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Short Communication

Anticaries Activity of *Usnea pictoides* G. Awasthi -A macrolichen from Western Ghats of Karnataka, India

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Abstract

The present study was conducted to determine anticaries activity of solvent extracts of a macrolichen *Usnea pictoides* G. Awasthi (Parmeliaceae) collected at Mullayanagiri, Western Ghats of Chikmagalur district, Karnataka, India. The lichen material was sequentially extracted using solvents *viz*., petroleum ether, ethyl acetate, chloroform and methanol in a soxhlet assembly. Anticaries activity of solvent extracts was determined against four clinical isolates of *Streptococcus mutans* (recovered from dental caries subjects) by Agar well diffusion assay. All solvent extracts were effective against the clinical isolates. High inhibitory potential was observed in case of chloroform extract. Thin layer chromatogram showed the presence of Usnic acid. Inhibitory effect could be ascribed to the bioactive secondary metabolites, mainly Usnic acid present in the lichen. Purification of bioactive principles and determination of their anticaries activity are to be conducted.

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INTRODUCTION

Lichens represent a close symbiotic association between two organisms namely a fungus (mycobiont) and an alga (phycobiont). The lichens are diverse with respect to their morphology (crustose, foliose and fruticose) and are functionally important in ecosystems. These lichens have been used as food, as a source of dye and as traditional medicine. The lichens are known to be the best bioindicator organisms of air pollution and are also as biomonitors for trace element and heavy metal accumulation and deposition in them. Moreover, these lichens also produce a huge number of chemical compounds (lichen substances), most of them are unique and are not produced by other organisms. n-alkane, unusual betaine and glycolipids, unsaturated, oxygenated, branched, and halogenated fatty acids are among the lichen substances. Usnic acid is a yellowish pigment produced by several species of lichens. It exists in two enantiomer forms which differ in the orientation of the methyl group located in position 9b. Usnic acid is responsible for several biological activities of lichens. In pure form, Usnic acid is formulated in creams, toothpaste, mouthwash, deodorants and sunscreen products. Many biological activities such as antimicrobial, antiviral, anti-inflammatory, analgesic, antipyretic, anti-proliferative, enzyme inhibitory, cytotoxic and other activities have been exhibited by lichen extracts and the compounds isolated by lichens (Cocchietto *et al*., 2002; Behera *et al*., 2009; Ribeiro-Costa *et al*., 2004; Yilmaz *et al*., 2004; Ul Haq *et al*., 2012; Pavithra *et al*., 2013).

The 'beard-like' lichenized ascomycete genus *Usnea* is a beloved genus for beginners in lichenology. This genus is cosmopolitan and shares a shrubby to pendant thallus, pale yellowish green branches with radial symmetry and having cartilaginous central axis and usnic acid present in the cortex (Clerc, 1998). *U. pictoides* G.Awasthi (Parmeliaceae) is fruticose lichen, endemic to Western Ghats and is found distributed in Kerala, Karnataka and Tamil Nadu. The lichen grows at high altitudes 1800-2200m. It has an erect, brown corticolous thallus with sympodial branching. Lateral branchlets are sparse, central axis is solid, surface has crackes, hypothecium is colorless, isidea, soredia and apothecia were absent. Iodine test for thallus shows blue color which later turns black. Usnic acid is present (Awasthi, 2000). In a previous study, we reported marked antimicrobial and antioxidant activity of solvent extracts of *U. pictoides* (Pavithra *et al*., 2013). In another study, we reported the mineral content of the powdered lichen material by ICP-OES technique (Vinayaka *et al*., 2013). The objective of the present study was to determine anticaries activity of solvent extracts of *U. pictoides* collected at Mullayanagiri, Western Ghats of Chikmagalur district, Karnataka against clinical isolates of *Streptococcus mutans* recovered from dental caries subjects.

MATERIALS AND METHODS

Collection and Identification of *U. pictoides*

The lichen *U. pictoides* was collected during June 2012 from Mullayanagiri, Western Ghats of Chikmagalur district, Karnataka. The place represents highest peak of Karnataka and is one of the best trekking places in Karnataka and South India. Identification of lichen was done based on morphological, anatomical, chemical tests. The presence of usnic acid in shade dried and powdered lichen material was detected by spotting the lichen sample on the silica plate and developing it with solvent system that consisted of 180 ml of toluene, 60 ml of 1-4, dioxine and 8 ml of acetic acid (Awasthi, 2000; Kumar *et al*., 2011).

Extraction

The powdered lichen material was subjected to sequential extraction using solvents *viz*., petroleum ether, ethyl acetate, chloroform and methanol in a soxhlet assembly. The solvent extracts were filtered through Whatman No. 1 and were concentrated in vacuum under reduced pressure and dried in the desiccator (Pavithra *et al*., 2013).

Anticaries Activity of Solvent Extracts

Agar well diffusion assay was performed to determine anticaries activity of solvent extracts against four clinical isolates of *Streptococcus mutans* (Sm-01 to Sm-04). The test bacteria were inoculated into Brain heart infusion broth (HiMedia, Mumbai) tubes and incubated at 37ºC for 24 hours. The broth cultures were inoculated on sterile Brain heart infusion agar (HiMedia, Mumbai) plates using sterile cotton swabs. Using a sterile cork borer, wells of 6mm diameter were punched in the inoculated plates and 100µl of solvent extracts (20mg/ml of 25% dimethyl sulfoxide [DMSO]), reference antibiotic (Streptomycin, 1mg/ml of sterile water) and DMSO (25%, in sterile water) were transferred into respectively labelled wells. The plates were incubated at 37^{o}C for 24 hours in upright position and the zones of inhibition formed around the wells were measured (Vivek *et al*., 2013).

Statistical Analysis

The experiment was conducted in triplicates. The results are presented as Mean±Standard deviation (SD).

RESULTS

In the present study, thin layer chromatogram revealed the presence of Usnic acid in the lichen *U. pictoides*. The result of inhibitory effect of solvent extracts of *U. pictoides* against cariogenic isolates of *S. mutans* is shown in Table 1. All test bacteria were found to be susceptible to all solvent extracts. Among extracts, high inhibitory activity was observed in case of chloroform extract. Overall, the isolate Sm-04 was inhibited to higher extent than other isolates. Reference antibiotic caused higher inhibition of test bacteria when compared to solvent extracts. DMSO did not cause inhibition of any test bacteria.

DISCUSSION

Western Ghats of India constitute one of the global biodiversity hotspots, covers an area of 1,80,000 km^2 (just under 6% of the land area of India) and contain >30% of all plant, fish, herpeto-fauna, birds, and mammal species found in India. The Western Ghats mountain range runs through various states viz., Gujarat, Maharashtra, Goa, Karnataka and Kerala. The area represents the 'gene pool' and harbors numerous species of plants, animals and microbes. The Western Ghats are known to harbor a number of globally threatened and endemic species of plants and animals (Nampoothiri *et al*., 2013). Western Ghats of Karnataka harbors a variety of lichens species (Vinayaka *et al*., 2010a; Vinayaka *et al*., 2011; Vinayaka *et al*., 2012a; Vinayaka *et al*., 2012b). It has been found that lichens of Western Ghat possess several biological activities such as antimicrobial, insecticidal, anthelmintic, antioxidant, enzyme inhibitory and others (Kekuda *et al*., 2009; Kumar *et al*., 2009; Vinayaka *et al*., 2009a; Vinayaka *et al*., 2009b; Swathi *et al*., 2010; Kumar *et al*., 2010a; Kumar *et al*., 2010b; Vinayaka *et al*., 2010b; Kumar *et al*., 2011; Kekuda *et al*., 2011; Karthik *et al*., 2011; Kekuda *et al*., 2012; Pavithra *et al*., 2013).

Table 1: Anticaries activity of solvent extracts of *U. pictoides*.

Extract	Zone of inhibition in cm (Mean±SD)			
	Sm-01	Sm-02	Sm-03	Sm-04
Petroleum ether extract	2.0±0.2	2.1±0.1	1.6±0.2	2.5±0.1
Chloroform extract	2.2±0.1	2.5±0.0	2.3±0.1	2.6±0.0
Ethyl acetate extract	1.9±0.0	2.2±0.1	2.2±0.0	2.5±0.1
Methanol extract	1.7±0.1	2.3±0.1	2.2±0.0	1.9±0.0
Streptomycin	3.2±0.2	3.6±0.3	3.9±0.1	2.9±0.1
DMSO	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Dental caries is the common and most important infections of the oral cavity. It affects people of all age groups worldwide. Among microflora, mutans streptococci, in particular *S. mutans* is considered as the primary aetiological agent of dental caries. Several agents such as antimicrobial mouth rinses (chlorhexidine and others) and antibiotics are used for prevention and treatment of dental caries. However, these chemicals agents are costly and are shown to possess some undesirable side effects such as tooth staining, taste alteration, development of hypersensitivity reactions, development of resistance by cariogenic flora. Natural products are used routinely for oral hygiene and treatment of dental caries (Aneja *et al*., 2010; Venugopal *et al*., 2011; Fani and Kohanteb, 2012; Chaiya *et al*., 2013; Junaid *et al*., 2013; Vivek *et al*., 2012; Vivek *et al*., 2013). In our study, the solvent extracts of *U. pictoides* were found to possess inhibitory activity against clinical isolates of *S. mutans*. It has been found that lichen extracts and the metabolites of lichens possess inhibitory activity against *S. mutans*. Ghione *et al*. (1988) observed that the D (+) enantiomer of usnic acid was more active against *S. mutans* than the L(+) form. In a study, ethanolic extract of *Cladonia* sp. and ethanolic and chloroform extracts of *Parmelia* sp. showed a marginal inhibition of *S. mutans* (Sharma *et al*., 2012). In another study, Sisodia *et al*. (2013) observed high inhibitory activity of hexane extract of *Ramalina roesleri* against *S. mutans*.

CONCLUSION

A marked inhibitory activity of solvent extracts of *U. pictoides* against clinical isolates of *S. mutans* was observed in this study. The inhibitory efficacy might be attributed to the presence of secondary metabolites in the

lichen mainly Usnic acid. Further studies on purification of active principles from solvent extracts and their bioactivity determination are to be conducted.

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