

## Larvicidal efficacy of medicinal plant extracts against *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae)

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**Abstract.** Mosquitoes transmit serious human diseases, causing millions of deaths every year. Natural products of plant origin with insecticidal properties have been used in recent years for control of a variety of pest insects and vectors. The present study was based on assessments of the larvicidal activity to determine the efficacies of hexane, chloroform, ethyl acetate, acetone and methanol extracts of ten medicinal plants tested against fourth instar larvae of malaria vector, *Anopheles stephensi* Liston and lymphatic filariasis vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). The larvicidal activity was assessed by the procedure of WHO with some modification. The highest larval mortality was found in leaf acetone of *Adhatoda vasica*, bark ethyl acetate of *Annona squamosa*, methanol leaf and flower of *Cassia auriculata*, leaf ethyl acetate of *Hydrocotyle javanica*, methanol leaf and seed of *Solanum torvum* and leaf hexane extracts of *Vitex negundo* against the fourth instar larvae of *An. stephensi* and *Cx. quinquefasciatus*. The calculated LC<sub>90</sub> for acetone, ethyl acetate, methanol and hexane extracts of dried leaf and bark of *A. vasica*, *A. squamosa*, *S. torvum*, and *V. negundo* were in the range of 70.38-210.68 ppm. Our results suggest that the leaf methanol extract of *S.torvum* and bark ethyl acetate extract of *A. squamosa* from Southern India have the potential for use to control mosquitoes. Therefore, this study provides the larvicidal activity against *An. stephensi* and *Cx. quinquefasciatus* of plant extracts.

### INTRODUCTION

*Anopheles stephensi* transmits malaria in the plains of rural and urban areas of India. Malaria afflicts 36% of the world population i.e. 2020 million in 107 countries and territories situated in the tropical and subtropical regions. In the South East Asian Region of WHO, out of about 1.4 billion people living in 11 countries, 1.2 billion (85.7%) are exposed to the risk of malaria and most of whom live in India. Of the 2.5 million reported cases in the South East Asia, India alone contributes about 70% of the total cases (Kondrachine, 1992). *Culex quinquefasciatus*, a vector of lymphatic

filariasis and it is widely distributed tropical diseases with around 120 million people infected worldwide and 44 million people have common chronic manifestation (Bernhard *et al.*, 2003)

Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents. Natural products are generally preferred because of their less harmful nature to non-target organisms and due to their innate biodegradability.

Many studies on plant extracts against mosquito larvae have been conducted around the world. The leaf powdered preparation of *Adhatoda vasica* (adhatoda), *Azadirachta indica* (neem) and *Ocimum sanctum* (tulsi), which on burning with charcoal produced smoke that repelled *Armigeres subalbatus* and *Cx. quinquefasciatus* to prevent their biting activity for 6–8 h (Pandian *et al.*, 1995). The seeds petroleum ether extract of *Annona squamosa* showed larvicidal activity against *Aedes aegypti*, *Cx. quinquefasciatus* and *An. stephensi* (George & Vincent, 2005). 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).

*Hemidesmus indicus*, commonly called Indian sarsaparilla is a climbing vine found throughout India which belongs to the family Asclepiadaceae and the extracts showed antibacterial properties (Aqil & Ahmad, 2007). The methanolic extract *Hydrocotyle javanica* exhibited larvicidal activity against *Cx. quinquefasciatus* (Venkatachalam *et al.*, 2001). The toxicity of *S. torvum* extracts was found effective against the early fourth-instar larvae of *Cx. quinquefasciatus* (Rahuman *et al.*, 2008). Acetone, chloroform, ethyl acetate, hexane, and methanol dried leaf, and seed extracts of *S. trilobatum* were tested against the fourth instar larvae of *An. subpictus* and *Cx. tritaeniorhynchus* (Zahir *et al.*, 2009). The oil obtained from leaves of *V. negundo* was evaluated against *Ae. aegypti* (Hebbalkar *et al.*, 1992). In all probability, the plants contained insecticidal phytochemicals that were predominantly secondary compounds produced by plants to protect themselves against herbivorous insects. The effective mosquito larvicidal plant extracts and their isolated compounds are essential to combat increasing resistance rates,

concern for the environment and food safety, the unacceptability and the high cost of synthetic pyrethroids. These studies have exposed an array of botanical insecticides containing a wide spectrum of bioactive insecticides, larvicides and carcinogenic inhibitors.

In the light of earlier literature, it is known that larvicides play a vital role in controlling mosquitoes in their breeding sites, but still vectors resistance to them remains unanswered. In addition they show a negative impact in areas of beneficial and non-target organisms. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal potential of the extracts from the medicinal plant against two medically important species of malaria vector, *An. stephensi* and filarial vector, *Cx. quinquefasciatus*.

## MATERIALS AND METHODS

### *Collection of plant materials*

The leaf of *A. vasica* Nees (Acanthaceae), bark of *A. squamosa* Linn. (Annonaceae), leaf and flower of *C. auriculata* Linn. (Cesalpiniaceae), leaf and root of *H. indicus* R.Br. (Asclepiadaceae), leaf of *H. javanica* Linn. (Apiaceae), leaf of *Papaver somniferum* Linn. (Papaveraceae), *Pavonia zeylanica* Cav. (Malvaceae), *Solanum indicum* Linn. (Solanaceae), *S. torvum* Swartz (Solanaceae), and *V. negundo* Linn. (Verbenaceae) were collected from Javadhu Hills, Tiruvannamalai district (12°36'10N, 078°53'07E, altitude 705 m) and Chitheri Hills, Dharmapuri district (11°53'28"N, 078°30'26"E, altitude 959), Tamil Nadu, India in March 2008 and were authenticated by Dr. C. Hema, Department of Botany, Arignar Anna Govt. Arts College for Women, Walajapet, Vellore, India. Voucher specimens have been deposited in the laboratory of Zoology, C. Abdul Hakeem College, Melvisharam.

### **Preparation of stock solution of plant extracts**

The dried (7-10 days in the shade at the environmental temperatures, 27-37°C day time) stem bark (750g), leaf (550g), flower (450g) and seed (600g) were powdered mechanically using commercial electrical stainless steel blender (Model:ICB-500. 1.5L Capacity, 6 angle blades, 2 speed w/pulse and made of Stainless Steel Blade. Libra Appliances pvt.ltd, Baroda, India) and extracted with hexane 95% 1400 ml, (Fine chemical manufactures Mumbai, India), chloroform 99% 2800 ml, (SRL-Sisco Research Laboratories pvt.ltd, Mumbai), ethyl acetate 98% 3100 ml, (Qualigens Thermo Electron LLS India pvt. Ltd, Navi Mumbai), acetone 99% 1600 ml, (Qualigens Thermo Electron LLS India pvt. Ltd, Navi Mumbai) and methanol 99.5% 4400ml, (Qualigens GlaxoSmithKline pharmaceuticals limited, Mumbai) in a soxhlet apparatus (boiling point range 60–80°C) for 8 h. The extract was concentrated under reduced pressure 22 – 26 mm Hg at 45°C and the residue obtained was stored at 4°C. One gram of crude extract was first dissolved in 100 ml of acetone (stock solution). From the stock solution, 400 – 1.25 ppm were prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05% in the final test solution. The control was set up with acetone and polysorbate 80.

### **Mosquito culture**

*Anopheles stephensi* and *Cx. quinquefasciatus* larvae were collected from stagnant water area of Melvisharam (12° 56' 23" N, 79° 14' 23" E) and identified Dr. V. Rajagopal, Senior Entomologist, Zonal Entomological Research Centre, Vellore (12° 55' 48" N, 79° 7' 48" E), Tamil Nadu, to start the colony, and larvae were kept in plastic and enamel trays containing tap water. They were maintained, and the experiments were carried out, at 27 ± 2°C and 75–85% relative humidity under 14:10 light and dark cycles. Larvae were fed a diet of Brewers yeast, dog biscuits and algae collected from ponds in a ratio of

3:1:1, respectively. Pupae were transferred from the trays to a cup containing tap water and were maintained in our insectary (45 x 45 x 40cm) where adults emerged. Adults were maintained in glass cages and continuously provided with 10% sucrose solution in a jar with a cotton wick. On day five, the adults were given a blood meal from a pigeon placed in resting cages overnight for blood feeding by females. Glass petridishes with 50ml of tap water lined with filter paper were kept inside the cage for oviposition (Kamaraj *et al.*, 2009).

### **Larvicidal bioassay**

During screening in the laboratory trial, the larvae of *An. stephensi* and *Cx. quinquefasciatus* were collected from the insect rearing cage and identified in Zonal Entomological Research Centre, Vellore. One gram of crude extract was first dissolved in 100 ml of acetone (stock solution). From the stock solution, 1000 ppm was prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05% in the final test solution. The larvicidal activity was assessed by the procedure of WHO (1996) with some modification and as per the method of Rahuman *et al.* (2000). For bioassay test, larvae were taken in five batches of twenty in 249 ml of water and 1.0 ml of the desired plant extract concentration. The control was set up with acetone and polysorbate 80. The numbers of dead larvae were counted after 24 hrs of exposure and the percentage mortality was reported from the average of five replicates. The control was set up with acetone, polysorbate 80 and distilled water. The experimental media, in which 100% mortality of larvae occurs alone, were selected for a dose response bioassay.

### **Dose - response bioassay**

From the stock solution, different concentrations ranging from 1.25 to 400 ppm were prepared. Based on the screening results, crude different solvent leaf, flower, seed, bark, and root extracts of *A. vasica*, *A. squamosa*, *C. auriculata*, *H.*

*indicus*, *H. javanica*, *P. somniferum*, *P. zeylanica*, *S. indicum*, *S. torvum*, and *V. negundo* were subjected to dose response bioassay for larvicidal activity against the larvae of *An. stephensi* and *Cx. quinquefasciatus*. The numbers of dead larvae were counted after 24 hrs of exposure, and the percentage mortality was reported from the average of five replicates. However, at the end of 24 hrs the selected test samples turned out to be equal in their toxic potential.

### Statistical analysis

The average larval 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 software developed (Reddy *et al.*, 1992). Results with  $p < 0.05$  were considered to be statistically significant.

## RESULTS AND DISCUSSION

The screening is a better means of evaluation of the potential larvicidal activity of plants popularly used for this purpose. The effect of the leaf, flower, bark and root hexane, chloroform, ethyl acetate, acetone and methanol extracts of *A. vasica*, *A. squamosa*, *C. auriculata*, *H. indicus*, *H. javanica*, *P. somniferum*, *P. zeylanica*, *S. indicum*, *S. torvum*, and *V. negundo* were tested at 1000 ppm and showed activity against the fourth instar larvae of *An. stephensi* and *Cx. quinquefasciatus* (Table 1). All plant extracts showed moderate larvicidal effects after 24 h; however, the highest larval mortality was found in leaf acetone of *An. vasica*, bark ethyl acetate of *An. squamosa* against the fourth instar larvae of *An. stephensi* ( $LC_{50} = 18.20$  and  $25.18$  ppm;  $LC_{90} = 96.33$  and  $94.04$  ppm) and against the fourth instar larvae of *Cx. quinquefasciatus* ( $LC_{50} = 27.24$  and  $20.56$  ppm;  $LC_{90} = 70.38$  and  $82.99$  ppm) (Table 2).

Nowadays, mosquito control is mostly directed against larvae and only against adults when necessary. This is because the fight against adult is temporary, unsatisfactory and pollutes the environment, while larval treatment is more localized in time and space resulting in less-dangerous outcomes. Larval control can be an effective control tool due to the low mobility of larval mosquitoes, especially where the principal breeding habitats are man-made and can be easily identified (Howard *et al.*, 2007).

The screening of plant extract is a better means of evaluation of the potential parasitic activity of plants popularly used for this purpose. The mode of treatment and observations concerning mosquito larvicidal activity are given in Tables 1 and 2. It is evident from our results that a rise in the concentration of plant extracts was the main cause of mortality in *An. stephensi* and *Cx. quinquefasciatus* larvae. In the present observation the leaf acetone extracts of *A. vasica* and bark ethyl acetate extract of *A. squamosa* showed the  $LC_{50}$  values of 18.20, 28.18, 27.24 and 43.07 ppm against *An. stephensi* and *Cx. quinquefasciatus* respectively.

The obtained results revealed the larvicidal effect of ten plants corresponding to different botanical families on *An. stephensi* and *Cx. quinquefasciatus*. The highest larval mortality was found in leaf acetone and methanol of *Canna indica* ( $LC_{50} = 29.62$  and  $40.77$  ppm;  $LC_{90} = 148.55$  and  $165.00$  ppm) against second instar larvae ( $LC_{50} = 121.88$  and  $69.76$ , ppm;  $LC_{90} = 624.35$  and  $304.27$  ppm) and against fourth instar larvae of methanol and petroleum ether extracts of *Ipomoea carnea* ( $LC_{50} = 41.82$  and  $39.32$  ppm;  $LC_{90} = 423.76$  and  $176.39$  ppm) against second instar larvae ( $LC_{50} = 163.81$  and  $41.75$  ppm;  $LC_{90} = 627.38$  and  $162.63$  ppm) and against fourth instar larvae of *Cx. quinquefasciatus*, respectively (Rahuman *et al.*, 2009). Kihampa *et al.* (2009) reported that the stem bark petroleum ether, chloroform and methanol crude extracts of *A. squamosa* showed activity against

III/IV instar larvae of *Anopheles gambiae* with LC<sub>50</sub> values of 50, 17 and 24 ppm respectively. Previous studies have shown that the alkaloids isolated from *A. squamosa* have shown larvicidal growth-regulating activities against *An. stephensi* at concentrations of 50 to 200 ppm and the mortality in the larvae, pupae and adults produced about a 52-92% decrease in the laboratory experiment (Saxena *et al.*, 1993). The secondary compounds of *An. squamosa* are a vast repository of compounds with a wide range of biological activities, such as palmitone and isomeric hydroxy ketones Shanker *et al.* (2007), annonaceous acetogenins Hopp *et al.*

(1998) were well documented. Therefore, the observed larvicidal activity of the stem bark extract was considered to have been exerted by such compounds.

The results from these trials indicate that the flower methanol extract of *C. auriculata* with LC<sub>50</sub> values of 33.74 and 51.22 ppm against *An. stephensi* and *Cx. quinquefasciatus*.

The ethanolic leaf extract of *Cassia obtusifolia* had significant larvicidal effect against *An. stephensi* with LC<sub>50</sub> and LC<sub>90</sub> values were 52.2 and 108.7 mg/l, respectively (Rajkumar & Jebanesan, 2009) and the emodin compound was isolated from seeds and showed the LC<sub>50</sub> values of

Table 1. Larvicidal activity of crude plant extracts against fourth instar larvae of *Anopheles stephensi* and *Culex quinquefasciatus* at 1000 ppm

Botanical names	Plant Parts	Species	% Mortality * ± SD				
			HE	CH	EA	AC	ME
<i>Adhatoda vasica</i>	LS	<i>A. stephensi</i>	78±2.824	88±2.742	71±3.051	100±0.000	100±0.000
		<i>C. quinquefasciatus</i>	78±2.126	91±2.144	62±2.169	100±0.000	96±2.842
<i>Annona squamosa</i>	BK	<i>A. stephensi</i>	84±2.280	100±0.000	68±3.049	79±0.577	81±1.924
		<i>C. quinquefasciatus</i>	81±2.588	100±0.000	82±1.140	75±1.581	80±1.673
<i>Cassia auriculata</i>	LS	<i>A. stephensi</i>	77±2.702	87±1.140	85±1.581	78±2.408	100±0.000
		<i>C. quinquefasciatus</i>	87±1.140	85±1.581	74±1.304	76±2.863	100±0.000
	FL	<i>A. stephensi</i>	69±1.789	79±2.588	81±1.924	83±1.957	100±0.000
		<i>C. quinquefasciatus</i>	82±1.172	80±1.581	75±2.915	73±2.766	100±0.000
<i>Hemidesmus indicus</i>	LS	<i>A. stephensi</i>	42±2.302	64±2.716	89±2.025	90±1.672	100±0.000
		<i>C. quinquefasciatus</i>	53±1.601	44±1.624	72±1.462	100±0.000	100±0.000
	RT	<i>A. stephensi</i>	74±2.842	82±3.422	100±0.000	94±2.264	88±3.262
		<i>C. quinquefasciatus</i>	84±3.262	76±2.354	96±2.524	88±3.269	92±2.846
<i>Hydrocotyle javanica</i>	LS	<i>A. stephensi</i>	79±2.147	69±2.212	100±0.000	68±2.274	88±1.517
		<i>C. quinquefasciatus</i>	72±2.322	71±2.375	100±0.000	78±2.363	75±2.333
<i>Papaver somniferum</i>	LS	<i>A. stephensi</i>	42±2.570	66±1.638	80±3.462	34±3.358	43±2.563
		<i>C. quinquefasciatus</i>	31±2.023	40±2.426	74±2.949	54±1.452	45±1.563
<i>Pavonia zeylanica</i>	LS	<i>A. stephensi</i>	93±2.129	67±3.135	82±2.624	96±3.526	100±0.000
		<i>C. quinquefasciatus</i>	92±2.628	82±1.681	74±2.652	100±0.000	100±0.000
<i>Solanum indicum</i>	LS	<i>A. stephensi</i>	22±1.342	48±1.615	47±1.703	65±2.949	85±3.272
		<i>C. quinquefasciatus</i>	67±1.629	53±2.483	64±2.824	75±2.487	93±3.224
<i>Solanum torvum</i>	LS	<i>A. stephensi</i>	72±1.140	84±1.924	87±2.074	85±1.581	100±0.000
		<i>C. quinquefasciatus</i>	80±2.236	78±2.408	74±1.924	83±1.517	100±0.000
	SD	<i>A. stephensi</i>	84±2.280	73±2.966	82±2.408	87±2.074	100±0.000
		<i>C. quinquefasciatus</i>	78±2.302	66±1.861	73±1.844	86±0.837	100±0.000
<i>Vitex negundo</i>	LS	<i>A. stephensi</i>	100±0.000	81±0.837	79±2.280	80±1.663	83±2.014
		<i>C. quinquefasciatus</i>	100±0.000	75±1.647	80±1.581	70±1.846	78±2.119

LS=Leaves; BK=Bark; FL=Flower; RT=Root; SD=Seed; Control= Nil mortality.

\* Mean value of five replicates. HE=Hexane; CH=Chloroform; EA =Ethyl acetate; AC=Acetone; ME = Methanol.

Table 2. Larvicidal activity of different solvent crude extracts against fourth instar larvae of *Anopheles stephensi* and *Culex quinquefasciatus*

Plant species	Plant Parts	Solvents	Species	LC <sub>50</sub> (ppm)	(UCL-LCL)	LC <sub>90</sub> (ppm)	(UCL-LCL)	Slope±SE	χ <sup>2</sup> (df = 4)
<i>Adhatoda vasica</i>	LS	Acetone	<i>A. stephensi</i>	18.20	(22.11-14.28)	96.33	(131.75-60.91)	4.881±2.521	9.04
			<i>C. quinquefasciatus</i>	27.24	(31.29-23.17)	70.38	(87.49-53.25)	1.325±0.211	4.16
<i>Annona squamosa</i>	BK	Methanol	<i>A. stephensi</i>	41.42	(49.46-33.38)	192.77	(256.36-129.17)	1.827±0.732	11.27
			<i>C. quinquefasciatus</i>	25.18	(28.34-22.03)	94.04	(112.27-75.81)	2.157±0.584	5.93
<i>Cassia auriculata</i>	LS	Methanol	<i>C. quinquefasciatus</i>	43.07	(48.62-37.53)	172.27	(208.22-136.31)	2.680±0.872	13.63
			<i>A. stephensi</i>	43.66	(49.13-38.18)	164.70	(197.06-132.34)	3.839±0.475	10.28
<i>Hemidesmus indicus</i>	FL	Methanol	<i>C. quinquefasciatus</i>	58.30	(65.41-51.19)	210.69	(252.26-169.13)	7.678±0.652	12.2
			<i>A. stephensi</i>	33.74	(38.03-29.46)	133.07	(160.23-105.92)	4.629±0.258	8.54
<i>Solanum torvum</i>	LS	Methanol	<i>C. quinquefasciatus</i>	51.22	(5782-44.61)	205.12	(248.58-61.67)	5.816±2.365	5.48
			<i>A. stephensi</i>	108.50	(124.72-96.14)	646.36	(743.15-579.63)	7.198±1.585	9.61
<i>Hydrocotyle javanica</i>	RT	Ethyl acetate	<i>A. stephensi</i>	155.19	(219.94-106.45)	607.86	(726.04-489.69)	4.650±0.698	11.46
			<i>C. quinquefasciatus</i>	192.83	(224.31-174.35)	760.01	(955.16-605.24)	4.289±1.332	10.80
<i>Pavonia zeylanica</i>	LS	Acetone	<i>A. stephensi</i>	217.61	(234.07-87.15)	784.16	(985.16-645.64)	4.182±0.582	12.89
			<i>C. quinquefasciatus</i>	88.28	(109.05-57.55)	560.75	(678.92-484.84)	3.907±0.852	7.46
<i>Vitex negundo</i>	SD	Hexane	<i>C. quinquefasciatus</i>	96.72	(126.92-74.51)	303.20	(420.79-215.62)	6.512±0.678	13.82
			<i>A. stephensi</i>	348.62	(398.64-268.63)	1497.09	(1782.43-1011.72)	5.079±0.563	9.49
<i>Solanum torvum</i>	LS	Methanol	<i>A. stephensi</i>	142.63	(185.54-69.11)	495.82	(584.00-336.46)	4.063±0.254	10.72
			<i>C. quinquefasciatus</i>	192.84	(272.99-82.79)	565.40	(746.54-454.28)	5.093±2.874	10.57
<i>Vitex negundo</i>	LS	Methanol	<i>A. stephensi</i>	29.65	(33.70-25.61)	133.01	(102.71-103.32)	2.547±0.421	11.72
			<i>C. quinquefasciatus</i>	20.56	(23.25-17.87)	82.99	(100.18-65.79)	3.599±0.365	7.36
<i>Vitex negundo</i>	LS	Hexane	<i>A. stephensi</i>	31.30	(35.42-27.17)	130.65	(157.20-102.89)	4.798±1.235	4.75
			<i>C. quinquefasciatus</i>	25.60	(28.96-22.24)	105.52	(127.45-83.59)	5.998±0.842	5.82
<i>Vitex negundo</i>	LS	Hexane	<i>A. stephensi</i>	33.83	(38.03-29.47)	127.47	(152.53-102.40)	5.502±0.541	7.65
			<i>C. quinquefasciatus</i>	56.981	(64.06-49.91)	210.68	(251.96-169.41)	4.763±2.365	9.26

LS=Leaves; BK=Bank; FL=Flower; RT=Root; SD=Seed; Control = Nil mortality. Significant at P< 0.05 level.LC<sub>50</sub>= Lethal concentration that kills 50% of the exposed larvae; LC<sub>90</sub> =Lethal concentration that kills 90% of the exposed larvae; UCL=Upper confidence Limit; LCL=Lower confidence Limit; χ<sup>2</sup> =Chi-square; df = degree of freedom.

1.4, 1.9, and 2.2 mg/L against *Culex pipiens pallens*, *Ae. aegypti*, and *Aedes togoi*, respectively (Yang *et al.*, 2003). Compared with earlier authors report, our results revealed that the experimental plant extracts were effective to control *An. stephensi* and *Cx. quinquefasciatus*.

Larval mortality was 100% with the use of 5% concentration of root extract of *H. indicus*, leaves extracts of *Gymnema sylvestre* and *Eclipta prostrata* after 2 days (Khanna & Kannabiran, 2007). 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<sub>50</sub> values of 2214.7 and 5362.6 ppm, respectively (Vahitha *et al.*, 2002). The peel methanol extract of *Citrus sinensis* and the leaf and flower ethyl acetate extracts of *Ocimum canum* were tested against the larvae of *An. stephensi* (LC<sub>50</sub> = 95.74, 101.53, 28.96, LC<sub>90</sub> = 303.20, 492.43 and 168.05 ppm), respectively (Kamaraj *et al.*, 2008). The present findings revealed that the methanol extract of *S. torvum* was less active compared with the petroleum ether extracts of *S. xanthocarpum*.

Karunamoorthi *et al.* (2008) reported that the petroleum ether (60-80 degrees C) extracts of the leaves of *V. negundo* were evaluated for larvicidal activity against larval stages of *C. tritaeniorhynchus* in the laboratory with LC<sub>50</sub> and LC<sub>90</sub> values of 2.4883 and 5.1883 mg/l, respectively. The methanol leaf extracts of *V. negundo*, *V. trifolia*, *V. peduncularis* and *V. altissima* possessed varying levels of larvicidal activity on *Cx. quinquefasciatus* and *An. stephensi* and found with LC<sub>50</sub> value of 212.57, 41.41, 76.28 and 128.04 ppm respectively (Pushpalatha & Muthukrishnan, 1995).

The results of this study indicate that the plant extracts would likely to be toxic to the target species tested at the concentrations necessary for controlling the fourth instar mosquito larvae. An attempt has been made to evaluate the toxic effect of plant extracts against *An. stephensi* and *Cx. quinquefasciatus* larvae. Our results suggest that the leaf

methanol extract of *S. torvum* and bark ethyl acetate extract of *A. squamosa* have the potential for use to control mosquitoes. Further studies are in progress to evaluate the effect of purified extract on larvicidal activity. The purified plant metabolite of the leaf methanol extracts of *S. torvum* and bark ethyl acetate extract of *A. squamosa* may be used as environment friendly and sustainable insecticides to combat mosquitoes.

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## REFERENCES

- Aqil, F. & Ahmad, I. (2007). Antibacterial properties of traditionally used Indian medicinal plants. *Methods and Findings in Experimental and Clinical Pharmacology* **29**: 79–92.
- Bernhard, L., Bernhard, P. & Magnussen, P. (2003). Management of patients with lymphoedema caused by filariasis in north-eastern Tanzania: alternative approaches. *Physiotherapy* **89**: 743–749.
- George, S. & Vincent, S. (2005). Comparative efficacy of *Annona squamosa* Linn and *Pongamia glabra* Vent to *Azadirachta indica* A juss against mosquitoes. *Journal of Vector Borne Disease* **42**: 159–163.
- Hebbalkar, D.S., Hebbalkar, G.D., Sharma, R.N., Joshi, V.S. & Bhat, V.S. (1992). Mosquito repellent activity of oils from *Vitex negundo* Linn leaves. *Indian Journal of Medicinal Research* **95**: 200–203.
- Hopp, D., Alali, F.Q., Gu, Z.M. & McLaughlin, J.L. (1998). Three new bioactive bis-adjacent THF-ring acetogenins from the bark of *Annona squamosa*. *Bio Organic and Medicinal Chemistry* **6**: 569–575.

- Howard, A.F.B., Zhou, G. & Omlin, F.X. (2007). Malaria mosquito control using edible fish in western Kenya: preliminary findings of a controlled study. *BMC Public Health* **7**: 199–204.
- Kamaraj, C., Bagavan, A., Rahuman, A.A., Zahir, A.A., Elango, G. & Pandiyan, G. (2009). Larvicidal potential of medicinal plant extracts against *Anopheles subpictus* Grassi and *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). *Parasitology Research* **104**: 1163–1171.
- Kamaraj, C., Rahuman, A.A. & Bagavan, A. (2008). Screening for antifeedant and larvicidal activity of plant extracts against *Helicoverpa armigera* (Hübner), *Sylepta derogata* (F.) and *Anopheles stephensi* (Liston). *Parasitology Research* **103**: 1361–1368.
- Karunamoorthi, K., Ramanujam, S. & Rathinasamy, R. (2008). Evaluation of leaf extracts of *Vitex negundo* L (Family: Verbenaceae) against larvae of *Culex tritaeniorhynchus* and repellent activity on adult vector mosquitoes. *Parasitology Research* **103**: 545–550.
- Khanna, V.G. & Kannabiran, K. (2007). Larvicidal effect of *Hemidesmus indicus*, *Gymnema sylvestre*, and *Eclipta prostrata* against *Culex quinquefasciatus* mosquito larvae. *African Journal of Biotechnology* **6**: 307–311.
- Kihampa, C., Joseph, C.C., Nkunya, M.H., Magesa, S.M., Hassanali, A., Heydenreich, M. & Kleinpeter, E. (2009). Larvicidal and IGR activity of extract of Tanzanian plants against malaria vector mosquitoes. *Journal of Vector Borne Disease* **46**: 145–152.
- Kondrachine, A.V. (1992). Malaria in WHO Southeast Asia Region. *Indian Journal of Malaria Research* **29**: 129–160.
- Pandian, R.S., Manoharan, A.C. & Pandian, R.S. (1995). Herbal smoke a potential repellent and adulticide for mosquitoes. *Insect Environment* **1**: 14–15.
- Pushpalatha, E. & Muthukrishnan, J. (1995). Larvicidal activity of a few plant extracts against *Culex quinquefasciatus* and *Anopheles stephensi*. *Indian Journal of Malaria Research* **32**: 14–23.
- Rahuman, A.A., Bagavan, A., Kamaraj, C., Saravanan, E., Zahir, A.A. & Elango G. (2009). Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitology Research* **104**: 1365–1372.
- Rahuman, A.A., Gopalakrishnan, G., Ghouse, B.S., Arumugam, S. & Himalayan, B. (2000). Effect of *Feronia limonia* on mosquito larvae. *Fitoterapia* **71**: 553–555.
- Rahuman, A.A., Gopalakrishnan, G., Venkatesan, P. & Geetha K. (2008). Isolation and identification of mosquito larvicidal compound from *Abutilon indicum* (Linn) Sweet. *Parasitology Research* **102**: 981–988.
- Rajkumar, S. & Jebanesan, A. (2009). Larvicidal and oviposition activity of *Cassia obtusifolia* Linn (Family: Leguminosae) leaf extract against malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitology Research* **104**: 337–340.
- Reddy, P.J., Krishna, D., Murthy, U.S. & Jamil, K. (1992). A microcomputer FORTRAN program for rapid determination of lethal concentration of biocides in mosquito control. *CABIOS* **8**: 209–213.
- Saxena, R.C., Harshan, V., Saxena, A., Sukumaran, P., Sharma, M.C. & Kumar, M.L. (1993). Larvicidal and chemosterilant activity of *Annona squamosa* alkaloids against *Anopheles stephensi*. *Journal of the American Mosquito Control Association* **9**: 84–87.
- Shanker, K.S., Kanjilal, S., Rao, B.V., Kishore, K.H., Misra, S. & Prasad, R.B. (2007). Isolation and antimicrobial evaluation of isomeric hydroxy ketones in leaf cuticular waxes of *Annona squamosa*. *Phytochemical Analysis* **18**: 7–12.

- Vahitha, R., Venkatachalam, M.R., Murugan, K. & Jebanesan, A. (2002). Larvicidal efficacy of *Pavonia zeylanica* L. and *Acacia ferruginea* D.C. against *Culex quinquefasciatus* Say. *Bioresource Technology* **82**: 203–204.
- Venkatachalam, M.R. & Jebanesan, A. (2001). Larvicidal activity of *Hydrocotyl javanica* Thumb. (Apiaceae) extract against *Culex quinquefasciatus*. *Journal of Experimental Zoology* **4**: 99–101.
- WHO. (1996). Report of the WHO informal consultation on the evaluation on the testing of insecticides. Geneva: World Health Organization CTD/WHO PES/IC/ p.961–969.
- Yang, Y.C., Lim, M.Y. & Lee, H.S. (2003). Emodin isolated from *Cassia obtusifolia* (Leguminosae) seed shows larvicidal activity against three mosquito species. *Journal of Agricultural and Food Chemistry* **51**: 7629–7631.
- Zahir, A.A., Rahuman, A.A., Kamaraj, C., Bagavan, A., Elango, G., Sangaran, A. & Kumar, B.S. (2009). Laboratory determination of efficacy of indigenous plant extracts for parasites control. *Parasitology Research* **105**: 453–461.