Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

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

Document heading doi: 10.1016/S2222-1808(14)60729-5

Evaluation of larvicidal efficacy of *Cleome viscosa* L. (Capparaceae) aerial extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae)

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ARTICLE INFO

Article history:
Received 15 Jul 2014
Received in revised form 11 Aug 2014
Accepted 29 Aug 2014
Available online 14 Sep 2014

Keywords:
Cleome viscosa
Crude aerial extracts
Culex quinquefasciatus
Larvicidal efficacy

ABSTRACT

Objective: To evaluate the larvicidal efficacy of crude petroleum ether, chloroform, acetone and aqueous aerial extracts of *Cleome viscosa* against the filarial vector, *Culex quinquefasciatus*.
Methods: Standard World Health Organization protocols with minor modifications was adopted for the larvicidal bioassay. Larvicidal activity of exyracts was evaluated at concentrations of 62.5, 125, 250, 500 and 1 000 mg/L. Larval mortality was observed after 24 and 48 h.
Results: Amongst the solvent extracts tested, petroleum ether exhibited the highest larvicidal activity and LC_{50} values was 52.62 and 43.16 mg/L followed by acetone, aqueous and chloroform extract with LC_{50} values of 328.64 and 280.58; 493.44 and 298.76; 509.27 and 434.40 mg/L after 24 and 48 h respectively.
Conclusions: Further investigations are needed to explore the larvicidal activity of the petroleum ether aerial extract of this plant against a wide range of mosquito species and also the active ingredient(s) of the extract responsible for larvicidal activity should be identified.

1. Introduction

Mosquito is the most indisputable medicinal significant arthropod vector of diseases[1]. The application of chemical insecticides for decades has met with tremendous setbacks in the light of the development of vector resistance and some attendant environmental hazards[2]. The popularity of botanical pesticides is once again increasing and some plant products are being used globally as green pesticides. The body of scientific literature documenting bioactivity of plant derivatives to different pests continues to expand, yet only a handful of botanicals are currently used[3]. Natural insecticides of plant origin have been regarded important due to their ecofriendly nature and biodegradability as a substitute of synthetic insecticides for the control of vectors of public health importance. Botanical larvicides can contribute remarkably to the lowering of vector mosquito population[4–9]. Different types of phytochemicals of plant either from the whole part or from the specific parts come out with solvent during chemical extraction depending on the polarity of the solvent[10]. These phytochemicals generally act as a toxicant towards adult, as well as larval form of mosquitoes, while some interfere with the growth (growth inhibitory activity). Insecticides of botanical origin are attractive alternatives because they contain rich sources and various bioactive compounds, many of which are selective and have little or no harmful effect on non-target organisms and the environment[6]. Furthermore, the complex and variable mixtures of bioactive constituents with different modes of action may lessen the chance of resistance in mosquito populations[11].

Cleome viscosa L. (*C. viscosa*) (Capparaceae) is an annual, herbaceous plant present in Northern and North Eastern parts[12,13], over the plains of India, and throughout the tropics of the world[12]. The plant is also found distributed in the plains of Arabia, Egypt, Pakistan and also in Asia and Africa[14]. *C. viscosa* is commonly known as wild or dog mustard[12], tickweed, wild mustard and spider plant in English[15], nayikkadugu in Tamil and hurhuria in Bengali[15,16]. *C. viscosa* is used in Indian system of medicine for treating fever, inflammations, liver diseases,

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Foundation Project: Supported by Council for Scientific and Industrial Research (CSIR), Government of India (Grant No. 9/656(12)/2004-EMR-I).

bronchitis and diarrhoea[17]. The whole herb is used in treatment of ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis and cardiac disorders[18]. *C. viscosa* is reported to possess pharmacological properties *viz.*, antihelmintic, antipyretic, antidiarrhoeal, anti-inflammatory, hepatoprotective, antiseptic, carminative, febrifuge, sudorific, cardiac stimulant and immunomodulatory, and the phytochemical constituents/compounds including alkaloids, flavanoids, saponins, tannins and steroids and diterpenoids[19]. Very few reports are present pertaining to the insecticidal property of *C. viscosa*. *C. viscosa* leaves was found to possess insecticidal activity when tested against the larva of *Culex quinquefasciatus (Cx. quinquefasciatus)* [20]. Recently, Bansal *et al*. has reported on the larvicidal potential of crude petroleum ether, acetone and methanol seed extracts of *C. viscosa* against *Anopheles stephensi*, *Aedes aegypti* and *Cx. quinquefasciatus*[21]. Therefore, in the present investigation, the crude aerial extracts of *C. viscosa* has been evaluated for its larvicidal activity against *Cx. quinquefasciatus*.

2. Materials and methods

2.1. Plant collection and extraction

Mature aerial parts of *C. viscosa* collected from places in and around Chennai, Tamil Nadu, India were brought to the laboratory, shade dried at room temperature and powdered. Dried and powdered aerial parts (1 kg) was macerated sequentially with 3 L of petroleum ether, chloroform, acetone and distilled water for a period of 96 h and filtered. The filtrate was then concentrated at reduced temperature on a rotary evaporator. The crude petroleum ether, chloroform, acetone and aqueous aerial extracts thus obtained were lyophilized and a stock solution of 100 000 mg/L prepared by adding adequate volume of Tween 80 was refrigerated at 4 °C until testing for bioassay.

2.2. Larvicidal bioassay

Bioassay was carried out against laboratory reared vector mosquitoes with free exposure to insecticides. Standard World Health Organization protocol with slight modifications was adopted for the study[22]. The tests were conducted in glass beakers. Mosquito immature particularly third instar larvae were obtained from laboratory colonized mosquitoes of F_1 generation. From the stock solutions, concentrations of 62.5, 125, 250, 500 and 1 000 mg/L was prepared. Twenty healthy larvae were released into each 250 mL glass beaker containing 200 mL of water and extracts with test concentrations. Larval mortality was observed for 24 and 48 h after treatment. Larvae were considered dead if they showed no signs of movement when provoked on their respiratory siphon by a needle. A total of three trials with three replicates per trial for each concentration were carried out. Controls were run simultaneously. Treated control was prepared by the addition of Tween 80 to water. Distilled water served as untreated control. The larval per cent mortality was calculated and when control mortality ranged from 5%-20% it was corrected using Abbott's formula[23]. SPSS 11.5 version package was used for determination of LC_{50} and LC_{90} values. One way ANOVA followed by Duncan multiple range test (DMRT) was performed to determine the difference in larval mortality between concentrations.

3. Results

The results of *Cx. quinquefasciatus* larval mortality tested with *C. viscosa* crude petroleum ether, chloroform, acetone and aqueous aerial extracts are presented in Tables 1 and 2. No larval mortality was observed in untreated control and treated control after 24 and 48 h. The results of the present study revealed that among the solvent extracts tested, petroleum ether exhibited the highest larvicidal activity and LC_{50} values were 52.62 and 43.16 mg/L followed by those of

Table 1

Larval mortality of *Cx. quinquefasciatus* by crude aerial extracts of *C. viscosa* at 24 h.

Solvent	Larval mortality				
	62.5 (mg/L)	125 (mg/L)	250 (mg/L)	500 (mg/L)	1 000 (mg/L)
Petroleum ether	7.66 ± 0.57^{b} (76.6)	9.66 ± 0.57^{c} (96.6)	10.00 ± 0.00^{c} (100.0)	10.00 ± 0.00^{c} (100.0)	10.00 ± 0.00^{c} (100.0)
Chloroform	0.00 ± 0.00^{a} (0.0)	0.00 ± 0.00^{a} (0.0)	4.33 ± 1.15^{b} (43.3)	4.66 ± 1.15^{b} (46.6)	9.33 ± 0.57^{c} (93.3)
Acetone	1.00 ± 0.00^{a} (10.0)	3.00 ± 1.00^{b} (30.0)	5.33 ± 0.57^{c} (53.3)	6.66 ± 1.15^{d} (66.6)	9.66 ± 0.57^{e} (96.6)
Aqueous	0.33 ± 0.57^{a} (3.3)	2.00 ± 1.00^{b} (20.0)	4.00 ± 1.00^{c} (40.0)	5.33 ± 1.52^{c} (53.3)	9.66 ± 0.57^{d} (96.6)

Values are mean of the three replicates of three trials±standard deviation. Figures in paranthesis denote per cent larval mortality.
Different superscript alphabets within same row indicate statistical significant difference at $P<0.05$ level by One way ANOVA followed by DMRT.

Table 2

Larval mortality of *Cx. quinquefasciatus* against crude aerial extracts of *C. viscosa* at 48 h.

Solvent	Larval mortality				
	62.5 (mg/L)	125 (mg/L)	250 (mg/L)	500 (mg/L)	1 000 (mg/L)
Petroleum ether	9.66 ± 0.57^{b} (96.6)	10.00 ± 0.00^{b} (100.0)	10.00 ± 0.00^{b} (100.0)	10.00 ± 0.00^{b} (100.0)	10.00 ± 0.00^{b} (100.0)
Chloroform	0.00 ± 0.00^{a} (0.0)	1.00 ± 1.00^{a} (10.0)	4.66 ± 0.57^{b} (46.6)	5.66 ± 1.15^{b} (56.6)	9.66 ± 0.57^{c} (96.6)
Acetone	1.66 ± 0.57^{b} (16.6)	3.66 ± 1.15^{c} (36.6)	6.33 ± 0.57^{d} (63.3)	7.33 ± 1.15^{d} (73.3)	10.00 ± 0.00^{e} (100.0)
Aqueous	0.66 ± 0.57^{a} (6.6)	2.33 ± 0.57^{b} (23.3)	4.00 ± 1.00^{c} (40.0)	6.33 ± 1.15^{d} (63.3)	10.00 ± 0.00^{e} (100.0)

Values are mean of the three replicates of three trials±standard deviation. Figures in paranthesis denote per cent larval mortality.
Different superscript alphabets within same row indicate statistical significant difference at $P<0.05$ level by One way ANOVA followed by DMRT.

acetone, aqueous and chloroform extract which were 328.64 and 280.58 mg/L; 493.44 and 298.76 mg/L; 509.27 and 434.40 mg/L after 24 and 48 h respectively (Table 3).

Table 3
Probit analysis of larvicidal efficacy of crude aerial extracts of *C. viscosa* against *Cx. quinquefasciatus*.

Solvent	LC_{50} (mg/L)		LC_{90} (mg/L)	
	24 h	48 h	24 h	48 h
Petroleum ether	52.62	43.16	85.07	56.67
Chloroform	509.27	434.40	847.36	743.24
Acetone	328.64	280.58	610.23	549.09
Aqueous	493.44	298.76	875.75	511.70

4. Discussion

Mosquitoes are a serious threat to public health, transmitting several dangerous diseases for over two billion people in the tropics. Vector-borne diseases caused by *Cx. quinquefasciatus* and other mosquitoes have become a global health problem[24]. Botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and may act as suitable alternative product to fight against mosquito-borne diseases[6]. In the past years, the plant kingdom has been of great interest as a potential source of insecticidal products. In addition, natural insecticides can provide core structures from which new insecticidal agents can be synthesized[25]. Many species in the plant kingdom synthesize a variety of secondary metabolites which play an active role in defense of plants against insects/mosquitoes. Phytochemicals obtained from plants are usually less environmentally harmful than synthetic chemicals and it has renewed the interest in the research on these compounds, considering them as an ecologically safe alternative for synthetic insecticides[26]. Phytochemicals obtained from plants with proven mosquito control potential can be used as an alternative to synthetic potential insecticides or along with other insecticides under the integrated vector control. Plant products can be used as an insecticide for killing mosquito larvae[27]. Products of secondary plant metabolisms may be responsible for the chemical communication between plants and insects. Allelochemicals have been considered as potential natural insecticides and can be used for insect/mosquito management in integrated control. Reviews on botanical phytochemicals with mosquitocidal potential published by Shaalan *et al*. and Ghosh *et al*. demonstrated identification of novel effective mosquitocidal agents from botanicals containing active phytochemicals[6,28]. The review gives current state of knowledge on larvicidal plant species, extraction processes, residual capacity, effects on non-target organisms, resistance, screening methodologies, and discusses promising advances made in phytochemical research for vector control.

Plants have been screened for their larvicidal activity against *Cx. quinquefasciatus*[8]. The results of the present study indicated that petroleum ether extract was found to be effective and LC_{50} value was 52.62 and 43.16 mg/L after 24 and 48 h followed by those of acetone, aqueous and chloroform which were 328.64 and 280.58, 493.44 and 298.76, 509.27 and 434.40 mg/L respectively. The results of the present study were comparable with the reports of Bansal *et al*. who found that the crude petroleum ether, acetone and methanolic seed extracts of *C. viscosa* exhibited larvicidal activity against *Anopheles stephensi*, *Aedes aegypti* and *Cx. quinquefasciatus* and LC_{50} values was 166.4, 106.3, 144.1, and 51.4, 138.9, 99.5 and 301.9, 118.5, 127.1 mg/L respectively by 24 h exposure[21]. Pavela *et al.* reported that the methanolic stem extract of *Artemisia campestris* exhibitd larvicidal activity against *Cx. quinquefasciatus* and LC_{50} value was 23 mg/L[29]. Besides these, the methanolic extracts of *Gleoonis coronarium* flowers (LC_{50} 53 mg/L), *Sonchus arvensis* stem (LC_{50} 68 mg/L), *Matricaria maritima* flowers (LC_{50} 72 mg/L)[30], *Tagetes erectes* petroleum ether leaf extract (LC_{50} 100 mg/L) were found to exhibit larvicidal activity against *Cx. quinquefasciatus*[31]. Crude extracts of plants which showed larvicidal activity with LC_{50} value above 100 mg/L against *Cx. quinquefasciatus* were *Achilea millefolium* methanolic stem extract (LC_{50} 120 mg/L), *Tanacetum vulgare* methanolic flower extract (LC_{50} 178 mg/L) and methanolic stem extract of *Otanthus maritimus* (LC_{50} 195 mg/L)[29].

Insecticidal effects of plant extracts vary not only according to plant species, geographical distribution and parts used, but also the extraction methodology adopted and the polarity of the solvents used during extraction. It has been shown that the extraction of active biochemicals from plant depends upon the polarity of solvents used. In most of the studies solvent with minimum (hexane or petroleum ether) and maximum polarity (aqueous) was used[6,28,32]. Therefore, most effective alternative approaches under the biological control program are to explore the floral biodiversity and use safer insecticides of botanical origin as a simple and sustainable method of mosquito control. Biopesticides may serve as suitable alternative to chemical insecticides in future as they are relatively safe and are mostly directed against larvae since larval treatment is more localized in time and space resulting in less-dangerous outcomes. Larval control can be effective due to the low mobility of larval mosquitoes, especially where the principal breeding habitats are man-made and can be easily identified[33]. The findings of the present investigation revealed that the crude petroleum ether aerial extract of *C. viscosa* possess larvicidal activity against *Cx. quinquefasciatus*. It may concluded that natural products like extracts from parts of plants with insecticidal and medicinal values have higher efficiency in reducing mosquito menace due to their larvicidal toxicity. Further studies on the screening, isolation and purification of bioactive phytochemical constituents/compounds followed by in-depth laboratory and field bioassays are needed as the present study shows that *C. viscosa* petroleum ether aerial extracts can be used to control the immature stages of vector mosquitoes.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors thank the Director, King Institute of Preventive Medicine and Research for the facilities provided.

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