HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Entomological research doi: 10.1016/S2222-1808(16)61070-8

Ovicidal and larvicidal activities of some plant extracts against *Aedes aegypti* L. and *Culex quinquefasciatus* Say (Diptera: Culicidae)

Rajiv Gandhi Munusamy[1#], Daniel Reegan Appadurai[2#], Sivasankaran Kuppusamy[1], Gabriel Paulraj Michael[1], Ignacimuthu Savarimuthu[1*]

[1]*Division of Vector Control, Entomology Research Institute, Loyola College, Chennai 600 034, India*

[2]*National Vector Borne Disease Control Programme, ROHFW, Government of India, Chennai 600 090, India*

ARTICLE INFO

Article history:
Received 31 Aug 2015
Received in revised form 19 Oct, 2nd revised form 15 Dec 2015
Accepted 20 Mar 2016
Available online 15 Jun 2016

Keywords:
Bioassay
Culex quinquefasciatus
Aedes aegypti
Ovicidal and larvicidal activities

ABSTRACT

Objective: To evaluate the ovicidal and larvicidal activities of hexane, chloroform and methanol extracts of *Gymnema sylvestre*, *Scilla peruvina*, *Rubia cordifolia* (*R. cordifolia*) and *Elytraria acaulis* roots against the eggs and larvae of *Aedes aegypti* L. (*Ae. aegypti*) and *Culex quinquefasciatus* Say (*Cx. quinquefasciatus*) at different concentrations of 62.5, 125, 250 and 500 mg/L.

Methods: The plant materials were shade dried in the laboratory for one week and then coarsely powdered. The root powder of each plant (500 g) was sequentially soaked in hexane, chloroform and methanol for 96 h with intermittent shaking. After 96 h, the solution was filtered and the filtrate was concentrated under reduced pressure by using rotary vacuum evaporator. All the crude extracts thus obtained were stored in air tight glass vials and Petri dishes.

Results: The ovicidal activity results showed that the methanol extract of *R. cordifolia* root was the most potent compared to other with 82.40% and 70.40% activity against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively, at 500 mg/L concentration similarly, methanol extract of *R. cordifolia* root also recorded the highest larvicidal activity with LC_{50} and LC_{90} values of 95.69, 347.96 mg/L against *Cx. quinquefasciatus* and 102.23, 350.20 mg/L against *Ae. aegypti* larvae, respectively.

Conclusions: Hence, methanol extract of *R. cordifolia* root can be probed further for effective biological control of mosquitoes.

1. Introduction

Mosquitoes are well established in tropical regions and serve as vectors responsible for transmission of several pathogens to human. Some pathogens are transmitted by the day-biting mosquitoes and some are transmitted by the night-biting mosquitoes. Particularly, in recent years thousands of dengue and chikungunya positive cases have been reported from several countries[1-4]. *Aedes aegypti* (*Ae. aegypti*), a day-biting mosquito is the primary vector of dengue and chikungunya. Similarly, several thousands of people suffer from filariasis[5,6]. *Culex quinquefasciatus* (*Cx. quinquefasciatus*) is a night-biting mosquito involved in the transmission of filarial nematode. Targeting the immature stages is the key factor in controlling these two species of mosquitoes[7,8]. For this, chemical larvicides such as organophosphates are being used; but several disadvantages have been reported due to harmful effects to human and other associated organisms[9-14]. Further, many factors involved in the increase of mosquito population[15], particularly development of resistance to synthetic insecticides lead to greater increase of vector mosquitoes.

Plant extracts are good alternatives to chemical insecticides to control mosquito population[16]. Many earlier reports have confirmed the bioactivity of several plant extracts. In the present study, the roots of four plants *viz*., *Gymnema sylvestre* (*G. sylvestre*), *Scilla peruvina* (*S. peruvina*), *Rubia cordifolia* (*R. cordifolia*) and *Elytraria acaulis* (*E. acaulis*) were used for solvent extraction. *G. sylvestre* is used in the treatment of diabetes, besides being used for arthritis, diuretic, anemia, osteoporosis, hypercholesterolemia[17]. Species under the genus *Scilla* reported to possess antidote effect, blood circulatory activation, cough control and abscess reduction[18]. *R. cordifolia* decoction from roots is prescribed to cure jaundice, paralytic affections and urinary troubles[19]. Roots of *R. cordifolia* have also been used as astringent, thermogenic, febrifuge, antidysenteric, antihelmintic, galactopurifier, ophthalmic and rejuvenant[20]. The extracts of *E. acaulis* are reported to possess decreasing effect on blood glucose level, reduction in the liver glycogen level and reduction in glycated hemoglobin levels[21]. The leaves decoction of *E. acaulis* is prescribed to treat fever, venereal diseases and its root is used in traditional medicine against tumor,

*Corresponding author: Dr. Savarimuthu Ignacimuthu, PhD, DSc, Division of Vector Control, Entomology Research Institute, Loyola College, Chennai 600 034, India.
Tel: +91 44 28178348
Fax: +91 44 28175566
E-mail: eriloyola@hotmail.com
Foundation Project: Supported by Entomology Research Institute (Grant No. FS8).
The journal implements double-blind peer review practiced by specially invited international editorial board members.
#These authors contributed equally to this work.

pneumonia, asthma, migraine, leucorrhoea, snake bite and diarrhea. Leaves are also used as antidiabetic[22,23]. In this paper, in order to evaluate the ovicidal and larvicidal activities of hexane, chloroform and methanol extracts of four plants, the crude extracts of the roots of the above mentioned plants were tested against the eggs and larvae of *Ae. aegypti* and *Cx. quinquefasciatus* under laboratory conditions.

2. Materials and methods

2.1. Sample collection and preparation of solvent extracts

Root of *R. cordifolia* was purchased from local market, Parrys. Roots of other three plants were collected naturally from the field in Thiruvallur District, India. The plant materials were shade dried in the laboratory for one week and then coarsely powdered. The root powder of each plant (500 g) was sequentially soaked in hexane, chloroform and methanol for 96 h with intermittent shaking. After 96 h, the solution was filtered and the filtrate was concentrated under reduced pressure using rotary vacuum evaporator. All the crude extracts thus obtained were stored in air tight glass vials and Petri dishes.

2.2. Preparation of various concentrations and test mosquitoes

From the crude extract, different concentrations (62.5, 125, 250 and 500 mg/L) were prepared by using acetone. The mosquitoes were reared at (27 ± 2) ℃, 75%–85% relative humidity under a photoperiod of 14:10 h as previously reported in our laboratory[24].

2.3. Ovicidal assay

Ovicidal activity was studied following the method of Reegan *et al.*[25]. Twenty five freshly laid eggs of *Ae. aegypti* and *Cx. quinquefasciatus* were separately exposed to four different concentrations *viz*., 62.5, 125, 250 and 500 mg/L prepared using acetone. Each concentration was replicated five times. Azadirachtin was used as positive control with the concentration of 10.0 mg/L for comparison. Control was maintained separately with five replicates and egg mortality was assessed after 120 h post treatment using the following formula:

$$\text{Percent ovicidal activity} = \frac{\text{Number of unhatched eggs}}{\text{Total number of eggs introduced}} \times 100$$

2.4. Larvicidal activity

The larvicidal activities of the crude extracts were assessed following the protocol of World Health Organization[26]. The early third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were exposed to the concentrations of 62.5, 125, 250 and 500 mg/L. Five replicates were maintained for every concentration of each extract. Control was maintained separately with five replicates. Larval mortality was recorded after 24 h. Larvae were considered dead when they did not move to the surface of the solution. Azadirachtin was used as positive control with the concentrations of 2.5, 5.0, 7.5 and 10.0 mg/L for comparison.

2.5. Statistical analysis

The mean values and SD were calculated from five replications. The calculated percent ovicidal means were separated by Tukey's test of multiple comparisons, One-way ANOVA. The larvicidal mortality was corrected by Abbott's formula[27], and the lethal concentration values of LC_{50} and LC_{90} were calculated by using EPA probit analysis program (version 1.5).

3. Results

3.1. Ovicidal activity

The ovicidal activity varied among the different extracts of four plants. The methanol extract of *R. cordifolia* root recorded the highest ovicidal activities of 82.40% and 70.40% against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively, at 500 mg/L concentration (Tables 1 and 2). It was followed by hexane extract of *S. peruvina* root which recorded ovicidal activities of 44.80% and 43.20% against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively, at 500 mg/L concentration. Further, the hexane extract of *R. cordifolia* root recorded moderate ovicidal activities of 26.40% and 25.60% against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively, at 500 mg/L concentration. All the other plant extracts showed low ovicidal activities. The positive control, azadirachtin recorded ovicidal activities of 95.20% and 92.80% against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively, at 10.0 mg/L concentration (Tables 1 and 2).

Table 1

Percent ovicidal activity of crude extracts against *Cx. quinquefasciatus* eggs.

Plant species	Treatment	Concentration (mg/L)			
		62.5	125	250	500
G. sylvestre	Hexane	0.00^{e}	0.00^{e}	5.60 ± 2.19^{ef}	8.00 ± 2.82^{e}
	Chloroform	0.80 ± 1.78^{de}	1.60 ± 2.19^{de}	2.40 ± 2.19^{fg}	4.00 ± 4.00^{ef}
	Methanol	0.80 ± 1.78^{de}	2.40 ± 2.19^{de}	3.20 ± 1.78^{fg}	7.20 ± 3.34^{e}
S. peruvina	Hexane	4.80 ± 1.78^{bcd}	9.60 ± 3.57^{bc}	22.40 ± 2.19^{b}	44.80 ± 1.78^{b}
	Chloroform	0.00^{e}	1.60 ± 2.19^{de}	3.20 ± 1.78^{fg}	8.80 ± 1.78^{e}
	Methanol	2.40 ± 2.19^{cde}	4.80 ± 1.78^{cde}	10.40 ± 2.19^{de}	23.20 ± 1.78^{cd}
R. cordifolia	Hexane	7.20 ± 1.78^{b}	10.40 ± 3.89^{bc}	16.80 ± 3.34^{c}	26.40 ± 3.57^{c}
	Chloroform	6.40 ± 2.19^{bc}	12.00 ± 4.00^{b}	18.40 ± 2.19^{bc}	24.80 ± 3.34^{c}
	Methanol	23.20 ± 1.78^{a}	39.20 ± 1.78^{a}	53.60 ± 3.57^{a}	82.40 ± 2.19^{a}
E. acaualis	Hexane	3.20 ± 1.78^{bcde}	6.40 ± 4.56^{bcd}	10.40 ± 3.57^{de}	20.80 ± 5.21^{cd}
	Chloroform	3.20 ± 3.34^{bcde}	6.40 ± 3.57^{bcd}	11.20 ± 1.78^{d}	16.80 ± 1.78^{d}
	Methanol	0.80 ± 1.78^{de}	1.60 ± 2.19^{de}	4.00 ± 2.82^{fg}	9.60 ± 3.57^{e}
Control		0.80 ± 1.78^{de}	0.00^{e}	0.00^{g}	0.00^{f}
Azadirachtin (10.0 mg/L)		95.20 ± 1.78			

Data were shown as mean ± SD; Means were separated by Tukey's test of multiple comparisons, One-way ANOVA. Data with same letters in the column are not significantly different.

Table 2

Percent ovicidal activity of crude extracts against *Ae. aegypti* eggs.

Plant species	Treatment	Concentration (mg/L)			
		62.5	125	250	500
G. sylvestre	Hexane	0.00 ± 0.00^{d}	0.80 ± 1.78^{e}	2.40 ± 2.19^{d}	8.80 ± 1.78^{fg}
	Chloroform	0.80 ± 1.78^{cd}	1.60 ± 2.19^{e}	2.40 ± 2.19^{d}	3.20 ± 3.34^{g}
	Methanol	2.40 ± 3.57^{bcd}	3.20 ± 1.78^{de}	4.80 ± 1.78^{cd}	8.00 ± 2.82^{fg}
S. peruvina	Hexane	5.60 ± 2.19^{bc}	9.60 ± 2.19^{bc}	20.00 ± 2.82^{b}	43.20 ± 1.78^{b}
	Chloroform	4.00 ± 2.82^{bcd}	4.80 ± 1.78^{cde}	5.60 ± 2.19^{cd}	10.40 ± 3.57^{ef}
	Methanol	1.60 ± 2.19^{cd}	2.40 ± 2.19^{de}	5.60 ± 2.19^{cd}	8.80 ± 1.78^{fg}
R. cordifolia	Hexane	7.20 ± 1.78^{b}	12.80 ± 1.78^{b}	18.40 ± 2.19^{b}	25.60 ± 4.56^{c}
	Chloroform	4.00 ± 2.82^{bcd}	7.20 ± 3.34^{cd}	10.40 ± 3.57^{c}	19.20 ± 3.34^{cd}
	Methanol	20.80 ± 3.34^{a}	34.40 ± 2.19^{a}	48.80 ± 4.38^{a}	70.40 ± 2.19^{a}
E. acaualis	Hexane	2.40 ± 3.57^{bcd}	3.20 ± 3.34^{de}	5.60 ± 4.56^{cd}	16.00 ± 3.57^{de}
	Chloroform	5.60 ± 2.19^{bc}	7.20 ± 3.34^{cd}	7.20 ± 1.78^{cd}	10.40 ± 3.57^{ef}
	Methanol	0.80 ± 0.44^{cd}	1.60 ± 0.54^{e}	2.40 ± 2.19^{d}	3.20 ± 1.78^{g}
Control		0.00^{d}	0.00^{f}	0.00^{e}	0.00^{h}
Azadirachtin (10.0 mg/L)		92.8 ± 0.81			

Data were shown as mean ± SD; Means were separated by Tukey's test of multiple comparisons, One-way ANOVA. Data with same letters in the column are not significantly different.

3.2. Larvicidal activity

The results clearly showed that the methanol extract of *R. cordifolia* root was the most effective against the larvae of both mosquitoes. The calculated LC_{50} and LC_{90} values of methanol extract for *R. cordifolia* were 95.69, 347.96 mg/L against *Cx. quinquefasciatus* and 102.23, 350.20 mg/L against *Ae. aegypti* larvae, respectively (Tables 3 and 4). This was followed by hexane extract of *S. peruvina*

Table 3

Lethal concentrations of crude extracts against the third instar larvae of *Cx. quinquefasciatus*.

Plant species	Treatment	LC_{50} (mg/L)	95% *CI*		LC_{90} (mg/L)	95% *CI*		Intercept ± SE	χ^2
			LL	UL		LL	UL		
G. sylvestre	Hexane	236.48	167.65	324.84	599.42	466.44	898.61	-0.8 ± 0.1	4.2*
	Chloroform	371.00	302.22	482.30	763.03	611.89	1066.92	-1.2 ± 0.1	2.8*
	Methanol	685.37	427.52	1013.00	1308.41	767.50	23686.00	-1.4 ± 0.1	7.6*
S. peruvina	Hexane	106.81	59.33	151.45	289.80	225.06	435.71	-0.7 ± 0.1	5.0*
	Chloroform	324.08	273.06	393.52	679.68	569.80	866.93	-0.1 ± 0.5	9.5*
	Methanol	211.97	12.24	498.05	799.45	507.02	388.01	-0.4 ± 0.1	7.5*
R. cordifolia	Hexane	178.52	127.79	232.14	463.49	378.90	617.57	-0.8 ± 0.1	7.5*
	Chloroform	240.69	181.35	313.93	600.38	482.16	834.29	-0.8 ± 0.1	3.2*
	Methanol	95.69	21.49	152.74	347.96	266.10	535.09	-0.4 ± 0.1	5.1*
E. acaualis	Hexane	207.39	179.77	241.27	801.65	609.76	1186.21	-0.5 ± 0.5	0.4*
	Chloroform	230.05	198.87	270.04	911.07	680.23	1391.40	-0.1 ± 0.5	0.7*
	Methanol	268.83	234.50	314.08	921.87	704.13	1353.18	-0.8 ± 0.5	3.1*
Positive control	Azadirachtin (2.5–10 mg/L)	3.00	2.61	3.35	6.64	5.91	7.71	3.2 ± 0.2	4.3*

95% *CI*: 95% Confidence interval; LL: Lower limit (95% *CI*); UL: Upper limit (95% *CI*). *: $P \leqslant 0.05$, level of significance of *Chi*-square values.

Table 4

Lethal concentrations of crude extracts against the third instar larvae of *Ae. aegypti*.

Plant species	Treatment	LC_{50} (mg/L)	95% *CI*		LC_{90} (mg/L)	95% *CI*		Intercept ± SE	χ^2
			LL	UL		LL	UL		
G. sylvestre	Hexane	310.79	270.02	361.77	580.73	504.43	679.39	-1.4 ± 0.1	1.6*
	Chloroform	280.55	131.90	711.77	891.95	567.11	3565.71	-0.5 ± 0.1	6.8*
	Methanol	290.27	245.96	346.47	581.38	495.49	720.21	-1.2 ± 0.1	2.3*
S. peruvina	Hexane	114.13	6.26	193.36	434.62	321.02	740.01	-0.4 ± 0.1	9.5*
	Chloroform	619.19	496.40	881.06	1095.00	846.37	1663.94	-1.6 ± 0.1	6.8*
	Methanol	896.04	658.87	1739.71	1414.30	994.80	2964.12	-2.2 ± 0.2	1.8*
R. cordifolia	Hexane	194.89	166.15	230.17	903.58	656.85	1453.02	0.5 ± 0.4	0.7*
	Chloroform	876.83	564.88	4986.35	1485.86	903.35	9619.06	-1.8 ± 0.1	4.7*
	Methanol	102.23	86.42	117.78	350.20	287.55	459.61	-0.1 ± 0.5	5.9*
E. acaualis	Hexane	219.98	188.84	259.82	948.90	694.53	1502.84	0.2 ± 0.4	1.4*
	Chloroform	261.73	227.53	306.90	941.57	711.68	1406.81	-0.5 ± 0.5	4.1*
	Methanol	316.23	212.02	548.83	880.74	613.36	888.88	-0.7 ± 0.1	4.3*
Positive control	Azadirachtin (2.5–10 mg/L)	3.17	2.59	3.65	9.67	7.76	14.10	3.6 ± 0.2	0.3*

95% *CI*: 95% Confidence interval; LL: Lower limit (95% *CI*); UL: Upper limit (95% *CI*); *: $P \leqslant 0.05$, level of significance of *Chi*-square values.

which recorded LC_{50} and LC_{90} values of 106.81, 289.80 mg/L against *Cx. quinquefasciatus* and 114.13, 434.62 mg/L against *Ae. aegypti* larvae, respectively. All the other extracts showed only moderate or least larvicidal activities (Tables 3 and 4). Further, it was noted that *Cx. quinquefasciatus* larvae were more susceptible than *Ae. aegypti*. The results were compared with positive control azadirachtin, which showed LC_{50} and LC_{90} values of 3.00, 6.64 mg/L against *Cx. quinquefasciatus* and 3.17, 9.67 mg/L against *Ae. aegypti* larvae, respectively.

4. Discussion

Mosquitoes are nuisance and most dangerous insects, since they transmit pathogens. Vector mosquitoes are well established in tropical and subtropical regions and they have also developed resistance to chemical insecticides. Hence biological control method would be a good approach in mosquito control program.

In the present study, the methanol extract of *R. cordifolia* recorded the highest ovicidal activities of 82.40% and 70.40% against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively, at 500 mg/L concentration. Our result is comparable to the report of Elango *et al*.[28], who had reported that *Cocculus hirsutus* methanol extract caused 86% ovicidal activity at 500 mg/L concentration against the eggs of *Anopheles subpictus*. In another study, Marimuthu *et al*.[29], have reported 100% ovicidal activity at 300 mg/L concentration with methanol extract of *Delonix elata* against the eggs of *Anopheles stephensi* (*An. stephensi*) and *Ae. aegypti*.

Further, the same methanol extract of *R. cordifolia* showed good larvicidal activity with LC_{50} and LC_{90} values of 95.69, 347.96 mg/L against *Cx. quinquefasciatus* and 102.23, 350.20 mg/L against *Ae. aegypti* larvae, respectively. Our results corroborate with the results of Aivazi and Vijayan[30], who had reported LC_{50} and LC_{90} values of 116.92, 144.77 mg/L with ethyl acetate extract of *Quercus infectoria* Gall against the fourth instar larvae of *Ae. stephensi*. The LC_{50} values of 177.14 and 513.387 mg/L were reported with methanol extracts of *Euphorbia tirucalli* latex and stem bark, respectively, against the larvae of *Cx. quinquefasciatus*[31].

Further, our results revealed that *Cx. quinquefasciatus* larvae were more susceptible than *Ae. aegypti*. Similar to our results, many investigators have reported varied results among different mosquito species[32] and evaluated the methanolic extracts from fruits and seeds of *Solanum xanthocarpum* against the larvae of *Anopheles culicifacies*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The LC_{50} values varied for fruits and seeds with 51.6, 52.2, 118.3, 157.1 mg/L and 66.9, 73.7, 123.8, 154.9 mg/L against *Anopheles culicifacies*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. Similarly, Patil *et al*.[33] recorded the highest larval mortality with methanol extracts of *Plumbago zeylanica* root with LC_{50} value of 169.61 mg/L against *Ae. aegypti* larvae than *An. stephensi*, which showed LC_{50} value of 222.34 mg/L.

Phytochemicals like anthraquinones, alkaloides, glycosides, flavanoids, tannins, saponins, phenols and triterpenoides have been reported earlier from leaves and roots of *R. cordifolia*[34-36].

In conclusion, the methanol extract of *R. cordifolia* was the

most potent against the eggs and larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. These results suggest that methanol extract of *R. cordifolia* can be probed further for effective mosquito control.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are thankful to Entomology Research Institute for financial assistance (Grant No. FS8).

References

[1] Pialoux G, Gaüzère BA, Jauréguiberry S, Strobel M. Chikungunya, an epidemic arbovirosis. *Lancet Infect Dis* 2007; **7**: 319-27.
[2] Simmons CP, Farrar JJ, Nguyen VV, Wills B. Dengue. *N Engl J Med* 2012; **366**: 1423-32.
[3] Yang CF, Hou JN, Chen TH, Chen WJ. Discriminable roles of *Aedes aegypti* and *Aedes albopictus* in establishment of dengue outbreaks in Taiwan. *Acta Trop* 2014; **130**: 17-23.
[4] Johansson MA, Powers AM, Pesik N, Cohen NJ, Staples JE. Nowcasting the spread of chikungunya virus in the Americas. *PLoS One* 2014; **9**(8): e104915.
[5] World Health Organization. Global programme to eliminate lymphatic filariasis. Geneva: World Health Organization; 2014. [Online] Available from: http://www.who.int/lymphatic_filariasis/disease/en/ [Accessed on 6th January, 2015]
[6] Reegan AD, Kinsalin AV, Paulraj MG, Ignacimuthu S. Larvicidal, ovicidal, and repellent activities of marine sponge *Cliona celata* (Grant) extracts against *Culex quinquefasciatus* Say and *Aedes aegypti* L. (Diptera: Culicidae). *ISRN Entomol* 2013; **2013**: 315389.
[7] Zahran HE, Abdelgaleil SA. Insecticidal and developmental inhibitory properties of monoterpenes on *Culex pipiens* L. (Diptera: Culicidae). *J Asia-Pac Entomol* 2011; **14**(1): 46-51.
[8] Muthu C, Reegan AD, Kingsley S, Ignacimuthu S. Larvicidal activity of pectolinaringenin from *Clerodendrum phlomidis* L. against *Culex quinquefasciatus* Say and *Aedes aegypt*i L. (Diptera: Culicidae). *Parasitol Res* 2012; **111**: 1059-65.
[9] Sutthanont N, Choochote W, Tuetun B, Junkum A, Jitpakdi A, Chaithong U, et al. Chemical composition and larvicidal activity of edible plant-derived essential oils against the pyrethroid-susceptible and -resistant strains of *Aedes aegypti* (Diptera: Culicidae). *J Vector Ecol* 2010; **35**: 106-15.
[10] Bayen S. Occurrence, bioavailability and toxic effects of trace metals and organic contaminants in mangrove ecosystems: a review. *Environ Int* 2012; **48**: 84-101.
[11] Mulyatno KC, Yamanaka A, Ngadino, Konishi E. Resistance of *Aedes aegypti* (L.) larvae to temephos in Surabaya, Indonesia. *Southeast Asian J Trop Med Public Health* 2012; **43**: 29-33.
[12] Grisales N, Poupardin R, Gomez S, Fonseca-Gonzalez I, Ranson H, Lenhart A. Temephos resistance in *Aedes aegypti* in Colombia compromises dengue vector control. *PLoS Negl Trop Dis* 2013; **7**: e2438.
[13] Chen CD, Nazni WA, Lee HL, Norma-Rashid Y, Lardizabal ML, Sofian-Azirun M. Temephos resistance in field *Aedes* (*Stegomyia*) *albopictus* (Skuse) from Selangor, Malaysia. *Trop Biomed* 2013; **30**: 220-30.
[14] Chavshin AR, Dabiri F, Vatandoost H, Bavani MM. Susceptibility of *Anopheles maculipennis* to different classes of insecticides in West Azarbaijan Province, Northwestern Iran. *Asian Pac J Trop Biomed* 2015; **5**(5): 403-6.
[15] Eida OM, Eida AM. Limited genetic diversity among *Plasmodium falciparium* isolates using nested PCR in Jazan Area, Saudi Arabia. *Asian Pac J Trop Biomed* 2015; **12**(5): 407-11.
[16] Ruiz-Guerrero R, Rodríguez-Pérez MA, Norzagaray-Campos M. Toxicity of Mexican native plant extracts against larvae of *Aedes aegypti* (Diptera: Culicidae) *Asian Pac J Trop Biomed* 2015; **5**(4): 287-91.
[17] Tiwari P, Mishra BN, Sangwan NS. Phytochemical and pharmacological properties of *Gymnema sylvestre*: an important medicinal plant. *Biomed Res Int* 2014; **2014**: 830285.
[18] Lee HB, Lee SM. Antimicrobial activity of eucosterol oligosaccharides isolated from bulb of squill (*Scilla scilloides*). *Pharmacol Pharm* 2013; **4**: 110-14.
[19] Devi Priya M, Siril EA. Traditional and modern use of Indian madder (*Rubia cordifolia* L.): an overview. *Int J Pharm Sci Rev Res* 2014; **25**(1): 154-64.
[20] Sivarajan VV, Balachandran I. *Ayurvedic drugs and their plant sources.* New Delhi: Oxford and International Book House Publishing Company; 1994, p. 496.
[21] Praveen Kumar R, Sukanyahdevi E, Shruthilavanya S, Vaishali C, Gospelia Nivetha L, Chozhavendhan S, et al. Evaluation of anti-septic and anti-inflammatory activity of *Elytraria acualis*. *Int J ChemTech Res* 2014; **6**(9): 4166-71.
[22] Koshy R, Raj Kapoor B, Azmathulla M. Acute and sub acute toxicity of ethanol extract of *Elytraria acaulis* Lindau. in rats. *Pharmacologyonline* 2011; **3**: 229-42.
[23] Kiruthika N, Dhivya D, Kalaiselvi K, Kanimozhi P, Panneerselvam K. Phytochemical studies on *Elytraria acaulis. Int J Pharm Bio Sci* 2012; **3**(3): 1054-62.
[24] Maheswaran R, Ignacimuthu S. A novel herbal formulation against dengue vector mosquitoes *Aedes aegypti* and *Aedes albopictus*. *Parasitol Res* 2012; **110**: 1801-13.
[25] Reegan AD, Gandhi MR, Paulraj MG, Balakrishna K, Ignacimuthu S. Effect of niloticin, a protolimonoid isolated from *Limonia acidissima* L. (Rutaceae) on the immature stages of dengue vector *Aedes aegypti* L. (Diptera: Culicidae). *Acta Trop* 2014; **139**: 67-76.
[26] World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization; 2005. [Online] Available from: http://apps.who.int/iris/bitstream/10665/69101/1/WHO_CDS_WHOPES_GCDPP_2005.13.pdf [Accessed on 12th January, 2015]
[27] Abbott WS. A method of computing the effectiveness of an insecticide. 1925. *J Am Mosq Control Assoc* 1987; **3**: 302-3.
[28] Elango G, Rahuman AA, Bagavan A, Kamaraj C, Zahir AA, Venkatesan C. Laboratory study on larvicidal activity of indigenous plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*. *Parasitol Res* 2009; **104**: 1381-8.
[29] Marimuthu G, Rajamohan S, Mohan R, Krishnamoorthy Y. Larvicidal and ovicidal properties of leaf and seed extracts of *Delonix elata* (L.) Gamble (Family: Fabaceae) against malaria (*Anopheles stephensi* Liston) and dengue (*Aedes aegypti* Linn.) (Diptera: Culicidae) vector mosquitoes. *Parasitol Res* 2012; **111**: 65-77.
[30] Aivazi AA, Vijayan VA. Larvicidal activity of oak *Quercus infectoria* Oliv. (Fagaceae) gall extracts against *Anopheles stephensi* Liston. *Parasitol Res* 2009; **104**: 1289-93.
[31] Yadav R, Srivastava VK, Chandra R, Singh A. Larvicidal activity of latex and stem bark of *Euphorbia tirucalli* plant on the mosquito *Culex quinquefasciatus*. *J Commun Dis* 2002; **34**(4): 264-9.
[32] Bansal SK, Singh KV, Kumar S. Larvicidal activity of the extracts from different parts of the plant *Solanum xanthocarpum* against important mosquito vectors in the arid region. *J Environ Biol* 2009; **30**(2): 221-6.
[33] Patil SV, Patil CD, Salunkhe RB, Salunke BK. Larvicidal activities of six plants extracts against two mosquito species, *Aedes aegypti* and *Anopheles stephensi*. *Trop Biomed* 2010; **27**(3): 360-5.
[34] Prajapati SN, Parmar KA. Anti-viral and *in-vitro* free radical scavenging activity of leaves of *Rubia cordifolia*. *Int J Phytomed* 2011; **3**: 98-107.
[35] Siddiqui A, Tajuddin, Amin KM, Zuberi RH, Jamal A. Standardization of Majith (*Rubia cordifolia* Linn.). *Indian J Tradit Know* 2011; **10**(2): 330-3.
[36] Devi Priya M, Siril EA. Pharmacognostic studies on Indian madder (*Rubia cordifolia* L.). *J Pharmacogn Phytochem* 2013; **1**(5): 112-9.