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Research Article

Phytochemical Investigation and Validation of Antioxidant Potential of β-Sitosterol from Tubers of *Eulophia herbacea* and *Eulophia ochreata*

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ABSTRACT

Many species of the genus *Eulophia* from family Orchidaceae are used as excellent health-promoting agent, in traditional medicine to treat diarrhoea, stomach pain, rheumatoid arthritis, cancer, asthma, bronchitis, gynaecological problems, paralysis, cough, anaemia, piles, impotency, tuberculosis, epilepsy, blood purification. Phytochemical investigation has been carried out qualitatively as well as quantitatively on tuber powder and extracts of *Eulophia herbacea* and *Eulophia ochreata* prepared in different solvents. The order of % of extracts obtained in different solvents is water > methanol > benzene > chloroform > petroleum ether for *E. herbacea* as well as *E. ochreata.* Secondary metabolites like phytosterol, mucilage, phenol, proanthocyanidine and flavonoid have been detected in both the test plant extracts. β-sitosterol has been isolated and purified from methanolic extract of both the plants using TLC, HPLC and spectroscopically, however β-sitosterol is more in *E. ochreata* than *E. herbacea*. Both the *Eulophia* species have been found to possess significant radical scavenging activity against 1, 1-diphenyl-2-picrylhydrazyl (DPPH). IC_{50} value of *E. herbacea* and *E. ochreata* was found to be 51.04 µg/ml and 50.13µg/ml respectively. Further validation of antioxidant potential of β-sitosterol confirmed its *in vitro* antioxidant property through DPPH free radical scavenging and reducing power properties.The medicinal property of *Eulophia* may be attributed due to the presence of phenol, flavonoids and β-sitosterol with profound antioxidant potential. The therapeutic effect of this plant may be accounted for its counteracting action on free radicals *in vivo*.

Keywords: secondary metabolites, β-sitosterol, antioxidant, DPPH, *Eulophia* species.

INTRODUCTION

Orchids such as *Eulophia* from the family *Orchidaceae* have attracted the worldwide researchers towards their ethnomedicinal potential. Phytochemical analysis of plant is very important commercially and has great interest in pharmaceutical industries for the production of the new drug for curing of various diseases[1]. Tribal people of India routinely consume tubers of *Eulophias* as food and use therapeutically for better health and longevity[2]. The tubers of *Eulophia* species are used for its rejuvenating, aphrodisiac and antirheumatic properties. The various biological activities of *Eulophia* tubers reported are: anabolic[3], anthelmintic[4], antidiarrheal[5,] antimicrobial[4], anti-inflammatory[6], antioxidant[7], antitumor[8,9], hypolipidomic[10] and fertility[3]. Very recently, spermatogenic parameters have been reported in herbal composition containing *Macuna pruriens* (Linn), *Chlorophytum borivilianum* (Sant and Fernand) and *Eulophia campstris* (Wall) and authors proved aphrodisiac[11] nature of these plants experimentally. Amongst large number of activities the fertility and aphrodisiac activity contribute 47 % and 35% respectively[12].

In Ayurvedic medicine, *Eulophia* is prescribed for the treatment of impotency, gynaecological problems, decreased sperm count[13] and blood purification[14]. The phytochemical analysis of *E. campestris*[15], *E. epidendraea*[16], *E. herbacea*[17], *E. ochreata*[18], *E. nuda*[19] are reported qualitatively along with proximate and mineral composition of *E. ochreata*[20]. The present study includes i) Physico-chemical analysis of tuber powder of *E. herbacea* and *E. ochreata* and chemical nature of extractives. ii) Phytochemical analysis of methanolic extracts of *E. herbacea* and *E. ochreata* for the detection of secondary metabolites qualitatively as well as quantitatively. iii) Chromatographic analysis of crude drug extracts. iv) Validation of β-sitosterol for antioxidant properties and other bioactivities.

MATERIAL AND METHOD

Extracts Preparation

The tubers of *E. herbacea* and *E. ochreata* were collected from Nasik and Jalgaon district respectively. Identified and shade dried tubers were extracted with solvents: petroleum ether, benzene, chloroform, methanol and water successively in a Soxhlet extractor. All the extracts were analyzed phytochemically and proceed for isolation

**Author for Correspondence:*

a

b

Figure 1: Photoplate showing a) flower(white) and b) tubers *Eulophia herbacea*.

a

b

Figure 2: Photoplate showing a) flowers(golden yellow) and b) tubers of *E. ochreata*.

of active ingredients.

Eulophia herbacea

Common name: Herbaceous Eulophia; English: Salep (var.); Ayurvedic: Munjaataka (substitute), Saalam-misri (substitute); Marathi: कुकुडकंद kukudkand, Kukad-kand, Kutri-kand or umarkand[21]; Hindi: Bilarikand, Vansingara

Eulophia ochreata

Common name: Golden-Yellow Eulophia; Marathi: अमरकंद amarkand, अमरकांदा amarkanda, Singadya-kand; Rajasthani: Gorakhamundi, Salam-mishri; English - Wild coco Golden-Yellow *Eulophia.*

Physico-chemical analysis of Tuber powder of Eulophia species

The crude drugs were tested for the following tests as per the USP[22] and IHP[23]. The results were reported in % Mean ± SD. Total ash content, acid insoluble ash, water soluble ash and sulphated ash, extract percentage, loss on drying was determined by methods of AOAC[24]. Qualitative screening was done as shown in Table-1 using the procedures of Harborne[25].

Qualitative and quantitative screening of metabolites

The chemical compounds synthesized by plants are classified on the basis of their biosynthetic origin, chemical class and functional groups into primary and secondary metabolites. Phytochemical (quantitative) detection of metabolites in *E. herbacea* and *E. ochreata* was done by following methods.

Detection of β-sitosterol in E. herbacea and E. ochreata on the basis of analytical (TLC) and spectral analysis (UV, HPLC)

The earlier results on various medicinal plants and allied activities of methanolic extracts (from five solvent extracts potentially isolated) inspired us to study the detection of β-sitosterol in methanolic extract of *E. herbacea* (MEEH) and methanolic extract of *E. ochreata* (MEEO) by applying various analytical and spectroscopic techniques namely: UV-Vis, TLC, HPLC.

Spectral analysis-UV-Vis spectrum

The dried extract dissolved in methanol (1mg/ml) and the UV-Vis spectrum was recorded.

Analytical Characterization-1. TLC

Thin layer chromatographic studies of MEEH and MEEO were carried out. A phytochemical standard sterol (beta-sitosterol) and phenols (gallic acid and tannic acid) were used for identification. The glass plates coated with silica gel (Loba), slightly warmed in hot air oven and used for TLC. Using a capillary, solution of standard and extracts were loaded gradually over the plate by concentrating the spot. The 10 ml mobile phase as required was added in the glass jar. The jars were allowed 30 min. for saturation with mobile phase. The TLC development was carried out for 20 min. and then the dried plate was exposed to the corresponding spraying agents (Iodine for 4**a**; Ciocalteu for **4b** and **4c**). The plate is viewed under normal light in TLC cabinet and R_f values were

Table 1: Tests used for qualitative screening of secondary metabolites.

Sr No	Metabolite	Test Name	Procedure	Observation
1	Alkaloids	Mayer's Test	Filtrate + 1-2 drops Mayer's reagent	White or creamy precipitate
		Wagner's test	Filtrate + 1-2 drops Wagner's reagent	Reddish – brown precipitate
		Hager's test	Filtrate + 1-2 ml Hager's reagent (saturated aqueous solution of picric acid)	Prominent yellow precipitate
		Dragendorff's test	Filtrate + 1–2 ml Dragendorff's reagent	Yellow precipitate
2	Flavonoids	Alkaline reagent test	Extract + dilute NaOH	Intense yellow colour+ dilute HCl → colourless
3	Glycosides	Borntrager's test	Filtered hydrolysate + 3 ml chloroform + 10% ammonia in chloroform layer	Pink colour
		Legal's test	Extract + pyridine + sodium nitroprusside	Pink colour
4	Phenolic compounds and Tannins	Ferric chloride test	Extract + DW + few drops of neutral 5 % ferric chloride	Dark green colour
		Lead acetates test	Extract + DW + 3 ml of 10 % lead acetate solution	white precipitate (Phenolic compounds)
		Alkaline reagent test	Extract + DW + 10 % ammonium hydroxide	Yellow fluorescence (flavonoids)
		Magnesium and hydrochloric acid reduction	Extract + alcohol + magnesium ribbon + conc. HCL (Drop wise)	pink to crimson colour (flavanol glycosides)
5	Phytosterols	Libermann – Burchard's test	Extract + 2 ml acetic anhydride + 1-2 drops conc. H_2SO_4	Colour change
		Salkowski's test	Extract + Salkowski's reagent	Yellowish colour with green fluorescence
6	Saponin	Foam Test	Extract + DW- Shake well	1 cm Foam layer
7	Tannins	Ferric chloride test	Extract + DW + Iron Chloride	Blue black colour
		Lead acetate test	Extract + 1% lead acetate	Yellow precipitate
8	Triterpenoid	-	Extract + chloroform + conc. H_2SO_4 + 1 ml acidic anhydride	Reddish-violet colour

calculated.

HPLC (High performance Liquid Chromatography)

HPLC technique is simple, perfect and powerful separation method available for analyst. With the help of this technique we have analyzed different phyto-constituents from test plant extracts.

The above chromatographic conditions were established by trial and error and were kept constant throughout the experimentation. According to reverse phase method HPLC was carried out.

In vitro antioxidant activity of β-sitosterol from MEEH and MEEO

Evaluation of free radical scavenging property

The free radical scavenging capacity of extracts was determined using DPPH method[38,39,40]. Freshly prepared DPPH solution (0.004 % w/v) was taken in test tubes and test samples of concentration ranging from 20-100 µg were added. After 10 min, the absorbance is measured at 517 nm. A blank was prepared without extract. The radical scavenging activity of Ascorbic acid (Vitamin C) was used as standard for validation of antioxidant property of β- sitosterol isolated from both plants. The capability to scavenge the DPPH radical was calculated using the following equation.

DPPH Scavenged (%) = [$(A_{control} - A_{test})/ A_{control}$] x 100

where, $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

Evaluation of Reducing Power Assay

The reducing power was determined by the method of Oyaizu[41]. Various concentrations of β-sitosterol from the plant extracts in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml) and incubated at 50^0C for 20 minutes. Aliquot of TCA (2.5 ml) was added to the mixture, which then centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 ml) was mixed with DW (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm using blank[42]. Ascorbic acid was used as standard.

% increase in Reducing Power = [1- (A_{test} /A_{blank}) x 100]……

Where, $\mathbf{A_{test}}$ is absorbance of test solution; $\mathbf{A_{blank}}$ is absorbance of blank. In this assay the yellow test solution changed to various shades of green and blue depending upon the reducing power of each compound. The presence of radicals (i.e. antioxidant) caused the conversion of the Fe^{3+}/ ferricyanide complex used in this method to the ferrous form. Therefore, by measuring the

Table 2: Metabolite and Test

SN	Metabolite	Test	Reference
1	Crude lipid content	AACC Approved Method	26
2	Total Flavonoid	Aluminium chloride method	27
3	Proanthocyanidin(condensed tannin)	vanillin-HCl assay	28
4	Proteins	Lowry's method	29
5	Carbohydrates	Anthrone method	30
6	Mucilage	Conventional method	31,32
7	Total phenol	Folin-Ciocalteu reagent	33
8	Saponin.	Foam test	34
9	Swelling Index	BP method	35,36
10	Crude fibers	Nitric acid method	37

Table 3: Parameters

Sr No	Parameter	Particulars/ Condition
1	HPLC Machine	Younglin (S.K), Gradient System, UV Detector
2	Software	Autochro -3000
3	Column	4.6 x 250 mm
4	Particle size packing	5 µm
5	Stationary phase	C_{18} (GRACE)
6	Mobile Phase (Acetonitrile : Water)	90:10 (0.05% OPA)
7	Detection Wavelength	254 nm
8	Flow rate	0.6 ml/min
9	Temperature	Ambient
10	Sample size	20 µl

formation of pearls Prussian blue at 700 nm, the Fe^{2+} concentration was monitored; a higher absorbance at 700 nm indicated a higher reducing power.

RESULTS

Physico-chemical analysis of Tuber powder

The ash content, moisture content and foreign organic matters in % w/w; solvent extract in % of the *Eulophia* tuber powder is given in Table 2.

Qualitative and quantitative screening of metabolites

Primary and secondary metabolites in different solvent extracts of *E. herbacea* and *E. ochreata* are detected qualitatively and quantitatively (Table 3 and 4).

The quantitative estimation of primary metabolites reveals various chemical constituents present in the test plants (Table-4). Carbohydrate content was found high (28.54 and 29.13% respectively) followed by protein (17.39 and 17.07% respectively) and lipid contents found very low i.e., 00.95 and 00.99%.

Detection of β-sitosterol and phenolic compounds in E. herbacea and E. ochreata on the basis of spectral (UV-Vis) and chromatographic-analysis (TLC and HPLC).

Spectral analysis-UV-Vis spectrum

The absorption spectra studied in the range 600-260 nm shows one absorption maxima. It has two shoulders at about 380 and 320 nm followed by one broad peak at about 270 nm which is the concomitant observation for both the *Eulophia* species. The absorption of the UV-Vis spectra of the standard phenols (Gallic and Tannic acid) and □-sitosterol in combination indicated that there is no absorption peak over the wavelength 265 nm. This observation indicated that the extract contains mainly □-sitosterol and the phenolic compounds, further, it is well supported by HPLC studies.

Chromatographic analysis

TLC

The presence of sterol (standard □-Sitosterol) and phenols (standard Gallic and Tannic acid) are confirmed by TLC using appropriate solvent system and spraying reagent.

Detection of □-Sitosterol

From Figure 4a, the results of chromatographic analysis indicate the circular spot for the □-sitosterol (standard) with R_f value 0.29. In case of MEEH and MEEO, two spots are observed with Rf values 0.32 and 0.29 respectively which are indicative of presence of □-sitosterol. The upper unknown spot with R_f value 0.48 and 0.46 are observed in MEEO and MEEH respectively. This result clearly indicates that MEEH and MEEO contain □-sitosterol in different concentration in both the plant extracts.

For the detection of Phenolic compounds

According to result analysis (Figure. 4b**)** one oval spot for GA (standard) with R_f value 0.26 and three separate spots for TA (standard) with R_f values 0.08, 0.26 and 0.43 of lower, middle and upper spot respectively. For the MEEH, similar R_f values for GA (0.26) and TA (0.08, 0.26 and 0.43) are observed as oval spots.

For the MEEO, (Figure 4c) similar R_f values for GA(0.26) and TA (0.09, 0.25 and 0.33) are observed as oval spots. One unknown spot with R_f value 0.86 and 0.72 is also observed in MEEH and MEEO respectively. This observation clearly indicates that MEEH and MEEO contain GA as well as TA in different concentration as noted during color development

HPLC (High performance Liquid Chromatography)

The qualitative HPLC profile of the studied *Eulophia* species at wavelength 210 nm due to peak sharpness and proper base line and recorded the retention time (R_t in min.)**.** HPLC chromatogram has shown only 4-5 peaks. However, only 2-3 peaks are prominent with significant per cent area and height (> 5 %).

The most abundant peak indicated, β-sitosterol content (Figure 5 and Table 5) of the studied plant species in MEEH is 79.7% and in MEEO is 94.6%, which coinsides with that of the standard retention time (R_t = 5.45 min.) of □-sitosterol. The other prominent peaks reported are due to the phenolic content of the extract as per the standard phenols like gallic acid (R_t = 5.01 min.) and tannic acid

Table 4. Physico-chemical parameters of *E. herbacea* and *E. ochreata* tuber powder

Parameters	Ash% w/w						Solvent extract%				
	Total Ash	Acid–insoluble Ash	Water-soluble Ash	Sulphated Ash	Moisture content (Fresh tubers)	Foreign Organic Matters	Pet. Ether soluble extractive value	Benzene soluble extractive value	Chloroform soluble extractive value	Methanol soluble extractive value	Water soluble extractive value
Eulophia herbacea	14.10 ± 0.19	5.35 ± 0.05	7.80 ± 0.02	2.06 ± 0.02	83.60 ± 0.96	0.23 ± 0.01	0.95 ± 0.02	1.67 ± 0.03	1.27 ± 0.03	4.30 ± 0.05	29.75 ± 0.09
Eulophia ochreata	12.20 ± 0.17	4.90 ± 0.06	6.60 ± 0.01	1.89 ± 0.01	76.60 ± 1.28	0.19 ± 0.01	0.99 ± 0.03	1.80 ± 0.02	1.34 ± 0.03	5.47 ± 0.04	15.50 ± 0.07

All values are expressed as mean ± SEM, + indicate presence and – indicate absence

secondary metabolites like flavonoids[43], total phenolic

Table 5. Preliminary phytochemical screening of tuber extracts of *E. herbacea* and *E. ochreata*

Sr No	Solvents → / Type of Secondary Metabolite ↓	*Eulophia herbacea*					*Eulophia ochreata*				
		PE	BZ	CH	MeOH	Aq.	PE	BZ	CH	MeOH	Aq.
1	Alkaloids	-	-	-	-	-	+	+	+	-	-
2	Flavonoids	+	+	+	+	-	+	-	-	+	+
3	Glycosides	+	+	+	+	+	-	-	-	-	-
4	Phenols	-	+	+	+	-	-	-	-	+	-
5	Phytosterol or Steroids	+	+	+	+	-	+	+	+	+	-
6	Saponins	+	+	+	-	-	-	+	-	-	-
7	Tannins	+	+	+	+	-	+	+	+	+	-
8	Triterpenes	+	+	+	+	-	+	+	+	+	-

PE = Pet. Ether; BZ = Benzene; CH = Chloroform; MeOH = Methanol; Aq. = Water.

(R_t = 4.26 min.).

Secondly, both these extracts contain some phytophenols like gallic acid 11.6% in MEEH and 0.8% in MEEO whereas MEEO contains additional phenol, Tannic acid (0.8%).

In vitro antioxidant activity of methanolic extract of Eulophia species

Evaluation of Free Radical scavenging property of various extracts of E. herbacea and E. ochreata by DPPH

For DPPH radical scavenging property, as concentration increases, absorbance decreases and hence % inhibition increases (Table 6). The antioxidant activity of β-sitosterol increased in a dose dependent manner. About 20, 40, 60, 80 and 100 µg of β- sitosterol from MEEH and MEEO showed 12.70, 23.83, 55.76, 62.53 and 67.58% inhibition respectively in DPPH free radical scavenging assay. The DPPH radical scavenging percentage in β-sitosterol from MEEH is very close to that of ascorbic acid while the percentage in β-sitosterol from MEEO is about 21% more than that of ascorbic acid. The positive correlation between antioxidant property and β-sitosterol in MEEH and MEEO was noted (Table 6). Total antioxidant potential maybe due to compounds[44] and β- sitosterol[45,46].

Reducing Power Assay

The reducing power of the β-sitosterol from MEEH and MEEO increased with increase in absorbance. The effect of β-sitosterol on reducing power increased in a dose dependent manner (Table 6). The reducing property for the methanolic extract in both the species is close to the property of positive control, ascorbic acid. The MEEO has strong reducing power than MEEH.

DISCUSSION

The detection tests of secondary metabolites of tuber powder of *E. herbacea* and *E. ochreata* are positive for steroids, tannin, anthroquinone glycoside, mucilege and starch however negative for alkaloids. The methanolic extracts have appreciable amount of flavonoids. The applied parameter used for the identification of plant material is estimation of ash value that determines the quality, purity and nature of material added to the drug as an adulterant[47]. The total ash and sulphated ash as reported earlier was almost 50% less than the present study. The % solvent extraction values of *E. herbacea* was in similar trend as in present study in the order: water > methanol > benzene > chloroform > petroleum ether for

Table 4. Quantification of primary metabolites from *E. herbacea* and *E. ochreata*

S. No	Primary Metabolites	*E. herbacea*	*E. ochreata*
1	Carbohydrates	28.54± 6.52	29.13 ± 5.43
2	Proteins	17.39 ± 2.15	17.07 ± 2.07
3	Lipids	00.95 ± 0.15	00.99 ± 0.35

Values are %means of three independent analyses ± SD (n=3)

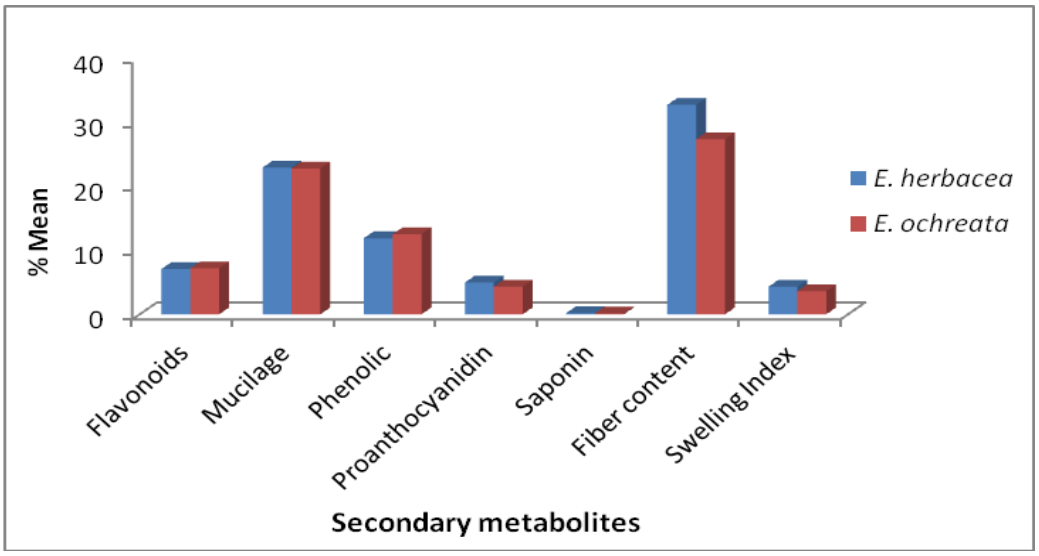


Figure 3. Quantification of secondary metabolites

E. herbacea as well as *E. ochreata* are 0.95, 1.67, 1.27, 4.30 and 29.75 in *E. herbacea* and 0.99, 1.80, 1.34, 5.47 and 15.30 in *E. ochreata* in petroleum ether, benzene, chloroform, methanol and water respectively. This indicates that extraction of soluble ingredients in the extract is dependent on the polarity of the solvent. Earlier workers observed the tubers of test plants have high carbohydrate content and concluded that they are very good source of energy[48, 49]. Protein supports nutritive value of tuber. Less than one per cent lipid content indicates that plant tissues are free of the oily substances. A diet providing 1-2% of its caloric energy as fat is said to be sufficient for human beings as excess fat consumption is implicated in certain cardiovascular disorders such as atherosclerosis, cancer and aging. Our result was in agreement with Tatiya et al.[17].

Secondary metabolite analysis is necessary for extraction, purification, separation, crystallization, identification of various phytocompounds. The methanolic extract showed higher level of phenols (118.70 mg/g in *E. herbacea*; 125.1 mg/g in *E. ochreata*) than the other secondary metabolites. The higher amount of phenol is important in regulation of plant growth, development and disease resistance. Usually *Eulophia* tubers contain highest amount of phenolic contents[50]. The flavonoid contents in *E. herbacea* and *E. ochreata* are noted 70.7 mg/g and 71.7 mg/g respectively. Earlier reports revealed that plant phenolic compounds including flavonoids are potent antioxidants with reported antimutagenic, anticarcinogenic, anti-inflammatory, antiallergic and antiviral effects[51]. The *Eulophia* tuber samples collected from different locations of India showed significant

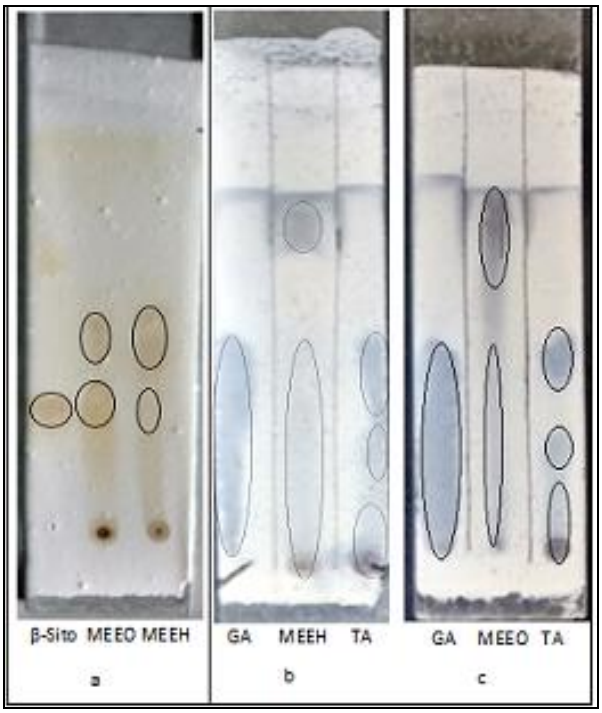
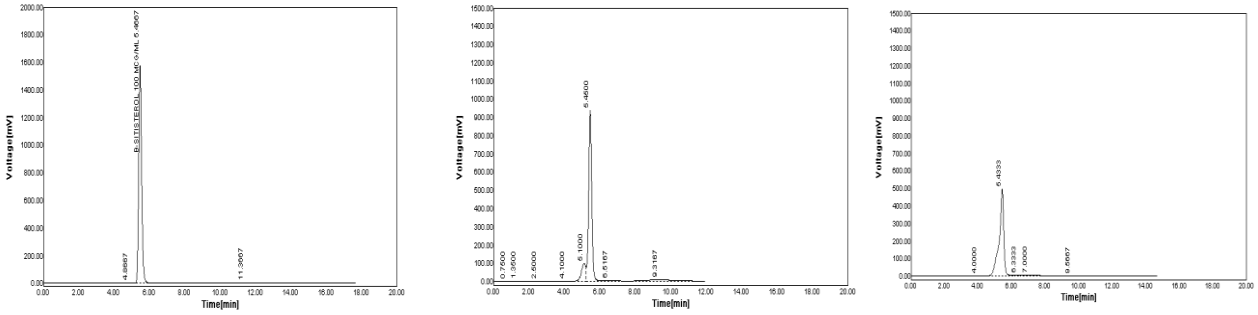


Figure 4. TLC analysis of MEEH and MEEO with standard sterol and phenols.

variations in the contents of sterols, phenols and flavonoids[52]. The present investigation showed significant variation in the contents like phenol, flavonoids, and tannin when compared to above mentioned reports. These variations are due to number of environmental factors such as climate, altitude, rainfall etc. as mentioned by



Standard (β-Sitosterol) 100 µg/ml MEEH (1mg/ml) MEEO (1mg/ml)

Figure 5. Detection of β-sitosterol in MEEH and MEEO on the basis of HPLC

Table 5. HPLC results showing percentage of content in MEEH and MEEO.

Sample	Retention time (R_t)	Content	Percentage
Standard	5.01	Gallic acid	93.13
	5.45	β-Sitosterol	100.00
	4.26	Tannic acid	93.43
MEEH	5.10	Gallic acid	11.56
	5.45	β-Sitosterol	79.69
MEEO	4.00	Tannic acid	00.79 *
	5.43	β-Sitosterol	94.56
	5.13	Gallic acid	00.82*

* Trace amount

Kokate et al.[53].

All secondary metabolites have specific functions. The presence of β-sitosterol is recorded in appreciable amount in both MEEH and MEEO extract. Sterols or steroids have hormonal like activities[54,55] and aphrodisiac activity in *Eulophias* may be attributed to the presence of β-sitosterol[11]. The polyphenols and flavonoides exhibit antioxidant properties in the extracts. The noticeable percentage of polyphenolic compounds may show beneficial effects on human health and also possess antiviral, anti-inflammatory, antitumour, antihaemolytic and antioxidative activity[56, 57]. The mucilage has a good potential as a binder for conventional tablet formulations[58] and such property of binder in MEEH and MEEO extracts may be due to high amount of mucilage.

The crude fibers content in *E. herbacea* is more than that of *E. ochreata* tubers, which could be a valuable source of dietary fibre in human nutrition. The crude fibre content in *E. herbacea* (42.7%) and *E. ochreata* (37.33%) tubers was higher than the reported values[17, 20] 31.5%, 22.90% respectively, may be due to source/ seasonal variation. Aberoumand and Deokule[20] have estimated the calorific value 288.25 kcal/100g (DW) in *E. ochreata* tubers which is an indication that it could be an important source of dietary calories. High calorific content of the tubers may be attributed to high total carbohydrates content. The considerable content of proanthocyanidin may attribute to antimicrobial, insecticidal activities, as in nature, proanthocyanidins serve among other chemical and induced defense mechanisms against plant pathogens and predators, as occurs in strawberries[59].

Phytochemical nature of MEEH and MEEO exhibit presence of β-sitosterol as detected quantitatively and confirmed chromatografically. These results are in accordance with the reported values of *E. epidendraea*[16] and *E. herbacea*[17] respectively. This study reveals that the marked percentage of β-sitosterol confers strong radical scavenging and reducing power ultimately to strengthen the natural antioxidant potential to the methanolic extract of test plants and that could be exploited for their medicinal properties and neutraceuticals. Presence of β-sitosterol may also be attributed to hormonal properties such as estrogenic[584,55], antifertility[60]. Such observations also recorded in our laboratory for MEEH and MEEO experimentally[3,11]. The higher concentration of β-sitosterol owes potency, aphrodisiac activity, prevention of heart disease, hypercholesterolemia, rheumatoid arthritis, tuberculosis, prostatic hyperplasia[61,62]; used for modulating the immune system[59], anticancer[63] (colon); antidiabetic[64].

Table-6. DPPH radical scavenging activity of extract of tubers of *E. herbacea* and *E. ochreata*

β- sito µg/mL	DPPH scavenging %			% Increase in reducing power		
	Ascorbic acid	β-sito (MEEH)	β-sito (MEEO)	Ascorbic acid	β- sito (MEEH)	β- sito (MEEO)
0	0.00	0.00	0.00	0.00	0.00	0.00
20	20.19	21.01	25.75	17.87	18.61	21.9
40	48.57	42.19	54.25	42.99	32.72	38.53
60	68.78	72.26	81.69	59.11	59.52	61.75
80	87.38	84.82	107.04	69.63	66.89	76.53
100	92.04	93.78	113.61	83.99	83.01	95.8
Avg	105.65	104.69	127.45	91.20	86.92	98.17

β-sito means β-sitosterol; MEEH- Methanolic extract of *E. herbacea*; MEEO- Methanolic extract of *E. ochreata*

High antioxidant property corresponds to high total phenolics in the tested extracts. The antioxidant property of extract is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and single oxygen quenchers[65]. The interests of phenolics are increasing in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food[66]. The extractability of phenol/flavonoids is dependent on nature of solvent and consequently the antioxidant property of the extracts also differs as well.

The DPPH free radical scavenging of antioxidants is due to their hydrogen donating ability. The plants with higher donating capacity have shown higher DPPH free radical scavenging activity[67]. Our results are agreed with recent findings[68] based on free radical quenching potential. In this study, the strong reducing power was seen in the methanolic extract than other solvent extracts of *E. herbacea* and *E. ochreata*, may be due to the biologically active compounds or phenolic compounds in the extract which possess potent donating abilities. This assay further confirmed the antioxidant properties of the extracts. The potential scope of these plants may serve the mankind for regulating fertility/remedy on reproductive health/ disorder in both men and women. Their medicinal uses are not yet fully established clinically. The output of present study provides a clue to find a prototype of compound to synthesize possible drugs with higher efficacy. *E. ochreata* possesses life encouraging activities for human and justify its name as "Amarkand", which in turn, comply this statement stated by local traditional medical practitioners of North Maharashtra region, Maharashtra.

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