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Full Length Research Paper

Larvicidal activity of crude extracts and fractions of native plants against *aedesaegyptil*. and *culexquinquefasciatussay*. (Diptera: Culicidae)

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Abstract

Several millions of people get affected by mosquito-borne sicknesses every year, and hence mosquito-borne sicknesses are regarded as major public health diseases. The vector mosquito *Aedesaegypti* transmits chikungunya fever, dengue fever, yellow fever viruses and *Culexquinquefasciatus* transmits filarial nematode and cause filariasis. In the present study, In the present study, the leaves of *Cymbopogoncitratus*, *Azadirachtaindica*,*Tageteserecta*, *Eucalyptus deglupta* and *Syzygiumaromaticum* were screened on *Cx. quinquefasciatus* and *Ae. aegypti* mosquito larvae. Ethanol extract of *Eucalyptus deglupta* leaves was found to be very active and the LC_{50} and LC_{90} results *were* 63.54ppm, 96.90ppm against *Cx. quinquefasciatus* and 72.19ppm, 137.90ppm against *Ae. aegypti* larvae, respectively. Eight fractions were obtained from the active extract and fraction 7 was identified to be very effective. The LC_{50} and LC_{90}results of fraction-7 were 5.07ppm, 12.64ppm against *Cx. quinquefasciatus* larvae and 5.50ppm, 17.18ppm against *Ae. aegypti* larvae, respectively. The active ethanol extract of *Eucalyptus deglupta* leaves may be used in mosquito control activities.

INTRODUCTION

Controlling all the adult mosquitoes is nearly impossible. Hence, public health programmer majorly concentrates controlling larval stage of the mosquitoes (Zahran,H.E.,Abdelgaleil,S.A., 2011). and organophosphates are the choice of chemicals in India, especially temephos is being extensively used. Nevertheless, these chemical insecticidesresulted in many unwanted effects in human and non-target organisms in the environment (Sutthanont. N et al. 2010), (Bayen, S.2012, Chen, C.D. et al.2013, Chavshin, A.R et al. 2015). Apart from this, insecticide resistance in vector mosquitoes due to the continuous application of chemical larvicides showed a more significant challenge in vector mosquito control. Because of insecticide resistance, the mosquito population also increased in the ecosystem and hence the mosquito-borne disease in human is highly prevalent, and the number of cases also shows increasing trend every year. Chemical insecticides also contaminate the environment (Ruiz-Guerrero, R. 2015)

Hence, plant extracts and plant-derived compounds will substitute to chemical insecticides to manage vector mosquitoes, and also, it is non-toxic to all the other organisms, including humans. Many researchers have studied the efficacy of several plant extracts in the recent past (Hayatie, L.2015, Pavela, R.2016, Subashini 2017)

In the present study, the leaves of *Cymbopogoncitratus*, *Azadirachtaindica*, *Tageteserecta*, *Eucalyptus deglupta* and *Syzygiumaromaticum*were used for solvent extraction such as hexane, chloroform, ethyl acetate, ethanol and aqueous and screened on *Cx. quinquefasciatus* and *Ae. aegypti* mosquito larvae. *Cymbopogoncitratus* is reported to possess antifungal, antibacterial, antiprotozoal, antioxidant, anti-inflammatory, anti-carcinogenic anti-rheumatic and anti-protective activities (Ekpenyong C.E.2015, Chukwuocha U.M.2016, Avoseh O.2015) *Cymbopogoncitratus*leaves have been used in folk and ayurvedic medicine (Tarkang P.A.2012) It has also been known for insecticidal, anti-malarial, and anti-pneumonic activities (Manvitha K. 2014, Chinsembu K.C.2015) *Azadirachtaindica* bark extracts showed antihyperglycemic activity, hepatoprotective (Costa G.et al.2016) The different solvent extracts of *Azadirachtaindica* showed to possess mosquito larvicidal activity (Okumu, F.O.2007)

Tageteserecta extracts are reported topossess antibacterial, nematicidal, antioxidant, wound healing, analgesic, hepatoprotective activities (Giri RK. et al. 2011, Farjana

Nikkon M.et al.2011, Hussain MA.et al.2011) It has also been known for insecticidal and mosquito larvicidal activities (Farjana Nikkon M. et al. 2005) Extracts and oil of *Eucalyptus deglupta* are used in perfumes as ingredients, disinfectants, fungicides, cleaning agents, medicines, and other medical purposes (MotiurRahman M. et al. 2009) The different solvent extracts of *Eucalyptus deglupta* is also reported to possess insecticidal and larvicidal properties (Kiplang'at KP.2013, Shooshtari MB. et al. 2013) *Syzygiumaromaticum* is reported to have anti-mutagenic, anti-inflammatory, antioxidant, anti-thrombotic, anti-carcinogenic, antiviral and anti-parasitic (Miyazawa M. 2003, Chaieb K. et al. 2007, Hussein G et al.2000, Yang YC . et.al. 2000) The raw extracts obtained from the leaves of the above given plants were screened on the fourth stage larvae of *Ae. aegypti* and *Cx. quinquefasciatus* in the laboratory settings.

These plants were selected based on their broad pharmacological importance. Solvent extractions using five solvents, namely hexane, chloroform, ethyl acetate, ethanol, and aqueous were done and they were screened on the fourth stage larvae of *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes.

MATERIALS AND METHODS

Collection of Plant material

Leaves of the selected five plants were brought from the fields in Chennai District, Tamil Nadu, India. A Botanist authenticated the plant species at Entomology Research Institute (ERI), Loyola College (Autonomous), Chennai. The voucher specimens (ERIL-MRG-VEC-350-355) of selected plants were preserved in the herbarium of the institution. Initially, the leave materials were shade dried in the laboratory for ten days continuously and then crushed with an electric mixer.

Preparation of Solvent extracts

The extracts were extracted from the crushed leaves of each plant by successive extraction technique using five different solvents in the order hexane-chloroform-ethyl acetate-ethanol-aqueous solvents. So, initially, 1.5Kg powdered leaf of each plant was soaked in 3 litres of hexane for 72h with three-time shaking in a day. The Whattman No:1 filter paper was used to filter the solvent, then concentrated using rotary instruments and finally allowed to dry. Then the remainder of the plant leaves was soaked in the subsequent solvents viz., chloroform-ethyl acetate-ethanol-aqueous and crude extracts were extracted similarly. All these crude extracts were kept open overnight at the laboratory to dry completely and then stored at 4°C in airtight glass vials in the refrigerator.

Test mosquitoes

The third instar stage larvae of *Ae. aegypti* and *Cx. quinquefasciatus* mosquito subjected in the present study were collected from Entomology Research Institute laboratory (ERI); mosquitoes did not expose to any pathogens or microorganisms, any insecticides, or repellent chemicals. The mosquito colony rearing conditions at the laboratory were: 28 ± 1°C; 70 - 75% Relative Humidity and 11 ± 0.5 hours photoperiod.

Larvicidal activity procedure

Larvicidal activities were carried out at the laboratory using the methodology prescribed by the World Health Organization (WHO 2005) with minor modifications. Test concentrations viz., 62.5ppm, 125ppm, 250 ppm, and 500ppm were prepared for each plant crude extract using DMSO and each concentration was replicated five times. In each replication, 99ml of water with twenty larvae of *Ae. aegypti* and 1ml of DMSO in which extract was dissolved (Total 100ml). Five controls with DMSO without plant extract was maintained along with the experiment. Similar experiments were carried out with *Cx. quinquefasciatus* mosquito larvae.

Similarly, the concentrations used to test the different fractions were 2.5 ppm, 5.0 ppm, 7.5 ppm and 10.0ppm produced using a dissolving agent called DMSO. Azadirachtin and temephos (positive control) were also tested with 2.5ppm, 5.0ppm, 7.5ppm and 10.0ppm concentration for comparison. Five replications of control (without any extract) were also maintained. The total dead larvaewere documented after 24 hoursof experimental time. The percentage of mortality was determined for each concentration of each plant extract using the following formula.

Percentage of Mortality = No. of Dead larvae/ No. of Larvae introduced x 100

Abbott's formula [30] was used to get corrected percentage mortality when control mortality was below 5%:

Corrected percentage of mortality: (1 – *n*) in Treatment/ *n* in Control x 100

Fractionation of active extract

The promising ethanol extract of *Eucalyptus deglupta* (86 g) was initially packed in column chromatography using silica gel (100–200 sized mesh) and ethyl acetate. Then the raw extract was separated with commercial solvents from low polar to high, i.e. hexane, chloroform, ethyl acetate, ethanol, and methanol and its mixtures. All the fractions were assessed on TLC and fractions were pooled together. Finally, 8 fractions had resulted. These fractions were screened for larvicidal activity at different concentrations i.e. 2.5ppm, 5.0ppm, 7.5ppm and 10ppm. Fraction 7 eluted with ethanol: methanol (90:10) showed significant larvicidal results against both the mosquito larvae.

Statistical analysis

Dose-response curves were prepared for each derivative

with larval and pupal mortality data. Further, larvicidal and pupicidal mortality data were subjected to probit analysis (US EPA probit; version 1.5) to find LC_{50} and LC_{90} values, and the differences were considered significant at $p \leq 0.05$.

RESULTS

Larvicidal activity result of crude extracts

The larvicidal assay results are given in tables 1 to 5, which evidenced that the ethanol extract of *Eucalyptus deglupta* leaves was the very active extract in killing the fourth stage larvae of *Ae. aegypti* and *Cx. Quinquefasciatus* mosquitoes (Tables 4).

The LC_{50} and LC_{90} results of ethanol extract of *Eucalyptus deglupta* were 63.54ppm, 96.90ppm against *Cx. Quinquefasciatus* and 72.19ppm, 137.90ppm against *Ae. aegypti* larvae, respectively (Tables 4). This was followed by ethyl acetate extract of the same plant *Eucalyptus deglupta* recorded to be good larvicide with LC_{50} and LC_{90} results of 109.07ppm, 258.53ppm on *Cx. Quinquefasciatus* and 113.87ppm, 243.27ppm on *Ae. aegypti* larvae, respectively (Tables 4). All the remaining raw extracts produced either modest or significantly less larvicidal results (Tables1 to 5).

Larvicidal activity of fractions

The Lethal Concentration values (lethal dose) of different fractions on the fourth stage larvae of *Cx. quinquefasciatus* and *Ae.aegypti* are given in (Tables 6 and 7).

Among the 8 fractions screened, fraction 7 was identified to be a very effective fraction, which showed LC_{50} and LC_{90} results of 5.07ppm, 12.64ppm against *Cx. Quinquefasciatus* larvae (Tables 6) and 5.50ppm, 17.18ppm against *Ae. aegypti* larvae (Tables 6), respectively. Following this, fraction 8 produced LC_{50} and LC_{90} results of 10.59ppm, 23.18ppm on *Cx. quinquefasciatus* and 10.73ppm, 21.46ppm on *Ae. aegypti* larvae, respectively. Remaining fractions recorded a modest or less larvicidal activity as given below.

Fraction 1 produced LC_{50} and LC_{90} results of 15.39ppm, 39.58ppm against *Cx. quinquefasciatus* and 18.42ppm, 54.96ppm against *Ae. aegypti* larvae, respectively. Fraction 2 produced LC_{50} and LC_{90} results of 17.56ppm, 49.32ppm against *Cx. quinquefasciatus* and 21.42ppm, 69.40ppm on *Ae. aegypti* larvae, respectively. Fraction 3 recorded LC_{50} and LC_{90} results of 20.78ppm, 156.28ppm against *Cx. quinquefasciatus* and 24.64ppm, 205.76ppm on *Ae. aegypti* larvae, respectively. Fraction 4 recorded LC_{50} and LC_{90} results

Table 1. Lethal concentrations (in ppm) of crude extracts of *Cymbopogoncitratus* against the fourth instar larvae of *Ae. aegypti*and *Cx. quinquefasciatus*.

Plant species	Mosquito species	Treatment	LC_{50} (ppm)	95% confidence Limit		LC_{90} (ppm)	95% confidence limit		Intercept ± SE	Slope ± SE	χ^2
				LL	UL		LL	UL			
Cymbopogoncitratus	*Aedesaegypti*	Hexane	196.37	174.21	218.44	446.58	413.34	461.51	2.5±0.4	3.0±0.3	19.0*
		Chloroform	256.59	132.47	873.50	710.30	364.19	831.73	-1.9±1.2	2.8±0.5	8.5*
		Ethyl acetate	244.24	81.69	607.30	675.69	322.28	989.71	-1.9±1.4	2.9±0.6	12.1*
		Ethanol	164.31	82.61	314.95	368.71	223.02	3769.57	-3.0±1.3	3.6±0.6	8.9*
		Aqueous	299.98	261.63	352.10	985.74	751.39	1453.78	-1.1±0.5	2.4±0.2	4.8*
	Culexquinquefasciatus	Hexane	184.46	5.73	1302.07	426.99	221.64	571.78	-2.9±1.8	3.5±0.8	16.1*
		Chloroform	233.08	86.47	1436.1	595.08	304.15	627.00	-2.4±1.5	3.1±0.6	11.9*
		Ethyl acetate	220.99	193.32	274.71	527.80	483.80	591.60	-2.9±1.8	3.3±0.8	16.7*
		Ethanol	140.27	63.51	262.02	338.08	201.30	3951.97	-2.2±1.2	3.3±0.5	8.4*
		Aqueous	270.37	242.14	304.80	683.96	565.57	884.75	-2.7±0.6	3.1±0.2	5.4*

LC_{50} - lethal concentration that kills 50% of the exposed larvae; LC_{90} - lethal concentration that kills 90% of the exposed larvae; LL-lower limit (95% confidence limit); UL-upper limit (95% confidence limit). *$p \leq 0.05$, level of significance of chi-square values.

Table 2. Lethal concentrations (in ppm) of crude extracts of *Azadirachtaindica*against the fourth instar larvae of *Ae. aegypti*and *Cx. Quinquefasciatus*

Plant species	Mosquito species	Treatment	LC_{50} (ppm)	95% confidence Limit		LC_{90} (ppm)	95% confidence limit		Intercept ± SE	Slope ± SE	χ^2
				LL	UL		LL	UL			
Azadirachtaindica	*Aedesaegypti*	Hexane	400.63	340.17	495.97	1418.03	1006.55	2382.99	-1.0±0.6	2.3±0.2	1.7*
		Chloroform	165.05	54.14	453.66	390.16	216.30	6989.40	-2.6±1.5	3.4±0.6	12.0*
		Ethyl acetate	185.30	86.85	410.46	436.41	251.36	9682.12	-2.8±1.4	3.4±0.6	9.7*
		Ethanol	130.37	59.69	230.09	321.88	194.09	2919.30	-1.9±1.1	3.2±0.5	7.6*
		Aqueous	663.06	515.98	986.96	2676.48	1592.66	6551.37	-0.9±0.7	2.1±0.2	0.3*
	Culexquinquefasciatus	Hexane	370.90	320.03	446.18	1200.49	889.14	1869.66	-1.4±0.6	2.5±0.2	2.1*
		Chloroform	143.61	74.10	251.09	335.33	206.32	2351.44	-2.5±1.2	3.4±0.5	7.6*
		Ethyl acetate	151.37	43.90	393.55	376.72	206.34	7814.35	-2.0±1.4	3.2±0.6	11.5*
		Ethanol	118.24	106.57	130.49	259.93	226.76	310.36	-2.7±0.6	3.7±0.3	4.6*
		Aqueous	424.57	361.00	525.93	1414.34	1011.39	2355.22	-1.4±0.6	2.4±0.2	0.9*

LC_{50} - lethal concentration that kills 50% of the exposed larvae; LC_{90} - lethal concentration that kills 90% of the exposed larvae; LL-lower limit (95% confidence limit); UL-upper limit (95% confidence limit). *$p \leq 0.05$, level of significance of chi-square values.

Table3. Lethalconcentrations (in ppm) of crude extracts of *Tageteserecta* against thefourth instar larvae of *Ae. Aegypti* and *Cx. quinquefasciatus*.

Plant species	Mosquito species	Treatment	LC_{50} (ppm)	95% confidence Limit		LC_{90} (ppm)	95% confidence limit		Intercept ± SE	Slope ± SE	$\chi 2$
				LL	UL		LL	UL			
Tageteserecta	*Aedesaegypti*	Hexane	135.62	68.71	233.96	316.28	195.62	2184.29	-2.4±1.2	3.4±0.5	7.5*
		Chloroform	167.16	19.51	1038.43	409.40	212.61	8165.00	-2.3±1.6	3.2±0.7	14.5*
		Ethyl acetate	288.37	255.50	330.48	810.61	648.44	1104.95	-2.0±0.6	2.8±0.2	4.8*
		Ethanol	141.44	67.91	257.52	332.41	201.16	3103.66	-2.4±1.2	3.4±0.5	8.2*
		Aqueous	160.29	38.10	525.84	394.61	211.41	621.75	-2.2±1.5	3.2±0.6	12.7*
	Culexquinquefasciatus	Hexane	117.14	45.31	223.35	250.80	154.35	4943.50	-3.0±1.5	3.8±0.7	9.4*
		Chloroform	137.21	63.11	249.12	337.09	201.50	3414.82	-2.0±1.2	3.2±0.5	8.0*
		Ethyl acetate	234.85	100.27	1073.50	639.03	322.29	910.42	-1.9±1.3	2.9±0.5	10.4*
		Ethanol	119.54	35.02	226.36	341.11	191.92	1031.74	-0.8±1.1	2.8±0.5	8.0*
		Aqueous	138.40	67.79	244.61	331.46	201.59	2670.31	-2.2±1.2	3.3±0.5	7.7*

LC_{50} - lethal concentration that kills 50% of the exposed larvae; LC_{90} - lethal concentration that kills 90% of the exposed larvae; LL-lower limit (95% confidence limit); UL-upper limit (95% confidence limit). *p ≤ 0.05, level of significance of chi-square values.

Table 4. Lethal concentrations (in ppm) of crude extracts of *Eucalyptus deglupta* against the fourth instar larvae of *Ae. Aegypti* and *Cx. quinquefasciatus*.

Plant species	Mosquito species	Treatment	LC_{50} (ppm)	95% confidence Limit		LC_{90} (ppm)	95% confidence limit		Intercept ± SE	Slope ± SE	χ^2
				LL	UL		LL	UL			
Eucalyptus deglupta	*Aedesaegypti*	Hexane	199.96	153.67	256.12	453.95	421.43	498.41	-3.2±2.2	3.5±0.9	21.8*
		Chloroform	219.72	181.01	246.18	581.41	514.24	625.03	-2.10±1.6	3.0±0.7	15.7*
		Ethyl acetate	113.87	43.21	214.68	243.27	150.31	4832.40	-2.9±1.5	3.8±0.7	9.2*
		Ethanol	72.19	64.12	79.58	137.90	122.01	164.05	-3.4±1.0	4.5±0.5	1.1*
		Aqueous	269.13	238.56	307.59	768.76	617.09	1040.73	-1.8±0.6	2.8±0.2	4.3*
	Culexquinquefasciatus	Hexane	186.76	132.54	231.22	469.36	412.86	498.66	-2.27±2.0	3.2±0.9	23.6*
		Chloroform	213.73	179.63	247.17	539.80	516.72	574.36	-2.4±1.8	3.1±0.7	17.9*
		Ethyl acetate	109.07	97.19	121.25	258.53	223.58	312.79	-1.9±0.6	3.4±0.3	5.2*
		Ethanol	63.54	57.96	68.45	96.90	87.69	113.85	-7.6±1.9	6.9±1.0	0.1*
		Aqueous	119.77	61.85	197.91	259.84	166.86	1539.74	-2.9±1.3	3.8±0.6	7.2*

LC_{50} - lethal concentration that kills 50% of the exposed larvae; LC_{90} - lethal concentration that kills 90% of the exposed larvae; LL-lower limit (95% confidence limit); UL-upper limit (95% confidence limit). *p ≤ 0.05, level of significance of chi-square values.

Table 5. Lethal concentrations (in ppm) of crude extracts of *Syzygiumaromaticum* against the fourth instar larvae of Ae. *Aegypti* and *Cx. quinquefasciatus*.

Plant species	Mosquito species	Treatment	LC_{50} (ppm)	95% confidence Limit		LC_{90} (ppm)	95% confidence limit		Intercept ± SE	Slope ± SE	χ^2
				LL	UL		LL	UL			
Syzygiumaromaticum	*Aedesaegypti*	Hexane	205.44	176.45	247.61	469.17	422.38	491.38	-3.2±2.3	3.5±1.0	25.1*
		Chloroform	213.42	179.34	268.44	494.75	456.88	534.67	-3.1±2.3	3.5±0.9	23.9*
		Ethyl acetate	213.89	187.24	265.67	497.0	455.71	562.23	-3.1±2.0	3.5±0.9	19.6*
		Ethanol	194.74	125.75	258.29	458.88	401.37	485.28	-2.8±2.1	3.4±0.9	21.7*
		Aqueous	205.71	144.83	273.28	450.61	407.36	494.46	-3.7±2.2	3.7±0.9	20.3*
	Culexquinquefasciatus	Hexane	200.65	167.37	244.27	494.69	461.33	513.55	-2.5±2.1	3.2±0.9	23.4*
		Chloroform	188.11	134.88	261.68	448.19	412.38	483.68	-2.7±1.9	3.3±0.8	20.0*
		Ethyl acetate	186.05	102.11	234.0	464.89	437.82	498.24	-2.3±1.7	3.2±0.7	17.5*
		Ethanol	177.94	113.46	248.99	420.97	215.32	479.33	-2.7±1.8	3.4±0.7	16.4*
		Aqueous	184.14	131.44	241.65	458.02	419.55	493.74	-2.3±2.0	3.2±0.8	21.9*

LC_{50} - lethal concentration that kills 50% of the exposed larvae; LC_{90} - lethal concentration that kills 90% of the exposed larvae; LL-lower limit (95% confidence limit); UL-upper limit (95% confidence limit). *P ≤ 0.05, level of significance of chi-square values.

of 11.40ppm, 23.88ppm against *Cx. quinquefasciatus* and 15.65ppm, 36.19ppm on *Ae. aegypti* larvae, respectively. Fraction 5 produced LC_{50} and LC_{90} results of 12.42ppm, 34.97ppm against *Cx. quinquefasciatus* and 14.86ppm, 114.46ppm on *Ae. aegypti* larvae, respectively. Fraction 6 produced LC_{50} and LC_{90} results of 25.50ppm, 128.92ppm against *Cx. quinquefasciatus* and 27.16ppm, 143.84ppm on *Ae. aegypti* larvae, respectively (Tables 6 and 7).

DISCUSSION

Mosquitoes are a highly risky organism because it transmits

Table 6. Lethal concentrations (in ppm) of different fractions of *Eucalyptus deglupta* ethanol extract against larvae of *Cx. quinquefasciatus*.

Mosquito species	Treatment	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Intercept ± SE	Slope ± SE	X^2
			LL	UL		LL	UL			
Culexquinquefasciatus	Fraction 1	15.39	12.18	23.91	39.58	25.08	100.46	1.2±0.4	3.1±0.5	4.6*
	Fraction 2	17.56	13.24	31.11	49.32	28.66	159.08	1.4±0.4	2.8±0.5	3.7*
	Fraction 3	20.78	13.43	48.15	156.28	61.99	1028.85	3.0± 0.1	1.4± 0.2	0.1*
	Fraction 4	11.40	9.92	14.14	23.88	18.08	39.18	0.7±0.5	3.9±0.6	1.2*
	Fraction 5	12.42	10.24	16.80	34.97	23.71	68.33	1.8±0.3	2.8±0.4	0.7*
	Fraction 6	25.50	16.16	65.84	128.92	53.75	865.84	2.4± 0.2	1.8± 0.3	0.05*
	Fraction 7	5.07	2.24	8.27	12.64	7.91	28.96	2.7± 0.4	3.2±0.5	6.5*
	Fraction 8	10.59	9.26	12.85	23.18	17.74	36.23	1.1±0.4	3.7±0.5	2.3*
	Azadirachtin	6.69	5.74	8.10	24.69	17.87	39.87	3.1±0.1	2.2±0.2	3.7*
	Temephos	3.91	2.30	6.75	7.60	4.98	41.64	2.36± 0.4	4.4±0.7	7.9*

LC_{50} - lethal concentration that kills 50% of the exposed larvae, LC_{90} - lethal concentration that kills 90 % of the exposed larvae, LL - lower limit (95 % confidence limit), UL - upper limit (95% confidence limit), *p≤0.05, level of significance of chi-square values.

Table 7. Lethal concentrations (in ppm) of different fractions of*Eucalyptus deglupta* ethanol extract against larvae of *Ae. aegypti*.

Mosquito species	Treatment	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Intercept ± SE	Slope ± SE	X^2
			LL	UL		LL	UL			
Aedesaegypti	Fraction 1	18.42	13.60	34.14	54.96	30.78	192.35	1.5±0.4	2.7±0.5	2.2*
	Fraction 2	21.42	14.89	47.71	69.40	35.11	337.22	1.6± 0.4	2.5±0.5	0.1*
	Fraction 3	24.64	14.96	68.50	205.76	72.52	1940.88	3.0± 0.1	1.3± 0.2	0.1*
	Fraction 4	15.65	12.45	25.22	36.19	23.18	98.57	0.7±0.6	3.5±0.7	3.4*
	Fraction 5	14.86	10.42	27.85	114.46	50.91	537.89	3.3± 0.1	1.4± 0.2	0.08*
	Fraction 6	27.16	16.76	76.01	143.84	57.18	1121.27	2.4± 0.2	1.7± 0.3	0.06*
	Fraction 7	5.50	4.83	6.37	17.18	13.43	24.26	3.0±0.1	2.5±0.2	4.9*
	Fraction 8	10.73	9.48	12.85	21.46	16.80	32.76	0.6±0.5	4.2±0.6	1.7*
	Azadirachtin	6.98	6.06	8.31	22.30	16.75	33.82	2.8±0.1	2.5±0.2	4.0*
	Temephos	4.04	2.05	8.25	7.83	4.94	107.97	2.2±0.5	4.4±0.8	10.0*

LC_{50} - lethal concentration that kills 50% of the exposed larvae, LC_{90} - lethal concentration that kills 90 % of the exposed larvae, LL - lower limit (95 % confidence limit), UL - upper limit (95% confidence limit), *p≤0.05, level of significance of chi-square values.

disease-causing pathogens to human. In recent years, the vector mosquito population has increased in several fold in tropical and subtropical countries, including India. On the other hand, mosquitoes have developed resistance to many available conventional chemical insecticides. Hence, mosquito control with plant extracts would be a good substitute for chemical pesticides.

In ourstudy, theethanolextract of *Eucalyptusdeglupta* leaves produced the maximum larvicidal results with LC_{50} and LC_{90} data of 63.54ppm, 96.90ppm in killing *Cx. quinquefasciatus* and 72.19ppm, 137.90ppm in killing *Ae. aegypti* larvae, respectively. Our results were similar to the results of (RajivGandhi.et al.2016) who screened different extracts from five plants and found that the raw methanol extract of *Rubiacordifolia* was very active with LC_{50} and LC_{90} results of 95.69mg/L, 347.96mg/L in killing *Cx. quinquefasciatus* and 102.mg/L, 350.20mg/L in killing *Ae. aegypti* larvae, respectively. Similarly, (Aivazi, A.2009) reported that the ethyl acetate extract of *Quercusinfectoria*was most effective with LC_{50} and LC_{90} results of 116.92ppm, 144.77ppm against the fourth instar larvae of *An. stephensi*. Likewise, (Yadav, R.2013) screened different plant extracts. They found that the methanol extracts of *Euphorbia tirucalli* latex and stem bark was most effective with LC_{50} values of 177.14mg/L and 513.387mg/L against the third instar *Cx. quinquefasciatus* larvae, respectively.

Besides, our study discovered that larvae of *Cx. Quinquefasciatus* mosquitowas more vulnerable than the larvae of *Ae. aegypti*. Similar to our report, numerous studies have reported earlier with wide-ranging larvicidal efficacy of plant extracts among different mosquito species. For example, the methanol extract from *Solanumxanthocarpum* seeds and fruits was tested on *An. culicifacies*, *An. stephensi*, *Ae. Aegypti* and *Cx. quinquefasciatus* mosquito larvae by (Bansal,Set al.2009). The results varied for fruits and seeds with LC_{50} values of 51.6 mg/L, 52.2 mg/L, 118.3 mg/L, 157.1mg/L and 66.9 mg/L, 73.7 mg/L, 123.8 mg/L, 154.9mg/L on *An. culicifacies*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. In the same way, (Patil,S.V. et al. 2010) reported that *Ae. aegypti*larvae were more susceptible to methanolic extract of *Plumbagozeylanica*root with an LC_{50} result of 169.61mg/l than *An. stephensi* larvae with an LC_{50} result of 222.34mg/L.

In recent years, numerous studies have been conducted with plant crude extracts for its effect on mosquito larvae, and many crude extracts were reported to be very effective on various species of mosquito larvae. For instance, methanolic extracts were prepared from leaves of *Moringaoleigera* by (Prabhu, K. et al. 2011) and tested on first to fourth-stage larvae of *Anopheles stephensi*. The LC_{50} and LC_{90}results were reported to be 57.79ppm 125.93ppm

for the first instar, 63.90ppm and 133.07ppm for the second instar, 72.45ppm and 139.82ppm for the third instar, and 78.93 ppm and 143.20 ppm for the fourth stage larvae, respectively (Prabhu, K.et al. 2011) In another experiment, different solvent extracts were prepared from the root of *Asparagus racemosus* and they were studied on the larvae of *Ae. aegypti*, *Cx. quinquefasciatus*, and *Anopheles stephensi* (Govindarajan,M.2014)

Their study displayed LC_{50} and LC_{90} results of 90.97ppm, 210.96ppm and 179.92ppm, 168.82ppm and 115.13ppm, 97.71ppm against *Aedesaegypti*, *Anopheles stephensi* and *Culexquinquefasciatus* mosquito larvae, respectively (Govindarajan, M. 2014) Similarly, different solvent extracts viz., acetone, chloroform, ethyl acetate, methanol and petroleum benzene were extracted from the leaf extracts of *Clausenadentate* and screened by (Manjari, M.S.et al.2014) on the fourth stage larvae of *Cx. quinquefasciatus*, *Ae. aegypti*and *An. stephensi*. Their study showed that the acetone extract was most active and the LC_{50} and LC_{90} results were 0.045694mg/ml, 0.045684mg/ml on *An. stephensi* larvae and 0.150278mg/ml, 7.302613mg/ml against*Cx. quinquefasciatus* larvae and 0.169495mg/ml, 1.10034mg/ml against *Ae. aegypti* larvae (Manjari,M.S.et al.2014)

CONCLUSION

Inthisstudy,thedifferentcrudeextractsoffiveplants,namely, *Cymbopogoncitratus*, *Azadirachtaindica***,** *Tageteserecta*, *Eucalyptus deglupta,* and *Syzygiumaromaticum* were tested for their larvicidal activity against the fourth stage larvae of *Ae. Aegypti* and *Cx. quinquefasciatus* vector mosquitoes. The results undoubtedly proved that the ethanol extract of *Eucalyptus deglupta* was very active in killing the fourth stage larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. Hence, based on these study results, the ethanol extract of *Eucalyptus deglupta* was further investigated to isolate the effective compound.

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COMPETING INTERESTS

Authors declare that there is no conflict of interest.

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