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Antioxidant activity by DPPH fundamental scavenging technique of aqueous ethanolic extract of *Ageratum albidum* leaves Linn

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ABSTRACT

Although the present survival and availability of synthetic drugs for the treatment of various diseases, these medications are neither cheap nor completely effective. Furthermore, the long-term utilization of synthetic drugs may cause adverse effects, despite the fact that those medications provided from natural sources are more affordable and shown lesser adverse effects. The existing belief is oxidative stress plays a considerable role in the pathogenesis of a range of diseases and its complications. Our aims to find and evaluate the combination of strong antioxidants are positively work various disease and its complications. They exhibited strong anti oxidant activity DPPH(2, 2-Diphenyl-1-picrylhydrazyl) scavenging activity with IC_{50} value of 66.35±1.2 and51.5±2 µg/ml for Ascorbic acid and Aqueous Ethanolic Extract of Ageratum albidum leaves (AEEAA) respectively. They strongest anti oxidant activity have Aqueous Ethanolic Extract due to may be presence of flavonoids and phenols.

Keywords: Synthetic drugs, Anti Oxidant, DPPH, IC_{50} and *Ageratum albidum.*

INTRODUCTION

Antioxidants participate significant responsibilities as physical condition protecting factor. Methodical confirmation suggested that antioxidants decrease the risk for chronic diseases including cancer and heart disease. Major sources of natural antioxidants are obtained from grains, fruits and leafy vegetables[1] Plant source antioxidants like vitamin C, vitamin E, carotenes, phenolic acids and flavonoids have been recognized as a potential to reduce risk diseases[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]. The DPPH method is rapid, simple, accurate and inexpensive assay for measuring the ability of different compounds to act as free radical scavengers or hydrogen donors to evaluate the antioxidant activity of foods and beverages [4]. The DPPH method is described as a simple, rapid and convenient method independent of sample polarity for screening for radical scavenging activity[5]. The method DPPH is widely used for measurement of free radical scavenging ability of antioxidants[6]. For determination of radical scavenging activity of different foods, beverages and substrates were elaborated a great variety of methods with utilization of DPPH. This plant spices has been reported to possess antioxidant properties [7]. So, this study has been undertaken to evaluate *Ageratum Albidum* Leaves Linn plant for their possible potential to antioxidant action by DPPH scavenging method [8].

MATERIALS AND METHODS

a) Extraction

Shade dried leaves part of this plant were pulverized and about 100 gms of powdered leaves and flowers were extracted with Ethanol via soxhlet apparatus by hot continuous percolation method. At last, all extracts were concentrated in a rotary flash evaporator and the residue were dried in a desiccator over Sodium sulphite[9].

b) Preliminary phytochemical screening of Aqueous Ethanolic Extract of *Ageratum Albidum* leaves[10]

The Aqueous Ethanolic Extract of *Ageratum Albidum* (AEEAA) leaves was used for testing preliminary phytochemical screening in order to detect major chemical groups.

The leaves of *Ageratum Albidum* lis taken 10g in 50ml methanol and subjected to extraction. The filtrate was subjected to different test

Molisch's test

1ml of sample added with few drops of Molisch's reagent & 2 drop of Conc H_2SO_4

Fehling's test

Dissolve a small portion of extract in water and treat with Fehling's solution.

Phenols test

The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours.

Test for flavonoids

Shinoda test

To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added.

Lead acetate test

To 5ml of extract 1ml of lead acetate solution was added. Flocculent white precipitate.

Test for tannins

Braemer's test

To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added.

Test for alkaloids

Draggendorof's test

A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Draggendorf's reagent.

Hager's test

The extract was treated with few ml of Hager's reagent.

Wagner's test

The extract was treated with few ml of Wagner's reagent.

Test for steroid

Liebermann-Burchard test

To 1ml of extract, 1ml of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added.

Tests for Glycosides

Legal's test

Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution.

Test for Saponins

Foam test

1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes.

Test for Amino acids

Ninhydrin test

Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent.

Test for terpenoid
Liebermann-Burchard test
To 1ml of extract, 1ml of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added.

c) Antioxidant activity[11- 14] In brief, 0.1 mM solution of DPPH in ethanol was prepared. DPPH dissolved with ethanol 1ml solution added to 3ml of extract and prepared different concentration such as 20, 40, 60, 80, 100,120 µg/ml by dilution method. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 520 nm. by using spectrophotometer (UV-VIS Shimadzu). Reference standard compound being used was ascorbic acid and experiment was done DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. Percentage of DPPH scavenging effect was calculated by using following equation:

DPPH scavenging effect (%) or Percent inhibition = A_0 - A_1 / A_0 × 100.
Where A_0 was the Absorbance of control reaction and A_1 was the Absorbance in presence of test or standard sample.

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RESULTS AND DISCUSSION

a) After the extraction, practical yield was weighed and calculated as 3 gm (10%) of ethanol extract.

b) Phytochemical studies

Table No: I, Results of AEEAA

Class of compounds	Tests performed	Results
Carbohydrates	Molisch's test Fehling's test	- -
Phenols	Phosphomolybdic acid test	+
Flavonoids	Shinoda test Lead acetate test	+ +
Tannins	Braemer's test	_
Alkaloids	Wagner's Mayer's Draggendrof's test	+ + +
Glycosides	Legal's test Brontranger's test	+ +
Saponins	Foam test	+
Sterols	Salkowski's test	_
Amino acids	Ninhydrin test	_
Terpenoids	Lieberman Burchard test	+

+ Present
-Absence

The phytochemical studies results (Table-I) revealed that the Molisch's test no characteristic observation indicated the absence of carbohydrates, by phosphomolybdic acid test Blue coloration of the spot indicated the presence of phenols. Shinoda test and Lead acetate test gave pink or red coloration of the solution indicated the presence of flavonoids. Formation of dark greenish grey coloration of the solution indicated the presence of tannins. Orange coloration of the spot indicated the presence of alkaloids. Yellow or reddish brown precipitation precipitation indicated the presence of alkaloids. Characteristic color changed indicated the presence of alkaloids. Pink to red colour solution indicates the presence of glycosides. No layer of foam formation indicates the absence of Saponins. No characteristic observation for steroids absence. No blue color formed which indicated absence of amino acids. Dark pink or red coloration of the solution indicated the presence of terpenoid

c) Antioxidant activity

Table No: II, AEEAA articulated % of Inhibition

S.No	Concentration µg/ml	% of Inhibition	
		Ascorbic acid µg/ml	AEEAA µg/ml
1	20	32.9±0.9	21.5±1.2
2	40	55.2±1.5	42.5±2.0
3	60	77.5±1.0	60.5±0.1
4	80	92.1±3.5	80.0±0.0
5	100	98.5±2.0	90.0±0.0
6	120	104±1.0	100±0.1

The values were expressed as Mean±SEM

Fig No: I, AEAA articulated % of Inhibition

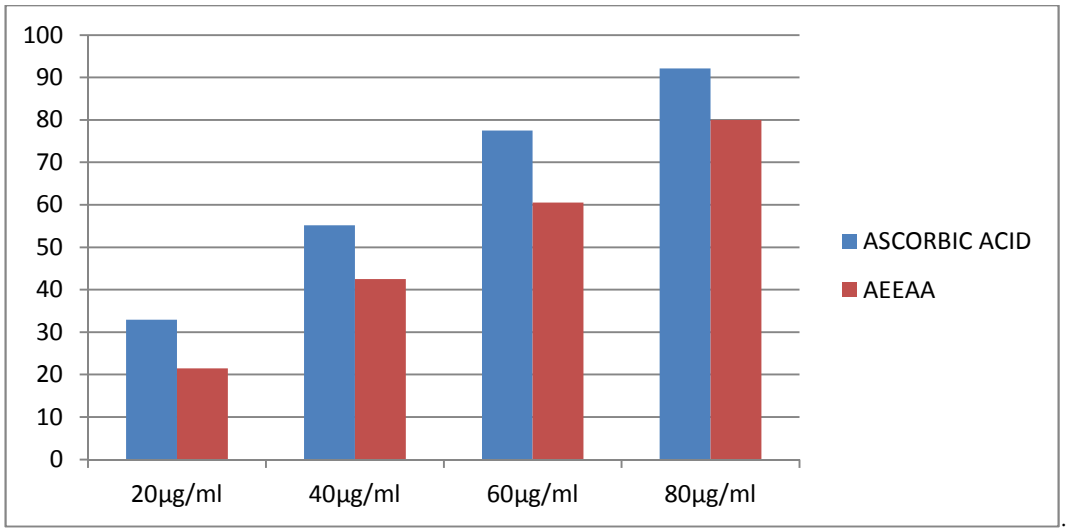

Table No: III, AEEAA expressed Absorbance

S.No	Concentration µg/ml	Absorbance	
		Ascorbic acid µg/ml	AEEAA µg/ml
1	20	0.032	0.021
2	40	0.071	0.034
3	60	0.162	0.042
4	80	0.252	0.048
5	100	0.401	0.061
6	120	0.810	0.084

The table –I &III & Fig –I have been expressed Aqueous Ethanolic Extract of *Ageratum Albidum* leaves shown better antioxidant potential when compare to standard ascorbic acid by DPPH scavenging assay method. The absorbance at 520 nm by UV spectrophotometer were found to be as0.1050 and 0.0390 for standard ascorbic acid and alcoholic extract respectively and IC $_{50}$ value obtained were as 66.35±1.2 and51.5±2_µg/ml. for same ascorbic acid and alcoholic extract respectively. So, we can say this plant having antioxidant activity.

CONCLUSION

The literature assessment of the methods for determination of free radical scavenging activity by DPPH exposed that considerable differences are used solvents, concentration of DPPH working solutions, relation between volumes of sample and reagent, duration of reaction, wave length of absorbance capacity, standard solutions and equations for calculation of the results. They strongest anti oxidant activity have Aqueous Ethanolic Extract of *Ageratum Albidum* leaves due to may be presence of flavonoids and phenols. This work will be useful for research worker to evaluate antioxidant with cancer and diabetes mellitus for Aqueous Ethanolic Extract *Ageratum Albidum* leaves.

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