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# Phytochemical screening, antimicrobial activity, antioxidant activity of chlorform and ethanol extract *Eulophia ochreata.*

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***Abstract***:-The present research work explores the phytochemical and biological properties of *Eulophia ochreata*, a terrestrial orchid species with significant ethnobotanical importance. The tubers of *Eulophia ochreata* were selected for the study, and the plant material was identified with the assistance of local residents, forest guards, and botanists. The study focuses on evaluating the antimicrobial and antioxidant activities of the bioactive compounds extracted from *Eulophia ochreata*, along with determining the phenolic and flavonoid content.

**Introduction**

*Eulophia ochreata,* commonly known as Amarkand or Singadyakand, is a perennial herb belonging to the Orchidaceae family. This plant species holds medicinal significance and has been traditionally used by various ethnic tribes for its therapeutic properties[1]. The genus *Eulophia* comprises terrestrial orchid species distributed across India, including Maharashtra, Andhra Pradesh, Chhattisgarh, and other regions. The study area, Amba Barwa forest in Buldhana district, Maharashtra, is rich in tropical evergreen plants, providing a diverse landscape for medicinal plants like E. ochreata. The research aims to investigate the phytochemical composition[2,3] and biological activities of *Eulophia ochreata* to uncover its potential as a source of bioactive compounds with therapeutic benefits[3,4,5].

**Material and Methods**

**3.1 Sample Collection**

The tubers of *Eulophia ochreata* were collected from the Amba Barwa forest area in Buldhana district, Maharashtra, during September-October 2019. The plant material ( Fig.1) was identified with the help of local residents, forest guards, and botanists.

**3.2 Isolation and Extraction**

The collected plant material was shade-dried, powdered, and extracted using Soxhlet apparatus with ethanol and chloroform as solvents.

**3.3 The authentication**

The authentication of the plant material was done by Dr. V. U. Pochhi, Professor, Department of Botany, Shri Shivaji Science and Arts college, Chikli, Buldhana district.


**Figure.1** Images of *Eulophia ochreata* plant, flowers and tuber.

### 3.4 Antimicrobial Activity and Phytochemicals

The antimicrobial activity of the extracts was evaluated against E. coli, ***K. pneumoniae, S. aureus and E. faecalis*** and the phytochemical analysis was conducted to identify bioactive compounds. 6 The results of the antimicrobial activity study of the crude extracts in ethanol A showed the most efficient antibacterial activity against *E. coli*, *K. pneumoniae*, *E. faecalis* except that of *S. aureus*. Gentamicin (NA, 29 mm, NA, 27 mm), Control (NA, 15 mm, NA, 17 mm) 50mg (NA, 12 mm, NA, 15 mm), 100 mg (10 mm, 13 mm, NA, 17 mm) 200 mg (13 mm, 13 mm, NA, 16 mm), Ethanol B Gentamicin (NA, 29 mm, NA, 27 mm), Control (NA, 10 mm, NA, 14 mm), Control (NA, 10 mm, NA, 14 mm), 50mg (NA, 11 mm, NA, 15 mm), 100 mg (NA, 20 mm, NA, 14 mm), 200 mg(13 mm, 25 mm, 10 mm, 25 mm), Chloroform A Gentamicin (19 mm, NA, 22 mm, NA), Control (13 mm, NA, NA, NA), 50mg(12 mm, NA,11 mm, NA), 100mg (12 mm, NA, 13 mm, 10 mm), 200mg (14 mm, 9 mm, 19 mm, 12 mm). While in case of chloroform extracts efficient activity was observed in case of *E. coli* and *S. aureus*. The given numbers are the mean value of the analysis carried out in triplicate.

| **Samples** | | **Zone of Inhibition (mm)** | | | |
|---|---|---|---|---|---|
| | | ***E. coli*** | ***K. pneumoniae*** | ***S. aureus*** | ***E. faecalis*** |
| **Ethanol extract** | **Gentamicin** | **NA** | **29 mm** | **NA** | **27 mm** |
| | **Control** | **NA** | **15 mm** | **NA** | **17 mm** |
| | **50** | **NA** | **12 mm** | **NA** | **15 mm** |
| | **100** | **10 mm** | **13 mm** | **NA** | **17 mm** |
| | **200** | **13 mm** | **13 mm** | **NA** | **16 mm** |
| **Chloroform extract** | **Gentamicin** | **19 mm** | **NA** | **22 mm** | **NA** |
| | **Control** | **13 mm** | **NA** | **NA** | **NA** |
| | **50** | **12 mm** | **NA** | **11 mm** | **NA** |
| | **100** | **12 mm** | **NA** | **13 mm** | **10 mm** |
| | **200** | **14 mm** | **9 mm** | **19 mm** | **12 mm** |

Table 1. Showing sensitivity results.

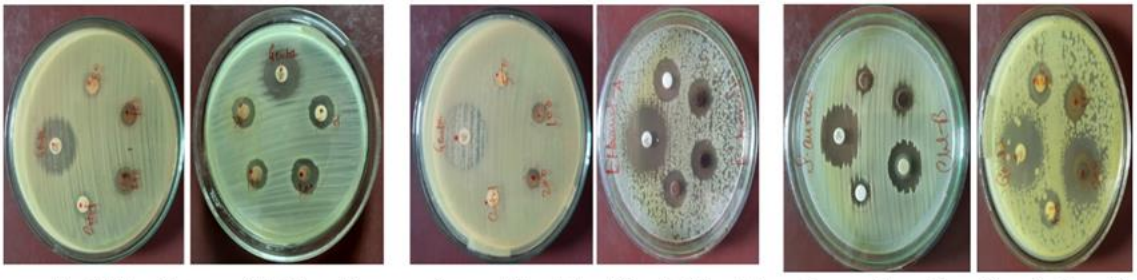


Fig. Image showing Antimicrobial Activity and Phytochemicals

### 3.5 Antioxidant Activity of Bioactive Compounds

. This assay measures the ability of test samples to donate hydrogen, quantifying their capacity to scavenge the stable organic free radical DPPH and induce reduction[7]. The evaluation involves measuring the absorption of the deep violet DPPH solution at 517 nm. After reduction, the absorption decreases, leading to decolorization from deep violet to a yellow-white hue. This reduction-induced decrease in absorption is directly proportional to the degree of reduction, as elucidated by Arulpriya *et al.,* (2010).The free-radical scavenging activity was estimated by DPPH assay.

| S. No. | Conc. | CN (Mean ±SD) | EN (Mean ±SD) | CO (Mean ±SD) | EO (Mean ±SD) | Ascorbic Acid (Mean ±SD) |
|---|---|---|---|---|---|---|
| 1 | 2 mg | 4.43± 2.14 | 5.40±3.92 | 15.20±3.21 | 30.20±3.21 | 40.49±2.23 |
| 2 | 4 mg | 18.85±3.3 | 27.78±8.80 | 19.17±0.74 | 39.17±0.74 | 55.33±2.20 |
| 3 | 6 mg | 20.83±5.0 | 35.23±2.66 | 25.81±4.09 | 45.81±4.09 | 80.06±0.51 |
| 4 | 8 mg | 21.99±6.32 | 47.33±2.76 | 28.30±0.72 | 60.30±2.72 | 84.49±2.23 |
| 5 | 10 mg | 23.17±0.82 | 63.17±1.82 | 30.90±12.29 | 68.20±2.78 | 84.33±2.20 |
| R2 | | 0.7009 | 0.9754 | 0.9659 | 0.9866 | 0.8549 |
| Y equation | | y = 4.062x + 5.668 | y = 13.509x - 4.745 | 4.053x + 11.717 | 9.713x + 19.597 | 11.684x + 33.888 |
| IC 50 | | 21.823 | 8.104 | 18.89 | 6.26 | 2.74 |

Table.2 Antioxidant Activity of Bioactive Compounds.

**Results and Discussion**

The phytochemical analysis revealed the presence of alkaloids, flavonoids, minerals, polyphenols, and saponins in the extracts of *Eulophia ochreata*. The antimicrobial and antioxidant activities of the extracts were significant, indicating their potential therapeutic value[8,9]. The high phenolic and flavonoid content correlates with the antioxidant activity observed.

**Conclusion**

In conclusion, the study highlights the promising bioactive compounds present in *Eulophia ochreata*, showcasing its potential as a valuable source of therapeutic agents. The significant antimicrobial and antioxidant activities, coupled with the rich phenolic and flavonoid content, underscore the medicinal importance of this orchid species. Further research on isolating and characterizing these bioactive compounds *could* lead to the development of novel pharmaceutical drugs with diverse health benefits.
*Eulophia ochreata.*

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