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Short Communication

In Vitro Anticaries Activity of Some Macrolichens of Karnataka, India

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ABSTRACT

Dental caries is the most common infectious diseases of the oral cavity. Among the oral microbiota, *Streptococcus mutans*, plays a major role in causing dental caries. The present study was carried out to determine inhibitory activity of ethanol extract of ten macrolichens *viz.*, *Everniastrum cirrhatum*, *Ramalina conduplicans*, *R.hossei*, *R. pacifica*, *Heterodermia leucomela*, *Parmotrema tinctorum*, *P.pseudotinctorum*, *Usnea sinensis*, *Dirinaria consimilis* and *Roccella montagnei* against 13 clinical isolates of *Streptococcus mutans* by Agar well diffusion method. All lichens were effective in inhibiting the caries isolates as evidenced by the formation of zone of inhibition around the wells. Extracts from lichens showed more or less similar inhibitory activity against bacterial isolates except extract of *R.montagnei* and *H.leucomela* which displayed lesser activity when compared to other lichens. The lichens of this study appear to be promising resources of bioactive agents with activity against cariogenic microflora. Further studies on isolation of bioactive principles from lichen extracts and their inhibitory activity against cariogenic flora are to be carried out.

Key words: Macrolichens, Dental caries, *S. mutans*, Agar well diffusion, Zone of inhibition

1. INTRODUCTION

Lichens are composite organisms and represent self-supporting symbiosis between a photobiont (alga/blue-green alga) and mycobiont (fungus). In this association, the photobiont provides the nutrients by photosynthetic activity while the mycobiont helps in absorption of water and nutrients from surroundings. Lichens are considered as primary colonizers of

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terrestrial habitat and they inhabit every possible place on this earth ranging from artic to tropical regions and from the plains to the highest mountains and occur in different growth forms such as crustose, foliose and fruticose. Lichens lack organs such as roots, leaves etc. Lichens grow on rocks (saxicolous), tree barks (corticolous), soil (terricolous) etc. Together with mosses, lichen covers 10% of terrestrial habitats. Lichens are well known as the indicators of air pollution. Lichens are valuable resources of medicine, food, fodder, perfume, spices and dyes. In various parts of the world, lichens are used by people as spice and flavoring agents and to treat various ailments such as dyspepsia, bleeding piles, diabetes, bronchitis, pulmonary tuberculosis, spermatorrhoea, bleeding piles, leprosy etc. Lichens are known to produce characteristic secondary metabolites called lichen substances, many of which do not occur in other organisms. Mycobiont of lichens is known to elaborate majority of these metabolites and these compounds are helpful in the lichen taxonomy (chemotaxonomy). Extracts and purified compounds from various lichens have shown to exhibit a wide variety of bioactivities [1-10]. The present study was performed to investigate anticaries activity of extract of five fruticose macrolichens *viz.*, *Ramalina conduplicans* Vain, *R. hossei* H.Magn & G. Awasthi, *R. pacifica* Asahina, *Usnea sinensis* Mot. and *Roccella montagnei* Bel. Em. D.D. Awasthi and five foliose macrolichens *viz.*, *Everniastrum cirrhatum* (Fr.) Hale, *Heterodermia leucomela* (L.) Poelt, *Parmotrema tinctorum* (Nyl.) Hale, *P. pseudotinctorum* (des. Abb.) Hale and *Dirinaria consimilis* (Stirt.) D.D. Awasthi.

2. MATERIALS AND METHODS

Collection and identification of lichens

The 8 corticolous macrolichens of this study were collected at different places of Karnataka (Table 1). The lichens were identified on the basis of morphological characteristics and the results of anatomical and color tests. The colour tests were carried out on cortex and medulla using 10% potassium hydroxide (K), Steiner's stable para-phenylenediamine solution (P) and calcium hypochlorite solution (C). Secondary metabolites i.e., lichen substances were detected by thin layer chromatography [11,12,13].

Table 1: Lichens used in this study

Name of lichen	Form	Family	Place of collection
U. sinensis	Fruticose	Parmeliaceae	Mullayanagiri
R. montagnei	Fruticose	Roccellaceae	Aynur
R. conduplicans	Fruticose	Ramalinaceae	Shikaripura
R. hossei	Fruticose	Ramalinaceae	Sagara
R. pacifica	Fruticose	Ramalinaceae	Tarikere
H. leucomela	Foliose	Physciaceae	Mullayanagiri
P. tinctorum	Foliose	Parmeliaceae	Thirthahalli
P. pseudotinctorum	Foliose	Parmeliaceae	Thirthahalli
E. cirrhatum	Foliose	Parmeliaceae	Bababuddangiri
D. consimilis	Foliose	Physciaceae	Tarikere

Extraction

Maceration process was used to extract powdered lichen materials. Here, a known quantity (10g) of each of the powdered lichen material was transferred to a conical flask containing 100ml of ethyl alcohol (HiMedia, Mumbai) and the flasks were left for 48 hours. The flasks were stirred occasionally. The contents were filtered through sterile muslin cloth followed by Whatman No. 1 filter paper. The filtrates were evaporated to dryness at 40^{o}C [8].

Preparation of bacterial inoculum

The inhibitory efficacy of extracts of selected lichens was tested against 13 clinical isolates of *S. mutans* (S1-S13) by agar well diffusion assay. The test bacteria were inoculated into sterile Brain Heart Infusion (BHI) broth (HiMedia, Mumbai) aseptically and incubated at 37^{o}C for 24 hours. The broth cultures were adjusted spectrophotometrically to match a turbidity of 10^{8} CFU/ml (equivalent to 0.5 McFarland standards) and used for antibacterial activity by Agar well diffusion assay.

Anticaries activity of lichen extracts

The broth cultures were seeded uniformly on the surface of sterile BHI agar (HiMedia, Mumbai) plates using sterile cotton swabs. Using a sterile cork borer, wells of 6mm diameter were punched in the inoculated plates. Extracts (20mg/ml of dimethyl sulfoxide [DMSO]), reference antibiotic (chloramphenicol, 1mg/ml of sterile distilled water) and DMSO were transferred aseptically into respectively labelled wells. The plates were left undisturbed for about 30 minutes and then incubated at 37^{o}C for 24 hours in upright position. The zones of inhibition formed around the wells were measured using a ruler [8].

3. RESULTS AND DISCUSSION

Dental caries and periodontal problems are among the most common and chronic diseases. Among oral bacteria, *Streptococcus mutans* is the most important pathogen (primary organism) having eight serotypes being highly stimulated by sucrose. All serotypes of *S. mutans* are acidogenic (acid producing) and aciduric (acid tolerant). Caries prevention is a complex process and is achieved by limiting substrate, preventing of plaque formation, modifying tooth surface, restoring cavitated tooth surface, use of antibiotics and bactericidal mouth rinses. However, the usage of antibiotics and oral rinses often suffer from certain drawbacks such as development of resistance in cariogenic flora, staining of teeth, impairment of taste perception, sore and dry mouth and sloughing of gingival tissue. Natural products appear to be promising against cariogenic flora. It has been shown that extracts of lichens possess marked antibacterial effect against *S. mutans* [8, 14-18]. In the present study, we evaluated the extracts of 8 macrolichens for inhibitory activity against 13 clinical isolates of *S. mutans* by agar well diffusion assay. Presence of zone of inhibition confirms antibacterial activity. All lichens were effective in inhibiting isolates as evidenced by absence of growth of isolates around the wells. Extract of all except *H. leucomela* and *R. montagnei* exhibited marked inhibition of *S. mutans* isolates. Standard antibiotic displayed higher inhibitory activity than lichen extracts. DMSO did not cause inhibition of any of the isolates (Table 2). Extract of *R. hossei* displayed stronger inhibitory effect when compared to *R. conduplicans*. An earlier study by Sisodia *et al*.[17] showed the efficacy of hexane extract of *Ramalina roesleri* to inhibit *S. mutans*. The study of Kekuda *et al*.[19], Kambar *et al*.[8] and Vivek *et al*.[20] showed the inhibitory potential against *S. mutans* isolates of extract of *Usnea pictoides*, *R. conduplicans* and three species of *Parmotrema* respectively.

4. CONCLUSION

The macrolichens used in this study have shown marked inhibitory activity against clinical isolates of *S. mutans*. The observed inhibitory effect could be attributed to the presence of lichen substances. In suitable formulation, these lichens can be employed in the prevention and control of dental caries. Further studies are warranted to isolate and characterize bioactive secondary metabolites from lichen extracts and determine their inhibitory effect against cariogenic flora.

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Table 2: Inhibition of *S. mutans* isolates by lichen extracts

Isolates	Zone of inhibition in cm (Mean±S.D)											
	Ec	***Rh***	***Rc***	***Hl***	***Pt***	***Pp***	***Us***	***Rm***	***Dc***	***Rp***	**Ab**	**DMSO**
S1	2.60±0.00	2.20±0.00	2.00±0.00	1.90±0.00	3.30±0.00	2.40±0.00	2.66±0.11	1.90±0.00	2.33±0.05	2.40±0.00	2.80±0.00	0.00±0.00
S2	2.33±0.05	2.33±0.05	2.10±0.00	1.60±0.00	2.10±0.00	2.33±0.05	2.40±0.00	1.70±0.00	2.10±0.00	2.10±0.00	2.93±0.05	0.00±0.00
S3	2.10±0.05	2.33±0.05	2.03±0.05	1.53±0.05	3.23±0.05	2.30±0.00	2.60±0.00	2.13±0.05	2.23±0.05	2.30±0.00	3.40±0.00	0.00±0.00
S4	2.88±0.11	2.11±0.11	1.96±0.11	1.86±0.11	2.76±0.11	2.13±0.05	2.53±0.05	2.00±0.00	2.33±0.23	2.03±0.05	2.83±0.11	0.00±0.00
S5	2.70±0.00	3.00±0.00	2.33±0.05	1.60±0.00	2.20±0.00	2.20±0.00	1.90±0.00	2.00±0.00	2.20±0.00	2.50±0.00	3.30±0.00	0.00±0.00
S6	2.66±0.05	2.43±0.05	2.20±0.00	1.63±0.05	2.66±0.11	2.83±0.05	2.00±0.00	1.90±0.00	2.46±0.11	2.20±0.00	3.00±0.00	0.00±0.00
S7	2.60±0.00	2.36±0.11	2.03±0.05	1.90±0.00	2.43±0.05	2.60±0.00	2.26±0.11	1.80±0.00	2.10±0.00	2.30±0.00	2.93±0.05	0.00±0.00
S8	2.53±0.11	2.03±0.05	2.03±0.05	1.70±0.00	2.10±0.00	2.40±0.00	2.13±0.05	2.03±0.05	2.00±0.00	2.56±0.11	3.00±0.00	0.00±0.00
S9	2.70±0.00	2.60±0.00	2.30±0.00	1.96±0.11	3.03±0.05	2.33±0.05	2.00±0.00	1.90±0.00	2.33±0.05	2.30±0.00	3.20±0.00	0.00±0.00
S10	2.43±0.05	2.46±0.11	2.13±0.05	1.90±0.00	2.60±0.00	2.30±0.00	2.60±0.00	2.20±0.00	2.00±0.00	2.23±0.05	3.00±0.00	0.00±0.00
S11	2.60±0.00	2.56±0.11	2.46±0.11	1.66±0.11	2.40±0.00	2.40±0.00	2.50±0.00	1.80±0.00	2.00±0.00	2.10±0.00	2.83±0.11	0.00±0.00
S12	2.33±0.05	2.30±0.00	1.90±0.00	1.53±0.05	2.13±0.05	2.70±0.00	2.46±0.11	1.93±0.05	2.50±0.00	2.30±0.00	2.83±0.11	0.00±0.00
S13	2.56±0.11	2.30±0.00	2.80±0.00	1.80±0.00	2.10±0.00	2.10±0.00	2.30±0.00	1.90±0.00	2.40±0.00	2.43±0.05	2.60±0.00	0.00±0.00

Ec- E. cirrhatum; *Rc- R. conduplicans*; Rh- *R. hossei*; Rp- *R. pacifica*; *Hl- H. leucomela*; *Pt- P. tinctorum*; *Pp- P. pseudotinctorum*; *Us- U. sinensis*; *Rm- R. montagnei*; *Dc- D. consimilis*; Ab- Antibiotic

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