WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 10, 1063-1073. Research Article ISSN 2277– 7105

PRELIMINARY PHYTOCHEMICAL AND PHARMACOGNOSTIC INVESTIGATION OF EULOPHIA OCHREATA LINDL. TUBERS (ORCHIDACEAE)

Akshada A. Koparde* and Dr. C. S. Magdum

Dept of Pharmaceutical Chemistry, Rajarambapu College of Pharmacy, Kasegaon.

Article Received on
20 July 2015,

Revised on 11 Aug 2015,
Accepted on 02 Sep 2015

***Correspondence for Author**
Akshada A. Koparde
Dept of Pharmaceutical Chemistry, Rajarambapu College of Pharmacy, Kasegaon.

ABSTRACT

Objective: Eulophia ochreata Lindl (Orchidaceae) is an important traditional medicinal plant,but unknown to most of the people and widely used in treatment of variety of disease by tribes of taluka maval. 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.ochreata Lindl **Method:** Systematic pharmacognostic evaluation of tubers of E.ochreata Lindl has been carried out with respect to macroscopy, microscopy, and followed by preliminary phytochemical investigation and estimation of various chemical standards.**Result:** Tubers are fibrous, woody and perennial with numerous rootlets. Microscopic study shows the presence of cork layer with alternate lignified cells pink in colour, cortex, layer circular, parenchymatus ground tissue, fibro vascular bundles, muciligenous cells and crystals. Qualitative phytochemical test revealed the presence of most important that is alkaloids then saponin glycosides, mucilage, tannins, flavonoids, steroids and triterpenoid. **Conclusions:** Morphological, and phyto-chemical parameters studied in this paper may be proposed to establish the authenticity of plant Eulophia ochreata, and most important differentiating characteristic is yellow flowers which can probably, helps to differentiate the crude drug from its other species with respect to quality, purity and identification and revealing its important constituents present in plant.

KEYWORDS: Eulophia ochreata, Orchidaceae, pharmacognostic, phytochemical, alkaloids, identification.

1. INTRODUCTION

India has heritage of traditional medicine, Materia medica of India provides a lot of information on the folkore practices and traditional aspects of therapeutically important natural products. The evaluation of these drugs is mostly based on phytochemical, pharmacological and allied approaches including various instrumental techniques like chromatography, microscopy and others.[1] The herbal medicine is based on traditional medicine, exists in every continent of the globe and in every cultural area of the world. Each of these traditional medicines has its own origin and an individual basic philosophy.[2] Exploration of the chemical constituents of the plants and pharmacological screening may provide us the basis for developing a lead molecule. Herbs have provided us some of the very important life saving drugs used in the armamentarium of modern medicine.

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.[3] 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,ablong-lanceolate to ovate- elliptic. Flowers in dense racemes clustered at top of scape, corolla yellow,capsules broadly ovoid,deflexed,strongly ridged,green.[4]

Ehanobotanical survey of the forest areas of Maharashtra revealed that these tubers are used as a specialty food, general tonic and as rejuvenating herb.[5] It has been used by the tribes for properties like astringent, antifatigue, aphrodisiac, anthelminthic, and as a blood purifier. The tubers are also used in cough, cold and heart troubles.[6] According to flora of kolhapur district, localities for *Eulophia ochreata* Lindl in kolhapur region are Mahalunge,Panhala, Turrukwadi.[7] The tribal as well as rural people have distinct traditions, beliefs, dialects, way of life and knowledge of local flora. They are intimately associated with the forests. These people depend on plants for their routine requirement. Pawara tribals of Toranmal region, Nandurbar, Maharashtra eat raw tubers of *E. ochreata* for rejuvenating and aphrodisiac properties and tuber sap is also applied externally for curing Rheumatism.[8] Tribals have been using tubers as a general tonic and as rejuvenating since long. The aqueous dispersion of *Eulophia ochreata* Lindl. powder was found to be a better binding agent, being used in local region of Bhimashankar region as secondary food material, free from toxicity and is also economic. The advantage of *Eulophia ochreata* Lindl. over starch as a binding agent was that

it could be used as a cold binder whereas starch has to be heated.[9] On the basis of these prominent uses of *E. ochreata*, tubers were selected for my further work. The lack of well-documented scientific evidence will predominantly impede the progress of plant in the avenue. 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 pharmaognostical screening.

2. MATERIALS AND METHODS

2.1. Collection of Plant material

The tubers of *Eulophia ochreata* Lindl. was obtained from the Bhimashankar region of Maharashatra, India. Plant material was identified and authenticated by Dr. G.G. Potdar, Department of Botany, Y.C. College of Science, Karad; Voucher specimen was deposited at the same college as number AAK1 and AAK2.

2.2 Processing of Plant material

After collection tubers were washed thoroughly, and tubers were separated from other parts, cleaned and dried for further use.

2.3 Macroscopic Examination

The detail macroscopic characters of fresh tubers were noted including special Features such as colour,odour,shape size etc.[10]

2.4 Microscopic Examination

Microscopic sections were cut 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 manully prepared photomicroscope.[11-12]

2.5 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.[10,12]

2.6 Fluorescence analysis of tuber powder

Powder material was analyzed under visible light, short UV light(254nm) 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.[13]

2.7 Physicochemical parameters

Physicochemical parameters such as percentage of total ash, extractive values and moisture content loss on drying, swelling index, were determined as per official method of the Indian pharmacopoeia and the WHO guidelines on the quality control methods of medicinal plant materials.[14-15]

2.8. Extraction and Preliminary phytochemical screening

Extraction forms the first basic step in medicinal plant research because the preparation of crude extracts from plants is the starting point for the isolation and purification of chemical constituents present in plants.[16]

Powdered drug was extracted with petroleum ether, chloroform, methanol and water successively by soxhlet extraction and microwave. Microwave extraction can be the better alternative to conventional extraction.

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.[10]

2.10 Thin layer chromatograpic studies.[17]

TLC studies of chloroform extract of *Eulophia ochreta* Lindl was studied.It was carried it on TLC plates using silica gel,which are manually prepared and mobile phase was developed with trail and error basis.[Tolune: Ethylacetate:Diethyl Amine}{7:2:1}. Plate was observed in visible light and it was also sprayed with anisaldehyde sulphuric acid spray reagent followed by heating the plate at 110^0 C. The colour and Rf value of resolved spots were noted.

3. RESULTS

3.1. Macroscopic characteristics

Leaves are green in colour,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

4-7 cm in width and 5-9 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 yellow most identificable charater, in lax racemes, scape stout, 20-30 cm long (Figure 1C).

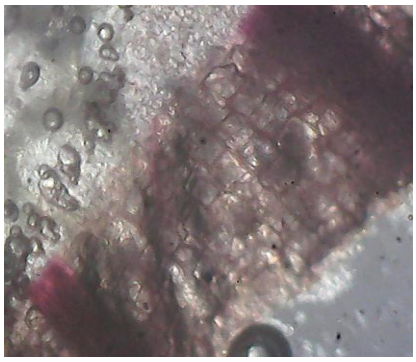
Fig 1A Leaves

Fig 1B Tubers

Fig 1C Yellow flowers

3.2. Microscopic characteristics of tubers

T.S. of *Eulophia ochreta* Lindl tubers shows the entire general characteristic with some prominent identification feature. In general it showed the presence of cork, cortex, scattered vascular bundle and calcium oxalate crystal. Cork represented the outermost layer of tubers, thin walled cork cells containing 7-8 layered rectangular(Figure 2A).,with alternate lignified cells stained pink in colour, (Figure 2B). Cortex layer consists of 6-10 layers of oval ,circular polygonal thin walled cellulosic parenchymatus cells in rows.(Figure 2C). 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 2D). 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(Figure 2E). occupied relatively large area than xylem, with thick walled and big parenchymatus 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.



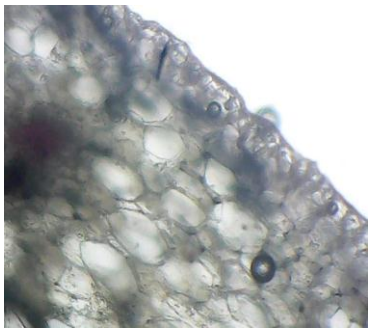


Fig No 2A- cork cells **Fig No 2B- lignified cells** **Fig No2C- parenchymatus cells**

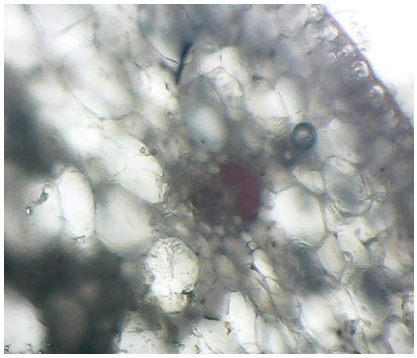
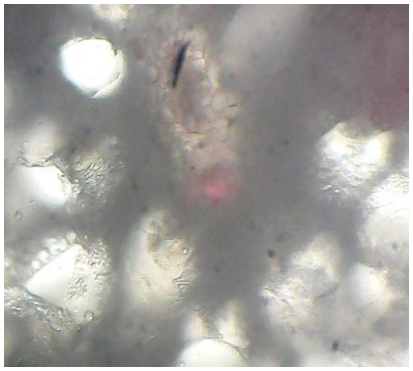
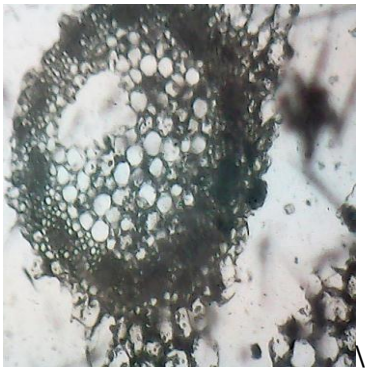




Fig No 2D Vascular bundle **Fig No 2E Pholem** **Fig No 2F TS of Eulophia**

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,Mucilage cells(Fig No 3A) were found to be present when stained with iodine. Pressing a little amount of powder between filter paper, no greasy stain was found, indicating absence of fatty oils. Strach Grains were present when stained with iodine solution (Fig No 3B). Microscopical examination the powder showed Acicular crystals (Fig No 3C)., Rosset crytals, (Fig No 3D).lignified cells(Fig No 3E).,pholem fiber surrounded with cork cells(Fig No 3F).,parencymatoues cells(Fig No 3G). Behavior of powder with different chemical reagents is shown in Table 1. The fluorescence analysis observed in visible, short and long ultra violet was depicted in Table 2.

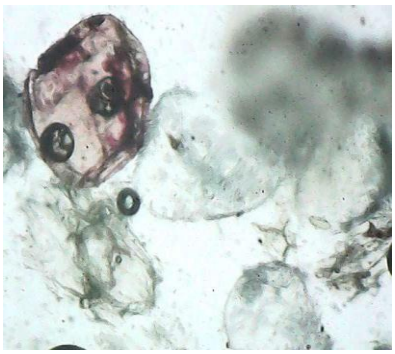
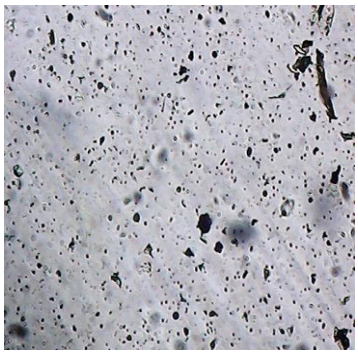
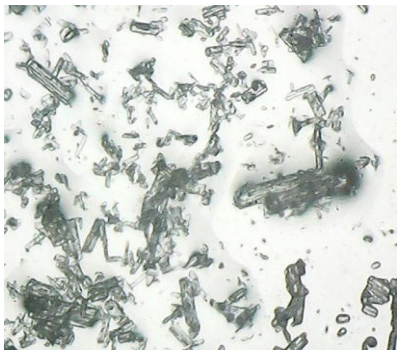
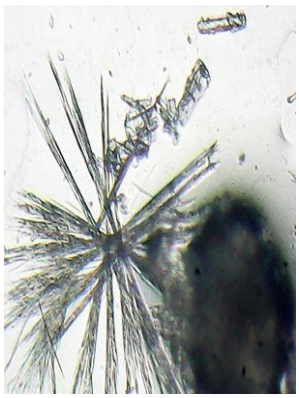




Fig No 3A- Mucilage cells **Fig No 3B- Strach Grains** **Fig No 3C-Acicular crystals**



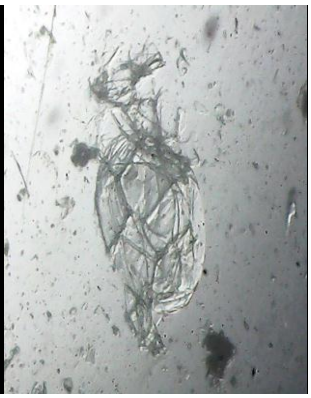



Fig No 3D-Rosset crystals **Fig No 3E-Lignified cells** **Fig No 3F- pholem fiber** **Fig No 3G- parencymatoues cells**

Table 1: Behavior of E. ochreata L tuber powder with different chemical reagents

Reagent	Colour/ Precipitate	Constituent
Conc. sulphuric acid	Reddish	Steroid present
Picric acid solution	Yellow ppt	Alkaloid present
Aq. silver nitrate solution	No ppt	Protein absent
Aq. ferric chloride (5%)	Black colour	Tannin present
Aq.mercuric chloride solution	Brown colour	Alkaloid present
Ammonical solution	No change	Anthraquinone glycoside absent
Iodine solution	Blue	Starch present
Ruthenium red	Red colour	Mucilage present
Magnesium- HCl	No change	Flavonoid present

Table 2: Fluorescence analysis of powdered tubers of E. ochreata L

Treatment	Visible Light	Short UV (254nm)
Powder as such	Light Brown	Brown
Powder + 10%NaOH	Green	Light Green
Powder+Ammonia	Light Green	Light brown
Powder+Acetic acid	Yellowish Green	Green
Powder+HNO3	Lemon Yellow	Yellowish Green
Powder+ 50%HCl	Pale Green	Slight Brown Turbid
Powder+Iodine Light Green	Light Yellow	Light Green
Powder+FeCl3	Dark Green	Dark Green

3.4. Physiochemical parameters

The physiochemical parameters such as total ash value was found to be 5%, water soluble ash 2.8%, acid insoluble ash 1.3%, swelling index 6 mL,Moisture content %LOD 5.33%. The extractive values are mainly useful for the determination of the exhausted or adulterated drug. water soluble extractive values 11.824% and alcohol soluble extractive values 6.36 % w/w .

3.5 Preliminary phytochemical examination

Table 3: Preliminary Phytochemical Investigation of tubers extracts

Test	Pet Ether Extract	Chloroform Extract	Methanol 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	+	+	+	+

3.6. TLC analysis of chloroform extract: TLC analysis of extract gives the idea about the presence of chemical compounds Fig No 6A. The Rf value and spot colour are informed in Table no 4.

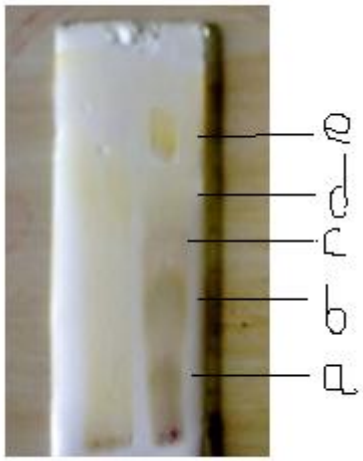


Fig No-6A TLC of Chloroform extract

Table 4: Rf values of chlorofrom extract

	Values	Spot colour	Mobile phase
Rfa	0.176	Redissh yellow	Tolune: Ethylacetate:Diethyl Amine
Rfb	0.470	brownish	
Rfc	0.564	brown	
Rfd	0.647	Yellow brown line	
Rfe	0.941	Yellowish green	

CONCULSION

The plant *Eulophia ochreta* Lindl have some important characteristic features In tubers in order to identifying the plant material. It find application in ayurvedic and other traditional system of medicine. The macroscopic and microscopic and macroscopic charaters reveal the presence of important diagnostic characters and structures which help in identification of plant material. The 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.[18] 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 percentage weight of loss on drying, which is an indication of the moisture content of the material .The phytochemical screening of the drug is very important to identify the different phytoconstituents present in plant material. It is a very important in the process of standardization and quality control because the constituent vary from plant to plant and also in different samples of the same species depending upon various atmospheric factors and storage conditions. TLC detection, has found a variety of analytical uses in the Pharmaceutical industries. TLC method has emerged as an important tool for the qualitative and quantitative phytochemical analysis of herbal drugs and formulations. Chromatographic analysis is the first step towards understanding the nature of active principles and their detailed phytochemistry.[20]

REFERENCES

1. Mukherjee P, Quality control of herbal drugs – An approach to evaluations of botanicals, (5th Edition) 2005: 2.
2. Vogel G.H, Drug discovery and evaluation, Pharmacological assays, 2003; 202.
3. Karthikeyan K. Flora of Maharashtra: Monocotyledon. Botanical Survey of India.1997; 23.

4. S.R.Yadav, M.M.Sardesai. Flora of kolhapur district .Published by Shivaji University, Kolhapur (1st edition) April 2002; 463.
5. Akshada A. Koparde, Dr. C.S. Magdum, Potentials of Eulophia ochreata Lindl: A Review, Research Journal of Pharmacognosy and Phytochemistry. July- September, 2014; 6(3): 1-5.
6. Jagtap S, Deokule S, Bhosale S. Some Unique ethnomedicinal uses of plants used by the Korku tribe of Amravati District of Maharashtra, India.Journal of Ethnopharmacology, 2005; 107: 463-469.
7. S.R.Yadav, M.M.Sardesai. Flora of kolhapur district .Published by Shivaji University, Kolhapur (1st edition) April 2002; 464.
8. Kshirsagar RD, Kanekar YB, Jagtap SD, Upadhyay SN, Rao R, Bhujbal SP, Kedia JN, Phenanthrenes of Eulophia ochreata Lindl. International Journal of Green Pharm, 2010; 2(2): 76-78.
9. A.A. Koparde and C.S. Magdum, Isolation of Natural Binding Agent From Eulophia Ochreata Lindl. And Its Evaluation, IJPSR, 2014; 5(6): 2546-2551.
10. Khandelwal KR. Practical Pharmacognosy. (16th ed.) Pune: Nirali Publication, 2010; 149-161.
11. Government of India. The Ayurvedic pharmacopoeia of India. 1st ed. New Delhi: Ministry of Health and Family Welfare, Department of Indian Systems of Medicines and Homeopathy, 2009; 242-244.
12. Kokate CK. Practical Pharmacognosy. 4th ed. New Delhi: Vallabh Prakashan, 2010; 17-26.
13. Kokoski CJ, Kokoski RJ, Salma FJ. Fluorescence of powdered vegetable drug under ultraviolet radiation. J Am Pharm Assoc, 1958; 47: 715-717.
14. WHO. Quality control methods for medicinal plant material. Geneva, 1992; 22-34.
15. Government of India. Indian Pharmacopeia. (4th ed.) New Delhi: Ministry of Health and Welfare, Controller of Publications, 2007; A53-54.
16. Akshada A. Koparde , Dr. C.S. Magdum, Conventional and Microwave Assisted Extraction of Eulophia ochreta Lindl and Zingiber cassumunar Roxb: An Comparitive Account. Research J. Pharm. and Tech, July 2014; 7(7): 746-748.
17. Dr.S.S.khadabadi, Dr.S.L.Deore, Dr.B.A.Baviskar , Experimental Phytopharmacognosy: A comprehensive Guide,Nirali Prakashan ,(first Edition): 2011; Annx-2: A2.1.

18. Kumar S, Kumar V, Prakash OM. Pharmacognostic study and antiinflammatory activity of Callistemon lanceolatus leaf. Asian Pac J Trop Biomed 2011; 1(3): 177-181.
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. 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; 6-10.