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Research Article

FREE RADICAL SCAVENGING ACTIVITY OF *GYROCARPUS ASIATICUS* BY USING DPPH AND ABTS METHOD

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ABSTRACT

The antioxidant capacity of the barks of *Gyrocarpusasiaticus* belonging to the family Hernandiaceae was assessed by using ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline -6-sulphonic acid) andDPPH (2, 2-diphenyl-1-picrylhydrazyl) methods. The extract showed a very good antioxidant property which was compared with the standard Ascorbic acid in both methods. The IC_{50} of the extract was found to be 24µg/ml and 6µg/ml respectively which was compared with the standard Ascorbic acid which showed IC_{50} value of 16µg and 5µg in respective methods. It was concluded thatthat *G.asiaticus* showed a very good antioxidant activity and showed a better result in DPPH method compared to the other method.

Key words: *Gyrocarpusasiaticus*, Antioxidant, DPPH, ABTS, UV visible spectrophotometer.

INTRODUCTION

Gyrocarpusasiaticus is one of the species in the genus *Gyrocarpus* belonging to the family Hernandiacea with the class Magnoliopside[1]. The availability and the taxonomy of the plant have not been established still. There are not many scientific articles on this plant extract thereby indicating lack of exploration into its pharmaceutical benefits.

The aim of the present study was to carry out the antioxidant activity with the methanolic extract of *Gyrocarpusasiaticus* bark by using DPPH and ABTS methods.

Free radicals are atoms or groups of atoms with an odd number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, cell membrane. To prevent free radicals attack body has a defense system of antioxidants. Antioxidants are intimately involved in the prevention of cellular damage which is the common pathway for cancer, ageing etc.

Although there are several enzyme systems within the body that scavenge free radicals, the important antioxidants are vitamin E, vitamin C and beta-carotene. Most of the antioxidant compounds are derived from the plant sources[2-4].

2. EXPERIMENT

2.1. Plant material

The bark of *Gyrocarpusasiaticus* was collected from Tirupathi, Andhra Pradesh in June 2010 and shade dried.

2.2. Extraction

Dried bark was soxhelet extracted with 99% methanol. The extract was concentrated by evaporation to yield a concentrated extract.

2.3. Chemicals

ABTS, DPPH was obtained from Sigma Chemicals. Ascorbic acid and other chemicals were purchased from S.D.Fine chemicals. All the chemicals used were analytical grade.

2.4. Phytochemical studies

From the preliminary Phytochemical studies, it showed high amount of phenolics, steroids, alkaloids, terpenoids, tannins and flavonoids present in the extract of *Gyrocarpusasiaticus*.

2.5. The DPPH radical scavenging assay

To a set of clean and dried test tubes 3 ml of methanol and 150µl of 0.1% DPPH reagent was added and mixed thoroughly, allow the solution to stand for 30 minutes. The initial absorbance of each test tube was measured at 517 nm. To these test tubes1 ml of aqueous solution of extracts were added in increasing concentration of 5-25µg/ml. The solution of ascorbic acid (5-25µg/ml) was taken as a standard. The solution was mixed and allowed to stand for 30 minutes at room temperature and the final absorbance was measured at 517nm using a spectrophotometer(UV-Visible 1700 pharma spec). The experiment was performed in triplicate. The percentage reduction in absorbance was calculated from initial and final absorbance at each level. Concentration of the substance required for 50% reduction in absorbance (IC_{50}) was calculated from the calibration curve (Concentration of extract in µg/mlV_s % reduction in absorbance)5,6. The result was tabulated in Table 1 and Figure 1.

2.6. The ABTS scavenging assay

ABTS and Ascorbic acid stock solution was prepared. From the stock solution various dilutions viz 2, 4, 8,16,32,54,128 µg/ml were prepared and used for antioxidant study. ABTS solution 2mM (0.0548g in 50 ml) was prepared in distilled water. Potassium persulphate solution 70mM (0.0189g in 1 ml) was prepared in distilled water. 200 ml of potassium persulphate solution and 50ml of ABTS solution were mixed and used after 2 hrs. This solution is called as ABTS radical cation, which was used for assay. To 0.5 ml of various concentrations of extract 0.3 ml of ABTS radical cation and 1.7 ml of phosphate buffer was added and the same was performed for the standard Ascorbic acid also. The absorbance was measured at 734 nm.The percentage reduction in absorbance was calculated from initial and final absorbance at each level. Concentration of the substance required for 50% reduction in absorbance (IC_{50}) was calculated from the calibration curve (Concentration of extract in µg/ml V_s % reduction in absorbance) [7-9]. The result was tabulated in Table 2 and Figure 2.

Table 1: DPPH assay

		Concentration(µg/ml)				
Drug	**Absorbance**	**5**	**0**	**15**	**20**	**25**
Ascorbic acid	Initial	0.303	0.303	0.303	0.303	0.303
	Final	0.145	0.120	0.109	0.065	0.040
	% Inhibition	52.14	60.39	64.02	78.54	86.79
G.asiaticus	Final	0.165	0.128	0.108	0.077	0.071
	% Inhibition	45.54	57.75	64.35	74.58	76.56

Table 2: ABTS Assay

		Concentration(µg/ml)						
Drug	**Absorbance**	**2**	**4**	**8**	**16**	**32**	**64**	**128**
Ascorbic acid	Initial	0.295	0.295	0.295	0.295	0.295	0.295	0.295
	Final	0.198	0.182	0.164	0.144	0.128	0.108	0.088
	% Inhibition	32.88	38.30	44.40	51.18	56.61	63.38	70.16
G.asiaticus	Final	0.230	0.198	0.186	0.168	0.132	0.120	0.108
	% Inhibition	22.0	32.88	36.94	43.05	55.25	59.32	63.38

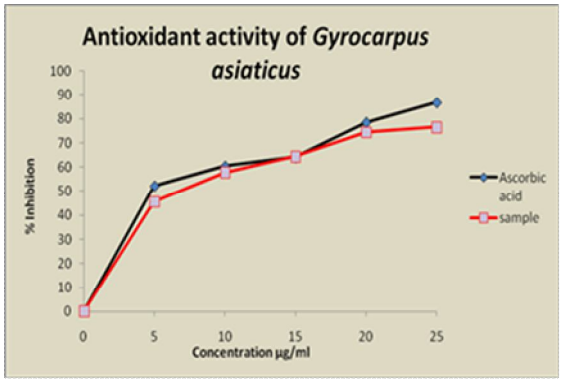

Fig. 1: DPPH method

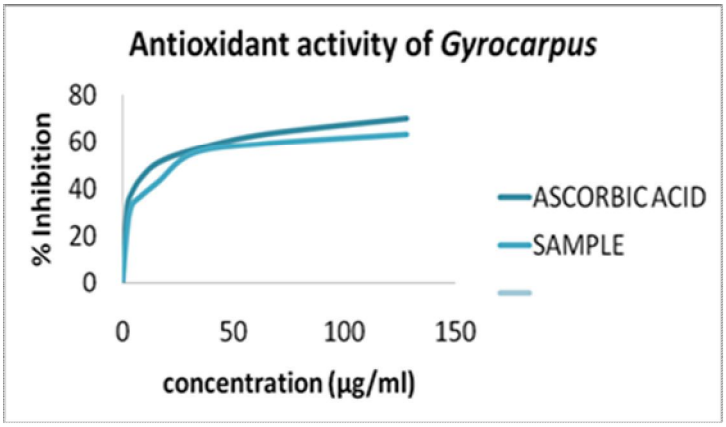

Fig. 2: ABTS method

3. RESULTS AND DISCUSSION

The DPPH antioxidant assay is best on the ability of 1-1-diphenyl-2-picrylhydrazyl, is a stable free radical to decolorize in the presence of antioxidants. The DPPH free radical contains an odd electron, which is responsible for the absorbance at 517nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance.The methanolic extract of *G.asiaticus*barks showed prominent IC_{50} value of 6µg/ml which was compared with ascorbic acid which showed an IC_{50} value of 5µg/ml which is a well-known antioxidant.

This is a measure of antioxidant activity. It also permits the measurement of antioxidant activity of mixtures of substances andhence helps to distinguish between additive and synergistic effects.

The methanolic extract of *G.asiaticus*barks showed prominent IC_{50} value of 24µg/ml which was compared with ascorbic acid which showed an IC_{50} value of 16µg/ml which is a well-known antioxidant.

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