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Evaluation of In -vitro Antioxidant Activity of Methanolic Extract of *Eclipta albaleaves*.

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Abstract

The present work was conducted to evaluate the antioxidant activity of the leaf extract of Eclipta alba by means of various methods [DPPH scavenging, ABTS radical scavenging, nitric oxide radical scavenging, hydroxyl radical scavenging and superoxide radical] using methanolic extract. The results showed that extracts of Eclipta alba possess significant Antioxidant property. Hence, it could be a source of natural antioxidant that could have greater importance for slowing oxidative stress related degenerative diseases.

Keywords:- Eclipta alba, Antioxidant, ABTS, DPPH.

1.Introduction:-

The complex biochemical reactions of the body and increased exposure to environmental toxicants and dietary xenobiotics result in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to oxidative stress under different pathophysiological conditions like Alzheimer's disease (AD), Parkinson's disease (PD) and Amyotrophic lateral sclerosis (ALS)[1] .Plants are valuable gift of nature providing naturally obtained Antioxidant that can be an effective and promising way to protect Human body against various harmful effects due to reactive Free radicals[2,3].

Eclipta alba Linn. occurs throughout the whole of India. It is widely distributed throughout India, China, Thailand, and Brazil. Eclipta alba (L.) has been used in various parts of tropical and sub-tropical regions like south America, Asia, Africa. In India, the plant is known as bhangra, "bhringraj" or bhringraja. The leaves are opposite, lance like with toothed edge and hairy[4].

2. Material and Methods

2.1Plant Material: -

Leaves of *Eclipta alba* was collected from the premises of the campus of Rajasthan University, Jaipur. Authenticated by Dept. of botany, Rajasthan University, Jaipur.

2.2 Extraction Procedure

The collected material was dried in the shade for 5 weeks away from sunlight. The dried material was ground to a coarse powder and extracted (100 g) successively with 600 ml methanol in a Soxhlet extractor at 130oC for 24-30 hrs. The extract was concentrated by using rotary evaporator to yield a light brown solid. The extract was preserved in a desiccator till further use.

2.3 Determination of DPPH radical scavenging activity

1, 1- diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity was measured by the spectrophotometric method. To a solution of DPPH (200µg) in methanol, 0.05ml of the test compounds was added at different concentration (100-500µg/ml). Control was also maintained. After 20min, the decrease in the absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517nm and percentage inhibition was calculated using the formula.

% inhibition = (control-test)/control×100.

2.4 Determination of ABTS radical activity

ABTS the oxidant is generated by per sulfate oxidation of 2,2-azinobis(3-ethylbenzoline-6- sulphonic acid). For the study, different concentration (100-500µg/ml)of the extract (0.5ml) were added to 0.3ml of ABTS solution and the final volume was made upto1ml. The absorbance was read at 745nm and the percentage inhibition was calculated [5, 6].

2.5 Scavenging of nitric oxide radical activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside (5mm) in standard phosphate buffer solution was incubated with different concentration (100-500µg/ml) of the extract dissolved in phosphate buffer (0.025M, pH7.4) and the tubes were incubated at 251ºC for 5hrs. Control experiments without the test compounds, but with equivalent amounts of buffer were conducted in an identical manner. After 5hrs, 0.5ml of incubation mixture was removed and diluted with 0.5 ml of Griess reagent (1% sulphanilamide, 2% O-phosphoric acid and 0.1% napthyl ethylene diamine dihydrochloride). Absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylene diamine was read at 546 nm[7].

2.6 Hydroxyl radical scavenging activity

It was measured by studying the competition between deoxyribose and test extract for hydroxyl radical generated by Fenton's reaction. The reaction mixture contained deoxy ribose (2.8mm), FeCl3 (0.1mm), EDTA (0.1mm),H2O2 (1mm), ascorbate (0.1mm), KH2PO4 KOH buffer (20mm, pH 7.4) and various concentrations of the sample extracts in a final volume of 1.0ml. The reaction mixture was incubated for 1hr at 370C. Deoxyribose degradation was measured as thiobarbituric acid reacting substances (TBARS) and the percentage inhibition was calculated[8].

2.7 Scavenging activity of superoxide

The scavenging activity towards the superoxide radical (O2-) was measured in terms of inhibition of generation of O2. The reaction mixture consisted of phosphate buffer (50mm, pH 7.6), riboflavin (20µg/ml), EDTA (12mm), NBT (0.1mg/3ml) and sodium cyanide (3µg/0.2ml). Test compounds of various concentrations of the extract 100-500µg/ml were added to make a total volume of 3ml. The absorbance was read at 530nm before and after illumination under UV lamp for 15min against a control instead of sample. The percentage inhibition was calculated[9]

2.9 Statistical analysis

The results derived in the study were expressed as the mean from three parallel measurements and for the calculation of IC50 values linear regression analysis was used.

3. Results and discussion

Results obtained showed that free radicals were scavenged by the test compound in a concentration (100-500ug/ml) dependent manner in all the different models used for the study.

DPPH radical was widely used as a model to investigate the scavenging potential of several natural compounds such as phenolic or crude mixtures such as methanol extracts of plants. The concentration of the sample necessary to reduce the initial concentration of DPPH by 50% (IC50) under the experimental conditions was determined.Free radical scavenging activity was obtained with the Methanolic extract with an IC50 value of 310.47 ug/ml.

ABTS assay is a decolorizing assay, which involves the direct generation of ABTS radical into mono cation,The antioxidant activity of the extract by this assay implies that the action may be either inhibiting or scavenging radicals. Highest ABTS radical activity was obtained with methanolic extract (74.38%) at a concentration of 500 ug/ml.

Excess concentration of NO is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which acts as free

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radicals. The results revealed that methanolic extract exhibit highest antioxidant activity 72.05% in a dose dependent manner.

In the present study, Hydroxyl radical scavenging activity observed was in range of 15.95% to 85.50% with an Ic 50 value of 294 (µg/ml).

For Superoxide radical ,the maximum inhibition was found to be 87.31% with an Ic 50 of 290 The Methanolic extract was found to be an effective scavenger of superoxide radical. Superoxide anion radical is one of the strongest ROS among the free radicals and get converted to other harmful reactive oxygen species such as hydrogen peroxide and hydroxyl radical, damaging biomolecules which results in chronic diseases[10]

4. Conclusion

The present study provides evidence that methanol extract of Eclipta alba leaves is a potential source of natural antioxidants.

Table 1. In vitro antioxidant activity of methanolic extract of Eclipta alba by various models (Values are given in mean±sem, n=3)

Concentration (µg/ml)	Inhibition (%)				
	DPPH radical	ABTS radical	Nitric oxide radical	Hydroxyl radical	Superoxide radical
100	22.34±0.92	37.25 ±.87	19.5±1.10	15.95±.56	18.81±0.60
200	39.44±1.47	42.12±1.00	32.34±1.14	34.59±1.06	34.21±1.38
300	43.50±1.67	50.44±.76	44.11±0.97	51.72±1.54	48.69±0.79
400	66.00±2.08	69.43±1.12	55.75±1.01	67.58±0.5	69.08± 1.58
500	72.05±1.84	74.38±.96	72.05±1.32	85.50± 1.80	87.31±1.34
IC 50 (ug/ml)	310.47	299	344.96	294.4	290.6

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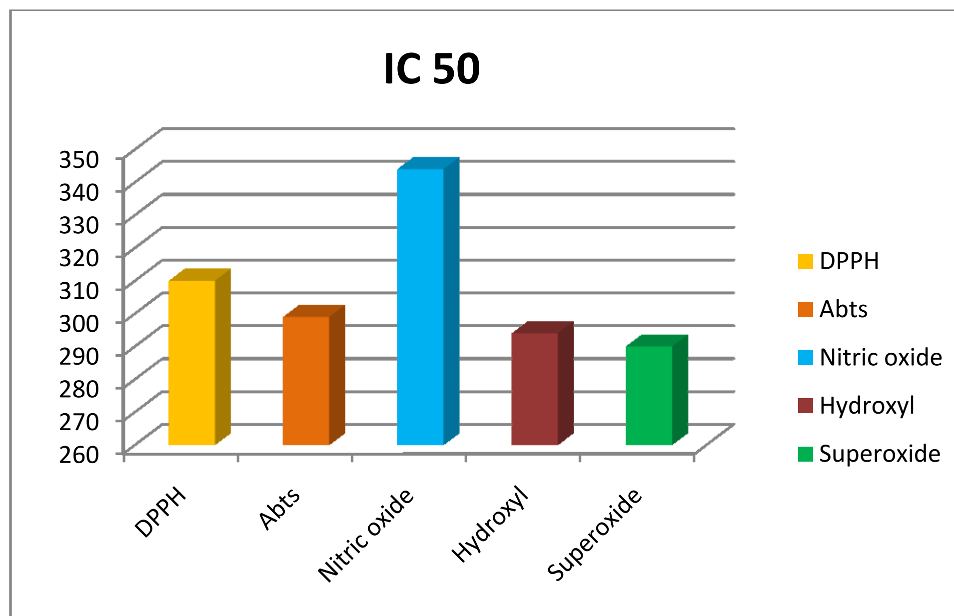
Graph1. In vitro antioxidant activity of methanolic extract of Eclipta alba by various models.



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