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Research Article

EVALUATION OF THE FREE RADICAL AND ANTIOXIDANT ABILITIES IN ETHANOLIC EXTRACT OF THE *BREYNIA PATENS (ROXB.)* BENTH. AND HOOK. F.

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AQ1 **ABSTRACT**

This study evaluates the free radical and antioxidant abilities in the extract of *Breynia patens*. Some *in vitro* antioxidant assays were employed, Such as 2, 2-diphenyl-1-picryl- hydrazyl (DPPH), 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), reducing power assay, ferrous ion chelating activity and hydroxyl radical scavenging assays. The plant extract shows excellent activity in DPPH (inhibitory concentration [IC_{50}]=43.47) and ABTS (6314.6±15.5 µmol/g). Reducing power assay indicates an increase in reductive ability, ferrous ion chelating activity (IC_{50}=303.03) and hydroxyl radical scavenging assays (). The results could be considered the plant as natural antioxidants and may be useful for curing diseases arising from oxidative deterioration and considered as a source of natural therapeutic agent.

Keywords: *Breynia patens, In vitro,* Antioxidant and free radical.

INTRODUCTION

There is a large interest in charting the biological activities of plants from geographically fragile habitats such as Western Ghats in India. *Breynia patens (Roxb.) Benth. & Hook. f.* is a shrub up to 2 m tall. It belongs to the family Euphorbiaceae. The plant bark and stem juice were used as a general tonic. The folklore used this plant because of the medicinal properties such as antipyretic, antitoxic, anti-swelling, and antipruritic; also used for fever, headache, hemorrhage, mumps, puerperium, stomach pain; antiseptic for cuts and sores, bruises, syphilis, abscesses, suppurating sores, lactagogue. Oxidative stress is believed to be the most important provider to the pathogenesis of a number of chronic diseases [2].

Living cells may create free radicals and other reactive oxygen species (ROS) as a by-product of physiological and biochemical processes. This Free radical has the capacity to join with nucleotide in DNA and cause strand breakage that contribute to carcinogenesis, mutagenesis and cytotoxicity in human beings [16]. Antioxidants interfere with the oxidative processes by scavenging the free radicals, chelating free catalytic metals and acting as electron donors [8]. This may act independently or in combination with anticancer or cardioprotective agents by an assortment of mechanisms. Hence, research has focused on the use of antioxidants, particularly, on the importance of naturally derived antioxidants, which may reduce free radicals, ROS production and may exhibit defensive property. Effective antioxidants are occurring in vegetables, fruits, nuts, seeds, roots, barks, and leaves [5] in the form of plant phenolic compounds, tannins, flavonoids, carotenoids, fiber, vitamins A, B, C, and E, tocopherols, calcium, and selenium [1,7,10]. Therefore, the antioxidant property is considered as a universally determined biological activity of a plant.

MATERIALS AND METHODS

Preparation of plant extracts

Fresh plant material was washed under running tap water, air-dried and powdered. About 50 g of coarsely powdered plant materials (50 g/250 ml) was extracted in a soxhlet extractor for 8-10 hrs, with ethanol. The extract obtained was then concentrated using a vacuum evaporator and weighted. For stock solutions, 1 mg/ml of ethanolic extract was dissolved in dimethyl sulfoxide (DMSO).

Free radical scavenging activity (2, 2-diphenyl-1-picryl- hydrazyl [DPPH$^{\bullet}$])

DPPH$^{\bullet}$ the free radical scavenging activity of ethanolic extract of *B. patens* was measured by the following method of Blois [3]. 0.2 mM solution of DPPH$^{\bullet}$ in methanol was prepared, and 100 µl of this solution was added to various concentrations of ethanolic extract at the concentrations of 50, 100, 150, 200 and 250 µg/ml. After 30 minutes, absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT) was used as a standard material. All the tests were performed in triplicate and percentage of inhibition was calculated by comparing the absorbance values of the control and test samples.

$$\text{Percentage of inhibition} = \frac{Abs_{cntrl} - Abs_{test}}{Abs_{cntrl}} \times 100$$

Free radical scavenging activity (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid [ABTS$^{\bullet+}$])

The total antioxidant activity of the samples was measured by ABTS$^{\bullet+}$ radical cation decolorization assay followed by the method of Re *et al.* [18]. ABTS$^{\bullet+}$ was produced by reacting 7 mM ABTS$^{\bullet+}$ aqueous solution with 2.4 mM potassium persulfate in the dark for 12-16 hrs at room temperature. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30°C to give an absorbance of 0.70±0.02 at 734 nm. Then, 2 ml of diluted ABTS$^{\bullet+}$ solution was added to the sample concentration at 20 µl (1 mg/ml). After 30 minutes of incubation at room temperature, the absorbance was recorded at 734 nm and percentage of inhibition was calculated. Trolox was used as a reference standard. Triplicates were performed.

Reducing power activity

Reducing power assay was determined according to the method of Yildirim *et al.,* [24]. Different concentrations of ethanolic extract (100, 200, 300, 400, 500 µg/ml) of the study species were mixed with 1 ml of 200 mM sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferric cyanide followed by incubation at 50°C for 20 minutes. After adding 1 ml of 10% trichloroacetic acid, the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant was taken out and mixed with 2 ml of distilled water and 0.5 ml of 1% ferric chloride. After incubation for 10 minutes, the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates reductive potential

of the extracts [12]. All the tests were performed in triplicates, and ascorbic acid was used as a reference standard.

Ferrous ion chelating activity

The chelating of ferrous ions by ethanolic extract was estimated by the method of Singh and Rajini [20]. Different concentrations of ethanolic extracts (100, 200, 300, 400 and 500 µg/ml) were mixed with 100 µl of 2 mM ferrous sulfate solution and 300 µl of 5 mM ferrozine. The mixture was incubated at room temperature for 10 minutes. The absorbance of the solution was measured at 562 nm. Ethylene diamine tetra acetate (EDTA) was used as standard. All the tests were performed in triplicate and percentage of inhibition was calculated by using the formula,

$$\text{Percentage of inhibition} = \frac{Abs_{cntrl} - Abs_{test}}{Abs_{cntrl}} \times 100$$

Hydroxyl radical scavenging activity (HRSA)

The scavenging activity for the sample extract on hydroxyl radical was measured according to the method of Klein *et al*., [11]. 20 µg concentration of the extract was added with 1.0 ml of iron – EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%), and 1.0 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) sequentially. The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22%) and incubated at 80-90°C for 15 minutes in a water bath. The reaction was terminated by the addition of 1.0 ml of ice-cold trichloroacetic acid (17.5% w/v). Then, 3.0 ml of nash reagent (75.0 g of ammonium acetate, 3.0 ml of glacial acetic acid, and 2.0 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added and left at laboratory temperature for 15 minutes. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectrophotometrically at 412 nm against the reagent blank. Results were compared with the activity of standard antioxidant BHT. The % HRSA was calculated using the following formula:

$$\text{HRSA (\%)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Statistical analysis

The statistical comparison among the groups were performed with one-way analysis of variance (ANOVA) test and the significance of the difference between means was determined by Duncan's Multiple Range Test at ($p<0.05$) significant level. (Statsoft Inc., Tulsa, USA).

RESULT AND DISCUSSION

Free radical is a molecule with an unpaired electron and involved in lung damage, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases [19]. They also involved in the autoimmune disorder like rheumatoid arthritis, etc. [17]. The five methods described in the experimental chapter were used for the assay purposes. A summary of the suitability of the methods for assay of antioxidants used throughout experiments is depicted in below. The ABTS test, the DPPH test, reducing power activity, ferrous ion chelating activity and HRSA were suitable for assay of all the tested antioxidants.

DPPH radical scavenging activity

The DPPH radical was widely used as a model system to investigate the scavenging activities of several natural compounds in plant extract. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. DPPH method allows estimation of hydrogen radical donating ability of the extract [21]. The result of the present study revealed that the ethanol extract of *B. patens* expressed concentration dependent scavenging effect against DPPH radical. The inhibitory concentration (IC_{50}) value of the ethanol extract was observed as 43.47 (Table 1). This showed that the *B. patens* contained high amount of radical scavenging compounds with proton-donating ability.

ABTS radical scavenging activity

The scavenging capacity of the extract for the ABTS radical was measured 6314.6±15.5 µmol/g, as in the result scavenging effect of the extract was increased. Which absorbs at 743 nm (giving a bluish-green color) is formed by the loss of an electron by the nitrogen atom of ABTS. In the presence of another hydrogen donating antioxidant and yielding the solution decolorization.

Reducing power assay

The reducing power was resolute by the Fe^{3+}-Fe^{2+} transformation in the presence of the extracts as described in the literature [6]. The reducing capacity of the extract is another significant indicator of antioxidant activity. The presence of antioxidants in the sample would result in the reduction of Fe^{3+}-Fe^{2+} by donating an electron. The results showed that an increase in reducing power of the plant extract as the extract concentration increases (Table 2). Increasing absorbance indicates an increase in reductive ability (Fig. 1).

Ferrous ion chelating activity

The ferrous ion chelating activity can be used to the antioxidant assay and measured by a decrease in the absorbance at 562 nm of the iron $FeCl_2$ and ferrozine complex [9]. It is one of the most effective pro-oxidants and their interaction with hydrogen peroxide in biological systems. In the current study, the percentage of incubation in plant extract was observed (Table 3). The IC_{50} value (303.03) has demonstrated presence of an excellent antioxidant activity in the plant extract.

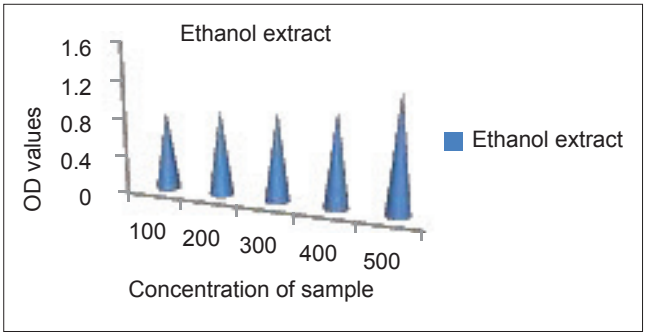

Fig. 1: Reducing power assay

Table 1: DPPH radical scavenging activity

S.No.	Concentration	Percentage of inhibition	IC_{50} value
1	100	76.68±0.43	43.47
2	200	80.04±0.17	
3	300	82.98±0.72	
4	400	83.92±0.36	

IC_{50}: Inhibitory concentration

Table 2: Reducing power assay

S.No.	Concentration	Absorbance	
		Plant extract	Ascorbic acid
1	100	0.823	0.549
2	200	0.895	0.598
3	300	0.934	0.635
4	400	0.992	0.682
5	500	1.036	0.691

Table 3: Ferrous ion chelating activity

S.No.	Concentration	Percentage of inhibition	IC_{50} value
1	100	46.39±0.27	303.0303
2	200	49.40±0.73	
3	300	57.45±0.13	
4	400	61.73±0.56	
5	500	68.89±0.30	

IC_{50}: Inhibitory concentration

Hydroxyl radical scavenging assay

The result of hydroxyl radical scavenging assay is 581.39 (IC_{50} value). The hydroxyl radical is extremely reactive in biological systems and has been implicated as highly damaging species in free radical pathology, capable of damaging bio-molecules of the living cells. These radical combines with nucleotides in DNA and cause strand breakage leading to carcinogenesis, mutagenesis and cytotoxicity [22]. Hydroxyl radical scavenging capacity of the extract is directly related to its antioxidant activity. It was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system [13]. From the present study, the Hydroxyl radical scavenging of *B. patens* was highly effective.

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