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RESEARCH ARTICLE

BIOCHEMICAL PROFILING OF MEDICINALLY IMPORTANT PLANT *BACOPA MONNIERI* (L.)

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ABSTRACT

Herbal medicines have gained global importance, both medically as well as economically. *Bacopa monnieri* (L.) is commonly and widely called as brahmi, belongs to the family Scrophulariaceae. It is a traditional plant in India, which has been used for centuries to increase mental capacity, improve mental and brain functions. The plant was reported to have anti-inflammatory, analgesic, antipyretic, antioxidant and anticancer activities. In the present study, a comparative analysis of antioxidant and phytochemical content difference is done among the *in vitro* and *in vivo* cultivated plants of three different cultivars of *Bacopa monnieri* (L.). In DPPH free radical assay, cultivar procured from Chuharpur showed 95.47% of inhibition at 70µg/ml concentration whereas 95.02% of inhibition of nitric oxide (at conc.140µg/ml) in nitric oxide free radical assay. Callus and *in vitro* grown plants of all the three cultivars of *Bacopa monnieri* showed positive test for amino acids, carbohydrates, alkaloids, glycosides, flavonoids, phenolic compounds, carotenoids and terpenoids.

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INTRODUCTION

Medicinal plants are the most important source of life saving drugs for the majority of the world's population (Mohan *et al.,* 2011).Today's medicinal plants are important to the global economy, approximately 80% of traditional medicine preparations involve the use of plants or plant extracts (Dhyani and Kala, 2005; Pandiyan and Selvaraj, 2012). The global demand for herbal medicine is not only large, but plant secondary metabolites are economically important as drugs, fragrances, food additives and pesticides. In the folklore of Indian medicine, certain herbs have been used traditionally as brain or nerve tonic (Mohan *et al.,* 2011). *Bacopa monnieri* is a herb which is most popular as neurotonic. It is a vegetatively propagated medicinal plant enlisted among the most endangered plant due to its over exploitation. *Bacopa monnieri*, commonly and widely called as brahmi, belongs to the family Scrophulariaceae and is a small herb, prostrate, succulent, with wide spreading or ascending branches and rooting at the nodes. Its leaves are ovate-oblong or spathulate and grows up to a height of about 18cm. Flowers of *Bacopa monnieri* blossoms during the month of August and continues till the month of October and are white in colour, companulate, axillary, solutary, short or long pedicilate. It commonly grows in damp and marshy places throughout India, ascending up to an altitude of 1320m (Sinha and Saxena, 2006). *Bacopa monnieri* is prescribed for a variety of therapeutic indications including antipyretic, anti-inflammatory, analgesic, epilepsy, insanity, anticancer, antioxidant activities and memory enhancement (Jain and Kulshreshtha, 1993; Satyavati *et al.,* 1976). It is used to treat asthma, hoarseness, snake bite, rheumatism, water retention, blood cleaning, eczema and ring worm (Basu and Walia, 1994). *Bacopa*'s antioxidant properties may offer protection from free radical damage in cardiovascular disease and certain types of cancer. It also helps to prevent induced lipid peroxidation (Tripathi *et al.,* 1998). Brahmi has ability to reduce NO-induced cellular alterations and thus has a therapeutic potential in treatment or prevention of neurological diseases (Russo *et al.,* 2003). Dammarane-type triterpenoid saponins, classified as pseudojujubogenin and jujubogenin glycosides were reported to be responsible for the cognition enhancing activity of this plant (Das *et al.,* 2002; Singh and Dhawan, 1997; Sumathi *et al.,* 2002; Stough *et al.,* 2001). It was also reported that *Bacopa* exerts a protective effect against DNA damage in astrocytes (Russo *et al.,* 2003) and human fibroblasts (Russo and Borrelli, 2005) in *in vitro* conditions. Also, *in vitro* studies shows that *Bacopa* extracts have an anticancer effect, possibly due to inhibition of DNA

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replication in cancer lines (Green *et al.,* 1982). In the present study, we compared the antioxidant and phytochemical content difference in the *in vitro* and *in vitro* cultivated plants of three different cultivars of *Bacopa monnieri*.

MATERIALS AND METHODS

Chemicals

All the chemicals used were of analytical grade. The chemicals were purchased from Sigma Chemical Company (St. Louis USA).

Collection of material

Bacopa monnieri L. Penn. plant material was purchased from three different places of North India *viz.* Devi Lal Herbal Park, Chuharpur, Yamunanagar, (Haryana), CIMAP Lucknow (U.P.) and CCS HAU, Hisar (Haryana). It was established in field during the months of July- August 2013.

Antioxidant Assays

Two types of antioxidant assays were performed *viz*. DPPH free radical scavenging assay and Nitric oxide radical scavenging assay.

DPPH free radical scavenging assay

DPPH [1,1-diphenyl-2-picryl hydrazyl] is a stable free radical. Its intensity is measured at 510 nm spectrophotometrically and it is purple in colour. Antioxidants reduces DPPH to 1,1-diphenyl-2-picryl hydrazine, which is a colourless compound.

Procedure

DPPH free radical scavenging assay was carried out using method described by Mohan *et al.* (2011). At 25°C, various concentrations of test solution and 1ml of DPPH (0.3 mM) solution were incubated for 20 min. Then at 510 nm absorbance was read. A control reaction was carried out without the test sample. According to the following equation, the % inhibition was calculated.

$$\% \text{ Inhibition} = (A_o - A_t) / A_o \times 100$$

Where, A_o = Absorbance of the control (blank, without extract); A_t = Absorbance in the presence of the extract.

Nitric oxide radical scavenging assay

At physiological pH, Sodium nitroprusside in aqueous solution generates nitric oxide which interacts with oxygen to produce nitrite ions. These nitrite ions can be measured at 540 nm spectrophotometrically in the presence of Griess reagent (1% sulphanilamide,0.1% naphthyethylene diamine dihydrochloride is dissolved in 2% phosphoric acid).

Procedure

Nitric oxide radical scavenging assay was performed as per the method illustrated by Jain and Kulshreshtha (1993). At room temperature (25-30°C), 2 ml test solution of various concentrations and 3 ml of 10 mM Sodium nitroprusside was added and incubated for 1hr. 5ml of Griess reagent was added and incubated for 10 min at room temperature. The colour developed was measured at 540 nm. According to the following equation, the % inhibition was calculated.

$$\% \text{ Inhibition} = (A_o - A_t) / A_o \times 100$$

Where, A_o = Absorbance of the control (blank, without extract); A_t = Absorbance in the presence of the extract.

Phytochemical Study

The Phytochemical investigations were carried out on alcoholic extract of callus, organogenetic and field grown plants for the presence or absence of primary and secondary metabolites *viz.,* phenolics, flavonoids, carotenoids, alkaloids, amino acids, tannins, carbohydrates, anthraquinones and terpenoids.

Estimation of Phenolics

The phenolic content was estimated using the method of Price *et al.* (1980). 5 g sample was homogenized in acetone and kept overnight in a flask. Supernatant was collected and residues were extracted with acetone followed by filtration and centrifugation. For estimation of phenolics, supernatant was used. Distilled water and ferric ammonium sulphate was added to extract and then kept at room temperature. Potassium ferricyanide was added and absorbance was measured at 720 nm (concentration in µg/ml of extract).

Estimation of Flavonoids

Flavonoid content was determined by the method described by Harborne *et al*. (1975); Sundriyal *et al*. (2013). 5 g sample was acid hydrolyzed with sulphuric acid and neutralized with sodium hydroxide. Then ethyl acetate was added and shaked well, ethyl acetate portion was collected (repeated thrice). Ethyl acetate was palled and evaporated to dryness. With methanol, residues were reconstituted and assayed for flavonoids content. Extract was mixed with methanolic $AlCl_3$ and at 430 nm absorbance was measured.

Estimation of Carotenoids

Carotenoids content was estimated by the method elucidated by Narayanswamy and Palanisami (1973). 5 g sample was homogenized in acetone and then filtered. This step was repeated until extract was not free from pigments. Filtrate was pooled and partitioned with equal volume of peroxide free ether thrice using separating funnel. The ether phase containing the carotenoids gets evaporated and residues were dissolved in ethanol. KOH was added to particles twice into peroxide free ether. Ether was evaporated and dissolved in ethanol. At 450 nm absorbance, carotenoids were measured by spectrophotometer.

Estimation of Alkaloids

Dragondorff's reagent test: To 2 ml of each sample, Dragondorff's reagent (solution of potassium bismuthiodide) was added.

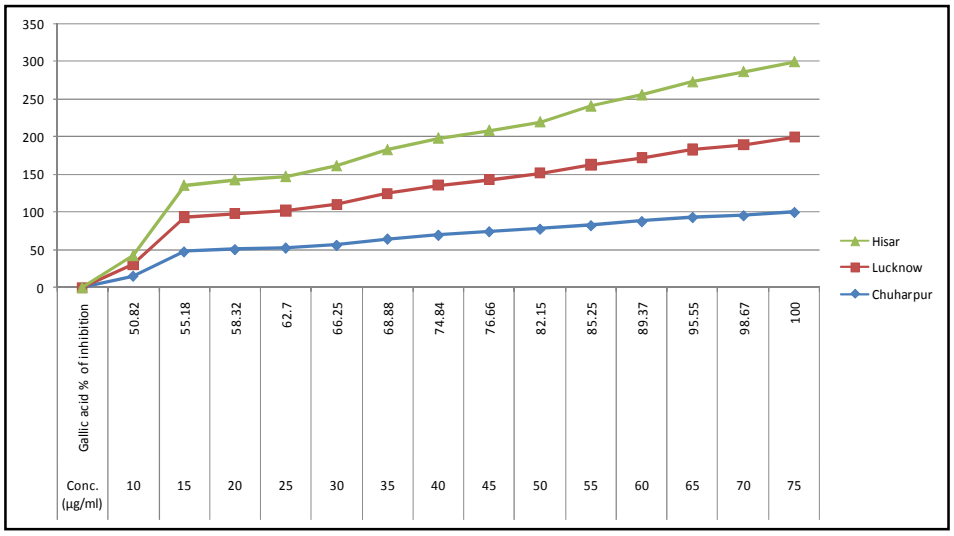

Fig. 1. % of inhibition of DPPH by the plant extracts of different cultivars of *Bacopa monnieri* (L.) W.R.T. Gallic acid as standard

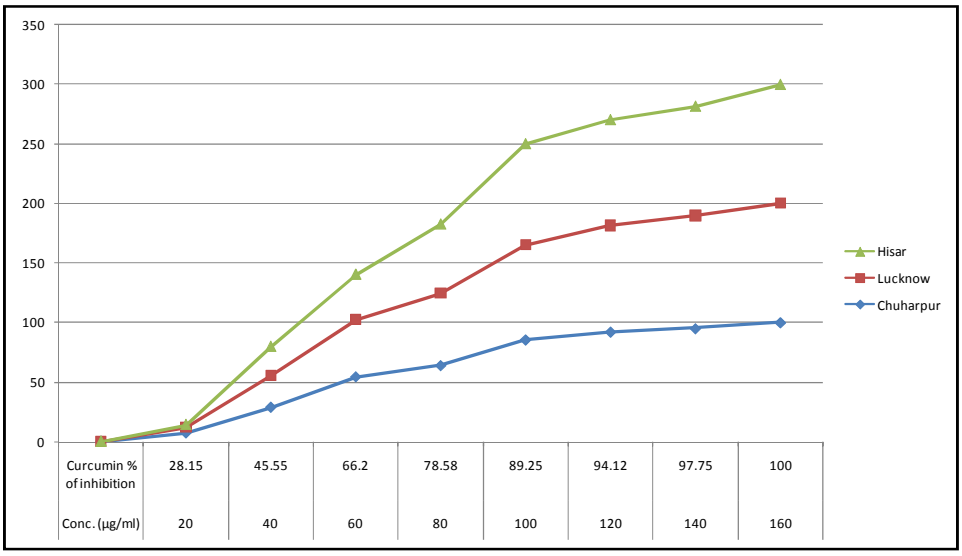

Fig.2. % of inhibition of nitric oxide by the plant extracts of different cultivars of *Bacopa monnieri* (L.) W.R.T. Curcumin as standard

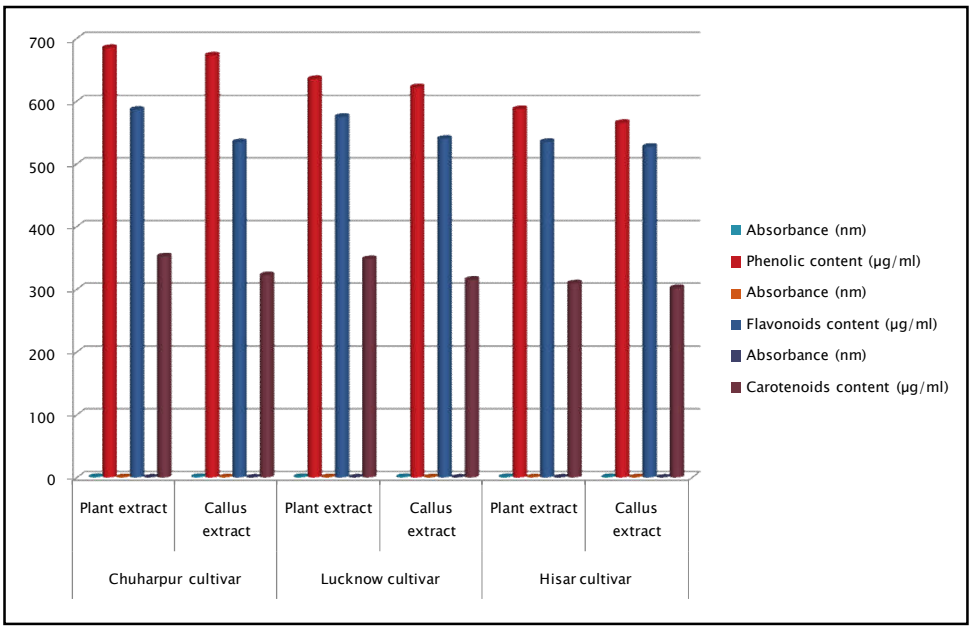

Fig.3. Comparison of phytochemical content in plant extracts and callus extracts of three cultivars of *Bacopa monnieri*

A reddish brown precipitate appeared in all the samples, indicating the presence of alkaloids. Mayer's reagent test: To 5 ml of filtrate, Mayer's reagent (KI + $HgCl_2$) was added. Appearance of a creamy precipitate confirmed the presence of alkaloids.

Estimation of Amino acid

Callus and plant materials were extracted using 90% ethanol. To concentrated extracts, a few drops of ninhydrin solution were added and were heated in water bath. The appearance of violet colour indicated the presence of amino acids.

Estimation of Tannins

Gelatin Test: 5 drops of 1% solution of gelatin was added to 2-3 ml of aqueous extract. If precipitate or turbidity appeared, it confirms the presence of tannins. Lead Acetate Test: To 2-3 ml of aqueous extract, 5 drops of lead acetate were added. If yellowish white precipitate appeared, then tannins were present.

Estimation of Carbohydrates

Fehling Solution Test: To the aqueous extracts of callus, organogenetic and field grown plant of *Bacopa monnieri*, 1ml of Fehling solution of 'A' and 'B' was added. Formation of brick red precipitate upon heating in water bath indicated the presence of reducing sugar.

Estimation of Anthraquinones

Borntrager Test: 0.1 g of powdered callus was boiled with 5 ml of dil. H_2SO_4 for 2 min. The extract was filtered while hot. The filtrate was cooled and shaken with equal volume of benzene. The benzene layer was allowed to separate completely. 10% ammonia solution was added to half of its volume, gently shaken and the layer was allowed to separate. Appearance of purple colour gives the confirmation of anthraquinones.

Estimation of Terpenoids

The plant material was extracted with chloroform and the extract was concentrated to $1/5^{th}$ of its volume. Few drops of acetic anhydride and concentrated H_2SO_4 were added to 1 ml of concentrated extract. Formation of blue to violet colour showed the presence of terpenoids.

RESULTS AND DISCUSSION

Herbal medicines have gained global importance, both medically as well as economically. In developing countries, 80% of the population depends on traditional systems of medicine as their primary source of healthcare (Saini *et al.,* 2012). *Bacopa monnieri* is a traditional plant in India which has been used for centuries to increase mental capacity, improve mental and brain functions. The plant was reported to have anti-inflammatory, analgesic, antipyretic, antioxidant and anticancer activities (Alam *et al.,* 2011).

Antioxidant Assays

DPPH free radical assay: DPPH free radical assay depicted the presence of antioxidants in *Bacopa monnieri* plant. The reduction capability of the DPPH radical was determined by the decrease in its absorbance at 517 nm induced by antioxidants. . It was reported that extract's scavenging effect increased with their concentrations to similar extent (Mohan *et al.,* 2011). The percentage inhibitions of Gallic acid (taken as standard) and plant extract showed the best results for Chuharpur cultivar of *Bacopa monnieri* followed by Lucknow and Hisar cultivars (Fig.1). At 70µg/ml concentration, Chuharpur cultivar showed 95.47% of inhibition followed by Lucknow (94.75%) and Hisar (96.74%), whereas, at 75µg/ml concentration, an absolute inhibition was reported in all the three cultivars of *Bacopa monnieri*. Nitric oxide free radical assay: Nitric oxide or reactive nitrogen species formed during its reaction with oxygen or with superoxide such as NO_2, N_2O_4, N_3O_4, nitrate and nitrite are very reactive. These compounds have the capability to alter the structure and function of many cellular components. Any natural or synthetic compound, having antioxidant properties might contribute towards the partial or total alleviation of this damage. Extract of *Bacopa monnieri* plant shows reduction in nitric oxide. In this assay, curcumin was taken as standard. A considerable increase in the scavenging effect of the plant extracts was reported with their increasing concentrations to similar extent by all the three cultivars of *Bacopa monnieri*. These results were supported by findings of Mohan *et al.* At 140µg/ml concentration, cultivar procured from Chuharpur showed 95.02% of inhibition of nitric oxide whereas at the same concentration Lucknow cultivar exhibited 94.45% inhibition and Hisar cultivar showed 92.28%. An absolute inhibition was reported at concentration 160 µg/ml by all the three cultivars. A comparative analysis was done for the % inhibition of nitric oxide by the plant extracts of various cultivars of Brahmi w.r.t. curcumin (Fig.2).

Phytochemical Study

For carrying out phytochemical analysis, methanolic extract of six month old field grown plant, *in vitro* grown plant and the callus after sixth subculturing of *Bacopa monnieri* were used to detect the presence and absence of primary and secondary metabolites. Field grown plants of Chuharpur and Hisar cultivars of *Bacopa monnieri* showed positive test for amino acids, alkaloids, glycosides, flavonoids, tannins, phenolic compounds, carotenoids and terpenoids whereas cultivar from Lucknow showed negative test for tannins (Fig. 3). In field grown plants of Lucknow cultivar, presence of carbohydrate was reported whereas it was absent in field grown plants of Chuharpur and Hisar cultivars (Fig. 3). Callus and *in vitro* grown plants of all the three cultivars of *Bacopa monnieri* showed positive test for amino acids, carbohydrates, alkaloids, glycosides, flavonoids, phenolic compounds, carotenoids and terpenoids (Fig. 3). Quercetin was taken as standard for the estimation of phenolics. The absorbance of Quercetin in different concentration was taken at 720 nm for preparing the standard curve. Plant and callus extracts of Chuharpur cultivar of *Bacopa monnieri* showed 685.4 µg/ml and 673.4 µg/ml phenolic content (Fig. 3) whereas in case of Lucknow cultivar the results were 635.7 µg/ml and 622.4 µg/ml respectively. In Hisar cultivar, phenolic content was reported as 587.6 µg/ml in plant extract and 565.7µg/ml in callus extract (Fig. 3). For the flavonoid estimation, Quercetin was taken as standard and its

absorbance was taken at 430 nm for preparing the standard curve. All the three cultivars of *Bacopa monnieri* showed slight variation in terms of flavonoid concentration. The flavonoid content in plant extract of Chuharpur cultivar was 586.6 µg/ml whereas it was 535.0 µg/ml in its callus extract. Similarly, flavonoid contents in plant and callus extract of Lucknow cultivar was reported as 575.4 µg/ml and 540.5 µg/ml respectively. The cultivar procured from Hisar showed flavonoid content in plant extract as 535.5 µg/ml and in callus extract as 527.7 µg/ml (Fig. 3).

For carotenoids content estimation also, the Quercetin was taken as standard. The absorbance of Quercetin in different concentration was taken at 430 nm for preparing the standard curve. In Chuharpur cultivar, the plant extract showed carotenoid content as 353.0 µg/ml whereas callus extract showed a value of 323.0 µg/ml. The carotenoid content was reported as 310.0 µg/ml in plant extract and 302.0 µg/ml in callus extract of Hisar cultivar (Fig. 3).Out of the three cultivars of *Bacopa monnieri* best results of phytochemical analysis were reported in Chuharpur cultivar (Fig.3) and this might be because the plant is natural habitat of muddy shores and wetlands.

Conclusion

In the present study, it has been observed that *Bacopa monnie*ri has antioxidant and phytochemical contents which might be responsible for its therapeutical potential in treatment or prevention of neurological diseases. The study also shows that there was an insignificant difference in these bioactivities between *in vitro* and *in vivo* grown plants. *In vivo* grown plants have higher antioxidant and phytochemical content as compared to *in vitro* growncallus.

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