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Research Article

IN-VITRO ANTIOXIDANT ACTIVITY OF *POLYGONUM BARBATUM* LEAF EXTRACT

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ABSTRACT

The *In-vitro* antioxidant activity of *Polygonum barbatum* Leaf Extract was investigated by different methods viz DPPH radical scavenging assay, Nitric oxide scavenging assay, Hydroxyl radical scavenging assay, Superoxide scavenging assay and Total phenolic content. The IC_{50} value found in DPPH method is more effective than other three methods. In addition, the PBLE was found to contain a noticeable amount of total phenols, which plays a major role in controlling antioxidants. Moreover, the results observed is in a concentration dependent manner. Thus the *in-vitro* studies clearly indicate that the ethanolic extract of *polygonum barbatum* has significant antioxidant activity and also a better source of natural antioxidant, which might be helpful in preventing the process of various oxidative stress.

Keywords: *Polygonum barbatum,* Antioxidant activity, Total phenolic content.

INTRODUCTION

Free radicals are reactive species generated in the body during normal metabolic functions. These species causes cellular damage by reacting with the various biomolecules of body such as membrane lipids, nucleic acid, proteins and enzymes. This damage is the major contributor of many disorders like Cancer, Hepatopathy, Cardiovascular disorders, inflammation, diabetes mellitus, renal failure and brain dysfunction. Body has itself antioxidant system which reacts with reactive specie and neutralizes them. This natural antioxidant system includes enzymes like catalase, superoxide dismutase and glutathione which protect the body from free radical species and prevent oxidative stress. Synthetic antioxidant like butylated hydroxyl toluene and butylated hydroxyl anisole are carcinogenic in nature. So there arises a need for natural antioxidant.

Polygonum barbatum Linn. (Family Polygonaceae) a stout, annual herb, distributed throughout the hotter parts of India. The leaves are lanceolate or non lanceolate. The decoction of the leaves and stalks were used for wash of ulcers in China[1].The roots are used as astringent and cooling remedy[2] . It has been reported that dichloromethane extract of *Polygonum barbatum* showed brine shrimp toxicity and spasmolypic activity .The methanolic extract of *Polygonum barbatum* was found to possess cholinergic activity[3]. The antinociceptive, anti-inflammatory and diuretic properties were also studied[4]. However, no data were available in literature on the antioxidant activity on leaf of *Polygonum barbatum.*

Therefore we undertook the present investigation to examine the antioxidant activity of *polygonum barbatum* through various *in-vitro* models.

MATERIAL AND METHODS

Plant material

The leaves of *Polygonum barbatum* were collected along the beds of Cauvery river, near Trichy, Tamil Nadu in February 2008. The plant was identified and authenticated by Dr.G.V.S.Moorthy, Joint Director, Botanical Survey of India (BIS), Agriculture University campus, Coimbatore, India. The voucher specimen number was BIS/SC/5/23/08-09/Tech-1614, and the specimen was deposited at herbarium.

Preparation of plant extract

Fresh leaves were collected, shade dried and powdered mechanically. About 100 g of the powder were extracted with 1000 ml of 70% ethanol by hot percolation method using soxhlet extractor for 4 h. The extract obtained was evaporated at 45°C to get a semisolid mass. The yield of ethanolic extract was found to be 30%. This extract was used for further studies.

Drugs and Chemicals

DPPH was obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. All other chemicals used in this study were of analytical grade.

DPPH radical scavenging activity (Hydrogen Donating Activity*)*

DPPH radical scavenging activity of PBLE was investigated by the method of Blois[5]. About 0.3 mM solution of DPPH in 100% ethanol was prepared and 1 ml of this solution was added to 3 ml of the extract dissolved in ethanol at different concentration (10, 20, 40, 60, 80 and 100 µg/ml).The mixture was shaken and allowed to stand at room temperature for 30 minutes, the absorbance was measured at 517 nm using a spectrometer. The IC_{50} values of the crude extract and standard ascorbic acid were calculated.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of PBLE was measured according to the method described in Yingming Pan et al[6] .The reaction mixture containing deoxyribose (3.75 mM), H_2O_2 (1 mM), $FeCl_3$ (100 µM), ethylene diamine tetra acetic acid (100 µM), ascorbic acid (100 µM) in potassium phosphate buffer (20 mM, pH 7.4) and PBLE at different concentration (10, 20, 40, 60, 80 and 100 µg/ml) were incubated for 60 min at 37 °C. The reaction was terminated by adding 1 ml of 2-thiobarbituric acid (1% w/v) and 1 ml of trichloroacetic acid (2% w/v) and then heating the tubes in a boiling water bath for 15 min. The contents were cooled and the absorbance of the mixture was measured at 535 nm. Scavenging effect was determined. The hydroxyl radical scavenging activity of butyl hydroxyl toluene (0.5 mg/ml) was also measured.

Nitric oxide scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of Griess Illosvoy reaction[7]. Scavengers of nitric oxide, compete with oxygen leading to reduced production of nitric oxide[8].

This experiment was performed according to the method of Umamaheswari *et al*[9]. The reaction mixture (3 ml) containing 10 mM sodium nitroprusside in phosphate buffer saline, the extract and the reference compound (Curcumin) at different concentrations (10, 20, 40, 60, 80 and 100 µg/ml) were incubated at 25°C for 150 min. 0.5 ml aliquot of the incubated sample was removed at 30 min intervals and 0.5 ml Griess reagent was added. The absorbance of the chromophore formed was measured. The IC_{50} values of the crude extract and reference compound curcumin were measured.

Superoxide scavenging activity (SOSCA)

Superoxide scavenging activity was carried out using alkaline dimethyl sulfoxide (DMSO) method[10]. Solid potassium superoxide (1.75 mg/ml) was allowed to stand in contact with dry DMSO for at least 24 h and the solution was filtered immediately before use. About 200 µl of filtrate was added to 2.8 ml of an aqueous solution containing nitroblue tetrazolium (56 µM), EDTA (10 µM) and potassium phosphate buffer (10 µM, pH 7.4). PBLE at the concentration of (10, 20, 40, 60, 80 and 100 µg/ml) was added and the absorbance was recorded at 560 nm against a control in which pure DMSO has been added instead of alkaline DMSO. Ascorbic acid (100 µg/ml) was used as a standard antioxidant.

Total phenolic content

The total phenolic content of the extract was determined with Folin Ciocalteu reagent[11]. A 0.1 ml aliquot of suspension of 1 mg of PBLE in water was totally transferred to a 100 ml Erlenmeyer flask and the final volume was adjusted to 46 ml by the addition of distilled water. Folin Ciocalteu reagent (1 ml) was added to the mixture, followed by 3 ml 2% sodium carbonate 3 min later. Subsequently, the mixture was shaken for 2 h at room temperature and absorbance measured at 760 nm. All tests were performed in triplicate. The concentration of total phenolic contents in the extract was determined as µg pyrocatechol equivalents by using the equation obtained from standard pyrocatechol graph.

Statistical analysis

The free radical scavenging activity was analyzed using linear regression analysis followed by the percentage inhibition calculated by using the formula, inhibition (%) = (control - test) x 100/control. Results are mean ± SEM (n= 3).

RESULT

In this present study, the antioxidant activity of the PBLE was investigated by using four methods. The extract has got profound antioxidant activity. In DPPH method, the IC_{50} of the extract was 35.62 µg where as IC_{50} for ascorbic acid was 23.35 µg (Fig. 1).

In nitric oxide scavenging method the IC_{50} value of PBLE and curcumin were recorded at 39.45 µg and 15.46 µg (Fig. 2) respectively. The scavenging activity of the extract, increase with increase in concentration. The hydroxyl radical scavenging activity of PBLE, increase in a dose dependent manner.

The IC_{50} value of the extract was found to be 82.05 µg where as IC_{50} value of standard BHT was 49.63 µg (Fig. 3). In superoxide method, the IC_{50} value of the extract was 49.71 µg and for the standard ascorbic acid value was 43.84 µg (Fig. 4). From the estimation of total phenol content, it was found that 4 µg/ml of the extract contains 3.61 µg equivalent pyrocatechol was detected.

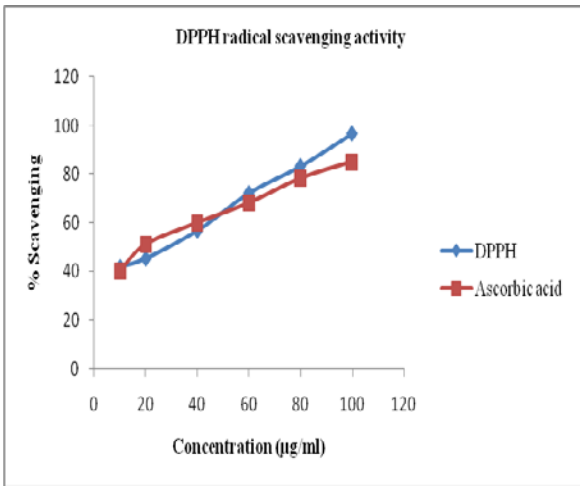

Fig. 1: DPPH radical scavenging activity

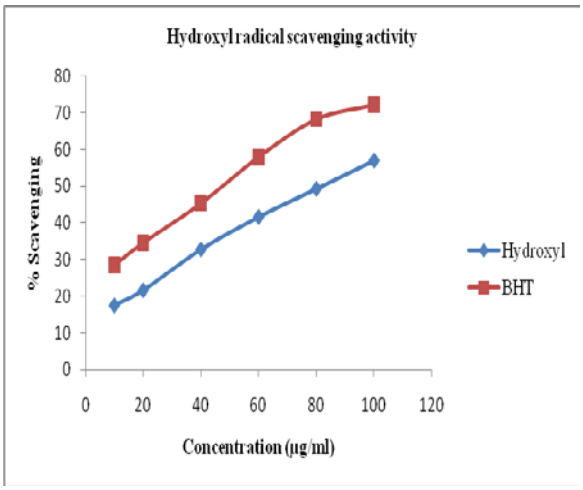

Fig. 2: Hydroxyl radical scavenging activity

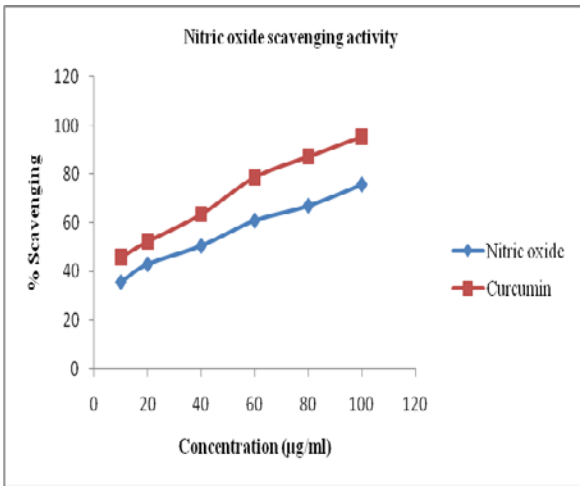

Fig. 3: Nitric oxide scavenging activity

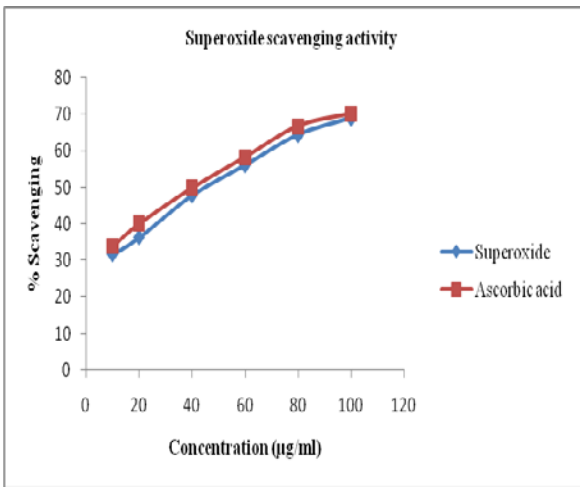

Fig. 4: Superoxide scavenging activity

DISCUSSION

Antioxidant compounds may function as free radical scavengers, indicator of the complex of pro-oxdant metals, reducing agents and quenchers of singlet oxygen formation.Various concentrations ranging from 10-100 µg/ml of ethanolic extract was tested for their free radical scavenging activity it was observed that free radical

scavenged by PBLE is in dose dependent manner. DPPH is one of the free radicals generally used for testing preliminary radical scavenging activity of a compound or a plant extract. DPPH radical is a free radical in an aqueous or ethanolic solution.

It accepts an electron or hydrogen radical to become stable diamagnetic molecule. Because of the odd electron of DPPH, it gives a strong absorption maximum at 517 nm by visible spectroscopy. The antioxidant activity measured by the capacity of odd electron of the radical becomes paired off in the presence of extract (hydrogen donar). When it becomes paired off, the absorption strength is decreased and the resulting decolorization is stiochiometric with respect to the number of electrons captured. Substances which are able to perform this reaction can be considered as antioxidants and hence radical scavengers. PBLE showed concentration dependent decrease in absorbance and increase in scavenging activity. Hydroxyl radical is highly reactive species formed in biological systems and have been implicated as highly damaging in free radical pathology, capable of damaging almost every molecule found in living cells.

In addition, this species is one of the quick initiators of lipid peroxidation process abstracting hydrogen atoms from unsaturated fatty acids. It will further disrupt biomembrane and causes cell damage. It is very important to scavenge hydroxyl radical. PBLE resists hydroxyl radical generation. The nitric oxide radical scavenging assay, the scavengers of nitric oxide competes with oxygen, leading to reduced production of nitrite ions. PBLE competes with oxygen which results in decreased production of nitric oxide. In Superoxide scavenging activity method, there is a decrease in absorbance at 560 nm which indicates the consumption of Superoxide anion in the reaction mixture. The amount of phenolic content estimated with Folin Ciocalteu reagent also witnesses the antioxidant activity in the medicinal plant *Polygonum barbatum.*

CONCLUSION

The ethanolic extract of *Polygonum barbatum* showed different levels of antioxidant activity in all the models. Therefore, it can be concluded that the antioxidant activity of the extract may be attributed to its phenolic content.

REFRENCES

1. Kirtikar KR, Basu BD. Indian Medicinal plants. 2nd ed. India: International Book Distributors1993; 3: 2100.
2. The Wealth of India. New Delhi: Publications & Information directorate, CSIR 1969; 8: 195-196.
3. Bashir Ahmad Chaudhry, Muhammad Younas Syad, Khalid Hussain Janbaz, Altaf Ahmed Dasti, Bashir Ahmad Loothar. Biological Activity of *Polygonum barbatum*. Journal of Research (Science) 2003; 14: 169-175.
4. Abdul Mazid M, Bidyut K Datta, Lutfun Nahar S, Khairul Bashar A M, Sitesh C Bachar, Satyajit D Sarker. Antinociceptive, Anti-inflammatory and Diuretic Properties of *Polygonum barbatum* (L.) Hara var.*barbata.* Brazilian Journal of Pharmacognosy 2009; 19: 749-754.
5. Blois M. Antioxidant determination by the use of stable free radical. Nature 1958; 181: 1199-1200
6. Yingming Pan, Xiaopu Zhang, Hengshan Wang, Ying Liang, Jinchan Zhu, Haiyun Li, Qingmao Wu. Antioxidant potential of ethanolic extract of *Polygonum cuspidatum* and application in peanut oil. Food Chemistry 2007; 105: 1518-1524.
7. Garrat DC. 1964. The Quantitative Analysis of Drugs, Chapman and Hall, Japan, 3, 456-458.
8. Marcocci, L., Packer, L., Sckaki, A., Albert, G.M., 1994. Antioxidant action of *Ginkgo biloba* extracts EGb 761. Methods in Enzymology 234, 462-475.
9. Umamaheswari, M., Asokkumar.K., Rathidevi.R., Shivasanmugam, A.T., Subhadradevi, V., Ravi.T.K., 2007. Antiulcer and *in vitro* antioxidant activities of *Jasminum grandiflorum* L. Journal of Ethnopharmacology, 110, 464-470.
10. Henry, L. E. A., Halliwell, B., Hall, D. O., 1976. Superoxide scavenging by alkaline DMSO method. Federation of European Biochemical Societies Letters 66**:** 303-306.
11. Gulcin, I., oktay, M., Kufrevioglu, O.I., Aslan.,A.,2002.D etermination of antioxidant activity of Lichen *Cetroria island* (L) Ach. Journal of Ethinopharmacology 79,325-329.