Indian J. Applied & Pure Bio. Vol. 39(1), 130-134 (2024). **ISSN: 0970-2091**
A Web of Science Journal

Estimation of Antimicrobial activity of Lichen *Ramalina hossei* (L.) Ach. from Darjeeling Hills and identification of its Active compounds by LCMS analysis

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Abstract

A fruticose lichen *Ramalina hossei* from Darjeeling Hills was screened for its antimicrobial activity against eight microorganisms. The ethanolic and methanolic extracts of *R. hossei* exhibited antimicrobial property against Gram positive bacteria, Gram negative bacteria and a fungus. Identification of active principle in lichen extract and the probable active compounds were identified by LCMS analysis. LCMS Chromatogram of methanolic extract of *Ramalina hossei* 7904 (SAIF) indicated the presence of occurrence of different group of phytochemicals which may be explored for the development of widely acceptable agents to combat disorders without any side effects.

Key words : Antimicrobial, LCMS analysis, *Ramalina hossei.*

The genus *Ramalina* belongs to the family Ramalinaceae and is widespread fruticose lichen. Although research has been conducted wide over the world on this lichen, but *R. hossei* from Darjeeling Hills has not been much explored. The main objective of this study is to screen the antimicrobial activity this hill lichen and identify the active principle active compound responsible for such activity.

Collection of lichen :

The samples of lichens *Ramalina hossei* were collected from profusely grown places of Darjeeling and surrounding areas. The sampling sites were selected on the basis of elevation, vegetation and population status.

Extraction of samples :

Lichen sample was washed to remove debris, dried and ground to powder and was stored in sterile glass bottle in the refrigerator. The 10g portions of sieved powder were added to 100 ml of solvents (ethanol and methanol), sonicated for 30 min and left overnight at room temperature. The crude extract was prepared by decanting, followed by filtration through

muslin cloth and further filtered with Whatman No.1 filter paper to obtain a clear filtrate. Fifty ml of the filtrate was evaporated to obtain 10 ml of concentrated extract and sterilized by membrane filtration using 450 nm bacteriological filters. Such sterilized filtrate was stored in screw capped airtight containers in the refrigerator and used for antimicrobial screening.

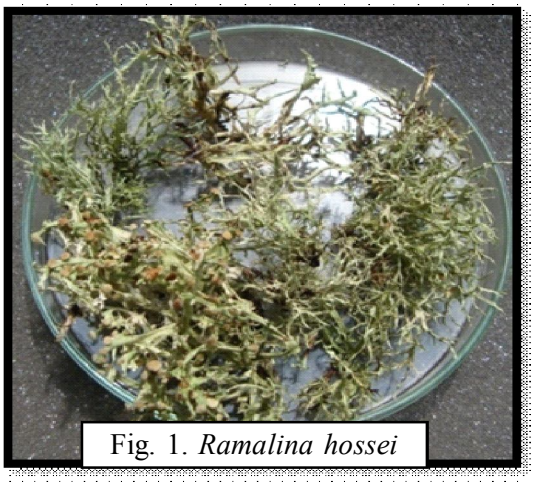

Fig. 1. *Ramalina hossei*

Table-1 Ethanol and methanol extract of lichen samples

Ramalina hossei	ethanol	RARE
	methanol	RARM

Table-2. Test microorganisms under study obtained from Institute of Microbial Technology, Chandigarh, India

Sl No.	Test Microorganisms	Gram nature	MTCC Code
1	*Alcaligenes faecalis*	Gram negative	MTCC9780
2	*Bacillus megaterium*	Gram positive	MTCC 7192
3	*Bacillus subtilis*	Gram positive	MTCC 3972
4	*Candida albicans*	-	MTCC 4748
5	*Escherichia coli*	Gram negative	MTCC 6365
6	*Enterobacter aerogenes*	Gram negative	MTCC 111
7	*Staphylococcus aureus*	Gram positive	MTCC 7443
8	*Pseudomonas aeruginosa*	Gram negative	MTCC 424

Test microorganisms (seven bacteria and one fungus) were obtained from Institute of Microbial Technology, Chandigarh, India (refer Table-2), the bacterial culture was preserved in N.A medium and fungal culture in King's medium B.

Screening of antimicrobial activity :

This procedure is based on disc diffusion method of Bauer *et. al.,*[1]. Overnight grown bacterial cultures of approximately (0.1ml) were spread plated on nutrient agar plates to achieve semi confluent growth. Sterile filter paper discs were soaked in concentrated extracts, allowed to dry between the applications and placed on plates which were then incubated at 37°C for 24 hrs. Streptomycin (10µg/ml) and sterile distilled water were taken as positive control and negative control respectively. Growth was evaluated and inhibition zone were measured. All the experiments were repeated thrice and data presented are average of three independent readings.

Identification of active principle in lichen extract :

Lichen sample was air dried at room

temperature (26°C) for until complete drying and then it was ground to powder. Powdered lichen material (10g) was added to 100ml methanol, sonicated and shaken for 7 days in shaking incubator at room temperature. The extract was filtered through whatman filter paper no 42 and was concentrated using a rotary evaporator the obtained extracts were sent to SAIF, CDRI, Lucknow for LCMS analysis.

The mass spectrum as LCMS chromatogram of RARM obtained from SAIF was studied following the literature - A catalogue of standardized chromatographic data of synthetic relationship for lichen substances[2] and lichen substances (refer Table-4) were determined.

Statistical analysis :

Experimental values are the mean ± standard deviation (SD) was calculated using EXCEL 2007. The graphs were designed using Excel software.

Ramalina hossei proved to be a potent antibacterial agent from this study as the ethanolic and the methanolic extract inhibited the growth of 4 gram(+)ve bacteria, three gram negative and a fungus. Both ethanolic and methanolic extract of *R. hossei* produced largest zones of inhibition measuring 17 mm and 18 mm respectively against *B. megatarium*. The extracts were also active against *C. albicans* with zones measuring 16 mm and17 mm. Both extracts were moderately active against *A. faecalis*, *B. subtilis*, *E. aerogenes* and *P. aeruginosa*. Ethanolic extract of *R. hossei* showed a higher antibacterial activity against *S. aureus* (15 mm) and *E. coli*. The ethanolic extract of *R. hossei* (refer table-3) and *R. pacifica* produced similar inhibition zones measuring 15 mm and 16.5 mm respectively against *S. aureus*. The ethanolic extract of *R. hossei* (Table-3) and *R. pacifica* (Hoskeri *et. al.*[3]) inhibited the growth of *E. coli* an opportunistic pathogenic. Study on phytochemical constituents, antibacterial, antifungal and cytotoxic properties of lichen member *Ramalina* sp was conducted by Kambar *et. al.,*[4]. Present study (refer Table-3) also revealed that ethanolic and methanolic extract of *R. hossei* inhibited the growth of all test bacteria and a fungus.

Table-3. Antimicrobial activity of extracts of *Ramalina hossei* by disc diffusion method

Sl No.	Test organisms	Inhibition zone(mm)			
		SDW	RARE	RARM	Streptomyc in
1	*A. faecalis*	0	10	12	16
2	*B. subtilis*	0	9	14	15
3	*B. megaterium*	0	17	18	14
4	*C. albicans*	0	16	17	9
5	*E. aerogenes*	0	16	12	16
6	*E. coli*	0	15	7	10
7	*P. aeruginosa*	0	12	0	12
8	*S. aureus*	0	15	11	16

SDW = sterile distilled water

Table-4. List of names, classes, mass spectrum and occurrence of lichen substances obtained from LCMS Chromatogram of methanolic extract of *Ramalina hossei* 7904 (SAIF)

Sl No	Compound	Class	Mass spectrum (nm)
1.	Gyrophoric acid	Orcinol Tridepsides	-1,318, 168, 150
2.	Calycin	Pulvinic acid derivatives	306,250,161,153
3.	2-Chlorolichexanthone	Xanthones	322, 321, 320, 319
4.	Coronatoquinone	Naphthaquinone	320, 318, 303, 302
5.	Pulvinic dilactone [Pulvinic acid lactone]	Pulvinic acid derivatives	290, 261, 234, 178
6.	(-)-Dihydropertusaric acid.	Aliphatic acids	368, 353, 326, 293
7.	20,24-Epoxydammarane-3â,12â,25-triol (Pyxinol)	Terpenoids	-1, 463, 417, 400, 381
8.	Methyl haematommate	Monocyclic aromatic derivatives	210, 179, 178

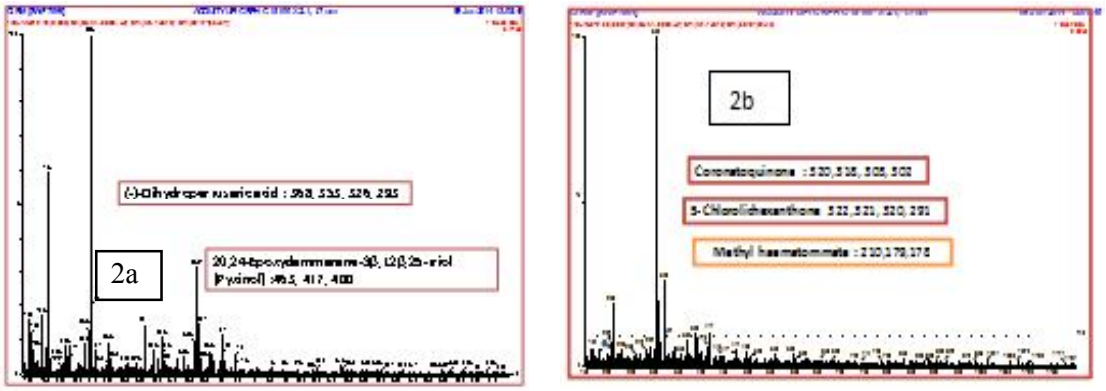

Fig. 2a, 2b LCMS spectral peaks and respective compounds of methanolic extract *Ramalina hossei*

Different classes of compounds namely Terpenoids, Pyxinol, Pulvinic acid derivatives Xanthones, Naphthaquinone, Pulvinic acid derivatives, Aliphatic acids, Orcinol Tridepsides could be identified from chromatograms of methanolic extract of *R. hossei*. The compounds identified are namely Gyrophoric acid, 24-Epoxydammarane-3β, 12β, 25-triol[Pyxinol], Coronatoquinone, 2-Chlorolichexanthone, Calycin, 2-Chlorolichexanthone, Coronato-quinone, ,(-)-Dihydropertusaric acid, (-)-Dihydropertusaric acid and Methyl haemato-mmate. *Ramalina hossei* showed the presence of xanthones. Xanthones occur in many species namely *Lecanora, Pertusaria, Melanaria, Lecidea* and *Buellia*, lichen xanthones contain larger amount of chlorinated substituent suggesting the availability of chloride in

environment may affect the production of these compounds. Lichen compounds occurring as phenolics with carbonyl as functional groups play an important role in withering of rocks due to complex metal ions which in turn leads to soil formation.

Lichen the various biological activities of lichen compounds also help in colonization of terrestrial areas as these compounds have been used by man during ancient Chinese and Egyptian evolution[5].

Ramalina hossei proved to be a potent antibacterial agent. Occurrence of different group of phytochemicals in lichen samples (refer Table-4.) may be exploited for the development new drugs which would be utilized for human welfare for treatment of various ailments.

This is a Conference Paper, presented by the author at National Level Conference entitled, "Ethnobiology Knowledge and Biodiversity Conservation-2022" held through virtual mode and physical mode (blended mode) on 25th to 26th Nov., 2022. I thank to OIC of the College and Dr. Debabrata Das, HOD, Department of Botany, GGDC Lalgarh, Jhargram for their support and help to make it complete in all respect. I convey sincere thanks to my guide Dr. Binod Chandra Sharma and Principal Sir Dr. Samir Bal of our present Institute.

References :

1. Bauer AW, WMM Kirby, JKC Scherris and M Turk (1966). *Am J of Clin Pathol 45*(4): 493-496.
2. Elix, JA (2014). A Catalogue of Standardized Chromatographic Data and Biosynthetic Relationships for Lichen Substances. (3rd ed.). Pub. Canberra.
3. Hoskeri HJ, V Krishna. and C Amruthavalli (2010) *Researcher. 2*(3): 81-85.
4. Kambar Y, MN Vivek, M Manasa, Prashith TR Kekuda, and R Onkarappa (2014) *Sci Technol Arts Res. J 3*(3): 57-62.
5. Karunaratne V, K. Bombuwela, S. Kathirgamanathar and V.M. Thadhani (2005). *J. Nat. Sc. Foundation of Sri Lanka. 33*(3): 169-186.