Parasitol Res (2012) 111:1059–1065
DOI 10.1007/s00436-012-2932-8

ORIGINAL PAPER

Larvicidal activity of pectolinaringenin from *Clerodendrum phlomidis* L. against *Culex quinquefasciatus* Say and *Aedes aegypti* L. (Diptera: Culicidae)

Chellaiah Muthu · Appadurai Daniel Reegan · Selvadurai Kingsley · Savarimuthu Ignacimuthu

Received: 6 October 2011 / Accepted: 17 April 2012 / Published online: 5 May 2012

Abstract Larvicidal activity of 12 fractions and a compound of chloroform extract of *Clerodendrum phlomidis* L. (Lamiaceae) was assayed for their toxicity against the early fourth-instar larvae of the filarial vector *Culex quinquefasciatus* Say and dengue vector *Aedes aegypti* L. The fractions were tested at 100-, 50-, 25- and 12.5-ppm concentrations. The compound pectolinaringenin was tested at 5-, 2.5-, 1.0- and 0.5-ppm concentrations. Among the different fractions, fraction 5 recorded the lowest LC_{50} and LC_{90} values of 5.02, 61.63 ppm and 32.86, 73.62 ppm against *C. quinquefasciatus* and *A. aegypti*, respectively. The compound pectolinaringenin showed the lowest LC_{50} and LC_{90} values of 0.62, 2.87 ppm and 0.79, 5.31 ppm against *C. quinquefasciatus* and *A. aegypti*, respectively. This is the first report on the mosquito larvicidal activity of the isolated compound pectolinaringenin from *C. phlomidis*. The results of this study show that the chloroform extract of *C. phlomidis* can be used as a potent source and pectolinaringenin as a new natural mosquito larvicidal agent.

Chellaiah Muthu and Appadurai Daniel Reegan contributed equally to this work.

C. Muthu · A. Daniel Reegan · S. Kingsley · S. Ignacimuthu (✉)
Entomology Research Institute, Loyola College,
Chennai 600 034, Tamil Nadu, India
e-mail: entolc@hotmail.com

C. Muthu
e-mail: muthu_mathi@yahoo.com

A. Daniel Reegan
e-mail: jack_reegan@yahoo.co.in

S. Kingsley
e-mail: kingsselvagrace@yahoo.co.in

Introduction

The prevalence of mosquito-borne diseases is one of the world's most important health problems. Mosquitoes are responsible for transmitting various infectious diseases; hence, the mosquito has been declared as 'Public Enemy Number One' (WHO 1996a). Mosquitoes belonging to the genera *Anopheles*, *Culex* and *Aedes* are the vectors for the pathogens of different diseases such as malaria, filariasis, Japanese encephalitis, dengue and dengue haemorrhagic fever, yellow fever and chickungunya (Rahuman et al. 2009; Borah et al. 2010). They cause allergic responses including local skin and systemic reactions, such as angioedema and urticaria (Peng et al. 1999). Tropical areas are more vulnerable to parasitic diseases, and the risk of contracting arthropod-borne illnesses has increased due to climate change and intensifying globalization (Karunamoorthy et al. 2010). It is necessary to prevent mosquito-borne diseases and improve public health by controlling mosquitoes.

Aedes aegypti is the vector of arboviruses responsible for major diseases like dengue, dengue haemorrhagic fever and chikungunya. Dengue fever is endemic in the Southeast Asia and India, Bangladesh and Pakistan (Akram and Ahmed 2005) and continues to increase with more severe forms of the disease such as dengue haemorrhagic fever and dengue shock syndrome or with unusual symptoms such as central nervous system involvement (Hendarto and Hadinegoro 1992; Pancharoen et al. 2002). *Culex quinquefasciatus* is an important vector of lymphatic filariasis in tropical and subtropical regions. It is a pantropical pest and urban vector of *Wuchereria bancrofti* (Holder 1999) and is possibly the most abundant house mosquito in towns and cities of tropical countries. Lymphatic filariasis is an arthropod-borne infectious disease affecting 128 million people over 78 countries around the world (WHO 2008). In India alone, 25 million

people harbour microfilaria, and 19 million people suffer from filarial disease manifestations (NICD 1990).

In recent years, mosquito control programmes had a setback because of the ever-increasing insecticide resistance (WHO 1992; Liu et al. 2005). Besides insecticidal resistance in arthropod vectors, the increased cost of insecticides and increased public concern over environmental contamination have demanded a continued search for alternative vector control strategies which would be environmentally safer and specific in their action (Coats 1994; Khan and Selman 1996; Peng et al. 1998). Studies on the effectiveness of the plant products as larvicides indicated that they could be used as alternatives to synthetic pesticides, and many studies of phytochemicals have been done to test their potential as a source of commercial mosquito control agents or as lead molecules (Pitasawat et al. 2007; Arnason et al. 1989; Pavela 2008; Park et al. 2002; Maheswaran 2010; Arivoli et al. 2011).

Clerodendrum L. [Family Lamiaceae (Verbanaceae)] is widely distributed in tropical and subtropical regions of the world and is comprised of small trees, shrubs and herbs. *Clerodendrum phlomidis* is an important and well-known medicinal plant and one of the highly traded plants from tropical forests as the leaves and roots are widely used in Unani, Siddha, Ayurveda and folklore medicines for the treatment of various diseases (Ved and Goraya 2008; Murugadoss et al. 2010). In ethnomedicine, it is used to treat diabetes (Dhanapal et al. 2008), fever, malaria, stomachache, dyspepsia, digestive disorders, eye disorders, lung diseases, rheumatism, asthma, inflammatory diseases and swelling (Pandey et al. 2005; Anonymous 1992) and to treat guinea worms (Katewa et al. 2004). The alcoholic extract of *C. phlomidis* exhibited antimalarial activity against *Plasmodium falciparum* (Simonsen et al. 2001). *Clerodendrum inerme* also inhibited the growth of larvae of *A. aegypti*, *C. quinquefasciatus* and *Culex pipiens* (Gayar and Shazll 1968; Kalyanasundaram and Das 1985). Our preliminary studies had indicated that among hexane, chloroform and ethyl acetate crude extracts of *C. phlomidis*, only the chloroform extract had shown mosquito larvicidal activity (Maheswaran et al. 2008). Hence, in the present study, the isolated fractions and the compound (pectolinaringenin) from the chloroform extract of *C. phlomidis* were evaluated for their larvicidal effect against *C. quinquefasciatus* and *A. aegypti*.

Materials and methods

Plant collection and extraction

The matured leaves of *C. phlomidis* were collected from Alanthurai in Coimbatore district, Tamil Nadu, India. Leaves were shade-dried and powdered using an electric blender. One kilogram of leaf powder was soaked in a glass jar containing 3 L of hexane, chloroform and ethyl acetate with intermittent shaking. The leaves were sequentially extracted, and the extracts were condensed using a rotary vacuum evaporator at 40 °C. The condensed materials were placed in a glass bottle and stored in a refrigerator at 4 °C.

Isolation of the active principle using column chromatography

The chloroform extract was selected for the isolation of fractions and identification of the compound for further study on the basis of the biological activities (Maheswaran et al. 2008). The chloroform extract (30 g) was mixed with 120 g of silica gel (60–100 mesh), and the mixture was packed with 300 g of silica gel (100–200 mesh) in the column and successively eluted with individual and a combination of solvents as follows: (1) hexane (100 %), (2) hexane/ethyl acetate (75:25), (3) hexane/ethyl acetate (50:50), (4) hexane/ethyl acetate (25:75), (5) ethyl acetate (100 %), (6) acetone (100 %) and (7) methanol (100 %). A total of 206 fractions were collected. Based on the thin-layer chromatography (TLC) profile, 12 fractions were obtained. Fraction 5 was selected for purification of the compound on the basis of the bioassay and was purified further using preparatory high-performance liquid chromatography (HPLC). One pure compound was obtained, and the purity of the compound was checked using HPLC.

Identification of the active principle

Melting points were taken by the open capillary method on a heating block apparatus. The compound was subjected to UV–visible spectrophotometer (Model–Hitachi 2010), IR spectrum (Perkin-Elmer FT-IR instrument in KBr disc), proton nuclear magnetic resonance (^{1}H NMR) spectrum (500 MHz) and carbon-13 nuclear magnetic resonance (^{13}C NMR; 75 MHz) spectrum on a JEOL AL-300 instrument in $CDCl_3$. Electron ionization mass spectrometry (EI-MS) was taken on a Shimadzu instrument at 70 ev by the direct inlet method. All the chemicals and solvents used were of analytical grade.

Insect rearing

C. quinquefasciatus eggs were collected from the Cooum river in Chennai. The eggs of *A. aegypti* were collected from water trays with filter paper lining the sides of the trays placed in and around the laboratory and in the mosquito breeding room for hatching. The hatched larvae were kept in enamel trays containing tap water. They were maintained at 27±2 °C, 75–85 % RH with 14:10 L/D photoperiod cycle. The larvae were fed with dog biscuits and Brewer's yeast in

the ratio of 3:2. Pupae were transferred from the trays to a cup containing tap water and placed in screened cages (23×23×32 cm in dimension) for the adult emergence. Adults were provided with 10 % sucrose solution in a jar with a cotton wick. On the day of post-emergence, the adult females were deprived of sucrose for 12 h and then provided with a mouse in a resting cage overnight for blood feeding. After 3 days, the ovitrap was kept in the cages, the eggs were collected, transferred to the enamel trays and maintained for further experimentation.

Larvicidal bioassay

Larvicidal activity was evaluated using a modified method prescribed by the WHO (1996b). Twenty early fourth-instar larvae of *C. quenquefasciatus* and *A. aegypti* were introduced into the containers. Concentrations were 100, 50, 25 and 12.5 ppm for fractions and 5, 2.5, 1.0 and 0.5 ppm for compounds. The volume in each container was increased to 250 ml using tap water. The test materials were dissolved in acetone, and Tween 80 was used as an emulsifier. Acetone with Tween 80 was used as negative control, and azadirachtin (40.86 % purity) was used as positive control. Mortality and survival rate were registered after a 24-h exposure period. The moribund and dead larvae were collected, and larval mortality was calculated for each concentration. Five replicates were maintained. Percent mortality was calculated using the formula (1), and corrections for mortality when necessary were done using Abbot's (1925) formula (2).

Percentage of mortality:

$$\frac{\text{no. of dead larvae}}{\text{no. of larvae introduced}} \times 100 \quad (1)$$

Corrected percentage of mortality:

$$\frac{(1 - n \text{ in T after treatment} \times 100}{n \text{ in C after treatment}} \times 100 \quad (2)$$

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

The corrected percentage mortality value for each concentration was considered to estimate LC_{50} and LC_{90} values using probit analysis (Finney 1971). Statistical package, SPSS version 11.5, was used for statistical analysis.

Results

Isolation and characterization of the compound

The fraction eluted with the solvent hexane/ethyl acetate (1:1) gave a compound as a pale yellow crystal crystallized from methanol (melting point, 210 °C; Lit. mp, 210–212 °C). The yield was 850 mg. It gave a positive ferric reaction (green colour) with alcoholic ferric chloride for phenol. It also was positive for the Shinoda test for flavonoids by giving a reddish pink colour with Mg/HCl. TLC over silica gel G with chloroform/ethyl acetate (9:1) as the developing system produced a single spot, pale yellow turning dark yellow, on exposure to ammonia (Rf=0.35). The purity of the compound was 98.2 % using HPLC. Spectral data using UV, IR, ^{1}H NMR, ^{13}C NMR and EI-MS were taken for structural elucidation of the isolated compound.

The ^{1}H NMR spectrum showed absence of substituent at C-3, H-3 appearing as singlet at δ=6.56. The lone aromatic proton H-8 appeared as singlet at δ=6.54. The A_2B_2 system in ring B was shown by two sets of ortho coupled protons at δ 7.85 and 7.03 (J=9.0 Hz) corresponding to H-2′, H-6′ and H-3′ and H-5′. 4′-OMe appeared as a three-proton singlet at δ 3.94, and 6-OMe appeared at 3.90. 7-OH appeared as a broad singlet at δ 9.39, and the chelated 5-OH appeared downfield at δ 12.96.

The ^{13}C NMR also confirmed the structure of the compound as pectolinaringenin in the present investigation. The flavonoid carbonyl appeared much downfield at δ 182.33. This suggested that C-3 is unsubstituted and hydroxyl is present at C-5. The single peak at δ 93.09 was assigned to C-8, and the absence of a slightly downfield peak around δ 97.0 found for C-6 in 5,7-oxygenated flavones showed that C-6 was substituted. C-6 OMe appeared at δ 131.08. C-5 attached to chelated OH appeared at δ 152.72. The δc values in the ring B corresponded to pectolinaringenin.

The EI-MS gave M^+, the molecular ion at m/z 314 corresponded to the molecular formula $C_{17}H_{14}O_6$. The peak at m/z 296 corresponded to $[M\text{-}H_2O]^+$. The above data confirmed the structure as pectolinaringenin (Fig. 1).

Larvicidal activity

All the 12 fractions eluted from the chloroform extract of *C. phlomidis* were evaluated for their larvicidal activity against *C. quinquefasciatus* and *A. aegypti*. Among the fractions, fraction 5 was found to be active against *C. quinquefasciatus* which recorded the lowest LC_{50} value of 5.02 ppm with the fiducial limits of 1.50 ppm (lower) and 12.67 ppm (upper) and

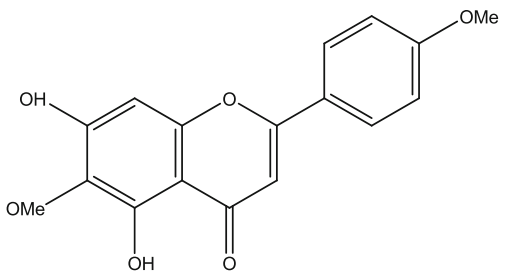

Fig. 1 Pectolinaringenin (5,7-dihydroxy-4′,6-dimethoxy-flavone)

the LC_{90} value of 61.63 ppm with the fiducial limits of 53.28 ppm (lower) and 74.56 ppm (upper). The chi-square values were significant in all the fractions except fractions 1, 8, 9 and 10. Fractions 4 and 6 also recorded good larvicidal activity with the LC_{50} and LC_{90} values of 13.0, 9.63 and 106.81, 113.95 ppm, respectively (Table 1).

In the case of *A. aegypti*, fraction 5 recorded the lowest LC_{50} and LC_{90} values of 32.86 and 91.70 ppm, respectively. The fiducial limits were 28.06, 73.62 and 37.43, 92.76 ppm, for LC_{50} and LC_{90}, respectively. Fractions 6 and 4 had also recorded a notable amount of larvicidal activity with the LC_{50} and LC_{90} values of 38.98, 50.35 ppm and 82.29, 133.63 ppm, respectively. Significant chi-square values were recorded in all the fractions (Table 2).

The bioassay of the compound revealed that the effective LC_{50} concentrations to kill the larvae were 0.62 and 0.79 ppm with the fiducial limits of 0.28, 0.14 ppm (lower) and 0.88, 1.26 ppm (upper) for *C. quinquefasciatus* and *A. aegypti*, respectively. The LC_{90} values were 2.87 and 5.31 ppm with the fiducial limits of 2.50, 4.50 ppm (lower) and 3.44, 6.66 ppm (upper) for *C. quinquefasciatus* and *A. aegypti*, respectively. The chi-square values were significant as 64.91 and 83.61 against *C. quinquefasciatus* and *A. aegypti*, respectively (Table 3). The negative control acetone with Tween 80 showed no activity, and the positive control Temephos at 0.1 ppm showed 100 % mortality. *C. quinquefasciatus* was more susceptible to the treated compound pectolinaringenin than *A. aegypti*; the longevity of the larvae of *A. aegypti* was found to be extended more than 7 days.

Discussion

In the present study, chloroform extracts of *C. phlomidis* eluted with hexane/ethyl acetate (1:1) fractions recorded good larvicidal activity against *C. quinquefasciatus* and *A. aegypti*. Fraction 5 was the most effective followed by fractions 4 and 6. Earlier, Maheswaran et al. (2008) studied the mosquito larvicidal activity of different crude extracts of *C. phlomidis* and observed 72 % larval mortality in the chloroform extract against *C. quinquefasciatus*. Leaf powder of *C. inerme* (200 mg) exhibited 100 % larval mortality in *A. aegypti* (Patil et al. 2006). The LC_{50} values for fraction 5 were 5.02 and 32.86 ppm against *C. quinquefasciatus* and *A. aegypti*, respectively. This result corroborated with the findings of Katade et al. (2006) who studied different fractions isolated from the methanol extract of *Sterculia guttata* seeds. They observed that the LC_{50} values for an active fraction A1 were 21.552 and 35.520 ppm against *C. quinquefasciatus* and *A. aegypti*, respectively. They further reported that the larvicidal activity was due to the presence of compounds like flavonoids, alkaloids, etc. in the fraction.

Our study revealed that the compound pectolinaringenin belonged to flavonoid group; it exhibited the lowest LC_{50} values of 0.62 and 0.79 ppm with the fiducial limits of 0.28, 0.14 ppm (lower) and 0.88, 1.26 ppm (upper) for *C. quinquefasciatus* and *A. aegypti*, respectively and the LC_{90} values of 2.87 and 5.31 ppm with the fiducial limits of 2.50, 4.50 ppm (lower) and 3.44, 6.66 ppm (upper) for *C. quinquefasciatus* and *A. aegypti*, respectively. Many researchers have reported the effects of flavonoid group on *C. quinquefasciatus* and *A.*

Table 1 Larvicidal activity (in parts per million) of isolated fractions from chloroform extract of *C. phlomidis* against *C. quinquefasciatus*

Fractions	LC_{50}	95 % fiducial limit		LC_{90}	95 % fiducial limit		Chi-square
		LL	UL		LL	UL	
1	179.27	143.51	253.01	440.95	337.37	659.08	25.87
2	61.62	52.52	72.97	206.96	172.12	266.87	31.47*
3	14.40	0.35	24.20	145.81	123.47	182.95	32.11*
4	13.0	2.58	20.78	106.81	93.77	126.17	35.68*
5	5.02	1.50	12.67	61.63	53.28	74.56	64.33*
6	9.63	1.88	15.81	113.95	103.25	128.23	16.42
7	32.59	22.58	40.92	139.49	118.65	173.81	47.90*
8	68.04	62.82	74.09	175.06	158.55	195.85	8.87
9	84.02	74.87	96.56	246.97	211.45	301.40	16.61
10	99.51	86.69	119.28	292.15	242.52	374.79	17.31
11	108.80	92.20	137.80	240.64	194.68	327.40	46.94*
12	126.37	105.97	163.33	283.84	227.33	391.46	33.41*

LC_{50} lethal concentration that kills 50 % of the exposed larvae, *LC_{90}* lethal concentration that kills 90 % of the exposed larvae, *LL* lower limit (95 % confidence limit), *UL* upper limit (95 % confidence limit)

*$p \leq 0.05$, level of significance of chi-square values

Table 2 Larvicidal activity (in parts per million) of isolated fractions from chloroform extract of *C. phlomidis* against *A. aegypti*

Fractions	LC_{50}	95 % fiducial limit		LC_{90}	95 % fiducial limit		Chi-square
		LL	UL		LL	UL	
1	100.06	84.96	126.45	187.98	153.29	256.12	107.65*
2	106.04	89.95	133.86	241.98	195.82	328.75	44.40*
3	70.36	62.90	79.72	164.27	143.60	195.18	41.18*
4	50.35	44.73	56.26	133.63	119.63	153.09	35.33*
5	32.86	28.06	37.43	81.70	73.62	92.76	58.038*
6	38.98	35.29	42.78	82.29	75.42	91.22	46.06*
7	80.82	68.67	99.81	200.97	163.66	271.27	59.97*
8	97.26	80.85	127.61	233.79	184.17	337.67	60.28*
9	121.10	98.50	170.18	229.72	177.70	351.75	102.92*
10	137.92	115.15	180.47	264.96	212.35	367.65	47.75*
11	118.53	101.11	149.15	211.95	173.98	283.24	76.15*
12	113.73	97.77	140.21	229.91	190.02	300.44	46.98*

LC_{50} lethal concentration that kills 50 % of the exposed larvae, LC_{90} lethal concentration that kills 90 % of the exposed larvae, *LL* lower limit (95 % confidence limit), *UL* upper limit (95 % confidence limit)

*$p \leq 0.05$, level of significance of chi-square values

aegypti. Ho et al. (2003) reported that the isolated compounds such as meliternatin (3, 5-dimethoxy-3′, 4′, 6, 7-bismethylendioxyflavone) (6) and six other minor polyoxygenated flavones derived from *Melicope subunifoliolata* showed larvicidal activity against *A. aegypti*. Isolated compounds such as prenylated flavonoids derived from *Dodonaea viscosa* showed larvicidal activity against *Aedes albopictus* and *C. pipiens* (Niu et al. 2010). Isoflavonoids neotenone and neorautanone and the pterocarpans neoduline, nepseudin and 4-methoxyneoduline isolated from *Neorautanenia mitis* showed larvicidal activity against *Anopheles gambiae* and *C. quinquefasciatus* (Joseph et al. 2004). Larvicidal activity of *Lantana camara* was reported against *C. quinquefasciatus* and *A. aegypti* by Sathish Kumar and Maneemegalai (2008). They reported that methanol and ethanol flower extracts exhibited 96 and 100 % larval mortality against the third instar; 88 and 100 % against the fourth instar of *A. aegypti*, respectively, at 0.75 and 1.00-mg/ml concentrations. They further reported that the higher activity was due to the presence of flavonoid (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one). Similarly, Gopleshkhanna and Kannabiran (2007) reported the presence of flavonoids, tannins and carbohydrates in the plant extracts having larvicidal property against mosquitoes. Rajkumar and Jebanesan (2008) reported the larvicidal activity of flavonoid compounds poncirin, rhoifolin, naringin and marmesin against *A. aegypti*.

In the present study, the longevity of the larvae extended up to 7 days, after which they died. This may be due to the effect of juvenile hormonal activity of the tested materials. This finding coincides with the earlier findings of Pereira and Gurudutt (1990) who observed that (−)-3-epicaryoptin isolated from *C. inerme* inhibited the growth of *C. quinquefasciatus*.

Table 3 LC_{50} and LC_{90} values for the larvicidal activity (in parts per million) of pectolinaringenin (98.2 % purity) from chloroform extract of *C. phlomidis* against *C. quinquefasciatus* and *A. aegypti*

Tested mosquito species	LC_{50}	95 % fiducial limit		LC_{90}	95 % fiducial limit		Chi-square
		Lower	Upper		Lower	Upper	
Pectolinaringenin (98.2 % purity)							
C. quinquefasciatus	0.62	0.28	0.88	2.87	2.50	3.44	64.91*
A. aegypti	0.79	0.14	1.26	5.31	4.50	6.66	83.61*
Azadirachtin (40.86 % purity)							
C. quinquefasciatus	0.49	0.21	0.70	3.04	2.75	3.45	31.41*
A. aegypti	0.61	0.17	1.33	4.68	4.13	5.46	39.27*

*$p \leq 0.05$, level of significance of chi-square values

Sukumar et al. (1991) studied the effect of extracts of *Cymbopogon citratus* against *A. aegypti*. They observed significant growth inhibition and mortality of *A. aegypti* since *C. citratus* had high quantity of flavonoid. This is the first report on the larvicidal activity of fractions and compound pectolinaringenin from *C. phlomidis* against vector mosquitoes.

In summary, in this study, fractions 5, 4 and 6 showed significant mosquito larvicidal activity, and the isolated compound from fraction 5 showed the highest larval mortality at the lowest concentration. The study clearly demonstrates that the compound pectolinaringenin isolated from *C. phlomidis* could be used as an effective agent to develop mosquito larvicidal formulation for the control of disease-causing vector mosquitoes.

Acknowledgments The authors are thankful to the Entomology Research Institute, Loyola College, Chennai, India for the financial assistance.

References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18:265–267

Akram DS, Ahmed S (2005) Dengue fever. Infect Dis J 14:124–125

Anonymous (1992) The useful plants of India. CSIR, New Delhi, pp. 161–164

Arivoli S, Tennyson S, Martin JJ (2011) Larvicidal efficacy of *Vernonia cinerea* (L.) (Asteraceae) leaf extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). J Bio Pestic 4:37–425

Arnason JT, Philogene BJR, Morand P (1989) Insecticides of plants origin. American Chemical Society, Washington, DC, American Chemical Society Symposium Series no. 387

Borah R, Kalita MC, Kar A, Talukdar AK (2010) Larvicidal efficacy of *Toddalia asiatica* (Linn.) Lam against two mosquito vector *Aedes aegypti* and *Culex quinquefasciatus*. Afr J Biotechnol 9:2527–2530

Coats JR (1994) Risks from natural versus synthetic insecticides. Annu Rev Ent 39:489–515

Dhanapal SP, Raja MM, Suresh B (2008) Antidiabetic activity of *Clerodendron phlomidis* leaf extract in alloxan-induced diabetic rats. Ind J Phar Sci 70:841–844

Finney DJ (1971) Probit analysis, 3rd edn. Cambridge University Press, London

Gayar R, Shazll A (1968) Toxicity of certain plants to *Culex pipiens* larvae. Bull Soc Entomol 52:458–467

Gopleshkhanna V, Kannabiran K (2007) Larvicidal effect of *Hemidesmus indicus*, *Gymnema sylvestre* and *Eclipta prostrata* against *Culex quinquefasciatus* mosquito larvae. Afr J Biotech 6:307–311

Hendarto SK, Hadinegoro SR (1992) Dengue encephalopathy. Acta Paediatr Jap 34:350–357

Ho SH, Wang J, Sim KY, Ee GC, Imiyabir Z, Yap KF, Shaari K, Hock Goh S (2003) Meliternatin: a feeding deterrent and larvicidal polyoxygenated flavone from *Melicope subunifoliolata*. Phytochem 62:1121–1124

Holder P (1999) The mosquitoes of New Zealand and their animal disease significance. Surveillance 26:12–15

Joseph CC, Ndoile MM, Malima RC, Nkunya MH (2004) Larvicidal and mosquitocidal extracts, a coumarin, isoflavonoids and pterocarpans from *Neorautanenia mitis*. Trans R Soc Trop Med Hyg 98:451–455

Kalyanasundaram M, Das PK (1985) Larvicidal and synergistic activity of plant extracts for mosquito control. Ind J Med Res 82:19–23

Karunamoorthy K, Ilango K, Murugan K (2010) Laboratory evaluation of traditionally used plant-based insect repellents against the malaria vector *Anopheles arabiensis* Patton. Parasitol Res 106:1217–1223

Katade SR, Pawar PV, Wakharkar RD, Deshpande NR (2006) *Sterculia guttata* seeds extractives—an effective mosquito larvicide. Ind J Exp Biol 44(8):662–665

Katewa SS, Chaudhary BL, Jain A (2004) Folk herbal medicines from tribal area of Rajasthan, India. J Ethnopharmacol 92:41–46

Khan AR, Selman BJ (1996) Microsporidian pathogens of mosquitoes and their potential of control agents. Agri Zool Rev 7:303–335

Liu H, Xu Q, Zhang L, Liu N (2005) Chlorpyrifos resistance mosquito *Culex quinquefasciatus*. J Med Entomol 42:815–820

Maheswaran R (2010) Bioefficacy of *Psoralea corylifolia* Linn. for the management of human vector mosquitoes *Anopheles stephensi* Liston, *Aedes aegypti* Linn. and *Culex quinquefasciatus* Say (Diptera: Culicidae). Ph.D. Thesis, University of Madras

Maheswaran R, Kingsley S, Ignacimuthu S (2008) Larvicidal and repellent activity of *Clerodendron phlomides* against *Culex quinquefasciatus* Say (Diptera: Culicidae). In: Ignacimuth S, Jayaraj S (eds) Proceedings of Recent Trends in Insect Pest Management. Elite Publishing House, New Delhi, pp 240–243

Murugadoss M, Raja MM, Mishra SH (2010) Comprehensive review of *Clerodendrum phlomidis*: a traditionally used bitter. J Chinese Integ Med 8:510–524

NICD (1990) Proceeding of the National Seminar on operation research on vector control in filariasis. New Delhi

Niu HM, Zeng DQ, Long CL, Peng YH, Wang YH, Luo JF, Wang HS, Shi YN, Tang GH, Zhao FW (2010) Clerodane diterpenoids and prenylated flavonoids from *Dodonaea viscosa*. J Asian Nat Prod Res 12:7–14

Pancharoen C, Kulwichit W, Tantawichien T, Thisyakorn U, Thisyakorn C (2002) Dengue infection: a global concern. J Med Assoc Thai 85:S25–S33

Pandey CN, Raval BR, Mali S, Salvi H (2005) Medicinal plants of Gujarat. Ecological Education and Research (GEER) Foundation, Ghandhinagar, p 156

Park IK, Lee SG, Shin SC, Park JD, Ahn YJ (2002) Larvicidal activity of isobutylamides identified in *Piper nigrum* fruits against three mosquito species. J Agric Food Chem 50:1866–1870

Patil PB, Holihosur SN, Kallapur VL (2006) Efficacy of natural product, *Clerodendron inerme* against dengue mosquito vector *Aedes aegypti*. Curr Sci 90:1064–1066

Pavela R (2008) Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). Parasitol Res 102:555–559

Peng Y, Song J, Tian G, Xue Q, Ge F, Yang J, Shi Q (1998) Field evaluation of *Romanomermis yunanensis* (Nematoda: Mermithidae) for control of culicinae mosquitoes in China. Fundam Appl Nematol 21:227–232

Peng Z, Yang J, Wang H, Simons FE (1999) Production and characterization of monoclonal antibodies to two new mosquitoes *Aedes aegypti* salivary proteins. Insect Biochem Mol Biol 29:909–914

Pereira J, Gurudutt KN (1990) Growth inhibition of *Musca domestica* L. and *Culex quinquefasciatus* (Say) by (−)-3-epicaryoptin isolated from leaves of *Clerodendron inerme* (Gaertn) (Verbenaeae). J Chem Ecol 16:2297–2306

Pitasawat B, Champakaew D, Choochote W, Jitpakdi A, Chaithong U, Kanjanapothi D, Rattanachanpichai E, Tippawankosal P, Riyong D, Tuetun B, Chaiyasit D (2007) Aromatic plant-derived essential oil: an alternative larvicide for mosquito control. Fitoterapia 78:205–210

Rahuman AA, Bagavan A, Kamaraj C, Saravanan E, Zahir AA, Elango G (2009) Efficacy of the larvicidal botanical extracts against *Culex quinquefasciatus* Say (Dipetera: Culicidae). Parasitol Res 104:1365–1372

Rajkumar S, Jebanesan A (2008) Bioactivity of flavonoid compounds from *Poncirus trifoliate* L. (Family: Rutaceae) against the dengue vector, *Aedes aegypti* L. (Diptera: Culicidae). Parasitol Res 104:19–25

Sathish Kumar M, Maneemegalai S (2008) Evaluation of larvicidal effect of *Lantana camara* Linn. against mosquito species *Aedes aegypti* and *Culex quinquefasciatus*. Adv Biol Res 2:39–43

Simonsen HT, Nordskjold JB, Smitt UW, Nyman W, Palpu P, Joshi P, Varughese G (2001) In vitro screening of Indian medicinal plants for antiplasmodial activity. J Ethnopharmacol 74:195–204

Sukumar K, Perich MJ, Boobar LR (1991) Botanical derivatives in mosquito control: a review. J Am Mosq Control Assoc 7:210–237

Ved DK, Goraya GS (2008) Demand and supply of medicinal plants in India. Bishen Singh Mahendra Pal Sing, Dehradun and FRLTH, Bangalore, India, pp 139–204

WHO (2008) The Global Programme to Eliminate Lymphatic Filariasis (GPELF). http://www.who.int/lymphatic_filariasis/disease/en/. Accessed Mar 2008

World Health Organization (1992) Lymphatic filariasis. The disease and its control. WHO, Geneva, p 821, Technical Report Series

World Health Organization (1996a) Report of the WHO informal Consultation on the evaluation and testing of insecticides. CTD/WHO PES/IC/96.1. WHO, Geneva, p 69

World Health Organization (1996b) The World Health Report 1996: fighting disease, fostering development. WHO, Geneva, p 48