

Short Communication

ANTIOXIDANT ACTIVITY OF FOURTEEN *EULOPHIA* SPECIES TRADITIONALLY KNOWN AS AMARKAND

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ABSTRACT

Objective: Amarkand is a group of medicinal plants, of which tubers are regularly consumed by the tribal people in India as food and medicine. It has a strong background of regional ethnopharmacological and food uses. Thus it can be effectively utilized as a source of natural antioxidants. The present study, of fourteen *Eulophia* species which are predominantly considered as "Amarkand", were focused for their antioxidant potential and polyphenolic content which facilitates the contribution of the traditional knowledge of plants from India.

Methods: Radical scavenging activity, ferric reducing antioxidant power and anti-lipid peroxidation assay were used to evaluate the antioxidant potential. In addition, total phenols, flavonoid and proanthocyanidins were estimated.

Results: Results showed that all the studied amarkand species have promising antioxidant potential. *Eulophia epidendrea* (J. Koenig ex. Retz.) C. E. C. Fisch. (EE) and *Eulophia mannii* (Rchb. f.) Hook. f. (EM) had highest free radical scavenging potential among all. *Eulophia epidendrea* have highest ferric reducing potential which may be due to high flavonoid and proanthocyanidin content present.

Conclusion: The data clearly demonstrated that EE and EM may serve as a good natural antioxidant source against oxidative stress and related diseases.

Keywords: *Eulophia*, Amarkand, Antioxidant activity, Polyphenolic content

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Free radicals are any atom, molecule or ion containing one or more unpaired electrons in the outer orbital which are produced in the normal cellular metabolism. These radicals are mainly included reactive oxygen species (ROS) and reactive nitrogenous system (RNS) which constantly produced in the sub-cellular organelles of living cells and its overproduction can lead to damage biomolecules such as DNA, proteins and lipids [1]. Antioxidants are our vital defense against free radical induced damage. These compounds are associated with a reduction of excess load of free radicals and maintain a redox balance of the body, which is essential for its normal functions [2]. Plant and their products such as fruits, vegetables, grain cereals, etc. are rich in antioxidants. Previously it was reported and identified that more than 3100 antioxidant-containing food, herbs, spices, beverages and supplements are regularly consumed by different cultures to decrease the oxidative stress and related cell damage [3]. Over the centuries, traditional medicines or folk medicines includes natural substances derived from plants for treatment of many diseases. These antioxidants are found to be responsible for several health benefits [4]. However, now a day's several plant's antioxidants are struggling to establish as sustainable nutraceuticals due to lack of quality control standards and also having confusion in the identification of exact plant species.

Amarkand is a group of medicinal plants, of which tubers are regularly consumed by the tribal people in India as a food and medicine. The word 'Amarkand' is commonly used for 30 plant species of *Eulophia* (Family-Orchidaceae) and *Dioscorea bulbifera* (Family-Dioscoraceae), out of these 21 species reported to possess food and medicinal values [5-7]. A study, on the lifestyle of the tribes in Maharashtra state, India, found that tribal people extensively use many species of amarkand as a major constituent in their diet [8]. *Eulophia* species reported to have various uses in Ayurveda such as astringent, digestive, expectorant, anabolic, tonic, diuretic, soft purgative [9]. These tubers are applied externally and given internally to cure disease. In northern India, especially in Punjab, tubers are used as a salep, which is considered as a general tonic [7]. Tribal people believe that these tubers would maintain their

endurance under severe conditions in their daily life [10]. Thus, finding out authentic species of amarkand having highest potential is necessary for societal benefit. Tubers of these species are rich in all nutrients such as starch, free sugars, oils, proteins, phenols and also a good source of almost all elements [11]. In our earlier study, we have reported the anti-inflammatory, antimicrobial, antiglycation, and antioxidant properties of *E. ochreatea* tubers [8, 12, 13]. It is evident that amarkand has exhibited both preventive and curative medicinal properties.

Recently, we have also investigated antioxidant potential of seven amarkand plants which includes six *Eulophia* species and *D. bulbifera* [6] and there is a need to study remaining amarkand species to know the best species among 21. In the present study, fourteen species of *Eulophia* were collected and assessed for their polyphenolic content and antioxidant efficacy. This study can help to identify most bioactive amarkand species and to investigate their antioxidant potential.

All reagents were of analytical grade and distilled water was used throughout. Plants were identified by Dr. Suresh Jagtap (Senior Taxonomist, IRSHA, Pune). Fresh tubers of fourteen *Eulophia* species (Listed in table 1.) were collected from different locations of India. Collected tubers were cut into small pieces, shed dried and subjected to size reduction to coarse powder using mixer grinder. Extracts were prepared by soxhlet apparatus using methanol at 45°C for 6 h and subsequently evaporated to dryness under reduced pressure at 45 °C in a rotary evaporator.

Polyphenolic content assays include determination of total phenol, flavonoid and proanthocyanidin contents. Total phenolic content of extracts was assessed using known folin-ciocalteu method [14]. It is expressed as mg of gallic acid equivalents (GAE) per gram of dry weight. Total flavonoid content was determined using an aluminum chloride colorimetric method adapted from [15] and it is expressed as mg of quercetin equivalents. The assay was performed using vanillin method [16]. Results were expressed as mg equivalent to catechin. All the analysis was performed in triplicates.

Free radical scavenging assays were used to determine the antioxidant capacity of amarkand extracts. Ferric reducing antioxidant power (FRAP) value was determined by modified method reported by [17]. Results were analyzed from the standard curve of ferrous sulphate and expressed in terms of $\mu\text{M Fe (II)}/\text{g dry mass}$. Scavenging of nitric oxide capacity was measured spectrophotometrically using Griess reagent method [18]. *In vitro*

DPPH radical scavenging activity was determined by the method as described previously in [19]. In addition, the lipid peroxidation inhibition potential of extracts was analyzed by Thiobarbituric Acid Reactive Substances method described by [20]. Percent inhibition of free radicals was calculated and IC_{50} values were compared. The observations were replicated thrice for each parameter; mean values were pooled and standard deviation (SD) was calculated.

Table 1: *Eulophia* species studied from amarkand group

S. No.	Plant name	Location	Code
1.	<i>E. andamanensis</i> Rchb. f.	Portblair (Andaman)	EA
2.	<i>E. bicallosa</i> (D. Don.) Summerh.	West Sikkim (Sikkim)	EB
3.	<i>E. densiflora</i> Lindl.	Nagaon (Assam)	EDe
4.	<i>E. dufosseii</i> Guillaumin.	Patna (Bihar)	EDu
5.	<i>E. epidendreaea</i> (J. Koenig ex. Retz.) C. E. C. Fisch.	Tirunelveli (Tamilnadu)	EE
6.	<i>E. explanata</i> Lindl.	Junagadh (Gujrat)	EEEx
7.	<i>E. flava</i> (Lindl.) Hook. f.	Nandurbar (Maharashtra)	EF
8.	<i>E. herbacea</i> Lindl.	Thane (Maharashtra)	EH
9.	<i>E. kamarupa</i> S. Chowdh.	Nagaon (Assam)	EK
10.	<i>E. mackinnonii</i> Duthie.	Tawang (Arunachal Pradesh)	EMk
11.	<i>E. mannii</i> (Rchb. f.) Hook. f.	West Sikkim (Sikkim)	EM
12.	<i>E. obtusa</i> (Lindl.) Hook. f.	Lakhimpur Kheri (Uttar Pradesh)	EOb
13.	<i>E. promensis</i> Lindl.	Sundarban (West Bengal)	EP
14.	<i>E. zollingeri</i> (Reichb. f.) J. J. Sm.	West Kameng (Arunachal Pradesh)	EZ

Table 2: Total phenol, flavonoid and proanthocyanidin content in *Eulophia* species

Name of the plant	Total phenol content (50 $\mu\text{g}/\text{ml}$ conc.)	Total flavonoid content (1 mg/ml conc.)	Total proanthocyanidin content (1 mg/ml conc.)
	mg/g of GAE equivalent ^{a,b}	mg/g of quercetin equivalent ^{a,c}	mg/g of catechin equivalent ^{a,d}
EA	0.25±0.017	0.06±0.007	0.14±0.016
EB	0.23±0.021	0.08±0.004	0.16±0.038
EDe	0.53±0.001	0.01±0.001	0.03±0.003
EDu	0.78±0.003	0.03±0.008	0.12±0.010
EE	0.21±0.090	0.63±0.003	0.78±0.080
EEEx	0.13±0.350	0.51±0.026	0.31±0.018
EF	0.31±0.078	0.08±0.050	0.41±0.038
EH	0.19±0.013	0.05±0.002	0.45±0.022
EK	0.41±0.081	0.22±0.070	0.29±0.019
EMk	0.29±0.014	0.08±0.002	0.31±0.056
EM	0.45±0.381	0.39±0.018	0.09±0.030
EOb	0.21±0.072	0.31±0.190	0.02±0.038
EP	0.29±0.003	0.30±0.083	0.07±0.010
EZ	0.51±0.075	0.29±0.008	0.31±0.090

^aAll values are mean±SD, n=3, ^bValues are expressed as equivalent to gallic acid(mg/gram of GAE), ^cValues are expressed as equivalent to quercetin (mg/gram of quercetin), ^dValues are expressed as equivalent to catechin (mg/gram of catechin)

Table 3: FRAP activity and NO scavenging ability of *Eulophia* species

Name of the plant	FRAP assay (1 mg/ml conc.)	NO scavenging assay (500 $\mu\text{g}/\text{ml}$ conc.)
	$\mu\text{M Fe (II)}/\text{g}$ of dry mass ^a	% inhibition
EA	0.06±0.001	11.02
EB	0.05±0.001	15.32
EDe	0.01±0.001	22.10
EDu	0.02±0.001	16.82
EE	1.00±0.381	41.12
EEEx	0.79±0.090	22.15
EF	0.32±0.082	16.22
EH	0.07±0.006	32.66
EK	0.65±0.018	18.10
EMk	0.07±0.003	29.10
EM	1.09±0.083	23.16
EOb	0.31±0.190	12.80
EP	0.09±0.005	14.44
EZ	0.89±0.080	32.80

^aAll values in FRAP assay are mean±SD and values are expressed as equivalent to Fe (II) ($\mu\text{M}/\text{gram}$ of Fe (II)), n=3

Plant polyphenols are the secondary metabolites; they show scavenging ability due to presences hydroxyl groups in their structures. These compounds are believed to be responsible for the antioxidant activity of plant [21]. Among plant phenolics, flavonoids are a major group of compounds which have potent antioxidant activities. Proanthocyanidins are condensed tannins, which help the body to remove free oxygen radicals from cells [22]. In the present study, all *Eulophia* species extracts showed the presence of polyphenols. Among 14 species, EDu, EZ and EM had highest phenolic content, whereas, EE was rich in flavonoids and proanthocyanidins as compared to other *Eulophia* species (table 2).

Reducing potential of antioxidant compounds present in the plants was measured by FRAP method. This method is directly related to the total antioxidant activity of hydrogen donating compounds present in the extracts. FRAP value was analyzed at 593 nm by monitoring the formation of intense blue colored ferrous tripyridyl triazine (Fe (II)-TPTZ) complex [23]. In the study, EM and EE were shown highest FRAP value which is 1.09 ± 0.083 and 1.00 ± 0.381 respectively than other species (table 3). This data suggests that EM and EE extracts have highest ferric reducing power which indicates their ability to donate an electron.

Endothelial cells, macrophages, neurons, etc. generate nitric oxide (NO) which is an important chemical mediator involved in many physiological processes especially in the pathogenesis of inflammation. Under aerobic conditions, NO is very unstable and reacts with oxygen to produce intermediates which is highly toxic to humans [24]. The present study showed that the methanol extract of EE had highest inhibition activity of 41.12%, followed by EZ and EH having inhibition of 32.80% and 32.66%, respectively than other *Eulophia* species at concentration 500 μ g/ml (table 3). Thus, EE may have the property to counteract the ill effects of excessive nitric oxide generation.

DPPH is nitrogen-centered, stable free radical, which can get readily destroyed by free radical quencher and shows a strong absorption band at 517 nm. The decrease in optical density of plant samples indicates its antioxidant activity [25]. Results revealed that EE and EM extracts had IC 50 value of 32 ± 0.5 , 57.2 ± 0.9 respectively) highest ability to scavenge DPPH free radical than other *Eulophia* species (fig. 1). These results suggest that EE and EM contain more hydrogen donor compounds to free radicals, which removes an odd electron.

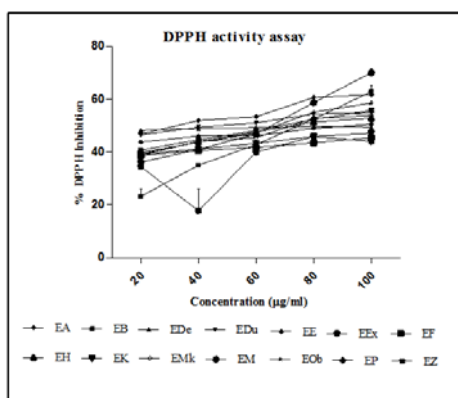


Fig. 1: DPPH scavenging activity of *Eulophia* species

Anti-lipid peroxidation assay was performed using Thiobarbituric acid reactive substances (TBARS) method using goat liver for lipid source [26]. Lipid peroxidation refers to the oxidative deterioration of lipids, mainly by the effect of different ROS. In this method, polyunsaturated fatty acids get oxidized and react with thiobarbituric acid to give malonaldehyde (MDA) in the form of pink chromogen [27]. Results revealed that all *Eulophia* species had shown anti-lipid peroxidation effect, but EMk, EE and EDu showed highest inhibition of peroxidation than other species which indicates their potential against oxidative stress (fig. 2). This activity against

peroxidation by amarkand species justifies their traditional use, against inflammation.

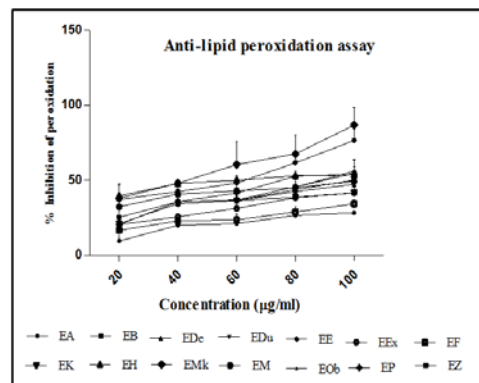


Fig. 2: Anti lipid peroxidation potential *Eulophia* species

In literature, medicinal properties in terms of ethnobotany and pharmacology have been reported only for 20 out of 32 known *Eulophia* species. Among the 20 species, six species have been proved to possess antioxidant activity [6]. In the present study, we have found that all investigated members of *Eulophia* have better antioxidant potential, but among them *Eulophia epidendrea* and *Eulophia mannii* possesses significant potential for scavenging free radical. Both these species revealed the presence of compounds like phenols, flavonoids, proanthocyanidins. Among both of these species, EE had highest ferric reducing potential and possess marked anti-lipid peroxidation potential. *Eulophia epidendrea* tubers possess high antioxidant potential which may be directly proportional to its polyphenolic content, and this activity may be contributed by the presence of sterols, triterpene and triterpenoids as reported previously [28]. Further, *in vivo* work is warranted to support our findings and conclusions, which bring a new natural product into cosmetic, food production and pharmaceutical industries.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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