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IMPACT OF *ECLIPTA ALBA* ROOT EXTRACT ON ANTIOXIDANT ACTIVITY IN EXPERIMENTAL MODELS

ANJALI SINGH, AYODHYA SINGH, VANDANA DWIVEDI

Deptt. of Chemistry, M. M. H. College, Ghaziabad (U.P.) India

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The herbal drugs have always been used in healing various diseases because of the wide safety profile they provide. *Eclipta alba* possesses various biological properties and used for the treatment of various disorders like general tonic, edema, digestion, hepatitis, enlarged spleen, antioxidant activity and skin disorders. Potentially reactive derivatives of oxygen like O_2., H_2O_2. and .OR, described as reactive oxygen species (ROS) are continuously generated inside the human bodies as a consequence of exposure to a large number of exogenol ˜s chemicals occurring in the ambient environment and a number of endogenous metabolic processes involving bioenergetic electron transfer and Redox enzymes. The present work is aimed to evaluate the effect of *Eclipta alba* on oxidative stress of streptozotocin (STZ) induced severely diabetic rats. The prolonged treatment for 40 days with the dose of 600 mg/kg b. w., identified as the most effective dose of *Eclipta alba* root extract was given orally to rats.˜ At the end of the treatment period, the tissues of brain, spleen, pancreas, kidney, liver of the rats were removed and analysed for the status of antioxidant parameters.

Diabetic rats showed increased level of lipid peroxidation (LPO) and decreased activities of antioxidative enzymes, for e.g., catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and glutathione-s-transferase (GST) in various organs of the rats. Interestingly, diabetic rats treated with the ethanolic root extract of *Eclipta alba* exhibited a marked recovery ($p>0.05$) in the reactivities of these antioxidant enzymes along with significantly abridbged LPO. The *Eclipta alba* root extract thus represented to have flavonoids, which substantiates its antioxidant efficiency.

KEYWORDS : Immunomodulatory, LPO (lipid peroxidation), GPx (glutathione peroxidase).

INTRODUCTION

The plant *Eclipta alba* selected for our study belongs to family Asteraceae and is popularly known as false daisy or Bhringaraj. It is a creeping and moisture loving herb commonly distributed on roadsides and wastelands throughout India. It is reported to contain

phytosterol, β-amyrin, triterpenes such as ecalbatin, echinocystic acid, flavones such as luteolin and coumarin such as wedelolactone [1-6]. The whole plant is used as a stimulant. The flowers are used for their analgesic, antispasmodic, fungicidal, digestive, bactericidal and vulnerary properties. The plant is known to have some important pharmacological activities such as hepatoprotective, antimicrobial, antioxidant, anti-inflammatory, antiviral, immunomodulatory and analgesic activity [7]. Morbidity and mortality resulting from liver diseases (such as hepatitis) is a major public health problem worldwide, especially in developing countries, the major abnormalities associated with hepatitis are lipidemia, peroxidation and loss of plasma membrane integrity. The plant derived natural products of many herbs includes my herb of interest such as flavanoids, terpenoid, and steroids possess many pharmacological properties including antioxidant activity [8]. Antioxidants are compounds that help to inhibit many oxidation reactions caused by free radicals, which damage to the cells and tissues. As antioxidants play an important role in inhibiting and scavenging radicals thereby providing protection to human beings against infection and the degenerative diseases [9]. But we need isolation and characterization of natural antioxidant having less or no side effects, for medicinal materials to replace synthetic antioxidant [10]. Our investigation aimed to study of the ethanolic extracts of *Eclipta alba*. It is subjected to analyze the antioxidant activity by utilising 2, 2 -diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method.

MATERIALS & METHODS

Collection of herbs and its identification

The whole plant of *Eclipta alba* was collected during September-October from the Botanical garden of the M.M.H. College, Ghaziabad Campus. Herbarium specimens were prepared and authenticated by Dr. R.M Johari, Associate Professor, and Head of the Department of Botany, M.M.H. College, Ghaziabad, affiliated to CCS University, Meerut, U.P. India.

Chemicals and Reagents

Sodium nitroprusside, Fehling's solution A & B, Benedict's reagent, Mayer's Reagent, Dragendorff 's Reagent, Biuret reagent, $FeCl_3$, H_2SO_4, $KMnO_4$ & KNO_3 employed in the entire study were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Preparation of Crude Extract of Plant

After collection and authentication, the plant's root was shade dried and coarsely powdered with the help of mechanical grinder. Crude ethanolic extracts of all the plant materials were made following a standard protocol. The plant root material was dipped in ethanol for 24 h with intermittent shaking 2-3 times then it was filtered using 125 mm Whattman qualitative filter paper under sterile conditions and the filtrate was collected in a separate conical flask. After percolation the filtrate was concentrated in a round bottom flask using rotavapour at 40^oC. This process is repeated 4 times unless the filtrate becomes colourless. Finally, the concentrates in a round bottom flask were stored in fridge at 4^oC.

Preliminary Investigation

Preliminary phytochemical investigation was done with the ethanolic extract of *Eclipta alba* using qualitative tests to identify the phyto-constituents in the extract by the method [11].

Total Phenolic Content Determination

The total phenolic content of the plant extracts was analyzed spectrophotometrically by the method [12]. 1 ml of each extract was taken. In it 1 ml of Folin-Ciocalteu reagent was added and incubated for 5 min. 10 mL of 7% (w/v) sodium carbonate solution was added in it followed by the addition of 13 ml distilled water. The mixture was kept in dark for 90 min at 23^0C. Finally, absorbance was measured at 750 nm. For each concentration the samples were prepared in triplicate. Then the content of phenolics in extracts was expressed in terms of Gallic acid equivalent (mg of GA/g of extract).

Table 1: Total Phenolic content in methanol and Ethanol extract of *Eclipta alba*

Plant extracts	Total Phenolic Content (mg Gallic acid Equivalent/g)
Ethanolic	2.85 ± 1.28
Methanolic	16.23 ± 2.7
Ethanol + Water	21.11 ± 1.95

DPPH radical scavenging assay

The DPPH radical scavenging assay was conducted [13]. In brief, 1 mL of DPPH solution (0.1 mM in methanol) was blended with plant extract solution at varying concentrations 1, 2, 5 and 10 μg/ml in triplicate. Mixture of 1 ml methanol and 1 ml of DPPH solution was used as control. The inhibition of the DPPH radical by the sample was computed according the formula given below.

$$\text{Inhibition \%} = (Ac - As \times 100)/Ac$$

(Ac = Absorbance of the control, As = Absorbance of the sample)

Catalase activity

Catalase activity was also assayed [14] with some recent modifications for the estimation of catalase activity. 5% extract of plant tissue was prepared. The catalase activity was assayed by taking two test tubes for each sample, one for catalase activity and the other for its blank. In each test tube 2 ml citrate phosphate buffer (pH 7.0) was added then 2 ml 0.5% H_2O_2, 2 ml D.W. 2 ml tissue extract was added in a series and incubated for 10 min. Thereafter, the reaction was stopped by adding 2 ml 4N H_2SO_4 and thus was done in the case of sample showing catalase activity and in the case of blank, H_2SO_4 was added before addition of H_2O_2, finally titrated against 0.01N $KMnO_4$.

Catalase activity was computed according to nhte formula:

$$\text{Catalase activity} = (\text{Blank} - \text{Sample}) \times 40$$

Activity was determined in terms of ml H_2O_2 hydrolyzed/g fresh weight.

Hydrogen peroxide (H_2O_2) scavenging activity

The capability of scavenging hydrogen peroxide by the extract was determined by the method [15]. In 3.4 ml of phosphate buffer pH 7.4 was added to 43 mM hydrogen peroxide solution to which 1 ml of each of plant extract in triplicate was added and tubes were vortexed and the absorbance was measured at 230 nm after 10 min, against a blank. The abilities to scavenge the hydrogen peroxide were calculated based on the following equation:

$$\text{\% Hydrogen peroxide inhibition} = (Ao - As \times 100)/Ao$$

(where Ao=absorbance of the blank without extract (control); As=absorbance in presence of extract)

Statistical analysis

Every experiment was repeated thrice and an average of the three data has been given in the tables. The given data has been statistically analyzed for the calculation of standard error (SE). $P < 0.05$ was considered as significant.

RESULTS & DISCUSSIONS

During the experiment, preliminary phytochemical investigation of the ethanolic extract showed the presence of saponins, alkaloids, phenolic compounds, tannins and glycosides as the phytoconstituents present in the plant. Total Phenolic Content of the different extracts of *Eclipta alba* was solvent dependant and expressed as mg of Gallic acid equivalent (GAE). The total phenolic compound in extract varied greatly ranging from 2.85±1.28 to 21.11±1.95 mg/g in *E. alba* expressed as Gallic acid equivalent (GAE) but ethanol extract exhibited the highest total phenolic content respectively as shown in the Table 1.

DPPH radical scavenging assay

In the present study, the percentage increase in scavenging effect was observed with increasing concentrations of the plant extract of *E.alba* taken as 25, 50, 75, 100 µg/ml respectively. *Eclipta alba* showed higher scavenging effect of 83.02±2.06% at higher conc. of 100 µg/ml with an IC_{50} of 39.85 µg/ml. The % of scavenging of the ascorbic acid was 82.12±0.05% at higher conc. with an IC_{50} of 18.23 µg/ml. The results obtained were comparative to standards used (methanol) in blank which served as control.

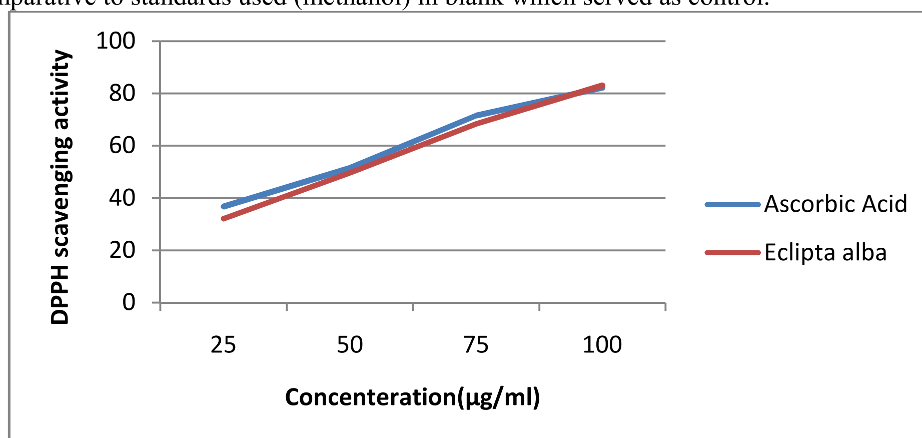

Fig. 1: DPPH radical scavenging activity in *Eclipta alba*

Hydrogen peroxide (H_2O_2) scavenging activity

The root extract of *Eclipta alba* exhibited scavenging activity for H_2O_2 in a dose-dependent manner. *Eclipta alba* showed 5.25% scavenging activity on H_2O_2 which are well correlated to the standard with highest scavenging effect of 6.35% at 10µg/ml with an IC_{50} value of 128.61 µg/ml.

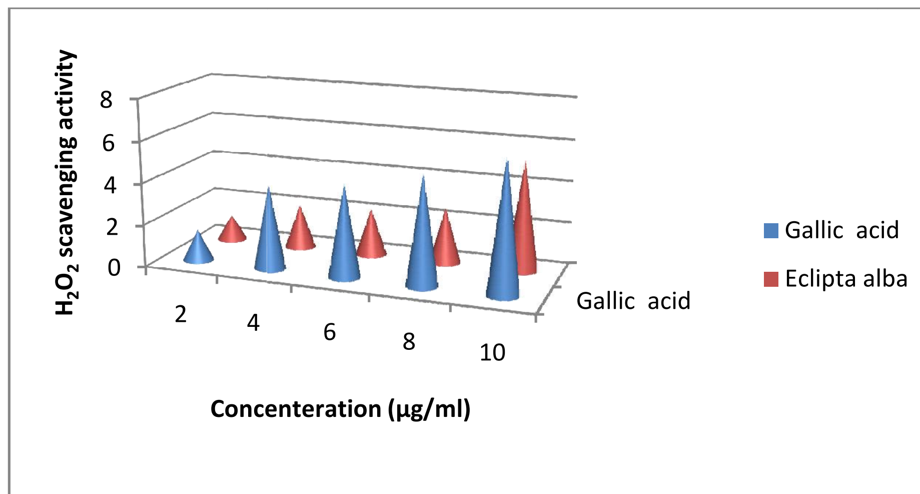

Fig. 2. H_2O_2 radical scavenging activity in *Eclipta alba*

Catalase activity

The results reveal an increase in the catalase activity of 30.10 and 39.35 at 0.5 and 1 mg/g fresh wt. respectively in *Eclipta alba* as compared to control.

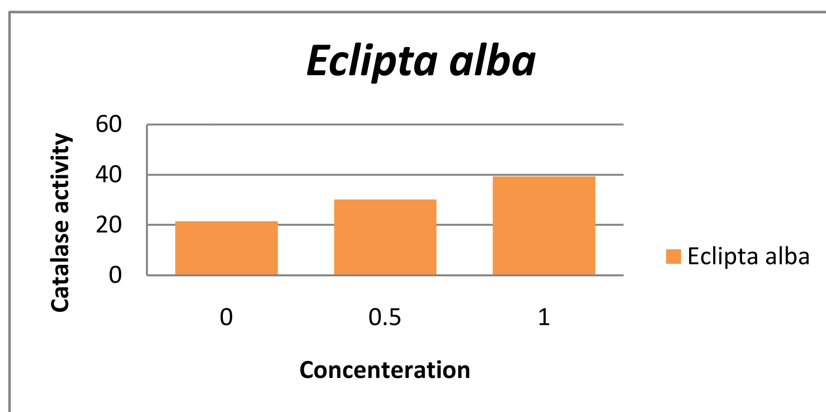

Fig. 3: Catalase activity in *Eclipta alba*

Antioxidant therapy is aimed at preventing and treating diseases caused by free radical derived oxidative stress. It is an exciting new concept which can be used to cure countless human diseases such as inflammation, cardiovascular disease, cancer and aging related disorders. Our study explores the antioxidative potential of the selected plant along with its herbal root extract which has shown a strong antioxidant activity and is now capable of mentioning its strong capability to cure these diseases without having any side effects. The strong antioxidant activity in the herbal root extract is directly correlated with suppression of free radical induced diseases i.e. cancer. This capacity of the plant could be due to synergistic effect and the key contribution of many phyto-constituents together in the root extract for achieving the desired therapeutic effects. Different studies have indicated that phenols play a

key role for the variation in the antioxidant activity of the plant [16,17]. They execute their antioxidant activity in two ways -- lipid free radicals are inactivated or hydroperoxides are prevented from decomposition into free radicals. The antioxidant effect of plant products is mainly due to radicals scavenging activity of phenolic compounds such as tannins, flavonoids, polyphenols, and phenolic terpenes [18-20]. Our analysis of total phenolic content showed that ethanol extract of the root of *Eclipta alba* can be potent source of natural antioxidant, while ethanolic extract exhibited highest total phenolic content in this plant.

On the basis of our results of the present study, it is concluded that the ethanol extracts of *Eclipta alba* is presented in Table 2, have significant antioxidant activity. Ethanol is preferred for the extraction of antioxidant compounds mainly because its lowers toxicity [21]. Hydrogen peroxide and super oxide can interact in the presence of certain transition metal ions to yield a highly reactive oxidizing species, the hydroxyl radical. The antioxidants react with the stable free radical DPPH (deep violet color) and convert it to 1,1-diphenyl-2-picryl hydrazine with decolouration. The scavenging effects of root extract increased with their concentrations to similar extend. *Eclipta alba* (83.02%) showed potent DPPH radical scavenging activity at the concentration of 100ug/ml than compared to standard ascorbic acid.

Table 2: Scavenging effects of ethanol extracts of *Eclipta alba* and standard ascorbic acid on DPPH radical

S. No.	Sample concentration (µg /ml)	Percentage of inhibition	
		Eclipta alba	Ascorbic acid(control)
1.	25	32.02 ±2.02	36.72±1.02
2.	50	49.50±0.05	51.36±0.05
3.	75	68.32±0.08	71.52±0.08
4.	100	83.02±2.06	82.12±0.05

Results are expressed as mean + SD of the three parallel measurements.

This discussion clearly mentioned about the DPPH radical, this radical is one of the best antioxidant and also suitable for the prevention of human diseases such as liver disorders.

Ethanol is a good solvent, due to its high polarity. Ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material. But the ethanol extract of the weed showed the alkaloid and steroid. This may be due to trace amount of these metabolites in the extract. ROS produced in vitro include superoxide radical, hydrogen peroxide and hypochlorous acid. Hydrogen peroxide and superoxide can interact in the presence of certain transition metal ions to yield a highly-reactive oxidizing species, the hydroxyl radical.

Conclusion

Eclipta alba strongly indicated potent antioxidant potential. Moreover, the study reveals that *Eclipta alba* root extract have shown a strong antioxidant activity which is directly correlated with suppression of free radical induced diseases such as cancer, inflammation, cardiovascular disease, etc. These findings encourage studying this plant and its root extract further as a potential agent against diseases mainly caused due to free radicals..

FUTURE SCOPE OF THE WORK

The present study leaves a tremendous scope for finding out the underlying mechanism responsible for this potential.

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