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Phyto-pharmacological activity of *Eulophia nuda* Lind: A review

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Abstract
Eulophia nuda Lind. (Orchidaceae) is a small perennial terrestrial herb that is mostly used by traditional healers in India and other parts of the world for ethnomedicine. It is widespread in central and southeast Asia. Tumors, scrofulous glands in the neck, rheumatoid arthritis, bronchitis, blood disorders, vermifuges, etc. have all been treated with E. nuda tubers. The phytochemical makeup of this plant has not been thoroughly studied. From this plant, only a small number of substances have been identified, including phenanthrene derivatives and benzylated phenanthrene derivatives. Pharmacologically, this plant has cytotoxic, antioxidant, antiglycation, and DNA-protective characteristics that are all active. *Eulophia nuda* Lind. is a safe and conventional medication that is used to cure illnesses and infections. They are safe to use, and experts from all over the world have been paying close attention to them.

Keywords: Introduction of *Eulophia nuda*, phytochemicals test, ethanomedicinal uses, pharmacological activity

1. Introduction
The family Orchidaceae includes the genus *Eulophia nuda*, which comprises between 210 and 230 species of orchids. Lind's nude Eulophia. A perennial terrestrial orchid that grows only from subterranean tubers. The stunning blossoms of this orchid species are the main reason it is utilized as an ornamental plant. For the past few decades, this plant has been utilized in the traditional medical system to treat a wide range of illnesses due to its significant medicinal potential. Extract from the tuber *Eulophia nuda* has been used as a vermifuge, blood thinner, and against scrofulous gland in the neck. *Eulophia nuda* contains a variety of phytochemically active compounds, including alkaloids, flavonoids, saponins, cardiac glycosides, terpenoids, and steroids, according to a qualitative phytochemical investigation [2]. *Eulophia nuda* is used as an antidote for snake bites, an anthelmintic, a treatment for tumors, bronchitis cases, scrofulous affliction of the neck glands, and blood diseases [3]. Additionally, it is asserted that the herb can treat tuberculosis [2].

2. Distribution
The Orchidaceae family includes the extremely diverse genus *Eulophia nuda*, which can be found in a variety of settings. From its underground tubers, this plant generates two shoots: vegetative and reproductive. A review of *Eulophia Nuda* The genus *Eulophia nuda* is widely distributed and includes approximately There are 3,230 species, which are found all throughout the neotropics, Madagascar, tropical and southern Africa, and tropical and subtropical regions of Asia and Australia. One species of them is found in tropical America. This genus is mostly found in the tropical Himalaya and Deccan Peninsula regions of India. In the International Plant Name Index, *Eulophia nuda* has around 723 records. But 500 are synonyms, and a large number of them are decorative. There are about 28 species known to exist in India [3]. The main 6 species namely; *E. nuda*, *E. ochreata*, *E. herbacea*, *E. graminea*, *E. epidendrea* and *E. ramentacea* are reported from Maharashtra. Of these 6 species, *E. nuda* has shown high biological activity [4].

3. Materials and Methods
3.1 Standardization
Collection of Kukadkand (Amarkand) tubers: *Eulophia nuda* tubers were collected randomly during the month of September from (Upavan Society for research and development activity) Nagpur, India.

Authentication of plant species: The leaves and tubers of *Eulophia nuda* plant commonly known as ―Kukadkand (Amarkand) Swas collected from area of Nagpur and authenticated by Dr. S.N. Malode from Govt. vidharbha institute of science and humanities Amravati, Department of Botany.
Reference no. GVISH/BOT/Report/12/2015 [5].

Fig 1: Amarkand Tubers

4. Morphology

Terrestrial herbs that are either autotrophic or infrequently heteromycotrophic make up the genus Eulophia species [Figure 1a]. The persistent organs can resemble tubers or pseudo bulbs. These pseudo bulbs typically have numerous nodes and thin or thick fibrous roots at the base. They might be underground or born above ground, corm-like, tuberous, or rhizomatous. A network of underground tubers forms under Eulophia [Figure 1b]. The leaves, which are basal and have petiole-like leaf bases and are one to many, lanceolate and plicate, develop at or after anthesis and are thin but durable, slender, and grass-like. They can occasionally overlap and form a pseudo stem. Some species are saprophytic and lack green leaves. The inflorescence can be laxly to sub-densely numerous, flowered, erect, lateral, racemose, paniculate, or occasionally reduced to a single flower. Most Eulophia species can be recognized by their flowers. Eulophia is home to two different kinds of flowers. The sepals and petals of the first kind are comparable in size, shape, and colour to those of the petals, while the sepals of the second type are smaller and frequently recurved. Both types of spurs have an extended lip that can take on a wide variety of shapes [6].

Fig 2: Representative photograph of (a) whole plant of Eulophia species and (b) chain of underground

Table 1: Macroscopic study of *Eulophia nuda*

Sr. no.	Parameters	Observation of *Eulophia nuda*
1.	Microscopic	
2.	Colour	Creamish white
3.	Odour	indistinct
4.	Taste	Bitter and mucilaginous
5.	Size	1.8-8cm Length, 2.5-5.6 width. 1.8-8cm Length, 2.5-5.6 width.

4.2. Microscopic Characteristic [7]

Epidermis: Single layered, made up of small tangentially elongated thin walled cells. Ground tissue: A broad region of parenchymatous cells. In ground tissue, some cells are filled with calcium oxalate crystals & starch grains, mucilage ducts and conjoint, collateral and closed vascular bundles are also seen scattered. Each vascular bundle is covered with a thick fibrous sheath. The sheath is thick on one side. Stone cells are also observed. Xylem vessels show spiral pitting.

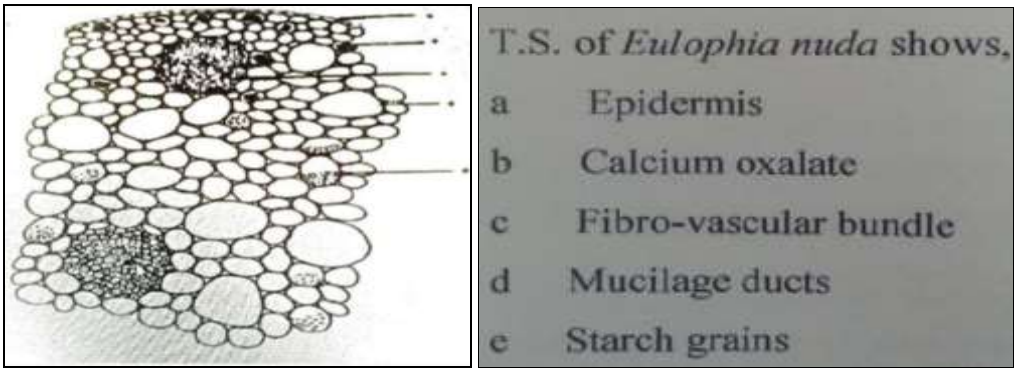

Fig 3: T.S. of *Eulophia nuda*

5. Taxonomy [8]

1	Kingdom	Plantae
2	Division	Magnoliophyta
3	Order	Asparagales
4	Family	Orichideaceae
5	Genus	Eulophia
6	Species	Eulophianuda
7	Class	Liliopsida

6. Phytochemicals Test

This involves various chemical tests to identify different phytochemicals. For example, alkaloids can be detected by Mayer's reagent, etc. Observation and results of qualitative chemical constituents of *Eulophia nuda* are shown in the table. A combined knowledge of chemical constituents is desirable for supportive evaluation of natural drugs or drug component as well as phytopharmaceuticals for knowledge of phytopharmacognostic and phytopharmacological screening. The plant may be considered a biosynthetic laboratory for a multitude of components like alkaloids, glycosides, volatile oils, tannins and Flavonoids, etc. which are termed secondary metabolites and responsible for therapeutic effects. Hence, various extracts of *Eulophia nuda* was subjected to phytochemicals screening for the identification of different constituents and plant metabolites present in the extract [9].

Chemical test for various extract are as follows
Phytochemicals test present in various extract

Table 2: Chemical Test for Alcoholic Extract (Ethanol) [9].

Sr. No.	(A) Test for alkaloid	Observation	Inference
1	Hagers Test: test solution + Hager's reagent (Saturated picric acid solution)	Yellow ppt	Alkaloid present
2	Dragendroff's: Test solution + Dragendroff's reagent (potassium bismuth Iodide).	Orange brown ppt	Alkaloid present
3	Mayer's Test: test solution + Mayer's reagent (Potassium mercuric iodide)	Cream coloured precipitate was formed	Alkaloid present
4	Wagner's Test: test solution + Wagner's reagent (Iodine Potassium Iodide)	Reddish brown precipitate was formed	Alkaloid present

Sr. No.	(B) Test for flavonoid	Observation	Inference
1	Alkali Test: Test solution with NaOH.	Yellow colour decolourised after addition of acid.	Flavonoid present
2	Lead test: Mix test solution + lead acetate	Yellow ppt	Flavonoid present
Sr. No.	**(c) Test for cardiac glycosides**	**Observation**	**Inference**
1	Baljet Test: 2-3 mg of sample + 2 ml Sodium picrate solution	Yellow, orange to deep red Colour.	Glycosides present
2	Killer killani Test: 2 ml of extract + glacial acetic acid + one drop 5% ferric Chloride and conc. sulphuric acid.	Yellow, orange to deep red Colour.	glycosides present

Sr. No.	Test for Steroids	Observation	Inference
1	Liebermann- Burchard Test: Mix 2 ml of extract with Chloroform, add 1-2 ml Acetic anhydride and two Drops of conc.H_2SO_4.	First red, then blue and finally Green colour appears**.**	Steroid present
2	Salkowaski Test: 1ml of conc. H_2SO_4 added to 10 mg of extract dissolved 1ml chloroform layer and green of chloroform. Fluorescence by the acid layer	Reddish-brown colour exhibited	Steroid present

Table 3: Chemical test for petroleum ether extract.

Sr. no.	(A)Test for alkaloid	Observation	Inference
1	**Wagner's Test:** Treat test solution with Wagner's reagent (Iodine Potassium Iodide	Reddish brown ppt	Alkaloid present
Sr. No.	**(B)Test for cardiac glycoside**	**Observation**	**Inference**
1	**Baljet Test:** Mix 2-3 mg of sample in 2 ml Sodium picrate solution	Yellow, orange to deep red Colour.	Glycoside present
2	**Legal Test:** To aqueous or alcoholic extract, add 1ml pyridine and 1ml sodium nitroprusside	Pink to red colour appears	Glycoside present
Sr. No.	**(C) Test for flavonoid**	**Observation**	**Inference**
1	**Sulphuric acid Test:** Use zinc instead of magnesium	Deep red to magenta colour.	Flavonoid present
2	**Lead acetate Test:** Mix test solution with lead Acetate.	Yellow precipitate	Flavonoid present
Sr. No.	**(D) Test for Steroid**	**Observation**	**Inference**
1	**Salkowaski Test:** 1ml of conc. H_2SO_4 added to.10 mg of extract dissolved 1ml chloroform.	Reddish brown colour exhibited	Steroid are present

7. Ethno-medicinal Uses [10]

Tubers: Rheumatoid arthritis, Exorcise intestinal worms, bronchitis, tonic, vitiated blood, blood purifier, tumours, scrofulous glands, vermifuge, Anti-aphrodisiac, Demulcent and anthelmintic, antidote for poisoning. Tumours and bronchitis or merely to be consumed as appetizers, Kill intestinal worms, or to treat abscesses and infected wounds. Worms and scrofula Acidity, piles and stomach complaints Intestinal worms, scrofula, blood disorders, bronchitis and as an appetizer.

Leaves: As vermifuge.

Whole Plant: Stomach-ache and related complaints snake bite.

Stem: To stop bleeding and pain from trauma or snake bite.

8. Pharmacological Activity

8.1 Cytotoxic Activity

The global burden of breast cancer is increasing with alarming rates among the women worldwide. The currently available therapies for breast cancer are associated with number of side effects. Hence there is a need to find the better alternative drugs for the treatment of breast cancer with fewer side effects. *Eulophia nuda* is one such terrestrial orchid which has been traditionally used for the treatment of

tumours. In the present study, the alcoholic extract of *E. nuda* tuber and its different fractions were studied *in-vitro* for their cytotoxic activity using MTT assay on the MCF7 cell line. The results showed that the chloroform fraction at the concentration of 1000 µg/ml showed maximum cytotoxicity of 73.50% among all the fractions which was less than the cytotoxic activity of the alcoholic extract of *E. nuda*, 80.77%. The results suggested that the cytotoxic activity could be due to the synergistic action of the phytoconstituents present in the plant. Further *in-vitro* and *in-vivo* studies are necessary to establish the use of *E. nuda* for the treatment and management of the breast cancer [11].

8.2. Anti-Glycation Effect

Non enzymatic glycation takes place when elevated levels of reduced sugars react with amino groups of proteins and is called as advanced glycation end products (AGEs) are responsible for Diabetes Mellitus. Hydro alcoholic extract of Whitton root (*Eulophia nuda*) was tested for in-vitro inhibition of non-enzymatic glycation of Immunoglobin G. Plant extracts have their own importance and now being studied extensively due to having little or no side effects in all aspects of life sciences from botany to medicine in biochemical research. In present study Whitton root was selected and used to check the glycation inhibitory activity *in-vitro* condition. Various combinations of glucose, protein and Whitton root extracts were made under *in vitro* conditions and their activity was monitored with Trichloro acetic acid treatment method at 350 nm. Glycated products/ AGEs were more with high glucose and high protein concentration and these were decreased by highest concentration of Whitton root extract i.e. 30 mg/mL or 300 µL. Lower concentrations of plant extract produced either no or least response against advanced glycation end products [12].

8.3 *In vitro* Cytotoxicity

The global burden of breast cancer is increasing with alarming rates. The current research trend in the cancer is focused towards finding the safe and effective therapy from the traditionally used medicinal plants. *Eulophia nuda* is one such terrestrial orchid which has been traditionally used for the treatment of tumours. In the present study, the alcoholic, hydro alcoholic and aqueous extracts of *E. nuda* tubers were studied *in-vitro* for their cytotoxic activity using two different models for cytotoxicity, brine shrimp lethality assay and MTT assay. The MTT assay was performed on the breast carcinoma cell line MCF7 and on the non-cancerous Vero cell line. The results suggested that the alcoholic extract of *E. nuda* showed the most significant cytotoxicity, 80.77% on the MCF7 cell line at the concentration of 1000 µg/ml. The extract did not show any cytotoxicity on the Vero cells, and hence it could be considered safe to the normal cells. Further *in-vitro* and *in-vivo* studies are necessary to establish the use of *E. nuda* for the treatment and management of the breast cancer [13].

8.4. *In vivo* Immunomodulatory Activity

The immune system is known to be involved in the etiology as well as Pathophysiological mechanisms of many diseases [1]. Ayurveda gives Emphasis on promotion of health - A concept of strengthening host Defences against different diseases [2]. Ramayana plants are particularly recommended for the treatment of immune disorder [3], Ayurveda (with particular reference to plants) may play an important role in Modern health care, particularly where satisfactory treatment is not available. There is need to evaluate the potential of Ayurveda remedies To the advantage of counteract side effect of modern therapy and Compare the cost-effectiveness of certain therapies vis-a-vis modern Therapeutic schedules [14].

8.5. Antioxidant Activities

Eulophia nuda, is a highly medicinal orchid with strong antioxidant and anticancer potentials in traditional systems of medicine. But few reports are available on the scientific validation. The aim of the study was to investigate phytochemical constituents, antioxidant and cytotoxic efficacies of extracts of *Eulophia nuda*, and the underlying mechanisms-of-action via upregulation of nuclear transcription factor-erythroid-2 related factor (Nrf2) and hemeoxygenase-1 (HO-1) pathways [15].

8.6. Antibacterial Activity

The antibacterial activity was carried out using various extracts such as alcoholic, acetone and chloroform extract of dried tuber powder of E. nuda compared with standard antibiotic drug ampicillin. Disc diffusion method was used against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. Among all extracts acetone extract was found more potent against S. aureus with maximum zone of inhibition 18 mm as compared to standard antibiotic drug ampicillin with 20 mm zone of inhibition [16].

8.7. Hepatoprotective Activity

Acetone, chloroform and ethanolic tuber extracts of *E. nuda* were used to evaluate acute toxicity study according to OECD guidelines 425 on Wistar albino rats. The activity was studied by using SGOT (Serum Glutamate Oxaloacetate Transaminase), SGPT (Serum Glutamate Peroxides Transaminase), and ALP (Alkaline Phosphates) parameters compared to standard LIV 52. The study showed that *E. nuda* tuber is active as hepatoprotective when compared with standard LIV 52 effect on Wistar albino rats [17].

9. Conclusion

The main topics of this review are the pharmacological and phytochemical applications of the E. nuda plant. The entire E. nuda plant is used medicinally to cure a variety of illnesses, including bronchitis, tuberculosis, antiaphrodisiacs, poisoning antidotes, and blood purifiers. Many experimental studies have demonstrated the wide range of biological activity exhibited by E. nuda. It is an example of a class of therapeutic herbs whose application is well grounded in theory. Herbal medications are made from nearly every aspect of E. nuda. It is known that this plant has antitumor, antioxidant, antitumor, hepatoprotective, antibacterial, antifungal, immunomodulatory, and DNA-protecting properties. Therefore, the pharmaceutical industry has a lot of potential to turn this plant into a medicine.

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