



**ANNUAL VARIATION IN BACOSIDE CONTENT OF  
*BACOPA MONNIERI* (L.) WETTST PLANTS**

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**ABSTRACT**

A study was conducted to evaluate bacoside (expressed as sum of bacoside A<sub>3</sub> and A<sub>2</sub>) production on a monthly basis in net house grown plants of *Bacopa monnieri* (accession BM001) collected from Jammu region. Bacoside content differed during the course of the whole year. The whole herb is used commercially for extracting the memory enhancing molecule - bacosides from the plant. The study significantly points out that the maximum bacoside yield was obtained during August to October (monsoon period) which also coincides with the favourable period for plant growth and would be suitable for plant harvest. Amongst the various individual plant parts, the highest content was recorded in the leaves (6.06 mg g<sup>-1</sup> DW) followed by stem (5.13 mg g<sup>-1</sup> DW) and least in the roots (3.19 mg g<sup>-1</sup> DW).

**KEYWORDS:** Brahmi, HPLC, Memory enhancing molecule, Seasonal variation, Secondary metabolite.



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## INTRODUCTION

*Bacopa monnieri* (L.) Wettst. (Commonly known as 'Brahmi', Scrophulariaceae) is a small creeping annual herb with numerous branches, small oblong leaves with light purple flowers. It is distributed widely throughout warmer regions of the world such as India (ascending to an altitude of 1,320 m), Nepal, Sri Lanka, China, Taiwan and Vietnam and other southern states of USA<sup>1</sup>. In India, it has been found in damp and marshy or sandy areas near streams in tropical regions. The plant species has been widely used in Ayurvedic system of Medicine for improving memory and intelligence<sup>2</sup>. Extracts of *Bacopa monnieri* have been reported for their anti-inflammatory, antipyretic, epilepsy, anticancer and antioxidant activities<sup>3,4</sup>. The compounds which are responsible for the pharmacological effects of *Bacopa* include; alkaloids, flavonoids, saponins, and sterols. The triterpenoid saponins especially bacoside A and B are considered as bioactive marker compounds of this plant amongst which, Bacoside A is a major chemical entity shown to be responsible for memory-facilitating action<sup>2</sup>. It comprises of a mixture of triglycosidic saponins, bacoside A<sub>3</sub>, bacoside II, jujubogenin and bacosaponin C<sup>5</sup>. Other compounds present in this plant are Brahmine, herpestatine, bacoside A<sub>1</sub>, monnierin, hersaponin, d-mannitol, betulinic acid, stigmasterol,  $\beta$ -sitosterol<sup>6,7</sup>. On acid hydrolysis, bacosides yield a mixture of aglycones, bacogenin A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub><sup>8,9,10</sup>. Knowledge of the presence of active components in medicinal plants as well as individual plant parts is an important aspect for the standardization of commercial herbal formulations. Different environmental factors such as temperature, humidity, altitude, rainfall may affect the presence secondary metabolites in plants<sup>11</sup>. Seasonal variation as an extrinsic factor in the antioxidant activities, total phenolic and flavonoid content of *Nothapodytes nimmoniana* bark has also been reported<sup>12</sup>. Therefore it is imperative to determine the suitable harvesting time for such plant materials used for preparation of herbal health care products. The purpose of the present study was to determine the bacoside content on a monthly basis in plants of *B. monnieri*

growing under net house conditions using HPLC analysis.

## MATERIALS AND METHODS

### (i) Plant material

Plants of *Bacopa monnieri* (L.) Wettst. (BM001) were collected from Indian Institute of Integrative Medicine, Jammu. The plants were transferred into a container filled with farm soil and farmyard manure (1:1) and placed under net house at 'Herbal Garden', SMVDU, Kakryal (Latitude = 28°66'67" North, Longitude = 77°21'67" East). Planting in beds was initiated in May 2009 and the cuttings were watered immediately after transplantation to maintain healthy growth for 6-8 months. Aerial parts (stem with leaves) of *B. monnieri* were harvested on monthly interval for two consecutive years (2010, 2011) with three replicates / collection for evaluation of bacoside content. Independently, sample of leaves, stems and roots were collected from the plants grown at 'Herbal Garden', SMVDU during the month of November 2010 in order to evaluate the individual bacoside content in various plant parts of *B. monnieri*.

### (ii) Extraction and analysis of bacosides

Aerial parts of healthy plants were collected, washed under running tap water and air dried to a constant weight. Air dried shoots were finely powdered and extracted in a Soxhlet apparatus with methanol for 5 h at a temperature of not more than 60°C. The methanolic extract was filtered with a 0.45  $\mu$ m PVDF syringe filter (Axiva Sicheem Biotech, India), air dried and further dissolved in HPLC grade methanol and subjected to HPLC analysis to estimate the total bacosides (A<sub>3</sub> and A<sub>2</sub>). Quantification of bacosides was carried out on Agilent- 1100 series HPLC system equipped with dual channel interface 35900E, quaternary pump, degasser, auto sampler, column oven and ultraviolet (UV) detector. HPLC analysis was conducted using Acetonitrile:Water (48:52, v/v) as the mobile phase with a flow rate of 0.4 ml min<sup>-1</sup> at wavelength of 210 nm on a column RP-18 (E-Merck, 5  $\mu$ m, 4.0  $\times$  250 mm). The column

temperature was maintained at 30° C for maximum peak efficiency with injection volume of 10  $\mu$ L and a run time of 30 min for each separation. Data acquisition was performed by Chemstation software. All solvents used for the HPLC analysis were of HPLC grade (Acetonitrile, Milli Q distilled water, Methanol). A 1.0 mg ml<sup>-1</sup> stock solution of standard bacoside (mixture of A<sub>3</sub> and A<sub>2</sub>) was prepared in methanol and 1.0 ml of this solution was diluted to 10 ml with methanol to get a 100  $\mu$ g ml<sup>-1</sup> working solution. Standard calibration curves were established by plotting the area of peaks against different concentrations of 2  $\mu$ l, 4  $\mu$ l, 6  $\mu$ l, 8  $\mu$ l and 10  $\mu$ l. Quantification of bacosides in the samples was determined using regression equation of calibration curves.

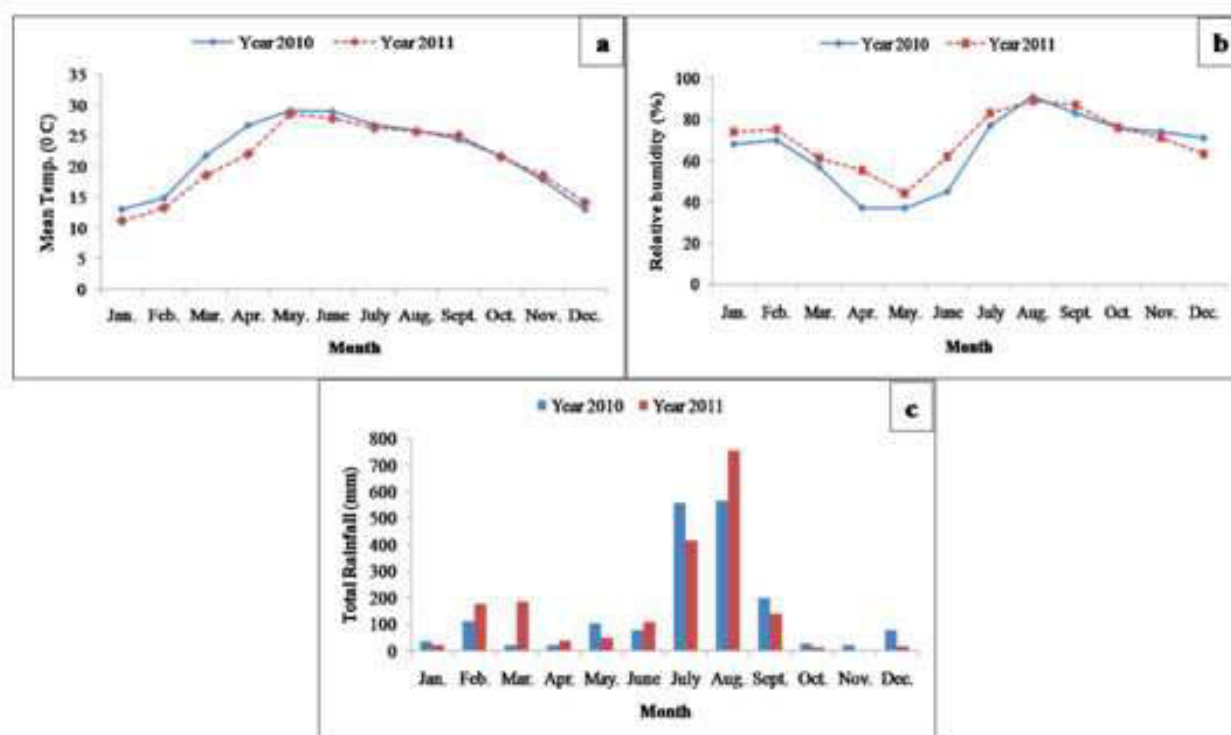
On the basis of the standard curves obtained, the quantity of bacoside A<sub>3</sub> and A<sub>2</sub> were determined in samples of shoot cultures. Data represents mean of two independent studies with three replicates per treatment and bacoside content is expressed as mg g<sup>-1</sup> DW.

## RESULTS AND DISCUSSION

Environmental conditions such as temperature, relative humidity and rainfall were recorded during the experimental period (2010-2011) for Katra station by the Regional Meteorological Centre, Srinagar (J&K) are graphically depicted (Graph 1).

**Graph 1**

**(a) Average Monthly Temperature (b) Humidity and (c) Total Rainfall for the year 2010-2011.**



The amount of bacoside present in *Bacopa monnieri* samples collected on monthly basis are tabulated (Table 1).

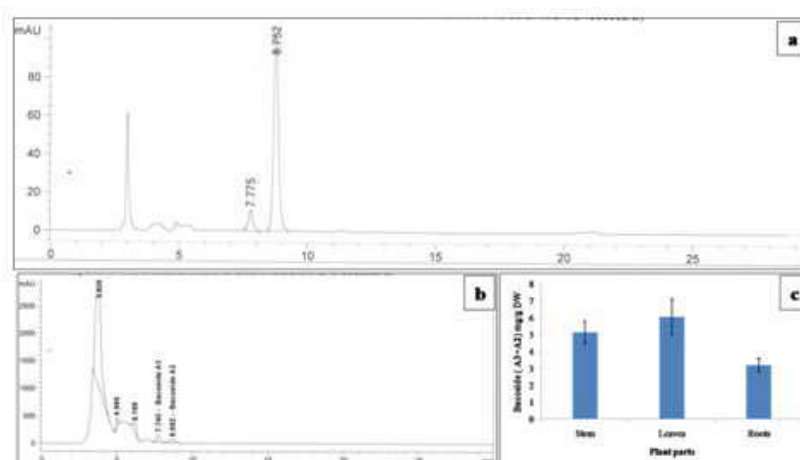
**Table 1**  
**Annual variation in bacoside content in *Bacopa monnieri* plants.**

Month	Bacoside mg g <sup>-1</sup> DW		Total Bacoside mg g <sup>-1</sup> DW
	A <sub>3</sub>	A <sub>2</sub>	
January	3.47±0.3	1.03±0.64	4.49±0.94
February	4.13±0.04	1.57±0.1	5.7±0.14
March	4.42±0.91	1.03±0.35	5.44±1.25
April	2.95±0.18	1.38±0.1	4.33±0.28
May	2.56±0.35	1.4±0.19	3.96±0.54
June	3.25±0.53	1.39±0.65	4.64±1.17
July	4.8±1.23	1.88±0.1	6.68±1.13
August	5.03±0.38	2.85±0.19	7.88±0.57
September	5.93±0.64	1.73±0.44	7.66±1.08
October	6.08±0.2	2.44±1.19	8.52±1.39
November	4.06±0.18	2.17±0.27	6.23±0.1
December	5.07±0.05	1.87±0.64	6.94±0.59

Values are mean± SE of two independent studies with 3 replicates/collection conducted for over two consecutive years 2010 and 2011.

Average bacoside content for two consecutive years (2010, 2011) of the samples collected during July-October (monsoon period) was 7.69 mg g<sup>-1</sup> DW while March-June (summer period) had the least bacoside content (4.59 mg g<sup>-1</sup> DW). Monsoon season favoured plant growth as well as bacoside accumulation. It might be due to high humidity, temperature and availability of large amount of water during monsoon period which are favourable conditions for the growth of *B. monnieri* plants. These observations were similar to those previously studied<sup>13,14,15</sup> wherein they have reported higher bacoside A content in different accessions of *B. monnieri* during monsoon period (June to September). The results for quantitative distribution of bacoside in different organs of *B. monnieri* collected during the month of November are presented (Fig 1c).

The HPLC chromatogram of bacoside standard and extract from *B. monnieri* leaves are shown (Fig 1a, b). Leaves contained the highest bacoside content (6.06 mg g<sup>-1</sup> DW) followed by stem and roots (5.13 and 3.19 mg g<sup>-1</sup> DW, respectively). Although the leaves contain slightly higher bacoside content than the stem, for large scale harvesting, collecting shoots (including leaves and stem) would be more practical than collecting only the leaves, which may be more time consuming. Higher saponin concentration was also observed in leaves than the shoots, stems and roots of *B. monnieri* during rainy season<sup>16</sup>. A similar report on variation in withanolide A content in different plant parts of *Withania somnifera* has been reported wherein higher withanolide A content was accumulated in the young leaves as compared to shoot tips of the plant<sup>17</sup>.



**Figure 1**

(a) HPLC Chromatogram of Standard bacoside (A<sub>3</sub>, A<sub>2</sub>) (b) HPLC Chromatogram of leaf extract collected from net house grown plants of *Bacopa monnieri* in the month of November (c) Distribution of bacoside in Plant parts. Data represents mean values ± SE of three replicates. Error bars represent Standard Error.

## CONCLUSION

Results of the present study showed that the highest bacoside content was obtained from *Bacopa monnieri* plants grown under net house condition during the monsoon period (July-October). In conclusion, our study might be useful for ascertaining the suitable time for commercial collection and harvesting of this important medicinal herb *B. monnieri* for getting maximum bacoside content for preparation of commercial formulations.

## CONFLICT OF INTEREST

Conflict of interest declared none.

## REFERENCES

1. Barrett SCH, Strother JL, Taxonomy and natural history of *Bacopa* in California. *Syst Bot.* 5: 408-419, (1978).
2. Singh HK, Dhawan BN, Neuropsychopharmacological effects of the Ayurvedic nootropic *Bacopa monniera* Linn (Brahmi). *Indian J. Pharmacol.* 29: S359-S365, (1997).
3. Russo A, Borrelli F, *Bacopa monnieri*, a reputed nootropic plant: An overview. *Phytochemistry.* 12: 305-317, (2005).
4. Sinha S, Saxena R, Effect of iron on lipid peroxidation and enzymatic and non-enzymatic antioxidants and bacoside A content in medicinal plant *Bacopa monnieri* L. *Chemosphere.* 62: 1340-1350, (2006).
5. Deepak M, Sangli GK, Arun PC, Amit A, Quantitative determination of the major saponin mixture bacoside A in *Bacopa monnieri* by HPLC. *Phytochem Anal.* 16:24-29, (2005).
6. Chatterji N, Rastogi RP, Dhar ML, Chemical examination of *Bacopa monnieri* Westtst.: Part I - isolation of chemical constituents. *Indian Journal of Chemistry.* 1: 212-215, (1963).
7. Jain P, Kulshreshtha DK, Bacoside A<sub>1</sub> a minor saponin from *Bacopa monniera*. *Phytochem.* 33: 449-451, (1993).
8. Kulshreshtha DK, Rastogi RP, Bacogenin [A.sub.1]: a novel dammerane triterpene sapogenin from *Bacopa monniera*. *Phytochem.* 12: 887-892, (1973).
9. Kulshreshtha DK, Rastogi RP, Bacogenin [A.sub.2]: a new sapogenin from bacosides. *Phytochem.* 13: 1205-1206, (1974).
10. Chandel RS, Kulshreshtha DK, Rastogi RP, Bacogenin-[A.sub.3]: A new sapogenin from *Bacopa monniera*. *Phytochemistry* 16: 141-143, (1977).
11. Kokate CK, Purohit AP, Gokhale SB, *Pharmacognosy*, 26th edition. Nirali Prakashan. 466-470, (2004).
12. Pai SR, Nimbalkar MS, Pawar NV, Patil RP, Dixit GB, Seasonal discrepancy in phenolic content and antioxidant properties from bark of *Nothapodytes nimmoniana* (Grah.) Mabb. *Int. J. Pharm. Bio. Sci.* 1(3): 1-17, (2010).
13. Ganjewala D, Srivastava AK, Luthra R, Ontogenic and seasonal variation in accumulation of bacoside-A in *Bacopa monnieri* (L.). *Journal of Medicinal and Aromatic Plant Sciences.* 22/23, (4A/1A): 233-237, (2001).
14. Mathur S, Gupta MM, Kumar S, Expression of growth and Bacoside-A in response to seasonal variation in *Bacopa monnieri* accessions. *Journal of Medicinal and Aromatic Plant Sciences.* 22/23, (4A/1A): 320-326, (2001).

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15. Mathur S, Gupta MM, Ram M, Sharma S, Kumar S, Herb yield and bacoside-A content of fieldgrown *Bacopa monnieri* accessions. Journal of Herbs, Spices & Medicinal Plants. 9(1): 11-18, (2002).
16. Phrompittayarat W, Jetiyanon K, Wittaya-areekul S, Putalun W, Tanaka H, Khan, Ingkaninan K, Influence of seasons, different plant parts, and plant growth stages on saponin quantity and distribution in *Bacopa monnieri*. Songklanakarin J. Sci. Technol. 33(2):193-199, (2011).
17. Praveen N, Naik PM, Manohar SH, Murthy HN, Distribution of withanolide A content in various organs of *Withania somnifera* (L.) Dunal. Int. J. Pharm. Bio. Sci. 1(3): 1-5, (2010).