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In vitro Antimicrobial Activity of *Parmotrema praesorediosum* Thallus Extracts

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Abstract: Hexane, dichloromethane, ethyl acetate, acetone and methanol extracts of the thalli of *Parmotrema praesorediosum* (Nyl.) Hale was tested for their antimicrobial activity against eleven human pathogenic microorganisms using standard disc diffusion method. The potential secondary metabolite constituents of ethyl acetate and dichloromethane extracts were active against the microorganisms viz., *Bacillus cereus*, *Corynebacterium diptheriae*, *Shigella flexnerii*, *Staphylococcus aureus*, *Vibrio cholerae* and *Candida albicans*. Solvents treated discs were used as negative control and discs treated with standard antibiotics served as positive controls in the experiment. The dichloromethane extract exhibited potential antibacterial and antifungal activity.

Key words: Lichen, *Parmotrema praesorediosum*, crude extracts, antibacterial, antifungal activity

INTRODUCTION

The search for novel natural bioactive compounds as a foundation to new drug discovery is receiving greater attention as previously reliable standard drugs become less effective against the emerging new strains of multi drug-resistant pathogens (Müller, 2001). In this backdrop, wide ranges of secondary metabolites of lichens (fungi that live symbiotically with algae or cyanobacteria) that were found to occur as extra cellular crystals on the cell walls of the mycobiont (Huneck and Yoshimura, 1996) are considered as a potential resource since these compounds function as chemical defense against biotic and abiotic stresses and they exhibit antibacterial (Lawrey, 1986), anti tubercular (Marshak and Kuschner, 1950; Hartwell, 1971), anti-cancer (Williams *et al*., 1998), anti-HIV (Huneck and Yoshimura, 1996) analgesic and antipyretic (Müller, 2001) properties. Lichen secondary compounds are Phenolic compounds, Dibenzofuranes and Usnic acids, Depsidones, Depsones, Lactones, Quinines and Pulvinic acid derivatives (Boustie and Grube, 2005). These compounds were derived from diverse biosynthetic pathways and mostly unique to lichens.

In India, species of *Parmelia* are extensively used in traditional medicinal systems and are being extensively collected (Kumar and Upreti, 2001). The present study reports antimicrobial activity exhibited by crude extracts of *P. praesorediosum* against ten human bacterial and fungal (*Candida albicans*) pathogens.

MATERIALS AND METHODS

Lichen Material

The lichen thalli of *P. praesorediosum* was collected from siliceous rocks at sites above 700 m MSL from Kalkotti, Bolampatti II Forest range (76° 33" to 76° 46" E and 11° 2" to 10° 54" N),

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Western Ghats, Tamil Nadu during June, 2004 (Balaji, 2005) and part of the thalli has been preserved as voucher specimens (India: Tamil Nadu, Coimbatore district, Kalkotti, Bolampatti II Forest range, on rock, alt. 750 m (MSSRF/Herb/993/04) G.N. Hariharan and P. Balaji) at the Lichen Ecology and Bioprospecting Laboratory, M. S. Swaminathan Research Foundation, Chennai.

Extraction of Lichen Material

The dried lichen thalli were powdered using a mixer grinder. Fifty grams of this powder was extracted with 250 mL of hexane, dichloromethane, ethyl acetate, acetone and methanol solvents, respectively. All extractions were carried out at specific boiling temperature of solvents using soxhlet apparatus for 48 h for complete extraction of secondary compounds (Balaji *et al*., 2006). The final filtrate-the crude extract of the respective solvents were concentrated using a Büchi Rotavapor (R-225).

Screening of Antimicrobial Activity

A total of ten bacterial (Gram + and -) (*Bacillus cereus*, *Corynebacterium diptheriae*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexnerii*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Vibrio cholerae*) and one fungal *Candida albicans*, cultures were used in this investigation. All the cultures were obtained from Institute of Basic Medical Sciences (IBMS), University of Madras, Chennai. Cultures were grown on nutrient broth (Himedia, Mumbai) at 37°C for 12-14 h and were maintained on nutrient agar slants (Himedia, Mumbai) at 4°C. Antimicrobial assays on selected pathogens were carried out using disc diffusion method (Bauer *et al*., 1966). Suspension of microbial cultures (0.1 mL) was inoculated on the entire surface of the culture media in a petridish using a sterilized glass spreader. The sterile discs (Himedia, SD067) were impregnated with varying concentrations i.e., 0.5, 2 and 5% of the sterile test material and placed onto nutrient agar surface spread with 0.1ml of culture (ca. 3.0×10^8 cells mL for bacteria and 2.0×10^5 spore mL^{-1} for fungal strain). The plates were incubated at 37°C for 12-14 h. The experiments were carried out in triplicate. The results (mean value n = 3) were recorded by measuring the zone of growth inhibition around the discs (Table 1). The statistical analysis was carried out using Student's t-test. The sterile discs soaked with respective solvents served as negative control, while standard antibiotic discs, Tetracycline-30 µg/disc, Chloramphenicol-50 µg/disc, Erythromycin-15 µg/disc, Streptomycin- 10 µg/disc and Kanamycin 30 µg disc (Himedia, Mumbai) were used as positive control.

RESULTS

Disc diffusion assays of crude extracts of *P. praesorediosum* indicated that dichloromethane (DCM), ethyl acetate, acetone and methanol showed antimicrobial activity against various pathogens tested, while crude hexane extract exhibited no activity (Table 1). Among the crude extracts, ethyl acetate showed activity against maximum number (6) of pathogens (*Bacillus cereus*, *C. diptheriae*, *Staphylococcus aureus*, *Shigella flexnerii*, *Vibrio cholerae* and *Candida albicans*) followed by DCM extract against 4 pathogens (*Bacillus cereus*, *Corynebacterium diptheriae*, *Staphylococcus aureus* and *Candida albicans*), Acetone extract against 3 pathogens (*Staphylococcus aureus*, *Shigella flexnerii* and *Candida albicans*) and Methanol extract against 3 pathogens (*Proteus mirabilis*, *Salmonella typhi* and *Shigella flexnerii*). The antibacterial assays revealed that both DCM and ethyl acetate extracts inhibited the growth of *Bacillus cereus*, *Corynebacterium diptheriae*, *Shigella flexnerii*, *Staphylococcus aureus*, *Vibrio cholerae* and *Candida albicans* in a concentration dependent manner. The inhibition was detected at 0.5-5% for both DCM and ethyl acetate extracts. The DCM extract of *P. praesorediosum* had a larger inhibition zone (14.1±0.2 mm) when compared to ethyl acetate extract (5.7±0.3 mm) against *C. albicans* at 5% concentration. However, the growth of microbial pathogens such as *Proteus vulgaris*, *P. mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and

Table 1: *In vitro* antimicrobial activity of the extractives from *Parmotrema praesorediosum*

		Diameter of zone inhibition (mm) #											
		Ethyl acetate extract			Dichloromethane extract			Acetone extract			Methanol extract		
Organism	+ve control	0.5%	2%	5%	0.5%	2%	5%	0.5%	2%	5%	0.5%	2%	5%
1	15(K)	4.1±0.1	4.9±0.1	5.5±0.1	6.1±0.1	7.1±0.1	6.3±0.6	-	-	-	-	-	-
2	16(S)	5.3±0.1	6.6±0.4	4.0±0.2	6.6±0.1	7.3±0.1	6.9±0.05	-	-	-	-	-	-
3	14(S)*	5.1±0.3	5.6±0.4	5.4±0.1	5.4±0.2	6.3±0.6	6.1±0.3	Tr	4.3±0.2	4.3±0.2	-	-	-
4	18(T)	-	-	-	-	-	-	-	Tr	Tr	-	-	-
5	16(E)*	-	-	-	-	-	-	-	-	-	Tr	4.3±0.4	4.4±0.2
6	18(E)	-	-	-	-	-	-	-	Tr	-	-	-	-
7	11(K)*	-	-	-	-	-	-	Tr	-	Tr	-	-	-
8	10(C)*	-	-	-	-	-	-	Tr	-	-	Tr	4.0±0.1	4.2±0.2
9	21(T)*	3.9±0.1	3.9±0.2	4.0±0.1	-	-	-	4.1±0.1	3.9±0.1	4.2±0.1	4.1±0.05	3.9±0.1	4.0±0.1
10	16(K)	4.2±0.1	5.1±0.1	6.8±0.2	-	-	-	-	-	-	-	-	-
11	15(C)	3.5±0.3	4.9±0.1	5.7±0.3	8±0.1	11.1±0.2	14.1±0.2	Tr	3.8±0.3	3.5±0.2	-	-	-

#Mean of triplicate, Zones represents diameter in mm, + ve Controls: T*-Tetracycline-30 µg/disc, C*-Chloramphenicol-50 µg/disc, E*-Erythromycin-15 µg/disc, S*- Streptomycin-10 µg/disc and K* - Kanamycin 30 µg/disc, Tr- Activity in trace; (-) no inhibition, Organisms coded as 1- *Bacillus cereus*, 2 - *Corynebacterium diptheriae*, 3 - *Staphylococcus aureus*, 4 - *Streptococcus pyogenes*, 5 - *Proteus mirabilis*, 6- *Proteus vulgaris*, 7 - *Pseudomonas aeruginosa*, 8 - *Salmonella typhi*, 9 - *Shigella flexnerii*, 10 - *Vibrio cholerae* and 11 - *Candida albicans*

Streptococcus pyogenes were not inhibited by both DCM and ethyl acetate extracts. There was a trace antibacterial activity of crude acetone extract against *Streptococcus pyogenes*, *Proteus vulgaris* and *Pseudomonas aeruginosa* and methanol extracts against few pathogens only. The methanol extract of *P. praesorediosum* was able to inhibit the growth of both *Proteus mirabilis* and *Salmonella typhi*. However except methanol extracts none of the extracts (DCM, ethyl acetate and acetone) were not able to inhibit the growth of *P. mirabilis* and *S. typhi*. The comparison of different concentrations of DCM extract against pathogens growth inhibition showed greater zone of inhibition 8, 11 and 14 mm in 0.5, 2 and 5% concentration respectively for *C. albicans* (Table 1). The inhibition zone (14.1±0.2 mm) for DCM extract of *P. praesorediosum* against the growth of *C. albicans* at 5% concentration was closer to the inhibition zone of commercially available antibiotic drug Chloramphenicol (15 mm) (50 µg/disc), where as the antibacterial properties of DCM extract of *P. praesorediosum* was not as affective as the commercial antibiotics like Tetracycline, Chloramphenicol, Erythromycin, Streptomycin and Kanamycin. Among the antimicrobial activities of various extracts of *P. praesorediosum*, the DCM extract showed both antibacterial and antifungal activity against *C. albicans* at various concentrations. The inhibitory effect of lichen extracts is obviously due to the presence of lichen secondary compounds.

DISCUSSION

The search for novel bioactive natural compounds to improve pharmaceutical, cosmetic and agriculture applications is an ancient one and currently it is regaining its importance and lichen compounds are not an exception. In the past, many lichen compounds including usnic acid were very much in use as a remedy for bacterial infections and it was the main ingredient of the antibiotic cream Usno (Richardson, 1988). Currently the interest on the lichen secondary compounds is again increasing because of previously reliable drug becoming ineffective and since lichens are the repositories of pharmacologically relevant unique polyketide compounds (Huneck, 1999; Müller, 2001). In the natural product research, drug-prospecting odds have been much better around 1% when polyketides were screened compared to other compounds. The polyketides are small, cyclized molecules produced by sharing biosynthetic pathways that produce a common ketone structure and between 5000 and 10,000 are known and about 1% of them possess bioactivity and lichens possess a rich array of these compounds (Miao *et al*., 2001; Balunas and Kinghorn, 2005).

The present bioassay with crude extracts of *P. praesorediosum* (Table 1) against Gram-positive and Gram-negative bacteria and a fungus are comparable to Dülger *et al*. (1998) where in the crude extracts of ethyl acetate, acetone, chloroform and ethanol of *Cetraria islandica* showed activity against gram-positive bacteria. The various solvent extracts of *Usnea florida* loaded on sterile paper discs showed antimicrobial activity against *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Bacillus cereus* var. *mycoides*, *Bacillus sphaericus*, *Bacillus megaterium* and *Mycobacterium smegmatis* (Dülger *et al*., 1997). Similarly the ethanol extract of *Parmelia kamstchandalis* tested using disc diffusion technique showed antibacterial activity (Mazid *et al*., 1999). The acetone extract of *Ramalina farinacea* showed activity against *Bacillus subtilis*, *L. monocytogenes*, *Proteus vulgaris*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Candida albicans* and *C. glabrata* (Tay *et al*., 2004). Ylmaz *et al*. (2004) reported that the chloroform, diethyl ether, acetone and petroleum extracts of *C. foliacea* showed activity against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Proteus vulgaris*, *Listeria monocytogenes*, *Aeromonas hydrophila*, *Candida albicans* and *C. glabrata*. Behera *et al*. (2005) reported that the acetone, methanol and light Petroleum extracts of *Usnea ghattensis* showed activity against *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus subtilis* and *Staphylococcus aureus*. Ray *et al*. (2003) reported that ethanol extracts of *Usnea articulate*, *Ramalina jamesii* and *Parmotrema tinctorum* showed activity against gram-positive and gram-negative bacteria. Ozturk and Guvenc (1995) reported that the chloroform extracts of *Pseudevernia furfuracea* (L.) Zopf. var *furfuracea* and *Ramalina farinacea* (Esimone and Adikwu, 1999) showed activity against *Bacillus subtilis* and *Staphylococcus aureus*. We found that 0.5% concentration of ethyl acetate; acetone and methanol crude extracts were adequate to inhibit the growth of *Shigella flexnerii* while 200 μg/disc of ethyl haematommate was adequate to inhibit the growth of *S. flexnerii*. The ethanolic extract of *Parmelia kamstchandalis* was active against *Salmonella typhi* at 200 μg/disc (Mazid *et al*., 1999) while methanol extract of *P. praesorediosum* was active at 5% concentration. The Dichloromethane extract found to be broad spectrum with higher zone of inhibition compared to ethyl acetate extracts. It is generally known that, the anti-bacterial action of metabolites isolated from lichenized fungi involves interference within the ATP machinery of the cell wall. Lichen derived scabrosin esters have been reported for their ATP synthase inhibition activity and direct influence on the production of ATP within the mitochondrial apparatus. When the mitochondrial ATP synthase is inhibited then the mitochondrial membrane becomes hyperpolarized and finally apoptotic cell death occurs (Gardiner *et al*., 2005). The above studies including ours on *P. praesorediosum* indicate the antimicrobial potentials of these extracts and that lichens are the potential source of novel bioactive molecules with high drug prospecting odds.

Natural products provide a starting point for new synthetic compounds with diverse structures and often with multiple stereocentres that can be challenged synthetically. Hence the lead molecule exhibiting antimicrobial activity has to be further characterized and further assays involving its derivative molecules to target sites through high throughput screening and drug discovery have to be carried out to increase its drug prospecting odds (Balunas and Kinghorn, 2005).

The antifungal compounds present in *P. praesorediosum* extracted with DCM is a non-polar compound. Hence this extract is currently subjected to further fractionation to identify the potential antimicrobial compounds. Therefore the leads that were described in this paper will provide the basis for future studies for the discovery of potential compounds from *P. praesorediosum*.

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REFERENCES

Balaji, P., 2005. Assessing the lichen diversity and its distribution pattern for its ecological and economic potentials of lichens of Bolampatti II range forest (Western ghats), Siruvani hill, India, Ph.D Thesis. University of Madras. Chennai, pp: 184.

Balaji, P., P. Bharath, R.S. Satyan and G.N. Hariharan, 2006. *In vitro* antimicrobial activity of *Roccella montagnei* thallus extracts. J. Trop. Med. Plants, (In Press).

Balunas, M.J. and A.D. Kinghorn, 2005. Drug discovery from medicinal plants. Life Sci., 78: 431-441.

Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Truck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Behera, B.C., N. Verma, A. Sonone and U. Makhija, 2005. Antioxidant and antibacterial activities of lichen *Usnea ghattensis in vitro*. Biotechnol. Lett., 27: 991-995.

Boustie, J.L. and M. Grube, 2005. Lichens-a promising source of bioactive secondary metabolites. Plant Genet. Res., 3: 273-287.

Dülger, B., F. Gücin, A. Kara and A. Aslan, 1997. Antimicrobial activity of the lichen *Usnea florida* (L.) Wigg. Turk. J. Biol., 21: 103-108.

Dülger, B., F. Gücin and A. Aslan, 1998. Antimicrobial activity of the lichen *Cetraria islandica* (L.) Ach. Turk. J. Biol., 22: 111-118.

Esimone, C.O. and M.U. Adikwu, 1999. Antimicrobial activity and cytotoxicity of *Ramalina farinacea*. Fitoterapia, 70: 428-431.

Gardiner, D.M., P. Waring and B.J. Howlett, 2005. The epipolythiodioxopiperazine (ETP) class of fungal toxins: Distribution, mode of action, functions and biosynthesis. Microbiology, 151: 1021-1032.

Hartwell, J.L., 1971. Plants used against Cancer. A survey. Lloydia, 32: 204-255.

Huneck, S. and I. Yoshimura, 1996. Identification of Lichen Substances. Springer-Verlag, Berlin, Heidelberg, pp: 493.

Huneck, S., 1999. The significance of lichens and their metabolites. Die Naturwissenschaften, 86: 559-570.

Kumar, K. and D.K. Upreti, 2001. *Parmelia* sp. (lichens) in ancient medicinal plant lore of India. Econ. Bot., 55: 458-459.

Lawrey, J.D., 1986. Biological role of lichen substances. The Bryologist, 89: 111-122.

Marshak, A. and M. Kuschner, 1950. The action of streptomycin and usnic acid on the development of tuberculosis in guinea pigs. Pub. Health Rep., 65: 131-144.

Mazid, M.A., C.M. Hasan and M.A. Rashi, 1999. Antibacterial activity of *Parmelia kamstchandalis*. Fitoterapia, 70: 615-617.

Miao, V., M.F. Coëffet-LeGal, D. Brown, S. Sinnemann, G. Donaldson and J. Davies, 2001. Genetic approaches to harvesting lichen products. Trends Biotechnol., 19: 349-355.

Müller, K., 2001. Pharmaceutically relevant metabolites from lichens. Applied Microbiol. Biot., 56: 9-16.

Ozturk, S. and S. Guvenc, 1995. Comparison of antimicrobial effects of lichen samples of *Pseudevernia furfuracea* (L.) Zopf. var *furfuracea* collected from different regions. Turk. J. Bot., 19: 145-148.

Ray, S., A. Sinhababu and N.C. Mandal, 2003. Antibacterial activity of three lichen specimen viz., *Usnea articulata*, *Ramalina jamesii* and *Parmelia tinctorum* from the Eastern Himalaya. J. Hill Res., 16: 66-69.

Richardson, D.H.S., 1988. Medicinal and Other Economic Aspects of Lichens. In: CRC Handbook of Lichenology, Vol, III. Galun, M. (Ed.), CRC Press, Inc., Boca Raton, pp: 93-108.

Tay, T., A.O. Turk, M. Ylmaz, H. Turk and M. Kvanc, 2004. Evaluation of the antimicrobial activity of the acetone extract of the lichen *Ramalina farinacea* and its (+)-usnic acid, norstictic acid and protocetraric acid constituents. Zeitschrift fur Naturforschung Section-C. Biosciences, 59: 384-388.

Williams, D.E., K. Bombuwala, E. Lobkovsky, E. Dilip de Silva, V. Karunaratne, T.M. Allen, J. Clardy and R.J. Andersen, 1998. Ambewelamides A and B, antineoplastic epidithiapiperazinediones isolated from the lichen *Usnea* sp. Tetrahedron Lett., 39: 9579-9582.

Ylmaz, M., A.O. Turk, T. Tay and M. Kvanc, 2004. The antimicrobial activity of extracts of the lichen *Cladonia foliacea* and its (-)-usnic acid, atranorin and fumarprotocetraric acid constituents. Zeitschrift fur Naturforschung Section-C. Biosciences, 59: 249-254.