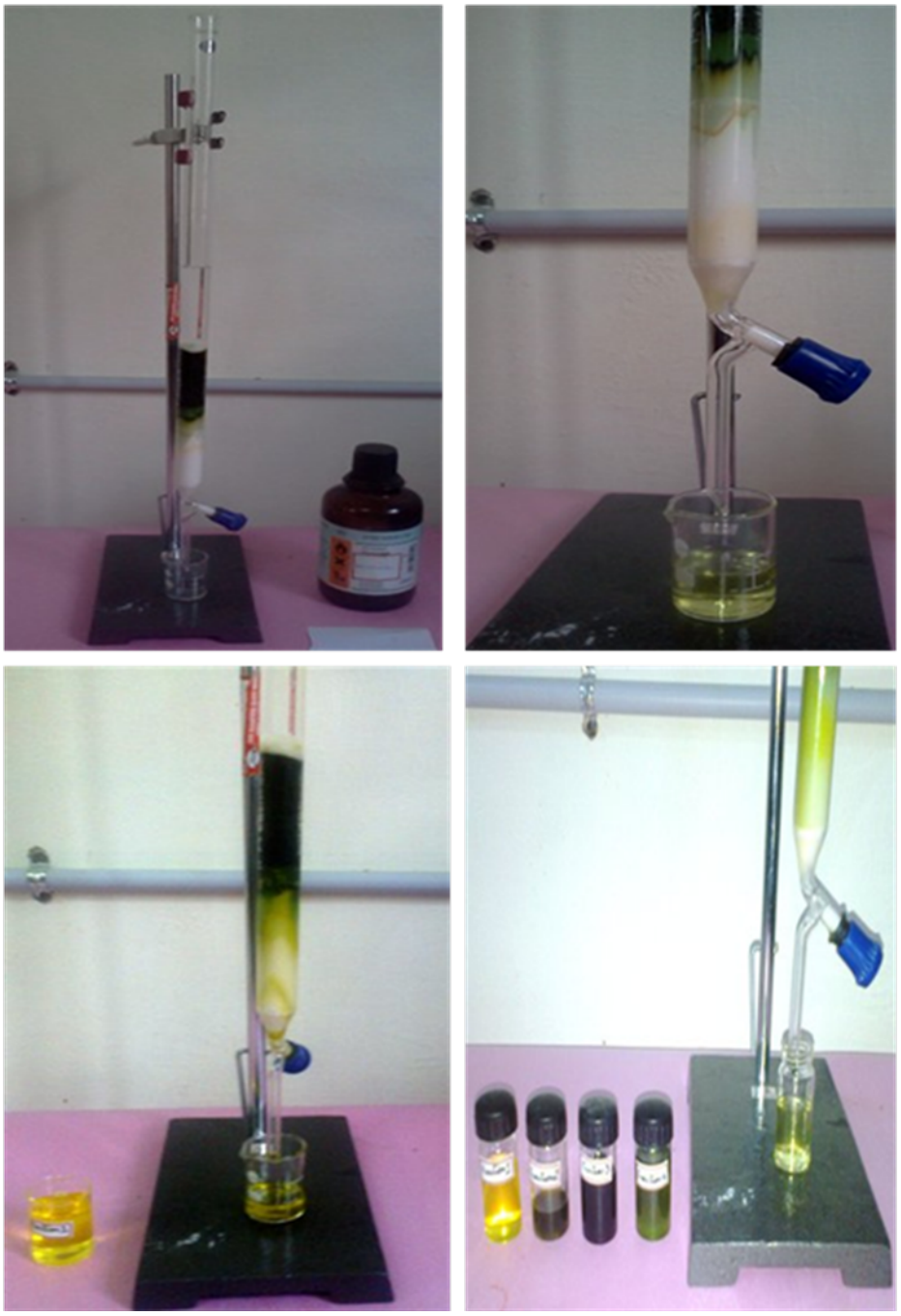
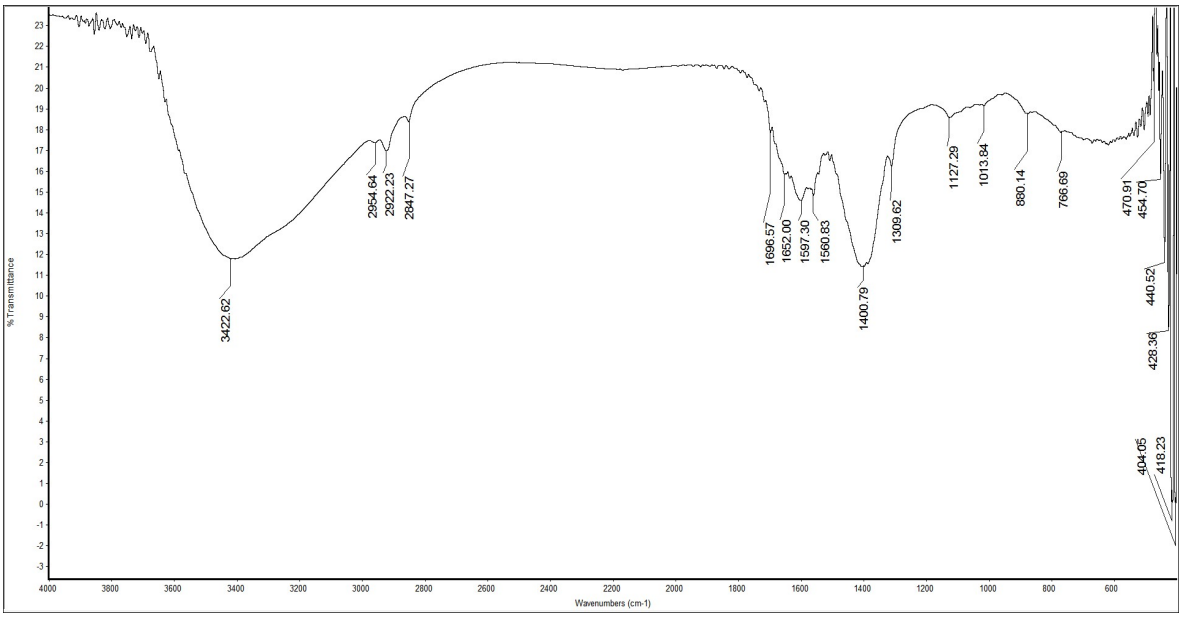


Figure 5a: FT-IR of *C. aromaticus* methanol extract



Gas Chromatography Mass Spectroscopy analysis for *Coleus aromaticus*

The leaf extracts of *C. aromaticus* hydrodistilled in a GC Clarus 500 Perkin Elmer apparatus and were further analyzed by GC–MS (Table 26 and Figure 5b). A total of 9 compounds were detected in the leaf extract. The major components present in the crude extract were copaene (4.49), caryophyllene (16.85), cedrene (2.80), 1-oxaspiro[2,5]octane,5,5-dimethyl-4-[3-methyl-1,3-butadienyl]- (2.24), tridecanoic acid, methyl ester (11.79), 1,4-methanoazulene-9-methanol, decahydro-4,8,8-trimethyl-, [1S-(1á,3aá,4á,8aá,9R×)]- (3.37), 11-octadecenoic acid, methyl ester (40.73) (Fig. 5c and 5d), 7,10-octadecadienoic acid, methyl ester (2.24) and flexinine (15.44).

Nuclear Magnetic Resonance (^{1}H and C^{13} NMR) analysis of *C. aromaticus* methanolic extract

Proton nuclear resonance (^{1}H NMR) spectra of 11-octadecenoic acid, methyl ester have been recorded in MeOH solvent. The signals obtained in the ^{1}H NMR spectra were assigned based on their position, multiplicities and integral values. The ^{1}H NMR chemical shifts are quoted after rounding off to two decimal points. The ^{1}H NMR spectrum of the compound *C. limetta* is recorded at 500.13 MH_Z. Generally, the aromatic proton signals appeared in the higher frequency region around at 11.20 ppm due to the magnetic anisotropic effect. In the ^{1}H NMR spectrum of compound *C. limetta* the signals appeared in the range between 9.45 – 12.14ppm. A singlet observed at 9.93 ppm is assigned to NH proton of the in dole moiety.

Table 26. Components identified in the *Coleus aromaticus* methanol extract using GC-MS (code ID: 364)

Peak	**Compound**	Retention time (min)	**Peak area (%)**
1	Copaene	10.57	4.49
2	Caryophyllene	11.17	16.85
3	Cedrene	12.55	2.80
4	1-Oxaspiro[2,5]octane,5,5-dimethyl-4-[3-methyl-1,3-butadienyl]-	13.38	2.24
5	Tridecanoic acid, methyl ester	17.1	11.79
6	1,4-Methanoazulene-9-methanol, decahydro-4,8,8-trimethyl-, [1S-(1á,3aá,4á,8aá,9R$^{\times}$)]-	18.1	3.37
7	11-Octadecenoic acid, methyl ester	18.8	40.73
8	7,10-Octadecadienoic acid, methyl ester	19.72	2.24
9	Flexinine	21.13	15.44

Figure 5b. GC-MS Chromatogram of *C. aromaticus* methanol extract

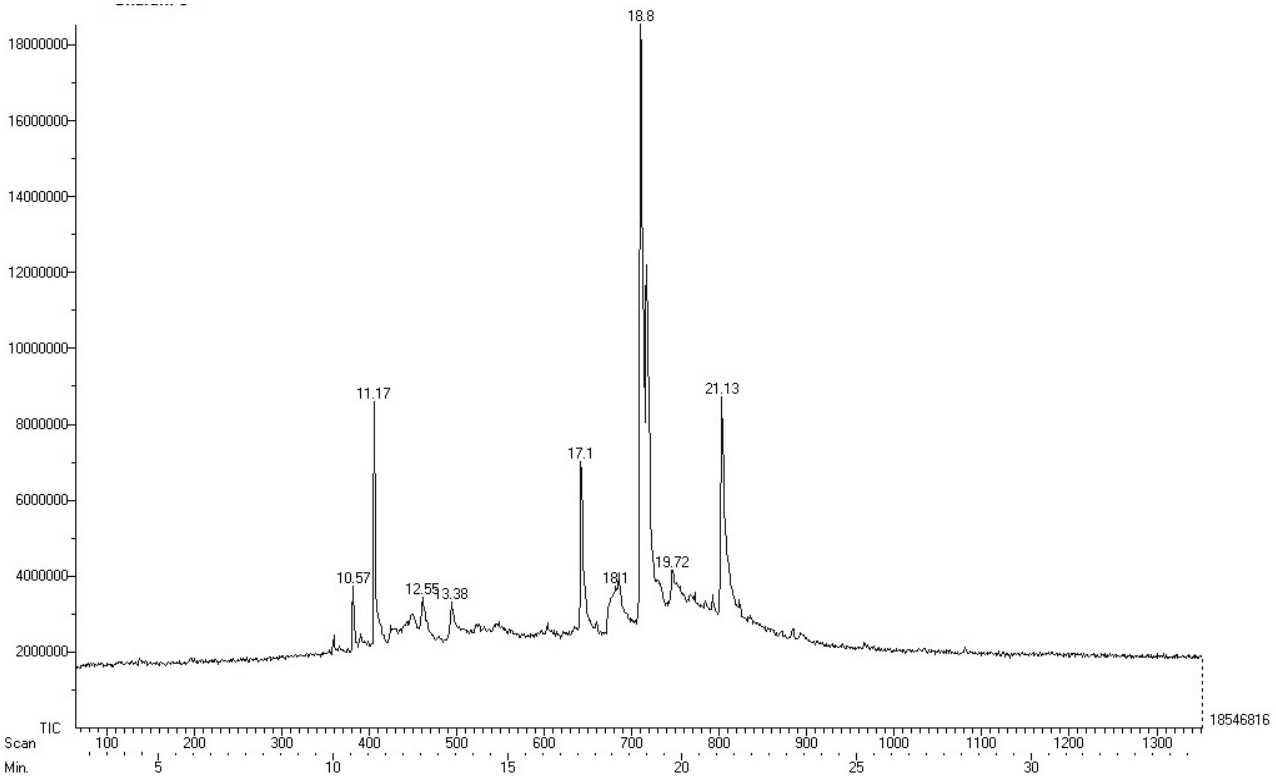


Figure 5c. GC-MS Chromatogram of 11-Octadecenoic acid, methyl ester compound in the *C. aromaticus* methanol leaf extract

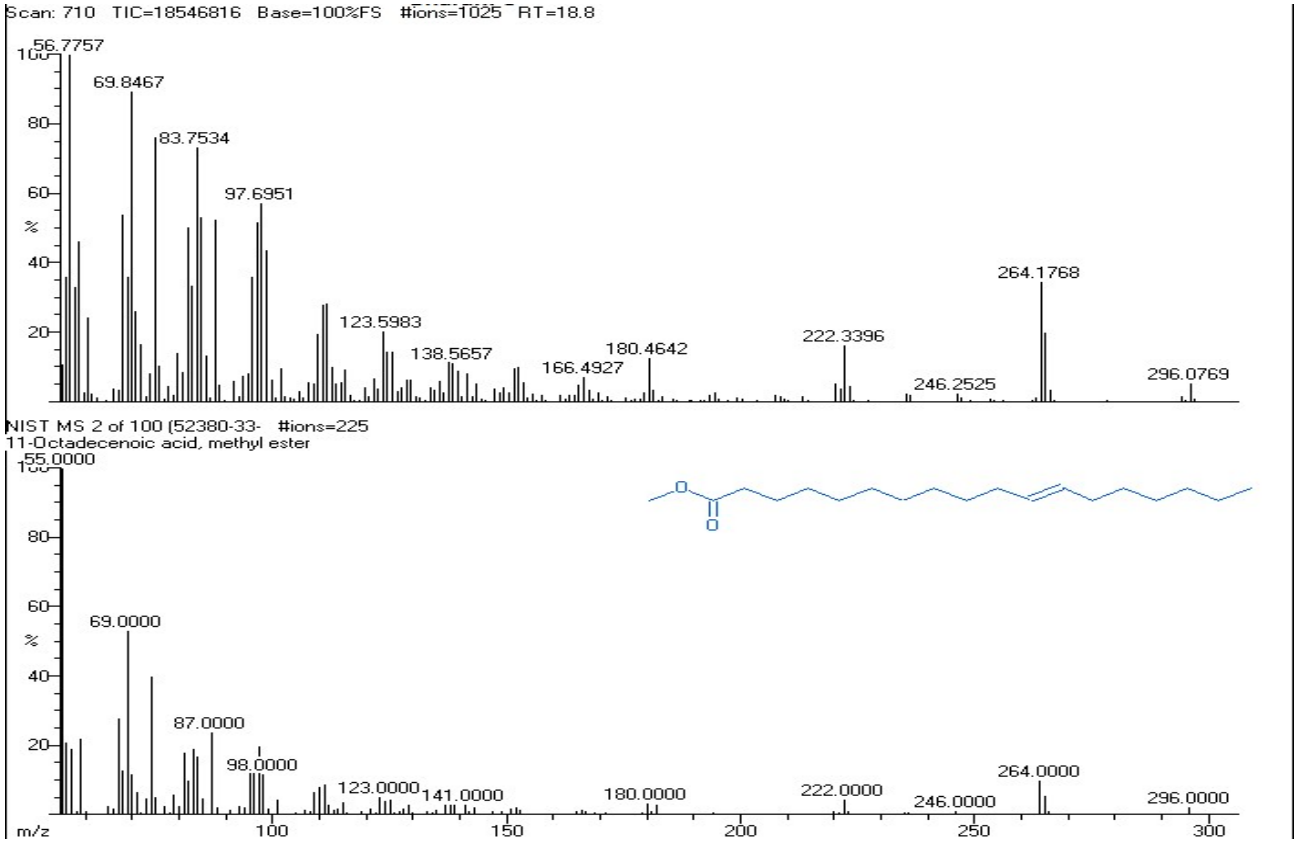
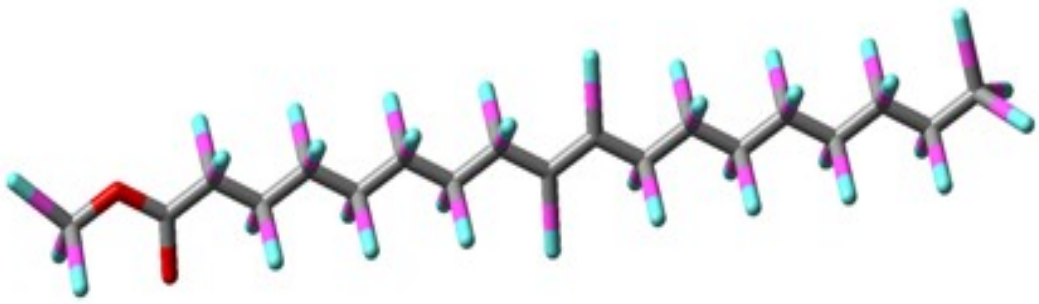


Figure 5d. Optimized *3D* structure of 11-Octadecenoic acid, methyl ester compound



Bio-activity of *Coleus aromaticus* fractions against mosquitoes

These six fractions were checked for their bioactivity against the selected mosquito species. Sixth fractions have been tested for their larvicidal activity of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. Fraction 4 showed the highest LC_{50} and LC_{90} values, 20.51 and 35.82 ppm, respectively on *Cx. quinquefasciatus* followed by LC_{50} and LC_{90} values of 22.32 and 39.03 ppm against *An. stephensi* than LC_{50} and LC_{90} values of 23.90 and 41.07 ppm against *Ae. aegypti* (Table 27). Fraction 4 also showed the highest ovicidal activity against *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti*. Further-more, there were no eggs hatchability recorded above 30 ppm(100% egg mortality) in the experimental group (Table 28). The repellent activity of fraction 4 was the highest, showing 100% protection up to 320 min against *Cx. quinquefasciatus*, *An.stephensi* and *Ae. Aegypti* (Table 29).

Table 27: Larvicidal activity of *Coleus aromaticus* selected fractions tested against freshly molted third instar larvae of three mosquitoes

Mosquito	Extract	Fraction	LC_{50} (ppm)	95% Confidence Limit (ppm)		LC_{90} (ppm)	χ^2
				LCL	UCL		
Ae. aegypti	Ethyl acetate	Fraction 1	35.58	33.58	37.74	55.62	1.076 n.s.
		Fraction 2	27.29	22.15	32.33	44.27	8.269 n.s.
	Methanol	Fraction 1	32.05	30.08	34.07	5192	1.041 n.s.
		Fraction 2	28.01	22.45	33.40	44.84	9.250 n.s.
		Fraction 3	26.06	24.18	27.87	43.76	7.380 n.s.
		Fraction 4	23.90	22.00	25.69	41.07	4.434 n.s.
An. stephensi	Ethyl acetate	Fraction 1	35.38	33.21	37.74	57.66	1.132 n.s.
		Fraction 2	25.71	23.87	27.49	42.94	6.223 n.s.
	Methanol	Fraction 1	30.04	28.13	31.96	49.06	4.411 n.s.
		Fraction 2	26.72	21.38	31.71	44.12	7.970 n.s.
		Fraction 3	24.81	22.92	26.61	42.30	5.647 n.s.
		Fraction 4	22.32	20.41	24.09	39.03	3.372 n.s.
Cx. quinquefasciatus	Ethyl acetate	Fraction 1	31.71	29.65	33.83	52.86	1.548 n.s.
		Fraction 2	24.32	22.41	26.14	41.93	5.333 n.s.
	Methanol	Fraction 1	27.83	22.33	33.12	45.39	8.413 n.s.
		Fraction 2	25.28	23.35	27.12	43.39	6.900 n.s.
		Fraction 3	23.41	21.49	25.22	40.77	4.421 n.s.
		Fraction 4	20.51	18.66	22.27	35.82	3.031 n.s.

Table 28. Ovicidal activity of *C. aromaticus* selected fractions tested against eggs of *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus.*

Mosquito	Extract	Fraction	Egg hatchability (%)						
			10 ppm	20 ppm	30 ppm	40 ppm	50 ppm	60 ppm	70ppm
Ae. aegypti	Ethyl acetate	Fraction 1	71.2±2.58 [a]	62.2±2.16 [b]	53.6±2.19 [c]	42.6±1.94 [d]	34.2±2.48 [e]	24.8±2.16 [f]	16.2±1.78 [g]
		Fraction 2	45.8±2.48 [a]	34.4±2.50 [b]	26.4±2.50 [c]	17.2±2.48 [d]	NH	NH	NH
	Methanol	Fraction 1	68.6±2.30 [a]	57.2±2.48 [b]	45.8±2.16 [c]	36.4±2.50 [d]	28.4±1.81 [e]	19.2±2.48 [f]	NH
		Fraction 2	53.6±2.19 [a]	42.2±2.48 [b]	31.4±2.88 [c]	20.6±2.19 [d]	13.2±2.16 [e]	NH	NH
		Fraction 3	44.2±2.16 [a]	36.2±2.48 [b]	27.6±2.19 [c]	19.6±2.60 [d]	NH	NH	NH
		Fraction 4	35.8±2.04 [a]	27.2±2.48 [b]	18.4±2.88 [c]	NH	NH	NH	NH
An. stephensi	Ethyl acetate	Fraction 1	66.2±1.92 [a]	58.4±2.30 [b]	48.4±1.81 [c]	40.6±2.60 [d]	28.2±1.92 [e]	19.4±2.88 [f]	NH
		Fraction 2	51.2±2.16 [a]	45.2±2.16 [b]	33.6±2.19 [c]	NH	NH	NH	NH
	Methanol	Fraction 1	58.2±2.28 [a]	44.8±1.92 [b]	36.2±1.92 [c]	26.8±1.64 [d]	17.8±2.28 [e]	NH	NH
		Fraction 2	47.6±1.94 [a]	38.8±2.16 [b]	31.8±2.58 [c]	18.4±1.81 [d]	NH	NH	NH
		Fraction 3	38.6±2.19 [a]	33.2±1.92 [b]	16.8±1.92 [c]	NH	NH	NH	NH
		Fraction 4	24.6±2.30 [a]	15.6±1.51 [b]	NH	NH	NH	NH	NH
Cx. quinquefasciatus	Ethyl acetate	Fraction 1	63.2±1.48 [b]	55.6±2.19 [b]	44.4±1.81 [c]	31.2±2.16 [d]	18.4±2.19 [e]	NH	NH
		Fraction 2	28.8±2.28 [a]	17.6±2.60 [b]	NH	NH	NH	NH	NH
	Methanol	Fraction 1	48.6±1.81 [a]	36.6±2.88 [b]	24.2±2.16 [c]	12.2±2.68 [d]	NH	NH	NH
		Fraction 2	37.6±2.19 [a]	26.4±2.88 [b]	15.2±2.28 [c]	NH	NH	NH	NH
		Fraction 3	25.2±2.28 [a]	13.4±1.81 [b]	NH	NH	NH	NH	NH
		Fraction 4	11.6±2.19 [a]	NH	NH	NH	NH	NH	NH

Each value was a Mean ± SD of five replicates

NH = No hatchability (100% mortality)

Within each row, different letters indicate significant differences (ANOVA, Tukey's HSD test, $P<0.05$)

Table 29. Repellent activity of *Coleus aromaticus* selected fractions tested against *Ae. aegypti, An. stephensi, Cx. quinquefasciatus* for 2.5 mg/cm^2

Extract	Fraction	Repellency (%)							
		40 min	80 min	120 min	160 min	200 min	240 min	280 min	320 min
Aedes aegypti									
Ethyl acetate	Fraction 1	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	94.4±1.81 ^{b}	84.2±1.92 ^{c}	74.6±2.19 ^{d}
	Fraction 2	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	92.6±1.94 ^{b}	83.4±2.19 ^{c}
Methanol	Fraction 1	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	98.6±1.34 ^{b}
	Fraction 2	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	96.2±1.78 ^{b}	83.6±1.94 ^{c}
	Fraction 3	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}
	Fraction 4	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}
Anopheles stephensi									
Ethyl acetate	Fraction 1	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	90.8±2.48 ^{b}	81.6±2.19 ^{c}
	Fraction 2	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	93.4±1.51 ^{b}
Methanol	Fraction 1	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}
	Fraction 2	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	95.2±2.16 ^{b}
	Fraction 3	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}
	Fraction 4	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}
Culex quinquefasciatus									
Ethyl acetate	Fraction 1	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	90.4±1.81 ^{b}	81.8±2.16 ^{c}	77.9±1.78 ^{d}	65.4±1.81 ^{e}
	Fraction 2	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	91.6±1.67 ^{b}	81.6±2.30 ^{c}	73.8±2.07 ^{d}
Methanol	Fraction 1	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	90.2±2.16 ^{b}	85.8±1.92 ^{c}
	Fraction 2	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	92.2±2.68 ^{b}	80.2±1.48 ^{c}	69.6±1.51 ^{d}
	Fraction 3	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	98.4±1.14 ^{b}
	Fraction 4	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}	100±0.00 ^{a}

Each value was a Mean ± SD of five replicates
Within each row, different letters indicate significant differences (ANOVA, Tukey's HSD test, $P<0.05$)

Phytochemical analysis of *A. adenophora* leaf extract

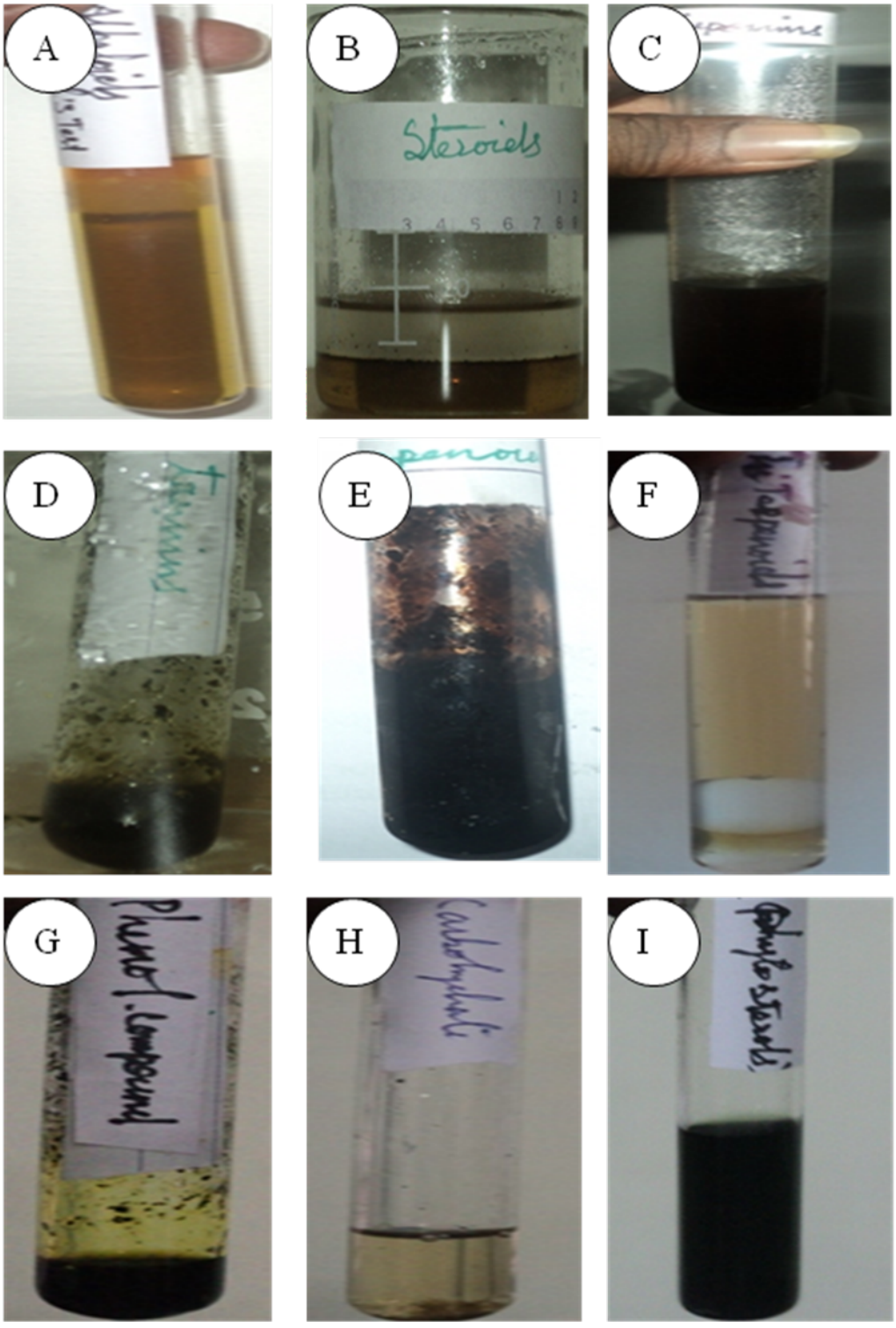
The *A. adenophora* leaf extract was screened for the presence of major phytochemical groups responsible of mosquitocidal activity. The results from the phytochemical screening of the *A. adenophora* leaf methanol, ethyl acetate, acetone and hexane extracts revealed the presence of a largest number of bioactive compounds, alkaloids, flavonoids, saponins, steroids, tannins, terpenoids, tri-terpenoids, phenol, carbohydrate, protein and phytosteroids except anthraquinones, amino acid and glycosides (Table 30 and Plate 9 A-I).

Table 30: Phytochemical screening of plant extract of *Ageratina adenophora*

S. No.	Phyto constituents	Methanol	Ethyl acetate	Acetone	Hexane
1	Alkaloids	+++	+++	+++	+++
2	Flavonoids	++	--	+	++
3	Saponins	+++	+++	++	++
4	Steroids	++	++	--	--
5	Tannins	--	--	+	+
6	Terpenoids	+++	++	+++	++
7	Tri-terpenoids	++	+++	++	+
8	Anthraquinones	--	--	--	--
9	Amino acid	--	--	--	--
10	Phenol	--	--	+	++
11	Glycosides	--	--	--	--
12	Carbohydrate	--	--	++	+
13	Protein	--	++	+	--
14	Phytosteroids	+++	+++	++	++

"+++" Strongly positive phytochemical group, "++"Positive phytochemical group, "+"Trace phytochemical group, "-" Absence of phytochemical group

Plate 9: Phytochemical present in the *A. adenophora* leaves



Fourier transforms infrared spectroscopy analysis (FT-IR)

Plant extracts were analyzed by using Thin Layer Chromatography with varying solvent systems (Plate 10). Hexane: ethyl acetate (2:8) gave 2 & 4 fraction, two fractions have been obtained in hexane: ethyl acetate (1.5:8.5), four fractions have been obtained in ethyl acetate: ethanol (1:9) and the maximum of five fractions have been obtained in hexane: ethyl acetate (0.5:9.5). FT-IR analysis was carried out, to identify the functional groups of the methanol extract, *A. adenophora*. FI-IR spectrum indicated the clear peaks with (3422, 2954, 2922, 2847, 1696, 1652, 1597, 1560, 1400, 1309, 1127, 1013, 880, 766, 470, 454, 440, 428, 418, and 404 cm^{-1}) different values (Figure 6a). Above the peak value they corresponded to functional groups like, alcohols and phenols groups (strong and broad, O-H, H-bonded 3422 cm^{-1}), alkenes group (medium, C-H stretching 2954 and 2922 cm^{-1}), carboxylic acid group (strong and very broad, O-H stretching 2847 cm^{-1}), carbonyls (general) group (strong, C=O stretching 1696 cm^{-1}), alkenes group (medium, -C=C stretching 1652 cm^{-1}), 1* amines group (medium, N-H bend 1597 and 1560 cm^{-1}), aromatics group (medium, -C-C stretching (in-ring) 1400 cm^{-1}), alcohols, carboxylic acids, esters and ethers groups (strong, C-O stretching 1309, 1127 and 1013 cm^{-1}), and 1*,2* amines groups (strong and broad, N-H wag 880 and 766 cm^{-1}). The functional groups such as alcohols, phenols, alkenes, carboxylic acids, carbonyls, 1* amines, aromatics, esters, ethers and 1*,2* amines confirmed their presence in methanol extract.

Gas chromatography mass spectroscopy analysis for *Ageratina adenophora*

The chemical components of *A. adenphora* leaf extract the retention indices and the percentage of the individual components is summarized in table 31 and figure 6b. The *A. adenophora* leaf extracts was in a GC Clarus 500 Perkin Elmer apparatus and was analyzed by GC-MS. A total of 21 compounds were detected representing 100%. The major components in extract are 3d structure of Phenol, 2-methyl-5-(1-methylethyl)- (32.32%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (5.92%), Squalene (3.86), Phytol (4.92%), n-Hexadecanoic acid (11.32%) and 4,4,6a,6b,8a,11,11,14b-Octamethyl-,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one 7.18%), this compound was otherwise called Hancolupenone. Furthermore, these six compounds were checked for their bio-efficacy against the selected mosquito species.

Plate 10: Thin Layer Chromatography

Fraction

Figure 6a: FT-IR analysis of *A. adenophora* methanol extract

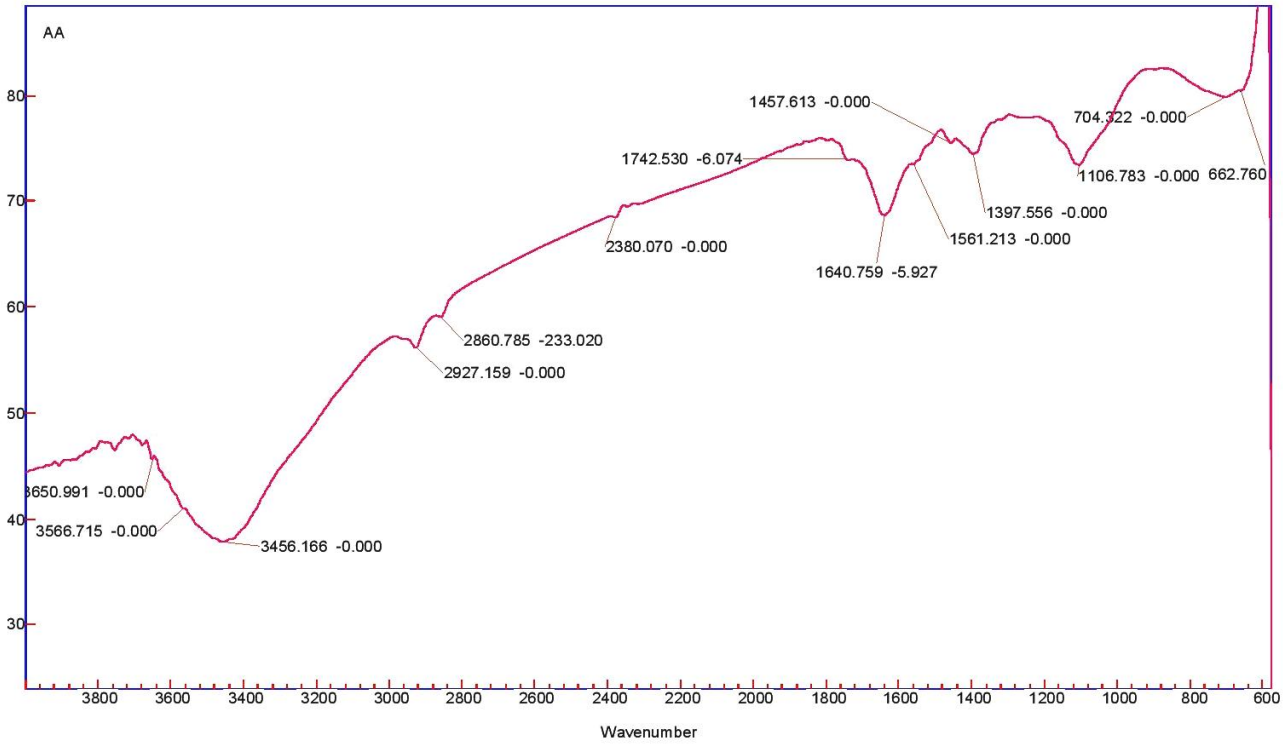
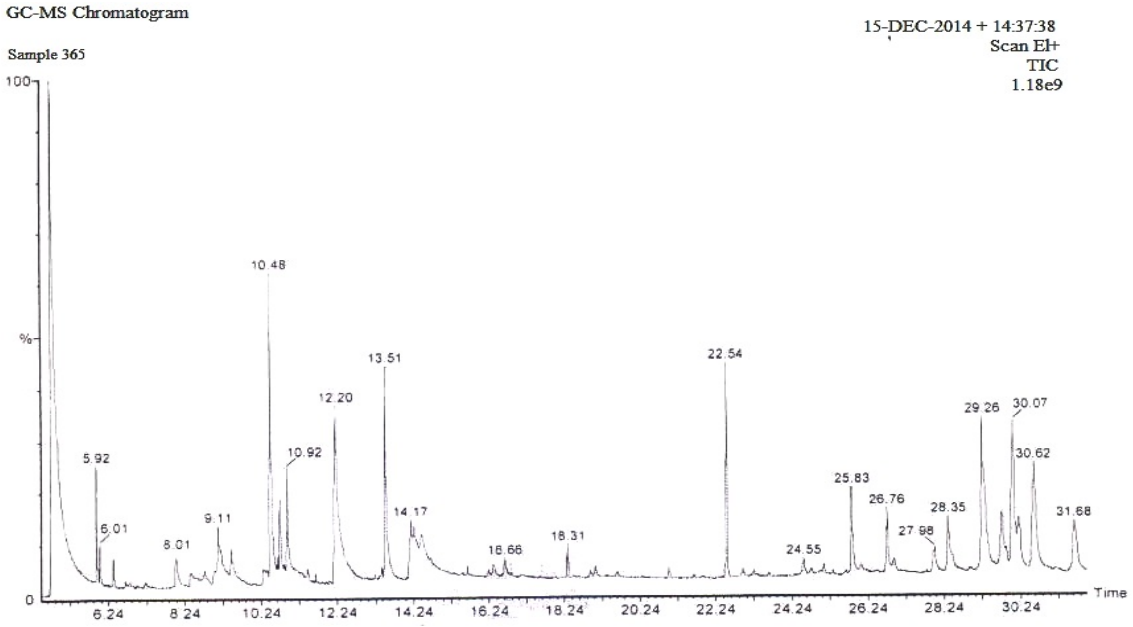


Table 31. Components identified in *Ageratina adenophora* by GC-MS (Code No. 365)

Peak	Name of Compound	RT (min)*	Peak Area (%)	MW	Molecular formula
1	Phenol, 2-methyl-5-(1-methylethyl)-	4.14	32.32	150	$C_{10}H_{14}O$
2	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene,[1R-(1R*,4Z,9S*)]-	5.92	1.34	204	$C_{15}H_{24}$
3	Trans-á-Bergamotene	6.01	0.43	204	$C_{15}H_{24}$
4	Caryophyllene oxide	8.01	1.16	220	$C_{15}H_{24}O$
5	Ar-tumerone	9.11	3.81	216	$C_{15}H_{20}O$
6	3,7,11,15-Tetramethyl-2-hexadecen-1-o1	10.48	5.92	296	$C_{20}H_{40}O$
7	n-Hexadecanoic acid	12.20	11.32	256	$C_{16}H_{32}O_2$
8	Phytol	13.51	4.92	296	$C_{20}H_{40}O$
9	9,12-Octadecadienoic acid (Z,Z)-	14.17	2.06	280	$C_{18}H_{32}O_2$
10	Z-8-Methyl-9-tetradecanoic acid	16.66	0.58	240	$C_{15}H_{28}O_2$
11	Methoxyacetic acid, 4-tridecyl ester	18.31	0.53	272	$C_{16}H_{32}O_3$
12	Squalene	22.54	3.86	410	$C_{30}H_{50}$
13	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]-	24.55	0.48	402	$C_{27}H_{46}O_2$
14	Ç-Tocopherol	25.83	2.48	416	$C_{28}H_{48}O_2$
15	Vitamin E	26.76	1.67	430	$C_{29}H_{50}O_2$
16	Cholestan-3-ol, 2-methylene-, (3á,5á)-	27.98	1.07	400	$C_{28}H_{48}O_2$
17	Cholesta-22,24-dien-5-ol, 4,4-dimethyl-	28.35	2.64	412	$C_{29}H_{48}O_2$
18	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	29.26	7.81	424	$C_{30}H_{48}O$
19	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	30.07	6.33	468	$C_{31}H_{48}O_3$
20	á-Amyrin	30.62	5.69	426	$C_{30}H_{50}O$
21	Cholest-4-en-3-one	31.68	4.21	384	$C_{27}H_{44}O$

*RT = Retention time (min). MW = molecular weight

Figure 6b: GC-MS chromatography of *A. adenophora* methanol extract



Bio-activity of *Ageratina adenophora* fractions

These six fractions were checked for their bioactivity against the selected mosquito species. Sixth fractions have been tested for their larvicidal activity of *Ae. Aegypti, An. stephensi* and *Cx. quinquefasciatus.* Fraction 4 showed the highest LC_{50} and LC_{90} values, 25.81 and 68.45ppm, respectively on *An.stephensi* followed by LC_{50} and LC_{90} values of *An.stephensi* 30.01 and 44.50ppm against *Ae. Aegypti* than LC_{50} and LC_{90} values of 33.60 and 49.85 ppm against *Cx. quinquefasciatus* (Table 32). Fraction 4 also showed the highest ovicidal activity against *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti*. Further-more, there were no eggs hatchability recorded above 30 ppm(100% egg mortality), 40 ppm, 50 ppm against *Ae. Aegypti, An.stephensi* and *Cx. quinquefasciatus* (Table 33). The repellent activity of fraction 4 was the highest, showing 100% protection up to 320 min against *Cx. quinquefasciatus*, *An.stephensi* and *Ae. Aegypti* (Table 34).

Table 32. Larvicidal activity of *Ageratina adenophora* selected fractions tested against freshly molted third instar larvae of three mosquitoes

Mosquito	Extract	Fraction	LC_{50} (ppm)	95% Confidence Limit (ppm)		LC_{90} (ppm)	χ^2
				LCL	UCL		
Ae. aegypti	Ethyl acetate	Fraction 1	94.82	88.94	102.58	140.79	2.729
		Fraction 2	104.56	96.26	116.13	157.49	1.176
	Methanol	Fraction 1	36.76	31.54	43.40	54.41	8.884
		Fraction 2	40.90	38.94	43.14	58.62	4.719
		Fraction 3	34.21	28.14	41.48	51.01	12.156
		Fraction 4	30.01	28.42	31.61	44.50	7.197
An. stephensi	Ethyl acetate	Fraction 1	86.49	75.12	105.86	128.54	11.291
		Fraction 2	98.21	91.64	108.29	135.77	10.457
	Methanol	Fraction 1	31.46	22.68	44.59	104.88	12.147
		Fraction 2	39.72	31.55	49.18	115.25	14.509
		Fraction 3	27.19	22.08	39.54	85.26	13.177
		Fraction 4	25.80	22.62	34.56	68.45	12.512
Cx. quinquefasciatus	Ethyl acetate	Fraction 1	108.67	99.74	122.27	164.09	0.164
		Fraction 2	113.68	103.05	130.77	175.26	0.287
	Methanol	Fraction 1	37.61	32.66	44.02	55.42	7.968
		Fraction 2	42.67	40.62	45.07	60.49	2.701
		Fraction 3	35.44	30.14	41.29	56.36	10.956
		Fraction 4	33.60	28.75	39.00	49.85	8.421

Table 33. Ovicidal activity of *A. adenophora* selected fractions tested against eggs of *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus*.

Species	Solvents	Fractions	Percentage of egg hatch ability, Concentration (ppm), 48 hrs post treatment					
			Control	10 ppm	20 ppm	30 ppm	40 ppm	50 ppm
Ae. Aegypti	Methanol	I	100±0.0	19.4±1.51	37.2±1.30	59.4±1.14	81.2±1.30	NH
		II	100±0.0	24.2±1.48	44.2±1.64	65.4±2.19	89.6±2.07	NH
		III	100±0.0	29.4±2.07	54.2±1.92	76.2±1.64	NH	NH
		IV	100±0.0	49.6±1.51	72.4±2.30	NH	NH	NH
	Ethyl acetate	I	100±0.0	16.4±1.81	31.4±1.51	53.2±2.38	76.2±1.64	98.4±1.14
		II	100±0.0	26.4±1.14	49.2±1.92	73.2±1.64	NH	NH
An. stephensi	Methanol	I	100±0.0	15.4±1.14	29.2±1.51	51.4±1.64	70.2±1.78	94.6±1.92
		II	100±0.0	18.2±0.83	35.2±2.38	56.4±0.54	77.4±1.67	99.2±0.74
		III	100±0.0	25.6±2.28	47.2±1.64	63.2±1.92	86.2±2.38	NH
		IV	100±0.0	36.2±2.38	65.8±1.92	90.6±1.81	NH	NH
	Ethyl acetate	I	100±0.0	13.2±1.30	27.4±1.81	48.2±2.30	67.2±1.09	88.6±1.64
		II	100±0.0	21.8±1.78	42.4±1.48	59.2±1.92	82.4±2.07	NH
Cx. quinquefasciatus	Methanol	I	100±0.0	12.8±2.07	24.2±2.30	45.4±1.51	66.2±1.92	87.8±1.30
		II	100±0.0	16.2±1.64	31.4±1.30	52.8±1.64	73.6±1.30	95.4±1.09
		III	100±0.0	21.4±1.30	40.8±1.14	59.6±1.34	82.6±1.51	NH
		IV	100±0.0	28.8±0.83	55.2±1.92	76.2±1.48	98.6±1.51	NH
	Ethyl acetate	I	100±0.0	10.6±1.14	21.2±1.67	40.4±1.14	62.2±2.61	84.6±1.14
		II	100±0.0	18.8±1.64	35.2±2.86	56.4±2.38	89.6±2.30	NH

Table 34. Repellent activity of *A. adenophora* selected fractions tested against *Ae. aegypti, An. stephensi, Cx. quinquefasciatus* for 0.75 mg/cm^2

Extract	Fraction	Repellency (%)							
		40 min	80 min	120 min	160 min	200 min	240 min	280 min	320 min
Aedes aegypti									
Ethyl acetate	Fraction 1	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	92.4±2.2^b	86.8±2.16^a	75.4±2.50^a
	Fraction 2	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
Methanol	Fraction 1	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	94.4±2.19^b	87.2±2.16^a
	Fraction 2	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
	Fraction 3	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
	Fraction 4	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
Anopheles stephensi									
Ethyl acetate	Fraction 1	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	97.4±1.67^b	88.8±2.77^a
	Fraction 2	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
Methanol	Fraction 1	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	95.8±2.28^b
	Fraction 2	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
	Fraction 3	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
	Fraction 4	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
Culex quinquefasciatus									
Ethyl acetate	Fraction 1	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	99.8±0.83^b
	Fraction 2	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
Methanol	Fraction 1	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
	Fraction 2	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
	Fraction 3	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
	Fraction 4	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

Each value was a Mean ± SD of five replicates

Within each row, different letters indicate significant differences (ANOVA, Tukey's HSD test, P<0.05)

SUMMARY

The medicinal plants of *Coleus aromaticus* and *Ageratina adenophora* were collected from in and around Yelagiri hills, Salem district, Tamil Nadu, India. Collected medicinal plants were air dried, powdered and extracted using various solvents such as hexane, diethyl ether, dichloromethane, ethyl acetate and methanol in Soxhlet apparatus then followed by 'Rota-vapour'. Eggs of *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* were collected from ICMR centre, Virudachalam, Cuddalore District, Tamilnadu and reared under laboratory conditions for toxicity assays. The larvicidal and repellent activity of plant crude extract was assessed by using the standard method as prescribed by WHO (2005). The method of Su and Mulla (1998) were followed for the ovicidal activity of the plant extracts. DMSO (emulsifier) in water was treated as control. The present results revealed that the highest larvicidal activity was recorded with methanol extract of *C. aromaticus* than *A. adenophora* for all the mosquitoes species tested. The reported LC_{50} and LC_{90} value of *C. aromaticus* with methanol extract were derived to be 28.66 and 69.19 ppm for *A. aegypti*, 22.20 and 58.80 ppm for *An. Stephensi*, 31.10 and 74.31 ppm for *C. quinquefasciatus*. The LC_{50} and LC_{90} value of *A. adenophora* with methanol extract were derived to be 137.02 and 243.99 ppm for *A. aegypti*, 108.52 and 185.91 ppm for *An. Stephensi*, 161.22 and 280.47 ppm for *C. quinquefasciatus*. Among the extracts tested, maximum ovicidal activity with methanol extract of *A. adenophora* exerted 100% mortality (*i.e.,* no hatchability) was recorded at 300 ppm against *An. stephensi* and *C. quinquefasciatus*. Maximum ovicidal activity with methanol extract of *C.aromaticus* exerted 100% mortality (*i.e.,* no hatchability) was recorded at 250ppm against tested species. The repellent activity of methanol extract *A. adenophora* was found to be most effective and at higher concentration (3.0 & 4.5) provided 100% protections up to 320 min against *C. quinquefasciatus* and *Ae. Aegypti*, respectively and up tp 280 min against *Ae. Aegypti.* In case of *C. aromaticus* at 5.0 mg/cm^2 provided 100% protection upto 240 min against *Ae. aegypti,* followed by 210 min. against *An. stephensi* and *Cx. quinquefasciatus*. The insecticidal compounds present in the crude extract of *C. aromaticus* and *A. adenophora* was isolated and identified using the thin layer chromatography and GC-MS techniques.

Conclusion

Eco-friendly tools to manage vector mosquitoes population in an Integrated Vector Management Programme are urgently required in the present situation. In this contest, medicinal plants are excellent resources as natural insecticides which have been traditionally utilized by human community in different rural areas worldwide against insect vectors and parasites. In this project, a novel approach on isolated and identified bioactive compounds such as 11-octadecenoicacid, methyl ester from *C. aromaticus* and Phenol, 2-methyl-5-(1-methylethyl) from *A. adenophora*, these compounds have been tested against three mosquitoes viz. *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Hence, it is concluded that it is recommended to use these two plants for the effective control of mosquitoes.

List of Publications

1. Baranitharan M , **Dhanasekaran S** , Mahesh Babu S Sridhar N. and Krishnappa K., 2014. Larvicidal activity of *Coleus aromaticus* Benth (Lamiaceae) *International Journal of Research in Biological Research* leaf extracts against three vector mosquitoes.. **4(2):** 55-59
2. M.Baranitharan and **S.Dhanasekaran. 2014.** Mosquitocidal effects of medicinal plant of *Coleus aromaticus* Benth (Lamiaceae) leaf extracts against chikungunya vector, *Aedes aegypti* (Linn.) (Diptera: Culicidae). *International Journal of Current Research in chemistry and pharmaceutical sciences*. **1(5):** 61-67.
3. M.Baranitharan and **S. Dhanasekaran. 2014.** Evaluation of larvicidal activity of *Ageratina adinophora* (Spreng)King & H.Rob against *Culex quinquefasciatus* (Say). International Journal of pharmaceutical and biological archive. 5(2): 120-125.
4. Mathalaimuthu Baranitharana, Shanmugam Dhanasekarana, Kalimuthu Kovendan, Kadarkarai Murugan, Jayapal Gokulakrishnan, Giovanni Benelli, 2017. *Coleus aromaticus* leaf extract fractions: A source of novel ovicides, larvicides and repellents against Anopheles, Aedes and Culex mosquito vectors?. *Process Safety and Environmental Protection* **1 0 6:** 23–33.

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