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SHORT COMMUNICATION

EFFECT OF SOME LICHEN EXTRACTS FROM SRI LANKA ON LARVAE OF *AEDES AEGYPTI* AND THE FUNGUS *CLADOSPORIUM CLADOSPORIOIDES*

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Abstract: Dichloromethane and methanol extracts of 48 lichens collected from six areas in Sri Lanka were subjected to mosquitolarvicidal assay against the second instar larvae of *Aedes aegypti* and antifungal assay against *Cladosporium cladosporioides*. The following extracts showed significant mosquitolarvicidal activity. CH_2Cl_2: *Cladonia coniocraea*, *Everniastrum* sp., *Parmelina tiliaceae*, *Parmotrema chinense*, *Parmotrema tinctorum*, *Rimelia reticulata*, *Dirinaria applanta*, *Heterodermia leucomelos*, *Roccella montagnei*, *Stereocaulon* sp., *Ocellularia* sp., two species of *Myriotrema*, and *Usnea* sp. MeOH: *Hypogymnia* sp., *H. leucomelos*, *Lepraria atrotomentosa*. The CH_2Cl_2 extracts of *C. coniocraea*, *Hypogymnia* sp., *Lecanora* sp., *Parmeliella* sp., *Everniastrum* sp., *Heterodermia diademata*, *H. leucomelos*, *R. montagnei*, and *Usnea* sp., exhibited potent antifungal activity.

Keywords: *Aedes aegypti*, *Cladosporium cladosporioides*, Lichen extracts, mosquito larvicidal and antifungal activity, Sri Lankan lichens.

INTRODUCTION

Lichens are symbiotic organisms of fungi (mycobionts) and algae (phycobionts) comprising of about 17,000 species recorded worldwide.[1] They commonly grow on rock surfaces, poorly developed soils such as those found in arid lands and boreal-arctic regions and as epiphytes on trees and shrubs.[2] Lichens synthesize a variety of metabolites with diverse biological activities. For example, lichens possess antibiotic activity, antitumour and antimutagenic activity, anti-HIV activity and plant growth and enzyme inhibitory activity.[3] The majority of the compounds responsible for these activities originate from the fungal mycobiont. The general resistance of lichens to insects and microbial attack is attributed to the presence of lichen metabolites . Lichens have not been subjected to comprehensive bioassays unlike the flowering plants. Recently, however, there have been some reports of screening of lichen metabolites, particularly for antibiotic activity.[4,5,6,7] Large pharmaceutical companies have initiated screening programs for application of these metabolites and their derivatives in medicine and crop protection.[2] In this investigation we report the bioactivities of some lichens of Sri Lanka, against the second instar larvae of *Aedes aegypti* and the fungus *Cladosporium cladosporioides*.

METHODOLOGY

Lichen species collected from six selected areas were used in this study. Specimens of lichens were deposited in the National Herbarium at the Royal Botanic Gardens, Peradeniya. Each lichen species (100 g) was washed thoroughly under running water, air-dried and extracted into CH_2Cl_2 (2.5 L) separately in bottle shakers for 24 h at room temperature. The dichloromethane extracts were filtered through a fluted filter paper and the solvents were evaporated under reduced pressure using a rotavapor below 40° C to yield crude extracts. The lichen residues after the extraction into CH_2Cl_2 were extracted into MeOH following the same procedure.

Laboratory cultured larvae of *A. aegypti* were used for this assay. [8] Five-second instar larvae were introduced into the test solutions (25 ml). Four replicates were used for each experiment. Test solutions were prepared by

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Table 1: Mosquito larvicidal activity and antifungal activity of the extracts of some lichen species of Sri Lanka

Lichen species	Collection	Mosquito larvicidal activity		Antifungal activity	
		CH_2Cl_2	MeOH	CH_2Cl_2	MeOH
Arthoniaceae					
Arthonia sp. (90) #	PD	-	-	*	*
Cladoniaceae					
Cladonia sp. (31)	PT	1	-	2	-
Cladonia coniocraea (Flörke) Spreng. (55)	HG, HR	3	-	2	-
Collemataceae					
Collema sp. *(02)*	HG	-	-	*	*
*Leptogium denticulatum** (104)	HG, HR, PD	1	-	-	-
Leptogium saturninum (Dicks.) Nyl. (11)	HG	-	-	*	*
Graphidaceae					
Graphina sp. (100)	PD	1	-	-	-
Parmeliaceae					
Hypogymnia sp. (30)	HG, HR	2	3	3	1
Menegazzia sp. (71)	HG, HR	-	1	*	*
Lecanoraceae					
Lecanora sp. (103)	HG, HR	-	-	*	*
Lecanora sp. (52)	HG, HR	-	1	-	-
Lecanora sp. (80)	HG, HR, PD	2	-	3	1
Letrouitiaceae					
Letrouitia domingensis (Pers.) Haf. & Bellem.(44)	HG, HR, PD	1	-	*	*
Lobariaceae					
Lobaria retigera (Bory) Trevis. (74)	HG	1	1	1	-
Lobaria sp. (73)	HG, HR	1	1	*	*
Pseudocyphellaria argyracea (Bory) Vain. (15)	HG	1	1*	*	
Pseudocyphellaria sp. (76)	HG, HR	2	-	1	-
Sticta ciliaris Mont. et v.d. Bosch (18)	HG	2	-	-	-
Megalosporaceae					
Megalospora sp. (47)	AB, PT, HR	-	1	*	*
Pannariaceae					
Leproloma sipmanianum Kümmerl & Leuckert (79)	BE	1	-	1	-
Pannaria sp. (08)	HG	2	2	1	-
Parmeliella sp. (07)	HG	3	1	2	-
Parmeliaceae					
Everniastrum sp.	HG	3	2	2	1
Hypotrachyna sp. (39)	PT, HG	1	1	1	-
Parmelina tiliacea (Hoffm.) Hale (Elix & McCarthy)	HG, HR	3	2	1	-
Parmotrema chinense (Osbeck) Hale & Ahti	HG, HR	3	1	1	1
Parmotrema crinitum (Ach.) M.Choisy. (24)	HG	2	1	-	-
Parmotrema tinctorum (Nyl.) Hale (72)	HG, HR, PD	3	2	1	-
Rimelia reticulata (63) (Taylor) Hale & A. Fletcher	HG	3	-	-	-
Pertusariaceae					
Pertusaria sp. (77)	BE	1	-	*	*
Pertusaria sp. (105)	HG, PD	2	1	*	*
Physciaceae					
Dirinaria applanta (Fée) Awasthi (78)	HG, HR	3	-	1	-

Lichen Species	Collection	Mosquito larvicidal activity		Antifungal activity	
		CH_2Cl_2	MeOH	CH_2Cl_2	MeOH
Dirinaria confluens (Fr.) Awasthi (99)	HG, HR, PD	2	1	-	1
Heterodermia diademata (Tayl.) Awas. (23)	HR	1	-	2	1
Heterodermia leucomelos (L.) Poelt (46)	HR	3	3	2	1
Heterodermia comosa (Eschw.) Follm. & Redón (4)	HG, HR	1	1	1	-
Pyxine coccifera (Fée) Nyl. (84)	HG, PD	1	1	*	*
Ramalinaceae					
Ramalina sp. (61)	HG, HR	2	1	*	*
Roccellaceae					
Roccella montagnei (Del.) (81)	KU, PD	3	-	2	-
Sphaerophoraceae					
Sphaerophorus sp. (61)	HR	-	-	-	2
Stereocaulaceae					
Stereocaulon sp. (101)	HG	3	-	1	1
Teloschistaceae					
Caloplaca sp. (93)	PD	1	1	*	*
Thelotremataceae					
Ocellularia sp. (09)	HG, HR, PT	3	2	1	-
Myriotrema sp. (48)	HG, HR	3	1	-	-
Myriotrema sp. (14)	HG, HR	3	2	-	-
Trichotheliaceae					
Porina sp. (19)	HG, HR, PT	2	1	*	*
Usneaceae					
Usnea sp. (51)	AB	3	-	3	2
Usnea plicata (L.) (27)	HG, HR	2	-	1	-
(Uncertain)					
Lepraria atrotomentosa Orange & Wolseley (49)	RB	2	3	1	*

AB: Ambewela; BE: Beragala; HG: Hakgala; HR: Horton Plains; KU: Kurunegala; PD: Peradeniya; PT: Pattipola; RB: Ramboda; #: Herbarium specimen number; *: Not studied; -: No activity; *For antifungal activity*- 1: Low activity (3 or <3mm); 2: Moderate activity (3-20mm); 3: High activity (>20mm). *For mosquito larvicidal activity*- 1: Low activity (1-40 %); 2: Moderate activity (41-90 %); 3: High activity (91-100 %).

dissolving the extract (100 mg) in a minimum volume (< 1 ml) of acetone and diluting with water up to 200 ml (500 ppm). Polyethyleneglycol (PEG) (120 µL) was added to the mixture to homogenise the test solution. A series of concentrations were prepared by diluting the 500 ppm solution with water. After the introduction of larvae, a small portion of powdered fish food was added to each beaker and the beakers were covered with watch glasses. In all experiments an untreated control, which contained all the ingredients except the lichen extract, was used. The number of moribund or dead larvae was recorded after 24 and 48 h.

Antifungal assays were carried out on extracts by dissolving the extract (2 mg) in either CH_2Cl_2 or MeOH (1 ml). Benlate (methyl-1-(butylcarbonyl)-2-benzamidizolecarbamate), a commercial fungicide (1 mg, 50% active ingredient), was also dissolved in water (1 ml) and used in the assays for comparison with the extracts.[9]

TLC plates (silica gel 60 $PF_{254+366}$, 0.5 mm ×20 cm ×20 cm) were spotted with extracts at about one inch from the base. The plates were developed in CH_2Cl_2 or 5% MeOH in CH_2Cl_2 and then air-dried (overnight) to evaporate all traces of remaining solvents. A spore suspension of *Cladosporium cladosporioides* in Czapek-dox nutrient solution (CNS) was sprayed onto the plates and the plates were incubated in a moist

chamber at 28 ± 2 °C for two days. On every plate run, Benlate was spotted as the standard. Inhibition zones appeared white against a background of green mycelia. The diameter of zones in which the growth was inhibited, which were approximately circular, was measured.

RESULTS

Dichloromethane and methanol extracts of 48 species of lichens collected from eight locations in Sri Lanka were subjected to mosquito larvicidal assay against the second instar larvae of *Aedes aegypti* and antifungal assay against *Cladosporium cladosporioides*. The CH_2Cl_2 extracts of *Cladonia coniocraea*, *Hypogymnia* sp., *Parmeliella* sp., *Everniastrum* sp., *Parmelina tiliaceae*, *Parmotrema chinense*, *Parmotrema tinctorum*, *Rimelia reticulata*, *Dirinaria applanta*, *Heterodermia leucomelos*, *Roccella montagnei*, *Stereocaulon* sp., *Ocellularia* sp., two species of *Myriotrema* and *Usnea* sp., and MeOH extracts of *Hypogymnia* sp., *H. leucomelos*, *Lepraria atrotomentosa* showed highly significant mosquito larvicidal activity. The CH_2Cl_2 extracts of *C. coniocraea*, *Hypogymnia* sp., *Lecanora* sp., *Parmeliella* sp., *Everniastrum* sp., *Heterodermia diademata*, *H. leucomelos*, *R. montagnei*, and *Usnea* sp., exhibited potent antifungal activity. *C. coniocraea*, *Hypogymnia* sp., *Everniastrum* sp., *Parmeliella* sp., *Lecanora* sp., *H. leucomelos*, *R. montagnei* and *Usnea* sp., showed both mosquito larvicidal and antifungal activity.

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