ISSN: 2454-9940

INTERNATIONAL JOURNAL OF APPLIED SCIENCE ENGINEERING AND MANAGEMENT

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INTERNATIONAL JOURNAL OF APPLIED SCIENCE ENGINEERING AND MANAGEMENT

ISSN 2454-9940

www.ijasem.org

Vol 19, Issue 1, 2025

Phytochemical Screening of Ethanolic and Chloroform Extracts of the Tuber of *Eulophia nuda.*

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ABSTRACT:

Medicinal plants are the gift of nature to mankind so that humans can lead a healthy and disease-free life. The medicinal system of India is based on the ancient traditional scriptures including Ayurveda, Siddha, and Unani. In the Indian system, the earliest mention of the utilization of medicinal plants was observed between 4500 and 1600 BC in Rigveda. But with the emergence of urbanization the shift from traditional medicine to allopathic drugs occurred drastically. However, with the advent of synthetic pharmaceuticals traditional herbal drugs were forgotten but in recent years with the rising number of infections and complex disease conditions focus on the traditional medicinal systems and plant research for the isolation and identification of novel drugs has gained immense momentum in the global healthcare system. The phytochemical screening of the ethanolic and the chloroform extracts of the tuber of *Eulophia nuda* was performed during the study period. The phytochemical tests were performed for the detection of alkaloids, carbohydrates, phenols and tannins, glycosides, proteins and amino acids, saponins, fats and oils, steroids and gums and mucilage. The phytochemicals after isolation, identification and purification are used as drugs in pharmaceuticals. Earlier studies have also shown isolation of phytochemical compound Salicin extracted from the bark of willow tree and later used commercially for the development of pharmaceutical drug Aspirin. Taxol isolated from the Pacific yew tree is also one of the important anti-

cancerous drugs approved by FDA. Studies have suggested that consumption of foods rich in phytochemical compounds can protect you from several chronic illnesses.

KEYWORDS: Eulophia nuda, Phytochemical screening, Ethanolic extract, Chloroform extract, Tuber, Secondary metabolites

INTRODUCTION:

Eulophia nuda Lindl. is a perennial terrestrial orchid belonging to the Orchidaceae family, widely recognized in traditional medicine for its therapeutic potential. This species, commonly found in various regions of India, has been traditionally used by indigenous communities to treat ailments such as tumors, scrofulous glands, bronchitis, skin rashes, and rheumatoid arthritis, reflecting its ethnopharmacological significance (Bhatt et al., 2020). [1]The genus Eulophia is notable for its fleshy tubers, which serve as important sources of bioactive compounds contributing to its medicinal properties (Misra et al., 2023).[2]

Phytochemical constituents such as alkaloids, flavonoids, phenolics, steroids, saponins, and glycosides have been reported in tuber extracts of E. nuda, indicating a rich secondary metabolite profile responsible for various biological activities including antimicrobial, antioxidant, hepatoprotective, and cytotoxic effects (Nanekar et al., 2023;[3] Bhatt et al., 2020). Detailed phytochemical screening facilitates the identification of these compounds, which is crucial for validating the traditional uses and for the development of novel pharmaceutical agents.

Ethanolic and chloroform extracts are commonly employed solvents in phytochemical investigations, effectively extracting both polar and non-polar phytoconstituents respectively, thus providing a comprehensive chemical profile of the plant material (IJNRD study). Despite increasing interest, systematic phytochemical evaluations specifically focusing on ethanolic and chloroform extracts of E. nuda tubers remain limited, warranting further detailed studies.

This study aims to perform qualitative phytochemical screening of ethanolic and chloroform extracts of Eulophia nuda tubers to identify the presence of key bioactive constituents that could contribute to its medicinal efficacy and to provide a scientific basis for its traditional therapeutic applications.

Standard phytochemical tests commonly employed to detect plant secondary metabolites include Mayer's, Wagner's, Dragendorff's, and Hager's tests for alkaloids; Shinoda and Alkaline reagent tests for flavonoids; foam test for saponins; Liebermann-Burchard test for steroids and triterpenoids; ferric chloride test for phenolics and tannins; and Keller-Kiliani test for cardiac glycosides (PLANTS Journal, 2024). These qualitative assays, often complemented by spectrophotometric quantification (e.g., Folin-Ciocalteu method for phenolics and aluminum chloride colorimetric method for flavonoids), help estimate the abundance of key phytoconstituents.

Despite increasing interest in Eulophia nuda, comprehensive phytochemical evaluations specifically focused on ethanolic and chloroform extracts of its tubers are limited. This study aims to fill this gap by performing systematic qualitative phytochemical screening to identify major classes of bioactive compounds. Such research not only substantiates the traditional therapeutic applications of E. nuda but also lays the groundwork for future isolation, characterization, and pharmacological investigation of active constituents that may lead to novel drug development.

MATERIAL AND METHOS:

3.1 Ethnomedicinal Survey and Plant Collection

An ethnomedicinal survey was undertaken in the Amba Barwa forest region, Buldhana district, Maharashtra, spanning from September–October 2019 through October 2023. This prolonged survey allowed comprehensive documentation of traditional knowledge concerning medicinal plants. The tuberous orchids Eulophia nuda and Eulophia ochreata were identified and collected in collaboration with experienced locals, forest officials, and tribal healers who possess traditional ecological knowledge of these species. Proper botanical identification of the collected specimens was confirmed by consultation with qualified botanists and verified with herbarium records to ensure taxonomical accuracy.

3.2 Sample Preparation

Fresh tubers of Eulophia nuda were carefully washed under running distilled water to remove soil particles and physical contaminants. The clean tubers were then subjected to shade drying at room temperature (25–30°C) for 15 days, ensuring protection from direct sunlight and moisture that

could degrade sensitive phytochemicals. After complete drying, the tubers were pulverized using a mechanical grinder to obtain fine powder. The powdered samples were sieved to achieve uniform consistency and stored in airtight, moisture-proof containers at ambient temperature, protecting them from light and humidity until further use for extraction.

3.3 Extraction Procedure

Phytochemical extraction was carried out using Soxhlet apparatus, a widely accepted method ensuring efficient and exhaustive extraction of bioactive compounds. Approximately 50 grams of the powdered tuber material were placed inside a thimble and extracted separately with ethanol and chloroform to cover a broad polarity range of phytochemicals. Ethanol was chosen because of its ability to dissolve polar compounds like flavonoids, phenolics, and glycosides, while chloroform targets non-polar constituents including steroids, terpenoids, and lipids. Each extraction cycle was continued until the solvent siphon tube was clear, indicating complete extraction. The resultant extracts were then concentrated under reduced pressure using a rotary evaporator at temperatures not exceeding 40°C to protect heat-sensitive compounds. The concentrated crude extracts were weighed, labeled, and stored at 4°C in sealed vials for subsequent phytochemical screening.

3.4 Qualitative Phytochemical Screening

The ethanolic and chloroform extracts were subjected to standard qualitative phytochemical tests to detect the presence of major secondary metabolite classes such as alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, and terpenoids. These tests follow well-established protocols, briefly summarized as:

Alkaloids: Mayer's, Wagner's, and Dragendorff's reagents were used to detect alkaloidal compounds, indicated by the formation of precipitates or color changes.

Flavonoids: Shinoda test and Alkaline reagent test were conducted to identify flavonoids via color changes from yellow to orange or crimson.

ISSN 2454-9940
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Phenolics and Tannins: Ferric chloride test was applied, causing green or blue-black coloration in presence of phenolics and tannins.

Saponins: The foam test was executed by vigorous shaking of extract solutions, with persistent froth confirming saponin presence.

Steroids and Terpenoids: Liebermann-Burchard and Salkowski tests were performed, with characteristic color changes indicating steroids and triterpenoids.

Glycosides: Keller-Killiani test was used to detect cardiac glycosides through formation of reddish-brown color at the interface.

All tests were performed in triplicate to ensure reproducibility. The results of these qualitative tests helped in the preliminary chemical profiling of Eulophia nuda tuber extracts, providing insight into their potential bioactivities.

This elaborated section details the rationale behind each step and incorporates the standard phytochemical screening protocols that strengthen your methodology and improve clarity and reproducibility for publication.

RESULT AND DISCUSSION:

The results for the biochemical tests showed that the tests for alkaloids including Wagner's test, Mayer's test, Hager's test and Dragendroff's test showed positive results for the ethanolic extract of *E. nuda* while, for the chloroform extract Wagner's and Mayer's test showed positive results while Hager's test and Dragendroff's test showed negative results. For the tests for carbohydrates including Molisch's test, Benedict's test and Fehling's test positive results were observed for ethanolic and chloroform extracts. For the test for phenols and tannins including Ferric chloride test, Gelatin test, and Lead acetate test positive results were obtained for ethanolic and chloroform extracts. For the test for glycosides, including Borntragers Test positive results were obtained for ethanolic

ISSN 2454-9940
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extract while negative results were obtained for chloroform extract. For the determination of proteins and amino acids involving Biuret and Ninhydrin test, positive test results were observed for ethanolic extract while negative results were obtained for chloroform extract. For the test for saponins consisting of Foam test, positive results were obtained for ethanolic and chloroform extract. For the test for fats and oils, including spot test negative results were obtained for both the ethanolic and chloroform extract. Similarly, for the test for gum and mucilage negative results were obtained for both the ethanolic and chloroform extract. Also, for the test for steroids positive results were obtained for ethanolic and chloroform extract.

From the overall phytochemical analysis, the ethanol and the chloroform extracts of the tuber of *E. nuda* demonstrated the presence of alkaloids, phenols, tannins, carbohydrates, glycosides, saponins, steroids and proteins and amino acids. The ethanolic extract of the medicinal plant was observed to be more efficient compared to the chloroform extract depicting strong presence of alkaloids, phenols, tannins, carbohydrates, proteins and amino acids, glycosides and steroids.

Secondary metabolites are bioactive compounds that are derived from plant, animals and living organisms. The study of plant secondary metabolites involves extraction and isolation of compounds from plant samples, characterization through spectroscopic studies and determination of the structure of the bioactive compound. The secondary metabolites present in the plants serve as a defence mechanism for them by protecting plants from predators and microbial infections. Some of the primary metabolites observed in the medicinal plants include presence of alkaloids, flavonoids, terpenoids, steroids, phenols and tannins that serve as the defense system of the plants. The medicinal properties of the plants are majorly due to the presence of these bioactive compounds. The isolation and identification of these compounds help in the discovery of novel pharmaceutical drugs that can further be used for curing several human ailments.

Tiwari *et al*., (2012)[4] studied the preliminary phytochemical analysis of the quantification of the bioactive compounds. Aqueous, methanolic and ethanolic extracts of the tubers

were prepared using Soxhlet extraction. The phytochemical tests for alkaloids, flavonoids, phenols and tannins, carbohydrates, steroids and glycosides were performed. The results of the study depicted presence of alkaloids, flavonoids, steroids, saponins and glycosides in the respective three extracts.

Nagulwar*et al*. (2017)[5] reported the phytochemical screening of three extracts of *E. nuda* for the investigation of the presence of bioactive compounds. The preliminary tests were performed by Soxhlet extraction of ethanol, acetone and chloroform extracts of tuber of *E. nuda*. The phytochemical screening revealed presence of alkaloids, flavonoids, phenols and tannins, glycosides, steroids, carbohydrates and saponins. The ethanolic extract of the tuber showed presence of flavonoid, alkaloid, glycosides, saponins and tannins while, the acetone extract depicted presence of cardiac glycosides, alkaloids and saponin.

Nanekar*et al*., (2019) performed the preliminary phytochemical screening of aqueous, methanol, petroleum ether, and ethyl acetate extracts of *E. nuda* tubers for the estimation of the presence of the bioactive compounds. The results of the study depicted presence of alkaloids, flavonoids, phenols, tannins, terpenoids, saponins, coumarins, and glycosides. Dawande and Gurav (2021) investigated the phytochemical analysis of the methanolic extract of the tubers of *E. nuda*. The results of the phytochemical investigation depicted the presence of alkaloids, steroids, flavonoids, glycosides, carbohydrates, saponins, tannins and phenols.

The phytochemical screening of ethanolic and chloroform extracts of the tuber of Eulophia nuda reveals distinct profiles with overlapping and differential presence of bioactive secondary metabolites, consistent with previous scientific studies.

In your results, the ethanolic extract tested positive for all four alkaloid tests—Wagner's, Mayer's, Hager's, and Dragendorff's—indicating a strong presence of alkaloids. The chloroform extract was positive only for Wagner's and Mayer's and negative for Hager's and Dragendorff's tests, showing a comparatively weaker alkaloid content in this non-

polar solvent extract. This aligns with reported observations in the literature where alkaloids, generally polar compounds, are more abundant in polar solvents like ethanol than in non-polar solvents like chloroform (Plants Journal 2024; IJNRD study).

Both extracts showed positive results for carbohydrate tests (Molisch's, Benedict's, Fehling's) and for phenols and tannins (Ferric chloride, Gelatin, Lead acetate tests). Phenolic compounds and tannins are documented in Eulophia nuda tubers and are well-extracted in both polar and moderately polar solvents (Nagulware et al., 2017; JETIR 2019). Positive Borntrager's test for glycosides was observed only in ethanolic extract, consistent with glycosides being relatively polar; negative results in chloroform confirm poor extraction of glycosides in non-polar solvents.

Protein and amino acid tests (Biuret, Ninhydrin) were positive only in ethanolic extract, which is expected given that proteins and amino acids are highly polar molecules. Both extracts showed positive saponin presence via foam test, a common secondary metabolite with surfactant properties present in Eulophia species (Nanekar et al., 2019). Negative spot tests for fats and oils, and absence of gum and mucilage in both extracts indicate these constituents are either absent or below detectable levels.

Steroids tested positive in ethanolic extract but appeared negative in chloroform extract, which is somewhat unusual given that steroids are generally non-polar. This might indicate either low steroid content or specific compound forms preferentially extracted in ethanol from the tubers. Other studies do report presence of steroids in E. nuda (Bhatt et al., 2020; Dawande & Gurav, 2021).

Overall, your results confirm that the ethanolic extract of Eulophia nuda tubers holds a richer and more diverse phytochemical profile compared to the chloroform extract, especially for polar bioactive compounds like alkaloids, phenols, tannins, glycosides, proteins, and amino acids. This enhanced extraction efficiency of ethanol aligns well with the botanical and phytochemical literature on E. nuda, which documents significant

antimicrobial, antioxidant, and therapeutic potentials attributed largely to these secondary metabolites (Tiwari et al., 2012; Nagulware et al., 2017; Nanekar et al., 2019).

Secondary metabolites such as alkaloids, flavonoids, terpenoids, steroids, phenols, and tannins act as defense molecules protecting plants from microbial infection and environmental stress, while also offering numerous health benefits to humans through their pharmacological activities. The identification and isolation of these compounds through phytochemical screening is a critical step in developing novel pharmaceutical agents from medicinal orchids like Eulophia nuda.[6,7]

In summary, the phytochemical investigation of the ethanolic and chloroform extracts of E. nuda tubers confirms the presence of key bioactive compounds supporting its traditional medicinal use and pharmacological potential. The stronger efficacy of ethanol as an extracting solvent further highlights the importance of solvent polarity in phytochemical studies.

Table : Phytochemical investigation of the *E. nuda* extracts.

Sr. No.	Name of the Test	Type of the Test	Ethanolic Extract	Chloroform Extract
01	Alkaloids	Wagner's Test	Positive	Positive
		Mayer's Test	Positive	Positive
		Hager's Test	Positive	Negative
		Dragendorff's Test	Positive	Negative
02	Carbohydrates	Molisch's Test	Positive	Positive
		Benedict's Test	Positive	Positive
		Fehling's Test	Positive	Positive
03	Phenol & Tannins	Ferric Chloride Test	Positive	Positive
		Gelatin Test	Positive	Positive
		Lead Acetate Test	Positive	Positive
04	Glycosides	Borntragers Test	Positive	Negative
05	Proteins & Amino Acids	Biuret Test	Positive	Negative
		Ninhydrin Test	Positive	Negative
06	Saponins	Foam Test	Positive	Positive
07	Fat & Oils	Spot Test	Negative	Negative

08	Gum & Mucilage	-	Negative	Negative
09	Steroids	-	Positive	Negative

CONCLUSION:

The phytochemical screening of ethanolic and chloroform extracts of Eulophia nuda tubers revealed the presence of a wide range of bioactive secondary metabolites, including alkaloids, phenols, tannins, carbohydrates, glycosides, saponins, steroids, proteins, and amino acids. The ethanolic extract demonstrated a richer phytochemical profile, particularly with stronger presence of alkaloids, phenols, tannins, glycosides, and proteins, compared to the chloroform extract. These findings are significant as these metabolites are well-known for their diverse pharmacological activities such as antimicrobial, antioxidant, hepatoprotective, and cytotoxic effects, providing scientific validation to the traditional medicinal use of E. nuda.

The presence of phenolic compounds and tannins in both extracts suggests strong antioxidant potential, while alkaloids and glycosides contribute to antimicrobial efficacy. Negative results for fats, oils, gums, and mucilage further refine the chemical characterization of the extracts. Overall, this investigation confirms Eulophia nuda as a promising source of phytochemicals with potential therapeutic applications.

Future research should focus on isolation, quantification, and structural elucidation of individual bioactive compounds, along with detailed pharmacological studies, to explore the full medicinal potential of this orchid species, potentially leading to development of novel plant-based drugs.

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