Research Article

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EVALUATION OF ANTHELMINTIC ACTIVITY OF LICHEN *USNEA UNDULATA* STIRT. (PARMELIACEAE)

Vinayaka S Kanivebagilu * and Archana R. Mesta
Department of Botany, Kumadvathi First Grade College, Shimoga Road, Shikaripura, Shimoga, Karnataka, India

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***Corresponding author**
E-mail: ks.vinayaka@gmail.com

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ABSTRACT

Lichen compounds are also known to have biological activities. Traditionally *Usnea* used as antibacterial, antidermatophytic and anthelmintic. The present study was carried to determine the anthelmintic activity of a macrolichen *Usnea undulata* Stirt. (Parmeliaceae) collected from forest area of Chikmagalur district. The lichen was powdered and extracted sequentially using solvents of increasing polarity *viz*., acetone, methanol and water. Thin layer chromatography showed the presence of usnic acid. The anthelmintic assay was performed on *Pheretima pasthuma*. High inhibitory potential was observed in case of acetone extract. Inhibitory effect could be ascribed to the bioactive secondary metabolites, mainly Usnic acid present in the lichen. Purification of bioactive principles is to be conducted.

Key word: lichen, anthelmintic, *Usnea*, bioactivity

INTRODUCTION

Lichens are self-supporting symbiotic associations of a fungus and one or several algal or cyanobacterial components. Since the fungal constituent is unique in that symbiosis. Lichens and lichen products have been used in traditional medicines for centuries and still hold considerable interest as alternative treatments in various parts of the world. In various systems of traditional medicine worldwide, including the Indian system of medicine, these lichen species are said to effectively cure dyspepsia, bleeding piles, bronchitis, scabies, stomach disorders, and many disorders of blood and heart[1-3]. The utility of lichens is due to range of secondary compounds produced by them[4]. The genus *Usnea* has wide range of traditional and ethno medicinal value. *U. longissima* is used as an ingredient of a poultice for bone setting[5]. A wide range of secondary metabolites of lichens were characterized. According to their chemical structure, most lichen substances are phenolic compounds, dibenzofuranes, Usnic acids, depsidones, depsones, lactones, quinines and pulvunic acid derivatives[6]. Usnic acid is one of the most common and investigated lichen compounds. Its antimicrobial, antiprotozoal, antiviral, antiproliferative, anti-inflammatory, analgesic, antipyretic, and anti-tumour activities as well as some other properties such as UV protection, allergen and toxicity have been summarized[7-8]. Infectious diseases caused by bacteria, fungi, viruses, and parasites remains a major threat to public health, despite tremendous progress in human medicine. Their impact is particularly great in developing countries because of the relative unavailability of medicines and the emergence of widespread drug resistance[9]. Helminthes are recognized as a major problem to livestock production throughout the tropics. Parasitic helminthes affect human being and animals by causing considerable hardship and stunted growth. Most diseases caused by helminthes are of a chronic and debilitating in nature[10]. Helminthic infections are among the most common infections in man, affecting a large proportion of the world's population. Today, the principal mode for control of gastrointestinal parasites is based on the commercial anthelmintics. Because of the increasing anthelmintic resistance and the impact of conventional anthelmintics on the environment, it is important to look for alternative strategies against gastrointestinal nematodes[11].

Lichens are one of the lesser known nutritive sources to reduce the malnourishment problems in most of the countries. Forests of Chikmaglur has $75^0$15'-$75^0$50' E and $13^0$25'-$13^0$50' N latitude. The area comprises the forests of Western Ghats and its fringes with cool climate throughout the year and affords pleasant days during the hot months. *U. undulata* Stirt. (Parmrliaceae) genus is recognized based on the fruticose thallus, branches with a cartilaginous central axis and the presence of Usnic acid in the cortex[12].

MATERIALS AND METHODS

Collection and identification of Lichen material

The lichen *U. undulata* was collected from the forest of Chikmaglur dist., Karnataka. The voucher specimen of lichen (Voucher no. LHKFGC0005) was deposited in the Department of Botany, Kumadvathi First Grade College, Shikaripura for future reference. The dried lichen material was identified based on morphological, anatomical and colour tests[13-14]. Thin layer chromatography in solvent A (180 ml toluene: 60 ml 1, 4, dioxine: 8 ml acetic acid) was performed to detect secondary metabolites[15-16].

Figure 1: *Usnea undulata* Stirt.
Source of photo: Original photo captured by Vinayaka K. S.

Extraction and phytochemical analysis

For extraction, 20g of powdered lichen material was added to 100 ml solvent, sonicated for 30 minutes and left at room temperature overnight. The extract was filtered over Whatman No 1 filter paper and the filtrate was concentrated under reduced pressure to pasty mass[17]. The condensed solvent extract was subjected to phytochemical screening[18-19].

Anthelmintic activity of solvent extract

The anthelmintic assay was performed on adult Indian earthworm (*Pheretima pasthuma*) due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Standard drug (Piperazine citrate, 1%) and different concentrations of solvent extract of lichen (10, 25 and 50mg/ml) were prepared in normal saline (0.85%) and poured into respective labelled petriplates (50 ml). Six worms of nearly equal size were introduced into each of the plates. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors[20]. Death was also confirmed by dipping the worms in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had ceased[11].

Statistical Analysis

The data was presented as mean ± SEM**.** The activities of the extract were compared with the control**.** All the extracts showed significantly higher duration of the paralysis and death**.** Value of $P<0.05$ considered statistical significant[21]**.**

Table 1: Yield of different solvent extract of *U. undulata* Stirt.

Solvent used for extraction	Yield of lichen extract (in mg)
Acetone	0.45
Methanol	0.30
Aqueous	0.37

Table 2: Anthelmintic activity of different solvent extract *U. undulata* Stirt.and standard drug

Extract	Concentration	Paralysis time	Death time
Control (Normal saline)	10 mg/ml	-	-
	25 mg/ml	-	-
	50 mg/ml	-	-
Piperazine citrate	10 mg/ml	30±0.61	75±0.44
Aqueous extract	10 mg/ml	45±0.30	81±0.51
	25 mg/ml	45±0.63	63±0.42
	50 mg/ml	39±0.71	48±0.34
Methanol extract	10 mg/ml	59±1.00	96±0.90
	25 mg/ml	41±0.65	58±0.41
	50 mg/ml	32±0.42	39±0.48
Acetone extract	10 mg/ml	49±1.00	62±0.92
	25 mg/ml	38±0.84	48±0.37
	50 mg/ml	29±0.47	36±0.46

RESULTS AND DISCUSSION

We have extracted the lichen in successive solvents such as acetone, methanol and water**.** Acetone has yield more extract when compared to other two solvents **(**Table 1**).** In this study, the different concentrations of all the solvent extracts of *U. undulata* were evaluated for anthelmintic activity using adult Indian earthworm model. The extract exhibited a dose-dependent inhibition of spontaneous motility (paralysis). With higher doses (10mg/ml and more) the effects were comparable with that of 1% piperazine (Table 2). Among different solvent extracts, acetone extract showed potent anthelmintic activity by causing paralysis and death of worms in shorter time as compared to other solvents. Lichen metabolites exert a wide variety of biological actions. Even though these manifold activities of lichen metabolites have now been recognized, their therapeutic potential has not yet been fully explored and thus remains pharmaceutically unexploited. According to their chemical structure, most lichen substances are phenolic compounds, depsidones, depsones, lactones, quinines and pulvunic acid derivatives[6]. Thin layer Chromatographic (TLC) study of *U. undulata* showed that secondary metabolites such as usnic acid, sekikaic acid were present.

Helminth infections are among the most common infections in man, affecting a large proportion of the world's population. Parasites have been of concern to the medical field for centuries and the helminthes still cause considerable problems for human beings and animals. During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminthes and the indiscriminate use of some drugs has generated several cases of resistance[22]. Furthermore, it has been recognized recently that anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health. Consequently, the discovery and development of new chemical substances for helminth control is greatly needed and has promoted studies of traditionally used anthelmintic plants, which are generally considered to be very important sources of bioactive substances[23]. In this study, the anthelmintic activity of solvent extract of *U. undulata* may be due to the presence of different chemical constituents like Usnic acid and sekkic acid.

CONCLUSION

The results of this study highlighted the significance of the macrolichen *U. undulata.* The various extracts of the lichen selected for this study have shown a good activity against the tested helminth. The extracts could be used to treat infections caused by helminthes. The presence of various constituents in the extracts could be responsible for the anthelmintic efficacy.

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