

DPPH Free Radical Scavenging Activity of Different Extract of Eclipta alba.



Chemistry

KEYWORDS : free radicals, reactive oxygen species (ROS), super oxide (O₂⁻) anion, per oxy (ROO⁻)-hydroxyl(OH⁻)

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ABSTRACT

Plant & plant products are being used as a source of medicine since a long period in our country. Natural products are organic compound that are synthesized by the plants as secondary metabolites to protect them from insect, pest, or other environmental conditions. The medicinal value of plants was to see chemical substances that produce a definite psychological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannis, and phenolic compounds. Flavonoids have been reported as an antioxidant. Majority of the disease are mainly linked to the oxidative stress due to free radicals. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life & metabolism the most common reactive oxygen species (ROS) include (O₂⁻) anion, hydrogen peroxide (H₂O₂), (ROO⁻) radicals & reactive (OH⁻) radicals. The nitrogen derived free radicals are, nitric oxide (NO) & (ONOO⁻) anion. ROS have been implicated in over a 100 of disease which range from arthritis & connective tissue disorder to carcinogenesis, ageing physical energy infection & acquired immune deficiency syndrome. Antioxidants play a very important role in the therapy of these diseases. The DPPH method applies to the overall antioxidant capacity of the sample. DPPH is relatively stable free radical and the method determines the ability of acetone extract of plant to reduce the DPPH radical to the corresponding hydrazine by reacting with the hydrogen donors in the antioxidant principles. . Ethanolic extracts of E. alba exhibited better antioxidant potential in comparison to ascorbic acid as evidenced by lower IC₅₀ values respectively in DPPH assay.

The medicinal value of plants was to see chemical substances that produce a definite psychological action on the human body. The most important of these bioactive compounds of plants are **alkaloids, flavonoids, tannis, and phenolic** Compounds. Plant & plant products are being used as a source of medicine since a long period in our country. The medicinal property of plant have been investigated in the recent scientific development not only in India and throughout of the world, due to their potent antioxidant activities. No sideeffects and economic viability are two others parameters for the search of antioxidant from botanicals. Natural products are organic compound that are synthesized by the plants as secondary metabolites to protect them from insect, pest, or other environmental conditions. The rediscovery of various medicinal plant & their products in quite evident the developed world form new herbal products coming in the market. Natural products are organic compounds that are formed by living cells of the plants from primary plant products or metabolites elucidation of their structure and chemistry extraction and biosynthesis are major areas of organic chemistry, natural products may be divided in three broad categories.

Primary Metabolites: These compounds which occur in all cells of the plant and play a central role in the metabolism and reproduction of the cells.

Polymeric compounds: These are high molecular weight compound such as cellulose, lignin and protein which forms the cellular structure of the plants.

Secondary Metabolites: These are formed by primary metabolites. They have more interest because 40% of medicine has origin from natural products.

Many Indian medicinal plants considered potential sources of antioxidant compound. There is an increased quest to obtained natural antioxidants with broad spectrum action.

Antioxidants have been reported to prevent oxidative damage by free radical and ROS may prevent the occurrence of disease such as cancer & aging. Flavonoids have been referred to as "**Nature's biological response modifiers**" because of strong experimental evidence of their inherent ability to modify the body's reactions to allergens, viruses, and carcinogens.

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Material and Method

Collection and Extraction of plant material

The plants Eclipta alba. Were collected from the local surrounding at Bhopal district of (M.P) during the month of October-November. The plants were identified by DrJagratiTripathi Professor of Botany and a voucher specimen was procured in herbarium record maintained at Research Centre P.G Department of Chemistry Govt. Geetanjali College Bhopal.

The plant material was thoroughly washed with tap water and kept for drying in shade at room temperature and thoroughly air dried plant material was grinded to powder(40- 60) weighted and stored(The crude extracts obtained through sox let apparatus from the plants were subjected to purification process by different chromatographic techniques followed by spectroscopic methods.

Chemical Testing of Flavonoids in crude extractions of Eclipta alba. DPPH free radical scavenging activity (1, 1-diphenyl-2 picrylhydrazyl radical)

Preparation of standard solution

Required quantity of ascorbic acid was dissolved in methanol to give the concentration of 5 , 10 ,20 , 30 , 40 and 50 mg/ml

Preparation of test sample

Stock solutions of samples were prepared by dissolving 10mg of dried methanolic extract in 10 ml of methanol to give concentration of 1mg/ml.

Preparation of DPPH solutions

4.3 mg of DPPH was dissolved in 3.3 ml methanol : It was protected from light by covering the test tubes with aluminum foil

Protocol for estimation of DPPH scavenging activity.

150 ul of DPPH solution was added to 3ml methanol and absorbance was taken immediately at 516 nm for control reading.

Different concentrations of each extracts were added at an equal volume, to methanolic solution of DPPH. After 15 minutes at room temperature ,the absorbance was recorded at 517nm. The experiment was repeated for three days .Vitamin C, BHA and quercetin were used as standard control IC₅₀ values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals.

Statistical Analysis

Experimental results are expressed as means + SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($P < 0.05$) and the means separated by Duncan's multiple range test. The IC₅₀ values were calculated from linear regression analysis

Table Showing DPPH Activity of Different Extracts of *Eclipta alba*.

Sl. No	Extract of DPPH in different solution	IC ₅₀ value in ug/ml
1.	Pet. Ether	245.45
2.	Benzene	345.25
3.	Chloroform	122.03
4.	Methanol	79.03
5.	Ethanol	84.08
6.	Ascorbic Acid	78.17
7.	Quercetin	53.60

In present study, DPPH radical scavenging capacity, were used for establishment of antioxidant potential of *Eclipta alba*. In comparison to ascorbic acid as standard compound within the concentration range of 5-100ug/ml. The DPPH method applies to the overall antioxidant capacity of the sample. DPPH is relatively stable free radical and the method determines the ability of acetone extract of plant to reduce the DPPH radical to the corresponding hydrazine by reacting with the hydrogen donors in the antioxidant principles. DPPH radicals convert the unpaired one to di phenyl hydrazine and the solution loses colour stoichiometrically depending on the number of electrons taken part.



IC₅₀ values of 21.35ug/ml and 40.87ug/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 IC₅₀ values respectively in DPPH assay. The use of DPPH for a radical scavenging measuring method is described e.g. DPPH is a stable free radical in a methanolic solution. In its oxidized form, the DPPH radical has an absorbance maximum centered at about 520nm. The DPPH method is described as a simple, rapid and convenient method independent of simple polarity for screening of many samples for radical scavenging activity. The use of methanol as extract solvent restricts the cellular compounds responsible for scavenging the DPPH radical. Only methanolic soluble substances (like e.g. carotenoids, fatty acids) are involved in this scavenging process. Carotenoids and fatty acids are known to possess antioxidative properties.

Oxidation is one of the most important chemical reactions involved in various metabolic activities in living organisms. However during their biological processes, oxygen centered free radicals and ROS are continuously produced which may cause tissue damage and even cell death. In living system, varieties of antioxidant mechanism play an important role in combining ROS.

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