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Antioxidant Activity by DPPH Radical Scavenging Method of *Ageratum conyzoides* Linn. Leaves

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ABSTRACT

In this study Antioxidant activity was performed by DPPH (1, 1diphenyl-2-picryl hydrazyl) radical scavenging method for different extracts of aerial parts like leaves and flowers of *Ageratum conyzoides* Linn. plant species which showed that alcoholic extract of leaves of this plant on higher concentration possess better antioxidant potential when compare to reference standard ascorbic acid. They exhibited strong antioxidant DPPH radical scavenging activity with IC50 value of 9.3 and 24.8 µg/ml for ascorbic acid and alcoholic leaves extract respectively. The absorbance for reducing power was found to be 0.0390, 0.0989 for ascorbic acid and alcoholic leaves extract respectively. The strongest antioxidant activity of ethanol extract could be due to the presence of flavonoids and phenols.

Keywords- *Ageratum conyzoides* linn., Antioxidants, DPPH, Flavonoids etc.

INTRODUCTION

Antioxidants play an important role as health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables.[1] Plant sourced antioxidants like vitamin C, vitamin E, carotenes, phenolic acids etc. have been recognized as having the potential to reduce disease risk.[2] Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties.[3] A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity.[4]

The DPPH assay method is based on the reduction of DPPH, a stable free radical.[5] The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When Antioxidants react with DPPH, which is a stable free

radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical-scavenging antioxidant) and is reduced to the DPPHH and as consequence the absorbance's decreased from the DPPH.[6] Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured.[7] More the decolorization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug.[8] When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Diphenylpicrylhydrazine; non radical) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present).[9]

This plant has been reported to possess antioxidant properties.[10] So, this study has been undertaken to evaluate *Ageratum conyzoides* Linn. plant for their possible potential to antioxidant action by DPPH scavenging method.[11]

MATERIALS AND METHODS

Ageratum conyzoides Linn. plant species along with leaves and flowers were collected from the local areas of dehradun along road side. Then, this plant was identified and authenticated from Botanical survey of india, Dehradun (Uttarakhand) under accession no.- 114004.

Extraction

Shade dried leaves and flowers part of this plant were pulverized and about 100 gms of powdered leaves and flowers were extracted with increasing order of polarity solvents series starting from Pet. ether, Chloroform, Ethanol via soxhlet apparatus by successive hot continuous percolation method.[13] At last, all extracts were concentrated in a rotary flash evaporator and the residue were dried in a desiccator over Sodium sulphite. After this, practical yield was weighed and calculated as 2.5 gm, 1.5 gm and 3 gm for pet. ether, chloroform and ethanol extracts respectively.

Evaluation of antioxidant activity by DPPH radical scavenging method

Free radical scavenging activity of different extracts of leaves and flowers of *Ageratum conyzoides* Linn. plant were measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH). In brief, 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts in ethanol at different concentration (5, 10, 15, 20, 25, 30 µg/ml). Here, only those extracts are used which are Solubilise in ethanol and their various concentrations were prepared by dilution method.[14] The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm. by using spectrophotometer (UV-VIS Shimadzu).[15] Reference standard compound being used was ascorbic acid and experiment was done in triplicate.[16] The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity.[17] The percent DPPH scavenging effect was calculated by using following equation:

DPPH scavenging effect (%) or Percent inhibition = $A_0 - A_1 / A_0 \times 100$.

Where A_0 was the Absorbance of control reaction and A_1 was the Absorbance in presence of test or standard sample.[18]

RESULTS

The leaves and flowers ethanolic extract of this plant showed better antioxidant potential when compare to

standard ascorbic acid by DPPH scavenging assay method. The absorbance at 517 nm by UV visible spectrophotometer were found to be as 0.0390 and 0.0989 for standard ascorbic acid and alcoholic extract respectively and IC 50 value obtained were as 9.3 and 24.8 µg/ml. for same ascorbic acid and alcoholic extract respectively. It means alcoholic extract of plant at higher concentration captured more free radicals formed by DPPH resulting into decrease in absorbance and increase in IC 50 value.

DISCUSSION

This study determined that Ethanolic extract of leaves & flowers of *Ageratum* plant species showed better antioxidant potential by DPPH radical scavenging method when compare to standard ascorbic acid[19] and IC 50 value found to be as 9.3 and 24.8 µg/ml for ascorbic acid and alcoholic extract respectively.[20] So, we can say this plant is having antioxidant activity.

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Table 1. Absorbance of different extract of *Ageratum conyzoides.* linn with standard ascorbic acid at 517 nm by uv visible spectorphotometer (dpph scavenging assay method)

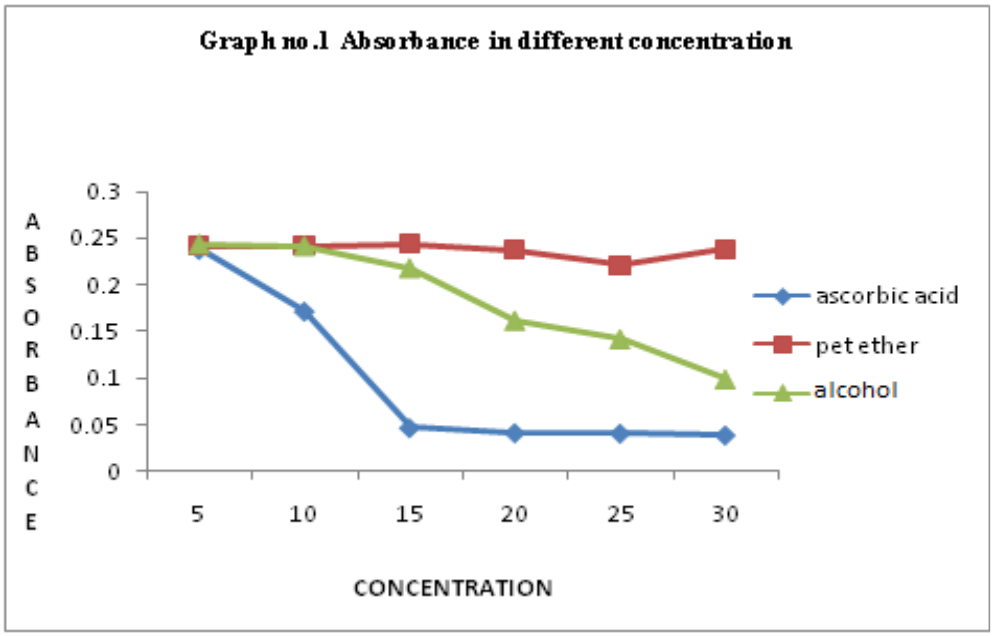
Concentration (µg/ml)	Ascorbic acid (Abs)	Pet ether (Abs)	AlcE (Abs)
5	0.2380	0.2421	0.2440
10	0.1719	0.2428	0.2420
15	0.0469	0.2440	0.2180
20	0.0415	0.2379	0.1619
25	0.0410	0.2218	0.1420
30	0.0390	0.2386	0.0989

Control: 0.2444

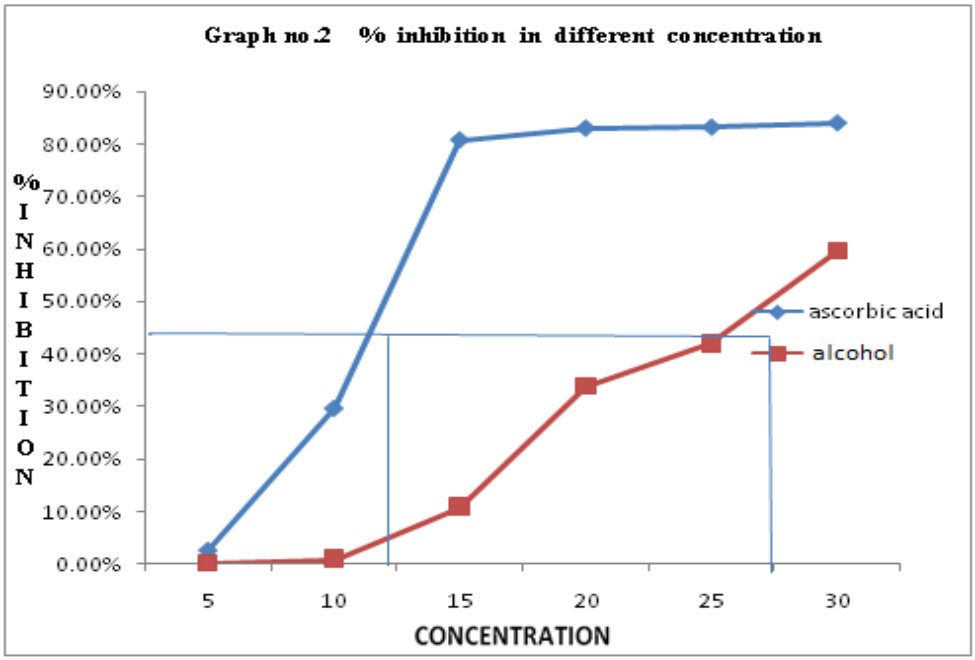
Table 2. % inhibition of different extract of *Ageratum conyzoides* Linn. with ascorbic acid

Concentration (µg/ml)	Ascorbic acid (% Inhibition)	Pet. ether (% Inhibition)	Alc. E (% Inhibition)
5	2.61%	0.99%	0.16%
10	29.66%	0.65%	0.98%
15	80.8%	0.16%	10.80%
20	83.01%	2.65%	33.75%
25	83.22%	9.24%	41.89%
30	84.02%	2.37%	59.53%

Figure 1. *Ageratum conyzoides*- full plant species[12]



Graph 1. Absorbance in different concentration



Graph 2. % inhibition in different concentration