



Research Article

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ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACTION OF *USNEA GHATTENSIS* AND *USNEA UNdulata*

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ABSTRACT

The fungal partner of lichens produces a great variety of biologically active metabolites such as antibiotic, antiviral, anti-inflammatory and many others. Antimicrobial is a substance which either inhibits the growth of micro-organism or kills the microbes. The pathogenic microorganisms causing number of diseases in plants and animals have developed resistance against many commonly available chemicals and antibiotics. Lichens are the combination of two life forms algae and fungi. A large number of lichens are known for the source of important secondary metabolites. The present study aims at the antimicrobial properties of two species of *Usnea* belonging to the largest family Parmeliaceae. The antibacterial activity of two species of *Usnea* was tested against six organisms such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella typhi*, *Escherichia coli*, *Vibrio cholerae*, *Bacillus coagulans*. Both species of *Usnea* had shown inhibitory activity against all pathogen but to different extent. These two species of *Usnea* are found in large quantity in Mullayangiri of Karnataka and these two species can be used sustainably in the preparation of antimicrobials.

Keywords: *Usnea*, antimicrobial, pathogen, Parmeliaceae

INTRODUCTION

The symbiotic association of fungi and algae or cyanobacteria forms the slow growing life forms called lichens. Lichens are very sensitive organisms and show poor growth in industrial area and big cities. Hence they are considered as indicators of air pollution¹. More than 50% of lichens that have been studied synthesize substances with some degree of antimicrobial activity². Many researches have been carried out to prove the antifungal and antimicrobial activity of the lichens. Different concentrations of lichen extracts have a variable degree of lichen activity³. Antifungal and antimicrobial activity of extracts from *Usnea* species is extremely proved. Secondary metabolites such as Usnic acid, Pulvinic acid derivatives, aliphatic acids, orcinol type depsides and depsidones are well known for its effective antimicrobial activity.

Lichens and their products are used in traditional medicines for centuries and used as an alternative treatment in various parts of the world. The secondary metabolites of lichens are unique with respect to higher plants and are found their uses in many fields such as medicines, dyes, cosmetics, deodorants, preservatives and also in biopesticides. These lichens grow slowly but still they are resistant to decaying microorganisms. This fact suggests that the lichen substances are very effective antibiotics⁴.

The secondary metabolites from the lichens have the capacity to inhibit the seed germination in vascular plants and spore germination in mosses⁴. These antimicrobial roles of the secondary metabolites of lichens have made possible for the lichens to live for hundreds of years⁴. *Usnea ghattensis* and *Usnea undulate* are fruticose lichens with bushy thallus. *U. ghattensis* is erected bushy but *U. undulate* shows sub-pendant thallus. *U.*

ghattensis is found growing in Karnataka and Maharashtra^{5,6} whereas *U. undulate* is distributed in states of Arunachal Pradesh, Meghalaya, Nagaland, Karnataka, Kerala and Tamil Nadu^{6,7}.

MATERIAL AND METHODS

Collection of the sample

Lichen *Usnea ghattensis* is collected from the Mullayangiri and Kemmangundi hills of Karnataka and Mahabaleshwar hills of Maharashtra. The lichen *U. undulate* was collected from Mullayangiri peaks of Karnataka, India. The collected lichens were dried and stored in lichen herbarium of K.F.G.C. Shikaripura. The lichens were identified up to species, based on its anatomy, morphology and chemical characters⁸⁻¹². The herbarium voucher specimen number of *U. ghattensis* is LHKFGC0012 and *U. undulate* is LHKFGC0015, are used for preparing the lichen extracts.

Test organisms

Six bacterial cultures were procured from Microbiology Department, Sahyadri Science College, Shimoga, India. *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella typhi*, *Escherichia coli*, *Vibrio cholerae*, *Bacillus coagulans* are the 6 test organisms.

The lichens were washed, dried and ground to powder. 10 g portion of powder was added to 100 ml of ethanol, mixed well, sonicated and left for 48 hours at room temperature with occasional stirring. The extracts were prepared by decanting the contents of flask through a clean muslin cloth and again with Whatman No.1 filter paper to get a clean filtrate. The filtrate was

evaporated in oven at 40°C to get dried and concentrated extract. Thus obtained sterilized filtrate was stored in refrigerator¹³.

Antibacterial Activity

The bacterial activity of lichen extracts was evaluated by standard agar well diffusion method. 0.1 ml of bacterial culture of 24 hours was evenly spread on sterile Mueller-Hinton agar plates with the help of sterile cotton swabs. The seeded plates were allowed to dry. The alcohol sterilized cork borer of 6 mm was used to make

wells in plates. 100 µl of different concentration (10 mg/ml, 15 mg/ml, and 25 mg/ml) of lichen extract was loaded to the well. 10 µg/ml of tetracycline was taken as positive control and 10% DMSO was taken as negative control. Further the plates were kept for incubation at 37°C for 24 hours. After incubation the plates were observed for zone of inhibition and results were recorded. All the experiments were repeated and the data presented is the average of 3 replications.

Table 1: Zone of inhibition of ethanolic extracts of two Lichen species

Test Sample	Concentration of Test sample	Zone of inhibition in mm					
		<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Vibrio cholerae</i>	<i>Bacillus coagulans</i>
<i>Usnea ghattensis</i>	10 mg/ml	10	11	12	13	13	13
	15 mg/ml	12	12	14	15	15	14
	25 mg/ml	16	17	18	17	18	16
<i>Usnea undulata</i>	10 mg/ml	14	16	12	15	14	14
	15 mg/ml	16	18	16	17	17	17
	25 mg/ml	20	20	19	20	20	20
Tetracycline	10 mg/ml	26	24	26	26	26	26



Usnea ghattensis



Usnea undulata

RESULT AND DISCUSSION

The result of inhibitory activity of extracts of *Usnea ghattensis* and *U. undulate* were observed on the growth of various microorganisms such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella typhi*, *Escherichia coli*, *Vibrio cholerae*, *Bacillus coagulans*. Lichen extracts of both species had shown inhibitory activity against all pathogen but to different extent (Table 1).

U. ghattensis shows maximum zone of inhibition against 3 microorganisms that is, *Escherichia coli*, *Vibrio cholera* and *Bacillus coagulans* of 13 mm.

U. undulate shows maximum zone of inhibition against *Streptococcus pneumonia* with 16 mm of zone followed by *Escherichia coli* with 15 mm. The more concentrated test samples shows the more zone of inhibition. At lower concentration of 10 mg/ml *U. ghattensis* shows minimum inhibition for *Streptococcus aureus* and *Salmonella typhi* and *U. undulata* shows for *Salmonella typhi* with 10 mm and 16 mm respectively. The results of present study was compared with the findings of^{13,14} who worked on the inhibitory activity of ethanolic extracts of *U. longissima* and *U. barbata* against gram positive and gram negative bacteria and found in accordance with their results.

CONCLUSION

Usnea produces a number of secondary metabolites. The ethanolic extracts of *U. Ghattensis* and *U. undulate* have high antibacterial potential. These two species of lichens are found in more number in Karnataka. The unscientific methods of gathering lichens may have negative impact on the ecology. Hence sustainable harvesting of lichens may result in the preparation of a potential antibacterial drug.

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