Evaluation of Free Radical Scavenging Activity of Hydroethanolic Extract of *Bacopa monnieri* Through DPPH Assay

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Abstract

The antioxidant potential of *Bacopa monnieri in vitro* through DPPH free radical scavenging assay, was evaluated using ascorbic acid as standard. There was a concentration dependent increase in percentage inhibition of DPPH free radical by ascorbic acid and hydroethanolic extract of *Bacopa monnieri*. The IC_{50} of the extract was found to be 28µg/ml and 270µg/ml for ascorbic acid and plant extract, with regression coefficients (r^2) 0.94 and 0.89, respectively. The hydroethanolic extract of *Bacopa monnieri* has a significant antioxidant potentiality comparable to a standard antioxidant like ascorbic acid.

Introduction

Bacopa monnieri, (Family: S*crophulariaceae*) commonly known as Brahmi has been used in Ayurveda since ancient times as a medicinal herb to improve memory, intellect, respiratory function in cases of bronchoconstriction as well as a cardiotonic and digestant **(Nadkarni, 1988).** The plant is a profusely branched creeping herb rooting at the nodes and form dense mats with succulent stem and leaves and small-white flowers and propagates through cuttings. The plant has no distinct odour and tastes slightly bitter **(Chopra *et al.*, 1956; Aiyer and Kolammal, 1964).** It commonly grows in marshy areas throughout India in all plain districts ascending to an altitude of 1,320m (asl)

As per the available literature, *Bacopa monnieri* is rich in many phytocompounds including alkaloids like brahmine and herpestine, saponins like d-mannitol, hersaponin, acid A and monnierin, flavonoids (luteolin and apigenin) and sterols responsible for its potent antioxidant activity.

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a free radical which reacts with an antioxidant compound that can donate hydrogen and gets reduced. DPPH, when acted upon by an antioxidant, is converted into diphenylpicryl hydrazine. This can be identified by the conversion of its colour from purple to light yellow.

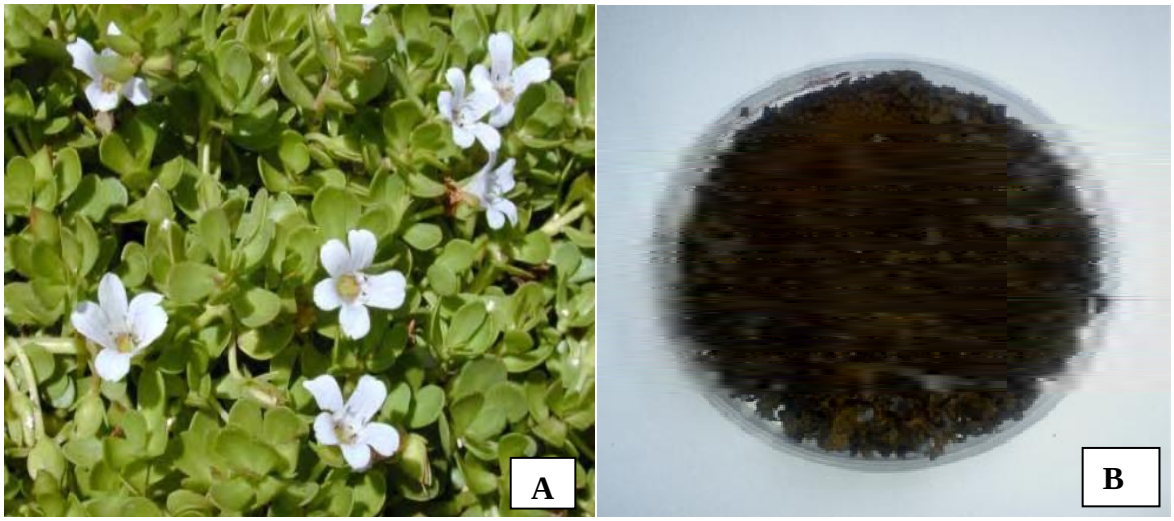


Figure 1. A. *Bacopa monnieri* with flower; B- Hydroethanolic extract of *B. monnieri*

Material and Method

Collection and authentification of the plant

Plant material was collected, identified and authenticated from Medicinal Plant Research and Developmental Centre, MRDC, Pantnagar. Aerial part of the whole plant was used for preparing extract (Fig. 1 A).

Preparation of hydroethanolic extract

Hydroethanolic extract of *Bacopa monnieri* was prepared using 50% ethanol. The cold extracts were prepared by the method described by **Singh (2008)** with slight modifications. According to this technique, powdered plant was soaked in 50% hydroethanolic solution (1gm/10ml of 50% hydroethanolic solution) for 24 hours with continuous stirring at 37 $^{\circ}$C. The mixture was filtered through several layered muslin cloth and centrifuged to separate the supernatant. The final extract was produced after drying the filtrate in fan incubator (JSGW, India) at 35°C. The percentage yield of extract was calculated. The dried extract was removed and kept in air tight bottles in refrigerator at $4\pm1^{\circ}$C (Fig. 1 B).

Phytochemical analysis

Qualitative chemical analysis of various extracts of *Bacopa monnieri* was done to detect major phytochemical groups viz., alkaloids, anthraquinones, flavonoids, saponins, tannins, sterols, reducing sugars, glycosides, resins, triterpenes and proteins by employing standard methods **(Harborne, 1973; Sofawara, 1982).**

DPPH free radical scavenging assay

The antioxidant activity of 50% hydroethanolic extract of *Bacopa monnieri* was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. DPPH, ascorbic acid and methanol were purchased from Hi-media.

Procedure

The ability of the plant extract to scavenge DPPH free radicals was assessed by the method of **Tekao *et al.* (1994) and Kumarasamy *et al.* (2007)** with slight modifications.

DPPH free radical scavenging assay in ascorbic acid: Ascorbic acid was taken as the standard antioxidant compound in this study. A stock solution of ascorbic acid as 1mg/ml was prepared in methanol and different concentrations ranging from 10-100µg/ml methanol (10µg/ml, 20µg/ml, 30µg/ml, 40 µg/ml, 50 µg/ml, 70 µg/ml, 80 µg/ml and 100 µg/ml) were made from this stock solution.

A 0.004% w/v solution of freshly prepared DPPH in methanol as a solvent was prepared. This solution also served as control. Each concentration of ascorbic acid was applied in triplicates. To each concentration of ascorbic acid, 1ml of freshly prepared 0.004% DPPH solution was added, mixed well and kept for 30 minutes incubation in darkness at 23°C.After incubation, optical density was determined against methanol as a blank at a wavelength of 517 nm.

DPPH free radical scavenging assay in Bacopa monnieri plant extract: A stock solution of plant extract as 1mg/ml was prepared in methanol and different concentrations ranging from 10-2000µg/ml methanol (10µg/ml, 20µg/ml, 50 µg/ml, 100 µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml and 2000 µg/ml) were made from this stock.

A 0.004% w/v solution of freshly prepared DPPH in methanol as a solvent was prepared. This solution also served as control. Each concentration of extract was applied in triplicates. To each concentration of extract, 1ml of freshly prepared 0.004% DPPH solution was added, mixed well and kept for 30 minutes incubation in darkness at 23°C. After incubation, optical density was determined against methanol as a blank at a wavelength of 517 nm.

Percentage inhibition of DPPH free radical was calculated as per the following formula:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{(\text{Absorbance of Control})} \times 100$$

Results

Phytochemical Analysis

The recovery of the extract as percent yield was found 22.33%. The result of phytochemical analysis of hydroethanolic extract are presented in table 1.As evident from the table, alkaloids, flavonoids, glycosides, proteins, resins, sterols, saponins, tannins and terpenes are present in HEBM. However, Anthraquinones and reducing sugars were found absent (Table 1).

DPPH free radical scavenging assay

The observations on DPPH free radical scavenging are summarized in the table 2. There was a concentration dependent increase in percentage inhibition of DPPH free radical by ascorbic acid and hydroethanolic extract of *Bacopa monnieri*. The IC_{50} values of ascorbic acid and hydroethanolic extract of *Bacopa monnieri* were approximately 28µg/ml and 270µg/ml, respectively (Table 2 and figure 3).

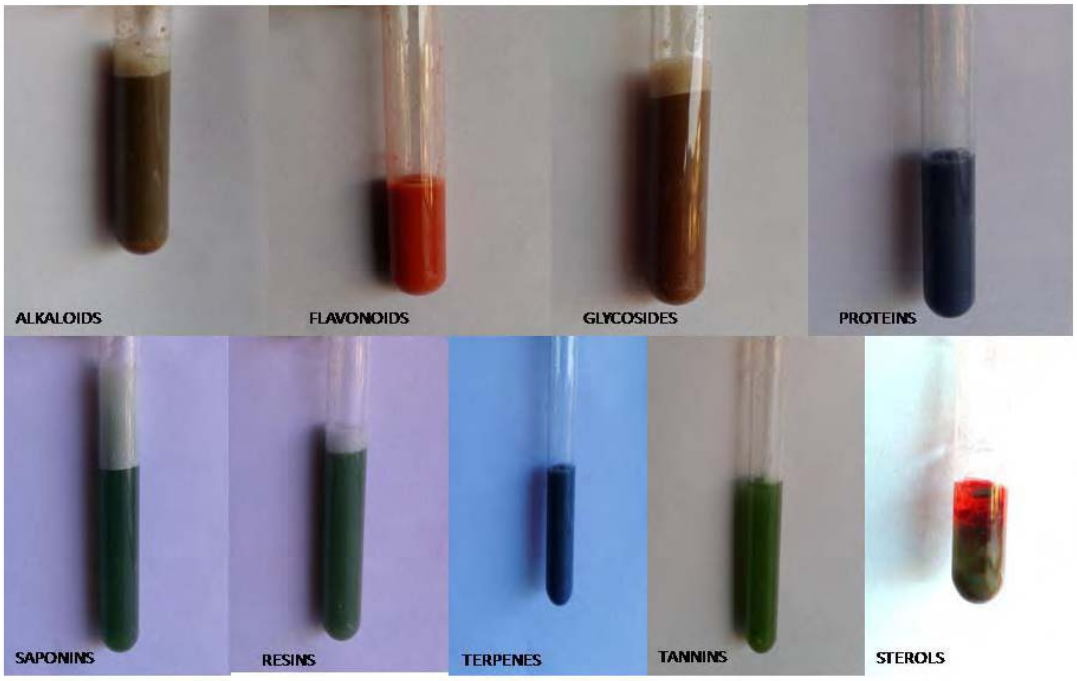


Figure 2. Phytochemical screening of *B. mommieri* extract.

Table 1. Phytochemical analysis of hydroethanolic extract of *Bacopa monnieri* (HEBM)

PHYTOCHEMICALS	OBSERVATIONS
Alkaloids	+
Anthraquinones	-
Flavonoids	+
Glycosides	+
Proteins	+
Reducing sugars	-
Resins	+
Sterols	+
Saponins	+
Tannins	+
Terpenes	+

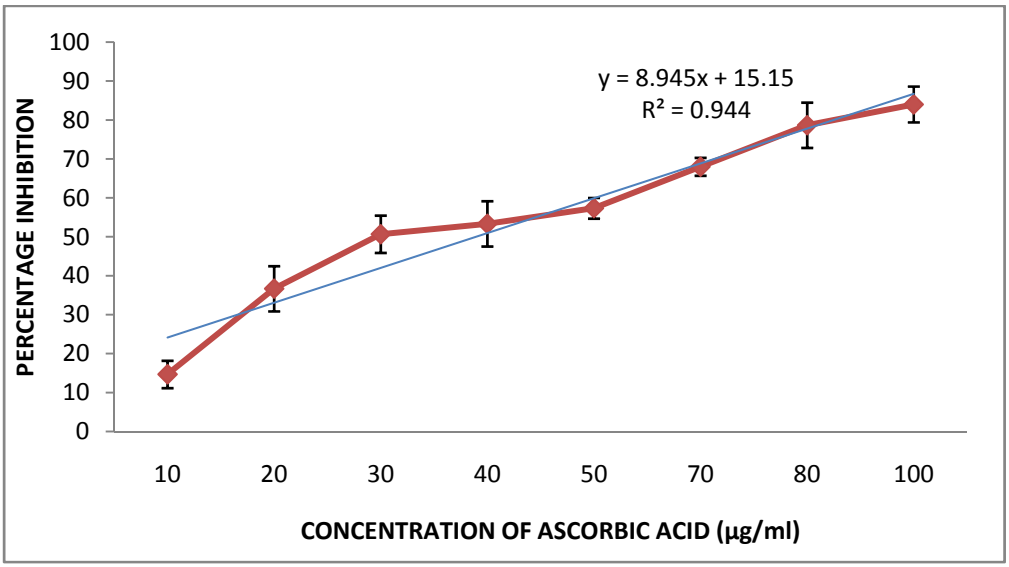
Table 2: Percentage inhibition of DPPH free radical by ascorbic extract and hydroethanolic extract of *Bacopa monnieri*

Conc. .(µg/ml)	Percentage inhibition by Ascorbic acid	Percentage inhibition by HEBM
10	14.66±3.52	20.00±2.30
20	34.66±5.81	21.33±2.66
30	50.66±4.80	-------
40	53.33±5.81	-------
50	57.33±2.66	26.66±2.66
70	68.00±2.30	-------
80	78.66±5.81	-------
100	84.00±4.61	40.00±4.00
250	-------	46.66±5.81
500	-------	89.33±4.80
1000	-------	117.33±4.80
2000	-------	141.33±5.81

Discussion

In the modern time people are actively looking for herbal drugs. Many plants are also being evaluated for their antioxidant property and many phytoconstituents have been reported to exhibit free radical scavenging property. Reactive oxygen species (ROS) are formed as a natural by-product of the normal metabolism of oxygen and have important role in cell signaling. However, during stress ROS levels can increase dramatically, resulting in significant damage to cell structures. Hence antioxidants are employed to scavenge the excess ROS.

DPPH acts as a stable free radical in methanol that easily accepts an electron or hydride radical and converts to a stable diamagnetic molecule by reacting with suitable reducing agents DPPH radicals formed into the corresponding hydrazine. Depending on the number of electrons taken up, the solution looses colour stoichometrically **(Alam *et al.*, 2012).** The IC_{50} values of ascorbic acid and hydroethanolic extract of *Bacopa monnieri* were approximately 28µg/ml and 270µg/ml with regression coefficients (r^2) of 0.93 and 0.89, respectively. The values of regression coefficients indicate a strong antioxidant potential of HEBM comparable to that of ascorbic acid (Fig. 3). The antioxidant activity might be attributed to phenolic content, triterpenoids and flavonoid content of the tested plant. Somewhat similar finding were also reported by other workers in their *in vitro* studies **(Ghosh *et al.*, 2007; Volluri *et al.*, 2011).** Thus it can be concluded that the plant extract of *Bacopa monnieri* has a significant antioxidant potential comparable to a standard antioxidant like ascorbic acid.



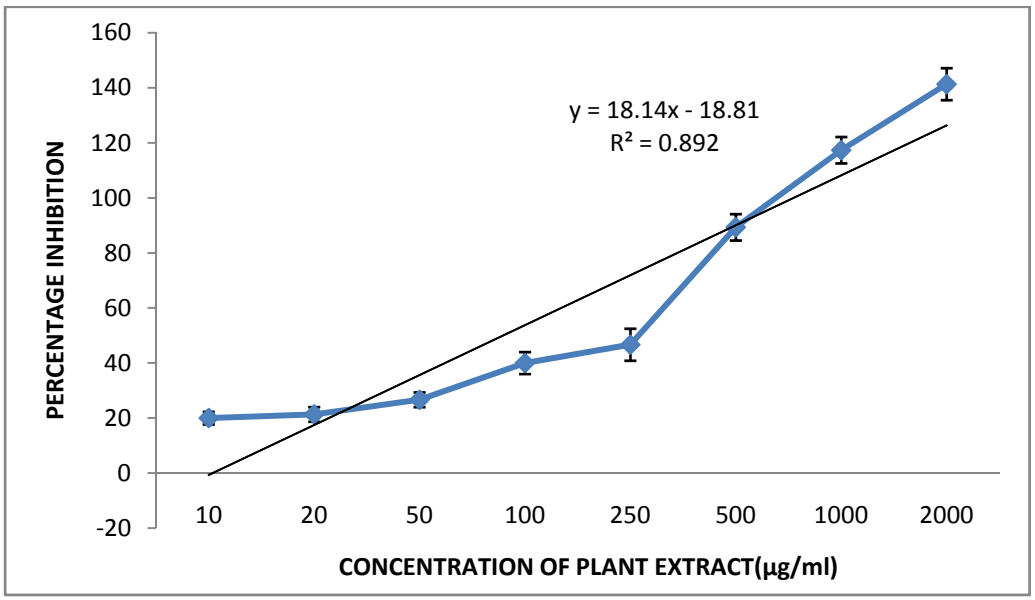


Figure 3: DPPH free radical scavenging assay

References

[1] Aiyer, K.N. and Kolammal, M.1964. Pharmacognosy of Ayurvedic drugs. Department of pharmacognosy, Trivendrum, University of Kerela. pp 27.

[2] Alam, M.B., Hossain, M.S., Asadujjaman, M., Islam, M. M., Mazumder, M.E.H. and Haque, M.E. 2010. Peroxynitrite scavenging and toxicity potential of different fractions of the aerial parts of Bacopa monniera linn. IJPSR. 1(10): 78-83.

[3] Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

[4] Chopra, R.N., Nayar, S.L. and Chopra, I.C. 1956. Glossary of Indian medicinal plants. Calcutta. pp 32.

[5] Ghosh, T., Maity, T.K., Das, M., Bose, A. and Dash D.K. 2007. In vitro antioxidant and hepatoprotective activity of ethanolic extract of Bacopa monnieri Linn. aerial parts. IJPT.6: 77-85.

[6] Harborne, J.B. 1973. Phytochemical methods. Chapman and Hall, London. pp 117.

[7] Kumarasamy, Y., Byres, M., Cox, P.J., Jasapars, M., Nahar, L. and Sarker, S.D. 2007. Screening seeds of some Scottish plants for free-radical scavenging activity. Phytother. Res. 21: 615-621.

[8] Nadkarni, K.M. 1988. The Indian Materia Medica. South Asia Books, Columbia. pp. 624-625.

[9] Singh, K.P. 2008. Elucidation of antioxidative and hepatoprotective properties of Emblica officinalis against mercury induced toxicity in rats and HepG2 cell line. Ph.D. Thesis, G.B. Pant University of Agriculture and Technology, Pantnagar. p 40.

[10] Sofawara, A. 1982. Medicinal plants and traditional medicine in Africa. Chichester, John Wiley.

[11] Tekao, T., Watanabe, N., Yagi, I. and Sakata, K. 1994. A simple screening method for antioxidant and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci. Biotechnol. Biochem. 58: 1780-1783.

[12] Volluri, S.S. Bammidi, S.R., Chaitanya, S. C. and Vangalapati, M. 2011. In vitro antioxidant activity and estimation of total phenolic content in methanolic extract of Bacopa monnieri. Rasayan J. chem.. 4(2): 381-386.