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IN VITRO ANTIMICROBIAL ACTIVITY OF *RAMALINA PACIFICA* AND *ROCCELLA MONTAGNEI* LICHENS

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ABSTRACT

In the present study antimicrobial activity of two lichen extracts, namely; *Ramalina pacifica* and *Roccella montagnei* against four bacteria (*Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus subtilis*) and four fungi (*Fusarium oxysporum, Fusarium moniliforme, Aspergillus flavus,* and *Alternaria solani*) was evaluated by disc diffusion method. The results revealed that, ethyl acetate and methanol extracts of *R. pacifica* were effective in inhibiting the growth of *B. substillis* and *S. aureus* and ethyl acetate extract of *R. montagnei* exihibited good zone of inhibition against the *E. coli* and *S. aureus*. Ethyl acetate extract of both *R. montagnei* and *R. pacifica* showed good antifungal potential against the *F. moniliforme* compared to control. These results cleary showed that ethanol extract and ethyl acetate extract of the *R. pacifica* and *R. montagnei* proved be best against test pathogens which was on par with synthetic chemicals and can be use as a broad spectrum antibacterial and antifungal agents.

KEYWORDS

Lichen, *Ramalina Pacifica, Roccella montagnei,* Antibacterial and Antifungal.

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INTRODUCTON

Development of antibiotic resistance in microorganisms is multi-factorial, containing the relationship between the specific nature of bacteria to antibiotics, the usage of antibacterial agent and characteristics of the host and environmental factors. This situation has approached to curious for new antimicrobial components from different sources as novel antimicrobial chemotherapeutic agents, but the cost of synthetic chemical production of medicine is high and also have adverse effects compared to natural drugs which are derived from the herbal products[1]. Nowadays most of the drugs are procured by the natural or semi

synthetic derivatives of non-chemical products and used in the traditional systems of medicine. Many of the diseases, caused by the bacteria, fungi, viruses, are treated with synthetic drugs and continuous consuming of synthetic drugs may leads various side effects[2]. Due to the concern regarding good human health, natural products are preferred over the synthetic drugs which require an advance approach in dealing with diseases caused by pathogens. The newer approaches that have low toxicity while still being effective against diseases would be natural products that have received much attention in recent years. Lichens are one such group of natural source which can be looked upon to treat various diseases[3] and still have great importance as possible substitute treatment in many countries of the world[4-5].

Lichens are unique and specialized organisms formed by the union of fungi and algae, which exist together intimately[6]. The fungus produce a thallus and known to synthesize more than thousands of characteristic secondary metabolites in all lichens[7-9]. These secondary metabolites are known to exhibit various biological activities such as antimicrobial, antiviral, antibacterial, antifungal, antiprotozoal, antiherbivore, antimutagenic, antioxidant, antiulcerogenic, antinociceptive, antipyretic and anti-inflammatory[10-16]. Aim of the present study was therefore to test the antimicrobial properties through proper knowledge and scientific investigation using the extract obtained from two lichens, *R. pacifica* and *R. montagnei.*

MATERIAL AND METHODS

Collection and identification of lichen

Lichens specimens were collected from Chamundi Hill, Mysuru, Karnataka. The samples were collected with the help of chisel and hammer along with their ecological notes for the identification, from different locations in different habitat types. The lichens were identified based on the morphology, anatomy of thallus structure and by studying the reproductive structures. External morphological studies were carried out under stereo-binocular microscope. The anatomy of thallus and reproductive structures were studied under compound microscope. Further, confirmation was done by the colour spot test[17].

Extraction of lichen samples

Lichens samples were cleaned and shade dried and made into fine powder. 10 g of air-dried powder of lichen was taken in the Soxhlet apparatus and subjected to successive solvent extractions in 100 ml non-polar to polar solvents (Chloroform, Ethyl acetate, Ethanol and Methanol). The Soxhlet was allowed to run for 48 hrs till the colour of the lichen material disappeared. The extract was collected and stored at 4°C. Qualitative tests for the presence of various lichen compounds were carried out.

Microorganisms

Antibacterial activity was conducted on four test organism, two gram-negative bacteria (*Pseudomonas aeruginosa* (MTCC1688) and *Escherichia coli* (MTCC7410) and two gram-positive bacteria (*Staphylococcus aureus* (MTCC7443) and *Bacillus subtilis* (MTCC121), which were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India and used throughout the study. All the bacterial cultures were adjusted to 0.5 McFarland standards containing approximately $1.5x10^8$cfu/ ml[18]. Antifungal activity was conducted on four fungal species viz. *Fusarium oxysporum, Fusarium moniliforme, Aspergillus flavus* and *Alternaria solani,* obtained from Departmental Studies of Botany, University of Mysore, Mysore.

Disc-diffusion assay

Antibacterial activity was carried out by the disc-diffusion method[19]. Lichen extracts were dissolved in the same solvent(s) to a final concentration of 50 mg/ml. The bacterial suspension ($1.5x10^8$cfu/ml) was seeded on nutrient agar (NA) medium in petri plate. Sterile discs (HIMEDIA SD067-1VL) of 6mm in diameter loaded with 0.5mg/ml, 1.25mg/ml and 2.5mg/ml of lichen extracts were placed on the bacteria seeded media. Negative control was prepared by using the same solvents used to dissolve the plant extracts. Ofloxacin (0.2 mcg/disc) was used as a positive reference standards to determine the sensitivity. Inoculated plates were incubated at 37°C for 24 hrs. Antimicrobial activity was evaluated by measuring the zone of inhibition

against the test organisms[20]. Each assay in this experiment was repeated thrice.

Antifungal activity was also carried out by the disc-diffusion method[19]. Lichen extracts were dissolved in the same solvent(s) to a final concentration of 50 mg/ml. Fungal spore suspension ($1x10^6$cfu/ ml) was seeded onto the potato dextrose agar (PDA) medium in petri plate. Sterile discs (HIMEDIA SD067-1VL) of 6mm diameter were loaded with 2.5mg/ml of lichen extracts were placed on the fungal spore seeded media. A negative control was prepared by using the same solvents used to dissolve the plant extracts. Mancozeb (25µl/ml) was used as a positive reference standard to determine the sensitivity. The inoculated plates were incubated at 25°C for 3-4 days. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms[20]. Each assay in this experiment was also repeated thrice.

Microdilution assay against bacterial strains

Determination of Minimum inhibitory concentration [MIC] was carried out by microdilution method[21]. The MIC was used to determine the lowest concentration at which the growth of bacteria and / or fungi was completely inhibited. MICs were calculated for the extract that had antibacterial and antifungal activity. A series of dilutions with concentrations ranging from 2.5- 0.001mg/mL was used in the experiment for each extract against every tested microorganism. Residues of lichen extracts were dissolved in respective solvents to a concentration of 50mg/ml. The lichen extracts (100µl) were serially diluted to 50% with solvents in 96 well flat bottomed microtitre plates. 24 hours old bacterial culture of each strains were transferred into fresh nutrient broth and 100 µl of this culture was added to each well and incubated at 37°C for 24 hrs. MIC was detected by adding 10 µl of TTC (2, 3, 5-triphenyl tetrazolium chloride) at 2mg/ml and incubated for 1 hour. Ofloxacin (HiMediaSD069-1VL Antimicrobial Susceptibility Discs) was used as a positive control. The MIC value was taken at the lowest concentration at which the colour was not changed.

Microdilution assay against fungal strains

The lichen extracts (100µl) were serially diluted to 50% with solvents in 96 well flat bottomed microtitre plates. Fungal cultures were transferred into fresh Potato dextrose broth, and 100µl of this was added to each well, 40µl of MTT dissolved in water was added to each of the microtitre plate wells, as growth indicator. Appropriate solvent blanks as control were included. The microtitre plates were covered with a cling film and incubated for 2-3 days at 27°C and at 100% relative humidity[22]. Mancozeb (Indofil M-45) was used as a positive control. The MIC was recorded by visual analysis in microtitre plate wells, where the lowest concentration of the lichen extracts that inhibited fungal growth after 48 to 72 hours of incubation was recorded. The MIC value taken at the lowest concentration at which the color was not changed.

RESULTS AND DISCUSSION

Antibacterial activity of *R. pacifica* and *R. montagnei* lichen extracts

Antibacterial potentiality of lichen extracts of *R. pacifica* and *R. montagnei* was assessed against the gram positive and gram negative bacteria at 0.5, 1.25 and 2.5 mg/ml concentrations obtained from chloroform, ethyl acetate, ethanol and methanol solvent extracts.

All the extracts of *R. pacifica* were effective against gram positive bacteria and did not show any activity against gram negative bacteria (Table No.1). However, the degree of percent inhibition varied between the lichen extracts. Among the lichen extracts of *R. pacifica*, methanolic extract proved to be the potent extract at all the tree concentrations in inhibited the growth of both *B. subtilis* and *S. aureus* with 23±0.56mm and 22±0.00mm zone of inhibition respectively, when compared to other lichen extracts obtained from *R. pacifica*. However, all the lichen extracts were not on par with standard antibiotic, used in the present study, which showed comparatively high degree of inhibition zone compared to all the lichen extracts (Table No.1). Ethyl acetate extract exhibited good activity at 2.5mg concentration against *S. aureus* with 21±0.57mm of zone inhibition. Moderate activity

was observed in chloroform and ethanol extracts against both *B. subtilis* and *S. aureus*. Both *E. coli* and *P. aeruginosa* were not sensitive to lichen extracts of *R. pacifica* (Table No.1, Figure No.1 and 2).

The antimicrobial activity was apparent with ethyl acetate and ethanol extracts of *R. montagnei* against all the four bacteria used in the present study. Among the two extracts the ethylacetate exhibited good zone of inhibition with 18.00±0.39mm against both *E.coli* and *S. aureus* at 2.5mg/ml concentration, whereas 13mm and 11.33mm zone of inhibition was recorded in *P. aeruginosa* and *B. subtilis* respectively. Ethanol extract proved to be less effective against all the four bacteria compared to ethyl acetate extract at 2.5mg/ml concentration (Table No.2, Figure No.1 and 2). Chloroform and methanol extracts did not show any activity.

The MIC of both the lichen extracts ranged between0.078 – 1.25 mg/ml. The maximum antibacterial activity was found in the ethyl acetate extract of lichen *R. montagnei* with the low MIC value of 0.078 mg/ml against *E. coli* and did not show any effect in other extracts. However, this was not on par with positive control where the lowest MIC was recorded at 0.009 mg/ml against *E. coli.* Both the lichen extracts were found to be ineffective against *P. aeruginosa* as against standard fungicide which was effective at the lowest possible MIC of 0.019mg/ml.

The MIC against *B. sutilis* varied between the different solvent extracts with the lowest MIC of 0.625mg/ml in the chloroform extract of *R. pacifica* and in ethanol extract of *R. montagnei,* the MIC was 0.156mg/ml. The lowest possible MIC against *S. aureus* was recorded in *R. montagnei* ethanol extract of 0.156mg/ml and in case of *R. pacifica*, the lowest MIC 0. 625mg/ml was recorded in both methanol and ethanol extracts. There was no inhibition of growth in wells of chloroform, ethyl acetate, ethanol and methanol used as solvent blank, which means that these solvents did not have any effect on the test organisms, proving them as a good solvent systems for bioassays (Table No.3).

Antifungal activity of lichen extracts of *R. pacifica* and *R. montagnei* against test fungal species

In the present study, efficacies of different solvent extract of lichens were tested against *F. oxysporum, F. moniliforme A. flavus* and *A. solani.* Ethyl acetate extracts of *R. montagnei* and *R. pacifica* were proved to be good against *F. moniliforme* with16.00±0.00mm and 14.66±0.33mm zone of inhibition respectively. However, the ethanol extract of *R. montagnei* exhibited moderate activity against *F. oxysporum, F. moniliforme* and *A. flavus*. Less activity was observed in methanolic extract of *R. montagnei* against *F. moniliforme*, and no activity was observed in chloroform extract of both lichen species. Ethanol and methanol extracts of *R. pacifica* also did not show any significant zone of inhibition (Table No.4 and Figure No.3 and 4).

The minimum inhibitory concentration was carried out to know the efficiency of lichen extracts at which the activity of the pathogen was completely inhibited. The minimum inhibitory concentration of all the tested lichen extracts ranged between 0.078 - 1.25mg/ml. Different solvent extracts revealed different levels of antibacterial activity depending on the tested species. The lowest concentration of MIC was observed in ethyl acetate extract of *R. montagnei* against *E. coli* which completely inhibited the growth at 0.078mg/ml concentration, followed by the chloroform extract of *R. pacifica* at 0.625mg/ml concentration (Table No.3). The highest minimum inhibitory concentration was recorded in ethanol and ethyl acetate extract of *R. montagnei* and *R. pacifica*, where both the extracts were able to inhibit the growth of *F. moniliforme* at 1.25mg/ml concentration (Table No.5).

The tested lichen extracts showed comparatively effective antimicrobial potentiality as the lichens are known to produce wide range of secondary metabolite having medicinal properties with manifold biological activities[23]. In the present study, antimicrobial activity was manifest with chloroform, ethyl acetate, ethanol and methanol extracts of *R. pacifica* and *R. montagnei* against *E. coli, B. subtilis, P. aeruginosa* which corroborates earlier report[24]. Ethyl acetate and methanol extracts

showed maximum zone of inhibition against all the bacterial strains used, whereas the remaining extracts inhibited mostly gram positive bacteria. The difference in antibacterial activity between lichen extracts can be attributed to solvents used for the extraction[25-26]. Ethyl acetate extract of *R. montagnei* showed maximum activity in both gram positive and gram negative bacteria which corroborates earlier report[27]. Between the lichen species, *R. pacifica* was found to be more effective than *R. montagnei* in different solvent extract. It was found that, the antibacterial activity was dose dependent, as at high concatenation, the zone of inhibition was high.

R. pacifica showed good antibacterial activity against the *B. subtillis* and *S. aureus* was corroborates with earlier reports[28-30]. Minimum inhibitory concentration was observed in methanol extract with 0.0785mg/ml, which was enough to inhibit the bacterial growth and which was much lesser concentration than earlier report[31]. Further, *R. montagnei* proved to be good antifungal agent against *F. moniliforme*.

Table No.1: Antibacterial activity of lichen extracts of *R. pacifica* against bacterial strains

S.No	Lichen extract	Concentration (mg/ml)	Zone of Inhibition (mm)			
			E. coli	***P. aeruginosa***	***B. subtilis***	***S. aureus***
1	Chloroform	0.5	-	-	16.00±0.57	15.00±0.52
		1.25	-	-	19.00±0.39	16.00±0.28
		2.5	-	-	19.33±0.66	17.00±0.01
2	Ethyl acetate	0.5	-	-	16.33±0.88	17.00±0.5
		1.25	-	-	17.00±0.57	18.00±0.57
		2.5	-	-	18.67±0.32	21.00±0.37
3	Ethanol	0.5	-	-	13.66±2.02	12.00±0.50
		1.25	-	-	19.00±0.00	19.00±1.52
		2.5	-	-	20.00±0.00	20.00±0.18
4	Methanol	0.5	-	-	19.33±0.33	17.00±0.57
		1.25	-	-	21.00±0.00	20.00±0.27
		2.5	-	-	23.00±0.56	22.00±0.00
5	Antibiotic	2mcg/disc	31±0.30	25.0±0.00	29.00±0.00	26.00±0.00
6	Solvent control	-	-	-	-	-

Values are means of three replicates, ± indicate standard error.

Table No.2: Antibacterial activity of lichen extracts of *R. montagnei* against bacterial strains

S.No	Lichen extract	Concentration (mg/ml)	Zone of Inhibition (mm)			
			E. coli	***P. aeruginosa***	***B. subtilis***	***S. aureus***
1	Chloroform	0.5	-	-	-	-
		1.25	-	-	-	-
		2.5	-	-	-	-
2	Ethyl acetate	0.5	15.00±0.37	8.00±0.82	7.60±0.33	12.00±0.57
		1.25	17.00±0.39	10.00±0.4	8.66±0.88	13.00±0.00
		2.5	18.00±0.39	13.00±0.34	11.33±0.82	18.00±0.00
3	Ethanol	0.5	-	-	-	7.60±0.88
		1.25	-	-	-	10.66±0.33
		2.5	-	11.00±0.52	8.00±0.54	12.00±0.57
4	Methanol	0.5	-	-	-	-
		1.25	-	-	-	-
		2.5	-	-	-	-
5	Antibiotic	2mcg/disc	31.00±0.30	28.00±0.12	28.00±0.00	28.00±0.00
6	Control (-ve)	-	-	-	-	-

Values are means of three replicates, ± indicate standard error.

Table No.3: Minimum inhibitory concentration of lichen extracts against bacteria strains

S.No	Lichen extract	(MIC) in mg/ml			
		E.coli	***P. auriginosa***	***B. subtilis***	***S. aureus***
Ramalina pacifica					
1	Chloroform	-	-	0.625	1.25
2	Ethyl acetate	-	-	1.25	1.25
3	Ethanol	-	-	1.25	0.625
4	Methanol	-	-	1.25	0.625
Roccella montagnei					
5	Chloroform	-	-	-	-
6	Ethyl acetate	0.078	-	0.625	1.25
7	Ethanol	-	-	0.156	0.156
8	Methanol	-	-	-	-
9	Control (+ve)	0.009	0.019	0.002	0.002
10	Control (-ve)	-	-	-	-

Table No.4: Antifungal activity of lichen extracts of *Ramalina pacifica* and *Roccella montagnei* against fungal species at 2.5 mg/ml concentration

S.No	Lichen sp.	Lichen extract	Zone of Inhibition (mm)			
			F.O	***F.M***	***A.F***	***A.S***
1	*Ramalina Pacifica*	Chloroform	-	-	-	-
		Ethyl acetate	12.60±0.66	14.66±0.33	-	-
		Ethanol	-	-	-	-
		Methanol	-	-	-	-
2	*Roccella montagnei*	Chloroform	-	-	-	-
		Ethyl acetate	12.66±0.00	16.00±0.00	15.00±0.00	14.00±0.00
		Ethanol	12.33±0.33	12.00±0.00	14.00±0.00	-
		Methanol	-	11.00±0.00	-	-
3	+ve control		30.00±0.00	16.00±0.00	19.00±0.00	24.00±0.00
4	-ve control		-	-	-	-

F.O-Fusarium oxysporum, F.M-Fusarium moniliforme, A.F-Aspergillus flavus, A.S-Alternaria solani. Values are means of three replicates, ± indicate standard error.

Table No.5: Minimum inhibitory concentration of lichen extracts against fungal species

S.No	Lichen sp.	Lichen extract	(MIC) in mg/ml			
			F.O	***F.M***	***A.F***	***A.S***
1	*Ramalina pacifica*	Chloroform	-	-	-	-
		Ethyl acetate	0.312	1.25	-	-
		Ethanol	-	-	-	-
		Methanol	-	-	-	-
2	*Roccella montagnei*	Chloroform	-	-	-	-
		Ethyl acetate	0.156	0.312	0.156	
		Ethanol	0.078	1.25	1.25	-
		Methanol	-	0.625	-	-
3	Control (+ve)		0.009	0.004	0.0024	0.039
4	Control (-ve)		-	-	-	-

F.O-Fusarium oxysporum, F.M-Fusarium moniliforme, A.F-Aspergillus flavus, A.S-Alternaria solani. Values are means of three replicates.

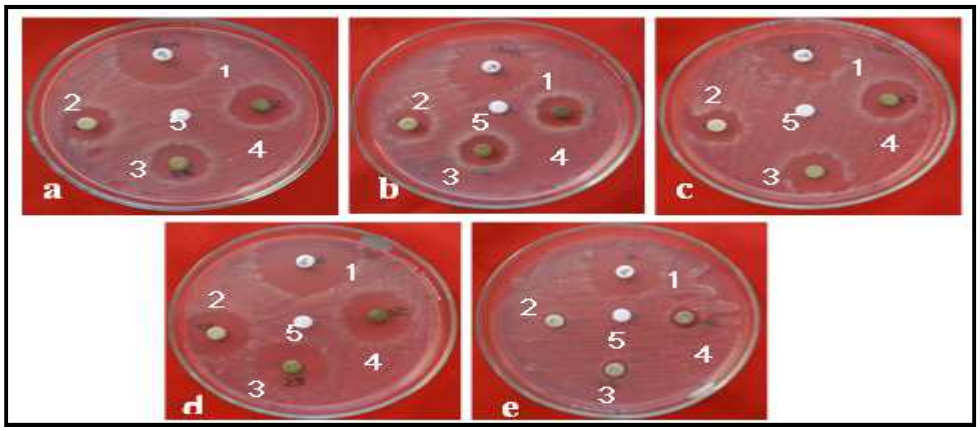

Figure No.1: Antibacterial activity of lichens extract against *B. subtilis:* a) Chloroform extracts of *R. pacifica*, b) Ethyl acetate extracts of *R. pacifica*, c) Ethanol extracts of *R. pacifica*, d) Methanol extracts of *R. pacifica*, e) Ethyl acetate extract of *R. montagnei*. 1- Antibiotic, 2-0.5mg/ml, 3-1.25mg/ml, 4-2.5mg/ml, 5-solvent

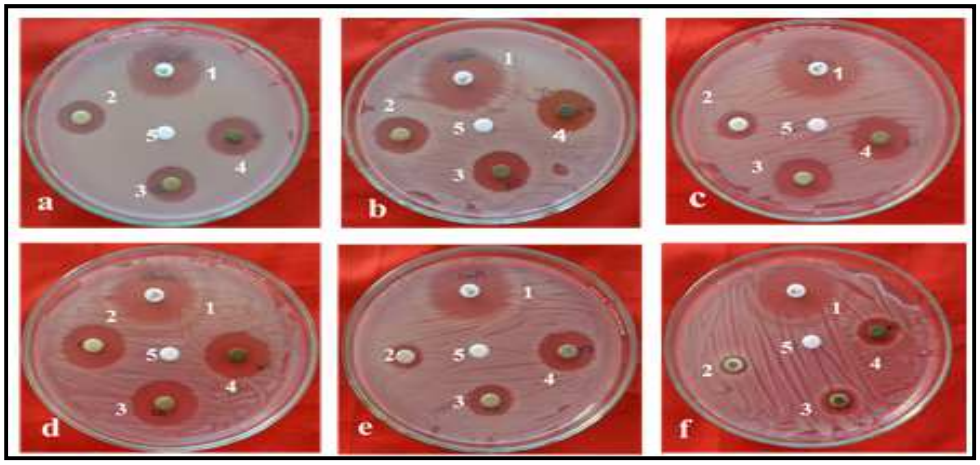

Figure No.2: Antibacterial activity of lichens extract against *S. aureus:* a) Chloroform extract of *R. pacifica*, b) Ethyl acetate extract of *R. pacifica*, c) Ethanol extract of *R. pacifica*, d) Methanol extract of *R. pacifica*. e) Ethyl acetate of *R. montagnei*, f) Ethanol extract of *R. montagnei,* 1-Antibiotic, 2 0.5mg/ml, 3-1.25mg/ml, 4-2.5mg/ml, 5-solvent

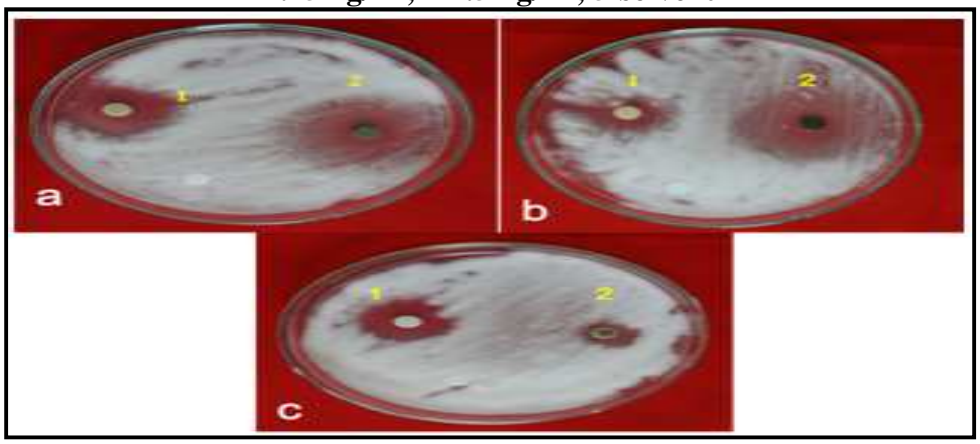

Figure No.3: Antifungal activity of *R. montagnei* species against the *F. moniliforme*: a) Ethyl acetate extract, b) Ethanol and c) Methanol extract, 1-positive control, 2-lichen extract

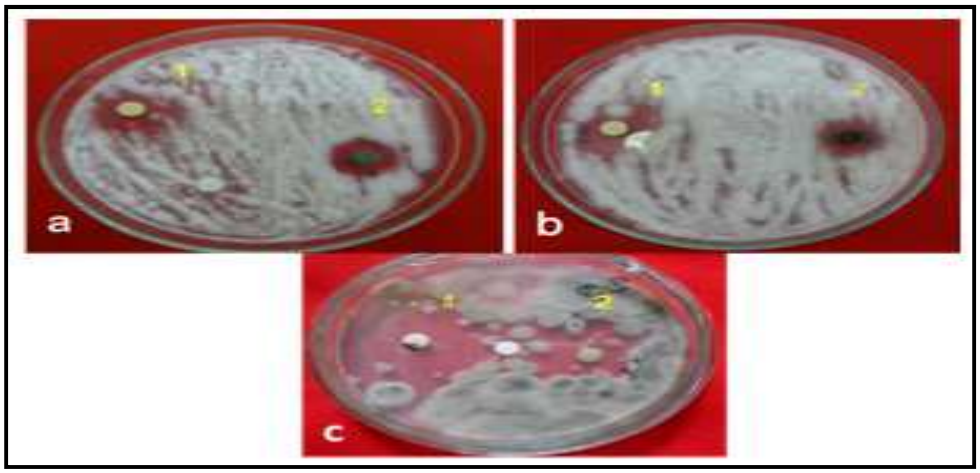

Figure No.4*:* Antifungal activity of *R. montagnei* species: against *A. flavus*- a) Ethyl acetate, b) Ethanol extract and against *A. solani* - c) Ethyl acetate extract

CONCLUSION

Search for novel bioactive compounds from natural resources to improve pharmaceutical, cosmetic and agriculture applications is an ancient practice and currently regaining more rapid importance. Lichens are inherently resistant to microbial infection due to the production of large numbers of unique secondary metabolites. Therefore, in the present study an attempt was made to evaluate the efficacy of two lichen extract in managing microbes of both plants and human concern. The present findings revealed that, solvent extracts of *R. pacifica* and *R. montagnei* could be the promising antimicrobial source in managing the diseases caused by both gram positive and gram negative bacteria as well as fungal phytopathogens. However, further studies are necessary to identify the biologically active compounds using analytical tools which may lead to the discovery of new antibiotic drug for diseases caused by the bacterial strains used in the present study.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBILIOGRAPHY

1. Abiramasundari P, Priya V, Jeyanthi G P, Gayathri D. Evaluation of the Antibacterial activity of *Cocculus hirsutus*, *Journal of Drugs Medicines,* 3(2), 2011, 26-31.
2. Karaman I, Sanin F, Gullice M, Ogutcu H, Sengul M, Adiguzel A. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxicedrus*, *Journal of Ethnopharmacology,* 85(2-3), 2003, 231-235.
3. Vivek M N, Kambar Y, Manasa M, Kekuda P T R, Vinayaka K S. Radical scavenging and antibacterial activity of three *Parmotrema species* from Western Ghats of Karnataka, India, *Journal of Applied Pharmaceutical Science,* 4(03), 2014, 086-091.
4. Kekuda P T R, Vivek M N, Kambar Y, Manasa N. Biocontrol potential of *Parmotrema* species against *colletotrichum capsici* isolated from Anthracnose of Chilli, *Journal of Biological and Scientific Opinion,* 2(2), 2014, 166-169.
5. Huneck S, Yoshimura I. Identification of lichen substances, *Springer Publication,*

Berlin, *Heidelberg,* 1st Edition, 1996, 11-123.

6. Nash T H. Lichen biology, *Cambridge University Press,* 2nd Edition, 2008, 1-8.
7. Ahmadijan V. The lichen symbiosis, *John Wiley and Sons, New york, NY, USA,* 1993.
8. Elix J A. Biochemistry and secondary metabolites, *In:* Lichen biology, *NASH T. H. III (Ed.), Cambridge University Press,* 1st Edition, 1996, 155-180.
9. Huneck S. The significance of lichens and their metabolites, *Naturwissenschaften,* 86(12), 1999, 559-570.
10. Perry N B, Benn M H, Brennana N J, Burgess E J, Ellis G, Galloway D J, Lorimer S D, Tangney D J. Antimicrobial, antiviral and cytotoxic activity of New Zealand lichens, *Lichenologist,* 31(6), 1999, 627-636.
11. Bombuwala K, Subramaniam V, De Silva K S, Karunaratne V, Adikaram N K B, Herath H M T B. Antitermite and antifungal activity of usnic acid and atranorin, *Ceylon Journal of Science, (Physical Science),* 6(1), 1999, 8-12.
12. Kekuda T R, Vinayaka K S, Swathi D, Suchitha Y, Venugopal T M, Mallikarjun N. Mineral composition, total phenol content and antioxidant activity of a macrolichen*Everniastrum cirrhatum* (Fr.) Hale (Parmeliaceae), *Journal of Chemistry,* 8(4), 2011, 1886-1894.
13. Kumar A H S, Kekuda P T R, VInayaka K S, Swathi D, Venugopal T M. Anti-obesity (Pancreatic lipase inhibitory) activity of *Everniastrum cirrhatum* (Fr.) Hale (Parmeliaceae), *Pharmcognosy Journal,* 3(19), 2011, 65-68.
14. Mitrovic T, Stamenkovic S, Cvetkovic V, Tosic S, Stankovic M, Radojevic I, Stefanovi O, Comic L, Dacci D, Curcic M. Markovic S. Antioxidant, antimicrobial and antiproliferative activities of five lichen species, *International Journal of Molecular Science,* 12(8), 2011, 5428-5448.
15. Kekuda P T R, Raghavendra H L, Swathi D, Venugopal T M, Vinayaka K S. Antifungal and cytotoxic activity of *Everniastrum cirrhatum* (Fr.) Hale Chiang Mai, *Journal of Science,* 39(1), 2012, 76-83.
16. Barreto R S S, Albuquerque-Junior R L C, Pereira-Filho R S, Quintans J S S, Barreto A S, De Santana J M, Santana-Filho V J, Santos M R V, Bonjardim L R, Araujo A A S, Quintas-Junior L J. Evaluation of wound healing activity of atranorin, a lichen secondary metabolite, on rodents, *Brazil Journal of Pharmacology,* 23(2), 2013, 310-319.
17. Awasthi D D. A key to the macrolichens of India and Nepal, *Journal- Hattori Botanical Laboratory,* 65, 1988, 207-302.
18. Schwalbe R, Moore L S, Goodwin A C. Antimicrobial susceptibility testing protocols, *CRC Press, Taylor and Francis Group, Boca Raton, London, New York,* 1st Edition, 2007, 432.
19. Murray P R, Baron E J, Pfaller M A, Tenover F C, Yolke R H. Manual of Clinical Microbiology, *ASM, Washington DC, USA,* 6th Edition, 1995, 1482.
20. Gulluce M, Aslan A, Sokmen M, Sahin F, Adiguzel A, Agar G, Sokmen A. Screening the antioxidant and antimicrobial properties of the lichens *Parmelia saxatilis*, *Platismatia glauca, Ramalina pollinaria*, *Ramalina polymorpha* and *Umbilicaria nylanderiana*, *Phytomedicine,* 13(7), 2006, 515-521.
21. Sarker S D, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals, *Methods,* 42(4), 2007, 321-324.
22. Ellof J N. Which extractant should be used for the screening and isolation of antimicrobial components from plants, *Journal of Ethanopharmocolgy,* 60(1), 1998, 1-8.

23. Elix J A. Biochemistry and Secondary Metabolites, *In: Lichen Biology (Nash III, T. H., Ed.), Cambridge University Press, Cambridge,* 2nd Edition, 2008, 154-181.
24. Behera B C, Verma N, Sonone A, Makhija U. Antioxidant and antibacterial activities of lichen *Usnea ghattensis*, *Biotechnology Letters,* 27(14), 2005, 991-995.
25. Rowe J G, Saenz M T, Garcia. Antibacterial activity of some lichens from southern Spain, *Annals of Pharmfrancaises,* 47, 1989, 89-94.
26. Karthikaidevi G, Thirumaran G, Anantharaman P, Kathiresan K, Balasubramanian T. Screening of the antibacterial properties of lichen *Rocella belangeriana* (Awasthi) from Pichavaram Mangoves (Rhizophora sp.), *Advanced Biological Research,* 3(3-4), 2009, 127-131.
27. Balaji P, Bharth P, Satyan R S, Hariharan G N. *In vitro* antimicrobial activity of *Rocella montagnei* thallus extract, *Journal of Tropical medicinal Plant,* 7(2), 2006, 169-173.
28. Ankith G N, Kekuda P T, Rajesh M R, Karthik K N, Avinash H C, Vinayaka, K S. Antibacterial and antifungal activity of three *Ramalina* species, *Journal of Drug Development Therapeutics,* 7(5), 2017, 27-32.
29. Hoskeri H J, Krishna V, Amruthavalli C. Effects of extracts from lichen *Ramalina pacifica* against clinically infectious bacteria, *Researcher,* 2(3), 2010, 81-85.
30. Kambar Y, Vivek M N, Manasa M, Mallikarjun N. Antimicrobial activity of *Leptogium burnetiae*, *Ramalina hossei*, *Roccella montagnei* and *Heterodermia diademata*. *International Journal of Pharmaceutical and Phytopharmacological Research,* 4(3), 2017, 164-168.
31. Rankovic B and Kosanic M. Antimicrobial activities of different extracts of *Lecanora atra, Lecanora muralis, Parmelia saxatilis, Parmelia sulcata* and *Parmeliopsis ambigua, Pakistan Journal of Botany,* 44(1), 2012, 429-433.

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