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Studies on chemical composition and *in vitro* antibacterial activity of solvent extracts of lichen *Ramalina hossei* Vain. (Ramalinaceae)

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ABSTRACT

Lichen compounds are also known to show some biological activities against microorganisms. The present paper describes the extraction and antibacterial activity of various solvent extracts of lichen *Ramalina hossei* Vain. (Ramalinaceae), against Gram positive and Gram negative bacteria. The dried and powdered lichen material was extracted with solvents namely methanol, chloroform and petroleum ether and the solvent extracts were subjected to antibacterial activity using Kirby Bauer method. Chemical tests revealed the presence of Usnic acid and sekikaic acid aggregates. The extracts were found to possess more activity against Gram positive bacteria than Gram negative bacteria. Among solvent extracts, methanol extract showed more inhibition of test bacteria than others. From the results, it could be concluded that the lichen selected for the study possess active antimicrobial principles. The results are in justification with the folkloric uses of lichen extracts.

KEYWORDS

Lichen;
Ramalina hossei Vain;
Usnic acid;
Antibacterial activity;
Kirby Bauer method.

INTRODUCTION

Lichen is one of the most widely distributed eukaryotic organisms in the world. There are about 20,000 known lichen species, which account for approximately 25% of all the fungi described[1]. Lichens produce a wide range of organic compounds that can be grouped as primary metabolites and secondary metabolites[2]. Secondary metabolites are produced by the fungus alone and secreted onto the surface of lichen's hyphae either in amorphous forms or as crystals. About 350 secondary lichen metabolites have been found and the chemical structures of approximately 200 of them have been established[3]. Lichen compounds are also known to show some biological activities against microorganisms. 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, toxicity have been summarized[4,5]. *Ramalina hossei* Vain. (Ramalinaceae) is a fruticose lichen, thallus corticolous, tufted, erect, yellowish grey in color, branched, rim of soralia are present[6]. The present paper describes the

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extraction and antibacterial activity of various solvent extracts of lichen against Gram positive and Gram negative bacteria.

***Ramalina hossei* Vain.**

MATERIALS AND METHODS

Collection and identification of lichen

The lichen, *Ramalina hossei* Vain. growing on barks of areca tree, was collected. The collected lichen specimens were dried identified by using standard manual[6], and also by morphological, anatomical, chemical tests. The color tests were performed with the usual reagent, i.e., K (5% Potassium hydroxide), C (aqueous solution of Calcium hypochlorite) and PD (Paraphenylene diamine). Thin layer chromatography (TLC) in solvent A (180 toluene: 60 1-4, dioxine: 8 acetic acid) using technique of Culberson[7] and Walker and James[8]. The lichen specimens were preserved in Department of Botany (KSV/KU00905).

Extraction of powdered material using solvents

The dried and powdered lichen material was extracted with solvents namely petroleum ether, chloroform and methanol. For extraction, air-dried lichen sample was first ground, then 20 g portions were taken and added to 100 ml of solvents namely petroleum ether, chloroform and methanol. The mixtures were sonicated for 30 min, and then left at room temperature overnight. The extracts were filtered over Whatman No 1 filter paper, and the filtrates were concentrated under reduced pressure to pasty mass. The condensed extract was used for antibacterial assay[9].

Antibacterial assay

Gram positive bacteria namely *Staphylococcus aureus* and *Bacillus subtilis* and Gram negative bacteria namely *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were used as target bacteria. The bacteria were obtained from the Dept. of Microbiology. The extracts were dissolved in DMSO to get a concentration of 100mg/ml. The Kirby and Bauer disk diffusion method was used to determine the antimicrobial activity of lichen extracts against test bacteria[9]. Bacteria strains were inoculated onto nutrient agar plate (10^8 cells/ml) by swabbing the broth culture of test bacteria. The extracts were screened for their activity by placing sterile Whatman filter paper discs of 6mm diameter saturated with extracts (10mg/disc). Filter paper disc with DMSO served as negative control. Commercial antibiotic chloramphenicol was used as positive control. The inoculated plates were incubated for 24h at 37°C. Zones of inhibition formed around the discs were measured with the help of ruler and the growth was evaluated visually by comparing the extract-containing disks with the control disks. The experiment was conducted in triplicate to arrive concordant reading.

RESULTS AND DISCUSSION

TABLE 1 : Antibacterial activity

Treatment	Average inhibition zone in cm				
	E. coli	*B subtilis*	*S. aureus*	*K. pneumoniae*	*P. aeruginosa*
DMSO	-	-	-	-	-
Chloramphenicol	1.7	1.9	1.9	1.7	1.7
Methanol extract	1.5	1.6	1.6	1.5	1.3
Chloroform extract	0.9	1.1	1.1	1.0	1.0
Pet.ether extract	0.8	0.9	1.0	0.8	0.8

The color tests reveals cortex K+ yellow, Medula K-. Thin layer chromatography shows the presence of Usnic acid and sekikaic acid aggregates. The antibacterial activity of various solvent extracts of *Ramalina hossei* Vain. showed higher inhibitory potential of the methanol extract followed by chloroform and petroleum ether extracts. There was no inhibition in case of

DMSO which was used as negative control. Among bacteria tested, Gram positive bacteria were found to be more susceptible as compared to Gram negative bacteria to extracts and standard antibiotic. The higher resistance of Gram-negative bacteria to plant extracts has previously been documented and related to thick murein layer in their outer membrane, which prevents the entry of inhibitor substances into the cell[10]. The inhibitory potential of methanol extract was found to be more in case of *B. subtilis* and *S. aureus* followed by *E. coli*, *K. pneumoniae* and *P. aeruginosa*. The antibacterial activity of the extracts tested could be due to the presence of inhibitory principles in the extracts. There are plenty of literatures available on antimicrobial activity of lichens and their purified compounds. The antimicrobial activity of the chloroform, diethyl ether, acetone, petroleum ether, and ethanol extracts of the lichen *Cladonia foliacea* and its (-)-usnic acid, atranorin, and fumarprotocetraric acid constituents against 9 bacteria and fungi has been investigated[9]. Usnic acid (60 μg per disk) showed antimicrobial activity against *Bacillus subtilis*, *Candida albicans* and *Trichophyton mentagrophytes*[11]. The antibacterial activity of usnic acid against *Streptococcus mutans* has been examined[12]. *In vitro* activities of (+)-usnic acid, (-)-usnic acid, and vulpinic acid against aerobic and anaerobic microorganisms[13]. Both forms of usnic acid inhibited the growth of *Mycobacterium tuberculosis* and *Mycobacterium tufu in vitro* at a relatively low concentration[14]. The most common five lichen compounds were screened for *in vitro* activity against *Mycobacterium aurum*. Among these compounds, (+)-usnic acid from *Cladonia arbuscula* exhibited the highest activity against *M. aurum* with a MIC value of 32 μg/ml[15].

CONCLUSION

The various extracts of the lichen selected for this study have shown a good activity against the tested bacteria. The extracts could be used to treat infections caused by these bacteria. The presence of various constituents in the extracts could be responsible for the antibacterial efficacy. The results of the study have justified the folkloric significance of lichens in curing diseases. Further studies in animal models have to be carried out.

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