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DRUG DISCOVERY

Larvicidal activity of various solvent extracts of Lichen *Roccella Montagnei* against Filarial Vector *Culex Quinquefasciatus*

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ABSTRACT

Polarity based different solvent extracts (at four different conc.) of lichen, *Roccella montagnei* Bél. emend. Awas. (Roccellaceae) were screened for mosquito larvicidal activity against the 3rd instar larvae of the filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). The Dichloromethane (DCM) extract showed highest toxic effect and hexane extract showed least effect. DCM extract showed significant lowest ranges of LC_{50} 126.16 at 24 h and 83.72 at 48 h. This study is the first to report on the larvicidal activity of crude extracts of *R. montagnei* lichen species against *C. quinquefasciatus*..

Key words: Lichens, *Culex quinquefasciatus*, crude extracts, *Roccella montagnei*, larvicidal activity, LC_{50}, LC_{90}.

Abbreviations: DCM – Dichloromethane, LC- Lethal Concentration, NMR-Nuclear magnetic resonance.

1. INTRODUCTION

Mosquito borne diseases such as malaria, filariasis, dengue, yellow fever and encephalities are continuing to be major health problems for the people (Das & Ansari, 2003). At least one hundred and twenty million people in 80 countries of the world are infected with lymphatic filarial parasites (MATENDE), and it is estimated that 1 billion (20% of the world's population) are at risk of acquiring the infection. *Culex quinquefasciatus* Say (Diptera: Culicidae), an ubiquitous urban mosquito breeds mainly in drains, cesspits and cesspools containing domestic effluents and act as a major vector for filariasis.

Current control for *C. quinquefasciatus* is based on the use of insecticides namely chlorpyrifos, dichlorovs, cypermethrin etc. However, synthetic chemical larvicides continue to be problematic in maintaining control, especially with organophosphate and pyrethroid larvicides and have a potential toxic effect on public health and the environment (Pidiyar *et al*, 2004). Biological control at the larval stage of mosquitoes is one of the suitable techniques, which offers a cheap, easy to use, and environment friendly method of mosquito control. There is considerable international interest in developing benign natural products as an alternative to synthetic pesticides to control invertebrate pests of medical and economic importance as they are easily degradable, safer than synthetic (Cetin *et al.*, 2006; Moretti *et al*., 2002). In this regard, thousands of plants have been tested as potential sources of insect repellents. Phytochemicals with mosquito larvicidial activity occur in oils, leaves and roots of plants (Ojewole *et al.*, 2000; Sharma *et al*., 1998; Sosan *et al*., 2001). For example, *Azadirachta indica* and other plants have proven mosquito control potential (Mittal & Subbarao, 2003).

2. STATEMENT OF THE PROBLEM

Secondary compounds from organisms especially lichens have not been explored for mosquito larvicidal properties. Lichens are known to contain unique secondary compounds such as aliphatic acids, pulvinic derivatives, hydroxybenzoic acids, depsides, depsidones, dibenzofurans, anthroquinones, naphthaquinones which are known to exert antipest, antiherbivore, antibiotic and other effects (Balaji & Hariharan, 2007; Huneck, 1999; Lawrey, 1984; Müller, 2001; Rundel, 1978).

2.1 Scope of the Study

Hence this paper reports the mosquito *Culex quinquefasciatus* larvicidal properties by different concentrations of crude organic solvent extracts of *Roccella montagnei*, a common lichen along the Coromandel Coast, Tamil Nadu. *R. montagnei* possesses secondary compounds such as Roccellic acid, Orcinol, Lecanoric acid, Montagnetol, Methylorsellinate, Meso-erythritol, Erythritol, β carotene, β sitosterol (Balaji & Hariharan, 2007).

3. MATERIALS AND METHODS

The thalli of *R. montagnei* were collected from *Rhizophora apiculata* trees in Pichavaram mangroves (11º 23´ N to 11º 30´ N latitude and 79º 45´ E to 79º 50´ E longitude), Tamil Nadu, India. Voucher specimens (MSSRF/Herb/0456/04, 0458/04) were deposited at the Lichen Ecology and Bioprospecting Laboratory, M. S. Swaminathan Research Foundation, Chennai. The collected material was shade dried for 2 days at room temperature. The dried powdered lichen thallus (950g) was kept in wrapped in a 8 x 6 cm cylindrical pouch (Whatmann filter paper grade 1) and extraction with Soxhlet apparatus (Balaji *et al*, 2006; Balaji &

Hariharan, 2007; Balaji, 2005) with the series of solvents based on their polarity hexane, dichloromethane (DCM), ethyl acetate, acetone and methanol and each extraction was carried out at the specific boiling temperature for a period of 48 hrs for the complete extraction of secondary compounds. The pooled solution was evaporated using a rotary evaporator (Buchi Rotary Evaporator R-200) to obtain the crude extract. The crude extract was lyophilized to powder (Virtis bench top model) and stored in glass ampules until use.The 250 mg of each extracts was dissolved in 5 ml of acetone and made up to 250 ml using distilled water, as 1000 ppm. One milligram of the extract in 1ml of water was considered to be the 1000 ppm solution. So, 125 ml of this stock solution was used as test solution for the bioassay test. The remaining 125 ml was made up to 250 ml by addition distilled water to prepare 500 ppm solution. 125 ml of this 500 ppm solution was used as test solution for bioassay test and the remaining 125 ml was used to prepare 250 ppm solution. In the same way test solutions of 125 and 62.5 ppm were prepared by adding equal proportions of distilled water. Two types of control solutions, i.e. with and without acetone and emulsifier (Tween 80), were also prepared simultaneously. Three replicates were maintained for all the tests in this study (Cetin *et al*, 2006).

The cyclic laboratory reared (temperature 25±2°C; moisture 70-85% and 14:10h light and dark photoperiod cycle) identified *C. quinquefasciatus* colony were maintained in Insect Pest Management Laboratory, M.S. Swaminathan Research Foundation, Chennai. The larvae were fed with powdered mixture of dog biscuits and yeast tablets in the 3:1 ratio. The emerged adults were fed with 10% glucose solution and with blood meal with restrainer chick (Susan & Vincent, 2005). Healthy third instar larva was chosen for the experimentation.

Bioassay studies (24h, 48h) were carried out using 10 laboratory reared third instar larvae of *C. quinquefasciatus* for each experiment under laboratory condition. The LC_{50} and LC_{90} values were calculated. The results obtained in the bioassay studies were subjected to statistical analysis made with Probit analysis (Cetin *et al*, 2006).

4. RESULTS AND DISCUSSION

Mortality was not observed in the control set up for all growth stages of larvae, pupa and adult. In experiments at various concentrations of the organic solvents, the following were observed: larval mortality, larval duration, formation of larval-pupal intermediates, pupal morality, pupal-adult intermediates and adult mortality. The parameter larval-pupal intermediate was observed in hexane extract. 100% mortality occurred at 3 days for 1000 ppm while Sakthivadivel and Thilagavathy (2003) reported that acetone fraction of the petroleum ether extract of *Argemone mexicana* showed 82% mortality was occurred at 11 days for 10 ppm.

Among the polarity based serial crude extracts tested, high mortality was observed in both DCM and ethyl acetate extracts followed by acetone extract, while least activity was found in hexane and nil activity in Methanol extract (Fig. 1 and 2). Mortality was observed at test concentrations from 125 to 1000 ppm, showing their toxic effect on the insect. At 250 ppm concentration the mortality was 60% in DCM and 50% in acetone extracts. In the present study, abnormal behaviors of the larvae such as circular movements near the periphery of the beaker as opposed to normal zig-zag motion in the control sets were also observed. Such movements indicate that the toxic effect of the test solution is on the nervous system of the larvae (Sakthivadivel & Thilagavathy, 2003).

The hexane extract showed mortality only at 48 hrs with 10% of larval-pupal intermediate, 60% of pupal mortality was observed at 1000 ppm and 10% of adult mortality, 30% pupal morality also observed at 500 ppm. The acetone and ethyl acetate extracts showed 10% pupal morality at 125 and 250 ppm respectively. The lower concentrations of 125 and 250 ppm of DCM extract prolonged the larval duration more than 3-5 days than the control and untreated. The lowest concentration 125 ppm of ethyl acetate and acetone also prolonged the larval duration of 2-4 days.

Sujatha *et al*. (1988) reported on the morphogenetic abnormalities among the developmental stages of *C. quinquefastiatus*, *Aedes aegypti* and *Anopheles stephensi* at lower concentrations of plant extracts viz. *Madhuca longifolia*, *Acorus calumus* and *Ageratum conyzoides*. Prolongation of larval and pupal periods and occurrence of larval-pupal and pupal-adult intermediates indicates the presence of the bioactive compounds on the normal hormonal activity of coordination of the metabolitc processes of the developing stages (Supavarn, 1974).

LC_{50}, LC_{90} and other associated statistical analysis of 24 h and 48 h given in Table 1. The LC_{50} ranges vary from

Table 1: Probit analysis of various crude extracts of *Roccella montagnei* against *Culex quinquefasciatus*

Extracts	LC_{50} (LC_{90})	Upper	Lower	Regression equation	Slope
		(95% confidence level)			
Hexane					
24 hours	0	0	0	0	0
48 hours	787.72 (4883.44)	2555.85 (115537.9)	242.78 (206.40)	Y= 0.31+1.61 X	4.120071
Ethyl acetate					
24 hours	974.07 (7762.39)	1362.47 (19064.43)	696.38 (3160.58)	Y=0.7+1.42 X	5.006534
48 hours	216.84 (1353.32)	519.36 (7826.90)	90.53 (233.99)	Y= 3.24+0.91 X	4.141564
DCM					
24 hours	126.16 (11635.05)	3030.28 (1.078937)	5.25 (1.254702)	Y=3.6+0.65 X	33.48144
48 hours	83.72 (2128.53)	164.60 (5205.24)	42.58 (870.39)	Y= 3.24+0.91 X	12.31778
Acetone					
24 hours	241.91 (3182.386)	721.10 (5602.177)	81.15 (1807.792)	Y=0.65+1.60 X	0.2152721
48 hours	507.01 (3182.38)	619.67 (5602.17)	414.82 (1807.79)	Y= .65+1.60 X	386.7915
Methanol					
24 hours	0	0	0	0	0
48 hours	0	0	0	0	0

Balaji et al.
Larvicidal Activity Of Various Solvent Extracts Of Lichen *Roccella Montagnei* Against Filarial Vector *Culex Quinquefasciatus,*
Drug discovery, 2012, 2(6), 36-39,

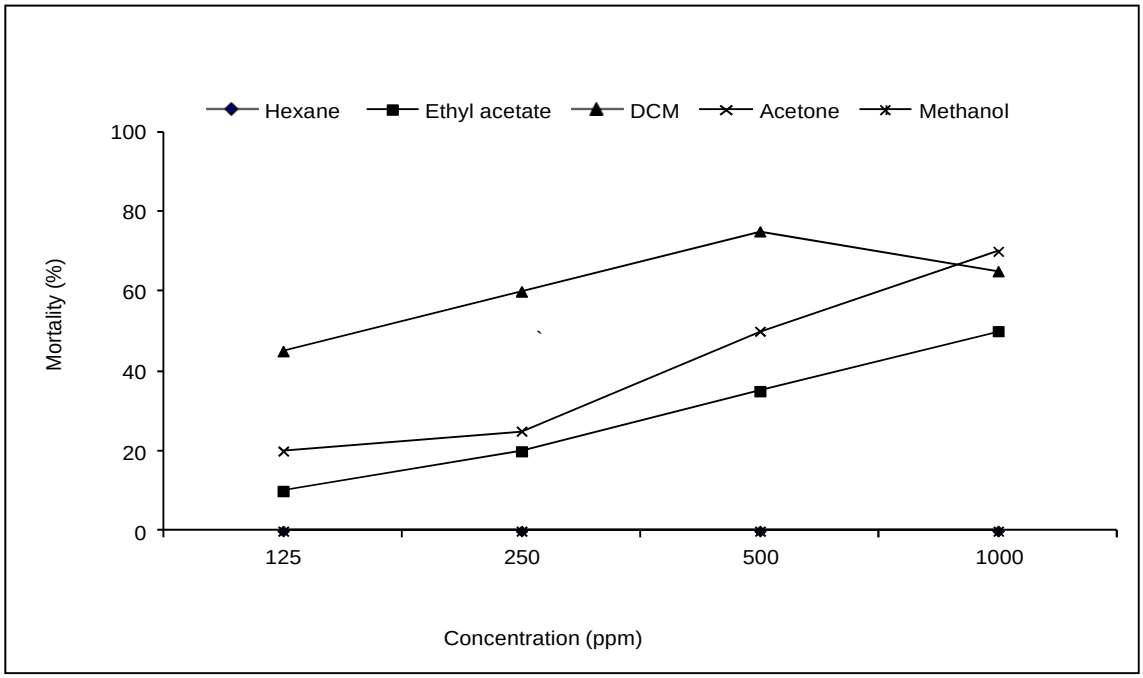

Figure 1

The percentage mortality (24 h) of various solvent extracts of R. montagnei on C. quinquefasciatus

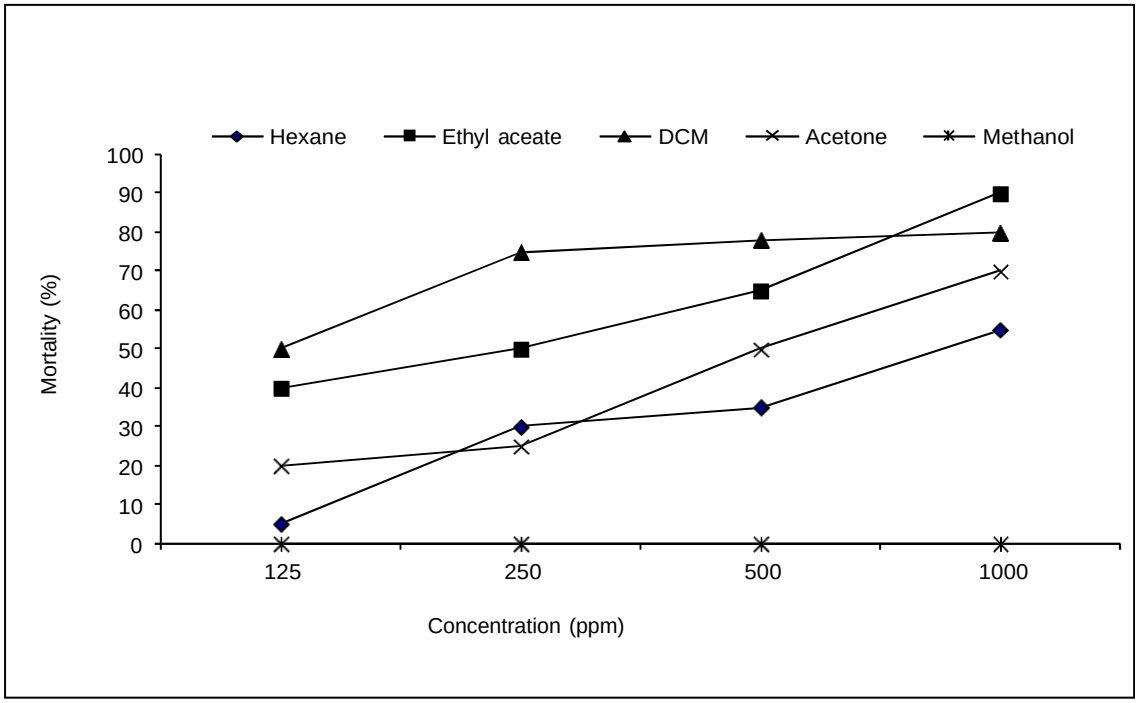

Figure 2

The percentage mortality (48 h) of various solvent extracts of R. montagnei on C. quinquefasciatus

126.16 to 974.07 in 24 h and 83.72 to 787.71 in 48 hr. Polarity based different solvent extracts exhibited DCM showed significant lowest ranges of LC_{50} 126.16 at 24 h and 83.72 at 48 h.

Similarly, crude extracts of *Quercus lusitania* var. *infrectoria* galls (Oliv.), *Mentha piperita*, lemon, grape fruit, navel orange, *Pavonia Zeylanica* L. and *Acacia ferruginea* D.C. was most active against *Culex mosquitoes* (LC_{50}: 256 ppm) (Dakhil and Morsy, 1999; Ansari *et al*., 2000; Redwane *et al*., 2002; Vahitha *et al*., 2002). Park *et al*. (2005) showed that a methanol extract of *Phryma leptostachya* var. *asiatica* roots exhibited quite an effective larvicidal activity against *Culex pipiens pallens*, *Aedes aegypti* and *Ocheratatos togoi*. Earlier reports on antibacterial, antifungal and antipest properties of *R. montagnei* thallus extracts (Balaji *et al*., 2006; Balaji & Hariharan, 2007) are noteworthy. This is the first study to test these extracts against *Culex quinquefasciatus*.

Balaji et al.
Larvicidal Activity Of Various Solvent Extracts Of Lichen *Roccella Montagnei* Against Filarial Vector *Culex Quinquefasciatus,*
Drug discovery, 2012, 2(6), 36-39,

SUMMARY OF RESEARCH

1. Different solvent extracts of lichen, *Roccella montagnei* were screened for mosquito larvicidal activity (*Culex quinquefasciatus* Say)..
2. Dichloromethane (DCM) extract showed highest toxic effect and hexane extract showed least effect
3. The study indicates that the secondary compounds of *Roccella montagnei* can cause mortality at higher concentrations on larvae of *C. quinquefasciatus*.

FUTURE ISSUES

The study using lichen extracts to investigate mosquito larvicidal activity is the first of its kind from India. Lichen extracts could be further purified and characterized using NMR, Mass Spec and X-ray diffraction for resolution of their structure in order to identify novel molecules that can be developed as products in larvicidal control.

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