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Antioxidant status and radical scavenging effects of *Bacopa monnieri* (L.) Pennell

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Abstract

Bacopa monnieri (L.) Pennell, a medicinal herb, commonly known as "Brahmi", has been used in medicine since times immemorial.The present study was formulated to assess the antioxidant properties of *B. monnieri* leaves, stolon and roots. The enzymic antioxidants, namely; catalase, peroxidase, glutathione S-transferase, superoxide dismutase and polyphenol oxidase, and the non-enzymic antioxidants vitamin C, vitamin E, total carotenoids, lycopene, glutathione, total phenols, chlorophyll and flavonoids were analysed. The antioxidants were found to be maximum in the leaves, followed by the stolon and the roots. Therefore, the leaf extracts alone were prepared in solvents of differing polarity (aqueous, methanol and chloroform), to find out the component that renders maximum protection by testing their radical quenching ability against a battery of radicals, namely; DPPH, ABTS, H_2O_2, $O_2^{\bullet -}$, NO and $^{\bullet}OH$. All the radicals were effectively scavenged by all the three extracts. The methanolic extract showed maximum scavenging activity, followed by the chloroform extract. The results of the present study showed that due to its antioxidant properties, *B. monnieri* leaves exhibited effective antioxidant and radical scavenging activity *in vitro*, thus, can be used in food and pharmacological industries due to its antioxidant properties.

Keywords: *Bacopa monnieri* (L.) Pennell, enzymic and non-enzymic antioxidants, antioxidant activity

1. Introduction

The antioxidant compounds function as free radical scavengers. The antioxidants form a complex with pro-oxidant metals and quench singlet oxygen. Antioxidants may offer resistance by inhibiting lipid peroxidation and by other mechanism and, thus, prevent diseases (Umamaheswari and Chatterjee, 2008). The plant kingdom is an abundant source of phytochemicals, possessing important properties. Naturally occurring antioxidant compounds from plants have developed a complex antioxidative defence, and are considered to be as free radical or active oxygen scavenger (Youwei *et al.*, 2008). Due to the production of antioxidants in a significant amount, thus, preventing oxidative stress, plants represents a prospective source of new compounds with antioxidant activity.

Dietetic antioxidants have lately gathered increased research interest due to the fact that their consumption is being associated with a lower incidence of diseases, associated with oxidative stress (Wojchikowski, 2008). Traditional herbal medications form a vital part of the healthcare system of India. Ayurveda, the oldest medical system in the world, affords possible clues to find dynamic and medicinally useful compounds from plants (Hazra *et al*., 2009). One of the most interesting searches in the realm of therapeutical and medical success, is the search for newer and more potent drugs with diminutive toxic effects, self-administrable, cheap and absolutely revocable. Much of these properties are observed in the drugs of plant origin (Riaz *et al*., 2011).

One such popularly used plant *B. monnieri*, commonly known as "Brahmi", is a member of the family, Scrophulariaceae. *B. monnieri* is a standard brain and nerve stimulant, used for the management of perceptive disorders of ageing. It aids to increase protein activity and protein synthesis, exclusively in brain cells, which can influence intellect and memory, and reduction in senility (Kapoor *et al*., 2009). Regardless of numerous scientific confirmations, endeavoured and described on *B. monnieri*, not voluminous studies highlighting its antioxidant properties have been reported. The present study was undertaken to analyze the antioxidant status and radical scavenging effects of *B. monnieri*.

2. Materials and Methods

The different parts of *B. monnieri* plant was evaluated for their antioxidant status. The parts of the plant, namely; leaves, stolon and roots were collected from the plants, grown in pots. The adhering dirt or soil particles were washed thoroughly in running tap water and any water droplets were removed by blotting gently between folds of tissue paper.

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2.1 Superoxide dismutase (SOD)

The leaves, stolon and roots (0.5 g) were ground with 3.0 ml of potassium phosphate buffer, centrifuged and used the supernatant. The assay mixture contained 1.2 ml sodium pyrophosphate buffer, 0.1 ml phenazine methosulphate (PMS), 0.3 ml nitroblue tetrazolium (NBT), 0.2 ml 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, incubated at 30°C for 90 sec. and arrested by the addition of 1.0 ml glacial acetic acid. The reaction mixture was then shaken with 4.0 ml n-butanol, allowed to stand for 10 min. and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560 nm (Kakkar *et al.*, 1984).

2.2 Catalase (CAT)

To 3.0 ml of H_2O_2-phosphate buffer, added 40 µl of enzyme extract (20% homogenate in phosphate buffer) and mixed thoroughly. The time essential for a reduction in absorbance by 0.05 units was documented at 240 nm (Luck, 1974).

2.3 Peroxidase (POD)

Enzyme extract (0.1 ml) (20% homogenate in phosphate buffer) was added to 3.0 ml pyrogallol solution, and adjusted the spectrophotometer to read zero at 430 nm. Added 0.5 ml of H_2O_2 and the change in absorbance was recorded every 30 sec. up to 3 min. (Reddy *et al.*, 1995).

2.4 Glutathione S-transferase (GST)

Enzyme extract - homogenized 0.5 g of the samples with 5.0 ml of phosphate buffer, centrifuged and used the supernatants for the assay. The reaction mixture in a total volume of 2.9 ml contained 0.1 ml GSH, 0.1 ml CDNB and phosphate buffer. The reaction was initiated by adding 0.1 ml of enzyme extract and observed the readings for every 15 sec. at 340 nm for a minimum of 3 min. (Habig *et al*., 1974).

2.5 Polyphenol oxidase (PPO)

The plant tissue (0.5 g) was homogenized in extraction medium (2.0 ml) containing HCl, sorbitol and NaCl. To 2.5 ml of phosphate buffer in the test cuvette, added 0.3 ml of catechol solution and set the spectrophotometer at 495 nm. Then added 0.2 ml of enzyme extract and recorded the change in absorbance for every 30 sec. up to 5 min. (Esterbauer *et al*., 1977).

2.6 Ascorbic acid

Plant sample (1g) was extracted using 4% TCA (10 ml), centrifuged and the supernatant was treated with a pinch of charcoal, shaken vigorously and allowed to stand for 5 min. Removed the charcoal particles by centrifugation and used the aliquots. Standard ascorbate (0.2-1.0 ml) and 0.5 ml and 1.0 ml of the supernatant were taken and the volume was made up to 2.0 ml with 4% TCA. 2,4-dinitrophenyl hydrazine (DNPH) reagent (0.5 ml), 2 drops of 10% thiourea solution were added and incubated at 37°C for 3 h., resulting in the formation of osazone crystals. Further, crystals were liquefied in 2.5 ml of 85% sulphuric acid in cold. DNPH reagent and thiourea were added to the blank after the addition of sulphuric acid, cooled and read at 540 nm (Roe and Kuether, 1943).

2.7 Tocopherol

Homogenized 2.5 g of the plant sample in 50 ml of 0.1N sulphuric acid and was allowed to stand overnight. The contents were shaken vigorously, filtered through Whatman No.1 filter paper and the aliquots of the filtrate were used for the estimation. 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. Ethanol (1.5 ml) and xylene (1.5 ml) were added to all the tubes and centrifuged. 1.0 ml of the xylene layer was transferred into another tube. Dipyridyl reagent (1.0 ml) was added to each tube. The extinction of the mixture (1.5 ml) was read at 460 nm. Ferric chloride (0.33 ml) solution was added to the tubes (plant extract, standard and water) and read after 15 min. at 520 nm (Emmerie-Engel reaction as reported by Rosenberg, 1992).

2.8 Total carotenoids and lycopene

The sample (leaves, stolon and roots) (0.5 g) was homogenized and saponified with alcoholic KOH in a water bath (60ºC) for 30 min. The extract after saponification was transferred to a separating funnel containing 10-15 ml of petroleum ether, mixed well. The upper petroleum ether layer containing the carotenoids was collected and the extraction of the lower aqueous layer was repeated until the aqueous layer became colourless. The volume of the petroleum ether layer was noted and read the absorbance at 450 nm and 503 nm (Zakaria *et al*., 1979).

The amount of total carotenoids and lycopene was calculated using the following formulae:

$$\text{Amount of total carotenoids} = \frac{A_{450} \times \text{Volume of the sample} \times 100 \times 4}{\text{Weight of the sample}}$$

$$\text{Amount of lycopene} = \frac{3.12 \times A_{503} \times \text{Volume of the sample} \times 100}{\text{Weight of the sample}}$$

2.9 Reduced glutathione

Plant sample (leaves, stolon and roots) (0.5 g) was homogenised with 2.5 ml of 5% TCA. The protein present in the samples was precipitated by centrifugation at 1000 rpm for 10 min. and the supernatant (0.1 ml) was used for the estimation of GSH. The supernatant (0.1 ml) was made up to 1.0 ml with 0.2 M sodium phosphate buffer (pH 8.0). Standard GSH (concentrations ranging between 2 and 10 nmoles) was also prepared. To the standard and sample extracts, 2 ml of 5,5'-dithiobis nitro benzoic acid (DTNB) solution was added and the colour intensity was measured at 412 nm after 10 min. (Moron *et al*., 1979).

2.10 Total phenols

The sample (0.5 g) was homogenized in 10 × volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min. The extraction was repeated with 80% ethanol. The supernatants were pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water. Different aliquots of the extract were pipetted out and made up to 3.0 ml with distilled water, added Folin-Ciocalteau reagent (0.5 ml), boiled, cooled and read at 650 nm. Standard catechol solutions (0.2-1 ml) ranging in concentration from 2.0-10 µg were also treated as above (Mallick and Singh, 1980).

2.11 Flavonoids

The different parts of *B.monnieri*, namely; leaves, stolon and roots (0.5 g) were extracted with methanol : water mixture (2:1) and secondly with the same mixture in the ratio 1:1. The extracts were shaken well and allowed to stand overnight, pooled the supernatants and measured the volume. This supernatant was concentrated and then used for the assay. A known volume of the extract was vaporized to dryness. To the extract, added 4.0 ml of vanillin reagent, heated in a boiling water bath for 15 min. Varying concentrations of the standard (catechin) were also treated in the same manner and read at 340 nm (Cameron et *al*., 1943).

2.12 Chlorophyll

Plant sample (leaves, stolon and roots) (1 g) was extracted, using 20 ml of 80% acetone. The absorbance of the acetone extracts was read at 645 and 663 nm against 80% acetone blank (Witham *et al*., 1971).

Among the three parts of *B.monnieri* plant (leaves, stolon and roots), which were analysed for the enzymic and non-enzymic antioxidants, leaves were found to be the richest source of antioxidants. Hence, in further study, only the leaves were analyzed. The leaves were extracted separately with two solvents of differing polarity, namely; methanol and chloroform. A crude aqueous extract was also prepared and the radical scavenging ability of the three different extracts were analysed.

2.13 Preparation of plant extracts

Three different extracts of the leaves (1 g in 10 ml of the solvent) were prepared using methanol, chloroform and water. Dried the organic extracts at 60^{o}C protected from light. The residue was weighed and the desired concentration was obtained by dissolving in dimethyl sulfoxide (DMSO). Aqueous extracts were prepared fresh.

2.14 Determination of DPPH (2,2-diphenyl-2-picryl hydrazyl hydrate) radical scavenging activity

The reaction mixture contains leaf extracts (20 µl), 0.5 ml of methanolic solution of DPPH (0.3 mM) and 0.48 ml of methanol. The purple colour after decolourisation was measured at 518 nm after 30 min. (Mensor *et al*., 2001).

2.15 ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical scavenging activity:

ABTS radical cations (ABTS^{+}) were produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulphate and was incubated at room temperature (dark) for 12-16 h. before use. To 0.3 ml of ABTS solution added aliquots (0.5 ml) of the three different extracts, the final volume was made up to 1 ml with ethanol and read at 745 nm (Shirwaikar *et al*., 2006).

2.16 Hydrogen peroxide scavenging effects

Leaf extracts (10 mg / 10µl) were added to 0.6 ml of H_2O_2 solution (40 mM in phosphate buffer); made up the total volume to 3 ml and read the absorbance at 230 nm (Ruch *et al*., 1989).

2.17 Superoxide scavenging activity

Generation of superoxide anions were done, using leaf extracts (20 mg / 0.2 ml), EDTA (0.2 ml), NBT (0.1 ml), riboflavin (0.05 ml) and phosphate buffer (2.64 ml) in a total volume of 3.0 ml. The tubes where DMSO was added instead of the plant extracts, served as the control. Vortexed all the tubes and the initial optical density was measured at 560 nm. The tubes were illuminated for 30 min, using a fluorescent lamp and measured the absorbance again at 560 nm (Winterbourne *et al*., 1975).

2.18 Measurement of nitric oxide scavenging activity

Initiated the reaction by incubating 2.0 ml sodium nitroprusside (100 mM), 0.5 ml PBS, 0.5 ml leaf extracts (50 mg) at 25°C for 30 min. Griess reagent (0.5 ml) was added, incubated for another 30 min., and read at 546 nm against a reagent blank (Green *et al*., 1982).

2.19 Hydroxyl radical (OH) scavenging activity

0.1ml deoxyribose, 0.1 ml $FeCl_3$, 0.1 ml EDTA, 0.1 ml H_2O_2, 0.1 ml ascorbate, 0.1 ml KH_2PO_4-KOH buffer and 20 µl of plant extracts were incubated at 37°C for 1 h. After incubation, 1.0 ml of TBA was added, heated at 95°C for 20 min. and the TBARS formed was measured at 532 nm (Elizabeth and Rao, 1990).

3. Results and Discussion

Medicinal plants are excellent sources of antioxidants. The antioxidant activity of number of spices and aromatic herbs has been tested. However, there are many plants, whose antioxidative property have not yet been examined or known (Djeridane *et al*., 2007). One such medicinal plant is *B. monnieri*, which has been widely used in folklore medication as a nerve energizer, diuretic and cardiotonic. The present study was formulated to assess the antioxidant potential of *B. monnieri*. The different parts of *B. monnieri*, namely, leaves, stolon and roots, were screened for their antioxidant status.

3.1 Activities of enzymic antioxidants in *B. monnieri*

The different parts of *B. monnieri*, namely; leaves, stolon and roots were assessed for the enzymic antioxidants SOD, CAT, POD, GST and PPO. The activities obtained are presented in Table 1.

Table 1: Activities of enzymic antioxidants in the different parts of *B. monnieri*

Enzyme	Leaves	Stolon	Roots
SOD (U$^{\#}$/g)	20.07 ± 0.53	5.11 ± 0.29	22.03 ± 0.33
CAT (U*/g)	340 ± 0.42	153 ± 0.55	84 ± 0.40
POD (U$^{\$}$/g)	3.12 ± 0.52	3.00 ± 0.31	2.70 ± 0.53
GST (U$^{\&}$/g)	0.135 ± 0.09	0.002 ± 0.0	0.004 ± 0.0003
PPO (U$^{@}$x10^{-3}/g)			
Catechol oxidase	1.36 ± 0.02	6.8 ± 0.5	1.22 ± 0.01
Laccase	1.21 ± 0.01	6.05 ± 0.04	1.09 ± 0.06

The values are Mean ± SD of triplicates

\# 1 Unit: Amount of enzyme that gives 50% inhibition of the extent of NBT reduction in 1 minute

* 1 Unit: Amount of enzyme required to decrease the absorbance at 240nm by 0.05 units / minute

$ 1 Unit: Change in absorbance / minute at 430nm

& 1 Unit: nmoles of CDNB conjugated / minute

@ 1 Unit: Amount of catechol oxidase/laccase, which transforms 1 unit of dihydrophenol to quinine/minute

From the results obtained, it was clear that the leaves possess considerably higher activities of all the enzymic antioxidants analysed, compared to the stolon and roots.

3.2 Levels of non-enzymic antioxidants in *B. monnieri*

The non-enzymic antioxidants analysed in the different parts of the plant are presented in Table 2.

Table 2: Levels of non-enzymic antioxidants in *B. monnieri*

Parameter	Leaves	Stolon	Roots
Ascorbic acid (mg/g)	2.0 ± 0.30	1.8 ± 0.20	1.3 ± 0.20
Tocopherol (µg/g)	2.4 ± 0.26	2.8 ± 0.15	3.4 ± 0.25
Total carotenoids (mg/g)	14.6 ± 0.40	0.6 ± 0.05	BD
Lycopene (mg/g)	13.0 ± 0.10	BD	BD
Total phenols (mg/g)	0.07 ± 0.02	0.04 ± 0.01	0.02 ± 0.02
Reduced glutathione (nmoles/g)	108 ± 0.50	12.0 ± 0.43	20.0 ± 0.51
Flavonoids (mg/g)	2.30 ± 0.21	2.26 ± 0.37	2.0 ± 0.47
Chlorophyll (mg/g)	0.80 ± 0.21	BD	BD

The values are Mean ± SD of triplicates
BD-below detectable levels

From the results, it can be inferred that the leaves possessed maximum levels of non-enzymic antioxidants than stolon and roots except tocopherol.

The antioxidant status of several plants has been studied. Moderate activities of all the enzymic antioxidants were found in three under-exploited medicinal plants; *Pergulari adaemia, Rhinacanthus nasutus and Ruellia strepens* (Nirmaladevi and Padma, 2008). High carotenoid content was observed in the leaves of *Allium ursinum* (Stajner and Popovic, 2009). Vitamins C, E, A and GSH were found in the fresh and dried forms of *Pleurotus florida* and *Calocybe indica* (Selvi *et al*., 2007). The highest total phenolic and total flavonoid content were shown by the methanolic extract of cantaloupe leaf than the stem, skin, seed and flesh extracts (Ismail *et al*., 2010). *Gemelia* plant leaves showed maximum content of phenols, while *Hibiscus* leaf possessed the maximum flavonoid content (Patel *et al*., 2010).

Peltophorum pterocarpum leaves showed high content of total phenolic compounds, total flavonoids and polyphenolic compounds (Satapathy and Swamy, 2012). *Moringa oleifera* leaves have exhibited high phenol content, rich polyphenol profile and strong antioxidant capacity. The antioxidant potential and radical scavenging activities vary considerably among different scavenging assays, and the results showed a promising source of natural antioxidant, which can prevent and protect from various diseases (Sravanthi and Rao, 2014).

The results showed that the leaves of *B. monnieri* are rich sources of both enzymic and non-enzymic antioxidants. Therefore, the leaves were used further to assess the antioxidant activity of *B.monnieri*.

3.3 Free radical scavenging effects of *B. monnieri* leaves

Three different extracts of the leaves were prepared in solvents of differing polarity, namely; aqueous, methanol and chloroform. The radical scavenging ability of the extracts were then tested against a battery of radicals such as DPPH, ABTS, H_2O_2, $O_2^{\bullet-}$, NO and •OH.

The three different extracts of *B. monnieri* leaves were assessed for their DPPH scavenging ability. It is found from the results that the methanolic extract exhibited the highest activity implicating the strong medicinal properties of the plant. The total amount of radicals that can be scavenged by an antioxidant was assessed by ABTS radical cation decolorization assay showing results similar to that of DPPH assay. The hydrogen peroxide scavenging ability of *B. monnieri* leaf extracts in *in vitro* system was studied, revealing the strong scavenging effect of the methanolic extract against hydrogen peroxide.

Figure 1 represents the extent of DPPH, ABTS and H_2O_2 scavenging ability of the three extracts of *B. monnieri*. The methanolic extract showed maximum scavenging effect, followed by the chloroform extract. All the three extracts were screened for their ability to inhibit *in vitro* generation of superoxide and nitric oxide (Figure 2). From the results, it can be inferred that all the extracts exhibited good superoxide and nitric oxide radical scavenging activity and the maximum inhibition was mediated by the methanolic extract. The results reiterated the strong antioxidant potential of the leaves.

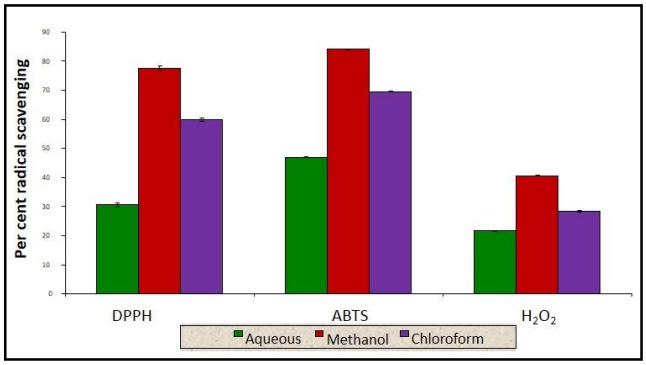


Figure 1: Radical scavenging effects of *B. monnieri* leaf extracts

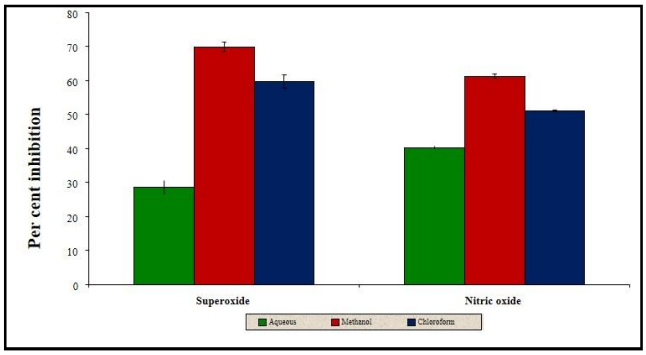
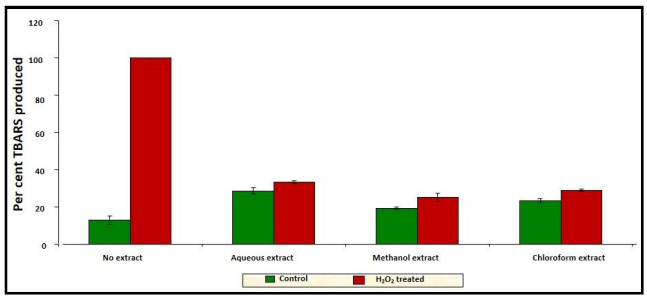


Figure 2: Superoxide and nitric oxide scavenging effects of *B. monnieri* leaf extracts

The effect of *B. monnieri* leaf extracts on the damage induced by H_2O_2 to deoxyribose was quantified as the amount of TBARS formed (Figure 3). The extent of damage was found to be maximum on H_2O_2 exposure where the methanolic extract of *B. monnieri* was found to be an effective hydroxyl radical scavenger, followed by the chloroform extract, indicating the very good hydroxyl radical scavenging activity of the leaves of *B. monnieri.*

The antioxidant efficacy of herbal extracts and their components are assessed by the DPPH scavenging assay (Mothana *et al*., 2008). The antioxidant effects of aqueous, methanolic and ethanolic extracts of *Melissa officinalis*, *Matricaria recuttia* and *Cymbopogan citrates* were reported by Pereira *et al*. (2009).



The values of the positive control (H_2O_2 treated) group were fixed as 100% damage and the per cent damage in the other groups were calculated relative to this

Figure 3: Effects of *B. monnieri* leaf extracts against oxidative damage to deoxyribose

An excellent substrate for peroxidases is ABTS and is frequently used to study the antioxidant properties of natural compounds (Reszka and Britigan, 2007). Of the successively extracted *Aphanamixis polystachya* bark with hexane, ethyl acetate, methanol and water, the methanolic extract possessed potent ABTS scavenging activity (Krishnaraju *et al*., 2009).

H_2O_2, a normal cellular metabolite when produced in excess, causes cell dysfunction and generation of diseases (Gonzalez *et al*., 2005). The aqueous extract of *Melothria maderaspatana* scavenged H_2O_2 in a dose-dependent manner (Raja and Pugalendi, 2010). The strong antioxidant activity and H_2O_2 scavenging effect of water and ethanolic extract of *Ocimum basilicum* was reported by Gulcin *et al*. (2007).

The effect of *B. monnieri* leaf extracts on the inhibition of *in vitro* generation of $O_2^{\bullet-}$ were studied. The results of the study revealed that the methanolic extract exhibited the highest $O_2^{\bullet-}$ scavenging activity. Anandjiwala *et al*. (2007) reported that the *Bergia suffruticosa* (methanolic extract) showed inhibition of superoxide generation. The antioxidant potential of *Trigonella foenum graecum* L. varies considerably among different radical scavenging assay, and the results showed promising source of natural antioxidant (Sravanthi *et al*., 2013).

Inhibition of NO generation was exhibited by the aqueous extract of *Wasabia japonica* (Lee *et al*., 2010). The aqueous extract of *Strychno shenningsii* caused a moderate dose-dependent inhibition of nitric oxide generation (Oyedemi *et al*., 2010). Free radical scavenging activity was observed in the crude ethanolic bark extract of *Terminalia arjuna* (Sree *et al*., 2007).

The most reactive among the ROS was the hydroxyl radical which has the shortest life and is responsible for much of the biological damage.The rate constant of hydroxyl radical reactions deoxyribose degradation is the common method for determining deoxyribose degradation (Jelili *et al*., 2010). It is difficult to directly determine the hydroxyl radical. The scavenging activity of hydroxyl radical is measured as the formation of $^{\bullet}OH$ induced TBA-reactive substance (TBARS) formation. The reaction of deoxyribose with hydroxyl radical influences the rate of TBARS formation.

Higher hydroxyl radical scavenging activity was shown by the methanol extract of *Lagerstroemia speciosa* L. (Priya *et al*., 2008). The methanolic extract of *Picrasma quassiades* (Yin *et al*., 2009), the ethanol extract of the leaves of *Stachytarpheta angustifolia* (Awah *et al*., 2010) and the aqueous extract of *Wagatea spicata* flowers (Samak*et al*., 2009) efficiently inhibited $^{\bullet}OH$.

4. Conclusion

In the present study, different parts of *B. monnieri* plant, namely; leaves, stolon and roots, were analysed for their antioxidant content. All the three parts, namely; leaves, stolon and roots possessed considerable quantities of enzymic and non-enzymic antioxidants among which, the leaves possessed the maximum quantity of antioxidants analyzed, followed by the stolon and the roots. In order to identify the nature of the leaves component, rendering the maximum protection, three different extracts, namely; aqueous, methanol and chloroform were prepared and analysed for their radical quenching ability against a battery of radicals, namely; DPPH, ABTS, H_2O_2, $O_2^{\bullet-}$, NO and $^{\bullet}OH$. All the three extracts of the leaves effectively scavenged or inhibited all the radicals tested. Of the three, the methanolic extract effectively scavenged the free radicals, followed by the chloroform extract. Thus, from the results, it can be concluded that *B. monnieri* leaves possess good antioxidant potential and, thus, an effective radical scavenger, implicating their use in pharmacological and food industries due to its antioxidant properties.

Conflict of interest

We declare that we have no conflict of interest.

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