Larvicidal efficacy of *Vernonia cinerea* (L.) (Asteraceae) leaf extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae)

S. Arivoli*, Samuel Tennyson and J. Jesudoss Martin

ABSTRACT

The larvicidal efficacy of *Vernonia cinerea* leaf extracts against the common filarial vector, *Culex quinquefasciatus* was determined. Ground *Vernonia cinerea* leaves were sequentially extracted with ethyl acetate, chloroform, acetone and methanol. A series of concentrations of the extracts ranging from 0.05 – 2 mg/ml were tested against third instar larvae using WHO protocol and their percentage mortalities, LC_{50} values were determined. The ethyl acetate leaf extract of *Vernonia cinerea* was found to be effective with an LC_{50} value of 1.63 mg/ml after 24 hr. Application of these extracts to larval habits may lead to promising results in filarial and mosquito management programmes.

Key words: *Vernonia cinerea*, leaf extracts, *Culex quinquefasciatus*, larvicidal activity.

INTRODUCTION

Among the various groups of invertebrate animals, insects have a very close relationship with life and existence of mankind (Venkitaraman, 1983). In the insect group, many insects of the Order Diptera act as vectors and play a role in spreading diseases among man. Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases such as Malaria, Filariasis, Dengue, Japanese encephalitis, etc., causing millions of deaths every year (Rahuman *et al*., 2009; Borah *et al*., 2010). Filariasis is an endemic, disabling, disfiguring disease and the filarial worm, *Wuchereria bancrofti* responsible for human filariasis is carried by *Culex quinquefasciatus* (Mani, 1982; Kettle, 1984). *Culex quinquefasciatus* Say (Diptera: Culicidae) is a pantropical pest and urban vector of *Wuchereria bancrofti* (Holder, 1999) and is probably the most abundant house mosquito in towns and cities of the tropical countries (Samuel *et al*., 2007). Interest in the control of *Culex quinquefasciatus* lies in the fact that it acts as a vector of filarial fever, a serious public health problem in India and many developing countries. One of the strategies of the WHO in combating tropical diseases is to destroy their vectors or intermediate hosts. Since no effective vaccine is available for filarial fever, the only efficacious approach of minimizing the incidence of this disease is to eradicate and control mosquito vectors mainly by application of insecticides to larval habitats.

In the past few decades, synthetic insecticides were used as mosquito controlling agents but have produced a negative feedback of environment ill effect, non-target organisms being affected and most of mosquito species becoming physiologically resistant (VCRC, 1989; Severini *et al*., 1993). These factors have created a search for eco-friendly, biodegradable and target specific insecticides against the mosquitoes. In recent years, the emphasis to control the mosquito population has shifted steadily from the use of conventional chemicals towards more specific and environmentally friendly materials, which are generally of botanical origin. Plant products have been used traditionally by human communities in many parts of the world against the vector and pest species of insects (Jacobson, 1958; Pavela, 2007). The plant derived natural products as larvicides have the advantage of being harmless to beneficial non-target organisms and environment when compared to synthetic insecticides (Pitasawat *et al*., 2007). For this purpose, a lot of phytochemicals extracted from various plant species have been tested for their larvicidal actions against mosquitoes (Ciccia *et al*., 2000; Pavela, 2008a).

Members of the plant family Asteraceae possess various types of activity against many species of mosquitoes (Johnson, 1998). Thiophene derivatives and flavonoids present in some species of Asteraceae family have been found to be toxic to insects including mosquito larvae (Bohlmann *et al*., 1981; Perich *et al*., 1994; Ribeiro *et al*., 1994). *Vernonia cinerea* (L.) belonging to the family Asteraceae is an annual plant widely distributed in India, Bangladesh, Sri Lanka and Malay island (Harborne, 1998). It is commonly known as 'little ironweed' in English, 'joanbeer', 'kukshim' in Bengali, 'puvamkurunnel' in Malayalam and 'sahadevi' in Sanskrit and Hindi (Pattanayak

and Datta, 2008; Varghese *et al*., 2010). The plant is extensively used in indigenous medicine as stomachic and for cold, asthma and bronchitis (Kirtikar and Basu, 2000). The Ayurvedha Pharmacopoeia of India recommends the plant to treat intermittent fever, filariasis, blisters, boils and vaginal discharges. The roots of the plant are used traditionally for the treatment of all types of eruptive boils and the juice is used for quicker healing of accidental wounds, filariasis and toxic viral fevers. The seeds are used in dysuria and to treat colic in the form of decoction (Varghese *et al*., 2010). The young leaves of this plant are used for the treatment of tonsillitis (Hamill *et al*., 2003). The leaf juice extract is used to treat skin diseases and the leaf extract for treating dysentery in children (Maruthupandian and Mohan, 2010). Besides these, the plant is used in smoking cessation, cough, fever, malaria, urinary calculi, arthritis (Lhieochaiphant, 1985; Bunyapraphatsara, 2005; Lin, 2005) and leprosy (Vijayan *et al*., 2010). The plant possess antimicrobial (Yoga *et al*., 2009), antibacterial (Gupta *et al*., 2003), antioxidant (Mishra *et al*., 1984), antihelmentic (Anonymous, 2001), anti-inflammatory, analgesic, antipyretic (Gupta *et al*., 2003; Iwalewa *et al*., 2003; Mazumder *et al*., 2003), antiflautulent, antispasmodic (Varghese *et al*., 2010) and antidiuretic properties (Herrera *et al*., 1998). Some of the phytochemical compounds present are sterols, flavonoids, sesquiterpene lactones (Chopra *et al*., 1992) and a terpenoid, 'leupeol acetate' which shows antihyperglycaemic and antiulcer properties (Harborne and Baxter, 1996). Furthermore, *Vernonia cinerea* also possesses insect-antifeedant property (Tandon *et al*., 1999). Therefore, the present study was taken up to evaluate the crude leaf extracts of *Vernonia cinerea* for its larvicidal activity against *Culex quinquefasciatus.*

MATERIALS AND METHODS

Plant extracts

Vernonia cinerea leaves collected in and around Kancheepuram, Tamilnadu, India were brought to the laboratory, shade dried under room temperature and powdered using an electric blender. Dried and powdered leaves (1 kg) was subjected to sequential extraction using 3 L of ethyl acetate, chloroform, acetone and methanol for a period of 72 h r to obtain the crude extracts using rotary vacuum evaporator. The ethyl acetate, chloroform, acetone and methanol crude extracts thus obtained were lyophilized and a stock solution prepared from each crude extract by adding adequate volume of DMSO (Dimethyl-sulphoxide) and was refrigerated at 4 ^{0}C until testing for larvicidal bioassay.

Test animals

Experimental tests were carried out against laboratory reared vector mosquito *viz*., *Culex quinquefasciatus* free of exposure to insecticides and pathogens. Cyclic generation of *Culex quinquefasciatus* was maintained at 25 – 29^o C and 80 – 90 per cent R.H. in the insectarium. Larvae were fed on larval food (powdered dog biscuit and yeast in the ratio 3:1) and adult mosquitoes on 10 per cent glucose solution. Adult female mosquitoes were periodically blood-fed on restrained albino mice for egg production.

Larvicidal activity

Bioassay for the larvicidal activity was carried out using WHO (1981) procedure with slight modifications. From the stock solution, a series of concentrations *viz*., 0.05, 0.1, 0.2, 0.3, 0.5, 1.0, 1.5 and 2.0 mg/ml were prepared. Twenty early third instar larvae were introduced in 250 ml beaker containing 200 ml of distilled water with each concentration. A control was prepared by the addition of DMSO to distilled water. A total of three trials were carried out with five replicates per trial. Mortality was recorded after 24 and 48 hr and the control mortality was corrected using Abbott's (1925) formula when the control mortality ranged between 5-20 per cent,

$$\text{Per cent mortality} = \frac{\text{\% Mortality in treated} - \text{\% Mortality in control}}{100 - \text{\% Mortality in control}} \times 100$$

Statistical analysis

Probit analysis (Finney, 1971) was used for determination of LC_{50} and LC_{90}. Data from mortality and effect of concentrations were subjected to analysis of variance. Difference between the treatments was determined by Duncan multiple range test ($P < 0.05$). The highest different values from average detected by statistical testing are marked with letter "a" the next text lower with "b" and continued accordingly (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

The results of the larval susceptibility of *Culex quinquefasciatus* using *Vernonia cinerea* leaf extracts are presented in Table 1 and 2. The results of the present study revealed that among the four extracts tested, the ethyl acetate extract was found to be effective followed by chloroform, acetone and methanol. Furthermore, the effect of larval mortality was dependent on the concentration of leaf extract. The LC_{50} and LC_{90} of third instar larvae of *Culex quinquefasciatus* were determined to be 1.63 and 4.25 mg/ml after 24 hr. *Vernonia cinerea* leaf extracts can kill more than 50 per cent of the larvae population tested indicating that this plant species can certainly help reduce the population of mosquito. These results clearly indicate that the plant-based

Table 1. Per cent larval mortality of *Culex quinquefasciatus* against the leaf extracts of *Vernonia cinerea*

Extracts	Concentration (mg/ml)															
	0.05		0.1		0.2		0.3		0.5		1.0		1.5		2.0	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
Ethyl acetate	21.67 ±2.89^{b}	71.67 ±2.89^{b}	26.67 ±2.89^{c}	76.67 ±2.89^{bc}	28.83 ±2.89^{c}	81.67 ±2.89^{cd}	33.33 ±2.89^{d}	85.00 ±5.00^{de}	36.67 ±2.89^{d}	88.33 ±2.89^{ef}	41.67 ±2.89^{e}	93.33 ±2.89^{fg}	46.67 ±2.89^{f}	95.00 ±0.00^{g}	51.67 ±2.89^{g}	98.33 ±2.89^{g}
Chloro - form	16.67 ±2.89^{b}	63.33 ±2.89^{b}	20.00 ±0.00^{bc}	68.33 ±2.89^{c}	23.33 ±2.89^{cd}	73.33 ±2.89^{d}	26.67 ±2.89^{d}	78.33 ±2.89^{e}	31.67 ±2.89^{e}	80.00 ±0.00^{ef}	38.33 ±2.89^{f}	81.67 ±2.89^{fg}	43.33 ±2.89^{g}	83.33 ±2.89^{gh}	46.67 ±2.89^{g}	90.00 ±0.00^{h}
Acet - one	11.67 ±2.89^{b}	71.67 ±2.89^{b}	16.67 ±2.89^{c}	76.67x ±2.89^{c}	23.33 ±2.89^{d}	80.00 ±0.00^{de}	26.67 ±2.89^{d}	83.33 ±2.89^{ef}	31.67 ±2.89^{e}	86.67 ±2.89^{fg}	40.00 ±0.00^{f}	88.33 ±2.89^{fg}	41.67 ±2.89^{fg}	90.00 ±5.00^{g}	45.00 ±0.00^{g}	100.00 ±2.89^{h}
Metha - nol	15.00 ±0.00^{b}	61.67 ±2.89^{b}	16.67 ±2.89^{b}	63.33 ±2.89^{b}	23.33 ±2.89^{c}	66.67 ±2.89^{b}	26.67 ±2.89^{cd}	73.33 ±2.89^{c}	31.67 ±2.89^{de}	78.33 ±2.89^{d}	38.33 ±2.89^{ef}	81.67 ±2.89^{de}	43.33 ±2.89^{fg}	83.33 ±2.89^{de}	46.67 ±2.89^{g}	86.67 ±2.89^{e}
Control	0 ±0^{a}	0 ±0^{a}	0 ±0^{a}	0 ±0^{a}	0 ±0^{a}	0 ±0^{a}	0 ±0^{a}	0 ±0^{a}	0 ±0^{a}	0 ±0^{a}	0 ±0^{a}	0 ±0^{a}	0 ±0^{a}	0 ±0^{a}	0 ±0^{a}	0 ±0^{a}

Values are mean (%) of the five-replication of three trials ± standard deviation. ANOVA followed by DMRT test performed.
Different superscripts in the column indicate significance difference at P <0.05 levels.

Table 2. Probit analysis of larvicidal efficacy of leaf extracts of *Vernonia cinerea* against *Culex quinquefasciatus*

Extracts	LC_{50}(mg/ml)		LC_{90}(mg/ml)		F value	
	24h	48h	24h	48h	24h	48h
Ethyl acetate	1.63	-.13	4.25	0.83	38.57*	27.61*
Chloro form	1.84	-.12	4.30	1.54	50.28*	39.90*
Acet - one	1.89	-.15	4.29	0.98	70.76*	27.64*
Metha -nol	2.08	-.03	4.74	1.77	31.74*	33.12*

$LC_{50:}$ Lethal concentration required to kill 50 per cent of the population exposed
$LC_{90:}$ Lethal concentration required to kill 90 per cent of the population exposed
* Significant at P < 0.05 level

insecticides, which are less expensive than synthetic insecticides, exert high larvicidal effect.

There is no vaccine to prevent infection caused by *Culex quinquefasciatus* mosquito and the filarial parasite is continually developing resistance to the available drugs, so vector control is the best option. Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides or development of newer insecticides (Cisneros *et al*., 2002). However due to the continuous increase in resistance of mosquitoes to familiar synthetic insecticides better alternative means are sought (Hag *et al*., 1999). A considerable number of plant derivatives have shown to be effective against mosquitoes with a safe manner. Though several plant species from different families have been reported for mosquitocidal activity, only a few botanicals have moved from laboratory to field use which might be due to the presence of phytochemicals when compared to synthetic insecticides (Green *et al*., 1991).

Plants belonging to the family Asteraceae have been extensively screened/studied for their larvicidal activity ever since the discovery of the larvicidal potential of the extract of *Chrysanthemum cinnerariaefolium* (Omena *et al*., 2007). Plants that showed promising larvicidal activity are *Otanthus maritimus* methanolic stem extract (LC_{50} = 7.0 ppm) (Pavela, 2008b), *Artemisia campestris* methanolic stem extract (LC_{50} = 23.0ppm) (Pavela *et al*., 2009), methanolic extracts of flowers of *Gleoonis coronarium* (LC_{50} = 53.0 ppm), stem of *Sonchus arvensis* (LC_{50} = 68.0 ppm), flowers of *Matricaria maritima*

(LC_{50} = 72.0 ppm), (Pavela, 2008b), *Tagetes erectes* petroleum ether leaf extract (LC_{50} = 100.0 ppm) (Sakthivadivel and Daniel, 2008) against *Culex quinquefasciatus*. Crude extracts of many other plants showed moderate larvicidal activity and their LC_{50} values ranged between 100 to 200 ppm. They include ethyl acetate leaf extract of *Eclipta prostrata* (LC_{50} = 119.89 ppm) against *Culex tritaeniorhynchus* (Elango *et al*., 2009), *Achilea millefolium* methanolic stem extract (LC_{50} 120.0 ppm) (Pavela, 2008b), *Tanacetum vulgare* methanolic flower extract (LC_{50} = 178.0 ppm) and methanolic stem extract of *Otanthus maritimus* (LC_{50} = 195.0 ppm) (Pavela *et al*., 2009) and LC_{50} values ranging above 100 ppm were found in petroleum ether leaf extracts of *Artemisia nilagirica* and *Galinsoga quadriradiata* (Sakthivadivel and Daniel, 2008), *Ageratum conyzoides* (Sharma *et al*., 2009), and ethyl acetate leaf extracts of *Ageratum houstonianum* (Samuel, 2010) and dichloromethane whole plant extracts of *Citrullus colocynthis* (Arivoli and Samuel, 2011) against *Culex quinquefasciatus*. Therefore, the results of the present and previous studies mentioned above indicate that the leaf extract of *Vernonia cinerea* possesses larvicidal activity against *Culex quinquefasciatus*. Therefore, further in-depth investigations on the crude extract/phytotoxic compounds of *Vernonia cinerea* are needed to elucidate the larvicidal activity against a wide range of all stages of mosquito species and also the active ingredients of the extract responsible for larvicidal activity in *Culex quinquefasciatus* should be identified, and small scale field trials are needed for usage of this plant as a mosquitocidal agent.

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S. Arivoli*, Samuel Tennyson and J. Jesudoss Martin
P.G. & Research Department of Advanced Zoology and Biotechnology, Loyola College, Chennai 600 034 Tamilnadu, India. Mobile: +91 9444173931, *Corresponding author E-mail: rmsarivoli@gmail.com

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