EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 11
338-341
Year: 2017

IN VITRO ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF ERIA ALBA AGAINST HUMAN PATHOGENS

Darshan Singh*, Satish C. Sati and Manisha D. Sati

Department of Chemistry, HNB Garhwal University Srinagar, 246174, Uttrakhand, India.

***Corresponding Author: Darshan Singh**
Department of Chemistry, HNB Garhwal University Srinagar, 246174, Uttrakhand, India.

Article Received on 04/09/2017 **Article Revised on 25/09/2017** **Article Accepted on 16/10/2017**

ABSTRACT
The antibacterial, antifungal and antioxidant study is done for Eria alba crude extract and its various fractions. The E. alba Crude extract and methanolic extract showed more potent against E. coli, P. aeruginosa, and B. aureus. The ethyl acetate and petroleum ether extract showed potent against E. coli and B. aureus. The crude extact and methanolic extract showed low activity and ethyl acetate and petroleum ether extract did not showed against P. aeruginosa baeteria. Antifungal activity is significant forE. alba crude extract and methanolic extract. while poor for Ethyl acetate and petroleum ether fractions. Antioxidant activity was shown by petroleum ether, crude ethanolic extract, ethyl acetate and methanolic fractions. The methanolic extract of E. alba and ethyl acetate fraction had significant scavenging effects with increasing concentration in the range of 100-400µg/mL

KEYWORDS: Eria alba, Orchidaceae, antibacterial, antioxidant, 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).

MATERIALS AND METHODS
Collection and identification of plant materials: Eria alba (Orchidaceae) whole plants were collected from the Guptakashi, Rudraprayag Uttrakhand, India in September-October 2013. The plant was identified from 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 pharmacological /biological activities.

Determination of Antibacterial activity
Collection of test organism and preparation of stock culture: The Four species of bacteria, one gram-positive (B. aureus) and three gram-negative (E. coli, P. aeruginosa and P. vulgaris) 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 corn 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).

Table1: Zone of Inhibition (mm) of E. alba extract and its various fractions tested for antibacterial activity.

Microorganisms(0.1ml)	Zone of Inhibition (mm)					
	MAEA (10mg/ml)	EAEA (10mg/ml)	PEEA (10mg/ml)	CEEA (10mg/ml)	Streptomycin (1mg/ml)	Ampicillin (1mg/ml)
E. coli (Ec)	19	16	14	22	19.3	15.1
P. vulgaris (Pv)	17	7	6	20	16.6	20.6
P. aeruginosa (Pa)	8	-	-	6	-	13.8
B. aureus (Ba)	23	18	12	19	-	18.5

Abbrevation: MAEA = Methyl alcohol E.alba soluble extract; EAEA = Ethyl acetate E.alba soluble extract; PEEA = Petrolium ether E. alba soluble extract; CEEA = Ethyl alcohol Crude extract E.alba; Genta=gentamicin; Ampi= Ampicillin; EC= Escherichia coli, PV = P. vulgaris, BA = Bacillus aureus, PA= P. aeruginosa

Determination of Antifungal Activity: The minimum inhibitory concentration (MIC), of plant extracts against the toxigenic strain of A. flavus (LHPpv1) was determined by poisoned food technique reported (I. Suln, et al 2011). Requisite amount of the extracts were prepared in different solvent system and incorporated to 9.5 mL PDA (potato dextrose Agar) Petri dish to achieve different concentrations at (0.50 to 5.0 µL mL-1). Thereafter, 25µL spore suspension (containing 106spores mL-1) of toxigenic strain of A. flavus (LHPpv1) was added to the control as well as to the treated sets. The PDA plate, without extract was treated as control set. The Petri dishes were kept in BOD incubator for 10 days incubation period (27±2 °C). The lowest concentration of extracts that inhibited the complete growth of test mould was taken as MIC.

Determination of Antioxidant Activity: The radical-scavenging capacity of E.alba Cr. extract and its various fractions of E.alba was determined using the DPPH radical method. A 2 mL aliquot of test solutions was added to 2 mL of 2 x 10-4 mol L-1 ethanolic DPPH solution. The mixture was shaken vigorously and the absorbance was measured at 517 nm immediately. All the tests were performed in triplicate and mean values calculated. The antioxidant activity was expressed according to the ability of an extract to scavenge DPPH free radicals and was determined using the following equation: % Inhibition = [1- (A1-A2)/A0] x 100

Where A0 is the absorbance of negative control (original DPPH sample without sample), A1 is the absorbance of test sample (DPPH sample in presence of sample) and A2 is the absorbance of sample without DPPH [B. Halliwell, et al 1985]. The IC50 value is the concentration (µg mL-1) of extract/standard necessary to reduce the absorbance of DPPH by 50% compared to the negative control. The IC50 was determined by interpolation from linear regression analysis of the antioxidant activity (% Inhibition) against sample concentration (µg mL-1) and the IC50 value decreases as a function of increasing antioxidant activity of samples.

RESULTS AND DISCUSSION

Antibacterial activity: The antibacterial activities of E.alba Crude extract and its various fractions gave different zones of inhibition on the organisms tested (Table 1).The E.alba crude extract and methanolic extract inhibited the growth of four isolates of bacteria. The E.alba Crude extract and methanolic extract showed more potent against E. coli, P.aeruginosa, and B. aureus.The ethyl acetate and petroleum ether extract showed potent against E. coli and B. aureus.The crude extact and methanolic extract showed low activity and ethyl acetate and petroleum ether extract did not showed against P.aeruginosa baeteria. The antibacterial activities of various fractions of E.alba compare with different standard shown in (Figure 1).

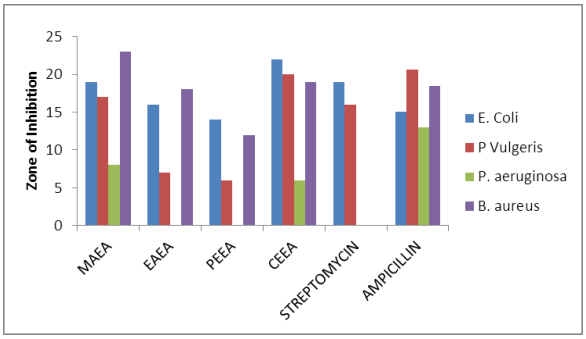

Figure 1: Antibacterial activity of E.alba Crude extract and its various fractions against the test organisms.

Antifungal Activity: All the concentration of crude extract of E.alba and it various fraction were found significantly effective over control after Ten days according to ANOVA and tukeys comparison test (Table 2), during antifungal assay . A corresponding decrease in mycelia growth was recorded with increased concentration of crude extract of E. alba and its various fraction. At 1000 ppm, A flavus $LHP_{PV}1$ was inhibited 62.202%, 58.464% and 8.532% and 8.431% against CEEA, MEEA, EAEA and PAEA respectively and at different concentration is given in Table 2. Complete inhibition of A.Flavus for crude extract of E.alba and its various fraction are given in Table 3.

Table 2: Average dimeters of colony of Aspergillus flavus $LHP_{PV}1$ and percentage mycelia inhibition by different plant extracts.

Conc. (ppm)	Dimeter (Mean ± S.E) of colony of Aspergillus Flavus $LHP_{PV}1$ (mm)				% Mycelial Inhibition			
	CEEA	MEEA	EAEA	PAEA	CEEA	MEEA	EAEA	PAEA
Control	6.936±0.053	6.900±0.152	7.033±0.088	7.011±0.066	-	-	-	-
500	5.033±0.033	5.866±0.088	6.700±0.000	6.500±0.000	27.742	14.984	4.734	4.657
1000	0.000±0.000	2.866±0.120	6.433±0.033	6.411±0.044	62.203	58.462	8.531	8.164
1500	0.000±0.000	0.533±0.088	6.123±0.021	6.122±0.022	100.000	95.325	11.789	12.523
2000	0.000±0.000	0.000±0.000	5.900±0.057	5.890±0.047	-	100.00	16.524	16.005
2500	0.000±0.000	0.000±0.000	5.333±0.033	5.311±0.001	-	-	24.523	24.071
3000	0.000±0.000	0.000±0.000	4.833±0.066	4.814±0.056	-	-	32.245	31.181
3500	0.000±0.000	0.000±0.000	4.200±0.100	4.100±0.000	-	-	40.281	40.521
4000	0.000±0.000	0.000±0.000	3.733±0.066	3.722±0.052	-	-	46.927	46.625
4500	0.000±0.000	0.000±0.000	3.300±0.152	3.200±0.052	-	-	53.072	53.054
5000	0.000±0.000	0.000±0.000	2.833±0.007	2.811±0.005	-	-	59.715	59.625

Table 3: MIC values of E. alba Crude extract and its various fraction.

Extract	E. alba Crude Extract	MEEA	EAEA	PAEA
MIC (ppm.)	1500	2000	>5000	>5000

Antioxidant activity

Our results found that the various plant extract of E.alba is effective at reducing the stable radical DPPH to the yellow- coloured di phenyl picryl hydrazyl indicating that the extract is active in DPPH radical scavenging. The methanolic extract of E.alba and ethyl acetate fraction had significant scavenging effects with increasing concentration in the range of 100-400µg/mL. At a concentration of 400µg/mL, the scavenging activity of methanolic extract of E.alba and ethyl acetate reached 85.664 % and 90.035% respectively. which is better than BHT, while at the concentration that of crude extract and petroleum ether extract was 50.452% and 25.125% (figure 2).The IC_{50} values (µg/mL) of different extract are given in Table 4.

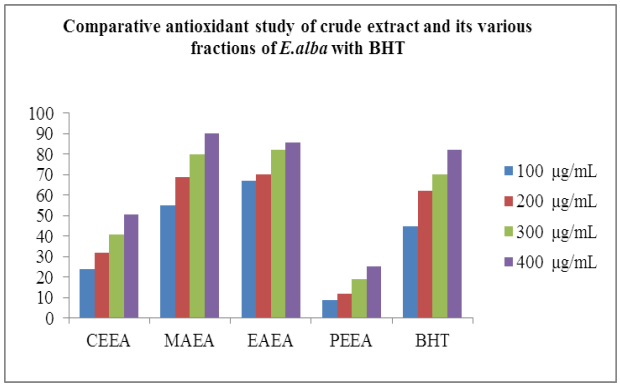

Figure 2: DPPH scavenging activities of the different solvent extracts of Eria alba.

Table 4: Inhibitory concentration (IC_{50}) of E.alba crude extract and its various fraction.

Concentration (400µg/mL)	CEEA	MEEA	EAEA	PE EA	BHT
IC_{50}	408.047	68.725	54.135	838.645	171.054

Applications

Antimicrobial medicinally plants are most potential candidate for providing novel drug with new mechanism of action. They kill or inhibit the growth of micro-organism such as bacteria, fungi or protozoan's. The antioxidant supplements or foods rich in antioxidants may be used to help the human body in reducing oxidative damage by free radicals and active oxygen. The role of active oxygen and free radicals in tissue damage in such diseases, are becoming increasingly recognized [K. Yagi et al 1987].

CONCLUSIONS

E.alba was screened for the first time for antioxidant activity. The Crude extract and methanolic fractions were found strong DPPH scavenging activity. We found strong antibacterial activity specifically in the Crude extract and methanolic extract showed against E. coli, P.aeruginosa, and B. aureus and strong antifungal activity for methanolic, E.alba Crude extract against Aspergillus flavus LHPpv1. The present work revealed that the plant could be used for Herbal medicine. In conclusion, E.alba 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.

ACKNOWLEDGEMENTS

This work was financially supported by UGC New Delhi under the Fellowship Scheme. The authors pay their sincere thanks to my supervisor for their valuable suggestions to improve this article.

REFERENCE

1. Gaur. R. D, " Flora of the District Garhwal North West Himalaya Trans media , media house Srinagar Garhwal, 1999; 741.
2. Agrawala, D.K., Taxonomic studies on the genus Eria Lindl. (Orchidaceae) in India. Ph D. Thesis submitted to Kalyani University, West Bengal, 2009.
3. I. Suln, J. Zhang, X. Lu, L. Zhang, Y. Zhang. Evaluation to the antioxidant activity of total flavonoids extract from persimmon (Diospyros kaki L.) leaves, Food and Chemical Toxicology, 2011; 49: 2689-2696.
4. A. Hasan, M.A. Khan, M. Ahmad. Authenticity of folk medicinal plants of Pakistan, Taxon Chem Methods, 2007; 1: 1-5.
5. U. Dhar, R. S. Rawal, J. Upreti, Setting priorities for conservation of medicinal plants - A case study in the Indian Himalaya, 2000; 57-65.
6. T.K. Mukherjee, Protection of Indian traditional knowledge. Editors. Trivedi PC, Sharma Etnomed NK. Palnt, 2004; 18-33.
7. K. Yagi, Lipid peroxides and human disease, Chemistry and Physics of Lipids, 1987; 45: 337–341.
8. B. Halliwell, J. M. C Gutteridge, Free Radicals in Biology and Medicine, Oxford University Press, Oxford, UK, 1985.