In vitro antimicrobial activity of *Roccella montagnei* thallus extracts

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Abstract: The antimicrobial activities of Hexane, Ethyl acetate, Acetone, and Methanol extracts of *Roccella montagnei* Bél. emend. Awas. were assayed for their antimicrobial activity against six human pathogenic microorganisms using standard disc diffusion method. The methanolic extract was found antimicrobial against most of the tested organisms. The activities of the extracts were compared with reference to standard antibiotics for their effectiveness and minimum Inhibitory concentrations (MIC). The drug prospecting odds of methanol extract of *R. montagnei* were better compared to other solvent extracts, as potential antibacterial and antifungal agents screened against human pathogenic microorganisms.

Keywords: Lichens; *Roccella montagnei*; crude extracts; Antibacterial activity; Antifungal activity

INTRODUCTION

The search for novel natural bioactive compounds leading to new drug discovery is increasing as previously reliable standard drugs become less effective against 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) were found to occur as extra cellular crystals on the cell walls of the mycobiont (Huneck and Yoshimura, 1996). Lichens are considered as a potential resource since these compounds function as chemical defense against biotic and abiotic stresses and they are 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). According to their chemical structures, most lichen substances 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 there are other unusual compounds among these organisms. Uncommon features are also detected in residues of common substance classes, in the form of other intramolecular arrangements, or in the binding with other compounds such as sugars (Boustie and Grube, 2005).

The lichen species *Roccella montagnei* Bél. emend. Awas. (Roccellaceae) (Fig-A), is a fruticose growth form, found common as epiphytes along the Coromandel Coast, Tamil Nadu, India and it is abundant in Pichavaram mangrove forests (Awasthi, 1988, Mohan and Hariharan, 1999). This lichen possesses a wide array of secondary compounds such as Roccellic acid, Orcinol, Lecanoric acid, Montagnetol, Methylorsellinate, Meso-erythritol, Erythritol, β carotene, and β sitosterol (Neelakantan and Seshadri, 1952; Bambuwala, 2000). The present study reports the extraction of secondary compounds of *R. montagnei* using organic solvent gradients and the antimicrobial activity exhibited by different concentrations of crude extracts (including establishing the Minimum Inhibitory Concentration) against human bacterial and fungal (*Candida albicans*) pathogens.

MATERIALS AND METHODS

Lichen material: The thalli of *R. montagnei* was collected in the month of June from the *Rhizophora apiculata* trees in Pichavaram mangroves (N 11° 25'52. 2" E 79° 47'35"), Tamil Nadu, India, and identified based on standard literature (Awasthi, 1988; Swinscow and Krog, 1988). Voucher specimens (MSSRF 207/02, 208/02) were deposited at the Lichen Ecology and Bioprospecting Laboratory, M. S. Swaminathan Research Foundation, Chennai. The collected material was shade dried for 2 days at room temperature. The dried material was powdered and used for the organic extraction process.

Extraction of Lichen material: The powdered lichen (30gm) was wrapped in a 8 x 6 cm cylindrical pouch (Whatmann filter paper grade 1) and kept inside the extractor arm of the Soxhlet apparatus (Balaji, 2005). A series of solvents (Hexane, Ethyl acetate, Acetone and Methanol) were used for extraction based on their polarity and each extraction was carried out at the specific boiling temperature for a period of 48 hrs for the complete extraction of secondary compounds. The final filtrate of each of the extraction obtained was concentrated using a Buchi Rotary Evaporator (Switzerland).

Culture media: Nutrient Agar (NA) and Nutrient Broth (NB) medium were used to culture pathogens and for bacterial and fungal susceptibility test (Balaji, 2005).

Microorganism source: A total of five bacterial cultures (*Staphylococcus aureus, Salmonella typhi, Salmonella para typhi - B, Proteus vulgaris, Klebsiella pneumoniae*) and a fungal culture of *Candida albicans* were used in this investigation. All the cultures were obtained from Institute of Basic Medical Sciences (IBMS), University of Madras, Chennai. The cultures were maintained at 4 °C and subcultured frequently both in semisolid and solid nutrient agar slants.

Inoculum preparation: The starter cultures, in tubes with 2ml of nutrient broth were inoculated with the organisms for bioassay, and incubated for 24 hr time period at 37 ± 1°C. The bacteria grew in liquid broth making it turbid. The turbidity was adjusted to that of standard level by adding more sterile fluid.

Determination of antimicrobial activity: Antimicrobial activity was tested using disc-diffusion assay (Bauer et al., 1966). The nutrient agar medium was transferred in to one-fourth volume of a petri plate. Inoculation of cultures (100µl) to this medium was carried out uniformly using a glass spreader. Different concentrations of crude extracts of Hexane, Ethyl acetate, Acetone, and Methanol (i.e., 5%, 10%, 15% and 20%) were prepared as individual stocks in sterile vials. Whatman filter paper discs (Himedia, SD067) were soaked for 2 hrs and air-dried thoroughly for 1 hr before the assay. The plates were incubated for 24 hrs at 37°C. The inhibition of bacterial and fungal growth was determined by measuring the diameter of the clear zone around each disc. The sterile discs were soaked with respective solvents, that served as control. Standard antibiotic discs Tetracycline-30µg/disc; Chloramphenicol-50µg/disc; Erythromycin-15µg/disc and Streptomycin-10µg/disc were used as reference or positive control. Average of triple impendent readings for each microorganism was recorded.

RESULTS AND DISCUSSION

The results of antimicrobial activity of extracts are given in Table 1. The inhibitory effects of solvents alone on microorganisms were negligible and hence were not included in the Table 1. Among the four different extracts, Methanol extract exhibited growth inhibition on six organisms (Figs-B-F) whereas the Hexane and Ethyl acetate extracts exhibited inhibition on *Klebsiella pneumoniae, Salmonella typhi* and *Salmonella para typhi* – B, but no inhibition against *Candida albicans* and *Proteus vulgaris*. There was no inhibitory activ for Acetone extract. The reactions of Bacteria agai extract were of different degree (Table 1).

The Methanolic extract inhibited the growth of all organisms tested and especially exhibited 10-15 n zone of inhibition against *K. pneumoniae, P. vulgar S. typhi, S. para typhi* - B and *C. albicans*. The vari concentrations (5% – 20%) of Methanolic extr exhibited lesser inhibition compared to the antibio standard Tetracycline (21mm), which showed high inhibitory activity (15-18mm at 10,15 and 20 concentrations) compared to the Chloramphenicol 50µg / disc (10mm) against Gram - negative bacter *S. typhi*.

The antimicrobial potential of Methanolic extracts of *R montagnei* (inhibition > than 15 mm) are in conformit and comparable with similar assays using Methanoli extracts (Soxhlet extraction) of *Cladonia unciali* (Concentration 4.2mg/ml), *Peltigera canina* (6.6mg ml) which showed significant inhibition (> than 13mm against *S. aureus* and *C. albicans* (Ingólfsóttir et al. 1985), and also similar to methanolic extract of *Usnea ghattensis* against *S. aureus, Bacillus licheniformis, B subtilis* and *B. megaterium* (Behera et al., 2005).

The antimicrobial potential of Ethyl acetate and Acetone extracts of *R. montagnei* are in conformity and comparable with similar assays by Dülger et al., (1998), Tay et al., (2004) and Ylmaz et al., (2004) using ethyl acetate, acetone, and ethanol extracts of *Cetraria islandica, Ramalina farinacea* and *Cladonia foliacea* showing distinguishable activity against *P. vulgaris, S. aureus* and *C. albicans*.

The thallus of *R. montagnei* is known to contain class of compounds Depsides and Terpenes, (Rundel, 1978; Huneck and Yoshimura, 1996) while Acetone and Methanol extracts contain Terpenoids, Depsides, polyols, aromatic, aliphatic and cycloaliphatic compounds (Bombuwala, 2000).

CONCLUSION

The acetone extract (standard lichenological procedure) showed no inhibitory activity against the pathogens tested. However the methanolic extracts (Soxhlet) showed broad spectrum and significant antimicrobial (bacterial and fungal) potential. Therefore, this study proved 1.the antimicrobial potential of methanolic extracts of *R. montagnei* and 2. in the discovery of the novel potential biomolecules from lichens, application of different solvents in combination with extraction procedures. Further investigation into the fractionation and purification of methanolic extract may result in the

isolation of a viable alternate source to the presently available antibiotics.

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Table 1. Antimicrobial activity of crude extracts of *R. montagnei*

Organism	(Value) + ve Control	Diameter of zone inhibition (mm) #															
		Hexane Extract (%)				Ethyl acetate extract (%)				Acetone extract (%)				Methanol extract (%)			
		5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	2(
A	21(T)	-	-	-	-	-	-	-	-	-	-	-	-	9	9	10	12
B	15 (T)	6	7	7	8	5	5	7	8	-	-	-	-	5	9	9	10
C	18 (E)	-	-	-	-	-	-	-	-	-	-	-	-	13	16	14	16
D	10 (C)	-	-	-	-	4	4	4	5	-	-	-	-	10	18	18	15
E	16 (T)	-	-	-	-	-	-	-	-	-	-	-	-	12	13	14	15
F	13 (S)	-	-	-	-	5	5	6	7	-	-	-	-	9	10	9	10

#Results were the mean of triplicates. Zones represents diameter in mm. + ve Controls: T-Tetracycline-30µg/disc, C-Chloramphenicol-50µg/disc, E-Erythromycin-15µg/disc and S- Streptomycin-10µg/disc. Organisms coded as A *Candida albicans*; B- *Klebsiella pneumoniae*; C- *Proteus vulgaris*; D- *Salmonella typhi*; E- *Salmonella para typh* –B; F- *Staphylococcus aureus*; (-) indicates no activity.

Figure A. Habit - *Rocella montagnei* growing on *Rhizophora apiculata* tree

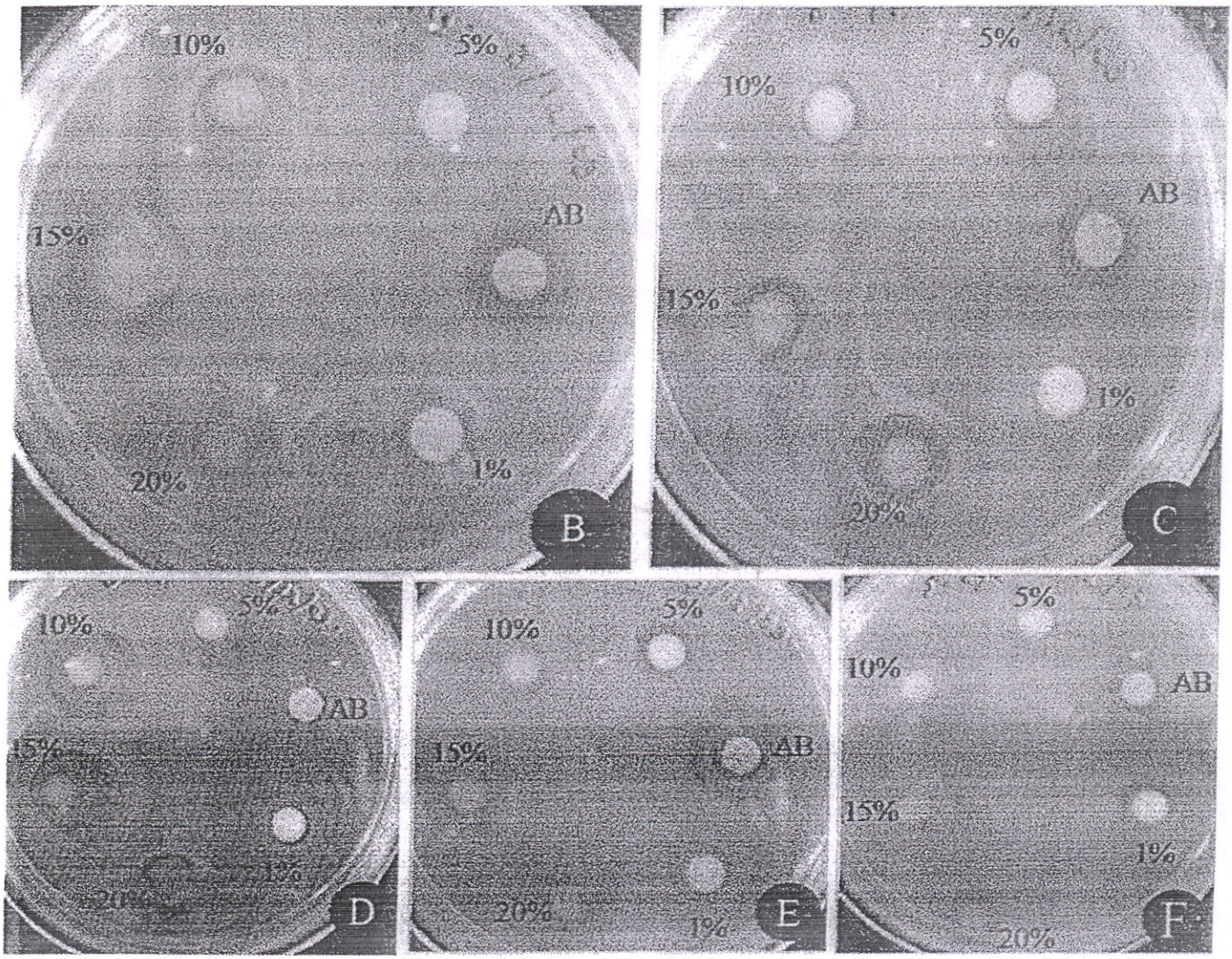

Figure B-F. **Anti-microbial activity of crude Methanolic extracts of *R. montagnei* against various pathogens - B.** *Klebsiella penumoniae* C. *Proteus vulgaris* **D.** *Salmonella typhi* **E.** *Salmonella para typhi* - B and F. *Staphylococcus aureus.* AB - Antibiotic disc