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IN-VITRO ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY SCREENING OF MEDICINALLY USED *ERIA ALBA*

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ABSTRACT
The antibacterial and antifungal study is done for Eria alba crude extract and its various fractions. The *Eria* alba ethyl acetate extract showed more potent against all isolates of bacteria. The methanolic extract showed good activity against K. pneumonia, B. aureus, and S. typhi and did not show any effect of E.coli. All the plant extract fraction showed more activity against B. aureus. The methanolic and petroleum ether extract did not show any effect of E. coli. 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.

KEYWORDS: *Eria alba*, Orchidaceae, Antibacterial, Antifungal activity.

INTRODUCTION
The genus *Eria* (Orchidaceae) belongs to the tribe coelogyneae, It contain about 375 species in tropical Asia,Polynesia and Australia.50 species are found in india.Commonly found througout the Himalayan region at the altitude of 2200-3000m (Gaur.R.D et al 1999). Most plants of the genus *Eria* found in India grow as epiphytes. Some are also found growing on moist, moss covered rock structures on large, hilly slopes (Agrawala, D.K et al 2009). 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 this genus have been studied extensively because of the traditional medicinal uses associated with them. The leaves, stems and flowers are used mostly in folk medicine for the treatment of dysentery, treatment of asthma, coughs, bronchitis, eczema and wound healing. The plant leaves used as remedy for skin diseases to reduce swelling and pain. (Gaur.R.D et al 1999). Plants are used medicinally in different countries and are a source of many powerful and potent drugs (Srivastava et al., 1996). A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts of plants used include flower, root, stem, fruits and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Uniyal et al., 2006).

MATERIAL AND METHODS
Materials and reagents used were of Analytical grade and were obtained from Ranchem and CDH, India. The media used for the growth of bacterial and fungal cultures and the reagent 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were procured from Hi-Media Pvt. Ltd., Bombay, India. Microbial Cultures (pure bacterial and fungal) were obtained from NCL, Pune, India and were revived for further use.

Collection and identification of plant materials: *Eria* alba (Orchidaceae) whole plants were collected from the Ukhimath, Rudraprayag Uttrakhand, India in October 2014. The plant was identified from Department of Botany, HNB Garhwal University Uttrakhand.

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

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.

RESULTS AND DISCUSSION

Antibacterial Activity of *Eria* alba Crude extract and its various fractions

The antibacterial activities of *Eria* alba crude extract and its various fraction give different zone of inhibition on the organisms tested (Table 1). The *Eria* alba ethyl acetate extract showed more potent against all isolates of bacteria. The methanolic extract showed good activity against K. pneumonia, B. aureus, and S. typhi and did not show any effect of E.coli. All the plant extract fraction showed more activity against B. aureus. The methanolic and petroleum ether extract did not show any effect of E.coli. The *Eria* alba crude extract shown potent activity against E.coli, K. pneumonia and B. aureus and did not show any effect of Salmonella typhi. The antibacterial activities of various fractions of *Eria* alba compared with different standard shown in (Figure 1).

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

Microorganism MS (0.1ml)	Zone of Inhibition (mm)					
	EASC (10mg/ml)	PESC (10mg/ml)	MASC (10mg/ml)	ECSC (10mg/ml)	Streptomycine (1mg/ml)	Ampicilline (1mg/ml)
Ec	15	-	-	13	09	20
KP	13	10	25	26	17	10
BA	18	12	19	04	10	08
ST	06	02	12	-	10	-

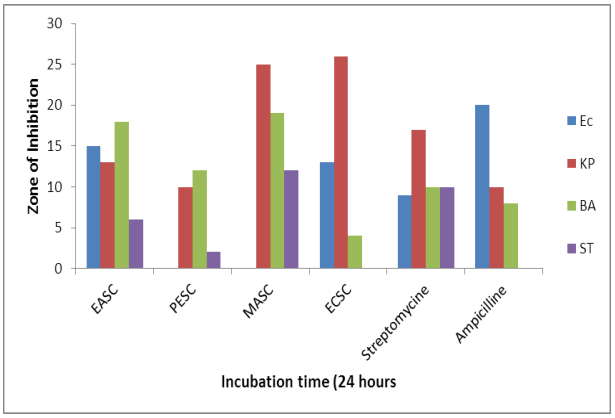

Figure 1: Comparative Antibacterial Activity of Eria alba Crude extract and its various fractions against the test organisms.

Antifungal activity of *Eria* alba Crude extract and its various fractions

The antifungal activities of *Eria* alba crude extract and its various fractions gave different zone of inhibition on the fungal organisms tested (Table 2). 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 *Eria* alba compared with different standard shown in (Figure 2).

Table 2: Zone of Inhibition (mm) of *Eria* alba 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

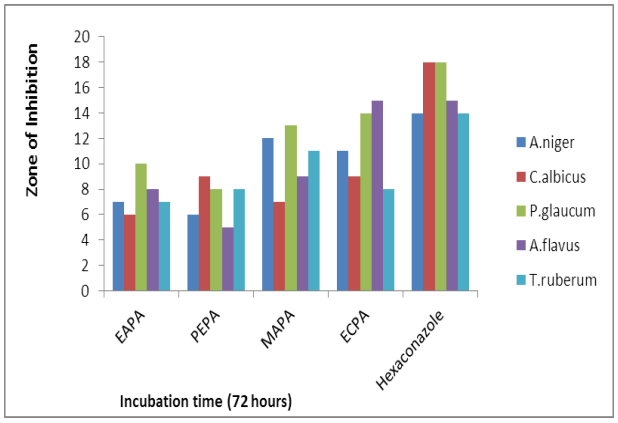

Figure 2: Comparative Antifungal Activity of *Eria* alba Crude extract and its various fractions against the fungal species.

CONCLUSIONS

The antibacterial and antifungal study is done for Eria alba crude extract and its various fractions. The *Eria* alba ethyl acetate extract showed more potent against all isolates of bacteria. The methanolic extract showed good activity against K. pneumonia, B. aureus, and S. typhi and did not show any effect of E. coli. The crude extract and methanolic extract showed more potent against T. ruberum, A. flavus and A. niger.

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