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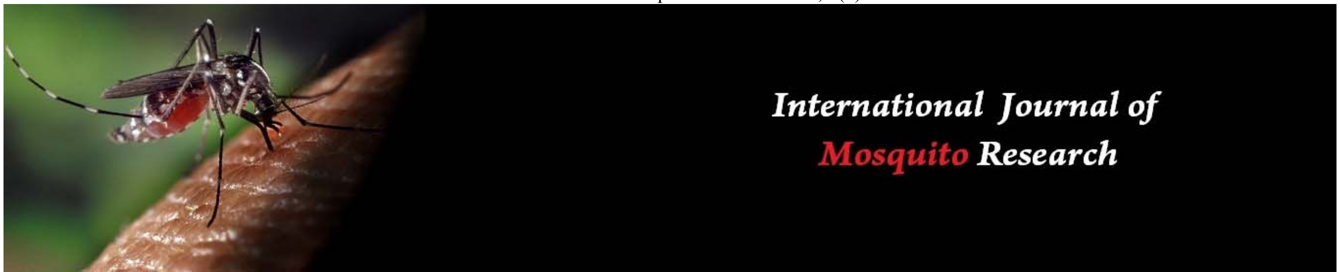

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Bioefficacy of *Nerium oleander* Linnaeus (Apocynaceae) floral extracts on the larva of three vector mosquitoes of medical importance

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Abstract
Mosquitoes are the major vectors of many pathogens which cause diseases like dengue, chikungunya, yellow fever, Zika virus fever, filariasis, Japanese encephalitis and malaria. Vector mosquito control or management is important in order to prevent mosquito-borne diseases as well as to improve public health. Repeated use of synthetic insecticides induces ecological imbalance, affects non-target organisms and poses severe health risk to humans. Plant extracts are believed to be a good alternative to chemical insecticides. In the present study, *Nerium oleander* flower (pink, red and white) extracts were evaluated for its bioefficacy on the larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* mosquitoes. The larvae of each species were exposed to different concentrations *viz*., 62.5, 125, 250, 500 and 1000mg/L and larval mortality was assessed 24 hours after exposure and LC_{50} and LC_{90} values were calculated. Amongst the different floral extracts tested, the acetone extract of *Nerium oleander* red flowers exhibited the highest larvicidal activity against all the three mosquito species and their LC_{50} values were 94.60, 101.21 and 121.79mg/L against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* respectively. Identifying plant based insecticides that are efficient as well as suitable and adaptive to local ecological conditions, biodegradable and having wide spread mosquito larvicidal chattels will work as a new weapon in the arsenal of insecticides and in the future may add to a suitable alternative tool to fight against mosquito-borne diseases.

Keywords: *Nerium oleander*, flower extracts, larvicidal activity, *Aedes aegypti, Anopheles stephensi*, *Culex quinquefasciatus*

1. Introduction
Mosquitoes are the major vectors of many pathogens which cause diseases like dengue, chikungunya, yellow fever, Zika virus fever, filariasis, Japanese encephalitis and malaria [1, 2]. These vector-borne diseases continue to have devastating impact on human beings for the past several decades [3, 4], besides causing high levels of economic impact throughout the world [5-7]. Mosquito-borne diseases affect over 700,000,000 people every year globally and 40,000,000 of the Indian population [8]. *Aedes aegypti* Linnaeus is generally known as vector of arboviruses and is responsible for dengue and chikungunya [9, 10], and is endemic to Southeast Asia, the Pacific island area, Africa and Americas [11, 12]. *Anopheles stephensi* Liston is widespread in tropical and subtropical regions and is the primary vector of malaria in India and other West Asian countries [13]. *Culex quinquefasciatus* Say is found in pan and subtropical Americas, the neotropics, Afrotropics, Indo-malayan, Australasian, East Asia, UK and parts of Middle East [14]. It is commonly known as southern house mosquito and is probably the most abundant house mosquito in towns and cities of the tropical countries [15] acting as a primary vector of lymphatic filariasis with worldwide distribution.
Vector mosquito control or management is important in order to prevent mosquito-borne diseases as well as to improve public health. Repeated use of synthetic insecticides has created a number of ecological problems, such as development of resistant strain, ecological imbalance, effects on non-target organisms and health risk to humans [16]. Plant extracts are believed to be a good alternative to chemical insecticides. A brief delve into the literature reveals many investigations have been made towards the biological screening of botanical

extracts and the activity of many plant derived components against mosquitoes [8, 17-29] and in the current scenario, several researchers are searching locally available plant materials in order to find out eco-friendly products to manage different mosquito species [30-39]. *Nerium oleander* Linnaeus (Apocynaceae) is an evergreen flowering shrub which grows in Mediterranean tropical and subtropical regions [40, 41]. The plant called '*Arali*' in Tamil [42, 43] is known for its ethnomedicinal value. The plant possess antibacterial [41, 44], antifungal [45], antimicrobial [46, 47], antiinflammatory, antinociceptive [48], antioxidant [49], hepatoprotective [50] and antitumor [47] activities. The phytocompounds like flavonoids, coumarins, triterpenes, anthraquinones [51], cardiac steroid, arabinogalactan and cardenolides, cardiac glycosides like oleandrin and nerine, rosagenin [44], kanersoide, neriumoside, karenin, oleandrin, folinrin, adenerin, nerine, digitoxigenin, bufadienolides, ouabain, proscillaridin, 4-oxooctyl-2-hydroxy-undecanoate, heptacosane-3-enyl-5-hydroxyhexanoate, betulin, betulinic acid, stigmasterol, quercetin-5-O-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside and kaempferol-5-O-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside [52] are present in this plant. Al-Yahya *et al*. [53] reported *Nerium oleander* to possess insecticidal properties. Toxic effects of *Nerium oleander* leaves [54] and essential oil [55] were found to have an exerted effect on the desert locust *Schistocerca gregaria*. The ethanolic leaf extract of *Nerium oleander* exhibited insecticidal activity against *Trogoderma granarium* and *Drosophila rufa* [56]. The roots, bark, stem, leaves and flowers of *Nerium oleander* are reported to possess insecticidal and antifeedant activity against diamondback moth (*Plutella xylostella*) [57-59]. Rathi and Al-Zubaidi [60] evaluated the phenolic leaf extract of *Nerium oleander* against whitefly, *Bemisia tabaci* population and observed adult mortality.

Noteworthy mosquitocidal researches were reported in *Nerium oleander* which has been evidenced by the following reports. Consoli *et al*. [61] tested the stem and leaves for larvicidal activity against *Aedes fluviatilis*. Komalamisra *et al*. [62] reported its crude ethyl acetate leaf extract for insect growth regulatory activity against *Aedes aegypti* and Pushpalatha and Muthukrishnan [63] for larvicidal activity against *Anopheles stephensi* and *Culex quinquefasciatus*. The aqueous and methanol leaf extract possessed larvicidal activity against *Aedes*, *Anopheles* and *Culex* species [64, 65]. The aqueous leaf extract were tested for ovicidal, larvicidal and repellent activity against *Culex tritaeniorhynchus* and *Culex gelidus* [66] and against *Anopheles stephensi* for ovicidal and adulticidal activity [51] and were found to be active. Raveen *et al*. [36] reported the crude hexane flower extract and Karlekar and Andrew [67] the ethanolic leaf and seed extract to possess larvicidal activity against *Culex quinquefasciatus*. The plant was reported for larvicidal activity against *Culex pipiens* on exposure to hydroethanolic extract [68] and diethyl ether extract [69], and also against *Anopheles stephensi* to the crude chloroform, petroleum, benzene, aqueous and acetone leaf extracts [70]. Thus, in continuation to the work by Raveen *et al*. [36], the present investigation was carried out to explore the larvicidal properties of *Nerium oleander* crude flower (pink, red and white) extracts against the mosquitoes of medical importance *viz*., *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* under laboratory conditions.

2. Materials and methods

2.1. Plant collection and preparation of floral extracts

Mature and healthy *Nerium oleander* flowers (pink, red and white) (Figure 1) collected from Chennai, Tamil Nadu, India were taxonomically identified and confirmed at Department of Plant Biology and Plant Biotechnology, Madras Christian College, Chennai, Tamil Nadu, India. The pink flowers were then brought to the laboratory, washed in dechlorinated water, shade dried and powdered with the aid of an electric blender. The powdered pink flowers (1Kg) were extracted with different solvents (3L) each (low polar: hexane, petroleum ether, dichloromethane; mid polar: chloroform, ethyl acetate, acetone, methanol; and high polar: distilled water) in a Soxhlet apparatus with minor modifications [71] and air dried to obtain the crude pink flower extracts. Likewise, the same methodology was adopted to obtain the crude red and white flower extracts. The crude pink, red and white flower extracts thus obtained were stored in air tight amber coloured bottles at 4°C for bioassays.

2.2. Test mosquitoes

The larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* were obtained from Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India which were free of exposure to insecticides. Cyclic generations of the above mentioned vector mosquitoes were maintained separately in mosquito cages (2'x2'x2') in an insectary with a mean room temperature of 27±2°C and a relative humidity of 70-80%. The adult mosquitoes were fed on ten per cent glucose solution in water. The eggs laid in ovitraps placed inside the mosquito cages were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with larval food (dog biscuits and yeast in the ratio 3:1). The larvae on becoming pupae were collected, transferred to plastic bowls and kept inside another mosquito cage for adult emergence.

2.3. Larvicidal bioassay

Larvicidal bioassay was carried out as per the guidelines of World Health Organization (WHO) [72] with minor modifications. Larvicidal activity at test concentrations of 62.5, 125, 250, 500 and 1000mg/L of each crude floral extract was assessed. The required test concentrations and quantity of test solution was prepared by serially diluting one per cent stock solution of the crude extract. Twenty early third instar larvae of each species from laboratory colonized mosquitoes of F_1 generation were introduced into glass beakers (250mL) containing 200mL of distilled water and test concentration. Untreated control (distilled water only) and treated control (Tween 80 added to distilled water) were maintained separately and run simultaneously. Mortality was observed 24 hours after treatment. Moribund larvae were scored dead when they showed no signs of movement when probed by a needle at their respiratory siphon. The per cent larval mortality was calculated using the formula (1) and corrections for control mortality (5-20%) when necessary was done using formula (2) of Abbott [73]. A total of five replicates per trial for each concentration were carried out. Statistical analysis of all mortality data of larvicidal activity were subjected to probit analysis [74]. The differences were considered as significant at $P \leq 0.05$ level.

Fig 1.

A: *Nerium oleander* plant
B: *Nerium oleander* pink flowers
C: *Nerium oleander* red flowers
D: *Nerium oleander* white flowers

Per cent larval mortality (1):

$$\frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Corrected percentage of control mortality (2):

$$\frac{1 - n \text{ in T after treatment}}{n \text{ in C after treatment}} \times 100$$

Where, n is the number of larvae, T: treated and C: control.

3. Results

The crude solvent extracts of *Nerium oleander* pink, red and white flowers exhibited larvicidal activity against vector mosquitoes after 24 hours of exposure. No larval mortality was observed in treated and untreated control. The crude pink flower extracts of *Nerium oleander* when tested on the larvae of vector mosquitoes showed that the chloroform extract exhibited the highest activity against *Aedes aegypti* followed by methanol with respective LC_{50} and LC_{90} values of 424.58 and 1123.69; 436.86 and 1319.12mg/L. In *Anopheles stephensi*, it was the acetone extract which showed the highest activity followed by methanol and the corresponding LC_{50} and LC_{90} values were 180.81 and 621.32; 201.44 and 663.39mg/L. Likewise, the acetone extract exhibited larvicidal activity against *Culex quinquefasciatus* but was followed by ethyl acetate. The respective LC_{50} and LC_{90} values were 196.98 and 509.78; 211.97 and 551.87mg/L (Table 1; Figure 2). In the case of red flower extracts, the acetone extract exhibited pronounced activity on the larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* and their LC_{50} and LC_{90} values were 121.79 and 476.68; 94.60 and 286.36; 101.21 and 303.37 followed by ethyl acetate with 150.02 and 437.25; 125.99 and 370.46; 147.77 and 526.49mg/L respectively (Table 2). One hundred per cent larval mortality was observed in *Aedes aegypti* and *Culex quinquefasciatus* by ethyl acetate and methanol extract and in *Anopheles stephensi* by ethyl acetate extract at 1000mg/L (Figure 2). Similarly, the same trend followed when the white flower extracts where tested against the larvae of vector mosquitoes and their respective LC_{50} and LC_{90} values were 155.20 and 546.82; 147.50 and 538.60; 128.72 and 448.49; 168.29 and 650.43; 164.46 and 437.95; 157.83 and 617.31mg/L (Table 3). One hundred per cent larval mortality was observed in *Culex quinquefasciatus* at 500mg/L and at 1000mg/L in *Aedes aegypti* and *Anopheles stephensi* by ethyl acetate extract (Figure 2). Overall assessment indicated that the acetone extract of red flowers exhibited the highest larvicidal activity against *Anopheles stephensi* followed by *Culex quinquefasciatus* and *Aedes aegypti*.

4. Discussion

Larval control is regarded as the best approach to diminish mosquito population at a very early stage as imprisonment to water bodies and very stumpy rate of scattering make the mosquito larvae most susceptible. Hence, mosquito control is primarily aimed at wrigglers and secondarily against matures when necessary. Development of resistance to insecticides by mosquitoes expresses the need of new agents from plant sources as they are chemical factories of nature. Literature displays many such studies on bioefficacy of plant extracts against mosquito larvae. Botanicals are proved to be efficient biopesticides not only as crude extract but as solvent extracts also [75]. Plant species belonging to the Apocynaceae family are reported to possess larvicidal property against vector mosquitoes (Table 4). Research on the use of phytochemicals against mosquitoes should consider such factors as mosquito species, the geographical origin of a plant, the plant parts and solvents used for extraction, and specificity of a phytocompound. Further, the larvicidal activity of crude solvent extracts depends upon the mixture of active compounds. In the present study, eight different crude solvent extracts of *Nerium oleander* flower (pink, red and white colour) were tested against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* larvae and the *Nerium oleander* red flower extracts exhibited the highest larvicidal activity followed by white flowers. The LC_{50} values for acetone extract of *Nerium oleander* red flowers were 94.60, 101.21 and 121.79mg/L against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* larvae. These results were comparable with the earlier reports. The geographical distribution of plants may possibly influence their toxicity. In an earlier study, Komalamisra *et al*. [62] reported the ethanolic leaf extracts of *Nerium oleander* to exhibit larvicidal activity against *Aedes aegypti* and LC_{50} value was 197.97mg/L. Recently, Raveen *et al*. [36] screened the hexane and aqueous flower extracts of *Nerium oleander* and found the hexane extract to exhibit larvicidal activity against *Culex quinquefasciatus* and LC_{50} value was 102.54ppm whereas the aqueous extract exhibited LC_{50} of 2758.87ppm after 24 hours of exposure. Contradictory to the study by Raveen *et al*. [36], El-Akhal *et al*. [68] reported that the aqueous extract obtained from the mixture of leaves, stems and roots of *Nerium oleander* showed LC_{50} of 57.57mg/mL and LC_{90} of 166.35mg/mL against the larvae of *Culex pipiens*. In another study, El-Sayed and El-Bassiony [69] evaluated the larvicidal activity of *Nerium oleander* leaf extracts and showed that the diethyl ether extract of *Nerium oleander* leaves exhibited LC_{50} value of 10500mg/L against the larvae of *Culex pipiens*.

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent and the choice of solvent is influenced by what is intended with the extract [90]. Of primary consideration is the type of solvent used since non-polar solvents will extract non-polar molecules and polar solvents will extract polar molecules. This is achieved by using solvents ranging from hexane the least polar with a polarity index (P) of 0.1 to chloroform (relatively mid polar; P=4.1) and water the most polar (P=10.2) [91]. Hartzell [92] tested acetone and aqueous extracts of certain plant products against the larvae of *Culex quinquefasciatus* and found acetone to be a better solvent. Noteworthy crude acetone plant extracts have been reported for larvicidal properties against vector mosquitoes (Table 5).

It is found that botanical derivatives possessing mosquito larvicidal properties, directly attack the nervous system and damage it, primarily affect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules of mosquito larvae [120]. Further, they also act as mitochondrial poison [121] and work by interacting with cuticle membrane of the larvae ultimately disarranging the membrane which is the most probable reason for larval death [122]. This could be due to the presence of alkaloids, flavonoids, steroids, tannins, terpenes and terpenoids and it is said that several

Table 1: Larvicidal activity of *Nerium oleander* pink flower extracts against vector mosquitoes

Solvents	LC_{50} (mg/L)	95% confidence limit		LC_{90} (mg/L)	95% confidence limit		Slope ±SE	Intercept ±SE	χ^2
		LL	UL		LL	UL			
Aedes aegypti									
Hexane	558.43	461.29	705.19	1909.63	1346.45	3289.16	2.4 ±0.2	-1.5 ±0.7	3.9*
Petroleum ether	542.34	446.69	686.86	1923.31	1346.55	3346.29	2.3 ±0.2	-1.3 ±0.7	6.8*
Dichloromethane	616.31	414.98	932.08	1445.79	950.87	3931.09	3.4 ±0.6	-4.6 ±1.7	12.3*
Chloroform	424.58	214.86	1123.69	1118.78	593.94	31745.22	3.0 ±0.7	-3.0 ±1.8	13.6*
Ethyl acetate	449.73	241.13	1098.43	1151.00	628.83	22325.30	3.1 ±0.7	3.3 ±1.8	12.4*
Acetone	553.09	278.46	2716.19	1482.45	737.81	505598.12	2.9 ±0.7	-3.2 ±2.0	13.8*
Methanol	436.86	191.66	2117.67	1319.12	614.12	593452.56	2.6 ±0.6	-2.0 ±1.7	15.9*
Aqueous	451.66	98.89	3645.00	1291.21	563.27	5375.00	2.8 ±0.8	-2.4 ±2.2	22.7*
Anopheles stephensi									
Hexane	338.86	282.55	411.53	1194.20	887.16	1841.07	2.3 ±0.2	-0.9 ±0.6	2.9*
Petroleum ether	370.07	308.20	451.96	1311.33	964.65	2059.48	2.3 ±0.2	-0.9 ±0.6	5.3*
Dichloromethane	350.25	153.91	1100.00	1091.27	524.26	79679.86	2.5 ±0.6	-1.6 ±1.5	15.1*
Chloroform	228.43	190.06	273.62	788.59	604.57	1150.20	2.3 ±0.2	-0.6 ±0.6	6.1*
Ethyl acetate	219.61	181.74	263.96	787.35	599.68	1161.72	2.3 ±0.2	-0.4 ±0.6	2.9*
Acetone	180.81	149.22	216.32	621.32	481.29	892.51	2.3 ±0.2	-0.3 ±0.6	3.3*
Methanol	201.44	167.87	239.95	663.39	516.36	944.02	2.4 ±0.2	-0.7 ±0.6	3.7*
Aqueous	363.84	194.23	787.55	982.37	537.95	11251.72	2.9 ±0.6	-2.6 ±1.6	12.1*
Culex quinquefasciatus									
Hexane	419.92	258.08	775.82	1101.68	642.48	6373.43	3.0 ±0.5	-3.0 ±1.4	8.9*
Petroleum ether	378.69	172.73	1133.41	1057.54	535.20	57868.23	2.8 ±0.6	-2.4 ±1.7	15.3*
Dichloromethane	285.31	165.90	508.89	836.90	478.96	4360.41	2.7 ±0.4	-1.7 ±1.2	9.1*
Chloroform	236.42	197.50	282.45	792.48	610.55	1146.08	2.4 ±0.2	-0.7 ±0.6	2.9*
Ethyl acetate	211.97	131.45	333.88	551.87	346.82	1789.41	3.0 ±0.5	-2.1 ±1.2	7.8*
Acetone	196.98	105.78	347.04	509.78	302.51	2715.79	3.1 ±0.6	-2.1 ±1.4	10.7*
Methanol	275.45	229.25	332.26	980.74	738.39	1475.10	2.3 ±0.2	-0.6 ±0.6	1.5*
Aqueous	383.45	199.55	878.88	1002.62	547.75	15061.80	3.0 ±0.6	-2.9 ±1.7	12.9*

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; UL-upper limit; *$P \leq 0.05$ level of statistical significance of (χ^2) chi-square values

Table 2: Larvicidal activity of *Nerium oleander* red flower extracts against vector mosquitoes

Solvents	LC_{50} (mg/L)	95% confidence limits		LC_{90} (mg/L)	95% confidence limits		Slope ±SE	Intercept ±SE	χ^2
		LL	UL		LL	UL			
Aedes aegypti									
Hexane	378.88	166.95	1240.05	1081.18	535.44	93185.72	2.8 ±0.6	-2.2 ±1.7	15.8*
Petroleum ether	311.78	157.20	699.63	981.79	505.21	14272.78	2.5 ±0.5	-1.4 ±1.3	11.9*
Dichloromethane	338.83	166.75	825.35	1012.73	518.97	20542.85	2.6 ±0.5	-1.8 ±1.5	13.1*
Chloroform	2696.57	1471.44	13140.35	15590.34	5002.02	36749.09	1.6 ±0.4	-0.7 ±1.1	0.4*
Ethyl acetate	150.02	79.73	242.58	437.25	264.54	1815.41	2.7 ±0.5	-1.0 ±1.1	8.1*
Acetone	121.79	95.12	149.19	476.68	364.82	705.17	2.1 ±0.2	0.4 ±0.6	2.2*
Methanol	958.48	726.77	1475.41	4137.2	2369.32	11337.72	2.0 ±0.3	-1.0 ±0.8	1.8*
Aqueous	1239.03	885.60	2230.48	5785.19	2948.15	21784.09	1.9 ±0.3	-0.9 ±0.8	0.8*
Anopheles stephensi									
Hexane	304.32	153.61	675.81	996.84	508.28	14242.34	2.4 ±0.5	-1.1 ±1.2	11.4*
Petroleum ether	305.64	263.17	355.93	769.09	623.10	1029.18	3.1 ±0.3	-2.9 ±0.8	7.2*
Dichloromethane	351.11	146.86	1236.44	1112.52	522.18	152497.53	2.5 ±0.6	-1.5 ±1.5	15.8*
Chloroform	2139.51	1335.78	8128.31	7806.30	3243.44	113812.01	2.2 ±0.5	-2.5 ±1.6	0.1*
Ethyl acetate	125.99	103.87	149.22	370.46	296.45	508.77	2.7 ±0.3	-0.7 ±0.7	3.4*
Acetone	94.60	74.78	113.84	286.36	228.63	398.76	2.6 ±0.3	-0.2 ±0.7	0.6*
Methanol	928.86	742.49	1303.51	2928.71	1893.06	6444.41	2.5 ±0.4	-2.6 ±1.0	1.0*
Aqueous	1094.67	796.47	1856.19	5346.55	2799.87	18134.64	1.8 ±0.3	-0.6 ±0.8	1.2*
Culex quinquefasciatus									
Hexane	352.97	160.51	1013.62	1038.54	517.57	47471.72	2.7 ±0.6	-1.9 ±1.6	14.9*
Petroleum ether	358.43	156.09	1147.60	1067.05	520.35	85462.96	2.7 ±0.6	-1.9 ±1.6	15.6*
Dichloromethane	337.71	196.81	625.51	911.07	525.06	5361.26	2.9 ±0.5	-2.5 ±1.4	9.9*
Chloroform	2678.85	1497.28	19414.59	10770.44	3737.33	495886.84	2.1 ±0.6	-2.2 ±1.7	0.2*
Ethyl acetate	147.77	119.75	177.94	526.49	407.51	760.09	2.3 ±0.2	-0.1 ±0.6	2.6*
Acetone	101.21	81.06	121.17	303.37	242.43	420.71	2.6 ±0.3	-1.3 ±0.7	0.9*
Methanol	886.99	725.89	1189.56	2500.03	1702.27	4976.09	2.8 ±0.4	-3.3 ±1.2	2.3*
Aqueous	855.98	670.31	1226.55	3313.36	2040.25	7718.23	2.1 ±0.3	-1.3 ±0.8	3.4*

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; UL-upper limit; *$P \leq 0.05$ level of statistical significance of (χ^2) chi-square values

Table 3: **Larvicidal activity of *Nerium oleander* white flower extracts against vector mosquitoes**

Solvents	LC_{50} (mg/L)	95% confidence limit		LC_{90} (mg/L)	95% confidence limit		Slope ±SE	Intercept ±SE	χ^2
		LL	UL		LL	UL			
Aedes aegypti									
Hexane	538.41	312.56	1574.73	1867.99	876.26	49431.80	2.3 ±0.4	-1.4 ±1.2	8.8*
Petroleum ether	694.99	551.32	953.60	2885.95	1824.11	6186.27	2.0 ±0.2	-0.8 ±0.7	1.9*
Dichloromethane	173.66	87.83	312.55	435.18	258.02	2767.99	3.2 ±0.6	-2.1 ±1.5	11.6*
Chloroform	424.35	354.18	519.91	1440.24	1057.24	2279.0	2.4 ±0.2	-1.3 ±0.7	3.7*
Ethyl acetate	168.29	136.10	203.90	650.43	493.35	970.34	2.1 ±0.2	0.1 ±0.5	4.0*
Acetone	155.20	126.46	186.48	546.82	423.53	787.71	2.3 ±0.2	-0.1 ±0.6	2.4*
Methanol	358.75	293.60	447.82	1509.77	1058.74	2582.59	2.0 ±0.2	-0.2 ±0.6	0.4*
Aqueous	2145.13	1226.91	7351.21	16737.02	5485.36	235409.39	1.4 ±0.3	0.2 ±0.8	0.4*
Anopheles stephensi									
Hexane	414.31	176.88	1754.67	1191.75	573.25	346503.15	2.7 ±0.7	-2.3 ±1.8	16.5*
Petroleum ether	401.90	240.13	823.24	1370.45	711.26	11636.46	2.4 ±0.4	-1.2 ±1.1	8.0*
Dichloromethane	179.09	152.46	209.05	476.20	385.90	636.67	3.0 ±0.3	-1.7 ±0.7	7.1*
Chloroform	310.25	179.11	570.08	872.40	498.35	5023.53	2.8 ±0.5	-2.1 ±1.3	9.7*
Ethyl acetate	164.46	139.64	192.08	437.95	355.03	586.05	3.0 ±0.3	-1.6 ±0.7	7.4*
Acetone	147.50	119.06	178.09	538.60	415.04	783.55	2.2 ±0.2	0.1 ±0.6	2.3*
Methanol	244.13	207.05	287.69	711.16	563.72	981.72	2.7 ±0.2	-1.5 ±0.6	7.3*
Aqueous	757.79	580.57	1115.10	3796.40	2190.91	9825.36	1.8 ±0.2	-0.2 ±0.6	1.9*
Culex quinquefasciatus									
Hexane	407.98	183.80	1431.23	1162.54	572.49	128483.29	2.8 ±0.7	-2.3 ±1.8	15.5*
Petroleum ether	513.09	424.98	642.03	1771.05	1261.59	2978.75	2.3 ±0.2	-1.4 ±0.7	6.4*
Dichloromethane	196.98	105.78	347.04	509.78	302.51	2715.79	3.1 ±0.6	-2.1 ±1.4	10.7*
Chloroform	404.65	332.78	505.22	1595.14	1125.25	2703.80	2.1 ±0.2	-0.6 ±0.6	0.4*
Ethyl acetate	157.83	126.80	191.67	617.31	468.32	921.79	2.1 ±0.2	0.2 ±0.5	3.6*
Acetone	128.72	103.33	155.26	448.89	349.87	642.54	2.3 ±0.2	0.1 ±0.6	1.9*
Methanol	254.76	217.32	298.75	702.77	562.37	956.09	2.9 ±0.2	-1.9 ±0.7	7.0*
Aqueous	1843.97	1152.31	4925.11	10674.62	4231.55	86931.46	1.6 ±0.3	-0.4 ±0.9	5.5*

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; UL-upper limit; *$P \leq 0.05$ level of statistical significance of (χ^2) chi-square values

Table 4: Plant species of Apocyanaceae family reported for mosquito larvicidal activity

Plant species	Parts	Mosquito species	Work cited
Apocynum androsaemifolium	Roots, stem, leaves, flowers	*Aedes aegypti*	Patterson *et al*. [76]
Catharanthus roseus	Leaves	*Aedes aegypti*	Remia and Logaswamy [77]
Ervatamia coronaria	Whole plants	*Aedes aegypti* *Anopheles stephensi* *Culex quinquefasciatus*	Qureshi *et al*. [78]; Mathivanan *et al*. [79]; Govindarajan *et al*. [80]
Malovetia obtusiloba	Bark, branchlets, leaves	*Aedes* & *Anopheles* species	Jacobson [81]
Nerium indicum	Roots, leaves	*Anopheles stephensi* *Culex quinquefasciatus*	Chavan and Nikan [82]; Evans and Kaleysaraj [83]; Srivastava *et al*. [84]; Sharma *et al*. [85]; Rahuman *et al*. [86]; Tandon and Sirohi [87]
Nerium oleander	Stem, leaves, flowers	*Aedes aegypti* *Aedes fluviatilis* *Anopheles stephensi* *Culex gelidus* *Culex pipiens* *Culex quinquefasciatus* *Culex tritaeniorhynchus*	Consoli *et al*. [61]; Pushpalatha and Muthukrishnan [63]; Komalamisra *et al*. [62]; Lokesh *et al*. [64]; Kumar *et al*. [66]; Madhuri *et al*. [65]; Raveen *et al*. [36]; El-Akhal *et al*. [68]; Fakoorziba *et al*. [70]; Karlekar and Andrew [67]; El-Sayed and El-Bassiony [69]
Rauvolfia canescens	Whole plants	*Aedes aegypti* *Anopheles stephensi* *Culex quinquefasciatus*	Kalyanasundaram and Das [88]
Rhazya stricta	Seeds	*Culex pipiens*	Hag *et al*. [89]
Thevetia nerifolia	Cotyledons	*Culex quinquefasciatus*	Evans and Kaleysaraj [83]
Vinca rosea	Whole plants	*Aedes aegypti* *Anopheles stephensi* *Culex quinquefasciatus*	Kalyanasundaram and Das [88]

HE: Hexane; PE: Petroleum ether; DM: Dichloromethane; CH: Chloroform; EA: Ethyl acetate; AC: Acetone; ME: Methanol; AQ: Aqueous

Fig 2: Per cent larval mortality of vector mosquitoes on treatment to *Nerium oleander* crude solvent (A): pink; (B): red; and (C) white flower extracts. LC_{50} values of *Nerium oleander* crude solvent (D): pink; (E): red; and (F): white flower extracts against the larvae of vector mosquitoes

Table 5: List of acetonic plant extracts reported for mosquito larvicidal activity

Plant species	Part	LC_{50}	Mosquito species	Work cited
Melia azedarach	Seed	30-40mg/L	*Culex pipiens*	Al-Sharook *et al*. [93]
Melia volkensii	Seed	20-30mg/L	*Culex pipiens*	Al-Sharook *et al*. [93]
Caulerpa scalpelliformis	Whole	53.7mg/L	*Aedes aegypti*	Thangam and Kathiresan [94]
Dictyota dichotoma	Whole	61.7mg/L	*Aedes aegypti*	Thangam and Kathiresan [94]
Feronia limonia	Leaf	57.23ppm	*Aedes aegypti*	Rahuman *et al*. [95]
Feronia limonia	Leaf	79.58ppm	*Anopheles stephensi*	Rahuman *et al*. [95]
Feronia limonia	Leaf	129.24ppm	*Culex quinquefasciatus*	Rahuman *et al*. [95]
Murraya koenigii	Leaf	24.21ppm	*Aedes aegypti*	Harve and Kamath [96]
Ferula asafoetida	Whole plant	65.02ppm	*Aedes aegypti*	Harve and Kamath [96]
Coriandrum sativum	Seed	273.23ppm	*Aedes aegypti*	Harve and Kamath [96]
Trigonella foenum graceum	Seed	92.84ppm	*Aedes aegypti*	Harve and Kamath [96]
Nerium indicum	Leaf	185.99 ppm	*Anopheles stephensi*	Sharma *et al*. [85]
Ageratina adenophora	Twigs	356.70ppm	*Aedes aegypti*	Rajmohan and Ramaswamy [97]
Ageratina adenophora	Twigs	227.20ppm	*Culex quinquefasciatus*	Rajmohan and Ramaswamy [97]
Ocimum sanctum	Leaf	425.94ppm	*Aedes aegypti*	Anees [98]
Ocimum sanctum	Leaf	592.60ppm	*Culex quinquefasciatus*	Anees [98]
Millingtonia hortensis	Leaf	104.70ppm	*Aedes aegypti*	Kaushik and Saini [99]
Millingtonia hortensis	Leaf	138.00ppm	*Anopheles stephensi*	Kaushik and Saini [99]
Millingtonia hortensis	Leaf	83.18ppm	*Culex quinquefasciatus*	Kaushik and Saini [99]
Piper cubeba	Bud	1.83 mg/mL	*Aedes aegypti*	Murthy and Rani [100]
Capparis spinosa	Bud	1.77 mg/mL	*Aedes aegypti*	Murthy and Rani [100]
Syzgium cumini	Bud	0.86mg/mL	*Aedes aegypti*	Murthy and Rani [100]
Millingtonia hortensis	Flower	1.61 mg/mL	*Aedes aegypti*	Murthy and Rani [100]
Nerium indicum	Flower	2.00mg/mL	*Aedes aegypti*	Murthy and Rani [100]
Delonix regia	Flower	1.07 mg/mL	*Aedes aegypti*	Murthy and Rani [100]
Limonia acidissima	Leaf	1.24 mg/mL	*Aedes aegypti*	Murthy and Rani [100]
Jatropha curcas	Leaf	0.85 mg/mL	*Aedes aegypti*	Murthy and Rani [100]
Tridax procumbens	Leaf	39.98mg/L	*Anopheles subpictus*	Kamaraj *et al*. [101]
Ulva fasciata	Seaweed	504.47mg/L	*Culex quinquefasciatus*	Poonguzhali and Nisha [102]
Grateloupia lithophila	Seaweed	349.74mg/L	*Culex quinquefasciatus*	Poonguzhali and Nisha [102]
Biophytum sensitivum	Leaf	21.79 µg/mL	*Aedes aegypti*	Shivakumar *et al*. [103]
Elaeagnus indica	Leaf	90.8ppm	*Aedes aegypti*	Shivakumar *et al*. [104]
Maesa indica	Leaf	173.2ppm	*Aedes aegypti*	Shivakumar *et al*. [104]
Vernonia cinerea	Leaf	81.30mg/L	*Anopheles stephensi*	Varun *et al*. [105]
Prosopis juliflora	Leaf	37.55mg/L	*Anopheles stephensi*	Varun *et al*. [105]
Cassia tora	Leaf	140.98mg/L	*Anopheles stephensi*	Varun *et al*. [105]
Ipomoea carnea	Stem	119.61ppm	*Aedes aegypti*	Khatiwora *et al*. [106]
Ipomoea carnea	Stem	166.76ppm	*Culex quinquefasciatus*	Khatiwora *et al*. [106]
Callistemon citrinus	Leaf	327.95mg/L	*Culex quinquefasciatus*	Ali and Aneesh [107]
Elaeagnus indica	Leaf	2.97mg/mL	*Aedes aegypti*	Srinivasan *et al*. [108]
Elaeagnus indica	Leaf	3.92mg/mL	*Anopheles stephensi*	Srinivasan *et al*. [108]
Coriandrum sativum	Leaf	14.99mg/L	*Culex quinquefasciatus*	Thangaraj *et al*. [109]
Brassica nigra	Leaf	90.01mg/L	*Culex quinquefasciatus*	Thangaraj *et al*. [109]
Terminalia catappa	Leaf	177.80ppm	*Aedes aegypti*	Unnikrishnan [110]
Alstonia scholaris	Leaf	459.81ppm	*Aedes albopictus*	Yadav *et al*. [111]
Callistemon viminalis	Leaf	110.35ppm	*Aedes albopictus*	Yadav *et al*. [111]
Hyptis suaveolens	Leaf	258.39ppm	*Aedes albopictus*	Yadav *et al*. [111]
Malvastrum coromandelianum	Leaf	371.45ppm	*Aedes albopictus*	Yadav *et al*. [111]
Prosopis juliflora	Leaf	130.74ppm	*Aedes albopictus*	Yadav *et al*. [111]
Vernonia cinerea	Leaf	64.57ppm	*Aedes albopictus*	Yadav *et al*. [111]
Lantana camara	Whole plant	60.64pppm	*Aedes aegypti*	Hemalatha *et al*. [112]
Lantana camara	Whole plant	80.86ppm	*Anopheles stephensi*	Hemalatha *et al*. [112]
Lantana camara	Whole plant	64.24ppm	*Culex quinquefasciatus*	Hemalatha *et al*. [112]
Ocimum gratissimum	Leaf	3.05mg/L	*Culex quinquefasciatus*	Pratheeba *et al*. [113]
Sphagneticola trilobata	Stem with leaf	189.24ppm	*Culex pipiens*	Pushpalatha *et al*. [114]
Acalypha fruticosa	Leaf	461.23ppm	*Culex pipiens*	Pushpalatha *et al*. [114]
Quassia indica	Seed	395.85ppm	*Culex pipiens*	Pushpalatha *et al*. [114]
Anamirta cocculus	Fruit	29.52ppm	*Culex pipiens*	Pushpalatha *et al*. [114]
Piper longum	Fruit	611.96mg/L	*Aedes aegypti*	Rajan *et al*. [115]
Hyptis suaveolens	Aerial	485.61mg/L	*Culex quinquefasciatus*	Sakthivadivel *et al*. [116]
Turbinaria conoides	Seaweed	100.07mg/L	*Aedes aegypti*	Valentina *et al*. [117]
Turbinaria conoides	Seaweed	76.35mg/L	*Anopheles stephensi*	Valentina *et al*. [117]
Turbinaria conoides	Seaweed	62.12mg/L	*Culex quinquefasciatus*	Valentina *et al*. [117]

Glycosmis pentaphylla	Leaf	0.0585mg/mL	*Aedes aegypti*	Ramkumar *et al*. [118]
		0.480mg/mL	*Anopheles stephensi*	
		0.00045mg/mL	*Culex quinquefasciatus*	
Annona reticulata	Leaf	150.61ppm	*Aedes aegypti*	Mohankumar and Vijayan [119]
		157.87ppm	*Anopheles stephensi*	
		70.30 ppm	*Culex quinquefasciatus*	
Psoralea corylifolia	Seed	114.13ppm	*Aedes aegypti*	
		108.16ppm	*Anopheles stephensi*	
		110.02ppm	*Culex quinquefasciatus*	

groups of the above mentioned phytochemicals from different plants have been reported for their insecticidal activities particularly on mosquito larvae [18]. This principle would have been responsible for causing the larval death in the present study also because El-Akhal *et al*. [68] reported that the larvicidal activity of *Nerium oleander* on *Culex pipiens* could be due to the major components which include triterpenes, coumarins and flavonoids and further, Roni *et al*. [51] reported the presence of these phytocompounds in *Nerium oleander*. Studies publicized that the triterpenes isolated from *Dysoxylum malabaricum* and *Dysoxylum beddomei* exhibited 95% larval mortality in *Anopheles stephensi* [123]; from *Azadirachta indica* leaves, significant larval mortality was observed in the fourth instar larvae of *Anopheles stephensi* and LC_{50} value was 60ppm [124]; from stem of *Duranta repens* which displayed strong activity against first to fourth instars larvae of *Culex quinquefasciatus* with LC_{50} value of 7.75, 16.11, 28.63 and 26.53ppm, respectively after 24 hours [125]; from *Gymnema sylvestre* which showed toxicity against the fourth instar larvae of *Anopheles subpitcus* and *Culex quinquefasciatus* with LC_{50} values of 22.99 and 15.92ppm, respectively [126]. Coumarins isolated from *Cnidiummon nieri* fruit showed activity against *Culex pipiens pallens* and *Aedes aegypti* larvae [127]. Deshmukh *et al*. [128] documented LC_{50} values of 1.49 and 2.23ppm against the fourth instar larvae of *Culex quinquefasciatus* and *Aedes aegypti*, respectively, and 100% larval mortality at a concentration of 25ppm against *Aedes aegypti*, and at 10ppm complete lysis of *Culex quinquefasciatus* larvae. The flavonoids isolated from *Poncirus trifoliata* showed larvicidal activity against *Aedes aegypti* and LC_{50} values ranged from 0.082 to 0.122mg/L after 24 hours [129].

In conclusion, the present study revealed that the crude acetone extract of *Nerium oleander* red flowers displayed mosquito larvicidal activity against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* which further encourage investigation on its bioactive compounds that might own virtuous larvicidal properties when isolated in pure form. Identifying plant based insecticides that are efficient as well as suitable and adaptive to local ecological conditions, biodegradable and have the wide spread mosquito larvicidal chattels will work as a new weapon in the arsenal of insecticides and in the future may add to a suitable alternative tool to fight against mosquito-borne diseases.

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