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Nagapattinam medicinal plants against the dengue fever mosquito, *Aedes aegypti*

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Abstract
In perspective of its expanding interest, an endeavor was made in the present study to evaluate the larvicidal capability of essential Nagapattinam therapeutic plants like *Pelargonium graveolens, Commiphora berryi* and *Ceiba pentandra* against *Aedes aegypti*. Twenty five early third instar larvae of *Ae. aegypti* were exposed to various concentrations (50-250 ppm) and the LC_{50} and LC_{90} values of the *P. graveolens, C. berryi* and *C. pentandra* extracts was analysed by probit analysis. The oviposition deterrent activity was determined against *Ae. aegypti* to various concentrations ranning from 75-350 ppm under research facility conditions. The present examination revealed that the most elevated LC_{50} and LC_{90} values methanol concentrate of *P. graveolens, C. berryi* and *C. pentandra* against *Ae. aegypti* hatchlings were 112.01, 122.02 and 137.97 ppm, respectively and 205.97, 220.74 and 244.47 ppm, individually. Thus, the methanol extract of *P. graveolens, C. berryi* and *C. pentandra* showed 100%, 87.4% and 58.8% oviposition deterrent activity at 350 ppm concentration against *Ae. aegypti* adult females. The outcomes plainly demonstrate that most extreme larvicidal activity against *Ae. aegypti* was acquired with methanol concentrate of *P. graveolens*.

Keywords: *Pelargonium graveolens, Commiphora berryi, Ceiba pentandra, Aedes aegypti,* Larvicidal activity

1. Introduction
Man Mosquitoes area unit dangerous vectors of deadly pathogens and parasites, which can it as epidemics or pandemics within the expanding world populace of people and animals. They are therapeutically critical bugs and are viewed as real general wellbeing bugs, and numerous tropical and subtropical diseases area unit the foremost necessary single cluster of insect well-known for the general public health importance [1]. It is transmit numerous terrifying ailments like yellow fever, filariasis, malaria, dengue and Japanese encephalitis to people and different vertebrates; thus, they have been declared "Open Enemy Number 1" [2]. *A. aegypti* L., a vector dengue that carries the arbovirus responsible for these diseases, is widely distributed in the tropical and subtropical zones [3]. Dengue fever has become a vital public unhealthiness because the variety of reported cases continues to extend, particularly with a lot of severe varieties of the illness, virus infection and shock syndrome or with uncommon manifestation like central system involvement [4]. *Ae. aegypti* and *Ae. albopictus* are two primary types of mosquitoes in charge of dengue and yellow fever mosquito in Taiwan, where the quantity of dengue fever cases has expanded significantly lately. The World Health Organization evaluates that around 2.5 billion individuals are at danger of dengue. No powerful medication or immunization is accessible in this way [5]. It's indications range from gentle fever to a serious and conceivably life-undermining hemorrhagic disease [6].
The control of mosquito hatchlings overall depends principally on proceeded with uses of organophosphates, for example, temephos, fenthion and creepy crawly development controllers, for example, diflubenzuron and methoprene. A late gauge demonstrates that more than 50 million individuals are at danger of dengue infection introduction worldwide and dengue fever can show as the great type of the illness, which weakens the patient for a week or increasingly, or as the hemorrhagic structure which, much of the time, prompts passing [7]. Measures to control the mosquito shape a vital segment of illness counteractive action programs in endemic nations. Notwithstanding, the continually expanding resistance of mosquitoes to the bug sprays that have been most usually utilized over a decade ago [8-9] has centered enthusiasm on option mixes for mosquito control.

The war against mosquitoes by method for concoction pesticides has fizzled because of the resistance created by mosquitoes [10]. Numerous therapeutic plants have been found to contain chemicals which are useful for the control of insects and are valuable for field applications in mosquito control programs [11]. *P. graveolens* are rich source of monoterpenes, sesquiterpenes, coumarins, tannins, phenolic acids, cinnamic acids, flavones, flavonoids and flavonols derivatives. *C. berryi* has been reported to have potential use in folklore medicine to treat various ailments such as ulcer, infection, loss of appetite etc. *C. pentandra* have been recommended for the treatment of bronchitis, diabetics, diarrhoea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, insect bite etc. Compound control of mosquitoes is likewise bringing on numerous undesirable impacts on human wellbeing and nontarget creatures. By comprehension these symptoms of chemicals, individuals are presently indicating interest towards biopesticides and plant details, which are considered as eco-accommodating [12] however people, regularly the different courses of organization of natural pharmaceuticals are chosen by consistency of the readiness and as per the infection that is being dealt with. Plant concentrates are likewise prepared or refined to create helpful tinctures, syrups, sauces, oral showers, tablets, typified powders, snuffs and capsules [13]. Therefore, in the present investigation, the crude extracts of *P. graveolens, C. berryi* and *C. pentandra* has been tested for its larvicidal activity against *Ae. aegypti*.

2. Material and Methods

2.1 Plant Material

Leaves of *P. graveolens* (Geraniaceae), *C. berryi* (Burseraceae) and *C. pentandra* (Malvaceae) were gathered from Velankanni (10.688610ºN-79.849598ºE), South poigainallur (10.695463ºN-79.839813ºE) and Nagapattinam (10.770115ºN-79.846485ºE), Nagapattinam District, Tamilnadu in India. These were ground to fine powder. At the season of gathering, voucher herbarium examples were arranged and related to the assistance of Plant Taxonomist, Department of Botany, Annamalai University, Chidambaram.

2.2 Extraction method

Leaves were air dried in shaded spot for 15 days at room of temperature. Dried materials were powdered by utilizing an electric blender. Powdered plant material (100 g) was dissolved in methanol, ethanol, benzene and acetone (500 ml). Then water/air dried wide mouth bottle (1000 ml) and kept for 7 days with occasional shaking. After that, the icy concentrates were sifted utilizing Whatman channel paper and unbroken in Petri dishes for drying at temperature [14]. Dried concentrates were utilized for the arrangement of stock arrangement (2%).

2.3 Test mosquito larvae

Ae. aegypti hatchlings were gathered from restorative field and stagnant water zones of Chidambaram, Tamilnadu in India. It was kept up at 27±20 ºC, 75-85 relative mugginess. The hatchlings were bolstered with dog biscuits and yeast at 3:1 proportion.

2.4 Larvicidal activity

The larvicidal activity of chose medicinal plants concentrates were assessed according to the convention beforehand portrayed [15]. In view of the wide range and thin range tests, all concentrates tried going from 50-250 ppm were readied and they were tried against the newly shed (0-6 shrs) third instar hatchlings of chose mosquito species. The plants concentrates were disintegrated in 1 ml DMSO (Dimethyl sulfoxide) and afterward weakened in 249 ml of dechlorinated faucet water to acquire each of the fancied focuses. The control was readied utilizing 1ml of DMSO as a part of 249 ml of dechlorinated water. The hatchlings of test species (25) were presented in 250 ml plastic glass containing 250 ml of fluid medium (249 ml of dechlorinated water + 1ml of Dimethyl Sulfoxide) and the required measure of compound sytheses was included. The larval mortality was watched and recorded after 24 h of post treatment. For every examination, five recreates were kept up at once. Percent mortality was rectified for control mortality utilizing [16].

2.5 Oviposition deterrence activity

To ponder the ovipositional discouragement impact and the quantity of eggs saved in the vicinity of various dissolvable concentrates of trial plants, a numerous focus test was done. For bioassay test, 20 females were isolated in the pupal stage (by size of the pupae) and were brought into screen confines (45×45×40 cm) in a room at 27±2 ºC and 75-85% relative mugginess with a photoperiod of 14:10 h light and dim cycles. The pupae were permitted to rise into grown-ups in the test confines. Arrangement in a plastic glass with a cotton wick. They were blood nourished (from chicken) on day five glasses, each containing 100 mL refined water with a 9-cm bit of white channel paper for oviposition and in addition dissolvable concentrates at a convergence of 75, 150, 250 and 350 ppm were spot in every confine. A 6th container without concentrate served as a control. The positions of the plastic glasses were substituted between the distinctive recreates so to invalidate any impact of position on oviposition. Five reproduces for every fixation were keep running with enclosures set one next to the other for every bioassay. After 24 h, the quantity of eggs laid in treated and control glasses were numbered under a stereomicroscope. The percent successful repellency for every focus was computed utilizing the accompanying equation.

$$ER\% = \frac{NC\text{-}NT}{NC} \times 100$$

Where *ER* = effective repellency, *NC* = number of egg in control and *NT* = number of eggs in treatment [17].

2.6 Statistical study

The LC_{50} value was figured by utilizing probit analysis [18]. The normal mortality information were subjected to probit investigation for computing LC_{50}, LC_{90} insights qualities were ascertained by utilizing the product utilizing factual bundle of sociology (SPSS) rendition 16.0 for windows.

3. Results

3.1 Larvicidal activity

Information of the larvicidal movement of four distinct concentrates (methanol, ethanol, benzene and acetone of *P. graveolens, C. berryi* and *C. pentandra* against the hatchlings of *Ae. aegypti* was performed under research center assessment. As showed from the table 1, for the most part expanded larval mortality was seen with expanded centralization of the extracts tested against *Ae. aegypti*. As far as mortality fixation for 50% and 90% mortality (LC_{50} and LC_{90}) qualities were spoken to as takes after: For *P. graveolens* LC_{50} estimation of the methanol, ethanol, benzene and acetone concentrate was 112.0, 121.11, 138.14 and 156.91

ppm; LC_{90} estimation of the methanol, ethanol, benzene and acetone concentrate of was 205.97, 218.89, 240.47 and 264.89 ppm, respectively. As appeared from the table 1, generally extended larval mortality was seen with extended centralization of the extracts tested against Ae. aegypti. To the extent mortality fixation for 50% and 90% mortality (LC_{50} and LC_{90}) qualities were addressed as takes after: For P. graveolens LC50 values of the methanol, ethanol, benzene and CH3)2CO concentrate was 112.0, 121.11, 138.14 and 156.91 ppm; LC_{90} estimation of the methanol, ethanol, benzene and CH3)2CO concentrate of was 205.97, 218.89, 240.47 and 264.89 ppm, separately

C. berryi LC_{50} estimation of the methanol, ethanol, benzene and acetone concentrate was 122.00, 128.01, 156.20 and 175.47 ppm, respectively; LC_{90} estimation of the methanol, ethanol, benzene and acetone concentrate was 220.74, 229.83, 264.48 and 282.50 ppm, respectively. *C. pentandra* LC_{50} estimation of the methanol, ethanol, benzene and acetone concentrate was 137.97, 150.17, 175.23 and 219.90 ppm, respectively; LC_{90} estimation of the methanol, ethanol, benzene and acetone concentrate was 244.47, 271.31, 282.99 and 338.36 ppm, respectively in Table 2 and Figure 1-3.

3.2 Oviposition deterrent activity

These outcomes recommended that *P. graveolens, C. berryi* and *C. pentandra*, leaves displayed imperative action and could be considered as an important regular larvicidal activity against vector mosquito in Table 3 and Figures 1-3.

Table 1: Percentage mortality of mosquito larvae of *Ae. aegypti* exposed to different concentrations of different leaf extracts

Treatment	Concentrations	*P. graveolens*	*C. berryi*	*C. pentandra*
		% of mortality±SE[a]	% of mortality±SE[a]	% of mortality±SE[a]
Methanol	50	22.6±0.87	19.6±1.16	16.2±1.01
	100	43.8±0.73	39.8±1.31	32.2±1.11
	150	64.8±1.01	60.6±0.87	54.8±1.24
	200	86.4±1.02	79.8±1.01	72.2±1.49
	250	100.0±0.0	99.2±0.37	94.8±1.01
Ethanol	50	20.2±1.11	18.8±1.31	15.2±1.01
	100	39.8±1.31	36.6±0.97	29.6±0.97
	150	60.6±0.97	57.8±1.62	50.2±1.49
	200	80.2±1.49	76.2±1.31	66.8±0.73
	250	99.8±0.2	98.4±0.81	87.6±1.16
Benzene	50	14.8±1.77	11.4±1.12	8.4±1.32
	100	32.6±1.16	24.8±0.96	18.2±0.96
	150	54.2±1.49	47.6±0.97	35.6±1.16
	200	72.8±1.52	65.8±0.73	59.8±1.52
	250	95.8±1.31	89.2±1.28	83.8±1.49
Hexane	50	10.4±1.63	7.2±0.8	4.4±0.50
	100	24.8±1.24	18.6±1.28	9.8±0.86
	150	48.6±1.69	36.8±0.86	20.2±0.96
	200	65.6±0.97	60.2±1.49	39.8±1.24
	250	88.4±1.28	82.8±1.24	65.2±0.66

SE= Standard Error, [a] Values are mean ± SE of five replicates

Table 2: Larvicidal activity of three medicinal plants against third instars larvae of *Ae. aegypti*.

Plants name	Treatment	LC_{50} (mg/L)	95% Confidence limits		LC_{90} (mg/L)	95% Confidence limits		χ^2
			LCL	UCL		LCL	UCL	
	Methanol	112.01	101.45	121.67	205.97	191.85	224.30	5.159
P. graveolens	Ethanol	121.11	110.62	130.91	218.89	203.93	238.39	7.379
	Benzene	138.14	127.81	148.20	240.47	224.12	261.92	3.946
	Acetone	156.91	146.42	167.63	264.89	246.38	289.55	1.178
	Methanol	122.02	111.46	131.87	220.74	205.63	240.47	6.077
C. berryi	Ethanol	128.01	117.43	138.05	229.83	213.97	250.64	6.607
	Benzene	156.20	145.68	166.93	264.48	245.92	289.20	1.439
	Acetone	175.47	164.98	186.76	282.50	262.71	309.09	0.326
	Methanol	137.97	127.29	148.34	244.47	227.35	267.09	3.388
C. pentandra	Ethanol	150.17	138.58	161.80	271.31	250.31	299.98	0.937
	Benzene	175.23	164.68	186.61	282.99	263.03	309.83	1.233
	Acetone	219.90	206.44	236.80	338.36	309.77	379.57	1.044

Values represent mean of five replications. Mortality of the after 24 h of exposure period LC_{50}= Lethal Concentration brings out 50% mortality and LC_{90}= Lethal Concentration brings out 90% mortality. LCL- Lower Confident Limit, UCL- Upper Confident Limit, χ^2= Chi-squire, Significant at $p<0.05$

Table 3: Percent oviposition deterrent activity of crude extracts against *Aedes aegypti* adult females.

Plant name	Treatment	Concentration (ppm)			
		75	150	250	350
P. graveolens	Methanol	34.8±0.96	57.8±1.46	78.6±1.28	100.0±0.0
	Ethanol	29.8±1.31	52.2±1.28	71.4±1.12	96.6±1.02
	Benzene	21.2±1.20	39.4±1.12	58.8±1.24	80.2±0.96
	Acetone	18.4±1.02	34.2±0.96	51.2±1.20	71.6±1.12
C. berryi	Methanol	27.6±0.97	45.6±0.87	62.2±0.96	87.4±1.12
	Ethanol	22.4±1.28	36.2±1.20	54.8±1.01	76.6±0.87
	Benzene	15.2±0.96	28.2±1.20	40.6±1.12	57.8±1.15
	Acetone	10.8±1.31	18.4±1.12	29.2±0.96	36.6±0.97
C. pentandra	Methanol	20.8±1.24	31.6±0.92	43.2±0.80	58.8±1.24
	Ethanol	17.4±1.14	26.8±0.86	34.6±0.81	47.8±0.73
	Benzene	11.4±1.02	18.8±1.31	26.8±1.24	37.4±1.16
	Acetone	7.4±1.02	14.2±0.96	21.4±1.12	32.2±0.96

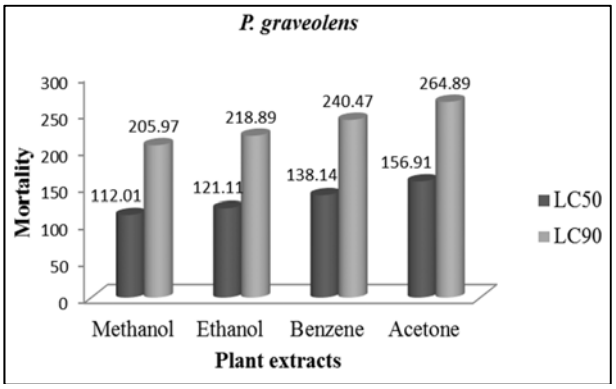

Fig 1: LC_{50} and LC_{90} values of different extracts of *P. granatum* against *Ae. aegypti*

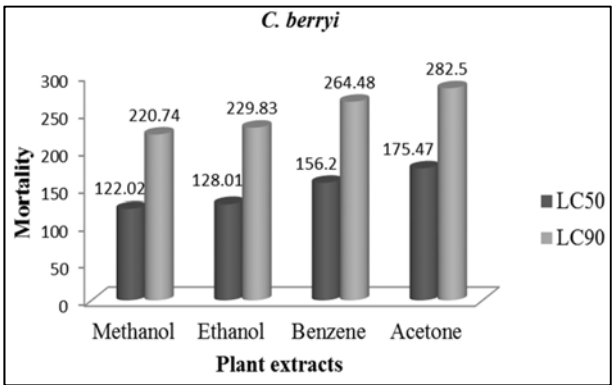

Fig 2: LC_{50} and LC_{90} values of different extracts of *C. berryi* against *Ae. aegypti*

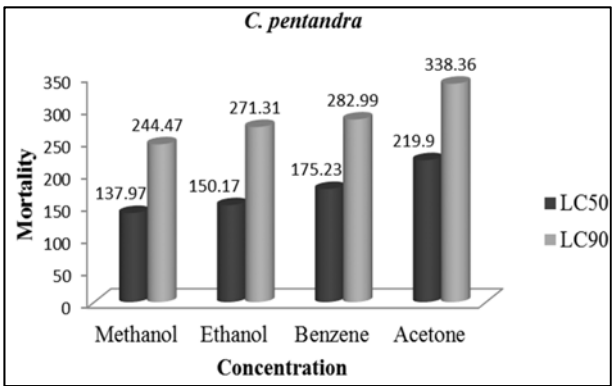

Fig 3: LC_{50} and LC_{90} values of different extracts of *C. berryi* against *Ae. aegypti*

4. Discussion

The present study demonstrates that each of the three medicinal plant species *P. graveolens, C. berryi and C. pentandra* have the potential for the change of new and safe control items against *Ae. aegypti*. Those plants species collected from Velankanni, South poigainallur and Nagapattinam, Nagapattinam District, Tamilnadu in India. Further studies on the larvicidal method of activity, their consequences for non-target creatures and definitions for enhancing the insecticidal strength of all the three plant concentrate are in advancement. Distinctive parts of plants contain a complex of chemicals with novel natural action which is thought to be because of poisons and auxiliary metabolites. Which go about as attractants or impediment our result exhibited that the crude extracts of *P. graveolens, C. berryi* and *C. pentandra* have basic larvicidal activity.

Deniel *et al.* [19] have reported that the 5 medicative plants, *Aegle marmelos, Limonia acidissima, Sphaeranthus indica, Sphaeranthus amaranthoides* and *Chromolaena odorata* for ovicidal and oviposition deterrent activities against *A. aegypti* and *C. quinquefasciatus*. The best ovicidal activity of resolvent extract of *L. acidissima* were 79.2% and 60% at 500 ppm concentration against *C. quinquefasciatus* and *A. aegypti*. Similarly, an equivalent of *L. acidissima* hexane extract showed 100% oviposition deterrent activity in any respect concentrations against *C. quinquefasciatus* and *A. aegypti*. The larvicidal, ovicidal, repellent and oviposition deterrent activity of essential oil and isolated compound from *Polygonum hydropiper* against II and IV instar larvae of *Ae. albopictus*. The essential oil tested LC_{50} values of 194.63 and 199.65; confertifolin tested LC_{50} values of 2.02 and 3.16 against II and IV instar larvae of *Ae. albopictus*. The highest ovicidal activity of 100% on 0 to 6 h old eggs, repellent activity of 320.6 min, oviposition deterrent activity of 98.51% and adulticidal activity of 100% at 10 ppm concentration of confertifolin [20]. Ke-Xin Yu *et al.* [21] demonstrated the evaluation of larvicidal, ovicidal and oviposition repellent activities of *Bryopsis pennata* against *Ae. aegypti* and *Ae. albopictus*. The chloroform extract of *Bryopsis pennata* have both ovicidal and larvicidal activity against *Ae. aegypti* and *Ae. albopictus* with LC_{50} values of 4.7 µg/mL and 5.3 µg/mL. Methanol extract showed sturdy repellent result against female oviposition, beside weak adulticidal activity against *Ae. aegypti* and *Ae. aegypti*. Jayaprasad *et al.* [22] aftereffects of the present study uncover that the methanol and aqueous bark concentrate of *Ch. swietenia* has a promising larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus*. With LC_{50} and LC_{90} values of methanol and ethanol concentrates of 124.70, 133.10 ppm

and 226.26, 238.93 ppm respectively against *Ae. aegypti*. Larvicidal activity of seed essential oil from *Nigella sativa* against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* at 24 h and 48 h. The LC_{50} and LC_{90} values of 196.9 and 523.5 ppm, at 24 h and 99.9 and 300.8 ppm, at 48 h against *Ae. aegypti* [23]. Shanmugam *et al.* [24] examination that the larvicidal potential of *Murraya exotica* essential oil against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*. After 12 h of introduction period, the larvicidal action are LC_{50}= 74.7 and LC_{90}= 152.7 ppm; after 24 h presentation period were LC_{50}= 35.8 and LC_{90}= 85.4 ppm, respectively against *Ae. aegypti*.

The larvicidal, ovicidal, repellent, oviposition detterency and adulticidal action of *Polygonum hydropiper* and confertifolin compound against II and IV instars hatchlings of *Aedes albopictus*. The LC_{50} activities are 194.63 and 199.65 ppm, separately. The ovicidal action of 100% on 0-to 6 h old eggs, repellent action of 230.6 min, ovipositoin deterrent action of 98.51% and adulticidal action of 100% at 10 ppm centralization of confertifolin were recorded [25]. Vijaya kumar *et al.* [26] analysis of larvicidal, oviposition deterrent and repellent activity of *Annona squamosa* against *Ae. aegypti, An. stephensi* and *Cx.. quinquefasciatus*. The LC_{50} and LC_{90} values of 219.41 and 394.87 ppm, severally. In oviposition deterrent activity the best concentration of 0.1%, *Annona squamosa* manufacture 92.4% against *Ae. aegypti*. Skin repellent check at 0.02 ppm concentration of *Annona squamosa* offers the entire protection time renges from 50.4 to 271 minutes. The *Annona squamosa* exerted the best protection time of 126.2 minutes. The most noteworthy mortality was found in acetone extract against *Ae. aegypti* with LC_{50} and LC_{90} estimations of 4.1783 and 9.3884 mg/ml, individually. Smoke poisonous quality was seen at 10 min interim for 40 min, and the mortality information were recorded [27]. The grown-up mortality was found in ethanol concentrate of *Citrus sinensis* with the LC_{50} and LC_{90} estimations of 320.38 and 524.57 ppm, individually against *Ae. aegypti* [28]. The adequacy of ovicidal and ovipositon reaction of plant volatile oils against *Cx. quinquefasciatus*. Among the lemon oil highest (97.5%) ovicidal activity at 200 ppm. most oviposition response activity (100%) was obtained in essential oil against *Cx. quinquefasciatus* [29]. An attempt has been made to evaluate the role of *P. graveolens, C. berryi* and *C. pentandra* extracts for their larvicidal bioassay against *Ae. aegypti*. The results reported in the present study open the possibility for further investigations of the efficacy of larvicidal properties of natural product extracts as a potential agent for combating mosquitoes.

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