



Qualitative Phytochemical and Physicochemical Analysis of Aqueous Bulb Extracts of *Eulophia graminea* Lindl. - Terrestrial orchid

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Abstract

The present research was carried out to investigate the Preliminary phytochemical screening, and physicochemical analysis of aqueous extracts bulb of *Eulophia graminea*. The phytochemical screening showed the presence of alkaloids, Flavonoids, Phenols, Terpenoids, Anthocyanidins, Saponins, Tannins and Glycosides in different extracts of bulb. Results of physicochemical analysis shows the percent of water soluble as 32.29 gm alcohol soluble ash 12.10 gm; whereas acid insoluble soluble ash 1.27gm the loss of weight on drying at found to be 6.29 gm. Qualitative analysis of phytoconstituents and physicochemical analysis of *Eulophia graminea* was not reported earlier and in other species *Eulophia* also not reported. Presence of bioactive constituents may exhibit various biological activities in support as represented in the herbal usage against vermifuge and as ear drops.

Keywords

Flavonoids, phytochemical screening, physicochemical analysis, *Eulophia graminea*.

INTRODUCTION

Medicinal values of many plants still remain unexplored for their therapeutic activities of compounds. Pharmacognostic investigations of plants are carried out to find novel drugs for the development of new therapeutic agents [1]. Many plant extracts have been shown to inhibit the growth of microorganisms usually considered to play a role in defense reactions of plants, towards infections by pathogenic microorganisms [2]. In India, genus *Eulophia* (Orchidaceae) is distributed in tropical Himalaya and Deccan Peninsular region. *Eulophia ochreatea* bulb extract were used for several therapeutic purposes in different parts of India [3-4].

Crushed bulb extract is used as vermifuge [5]. Bulb extract used as Ear drops.[6]. Hence the extracts were subjected to analysis for organoleptic and physicochemical parameters. Results exhibit in the ash values percentage more in water soluble extractives 32.29 gm followed by alcohol 12-10gm total ash 7.48gm, and moisture content of the bulb shows 6.29 gm. Qualitative Phytochemical screening shows the presence of more secondary metabolites in aqueous, alcohol and acetone extracts like alkaloids, flavonoids, phenols, terpenoids saponins and glycosides. Steroids present only in alcohol and anthocyanidins in ethyl acetate extract. Hence a further study like antimicrobial, antihelminthic activities has to be

carried out. Application of these bio activities constituents in the preparation of ear drops and against vermifuge by the pharmaceutical industries is recommended.

MATERIALS ANDMETHODS

Plant material collection:

Eulophia graminea bulbs are collected from Jaapali area of Tirumala, Chittoor District of Andhra Pradesh, India. Species was identified and herbarium Specimen deposited With Voucher No.SR:01 in the Department of Biotechnology, Rayalaseema University, Kurnool District, AP. The bulbs were rinsed with clean tap water to make it dust free, sliced and subjected to shade drying kept at room temperature for few days. The dried bulbs were grounded into fine powder using the mixture grinder and stored in airtight bottles. Preparation of plant extracts for phytochemical studies. The bulb powder was subjected to successive extraction with Cold and Hot water, Methanol, Chloroform, Ethyl Acetate and Acetone using soxhlet apparatus. The collected extracts were concentrated by heating on water bath and further used for phytochemical studies; by standard method [7]; [8]; [9].

1. Test for Alkaloids:

To the plant extract chloroform was added and the residue obtained was digested with 1% HCl. The resulting acidic solution was divided into 2 parts. To one part was added the Mayer's reagent and to the second part Wagner's reagent was added.

- i. Mayer's reagent test: Mayer's reagent was prepared by adding 1.3g of Mercuric chloride and 5g of Potassium Iodide which were dissolved separately in 60ml and 10ml of double distilled water then both the solutions were mixed and diluted to 100ml. Development of precipitation and turbidity shows the presence of alkaloids.
- ii. Wagner's reagent test: Wagner's reagent was prepared with 2g of Potassium Iodide and 1.27g of Iodine dissolved in distilled water and they were diluted to 100ml distilled water. Development of yellowish white precipitate shows the presence of alkaloids.

2. Test for Flavonoids

- i. **Shinodon's test:** To few ml of the extract few drops of Conc. HCl is added and followed by addition of small pieces of magnesium ribbons. Development of pinkish red colour shows the presence of Flavonoids.
- ii. **Ferric chloride test:** To few ml of the extract, few drops of Ferric Chloride solution were added. Formation of blackish red color indicates the presence of Flavonoids.

3. Test for Phenolic Compounds

- i. **Phenols test:** To few ml of plant extract 1 or 2 drops of 1% Ferric chloride solution is added. Formation of Intense blue colour indicates the presence of phenols.
- ii. **Ellagic acid test:** Few ml of the extract was treated with a few drops of 5 % acetic acid and few drops of 5% sodium nitrate solution. If muddy or brown precipitate indicates the presence of Phenols.

4. Test for Glycosides

Keller Kilani test: To 5 ml of the extract glacial acetic acid and 2 drops of ferric chloride is added. The contents were transferred to test tube containing 2ml of Conc. H₂SO₄. A reddish brown colour ring was observed at the junction of two layers considered as positive test for Glycosides.

5. Test for Tannins

- i. **Gelatin test:** The methanolic extract was concentrated and the residue was dissolved in water and tested with 1% gelatin solution (1 g of gelatin dissolved in 10g NaCl w/v solution). Appearance of white precipitate is taken as positive test for Tannins.
- ii. **Ferric chloride test:** To 5 ml of the extract a few drops of ferric chloride was added. A blackish precipitate indicates the presence of Tannins.

6. Test for Steroids

- i. **Salkowski test:** Few ml of the extract CHCl₃ was added followed by the addition of Conc.H₂SO₄. Formation of red colour shows the positive test for steroidal compounds.
- ii. **Liebermann's Burchard test:** Few ml of the extract is treated with 0.5 ml of CHCl₃ followed by adding Conc. H₂SO₄ along the sides of the test tube. Formation of green colour indicates the presence of steroids.

7. Test for Lignins

- i. **Lignin test:** The plant extract was tested by adding conc. HCl and 2% furfuraldehyde. Development of red color indicates the presence of Lignin.
- ii. **Labat test:** The extract was mixed with Gallic acid. It develops olive green colour indicates the positive test for Lignins.

8. Test for Saponins

The plant extract was evaporated to dryness; Tap water is added and shaken vigorously in the graduated cylinder for 15 minutes. Formation of persistent 2 cm honeycomb froth was taken as positive test for Saponins [10]

9. Test for Terpenoids:

Liebermann–burchard's test: Few ml of extract is treated with 0.5ml acetic anhydride and 0.5ml of CHCl_3 followed by adding 0.5 ml of Conc. H_2SO_4 . Formation of reddish violet color shows the positive test for terpenoids.

10. Test for Anthocyanidins

To 5 ml of the extract equal volume of methanolic HCl was added. The Formation of red or purple color considered as positive test for Anthocyanidins. Physicochemical parameters. Physicochemical analysis of the *Eulophia graminea* was done by the standard procedures of [11]; [12];[13]; [14]. Such as total ash, acid insoluble ash, solubility matter in alcohol and water, loss on drying at 105°C as per methods described above.

Determination of Ash value

Ash values such as total ash, acid insoluble ash, water-soluble ash, and sulfated ash were determined. For determination of ash values, powders of leaf, scape, rhizome, flower and roots of selected plant were passed through sieve no. 20 and following tests were performed.

a) Total ash

About 3 g of dried powders were taken separately in a previously ignited and weighed silica crucible. The powders were spreaded in an even layer and ignited gradually by increasing temperature up to $500\text{--}600^\circ\text{C}$ to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air-dried powder.

$$\% \text{ of Total Ash Value} = \frac{\text{Weight of the Ash}}{\text{Weight of the crude drug taken}} \times 100$$

b) Acid insoluble ash

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get a constant weight. The

percentage of acid insoluble ash was calculated with reference to the air-dried drug.

$$\% \text{ of Acid insoluble Ash} = \frac{\text{Weight of the acid insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

c) Water soluble ash

The ash obtained as described for the total ash, was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 min. and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of total ash. The difference of weight was considered as water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried parts respectively.

$$\% \text{ of Water Soluble Ash Value} = \frac{\text{Weight of the Total Ash} - \text{Weight of the water insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

d) Moisture content

Air dried material was accurately weighed (10g) in a previously dried and stored in weighing bottle. The sample was heated in an oven at 105°C . The loss of weight in mg material was calculated and values were tabulated.

$$\% \text{ of Moisture Content} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample taken}} \times 100$$

RESULTS AND DISCUSSION

Phytochemical studies

The results of the phytochemical analysis of bulb extracts are presented in (Table 1). Cold and Hot water, Alcohol and Acetone extracts shows nearly 10 Phytoconstituents and lowest constituents represent in methanol and ethyl acetate extracts. Main constituents are Alkaloids, Flavonoids, Phenols; Terpenoids, Saponins, Tanins and Glycosides, but Steroids are present in alcohol extracts, Anthocynadin in Ethyl acetate extract; and lignins in Acetone extract only.

Methanol extracts represents only Flavonoids. It is observed that the presence of alkaloids, flavonoids, steroids terpenoids and saponins in *Eulophia nuda* common constituents [15].

Table 1: PHYTOCHEMICAL SCREENING OF *Eulophia graminea*

Name of the Test	Bulb						
	CW	HW	A	M	C	E. A	AC
1 Alkaloids							
a) Mayer's test	+	-	++	-	-	-	++
b) Wagner's test	-	-	++	-	-	-	+
2 Flavonoids							
a) Shinodons test	-	-	-	+	-	-	-
b) FeCl ₃ test	+	+	-	+	-	-	-
3 Phenols							
a) FeCl ₃ test	-	+	-	-	-	-	-
b) Ellagic acid test	+	-	+	-	++	-	-
4 Terpenoids							
a) Salkowski test	-	+	-	-	-	+	+
5 Steroids							
a) Salkowski test	-	-	+	-	-	-	-
b) Liebermann's Burchard test	-	-	-	-	-	-	-
6 Anthocyanidins	-	-	-	-	-	+	-
7 Saponins	+	+	+	-	-	-	+
8 Tannins							
a) Gelatin test	-	-	-	-	-	-	-
b) FeCl ₃ test	+	+	-	-	+	-	+
9 Lignins	-	-	-	-	-	-	+
10 Glycosides							
a) Keller-kilani test	-	++	-	-	+	-	-

- : absent +: Present

CW: Cold water HW: Hot water A: Alcohol M: Methanol C: Chloroform E.A: Ethyl Acetate AC: Acetone

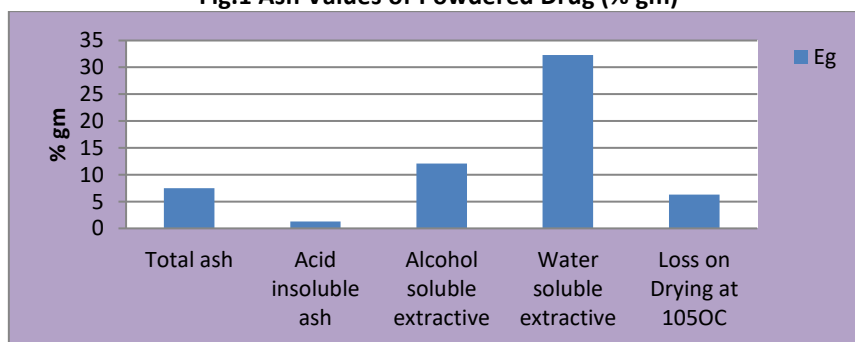
Table 2. Ash Values of bulb Powder (%gm)

S.no	Parameters	Eg
1	Total ash	7.48
2	Acid insoluble ash	1.27
3	Alcohol soluble extractive	12.10
4	Water soluble extractive	32.29
5	Loss on Drying at 105°C (Moisture content)	6.29

Physico-Chemical Standards

The powder extracts microscopic observations show that colour represents green; without any specific odour and texture is fibrous. The physico-chemical parameters (Table-2 and Fig 1) in the percentage of

water soluble as 32.29 gm, alcohol soluble ash 12.10 gm; total ash 7.48 gm, acid insoluble ash 1.27 and the loss of weight on drying moisture content found to be 6.29 gm.

Fig.1 Ash Values of Powdered Drug (% gm)


Physicochemical parameter was carried out only in *Eulophia graminea* so far. Not in any other orchids.

CONCLUSION:

Due to the presence of high amounts of alkaloids, flavonoids, phenols, further studies like antimicrobial, anthelmintic activities have to be screened out for their biological activity against vermifuge action and also as ear drops. For the pharmaceutical industries drug preparation against ear drops and as antihelmintic is recommended.

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