World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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Extraction, phytochemical screening and antioxidant potential of extract of *Eulophia herbacea*

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World Journal of Biology Pharmacy and Health Sciences, 2023, 13(02), 339–344

Publication history: Received on 13 January 2023; revised on 20 February 2023; accepted on 23 February 2023

Article DOI: https://doi.org/10.30574/wjbphs.2023.13.2.0109

Abstract

A family of extremely reactive molecules called reactive oxygen species (ROS) are produced during the metabolism of oxygen. Superoxide radicals, hydroxyl radicals, and hydrogen peroxide molecules are among the ROS that are frequently produced as byproducts of biological processes or as a result of external stimuli. There are several there is evidence that ROS play a role in the emergence of degenerative illnesses. There is evidence that substances, particularly those derived from natural sources, can protect against free radicals. Traditional medicines with therapeutic utility have been used since antiquity and are still contributing a significant role in the primary health-care system. Thus this article focused on the extraction, phytochemical and detection of antioxidant potential of *Eulophia herbacea* plant extract. The phytochemical screening revealed that the plant is a rich source of different secondary metabolites like flavonoids, saponins, carbohydrates, proteins phenol, glycosides, diterpenes and tannins. The extract only has flavonoid content with value 0.385 mg/100 mg of dried extract. The total phenolic content is only estimated in hydroalcoholic extract of *Eulophia herbacea* which is found to be 0.674mg/100mg of dried extract. The anti-oxidant activity was performed using DPPH method. The percentage inhibition seen to be highest this is 79.63% at maximum concentration of 100µg/ml. Beyond that IC_{50} value calculations reveal that *Eulophia herbacea* extract have comparable antioxidant potential with IC_{50} value for ascorbic acid is 18.69 and for plant extract it is 107.42.

Keywords: Anti- oxidants; Free radicals; Extraction; DPPH Method; Phytochemical Screening; *Eulophia herbacea*

1. Introduction

Free radicals and reactive oxygen species (ROS), which occur in the body under normal physiological circumstances, are results of oxidative stress. When endogenous mechanisms fail to remove an agent, it becomes harmful. In actuality, oxidative stress is brought on by an imbalance between endogenous antioxidant mechanisms and the production of reactive oxygen species. For example, cancer (Kinnula and Crapo, 2004), cardiovascular disease (Singh and Jialal, 2006), neural disorders (Sas *et al.,* 2007), Alzheimer's disease (Smith *et al.,* 2000), and mild cognitive impairment are all caused by oxidative stress, which is a major source of primary catalysts that start oxidation in vivo and in vitro (Guidi *et al.,* 2006). A chemical molecule known as a free radical has an unpaired electron spinning on the outermost layer around the nucleus. ROS are different types of activated oxygen, including free radicals like superoxide anion radicals (.O2 -) and hydroxyl radicals (OH), as well as non-free radicals such singlet oxygen and hydroxyl radicals (H2O2) (Halliwell, 1995). In the body, free radicals come from two different sources: exogenous sources like tobacco smoking, ionising radiation, air pollution, organic solvents, pesticides, etc. and endogenous ones like food metabolism, the ageing process, etc.

Antioxidants are chemicals, either man-made or natural, that may stop or postpone certain types of cell damage. Diets rich in fruits and vegetables, which are excellent sources of antioxidants, have been found to be healthful; however, studies have not demonstrated the use of antioxidant supplementation in avoiding disease. Natural antioxidants boost

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the plasma's antioxidant capacity and lower the risk of developing certain diseases like cancer, heart disease, and stroke (Prior and Cao, 2000). (Prior and Cao, 2000). There have been claims that plant secondary metabolites like phenolics and flavonoids are effective free radical scavengers. They are present in every component of a plant, including the leaves, fruits, seeds, roots, and bark (Mathew and Abraham, 2006). The use of synthetic antioxidants is widespread. However, it has been claimed that they have a number of negative consequences (Ito *et al.,* 1983), including the potential for liver damage and the development of cancer in experimental animals (Gao *et al.,* 1999; Williams *et al.,* 1999; Osawa and Namiki, 1981). So, there is a need for antioxidants that are more potent, safer, and less expensive.

Traditional medicines with therapeutic utility have been used since antiquity and are still contributing a significant role in the primary health-care system. It is estimated that 70-80% of the world's population relies on traditional herbal medicines for their primary health care. *Eulophia herbacea* Lindl (Orchidaceae) is a significant traditional medicinal plant that is used extensively by tribes to cure a wide range of diseases. Amarkand is typically advised for the treatment of ear discharge, blood clotting, joint edoema, and debility in Ayurveda medicine (Vaidya, 2004). It is also indicated as an expectorant, anabolic, tonic, diuretic, astringent, digestive, and mild purgative. Moreover, it is regarded as a general tonic that strengthens the body and balances all three "doshas" (Hossain, 2011). Moreover, these are utilised for stomatitis, purulent coughs, heart issues, blood disorders, and neck scrofulous diseases, as well as for bronchitis, blood diseases, and as a vermifuge. In order to conduct a thorough phytochemical investigation of E. herbacea and evaluation of antioxidation activity of this plant, the present study was conducted.

2. Material and methods

The plants have been selected on the basis of its availability and folk use of the plant. The leaves of *Eulophia herbacea* were collected from local area of Bhopal in the month of May, 2022. Drying of fresh plant parts was carried out in sun but under the shade. Dried leaves of *Eulophia herbacea* were preserved in plastic bags, closed tightly and powdered as per the requirements.

2.1. Method

2.1.1. Extraction Procedure

56.83 gram shade dried leaves were coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

2.1.2. Extraction by maceration process

Defatted leaves powdered of *Eulophia herbacea* has been extracted with hydroalcoholic solvent (ethanol: water; 75:25) solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40 °C (Mukherjee, 2007).

2.1.3. Determination of Percentage Yield

The yield of the collected plant extracts was measured in grams after extraction, and then converted into percentage. For calculating the percentage yield of selected plant products, formula following was introduced. By using the following formula the percentage yield of extract was calculated:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}}\ x\ 100$$

2.1.4. Phytochemical Screening

Phytochemical analyses were performed according to the normal protocols for extract. Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

2.2. Quantitative estimation of bioactive compounds

2.2.1. Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Gaur Mishra *et al.,* 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate.

The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

2.2.2. Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 10-50µg/ml were prepared in methanol (Gaur Mishra *et al.,* 2017). 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

2.3. *In vitro* anti- oxidant activity by DPPH Method

DPPH free radical scavenging assay DPPH scavenging activity was measured by modified method. DPPH scavenging activity was measured by the spectrophotometer (Gaur Mishra *et al.,* 2017). Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance.

Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly.

Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation:

$$\% \text{ inhibition} = [(\text{absorbance of control} - \text{absorbance of sample})/\text{absorbance of control}] \times 100\%.$$

3. Results and discussion

The plant *Eulophia herbacea* extract is prepared with two solvents namely Pet. ether and hydroalcoholic. The extractive values are represented in terms of percentage yield. For Pet. ether and hydroalcoholic extract it is 1.83% and 7.96% respectively. The phytochemical screening revealed that the plant is a rich source of different secondary metabolites like flavonoids, saponins, carbohydrates, proteins phenol, glycosides, diterpenes and tannins. The extract only has flavonoid content with value 0.385mg/100mg of dried extract. The total phenolic content is only estimated in hydroalcoholic extract of *Eulophia herbacea* which is found to be 0.674mg/100mg of dried extract. The anti-oxidant activity was performed using DPPH method. The percentage inhibition seen to be highest this is 79.63% at maximum concentration of 100 µg/ml. Beyond that IC_{50} value calculations reveal that *Eulophia herbacea* extract have comparable antioxidant potential with IC_{50} value for ascorbic acid is 18.69 and for plant extract it is 107.42.

Table 1 % Yield of hydroalcoholic extract of leaves of *Eulophia herbacea*

S. No.	Extracts	% Yield (w/w)
1.	Pet. ether	1.83%
2.	Hydroalcoholic	7.96%

Table 2 Phytochemical screening of extract of *Eulophia herbacea*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's Test	-ve
	Hager's Test	-ve
2.	**Glycosides**	
	Modified Borntrager's Test	-ve
	Legal's Test	+ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenol**	
	Ferric chloride test	+ve
5.	Proteins	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Molisch's Test	-ve
	Benedict's Test	-ve
	Fehling's Test	+ve
7.	**Saponins**	
	Froth Test	+ve
	Foam Test	+ve
8.	**Diterpenes**	
	Copper acetate test	+ve
9.	Tannins	
	Gelatin Test	+ve

Table 3 Estimation of total phenolic and flavonoids content of *Eulophia herbacea*

S. No.	Hydroalcoholic extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	*Eulophia herbacea*	0.674	0.385

Table 4 % Inhibition of ascorbic acid and hydroalcoholic extract of *Eulophia herbacea*

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	30.42	10.74
2	20	59.11	15.87
3	40	67.48	26.89
4	60	75.25	30.65
5	80	77.58	38.54
6	100	79.63	47.27
IC50 value		18.69	107.42

4. Conclusion

Eulophia herbacea Lindl (Orchidaceae) is a significant traditional medicinal plant that is used extensively by tribes to cure a wide range of diseases. The study concluded that the plant is is a rich source of different secondary metabolites like flavonoids, saponins, carbohydrates, proteins phenol, glycosides, diterpenes and tannins. Quantitative analysis revealed that the plant shows a good amount of flavonoid and phenols. This study confirms that plant showed a great potential of anti oxidant activity. The Antioxidant studies on the *Eulophia herbacea* have scientifically shown its rich antioxidant potentials which in addition to other factors could be helpful in validating the traditional uses of the plant in the treatment of several ailments.

Compliance with ethical standards

Acknowledgments

The authors would like to thank the Dr. Vivek Gupta, Principal, Shri Rawatpura Sarkar Institute of Pharmacy, Datia (M.P), India. Dr. Prabhat Kumar Jain and All supporting staff of Scan Research Laboratories, Bhopal (M.P.) who helped in the experiments during research work.

Disclosure of conflict of interest

No conflict of interest.

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