Pharmacognostic and preliminary phytochemical investigation of *Eulophia herbacea* Lindl. Tubers (Orchidaceae)

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**Objective:** *Eulophia herbacea* Lindl. (Orchidaceae) is an important traditional medicinal plant and widely used in treatment of variety of disease by tribes. Plant is unexplored scientifically yet for their identification and use. Therefore, the current study was carried out to perform detailed pharmacognostical and phytochemical analysis of *E. herbacea*. **Method:** Systematic pharmacognostic evaluation of tubers of *E. herbacea* has been carried out with respect to macroscopy, microscopy, WHO recommended physico-chemical parameters, florescence analysis of powder and estimation of different chemical standards, HPTLC fingerprinting of amino acid was also developed. **Result:** Tubers are fibrous, woody and perennial with numerous rootlets. Microscopic study shows the presence of cork, cortex, parenchymatus ground tissue, fibro vascular bundles and calcium oxalate crystals. Qualitative phytochemical test revealed the presence of acidic compounds, carbohydrates, amino acids, mucilage, tannins, steroids and triterpenoid. **Conclusions:** Morphological, histological and physico-chemical parameters studied in this paper may be proposed to establish the authenticity of plant *Eulophia herbacea*, which can probably, helps to differentiate the crude drug from its other species with respect to quality, purity and identification.

1. Introduction

*Eulophia*, a genus of perennial terrestrial orchids with fleshy tubers, rarely pseudobulbs, is distributed in the warm parts of the world, especially in Asia and Africa. About 28 species occur in India [1], several species are ornamental. *Eulophia herbacea* Lindl. (Orchidaceae) is commonly known as Kutri-kand, Kukad-kand, or umarkand [2] which is occurred in terrestrial, on hill slopes as forest undergrowth found in the area of Himalaya, Bengal, western parts of deccan peninsula[3]. Leaves linear-lanceolate or elliptic-lanceolate, glabrous, multi-nerved, plicate, 12–30 cm x 2.5–8.5 cm. Flowers are white, purple-nerved, plicate, 12–30 cm x 2.5–8.5 cm. Flowers are white, purple-nerved, in lax racemes, scape stout, 22–35 cm long. Capsules ellipsoid, obscurely ribbed. It bears flower & fruits in July–September [4]. It is traditionally used in the treatment of tumors of scrofulous glands of neck [5]. It is used as salka, dried tubers of various species of Orchids, and *Eulophia* used to make a nutritious beverage by treating the powdered preparation with hot water as tonic. Decocction of tuber is used on spermatorrhoea, urinary complaints, and menses [6]. *Eulophia herbacea* Lindl also shows multiple activities such as anti-cancer, nutritional, anti-hyperlipidemic, anti-oxidant, anti-arthritis, anti-inflammatory, antimicrobial and immunomodulator.

There is no report of systematic pharmacognostic and phytochemical studies on the tubers. In order to secure some standard for its identification; this study was carried out for pharmacognostical screening.

2. Materials and Methods

2.1. Plant material

The tubers of *Eulophia herbacea* Lindl. Orchidaceae, were collected from the subtropical hilly area of Toranal...
region, Maharashtra, India, in the month of July–August. They were identified and authenticated by Dr. D.A.Patil, Taxonomist, Department of Botany, S.S.V.P.S College of science, Dhule, Maharashtra, India. A Herbarium specimen was deposited in Dept of Pharmacognosy, R. C Patel institute of Pharmaceutical Education and Research, Shirpur, Dhule. With the number (FIH 107154).

2.2. Macroscopic and Microscopic characters of tubers

The tubers were separated from other parts, washed, cleaned and dried for further use. The detail macroscopic characters of fresh tubers were noted including special features. Microscopic sections were cut on a microtome & by free hand sectioning. Numerous temporary and permanent mounts of the microscopical sections of the tubers were made and examined microscopically. Histochemical reactions were observed with different staining agent for the general and specific microscopic characteristic of tubers. Photomicrographs of the microscopical sections were taken with the help of MOTIC photomicroscope provided with Motic Images plus 2.0 software [7].

2.3. Powder characteristics

Preliminary examination like behavior of powder with different chemical reagents and microscopically examination was carried out according to the method given in Khandelwal and Kokate [8, 9].

2.4. Fluorescence analysis of tuber powder

Powder material was analyzed under visible light, short and long UV light after treatment with various organic/inorganic solvents / reagents like Petroleum ether, methanol, water, 10% aqueous NaOH, 50%HCl, 50% H2SO4 , acetic acid, 50% HNO3 etc [10,11].

2.5. Physicochemical parameters

Physicochemical parameters such as percentage of total ash, extractive values and moisture content, foreign matter content, loss on drying, swelling index, foaming index were determined as per official method of the Indian pharmacopoeia and the WHO guidelines on the quality control methods of medicinal plant materials [12, 13].

2.6. Quantitative determination of heavy metal and minerals

Air dried tuber powder was kept in muffle furnace for preparing ash. Heavy metal and inorganic content of ash was determined quantitatively by atomic absorption spectrometer (AAS; Perkin Elmer 400) [19].

2.7. Preliminary phytochemical screening

Powdered drug was extracted with petroleum ether, methanol and water successively. The extracts were dried and weighed. The presence or absence of different phytoconstituents viz. triterpenoid, steroids, alkaloids, vitamins, tannins, glycosides and flavonoids, etc. were detected by usual given method [8].

2.8. Phytochemical standards

Total protein content by lowery method [14], phenolic content by folin–catechu reagent method [15], total flavonoid by the aluminum chloride colorimetric method [16] and proanthocyanidin by vanillin–sulfuric acid method [17] was determined. Available carbohydrate, crude lipid, crude fiber and total mucilage were estimated by official method [18,19].

2.9. HPTLC study of amino acids

Methanolic extracts of E. Herbacea (MEEH) studied for amino acid finger printing pattern using HPTLC. The plate was developed in n–Butanol: Water: acetic acid (4:1:1 v/v) as mobile phase in Camag twin trough TLC chamber with lid up to 8 cm. Derivatization of plate was done by dipping the plate in to 0.25% Ninhydrin in acetone. The plate was scanned at 366 nm using Scanner 3. In this study eleven numbers of amino acids was detected in MEEH, from that four amino acids were match with standard amino acids such as alanine, threonine, serine and aminobutyric acid.

2.10. GC–MS analysis of petroleum ether extract

An Agilent model 6890 GC interfaced to a 5973 mass selective detector was used for mass spectral identification of petroleum ether extract. HP–5MS capillary columns (30 m×0.25 mm×0.25 μm film thickness) were used for GC. The oven temperature was maintained at 60°C for 6 min then programmed to 240°C at 5°C min−1. The carrier gas was helium, at a flow rate of 0.9 mL min−1, and the injection volume was 1 μL. In mass spectrometry electron–impact ionization was performed at electron energy of 70 eV. Components of PE were identified by comparison of their mass spectra and retention indices with those published in and contained in the NIST ’98 MS computer library.

3. Results

3.1. Macroscopic characteristics

Leaves are linear–lanceolate or elliptic–lanceolate, glabrous, multi–nerved, plicate, 12–30cm x 2.5–8.5cm (Figure 1A). Fresh tubers are light brown colored, odorless with a slightly acrid taste. The tubers are found as napiform (Figure 1B) with average size of 4–6 cm in width and 5–8 cm in length. It shows prominent node like structure over the surface. The tubers are stout, shows the presence of numerous rootlets and root scars in upper parts, fractured surface is fibrous. Flowers white, purple–nerved, in lax...
racemes, scape stout, 22–35 cm long (Figure 1C).

3.2. Microscopic characteristics of tubers

T.S. of *Eulophia herbacea* tubers shows the entire general characteristic with some prominent identification feature. In general it showed the presence of cork, cortex, endodermis ground tissue, scattered vascular bundle and calcium oxalate crystal. Cork represented the outermost layer of tubers, containing 2–3 layered rectangular, thin walled cork cells (Figure 2A). Cortex consists of 6–15 layers of polygonal thin walled cellulosic parenchymatous cells (Figure 2B). Cells showed the presence of starch and acicular calcium oxalate crystals. The vascular bundles were found to be scattered in ground tissue and cortex. These vascular bundles were collateral closed and partially covered with lignified fibers i.e. fibro vascular bundle (Figure 2C). Xylem represented discontinuous groups of vessels. The vessels showed largely reticulate & pitted thickenings, responsible for water conduction. Phloem of vascular bundle consists of sieve tube along with companion cells, responsible for conduction of food. Phloem occupied relatively large area than xylem, with thick walled and big parenchymatous cells. The parenchyma in the phloem region is highly lignified cells. The calcium oxalates needles were abundant throughout the section. The mucilage cells were scattered in ground tissue and also deposited in cork cells.

3.3. Powder characteristics

Macroscopic and Microscopic

The tuber powder is light brown in color, slightly rough in touch with slight aromatic odour. Addition of small quantity of water, a mucilaginous mass was formed which indicates presence of considerable amount of mucilage. Pressing a little amount of powder between filter paper, no greasy stain was found, indicating absence of fatty oils. Behavior of powder with different chemical reagents is shown in Table 1. Microscopical examination the powder showed lignified fibers, Fibro vascular bundles, xylem, and phloem, as shown in Figure 3A, B, and C. The fluorescence analysis observed in visible, short and long ultra violet was depicted in Table 2.

![Figure 1](image1.png)

**Figure 1.** a) *E. herbacea* plant; b) tuber; c) flowers and leaves

![Figure 2](image2.png)

**Figure 2.** Microscopic characteristics of *E. herbacea* tubers
a) Fibro vascular bundles; b) cork cells; c) parenchymatous cells in cortex region

| Table 1 |
| --- |
| Behavior of *E. herbacea* tuber powder with different chemical reagents |
| Reagent | Colour/ Precipitate | Constituent |
| Conc. sulphuric acid | Reddish Steroid present |
| Aq. ferric chloride (5%) | Black colour Tannin present |
| Iodine solution | Blue Starch present |
| Picric acid solution | No Yellow ppt Alkaloid absent |
| Aq. mercuric chloride solution | No Brown colour Alkaloid absent |
| Magnesium– HCl | No change Flavonoid absent |
| Aq. silver nitrate solution | No ppt Proteins absent |
| Ammonical solution | No change Anthraquinone glycoside absent |
| Ruthenium red | Red colour Mucilage present |
3.4. Physicochemical parameters

The physiochemical parameters were shown in Table 2, such as total ash value was found to be 7.6%, water soluble ash 9.07%, acid insoluble ash 0.98%, swelling index 4 mL, Moisture content 84% and foreign matter was 0.20% w/w. The extractive values are mainly useful for the determination of the exhausted or adulterated drug. Petroleum ether soluble drug was 2%, water soluble 18% and methanol soluble 14% w/w (Table 3).

3.5. Heavy metal and mineral analysis

AAS analysis of tuber powder showed the presence of heavy metal namely cadmium and lead; and minerals such as calcium, potassium, magnesium, iron and sodium.

Table 3

| Parameters                      | Mean±SEM (%) |
|--------------------------------|--------------|
| Total ash                      | 7.6±0.208    |
| Acid-insoluble ash             | 0.98±0.023   |
| Water-soluble ash              | 9.07±0.231   |
| Sulphated ash                  | 1.02±0.067   |
| Moisture content (Fresh tubers)| 83.67±0.86   |
| Foreign organic matters        | 0.20±0.002   |
| Crude fiber content            | 31.5±1.322   |
| Swelling index                 | 4±0.001      |
| Pet. ether soluble extractive  | 2±0.001      |
| Methanol soluble extractive    | 14±0.021     |
| Water soluble extractive       | 18±0.002     |

3.6. Phytochemical screening and standards

Preliminary phytochemical screening mainly revealed the presence of amino acid, carbohydrates, flavonoids, fixed oils, proteins, Saponins, steroids, tannins and vitamins. Different chemical standard such as Total protein content, phenolic content, flavonoid, Proanthocyanidin, available carbohydrate, crude lipid, crude fiber and total mucilage (Table 4 and 5).

3.7. HPTLC study of amino acid

HPTLC study of various amino acids in methanol extracts of E. herbacea are presented in Figure 4; Ninhydrin spraying reagent was used as a developer. HPTLC studies of methanol extract revealed thirteen peaks at various Rf. Out of these, the most prominent peak of maximum area was at Rf 0.18, 0.20, 0.23 and 0.90 corresponding to that of marker
compound alanine, Threonine, serine and aminobutyric acid. The other peaks at Rf 0.36, 0.45, 0.60, 0.70, 0.72, 0.74, and 0.80 were also significantly prominent.

3.8. GC–MS analysis of Petroleum ether extract

GC–MS analysis of extract gives the idea about the presence of nature of chemical compound in the extracts. The results showed the presence of Dehydroabietane, Dehydroabietic acid, 1–Hexadecanol, 2–methyl–3',8,8'-Trimethoxy–3–piperidyl–2,2'–binaphthalene–1,1',4,4'-tetrone, β–sitosterol, tert–hexadecanethiol, stigmasterol, 3–eicosene (Table 6).

### Table 4
Preliminary Phytochemical Investigation of tubers extracts

| Test                          | Pet. Ether Extract | Methanolic Extract | Aqueous Extract |
|-------------------------------|-------------------|--------------------|----------------|
| Test for Carbohydrates        | –                 | +                  | +              |
| Test for Proteins             | –                 | +                  | +              |
| Test for Alkaloids            | –                 | –                  | –              |
| Test for Glycosides           | –                 | –                  | –              |
| Test for Saponins             | –                 | –                  | +              |
| Test for Flavonoids           | –                 | +                  | +              |
| Test for Tannins &phenolic    | –                 | +                  | +              |
| Test for Amino acids          | –                 | +                  | +              |
| Test for Steroids             | +                 | +                  | –              |
| Test for Fat & oil            | +                 | –                  | –              |
| Test for Mucilage             | –                 | –                  | +              |
| Test for Vitamins             |                   |                    |                |
| B1 (Thiamine)                | –                 | +                  | +              |
| C (Ascorbic acid)            | –                 | +                  | +              |
| E (Tocopherol)               | –                 | +                  | +              |

(+) present, (−) absent

### Table 6
GC–MS analysis of methanolic extract of *Eulophia herbacea* tubers

| Compound                                                                 | Mol. Formula | Rt (min.) | Conc. | M<sub>+</sub> | Base |
|-------------------------------------------------------------------------|--------------|-----------|-------|--------------|------|
| Dehydroabietane                                                         | C<sub>20</sub>H<sub>30</sub> | 24.76     | 9.11  | 270          | 255  |
| Dehydroabietic acid                                                     | C<sub>20</sub>H<sub>28</sub>O<sub>2</sub> | 25.90     | 8.23  | 300          | 285  |
| 1–Hexadecanol, 2–methyl–3',8,8'-Trimethoxy–3–piperidyl–2,2'–binaphthalene–1,1',4,4'-tetrone | C<sub>28</sub>H<sub>25</sub>NO<sub>7</sub> | 26.75     | 7.48  | 487          | 149  |
| β–sitosterol                                                             | C<sub>29</sub>H<sub>50</sub>O | 29.36     | 8.19  | 414          | 43   |
| tert–hexadecanethiol                                                    | C<sub>16</sub>H<sub>34</sub>S | 30.78     | 7.86  | 257          | 57   |
| Stigmasterol                                                            | C<sub>29</sub>H<sub>48</sub>O | 32.17     | 6.38  | 412          | 55   |
| 3–eicosene                                                              | C<sub>20</sub>H<sub>40</sub> | 35.48     | 2.10  | 280          | 57   |

Figure 4. HPTLC chromatogram of amino acid; alanine, threonine, serine and aminobutyric acid present in *E.herbacea* extract recorded at 366 nm after treatment with Ninhydrin reagent, n–butanol: acetic acid: water (BAW) as solvent system.

### Table 5
Phytochemical Standards of *Eulophia herbacea* tubers

| Parameter                   | % (Mean ± SEM) |
|-----------------------------|----------------|
| Total protein               | 5.238±0.023    |
| Total mucilage              | 22.000±1.45    |
| Total carbohydrate          | 43.420±0.004   |
| Total phenolic              | 12.600±0.028   |
| Total flavonoids            | 7.746±0.023    |
| Total proanthocyanidin      | 2.100±0.029    |
| Total saponin               | 3.500±0.011    |
| Crude lipid content         | 1.531±0.025    |

4. Discussion

In tubers some diagnostic characters are present in order to identifying the plant material. Some reliable characters like fibro vascular bundle, xylem and phloem were found in plant. The other commonly applied parameter for the identification is estimation of ash value, which establishes the quality and the purity of the drug. Ash value can also detect the nature of the material added to the drug for the purpose of adulteration [19]. The extractive values in different organic solvents is based on the quantity, which are soluble in them. It finds variation in the chemical constituents may cause a change in the extractive values. The variation in the extractive values may be possible due to the presence of specific compound, according to the solubility. The percentage weight of loss on drying, which is an indication of the moisture content of the material. The crude fiber is composed of many different compounds like cellulose, hemicelluloses and wood wool. Most of them are
polysaccharides. Generally, the herbal drugs are used in powder form and adulteration in the powdered drug can be detected by observing the powder under the ultraviolet light, because the fluorescence characteristic of any powder drug is very distinctive and helpful in distinguishing features for the determination of a drug. Microscopic and physicochemical studies are carried out on herbal crude drugs sample in order to establish appropriate data that may be utilized not only for identification but also to establish the purity and standard of plant sample, those supplied in powder form [20]. They are standard pharmacognostic parameters that can be used to differentiate closely related plant species or varieties with similar constituents or pharmacological activities. The phytochemical screening of the drug is a very responsive feature in the process of standardization and quality control because the constituent vary qualitatively and quantitatively not only from plant to plant but also in different samples of the same species depending upon various atmospheric factors and storage conditions. GC–MS detection, has found a variety of analytical uses, which are performing quality control analysis in the Pharmaceutical industries. In the last two decades HPTLC method has emerged as an important tool for the qualitative and quantitative phytochemical analysis of herbal drugs and formulations. This analysis is the first step towards understanding the nature of active principles and their detailed phytochemistry [21].

In the conclusion, present study on pharmacognostic evaluation of *E. herbacea* will provide useful data for identification, macroscopy, microscopy and physicochemical standards and phytochemical investigation discussed here which may help in authenticating the genuine plant along with nature of phytoconstituents present in the single drug and in polyherbal formulation.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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**References**

[1] Bhattacharjee SK. Hand book of medicinal plants. 3rd ed. Jaipur: Pointer Publisher; 2001, p.150–151
[2] Patil DA. Flora of Dhule and Nandurbar District. Dehradun: Bishan Singh and Mahender pal Singh Publication; 1992, p.566
[3] Kirtikar KR, Basu BD. Indian Medicinal Plants, 2nd ed. Vol.I.
[4] Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. New Delhi: Spring Science Business Media, LLC; 2007, p. 248–249
[5] Anonymous. The wealth of India: a dictionary of India raw material and industrial products. Vol.–III (D–E) New Delhi: Council of Scientific and Industrial Research; 2003, p. 221–222
[6] Patil DA, Patil SL. Ethnomedicinal plants of Dhule districts, Maharashtra. *Nat Prod Radiance*. 2007; 9:148–151
[7] Government of India. The Ayurvedic pharmacopoeia of India. 1st ed. New Delhi: Ministry of Health and Family Welfare, Department of Indian Systems of Medicine and Homeopathy; 2009, p. 242–244
[8] Khandelwal KR. Practical Pharmacognosy. 16th ed. Pune: Nirali Publication; 2010, p. 149–161
[9] Kokate CK. Practical Pharmacognosy. 4th ed. New Delhi: Vallabh Prakashan; 2010, p. 17–26
[10] Singh S, Manchawal I, Chauhan MG. Pharmacognostic study of male leaves of *Trichosanthes dioica* Roxb. with special emphasis on microscopic technique. *J Pharmach Soc Phytother* 2010; 2(5):71–75.
[11] Kokoski C, Kokoski RJ, Salma FJ. Fluorescence of powdered vegetable drug under ultraviolet radiation. *J Am Pharm Assoc* 1998; 47: 715–717.
[12] WHO. Quality control methods for medicinal plant material. Geneva;1992, p. 22–34.
[13] Government of India. Indian Pharmacopoeia. 4th ed. New Delhi: Ministry of Health and Welfare, Controller of Publications; 2007, p.A53–54
[14] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Method for protein estimation, *J Biol Chem* 1951; 193: 265–268
[15] Feng–LS, Ren YG, Yuan ZQ, Lei K, Hua–BL. Total Phenolic Contents and Antioxidant Capacities of Selected Chinese Medicinal Plants, *Int J Mol Sci* 2010; 11(6): 2362–2372
[16] Atanassova M, Georgieva S, Ivancheva K. Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. *J Univer Chem Tech and Metallurg* 2011; 46(1): 81–88
[17] Min–Ji Bak, Min Ju, Woo–Sik Jeong. Procyanidins from Wild Grape (Vitis amurensis) Seeds Regulate ARE–Mediated Enzyme Expression via Nrf2 Coupled with p38 and PI3K/Akt Pathway in HepG2 Cells. *Int. J. Mol. Sci.* 2012; 13: 801–818
[18] Ravi Kumar, Patil S .Patil MB, Patil SR, Paschapur MS. Isolation and evaluation of disintegrant Properties of Fenugreek Seed Mucilage. *Int J PharmTech Res* 2009; 1(4): 982–996
[19] Jain S, Koka S, Gupta A, Barik R, Malviya N. Standardization of “Chopchiniyadi Churna”: An Ayurvedic polyherbal formulation. *Phcog J* 2010; 2(5): 60–64
[20] Kumar S, Kumar V, Prakash OM. Pharmacognostic study and anti-inflammatory activity of Callistemon lanceolatus leaf. *Asian Pac J Trop Biomed* 2011; 1(3): 177–181
[21] Dinesh K, Gupta J, Sunil K, Renu A, Tarun K, Ankit G. Pharmacognostic evaluation of *Cayratia trifolia* (Linn.) leaf. *Asian Pac J Trop Biomed* 2012; 2(5): 60–64
[22] Chamblake DS, Upasani CD. Pharmacognostic standardization of stems of *Thespesia lampas* (Cav.) Dalz & Gilb. *Asin Pac J Trop Biomed* 2012;357–363