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ANTIOXIDANT ACTIVITY OF ECLIPTA ALBA BY IN-VITRO METHOD

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***Abstract*— Living Organism have antioxidant defense system that protects oxidation damage by removal or repair of damaged molecule. Antioxidant play an important role against this oxidative stress to protect our body. It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and incidence of human disease. The present study was carried out to assess the in-vitro antioxidant properties of methanol extract of Eclipta Alba. Antioxidant activity of Eclipta Alba was assayed on different in vitro free radical models like, DPPH, Nitro oxide, Superoxide, Hydrogen peroxide, and Lipid peroxide radical models. The Aim of present study was to examine the possible antioxidant activity of methanolic extract of Eclipta Alba by using invitro antioxidant methods. This was compared with its marketed product. Ascorbic acid is used as a standard. Result are produce in the form of graphical structure .concentration of methanolic extract , ascorbic acid, marketed product are increases as well as DPPH scavenging activity and Hydrogen peroxide scavenging activity also increases . Ascorbic acid shows stronger antioxidant activity as compared to methanolic extract and marketed product of Eclipta Alba.**

***Index Terms*— Antioxidant, In-Vitro, Eclipta Alba, Bhringraj.**

I. INTRODUCTION

Living organism have antioxidant defense system that protects oxidation damage removal or repair damaged molecule1.antixidants interfere with oxidative process by scavenging free radical chelating free catalytic metal and by acting as electron donors(2).it has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and incidence of human disease3.The antioxidant action of herbs use in Turkey for treading ailments was evaluated in vitro. Fruits, vegetables and herbs are recommended at present as optimal source of chemical constituents with antioxidant activity and supplementation of human diet with plant containing high amount of compounds capable of deactivating free radical may have beneficial effect. Antioxidants are compounds that can delay or inhibit the initiation or propagation of oxidizing chain reaction. They can scavenge free radicals and increase shelf life by retarding the process lipid peroxidation, which is one of the major reason for deterioration of food and the pharmaceutical products (4).Many plant contain substantial amount of antioxidant and can be utilized to scavenge the excess free radicals. Antioxidant compound play an important role as a health protecting factors. Plants are one of the most sources of medicines. Antioxidant: Antioxidant are the compound which prevent the oxidation and in general prolong the life or oxidizable matter. Antioxidant compound in food play an important role as health protecting factor. Scientific evidence suggests that antioxidant reduce the risk for chronic diseases including the cancer and heart disease.

Primary source naturally occurring antioxidant are whole grain. fruits, vegetables. Antioxidant are our First line of defense against free radical damage and are critical for maintaining optimum health and wellbeing(5).Free Radicals: An atom or group of atom that has at list one unpaired electron and is therefore unstable and highly reactive atom i;e capable of becoming part of potentially damaging molecules commonly called free radicals. Free radical are capable of attacking the healthy cell of the body,causing them to lose their structure and function.(6)

II. PLANT PROFILE

Plant: Eclipta Alba

Figure 1:Plant of Eclipta Alba

Taxonomy:
Kingdom: Plantea
Subkingdom: Tracheobionta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Asteridae
Order: Asterales
Family: Asteraceae
Genus: Eclipta
Species: Eclipta Alba

The plant has traditional uses in Ayurveda.It is bitter,hot,sharp,and dry in taste.In India,it is known as bhrigaraj.In Southeast Asia, the dried whole plant is used in traditional medicine, although there is no high quality clinical research to indicate such uses are effective.

Synonyms: Eclipta prostrata .

Ayurvedic Name: Bhringraj.

Biological Source: It consist of the fresh dried whole plant of Eclipta Alba belonging to family Asteraceae(Sunflower family).

Morphological characteristics: It is an erect or prostrate, branched,occasionally rooting at nodes,annual herb upto 30-40 cm high.Stem is cylindrical or flat, rough due to appressed white hairs,nodes distict and greenish occasionally brownish.Leaves are opposite,sessile 2 to 6.2 cm long,1.5-1.9cm wide,oblong,lanceolate,sub-entire,acute to sub-acute and strigose with appressed hairs on both surfaces. Fruit achene,cuneate, compressed and with a narrow wing. Flowering in rainy and fruiting till winter.Flowers are white,solitary or two on unequal axillary penduncles involucral bracts are about 8 in number,ovate,obtuse or acute and strigose with appressed hairs.Disc flowers are tubular and corolla is often 4 toothed.Stamens are 5,Filament epipetalous, free,anther united into a tube with base obtuse.Pistil is bicarpellary.Ovary is inferior and unilocular with one basal ovule.Fruit is achenial cypsela,one seeded,cuneate,with a narrow wing and brown in colour.

Figure 2: Flower of Eclipta alba

Figure 3: Seed of Eclipta alba

Chemical Contsituents:

Leaves: Wedelolactone[1.6],Desmethylwedelolactone-7-glucoside,stigmasterol.

Roots: Hentriacontanol,Heptacosanol and Stigmasterol,Ecliptal,Eclalbatin.

Aerial parts: Beta-Amyrin and Luteolin -7-O-Glucoside,Apigenin,Cinnaroside,Sulphur compounds,Eclalbasaponins 1-4.

Stems: Wedelolactone.

Seeds: Seroids,Ecliptalbine(alkaloid)

Whole Plant: Resin,Ecliptine, Reducing sugar, Nicotine, Stigmasterol, Triterpene saponin, Eclalbatin,Ursolic acid, Oleanolic acid.

Therapeutic Uses: The whole plant is used as antiseptic,febrifuge,tonic,deobstruent in hepatic and spleen enlargement and is emetic.In combination with aromatics,the juice is giving in anemia,cataeeh and cough.The plant is also used as scalp tonic for promoting hair growth.

Eclipta alba is commonly used as deobstruent to promote blie flow and to protect the liver parenchymatous tissue in viral hepatitis and other conditions involving hepatic enlargement. The fresh juice of the leaves is given in the treatment of edema, fevers,liver disorders,and rheumatic joint pains,It is also used to improve the appetite and to stimulate digestion.The juice is giving with honey to treat upper respiratory congestion in children.The hair oil is prepared from boiling the fresh leaves with either coconut or sesame oil renders the hair black and lustrous.

Uses of Eclipta Alba: It reduces kapha, Use of Eclipta alba in increases heamoglobin level,strengthering of bones,Act as tonic for eyes,brain,liver intestine, spleen,Eclipta alba is an important Ayurveda herb,widely used in hair fail treatment,liver disorder,skin diseases etc.

Medical uses: Eclipta alba is mainlt use in hair oils,but ita has been considered a good drug in hepatotoxicity.In hair oils,it may be used along with centela asiatica(Bramhi)and Phyllanthus emblica(Amla).It may be used to prevent habitual abortion and miscarriage and also in cass of post delivery uterine pain.Adecoction of leaves is used in uterine haemorrhage.The juice of the plant with honey is given to inflant with castor oil for explusion of worms.For the relief in

piles, fumigation with Ecliptaalba is considered beneficial.The paste prepared by mincing fresh plant has got an anti-inflammatory effect and may be applied to insect bites, stings,sweeling and other skin diseases.

Use of Bhringraj Powder:

For Healthy Function of Liver: Powder of Bhringraj mixed with"Black Pepper" And"Mishri".Take 1 teaspoon twice a day with water.

As a Tonic: Take 1 teaspoon or 3 gm of "Bhringraj Powder"daily with water for several days.It acts as reasayana and provides rejuvenating effects.

For Healthy Skin:"Bhringraj Powder"paste with water when apllied on skin, it helps various skin problems.

For Burning Sensation during Urine Pass Out: Mix "bhringraj Powder "and "Salt".Take it with water.it gives relief in Burning sensation in Urine.

Headache: Mix "Bhringraj Powder"in little of (any oil)and apply on the forehead.It helps in relieving the headache.

Figure 4:Marketed Product of Eclipta Alba

III. Plant Extract Preparation

The extract was prepared according to Word Health Organisation protocol [G.06(1983)] with slight modification8 .The fresh whole plant of Eclipta alba was wash with distilled water separately to remove unwanted foregin material like durts and soils.After washed plant material was dried under shake at room temperature without direct exposure of sunray then it coarsely griended by using mechanical device. The powdered plant material was passed through sieve no. 40 and stored in an airtight container for further use.The coarsely powdered plant materials of Ec;ipta Alba (2000 g)were exracted separately to exhaustion in soxhlet appertus for 72 hrs.by using methanol solvent. The content was filtered successively through ordinary and then Watman filter paper no.1. The process was repeated 5 times and then the solvent thus, collected was evaporated to dryness under reduced pressure using a rotatory evaporation below 500c.The Residue were furthure subjected to dryness by incubating them for 8 days at 370c and extract was kept at 40c until use.

Figure 5:Extract of Eclipta Alba

In the three dimensional finite element analysis adopted in this paper, a regular polyhedron element, with dense meshes at places where the field variations are being changed rapidly has been used. Each phase of the motor has two coils which are shown in different colours as presented in figure 1. There are 150 turns which are made up of stranded conductors. The windings were excited using voltage type of excitation. Also, the usual assumption that the magnetic field outside of an air box in which the motor is placed is considered to be zero. Figure 2 shows the shape of the boundary with the model while figure 3 shows the finite element mesh of the SRM.

IV. IN VITRO ANTIOXIDANT METHODS

Determination of DPPH Radical Scavenging Activity

- Determination of Hydrogen Peroxide (H2O2) Scavenging Activity
- Determination of Nitric Oxide (NO) Scavenging Activity
- Determination of Superoxide (SO) Radical Scavenging Activity
- Determination of Lipid Peroxide (LPO) Radical Scavenging Activity
- Determination of Reductive Ability
- Determination of Total Phenolic Content
- Determination of Total Flavonoid Content

V. IN VITRO ANTIOXIDANT ACTIVITY

Various concentrations of MECM (10-320 mg/ml in methanol) were used for the antioxidant studies on different in vitro models. For reductive ability study, 100-800 mg/ml concentration of the extract was used. Butylated hydroxy toluene (BHT) was used as standard.

A. Determination of DPPH Radical Scavenging Activity:

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was measured using the method of Cotelle et al[16]

with some modifications. 3 ml of reaction mixture containing 0.2 ml of DPPH (100 mM in methanol) and 2.8 ml of test or standard solution of various concentrations was incubated at 37°C for 30 min and absorbance of the resulting solution was measured at 517 nm using Beckman model DU-40 spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the formula:[9]

B. Determination of Hydrogen Peroxide (H_2O_2) Scavenging Activity:

The hydrogen peroxide scavenging ability of the extract was determined according to the method of Ruch et al[19]. A solution of H2O2 (40 mM) was prepared in phosphate buffer (pH 7.4). Extract or standards (of different concentrations) in phosphate buffer (3.4 ml) was added to the H2O2 solution (0.6 ml, 40 mM). The absorbance of the reaction mixture was recorded at 230 nm after 10 min against a blank solution of phosphate buffer. Percentage of H2O2 scavenging was calculated using the above formula.[9]

C. Determination of Nitric Oxide (NO) Scavenging Activity:

At physiological pH, sodium nitroprusside generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be measured by the Griess reaction[17]. 1ml of 10 mM sodium nitroprusside was mixed with 1 ml of test or standard solution of different concentrations in phosphate buffer (pH7.4) and the mixture was incubated at 25°C for 150 min. From the incubated mixture, 1 ml was taken out and 1 ml of Griess' reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added to it. Absorbance of the chromophore formed by the diazotization of nitrite with sulfanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was read at 546 nm and percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.[9]

D. Determination of Superoxide (SO) Radical Scavenging Activity:

Superoxide anion scavenging activity was measured according to the method of Robak and Gryglewski[18] with some modifications. All the solutions were prepared in 100 mM phosphate buffer (pH 7.4). 1ml of nitroblue tetrazolium (NBT, 156 µM), 1 ml of reduced nicotinamide adenine dinucleotide (NADH, 468 µM) and 3 ml of test/ standard solution were mixed. The reaction was initiated by adding 100 µl of phenazine methosulphate (PMS, 60 µM). The reaction mixture was incubated at 25◦C for 5 min, Free Radicals and Antioxidants 44 Vol 1, Issue 1, Jan-Mar, 2011 In vitro antioxidant activity of C.maxima followed by measurement of absorbance at 560 nm. The percentage inhibition was calculated from the above formula.[9]

E. Determination of Lipid Peroxide (LPO) Radical Scavenging Activity :

Rat liver homogenate was used as the source of polyunsaturated fatty acids for determining the extent of lipid peroxidation. Reaction mixture (0.5 ml) containing rat liver homogenate (0.1 ml, 25% w/v) in Tris–HCl buffer (40 mM, pH 7.0), KCl (30 mM), ferrous ion (0.16 mM) and ascorbic acid (0.06 mM) were incubated at 37°C for 1 h in the presence or absence of the extracts or standards. The lipid peroxide formed was measured according to the method of Ohkawa et al[20]. Incubation mixtures were treated with sodium dodecyl sulphate (SDS; 8.1%, 0.2ml), thiobarbituric acid (TBA; 0.8%, 1.5 ml) and acetic acid (20%, 1.5ml). The total volume was then made up to 4 ml with distilled water and kept on water bath for 30 min. After cooling, 1 ml of distilled water and 5 ml of a mixture of n-butanol and pyridine (15:1 v/v) were added and centrifuged at 4000 rpm for 10 min. The absorbance of the organic layer, containing the colored TBA-MDA complex, was measured at 532 nm. The percentage inhibition of lipid peroxidation was determined by comparing the results of the test compound with those of the control, using the formula mentioned above.[9]

F. Determination of Reductive Ability :

Reducing power of the extract and the standard was determined on the basis of the ability of their antioxidant principles to form colored complex with potassium ferricyanide, trichloro acetic acid (TCA) and FeCl3, which is measured at 700 nm[21]. 1 ml of different concentrations of the extract or standard solution was mixed with potassium ferricyanide (2.5 ml, 1%) and 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50°C for 20 min. 2.5 ml TCA (10%) was added to it and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was taken out and to this 2.5 ml water and 0.5 ml FeCl3 (0.1%) were added and absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power.[9]

G. Determination of Total Phenolic Content :

The amount of total phenolic compounds in MECM was determined using Folin-Ciocalteu's reagent and sodium carbonate solution and the absorbence was measured at 760 nm[22]. A calibration curve of standard pyrocatechol was prepared and the results were expressed as mg of pyrocatechol equivalents /g of dry extract.[9]

H. Determination of Total Flavonoid Content :

The total flavonoid content of MECM was determined spectrophotometrically[23]. Briefly 0.5 ml of 2% aluminium chloride in ethanol was mixed with same volume of extract (1.0 mg/ml). Absorption readings at 415 nm were taken after 1 h against a blank (ethanol). The total flavonoid content was determined using a standard curve with quercetin (0-50 mg/L). The mean of three readings was used and expressed as mg of quercetin equivalents/ g of dry extract.[9]

VI. EXPERIMENTAL WORK

Aim: To estimate antioxidant activity of Eclipta Alba by In-vitro methods.

Chemicals: DDPH solution, Nitric acid, methanol, Phosphate buffer(7.4),Eclipta Alba extract, Marketed preparation and Ascorbic acid.

Materials: Chemical are issued from the laboratory. All the chemicals and reagents are analytical grade.

Apparatus: Measuring cylinder, Test tube ,Volumetric flask, Beaker, Test tube stand ,Pippete etc.

Instrument	Company Name
UV –Visible Spectrophotometer	Shimadzu
Colorimeter	Aparna
Digital Weighing Balance	Dolphin

Figure no:1 List of Instrument

A. Preparation of sample extraxt:

1 gm of plant extract was dissolved in 10 ml of respective solvent(Methanol) to make a stock solution of 100 mg/ml. Then extract were further diluted as per requirement in different experiment.

B. Preparation of sample marketed product:

1gm of marketed preparation was dissolve in 10 ml of respective solvent(Methanol) tomake a stock solution of 100 mg/ml.

C. Preparation of sample of Ascorbic acid:

1gm of Ascorbic acid was dissolve in 10 ml of respective solvent(Methanol) to make a stock solution.

VII. METHODS

In-Vitro Antioxidant Activity:

A. Determination of DPPH Radical Scavenging Activity:

- 0.1M solution of DPPH in ethanol was prepared.
- This solution (1 ml) was added to 3ml of different extract in ethanol at a different concentration (10,20,40,60,80,100 µg/ml).
- Here, only those extract are used which are soluble in ethanol and their various concentration were prepared by dilution method.
- The mixture was shakend vigorously and stand to room temperature for 30 min.
- Absorbance measured at 517nm by using spectrophotometer.
- Reference standard solution being used was ascorbic acid

B. Determination of Hydrogen Peroxide (H2O2) Scavenging Activity :

- The hydrogen peroxide scavenging ability of the extract was determined according to the method of Rich et al.
- A solution of H2O2 (40 mm) was prepared in phosphate buffer (pH 7.4).
- Extract or standards (of different concentrations) in phosphate buffer (3.4 ml) was added to the H2O2 solution (0.6 ml, 40 mm).

- The absorbance of the reaction mixture was recorded at 230 nm after 10 min against a blank solution of phosphate buffer.

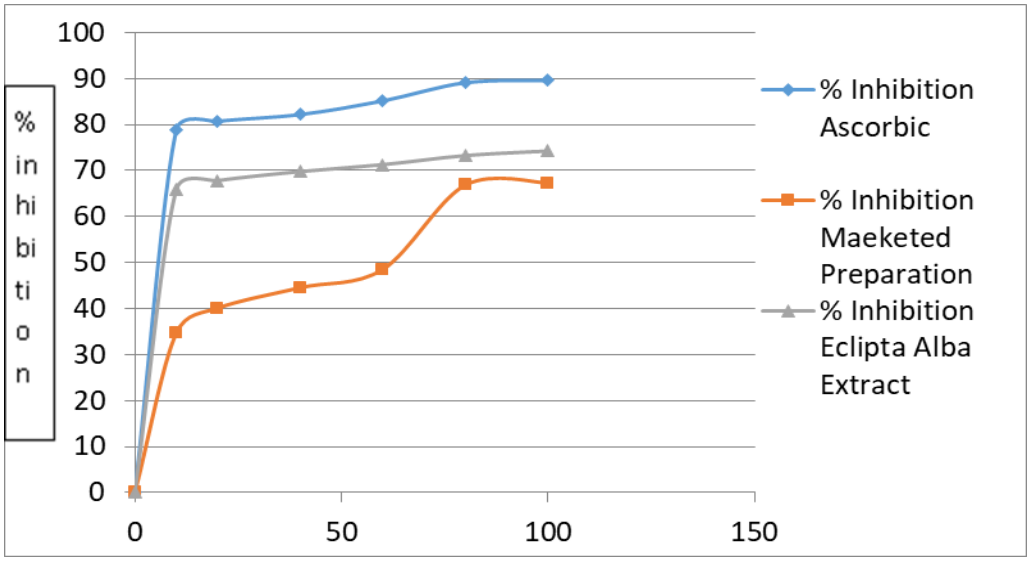
VIII. RESULT AND DISUCSSION:

1) Determination DPPH Radical Scavenging Activity:

Percentage inhibition= (Absorbance of control-Absorbance of test × 100)/Absorbance of control

TABLE NO:1 Observation of DPPH Radical Scavenging Activity

Sample	Percent inhibition					
	10µg/ml	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml
Extract(Eclipta Alba)	65.84%	67.82%	69.80%	71.29%	73.26%	74.25%
Marketed preparation	34.65%	40.09%	44.55%	48.51%	66.85%	67.32%
Ascorbic acid	78.71%	80.69%	82.17%	85.14%	89.10%	89.60%



Concentration(µg/ml)

Graph 1 : Concentration vs. percent inhibition

DISCUSSION:

The DPPH scavenging activity of extract, standard marketed antioxidant & ascorbic acid was given in above table. It is revealed that as concentration extract, standard marketed antioxidant & ascorbic acid was increase, the DPPH scavenging activity.

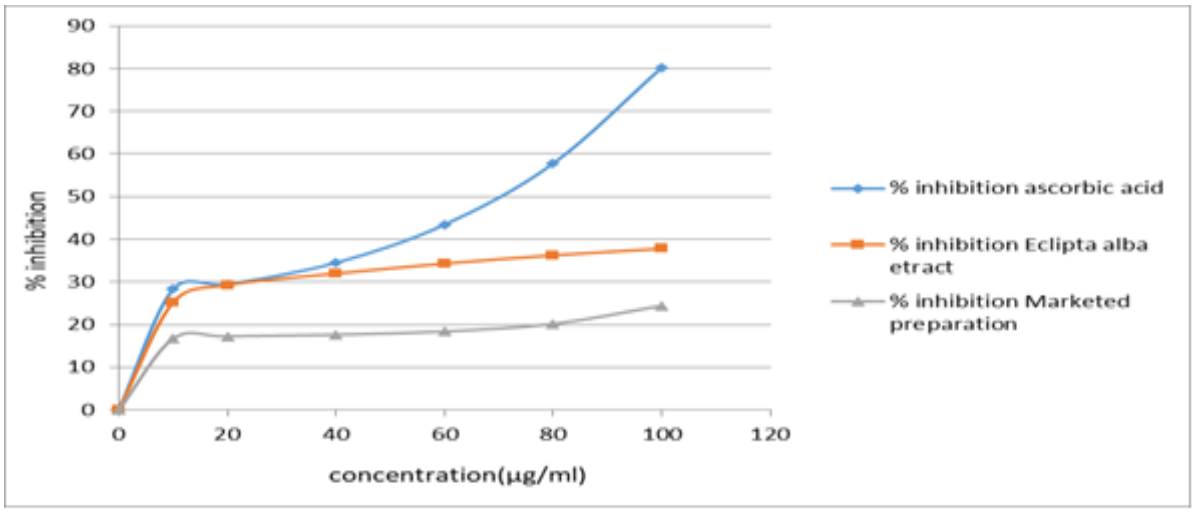
DPPH radical scavenging assay is one of the most widely used methods for screening of antioxidant property of plant products. DPPH is a stable nitrogen centered free radical and can easily abstract an electron or hydrogen radical from the suitable reducing agents to become a stable diamagnetic molecule. The unpaired electron of DPPH thus gets paired off forming the corresponding non-radical hydrazine. The radical scavenging property of the sample was determined by measuring the decrease in absorbance of DPPH.

2) Determination of Hydrogen Peroxide (H2O2) Scavenging Activity :

Percentage inhibition= (Absorbance of control-Absorbance of test × 100)/Absorbance of control

TABLE NO: 2 Observation of Hydrogen Peroxide Scavenging Activity

Sample	Percent inhibition					
	10µg/ml	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml
Extract (ecliptic alba)	25.19%	29.26%	31.97%	34.30%	36.24%	37.79%
Marketed preparation	16.66%	17.24%	17.63%	18.41%	20.15%	24.41%
Ascorbic acid	28.29 %	29.45%	34.49%	43.42%	57.75%	80.23%



Graph 2: Concentration vs. percent inhibition

DISCUSSION:

The H_2O_2 scavenging activity of extract, standard marketed antioxidant and ascorbic acid was given in above table.It is revealed that as concentration extract, standard marketed antioxidant and ascorbic acid was increases,the H2O2 scavenging activity was also increases.

IX. CONCLUSION:

Eclipta Alba plant is used as antiseptic, febrifuge, tonic, DE obstruent in hepatic and spleen enlargement, emetic & Antioxidant property. The antioxidant capacity of Eclipta Alba extract showed stronger activity. The Ascorbic acid shows stronger antioxidant activity as compared to Eclipta Alba Extract. These antioxidant activity depended on concentration of sample. Marketed preparation also shows greater antioxidant activity. It is believed that detection of natural antioxidant source & proper consumption of them in daily diet or use of isolated compounds in clinical practice would be beneficial for healthy life. The marketed preparation also show the stronger antioxidant activity[10].

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