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Evaluation of Anti-Oxidant Activity by DPPH Radical Scavenging Method of *Eulophia ochreatea Lindl.*



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ABSTRACT

The *Eulophia ochreatea Lindl* is an important medicinal plant from Orchidaceae family and it has diverse biological activity. It is used in traditional folk medicine system in most part of the Maharashtra district. Based on survey, herbs used for the review has well-established histories of human use for the treatment of rheumatic and arthritic conditions. Antioxidants play an important role as health protecting factor. Hence, there is urgent need to utilize antioxidant activity knowledge of *Eulophia ochreatea L.* to bring its maximum potential in the field of medical and pharmaceutical sciences in novel herbal drug development which will economically benefited for common man. So this research paper shows the antioxidant activity of *Eulophia Ochreatea Lindl.* In this study, Antioxidant activity was performed by DPPH (1, 1diphenyl-2-picrylhydrazyl) radical scavenging method for Chloroform extract of tubers and rhizomes of *Eulophia Ochreatea Lindl.* Plant species which showed that chloroform extract of this plant on higher concentration possess better antioxidant potential when compare to reference standard ascorbic acid. The strongest antioxidant activity of chloroform extract could be due to the presence of flavonoids, phenolic compounds and alkaloids.

INTRODUCTION

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological roles in the body. There is a global interest in non-synthetic, natural drugs derived from plant sources, because of low cost, nontoxic nature, and availability. Many plants with antioxidant potential possess flavonoids and phenolic compounds¹. Antioxidants play an important role as health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables². Plant sourced antioxidants like vitamin C, vitamin E, carotenes, phenolic acids etc. have been recognized as having the potential to reduce disease risk³. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties.⁴ A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity⁵. The DPPH assay method is based on the reduction of DPPH, a stable free radical.⁶ The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple color). When Antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical scavenging antioxidant) and is reduced to the DPPHH and as consequence, the absorbance's decreased from the DPPH.⁷

Antioxidant-based drugs and formulations for the prevention and treatment of complex diseases like Alzheimer's disease and cancer have appeared during last three decades⁸. Recent studies have shown that anumber of plant products including polyphenols, terpenes and various plant extracts exerted an antioxidant action⁹⁻¹².

About *Eulophia ochreata* L¹³

E. ochreata, commonly known as 'Amarkand' or 'Singadyakand', is a ground orchid in the family Orchidaceae. It is a perennial tuberous herb and usually appears in the forest during rainy season in shady rainforests. It is a herb growing in dry deciduous forest in huge patches and on slope where the soil is deep. It is terrestrial, perennial herbs, pseudobulbs ovoid-conical, marking

irregular, transverse longitudinal, leaves 2-5, sheathing at base, oblong-lanceolate to ovate-elliptic. Flowers in dense racemes clustered at top of scape, corolla yellow, and capsules broadly ovoid, deflexed, strongly ridged, and green. Ehao botanical survey of the forest areas of Maharashtra revealed that these tubers are used as a specialty food, general tonic and as rejuvenating herb. It has been used by the tribes for properties like astringent, anti-fatigue, aphrodisiac, anti-helminthic, and as a blood purifier. The tubers are also used in cough, cold and heart troubles.

Tribals have been using tubers as a general tonic and as rejuvenating since long. On the basis of these prominent uses of *E. ochreatea*, tubers were selected for my further work. The lack of well-documented scientific evidence will predominantly impede the progress of isolated molecule in the avenue.

Photos of *Eulophia ochreatea* Lindl¹³



Fig No.1 Single Tuber with Rhizomes

Phytochemical constituents^{13,14,15}:

Orchids have been used as a source of medicine for millennia to treat different diseases and ailments including tuberculosis, paralysis, stomach disorders, chest pain, arthritis, syphilis, jaundice, cholera, acidity, eczema, tumour, piles, boils, inflammations, menstrual disorder, spermatorrhea, leucoderma, diarrhea, muscular pain, blood dysentery, hepatitis, dyspepsia, bone fractures, rheumatism, asthma, malaria earache, sexually transmitted diseases, wounds and sores. Besides, many orchidaceous preparations are used as emetic, purgative, aphrodisiac, vermifuge, bronchodilator, sex stimulator, contraceptive, cooling agent and remedies in scorpion sting and

snake bite. Some of the preparations are supposed to have miraculous curative properties but rare scientific demonstration available which is a primary requirement for clinical implementations. Incredible diversity, high alkaloids and glycosides content, research on orchids is full of potential. The *Eulophia* species reported containing eullophiol, Nudol¹⁷, β - sitosterol¹⁶, ephemeranthal, fimbriol etc. as active phyto constituents¹⁸

Therapeutic uses^{13,14,15}:

Recently, established an evidence for the usage of *E. ochreatea* as an antioxidant and two active molecules responsible for it since the tubers of *E.ochreatea* have been used in folk medicine for rejuvenating, aphrodisiac and anti-rheumatic properties. Also used as for antiproliferative activity against human breast cancer. They are also used as Antipyretic, Immunomodulatory, Antianaemic, Nutritional, Anti-Diarrhoeal, Anti-Dysentery, Belly-ache, Anti- Asthamatic, Anti Bronchitis, Aphrodisiac, Anti-rheumatic, Anti fatigue, Skin Dis. Protective, Wound Healing, Antibacterial, Anti-Tumor Rejuvenating in general health.

MATERIALS AND METHODS

1) Plant material :

According to flora of Kolhapur district, localities for *Eulophia ochreatea* Lindl in Kolhapur region are Mahalunge, Panhala Turrukwadi¹⁹. *Eulophia Ochreatea* Lindl. Plant species along with tubers and rhizomes were collected from the Bhimashankar region. The plant was identified and authenticated by Dr. G. G. Podar, HOD, Department of Botany, Y. C. College of Science, Karad and Voucher specimen was deposited at the same college as number AAK1.

2) Preparation of the extract:

The 60 gm dried plant was extracted with the help of soxhlet extraction using solvent pet ether, chloroform, methanol, water. Chloroform extract was tested in vitro models for evaluation of antioxidant activity of plant.

3) Drugs and Chemicals:

DPPH was procured from the Research lab Islampur. All the other chemicals were of analytical grade obtained commercially. Double distilled water was used throughout the study.

4) Evaluation of Anti-Oxidant effects by DPPH Radical Scavenging Method²⁰

Free radical scavenging activity of different extracts of rhizomes of *Eulophia ochreatea Lindl plant* was measured by 1, 1- diphenyl-2-picrylhydrazyl (DPPH). In brief, 0.1 mM solution of DPPH in ethanol was prepared. This solution (3 ml) was added to 3 ml. of different extracts in ethanol at different concentration (5, 10, 15, 20, 25, 30 µg/ml). Here, only those extracts are used which are solubilise in ethanol (Chloroform extract was selected for activity) and their various concentrations were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. Then, absorbance was measured at 517 nm, by using spectrophotometer (UV-VIS Shimadzu). Reference standard compound being used was ascorbic acid and experiment was done in triplicate. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated by using following equation:

$$\text{DPPH scavenging effect (\%)} \text{ or Percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100.$$

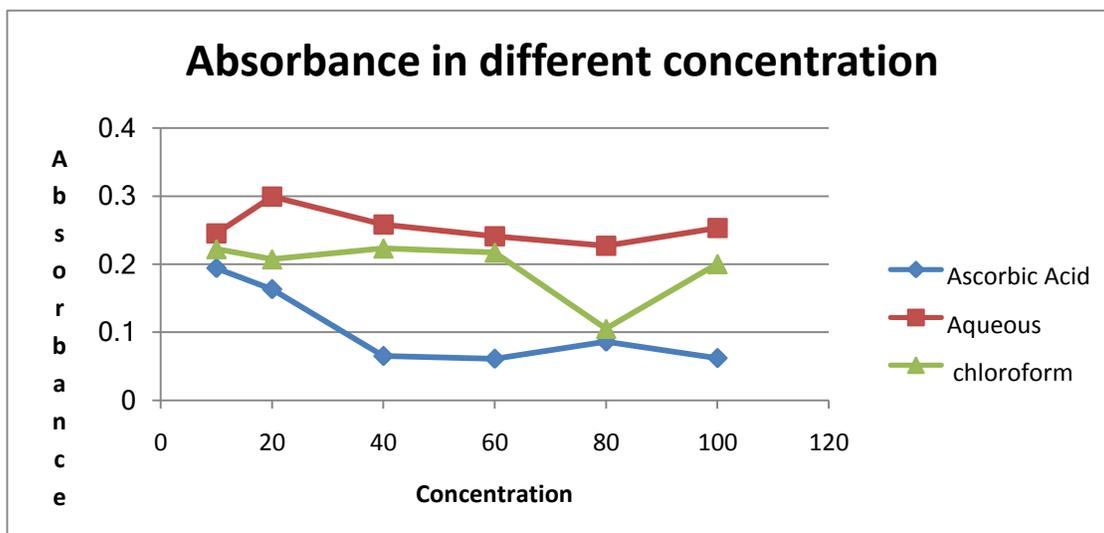
Where

A₀ was the Absorbance of control reaction and

A₁ was the Absorbance in presence of test or standard sample.

Table 1 – Absorbance of Chloroform Extract of *Eulophia Ochreatea Lindl* with standard Ascorbic Acid at 517 nm by UV-visible spectrophotometer (DPPH scavenging method)

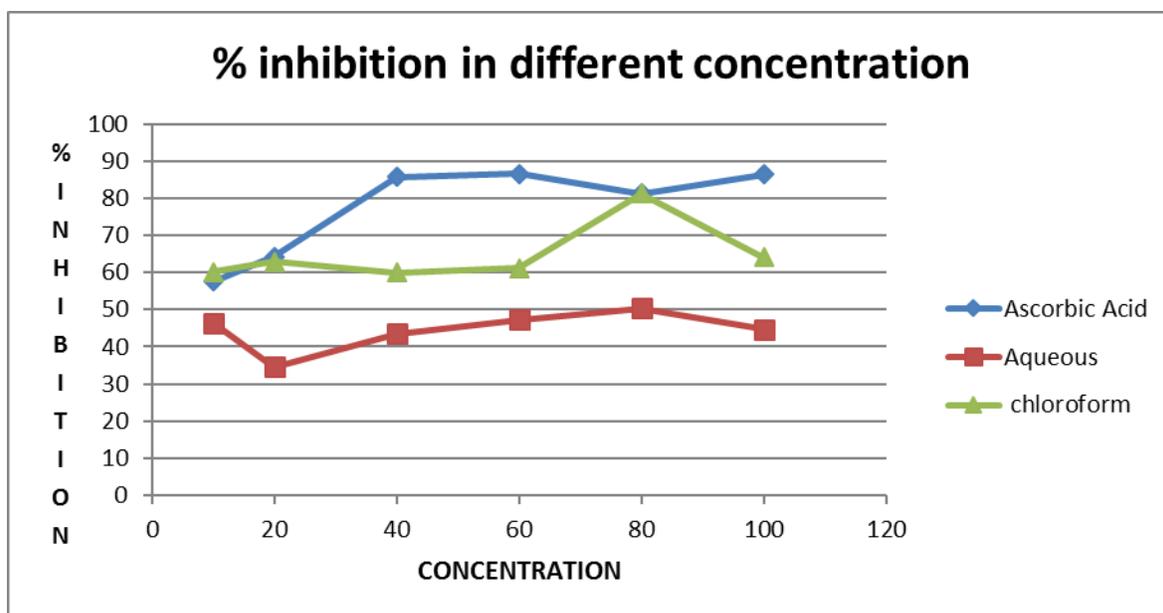
Concentration (µg/ml)	Ascorbic Acid (Abs)	Aqueous (Abs)	Chloroform (Abs)
10	0.194	0.245	0.222
20	0.163	0.299	0.207
40	0.065	0.258	0.223
60	0.061	0.241	0.217
80	0.086	0.227	0.105
100	0.062	0.253	0.2



Graph1.Absorbance in different concentration

Table 2. % inhibition of different extract of *Eulophia Ochreta Lindl.* with Ascorbic acid

Concentration (µg/ml)	Ascorbic Acid (% inhibition)	Aqueous (%inhibition)	Chloroform (% inhibition)
10	57.54	46.38	60.21
20	64.33	34.57	62.9
40	85.77	43.54	60.03
60	86.65	47.26	61.11
80	81.18	50.32	81.18
100	86.43	44.63	64.15



Graph 2. % inhibition in different concentration

RESULT

In the current study in-vitro results confirm the antioxidant activity of *Eulophia Ochrata Lindl.*

The rhizomes of chloroform extract of this plant showed better antioxidant potential when compare to standard ascorbic acid by DPPH scavenging assay method. % inhibition obtained was 81.18 % and 81.18 % µg/ml. same for ascorbic acid and chloroform extract respectively. The % inhibition of chloroform extract is similar to ascorbic acid. It means chloroform extract of plant captured same free radicals formed by DPPH resulting into decrease in absorbance and increase in % inhibition.

CONCLUSION

This study determined that Chloroform extract of rhizomes of *Eulophia Ochreata Lindl* plant species showed better antioxidant potential by DPPH radical scavenging method when compare to standard ascorbic acid and % Inhibition was found to be as 81.18 and 81.18 µg/ml for ascorbic acid and chloroform extract respectively. So, we can say this plant is having antioxidant activity.

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