Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Botany

Elixir Appl. Botany 65 (2013) 20103-20104

ISSN: 2229-712X

Antioxidant activity of *Barbula javanica* Doz. et Molk.: A relatively unexplored bryophyte

Sharad Vats* and Afroz Alam

Department of Bioscience & Biotechnology, Banasthali University-304022, Rajasthan, India.

ARTICLE INFO

Article history:

Received: 23 October 2013;

Received in revised form:

10 December 2013;

Accepted: 20 December 2013;

Keywords

Moss,

Barbula javanica,

Antioxidant potential,

Novel antioxidant.

ABSTRACT

In the present study antioxidant potential of the moss *Barbula javanica* Doz. et Molk was evaluated. The total phenolic content (TPC) was found to be 30mg±0.96 GAE/gdw. The radical scavenging activity as IC_{50} against DPPH (2, 2-diphenyl-1-picrylhydrazyl), NOSA (Nitric oxide scavenging assay) and DDA (deoxyribose degradation assay) was evaluated to be 100±1.12, 80±0.90 and 35±0.50 µg/ml, respectively. The reducing activity was assayed using FRAP (Ferric Reduction Antioxidant Potential) was found to be 1259±1.56 µM/l. The results show the potential of *B. javanica* as novel antioxidant, which has been reported for the first time.

Introduction

Free radicals have one or more unpaired electrons in the outer shell which makes them highly reactive. Reactive oxygen species (ROS) like superoxide anion ($O_2^{\bullet-}$), peroxyl ($ROO^{\bullet}$), hydroxyl ($HO^{\bullet}$), alkoxyl ($RO^{\bullet}$) and nitric oxide are oxygen-centered free radicals. These free radicals are short lived but are able to initiate a chain reaction wherein more free radical are formed by destabilizing other molecules. Human body is equipped with molecules that can combat adverse effects of free radicals but excessive generation of free radicals leads to/aggravates pathological events in living organisms such as cellular aging, carcinogenesis, mutagenesis, coronary heart disease, diabetes and neural disorders.

Plants possess myriads of bioactive compounds (Bhatia *et al*., 2008; Vats and Alam, 2013; Vats and Kamal, 2013) having potential antioxidant and free radical scavenging activity (Sharma *et al*., 2009; Vats, 2012). There is an ongoing search to explore new sources of natural antioxidants of therapeutic use viewing side effects associated with synthetic drugs.

Bryophytes (mosses, liverworts and hornworts) are archegoniate, atracheate cryptogams. About 15000 species are present globally making them the third largest group of land plants (Pejin and Bogdanović-Pristov, 2012). They are not as well explored as fruits and other angiosperms in terms of antioxidant potential. There are few reports which details about the exclusive bioactive principles and antioxidant activity of mosses (Chobot *et al*., 2008), still much is left to unearth. In the present study *Barbula javanica* collected from Ranthambore National Park, Rajasthan, India was evaluated for its total phenolic content and *in vitro* antioxidant activities.

Materaial and Methods

Plant material

The sample of *Barbula javanica* Doz. et Molk. (Pottiaceae Schimp.) was collected from Ranthambhore National Park, Rajasthan (India) in September 2011. Voucher specimen has been deposited in the Herbarium of Bioscience and Biotechnology Department, Banasthali University, India, BVH-786024- - 786030/2011.

Extraction

Initially soil and other plant material were carefully removed from the moss. Air-dried sample of *B. javanica* (1 g) was extracted in ethanol in orbital shaker at 50°C overnight. The extract was filtered and kept at 4°C for further use.

Total Phenolic content (TPC)

The total phenolics were determined colorimetrically according to the Folin- Ciocalteaeu method (Vats, 2012a). Briefly, 0.5 mL of water and 0.125 mL of the methanolic extract was added to a test tube. Folin-Ciocalteu reagent (0.125 mL), 1.25 mL of the sodium carbonate solution and 3 mL of water was added successively and allowed to stand for 90 minutes. The absorbance was measured at 760nm. Total phenol content was expressed as gallic acid equivalents (GAE) in (mg GAE/g dry weight of sample) dry material. Values are expressed as Mean ± S.D.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

Plant extract (1ml) were mixed with 1ml of 0.3mM. DPPH reagent and allowed to stand at room temperature for 30 minutes in dark. The absorbance was taken at 517nm. Radical scavenging activity was expressed as IC 50 (Mean ± S.D) value (Vats *et al*., 2012). **FRAP (Ferric Reduction Antioxidant Potential)**

Acetate buffer, 300mmol/l, 10 mmol/l 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mmol/l HCl and 20 mmol/l $FeCl_3x6H_2O$ in distilled water was prepared. 25ml of acetate buffer, 2.5ml TPTZ solution and 2.5ml $FeCl_3x6H_2O$ solution was mixed to make working solution. 50µl of sample extract was mixed with 1.5ml of FRAP reagent and monitored up to 5 min at 593nm. Absorbance was compared with calibration curve of aqueous solution of known Fe (II) concentration (µM/l). Values are expressed as Mean ± S.D (Vats *et al*., 2012).

Nitric oxide scavenging assay (NOSA)

2ml of Sodium nitroprusside in 0.5ml phosphate buffer saline was mixed with 0.5ml of extract and the mixture was incubated at 25ºC for 150 min. From the incubated mixture 0.5ml was taken out and added into 1.0ml of sulfanilic acid

Tele:

E-mail addresses: vats_sharad@yahoo.co.in

reagent. Finally 1.0ml of Naphthylethylenediamine dihydrochloride was mixed and left at room temperature for 10 min before measuring the absorbance at 540nm. NOSA was determined in triplicate according to the method of Badami *et al*. (2003) and expressed as IC_{50} (µg/ml).

Deoxyribose degradation assay (DDA)

The reaction mixture contained 0.8ml of phosphate buffer solution, 0.2ml of sample, 0.25ml of EDTA, 0.2ml of $FeCl_3$ and 0.2ml of 2-deoxy-ribose. The mixture was kept in water bath at 37ºC and the reaction was started by adding 0.2ml of ascorbic acid and 0.2ml of H_2O_2. After incubation at 37ºC for 1 h, 2ml of cold thiobarbituric acid was added into the reaction mixture followed by 2ml of HCl. Mixture were heated at 100ºC for 15min and then cooled down into water. The absorbance is then taken at 532nm. The assay was determined in triplicate according to the method of Elizabeth and Rao (1990) and expressed as IC_{50} (µg/ml).

Results and Discussion

Phenolics are universally present in plants and compounds possess antioxidant potential. They neutralize lipid free radicals and also prevent decomposition of hydroperoxides into free radicals, contributing to the overall antioxidant activities of plants (Li *et al*., 2009). In the present study a linear calibration curve of Gallic acid with coefficient of determination R2 =0.98 was obtained. The TPC of *B. javanica* extract was found to be 30mg±0.96 GAE/gdw (Mean±S.D, n=3).

DPPH is free radical and its absorbance decreases as a result of a colour change from purple to yellow due to radical scavenging effect of antioxidants. NO is a signaling molecule and act as vasodilator, neuronal messenger and others. Overproduction of this free radical adversely affect the metabolism and might lead to inflammation, cancer etc. NO can lead to generation of hydroxyl radical and nitric dioxide (Halliwell, 1997). The ·OH radical attacks biomolecules like polysaccharides, proteins and nucleic acids, in a diffusion-limited reaction. Hydroxyl radical is produced during stress and aggravates various disorders (Chen and Schopfer, 1999).

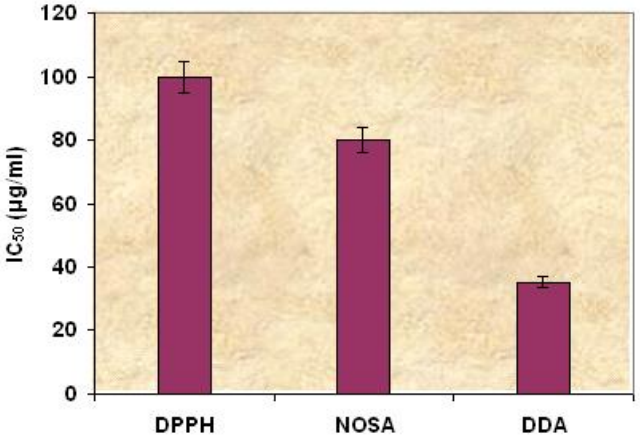

Fig 1: Antioxidant potential *B. javanica* extract. DPPH, NOSA and DDA

The antioxidant activity (Fig. 1) of extract as IC_{50} value against DPPH, NOSA and DDA was found to be 100±1.12, 80±0.90 and 35±0.50 (µg/ml; Mean±S.D, n=3), respectively. Higher the value of IC_{50} lower is the antioxidant activity. The extract was found to be best against hydroxyl radicals. In FRAP antioxidants reduce ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ), which imparts blue color in the reaction mixture at low pH. The FRAP was evaluated to be 1259±1.56 µM/l (Mean±S.D, n=3).

The significant antioxidant activities of the plant extract might be due presence of good phenolic content. The antioxidant activity of extract might be due to good phenolic content. Phenolic compounds include flavonoids which have diverse therapeutic potential mainly due to their antioxidant activity (Pietta, 2000). Moreover phenolic compounds are known to inhibit the oxidation activity of free radicals and enhance activity of antioxidative enzymes (Bandy *et al*., 2001; Ross and Kasum, 2002).

This is first ever report, to authors' best knowledge, indicating the potential antioxidant activity of *B. javanica.* The work is a step ahead in search for novel natural antioxidants, which could be of therapeutic and commercial value.

Acknowledgements

The authors are grateful to Prof. Aditya Shastri, Vice Chancellor, Banasthali University, Rajasthan for his encouragement and support.

References

1. Badami S, Moorkoth S, Rai SR, Kannan E, Bhojraj S. Antioxidant activity of Caesalpinia sappan heartwood. Biol Pharm Bull. 2003; 26(11): 1534-1537.
2. Bandy B, Bechara EJH. Bioflavonoid rescue of ascorbate at a membrane interface. J Bioenerg Biomembr. 2001; 33(4): 269-77.
3. Bhatia A.L, Kamal R, Verma G, Sharma KV, Vats S, Jain M. Radioprotective role of gymnemic acid on mice: study on hepatic biochemical alterations. Asian J Exp Sci. 2008; 22(3): 427-432.
4. Chen S, Schopfer P. Hydroxyl-radical production in physiological reactions- A novel function of peroxidase. Eur J Biochem. 1999; 260: 726-735.
5. Chobot V, Kubicová L, Nabbout S, Jahodář L, Hadacek F. Evaluation of antioxidant activity of some common moss species. Z Naturforsch C. 2008; 63**:** 476–482.
6. Elizabeth K, Rao MNA. Oxygen radical scavenging activity of curcumin. Int J Pharm 1990; 58(3): 237-240.
7. Halliwell B. Antioxidants and human disease: A general introduction. Nutr Rev. 1997; 55: 44–49.
8. Li H, Hao Z, Wang X, Huang L, Li J. Antioxidant activities of extracts and fractions from *Lysimachia foenum-graecum* Hance. Biores Technol. 2009; 100: 970–974.
9. Pejin B, Bogdanović-Pristov J. ABTS cation scavenging activity and total phenolic content of three moss species. Hemijska industrija 2012; 66(5): 22-22.
10. Pietta PG. Flavonoids as antioxidants. 2000; J Nat Prod. 63(7): 1035-42.
11. Ross JA, Kasum CM. Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu Rev Nutr. 2002; 22: 19-34.
12. Sharma KV, Singh U, Vats S, Priyadarsini KI, Bhatia AL, Kamal R. Evaluation of evidenced-based radioprotective efficacy of *Gymnema sylvestre* leaves in mice brain. J Environ Pathol Toxicol Oncol**.** 2009; 28(4): 311-323.
13. Vats S. Antioxidant Activity of Callus Culture of *Vigna unguiculata* (L.) Walp. Researcher 2012; 4(6): 22-24.
14. Vats S, Alam A. Antibacterial Activity of *Atrichum undulatum* (Hedw.) P. Beauv. against some Pathogenic Bacteria. 2013; J Bio Sci. 13(5): 427-431.
15. Vats S, Kamal R. *In vivo* and *in vitro* Evaluation of Sterols from *Gymnema sylvestrte* R. Br. Pak J Bio Sci. 2013; 16(23): 1771-1775.
16. Vats S, Tiwari R, Alam A, Behera KK, Pareek R. Evaluation of Phytochemicals, antioxidant and antimicrobial activity of *in vitro* culture of *Vigna unguiculata* L. Walp. Researcher 2012; 4(11): 70-74.