RESEARCH ARTICLE

In-vitro Study on Anthelmintic and Insecticidal Activity of Macrolichens Collected from South Karnataka

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Received: 01st January, 2024; Revised: 27th January, 2024; Accepted: 16th May, 2024; Available Online: 25th June, 2024

ABSTRACT

In the present study, lichen samples were collected from the southern Karnataka state and were used for *in-vitro* anthelmintic and insecticidal activity. The three lichen samples *Parmotrema tinctorum* (Nyl.) Hale, *Roccella montagnei* Bèl. Emend. Awas, *Everniastrum cirrhatum* (Fr.) Hale was subjected to the extraction with solvents. The prepared extracts from the lichen species were evaluated for anthelmintic activity against *Pheretima posthuma*. 100% mortality was obtained from all the samples and it was compared with the reference drug albendazole for positive control. *P. tinctorum* methanolic extract showed the fastest result but on the other side, the slowest result was observed in the hexane extract. The insecticidal activity of the crude extracts of the lichens was tested against two insects *Macrosiphum rosae* (L.) and *Sitophilus oryzae* (L.). There was a 100% mortality rate observed in *R. montagnei* and E. *cirrhatum* methanolic extracts against *M. rosae.* In *S. oryzae* the methanolic extracts of *P. tinctorum* and *E. cirrhatum* with 75 and 50% mortality rates at 42 and 48 hours was observed. The promising results of lichen extracts towards insecticidal and anthelmintic activity were found to be a potential candidate for drug discovery and development against biopesticides.

Keywords: *Parmotrema tinctorum, Roccella montagnei*, Biopesticides.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.2.14

How to cite this article: Kavana NL, Rashmi S. *In-vitro* Study on Anthelmintic and Insecticidal Activity of Macrolichens Collected from South Karnataka. International Journal of Drug Delivery Technology. 2024;14(2):699-702.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Lichens are the most unique form of perennial cryptogamic organisms; they are symbiotic associates of fungi and algae.[1] There are approximately 20,000 species all over the world, where in India there is about 10% of lichens identified.[2] The nature of the lichens is generally recognized for their inherent properties and their products have been used in the treatment as traditional medicine for ages, but all these lichens are less studied and understood.[3] There are great varieties of secondary metabolites that are present in the lichens, which have distinct biological properties, i.e., anticancer, antimicrobial, anti-inflammatory, antipyretic, analgesic, antiproliferative and, cytotoxic, antioxidant, and anthelminthic activities.[4]

The helminthic infection is being increasingly recognized as a contributing cause of chronic illness and sluggishness amongst children. About 50% of the population in the world suffers from various types of worm infestation, which will impact domestic animals and livestock, causing considerable economic losses.[5] The lichen extracts show anthelmintic toxicity over the presence of drug residues in animal products has led to the renewal of interest in the use of lichen-based drugs. *In-vitro* techniques are preferred to *in-vivo* methods due to their low cost, simplicity, and rapid turnover.[6] Insects are the main source of pollination in plants, but they may also be considered potentially dangerous because many insects can cause damage to crops both in pre and post-harvest conditions.[7] Lichens are most likely studied and hard to understand because they are so different from the organism, which is generally familiar to us. Although many uses of lichens have been mentioned, they are less studied when compared to other organisms. Therefore, the study is undertaken to discover the bio-pesticidal and helminthic efficacy of lichens.

MATERIALS AND METHODS

Sample Collection

The lichen materials were basically collected from Southern Karnataka, from districts of Mysore, Chamarajanagara and Kodagu. Abundantly available lichen specimens *Paramatrema*

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tinctorum (Nyl.) Hale*, Roccella montagnei* Bèl. Emend. Awas*, Everniastrum cirrhatum* (Fr.) Hale. were selected for the study of anthelminthic and insecticidal activities.

Extract Preparation

Lichen extract was prepared using the following organic solvents: Methanol as a polar solvent and Hexane as the nonpolar solvent. Firstly, the lichen materials were washed thoroughly using distilled water and dried under room temperature. After complete drying, the lichens were powdered and the lichen constituents were extracted by cold extraction method. About 1 g of lichen powder was added to 10 mL of the organic solvents separately in beakers, covered with aluminum foil, and left behind for 24 hours at room temperature. The solutions were filtered through Whatmann No.1 filter paper and the filtrate obtained was left for evaporation.[8]

Determination of Anthelmintic Activity

The anthelmintic assay was carried out using *Pheretima posthuma* (earthworms). The earthworms were thoroughly washed in tap water and then used for experiments.[9] A single earthworm was placed in the petri dishes treated with crude extracts of all three different lichen samples. The time of paralysis was taken down when there were no movements observed. The time of death was noted after ascertaining that the worm didn't move when shaken vigorously. The albendazole (0.1 mg/mL) was taken as the standard reference drug or positive control and the respective solvents were used as the negative control.[10]

Determination of Insecticidal Activity

The pest causing disease for the rosebuds (Aphids like insects), the *Macrosiphum rosae* (L.) and the primary post-harvest pest of rice, the *Sitophilus oryzae* (L.) (Rice Weevils) were collected for the insecticidal activity. The crude extracts of three lichens were loaded in the petri plates containing Whatman filter paper and the insects were placed inside. For positive control, the insects were treated with chemical pesticides and the negative control was done using respective solvents.[11] The time of death and paralysis was noted and the mortality rate was calculated.[12]

RESULTS AND DISCUSSION

The diverse chemical structures and broad range of biological activities shown by lichen compounds have attracted significant interest from many researchers over the years for the development of novel drugs.[13] Natural compounds are being used to be considered as alternatives to the arsenal of synthetic compounds currently available. When coming to lichens have a potential source of natural compounds for management and are known as a biological indicator organism. These metabolites are not harmful to the environment, soo they can be used as potential metabolites for biological activities. As per the phytochemical tests, lichen *P. tinctorum* contains tannins, alkaloids, proteins and carbohydrates in methanol extract, *R. montagnei* contains tannins, proteins, triterpenes and steroids.[14] *E. cirrrhatum* revealed the presence of alkaloids, saponins, tannins and terpenoids.[15]

Table 1: Anthelmintic activities of the different lichenic extracts

Lichens	*Solvents*	*Time taken for paralysis (P) in minutes*	*Time taken for death (D) in minutes*
P. tinctorum	Methanol	0:50	1:18
	Hexane	2:51	5:32
R. montagnei	Methanol	0:48	1:20
	Hexane	1:20	4:15
E. cirrhatum	Methanol	2:58	4:34
	Hexane	0:45	2:35
Positive control	Albendazole	3:35	30:34
Negative control	Methanol	4:48	-
	Hexane	3:36	-

Table 2: Insecticidal activity of *M. rosae* (Rose bud aphids)

Lichens	*Solvents*	*Time taken for paralysis (P) in minutes*	*Time taken for death (D) in minutes*	*Mortality rate (%)*
P. tinctorum	Methanol	5:00	15:45	66.66
	Hexane	1:41	16:35	66.66
R. montagnei	Methanol	4:45	14:10	100
	Hexane	2:23	14:35	33.33
E. cirrhatum	Methanol	3:25	11:25	100
	Hexane	5:47	18:55	66.66
Positive control	Chemical pesticide	0:21	0:25	100
Negative control	Methanol	8:35	-	0
	Hexane	6:20	-	0

The anthelmintic activity was carried out on the earthworms which has obtained significant results by all three lichen extracts. The methanolic extract showed more potential than the hexane extract. *P. tinctorum* methanol extract showed the fastest death rate at 1.18 minutes followed by *R. montagnei* methanol extract with 1.20 minutes compared to other extracts. To the surprise, the slowest death rate was observed in the hexane extract of *P. tinctorum* with 5.32 minutes (Table 1). The adult Indian earthworm experimented against macrolichen *Heterodermia boryi* which were collected from the Western Ghats, Nilgiris, and Tamil Nadu. Different concentrations of solvent extract of lichen in the solvents of methanol, acetone, petroleum ether, chloroform and aqueous. The various extracts of the lichen showed dose-dependent paralysis and death. There was a significant result obtained in the extracts of acetone and methanol when compared to the other solvents.[16] Similar results were shown in the anthelmintic activity of macrolichen *E. cirrhatum.* The activity was higher in the lichen extract than of the reference drug due to the presence of tannins in the metholic extract which could be responsible for the anthelmintic effect of the extract.[17]

Table 3: Insecticidal activity in the *Sitophilus ozyzae* (Rice weevil)

Lichens	*Solvents*	*Time taken for paralysis (P) in minutes*	*Time taken for death (D) in minutes*	*Mortality rate (%)*
Parmatrema tinctorum	Methanol	30:00	42:00	75
	Hexane	90:00	-	0
R. montagnei	Methanol	45:00	-	0
	Hexane	50:00	-	0
E. cirrhatum	Methanol	20:00	48:00	50
	Hexane	45:00	-	0
Positive control	Chemical pesticide	0:21	0:25	100
Negative control	Methanol	-	-	0
	Hexane	-	-	0

The insects *M. rosae* and *S. oryzae* were collected to perform insecticidal activity against lichen extracts. The significant insecticidal activity was shown by all the lichen extracts against the *M. rosae* with 100% mortality rate (Table 2). In *S. oryzae* there was no significant result observed. Only *P. tinctorum* and *E. cirrrhatum* methanolic extract showed 75 and 50% mortality, respectively (Table 3). The fumigant toxicity of essential oils was reported against *S. ozyzae*. The essential oils of *Piper nigrum* and *Cuminum cyminum* were used for the fumigant toxicity, repellant, and for seeing the effect on acetylcholinesterase enzyme.[18] Similarly, *S. oryzae* was treated with fruit extract of *Xylopia aethiopica* and *Dennettia tripetala*. The extracts were prepared using hexane extract and the productive rate of mortality was in higher concentrations.[19] The insecticidal activity was reported in Maize Weevil (*Sitophilus zeamais*) against lichens *Letharia vulpina* and *Peltigera rufescens* and using two compounds of *Usnea longissima*, the sample extract was prepared using n-hexane, diethyl ether, acetone, and methanol. At higher concentrations and, maximum toxicity was observed in adults. Mortality rates were above 90% after the exposure after 24, 48, 72, and 96 hours.[20]

CONCLUSION

Lichen extracts are eco-friendly, biodegradable and safe to humans mankind. The present study successfully established the effects of the methanolic extracts of the tested lichen species which were satisfactory as they had strong insecticidal activities and can be used as a potential biopesticide. It would be fascinating to determine the active compound responsible for the aforementioned biological activity and to investigate its pharmacological actions.

REFERENCES

1. Devi RS. Exploration of lichen biodiversity from central part of Manipur with special reference to bioprospection. Ph.D. Thesis, Assam University, Silchar 2016.
2. Upreti DK, Rajesh B, Nayaka S. Lichenology: current research in India. Springer India 2015; 1: 1-3.
3. Zambare VP, Christopher LP. Biopharmaceutical potential of lichens. Pharmaceutical biology 2012; 50(6): 2-7.
4. Anjali DB, Satish M, Madhusudhana AR, Akkulanna S, Sanjeeva N. Qualitative phytochemical analysis of three solvents extracts of some selected macrolichens from Seshachalam biosphere reserve, Andhra Pradesh. Cryptogam biodiversity and assessment 2017; 02(01): 03- 06.
5. Raghavamma STV, Rao NR. *In vitro* evaluation of anthelmintic activity of *Nauclea orientalis* leaves. Indian J pharm sci 2010; 72(4): 520-521.
6. Guimaraes MA, Oliveira RN, Veras LMC, Lima DF, Campelo YDM, Campos SA. Anthelmintic activity in vivo of *Epiisopiloturine* against junvenile and adult worms of *Schistosoma mansoni*. PLOS neglected tropical diseases 2015; 9(3): 1-8.
7. Sachin MB, Mahalakhmi SN, Prashith TRK Insecticidal efficacy of lichens and their metabolites- A mini review. Journal of applied pharmaceutical science 2018; 8(10): 159-164.
8. Nisha SP. Evaluation of wound healing property of *Parmelia sp.* against diabetic foot ulcer causing pathogens. Journal of pharmaceutical science 2018; 12(3): 780-789.
9. Rahman SMM, Mohaimenul I, Rubel H, Imran H, Atlkullah, Koushik A, Asraful I, Susmita MJ, Omar F. Cytotoxic and anthelmintic activity of three selected medicinal plants used in Bangladesh. International journal of pharmaceutical science 2019; 55(1): 114-119.
10. Ajaiyeoba EO, Onocha PA, Olarenwaju OT. *In-vitro* anthelmintic properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* extract. Pharmaceutical Biology. 2001; 39(3): 217-220.
11. Ayalew AA. Insecticidal activity of *Lantana camara* extract oil on controlling maize grain weevils. Toxicology research and application 2020; 4: 1-10.
12. Pitan, Olufemi R, Kehinde, Adesoji T, Osipitan, Adebole A, Ademolu AA, Lawal OA. Laboratory evaluation of insecticidal activity of some botanicals against four insect pests of honeybees (*Apis mellifera* L.). International journal of applied agricultural and apicultural research 2015; 11(2): 172-182.
13. Selamawit Z, Teka F, Solomon A. *In vitro* anthelmintic activity of crude extracts of aerial parts of *Cissus quadrangularis* L. and leaves of *Schinus molle* L. against *Haemonchus contortus*. BioMed research International 2017; 1-6.
14. Rashmi S and Rajkumar HG. Preliminary phytochemical screening of different solvent extracts of lichens from Kodagu district, Karnataka. Journal of Pharmacognosy and Phytochemistry 2014; 3(4): 209-212
15. Kekuda PTR, Raghavendra HL, Swathi D, Venugopal TM, Vinayaka KS. Antifungal and Cytotoxic Activity of *Everniastrum cirrhatum* (Fr.) Hale Chiang Mai J. Sci. 2012; 39(1): 76-83
16. Prabhu SS, Sudha SS. *In-vitro* study on Anthelmintic activity of *Heterodermia boryi* macrolichen collected from the Nilgiris, Tamilnadu, India. International journal of current microbiology and applied sciences 2016; 5(9): 764-768.
17. Swathi D, Suchitha Y, Prashith KTR, Venugopal TM, Vinayaka KS, Mallikarjun N, Raghavendra HL. Antimicrobial, Anthelmintic and Insecticidal activity of a Macrolichen *Everniastrum cirrhatum*(Fr.) HALE. International journal of drug development and research 2010; 2(4):780-789.
18. Chaubey MK. Fumigant toxicity of essential oils against rice weevil *Stiophilus oryzae L.* (Coleoptera: Curculionidae). Journal of biological sciences 2011; 11(6): 411-416.
19. Donald AU, Ene EO, Idorenyin AU, Abo IN, Jude AU.

Insecticidal effect of fruit extract from *Xylopia aethiopica* and *Dennettia tripetala* (Annonaceae) against *Sitophilus oryzae* (Coleoptera: Curculionidae). Chilean journal of agricultural research 2012; 72(2): 195-200.

20. Yildrium E, Emsen B, Aslan A, Bulak Y, Ercisli S. Insecticidal activity of lichens against the maize weevil, *Sitophilus zeamais* Motschulsy (Coleoptera: Curuclionidae). Eqyptian journal of biological pest control 2012; 22(2):151-156.