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PHYTOPHARMACOGNOSTICAL STUDY OF TUBERS OF *EULOPHIA NUDA* LINDL.

Dhara Bhatt *, Khushboo Jethva and Maitreyi Zaveri

Department of Pharmacognosy, K. B. Institute of Pharmaceutical Education and Research, Sector-23, Gandhinagar - 382023, Gujarat, India.

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Correspondence to Author:
Dhara Bhatt

Department of Pharmacognosy, K. B. Institute of Pharmaceutical Education and Research, Sector-23, Gandhinagar - 382023, Gujarat, India.

E-mail: dharabhatt88@gmail.com

ABSTRACT: Plants and plants-based medicines have been used to treat many ailments since the ancient times. Family Orchidaceae to which orchids belongs is the largest family among monocotyledons. The genus Eulophia comprises perennial terrestrial orchids with fleshy tubers, rarely pseudobulbs. One such perennial herb having underground tubers, *Eulophia nuda,* belonging to family Orchidaceae, has been traditionally used for the treatment of tumours, scrofulous glands of the neck, bronchitis, skin rash and rheumatoid arthritis. In the present study the pharmacognostical and phytochemical evaluation of tubers of *E. nuda* was performed as it is helpful for the standardization and authentication of medicinal plant. The microscopical study showed the presence of xylem vessels, fibres, starch, raphides and acicular crystals. The phytochemical analysis of the tubers of E. nuda indicated the presence of carbohydrates, alkaloids, flavonoids, steroids, triterpenoids, *etc*. The results of the HPTLC fingerprinting confirmed the presence of the flavonoids like rutin, quercetin and kaempferol in the tubers of *E. nuda.*

INTRODUCTION: Plants, animals and minerals are used in medicines by man since prehistoric time. Plants provide avariety of potent drugs, to prevent and cure diseases where the synthetic drugs fail [1]. India has great variety of herbal wealth due to its ecological and climatic diversity. Art of herbal healing is deep rooted in Indian culture and folklore. Even today in most of the rural and urban areas also, people depend on local traditional healing system for their primary healthcare. Especially the tribes of remote areas of India are mostly dependent on herbs for their healthcare [2]. Orchids are the most beautiful flowers and comprise a unique group of plants.

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The family Orchidaceae to which orchids belongs is the largest family among monocotyledons containing 600-800 genera. Orchids include terrestrial, epiphytic and saprophytic forms. Most of the terrestrial forms are having valuable medicinal properties. The Genus *Eulophia comprises* perennial terrestrial orchids with fleshy tubers, rarely pseudobulbs. The Genus *Eulophia includes* about 230 species mostly terrestrial and distributed worldwide [3]. In, India there are about 30 Eulophia species which have been reported for their ethno-botanical uses [4].

One such perennial herb having underground tubers, *Eulophia nuda*, belonging to family Orchidaceae, has been traditionally used for the treatment of tumours. The herb is distributed in the central and Southeast Asian regions. In India, it is found throughout the Himalayan regions, from Nepal to Assam and in Deccan from Konkan southwards. These tubers are reported for having number of medicinal uses. The tubers are used

against tumours, bronchitis and scrofulous glands of the neck [5-9]. In Thailand, the tuber is traditionally used in the treatment of skin rash and rheumatoid arthritis [10]. *E. nuda* tuber is also reported for their anthelmintic and demulcent action [11]. The tubers *E. nuda* of also used as an aphrodisiac, for the treatment of acidity, piles and stomach ailments [12], [13]. The present study was undertaken to evaluate the phyto-pharma-cognostical parameters of tubers of *Eulophia nuda*.

MATERIALS AND METHOD:

Collection and Authentication: The fresh tubers of *Eulophia nuda* were collected from the forest regions of Dang district, Gujarat, India. The fresh tubers of *E. nuda* were washed and shade dried.

The dried tubers of *E. nuda* were coarsely powdered to 60 # and stored in an air tight container for present work. The voucher specimen (PH/13/04) was deposited at Department of Pharmacognosy, K. B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India.

Macroscopical and Microscopical Study:

Macroscopical Study: The tubers of *E. nuda* were studied and identified by comparing their morpho-logical characters as mentioned in the literature [14].

Microscopical Study: Powdered material of tubers of *E. nuda* was observed under microscope in 10 x resolution and identified by comparing their microscopical characters as mentioned in the literature.

Physico-chemical Parameters: The powder of tubers of *E. nuda* was used or thephysico-chemical studies of the powdered drug, such as determination of the ash values, extractive values and loss on drying were performed according to the WHO guidelines [15].

Determination of Ash Values: Ash values of tubers of *E. nuda* were determined by the following method:

Determination of Total Ash: Two gram of the accurately weighed powder of tubers of *E. nuda* was incinerated in a crucible at a temperature of 500-600 ºC in a muffle furnace till carbon free ash was obtained. It was then cooled, weighed and percentage of ash was calculated with reference to the air-dried drug.

Determination of Acid Insoluble Ash: The total ash was boiled for 5 min with 25 ml of 70 g/l hydrochloric acid and filtered using an ash-less filter paper to collect the insoluble matter. The ash obtained was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of the acid insoluble ash was calculated with reference to the air-dried powered drug 60.

Determination of Water Soluble Ash: Total ash was boiled for 5 min with 25 ml of water and insoluble matter was collected on an ash-less filter paper. It was washed with hot water and ignited for 15 min at a temperature not exceeding 450 ºC in a muffle furnace. Difference in weight of ash and weight of water insoluble matter gave the weight of water soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried powered drug (60#).

Determination of Extractive Values:

Extractive Values of the Powder Tubers of *E. nuda*, were Determined by the Following Method:

Determination of Water Soluble Extractive: Four gram of the air-dried powdered material (60#) of the tuber of *E. nuda* was soaked in 100 ml of water in a closed flask for 1 h with frequently shaking. It was then boiled gently for 1 h on water bath; cooled, weighed and readjusted the weight. Twenty-five ml of the filtrate was evaporated to dryness in a porcelain dish and dried at 105 ºC to a constant weight. The percentage of water-soluble extractive was calculated with reference to the air-dried powered drug (60#).

Determination of Alcohol Soluble Extractive: Four gram of the air-dried powdered material (60#) of the tuber of *E. nuda* was macerated with 100 ml of alcohol in a closed flask for 24 h by shaking the flask frequently at an interval of 6 h. It was then allowed to stand for 18 h and filtered rapidly to prevent any loss during evaporation. Twenty-five ml of the filtrate was evaporated to dryness in a porcelain dish and dried at 105 ºC to a constant weight.

The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

Determination of Moisture Content:

Loss on Drying: About 1 g of the dried crude material of tubers of *E. nuda* was taken and powdered. A glass stoppered bottle was dried for 30 min under the same conditions to be employed in the determination and the weight of the bottle was taken. The sample was transferred into the bottle and weight of the bottle was noted. The stopper of the glass bottle was removed and kept in the oven. The sample was distributed evenly and was placed in the oven for drying, at 100-105 ºC. Then, the bottle was removed from the oven, stoppered immediately and was allowed to cool at room temperature and weighed. The experiment was repeated till constant values were obtained.

Phytochemical Screening: The powder (60#) of tubers of *E. nuda* was subjected to chemical tests to check the presence of various phytoconstituents like, alkaloids [16], flavonoids [16, 17], saponins [18], [19], carbohydrates [20], steroids [19], triterpenoids [20], tannins [21, 22], phenolics [23, 24], coumarins [25, 26] and anthraquinone [27] using standard procedures.

HPTLC Fingerprinting of Alcoholic Extract of *E. nuda:* Chemicals and Instruments: Methanol, toluene, chloroform, anisaldehyde sulphuric acid, Rutin (Sigma, USA), Kaempferol (Sigma, USA), Quercetin (Sigma, USA), TLC plates (Merck, Darmstadt, Germany), U.V chamber, Oven, HPTLC (CAMAG, Muttens, Switzerland), Linomat 5 autosampler, TLC scanner 3 and win CATS software.

Standard Preparation: A solution of 1 mg/ml of Catechin, Rutin, Quercetin and Kaempferol, was prepared in methanol.

Preparation of Extract: The dried and finely grounded powder of tubers of *E. nuda* was taken to prepare the alcoholic extract of tubers of *E. nuda*. For the preparation of extract, the powder of tubers of *E. nuda* was macerated for 24 h with alcohol.

It was then refluxed for about 1 h with occasional shaking, consecutively three times and filtered. The filtrates were pooled and concentrated to dryness. The prepared extract was labelled and stored in an air tight container for further, use.

This alcoholic extract of tubers of *E. nuda* was dissolved in methanol to get the final concentration of 1 mg/ml of solution.

Experimental Conditions: The plates were prewashed by methanol and activated at 110 ºC for 5 min prior to chromatography. The standard solutions of catechin, rutin, kaem-pferol and quercetin and the alcoholic extract of tubers of *E. nuda* were spotted using Linomat 5 sample applicator. HPTLC Fingerprinting was performed as following.

Stationary Phase: Precoated silica gel 60 F254 plate (Merck)

Mobile Phase: Toluene: Ethyl acetate: Formic acid (5:4:1 v/v)

Chamber Saturation: 25 min

Temperature: 27 ± 3 ºC

Slit Dimension: 5 × 0.45 mm

Chamber: Camag flat bottom and twin –trough developing chamber

Separation Technique: Ascending

Migration Distance: 8.0 mm

Detection: Scanned at 450 nm after derivatization Anisaldehyde Sulphuric Reagent

RESULTS:

Macroscopical and Microscopical Study:

Macroscopy of the Selected Plants: The morphology of tubers of *Eulophia nuda* as shown in the **Fig. 1**. The organoleptic and macroscopical characters of the selected plants are as described in the **Table 1**.

FIG. 1: TUBERS OF *E. NUDA*

TABLE 1: ORGANOLEPTIC CHARACTERS AND MACROSCOPY TUBERS OF *E. NUDA*

Colour	Cream brown
Odour	Characteristic
Taste	Characteristic and mucilaginous
Shape	Conical and shell shaped
Surface	Slightly rough and hairy

Microscopy of the Selected Plants: The powder characters of the tubers of *Eulophia nuda* are as shown in the **Fig. 2**. The photographs of the powder characters are taken in 10 × resolution. The powder study of the

3.2. Physico-chemical Parameters: The ash values, extractive values and loos on drying of the powder of tubers of *E. nudais* as shown in **Table 2**.

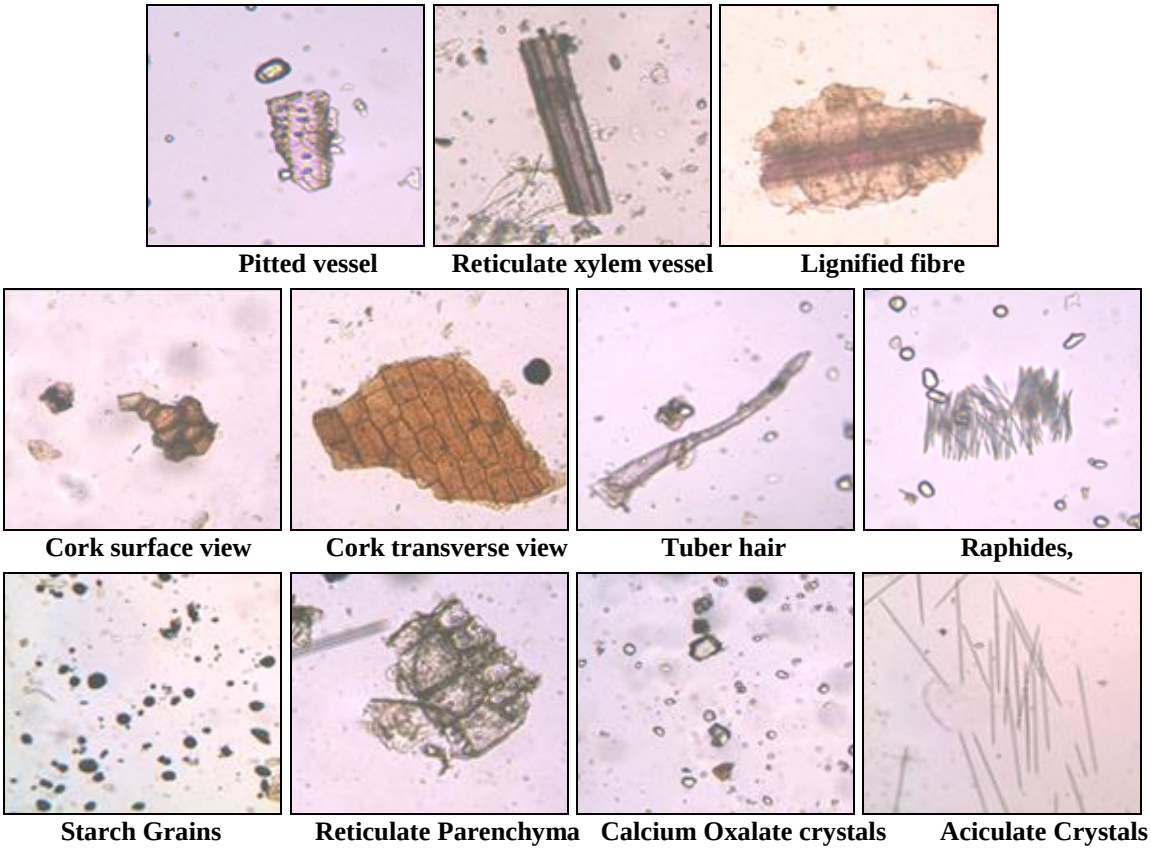

FIG. 2: POWDER CHARACTERS OF TUBERS OF *E. NUDA* IN 10X RESOLUTION

TABLE 2: PHYSICO-CHEMICAL PARAMETERS OF TUBERS OF *E. NUDA*

Ash Values (% w/w)	
Total ash	05.49 ± 0.45
Acid insoluble ash	01.24 ± 0.19
Water soluble ash	03.16 ± 0.62
Extractive Values (% w/v)	
Water extractive value	22.14 ± 0.35
Alcohol extractive value	18.40 ± 0.63
Loss on drying (%w/w)	05.21 ± 0.41

(n) = 3, Standard Deviation (SD) = ±SD

Phytochemical Screening: The powder of the tubers of *E. nuda* was subjected to chemical tests to check the presence of various phytoconstituents like, alkaloids, flavonoids, saponins, carbohydrates, steroids, triterpenoids, tannins, phenolics, coumarins and anthraquinones.

The results of the phyto-chemical screening are as described in **Table 3.**

TABLE 3: PHYTOCHEMICAL SCREENING OF TUBERS OF *E. NUDA*

Phyto-chemical Screening	*E. nuda* (Tubers)
Alkaloids	+
Flavonoids	+
Saponins	+
Carbohydrates	+
Steroids	+
Triterpenoids	+
Tannins	-
Phenolics	-
Coumarins	-
Anthraquinone glycosides	-

Where, (+) = present, (-) = absent

HPTLC Fingerprinting of Alcoholic Extract of *E. nuda*: The results of the HPTLC fingerprinting of the alcoholic extract of tubers of *E. nuda* are as shown in **Fig. 3** and **4**. They showed the presence of rutin (R_f 0.09), quercetin (R_f 0.61) and kaempferol (R_f 0.66) in the extract when compared the R_f of the standard rutin (R_f 0.09), Quercetin (0.60) and Kaempferol (0.66).

FIG. 3: CHROMATOGRAMSOF STANDARDS AND ALCOHOLIC EXTRACT OF TUBERS OF *E. NUDA* IN HPTLC ANALYSIS AFTER DERIVATIZATION: UNDER DAYLIGHT. THE TRACKS ARE AS FOLLOWS: 1. CATECHIN 2. RUTIN 3. QUERCETIN 4. KAEMPFEROL 5. ALCOHOLIC EXTRACT OF TUBERS OF *E. NUDA*

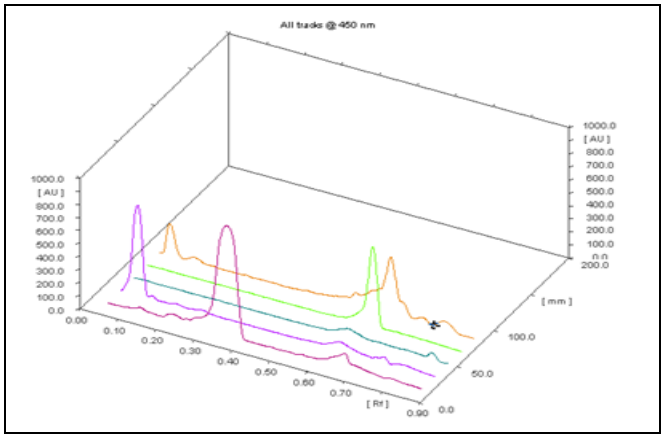

FIG. 4: HPTLC CHROMATOGRAM OFSTANDARDS AND ALCOHOLIC EXTRACT OF TUBERS OF *E. NUDA* IN HPTLC ANALYSIS AFTER DERIVATIZATION THE TRACKS ARE AS FOLLOWS: 1. CATECHIN 2. RUTIN 3. QUERCETIN 4. KAEMPFEROL 5. ALCOHOLIC EXTRACT OF TUBERS OF *E. NUDA*

CONCLUSION: In the present study the phytopharmacognostical evaluation of the tubers of *Eulophia nuda* was performed. Pharmacognostical study of the medicinal plants is very important as it gives the parameters for the standardization and authentication of the medicinal plant. Organoleptic evaluation and macroscopical description are the simplest and quickest methods to establish the identity and quality of a medicinal plant. The microscopical study of any plant part can be used to identify the distinguishing character of the plant. These parameters can be used for the identification purposes and also for the prevention of adulteration and substitution of the medicinal plants [28]. In the present study the organoleptic and the macroscopical and microscopical characters of the tubers of *E. nuda* were studied. The physicochemical analysis of the tubers of E. nuda was performed where the parameters such as ash values, extractive value and loss on drying were performed. Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water-soluble ash is used to estimate the amountofinorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. Moisture content of drugs should be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent [28]. Moreover, the phytochemical analysis of the tubers of E. nuda indicated the presence of carbohydrates, alkaloids, flavonoids, steroids, triterpenoids, *etc*. The results of the HPTLC fingerprinting confirmed the presence of the flavonoids like rutin, quercetin and kaempferol. Thephyto-pharmacognostical study of tubers of *E. nuda* would be helpful for carrying out further research and explore its therapeutic potential.

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CONFLICTS OF INTEREST: There are no conflicts of interest.

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