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Toxicity of *Vernonia anthelmintica* Linn. (Asteracea) seeds against mosquitoes vectors

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Abstract: The Toxicological activity (larvicidal, adulticidal and repellent toxicity) of *Vernonia anthelmintica* seeds fraction was tested against different species of mosquito vectors viz, malaria (*Anopheles culicifacies* and *Anopheles stephensi), filaria (Culex quinquefasciatus)* and *dengue (Aedes aegypti).* The larvicidal toxicity of *Vernonia anthelmintica* seeds fraction was evaluated against the early 4th instars larvae of different mosquitoes species. Mean LC_{50} value of the column fraction KAL-4 from seeds of *V. anthelmintica* against the larvae of *An. culicifacies, An. stephensi, Culex quinquifaciatus* and *Aedes aegpyti* were found to be 64 ppm, 70 ppm, 143 ppm and 166 ppm respectively. The larvicidal toxicity was more against *An. culicifacies, An. stephensi* than *Culex quinquifaciatus* and *Aedes aegypti.* The seed extracts did not show any adulticidal toxicity and repellent toxicity even at 10% concentrated impregnated paper and 5% on human hand, respectively.

Keywords: Toxicogical activity, *Vernonia anthelmintica*, Mosquitoes

INTRODUCTION

Mosquitoes are the single largest group of insects, which serve as vectors of several diseases causing various health problems to human beings. Although eradication of these vectors were considered by the use of chemical insecticides, which are now being seriously questioned due to environmental pollution (Edward *et al.,* 1978); multiple resistance (Brown, 1986) and high operational costs. These factors have created the need for environmentally safe, degradable and target specific insecticides against mosquitoes. The search for such compounds has been directed extensively to the plant kingdom. The phytochemicals derived from plant resources can be used as an alternative to the synthetic insecticides (Mittal and Subarao, 2003). A large number of plant extracts have been reported to have mosquitocidal or repellent Toxicity against mosquito vectors (Sukumar *et al.,* 1991). Recently Singh *et al.,* 2002 reported larvicidal properties of leaves extract of *Solanum nigrum* against *Aedes aegypti*. Das *et al.* (2003) reported the evaluation of botanicals as repellents against mosquitoes. While Rajkumar and Jebanesan (2005) reported larvicidal and adult emergence inhibition effects of *Centella asiatica* Brahmi (Umbelliferae) against mosquito *Culex quinquefasciatus* and adulticidal Toxicity against *An. stephensi*. Recently Dua *et al.* (2008) reported insecticidal activity of *Valeriana jatamansi* (Valerianaceae) against mosquitoes.

Veronia anthelmintica (Asteraceae) are annual robust, erect aromatic herbs (60-90 cm height) are distributed throughout warm parts of India as a weeds. *V. anthelmintica* seeds have been used in traditional ayurvedic medicine as anthelmintic, antipyretic and anti-inflammatory agents as well as for the treatment of skin diseases such as psoriasis (Kirtkar *et al.,* 1935). The present study was undertaken to investigate the biological Toxicity of different fractions of *Vernonia anthelmintica* seeds against *An. culicifacies, An. stephensi, Culex quinquifaciatus* and *Aedes aegypti,* which could be helpful in mosquito control.

MATERIALS AND METHODS

Vernonia anthelmintica Lin. (Asteraceae) seeds were procured from local herbal supplier Uttaranchal,(India). The seeds were washed properly, air dried in shade and powdered in Kitchen machine. 100g seeds were sequentially extracted using Soxhlet apparatus (6hrs) with petroleum ether, chloroform and methanol, which produced 10g (10%) petroleum fraction code KAL-a, 1.5g (1.5%) chloroform fraction code KAL-b and 3g (3%) methanol fraction code KAL-c respectively. The crude extracts thus obtained were evaporated to dryness under reduce pressure below 40°C. Larvicidal, adulticidal and repellent Toxicity of all coded fractions were carried out against different mosquito species in the laboratory of

Table 1. Percent yield of different extracts from seeds of *Vernonia anthelmintica* and their larvicidal toxicity against mosquito species.

Fraction code (% Yield)	Solvents	Mosquito species	Toxicity (ppm) LC^1_{50} (mean±sd)	LC_{90} (mean±sd)
KAL-a (10%)	Petroleum ether	*An.culicifacies*	125±5.0	225±8.2
		An. stephensi	150±3.5	230±9.4
		Culex quinquefasciatus	337±5.5	456±4.2
		Aedes aegypti	350±7.5	595±8.2
KAL-b (1.5%)	Chloroform	*An. culicifacies*	250±5.5	440±4.2
		An. stephensi	275±4.5	456±6.5
		Culex quinquefasciatus	612±6.2	1175±8.2
		Aedes aegypti	650±6.2	1050±7.3
KAL-c (3%)	Methanol	*An. culicifacies*	300±9.5	550±5.8
		An. stephensi	350±7.8	650±3.5
		Culex quinquefasciatus	625±12.5	1137±4.5
		Aedes aegypti	775±10.0	1237±3.7

[1]LC_{50}, median lethal concentration; Number of replicates = 5

National Institute of Malaria Research, Field Station, Haridwar, India.

Larvicidal toxicity was carried out using all the three fractions mentioned above against early 4th instars larvae of *An. culicifacies, An. stephensi, Culex quinquifaciatus and Aedes aegypti as* described earlier (WHO, 1996). Twenty larvae of mosquitoes were placed in to 500 ml capacity glass beaker containing 250ml water. The mosquito larvae were exposed to different concentration of the seed extracts and mixture of dog biscuit and yeast powder in the ratio of 3:2 were provided as nutrients. Mortality of larvae was monitored after 24hrs. Preliminary larvicidal test revealed that petroleum ether fraction (KAL-a) showed good larvicidal toxicity. Hence this fraction was further fractionated in Silica-gel column using petroleum ether; benzene; benzene: chloroform (1:1); chloroform and methanol as mobile phase. Silica gel (60-120m mesh size) was used as stationary phase. The different column fractions were collected and tested against different mosquito's larvae. All tests were carried out in five replicates along with untreated control in the laboratory.

Adulticidal toxicity of different fractions of *V. anthelmintica* seeds were evaluated against *An. culicifacies, An. stephensi, Cx. quinquifaciatus and Ae. aegypt*i. 2.5ml of each fraction with different concentration viz. 5% (0.69mg/cm^2) and 10% (1.38mg/cm^2) were tested on Whatman filter paper (12´15 cm size). Batches of 20 (one day old glucose fed) mosquitoes were exposed on treated papers into WHO susceptibility tube for one-hour exposure period and knocked down and then mortality was recorded. After one hour knocked or lived mosquitoes were transferred in the holding tube for further 24hrs of recovery period and the mortality was recorded. Similarly mosquitoes were exposed in control paper, knocked and then mortality was recoded.

The repellent toxicity of different fractions obtained from the seeds of *V. anthelmintica* were also carried out on the basis of the protection time against mosquito bites. In the experimental test with repellents only the upper surface of the arm was used, the lower surface was covered with aluminum foil. 0.6 ml of 5% v/v concentration in acetone and coconut oil of each fraction was applied on the upper arm and it was exposed in the mosquito cage (>100 mosquitoes). The number of mosquito bites was counted after every 15 min for 5 min until a confirm bite was received. Similarly, untreated arm was exposed for 5min. and the rate of mosquitoes biting was recorded. The adult mosquitoes and larvae obtained from well-established insectary at National Institute of Malaria Research, Haridwar (Uttranchal), India. All bioassay were performed according to standard methods (WHO, 1996) in the laboratory at constant temperature 26±2°C and relative humidity of (75±5)%.

RESULTS AND DISCUSSION

Larvicidal toxicity of isolated fraction code KAL-a, KAL-b and KAL-c from seeds of V*ernonia anthelmintica* was evaluated against mosquito larvae at different concentrations. The LC_{50} and LC_{90} values of all three fractions against different mosquito larvae are given in Table 1. The results revealed that only fraction code KAL-a was found most effective against mosquito larvae with LC_{50} range 125-350 ppm. Therefore, further study was confined to isolate the active molecule from KAL-a fraction by column chromatographic methods. The

Table 2. Larvicidal toxicity of different fraction isolated by column chromatography against *Anopheles stephensi.*

Column fraction	Solvents	Larvicidal toxicity[1] (LC_{50} ppm)
KAL-i	Petroleum ether	>250
KAL-ii	Benzene	>250
KAL-iii	Benzene and Chloroform (1:1)	>250
KAL-iv	Chloroform	71 ±8.5
KAL-v	Methanol	>250

[1] Value above 250 ppm was considered no toxicity.

Table 3. Larvicidal toxicity of fraction KAL-iv against different mosquito larvae.

Most active fraction	Mosquito species	Toxicity (ppm)	
		LC_{50} (mean±sd)	LC_{90} (mean±sd)
KAL-iv	*An.culicifacies*	64 ± 10.0	118 ± 8.0
	An.stephensi	71 ± 8.5	128 ± 9.0
	Culex quinquefasciatus	143 ± 13.0	260 ± 12.0
	Aedes aegypti	166 ± 12.0	288 ± 24.0

Number of replicates =4

bioassay test for larvicidal toxicity of five-column fractions coded as KAL-i (Petroleum ether), KAL-ii (Benzene), KAL-iii (Benzene and Chloroform 1:1), KAL-iv (Chloroform) and KAL-v (Methanol) were carried out against different species of mosquito larvae are given in Table 2. Among these, fraction code KAL-iv showed good larvicidal Toxicity at <170 ppm while other fraction did not show any larval mortality even at 250 ppm against different mosquito species within 24 hours. Therefore, further quantitative bioassay of fraction KAL-iv was carried out to find the median lethal concentration. The LC_{50} and LC_{90} value of fraction code KAL-iv against different mosquitoes species is given in Table 3. Further purification of the most efficient fraction KAL-iv in to pure compounds using TLC methods did not show any increase of larvicidal Toxicity suggesting that some synergistic factor may play a role regarding the larvicidal Toxicity. Therefore, further study was not carried out to purify the fraction code KAL-iv for insecticidal properties. Results revealed that larvae of *Anopheles stephensi* (LC_{50}=64±10.0ppm) and *Anopheles culicifacies* (LC_{50}=71±8.5ppm) are more susceptible than the larvae of *Culex quinquefasciatus* (LC_{50}=143±13.0ppm) and *Aedes aegypti* (LC_{50}=166±12.0ppm) against the fraction KAL-iv. However, the fraction of *V. anthelmintica* did not show any adulticidal toxicity and repellent toxicity against mosquitoes.

V. anthelmintica seeds have been used in traditional ayurvedic medicine as an antihelmintic, antipyretic and anti-inflammatory agent as well as treated for skin diseases such as psoriasis (Kirtkar *et al.,* 1935). Pires *et al.* (2002) reported the Anti-inflammatory toxicity of *V. anthelmintica* wild seeds extracts. However, there is no report of *V. anthelmintica* for larvicidal, adulticidal and repellent Toxicity against any mosquito species. The present study showed that seed extracts of *V. anthelmintica* can also be used as a potent larvicide against malaria vectors *Anopheles stephensi* and *Anopheles culicifacies.*

The literature indicates that seeds of *Syzygium jambolanum* (Pushpalatha and Muthukriishnan, 1995), saponin fraction of *Agave sisalana* (Pizzarro *et al.,* 1999), nine extract of four medicinal plant (Markouk *et al.,* 2000) and pine oil (Ansari *et al.,*2005) have been suggested for mosquito control.

The larvicidal toxicity of *V. anthelmintica* is comparable to other well established plant species. In earlier study, Pushpalatha and Muthukriishnan (1995) assessed the partially purified extracts of seeds of *Syzygium jambolanum* on different instars of *Culex quinquefasciatus* and *Anopheles stephensi* and concluded that petroleum ether (PE) : ethyl acetate(EA) in the ratio of 1:1 were most active with LC_{50} value 78.62, 43.87, 194.34 and 228.68ppm against I, II, III and IVth instars larvae of *Culex quinquefasciatus* while the LC_{50} value were 81.53, 84.61, 247.07 and 175.37 ppm against I, II, III and IV th instars larvae of *Anopheles stephensi*. Pizzarro *et al.* (1999) studied the toxicity of the saponin fraction of *Agave sisalana* and estimated the LC_{50} and LC_{90} value against 3rd instar larvae of *Culex quinquefasciatus*, that were 183 and 408 ppm, respectively. Markouk *et al.* (2000) evaluated the 16 extracts of four Moroccan medicinal plants for larvicidal properties against *Anopheles labranchiae* mosquito larvae and exhibited 9 extracts for high larvicidal toxicity with LC_{50} values (in the range 28 to 325ppm). Recently, Ansari *et al.* (2005) reported the larvicidal toxicity of pine oil against *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti* with LC_{50} value 112.6, 85.7 and 82.1 ppm, respectively. These concentrations were much higher than those reported in the present study for larvicidal toxicity against malaria vectors. Among different species of mosquitoes the Toxicity of *V. anthelmintica* was in the order of 64>71>143>166 ppm. Besides, the larvicidal toxicity of *V. anthelmintica* seed fraction KAL-iv is similar to various neem extracts, which are reported to be effective with LC_{50} values ranging from 55-65 ppm against mosquito larvae (Ascher and Meisner, 1989).

The present study indicates that seeds of *V. anthelmintica* fraction KAL-iv have potential larvicidal toxicity against malaria vector *An. stephenis* and *An. culicifacies* and can help in developing the mosquito larvicidal control agents with reduced environmental impact.

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