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In Vitro Antioxidant Activity of root extracts of *Heliotropium eichwaldi* Stued. ex DC

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ABSTRACT

In vitro antioxidant activity of methanolic and aqueous root extracts of *Heliotropium eichwaldi* Stued. ex DC. was determined by DPPH free radical scavenging assay and hydrogen peroxide scavenging activity. The extract revealed marked activity as a radical scavenger in a study indicating that extracts have ability to donate hydrogen. The absorption is stoichiometric in respect to the number of electrons taken up. Concentration of 0.1 mg/ml of methanolic extract (HME) and aqueous root extract (HAE) of *Heliotropium eichwaldi* exhibited 62.73% and 57.18% DPPH scavenging activity. The antioxidant activity of these extracts towards hydrogen peroxide was also reported. A 76.94% and 70.79% of inhibition of hydrogen peroxide was observed with HME and HAE respectively, when compared with control, at a concentration of 0.1 mg/ml using ascorbic acid as standard and positive control on analysis with UV-Visible Spectrophotometer. The results conclude that the extracts are a potential source of antioxidants of natural origin and may be a candidate for treating pathologies related to free radical oxidation due to its overall antioxidant effect in scavenging free radicals and active oxygen species.

Keywords: Antioxidant, DPPH, free radical, *Heliotropium eichwaldi,* Boraginaceae

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INTRODUCTION

Many human diseases are caused by oxidative stress which results from an imbalance between formation and neutralization of pro-oxidants [1, 2]. Oxidative stress initiated by free radicals, such as superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide and peroxynitrite, play a vital role in damaging various cellular macromolecules. This damage may result into many diseases, including nephrotoxicity, diabetes mellitus, atherosclerosis, myocardial infarction, arthritis, anemia, asthma, inflammation, neurodegenerative diseases and carcinogenesis [3]. However, human cells have an array of protecting mechanisms to prevent the production of free radicals and oxidative damage [4]. These mechanisms include both enzymic and non enzymic antioxidants such as superoxide dismutase, catalase, glutathione reductase, ascorbic acid and tocopherol [5]. The protective roles of these enzymes may be disrupted as a result of various pathological processes and thereby causes damage to the cells. Antioxidant supplement has been reported to reconcile the upshot of these radicals by directly reacting and quenching their catalytic metal ions [6]. Many synthetic antioxidant agents including BHA and BHT are commercially available. However, they are reported to be toxic to animals including human beings [7].

Furthermore, natural products of plant origin have been proposed as a potential source of natural antioxidants with strong activity [8-10], which is mainly due to the presence of phenolic compounds like flavonoids, phenols, flavonols and proanthocyanidins [11]. Today, a vast majority of plants used in traditional medicine in India have not been evaluated for their antioxidant potential. One of such plants is *Heliotropium eichwaldi* Stued. ex DC. The present investigation was therefore, undertaken to evaluate its antioxidant potential.

Heliotropium eichwaldi is a herbaceous weed and widely distributed in the state of Punjab, Haryana and Rajasthan [12]. The genus *Heliotropium* has been known to possess a number of medicinal properties which are chiefly attributed to pyrrolizidine alkaloids. Alkaloids, flavonoids and steroids have also been reported in this species [13].

The herb is used traditionally for earache, headache, cleaning and healing ulcers etc. [14, 15]. The plant has demonstrated hypotensive effect [16, 17] and antimicrobial [18] activities.

MATERIALS AND METHODS

Preparation of extracts of *Heliotropium eichwaldi*

The shade dried roots of the plant *Heliotropium eichwaldi* Stued. ex DC., Boraginaceae, was collected from waste land of districts Hisar and Sirsa, Haryana (India), in the month of October 2009 and authenticated by Dr. H. B. Singh, Head, Raw Materials, Herbarium and Museum division of NISCAIR, New Delhi [Ref. no. NISCAIR/RHM/F- 3/2005/consult/536/11] India. A voucher specimen no. PP-570 was deposited in the Department of Pharmacognosy, Guru Jambheshwar University of Science and Technology, Hisar. The plant material was further size reduced and stored until further use in an air tight container. The powdered material (200

g) was extracted with petroleum ether using a soxhlet apparatus. The defatted material was air-dried, then re-extracted with methanol using a Soxhlet apparatus. The extract was filtered through Whatman No. 1 filter paper and the supernatant was evaporated using rotary evaporator at 45^{o}C and the final liquid suspension was lyophilized to get a powder with 1.6% yield, hereafter referred as HME (*Heliotropium methanolic* extract). To acquire aqueous extract (HAE), marc was extracted four times in 1000 mL of hot water (80-83 ^{o}C) for 8 hrs. The extract was cooled down to 25^{o}C and filtered through Whatman No. 1 filter paper and filtrate dried under reduced pressure using rotary evaporator, 7.8% yield.

Antioxidant Assay

The antioxidant activity of plant extracts were determined by different *in vitro* methods such as, the DPPH free radical scavenging assay and hydrogen peroxide scavenging activity and reducing power methods. All the assays were carried out in triplicate and average values were considered.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity evaluation is a standard assay in antioxidant activity studies and offers a rapid technique for screening the radical scavenging activity (RSA) of specific compounds. The RSA of compound was tested using a methanolic solution of the 'stable' free radical, DPPH. A freshly prepared DPPH solution exhibits a deep purple color with absorption maximum at 517 nm. This purple color generally fades or disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably *via* a free-radical attack on the DPPH molecule) and convert them to a colorless/bleached product (i.e. 2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm. Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the compound.

The free radical scavenging capacity of the methanolic and aqueous extracts of *Heliotropium eichwaldi* root was determined using 3.94 mg of DPPH, a stable radical, dissolved in methanol (100 ml) to give 100 µM solution. To 3.0 ml of methanolic solution of DPPH, 0.5 ml of extract solution in methanol (0.1 g/100ml) was added. The decrease in absorption at 517 nm of DPPH was measured after 10 min. the actual decrease in absorption induced by the test extract was calculated by substracting that of control. The percentage inhibition was also calculated. All the tests and analysis were performed in triplicates and averaged [19].

Scavenging effects on hydrogen peroxide

The methanolic and aqueous extracts of *Heliotropium eichwaldi* root to scavenge hydrogen peroxide was subjected to spectroscopic determination at 285 nm using the method of Beers and Sizer [20]. A solution of 2 mM hydrogen peroxide was prepared in phosphate buffered saline (PBS) pH 7.4. The test extract solution at following concentrations, 0.005 mg/ml,

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0.01 mg/ml, 0.02 mg/ml, 0.05 mg/ml and 0.1 mg/ml was added to hydrogen peroxide solution. Absorbance of hydrogen peroxide at 285 nm was determined spectrophotometrically after 10 min against a blank solution containing test extract in phosphate buffered saline without hydrogen peroxide. All the tests and analysis were run in triplicates and averaged.

RESULTS AND DISCUSSION

Free radical Scavenging Activity

Table 1: DPPH radical scavenging activity of methanolic and aqueous root extracts of *Heliotropium eichwaldi* and standard Ascorbic acid

Compound	Concentration (mg/ml)	Absorbance at 517 nm	Inhibition %age
Control	0.1	1.387±0.09	--
HME	0.1	0.517±0.04	62.73
HAE	0.1	0.594±0.05	57.18
Ascorbic acid	0.1	0.207±0.01	85.08

All the tests and analysis were run in triplicates and averaged
Each value represents the mean ± standard deviation of triplicate analysis

The free radical scavenging activity was evaluated by the decrease in absorbtion of the stable radical DPPH at 517 nm. The HME and HAE decoulorized the DPPH due to its hydrogen donating ability. The absorption is stoichiometric with respect to the number of electron taken up. Scavenging activity of HME and HAE on DPPH is shown in table 1. Concentration of 0.1 mg/ml exhibited 62.73% and 57.18% scavenging activity respectively. However ascorbic acid at this concentration exhibited marked scavenging activity (85%).

Scavenging effects of HME and HAE on Hydrogen Peroxide

Table 2: Hydrogen peroxide radical scavenging activity of methanolic and aqueous root extracts of *Heliotropium eichwaldi* and standard Ascorbic acid

Compound	Concentration (mg/ml)	Absorbance at 285 nm	Inhibition %age
Control	0.1	1.756±0.12	--
HME	0.005	1.489±0.15	15.2
HME	0.01	1.218±0.13	30.64
HME	0.02	0.842±0.09	52.05
HME	0.05	0.634±0.07	63.90
HME	0.1	0.405±0.05	76.94
HAE	0.005	1.559±0.14	11.22
HAE	0.01	1.352±0.12	23.01
HAE	0.02	0.972±0.08	44.65
HAE	0.05	0.684±0.05	61.05
HAE	0.1	0.513±0.05	70.79
Ascorbic acid	0.1	0.287±0.02	83.66

All the tests and analysis were run in triplicates and averaged
Each value represents the mean ± standard deviation of triplicate analysis

The extracts were capable of scavenging the hydrogen peroxide in a concentration dependent manner after 10 min of incubation at 285 nm revealing 76.94% and 70.79% inhibition of hydrogen peroxide with HME and HAE, respectively, when compared with control at a concentration of 0.1 mg/ml. The scavenging activity of HME and HAE is shown in table 2. It has been shown that hydrogen peroxide has only a weak activity to initiate lipid peroxidation, but its activity as an active-oxygen species comes from its potential to produce the highly reactive hydroxyl radical through the Fenton reaction [21]. Removal of OH radical is one of the most important effective defenses of a living body against disease. Therefore any compound with antioxidant activity might contribute towards the total and partial alleviation of this damage [22].

CONCLUSIONS

It has been reported that reactive oxygen species contribute to various pathophysiological conditions and endogenous defense mechanisms offer protection in these conditions. An increase in the antioxidant reserves of the organism can reduce oxidative stress. Plant derived agents may act as source of antioxidants. Determination of the natural antioxidant compounds of plant extracts may help to develop new drug moiety for antioxidant therapy [23-26]. The plants may be considered as good sources of natural antioxidants for medicinal uses such as against aging and other diseases related to radical mechanisms [24,27,28]. Further investigation on the isolation and identification of antioxidant component(s) in the plant may lead to chemical entities with potential for clinical use.

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