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Research Article

Evaluation of the antibacterial properties of some Lichen species against human pathogens

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Abstract

Antibacterial activity of methanol, acetone, petroleum ether and chloroform extracts of lichens *Heterodermia boryi* (Fée) Kr. P. Singh & S.R. Singh, *Parmotrema stuppeum* (Taylor) Hale, *Usnea nilgirica* G. Awasthi, *Pyxine* sp. and *Parmotrema melanothrix* (Mont.) Hale has been screened in vitro against *E.coli, Pseudomonas sp, Klebsiella* sp, Coagulase negative *Staphylococcus aureus, Streptococcus viridians, Staphylococcus aureus, Acinetobacter* sp *and* MRSA by disc diffusion method. The solvent extracts of all the lichens exhibit variable range of antibacterial activity against all types of bacterial pathogens. This study indicates that lichens are rich source of antimicrobial agents.

Keywords: Antimicrobial agents.Antibacterial activity, Lichen, Western Ghats.

Introduction

Lichens are symbiotic organisms composed of a fungal partner (mycobiont) in association with one or more photosynthetic partners (photobiont). Lichens are important food source for many animals, and they do also play an important role for humans in some countries where they are part of their diet or used in traditional medicine (Upreti and Chatterjee, 2007). They usually grow on rocks, non-fertile ground, as well as epiphytes on the trees and leaves (Taylor *et al.*, 1995). About 8% of the terrestrial ecosystem consists of lichens and more than 20,000 lichen species are distributed throughout the world, their biological activities and biologically active compounds remain unexplored to a great extent (Toma *et al*., 2001).The total number of estimated species of lichens in India could vary between 2200 and 7200 species. Although inventorying of the Indian species of lichens is quite incomplete, India still emerges as the fifth richest country sharing 10.11% of species of lichens recorded in the world (Hans, 2003).

Lichen synthesize numerous metabolites called lichen substances including aliphatic, cycloaliphatic, aromatic and terpenic components. These metabolites exert a wide variety of biological actions including antibiotic, antimycobacterial, immunomodulatory, antioxidant, cytotoxic, antiherbivore, and antitumour effects (Chand *et. al.,* 2009). The first study of antibiotic properties of lichens was carried out by Burkholder (1944). Vartia (1973) reported antimicrobial properties of several lichens and other researchers have since then studied the antimicrobial activity of several lichens against gram-positive, gram-negative bacteria as well as several fungi. Gupta and Paul (1995) studied antimicrobial properties of five lichens and *Usnea floria* was found to be promising against *Bacillus megaterium* and *Staphylococcus aureus*. Ray *et. al.,* (2003) reported the antimicrobial activity of the extracts of *Usnea articulata, Ramalina jamisii and Parmelia tinctorum* against both Gram-positive and Gram-negative bacteria. According to wide screening of antimicrobial activity of lichen

extracts, it seems that bacterial inhibitions can vary within the lichen extract, solvent used for extraction and bacteria tested.

The search for novel natural bioactive compounds as a foundation to new drug discovery is receiving attention as previously reliable standard drugs become less effective against the emerging new strains of multiple drug resistant pathogens (Muller, 2001). Considering the above facts this present study was investigated the antibacterial potentials of various solvent extract of five lichen species collected from westernghats in vitro against bacterial strains which promotes the diseases in human.

Materials and Methods

Lichen Samples

Lichen samples were collected from various sites of Western Ghats, The Nilgiris, Tamil Nadu, and India. The collected samples identified as *Heterodermia boryi* (Fée) Kr. P. Singh & S.R. Singh, *Parmotrema stuppeum* (Taylor) Hale, *Usnea nilgirica* G. Awasthi, *Pyxine* sp. and *Parmotrema melanothrix* (Mont.) Hale by Dr. Biju Haridas Ph.D.

Technical Officer, Microbiology Division, JNTBGRI, Thiruvananthapuram, Kerala, India. Samples were dried at room temperature. Herbarium was prepared and voucher specimens were deposited in JNTBGR (Voucher specimen no. 1902, 1903, 1904a, 1905 and 1907b) for future reference.

Preparation of lichen Extracts

The fine dry ground thalli of lichen (50 g) were subjected for extraction using soxchlet apparatus. The extracts were filtered and concentrated under reduced pressure in rotary shaker evaporator and stored at 4°C.

Tested Bacteria

The antibacterial potential of lichens were tested against four gram positive bacteria (Coagulase negative *Staphylococcus aureus, Streptococcus viridians, Staphylococcus aureus, and* Methicillin resistant *Staphylococcus aureus*) and four gram negative bacteria (*E.coli, Pseudomonas sp, Klebsiella* sp. and *Acinetobacter* sp.) for this study. All the isolates were obtained from Kovai medical center and Hospitals (KMCH), Coimbatore, Tamil Nadu.

Determination of antibacterial activity

Antibacterial assay was done by paper disc diffusion using Kirby bauer method (Bauer, 1966). All the tests were performed on Mueller Hinton agar plates. Suspension of microbial cultures (0.5McFarlands) was inoculated on Mueller Hinton agar media in a petri plate using sterile swab. The sterile discs of diameter 6mm were impregnated with lichen extract (10 µg/ml) solutions were dissolved in Dimethyl Sulphoxide (DMSO) and placed onto the cultured Mueller Hinton agar plates. The negative control was maintained with DMSO and positive control was a Streptomycin (10µg/ml). Inoculated plates were incubated at 37 ^{O}C for 24 hrs. The antibacterial activity was measured by zone of inhibition. All the experiments were performed in triplicate

Results and Discussion

The lichens solvent extracts shows potential antibacterial activity against most of the tested organisms. The results of the antibacterial activity of investigated lichen extracts are summarized in Table 1. The highest antibacterial activities recorded in the extracts of Petroleum ether (24 mm), Chloroform (22mm) against *Staphylococcus aureus*, acetone extract (20mm) against *Streptococcus viridians by Parmotrema stuppeum*, The methanol extract of *Usnea nilgirica* G.aswathi showed maximum activity against *Acineobacter* sp.(18 mm). Acetone and chloroform extracts of exhibited most inhibitory activity against *Acinetobacter* sp .(16 mm). Chloroform extract of *Pyxine* sp. showed highest activity on *Pseudomonas* sp. (15 mm).

Heteroderma boryi methanol extract has the maximum activity against almost all the pathogens followed by the acetone extract. The petroleum ether and chloroform extracts of above lichens shows highest activity against the *Acinetobacter* sp. All the extracts of *Parmotrema stuppeum* has showed the activity against *Acinetobacter* sp. and it has no activity against *Klebsiella* sp. and CT-ve *Staphylococcus aureus.*

Usnea nilgiriga G.Aswathi has the activity against the various pathogens used. Acetone and methanol extracts of this lichen shows activity against most of

Table 1. Antibacterial activity of Lichen extracts against test organisms

		Zone of inhibition (diameter in mm)							
Lichens	**Solvent**	**EC**	**PS. Sp**	**KL. sp**	**C-ve SA**	**SV**	**SA**	**AC. sp**	**MRSA**
Heterodermia boryi	Met.	7.0± 1.0	8.0± 0.0	*	8.0± 1.0	*	8.0± 1.0	*	*
	Acetone	10 ± 0.0	7.0± 1.0	7.0±1.0	7.0± 0.0	10± 0.0	9.0± 1.0	16±1.0	*
	Pet Eth.	*	*	*	*	*	*	14±0.0	*
	Chlor.	*	*	*	*	*	*	16±2.0	*
	Aqueous	*	*	*	*	*	*	*	*
Parmotrema melanothrix	Met.	10 ± 1.0	8.0± 1.0	*	*	6.0±1.0	*	*	12±1.0
	Acetone	*	7.0± 0.0	*	*	7.0±0.0	*	7.0± 0.0	*
	Pet Eth.	*	*	*	*	*	*	12 ± 1.0	*
	Chlor.	*	*	*	*	*	*	11 ± 0.0	*
	Aqueous	*	*	*	*	*	*	*	*
Usnea nilgirica G. Aswathi	Met.	*	8.0± 0.0	7.0±1.0	10± 1.0	6.0± 2.0	9.0± 1.0	18± 0.8	*
	Acetone	*	7.0± 2.0	8.0±0.0	10± 1.0	7.0± 0.0	11± 1.0	14± 0.0	7.0± 0.0
	Pet Eth.	*	*	*	*	*	12± 0.6	10± 0.0	*
	Chlor.	*	*	*	*	*	10± 0.0	12± 1.0	14± 1.0
	Aqueous	*	*	*	*	*	*	*	*
Pyxine sp.	Met.	7.0± 0.0	8.0±0.0	*	*	*	*	*	*
	Acetone	*	10± 2.0	*	*	*	*	6.0±1.0	*
	Pet Eth.	11± 0.0	12± 0.0	10±0.0	*	*	12±0.0	11± 0.0	*
	Chlor.	11± 1.0	15± 0.0	10±1.0	*	*	11± 0.0	*	*
	Aqueous	*	*	*	*	*	NS	*	*
Parmotrema stuppeum	Met.	*	*	*	*	*	*	*	*
	Acetone	10±0.0	7.0±1.0	13± 0.0	*	20± 1.0	13± 2.0	12± 0.0	*
	Pet Eth.	10±1.5	10± 0.5	15± 1.0	*16± 0.0	*	24± 2.0	13± 1.0	10± 0.5
	Chlor.	10±0.0	10± 1.0	12± 1.0	*	*	22± 2.0	8.0±0.0	*
	Aqueous	*	*	*	*	*	*	*	*
					*				
	Strepto (10µg/ml)	20	19	20	19	21	22	20	18

EC - *E.coli* ; PS –*Pseudomonas*; KL- *Klebsiella*; C-ve SA- Coagulase –ve *Staph.aureus*; SV- *Streptococcus viridians*;SA- *Staphylococcuc aureus* AC – *Acinetobacter*; MRSA- Methicillin Resistant *S.aureus*, Strepto - Streptomycin

Met.- Methanol; Pet.Eth- Petroleum ether; Chlor.- Chloroform

- No sensitivity; (Values are mean ± by Std. deviation, n=3)

the pathogens. The petroleum ether and chloroform extracts exhibited highest activity against *Staphylococcus aureus* and *Acinetobacter sp.* The acetone and chloroform extracts of this lichen are found to be effective against MRSA, The *Usnea* has no activity on *E.coli.*

Pyxine sp. has no activity against CT-ve *Staphylococcus,Streptococcus viridians* and MRSA. However it is effective against *Pseudomonas sp.* and most of the extracts have good activity against *Klebsiella, Acinetobacter* and *E.coli.*

Compared with all the above samples *Parmotrema stuppeum* is found to have the most highest activity against all the pathogens. It is particularly effective against *Staphylococcus aureus* and MRSA. However the petroleum ether and chloroform extracts were found to be ineffective against *Streptococcus viridians* and CT-ve *Staphylococcus.* All the tested extracts were highly effective against *E.coli, Pseudomonas, Klebsiella* and *Acinetobacter sp.*

This present study reports the antibacterial activity of various solvent extracts of lichens *Heterodermia boryi* (Fée) Kr. P. Singh & S.R. Singh, *Parmotrema stuppeum* (Taylor) Hale, *Usnea nilgirica* G. Awasthi, *Pyxine* sp. and *Parmotrema melanothrix* (Mont.) Hale collected from The Nilgiris, Tamilnadu, India against eight bacterial pathogens. Lichens have been recognized as potent antimicrobial agents since ancient times and many studies conducted all over the world showed the potential of lichen extracts and purified metabolites to inhibit a wide range of bacteria and fungi (Yilmaz *et al*., 2004; Turk *et al*., 2006; Canarasan *et al*., 2006; Vinayaka *et al*., 2009; Kekuda *et al*., 2011).The results indicated differences in antimicrobial activity between extracts depending on the species of lichen and as a function of the type of extracting solvent and similar results were recorded by (Turk et al., 2003; Yilmaz et al., 2004 and Sanakara Narayan and Biswas, 2011). These results might be due to the fact that bioactive components of medicinal plants have different solubility in different extracting solvents (Oloke and Kolawole,1998).

Previous researches showed significant bioactive characteristics of similar lichens. Gulluce *et al*. (2006) found out that the methanol extract of the lichen *Parmelia saxatilis* had a strong antimicrobal influence. Similar results were reported by Candan *et al.* (2007) for different extracts extracted from the lichen *P. sulcata*. Ranković *at al.* (2007b) find an antimicrobial activity for the extracts of the lichens *P. caperata* and *P. pertusa*. This is the first study on antibacterial activity of Methanol, ethanol, petroleum ether and Chloroform extracts of *Usnea nilgirica* G.Aswathi.

The aqueous extracts of all lichens doesn't show any activity against tested organisms. The previous studies did not show any antibacterial activity of lichens extract in water (Yilmaz *et al*., 2004; Tay *et al*., 2004). The reason for weak or no activity of aqueous extracts is that active substances present in the thalli of lichens are insoluble or poorly soluble in water. (Kinoshita et al., 1994).

The differences in the antibacterial activity of extracts of different species of lichens are probably a consequence of the presence of different compounds with antibacterial activity. Therefore, further study is necessary to characterize the chemical constituents of the extracts of lichen species tested posses compounds with antibacterial properties and to determine antibacterial agents for therapy of human diseases.

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References

1.Bauer R.W, Kirby M.D.. Sherris J.C., and Turck M. 1966. Antibiotic susceptibility testing by standard single disc diffusion method, *American Journal of Clinical Pathology*, (45), pp.493–496.

2.Burkholder PR, Evans AW, McVeigh I and Thornton HK .1944. Antibiotic activity of lichens.
Proceedings on National Academic Science, USA. 30(9): 250-255.

3.Canarasan D, Kahya D, Yurdakulol E, Atakol O. 2006.Identification and quantitation of Usnic acid from the lichen *Usnea* species of Anatolia and antimicrobial activity. Z. Naturforsch. 61c: 773-776.

4.Candan, M., Yilmaz, M., Tay, T., Erdem, M., Türk, A.Ö. 2007. Antimicrobial Activity of Extracts of the Lichen *Parmelia sulcata* and its Salazinic Acid Constituent. *Z. Naturforsch*, 62: 619-621.

5.Chand P, Singh M and Rai Mayank .2009. Antibacterial activity of some Indian Lichens. *Journal of Ecophysiology and Occupational Health* 9: 23-29.

6.Gulluce, M., Aslan, A., Sokmen, M., Sahin, F., Adiguzel, A., Agar, G., Sokmen, A. 2006. Screening the antioxidant and antimicrobial properties of the lichens *Parmelia saxatilis, Platismatia glauca, Ramalina pollinaria, Ramalina poly*morpha and *Umbilicaria nylanderiana*. *Phytomedicine*, 13: 515–521.

7.Gupta, SK and Paul AK.1995. Studies on antimicrobial substances from lichens of Darjeeling Hills. *Journal of Hill Research* 8(1): 61-66

8.Hans Raj Negi.2003. Lichens: A Valuable Bioresource for Environmental Monitoring and Sustainable Development. Resonance. 51-58.

9.Kinoshita K, Matsubara H, Koyama K, Takahashi K, Yoshimura I, Yamamuto Y, MiuraY , Kinoshita Y, Kawai KI .1994. Topics in the chemistry of lichen compounds. J. Hattori. Bot. Lab. 76: 227- 233.

10.Muller K . 2002. Pharmaceutically relevant metabolites from lichens. *Applied Microbiology and Biotechnology* 56: 9-16.

11.Oloke J.O. & Kolawole D.O. 1998. The antibacterial and antifungal activities of certain components of Aframomum melegueteа fruits. Fitoterapia 59: 384– 388.

12.Ranković, B., Mišić, M., Sukdolak, S. 2007b: Antimicrobial activity of the lichens *Cladonia furcata, Parmelia caperata, Parmelia pertusa, Hypogymnia physodes* and *Umbilicaria polyphylla. British Jornal of Biomedical Science*, 64: 143-148.

13..Ray S, Sinhababu A and Mandal NC .2003. Antibacterial activity of three lichen specimens viz., *Usnea articulate, Ramalina jamesii, Parmelia tinctorum* from Eastern Himalaya. *Journal of Hill Research* 16(2): 66-69.

14.Sankar Narayan Sinha and Mrinal Biswas. 2011. Evaluation of Antibacterial Activity of Some Lichen From Ravangla, Sikkim, India. Int. J Pharm and Bio Sci 2(4): 24-28.

15.Taylor, T.N., Hass, H., Remy, W and Kerp, H. .1995. The oldest fossil lichen.*Nature*, 378: 244-244.

16.Toma N., Ghetea L., Nitu R., and Corol D. I.,2001. "Progress and perspectives in the biotechnology of lichens," *Romanian Biotechnological Letters*, 6: pp. 1–15.

17. Upreti D.K and Chatterjee S. 2007., Significance of lichens and theirsecondary metabolites: A review, In Fungi multifaceted microbes, (eds. Ganguli BN, Deshmukh SK), Anamaya Publishers, New Delhi, pp 169-188.

18.Tay, T., Turk, A. O., Yilmaz, M. and Kivac, M. 2004. Evaluation of the antimicrobial activity of the acetone extract of the lichen *Ramalina farinacea* and its (+)-usnic acid and procetraric acid constituents. *Verlag der Zeitschrift fur Naturforschung.* 59(C): 384-388.

19. Turk A.O., YilmazM., KivancM. & Turk H. 2003. The antimicrobial activity of extracts of the lichen Cetraria aculeata and its protolichesterinic acid constituent. Z.Naturforsch.58c:850–854.

20. Vartia KO .1973. Antibiotics in lichens. In: Ahmadijan V., Hale M.E. (eds): The Lichens. Academic Press, New York, pp. 547-561.

21.Vinayaka KS, Kumar PSV, Kekuda PTR, Krishnamurthy YL, Mallikarjun N, Swathi D. 2009. Proximate composition, antioxidant, anthelmintic and insecticidal activity of a macrolichen *Ramalina conduplicans* Vain. European Journal of Applied Sciences. 1(3): 40-46

22..Yilmaz M, Turk AO, Tay T and Kivanc M. 2004.The antimicrobial activity of extract of the lichen *Cladonia foliaceaandits* (-) Usnic acid, atranorin and fumarprotocetraic acid constituents. Z Naturforsch, 59c: 249-254.