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Mosquitocidal properties of *Pouzolzia bennettiana var. acuta* (Urticaceae) leaf extracts against three important human vector mosquitoes (Diptera: Culicidae)

[1] G Ramar, [2] A Jeyasankar

[1] PG & Research Department of Zoology, Arignar Anna Government Arts College, Musiri, Tamil Nadu, India
[2] PG & Research Department of Zoology, Government Arts College (Autonomous), Coimbatore, Tamil Nadu, India

Abstract
To determine the larvicidal and pupicidal activity of *Pouzolzia bennettiana var. acuta* leaf extracts against *Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus*. The larvicidal activity was determined against three vector mosquito species at concentrations of 50, 100, 150, 200 and 250 ppm. Larval and pupal mortality was assessed after 24 hours. The leaf extracts of *Pouzolzia bennettiana var. acuta* was found to be more susceptible against the larvae of *Ae. aegypti, An. stephensi and Cx. quinquefasciatus.* Insecticidal susceptibility tests were carried out using WHO standard method and the mortality was observed after 24-h exposure. All the tested oils showed moderate to good larvicidal and pupicidal activityes. However, the maximum larval mortality was detected in plant of Ethyl acetate extract against *An. Stephensi* 94.4% (LC_{50} 45.2 and LC_{95} 268.99). The maximum pupal mortality was detected in plant of Ethyl acetate extract in Ethyl acetate extract against *Cx. quinquefasciatus* 64.6 % 85.2%(LC_{50} 78.66 and LC_{95} 770.80). These results suggested that the leaf extracts of *Pouzolzia bennettiana var. acuta* showed potential to be used as an ideal ecofriendly approach for the control of the *Ae. aegypti, An. stephensi and Cx. quinquefasciatus.*

Keywords: larvicidal and pupicidal, *Pouzolzia bennettiana var. acuta*, Leaf extract, *Ae. aegypti, An. stephensi and Cx. quinquefasciatus*

1. Introduction
In India, Mosquito borne diseases constitute a major public health problem in the list of communicable diseases e.g., malaria, dengue, chikungunya, filariasis, Japanese encephalitis, and leishmaniasis, cause thousands of deaths per year. India reports 1.48 million malarial cases and about 1,173 deaths, 1.4 million suspected and 1,985 confirmed chikungunya cases, 5,000 Japanese encephalitis cases and approximately 1,000 deaths, and 383 dengue cases and 6 deaths during 2006 and 2007 (WHO 2007; Gopalan and Das 2009; Dhiman *et al.* 2010) [1, 2]. *Ae. aegypti* is the principal vector of dengue fever and dengue hemorrhagic fever and it is reported to infect more than 100 million people every year in more than 110 countries in the tropics. Thus one of the approaches for control of these mosquito-borne diseases is the interruption of disease transmission by killing or preventing mosquitoes to bite human beings (Rajmohan, *et al*.). *An. stephensi* (Liston) is the primary vector of malaria in India and other west Asian countries and improved methods of control are urgently needed (Bufield T and Reekie SL, 2005) [5]. Insect vector especially mosquitoes are responsible for spreading serious human diseases like malaria (Halsted SB, 2000) [6].
In India around 20,000 medicinal plants have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases (Kalaivani *et al*., 2012) [7]. Therefore the present study was carried out to determine the *larvicidal and pupicidal activity of Pouzolzia bennettiana var. acuta* leaf extracts against important vectors *Ae. aegypti, An. stephensi and Cx. quinquefasciatus.*

2. Materials and Methods
Plant material
The leaves of *Pouzolzia bennettiana var. acuta* were collected from Pulliansolai, Kolli hills, namakkal District, Tamil Nadu, India during the July 2013. Collecetd plant specimen was identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Sytematics, St' Joseph's College, Tiruchirapalli, Tamil Nadu, India and The Voucher specimen (IPH 4) was deposited in Entomology lab, Arignar Anna Government Arts College, Musiri, Tamil Nadu, India.

Extraction method
The dried leaves (100g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with hexane, chloroform, and ethyl acetate (500ml, Ranchem), in a soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure of 22-26mm hg at 45º C by 'Rotavapour' and the residue obtained was stored at 4ºC in an amber vial. Then the vials were named and covered with silver foil and transported to the laboratory. Until use those vials were kept in cool and dark place at 4ºC.

Vector rearing
The mosquito larvae of Ae. aegypti, *An. Stephensi and Cx. quinquefasciatus* were collected from National centre for disease control, Government of India ministry of health and family welfare, Southern India branch, field station, Mettupalayam. The larvae were kept in the plastic buckets half filled with tap water and fed with dog biscuit once a day initially and twice during the later stages of development. Water in rearing container was refreshed every day by removing a little quantity of water from the rearing buckets and replacing with fresh water. This was aimed at preventing scum from forming on the water surface.

Larvicidal bioassay

The larvicidal activity of selected plants extracts were evaluated as per the protocol previously described WHO, (2005) [8] Based on the wide range and narrow range tests, all extracts tested ranging 30-200ppm were prepared and they were tested against the freshly moulted (0-6 hrs) third instar larvae of selected mosquito species. The plants oils were dissolved in 2 drop twin20 and then diluted in 100ml of dechlorinated tap water to obtain each of the desired concentrations. The control was prepared using 2 drop tween 20 in 100ml of dechlorinated water. The larvae of test species (10) were introduced in 250-ml plastic cups containing 100ml of aqueous medium (100ml of dechlorinated + 2 drop tween 20) and the required amount of chemical compositions was added. The larval mortality was observed and recorded after 24 h of post treatment. For each experiment, five replicates were maintained at a time. The LC_{50} value was calculated by using probit analysis (Finney, 1971) [9]. The average mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics chi-square values were calculated by using the software using statistical package of social science (SPSS) version 18.0 for windows, significance level was set at $p \leq 0.05$.

Pupicidal bioassay

The pupicidal activity of plant crude extract will be assessed by using the standard method as prescribed by WHO (2005) [8]. Similar test concentrations as stated in the previous experiments will be prepared and they will be tested against the pupae of *Ae. aegypti*, *An. Stephensi and Cx. quinquefasciatus* Tween 20(emulsifier) in water will be treated as control. The pupae of these mosquito species (10 pupae) will be introduced in 250-ml plastic cups containing 100 ml of aqueous medium (100 ml of dechlorinated water + 2 drops of tween 20) and the required amount of plant extract will be added. The pupal mortality will be observed and recorded after 24 h of post treatment. For each experiment, five replicates will be maintained at a time. The percentage of mortality will be calculated by using Abbott's formula (Abbott, 1925) [10].

3. Results

As discussed in materials and methods, the results of relative toxicity of *Pouzolzia bennettiana var. acuta* against *Ae. Aegypti*, An. *stephensi* and *Cx. quinquefasciatus* after 24 hours of treatment are presented in Table 1, 2, 3 and 4. It was evident from table that all the tested essential oils demonstrated significant larvicidal and pupicidal activity against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus.* The tested plant Pouzolzia bennettiana var. acuta showed moderate to good larvicidal and pupicidal activities. The larval mortality was detected in Pouzolzia bennettiana var. acuta against *Ae. aegypt*, *An. stephensi and Cu. quinquefasciatus.* The *Ae. aegypti*, *An. stephensi and Cx. quinquefasciatus* highest larval mortality was found in Ethyl acetate extract 64.6 % (LC_{50} 112.05 and LC_{95} 6739.46),94.4% (LC_{50} 45.2 and LC_{95} 268.99) and 69.4% (LC_{50} 128.35 and LC_{95} 1857.53) followed by Hexane extract 56.8% (LC_{50} 199.35 and LC_{95} 9592.33), 92.2% (LC_{50} 35.72 and LC_{95} 587.51),58.2% (LC_{50} 220.58 and LC_{95} 3027.11) Chloroform extract 60.8% (LC_{50} 165.65 and LC_{95} 5264.96), 82.6% (LC_{50} 77.31 and LC_{95} 983.10) and 63.4% (LC_{50} 142.82 and LC_{95} 3103.87). The *Ae. aegypti An. stephensi and Cx. quinquefasciatus* highest pupal mortality was found in Ethyl acetate extract 64.6 % (LC_{50} 158.19 and LC_{95} 3031.72),84.2% (LC_{50} 58.58 and LC_{95} 894.22) and 85.2%(LC_{50} 78.66 and LC_{95} 770.80) followed by Hexane extract 55.2%(LC_{50} 218.06 and LC_{95} 8393.25),71.4% (LC_{50} 93.70 and LC_{95} 2621.20), 60.2% (LC_{50} 175.48 and LC_{95} 3305.76) Chloroform extract 62.2% (LC_{50} 170.63 and LC_{95} 3012.28), 80.4 (LC_{50} 59.44 and LC_{95} 1495.25) and 56.2%(LC_{50} 230.06 and LC_{95} 2890.46), respectively. Chi- square value was significant at $p<0.05$ level (Tables).

Table 1: Larvicidal activity of *Pouzolzia bennettiana var. acuta* against Mosquito vectors.

Plant extract	Concentration (ppm) % of larval mortality					LC50 (ppm)	95% Confidence Limit (ppm)		LC95 (ppm)	95% Confidence Limit (ppm)		$x2$ ($df = 4$)
	50 (ppm)	100 (ppm)	150 (ppm)	200 (ppm)	250 (ppm)		LCL	UCL		LCL	UCL	
Larvicidal activity of *Pouzolzia bennettiana var. acuta* against 4th instar larvae of Ae. aegypti												
Hexane extract	29.6±1.1	36.8±2.1	42.2 ±1.7	49.6±1.1	56.8±2.3	199.35	144.17	275.64	9592.33	1248.63	73690.62	1.009
Chloroform extract	30.2±1.4	38.4±3.0	46.4±2.6	52.2±2.7	60.8±2.3	165.65	128.73	213.15	5264.96	1092.46	25373.56	0.951
Ethyl acetate extract	39.2±1.9	45.6±2.5	52.4 ±1.6	59.8±1.7	64.6±1.6	112.05	84.21	149.10	6739.46	962.02	47213.27	0.768
Larvicidal activity of *Pouzolzia bennettiana var. acuta* against 4thinstar larvae of *An. stephensi*												
Hexane extract	61.8± 3.1	68.2± 2.3	76.8 ±2.3	83.5±3.8	92.2±3.1	35.72	22.00	57.99	587.51	312.66	1103.98	4.822
Chloroform extract	41.6±2.6	55.2±3.4	60.6±1.1	72.4±3.0	82.6±3.5	77.31	61.17	97.71	983.10	503.47	1919.61	3.425
Ethyl acetate extract	57.2±1.4	67.6±3.4	77.2 ±3.0	86.8±1.9	94.4±2.7	45.20	32.77	62.34	409.15	268.99	622.34	5.717
Larvicidal activity of *Pouzolzia bennettiana var. acuta* against 4thinstar larvae of *Cx. quinquefasciatus.*												
Hexane extract	20.4± 1.1	28.6± 2.6	35.2 ±2.6	47.2±2.1	58.2±1.3	220.58	173.38	280.63	3027.11	1054.66	8688.45	3.069
Chloroform extract	30.2±1.4	39.6±1.1	51.4±2.5	56.2±3.1	63.4±2.7	142.82	115.61	176.42	3103.87	927.26	10389.69	0.631
Ethyl acetate extract	31.2±2.2	39.8±1.4	50.6 ±2.3	61.2±2.7	69.4±1.1	128.35	106.88	154.12	1857.53	763.81	4517.39	2.231

Values are mean ± SD for five replications. Values not sharing a common superscript differ significantly at $p < 0.05$.

Table 2: Pupalcidal activity of *Pouzolzia bennettiana var. acuta* against Ae. aegypti.

Concentration (ppm)	Adult Emergence (%)	Pupal Mortality (%)	LC50 (ppm) Pupal Mortality	95% Confidence Limit (ppm)		LC95 (ppm) Pupal Mortality	95% Confidence Limit (ppm)		*x*2 (*df* = 4)
				LCL	UCL		LCL	UCL	
Hexane extract									
Control	94.6 ± 2.6	0± 0.0	218.06	156.61	303.62	8393.25	1274.83	55259.34	0.701
50	70.6± 1.6	26.6± 1.6							
100	62.6 ± 2.4	35.2± 2.1							
150	55.4 ± 1.5	41.2± 1.9							
200	50.6± 2.1	47.4± 2.1							
250	43.4± 3.2	55.2 ± 1.3							
Chloroform extract									
Control	90.8± 3.5	0± 0.0	170.63	137.81	211.26	3012.38	978.93	9269.75	1.222
50	71.6 ± 3.1	25.8± 2.1							
100	62.6 ± 2.9	36.4± 1.8							
150	54.4 ± 2.6	44.2 ± 3.1							
200	38.6 ± 1.1	52.4 ± 2.3							
250	29.4 ± 3.5	62.2 ± 3.8							
Ethyl acetate extract									
Control	90.4± 3.5	0± 0.0	158.19	128.15	195.28	3031.72	960.34	9570.90	1.765
50	61.6± 1.9	28.2 ±1.3							
100	59.4± 1.5	37.8 ±3.0							
150	50.6± 2.1	45.8± 2.7							
200	39.8 ± 1.3	53.2 ±2.7							
250	28.8 ± 1.3	64.6 ± 1.3							

Value represents mean ± S.D. of five replications. *Mortality of the larvae observed after 48h of exposure period. LC50=Lethal Concentration brings out 50% mortality and LC95 = Lethal Concentration brings out 95% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table 3: Pupalcidal activity of *Pouzolzia bennettiana var. acuta* against *An. stephensi.*

Concentration (ppm)	Adult Emergence (%)	Pupal Mortality (%)	LC50 (ppm) Pupal Mortality	95% Confidence Limit (ppm)		LC95 (ppm) Pupal Mortality	95% Confidence Limit (ppm)		*x*2 (*df* = 4)
				LCL	UCL		LCL	UCL	
Hexane extract									
Control	93.2 ± 2.3	0± 0.0	93.70	72.22	121.57	2621.20	766.28	8966.23	1.234
50	58.6± 2.0	40.2± 1.3							
100	51.2 ± 1.9	48.2± 1.3							
150	40.4± 1.1	56.6± 3.1							
200	33.4± 2.1	64.4± 2.1							
250	24.2± 3.3	71.4 ± 1.9							
Chloroform extract									
Control	92.4± 2.5	0± 0.0	59.44	41.05	86.05	1495.25	552.25	4048.50	1.899
50	48.4± 2.0	49.4± 1.8							
100	42.6 ± 2.7	56.2± 1.3							
150	31.2 ± 3.5	67.4 ± 2.3							
200	27.2 ± 3.3	71.8 ± 1.6							
250	19.2 ± 1.3	80.4 ± 2.7							
Ethyl acetate extract									
Control	88.2± 2.7	0± 0.0	58.58	42.66	80.44	894.22	443.56	1802.76	3.004
50	49.4± 1.1	49.8 ±2.3							
100	40.6± 2.1	58.6 ±1.5							
150	30.2± 1.3	67.2± 2.1							
200	20.4 ± 2.7	78.6 ±1.5							
250	12.4 ± 2.7	84.2 ± 2.3							

Value represents mean ± S.D. of five replications. *Mortality of the larvae observed after 48h of exposure period. LC50=Lethal Concentration brings out 50% mortality and LC95 = Lethal Concentration brings out 95% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table 4: Pupalcidal activity of *Pouzolzia bennettiana var. acuta* against *Cx. quinquefasciatus.*

Concentration (ppm)	Adult Emergence (%)	Pupal Mortality (%)	LC50 (ppm) Pupal Mortality	95% Confidence Limit (ppm)		LC95 (ppm) Pupal Mortality	95% Confidence Limit (ppm)		*x*2 (*df* = 4)
				LCL	UCL		LCL	UCL	
Hexane extract									
Control	93.6 ± 3.0	0± 0.0	175.48	140.45	219.25	3305.76	1012.98	10787.95	0.900
50	70.4± 2.6	25.8± 3.0							
100	62.2 ± 3.2	35.6± 2.0							
150	50.8 ± 2.1	43.8± 2.1							
200	41.2± 1.3	53.2± 2.7							
250	37.4± 3.2	60.2± 1.6							
Chloroform extract									
Control	91.2± 1.9	0± 0.0	230.06	180.55	293.13	2890.46	1042.94	8010.73	3.347
50	79.8 ± 2.3	19.4± 3.3							
100	71.2 ± 2.7	23.6± 2.7							
150	60.2 ± 2.2	39.4 ± 1.1							
200	51.4 ± 2.3	44.2 ± 3.5							
250	40.8 ± 1.9	56.2 ± 1.6							
Ethyl acetate extract									
Control	94.8± 3.1	0± 0.0	78.66	63.93	96.78	770.80	446.95	1329.30	5.797
50	56.6± 2.6	42.2 ±2.2							
100	41.4± 2.0	50.8 ±1.9							
150	32.2± 2.6	62.4± 3.0							
200	22.8 ± 2.5	75.6 ±3.7							
250	11.6 ± 2.0	85.2 ± 1.7							

Value represents mean ± S.D. of five replications. *Mortality of the larvae observed after 48h of exposure period. LC50=Lethal Concentration brings out 50% mortality and LC95 = Lethal Concentration brings out 95% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

4. Discussion

The results of present study are comparable with similar reports of earlier workers. Ramar and Jeyasankar (2014) [11, 12, 13] reported that, hexane extract of Trgia involucrata leaves exhibited larvicidal activity with LC_{50} value of 153.51ppm after 24 h of exposure.The toxicity to the third instar larvae of *Ae.aegypti, Cx.quinquefaciatus* and *An.stephensi* by the ethyl acetate leafe extract of Breyenia vitis –idaea showed the LC_{50} value of 98.2, 107.79 and 115.8ppm respectively Jeyasankar and Ramar (2014) [11, 12, 13]. Ramar *et al.* (2014) [11, 12, 13] have also reported that the present investigation was designed to determine the larvicidal activity of silver nanoparticles synthesized from aqueous leaf extract of *Cleistanthus collinus* against the larvae of *Aedes aegypti (Ae. aegypti*). *Ae. aegypti* larvae were exposed to varying concentrations of aqueous extract of *C. collinus* and synthesized silver nanoparticles for 24 h as per WHO protocols. Percentage of larval mortality was recorded. The synthesized nanoparticles exhibited significant larvicidal activity. This method is considered as an innovative alternative approach using green nanochemistry technique to control dengue vector parasites of *C. collinus* leaf mediated synthesized silver nanoparticles. Jeyasankar *et al.* (2012) [14] have reported that the ethyl acetate extract of *Phyllanthus Emblica* Linn. Exhibited more than 90% larval mortality at 250ppm on *Cx. quinquefaciatus.* The toxicity to the third instar larvae of *Ae.aegypti, Cx.quinquefaciatus* and *An.stephensi* by the ethyl acetate leafe extract of *Andrographis Paniculata* showed the LC_{50} value of 20.85 and LC_{95} 444.41ppm respectively Jeyasankar and Ramar (2015) [15, 16]. Jeyasankar and Ramar (2015) [15, 16] have reported that the Petroleum Ether extract of *Andrographis Paniculata* exhibited more than 85% Pupal mortality and 100% Ovicidal activity at 250ppm on *Ae.aegypti, Cx.quinquefaciatus* and *An.stephensi.*

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