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RESEARCH ARTICLE

LARVICIDAL, OVICIDAL, ADULTICIDAL AND REPELLENT ACTIVITY OF JUSTICIA ADHATODA LINN (ACANTHACEAE) AGAINST AEDES AEGYPTI LINN AND CULEX QUINQUEFASCIATUS SAY

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ABSTRACT

The Present study was undertaken to evaluate the effect of extraction from Justicia adhatoda leaves on four different solvents activity against the Aedes aegypti and Culex quinquefasciatus. Twenty five Ist to IVth instar larvae of Cx. quinquefasciatus and Ae. aegypti were exposed to various concentrations (60 - 300 ppm) and were assayed in the laboratory for 24 and 48 hours. Petroleum ether and all other solvent extracts such as chloroform, ethyl acetate and methanol leaf extracts of J. adhatoda showed significant results. Cx. quinquefasciatus larvae showed higher mortality than Ae. aegypti larvae among the solvents tested in the present study. The LC50 values of 75.39, 78.19, 84.50, 91.37 ppm were recorded against the Ist to IVth instar larvae of Cx. quinquefasciatus exposed to methanol extracts of J. adhatoda. In the same way LC50 values of 75.36, 79.62, 85.44, 96.06 ppm were recorded against the 1st to 4th instar larvae of Ae. aegypti were exposed to petroleum ether extracts of J. adhatoda respectively. The ovicidal activity was found to be more effective against the eggs of Cx. quinquefasciatus than Ae. aegypti. The adulticidal mortality was highly significant in petroleum ether extract of J. adhatoda leaf showed LC50 values of 73.50, 72.14 ppm and LC90 Values of 142.02, 225.07 in Cx.quinquefasciatus and Ae. aegypti. The petroleum ether extract had strong repellent action against mosquitoes as it provided 100% protection against Ae. aegypti for 210 min followed by Cx. quinquefasciatus 180 min. From the results it can be concluded the leaf extract of J. Adhatoda was an excellent potential for controlling Ae. aegypti and Cx. quinquefasciatus mosquitoes.

INTRODUCTION

Mosquitoes transmit serious human health diseases, causing millions of deaths every year. Lymphatic filariasis is endemic in 81 countries in tropical and subtropical regions of Asia, Africa, central and South America and Pacific Island nations, with more than 120 million people infected and 1.34 billion people at risk of infection. An estimated 25 million have genital disease and 15 million have lymph edema or elephantiasis caused by Wucheraria bancrofti or Brugia malayi. Lymphatic filariasis is caused mainly by W. bancrofti and transmitted by Culex quinquefasciatus (WHO, 2010).

Dengue fever is a most important re-emerging arboviral disease, causing an estimated 390 million infections every year worldwide (WHO, 2013). It is estimated that 34% of the global cases are from India (Bhatt S *et al*, 2013) and the country is known to be endemic, with all four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) circulating throughout the year in different parts (Gupta N *et al*, 2012). Aedes aegypti is regarded as the principal vector for this virus in India (WHO, 2009).

Vector control is an essential requirement in control of epidemic diseases such as malaria, filariasis, dengue, etc. that are transmitted by mosquitoes. Excessive use of synthetic pesticides causes emergence of pesticide resistance and harmful effect on non-target organisms (Sukumar K *et al*, 1991). Botanicals can be used as alternative to synthetic insecticides or along with other insecticides under integrated vector control programs. Plants may be a source of alternative agents for control of mosquitoes because they are rich in bioactive chemicals, are active against a limited number of species including specific target insects, and are biodegradable. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide (Elumalai K *et al*, 2012).

Acanthaceae, one of the 24 families in the mint order (Limiales) of flowering plants, containing approximately 220 genera and nearly 4,000 species distributed predominantly in tropical and subtropical regions of the world. The greater part of the Acanthaceae family are herbs or shrubs, but vines and trees occur as well. *Justicia adhatoda* is part of the Acanthaceae plant family. it is commonly known as adhatoda, pavettai or vasaka. It is otherwise called Adhatoda Vasica. It is a small evergreen, sub-herbaceous bush which grows commonly in open plains, especially in the lower Himalayas (up to 1300 meters above sea level), India, Sri Lanka, Burma and Malaysia. Adhatoda leaves have been used extensively in Ayurvedic Medicine for over

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2000 years primarily for respiratory disorders (Jain and Atal, 2014)

Plant constituents of *J. adhatoda* leaves contains: Quinazoline alkaloids, vasicine - 45-95% (the mucolytic drug bromhexine was developed from this alkaloid), N-oxides f, vasicine, vasicinone, deoxyvasicine, oxyvasicinine, maiontone, essential oil. The leaf extract, is considered safe and the oil has low toxicity .it is also used in the treatment of malaria, dysentery and diarrhea and has many other medicinal applications (Chakraborty and Brantner, 2001), it shows potent anti-inflammatory activity (Mulla and More, 2010) and *Adhatoda vasica* was traditionally used by midwives at the time of delivery because of its uterotonic activity. Due to its anti-implantation activity, *J. adhatoda* should not be used while pregnant (Gupta AP *et al*, 1978) *Adhatoda vasica* Linn.

It also has anti-inflammatory, analgesic, diarrhea, dysentery, antioxidant, hepatoprotective, Sedative, antispasmodic, antihelmintic properties Antimicrobial activity (Sheeba and Mohan, 2012), Anti-diabetic activity (Bhatt M *et al*, 2011), Wound healing effect (Vinothapoosan and Sundar, 2010), Infertility (Ganguli and Paramesh, 2010), Antibacterial (Kavitha G *et al, 2012*). The plant is recommended for first-aid medicine in primary health care and can be used in both adults and children and for a long period without any restriction of use.

Current research trends use plant extracts as alternative larvicide because they contain various phytochemical that are specific in killing mosquito larvae without harming other organisms and the environment. Botanical derivatives have drawn attention as potential insect control agents targeting only larval stages in the mosquito control programme in the last three decades (Anjali Rawani *et al*, 2010) Herbal insecticides of plant origin become a priority in this search. The present study was carried out to assess the role of larvicidal, ovicidal, adulticidal and repellent activities of the leaf extracts of Acanthaceae family plant against *Cx.quinquefasciatus* and *Ae.aegypti* in laboratory conditions.

MATERIALS AND METHODS

Plant Collection and authentication

Leaves of *Justicia Adhatoda* (Linn.) of Acanthaceae family were collected from the local areas of Vellore, Tamilnadu, India authenticated by professor P. Jayaraman, Botanist, Director, Plant anatomy research centre, Tambaram, Chennai, India in the month of May 2014 and registered Number of the Specimen is PARC/2014/2074.

Preparation of plant extracts

The leaves were washed with tap water, shade dried at room temperature (28 ± 2 ºC) for 5-8 days. The air dried materials were powdered separately using electrical blender. The finely ground plant material (3.0 kg/solvent) was loaded in Soxhlet apparatus and was extracted with four different solvents namely petroleum ether, chloroform, ethyl acetate and methanol individually (Vogel *et al*, 1978). The solvent from the extract was removed using a rotary vacuum evaporator to collect the crude extract. The crude residue of this plant varies with the solvents used. The *J. adhatoda* with four different solvents yielded 93.10, 99.26, 130.29 and 110.75 grams of crude residue. Standard stock solutions were prepared at 1% by dissolving the residues the universal solvents DMSO (dimethyl sulphoxide). From this stock solution, different concentrations (60-300 ppm) were prepared and these solutions were used for larvicidal, ovicidal, Adulticidal activity. In repellent activity the various range of stock solutions (1.0, 2.0, & 3.0 mg/cm2) were prepared by dissolving the residues in ethanol.

Culture of test organism

The Eggs of *Cx.quinquefasciatus* and *Ae.aegypti* were collected from zonal entomological team, Vellore, Tamil Nadu, India, using an "O"- type brush. These eggs were brought to the laboratory and transferred to (18×13×4 cm) enamel trays containing 500 ml of water for hatching. The mosquito larvae were fed with pedigree dog biscuits and yeast at 3:1 ratio. The feeding was continued until the larvae transformed into the pupal stage. The pupae were collected from the culture trays and transferred to plastic containers (12×12 cm) containing 500ml of water with the help of a dipper.

The plastic jars were kept in a (60×60×60 cm) mosquito cage for adult emergence. Mosquito larvae were maintained at 27 ± 2 °C, 75 – 85% relative humidity, under a photoperiod of 14:10 hrs light: dark. A 10% sugar solution was provided for a period of 3 days before blood feeding. The adult female mosquitoes were allowed to feed on the blood of a rabbit (a rabbit per day, exposed on the dorsal side) for 2 days to ensure adequate blood feeding for 5 days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

Larvicidal Bio-assay

Laboratory colonies of *Cx. quinquefasciatus* and *Ae. aegypti* Larvae were used for the larvicidal activity. Twenty five numbers of I^{st} to IV^{th} instars larvae were introduced into 500 ml glass beaker containing 249 ml of de-chlorinated water and 1 ml of desired concentrations of plant extract were added. Each tested concentration was five replicated. The control experiments were also run parallel with each replicate (WHO, 1996). The larval mortality was calculated after 24 hrs and 48 hrs of the exposure period. The control mortalities were corrected by using Abbott's formula (Abbott, 1925). The LC_{50} and LC_{90} were calculated from toxicity data by using Probit analysis (Finney, 1971).

Ovicidal Activity

The method of Su & Mulla, (1998) was followed to test the ovicidal activity. The leaf extract was diluted in the respective solvent to achieve different concentrations. The freshly laid egg raft containing 100 eggs. Ahead of treatment eggs of the *Ae. Aegypti and Cx. quinquefasciatus* was counted individually with the help of hand lens. Freshly hatched eggs (100) were exposed to each concentration of leaf extract until they hatched or died. Each concentration was replicated six times. Eggs exposed to respective solvents in water served as control. The hatch rate was assessed 48 hours post treatment by the following formula.

$$\% \text{ of egg mortality} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs in egg raft}} * 100$$

Repellent activity

The repellent study was followed by the method of WHO (2009). Three day old blood starved female *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes (100) were kept in a net cage (45 X 30 X 45 cm^2). The volunteer had no contact with lotions,

perfumes, oils or perfumed soaps on the day of the assay. The arm of volunteer, only 25 cm^2 dorsal side of the skin on each arms were exposed and the remaining area covered with rubber gloves. The Leaf extracts were applied at 1.0, 2.0 and 3.0 mg/cm2 separately in the exposed area of the forearm. Ethanol was served as the control. *Cx. quinquefasciatus* was tested during the night from 19.00 to 05.00 hours. *Ae. aegypti* was tested during the day time from 7.00 to 17.00 hours. The control and treated arm were introduced simultaneously into the mosquito cage, and gently tapping the sides on the experimental cages, the mosquitoes were activated. The volunteer conducted their test of each concentration by inserting the treated and control arm into the cages at a same time for one full minute for every 5 minutes. Until a confirmed bite was received the test was over after the conformation of mosquito bite in extract to be tested. The mosquito repellency of different extract was measured on the basis of the protection time (min) the time was introduced simultaneously into the cage. The numbers of bites were counted over 5 minutes for every 30 minutes. The experiment was conducted six times.

It was observed that there was no skin irritation from the plant extract. The percentage protection was calculated by using the following formula.

$$\% \text{ Repellency} = \frac{(Ta - Tb)}{Ta} * 100$$

Where Ta – Number of mosquitoes in the control group
Tb – Number of mosquitoes in the treated group.

Adulticidal activity

Six day old sugar fed adult female mosquitoes was used. *J. adhatoda* leaf extracts were diluted with ethanol to make different concentrations. The diluted plants extracts were impregnated on filter papers (140*120 mm). A blank paper consisting of only ethanol was used as control. The papers were left to dry at room temperature to evaporate off the ethanol overnight. Impregnated papers were prepared fresh prior to testing. The bioassay was conducted in an experimental kit consisting of two cylindrical plastic tubes both measuring 125*44 mm following the WHO method (WHO, 1981). One tube served to expose the mosquitoes to the plants extracts and another tube was used to hold the mosquitoes before and after the exposure periods. The impregnated papers were rolled and placed in the exposure tube. Each tube was closed at one end with a 16 mesh size wire screen. Sucrose-fed and blood starved mosquitoes (25) were released into the tube, and the mortality effects of the extracts were observed every 10 minutes for 3 hours exposure period. At the end of 1, 2, and 3 hrs exposure periods, the mosquitoes were placed in the holding tube. Cotton pads soaked in 10% sugar solution with vitamin-B complex were placed in the tube during the holding period of 24 hours and Mortality rate was recorded. The above procedure was carried out in triplicate for each concentration. Adulticidal activity was calculated by counting dead mosquito from the introduced mosquito. Any mosquito was considered to be dead if did not move when prodded repeatedly with a soft brush.

Statistical analysis

The average adult mortality data were subjected to Probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were calculated using the SPSS 20.0 version software. Results with P≤0.05 were considered to be statistically significant.

Table 1 Lethal Concentration values of J. Adhatoda leaf extract against Cx. Quinquefasciatus

Solvents	Instars	Period	LC_{50}, ppm (LFL - UFL)	LC_{90}, ppm (LFL - UFL)	X^2	Regression Equation
Petroleum Ether	1st instar	24	132.93 (102.74 - 171.99)	393.28 (148.14 - 1044.11)	1.19	Y = 2.72X - 0.78
		48	75.32 (39.45 - 143.79)	247.74 (125.39 - 489.47)	2.81	Y = 2.48X + 0.35
	2nd instar	24	139.38 (109.78 - 176.97)	403.33 (149.85 - 1085.59)	1.39	Y = 2.78X - 0.95
		48	81.87 (45.41 - 147.58)	289.10 (132.93 - 628.71)	2.77	Y = 2.34X + 0.53
	3rd instar	24	144.96 (115.99 - 181.17)	407.53 (149.74 - 1109.11)	1.47	Y = 2.85X - 1.17
		48	86.81 (50.04 - 150.61)	337.27 (137.884 - 824.96)	2.23	Y = 2.17X + 0.79
	4th instar	24	151.05 (122.90 - 185.65)	409.60 (148.55 - 1129.38)	1.44	Y = 2.96X - 1.45
		48	98.66 (61.16 - 159.15)	390.80 (150.45 - 1015.16)	1.54	Y = 2.14X + 0.73
Chloroform	1st instar	24	136.70 (106.37 - 175.68)	396.39 (149.88 - 1048.35)	1.45	Y = 2.77X - 0.92
		48	76.49 (40.39 - 144.86)	265.93 (127.73 - 553.66)	2.75	Y = 2.37X + 0.54
	2nd instar	24	142.99 (113.48 - 180.18)	403.63 (149.78 - 1087.76)	1.49	Y = 2.84X - 1.13
		48	82.42 (45.47 - 149.39)	312.22 (137.04 - 711.29)	2.35	Y = 2.22X + 0.75
	3rd instar	24	149.56 (121.88 - 183.53)	409.71 (150.03 - 1118.87)	1.51	Y = 2.93X - 1.37
		48	91.87 (54.69 - 154.34)	370.77 (142.77 - 962.87)	1.71	Y = 2.12X + 0.85
	4th instar	24	156.34 (128.07 - 190.85)	416.28 (150.15 - 1154.06)	1.51	Y = 3.01X - 1.61
		48	101.62 (65.03 - 158.79)	391.96 (147.63 - 1040.70)	1.20	Y = 2.19X + 0.61
Ethyl Acetate	1st instar	24	144.83 (116.39 - 180.21)	395.00 (150.09 - 1039.58)	4.42	Y = 2.94X - 1.36
		48	113.14 (78.66 - 162.74)	372.17 (147.35 - 939.98)	1.19	Y = 2.48X - 0.09
	2nd instar	24	148.81 (120.25 - 184.16)	402.29 (148.66 - 1088.67)	4.80	Y = 2.97X - 1.45
		48	117.64 (84.38 - 164.00)	383.82 (147.53 - 998.57)	1.23	Y = 2.50X - 0.17
	3rd instar	24	152.38 (124.85 - 185.97)	405.49 (148.75 - 1105.37)	5.02	Y = 3.02X - 1.58
		48	122.28 (89.17 - 167.68)	394.90 (148.49 - 1050.18)	1.28	Y = 2.52X - 0.25
	4th instar	24	156.04 (129.34 - 188.24)	407.82 (148.78 - 1117.85)	5.25	Y = 3.07X - 1.74
		48	127.06 (95.01 - 169.93)	405.31 (151.09 - 1087.26)	1.26	Y = 2.54X - 0.35
Methanol	1st instar	24	126.21 (93.71 - 169.96)	385.39 (147.96 - 1003.80)	0.98	Y = 2.64X - 0.55
		48	75.39 (40.81 - 139.28)	207.12 (112.87 - 380.09)	4.66	Y = 2.92X - 0.48
	2nd instar	24	134.41 (103.23 - 175.02)	397.45 (149.71 - 1055.15)	1.11	Y = 2.72X - 0.79
		48	78.19 (42.03 - 145.44)	259.67 (128.09 - 526.41)	2.82	Y = 2.46X + 0.35
	3rd instar	24	140.92 (111.57 - 177.99)	407.17 (150.89 - 1098.73)	1.40	Y = 2.78X - 0.98
		48	84.50 (47.62 - 149.94)	323.86 (137.68 - 761.79)	2.73	Y = 2.20X + 0.77
	4th instar	24	146.54 (117.95 - 182.07)	410.98 (149.71 - 1128.22)	1.48	Y = 2.86X - 1.19
		48	91.37 (54.116 - 154.26)	369.880 (142.73 - 958.54)	1.86	Y = 2.11X + 0.86

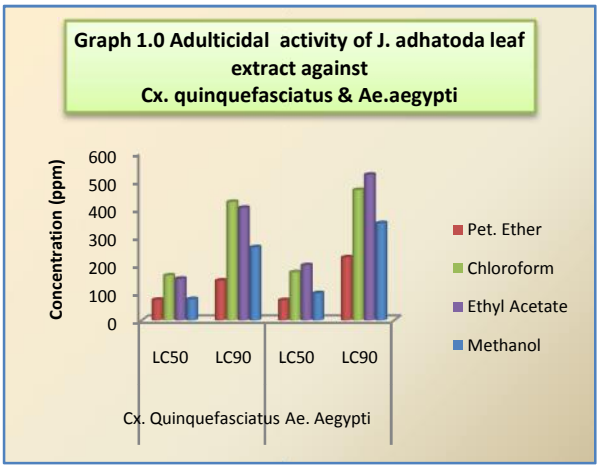
Control nil mortality significant at P≤0.05 level, *LFL* lower fiducidal limit, *UFL* upper fiducidal limit,
X^2chi-square value *(df = 4), df* degree of freedom.

RESULTS

The larvicidal activity of Petroleum ether, Chloroform, Ethyl acetate and Methanol extracts of *J.adhatoda* leaf against *Cx. quinquefasciatus* larvae reveals that the methanol extract indicates the higher mortality rates compared to the other solvent extracts. Table 1.1 indicates the larval mortality of I^{st} to IV^{th} instars. The highest larvicidal potency with LC_{50} and LC_{90} was depicted after 24 hours was 126.21, 134.41, 140.92, 146.55 ppm and 385.38, 397.45, 407.17, 410.98 ppm. While, after 48 hours the LC_{50} and LC_{90} indicated 75.39, 78.19, 84.50, 91.37 ppm and 207.12, 259.67, 323.86, 369.88 ppm.

The larvicidal activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of *J.adhatoda* leaf against *Ae. Aegypti* larvae revealed that the Petroleum ether extract indicates the higher mortality rates compared to the other solvent extracts. Table 1.2 indicates the larval mortality of I^{st} to IV^{th} instars. The highest larvicidal potency with LC_{50} and LC_{90} was depicted after 24 hours was 129.77, 136.96, 143.13, 149.96 ppm and 338.24, 401.45, 405.51, 409.72 ppm. While, after 48 hours the LC_{50} and LC_{90} values indicated 75.36, 79.62, 85.44, 96.06 ppm and 230.54, 275.77, 328.34, 376.80 ppm. The 95% confidence limits LC_{50} and LC_{90} (LFL – UFL) were also calculated the results of larvicidal activity clearly indicate that the percentage of mortality being directly proportional to the concentration of the extract. This proves that concentration plays important role in larvicidal activity. Chi-square values were significant at $p \leq 0.05$level each test included a control group with for each individual concentration.

chloroform, ethyl acetate and methanol extracts of J.adhatoda was found to be more effective against Cx.quinquefasciatus than Ae. aegypti. Among four tested solvents methanol and ethyl acetate crude extract was found to be most effective for ovicidal activity against the mosquito species. The extract of methanol and Petroleum ether exerted 100% mortality at 240 ppm against Cx.quinquefasciatus and Ae. aegypti respectively. From the results it can be concluded the crude extract of J. adhatoda was a potential for controlling Cx. quinquefasciatus and Ae. aegypti mosquitoes.



The results of the adulticidal activity of petroleum ether, chloroform, ethyl acetate and methanol leaf extract of *J.adhatoda* against the adult of two important vector

Table 2 Lethal Concentration values of J. Adhatoda leaf extract against Ae. Aegypti

Solvents	Instars	Period	LC_{50}, ppm (LFL - UFL)	LC_{90}, ppm (LFL - UFL)	X^2	Regression Equation
Petroleum Ether	1st instar	24	129.77 (98.56 - 170.87)	388.24 (148.08 - 1017.91)	1.06	Y = 2.69X - 0.69
		48	75.36 (39.79 - 142.74)	230.54 (121.12 - 438.82)	3.73	Y = 2.64X + 0.05
	2nd instar	24	136.96 (106.54 - 176.08)	401.45 (149.79 - 1075.90)	1.32	Y = 2.74X - 0.86
		48	79.62 (43.23 - 130.26)	275.78 (130.26 - 583.83)	2.57	Y = 2.38X + 0.48
	3rd instar	24	143.13 (114.73 - 178.55)	405.51 (149.95 - 1096.62)	1.41	Y = 2.83X - 1.11
		48	85.44 (48.61 - 150.17)	328.24 (137.77 - 782.53)	2.56	Y = 2.19X + 0.77
	4th instar	24	149.96 (121.44 - 185.17)	409.72 (148.51 - 1130.39)	1.30	Y = 2.94X - 1.39
		48	96.06 (59.52 - 155.04)	376.80 (143.11 - 992.09)	1.69	Y = 2.16X + 0.72
Chloroform	1st instar	24	147.06 (119.03 - 181.68)	400.41 (150.10 - 1068.14)	4.62	Y = 2.95X - 1.39
		48	115.17 (81.21 - 163.35)	379.33 (147.41 - 976.12)	1.25	Y = 2.48X - 0.10
	2nd instar	24	150.58 (122.57 - 185.00)	403.99 (148.74 - 1097.26)	4.91	Y = 2.99X - 1.51
		48	119.75 (87.00 - 164.84)	390.78 (147.87 - 1032.74)	1.25	Y = 2.49X - 0.19
	3rd instar	24	154.20 (127.11 - 187.05)	406.77 (148.76 - 1112.27)	5.13	Y = 3.04X - 1.66
		48	124.47 (91.94 - 168.51)	401.60 (148.54 - 1085.82)	1.34	Y = 2.52X - 0.28
	4th instar	24	157.90 (130.73 - 190.71)	408.61 (147.26 - 1133.83)	5.51	Y = 3.10X - 1.82
		48	129.33 (97.86 - 170.91)	411.70 (151.16 - 1121.33)	1.30	Y = 2.55X - 0.38
Ethyl Acetate	1st instar	24	139.64 (110.33 - 176.74)	398.66 (149.96 - 1059.83)	1.46	Y = 2.81X - 1.03
		48	77.92 (41.19 - 147.42)	285.11 (132.55 - 613.24)	2.41	Y = 2.27X + 0.70
	2nd instar	24	145.87 (117.38 - 181.28)	404.09 (149.87 - 1089.52)	1.56	Y = 2.90X - 1.27
		48	84.46 (47.47 - 150.27)	332.21 (137.68 - 801.60)	2.19	Y = 2.15X + 0.85
	3rd instar	24	152.65 (124.89 - 186.60)	411.58 (148.53 - 1140.49)	1.52	Y = 2.98X - 1.50
		48	94.54 (57.20 - 156.27)	391.20 (147.15 - 1039.98)	1.53	Y = 2.08X + 0.89
	4th instar	24	159.76 (132.38 - 192.80)	417.09 (150.23 - 1158.01)	1.49	Y = 3.08X - 1.78
		48	109.01 (72.42 - 164.08)	434.60 (151.43 - 1247.32)	1.06	Y = 2.13X + 0.65
Methanol	1st instar	24	135.46 (105.20 - 174.43)	392.76 (147.43 - 1046.33)	1.34	Y = 2.77X - 0.91
		48	76.05 (40.02 - 144.52)	257.31 (127.82 - 518.01)	2.79	Y = 2.42X + 0.45
	2nd instar	24	141.18 (112.08 - 177.84)	403.57 (150.97 - 1078.82)	1.43	Y = 2.81X - 1.04
		48	82.12 (45.55 - 148.05)	300.55 (132.86 - 679.86)	2.65	Y = 2.27X + 0.65
	3rd instar	24	147.46 (119.10 - 182.56)	409.81 (149.86 - 1120.73)	1.43	Y = 2.89X - 1.26
		48	89.17 (51.92 - 153.13)	345.90 (142.11 - 841.93)	2.13	Y = 2.18X + 0.76
	4th instar	24	153.46 (126.58 - 186.06)	412.85 (149.98 - 1136.42)	1.37	Y = 2.98X - 1.52
		48	100.81 (63.79 - 159.30)	407.09 (147.64 - 1122.50)	1.55	Y = 2.11X + 0.76

Control nil mortality significant at $P \leq 0.05$ level, LFL lower fiducidal limit, UFL upper fiducidal limit,
X2chi-square value (df = 4), df degree of freedom.

The ovicidal activity was determined against two mosquito species to various concentrations ranging from 60-300 ppm under the laboratory conditions. The petroleum ether, mosquitoes viz., *Cx.quinquefasciatus* and *Ae. aegypti* are presented in Graph 1.0. Among two vectors tested the highest

adulticidal activity was observed in Petroleum ether. At higher concentrations, the adult showed restless movement for sometimes with abnormal wagging and died. LC_{50} and LC_{90} values were calculated.

Table 3 Ovicidal activity of J. Adhatoda leaf extract against eggs of Cx. quinquefasciatus and Ae. aegypti

Species	Solvent	Percentage of egg hatching					
		Concentration (ppm)					
		Control	60	120	180	240	300
Culex quinquefaciatus	Pet. Ether	100.0 ± 0.0	79.7 ± 0.8	37.2 ± 1.2	23.7 ± 1.2	10.7 ± 0.5	NH
	Chloroform	99.3 ± 1.2	82.8 ± 0.8	40.5 ± 1.1	27.3 ± 0.8	13.0 ± 0.9	NH
	Ethyl Acetate	98.8 ± 1.2	84.3 ± 0.8	43.0 ± 0.9	30.8 ± 0.9	15.3 ± 0.5	NH
	Methanol	100.0 ± 0.0	76.2 ± 1.5	32.5 ± 0.8	17.3 ± 0.8	NH	NH
Aedes aegypti	Pet. Ether	100.0 ± 0.0	78.3 ± 0.5	34.3 ± 0.8	20.0 ± 0.9	NH	NH
	Chloroform	99.7 ± 0.5	86.7 ± 0.5	45.2 ± 0.8	32.3 ± 0.5	16.8 ± 0.4	NH
	Ethyl Acetate	99.8 ± 0.4	83.5 ± 0.6	41.7 ± 0.5	28.7 ± 0.8	14.0 ± 0.9	NH
	Methanol	100.0 ± 0.0	81.3 ± 1.0	38.7 ± 0.5	25.2 ± 0.8	12.3 ± 0.8	NH

The skin repellent activity of petroleum ether, chloroform, ethyl acetate and methanol extract of *J. adhatoda* against blood starved adult female of *Cx. quinquefasciatus* and *Ae. aegypti* are given in Table 3.1 and 3.2. The petroleum ether extract had strong repellent action against mosquitoes as it provided 100% protection against *Ae. aegypti* for 210 min followed by *Cu. quinquefasciatus* 180 min and methanol extract revealed strong repellent action against *Cu. quinquefasciatus* for 210 min followed by *Ae. aegypti* 180 min. It clearly shows that repellent activity was dose dependent. From the results it can be concluded the leaf extract of *J. Adhatoda* was an excellent potential for controlling *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes.

compounds included in the plant leaves. Among *A. vasica* leaf compounds that may interact with the feeding activity of insects (Rahman *et al*, 1997), hydroxylketones (Singh *et al*,1991) and alkaloids (Chowdhury and Bhattacharyya, 1985; Thappa *et al*, 1996; Shrivastava *et al*, 2006). Screening the effects of five alkaloids isolated from *A. vasica* leaves against four insect species (Saxena *et al*, 1993) found that the effects vary in extent and type (i.e. being toxic, feeding deterrent or sterilizing) depending on the compound and the species involved. Moreover the methanol extract of *J. adhatoda* exhibited positive antimicrobial activity for P. aeruginosa, S. aureus and B. subtilis while E. coli was not effectively inhibited by extracts of tested plant (Shinwari *et al*, 2009).

The leaf powdered preparation of *A. vasica* (adhatoda), Azadirachta indica (neem) and Ocimum sanctum (tulsi), which on burning with charcoal produced smoke that repelled

Table 4 Repellent activity of J. Adhatoda leaf extract against Culex quinquefasciatus

Extracts	Concentration mg/cm^2	Percentage of repellency, Time post application of repellent(min)							
		30	60	90	120	150	180	210	240
Pet. Ether	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	73.5 ± 1.4	61.0 ± 1.3
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	82.8 ± 1.2	69.3 ± 1.4
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	92.2 ± 1.8	78.2 ± 1.5
Chloroform	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	78.0 ± 1.4	70.2 ± 1.6	56.3 ± 1.4
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	84.5 ± 1.9	76.0 ± 1.4	61.3 ± 1.6
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	94.2 ± 1.5	83.3 ± 1.6	74.2 ± 1.8
E. Acetate	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	75.3 ± 1.4	67.3 ± 1.6	53.8 ± 1.2
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	81.5 ± 1.1	73.2 ± 1.5	57.3 ± 1.0
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	89.2 ± 1.5	80.5 ± 1.9	71.3 ± 1.2
Methanol	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	74.3 ± 1.4
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	79.7 ± 1.2
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	90.2 ± 1.2

Table 5 Repellent activity of J. Adhatoda leaf extract against Aedes Aegypti

Extracts	Concentration mg/cm^2	Percentage of repellency, Time post application of repellent(min)							
		30	60	90	120	150	180	210	240
Pet. Ether	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	72.5 ± 1.4
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	77.2 ± 1.2
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	88.3 ± 1.9
Chloroform	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	96.8 ± 1.9	72.3 ± 1.0	65.2 ± 1.2	52.3 ± 1.2
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	78.5 ± 1.9	71.8 ± 1.5	54.7 ± 1.2
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	87.2 ± 1.7	77.7 ± 1.6	68.3 ± 1.0
E. Acetate	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	98.2 ± 1.8	74.3 ± 1.6	68.5 ± 1.9	54.5 ± 1.2
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	81.7 ± 1.6	73.2 ± 1.9	58.5 ± 1.4
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	91.3 ± 1.0	80.8 ± 1.5	71.5 ± 1.9
Methanol	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	70.3 ± 1.9	59.2 ± 1.9
	2.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	80.7 ± 1.2	67.2 ± 1.5
	3.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	89.3 ± 1.2	75.5 ± 1.1

DISCUSSION

The results of present study are comparable with earlier reports. The toxic effects of *A. vasica* fraction from leaf and root extract studied in the present study and other studies (Bhaduri *et al*, 1985; Hiremath *et al*, 1997; Sadek MM, 2003; Lateef *et al*, 2003; Al-Shaibani *et al*, 2008; Govindappa *et al*, 2011) can be referred to one or more of the many bioactive

Armigeres subalbatus and *Cx. quinquefasciatus* to prevent their biting activity for 6–8 h (Pandian S R *et al*, 1995). The acetone, chloroform, ethyl acetate, hexane, methanol and petroleum ether extracts of leaf, flower and seed of *Cassia auriculata*, *Solanum torvum* and *Vitex negundo* were tested against fourth instar larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus* (Kamaraj *et al*, 2009)

Larvicidal efficacy of leaf methanol extracts of *P. zeylanica* and *Acacia ferruginea* were tested against the late third instar larvae of *Cx. quinquefasciatus* with LC_{50} values of 2214.7 and 5362.6 ppm, respectively (Vahitha *et al*, 2002). The bio-active compound azadiracta indica showed complete ovicidal activity in the eggs of *Cx. tarsalis* and *Cx. quinquefasciatus* exposed to 10 ppm concentration (Su & Mulla, 1998). Venkatachalam and Jebanesan (2001) have also reported that the repellent activity of methanol extract of *Feronia elephantum* leaves against Ae. aegypti activity at 1.0 and 2.5 mg/cm^2 concentrations gave 100% protection up to 2.14±0.16 h and 4.00±0.24 h, respectively, and the total percentage protection was 45.8% at 1.0 mg/cm2 and 59.0% at 2.5 mg/cm2 for 10 h.

The highest repellency was observed in *Zingiber officinale*, a higher concentration of 5.0 mg/cm^2 provided 100% protection up to 150 and 180 min against *Culex tritaeniorhynchus* and *Anopheles subpictus*, respectively (Govindarajan, 2011). Govindarajan *et al*, (2011) reported mosquito larvicidal, ovicidal, and repellent properties of botanical extracts against *An. stephensi, Ae. aegypti*, and *Cx. quinquefasciatus.* The benzene and ethyl acetate extracts of leaves of *E. coronaria* and *C. pulcherrima* show significant repellency against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*

Adulticidal activity, the LC_{50} and LC_{90} values of hexane, dichloromethane, ethyl acetate and methanol extracts of Barleria prionitis against *Cx. quinquefasciatus* larvae in 24 h were 280.25, 269.21, 253.12, 237.44 and 484.56, 473.39, 459.81 and 450.82ppm, respectively, (Janakan and Ramakrishnan, 2014). The present findings revealed that the petroleum ether and methanol extract of *J. adhathoda* was more active compared with the ethyl acetate, chloroform extracts against *Cx. quinquefasciatus* and *Ae. aegypti.*

CONCLUSION

The present investigation revealed that different extract of *Justicia adhatoda* possesses remarkable larvicidal, ovicidal, adulticidal and repellent activity against important vector mosquito. The extract might be used directly as adulticidal and larvicidal agent in small volume aquatic habitats or breeding sites of limited size around human dwellings. These results could encourage the search for new active natural compounds offering an alternative to synthetic repellents and insecticides from other medicinal plants.

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