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Antimicrobial activity of ethanolic extract of *Usnea longissima*

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Abstract
Introduction: *Usnea Longissima* is an epiphyte species of lichen belongs to the family *Parmeliaceae*. Lichenic acids isolated from *Usnea Longissima* are growth inhibitors. *Usnea Longissima* was used a dermatological aid for during wounds in the pecific North West. Methods: The ethanol extract of *Usnea Longissima* were screened for potential antibacterial activity and antifungal activity using Agar well diffusion method against six infectious strains and two dermatophytic fungi *Trichoderma viride* and *Candida albicans.* Results: Ethanol extract of *Usnea Longissima exhibited significant* antibacterial activity and antifungal activity with 1mg/ml Agar well diffusion method against the Gram positive Staphylococcus *aureus* (26 ± 0.5), and Gram negative *Pseudomonas aeruginosa* (18 ± 0.5), *Klebsiella pneumoniae* (21 ± 0.5), *Shigella dysenteriae* (10 ± 0.3), *Salmonella typhi* (14 ± 0.5), *Escherichia coli* (-) and two dermatophytic fungi *Trichoderma viride* (14 ± 0.5) and *Candida albicans* (11 ± 0.5). Conclusion The present study is justified the traditional use and the effect of ethanol extract of lichen *Usnea longissima* was screened their level of antimicrobial potential.

Keywords: *Usnea longissima,* Lichen, Antimicrobial activity, Ethanol

INTRODUCTION

Lichens are symbiotic associations of a fungus with a photosynthetic partner that can produce food for the lichen from sunlight. In recent years there has been a rising interest in the discovery of new antimicrobial compounds, due to alarming increase in the rate of infections with Multidrug resistance microorganisms (Crockett *et al*., 2003). The increased prevalence of antibiotic resistance bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control bacterial diseases. The investigation of new bioactive compounds is of utmost important in the control of antibiotic resistant microorganisms (Paulo, 2003). Many investigators have evaluated the bioactivity of lichen extracts and the isolated constituents against serious infectious organisms (Fournet *et al*., 1997). By investigating traditional uses of these lichens, modern science is given a foundation for exploration of lichen species and their chemical constituents. *Usnea* is a genus of lichen that has been classified under the family *Parmeliaceae* with around 1000 species worldwide (Galun, 1988). In recent years the genus *Usnea* has been divided into a number of smaller genera according to thallus morphology. Some of the members of genus *Usnea* are *Usnea barbata, Usnea dasypoga, Usnea florida*, *Usnea hirta, Usnea longissima, Usnea rubicunda, Usnea rubiginea* and *Usnea subfloridana* (Aslan *et al*., 2006). Various acids and Sterols have been isolated from *Usnea longissima* (Dubey and Dwivendi, 1991; Singh *et al*., 2007). Lichenic acids isolated from *Usnea longissima* are growth inhibitors. *Usnea Longissima* is an epiphyte species known to produce one or more secondary metabolic products which have been characterized as weak phenolic acids (Elix, 1996). *Usnea longissima* was used as a dermatological aid for dressing wounds in the Pacific North West. The metabolic products that have antibiotic activity may have function of protecting the organisms from attack by other fungi (Behera *et al*., 2005). Retarding action of extraction of lichen on the growth of lower phycomycetes and *Neurospora crassa* (Hale, 1974). Reported in Finland, complex derivative of usnic acid of lichen have been reported with enhanced antibiotic activity (Deacon, 1980). Sodium usnate have been successfully used for the control of various plant diseases in green house (Ark *et al*., 1960). In the present study, the extracts of *Usnea longissima* were investigated for their antimicrobial activity against the following pathogenic bacteria; *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella dysenteriae, Escherichia coli, Salmonella typhi* and some dermatophytic fungi like *Candida albicans* and *Trichoderma viride*

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MATERIALS AND METHODS

Collection of the sample

The vegetative bunches *Usnea longissima* were collected from Bhadra Wildlife Sanctuary of the Western Ghats region of Karnataka, India. The taxonomic identity was confirmed by comparing with the authenticated specimen deposited at Kuvempu University herbarium.

Extraction of lichens

Vegetative bunches of the lichens, *Usnea longissima* were air dried for four days and cleaned free of any other plant materials and washed under running tap water. They were oven-dried at 40°C for 72 h and ground into powder by the use of mechanical hand grinder. The powdered samples were stocked in sterilized specimen bottles until when needed. One fifty gram (150 g) of lichen powder was extracted in Soxhlet apparatus using 70% ethanol for 5 h. The

solvent was removed under reduced pressure at 40 ± 5ºC using rotary flash evaporator (Zechmeister, 2001).

Antimicrobial activity

Screening for antibacterial activity

The antibacterial activity of the crude extract was screened by the agar well diffusion method against six different bacterial species- *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella dysenteriae, Eschrichia coli* and *Salmonella typhi*. The clinical isolates were collected from the Department of Biotechnology, Kuvempu University which was previously identified following a standard method (Cown and Steel, 1993).

Screening for antifungal activity

The antifungal activity was screened against *Candida albicans* and *Tricoderma viride*. Spore suspension was prepared by washing one or two colonies using five milliliters of sterile phosphate buffer solution (pH 7.0). One milliliter of inoculum was added into 10±2 mL of Potato dextrose agar at 37 ± 3° C and mixed in Petridishes. After solidification at room temperature for a maximum of 20 minutes, wells were made in the agar with sterile stainless steel cork borer (d= 4 mm). 5 mg of ethanol extract were dissolved in each of 5 ml of 10% DMSO. 50 µg /100 ml of the extracts were loaded in the corresponding wells. Petri dishes were incubated for 48 hrs at 37° C. The standard fluconozole was used as reference antifungal substance (50µg /100µL of sterilized distilled water). Inhibition zones were expressed in millimeters as the diameters of clear zones around holes (Shahi *et al*., 2001).

Agar well diffusion method

A sensitive radial diffusion technique was used for the assessment of antibacterial activity of the test samples. Sterilized nutrient agar medium was poured into sterilized Petri dishes. Nutrient broth containing 100 µL of 24 h incubated cultures of the respective bacterial strains was spread separately on the agar medium. Wells were created using a stainless steel sterilized cork borer under aseptic conditions. 50 µg/100µL of crude extract were loaded into corresponding wells. The fluoroquinolone antibiotic Penicillin was used as standard (50µg/100 µL of sterile water). The plates were incubated for 24 h at 37° C and the diameter of the zone of complete inhibition of the bacteria was measured around each well and readings were recorded in millimeters.

RESULTS AND DISCUSSION

In the present study, the crude extract obtained from the lichen *Usnea longissima* using soxhlet apparatus. Ethanol was used as the extraction solvent and the crude was in the form of a greenish yellow paste with a characteristic odour (Öztürk *et al*., 1999). The crude extract was screened for antimicrobial activity by the agar well diffusion method against six different bacteria and two fungi (Halama and Haluwin, 2004; Vartia, 1973).

The overall data presented gives us the indication that the antimicrobial activity is present in crude extract of Lichen *Usnea longissima (*Rawat *et al*., 2006). In agar well diffusion method, the crude extract has shown high activity against *Pseudomonas aeruginosa* (18 ± 0.5), *Klebsiella pneumoniae* (21 ± 0.5) and *Staphylococcus aureus* (26 ± 0.5). Moderate activity was seen against *Salmonella typhi* (10 ± 0.3) and *Shigella dysenteriae* (10 ± 0.3) and no effect on *Escherichia coli* (Ingolfsdottir *et al*., 1997). Among fungi both *Trichoderma viride* (14±0.5) and *Candida albicans* (11 ± 0.5) were more susceptible to the crude extract (Table-1 & 2)

Table 1. Growth inhibition zone (mm) of different bacteria in agar well diffusion method

Test organisms	Inhibition zone (mm)		
	Ethanol extract	Penicillin	Control
Pseudomonas aeruginosa	18 ± 0.5	12 ± 0.1	-
Klebsiella pneumoniae	21 ± 0.5	18 ± 0.5	-
Staphylococcus aureus	26 ± 0.5	8 ± 0.2	-
Shigella dysenteriae	10 ± 0.3	6 ± 0.5	-
Salmonella typhi	14 ± 0.5	16 ± 0.1	-
Escherichia coli	-	6 ± 0.5	-

Table 2. Growth Inhibition Zone (mm) of different fungi in agar well diffusion Method

Test organisms	Zone of inhibition (mm)	
	Ethanol extract	Flucanozole
Trichoderma viride	14 ± 0.5	10 ± 0.1
Candida albicans	11 ± 0.5	12 ± 0.5

In the present study, the effect of ethanol extract of lichen *Usnea longissima* was screened against six bacteria and two dermatophytic fungi for their level of antimicrobial potential (Richardson, 1975). To determine the compounds present that may produce an inhibitory effect on different classes of bacteria and fungi, the crude extract were tested for their antimicrobial property and were found to be effective on all the test organisms except *Escherichia Coli* (Lauterwein *et al*., 1995).

CONCLUSION

The *Usnea longissima* is one of the important Lichen, which has antimicrobial activity against different bacteria and fungi. It would be advantageous to standardize the methods of extraction and *in vivo* testing so that the search could be more systematic and it may facilitate to control the pathogenic microorganisms, which have already become resistant to existing antibiotics. Hence, further

investigations on the antimicrobial activity as well as the economical and fast isolation of the metabolite from the Lichen are needed.

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