Journal of Environmental Biology
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September 2008, 29(5) 669-672 (2008)
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Larvicidal activity of an indigenous plant, *Centratherum anthelminticum*

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(Received: October 10, 2006; Revised received: April 09, 2007; Accepted: May 10, 2007)

Abstract: *Crude extracts of fruits and leaves of Centratherum anthelminticum in different solvents were tested for larvicidal activity against Anopheles stephensi, the vector of malaria. The petroleum ether crude extract of both fruits and leaves exhibited significant larvicidal activity against III instar larvae with LC_{50} values of 162.60 ppm and 522.94 ppm, respectively after 24 hr. The petroleum ether extract of fruit was 11.66, 2.15 and 1.32 times more toxic than that of leaf extract after 24 , 48 and 72 hr, respectively at LC_{90} level. However, at LC_{50} level the corresponding values were 3.22, 1.83 and 1.19, respectively. The petroleum ether extract of C. anthelminticum fruits is a promising source for the control of Anopheles larvae.*

Key words: *Centratherum anthelminticum, Anopheles stephensi, Probit analysis, Larvicidal activity*
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Introduction

Plants synthesize secondary metabolites that may possess insecticidal, antimicrobial (Leeja and Thoppil, 2007), herbicidal and other biological activities (Setia *et al.,* 2007; Tonk *et al.,* 2006). Phytochemicals such as nicotine, pyrethrins, rotenoids, brassinosteroids and azadirachtin obtained from plants have been evaluated and a few of them are also exploited commercially. Malaria is a major public health problem in more than 100 countries, inhabited by a total of some 2.4 billion people, or close to half of the world's population (Persidis, 2000). Female anopheline mosquitoes are involved in the transmission of malaria.

Many of the researches using plant derived products for controlling the malaria vector deal with ovicidal, larvicidal and adulticidal activities (Gbolade, 2001). The larval stage is the most vulnerable stage to attack mosquitoes as they are concentrated in smaller areas. Thus, one of the approaches for control of malaria transmission is by interrupting mosquito life cycle at larval stage.

Centratherum anthelminticum commonly known as kalijiri or Somraj is a robust leafy annual found throughout India ascending up to 5500 ft in Himalaya and Khasi Mountains, SriLanka, Afghanistan and Malaysia. The methanolic extract of seeds showed antiviral (Bhakuni *et al.,* 1969) and spermicidal activity (Setty *et al.,* 1977). Its anthelmintic, mild laxative, smooth muscle relaxant and mild hypotensive effects have also been demonstrated (Singh *et al.,* 1979). Antifilarial activity of acetone and ethyl acetate extracts of seeds against *Setaria cervi* have also been reported (Singhal *et al.,* 1992). The insecticidal activity of seed oil extracted in petroleum ether has been reported against *Bagrada cruciferanum* Kirk. 0.5% of its extract causes 100% mortality after 24 hr in laboratory condition and after 72 hr in field condition (Verma *et al.,* 2004).

Several plants of Asteraceae family are reported to have mosquito larvicidal activity due to presence of several thiophenes and flavanoids. *C. anthelminticum* is also a member of Asteraceae family and its phytochemical investigations shows presence of various sesquiterpene lactones, alkaloids (Johry and Singh, 1997), flavanoids (Yadava and Barsainya, 1997; Tiana *et al*., 2004), terpenoids (Mehta *et al*., 2004) and steroids (Mehta *et al*., 2005). Few reports are available to indicate pharmacological profile of this species (Johry and Singh, 1997) but none related to mosquito larvicidal property. In the present study the fruits and leaves of *C. anthelminticum* were extracted in various solvents successively and the extracts were evaluated against III instar larvae of *A. stephensi* to establish the most active extract.

Materials and Methods

Preparation of extracts: *C. anthelminticum* was cultivated in the botanical garden at Dayalbagh Educational Institute. Taxonomic identification was performed by Dr. H. B. Singh, Head, Raw Materials Herbarium and Museum, NISCAIR, New Delhi, India where voucher specimen has been deposited as RHMD 2264. Fruits and leaves were collected from these plants, shade dried and powdered. They were extracted for 48 hr successively in AR grade solvents *viz*., petroleum ether (60°-80°C), chloroform and methanol in soxhlet apparatus. Each solvent was distilled under reduced pressure to obtain crude extracts. The extracts obtained were stored at 4°C temperature. 1.0 g of each extract and fractions were dissolved in 10 ml acetone and the solution made up to 100 ml to give stock solution of 10,000 ppm. Test concentrations were prepared by serial dilution of each stock solution.

Bioassay: III instar larvae of *Anopheles stephensi*, collected from various places with stagnant water in Dayalbagh area, Agra, were used. They were cleaned several times with distilled water. The experiments were conducted according to WHO (1963).

Table - 1: Concentration mortality response data of different fruit and leaf extracts of *C . anthelminticum* against III instar larvae of *A. stephensi*

Extract / Concentrations (µgml^{-1})	Percentage mortality at different time interval (hr)		
	24 hr	48 hr	72 hr
Fruit extract			
Petroleum ether			
50	15	28	48
100	20	55	67
400	83	91	95
700	96	97	99
1000	97	98	100
Chloroform			
50	7	11	25
100	8	13	27
400	15	21	49
700	17	25	56
1000	19	27	58
Methanol			
50	2	6	23
100	10	11	26
400	34	39	44
700	48	53	62
1000	59	61	70
Leaf extracts			
Petroleum ether			
50	12	23	60
100	18	32	62
400	47	76	95
700	56	88	98
1000	62	92	99
Chloroform			
50	8	21	48
100	11	32	55
400	30	42	70
700	34	46	74
1000	37	49	77
Methanol			
50	3	20	48
100	7	28	60
400	18	45	85
700	23	47	91
1000	28	52	93

*No mortality was observed in control

The larvicidal activity of different concentrations (50 ppm to 1000 ppm) of each crude was tested with 20 III instar larvae of *A. stephensi* placed in beakers (250 ml) containing 200 ml distilled water and pinch of glucose (as larval food). Larvae exposed to 200 ml water containing 0.1 ml acetone served as control. Bioefficacy was determined in five replicates for each concentration. Laboratory temperature during the experiments was recorded as 26 $\pm$ 2°C. Mortality was evaluated after 24 , 48 and 72 hr of exposure. The whole set was discarded, if the mean mortality in the control was greater than 20%. The concentration - mortality response was determined by the lethal concentrations at LC_{50} and LC_{90} levels by log - probit analysis (Finney, 1971).

Results and Discussion

On successive extraction with petroleum ether, chloroform and methanol *Centratherum* fruits yielded 1.17 $\pm$ 0.28%, 1.99 $\pm$ 0.18% and 12.16 $\pm$ 2.19% and leaves yielded 3.18 $\pm$ 0.30%, 2.48 $\pm$ 0.32% and 2.04 $\pm$ 0.57% of crude, respectively. Bioassays conducted with both fruit and leaf extracts, indicated that the petroleum ether extract has maximum larvicidal activity followed by methanol and chloroform extracts. The petroleum ether extract of fruit showed

Table - 2: Log-probit analysis of larvicidal efficacy of petroleum ether extracts of fruits and leaves of *C. anthelminticum* against *A. stephensi* at different time intervals

Extract/Time interval	Regression coefficient	Regression equation	LC_{50} with fiducial limits	LC_{90} with fiducial limits
Fruit extract				
24 hr	0.9756	y = 2.4757x - 0.4741	162.60 (161.92 - 163.27)	534.7 (534.0 - 535.4)
48 hr	0.9970	y = 2.0474x + 0.9904	84.09 (83.47 - 84.72)	393.2 (392.6 - 392.8)
72 hr	0.9853	y= 2.2111x + 1.0806	59.23 (58.60 - 59.86)	224.61 (223.98 - 225.25)
Leaf extract				
24 hr	0.9947	y = 1.1894x + 1.7668	522.94 (522.38 - 523.51)	6232.45 (6231.9 - 6233.0)
48 hr	0.9910	y = 1.7337x + 1.2065	154.21 (153.63 - 154.79)	844.18 (843.60 - 844.18)
72 hr	0.9952	y = 2.0565x + 1.1983	70.57 (69.95 - 71.19)	295.83 (295.21 - 296.45)

Table - 3: Larvicidal activity of some of the members of Asteraceae family after 24 hr

Plant	Extract	Part	Mosquito species	Instar	LC_{50} (ppm)	Reference
Ageratum conizoides	Oil	-	*Aedes aegypti*	IV	120.0	Sosan *et al.* (2001)*
Artemisia annua	Petroleum ether	Leaves	*Anopheles stephensi*	III	263.0	Tonk *et al.* (2003)
Baccharis coridifolia	Dichloromethane	Aerial parts	*Aedes aegypti*	II	373.3	Ciccia *et al.* (2000)
Eclipta paniculata	Ethanol	Aerial parts	*Aedes fluviatilis*	IV	3.3	Macedo *et al.* (1997)
Eupatorium hecatanthum	Dichloromethane	Aerial parts	*Aedes aegypti*	II	317.4	Ciccia *et al.* (2000)
Melantheria albinervia	Dichloromethane	Roots	*Aedes aegypti*	II	500.0	Slimestad *et al.*(1995)*
Pterocaulon polystachium	Dichloromethane	Aerial parts	*Aedes aegypti*	II	149.2	Ciccia *et al.* (2000)
Pterocaulon purpurascens	Dichloromethane	Aerial parts	*Aedes aegypti*	II	>500	Ciccia *et al.* (2000)
Tagetes minuta	Ethanol	Aerial parts	*Aedes fluviatilis*	V	1.0	Macedo *et al.* (1997)
Xanthium spinosum	Dichloromethane	Aerial parts	*Aedes aegypti*	II	349.3	Ciccia *et al.* (2000)
C. anthelminticum	Petroleum ether	Fruits	*Anopheles stephensi*	III	162.60	Present study
C. anthelminticum	Petroleum ether	Leaves	*Anopheles stephensi*	III	522.94	Present study

* LC_{100} value

100 % mortality at 1000 ppm after 72 hr. It is clear from LC_{90} and LC_{50} values (Table 2) that the petroleum ether extract of fruit was 11.66, 2.15 and 1.32 times more toxic than that of leaf extract after 24, 48 and 72 hr, respectively at LC_{90} level. However, at LC_{50} level the corresponding values were 3.22, 1.83 and 1.19, respectively.

Literature indicates that members of Asteraceae (Table 3) have maximum mosquito larvicidal activity (Gbolade, 2001). Ethanol extract of aerial parts of *Tagetes minuta* shows the minimum LC_{50} value (1.0 ppm) followed by extract of *Eclipta paniculata* of 3.3 ppm against *Aedes fluviatilis*, while extracts of *Achryrocline satueoides, Ganaphalium spicatum, Senecio brasiliensis, Trixis vauthieri, Tagetes patula* and *Vernonia ammophila* were comparatively less active, killing more that 50% of the larvae at 100 ppm (Macedo *et al.,* 1997). 100% mortality has been reported against *A. aegypti* larvae using oil of *Ageratum conizoides* L. at 120 ppm (Sosan *et al*., 2001) and using dichloromethane extract of *Pterocaulon polystachium* at 149.2 ppm (Ciccia *et al.,* 2000). The dichloromethane extracts of *Xanthium spinosum*, *Baccharis coridifolia, Eupatorium hecatanthum* and *Pterocaulon purpurascens* (Ciccia *et al.,* 2000) and *Melantheria albinervia* (Slimestad *et al.,* 1995) also show larvicidal activity with higher LC_{50} values.

Table 3 shows *A. annua* (Tonk *et al.,* 2003) and present study to posess active component against *A. stephensi* III instar larvae. The larvicidal activity of petroleum ether extract of *C. anthelminticum* fruits is comparatively better than that of *Artemisia annua* leaves. However, the petroleum ether extracts of *C. anthelminticum* fruit and leaf show LC_{50} value of 162.60 ppm and 522.94 ppm, respectively after 24 hr. These extracts are comparable to other members of the Asteraceae and are very effective against the vector of malaria. Fruit extract is comparatively better that that of leaf extract.

In conclusion the petroleum ether extract of *C. anthelminticum* fruits is a promising source for the control of *Anopheles* larvae. It is an indigenous plant of India and is easily available to local people. It may be a safe alternative to synthetic chemicals.

Acknowledgments

We thank Prof. L. D. Khemani, Head, Department of Chemistry, Faculty of Science, Dayalbagh Educational Institute for the facilities provided. We are also thankful to Prof. V. P. Bhatnagar, Head, Department of Botany, Faculty of Science, Dayalbagh Educational Institute for providing garden space for cultivation of *C. anthelminticum*.

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