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Chemical analysis, nutritional content and antioxidant property of *Eulophia herbacea* Lindl. tubers: a medicinally versatile Indian tribal nutritional food supplement

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In the present study, chemical compositions, nutritional evaluation and antioxidant activity of orchid, *Eulophia herbacea* Lindl. tubers were investigated. Nutritional analysis was confirmed by presence of vitamins, amino acids, minerals, glucomannan and protein. The phenolic, flavonoid, proanthocynidin, and saponin content were also determined. The antioxidant capacity was evaluated by DPPH, reducing power, nitric oxide radical scavenging and total antioxidant capacity. The total caloric value was corresponded to 2742 Kcal/kg. HPTLC analysis of various amino acids revealed the presence alanine, threonine, serine and aminobuteric acid whereas HPLC quantify 2.84 & 0.22 µg/mL of ascorbic acid and pyridoxine respectively in tubers. The result indicated that *E. herbacea* contains polyphenolic compounds with good antioxidant properties. Thus *Eulophia herbacea* has a potential for developing value-added nutritional food products and could be used for the future therapeutic medicine as bioactive compound.

Keywords: Tubers, *Eulophia herbacea* Lindl., Nutritional value, Antioxidant, Glucomannan

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Horticultural plants are very important for human diet as sources of vitamins, minerals and dietary fiber and they become a significant part of human life moreover due to their medicinal and environmental uses as well as aesthetic and economic value. The stem, leaf, flowers, roots and the fruits of vegetables and fruit crops have the highest potential of export[1-4]. In developing nations, numerous types of edible wild plants are exploited as sources of food hence providing an adequate level of nutrition to the populations[5]. *Eulophia herbacea* Lindl. tubers are used as *salep* in Indian bazaar for many years for its nutritive and demulcent properties[6]. Tubers are widely used as cheap source of carbohydrate. The tubers and pseudo bulbs of several orchids like *Eulophia* are used for preparing *salep* which is valued as a restorative in the treatment of many diseases[7]. Decoction of tuber is used on spermatorrhoea, urinary complaints and menses[8]. It also shows antimicrobial and cytotoxic activity[9]. *Salep* is known to be a valuable source for glucomannan, contains natural neutral water soluble fibres, which help to normalize blood sugar, relieve stress on the pancreas and discourage blood sugar abnormalities such as hypoglycemia[6]. The tribal people of Satpudas are not aware about modern medicines and nutrition rich diet; still completely depend upon the conventional system of food habit and medicines in twenty first century. Nutritional information of this species will be useful for the nutritional education of the public and may act as a means to improve the nutritional status of the population. The objective of this study was to evaluate indigenous tuber of *E. herbacea* with the aim of preliminary phytochemical study that might serve as guide to exploit its potential and benefit for human nutrition. This data should be a starting point of a valuable knowledge resource, allowing better food selection and consequent improvement in nutritional status of the diet of local populations.

Materials and methods

Chemicals

1,1-diphenyl-2-picryl-hydrazyl (DPPH) was procured from Sigma Chemicals, USA; ferric chloride ($FeCl_3$),

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potassium ferricyanide [$K_3Fe(CN)_6$] were purchased from Loba Chemie, India; ascorbic acid, trichloro-acetic acid and ethylene diamine tetra acetic acid were purchased from S.D. Fine chemicals, India. α-tocopherol acetate and catechin were procured from Merck, India. Rutin was purchased from RRL, Jammu. All other reagents used were of analytical grade.

Plant material and extraction

The tubers of *Eulophia herbacea* Lindl. were collected in between July 2015 from the valley of Toranmal region, District Nandurbar, Maharashtra, India. The plant was authenticated by Dr T Chakraborty, Scientist D for Joint Director (voucher no. YOPA-EUHER2) Botanical Survey of India (BSI), Pune, Maharashtra, India.

For evaluating the antioxidant activity of plant, it is necessary to separate out the similar group of phytoconstituents in different solvent based on their solubility and polarity. The air dried tubers were coarsely powdered and batch of 150 g extracted successively with petroleum ether (60-80°) (PE), methanol (ME), aqueous (AE) by hot continuous extraction using Soxhlet apparatus. The hot continuous percolation was carried out as 25 cycles per hour for 6 hrs for each solvent. The Extracts after filtrations were dried using a rotary evaporator (BUCHI, Rotavapor R - 215) under reduced pressure and extract were freeze dried.

Preliminary phytochemical screening

Preliminary phytochemical investigation of PE, ME, AE extracts of *Eulophia herbacea* was carried out as per standard protocol[9]. Methanolic and aqueous extracts of *E. herbacea* were also evaluated for vitamins analysis.

Nutritional study

Moisture was determined using the drying oven methods, by drying a 2 g sample in an oven at 105 °C for 3 hrs. The ash content was determined by incineration of known amount of a sample (3 g) in a muffle furnace at 600 °C for 6 hrs. Reducing sugar was determined by 3, 5-dinitrosalicylic acid[10]. Fat was determined by petroleum ether extraction in a Soxhlet apparatus. Crude fibre[11] was estimated by acid-base digestion with 1.25 % sulphuric acid (W/V) and 1.25 % NaOH (W/V). Protein was determined by Lowry method[12]. Glucomannan in the sample was determined by konjac method[13]. Energy value of foods and energy required for the body are usually measured in calories.

Determination of minerals and heavy metals

The minerals calcium (Ca), magnesium (Mg), iron (Fe), sodium (Na) and potassium (K), heavy metals like arsenic (As), lead (Pb), mercury (Hg) and cadmium (Cd) were measured by atomic absorption spectroscopy.

Chromatographic study

The amino acid pattern of methanolic extract of *E. herbacea* plant was recorded using HPTLC (Camag, Switzerland) on pre-coated silica gel plates (Merck) using n-Butanol: Water: acetic acid (4:1:1v/v/v) as solvent system. Water soluble vitamins were estimated by an HPLC system (Agilent 1200) using C-18 column (150 cm x 4.6 mm) in isocratic mode with 0.1 mol L^{-1} of KH_2PO_4 (*p*H 7): methanol (90:10 v/v) as mobile phase.

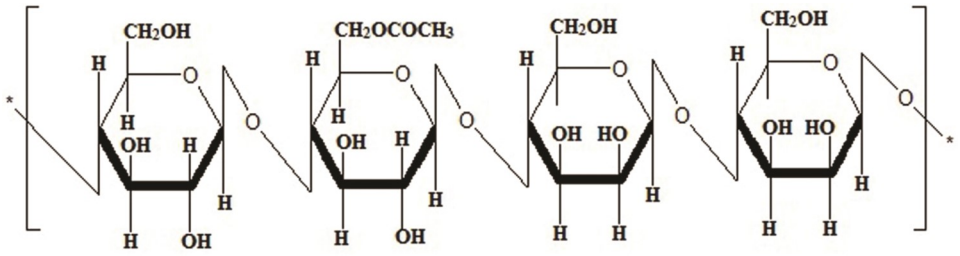
Determination of total phenolic, flavonoid, proantho-cynidin and saponin content

Total phenolic content of extracts was determined by Folin-Ciocalteau reagent using gallic acid as a standard. Absorbance was recorded at 750 nm using UV-visible spectrophotometer-[1601 (Shimadzu)] and the results were expressed as gallic acid equivalents. Total flavonoid content was determined by aluminium chloride colorimetric method using rutin as a standard. The absorbance of samples and blank was read at 520 nm. Total flavonoids were expressed as µg/mL rutin equivalent[14].

Total proanthocyanidins were determined using vanillin-H_2SO_4 method[15]. The absorbance was measured at 500 nm. Calibration curve was drawn using aqueous solution of epicatechin (20-100 µg/mL) as a reference standard. Saponin content was calculated as percentage according to method described by Rakhimov & Yuldasheva[16].

Isolation, purification and characterization of glu-comannan

Dried powder of *E. herbacea* tubers was extracted with water (1:25) by stirring at room temperature for 2 hrs. The extract was separated by centrifugation and treated with ethyl alcohol in the ratio 1:2, thus obtained crude glucomannan (EHG), dried in vacuum over P_2O_5, was subjected to spectral analysis[17] such as UV, FT-IR (8400S),^{13C} NMR



Structure of Glucomannan

analysis [Bruker DRX- 300] at 300MHz FT-NMR in DMSO using TMS as internal standard (see structure of Glucomannan).

Evaluation of antioxidant activity

1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity

The antioxidant activity of the plant extract was assessed on the basis of the radical scavenging effect of the stable DPPH free radical given by Chen *et al*., 2007[18]. Ascorbic acid was used as a standard compound in DPPH assay.

Reducing power

The reducing power of the extract of *Eulophia herbacea* was evaluated according to the method described by Adedapo[19]. The absorbance was measured at 700 nm.

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity of *Eulophia herbacea* was performed according to Chen *et al*., 2007[18].

Absorbance was measured at 546 nm against blank.

Total antioxidant capacity

The antioxidant capacity of the samples was evaluated according to Priet *et al*., 1999 and expressed as milligram of ascorbic acid equivalents per gram of dry weight[20]. The absorbance of the mixture was measured at 695 nm and standard curve was performed with ascorbic acid solution.

Statistical analysis

Results of *in vitro* studies were expressed as mean ± SD and n = 3.

Results

Preliminary phytochemical screening

The preliminary phytochemical screening of *E. herbacea* extracts revealed the presence of carbohydrate, protein, amino acids, flavonoids, tannins, phenolics, steroid, mucilage and vitamin C, B_1, B_6 and E.

Nutritional parameters

Nutritional study of *E. herbacea* tubers showed relatively higher moisture content (8.3±0.15, dry basis) (83.67±0.86, fresh tubers) and total ash content (7.6±0.208 %). These indicate that the tubers are a good source of minerals. These minerals form scaffolding of human body structure. Total ash value is lower as compared to different species of *E. ocherata* (9.1 ± 5.17)[21]. Fat content of *E. herbacea* (1.531 ± 0.25 %) was less than the other species reported in the literature is *E. ocherata* (3.25±3.81), One gram of fat on complete oxidation in the body gives rise to 9 kcal, more than twice that given by carbohydrates and proteins[22]. Crude fiber content of tubers (31.5±1.32 %) was found higher than the *Eulophia ocherata* (22.9±6.27). The protein content detected in tubers (5.23 ± 1.23) was somewhat lower than *E. ocherata* (5.44±10.23). According to result *E. herbacea* could be a good source of carbohydrates (43.42±0.004). The calorific value of *E. herbacea* tubers was found to be 2742 Kcal/kg. Total reducing sugar content of *E. herbacea* was found to be 1.467±0.03 %. Sugars are present in tubers in conjunction with various other macromolecules, which could decrease the radiation sensitivity of sugars to radiolytic attack. Total glucomannan content in *E. herbacea* was found to be 51.82 % and is shown in Table 1. Results of elemental analysis of *E. herbacea* are shown in Table 2.

Chromatographic study

Results of HPTLC analysis of methanolic extracts of *Eulophia herbacea,* confirmed the presence of alanine, threonine, serine and aminobuteric acid and is shown in Fig. 1.

The amount of Vitamin C and B_6 in methanolic extract of *E. herbacea* was detected by HPLC and it was found to be 2.84 and 0.22 (µg/mL) respectively as shown in Fig. 2 and Table 1.

Table 1 — Physicochemical, phytochemical and nutritional analysis of *Eulophia herbacea* Lindl. tubers

Parameters	Composition*
Physicochemical analysis	
Moisture	8.3 ± 0.152 %
Total ash	7.6 ± 0.208 %
Phytochemical analysis	
Phenolics	12.60 ± 0.028 µg equivalent gallic acid
Flavonoids	7.746 ± 0.023 µg equivalent rutin
Proanthocynidin	2.1 ± 0.002 µg equivalent catechin
Saponin	3.5 ± 0.011
Nutritional analysis	
Protein	5.23 ± 0.012 %
Fat	1.53 ± 0.250 %
Fibre	31.5 ± 1.322 %
Carbohydrate	43.42 ± 0.004 %
Reducing sugar	1.467 ± 0.001 %
Glucomannan	51.82 ±0.123 %
Calorific value	2747 Kcal/kg
Vitamin C	2.84 µg/mL
Vitamin B6	0.22 µg/mL

*values are mean ± s.e.m. of three replicates

Table 2 — Minerals and heavy metal composition of *Eulophia herbacea* Lindl. tubers

Parameters	Results (Unit)
Calcium (Ca)	1.71 %
Magnesium (Mg)	2.48 %
Iron (Fe)	164 mg/kg
Sodium (Na)	3.42 %
Potassium (K)	2.48 %
Arsenic(As)	N.D
Cadmium(Cd)	0.85 mg/kg
Lead (Pb)	2.5 mg/kg
Mercury	N.D.

n.d.- not detected

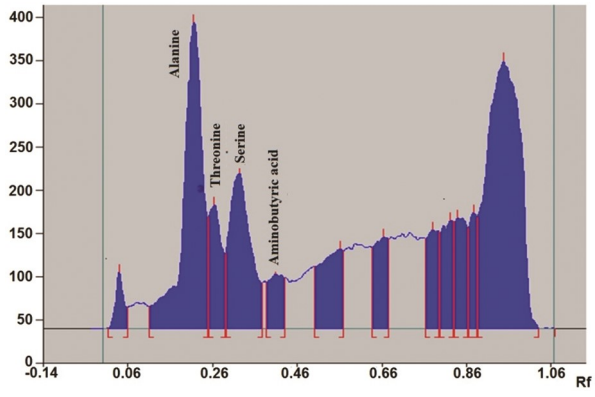


Fig. 1 — HPTLC chromatograms of amino acid pattern of *Eulophia herbacea* recorded at 366 nm after treatment with ninhydrin reagent showing alanine, threonine, serine and amino butyric acid

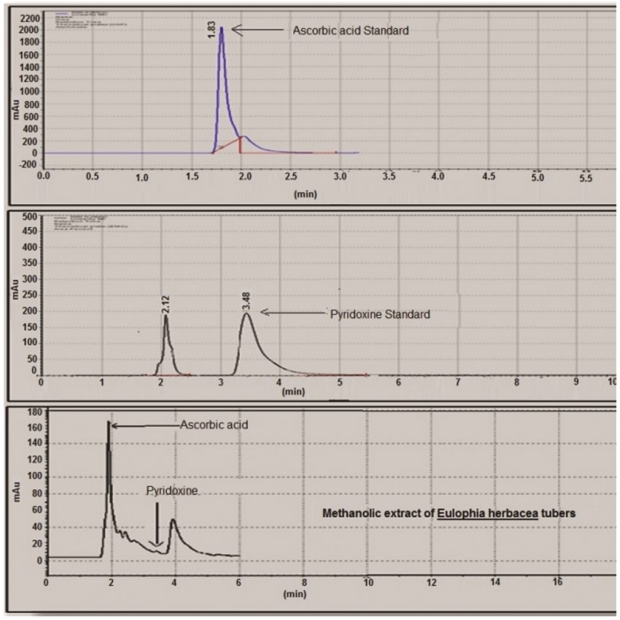


Fig. 2 — HPLC chromatogram of vitamins viz. ascorbic acid, pyridoxine and methanol extract of *Eulophia herbacea*

Determinations of phenolics, flavonoids, proanthocynidin, and saponin

The result of phenolic content of *E. herbacea* tubers was found to be 12.60±0.028 % gallic acid. *E. herbacea* showed the good amount, i.e., 7.75 ± 0.03 % of flavonoid equivalent to rutin whereas total proanthocynidins content of *E. herbacea* was found to be 2.1 ± 0.20 % equivalent to catechin; glycosylated derivative of anthocyanidin present in colorful flowers and fruits. Saponin content in *E. herbacea* was found to be 3.5 ± 0.01 % as shown in Table 1.

Characterization of glucomannan

The melting point of glucomannan was recorded as per standard method and it was found to be 220-221 °C**.** According to the results of mp, UV, IR, NMR data it was concluded that the isolated compound may be glucomannan[23].

Evaluation of antioxidant activity

Different extracts of *E. herbacea* were investigated for their *in vitro* antioxidant activity. The results indicate that extracts had comparable radical scavenging potential than reference standards due to the presence of phenolic acid, Vitamin B_1, B_6, C, and E. The DPPH radical scavenging effects of different extracts of EH have been shown in Fig. 3a. Lower IC_{50} value indicates high inhibitory activity. MEE < PEE < AEE 71.18, 75.80, 79.48 µg/mL suggested that MEE had the highest activity. *E. herbacea* showed

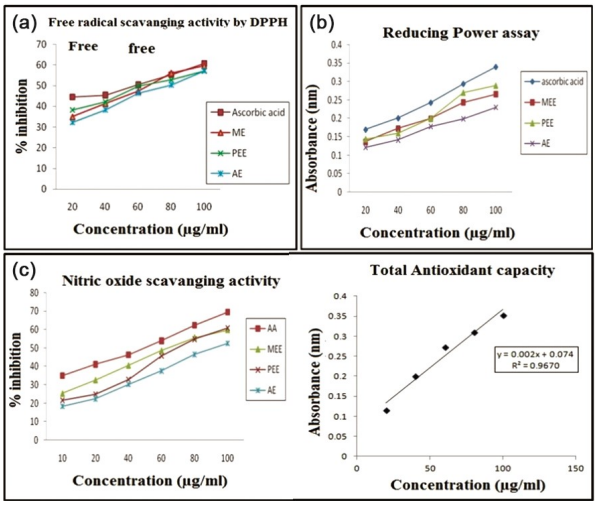


Fig. 3 — (a) DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity, (b) Reducing power assay, (c) Assay of Nitric oxide radical scavenging activity- Total antioxidant capacity

Table 3 — IC_{50} values for antioxidant activity of different extract of *Eulophia herbacea* Lindl. tubers

Test Material	IC_{50}(µg/mL)		Total antioxidant capacity. (µg/mL)
	Free Radical Scavenging Activity by DPPH	Nitric oxide radical scavenging activity	
Ascorbic Acid	59.41	55.59	-
MEEH	71.18	71.69	25
PEEH	75.80	72.88	66
AEEH	79.48	95.34	05

MEEE- Methanol extract of *E. herbacea;* PEEH - Petroleum ether extract; of *E. herbacea* AEEH – aqueous extract of *E. herbacea.*

lower reducing power than standards ascorbic acid. Reducing capabilities of different extracts of *E. herbacea* are shown in Fig. 3b. The nitric oxide radical scavenging activity of different extracts of *E. herbacea* is shown in Fig. 3c**.** Methanolic extract of *E. herbacea* displays good activity**.** The results of IC_{50} values for antioxidant activity are shown in Table 3.

Discussion

The presence of carbohydrate, protein, amino acids, flavonoids, tannins, phenolics, steroid, mucilage and vitamin C, B_1, B_6 and E in *E. herbacea* plant exhibited diverse pharmacological and nutritional action in human beings. The physiological effects of total dietary fiber, in the form of insoluble and soluble fractions of food, have a significant role in human nutrition. It plays an important role in decreasing the risk of many disorders such as constipation, diabetes, cardiovascular diseases, and obesity. Total protein and amino acids are essential for body cells[5]**.** Available carbohydrates provide the energy needed by the body to maintain its living activities, including working, walking and even sleeping. Glucomannan are natural water soluble fibres, which help to normalize blood sugar, relieve stress on the pancreas, and discourage blood sugar abnormalities, such as hypoglycemia[6]. Minerals are important in diet because they serve as cofactors for many physiological and metabolic functions and in their absence, clinical deficiencies may occur[24]. Calcium plays a crucial role in providing rigidity to the skeleton besides its involvement in neuromuscular function, blood clotting, and many other metabolic processes. Iron is an essential mineral and it is an important component of protein involved in oxygen transport and metabolism. Iron is also an essential cofactor in the synthesis of neurotransmitters such as dopamine and serotonin, and is important cellular growth. Environmental pollution is the main cause of heavy metal contamination in food chain[25].

There is increasing evidence that consumption of a variety of phenolic compounds present in natural foods may lower the risk of serious health disorders because of their antioxidant activity[26]. When added to foods, antioxidants minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality and increase shelf life[27]. Dietary flavonoids possess antiviral, anti-inflammatory, antihistamine, and antioxidant properties. Multiple benefits of eating flavonoid-rich plant foods for human health are well documented[28]. Saponin is known as anti-nutritional factor, which reduces the uptake of certain nutrients including glucose and cholesterol in the gut through intra-luminal physicochemical interaction. Hence, it has been reported to have hypocholesterolemic effects and thus may aid in lessening the metabolic burden that would have been placed on the liver[29].

Natural antioxidants with multifunctional potential are of high interest as alternatives for synthetic antioxidants to prevent oxidation in complex food systems like muscle food[30]. Briefly, the rationale for antioxidant activity in the present study has been mentioned as *E. herbacea* is popularly used for treatment of various diseases including diabetes, furthermore, it shows cytotoxic potential. The phytochemical analysis has shown to posses the significant amount of polyphenols and vitamins (known natural antioxidants). On these finding, the

extracts of *E. herbacea* were assessed for antioxidant activity through series of antioxidant assay. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. It has been widely accepted that the higher the absorbance at 700 nm, the greater is the reducing power. All extracts were found to have significant reducing capability, similarly mentioned by Kalaskar & Surana[31]. MEE showed appreciable reduction in absorbance than PEE and AEE indicating that it had higher reducing power. The phosphomolybdenum assay is a quantitative method to evaluate water-soluble and fat soluble antioxidant capacity (total antioxidant capacity), in which transforming of relative free radical species MO (VI) into more stable MO (V) non-reactive products occurs[32]. Total antioxidant activity is expressed as the number of equivalent of ascorbic acid (mg/g plant extract). The results of this work showed that the tubers of *E. herbacea* Lindl. are rich source of basic nutrients required by human and hence it is recommended for consumption by even economically weaker sections of populations to alleviate the problem of malnutrition or to overcome the nutritional disorders. Phenolic compound such as flavonoids, phenolic acid and proanthocynidin are considered to be the major contributor to the antioxidant activity of vegetables and medicinal plants, earlier studies reported similar findings[31], this confirms the results of present study. *Eulophia herbacea* tubers were found to be efficient free radical scavengers (60-70 % compared to ascorbic acid), therefore, these plant tubers could potentially be exploited as sources of antioxidants.

Conclusion

From the above results, it can be concluded that the antioxidant activity of *Eulophia* species is attributed to the presence of phenolic compounds acting as primary antioxidant or free radical terminators.

In the conclusion, the plant E. *herbacea* have good amount of carbohydrate, energy, adequate protein, low lipid content and phytochemicals revealed that it could be used as a food rich essential micronutrients for human. Further proper utilization and consumption of high amount of micro- and macro elements from such plants in human need to be exploited.

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