

Antibacterial Activity of Three *Parmotrema* Species from Western Ghats of Karnataka against Clinical Isolates of Burn and Dental Caries

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Abstract

The present study was conducted to evaluate the antibacterial efficacy of methanol extract of three species of macrolichen *Parmotrema* (Parmeliaceae) viz., *P.tinctorum* (Nyl.) Hale, *P. grayanum* (Hue.) Hale and *P. praesorediosum* (Nyl.) Hale from Western Ghats of Karnataka against clinical isolates of burn and dental caries. Identification of lichens was done by morphological, anatomical and color tests. The powdered lichen materials were extracted using methanol in Soxhlet apparatus. Inhibitory potential of lichen extracts was evaluated against two isolates of *Staphylococcus aureus* (Sa-01 and Sa-02 from burn subjects) and two isolates of *Streptococcus mutans* (Sm-01 and Sm-02 from dental caries) by agar well diffusion assay. Lichen extracts were found inhibitory against all clinical isolates and the inhibitory activity was dose dependent. Among *S. aureus* and *S. mutans* isolates, Sa-02 and Sm-02 were inhibited to higher extent respectively by lichen extracts. Extract of *P. praesorediosum* inhibited *S. aureus* isolates to higher extent. Extracts of *P. praesorediosum* and *P. tinctorum* inhibited *S. mutans* isolates to higher extent. The lichens of this study were found promising sources for development of agents active against clinical isolates. The observed activity of extracts could be ascribed to the presence of secondary metabolites.

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INTRODUCTION

Lichens represent a symbiotic association between an alga/cyanobacterium (photobiont) and a fungus (mycobiont). These lichens are distributed in almost all types of ecological habitats and are known to occur in different growth forms viz., crustose, foliose and fruticose. Lichens are considered as valuable resources of medicine, food, fodder, perfume, spices and dyes in various countries. Lichens are eaten by people in North America, Europe, Asia and Africa. Lichens are often merely famine foods and are eaten only in times of their needs. Lichens are used in folk medicine in India and other countries. The Ayurveda and Unani system of medicine describe the use of lichens (Vinayaka *et al.*, 2011; Vinayaka and Krishnamurthy, 2012; Kekuda *et al.*, 2012; Kekuda *et al.*, 2013). The central Western Ghats of Karnataka, known locally as 'Sahyadri', represents a long mountain chain along the west coast of India and encompass districts viz., Chikmagalur, Shivamogga, Udipi, Dakshina Kannada, Uttara Kannada, Hassan and Coorg. Ethno-lichenological studies of Karnataka revealed the utilization of lichens for various purposes such as treatment of common infections, flavoring food, healing wounds and others (Vinayaka *et al.*, 2011; Vinayaka and Krishnamurthy, 2012). Macrolichens of Western Ghats of Karnataka are shown to exhibit various bioactivities such as antimicrobial, antioxidant,

insecticidal, anthelmintic, cytotoxic, enzyme inhibitory (Vinayaka *et al.*, 2009; Kumar *et al.*, 2011; Kekuda *et al.*, 2011; Kekuda *et al.*, 2012; Pavithra *et al.*, 2013; Kekuda *et al.*, 2013). The objective of the study was to find out inhibitory potential of three species of Western Ghat macrolichen *Parmotrema* (Parmeliaceae) viz., *P.tinctorum* (Nyl.) Hale, *P. grayanum* (Hue.) Hale and *P. praesorediosum* (Nyl.) Hale against bacterial isolates from burn, dental caries and urinary tract infection.

MATERIALS AND METHODS

Collection of Lichens

P. grayanum (saxicolous) was collected at Guliguli Shankara, Hosanagara taluk, Shivamogga district, Karnataka. *P. tinctorum* (corticolous) and *P.praesorediosum* (saxicolous) were collected at Maragalale, Thirthahalli taluk of Shivamogga district, Karnataka. The lichens were collected during September 2013.

Identification of Lichens

Morphological, anatomical and chemical tests were used to identify lichens. Color tests were done on cortex and medulla by using 10% potassium hydroxide (K), Steiner's stable paraphenylenediamine solution (P) and

calcium hypochlorite solution (C). Thin layer chromatography (TLC) 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 (Awasthi, 2000; Culberson and Kristinsson, 1970; Culberson, 1972).

Extraction

The lichens were powdered in a blender. A known quantity (25g) of each lichen material was extracted using methanol (HiMedia, Mumbai) in a Soxhlet assembly. After extraction, the contents were filtered through sterile Whatman No. 1 filter paper and concentrated in vacuum under reduced pressure (Kekuda *et al.*, 2012).

Test Bacteria

Two isolates of *Staphylococcus aureus* (Sa-01 and Sa-02) isolated from pus of burn patients and two isolates of *Streptococcus mutans* (Sm-01 and Sm-02) isolated from plaque of dental caries subjects were used to screen their susceptibility to lichen extracts.

Preparation of Bacterial Inocula

The isolates of *S. mutans* and *S. aureus* were inoculated into sterile Brain heart infusion broth (HiMedia, Mumbai) and Nutrient broth (HiMedia, Mumbai) tubes respectively. The tubes were incubated at 37°C for 24 hours (Vivek *et al.*, 2013).

Antibacterial Activity of Lichen Extracts

Agar well diffusion assay was performed to investigate antibacterial efficacy of lichen extracts. The brain heart infusion broth cultures of *S. mutans* and nutrient broth cultures of *S. aureus* were swabbed uniformly on sterile Brain heart infusion agar (HiMedia, Mumbai) and Nutrient agar (HiMedia, Mumbai) plates respectively. Using sterile cork borer, wells of 6mm diameter were punched in the plates and 100µl of lichen extracts (10 and 20mg/ml of 25% dimethyl sulfoxide [DMSO; HiMedia, Mumbai]), reference antibiotic (Chloramphenicol, 1mg/ml of sterile water) and DMSO (25%, in sterile water) were transferred into labeled wells. The plates were incubated at 37°C for 24 hours in upright position and zones of inhibition were measured (Vivek *et al.*, 2013).

Statistical Analysis

The experiment was conducted in triplicate. Results are represented as Mean±Standard deviation (SD).

RESULTS

The lichen specimens were identified on the basis of morphological, anatomical and color tests. The lichen substances (secondary metabolites) were detected by TLC. The information about the thallus characteristics, color test results and secondary metabolites in the lichens is given in Table 1.

Table 2 and Figure 1 shows the inhibitory effect of *Parmotrema* extracts against clinical isolates. Lichen extracts were found effective against all tested clinical isolates. The inhibitory potential was found to be concentration dependent. Among *S. aureus* isolates, isolate Sa-02 was inhibited to maximum extent than Sa-01 by lichen extracts. In case of *S. mutans* isolates, isolate Sm-02 was inhibited to higher extent than Sm-01 by lichen extracts. Similar inhibitory effect was observed in case of standard antibiotic also. Extract of *P. praesorediosum* inhibited *S. aureus* isolates to higher extent followed by extract of *P. grayanum* and *P. tinctorum*. Extracts of *P. praesorediosum* and *P. tinctorum* inhibited *S. mutans* isolates to maximum extent when compared to extract of *P. grayanum*. Inhibition caused by antibiotic was higher than that of lichen extracts. There was no inhibition of bacteria in case of DMSO (not shown in table).

DISCUSSION

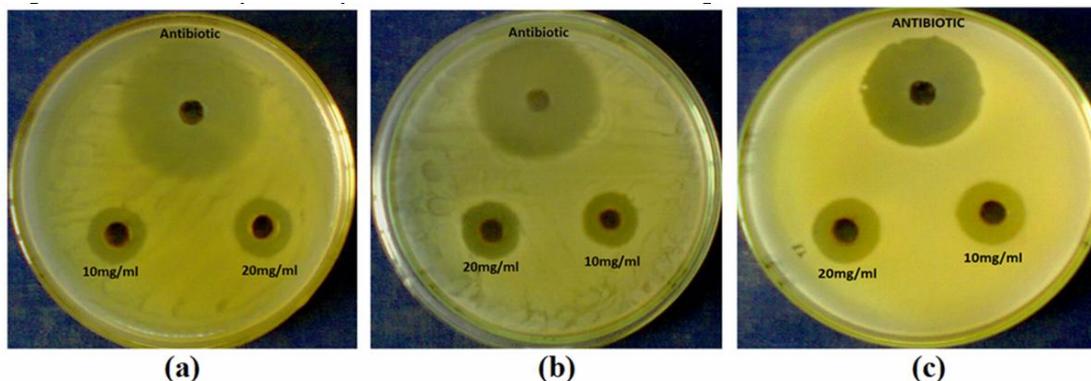
Infectious agents *viz.*, bacteria, fungi, viruses and parasites have threatened mankind throughout history and caused millions of deaths. Discovery of antibiotics during 20th century is one of the significant milestones in the history of preventive chemotherapy. The use of these wonder drugs saved countless lives during past years. However, overuse and abuse of these miracle drugs resulted in the development of resistance in pathogens. *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Mycobacterium tuberculosis*, *Candida albicans* and *Cryptococcus neoformans* are among the important drug resistant pathogens. The development of antimicrobial resistance presents a major threat to public health as it reduces the effectiveness of treatment, resulting in increased morbidity, mortality, and health care expenditure. Moreover, these pathogens have the ability to acquire and transmit resistance (Smith

Table 1: Details of thallus morphology, colour test and TLC of selected lichens

Lichen	Thallus	Colour test	TLC
<i>P. tinctorum</i>	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 the centre	Cortex K ⁺ yellow; Medulla K ⁻ , C ⁺ red, KC ⁺ red, Pd ⁻	Atranorin, Lecanoric acid, Orsellinic acid
<i>P. grayanum</i>	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 sparse, black and simple	Cortex K ⁺ yellow; Medulla K ⁻ , C ⁻ , KC ⁻ , Pd ⁻	Atranorin, Protolichestherinic acid
<i>P. praesorediosum</i>	Thallus coriaceous, adnate to substratum; lobes rotund; margins crenate; upper surface grey, smooth; lower surface minutely wrinkled, black; rhizines sparse, simple	Cortex K ⁺ yellow; Medulla K ⁻ , C ⁻ , KC ⁻ , Pd ⁻	Atranorin, Protopraesorediosic acid, Chloroatranorin, Praesorediosic acid

Table 2: Inhibitory activity of *Parmotrema* species against clinical isolates.

Treatment	Conc. (mg/ml)	Zone of inhibition in cm			
		Sa-01	Sa-02	Sm-01	Sm-02
<i>P. tinctorum</i>	10.0	0.0±0.0	1.6±0.0	1.6±0.0	1.8±0.1
	20.0	0.8±0.0	1.8±0.1	2.0±0.1	2.4±0.2
<i>P. grayanum</i>	10.0	1.5±0.0	1.9±0.0	1.5±0.0	1.5±0.0
	20.0	1.7±0.1	2.2±0.2	1.7±0.1	1.8±0.0
<i>P. praesorediosum</i>	10.0	1.7±0.0	2.2±0.1	1.9±0.2	1.9±0.2
	20.0	1.8±0.0	2.6±0.2	2.1±0.1	2.4±0.2
Antibiotic	1.0	2.5±0.1	3.8±0.2	2.6±0.0	3.2±0.1

**Figure 1:** Inhibitory activity of extracts of *Parmotrema* species.**Figure 1:** Inhibitory activity of extracts of *Parmotrema* species.

and Coast, 2002; Davies and Davies, 2010; Giedraitiene *et al.*, 2011; Kekuda *et al.*, 2012; Kekuda *et al.*, 2013). High cost, possible side effects and development of resistance in pathogens against antibiotics stimulated researchers to investigate antimicrobials from natural sources. Lichens are among the promising sources of chemotherapeutic agents that are active against pathogenic organisms including clinical and drug resistant strains (Kekuda *et al.*, 2012; Chauhan and Abraham, 2013; Javeria *et al.*, 2013; Kekuda *et al.*, 2013).

The genus *Parmotrema* (Parmeliaceae) is characterized by large foliose thalli with broad rotund lobe apices, the absence of pseudocyphellae, broad erhizinate marginal zone on the lower surface, marginal cilia, simple rhizines and thick walled ellipsoid ascospores. The members of this genus are best developed in tropical regions of the world. Over 220 species are known, out of which 46 species are distributed in India (Divakar and Upreti, 2005; Benatti *et al.*, 2013; Jayalal *et al.*, 2013). Studies have shown that *Parmotrema* species possess antibacterial activity. In a study, Balaji and Hariharan (2007) revealed antimicrobial activity of dichloromethane extract of *P. praesorediosum* from Western Ghats of Tamil Nadu. Kumar *et al.* (2010) observed antibacterial activity in methanol extract of *P. pseudotinctorum* from the Western Ghats of Karnataka. Sinha and Biswas (2011) showed antibacterial efficacy of solvent extracts of *P. reticulatum* from Sikkim, India. Verma *et al.* (2011) determined antibacterial activity of solvent extracts of *P. nilgherrensis* and *P. sancti-angelii* collected at different places of Karnataka, India. Chauhan and Abraham (2013) found inhibitory effect in methanol extract of *Parmotrema* sp. collected from Kodaikanal forest, India against clinical isolates of bacteria. Javeria *et al.* (2013) showed the inhibitory effect of solvent extracts of *P. nilgherrense* from Nainital, India against drug resistant bacteria. In our study, we found inhibitory potential of methanol extract of three *Parmotrema* species from Western Ghats of Karnataka

against clinical isolates previously recovered from burn and dental caries.

Lichens are known to produce characteristic secondary metabolites referred as lichen substances which seldom occur in other organisms. These metabolites possess a wide range of biological activities (Oh *et al.*, 2006). The secondary metabolites present in the *Parmotrema* species were detected by TLC. Atranorin was detected in all three specimens. Lecanoric acid and orsellinic acid were present in *P. tinctorum*. Protolichesterinic acid was detected in *P. grayanum*. Compounds *viz.*, Chloroatranorin, Protopraesorediosic acid and Praesorediosic acid were present in *P. praesorediosum*. Studies have shown that these secondary metabolites of lichens exhibit antimicrobial activity. Atranorin was shown to exhibit antibacterial activity (Thadhani *et al.*, 2012; Yilmaz *et al.*, 2004; Verma *et al.*, 2011). Chloroatranorin is found to exhibit inhibitory activity against bacteria and yeasts (Turk *et al.*, 2006). Lecanoric acid was found inhibitory to bacteria (Verma *et al.*, 2011). Protolichesterinic acid was shown to possess antibacterial activity (Turk *et al.*, 2003; Ingolfssdottir *et al.*, 1997).

CONCLUSION

In the present study, the antibacterial effect methanol extracts of three *Parmotrema* species collected at Western Ghats of Karnataka, India was determined against four clinical isolates. Lichen extracts showed dose dependent inhibitory activity and were found promising as potential sources of bioactive agents against clinical isolates. The observed inhibitory potential could be ascribed to the presence of secondary metabolites in the lichen extracts. Further studies on isolation of active principles from lichens and determination of their inhibitory activity against clinical isolates are to be conducted.

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