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Effective method for extraction of larvicidal component from leaves of *Azadirachta indica* and *Artemisia annua* Linn.

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Abstract: *Leaves of Artemisia annua linn. and Azadirachta indica were extracted in petroleum ether and hexane respectively by different methods of extraction i.e. cold extraction, reflux extraction and soxhlet extraction. The crude extract obtained was tested against third instar larvae of Anopheles stephensi. On comparison of larval mortality of crude extract obtained by these three methods, both soxhlet and reflux extraction method showed 100 % mortality at 200 ppm after 48 hr in case of A. annua. However LC_{50} (20 ppm) value of crude extract obtained by soxhlet extraction showed better results than reflux extraction (35 ppm) method after 72 hr. In case of A. indica, crude obtained by soxhlet showed 100% mortality (after 48 hr) at 250 ppm and LC_{50} of 69 ppm at 72 hr. Reflux extraction does not show any appreciable mortality even at 250 ppm concentration.*

Key words: *Anopheles stephensi, Larvicidal activity, Crude extract, Reflux, Hot extraction, Cold extraction.*

Introduction

Malaria is a highly prevalent mortality causing tropical disease which affects over 103 endemic countries with a combined population of over 2.5 billion people and causes one to three million deaths per year (Jain *et al*., 2000). The malaria vector *Anopheles* species and the larval stages thrive in stagnant waters in tropical climate and have been effectively controlled by the use of insecticidal sprays. However, the development of resistance by mosquito populations to chemical insecticides requires alternative approaches for sustainable vector control. Also growing awareness of environmental hazards of synthetic chemicals has promoted development of novel, alternative methods for mosquito control (Gbolade, 2001).

This led to a remarkable interest in plant products as insecticides in the last two decades. Unlike organic insecticides which are based on single active ingredient plant products comprise a number of chemicals which act concertedly on both behavioural and physiological processes. Various extracts of leaves, underground and aerial parts of plants are frequently investigated for mosquitocidal activities (Kalyanasundaram and Dass, 1985; Jalees *et al*., 1993). Among these plants, which, belonged to the Asteraeae, Rubiaceae, Ranunculaceae, Euphorbiaceae, Verbenaceae and Liliaceae (in decreasing order) rank high among the families that were screened. Among the high ranking larvicides are leaf extracts of *Gliricidia sepium* (Sharma *et al*., 1998), seed extract of *Atriplex cancescens* (Ouda *et al*., 1998), flower extract of *Tagetus minuta* (Perich *et al*., 1994) and others (Tonk *et al.,* 2003).

In the present study we have evaluated larvicidal activity of crude extracts obtained by different methods from leaves of *A. annua* and *A. indica*. Artemisinin is a secondary plant product of *A. annua* that has been found to have strong antimalarial properties, although reports on larvicidal properties are few (Tonk *et al*., 2003). Azadirachtin obtained from neem tree (*A. indica*) is widely used for treating malaria (Prakash *et al*., 2002).

The efficacy of these plant products as larvicides, depends upon the way, the plants (leaves) have been extracted. Several methods may be used for the extraction of plants in different solvents. In an attempt to develop an effective method for extraction of leaves, (*Artemisia annua* and *Azadirachta indica*) they were extracted by different methods (viz. extraction at room temperature, hot extraction method and soxhlet extraction method). The bioefficacy of crude extracts obtained were tested on *Anopheles stephensi* to determine the most effective method for extraction of larvicidal components.

Materials and Methods

Plant extracts: Crushed leaves of *Artemisia annua* and *A. indica* were extracted by the following methods.

Extraction at room temperature or cold extraction: 50 g of *Artemisia annua* and *A. indica* leaves were dipped in 500 ml petroleum ether and hexane respectively for 48 hr at room temperature in a stoppered conical flask and shaken periodically by electrical stirrer. The extracts were filtered and filtrate was evaporated under reduced pressure on water bath to obtain crude.

Hot extraction or reflux extraction: 50 g of *Artemisia annua* and *A. indica* leaves were refluxed in 500 ml petroleum ether and hexane respectively for 15 hr, in a round bottom flask. The extracts were filtered and filtrate was evaporated under reduced pressure on water bath to obtain crude.

Soxhlet extraction: In this method, 50 g of leaves were extracted in 500 ml petroleum ether by soxhlet extraction technique for 48 hr. The extracts were filtered and filtrate was evaporated under reduced pressure to obtain crude.

Table – 1 : Percentage mortality and LC_{50}(ppm) at 72 hr of *A. annua* and *A. indica* crude obtained by different methods on 3rd instar larvae of *A. stephensi.*

Method	Conc.(ppm)	24hr		48hr		LC_{50}(ppm) at 72 hr	
		A.annua	***A.indica***	***A.annua***	***A.indica***	***A.annua***	***A.indica***
Cold	50	30	3	5	6		
	100	3	5	10	10	316	500
	150	7	6	8	11		
	200	15	8	18	14		
	250	-	10	-	15		
Reflux	50	37	6	77	10	35	250
	100	52	10	83	15		
	150	51	12	80	20		
	200	77	16	93	25		
	250	-	20	-	30		
Soxhlet	50	5	44	17	32	19.9	69
	100	30	45	70	50		
	150	32	47	90	66		
	200	38	48	92	82		
	250	-	50	-	100		

(Larval mortality corrected, using Abbot's formula, Control mortality varied between 0-7%.

These crude extracts were subjected to bioassay.

Bioassay test: Toxicity assays of the crude extracts were conducted separately using the third instar larvae of *Anopheles stephensi*. Stock solution (10,000 ppm) was prepared by dissolving 1 g of crude in 10 ml acetone and volume raised to 100 ml with distilled water. From this different dilutions of 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm were prepared in 200 ml deionised water in 250 ml beaker and 20 third instar larvae were released in it and mortality was scored after 24 hr, 48 hr, and 72 hr. The beakers were kept in a temperature controlled room at 28°C ± 2°C. Larvae exposed to 200 ml water containing 0.1ml of acetone served as control. Each treatment was replicated four times. Percent larval mortality was calculated by using probit analysis technique (Finney, 1981).

Results and Discussion

In the present study *Artemisia annua* and *Azadirachta indica* leaves were extracted in petroleum ether and hexane respectively by using different extraction methods i.e. extraction at room temperature, hot extraction (reflux) and soxhlet extraction method. 50 g of *A. annua* leaves (500 ml petroleum ether) yield 1.0, 1.4 and 1.95 g crude respectively in the said methods, while 50 g of *A. indica* leaves (500 ml hexane) yield 0.8, 0.75 and 1.0 g crude respectively. Using different concentrations of crude extracts (50, 100, 150, 200, 250 ppm) larval mortality were scored and results are presented in Table 1.

Larval mortality of *A. annua* crude and *A. indica* crude against *A. stephensi* ranged from 10 to 100 % after 72 hr in extracts obtained by different methods as compared to 0 - 7% mortality in control (Abbot's formula was used to get correct mortality). There was a progressive increase in larval mortality with the increase in concentration but the extent of such increase was significantly lower in cold extraction method (Table 1)

In case of *A. indica*, crude obtained by soxhlet showed maximum larval efficacy. 100% mortality was observed at a concentration of 250 ppm after 48 hr. Cold extraction and reflux extraction however showed weak action against larvae of *A. stephensi.*

A. annua crude obtained by both soxhlet and reflux extraction method showed maximum larvicidal mortality (100%) at 200 ppm after 72 hr indicating the bioactive principle to be obtained in reflux or soxhlet extraction rather than cold extraction method.

Earlier studies report extraction of larvicidal components in other plant species by both reflux and soxhlet method. Pushplatha and Muthukrishnan (1995) report LC_{50} 72 ppm and 136 ppm for crude extract of *Vitex negundo* and *Nerium oleander* leaves obtained by methanol extraction using reflux method. Jalees *et al* (1993)., report a low LC_{50} of 1 ppm for *Cannabis sativa* Linn. extracted by soxhlet method.

In case of *A. annua,* although both soxhlet and reflux extraction method showed 100% mortality at 200 ppm after 72 hr, however LC_{50} value of crude obtained by soxhlet extraction showed higher (19.9 ppm) larval mortality than reflux extraction (35 ppm) indicating soxhlet method to be more effective for extraction of larvicidal components. In *A. indica* crude obtained by soxhlet showed 100 % mortality at 250 ppm after 48 hr however LC_{50} indicates a high value of 69 ppm after 72 hr. Hot extraction does not show any appreciable mortality even at 250 ppm concentration.

For both plants soxhlet extraction method seems to be more effective for extraction of larvicidal components.

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