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Preliminary phytochemical screening, antibacterial and antioxidant activity of *Eria pseudoclavicaulis* Blatt. -An endemic orchid of Western Ghats

Sahaya Shibu B[1*], Sarmad Moin[1], Servin Wesley P[1], Chitra Devi B[2]

1. Department of Biotechnology, Karpagam University, Coimbatore, India

2. Department of Botany, Karpagam University, Coimbatore, India

ABSTRACT

This study was performed to evaluate the presence of Phytochemical, antimicrobial and antioxidant activity of *Eria pseudoclavicaulis* Blatt. (Orchidaceae) leaf extracts. Preliminary phytochemical analysis revealed that the ethyl acetate extract shown the maximum phytochemical constituents followed by ethanol and water. Different extracts of *Eria pseudoclavicaulis* were tested for antimicrobial activity of this, ethyl acetate extract shown the maximum antibacterial activity against five microorganisms. The water extract possess strong 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (IC_{50} 318 µg/ml), This research findings suggest that *Eria pseudoclavicaulis* exhibits potential antimicrobial and antioxidant properties.

Key words: phytochemical analysis, antimicrobial, antioxidant, *Eria pseudoclavicaulis*

*Corresponding Author Email: shibubt@gmail.com

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INTRODUCTION

Plants are the oldest source of bio active compounds, and provided humankind with many medically useful compounds for centuries[1].These bioactive compounds in plants are produced as secondary metabolites such as alkaloids, triterpenoids, flavonoids, stilbenoids, steroids and phenolic compounds[2], which may be stage specific or organ specific or tissue specific properties[3]. Many orchid species are traditionally used in herbal medicine as a remedy for microbial infections and other ailments. However, the potential of most of the orchid species for therapeutic use is yet to be scientifically explored. The usage of orchids as old traditional and herbal medicine in different countries for their therapeutic uses.

As early as 200 BC the Chinese Materia medica (Shong Nung Pen – Gsao ching) mentions *Dendrobium* as a source of tonic, astringent, analgesic and anti-inflammatory substance, *Vanilla planifolia* used in treating hysteria, rheumatism and other low forms of fever.

Figure 1: *Eria pseudoclavicaulis*

In addition, this orchid contains alkaloids, flavonoids, glycosides, carbohydrates and other phytochemicals[4] which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids[5, 6]. Some plants like *Dendrobium crumenative*, *Eulopia campestris*, *Orchis latifolia*, *Vanda roxburghii* and *vanda tessellate* have been documented for their medicinal value. *Dendrobium nobile* is used in freshly cut wounds for quick healing .Leaves of *Chleisostoma williamsoni* are used for bone fracture by Monpa tribal of Arunachal Pradesh. *Eulophia noda* tuber extracts are used for blood purification. The native inhabitants of some areas use seeds of *Cymbidium madidum* and pseudobulbs of *Dendrobium tukai* as oral contraceptive[7]. Other orchid genera like *Oberonia*, *Eria*, *Bulbophyllum*, *Eulophia*, *Geodorum*, *Grammatophylum*, and *Hetaeria* are also reported to be used as medicine in different parts of the world to cure various diseases[8, 9].

In fact there are several studies which have revealed the presence of such compounds with antimicrobial and antioxidant properties. However, this plant has not been scientifically validated for its phytochemical, antimicrobial and antioxidant activities. Hence, the current study was initiated to determine antimicrobial and antioxidant potential of *Eria pseudoclavicaulis* for the first time.

MATERIALS AND METHODS

Plant material

Eria pseudoclavicaulis, leaves were collected from the National Orchidarium & Associated Garden, Botanical Survey of India (Southern Regional Centre), Yercaud, India.

Preparation of plant extracts

The *Eria pseudoclavicaulis* leaves were washed and shade dried, then ground into fine powder. The extraction was carried by soxhlet extraction technique. Different solvents were used successively with gradient polarity (petroleum ether, chloroform, ethyl acetate, ethanol and water). The extracts were completely evaporated by vacuum distillation and stored.

Phytochemical screening

Phytochemical screening was carried out according to the methods described by[10, 11, 12].

Test Microorganisms

The organisms included *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Enterococcus faecalis* ATCC 29212 (*E. faecalis*), *E.coli* (MTCC 40) *Staphylococcus aureus* ATCC 25923 (*S. aureus*), *Bacillus subtilis* MTCC 2393 (*B. subtilis*), *Salmonella enterica* MTCC 98 (*S. enterica*) and *Corynebacterium* sp. MTCC 3080 (*Corynebacterium* sp.). All the strains were collected from American Type Culture Collection, Manassas, USA and Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The microorganisms were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C.

Disc diffusion method

All the extracts of *Eria pseudoclavicaulis* leaves were screened for antimicrobial activity using disc diffusion method[13] using potato dextrose agar (Hi-media, Mumbai). 18 h old cultures of microorganisms maintained in Potato Dextrose Broth (Hi-media, Mumbai) of the test organisms were used. Sterilized discs (Hi media, 6 mm), soaked in a known concentration of the crude extracts of *Eria pseudoclavicaulis* (500 µg/ml DMSO of per disc). Soaked discs were applied over each of the culture plates previously seeded with the 0.5 McFarland (for bacteria) and antibiotic discs of Chloramphenicol (30 mcg/disc) were used as positive control and paper discs

with DMSO were used as negative controls. Incubations were done at 37°C for 24 - 48 h for bacteria. Zones of inhibition were measured, and the mean diameter was recorded.

Determination of Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was carried out according to the methods of[14] with little modification. A stock concentration 1mg/ml of the extract was prepared. From the stock, 50, 100, 150, 250, 500, 750 and 1000µg of each concentration were added to each 9ml of nutrient broth containing 0.1ml of standardized test organisms. The tubes were incubated at 37°C for 24h. A positive control was equally set up by using DMSO and test organisms without extract. The tube with least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration.

Antioxidant activity

All the five extracts of *Eria pseudoclavicaulis* and ascorbic acid were dissolved in 100% dimethyl sulfoxide (1 mg/ml) separately and used for the *in vitro* antioxidant assay. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) based on[15] to investigate the antioxidant properties.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of *Eria pseudoclavicaulis* showed a wide range of chemical constituents. The preliminary phytochemical analysis revealed that the ethyl acetate extract showed the maximum phytochemical constituents followed by ethanol and water. Similar results were observed during the phytochemical analysis of *Papilionanthe teres* and *Coelogyne stricta*[16, 17]. Since there was a lack of studies in the phytochemical analysis of orchids the present study revealed the presence of alkaloids, carbohydrates, saponins, phytosterol, phenolic compounds, terpenoids, and tannin in ethyl acetate extract (Table 1). The presence of saponin, alkaloids, and tannin enhanced the antimicrobial activity against the pathogenic microorganisms[18]. Various extracts of *Eria pseudoclavicaulis* were tested for the antimicrobial activity against various microorganisms, of this ethyl acetate and ethanol extract shows the maximum number of inhibition against the microbes used (Table 2). The diameter of inhibition zones for each extract with respect to microbes were compared with positive control standard antibiotic (Chloramphenicol 30 mcg/ disc) and with negative control. The highest activity and zone of inhibition were recorded when ethyl acetate extract used against *Enterococcus faecalis* (13 mm), *Bacillus subtilis* (13 mm), and *Pseudomonas aeruginosa* (12mm). This result is similar to the finding of Sarmad[17] in *Coelogyne stricta* orchid endemic to Western Ghats.

Table 1: Preliminary phytochemical screening of *Eria pseudoclavicaulis* leaf extracts

S.No	Phytochemical tests	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water
1.	Alkaloids	+	+	+	+	+
2.	Carbohydrates	-	+	+	+	+
3.	Glycosides	-	+	+	+	+
4.	Saponins	-	-	+	+	-
5.	Terpenoids	-	-	+	+	+
6.	Steroids	+	+	+	-	-
7.	Flavonoids	+	+	+	-	-
8.	Phenolic compounds	+	-	+	+	+
9.	Protein	-	-	-	-	-
10.	Amino acids	-	-	-	-	-
11.	Anthroquinones	-	-	-	-	-
12.	Fats and oils	+	+	+	-	-
13.	Carotenoids	-	-	-	-	-
14.	Gum and Mucilages	-	-	-	-	-
15.	Phytosterol	-	+	+	+	+
16.	Tannins	-	-	-	+	+
17.	phlobatannins	-	-	-	+	+

Where + Present, - Absent

Minimum Inhibitory Concentration of the ethyl acetate extract from *Eria pseudoclavicaulis* showed the strongest antibacterial activity with MIC value of 0.350mg/ml against *Enterococcus faecalis* and *Bacillus subtilis* (Table 3). These results states that the ethyl acetate extract of *Eria pseudoclavicaulis* shows strong antimicrobial activity. The antioxidant activity was determined based on the IC_{50} values in DPPH assay and it was calculated from the logarithmic regression curve. The IC_{50} values of the various extracts are shown in figure 2. The best free radical (DPPH) scavenging activity was observed in water extract of *Eria pseudoclavicaulis* leaves with IC_{50} value 318.0 µg, similarly, the free radical scavenging activity was tested by DPPH assay in various orchids by Guddadarangavvanahally[19]

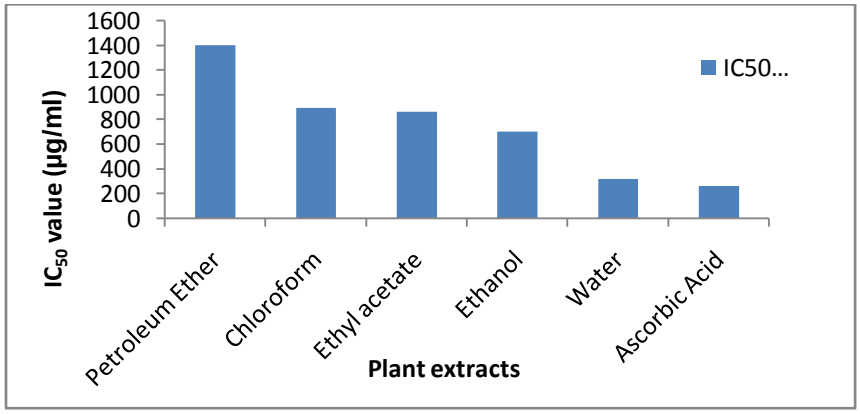

Figure 2: DPPH radical scavenging activity of *Eria pseudoclavicaulis*

Table 2: Antibacterial activity of *Eria pseudoclavicaulis* leaf extracts

S. no	Organism	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water	Positive control Chloramphenicol
1	*Pseudomonas aueroginosa* (ATCC 27853)	7 mm	10 mm	12 mm	11 mm	8 mm	32 mm
2	*Enterococcus facealis* (ATCC 29212)	-	10 mm	13 mm	6 mm	-	36 mm
3	*E.coli* (MTCC 40)	6 mm	5 mm	10 mm	9 mm	10 mm	35 mm
4	*Staphylococcus aureus* (ATCC 25923)	-	-	-	-	10 mm	32 mm
5	*Bacillus subtilis* (MTCC 2393)	6 mm	8 mm	13 mm	9 mm	-	33 mm
6	*Salmonella enterica* (MTCC 98)	-	-	-	-	-	29 mm
7	*Corynebacteria spp.* (MTCC 3080)	6 mm	9 mm	10 mm	11 mm	10 mm	32 mm

Table 3: Minimum Inhibitory Concentrations of ethyl acetate extract of *Eria pseudoclavicaulis*

S. No.	Organisms	Minimum Inhibitory Concentrations (µg/ml)
1	*Pseudomonas aueroginosa* (ATCC 27853)	400 µg
2	*Enterococcus facealis* (ATCC 29212)	350 µg
3	*E.coli* (MTCC 40)	450 µg
4	*Staphylococcus aureus* (ATCC 25923)	650 µg
5	*Bacillus subtilis* (MTCC 2393)	350 µg
6	*Salmonella enterica* (MTCC 98)	700 µg
7	*Corynebacteria spp*. (MTCC 3080)	400 µg

CONCLUSION

This study reports the phytochemical analysis, antimicrobial and antioxidant activities of *Eria pseudoclavicaulis* leaf extracts for the first time and the plant also shows high biologically active components, hence further more work is necessary to isolate the bioactive compounds from the plant to carry out for pharmaceutical use**.**

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