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## Short Communication

# Inhibitory activity of *Usnea pictoides* G. Awasthi (Parmeliaceae) against urinary tract isolates

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**Abstract:** The genus *Usnea* (Parmeliaceae) is one of the best studied lichen genera. The present study aimed at determining inhibitory potential of *Usnea pictoides* G. Awasthi against bacterial isolates from urinary tract infections. Susceptibility of urinary tract isolates namely *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli* to lichen extract was determined by Agar well diffusion assay. Minimum inhibitory concentration was determined by broth dilution method. The bacterial isolates were inhibited by lichen extract in a dose dependent manner. Among bacteria, marked susceptibility was recorded in case of *E. coli* followed by *E. faecalis* and *S. aureus* as revealed by MIC values. The lichen can be used for treatment of urinary tract infections.
**Keywords:** *Usnea pictoides*, Urinary tract infections, Agar well diffusion, MIC

## INTRODUCTION

Lichens represent self-supporting symbiotic association comprising of a Photobiont (algae or blue-green algae) and a Mycobiont (fungi). The lichens grow on rocks, roofs, tree trunks, leaves etc. and occur in different growth forms namely crustose, foliose and fruticose. They lack specialized organs such as roots, leaves etc., which allows them to survive in harsh environmental conditions. Lichens are considered as the primary colonizers of terrestrial ecosystem. Lichens are the indicators of air pollution and are valuable resources of medicine, food, fodder, perfume, spices and dyes. They are consumed in certain parts of the world as food especially during famine. Several lichen species are often used as spice and flavoring agents in certain foods. Lichens are traditionally used worldwide to treat various ailments. Lichens produce a number of secondary metabolites called lichen substances which seldom occur in other organisms. Most of these metabolites are of fungal origin. These metabolites are useful in the lichen taxonomy and are known to display diverse bioactivities[1-6]. The genus *Usnea* is one of the best known lichen genera. The diagnostic features of the genus include a fruticose shrubby to pendant thallus with a cortex, medulla, and a cartilaginous central axis, and the presence of usnic acid in the cortex[7]. *Usnea pictoides* G.Awasthi (Parmeliaceae) is an endemic fruticose lichen of Western Ghats and is found distributed in Kerala, Karnataka and Tamil Nadu. The lichen grows at high altitudes 1900-2200m and has an erect, brown corticolous thallus with sympodial branching[4]. The lichen is shown to contain various mineral elements such as calcium, iron, magnesium etc.[8]. In earlier studies, antimicrobial and radical scavenging activity of *U. pictoides* was reported[4,9,10]. The present study aimed at determining inhibitory potential of methanol extract of *U. pictoides* from Mullayanagiri, Western Ghats of Chikmagalore district, Karnataka, India against bacterial isolates from urinary tract infections.

## MATERIALS AND METHODS

### Collection and identification of lichen

The macrolichen *U. pictoides* was collected in our previous study from a place called Mullayanagiri, Western Ghats region of Chickmagalore district, Karnataka, India. The lichen was identified on the basis of morphological, anatomical and chemical tests. Usnic acid was detected in the lichen[4].

### Extraction

The lichen was dried under shade and powdered. About 25g of lichen material was extracted using methanol (HiMedia, Mumbai) in a soxhlet assembly. The content was filtered through sterile Whatman No. 1 filter paper, concentrated in vacuum under reduced pressure and stored in desiccator[4].

### Antibacterial activity of lichen extracts

Three urinary tract isolates namely *S. aureus*, *E. faecalis* and *E. coli* were tested for their susceptibility to extract of *U. pictoides* by Agar well

diffusion assay. The broth cultures of the isolates were prepared in sterile Nutrient broth tubes. The broth cultures were swabbed on sterile Nutrient agar plates using sterile cotton swabs. Using a sterile cork borer, wells of 6mm were punched in the inoculated plates. 100µl of lichen extract (10 and 20mg/ml of Dimethyl sulfoxide [DMSO]), Chloramphenicol (reference antibiotic, 1mg/ml of sterile water) and DMSO (25%, in sterile water) were filled into respective wells. The plates were incubated at 37$^{o}$C for 24 hours. The zones of inhibition formed were measured[4].

**Determination of Minimum inhibitory concentration (MIC)**

Broth dilution method was employed to determine the MIC of extract. In clean test tubes containing sterile Nutrient broth (5ml), a series of extract dilutions (2 fold dilutions; concentrations ranging from 20 to 0.0mg/ml) was prepared. The tubes were inoculated with 24 hours old Nutrient broth cultures of test bacteria (100µl) and incubated at 37$^{o}$C for 24 hours. The MIC was determined by observing the visible growth of the bacteria if any after incubation. The dilution tube in which visible growth was absent was considered as the MIC[11].

## RESULTS

**Inhibitory activity of extract of *U. pictoides***

The extract of *U. pictoides* was shown to inhibit all clinical isolates in a dose dependent manner. The extract was more effective in inhibiting Gram negative bacterium *E. coli* when compared to Gram positive bacteria *S. aureus* and *E. faecalis*. Among bacteria, least inhibition by extract was observed in case of *S. aureus*. Reference antibiotic was more inhibitory to clinical isolates. Moreover, antibiotic caused higher inhibition of Gram positive bacteria when compared to *E. coli*. DMSO did not cause inhibition of any isolate (Table 1).

**Table 1: Inhibitory activity of extract of *U. pictoides***

| Treatment | Concentration | Zone of inhibition in cm (Mean±S.D) | | |
|---|---|---|---|---|
| | | ***S. aureus*** | ***E. faecalis*** | ***E. coli*** |
| Extract | 10mg/ml | 1.5±0.0 | 1.7±0.1 | 2.2±0.1 |
| | 20mg/ml | 1.8±0.0 | 2.2±0.0 | 2.4±0.1 |
| Standard | 1mg/ml | 3.3±0.1 | 3.0±0.2 | 2.2±0.2 |
| DMSO | 25% | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |

**MIC of extract of *U. pictoides***

The MIC determination revealed that the lichen extract was more effective against *E. coli*. The MIC was found to be 78, 150 and 310µg/ml in case of *E. coli*, *E. faecalis* and *S. aureus* respectively.

## DISCUSSION

There are >20000 lichens described all over the world so far and 10% of them are found in India. In India, rich lichen diversity can be seen in Himalayas and Western Ghats[5]. Lichens are known to be promising sources of antibacterial agents and a number of studies have indicated their potential to inhibit a wide range of pathogenic bacteria including drug resistant clinical isolates[9,11,12,13]. In the present study, the antibacterial potential of methanol extract of *U. pictoides* was evaluated against three urinary tract bacteria. The extract was effective in inhibiting the test bacteria. *E. coli* was inhibited to higher extent while least inhibition was observed in case of *S. aureus* as indicated by results of agar well diffusion and MIC determination. In a previous study, the solvent extracts of *U. pictoides* inhibited reference strains of bacteria *viz*., *S. aureus* and *Pseudomonas aeruginosa*[4]. In another study, solvent extracts of *U. pictoides* was shown to inhibit *S. mutans* recovered from dental caries[9]. Recently, Vinayaka *et al*.[10] showed inhibitory potential of aqueous extract of *U. pictoides* against *Fusarium oxysporum* and *Pythium aphanidermatum* isolated from rhizome rot of ginger.

## CONCLUSION

The study showed marked inhibitory effect of extract of *U. pictoides* from Western Ghats of Karnataka, India against urinary tract isolates. The observed inhibitory effect could be ascribed to the presence of secondary metabolite Usnic acid. The lichen can be exploited for treatment of urinary tract infections.

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