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In vitro antimicrobial activity of Himalayan medicinal plant *Pholidota articulata*

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Abstract
The antimicrobial activity of the all extracts of *Pholidota articulata* were studied against five and three fungal stain. The results showed that the minimum inhibitory concentration (MIC) of *Pholidota articulata* extract was 50µg/ml against *Salmonella enterica* Typhim The ethyl acetate extract of *Pholidota articulata* showed significant activity 18±1mm, 16±1mm and 14±1mm against *Klebsiella pneumonia Salmonella enterica* Typhim and *E. coli* against food poisoning bacteria and the order of the species based on total antibacterial activity is as follows: *Klebsiella pneumonia* > *Salmonella enterica* Typhim > *Escherichia coli* and phytochemical screening for the presence of glycosides, alkaloids, phenols and tannins.

Keywords: Antibacterial, antifungal and phytochemical screening

1. Introduction
India has great wealth of medicinal plants and their traditional uses. The use of traditional medicinal plants as a source for relief from illness. Herbal medicine is the oldest form of health care known to mankind. Herbs have been used by all cultures throughout the history and they constitute an integral part of the development of modern civilization. Medicinal and aromatic plants and their derived are rich in antibacterial compounds which could be an alternate way to combat bacterial diseases even against some bacteria which are becoming resistant to certain synthetic medicines. The genus Pholidota (Orchidaceae) belongs to the tribe coelogyneae, and comprises 55 species with a distribution from tropical Asia to tropical Australia and china. Among them 9 species in India. Commonly distributed from submontane to montane Himalaya. The genus pholidota are epiphytic herbs generally grown on rocks and trees [1]. Most plants of the genus *P. articulata* found in India grow as epiphytes. Some are also found growing on moist, moss covered rock structures on large, hilly slopes .On the earth, out of 4, 22,127 plant species about 35,000 to 70,000 species are used as medicinal plants [2]. In the third world countries, 20,000 plants species are believed to be used medicinally [3]. At present, the pharmaceutical sector in India is making use of 280 medicinal plant species, of which 175 are found in the IHR [4]. The plants of the genus pholidota are used traditionally for medicinal purposes. The whole plant has long been used as a remedy for acute or chronic bronchitis, toothache, treatment of dysentery, infections, asthma, bronchitis, eczema and duodenal ulcer [5].

2. Materials and Methods
2.1 Plant Material
Pholidota articulata (Orchidaceae) whole plants were collected from the Ukhimath, Distt-Rudraprayag, Uttarakhand, in September-October 2014. The plant was authentic and identified by Dr. C. S. Rana, Department of Botany and the voucher specimen number is GUH 4325. H. N. B. Garhwal (A Central University) Srinagar Garhwal, Uttarakhand India.

2.2 Preparation of plant Extract
The plant material was separated into its selected part air dried ground to moderately fine powder and soxhlet extracted with increasing polarity solvent (petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water [6]. Each extract was evaporated to dryness under reduce pressure using rotary evaporator. The coarse powder of tuber were subjected to successive hot continuous extraction with various solvent each time before extracting with next solvent the powdered material will be air dried (weight of crude extract 500gm). The various concentrated extracts were stored in air tight container for further studies.

2.3 Media
Nutrient broth, agar, Muller Hinton agar, Malt extract broth and Sabouraud dextrose agar, Alcohol, Hydrochloric acid, alcohol, Distilled water etc. all product of Hi-media Laboratories Mumbai (India) were used in this study.

2.4 Bacterial Strains
Five bacterial strains were used namely *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella enterica* Typhim, The bacterial strains were supplied by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

2.5 Fungal Strains
Three fungal strains were used namely *Candida albicans*, *Aspergillus* flavus and Aspergillus parasiticus, The fungal strains were supplied by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

2.6 Antibacterial assay
The disc diffusion assay methods were used to determine the growth inhibition of bacteria by plant extracts [7-8]. Diluted bacterial culture (100µl) was spread over nutrient agar plates with a sterile glass L-rod. 10mg/ml and 50mg/ml of the each extracts were applied to each filter paper disc (Whatman No. 1, 5 mm diam.) and allowed to dry before being placed on the agar plate. Each extract was tested in triplicate (3 discs/ plate) and the plates were inoculated at 37 °C for 24 h. After incubation in the diameter of inhibition zones was measured with a caliper.

2.7 Antifungal assay
The antifungal activity was tested by disc diffusion method [9-10]. The Sabouraud dextrose agar plates were each similarly seeded with each fungal strain The 24 hrs. both culture of each bacterium and 7 days inoculated fungus culture were used to seed sterile Sabouraud dextrose agar at 45 °C respectively, and fungal plates were incubated at 25-28 °C for 7 days after which diameter of zones of inhibition were measured. Each disc filled with extract.

2.8 Phytochemical analysis
The qualitative phytochemical properties of the dried powdered sample were determined using standard methods [11].

3. Result and discussion
Plants are important source of potentially bioactive constituents for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antimicrobial activity. The results of antibacterial, antifungal and phytochemical screening activity table 1, 2 and 3 reveals that antibacterial, antifungal and phytochemical screening activity of *Pholidota articulata* was evaluated against five bacterial and three fungal human pathogenic strains.

3.1 Antibacterial and antifungal activity
The ethyl acetate extract of *Pholidota articulata* showed significant activity 18±1mm, 16±1mm and 14±1mm against *Klebsiella pneumonia*, *Salmonella enterica* Typhim and *E. coli* against food poisoning bacteria and the order of the species based on total antibacterial activity is as follows: *Klebsiella pneumonia* > *Salmonella enterica* Typhim > *Escherichia coli*.

3.2 Phytochemical screening
The phytochemical screening of plant for the presence of glycosides, flavonoids, phenols, resins and tannins, however flavonoids were minor or absent.

Table 1: Antibacterial activity of five bacterial strains against *Pholidota articulata* plant tubers extract, Disc size, 5 mm, Inhibitory zone size ±1 mm, mm means (millimetres) and – indicate (NIZ) No inhibitory zone.

Bacterial Name		Erythromycin	Petroleum ether Extract		Ethyl acetate Extract		Methanol Extract	
Genus /Species /Subspecies	**MTCC (Code)**	**10 Mg/ml**	**10 Mg/ml**	**50 Mg/ml**	**10 Mg/ml**	**50 Mg/ml**	**10 Mg/ml**	**50 Mg/ml**
Escherichia coli	443	14	6	-	12	14	8	6
Pseudomonas aeruginosa	854	13	10	8	10	9	7	8
Klebsiella pneumonia	432	11	7	9	10	18	10	11
Salmonella enterica Typhim	1255	10	-	7	9	16	12	9
Staphylococcus aureus	737	11	9	6	13	11	10	9

Table 2: Fungal activities of three fungal strains against *Pholidota articulata* plant extract, Disc size, 5 mm, Inhibitory zone size ±1 mm, mm means (millimetres) and – indicate (NIZ) No inhibitory zone.

Fungal Name		Ketoconazole	Petroleum ether Extract		Ethyl acetate Extract		Methanol Extract	
Genus /Species /Subspecies	**MTCC (Code)**	**10 Mg/ml**	**10 Mg/ml**	**50 Mg/ml**	**10 Mg/ml**	**50 Mg/ml**	**10 Mg/ml**	**50 Mg /ml**
Aspergillus parasiticus	2798	9	6	-	-	7	-	7
Aspergillus flavus	3017	7	8	6	-	8	-	8
Candida albicans	2796	9	-	9	-	-	-	9

Table 3: Phytochemical screening of *Pholidota articulata* different extract, (+) – Present, (-) – Absent.

	Test	Pt. ether Extract	Ethyl acetate Extract	Methanolic Extract	Water Extract
Alkaloid	(1) Mayer's test	(-)	(+)	(+)	(+)
	(2) Dragendorff test	(-)	(+)	(+)	(+)
Phenolics compound	(1) Ferric chloride	(-)	(+)	(-)	(-)
	(2) Nitric acid	(-)	(+)	(-)	(+)
Carbohydrates/ glycosides	(1) Molish test	(+)	(-)	(+)	(+)
	(2) Fehling test	(+)	(-)	(+)	(+)
	(3) Benedict test	(+)	(-)	(+)	(+)
Flavonoids	(1) Shinoda/pew	(-)	(-)	(-)	(-)
	(2) Ammonia	(-)	(-)	(-)	(-)
Saponins		(-)	(-)	(-)	(-)
Tannins	(1) Pyrogoll & catechol	(-)	(+)	(+)	(+)
	(2) Gallic acid	(-)	(+)	(+)	(+)
Resin		(-)	(-)	(-)	(-)

4. Conclusion

The present study results focused on antimicrobial activity and phytochemical screening of *Pholidota articulata* this investigation revealed that antimicrobial and antifungal activity against selected bacterial and fungal strains. Which encourage developing a novel broad spectrum antimicrobial formulation in future. Now our research will be directed to develop a broad spectrum antimicrobial herbal formulation with this plant. Even at low concentrations, these plant species contained potent antimicrobial and antifungal activity nearly equal to that of the commercial fungicide used as a positive control.

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