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IN - VITRO ANTIMICROBIAL ACTIVITY OF *PHOLIDOTA ARTICULATA* AGAINST HUMAN PATHOGENS

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ABSTRACT

The antibacterial and antifungal study is done for P.articulata crude extract and its various fractions. The *P.articulata* crude extract and methanolic extract showed more potent against B.aerues and S.typhi. The ethyl acetate and petroleum extract showed potent against E.coli and K.Pneumonia. The *Antifungal activity is significant for P.articulata* crude extract and methanolic extract showed more potent against T.ruberum, A. flavus and A.niger. The ethyl acetate and petroleum ether extract showed potent against C. albicans and P. glaucum.

KEYWORDS: *Pholidota articulata*, Orchidaceae, Antibacterial, Antifungal activity.

INTRODUCTION

The genus *Pholidota* (Orchidaceae) belongs to the tribe coelogyneae, and comprises 55 species with a distrubution 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 (**Gaur. R.D et al., 1999**). 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 **(A. Hasan, et al 2011).** In the third world countries, 20,000 plants species are believed to be used

medicinally (**T.K. Mukherjee, et al 2004).** At present, the pharmaceutical sector in India is making use of 280 medicinal plant species, of which 175 are found in the IHR **(U. Dhar, et al 2000).** 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 **(Zhong Hua et al., 1999).**

MATERIALS AND METHODS

Collection and identification of plant materials

Pholidota articulata (Orchidaceae) whole plants were collected from the ukhimath Rudraprayag Uttrakhand, India in September-October 2014. The plant was authentic and identified by Dr. C. S. Rana, Department of Botany, HNB Garhwal University Uttrakhand.

Preparation of crude extract

The shade dried whole plant was crushed and boiled in ethanol at 40-50 °C temperature for 16-18 h and then ethanol soluble fraction was filtered off. The filtrate was concentrated under vacuum at low temperature (40°C) with the help of a rotary evaporator (Perfit India). A crude extract (400 g) was obtained from the filtrate.

Fractionation: The crude extract was fractionated with petroleum ether and ethyl acetate by soxhlet apparatus to yield petroleum ether (20g), ethyl acetate (250g), ethyl acetate insoluble (200g) and 30g crude extract was reserved for the biological activities.

Determination of Antibacterial activity

Collection of test organism and preparation of stock culture

The Four species of bacteria, -Escherichia coli, klebsiella pneumonia, Bacillus aureus, Salmonella typhi were isolated from infected sites of patients attending SAI Institute and Science Dehradun, India for testing. These were cultured in nutrient broth for 24 hrs and the fresh inoculums were taken for the test and reconfirmed by gram staining and sub culturing in appropriate selective media.

Preparation of standard culture inoculums of test organism

Three to four isolated colonies were inoculated in 2 mL nutrient broth and incubated till the growth in the broth was equivalent with Mac-Farland standard (0.5%) as recommended by WHO at which the number of cells was assumed to be 1.5 x 108 cfu mL-1.

Determination of Zone of Inhibition (ZOI)

The antibacterial activity was assessed by agar well diffusion method. Muller Hinton agar medium was prepared by using 15g agar dissolved in 1L distilled water. Muller Hinton agar medium was poured into each Petri plate of 20 x 90mm and allowed to cool to 45°C to solidify. The freshly prepared inoculums were swabbed all over the surface of the MHA plate using sterile cotton swab. Wells of 8 mm diameter were made in the agar with a sterile cork borer. 100 µL of the working suspension/solution of different plant extracts were loaded in each well and same volume of extraction solvent for control was filled in the wells with the help of micropipette. Plates were left for some time till the extracts diffused in the medium with the lid closed and incubated at 37°C for 24 h. The tests were performed three times and the zones of inhibition were measured for each extract using a ruler and the results were recorded (Table 1).

Table. 1: Zone of Inhibition (mm) of *P.articulata* crude extract and its various fractions tested for antibacterial activity.

Microorganism Ms (0.1ml)	Zone of Inhibition (mm)					
	EAPA (10mg/ml)	PEPA (10mg/ml)	MAPA (10mg/ml)	ECPA (10mg/ml)	Streptomycin (1mg/ml)	Ampicillin (1mg/ml)
Ec	15	07	12	11	-	26
KP	13	08	09	09	15	-
BA	08	03	13	14	17	-
ST	06	0	17	15	-	24

Abbrevation: EAPA = Ethyl acetate P.articulata soluble extract; PEPA = Petrolium ether P.articulata soluble extract; MAPA = Methyl alcohol P.articulata soluble extract; ECPA = Ethyl alcohol Crude extract P.articulata; EC= Escherichia coli, KP = klebsiella pneumonia, BA = Bacillus aureus, ST= Salmonella typhi

Determination of Antifungal activity

The antifungal activity was tested by disc diffusion method **(Taylor., et al 1995; Espinel Ingroff et al 2002).** 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.

Table. 2: Zone of Inhibition (mm) of *P.articulata* crude extract and its various fractions tested for antifungal activity.

Test Fungal species	Zone of Inhibition (mm)				
	EAPA (10mg/ml)	PEPA (10mg/ml)	MAPA (10mg/ml)	ECPA (10mg/ml)	Hexaconazole (1mg/ml)
A.niger	07	06	12	11	14
C.albicus	06	09	07	09	18
P.glaucum	10	08	13	14	18
A.flavus	08	05	09	15	15
T.ruberum	07	08	11	08	14

RESULTS AND DISCUSSION

Antibacterial activity The antibacterial activities of P. articulata crude extract and its various fraction give different zone of inhibition on the organisms tested. The P.articulata crude extract and methanolic extract showed more potent against B.aerues and S.typhi. The ethyl acetate and petroleum extract showed potent against E.coli and K.Pneumonia. The petroleum ether extract did not show any effect of S.typhi. The antibacterial activities of various fractions of *P.articulata* compared with different standard shown in **(Figure 1).**

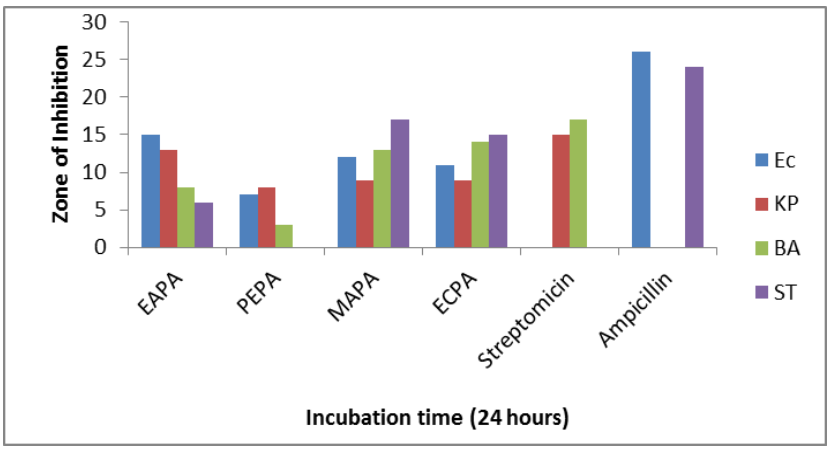

Figure. 1: Comparative Antibacterial Activity of *P.articulata* Crude extract and its various fractions against the test organisms.

Antifungal Activity: The antifungal activities of *P.articulata* crude extract and its various fractions gave different zone of inhibition on the fungal organisms tested. The crude extract and methanolic extract inhibited the growth of three isolates fungal. The crude extract and methanolic extract showed more potent against T.ruberum, A. flavus and A.niger. The ethyl acetate and petroleum ether extract showed potent against C.albicans and P. glaucum. All the extract showed low activity against A. niger. The antifungal activities of various fractions of P. articulate compared with different standard shown in **(FIGURE 2).**

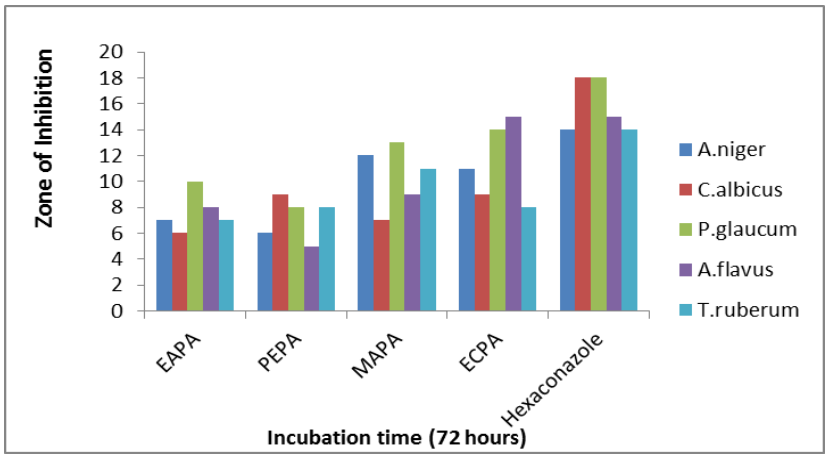

Figure. 2: Comparative Antifungal Activity of *P.articulata* Crude extract and its various fractions against the fungal species.

CONCLUSIONS

The *P.articulata* crude extract and methanolic extract showed more potent against B.aerues and S.typhi. The ethyl acetate and petroleum extract showed potent against E.coli and K.Pneumonia. The crude extract and methanolic extract showed more potent against T.ruberum, A. flavus and A.niger. *Antifungal activity is significant for P.articulata* ethyl acetate and petroleum ether extract showed potent against C.albicans and P.glaucum. The present work revealed that the plant could be used for Herbal medicine. In conclusion, *P.articulata* is an important medicinally plant and can be a potential candidate for further bio-assays which would lead to the synthesis of safe herbal drugs of global interests.

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