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Research Article

Antibacterial Activity of Three *Parmotrema* Species against Drug Resistant Uropathogens

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ABSTRACT

Urinary tract infections are the common infections in community and hospital settings and infect millions of people worldwide every year. The present study was undertaken to determine antibacterial efficacy of extracts of three *Parmotrema* species *viz*., *P. tinctorum*, *P. grayanum* and *P. praesorediosum*, macrolichens from Western Ghats of Karnataka, India. The powdered lichen materials were extracted using methanol in soxhlet assembly. Antibacterial activity of lichen extracts was determined against five clinical isolates *viz*., *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli* of urinary tract infection by agar well diffusion assay. The lichen extracts showed dose dependent inhibition of test bacteria. Inhibitory efficacy was highest against Gram positive bacteria than Gram negative bacteria. Overall, high and least inhibitory activity was observed against *E. faecalis* and *K. pneumoniae* respectively. The MIC of extracts was found to be least for Gram positive bacteria (0.3 to 0.6mg/ml) than Gram negative bacteria (0.6 to 2.5mg/ml). Thin layer chromatogram revealed the presence of Lecanoric acid, Atranorin, Orsellinic acid, Protolichesterinic acid, Chloroatranorin, Protopraesorediosic acid and Praesorediosic acid in lichen materials. The antibacterial potential of lichen extracts could be ascribed to the presence of secondary metabolites. The *Parmotrema* species appears to be promising source of agents with inhibitory activity against antibiotic resistant urinary tract pathogens.

INTRODUCTION

Urinary tract infection (UTI) is one of the common diseases in both community and hospital settings. An estimate of 150 million UTIs is reported each year worldwide. The severity of these infections depends on virulence of pathogens and the susceptibility of the host. The UTIs occur in both sexes but are more common in females than in males. The severity varies with gender, age and presence of associated genitourinary abnormalities. UTIs have also become the most common infections in ICU of hospital and are known to infect 2-3% of admitted patients. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus* species, *Enterococcus faecalis* and *Klebsiella pneumoniae* are among the important bacteria capable of causing urinary tract infections.

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The antibiotic susceptibility pattern of urinary pathogens in both community and hospitals has been continuously changing these years. Majority of uropathogens are gaining resistance against most commonly used antibiotic and are making the treatment more difficult. The antimicrobial resistance has become a major global problem. Hence, there is need for development of novel antimicrobials from natural sources [1-4].

Lichens are ecologically obligate, stable and self supporting composite organisms composed of mycobiont (fungus) and photobiont (alga or cyanobacterium). The lichens represent physiologically and taxonomically a diverse group of organisms. These lichens are known to be the earliest colonizers of terrestrial habitats. Lichens are distributed worldwide from arctic to tropical regions and from the plains to the highest mountains. Lichens have been widely used in food and in folk medicine in several countries over a considerable period of time.

These lichens are used to treat dyspepsia, bleeding piles, diabetes, bronchitis, pulmonary tuberculosis, spermatorrhoea and other diseases in many parts of the world. The lichens are also popular as they are bioindicators of air pollution. Lichens, in particular mycobiont produce a large number of secondary metabolites (lichen substances) which seldom occur in other organisms. The lichen extracts and their metabolites are known to exhibit various bioactivities such as antimicrobial, antiviral, antiprotozoal, enzyme inhibitory, insecticidal, antitermite, cytotoxic, antioxidant, antiherbivore, wound healing, analgesic and anti-inflammatory [5-14].

The genus *Parmotrema* (Parmeliaceae) is characterized by large foliose thalli with broad rotund lobe apices, the absence of pseudocyphellae and broad erhizinate marginal zone on the lower surface, marginal cilia, simple rhizines and thick walled ellipsoid ascospores. The species of *Parmotrema* are best developed in tropical regions. Over 220 species are known, out of which 46 species are distributed in India [15-17]. The *Parmotrema* species are known to possess antimicrobial activity [18-22]. The aim of the present study was to determine antibacterial activity of three *Parmotrema* species *viz*., *P. tinctorum*, *P. grayanum* and *P. praesorediosum* from Maragalale and Guliguli Shankara, Western Ghats of Karnataka, India against drug resistant urinary tract pathogens.

MATERIALS AND METHODS

Collection of lichens

The *Parmotrema* species of the present study were collected in the month of September 2013. *P. tinctorum* and *P. praesorediosum* were collected at a place called Maragalale, Thirthahalli taluk of Shivamogga district; Karnataka and *P. grayanum* was collected at Guliguli Shankara, Hosanagara taluk, Shivamogga district, Karnataka.

Identification of lichens

Identification of these lichens was done by morphological, anatomical and chemical tests. The color tests were performed on the cortex and medulla by using 10% potassium hydroxide (K), Steiner's stable paraphenylenediamine solution (P) and calcium hypochlorite solution (C). Thin layer chromatography (TLC) for lichen extracts was done using solvent system A (Benzene:1, 4-Dioxane:Acetic acid in the ratio 90:25:4). The spots were marked, Rf values were calculated and the compounds were identified [23-25].

Extraction

The powdered lichens (25g) were subjected to Soxhlet extraction and extracted using methanol (HiMedia, Mumbai). The lichen extracts were filtered through sterile Whatman No. 1 filter paper and concentrated in vacuum under reduced pressure [12].

Test bacteria

A panel of five urinary tract bacteria *viz*., *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* were used to assess their susceptibility to lichen extracts. These bacterial isolates were multidrug resistant [4].

Antibacterial activity of lichen extracts

The efficacy of lichen extracts to inhibit clinical isolates was tested by Agar well diffusion assay. The bacterial isolates were inoculated into sterile Nutrient broth (HiMedia, Mumbai) tubes and incubated for 24 h at 37°C. The broth cultures were then aseptically swabbed on sterile Nutrient agar (HiMedia, Mumbai) plates using sterile cotton swabs. Wells of 6mm diameter were punched in the inoculated plates using sterile cork borer. 100µl of lichen extracts (10 and 20mg/ml of 25% dimethyl sulfoxide [DMSO]), standard antibiotic (Chloramphenicol, 1mg/ml) and DMSO (25%, in sterile water) were transferred into labeled wells. The plates were incubated at 37°C for 24 h in upright position and the zone of inhibition was measured [4].

Determination of minimal inhibitory concentration (MIC)

The MIC of lichen extracts was determined by the broth tube dilutionmethod. Here, a series of dilutions (concentrations ranging from 20 to 0.0 mg/ml) of lichen extracts was used against each clinical isolate. Two-fold dilutions of extracts were prepared in Nutrient broth in test tubes. Test tubes containing different concentrations of lichen extracts were inoculated with test bacteria and incubated at 37°C for 24 hours. The MIC was determined by observing the visible growth of the isolates after incubation. The dilution tubes in which any visible growth was absent was considered as the MIC for the tested isolate at the given extract concentration [26].

Table 1: Habitat and thallus morphology of *Parmotrema* species

Lichen	Habitat	Thallus
P. tinctorum	Corticolous	Large loosely adnate, membranous, broad, lobes irregular, rotund; margins crenate, eciliate; upper surface grey, smooth, isidiate; lower surface minutely wrinkled, rough, black, erhizinate; rhizines sparse, coarse at centre
P. grayanum	Saxicolous	Adnate; lobes rotund; margins ascending, crenate, ciliate; cilia dense and thick; upper surface ashy grey; lower surface wrinkled, black, erhizinate, rhizinate at the centre; rhizines sparce, black and simple
P. praesorediosum	Saxicolous	Thallus coriaceous, adnate to substratum; lobes rotund; margins crenate; upper surface grey, smooth; lower surface minutely wrinkled, black; rhizines sparse, simple

Table 2: Color tests and TLC of *Parmotrema* species

Lichen	Color test	TLC
P. tinctorum	Cortex K+ yellow; Medulla K-, C +red, KC +red, Pd -	Lecanoric acid, Atranorin, Orsellinic acid
P. grayanum	Cortex K+ yellow; Medulla K-, C -, KC -, Pd -	Atranorin, Protolichesterinic acid
P. praesorediosum	Cortex K+ yellow; Medulla K-, C -, KC -, Pd -	Atranorin, Chloroatranorin, Protopraesorediosic acid, Praesorediosic acid

Table 3: Inhibitory activity of *Parmotrema* species against urinary tract isolates

Treatment	Conc. (mg/ml)	Zone of inhibition in cm (Mean±SD)				
		E. faecalis	***S. aureus***	***K. pneumoniae***	***P. aeruginosa***	***E. coli***
P. tinctorum	10.0	2.4±0.2	1.6±0.0	0.0±0.0	1.5±0.1	0.0±0.0
	20.0	2.8±0.2	1.8±0.1	1.0±0.0	1.7±0.1	0.8±0.0
P. grayanum	10.0	1.8±0.1	1.5±0.1	0.8±0.0	1.4±0.1	1.0±0.0
	20.0	2.0±0.1	1.9±0.1	1.2±0.0	1.5±0.1	1.3±0.0
P. praesorediosum	10.0	1.8±0.1	2.4±0.1	0.0±0.0	1.2±0.0	1.4±0.1
	20.0	2.1±0.1	2.7±0.1	0.8±0.0	1.4±0.1	1.8±0.2
Antibiotic	1.0	2.8±0.2	3.1±0.1	2.3±0.0	2.2±0.1	2.3±0.0

Table 4: MIC of *Parmotrema* species

Test bacteria	MIC of lichen extracts (mg/ml)		
	P. tinctorum	***P. grayanum***	***P. praesorediosum***
E. faecalis	0.3	0.6	0.6
S. aureus	0.6	0.6	0.3
K. pneumoniae	2.5	2.5	2.5
P. aeruginosa	1.2	1.2	1.2
E. coli	2.5	2.5	0.6

RESULTS

The information on the habitat, thallus nature, color tests and secondary metabolites (detected in TLC) of the *Parmotrema* species selected in this study are shown in Table 1 and 2. Table 3 and Figure 1 shows the result of inhibitory potential of extracts of *Parmotrema* species against urinary tract isolates. The lichen extracts displayed concentration dependent inhibition of test bacteria. Inhibitory potential was marked against Gram positive bacteria when compared to Gram negative bacteria.

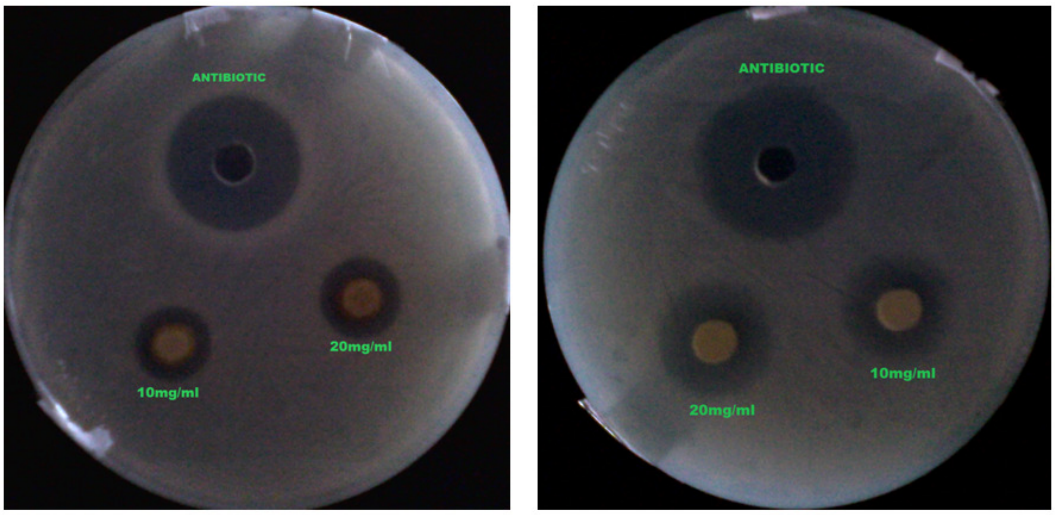

Figure 1: Inhibitory effect of (a) *P. praesorediosum* against *P. aeruginosa* (b) *P. grayanum* against *E. faecalis*

Among Gram positive bacteria, *E. faecalis* was inhibited to higher extent by extract of *P. tinctorum* and *P. praesorediosum* whereas the extract of *P. praesorediosum* caused higher inhibition of *S. aureus*. *P. aeruginosa* and *K. pneumoniae* were inhibited to higher and least extent respectively among Gram negative bacteria. Extract of *P. tinctorum* was least inhibitory to *K. pneumoniae* and *E. coli*. Reference antibiotic caused higher inhibition of clinical isolates when compared to lichen extracts. DMSO did not cause inhibition of clinical isolates. The MIC of lichen extracts was found to be lesser against Gram positive bacteria than Gram negative bacteria. The MIC ranged between 0.3 to 0.6 and 0.6 to 2.5mg/ml for Gram positive and Gram negative bacteria respectively (Table 4).

DISCUSSION

Western Ghats of India constitute one of the biodiversity hotspots in the world and harbors >30% of all plant, fish, herpeto-fauna, birds, and mammal species found in India. The mountain ranges of Western Ghats runs through five states *viz*., Gujarat, Maharashtra, Goa, Karnataka and Kerala. The area harbors numerous species of plants, animals and microbes including a number of globally threatened and endemic species of plants and animals [27]. Studies have been carried out on distribution and bioactivities of macrolichens of various places in Western Ghats of Karnataka [9, 10, 12, 14, 28-32]. Lichen extracts and their metabolites are shown to exhibit inhibitory activity against clinical isolates.Elo *et al*. **[33]** found potent inhibitory activity of (+)-usnic acid and its sodium salt against vancomycin resistant enterococci and methicillin resistant *S. aureus*.Esimone *et al*. [34] showed antibacterial activity of two bioactive fractions from *Ramalina farinacea* against clinical isolates of *S. aureus*. Sharma *et al*. [35] observed inhibitory activity ofmethanol extract of lichens *Parmelia* and *Dermatocarpon* against clinical isolates of *S. aureus*. Kekuda *et al*. [14] showed inhibitory effect of *Usnea pictoides* against *S. aureus* and *S. mutans* isolates from burn and dental caries respectively. In the present study, we evaluated antibacterial activity of three *Parmotrema* species from Western Ghats of Karnataka, India against clinical isolates from urinary tract infection. The lichen extracts exhibited dose dependent inhibition of bacterial isolates. The extracts were highly active against Gram positive bacteria when compared to Gram negative bacteria as revealed by lower MIC values for Gram positive bacteria. Similar results i.e., higher susceptibility of Gram positive bacteria to lichen extracts were observed in earlier studies by Srivastava *et al*. [36] and Srivastava *et al*. [37]. The lesser susceptibility of Gram negative bacteria to extracts could be related to their cell wall structure. In Gram negative bacteria, the presence of outer membrane forms an additional barrier for the entry of substances into the cells **[38, 39]**. It has been shown that species of *Parmotrema* are reported to possess inhibitory activity against clinical isolates. In a study, Chauhan and Abraham **[21]** showed the inhibitory effect of

methanol extract of *Parmotrema* sp. collected from Kodaikanal forest, India against clinical isolates of bacteria. In another study, Javeria *et al*. [22] showed the inhibitory efficacy of solvent extracts of *P. nilgherrense* collected from Nainital, India against drug resistant bacteria.

CONCLUSION

The present study revealed marked inhibitory activity of lichen extracts against drug resistant urinary tract pathogens. The observed inhibitory activity of lichen extracts could be ascribed to the presence of secondary metabolites. These species of *Parmotrema* appears to be promising sources of bioactive agents.

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