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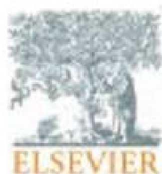

Asian Pacific Journal of Reproduction

Journal homepage: www.apjr.net

Document heading doi: 10.1016/S2305-0500(13)60158-X

Screening of antioxidant potential of the medicinal plant *Bacopa monnieri* (L.) Pennell

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ARTICLE INFO

Article history:
Received 2 September 2013
Received in revised form 10 September 2013
Accepted 10 September 2013
Available online 20 December 2013

Keywords:
Antioxidant enzymes
Ascorbic acid
Ecotypes
α-tocopherol
Reduced glutathione

ABSTRACT

Objective: To screen the antioxidant potential of leaf and stem of the various ecotypes of Brahmi. **Methods:** The medicinally important plant, *Bacopa monnieri* L (*B. monnieri*). to analyse the antioxidative enzymes, superoxide dismutase (EC 1.15.1.1) catalase (EC 1.11.1.6) and peroxidases (E.C. 1.11.1.7), and some non-enzymatic antioxidants. **Results:** The present study revealed that antioxidant enzyme activities higher in leaves and non-enzymatic antioxidants higher in leaves in all ecotypes except reduced glutathione. Among all the ecotypes, the Aliyar dam sample had more enzyme activities and quantity of non-enzymatic antioxidant contents. **Conclusion:** Both enzymatic and nonenzymatic antioxidants are rich in leaves when compared stem in the fresh plant of *B. monnieri*.

1. Introduction

The medicinal value of plants have assumed a more important dimension in the past few decades owing largely to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potential. Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease and in the aging process [1-3].

Antioxidants are obtained mainly from two major routes; chemical synthesis and natural living source extraction. According to scientific research, severe toxicity caused by chemical synthetic antioxidants such as genotoxicity, carcinogenicity [4] or hepatotoxicity [5] has been increasingly reported. Hence, the use of synthetic antioxidants is tending to decrease and needs replacement with other safer compounds.

These protective enzymes include antioxidant enzymes such as superoxide dismutase (SOD), peroxidases like ascorbate (APX) and guaiacol (POD) peroxidase. SOD is located in various cell compartments and catalyzes the disproportionation of two O_2 radicals to H_2O_2 and O_2. Ascorbate peroxidase is primarily located in chloroplast and cytosol and it is the key enzymes of the ascorbate-glutathione cycle that uses ascorbate as reducing substrate for H_2O_2 detoxification. However, hydrogen peroxide is also toxic to cells. In plant cells, the most important reducing substrate for H_2O_2 detoxification is ascorbate[6]. It eliminates peroxides by converting ascorbic acid to dehydroascorbate. Peroxidase participates in the lignin biosynthesis which may build up a physical barrier against poisoning of heavy metals. Besides enzymatic antioxidant reactions, active free radicals can be scavenged by small molecules such as carotenoids, ascorbate and thiol compounds[7]. Thus, the balance between free radical generation and free radical defense determines the survival of the system. However, ROS scavenging mechanism is crucial for identifying key component involved in oxidative tolerance. The present investigation is to study the enzymatic and non-enzymatic antioxidants of *Bacopa monnieri* (L.) (*B. monnieri*) Pennell collected from different geographical regions from Tamil nadu.

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Foundation Project: The authors gratefully acknowledge the University Grants Commission, New Delhi, Government of India: Grant. No. F37 - 30/2009 (SR) Dated - (12.01.2010) for financial support.

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2. Materials and methods

2.1. Plant material

B. monnieri[8] accessions were collected from the wild populations of the eight various locations of Tamil Nadu. The individual accessions show wide range of morphological variation including shape of the leaves, colour of the petal and type of growing. The plants were grown in Department Botanical Garden. Young leaves and stem from the plants were used for the enzymatic and non–enzymatic antioxidant analysis.

2.2. Assay of superoxide dismutase (SOD)

SOD was assayed according to the method of Kakkar *et al*[6]. The assay mixture contained 1.2 mL of sodium pyrophosphate buffer, 0.1 mL of PMS, 0.3 mL of NBT, 0.2 mL of the enzyme preparation and water in a total volume of 2.8 mL. The reaction was initiated by the addition of 0.2 mL of NADH. The mixture was incubated at 30 ℃ for 90 seconds and arrested by the addition of 1.0 mL of glacial acetic acid. The reaction mixture was then shaken with 4.0 mL of *n*–butanol, allowed to stand for 10 minutes and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560 nm in a spectrophotometer (Shimadzu– Japan).

Values are given as Mean ± S.D. of five replicates in each and expressed delta O.D per minute/mg of total soluble proteins from fresh tissue.

2.3. Assay of catalase (CAT)

Catalase activity was assayed following the method of Luck *et al*[9]. H_2O_2–phosphate buffer (3.0 mL) was taken in an experimental cuvette, followed by the rapid addition of 40 μL of enzyme extract and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240 nm in a spectrophotometer (Shimadzu–Japan). The enzyme solution containing H_2O_2–free phosphate buffer served as control.

Values are given as Mean ± S.D. of five replicates in each and expressed delta O.D per seconds/mg of total soluble proteins from fresh tissue.

2.4. Assay of peroxidase (POD)

The method proposed by Reddy[10] was adopted for assaying the activity of peroxidase. To 3.0 mL of pyrogallol solution, 0.1 mL of the enzyme extract was added and the spectrophotometer was adjusted to read zero at 430 nm. To the test cuvette, 0.5 mL of H_2O_2 was added and mixed. The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer (Shimadzu–Japan).

Values are given as Mean ± S.D. of five replicates in each and expressed delta O.D per minute/mg of total soluble proteins from fresh tissue.

2.5. Non–enzymic antioxidants

The non–enzymic antioxidants analyzed were ascorbic acid, α–tocopherol and reduced glutathione.

2.5.1. Estimation of ascorbic acid

Ascorbic acid was analysed by the spectrophotometric method described by Roe and Keuther[11]. Standard ascorbate ranging between 0.2–1.0 mL and 0.5 mL and 1.0 mL of the supernatant were taken. The volume was made up to 2.0 mL with 4% TCA. DNPH reagent (0.5 mL) was added to all the tubes, followed by 2 drops of 10% thiourea solution. The contents were mixed and incubated at 37 ℃ for 3 hours resulting in the formation of osazone crystals. The crystals were dissolved in 2.5 mL of 85% sulphuric acid, in cold. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. The tubes were cooled in ice and the absorbance was read at 540 nm in a spectrophotometer (Shimadzu– Japan).

A standard graph was constructed using an electronic calculator set to the linear regression mode. The concentration of ascorbate in the samples were calculated and expressed in terms of μg/g of sample.

2.5.2. Estimation of α–tocopherol

α–tocopherol was estimated in the plant samples by the Emmerie–Engel reaction as reported by Rosenberg[12]. Into 3 stoppered centrifuge tubes, 1.5 mL of plant extract, 1.5 mL of the standard and 1.5 mL of water were pipetted out separately. To all the tubes, 1.5 mL of ethanol and 1.5 mL of xylene were added, mixed well and centrifuged. Xylene (1.0 mL) layer was transferred into another stoppered tube. To each tube, 1.0 mL of dipyridyl reagent was added and mixed well. The mixture (1.5 mL) was pipetted out into a cuvette and the extinction was read at 460 nm. Ferric chloride solution (0.33 mL) was added to all the tubes and mixed well. The red colour developed was read exactly after 15 minutes at 520 nm in a spectrophotometer (Shimadzu–Japan).

A standard graph was constructed using an electronic calculator set to the linear regression mode. The concentration of α–tocopherol in the samples were calculated and expressed in terms of μg/g of sample.

2.6. Estimation of reduced glutathione

Reduced glutathione was determined by the method of Moron[13]. The supernatant (0.1 mL) was made up to 1.0 mL with 0.2 M sodium phosphate buffer (pH 8.0). Standard GSH corresponding to concentrations ranging between 2 and 10 nmoles were also prepared. Two mL of freshly prepared DTNB solution was added and the intensity of the yellow colour developed was measured in a spectrophotometer (Shimadzu– Japan) at 412 nm after 10 minutes. The values are expressed as μg GSH/g sample.

A standard graph was constructed using an electronic calculator set to the linear regression mode. The concentration of reduced glutathione in the samples were calculated and expressed in terms of μg/g of sample.

2.7. Statistics

Each parameter was analysed with at least five replicates and a standard deviation (SD) was calculated and data are expressed in mean ± SD of five replicates. All the statistical analyses were carried out using the SPSS statistical tool (SPSS for windows, release 16.0. SPSS Inc., Chicago, IL). The DMRT and one–way analysis of variance (ANOVA) were used to assess the differences. *P* values of <0.05 were considered as statistically significant.

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3. Results

The accessions of *B. monnieri* varied in the phenotypic characters indicating considerably variability among the accessions. Vattakottai and Kovai kutrallam stolon colour is green which differs from other ecotypes. Petel colour are almost the same in all ecotypes. Kovai kutrallam leaf shape varied from all other ecotypes (Table 1).

B. monnieri leaves from Aliyar dam showed the highest enzyme SOD activity of 30.119 and the least activity was noticed in Chidambaram sample. The highest stem enzyme activity was 30.316 in Nagar koil sample and the least activity was observed in Vatakottai sample (Table 2). The highest catalase enzyme activity was 1.965 noticed in the leaves of Aliyar dam and 1.186 observed in the stem of Vattakottai samples (Table 3). Maximum peroxidase enzyme activitie was 68.316 in leaves of Aliyar dam sample and lowest activity was found in Palani sample (5.645) when compared to other samples. In stem the enzyme activity was 33.497 in Urachikottai sample and least activity was 0.639 found in Palani sample (Table 4).

Table 1

Phenological characters of different ecotypes variant of *B. monnieri* (L.) Pennell.

Accession No.	Distinguishing phenotypic characters						
	Place of collection	Colour of stem	Colour of flower	Shape of leaves	Plant type	Latitude	Longitude
1	Palani	Pinkish green	Violet	Spatulate	Semi erect	10° 45 N	77° 50 E
2	Nagar koil	Pinkish green	Violet	Spatulate	Semi erect	08° 13 N	77° 43 E
3	Vattakottai	Green	Violet	Spatulate	Spreading	08° 32 N	77° 54 E
4	Chidambaram	Pinkish green	Violet	Spatulate	Semi erect	11° 47 N	79° 56 E
5	Urachikottai	Pinkish green	Violet	Spatulate	Semi erect	11° 28 N	76° 88 E
6	Athani	Pinkish green	Violet	Spatulate	Spreading	11° 57 N	77° 69 E
7	Aliyar dam	Pinkish green	Violet	Spatulate	Spreading	10° 32 N	76° 94 E
8	Kovai Kutralam	Green	Violet	Obovate	Semi erect	11^{o} 32 N	76^{o} 74 E

Table 2

SOD enzyme activity in leaves and stem of *B. monnieri.*

S. No	Places for collection	Leaves	Stem
1	Palani	$11.966^{b}\pm0.211$	$03.444^{b}\pm0.050$
2	Nagar Koil	$26.420^{d}\pm1.630$	$30.316^{h}\pm1.552$
3	Vattakottai	$29.560^{e}\pm1.725$	$0.884^{a}\pm0.448$
4	Chidambaram	$3.210^{a}\pm0.124$	$1.945^{a}\pm0.218$
5	Urachikottai	$16.955^{c}\pm0.100$	$08.458^{d}\pm0.423$
6	Athani	$27.625^{d}\pm0.574$	$06.224^{c}\pm0.119$
7	Aliyar Dam	$30.119^{e}\pm0.489$	$19.295^{g}\pm0.379$
8	Kovai Kutrallam	$17.901^{c}\pm0.236$	$11.993^{f}\pm0.313$

Values are given as Mean ± S.D of five replicates in each and expressed delta O.D per minute/mg of total soluble proteins from fresh tissue. Values, that are not sharing a common superscript (a,b,c,d,e,f,g,h), differ significantly at $P\leq 0.05$ (DMRT.)

Table 3

Catalase enzyme activity in leaves and stem of *B. monnieri.*

S. No	Places for collection	Leaves	Stem
1	Palani	$0.190^{a}\pm0.027$	$0.646^{ab}\pm0.098$
2	Nagar Koil	$0.910b^{c}\pm0.027$	$0.901^{c}\pm0.168$
3	Vattakottai	$1.361^{e}\pm0.166$	$1.186^{d}\pm0.219$
4	Chidambaram	$0.238^{a}\pm0.030$	$0.700^{ab}\pm0.124$
5	Urachikottai	$0.988^{cd}\pm0.091$	$1.150^{cd}\pm0.326$
6	Athani	$1.114^{d}\pm0.198$	$0.436^{a}\pm0.102$
7	Aliyar Dam	$1.965^{f}\pm0.175$	$0.668^{ab}\pm0.159$
8	Kovai Kutrallam	$0.740^{b}\pm0.106$	$0.628^{ab}\pm0.118$

Values are given as Mean ± S.D of five replicates in each and expressed delta O.D per seconds/mg of total soluble proteins from fresh tissue. Values, that are not sharing a common superscript (a,b,c,d,e,f,g,h), differ significantly at $P< 0.05$ (DMRT.)

Table 4

Peroxidase level in different ecotypes of *B. monnieri.*

S. No	Places for collection	Leaves	Stem
1	Palani	$5.645^{a}\pm0.150$	$0.639^{a}\pm0.030$
2	Nagar koil	$25.745^{c}\pm1.942$	$28.309^{e}\pm0.228$
3	Vattakottai	$27.304^{c}\pm0.298$	$9.977^{c}\pm1.004$
4	Chidambaram	$19.469^{b}\pm0.183$	$6.184^{b}\pm0.070$
5	Urachikottai	$36.032^{d}\pm1.784$	$33.497^{f}\pm0.360$
6	Athani	$38.808^{e}\pm1.535$	$9.570^{c}\pm3.101$
7	Aliyar dam	$68.316^{f}\pm1.967$	$27.658^{e}\pm0.242$
8	Kovai kutrallam	$26.334^{c}\pm1.097$	$21.899^{d}\pm1.126$

Values are given as Mean ± S.D. of five replicates in each and expressed delta O.D per minute/mg of total soluble proteins from fresh tissue. Values, that are not sharing a common superscript (a,b,c,d,e,f,g,h), differ significantly at $P< 0.05$ (DMRT.)

The non-enzymatic antioxidant ascorbic acid was maximum in Nagar koil sample (110.442 μg/g fresh weight of the sample) and the lowest ascorbic content was estimated in Palani leaf sample. On the other hand maximum ascorbic content of stem presented in Nagar koil sample (94.543) and the lowest ascorbic content was observed in Athani sample (31.430 μg/g of fresh stem tissue). Both leaf and stem samples of Nagar koil showed maximum level of ascorbic content. Reduced glutathione was maximum in Aliyar dam sample (162.76 μg/g) and and less amount was noticed in Athani leaves of fresh sample. Stem of the Aliyar dam sample had the maximum content of reduced glutathione and lowest content was found in Kovai kutrallam sample. Reduced glutathione was maximum in aliyar dam sample in both tissues (Figure 2). Vitamin E was maximum in Athani sample 88.883 μg/g and lowest amount was found in Urachikottai sample (37.263 μg/g fresh leaf samples).

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Maximum amount of 74.107 μg/g was obtained in stem of Athani sample and less quantity was found in Urachikottai sample. Both the tissues having the same type of manner for the content of α-tocopherol (Figure 3).

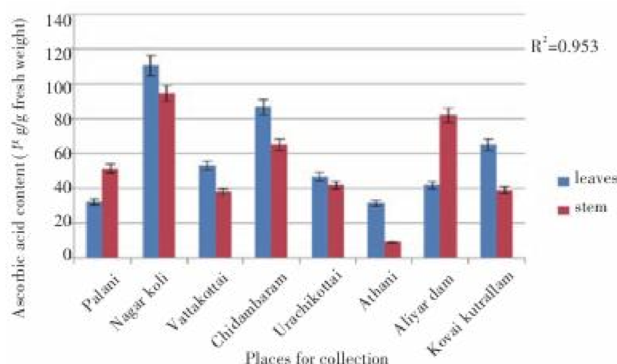

Figure 1. Ascorbic acid content of leaves and stem of *B. monnieri* (L.) Pennell ecotypes of Tamil Nadu.

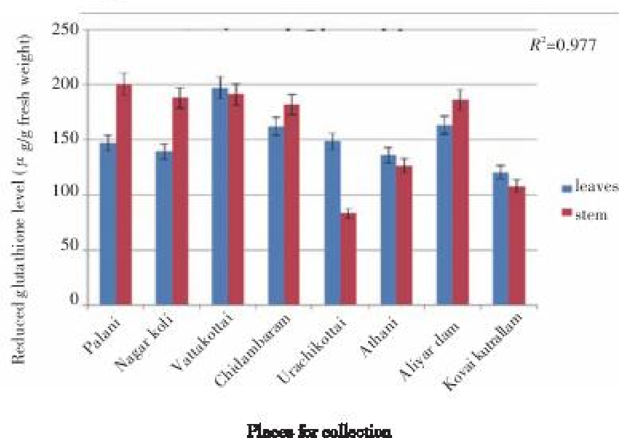

Figure 2. Reduced glutathione level of leaves and stem *B. monnieri* (L.) Pennell ecotypes of Tamil Nadu.

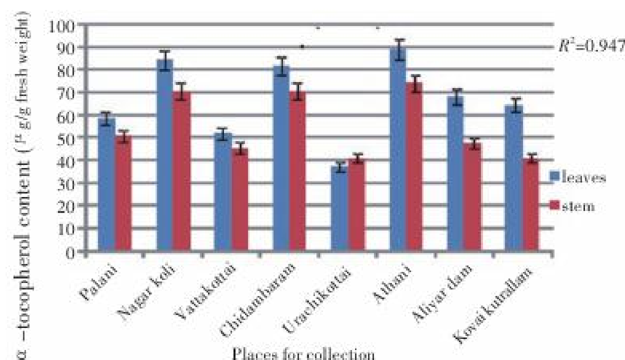

Figure 3. α– Tocopherol content in the leaves and stem *B. monnieri* (L.) Pennell ecotypes of Tamil Nadu.

4. Discussion

Phenological data of the study results are similar to previous results [14] observed 27 accessions of *B. monnieri* collected from semi–temperate, subtropical and tropical environments at geographically distinct locations in India were examined for genetic variability carried in them at Lucknow, India in a semi–temperate environment. It inferred that both the gross agroclimatic environment of a region and microenvironment in the vicinity of water bodies where a *B. monnieri* genotype occurred must have interacted for the natural selection of the concerned genotype.

Antioxidant enzymes are higher in leaves when compared to stem, this concept is negatively correlated to report of Mathur *et al.* [14] in which effect of Fe was investigated in medicinally important plant, *B. monnieri* L. and the response on malondialdehyde(MDA) content, superoxide dismutase (SOD), peroxidase (POD) and ascorbate peroxidase (APX) was found different in roots and leaves of the metal treated plants. Iron induced stress was observed as indicated by high level of lipid peroxidation, being more steep increase in leaves than roots. In roots, SOD activity was found to increase in metal treated plants except 80 and 160 μM at 72 h, whereas, it decreased in leaves except 10 and 40 μM after 48 h as compared to their respective controls. Among H_2O_2 eliminating enzymes, POD activity increased in roots, however, it decreased in leaves except at 10 and 40 μM Fe after 48 h as compared to control.

Apart from these enzymes, some non–enzymatic antioxidants like proline, ascorbic acid and carotenoids may play a role in inducing resistance to metals by protecting labile macromolecules against attack by free radicals which are formed during various metabolic reactions leading to oxidative stress [15]. Like enzymatic antioxidant (APX), ascorbic acid and non–protein thiol content also increased in the leaves of *B. monnieri*.

Detoxifying enzymes were observed in heat–treated plants, but the antioxidant enzymes were unable to operate in cold stress. This indicates that the plants have a higher capacity for scavenging oxygen radicals in heat stress than in cold stress. The different responses of antioxidant enzymes may be one of the possible mechanisms of the differences in temperature sensitivities of the two plant species [16]. The wild plants of *Catharanthus roseus* (*C. roseus*) from different geographical regions were collected with the hypothesis that compounds contribute in antioxidant activity are substantially affected by habitat temperature. With this hypothesis, plants were collected from different geographical regions considering temperature as leading factor of the study [17]. Severe deactivation of CAT and enhancement of POX accompanied by increased H_2O_2 during temperature rise in *C. roseus*, has also been observed in many other species [18]. Our results were also in agreement with heat stressed mustard [19], temperature stressed French bean[20] and drought stressed pea [21], which exhibited a significant increase in endogenous H_2O_2 and POX, and marked decline in CAT.

An increase in SOD activity with a decrease in CAT activity has been reported when the plants were subjected to abiotic stress [22, 23]. The diverse response of antioxidant enzymes such as SOD and other enzymes (CAT, POD, APX, MDAR, GR) due to thermal stress suggests the role of oxidative stress

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as a component of environmental stress on the two invasive species. It appears that the differences in SOD and other enzymes (CAT, POD, APX, MDAR, GR) has a direct relation to the sensitivity of the two plants to temperature. In rice, increased activity of SOD and decreased activities of CAT and POD were detected in cold–sensitive cultivars, whereas increased activities of these enzymes have been found in resistant cultivars exposed to chilling stress, while GR in both cultivars was stable to low temperature stress[24].

More than 4 000 phenol compounds (flavonoids, monophenols and polyphenols) are found in vascular plants. Phenolic compounds, such as querecetin, rutin, narigin, catechine, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents[25].

Declare of interest statement

We declare that we have no conflict of interest.

Acknowledgement

The authors gratefully acknowledge the University Grants Commission, New Delhi, Government of India: Grant No. F37 –30/2009 (SR) Dated – (12.01.2010) for financial support.

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