Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

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

Document heading doi:

Evaluation of larvicidal activity of medicinal plant extracts against three mosquito vectors

A Bagavan, A Abdul Rahuman*

Unit of Nanotechnology and Bioactive Natural Products, Post Graduate and Research Department of Zoology, C. Abdul Hakeem College, Melvisharam – 632 509, Vellore District, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 19 October 2010
Received in revised form 27 October 2010
Accepted 15 December 2010
Available online 20 January 2011

Keywords:
Anopheles vagus
Armigeres subalbatus
Culex vishnui
Plant extracts
Larvicidal activity

ABSTRACT

Objective: To evaluate the mosquito larvicidal activity of plant extracts. **Methods:** The hexane, chloroform, ethyl acetate, acetone, and methanol leaf, flower and seed extracts of *Abrus precatorius (A. precatorius), Croton bonplandianum (C. bonplandianum), Cynodon dactylon (C. dactylon), Musa paradisiaca (M. paradisiaca)* and *Syzygium aromaticum (S. aromaticum)* were tested against fourth instar larvae of *Anopheles vagus (An. vagus), Armigeres subalbatus (Ar. subalbatus)* and *Culex vishnui (Cx. vishnui)*. **Results:** The highest larval mortality was found in seed ethyl acetate extracts of *A. precatorius* and leaf extracts of *C. bonplandianum*, flower chloroform and methanol extracts of *M. paradisiaca*, and flower bud hexane extract of *S. aromaticum* against *An. vagus* with LC_{50} values of 19.31, 39.96, 35.18, 79.90 and 85.90 μg/mL; leaf ethyl acetate and methanol extracts of *C. dactylon*, flower methanol extract of *M. paradisiaca*, flower bud methanol extract of *S. aromaticum* against *Ar. subalbatus* with LC_{50} values of 21.67, 32.62, 48.90 and 78.28 μg/mL, and seed methanol of *A. precatorius*, flower methanol extract of *M. paradisiaca*, flower bud hexane extract of *S. aromaticum* against *Cx. vishnui* with LC_{50} values of 136.84, 103.36 and 149.56 μg/mL, respectively. **Conclusions:** These results suggest that the effective plant crude extracts have the potential to be used as an ideal ecofriendly approach for the control of disease vectors. This study provides the first report on the larvicidal activity of crude solvent extracts of different mosquitoes.

1. Introduction

Mosquitoes are responsible for the spread of more diseases than any other group of arthropods. Mosquito-borne diseases still remain a major health problem in both human and veterinary sectors. Diseases transmitted by mosquitoes include malaria, dengue hemorrhagic fever, japanese encephalitis, yellow fever and filariasis. One to two million deaths are reported annually due to malaria worldwide. Lymphatic filariasis affects at least 120 million people in 73 countries in Africa, India, Southeast Asia, and Pacific Islands. These diseases not only cause high levels of morbidity and mortality but also inflict great economic loss and social disruption on developing countries such as India, China, etc. India alone contributes around 40% of global filariasis burden and the estimated annual economic loss is about 720 corers[1]. The annual incidence and mortality estimates for Japanese encephalitis (JE) are 30 000–50 000 and 10 000, respectively[2]. The use of conventional pesticides in the water sources, however, introduces many risks to people and/or the environment. Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors[3]. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat. Many researchers have reported on the effectiveness of plant extracts against mosquito larvae[4,5].

Anopheles vagus (*An. vagus*) is a zoophilic species, which was more often collected in human–landing captures during the rainy season. The rainy season may be important in malaria transmission due to high biting populations[6,7]. A vector species that employs a unique, robust immune response against an invading pathogen is the *Armigeres subalbatus (Ar. subalbatus)*, a natural vector of the nematode parasites that cause lymphatic filariasis. It also serves as a

*Corresponding author: Dr. A. Abdul Rahuman, Associate Professor, Unit of Nanotechnology and Bioactive Natural Products, Post Graduate and Research Department of Zoology, C. Abdul Hakeem College, Melvisharam – 632 509, Vellore District, Tamil Nadu, India.
Tel: +91 94423 10155
Fax: +91 04172 269487
E-mail: abdulrahuman6@hotmail.com

competent laboratory vector of *Plasmodium gallinaceum* (*P. gallinaceum*), the causative agent of avian malaria in Asia[8] and also has been implicated in the transmission of JE virus in Taiwan[9,10]. *Culex vishnui (Cx. vishnui)* group to monitor JE virus activity in endemic areas of Tamil Nadu, southern India[11]. The *Cx. vishnui* subgroup mosquitoes comprising *Culex tritaeniorhynchus (Cx. tritaeniorhynchus), Cx. vishnui* and *Culex pseudovishnui (Cx. pseudovishnui)* have been implicated as major vectors of JE in India[12].

Many studies on plant extracts against mosquito larvae have been conducted around the world. *Abrus precatorius* (*A. precatorius*) locally known as "Kundu mani," is a slender woody vine plant.

Croton bonplandianum (C. bonplandianum) is a plant of considerable medicinal importance[13, 14]. The bioassay results showed that the essential oil of *Croton regelianus (C. regelianus)* and the isolated compound strongly effective against *Aedes aegypti (Ae. aegypti)*[15], and the essential oil obtained from stalks and leaves of *Croton argyrophylloides (C. argyrophylloides)*, *Croton nepetaefolius (C. nepetaefolius), Croton sonderianus (C. sonderianus)* and *Croton zehntneri (C. zehntneri)* were evaluated against *Aedes aegypti (Ae. aegypti) larvae*[16]. *Cynodon dactylon* (*C. dactylon)* infusions was effective attractant to ovipositing female mosquitoes of *Culex quinquefasciatus (Cx. quinquefasciatus), Culex nigripalpus (Cx. nigripalpus)*, and *Culex erraticus (Cx. Erraticus)*[17].

Musa paradisiacal (*M. paradisiacal)* (banana), is a perennial tree like herb widely distributed in moist tropics. Due to enriched food value and versatile medicinal value, banana is one of the most important fruits and vegetable crops of India. The leaves methanol and 95% ethanol extracts of *M. paradisiaca* were tested against the III instar larvae of *Anopheles stephensi (An. stephensi)* and L4 larvae of *Ae. aegypti*, respectively[18,19]. *Syzyium aromaticum* (*S. aromaticum)* (clove) undiluted oils was the most effective and provided 2 h of complete repellency against *Cx. quinquefasciatus* and *Anopheles dirus (An. dirus)*[20] and the methanol and ether extracts showed complete inhibition of adult emergence at 200 and 600 ppm, respectively against *Culex pipiens (Cx. pipiens)*[21].

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 view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess in the present communication, an attempt has been made to evaluate the larvicidal efficacy of the extracts from the medicinal plant against three medically important species of *Anopheles vagus (An. vagus)*, *Armigeres subalbatus (Ar. subalbatus*) and *Cx. vishnui*.

2. Materials and methods

2.1. Collection of plant materials

The seed of *A. precatorius* L. (Fabaceae), leaf of *C. bonplandianum* Baill. (Euphorbiaceae), *C. dactylon* L. (Poaceae), flower of *M. paradisiaca* L. (Musaceae), *S. aromaticum* L. Merr. et Perry (Myrtaceae) were collected from Javadhu Hills, Tiruvannamalai district and Nilgiri Mountain, Nilgiri district, Tamil Nadu, South India in February 2010. The plants 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.

2.2. Preparation of plant extracts

The dried (7-15 days in the shade at the environmental temperatures, 27-37 ℃ day time) leaf (500 g), flower (400 g) and seed (550 g) were powdered mechanically using commercial electrical stainless steel blender and extracted with hexane (1500 mL, Fine), chloroform (2 000 mL, Fine), ethyl acetate (2 800 mL, Qualigens), acetone (1 800 mL, Qualigens) and methanol (4 000 mL, Qualigens) in a soxhlet apparatus (boiling point range 60-80 ℃) for 8 h. The extract was concentrated under reduced pressure of 22-26 mmHg at 45 ℃ and the residue obtained was stored at 4 ℃. One gram of crude extract was first dissolved in 100 mL of acetone (stock solution). From the stock solution, 1000 - 3.125 μg/mL 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, dechlorinated tap water and polysorbate 80.

2.3. Mosquito culture

An. vagus larvae were collected from the rice fields, open muddy pools and ditches, *Ar. subalbatus* breeding in open and uncovered septic tanks and *Cx. vishnui* larvae were collected from rice fields stagnant water area of Melvisharam and identified Dr. V. Rajagopal, Senior Entomologist, Zonal Entomological Research Centre, Vellore, Tamil Nadu. To start the colony, the larvae were kept in plastic and enamel trays containing tap water. They were maintained at (27 ±2) ℃ 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 [(45×45×40) cm] 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. Glass petridishes with 50 mL of tap water lined with filter paper were kept inside the cage for oviposition[22].

2.4. Larvicidal bioassay

During screening with the laboratory trial, the fourth instar larvae of *An. vagus, Ar. subalbatus* and *Cx. vishnui* 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, 1 000 μg/mL 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[23] with some

modification[24]. 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 h of exposure and the percentage mortality was reported from the average of five replicates. The control was set up with acetone, dechlorinated tap water and polysorbate 80. The experimental media, in which 100% mortality of larvae occurs alone, were selected for a dose response bioassay.

2.5. Dose – response bioassay

From the stock solution, different concentrations ranging from 3.125 to 1 000 μg/mL were prepared. Based on the screening results, crude different solvent leaf, flower and seed extracts of *A. precatorius*, *C. bonplandianum*, *C. dactylon*, *M. paradisiaca*, and *S. aromaticum* were subjected to dose response bioassay for larvicidal activity against the larvae of *An. vagus*, *Ar. subalbatus* and *Cx. vishnui*. The numbers of dead larvae were counted after 24 h of exposure, and the per cent mortality was reported from the average of five replicates. However, at the end of 24 h the selected test samples turned out to be equal in their toxic potential.

3. Results

The preliminary screening is a good mean of evaluation of the potential larvicidal activity of plants popularly used for this purpose. The effect of the leaf, flower and seed hexane, chloroform, ethyl acetate, acetone and methanol extracts of *A. precatorius*, *C. bonplandianum*, *C. dactylon*, *M. paradisiaca*, *S. aromaticum* were tested at 1 000 μg/mL and showed activity against the fourth instar larvae of *An. vagus*, *Ar. subalbatus* and *Cx. vishnui* (Table 1). All plant extracts showed moderate larvicidal effects after 24 h; however, the highest larval mortality was found in seed ethyl acetate, acetone and methanol extracts of *A. precatorius*, leaf ethyl acetate extract of *C. bonplandianum*, leaf ethyl acetate and methanol extracts of *C. dactylon*, flower chloroform, acetone and methanol of *M. paradisiaca* and flower bud hexane extracts of *S. aromaticum* against the fourth instar larvae of *An. vagus* (LC_{50} = 19.31, 191.58, 90.69,39.96, 68.12, 41.13, 35.18, 119.33, 79.90 and 85.90 μg/mL; LC_{90} = 71.71,843.10, 372.15, 218.14, 241.46, 183.83, 311.55, 748.50, 381.93 and 408.89 μg/mL), seed ethyl acetate, acetone and methanol of *A. precatorius*, leaf ethyl acetate and methanol of *C. dactylon*, flower chloroform, acetone and methanol of *M. paradisiaca*, flower bud hexane, ethyl acetate and methanol extracts of *S. aromaticum* against the fourth instar larvae of *Ar. subalbatus* (LC_{50} = 93.94, 99.19, 88.72, 21.67, 32.62, 89.42, 167.19, 48.90,141.53, 48.90 and 78.28 μg/mL; LC_{90} = 413.27, 486.35, 662.61, 98.34, 267.21, 327.30, 1071.41, 187.82, 519.71, 187.82 and 641.32 μg/mL), seed methanol of *A. precatorius*, flower methanol of *M. paradisiaca*, flower bud hexane extract of *S. aromaticum* against the fourth instar larvae of *Cx. vishnui* (LC_{50} = 136.84, 103.36 and 149.56 μg/mL; LC_{90} = 1248.23, 340.44 and 995.56 μg/mL), respectively. Chi-square value was significant at $P< 0.05$ level (Table 2).

4. Discussion

The vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either counter measures or development of newer insecticides[25]. It is evident from our results that a rise in the concentration of plant extracts was the main cause of mortality in *An. vagus*, *Ar. subalbatus* and *Cx. vishnui* larvae. Similar study was conducted by Mathew *et al*[26] and reported that the seed methanol extract of *Clitoria ternatea (C. ternatea)* was effective against the larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* with LC_{50} values of 65.2, 154.5, and 54.4 ppm, respectively. The leaf extract of *Cassia obtusifolia (C. obtusifolia)* had significant larvicidal effect with LC_{50} and LC_{90} values were

Table 1
Larvicidal activity of crude plant extracts against fourth instar larvae of *An. vagus*, *Ar. subalbatus* and *Cx. vishnui* at 1 000 μg/mL.

Botanical name/ Family/ Vernacular names	Parts used	Species	% Mortality * (μg/mL)				
			Hexane	Chloroform	Ethyl acetate	Acetone	Methanol
A. precatorius L. / Fabaceae / Kundu mani	Seed	*An. vagus*	71.000±3.115	72.000±3.912	100.000±0.000	100.000±0.000	100.000±0.000
		Ar. subalbatus	87.000±1.140	78.000±2.325	100.000±0.000	100.000±0.000	100.000±0.000
		Cx. vishnui	82.000±2.191	85.000±1.581	81.000±1.923	89.000±1.302	100.000±0.000
C. bonplandianum Baill. / Euphorbiaceae / Reilpoondu	Leaf	*An. vagus*	62.000±2.170	84.000±1.483	100.000±0.000	53.000±3.240	41.000±2.588
		Ar. subalbatus	39.000±1.164	59.000±1.788	61.000±2.735	68.000±1.140	61.000±1.788
		Cx. vishnui	79.000±4.207	86.000±1.643	90.000±1.000	89.000±1.095	85.000±1.581
C. dactylon L. / Poaceae / Arugampallu	Leaf	*An. vagus*	73.000±2.408	75.000±2.549	100.000±0.000	86.000±1.923	100.000±0.000
		Ar. subalbatus	84.000±1.788	80.000±2.236	100.000±0.000	89.000±1.302	100.000±0.000
		Cx. vishnui	85.000±2.435	71.000±1.924	73.000±2.966	75.000±1.581	92.000±1.140
M. paradisiaca L. / Musaceae / Vaazhai	Flower	*An. vagus*	86.000±1.483	100.000±0.000	65.000±2.550	100.000±0.000	100.000±0.000
		Ar. subalbatus	64.000±1.883	100.000±0.000	76.000±2.775	100.000±0.000	100.000±0.000
		Cx. vishnui	84.000±1.517	77.000±3.209	75.000±2.916	76.000±2.588	100.000±0.000
S. aromaticum L. Merr. et Perry / Myrtaceae / Lavangam	Flower bud	*An. vagus*	100.000±0.000	89.000±1.095	78.000±3.095	75.000±2.916	76.000±2.775
		Ar. subalbatus	100.000±0.000	80.000±1.695	100.000±0.000	86.000±1.483	100.000±0.000
		Cx. vishnui	100.000±0.000	84.000±1.924	75.000±2.550	72.000±2.301	73.000±2.074

Control – Nil mortality, * Mean value of five replicates.

Table 2

LC_{50}, LC_{90}, and other statistical analysis of different solvent plant extracts against fourth instar larvae of *An. vagus*, *Ar. subalbatus* and *Cx. vishnui*.

Botanical name	Solvents	Species	LC_{50}±SE (μ g/mL)UCL – LCL	LC_{90}±SE (μ g/mL)UCL – LCL	χ^2(*df* = 4)
A. precatorius	Ethyl acetate	*An. vagus*	19.31±1.30(21.86–16.77)	71.71±7.43(86.28–57.15)	7.24
		Ar. subalbatus	93.94±6.43(106.55–81.34)	413.27±46.94(505.27–321.26)	6.71
	Acetone	*An. vagus*	191.58±13.45(217.95–165.20)	843.10±105.56(1050.01–636.18)	16.52
		Ar. subalbatus	99.20±7.70(114.29–84.10)	486.35±59.14(602.26–370.44)	5.39
	Methanol	*An. vagus*	90.69±6.55(103.53–77.85)	372.15±40.59(451.72–292.59)	3.88
		Ar. subalbatus	88.72±7.51(103.41–73.99)	662.61±107.22(872.77–452.45)	4.19
		Cx. vishnui	136.84±12.98(162.28–111.39)	1248.23±257.67(1753.27–743.20)	3.16
C. bonplandianum	Ethyl acetate	*An. vagus*	39.96±3.01(45.87–34.06)	218.14±27.58(272.20–164.09)	8.99
C. dactylon	Ethyl acetate	*An. vagus*	68.12±4.25(76.46–59.80)	241.46±24.79(290.04–192.88)	14.20
		Ar. subalbatus	21.67±1.51(24.63–18.72)	98.34±11.30(120.09–76.20)	12.38
	Methanol	*An. vagus*	41.13±2.83(46.68–35.59)	183.83±21.73(226.41–141.25)	11.17
		Ar. subalbatus	32.62±2.86(38.23–27.00)	267.21±45.92(357.22–177.23)	19.24
M. paradisiaca	Chloroform	*An. vagus*	35.18±3.19(41.43–28.93)	311.55±57.94(4.25–197.99)	4.04
		Ar. subalbatus	89.42±5.65(100.49–78.34)	327.30±32.57(391.15–263.47)	12.25
	Acetone	*An. vagus*	119.33±9.41(137.78–100.89)	748.50±107.35(958.91–538.09)	14.56
		Ar. subalbatus	167.19±13.38(193.42–140.96)	1071.41±163.83(1392.53–750.29)	21.76
	Methanol	*An. vagus*	79.90±5.64(90.95–68.85)	381.93±45.89(471.87–291.99)	10.73
		Ar. subalbatus	48.90±3.22(55.22–42.58)	187.82±20.74(228.47–147.17)	8.87
		Cx. vishnui	103.36±32.50(404.14–276.74)	340.44±32.50(404.14–276.74)	9.17
S. aromaticum	Hexane	*An. vagus*	85.90±6.50(98.63–73.17)	408.89±60.82(528.10–289.63)	7.80
		Ar. subalbatus	141.53±9.13(159.42–123.64)	519.71±58.68(634.73–404.70)	4.30
		Cx. vishnui	149.56±11.98(173.04–126.09)	995.56±148.85(1287.30–703.82)	22.54
	Ethyl acetate	*Ar. subalbatus*	48.90±3.22(55.22–42.58)	187.82±20.74(228.47–147.17)	8.87
	Methanol	*Ar. subalbatus*	78.28±6.87(91.74–64.82)	641.32±110.20(857–425.31)	19.23

Control – Nil mortality, LC_{50} – Lethal concentration that kills 50% of the exposed parasite, LC_{90} – Lethal concentration that kills 90% of the exposed parasite, UCL = upper confidence Limit, LCL = lower confidence Limit, χ^2 – *Chi*–square, *df* degree of freedom, Significant at $P< 0.05$ level.

52.2 and 108.7 mg/L, respectively against *An. stephensi*[27]. 100% mortality was observed in 1% concentration of petroleum ether and ethanolic extract of *Caesalpinia bonduc (C. bonduc)*, whereas it was 55% in 2.5% of aqueous extract and 92.6% in 2.5% of fixed oil against the fourth instar larvae of *Cx. quinquefasciatus*[28]. Earlier authors reported that the methanolic extract of *Cassia fistula (C. fistula)* was tested for larvicidal activity against *Cx. quinquefasciatus* and *An. stephensi* showed LC_{50} values of 17.97 and 20.57 mg/L, respectively[29]. Georges *et al*[30] reported that the emodin, the most abundant and active anthraquinone isolated from *Cassia nigricans (C. nigricans)* showed approximately 85% mortality on the larvae of *Anopheles gambiaea (An. gambiaea)*, in 24 h. The compounds diterpenoid furans 6alpha–hydroxyvouacapan–7beta,17beta–lactone (1), 6alpha,7beta–dihydroxyvouacapan– 17beta–oic acid (2) and methyl 6alpha,7beta–dihydroxyvouacapan–17beta–oate (3) isolated from the ethanolic extract from seeds of *Pterodon polygalaeflorus(P. polygalaeflorus)* exhibited LC_{50} values of 50.08, 14.69, and 21.76 μ g /mL against fourth–instar *Ae. aegypti* larvae[31]. Yenesew *et al*[32] reported that the crude chloroform extract of *Millettia dura (M. dura)* showed high activity (LC_{50} = 3.5 μ g/ mL at 24 h) against second–instar larvae of *Ae. aegypti*. Larvicidal efficacy of leaf extract of *Acacia ferruginea (A. ferruginea)* showed LC_{50} values of 5 362.6 ppm against late third instar larvae of *Cx. quinquefasciatus*[33].

The larvicidal activities of different crude solvent extracts of acetone, chloroform, ethyl acetate and methanol extracts of *Annona squamosa (A. squamosa)*, methanol extract of *Centella asiatica (C. asiatica)*, acetone and methanol extracts of *Gloriosa superba (G. superba)*, ethyl acetate, hexane and methanol extracts of *Pergularia daemia (P. daemia)* against *An. subpictus* (LC_{50} = 17.47, 76.04, 18.60, 119.93, 26.62, 18.43, 64.87, 34.06, 13.63, and 50.39 ppm) and acetone, chloroform, ethyl acetate and methanol extracts of *A. squamosa*, acetone and methanol extracts of *G. superba*, chloroform, ethyl acetate, methanol extract of *P. daemia*, ethyl acetate and methanol extracts of *P. emblica* against *Cx. tritaeniorhynchus* (LC_{50} = 106.41, 63.81, 60.01, 78.21, 87.25, 92.43, 124.73, 31.94, 76.64,69.09, and 54.82 ppm), respectively[34].

Karunamoorthi and Ilango[35] have reported that the LC_{50} and LC_{90} values of methanol leaf extracts of *Croton macrostachyus (C. macrostachyus)* were 89.25 and 224.98 ppm, respectively against late third instar larvae of malaria vector, *Anopheles arabiensis (An. arabiensis)*. Bagavan *et al*[36] have reported that peel chloroform extract of *Citrus sinensis (C. sinensis)*, leaf ethyl acetate extracts of *Ocimum canum (O. canum)*, and *Ocimum sanctum (O. sanctum)* and leaf chloroform extract of *Rhinacanthus nasutus (R. nasutus)* against the larvae of *An. subpictus* (LC_{50} = 58.25, 88.15, 21.67 and 40.46 ppm) and peel methanol extract of *C. sinensis*, leaf methanol extract of *O. canum*, ethyl acetate extracts of *O. sanctum* and *R. nasutus* against the larvae of *Cx. tritaeniorhynchus* (LC_{50} = 38.15, 72.40, 109.12 and 39.32 ppm),

respectively. The highest larval mortality was found in the leaf ethyl acetate of *Aegle marmelos (A. marmelos)*, *Eclipta prostrata (E. prostrata)*, hexane and methanol extracts of *Andrographis paniculata (A. paniculata)*, and *Cocculus hirsutus (C. hirsutus)* with LC_{50} values of 167.00, 78.28, 67.24, and 142.83 ppm, respectively[37]. The LC_{50} values for carbon tetrachloride, methanol and petroleum ether extracts of *Euphorbia hirta (E. hirta)* against the larvae of *An. stephensi* were 11 063.00, 19 280.00 and 9 693.90 ppm after 24 h, and the same extracts showed LC_{50} values of 10 922.00, 18 476.00 and 7 752.80 ppm after 48 h, respectively[38]. Batabyal *et al*[39] reported that the carbon tetrachloride extract of *Ricinus communis (R. communis)* with LC_{50} at 144.11 and 92.44 ppm and LC_{90} at 432.42 and 352.89 ppm after 24 and 48 h, respectively against the larvae of filarial vector, *Cx. quinquefasciatus*. Singh *et al*[40] have reported that the LC_{50} values of hexane extract obtained from leaves of *Eucalyptus citriodora (E. citriodora)* against IVth instar larvae of *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti* were 69.86, 81.12 and 91.76 ppm, respectively after 24 h, and 26.7, 29.9 and 38.8 ppm respectively after 72 h. The extracts of *Myrtus communis (M. communis)* was found to be the most toxic with LC_{50} values of 16 mg litre −1 against fourth-instar larvae of *Culex pipiens molestus (Cx. pipiens molestus)*[41].

The LC_{50} value of petroleum ether extracts of *Jatropha curcas (J. curcas), Pedilanthus tithymaloides (P. tithymaloides), Phyllanthus amarus (P. amarus), E. hirta* and *Euphorbia tirucalli (E. tirucalli)* were 8.79, 55.26, 90.92, 272.36, and 4.25 ppm, respectively, against *Ae. aegypti* and 11.34, 76.61, 113.40, 424.94, and 5.52 ppm, respectively, against *Cx. quinquefasciatus*[42]. This has been observed earlier by Kamaraj *et al*[22] that the highest larval mortality was found in leaf petroleum ether, flower methanol extracts of *Cassia auriculata (C. auriculata)*, flower methanol extracts of *Leucas aspera (L. aspera)* and *R. nasutus*, leaf and seed methanol extracts of *Solanum torvum (S. torvum)* and leaf hexane extract of *Vitex negundo (V. negundo)* against the larvae of *Anopheles subpictus(An. subpictus)* (LC_{50} = 44.21, 44.69, 53.16, 41.07, 35.32, 28.90 and 44.40 ppm) and against the larvae of *Cx. tritaeniorhynchus* (LC_{50} = 69.83, 51.29, 81.24, 71.79, 44.42, 84.47 and 65.35 ppm), respectively. The bioassay-guided fractionation of *Achyranthes aspera (A. aspera)* led to the separation and identification of a saponin as a potential mosquito larvicidal compound with LC_{50} value of 18.20 and 27.24 ppm against *Ae. aegypti* and *Cx. quinquefasciatus*, respectively[43].

Kamaraj *et al*[44] have reported that the peel methanol extract of *C. sinensis*, leaf and flower ethyl acetate extracts of *O. canum* against the larvae of *An. stephensi* (LC_{50} = 95.74, 101.53, 28.96; LC_{90} = 303.20, 492.43 and 168.05 ppm), respectively. The highest larval mortality was found in methanol extract of *O. canum, R. nasutus* and acetone extract of *O. sanctum* against the larvae of *Ae. aegypti* (LC_{50} = 99.42, 94.43 and 81.56 ppm) and against *Cx. quinquefasciatus* (LC_{50} = 44.54, 73.40 and 38.30 ppm), respectively[45]. The *R. communis* seed extract exhibited larvicidal effects with 100 % mortality at the concentrations of 32-64 μg/mL, and showed LC_{50} values 7.10, 11.64 and 16.84 μg/mL against the larvae of *Cx. quinquefasciatus, An. stephensi* and *Aedes albopictus (Ae. albopictus)*, respectively[46].

In conclusion, an attempt has been made to evaluate the larvicidal activity of plant extracts against *An. vagus*, *Ar. subalbatus* and *Cx. vishnui*. The results reported here open the possibility of further investigations of efficacy on their larvicidal properties of natural product extracts. The isolation and purification of crude and seed methanol of *A. precatorius*, flower methanol of *M. paradisiaca*, flower bud hexane extracts of *S. aromaticum* are in progress.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are grateful to C. Abdul Hakeem College Management, Dr. S. Mohammed Yousuff, Principal, Dr. K. Abdul Subhan, HOD of Zoology Department for their help and suggestion.

References

[1] Hotez PJ, Remme JHF, Buss P, Alleyne G, Morel C, Breman JG. Combating tropical infectious diseases: report of the disease control priorities in developing countries project. *Clin Infect Dis* 2004; **38**: 871-8.

[2] Solomon T. Flavivirus encephalitis. *N Engl J Med* 2007; **351**: 370-8.

[3] Das NG, Goswami D, Rabha B. Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. *J Vector Borne Dis* 2007; **44**(2): 145-8.

[4] Rahuman AA, Venkatesan P, Gopalakrishnan G. Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn) Schrad. *Parasitol Res* 2008; **103**(6): 1383-90.

[5] Rahuman AA, Gopalakrishnan G, Venkatesan P, Geetha K, Bagavan A. Mosquito larvicidal activity of isolated compounds from the rhizome of *Zingiber officinale*. *Phytother Res* 2008; **22**(8): 1035-9.

[6] Chow CY, Ibnoe RM, Josopoero ST. Bionomics of *Anopheles mosquitoes* in inland areas of Java with special reference to *Anopheles aconitus* Dön. *Bull Entomol Res* 1960; **50**: 647-60.

[7] Stoops CA, Rusmiarto S, Susapto D, Munif A, Andris H, Barbara K A, et al. Bionomics of *Anopheles* spp. (Diptera: Culicidae) in a malaria endemic region of Sukabumi, West Java, Indonesia. *J Vector Ecol* 2009; **34** (2): 200-7.

[8] Garnham PCC. *Malaria parasites and other haemosporidia*. Oxford: Blackwell Scientific; 1966.

[9] Chen WJ, Dong CF, Chiou LY, Chuang WL. Potential role of *Armigeres subalbatus* (Diptera: Culicidae) in the transmission of Japanese encephalitis virus in the absence of rice culture on Liu-chiu islet, Taiwan. *J Med Entomol* 2000; **37**(1):108-13.

[10] Kanojia PC, Geevarghese G. New mosquito records of an area known for Japanese encephalitis hyperendemicity, Gorakhpur District, Uttar Pradesh, India. *J Am Mosq Control Assoc* 2005; **21**(1):1-4.

[11] Tewari SC, Thenmozhi V, Arunachalam N, Philip Samuel P, Tyagi BK. Desiccated vector mosquitoes used for the surveillance of Japanese encephalitis virus activity in endemic southern India. *Trop Med Int Health* 2008; **13**(2):286-90.

[12] Reuben R, Tewari SC, Hiriyan J, Akiyama J. Illustrated keys to species of *Culex (Culex)* associated with Japanese encephalitis in Southeast Asia (Diptera: Culicidae). *Mosq Syst* 1994; **26**:75-96.

[13]Asolkar LV, Kakkar KK, Chakra OJ. *Second supplement to glossary of Indian medicinal plants with active principles*. New Delhi: Publication and Information Division (CSIR); 1992.

[14]Kumar S, Bagchi GD, Darokar MP. Antibacterial activity observed in the seeds of some coprophilous plants. *Int J Pharmacogn* 1997; **35** (3):179.

[15]Torres MC, Assunção JC, Santiago GM, Andrade-Neto M, Silveira ER, Costa-Lotufo LV, et al. Larvicidal and nematicidal activities of the leaf essential oil of *Croton regelianus*. *Chem Biodivers* 2008; **5**(12):2724-8.

[16]Lima MG, Maia IC, Sousa BD, Morais SM, Freitas SM. Effect of stalk and leaf extracts from *Euphorbiaceae* species on *Aedes aegypti* (Diptera, Culicidae) larvae. *Rev Inst Med Trop Sao Paulo* 2006; **48**(4):211-4.

[17]McPhatter LP, Debboun M. Attractiveness of botanical infusions to ovipositing *Culex quinquefasciatus*, *Cx. nigripalpus*, and *Cx. erraticus* in San Antonio, Texas. *J Am Mosq Control Assoc* 2009; **25**(4):508-10.

[18]Vinayagam A, Senthilkumar N, Umamaheswari A. Larvicidal activity of some medicinal plant extracts against malaria vector *Anopheles stephensi*. *Res J Parasitol* 2008; **3**: 50-8.

[19]de Omena MC, Navarro DM, de Paula JE, Luna JS, Ferreira de Lima MR, Sant′Ana AE. Larvicidal activities against *Aedes aegypti* of some Brazilian medicinal plants. *Bioresour Technol* 2007; **98**(13):2549-56.

[20]Trongtokit Y, Rongsriyam Y, Komalamisra N, Apiwathnasorn C. Comparative repellency of 38 essential oils against mosquito bites. *Phytother Res* 2005; **19**(4):303-9.

[21]El Hag EA, El Nadi AH, Zaitoon AA. Toxic and growth retarding effects of three plant extracts on *Culex pipiens* larvae (Diptera: Culicidae). *Phytother Res* 1999; **13**(5):388-92.

[22]Kamaraj C, Bagavan A, Rahuman AA, Zahir AA, Elango G, Pandiyan G. Larvicidal potential of medicinal plant extracts against *Anopheles subpictus* Grassi and *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). *Parasitol Res* 2009; **104**: 1163-71.

[23]World Health Organization. *Report of the WHO informal consultation on the evaluation on the testing of insecticides CTD/ WHO PES/IC/96.1*. Geneva: WHO; 1996, p. 69.

[24]Rahuman AA, Gopalakrishnan G, Ghouse BS, Arumugam S, Himalayan B. Effect of *Feronia limonia* on mosquito larvae. *Fitoterapia* 2000; **71**(5): 553-5.

[25]Chandre F, Darriet F, Darder M, Cuany A, Doannio JMC, Pasteur N, et al. Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. *Med Vet Entomol* 1998; **12**: 359-66.

[26]Mathew N, Anitha MG, Bala TS, Sivakumar SM, Narmadha R, Kalyanasundaram M. Larvicidal activity of *Saraca indica*, *Nyctanthes arbor-tristis*, and *Clitoria ternatea* extracts against three mosquito vector species. *Parasitol Res* 2009; **104**(5):1017-25.

[27]Rajkumar S, Jebanesan A. Larvicidal and oviposition activity of *Cassia obtusifolia* Linn (Family: Leguminosae) leaf extract against malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitol Res* 2009; **104**(2): 337-40.

[28]Saravanan KS, Periyanayagam K, Ismail M. Mosquito larvicidal properties of various extract of leaves and fixed oil from the seeds of *Caesalpinia bonduc* (L) Roxb. *J Commun Dis* 2007; **39**(3):153-7.

[29]Govindarajan M, Jebanesan A, Pushpanathan T. Larvicidal and ovicidal activity of *Cassia fistula* Linn leaf extract against filarial and malarial vector mosquitoes. *Parasitol Res* 2008; **102**(2): 289-92.

[30]Georges K, Jayaprakasam B, Dalavoy SS, Nair MG. Pest-managing activities of plant extracts and anthraquinones from *Cassia nigricans* from Burkina Faso. *Bioresour Technol* 2008; **99**(6):2037-45.

[31]De Omena MC, Bento ES, De Paula JE, Sant′Ana AE. Larvicidal diterpenes from *Pterodon polygalaeflorus*. *Vector Borne Zoonotic Dis* 2006; **6**(2):216-22.

[32]Yenesew A, Derese S, Midiwo JO, Heydenreich M, Peter MG. Effect of rotenoids from the seeds of *Millettia dura* on larvae of *Aedes aegypti*. *Pest Manag Sci* 2003; **59**(10):1159-61.

[33]Vahitha R, Venkatachalam MR, Murugan K, Jebanesan A. Larvicidal efficacy of *Pavonia zeylanica* L. and *Acacia ferruginea* D.C. against *Culex quinquefasciatus* Say. *Bioresour Technol* 2002; **82**(2):203-4.

[34]Bagavan A, Kamaraj C, Elango G, Zahir AA, Rahuman AA. Adulticidal and larvicidal efficacy of some medicinal plant extracts against tick, fluke and mosquitoes. *Vet Parasitol* 2009; **166**: 286-92.

[35]Karunamoorthi K, Ilango K. Larvicidal activity of *Cymbopogon citratus* (DC) Stapf. and *Croton macrostachyus* Del. against *Anopheles arabiensis* Patton, a potent malaria vector. *Eur Rev Med Pharmacol Sci* 2010; **14**(1):57-62.

[36]Bagavan A, Kamaraj C, Rahuman AA, Elango G, Zahir AA, Pandiyan G. Evaluation of larvicidal and nymphicidal potential of plant extracts against *Anopheles subpictus* Grassi, *Culex tritaeniorhynchus* Giles and *Aphis gossypii* Glover. *Parasitol Res* 2009; **104**: 1109-17.

[37]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.

[38]Sharma P, Mohan L, Srivastava CN. *Amaranthus oleracea* and *Euphorbia hirta*: natural potential larvicidal agents against the urban Indian malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitol Res* 2009; **106**(1):171-6.

[39]Batabyal L, Sharma P, Mohan L, Maurya P, Srivastava CN. Relative toxicity of neem fruit, bitter gourd, and castor seed extracts against the larvae of filaria vector, *Culex quinquefasciatus* (Say). *Parasitol Res* 2009; **105**(5):1205-10.

[40]Singh RK, Dhiman RC, Mittal PK. Studies on mosquito larvicidal properties of *Eucalyptus citriodora* Hook (family-Myrtaceae). *J Commun Dis* 2007; **39**(4):233-6.

[41]Traboulsi AF, Taoubi K, el-Haj S, Bessiere JM, Rammal S. Insecticidal properties of essential plant oils against the mosquito *Culex pipiens* molestus (Diptera: Culicidae). *Pest Manag Sci* 2002; **58**(5):491-5.

[42]Rahuman AA, Gopalakrishnan G, Venkatesan P, Geetha K. Larvicidal activity of some *Euphorbiaceae* plant extracts against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res* 2008; **1025**: 867-73.

[43]Bagavan A, Rahuman AA, Kamaraj C, Geetha K. Larvicidal activity of saponin from *Achyranthes aspera* against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res* 2008; **103**(1):223-9.

[44]Kamaraj C, Rahuman AA, Bagavan A. Screening for antifeedant and larvicidal activity of plant extracts against *Helicoverpa armigera* Hu¨ bner, *Sylepta derogata* F. and *Anopheles stephensi* Liston. *Parasitol Res* 2008; **1036**:1361-8.

[45]Kamaraj C, Rahuman AA, Bagavan A. Antifeedant and larvicidal effects of plant extracts against *Spodoptera litura* F., *Aedes aegypti* L. and *Culex quinquefasciatus* Say. *Parasitol Res* 2008; **1032**: 325-31.

[46]Mandal S. Exploration of larvicidal and adult emergence inhibition activities of *Ricinus communis* seed extract against three potential mosquito vectors in Kolkata, India. *Asian Paci J Trop Med* 2010; **3**: 605-9.