Original Research Article

Effect of extracts of lichens of different solvent races against clinically infectious bacteria

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Abstract

Background: Lichens are well-known prolific sources of biologically active natural products. They are symbiotic organisms of fungi and algae that synthesize numerous metabolites called the "lichen substances", which comprise aliphatic, cycloaliphatic, aromatic, and terpenic compounds. Lichens are good sources of biologically active secondary metabolites and have been used as medicine in treating wounds, stomach diseases, and whooping cough worldwide Bactericidal activity of crude extracts from lichens Usnea perplexans Stirton, Usnea spinocula Stirton, Usnea subsordiata Stirton, Ramalina conduplicans Vainio, Roccella montagnei Bel emend, Aswath were screened against pathogenic strains like gram negative bacteria such as Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and gram positive bacteria such as Staphylococcus aureus, Enterococci faecalis and Bacillus cereus. **Aims and Objectives:** To find out the Minimal inhibitory concentration (MIC) of the selected lichens against standard bacterial strains and to find out the Antibacterial activity of the selected lichen extracts **Methods:** The minimal inhibitory concentration of ethanol, methanol and acetone extracts of the selected lichens were determined against American type cell culture (ATCC) and Microbial type cell culture (MTCC) strains. All the three extracts of the selected lichens was potent in inhibiting bacterial growth which was tested by agar well diffusion method. **Results:** All the extracts exhibited bactericidal activity against the infectious sources and were dependent on the strain and solvent used. **Conclusion:** Lichen extracts of different solvent races showed significant antibacterial activity related to the tested gram positive and gram negative bacteria.

Key Word: Lichens, gram negative, gram positive, antibacterial.

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Received Date: 13/01/2019 Revised Date: 01/02/2019 Accepted Date: 26/02/2019
DOI: https://doi.org/10.26611/10081013

Access this article online	
Quick Response Code:	Website: www.medpulse.in
	Accessed Date: 20 April 2019

INTRODUCTION

Diseases caused by bacteria, fungi, viruses, and parasites are infectious and remain a foremost intimidation to public health, regardless of incredible evolution 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. The eternal more recurrent use of antibiotics in the treatment of bacterial infections is bringing on an increase of pathogen microorganisms that become resistant to common treatments. Consequently, it has become indispensable either to increase the administered doses or to provide new products [1.] Pathogenic microbes cause serious threats to human health and are increasing in prevalence in institutional health care settings[2]. New alternatives for combating the spread of infection by antibiotic resistant microbes in future are necessary tools for keeping pace with the evolution of 'super' pathogens. The most successful antibiotics that have been applied to combat disease are small molecule, secondary metabolites, including penicillin derivatives that were originally isolated from fungi[3].Natural products are proposed as a therapeutic alternative to conventional antimicrobial treatment, whose effectiveness is often limited by the resistance that the infectious agents have developed against antibiotics [4, 5.] Lichens are well-known prolific sources of

How to cite this article: N S Ravichandran, Daya Pauline S, Vishnu G Ashok. I Effect of extracts of lichens of different solvent races against clinically infectious bacteria. *MedPulse International Journal of Microbiology.* April 2019;10(1): 08-11. https://www.medpulse.in/Microbiology/

biologically active natural products. They are symbiotic organisms of fungi and algae that synthesize numerous metabolites called the "lichen substances", which comprise aliphatic, cycloaliphatic, aromatic, and terpenic compounds. Lichens are good sources of biologically active secondary metabolites and have been used as medicine in treating wounds, stomach diseases, and whooping cough worldwide [6, 7]. They are also reported to produce secondary metabolites with antimicrobial and anticancer activities[7,8,10]. The secondary compounds that are proposed to be present are Phenolic compounds, Dibenzofuranes and Usnic acids, Depsidones, Depsones, lactones, quinines and pulvinic acid derivatives. Out of all the secondary compounds extracted from lichens, the best known is usnic acid, an antibiotic with phenol structure.[11]. Numerous lichens were screened for antibacterial activity in the beginning of the antibiotic era in the 1950s[12]. Several lichen metabolites were found to be active against Gram-positive organisms[13,14,1].

MATERIALS AND METHODS

Study Design: Descriptive study

Study Setting: Department of Microbiology Sree Mookambika Institute Of Medical Sciences, Kulasekharam

Collection of lichens: Lichens specimens were collected from Kodayar of Western Ghats, Kanyakumari District, during summer season. Identification of collected lichens collected lichens were sent to NBRI Lucknow and identified by Dr. Upreti.

Preparation of the extracts of lichens: Collected lichens were cleaned of extraneous material, dried at room temperature and ground into a coarse powder. 100gms of the coarse powder was subjected to successive extraction with the three solvents namely diethyl ether (non polar), acetone (mid polar) and methanol (highly polar) by hot percolation method. The extractions were carried out for a period of 72 hours at a temperature not exceeding the boiling point of the solvents. At the end of the extraction, the respective solvents were concentrated by evaporation under reduced pressure. The crude extracts were then transferred to small glass bottles and placed in a desiccator containing fused calcium chloride. These crude extracts were redissolved in respective solvents and the antibacterial activity was carried.

Bacterial strains: In the 6 standard bacterial strains from ATCC and MTCC were used. Escherichia coli (ATCC – 25922), Klebsiella Pneumonia (ATCC – 700603), Pseudomonas aeruginosa (ATCC – 27853), Staphylococcus aureus (ATCC – 25923), Enterococci faecalis (ATCC – 29212) and Bacillus cereus (MTCC - 430) were used.

Antibacterial assays: The antibacterial activity of the various lichen extracts against test bacteria was determined according to the Kirby and Bauer disk diffusion method

RESULTS AND DISCUSSION

Table 1: Minimal inhibitory concentration (MIC) of the selected lichens against standard bacterial strains

Lichens	Extract	MIC in microgram					
		Staphylococcus aureus	*Enterococci faecalis*	*Bacillus cereus*	*Escherichia coli*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*
Usnea	E	40	40	40	1280	1280	640
subsordiata	M	80	160	160	1280	640	640
stirton	A	160	320	160	1280	2560	2560
Usnea	E	640	1280	640	1280	1280	1280
perplexans	M	2560	640	640	1280	640	1280
Stirton	A	640	640	320	640	2560	2560
Usnea	E	80	40	40	1280	640	1280
spinocula	M	80	160	160	1280	640	1280
Stirton	A	640	160	160	640	2560	1280
Ramalina	E	160	160	80	1280	640	1280
conduplicans	M	160	160	320	1280	640	640
Vainio	A	80	160	160	640	1280	1280
Roccella	E	80	80	160	1280	640	1280
montagnei	M	640	640	640	640	1280	640
Bel emend, Aswathi	A	640	640	640	1280	2560	2560
Amikacin							

E – Ethanol; M- Methanol; A- Acetone

Table 2: Antibacterial activity of the selected lichen extracts

Name of the lichens	Extracts	Zone of inhibition (mm)					
		Staphylococcus aureus	*Enterococci faecalis*	*Bacillus cereus*	*Escherichia coli*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*
Usnea	E	14	20	18	-	-	-
subsordiata	M	-	20	22	-	-	-
stirton	A	10	12	10	-	-	-
Usnea	E	-	-	-	-	-	-
perplexans	M	-	11	10	-	-	-
Stirton	A	-	10	10	-	-	-
Usnea	E	10	11	13	-	-	-
spinocula	M	-	11	11	-	-	-
Stirton	A	-	14	11	-	-	-
Ramalina	E	11	15	20	-	-	-
conduplicans	M	-	21	21	-	-	-
Vainio	A	14	21	20	-	-	-
Roccella	E	-	20	25	-	-	-
montagnei	M	-	-	10	-	-	-
Bel emend, Aswathi	A	-	-	11	-	-	-
Amikacin		20	13	24	20	13	28

E – Ethanol; M- Methanol; A- Acetone

The antibacterial activity of the investigated lichen substances against the tested gram positive and gram negative bacterial strains was determined by the values of MIC and zone of inhibition shown in table 1 and 2. All the investigated lichen substances showed antibacterial activity against gram positive bacterial strains and not against gram negative bacteria. The MIC values were different depending upon the sort of the lichen substances, its concentration and the tested strains. The MIC value for the different components against the tested bacterial strains was within the range of 0.0040 – 2.5mg/ml. The inhibition zones value of the selected lichen extracts varied between 10 – 25mm for gram positive bacteria. All the investigated lichen extracts were active inhibiting the growth of gram positive bacteria and not the growth of gram negative bacteria. Antibiotic activity of the lichen extracts were known in the earlier period. Pereira *et al* (1993) [16] studied the antibiotic property of crude extracts of Cladonia substellata against Mycobacterium smegnatis and Bacillus subtilis. The antibacterial properties of lichen extracts have been known for many years[17] and incorporation of usnic acid into medical devices inhibits bacterial biofilm formation on polymer surfaces[18]. A recent study by Krystle *et al* (2010)[19] showed the antibacterial activities of fruticose lichens collected from selected sites in Luzon Island, Philippines. Among the tested bacteria the Methanolic extract of Usnea subsordiata stirton was effective with the maximum zone of inhibition of 22mm and MIC of 0.16mg/ml against *Bacillus cereus. Also the ethanolic extract of* Roccella montagnei Bel emend, Aswathi showed highest active *inhibition against* Bacillus cereus with the zone of inhibition of 25mm at MIC 0.16mg/ml concentration. All the other lichen extracts also exhibited a potent antibacterial activity against gram positive bacteria with zone of inhibition ranging between 10 – 21mm. Yet, no lichen extract was active in inhibiting bacterial growth against gram negative bacteria. The permeability of single layered cell wall of gram positive bacteria would be increased. Consequently the biological enzymatic activities would be decreased once the lichen products entered each cell. The bacterial strains were incapable of producing antibodies against the lichen constituents. The gram negative bacteria have a double – layered cell wall. The constituents of such a cell wall would have impermeability. Because of the impermeability of such lichen extracts, gram negative bacteria show positive growth activities[20]. Despite the fact that gram negative bacteria has impermeable barrier provided by its cell wall the positive control Amikacin showed potent antibacterial activity against all the three gram positive and gram negative bacteria with the zone of inhibition ranging from 20 – 28mm with the highest being exhibited for Pseudomonas *aeruginosa*, a gram negative bacteria. The MIC of the lichen extracts was lowest (0.0040mg/ml) for the ethanol extract of Usnea subsordiata stirton and Usnea spinocula Stirton against all the three gram positive bacteria. This shows that the investigated lichen extracts are effective even at lower concentrations.

The obtained results showed that the tested lichen extracts of different solvent races showed significant antibacterial activity related to the tested gram positive and gram negative bacteria.

CONCLUSION

Lichens include not just the traditionally recognized algal and fungal symbionts but also diverse lichen-inhabiting (lichenicolous and endophytic) fungi as well as a surfeit of bacteria and harbor an enormous diversity of secondary metabolites. Investigating the bioactivities of such lichens may foretell for future research that ultimately might result in commercial production and development of interesting lichen substances.

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Source of Support: None Declared
Conflict of Interest: None Declared