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Research Article

EVALUATION OF ANTIOXIDANT ACTIVITY OF THE LEAVES OF *EUPATORIUM ODORATUM* LINN

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ABSTRACT

Eupatorium odoratum Linn is found all over tropical Asia, western Africa and in parts of Australia. It is also found in Koraput district and extensively used traditionally by the tribal people as anti-inflammatory, analgesic, antiprotozoal agent's e.t.c. Extraction of the leaves of the plant *Eupatorium Odoratum* using water and ethanol and evaluation of their antioxidant activity has been envisage in this present research work. Both the extracts exhibits significant antioxidant activity in DPPH, Nitric oxide and hydroxyl radical induced Invitro assay methods.

Keywords: *Eupatorium odoratum*, Extraction, Antioxidant

INTRODUCTION

Eupatorium Odoratum is a shrub of the sunflower family native to North America, from Florida and Texas to Mexico and the West Indies[1] (Family: Asteraceae). It is now found all over tropical Asia, Western Africa and in parts of Australia. It is sometimes grown as a medicinal and ornamental plant. It is used as a traditional medicine in Indonesia. It is now considered more closely related to other genera in the tribe Eupatorieae. Boneset although poisonous to humans and grazing livestock, has been used in folk medicine[2,3]. The literature survey reveals that various parts of Eupatorium odoratum possesses many activities like immunomodulator, antispasmodic, hepatoprotective, antiprotozoal, antidiabetic, antihypertensive, anti-inflammatory, antipyretic etc[4,5]. The aqueous extract of the leaves of *Eupatorium Odoratum* has been found to show numerous pharmacological activities such as anti-inflammatory, analgesic, antibacterial, antifungal, antiadrogenic etc[6,7]. Trolox equivalent antioxidant capacity and ferric-reducing antioxidant capacity power assays showed that the antioxidant activities were strongly correlated with total phenols[8]. In this research work the leaves of *Eupatorium Odoratum* were extracted using water and ethanol. A number of pharmacological activity studies have been reported on the leaves of *Eupatorium Odoratum Linn*. However, antioxidant activity has not been reported. The present study reports the free radical scavenging activity of the leaves of *Eupatorium Odoratum Linn*

MATERIALS AND METHODS

Plant material

Eupatorium odoratum leaves were collected from local area of koraput (Orissa) India. The taxonomical identification of the plant was done by Biju Patnaik Plant Garden and Research center, M, S. Swaminathan research foundation, Jeypore (K), Orissa.

Extraction of the leaves of *Eupatorium odoratum*

The dried leaves were powdered. 150g of powder was subjected to extraction using Soxhlet apparatus with various solvents like water and ethanol. The solvent was then removed under reduced pressure which will give a greenish-black coloured sticky residue. The prepared extracts were then subjected to antioxidant activity studies.

Evaluation of Antioxidant activity of the leaves of *Eupatorium odoratum*:

Scavenging of DPPH radical [9,10]

This assay is based on the measurement of the scavenging ability of the antioxidant test substances towards the stable radical. The free radical scavenging activity (Yokazawa et al.1998) of the extracts (EOA and EOM) were examined in vitro using DPPH radical. The test extracts were treated with different concentrations from a maximum of 250µg/ml to minimum of 4µg/ml. The reaction mixture consisted of 1 ml of 0.1mM DPPH in ethanol, 0.95 ml of 0.05 M Tris-HCl buffer (pH 7.4), 1 ml of ethanol and 0.05 ml of the extract. The absorbance of the mixture was measured at 517 nm exactly 30 sec after adding the extract. The experiment was performed in triplicate and the % of scavenging activity was calculated using the formula;

100-[100/blank absorbance × sample absorbance]

Scavenging of nitric oxide [11, 12]

Sodium nitroprusside (Sreejavan Rao, 1997) (5M) in standard phosphate buffer solution was incubated with different concentration of the test extracts dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25^0 C for 5 hrs. After 5 h, 0.5 ml of incubated solution was removed and diluted with 0.5 ml Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water). The absorbance of the chromophore formed was measured at 546 nm. The control was also carried out in similar manner using distilled water instead of extracts. The experiment was performed in triplicate and % scavenging activity was calculated using the formula;

100-[100/blank absorbance × sample absorbance]

The activity was compared with ascorbic acid, which was considered as standard antioxidant.

Hydroxyl radical scavenging activity[13]

The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. The reaction mixture contained deoxyribose (2-8mM), $FeCl_3$ (0.1mM), EDTA (0.1 mM), ascorbate (0.1 mM), H_2O_2 (1mM), KH_2PO_4-KOH buffer(20mM, pH 7.4) and various concentrations (25-400 µg/ml of extracts and standard 10-80 µg/ml) of standard drug in a final volume of 1 ml. The reaction mixture was incubated for 1 hr at 37°C, deoxyribose degradation was measured at 532 nm (Mary et al. 2002).

Statistical analysis

The data on *in-vitro* studies were reported as mean ± Standard deviation (n = 5). For determining the statistical significance, standard error mean and analysis of variance (ANOVA) at 5 % level significance was employed. The P values < 0.05 were considered as significant [14].

RESULTS

DPPH scavenging

The aqueous (EOA) and ethanolic extracts (EOM) of the leaves of *Euparotum Odoratum* showed promising free radical scavenging

effect of DPPH in a concentration dependent manner upto a concentration of 250µg/ml. The EOM showed more scavenging activity than EOA. The reference standard ascorbic acid also shows a significant radical scavenging potential in the concentration of 1 µg/ml. The DPPH radical inhibition (%) was 53.13, 55.12 and 79.12 for EOA, EOM and ascorbic acid respectively in table 1.

Nitric oxide scavenging

The Eupatorium Odoratum leaves extracts (EOA and EOM) showed significant free radical scavenging action against nitric oxide (NO) induced release of free radicals at the concentrations 250 µg/ml, showing 25.22% and 57.92% of NO inhibition, respectively. Ascorbic acid was used as reference standard. The % inhibition is shown in Table 2.

OH radical scavenging

The EOA and EOM extracts (25-400 µg/ml) significantly scavenged the hydroxyl radical generated by the EDTA/H_2O_2 system, when compared to that of ascorbic acid. The percentage scavenging of OH radicals by EOA and EOM was increased in a dose dependent manner. The standard ascorbic acid (10-80 µg/ml), also showed scavenging effect (Table 3).

Table 1: *In vitro* free radical scavenging activity of *Eupatorium Odoratum* leaves extracts by DPPH method.

Drug	% Scavenging (Mean ± SEM) of triplicates						
	4 µg/ml	8µg/ml	15 µg/ml	30 µg/ml	60µg/ml	150µg/ml	250µg/ml
EOA	22.35±0.002	23.54± 0.001	24.34±0.001	32.77±0.001	39.4± 0.001	47.22±0.002	53.13 ±0.002
EOM	27.02±0.002	29.86±0.002	32.85±0.001	37.3 ±0.001	45.44±0.002	46.03±0.002	55.13 ±0.002
	0.1 µg/ml	0.2 µg/ml	0.4 µg/ml	0.6 µg/ml	0.8 µg/ml	1 µg/ml	---
VitC	6.2 ±0.002	15.54±0.001	31.51±0.001	48.18±0.003	64.15±0.001	79.12±0.001	---

Table 2: *In vitro* free radical scavenging activity of *Eupatorium Odoratum* leaves extracts by nitric oxide scavenging method.

Drug	% Scavenging (Mean ± SEM) of triplicates						
	4 µg/ml	8µg/ml	15 µg/ml	30 µg/ml	60µg/ml	150µg/ml	250µg/ml
EOA	3.13 ±0.002	8..02± 0.001	9.49±0.001	10.53± 0.001	12.03± 0.001	17.68±0.002	25.22 ±0.002
EOM	52.61±0.002	52.71.±0.002	52.9± 0.001	53..19±0.001	53.77±0.002	56.28±0.002	57.92 ±0.002
	0.1 µg/ml	0.2 µg/ml	0.4 µg/ml	0.6 µg/ml	0.8 µg/ml	1 µg/ml	---
Vit C	6.2 ±0.002	15.54±0.001	31.51±0.001	48.18±0.003	64.15±0.001	79.12±0.001	---

Table 3: *In vitro* free radical scavenging activity of *Eupatorium Odoratum* leaves extracts by hydroxyl radical scavenging method.

Drug	% Scavenging (Mean ± SEM) triplicates				
	25 µg/ml	50µg/ml	100µg/ml	200 µg/ml	400µg/ml
EOA	3.13 ±0.002	8..02± 0.001	9.49±0.001	10.53± 0.001	12.03± 0.001
EOM	52.61±0.002	52.71.±0.002	52.9± 0.001	53..19 ±0.001	53.77±0.002
	10 µg/ml	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml
Vit-C	6.2 ±0.002	15.54±0.001	31.51 ±0.001	48.18± 0.003	64.15±0.001

DISCUSSION

The aqueous extracts of the leaves of *Euparotum Odoratum* possesses significant anti inflammatory activity. Reactive oxygen species (ROS) generated endogenously or exogenously are associated with the various diseases such as atherosclerosis, diabetes, cancer, arthritis and aging process. ROS plays an important role in the pathogenesis of inflammatory diseases. Thus antioxidants which can improve these disorders.

The free radical scavenging activity of the extracts was evaluated based on the ability to scavenge the DPPH. This assay is highly important to provide information about the reactivity of organic compounds with stable free radicals, because of the odd number of electrons. DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet color). As the electron became paired of in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stoichiometrically coincides with the number of electrons taken up. The bleaching of DPPH absorption is representative of the capacity of the test drugs to scavenge the free radicals independently. Hydroxyl radical is the principal contributor for tissue injury. The formation of hydroxyl radical from fenton reaction was quantified using 2, D-deoxyribose degradation. The extracts EOA and EOM inhibited hydroxyl scavenging activity.

Sodium nitroprusside serves as a chief source of free radicals. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine is used as a marker for NO scavenging activity (Mukherjee et al., 1989). The chromophore formation was not complete in the presence of extracts of the leaves of *Euparotum Odoratum* (EOA and EOM), which scavenges the NO thus formed from the sodium nitroprusside and hence the absorbance decreases as the concentration of the extracts (EOA and EOM) increases in the dose dependent manner.

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