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In vitro Antioxidant Activity of Extracts from *Eupatorium odoratum* L.

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ABSTRACT

The aim of this study was to assess the *in vitro* potential of ethanolic extracts of various parts of *Eupatorium odoratum* L. The antioxidant activities of ethanolic extracts of leaf, stem, root and defatted flower parts were evaluated by β-carotene bleaching and 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assays. Leaf and flower revealed the good antioxidant activities in both methods. In β-carotene bleaching assay, ethanol extracts of leaf and flower exhibited the relative antioxidant activity values as 0.95± 0.029 and 0.86±0.05, respectively. The DPPH activity of ethanol extracts of leaf and flower was increased in a dose dependent manner, which was found in the range of 5.65-87.93 and 13.98- 91.4% inhibition, respectively, as compared to butylated hydroxyanisole (23.29-91.05). The IC_{50} of leaf and flower extracts in DPPH radical was 44.5 and 50.9 μg mL^{-1}, respectively. This study suggests that leaf and flower parts of *E. odoratum* could be pharmaceutically exploited.

Key words: DPPH free radical scavenger, β-Carotene bleaching assay, *Eupatorium odoratum*, *Chromolaena odorata*, relative antioxidant activity, IC_{50}

INTRODUCTION

Free radicals such as Reactive Oxygen Species (ROS) in living organisms are known to cause damage to all the cell targets (proteins, lipids, DNA) implicating in the processes of aging as well as in wide range of degenerative diseases (Duan *et al*., 2006). A study shows that antioxidant substances with scavenge free radicals play an important role in the prevention of free radical-induced diseases. Since the natural antioxidant mechanism in humane body under some circumstances can be inefficient, a dietary intake of antioxidant compounds become an alternative. Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases (Malencic *et al*., 2000; Sanchez-Moreno *et al*., 1999; Stajner *et al*., 1998).

Eupatorium odoratum L. (synonym: *Chromolaena odorata*), a perennial shrub belonging to the Astereaceae family, invasive weed native to South and Central America. It has been introduced into the tropical regions of Asia, Africa and other parts of the world. Although, it is considered as one of the world's worst weeds, it has been used as folk medicine for the treatment of cough, malaria, diarrhea and skin infection (Iwu, 1993). The juice of aerial parts of this plant is used as a local antiseptic agent (Taylor *et al*., 1996). Many researchers reported

that its leaf exhibited antimicrobial (Iwu and Chiori, 1984), antifungal (Ngono Ngane *et al.*, 2006), anti-inflammatory, antipyretic and antispasmodic (Taiwo *et al.*, 2000), wound healing (Phan *et al.*, 2001), platelet-activating factor receptor binding inhibitory (Ling *et al.*, 2007) and haemostatic activity (Triratana *et al.*, 1991). Even though there are reports on the antioxidant activity of leaf of *E. odoratum*, different methods and or different solvents in extraction were used (Alisi and Onyeze, 2008; Rao *et al.*, 2010; Chomnawang *et al.*, 2007; Akinmoladun *et al.*, 2007). Therefore, the present study has been designed to evaluate the total antioxidant and free radical scavenging activities of the ethanol extracts of leaf, stem and root and flower parts of *Eupatorium odoratum* L. with a view to contributing to the search for beneficial uses of this plant.

MATERIALS AND METHODS

Chemicals: Chemicals and reagents, including 1,1-diphenyl-2-picrylhydrazyl (DPPH), β-carotene, linoleic acid were purchased from Sigma Chemical Co. (MO, USA). All other chemicals and solvents used in this study were of the reagent grade.

Plant materials: Root, leaf, flower and stem of *E. odoratum* were collected on July 2004 from TINPIPLAY, KAVRE district, Nepal. Plant was authenticated in the Central Department of Botany, Tribhuvan University (TU) and its voucher herbarium specimen (19-TUCH) was deposited at Tribhuvan University Central Herbarium, Central Department of Botany, TU, Nepal.

Root was washed with water and dried in sun for three days and finally dried in shade. Stem was dried in sunlight while leaf was dried in sunshade. These materials were ground to powder. Flower was shredded into coarse powder.

Preparation of the extracts: Powdered root, stem and leaf (50 g each) of *E. odoratum* were separately extracted with ethanol (400 mL) in a Soxhlet extractor for 7 h. On the other hand, the coarsely powdered flower parts (50 g) were first defatted with hexane. Thus defatted flowers (14 g) were extracted with ethanol (150 mL). Each extract liquid obtained from above plant materials was evaporated completely on Rotavapor in *vacuo* to obtain crude extract.

MEASUREMENT OF ANTIOXIDANT ACTIVITY

β-Carotene bleaching assay: Total antioxidant activity of ethanolic extracts of different parts of plant and standard (BHA) was measured according to literature (Bruni *et al.*, 2004). The Relative Antioxidant Activity (RAA) of extracts was calculated with the following formula:

$$RAA = A_s/A_{BHA}$$

Where:
A_S = Absorbance of sample
A_{BHA} = Absorbance of BHA

DPPH free radical scavenging assay: Effect of extracts on DPPH radical and standard (BHA) was measured by slightly modified method of Brand-Williams *et al.* (1995). The plant extract (0.5 g) was dissolved in methanol to make 10 mL stock solution. The volumes of 0.1, 0.2, 0.4 and 1 mL of stock solution were pipette out separately into 10 mL volumetric flasks and diluted further with methanol up to the mark to make four sample solutions corresponding to 0.5, 1, 2 and

5 mg mL^{-1} concentrations, respectively. Positive controls (BHA) of similar concentrations were also prepared by same method.

The test was performed in 4 mL reaction mixtures containing 3.9 mL methanolic DPPH solution (6×10^{-5} M) and 0.1 mL of sample solution of various concentrations (0.5, 1, 2 and 5 mg mL^{-1} corresponding to 50, 100, 200 and 500 μg extract or BHA) or 0.1 mL of methanol (as blank). Three replicates were made for each concentration of each extract and BHA. After 30 min of incubation in the dark at room temperature, absorbance of the reaction mixtures was measured at 517 nm using MeOH as reference. The blank reading was measured every time just before the experiment of each concentration of extract or BHA starts. The inhibitory effect of DPPH by the sample was calculated according to the formula (Yen and Duh, 1994):

$$\text{Percentage inhibition} = (A_{C(0)} - A_{S(t)}/A_{C(0)})\times100$$

Where:

$A_{C(0)}$ = Absorbance of the blank at t = 0 min

$A_{S(t)}$ = Absorbance of the sample at t

Inhibition concentration (IC_{50}) is the concentration (μg mL^{-1}) of antioxidant required to inhibit 50% of the DPPH free radicals. The IC_{50} was calculated by using linear regression equation at four different concentrations of sample

Statistical analysis: The RAA value was expressed as mean of three experiments with standard deviation. Percentage inhibition was expressed as mean of three experiments with standard error mean. Paired-sample Student's t-test was performed for the significant differences between sample and positive control, BHA using SPSS 11.5 software. The antioxidant activity of extract was considered not significantly different from BHA when $p>0.01$. The antioxidant activity was significantly different from BHA if $p<0.01$.

RESULTS AND DISCUSSION

The yield of ethanol extracts of stem, leaf, root and defatted flower were 28.82, 27.20, 23.34 and 4.64%, respectively. These extracts were evaluated and compared in their antioxidant effect by β-carotene bleaching and DPPH scavenging assays as discussed below:

β-Carotene bleaching assay: β-Carotene bleaching assay involves a reaction between a potential antioxidant, β-carotene and linoleic acid. β-Carotene undergoes rapid discoloration due to oxidation by free radicals generated from reaction between oxygen and linoleic acid in the absence of an antioxidant. The presence of antioxidant can hinder the extent of β-carotene destruction by neutralizing the linoleate free radical and any other free radicals formed within the system (Benabadji *et al*., 2004). β-Carotene destruction is measured spectrophotometrically at 470 nm against methanol as reference. The performances of methanol solution of leaf, defatted flower, root, stem extracts as well as BHA and negative control (linoleic acid plus carotene solution) in β-carotene discoloration model were presented in Fig. 1. The discoloration process in the model system progressed differently for the various samples which showed antioxidant activity in decreasing order:

BHA>leaf>flower>stem>root>-ve control

The Relative Antioxidant Activity (RAA) values at 60 and 120 min for leaf, flower, stem and root showed were 0.95±0.029, 0.86±0.050, 0.35±0.020, 0.22±0.015, respectively (Table 1). Similarly RAA values for leaf, flower, stem and root at 120 min were 0.89±0.032, 0.77±0.055, 0.21±0.020 and 0.16±0.011, respectively (Table 1). The RAA of root and stem at 60 min displayed significantly ($p<0.01$) lower RAA values than BHA and hence their extracts were considered as very weak antioxidants. After the same period, leaf and flower displayed their RAA values not significantly different ($p>0.01$) than BHA. The RAA values of leaf and flower reduced to 0.89, that is 6.3% and to 0.77 that is 10.4%, respectively, after 120 min which were again not significantly different ($p>0.01$) in comparison to BHA. Hence, this test revealed that leaf and flower have RAA values almost equivalent to BHA.

DPPH free radical scavenging assay: The results of free radical scavenging activity of ethanolic extracts of leaf, stem, root and defatted flower parts and positive control-BHA in DPPH free radical system were shown in Table 2. From the results it is evident that ethanolic extracts

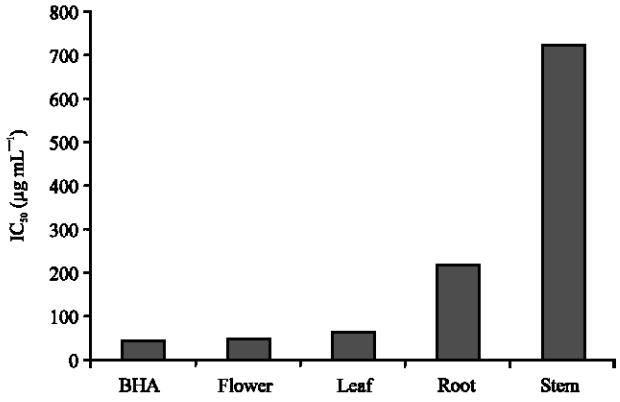

Fig. 1: Antioxidative effects of methanolic solution of different parts of *E. odoratum* and BHA in β-carotene linoleic acid model system at 50°C

Table 1: Relative Antioxidant Activity (RAA) of different parts of *E. odoratum*

Sample	RAA (60 min)[a]	RAA (120 min)[b]
Root	0.22±0.015*	0.16±0.0411*
Leaf	0.95±0.029**	0.89±0.032**
Stem	0.35±0.020*	0.21±0.020*
Flower	0.86±0.050**	0.77±0.055**
BHA	1.00	1.00
-ve Control	0.20±0.0*	0.13±0.011*

[a, b]are mean of three experiments, Mean±SD, *$p<0.01$ = significantly different from BHA, **$p>0.01$ = Not significantly different from BHA

Table 2: Percentage inhibition of different extracts on DPPH radicals

Amount of extracts (μg)	BHA[a]	Leaf	Flower	Stem	Root
50	23.29±0.07	5.65±0.33	13.98±1.04	<0	8.28±0.37
100	37.75±0.40	25.33±0.45	34.57±1.41	<0	9.18±0.37
200	67.39±0.04	47.21±0.75	65.66±1.25*	<0	10.29±0.04
500	91.05±0.33	87.93±0.08*	91.40±0.87*	7.59±0.79	31.25±1.01

[a]Positive control: butylated hydroxyanisole; *$p>0.01$ = Not significantly different from BHA

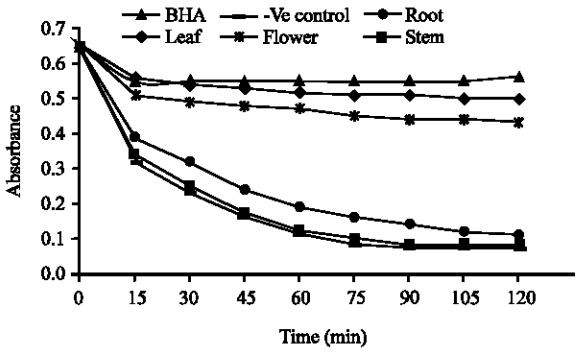

Fig. 2: IC_{50} (μg mL^{-1}) values of different parts of *E. odoratum* for free radical scavenging activity by DPPH radical. A lower value indicates higher antioxidant activity

of leaf and defatted flower at various doses (50-500 μg extract) exhibited scavenging activity in a dose dependent manner. At 500 μg extract, both leaf and flower exhibited 87.93±0.08 and 91.40±0.87% inhibitions, respectively, which were significantly not different ($p>0.01$) from activity shown by BHA (91.05±0.08%) of same dose. Ethanolic extracts of root and stem exhibited 31.25±1.01 and 7.59±0.79% inhibition, respectively, at 500 μg dose reflecting low scavenging activity. Figure 2 shows IC_{50} of DPPH free radical. IC_{50} of ethanolic extracts of defatted flower and leaf showed 50.9 and 65.4 μg mL^{-1}, respectively, indicating promising scavenging activity. Meanwhile, root and stem showed IC_{50} greater than 100 μg mL^{-1} indicating less scavenging activity on DPPH free radical. The effectiveness of antioxidants as DPPH radical scavengers ranged in the descending order: flower (defatted)>leaf>root>stem.

The results obtained for leaf and flower parts of *E. odoratum* on both β-carotene and free radical scavenging assays are meaningful because we are looking at the activity of the crude extracts, with no further purification of the compounds responsible for the antioxidant activity revealing as the most promising sources of natural antioxidants. Our present findings about leaf supports the earlier reported data (Chomnawang *et al.*, 2007) however, contradict with Akinmoladun *et al.* (2007). According to Akinmoladun *et al.* (2007), leaf showed weak antioxidant in DPPH method. This contradiction may be due to geographical variation. Antioxidant activity of flower from this plant is not reported yet so far in our knowledge. It is reported that leaf contains various phenolic compounds (Phan *et al.*, 2001) and flavonoids (Ling *et al.*, 2007; Wollenweber and Roitman, 1996) and flower also contains the various flavonoids (Suksamrarn *et al.*, 2004). These components may be responsible for antioxidant property of flower and leaf of this plant. Therefore, further study must be done to identify and isolate the active constitutes from *E. odoratum*.

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