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ORIGINAL RESEARCH ARTICLE

Evaluation of *In-vitro* Antioxidant Activity of *Eclipta alba*

Ashish Baldi[2], Rakesh Gupta[1]*, Mangal S. Panwar[2]

[1]*SDPS Ayurved Medical College, Bhanpura, Madhya Pradesh, India*
[2]*Mandsaur Institute of pharmacy, mandsaur (M.P.), India.*

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ABSTRACT

Antioxidant potential of ethanolic extract of *Eclipta alba* was evaluated within the concentration range of 5-100 µg/ml using *in-vitro* studies *viz.* free radical scavenging capacity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS) radical cation decolourization assay, scavenging of nitric oxide radical and total antioxidant capacity. Ascorbic acid was used as standard compound. Among antioxidant screening models tested, ethanolic extracts of *E. alba* had shown promising antioxidant potential in comparison to ascorbic acid. Therefore the results justify the free radical scavenging activity and therapeutic applications of *E. alba* in different systems of medicines.

Keywords: ABTS, DPPH, Nitric oxide, Free radical, scavenging capacity

INTRODUCTION:

The importance of free radicals and reactive oxygen species (ROS) has attracted increasing attention of researchers involved in the field of medical science. Generation of free radicals is known to be involved in the development of various ailments like cancer, diabetes, nephrotoxicity, liver cirrhosis, Parkinsonism, Alzheimer's disease and inflammatory responses [1, 2, 3, 4]. These free radicals are produced in body as by products of biological redox reactions [5] and their concentrations exist in a dynamic equilibrium with antioxidants to quench and/or scavenge then to protect the body against harmful effects of free radicals. Reactive oxygen species and free radicals mainly cause cumulative damage of DNA, proteins, lipids and membranes and thus oxidative stress. This may result in chromosomal aberrations and/or genetic transformations leading to carcinogenesis [6]. Furthermore imbalance between pro-oxidant and antioxidant homoeostasis generally results in various degenerative diseases. Presently, the use of complimentary and alternative therapy and especially the utilization of phytoconstituents have been significantly increasing worldwide. This is due to better acceptance of herbal products than synthetic drugs because of lesser side effects and better compatibility thus improving patient tolerance even on long term use [7]. Crude extracts from plants and a number of phytochemicals are known to posses' excellent antioxidant potential and may serve as successful lead molecules in therapeutic armamentarium against these diseases. Toxic or mutagenic effects of many synthetic antioxidants have also resulted in increased attention towards herbal medicines.Whole plant of *E. alba* Linn. belonging to family Asteraceae was used as a drug in present investigation. The plant is distributed throughout India in wet or moist lands, ascending up to 2000 m on the hills and is known to have hepatoprotective, antiviral, antibacterial, antinociceptive, antiinflammatory and bronchodilator properties. However no data is available in the literature on the antioxidant activity of *E. alba* against variety of free radicals. Therefore present study was designed to investigate the antioxidant activity of ethanolic extract of this drug through various *in vitro* models.

MATERIALS AND METHODS:

Collection of Plant Materials

Plant material of *E. alba* (whole plant with root), collected from Medicinal Plant Garden of SDPS Ayurved Medical College, Bhanpura, Madhya Pradesh, India was authenticated by Dr. Rakesh Gupta, Head, Department of Dravyaguna. The

***Corresponding Author:** Ashish Baldi**, Email:** baldiashish@gmail.com

specimen of the plant material was deposited vide voucher number SDPS/09/PS/118.

Preparation of Extracts

The collected plant material of *E. alba* was air dried in shade at 27±2 ^{o}C, powdered and stored in an air tight container at 27±2 ^{o}C till further use. These dried and powdered plant materials (1 Kg) were accurately weighed and defatted with 5 L petroleum ether (40-60). It was then extracted with 5 L ethanol in a soxhlet apparatus for 36 h. The extract was filtered, evaporated to dryness under vacuum and stored in the desiccator for use in subsequent experiments.

Phytochemical Tests

The qualitative chemical investigations of ethanolic extracts obtained from air dried plant material of *E. alba* were carried out to check the presence of various phytoconstituents [8].

Assessment of Antioxidant Activity

The assessment of antioxidant activity was done using following methods:

1. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity: For the present study, the samples were prepared in different concentrations i.e.5-100 µg/ml in methanol. The ethanolic extract of *E. alba* (samples) at above concentrations were mixed with 3 ml of 100 µM DPPH prepared in methanol and final volume was made up to 4 ml with methanol. The absorbance of the resulting solutions was recorded in triplicate after 20 min at 25±2 ^{o}C against ascorbic acid. The disappearance of color was read spectrophotometrically at 517 nm using a Shimadzu Visible spectrophotometer. Radical Scavenging Capacity (RSC) in percent was calculated by following equation:

$$RSC\ (\%) = 100 \times [A_{blank} - A_{sample} / A_{blank}]$$

Where,

RSC = Radical Scavenging Capacity,
A_{blank} = Absorbance of blank,
A_{sample} = Absorbance of sample,

From the obtained RSC values, the IC_{50} were calculated, which represents the concentration of the scavenging compound that caused 50% neutralization [9].

2. ABTS Radical Cation Decolorization Assay: ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulfate and the mixture was allowed to stand in dark at 25±2^{o} C for 12- 16 h before use. For this study, different concentrations (5-100 µg/ml) of the ethanolic extracts (2 ml) were added to 1.2 ml of ABTS solution and the final volume was made up with ethanol to 4 ml. The absorbance was read at 745 nm and the experiment was performed in triplicate [10].

3. Scavenging of Nitric Oxide Radical: Nitric oxide was generated from sodium nitroprusside and measured by Griess' reaction [11,12]. Sodium nitroprusside (5 mM) in standard phosphate buffer saline solution (0.025 M, pH: 7.4) was incubated with different concentrations (5 -100 µg/ml) of the ethanolic extracts dissolved in phosphate buffer saline and the tubes were incubated at 25±2^{o} C for 5 h. Control experiments without the test compounds but with equivalent amounts of buffer were conducted in an identical manner. After 5 h, 0.5 ml of incubation solution was removed and diluted with 0.5 ml of Griess' reagent (1 % sulphanilamide, 2 % *O*-phosphoric acid and 0.1 % naphthyl ethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylenediamine was recorded at 546 nm. The experiment was repeated in triplicate [13].

4. Total Antioxidant Capacity: Ethanolic extract of *E. alba* (100 µg) were added, in triplicate, to a mixture of ammonium molybdate (4 mM) and sodium phosphate (28 mM) in 0.6 M H_2SO_4 in total volume of 2 ml in eppendroff tubes and kept at 95 ±2^{o} C for 90 min and the absorbance was measured at 695 nm after cooling at 25±2^{o} C [14] .

IC_{50} was calculated by using formula:

$$b = \Sigma x.y / \Sigma x^2$$
$$a = \bar{y} - b\bar{x}$$
$$IC_{50} = a+b\ (50)$$

where, b = Regression coefficient of x on y ; a = Intercept of the line ; x = Concentration in µg/ml; y = % Scavenging; $\bar{x}$ = Mean of concentration; and $\bar{y}$ = Mean of % scavenging.

Statistical Analysis

The results were expressed as mean values. The significance statistical analysis was performed by ANOVA followed by Dunnett's test and P values (< 0.05 and < 0.01) implied significance.

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Table 1: Antioxidant activity of ethanolic extract of *E. alba#**

Conc. (µg/ml)	DPPH		ABTS		NO	
	Extract	Asc. acid	Extract	Asc. acid	Extract	Asc. Acid
5	8	22	9	12	10	22
25	14	36	26	38	13	30
50	24	48	34	49	20	45
75	32	54	51	65	27	55
100	38	58	63	76	35	63

* The values given are mean of triplicate readings in different experiments.
# All results are statistically significant with P<0.05.

RESULTS AND DISCUSSION:

Oxidation is one of the most important chemical reaction involved in various metabolic activities in living organisms. However during these biological processes, oxygen centered free radicals and ROS are continuously produced, which may cause tissue damage and even cell death [15]. In living system, varieties of antioxidant mechanisms play an important role in combating ROS. [16]. Few of the mechanisms are free radical scavenging, complexation of prooxidant metals, reduction and quenching of singlet oxygen formation. The antioxidants may also act by upregulating the expressions of genes encoding the enzymes such as superoxide dismutase (SOD), catalase or glutathione peroxidase and thus increasing the levels of endogenous defenses [17] .It is also reported that dietary intake of antioxidant – rich food/herbs decreases the incidence of a number of human disorders [18,19]. Hence research on evaluation and establishment of antioxidant potential has increased in recent times. Furthermore many methods for estimation of this bioactivity had reported in the literature [20, 21,22]. In present study, DPPH radical scavenging capacity, nitric oxide scavenging effect and ABTS assay were used for establishment of antioxidant potential of *E. alba* in comparison to ascorbic acid as standard compound within the concentration range of 5-100 µg/ml. Extraction of *E. alba* in soxhelet apparatus resulted in crude extract with yields of 5.2 % w/w. Preliminary phytochemical screening of ethanolic extracts revealed the presence of lactone derivative, tannins, steroids and saponins. The presence of a main constituent, wadelolactone, was further confirmed qualitatively by thin layer chromatographic studies using toluene:ethyl acetate:formic acid (5:4:1) as mobile phase and vanillin-sulphuric acid as spraying reagent. The DPPH method applies to the overall antioxidant capacity of the sample. DPPH is a relatively stable free radical and this method determines the ability of ethanolic extract of *E. alba* to reduce the DPPH radical to the corresponding hydrazine by reacting with the hydrogen donors in the antioxidant principles [23]. DPPH radicals convert the unpaired electrons to the paired one and the solution loses colour stoichiometrically depending on the number of electrons taken up [24]. The dose dependant inhibition of DPPH by ethanolic extract and ascorbic acid is given in Table-1. IC_{50} values of 21.35 µg/ml and 40.87 µg/ml were obtained from ethanolic extract and ascorbic acid respectively. Ethanolic extracts of *E. alba* exhibited better antioxidant potential in comparison to ascorbic acid as evidenced by lower IC_{50} values respectively in DPPH assay.The ABTS decolourization assay is based on the principle of inhibition/decrease in the absorbance of the radical cation ($ABTS^{\cdot+}$). This chemical reaction results in direct generation of ABTS radical mono cation prior to addition of antioxidant components instead of in presence of antioxidant. The concentration dependent inhibition/scavenging properties of ethanolic extracts of *E. alba* and ascorbic acid towards $ABTS^{\cdot+}$ are given in Table-1. *E. alba* extract had exhibited comparatively higher antioxidant potential with IC_{50} value of 41.8 µg/ml in comparison to ascorbic acid with IC_{50} value of 51.56 µg/ml.Nitric oxide (NO) is produced by endothelial cells, macrophages, neurons etc. It acts mainly as signaling molecule in regulation of various physiological processes. Although excess production of this compound is associated with several body disorders [25]. Chemically NO is very unstable under aerobic condition and it produces nitrite and nitrate upon reacting with O_2 through intermediates like NO_2, N_2O_4 and N_3O_4. In the present study, incubation of sodium nitroprusside in phosphate saline buffer had resulted in generation of nitrite, which was reduced by ethanolic extracts of *E. alba*. This effect may be due to competition of antioxidant compounds present in extract with oxygen to react with nitric oxide [26], which ultimately leads to inhibition of generation of nitrite.Effect of different concentrations of ethanolic extract on nitric oxide

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scavenging capacity was determined and results were presented in Table-1. The extract of *E. alba*, exhibited scavanging potential with IC_{50} values of 17.55 µg/ml. These values were significantly lower than ascorbic acid (46.67 µg/ml) used as standard in the assay indicating higher antioxidant activity of extracts of *E. alba.* Total antioxidant potential of extracts was also determined by formation of phosphomolybdenum complex under acidic conditions. Ethanolic extract of *in-vitro* cultured cells exhibited slightly higher total antioxidant activity (298.0) in comparison to that of ascorbic acid (264.0).

CONCLUSION:

The present study proved promising antioxidant potential of ethanolic extracts of *E. alba* against a variety of free radicals. It is reported that tannins and steroids are natural products which have been shown to possess various biological properties related to antioxidant mechanisms. Thus the antioxidant activity of *E. alba* may be attributed to the presence of these compounds as confirmed by qualitative phytochemical analysis. Hence these results support the view that some traditionally used Indian medicinal plants are promising source of potential antioxidants.

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