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Comparative Analysis of Phytochemical Contents In Hydroponically Grown *Bacopa monnieri* (L.) Pennel With The Soil Grown Plants

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Abstract: In Ayurvedic medicine, *Bacopa monnieri* (L) is used as a nervine tonic to support mental health and enhance brain function. The main bacopasaponin component of *B. monnieri* is called bacoside A. By 2050, the world will witness a population of 9.7 billion, out of which the urban settlements will be 75%, as reported by the Food and Agriculture Organization (FAO) of the United Nations Organizations. Meeting the global demand for plants (food and medicinal) will be a major challenge as geoponic agriculture is facing turbulence due to rapid urbanization, climate change, natural disaster, soil pollution, poor soil fertility, dearth of water, frequent drought conditions, natural calamities, indiscriminate use of chemicals and pesticides leading to soil pollution, among various other aspects. Therefore, to increase the production of plants under the given condition, it is imperative to change our existing traditional cultivation practices and switch to the "soilless" mode of cultivation known as 'hydroponics'. Soilless cultivation with ubiquity and space efficiency, quantity and quality assurance, cost-effectiveness, limited water requirement, and sustainability has shown promising results across the globe, particularly on a large scale in urban and suburban scenarios. The current study was embarked to compare the phytochemical contents between the *B. monnieri* grown under hydroponic condition and soil grown. Results have shown higher content of bacoside and other active compounds under hydroponic condition than compared to soil grown plants. Therefore, medicinal plants can be cultivated under hydroponic conditions as an alternative to geoponic cultivation and avoid the problems unique to traditional practices.

Keywords: *Bacopa monnieri* • bacosides • hydroponics • HPTLC • HPTLC

Introduction

A medicinal herb *Bacopa monnieri* or commonly known as Brahmi, belonging to family Scrophulariaceae is found throughout the Indian subcontinent in wet, damp, marshy environments, and river banks sides (Kapoor, 1990). About 100 species are found in the warmer parts of the world, out of which 4 species, namely- *B. monnieri*, *B. hamiltoniana*, and *B. procumbens* are found in the Indian states.

It is used in Ayurvedic, or traditional Indian medicine, to relieve anxiety as well as to sharpen the mind and memory (Singh and Dhawan, 1997). In addition to being a memory-improving activity, it is also asserted to be beneficial in the treatment of cardiac, respiratory, and neuro-pharmacological problems, including stress, sleeplessness, insanity, depression, psychosis, and epilepsy (Russo and Borrelli, 1995). It was also claimed to have anti-inflammatory, analgesic, antipyretic, sedative, free radical scavenging, and anti-lipid peroxidative properties (Kishore and Singh, 2005; Anbarasi *et al.*, 2005). *B. monnieri*'s pharmacological effects have undergone substantial study (Deepak and Amit, 2004), and the actions are mostly related to the presence of distinctive saponins known as "bacosides." Bacopasaponins and other minor components like bacopasaponin F, bacopasaponin E, bacopaside N1, bacopaside III, IV, and V are among the phytoconstituents that have been reported to be present in the plant, including flavonoids (luteolin and apigenin), betulic acid, stigmastarol, beta-sitosterol, and flavonoids. The complex mixture of chemicals known as bacosides,

which are either glycosides of jujubogenin or pseudo-jujubogenin, are closely linked structurally. According to Kishore and Singh (2005), bacosides have been revealed to play a protective role in the hippocampus' synaptic functioning of the nerves.

For formulation today, standardisation and quantification of medicinal plant extracts are crucial (Shahare and D'Mello, 2010). The necessity to create sensitive and dependable quality control measures to prove the authenticity and purity of memory-boosting medications has become critical as the number of elderly persons experiencing cognitive impairments has increased (Om *et al.,* 2008). Both raw and prepared medicinal herbs are being used much more frequently. The WHO introduced the use of chromatography for standardising plant products, and it is now acknowledged as a method for identifying and assessing the quality of plant products (Farnsworth *et al.,* 1985; Brun, 1989; Quality control, 1992). The majority of bacoside analysis techniques published in the literature are based on UV spectroscopy (Singh *et al.,* 1988; Pal and Sarin, 1992), thin layer chromatography (Gupta *et al.,* 1998), and high-performance liquid chromatography (Deepak and Amit, 2004; Renukappa *et al.,* 1999; Ganzera *et al.,* 2004).

Saline stress is more easily managed in hydroponics than other environmental elements that affect plant secondary metabolites (such as light and temperature). Additionally, it is economical and simple to integrate the management of the electric conductivity of the nutrient solution in the growth systems that are already in use (Corrado *et al.,* 2021). In particular, on a commercial scale, soilless agriculture (hydroponics), with its ubiquity and space efficiency, quantity and quality assurance, cost-effectiveness, and minimal water requirement, has demonstrated encouraging results all over the world. The introduction of hydroponic technology to the artificial growing has been widely used to develop and utilise the medicinal potential of *B. monnerii* and to increase the output. Growing commercial crops, studying plants in the lab, and quickly modifying a plant's secondary metabolism to enhance the pharmacological effects of plant components are all made possible by the use of hydroponics. The year-round production of similar plant material in a controlled environment, the potential for improved phytochemicals, and protection against agrochemicals (pesticides, fungicides, etc.) used to manage various diseases in the open environment are all benefits of hydroponics. In order to cultivate *B. monnerii*, a straightforward, affordable, adaptable, and reliable hydroponics system was designed that takes into account the aforementioned factors. Thus, the aim of the present study is to estimate and compare the phytochemical marker Bacoside A from ethanolic extracts of soil and hydroponically produced *B. monnieri*, an appropriate and trustworthy quantitative High Performance Thin Layer Chromatography method.

Methods and Materials

Chemicals and Reagents: HPLC grade methanol, acetonitrile (ACN), orthophosphoric acid and potassium dihydrogen orthophosphate (KH_2PO_4) used in the study were obtained from Merck. HPLC grade water was obtained from PALL life sciences water purification system. Durapore PVDF 0.45 µm membrane filter was obtained from Merck. Solvent used for extraction is AR grade procured from Rankem. For the hydroponic cultivation study demineralized water and greenhouse nutrient solutions were used.

Plant collection and identification: The plant specimens of *B. monnieri* (Brahmi) was collected from cultivated sources of Uttar Pradesh (Sandila, Hardoi). Collected plant species made into herbarium specimens as per standard method and given in-house

voucher/field collection number (accession number-1129762).

Fig 1: *B. monnieri* in natural habitat (Chywan vatika)

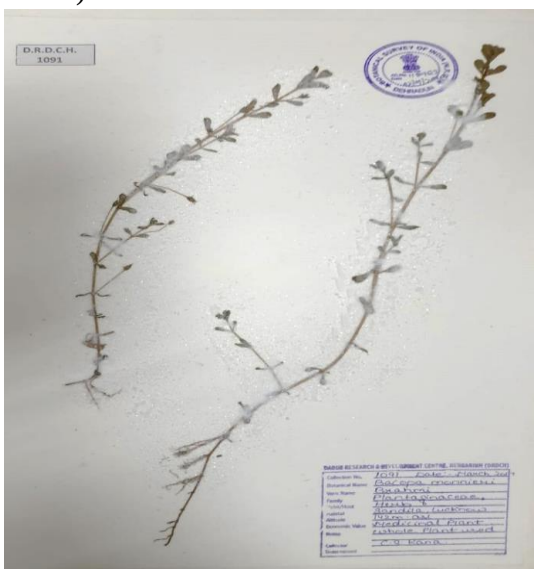

Fig 2. In-house herbarium specimen (accession number-) of *B. monnieri*

The plant species were identified by consulting the Herbarium of Botanical Survey of India, (BSD) Dehradun, Forest Research Institute (DD) Dehradun and Garhwal University Herbarium (GUH) Srinagar Garhwal, Uttarakhand. Raw material (RM) of the same has also been identified through powder microscopy and anatomical section as per standard methods. The whole plant parts of the plants (as a raw material) along with herbarium specimens (accession number-1129762) have kept in the museum as reference sample or as an in house standards for future references (Fig 2.).

Hydroponic cultivation system

The plants were collected from Sandila, UP hydroponically grown in 2.5-inch plastic containers (1 plants per container) filled with inert pot mixture containing neopeat and perlite (4:1), playing the role of holding the plant and providing aeration. Thirty-three such plastic containers were placed over three water pipes of 8 feet long as a hydroponic setup. The water pipes connected to 100 litre capacity water tanks were filled with demineralized water and greenhouse nutrient solution i.e. $Ca(NO_3)_2 \cdot 4H_2O$ (467.6 mg/l^{-1}), KNO_3 (232.3 mg/l^{-1}), KH_2PO_4 (272.0 mg/l^{-1}), K_2SO_4 (17.4 mg/l^{-1}), $MgSO_4 \cdot H_2O_2$ (209.1 mg/l^{-1}), NH_4NO_3 (80.0 mg/l^{-1}), Fe-EDTA (15.0 mg/l^{-1}), H_3BO_3 (1.4 mg/l^{-1}), $NaMoO_4 \cdot 2H_2O$ (0.12 mg/l^{-1}), $MnSO_4 \cdot 4H_2O$ (2.10 mg/l^{-1}), $ZnSO_4 \cdot 7H_2O$ (0.80 mg/l^{-1}), $CuSO_4 \cdot 5H_2O$ (0.20 mg/l^{-1}). The pH and Electrical Conductivity (EC) of these nutrient solutions were adjusted to 5.8 and 1.5 s/m, respectively, and the nutrient solution was renewed weekly. The nutrient solutions were weekly renewed in the tanks to prevent large fluctuations in EC, pH, and ionic concentrations. The plants were grown and maintained in greenhouse (Dabur Research and Development Centre, Dabur India Limited, Sahibabad, Ghaziabad-201010, Uttar Pradesh, India) with a controlled temperature of 25°C ± 2, relative humidity of 75%, and light intensity of approximately 200 µmol $m^{-2}s^{-1}$ provided by fluorescents lamps with a light to dark cycle of 16:8 h (Fig 3.). The whole plants were harvested after 3 weeks to determine the growth and secondary metabolite content. The experiments were set up in triplicates. For the phytochemical assays, the whole plant were harvested after a 3-week, sun-dried and extracted with methanol according to mention reference (year).

Preparation of alcohol extract: Dried whole plant part of *B. monnieri* (100 gm) were pulverised to get coarse ground powder.

Coarse ground powder taken in round bottom flask fitted with condenser and refluxed for 1 hr using water (6 volume w.r.t. batch size) as solvent at 80^0C.

Fig 3. *B. monnieri* under hydroponic conditions inside greenhouse

Then filtered through Whatman filter paper 4 (125 mm) and mark was again treated with ethanol and refluxed for 3 hrs. (4 volume w.r.t. batch size) at 80^0C. Then filtered through Whatman filter paper 4 (125 mm) to get the filtrate. The process repeated twice for 3 hrs by using fresh solvent till drug appear exhaust. Obtained filtrate was concentrated by rotary evaporator under vacuum to get fine powder.

Chromatographic methods HPLC Analysis

Preparation of Standard Solution

Stock solution of Bacoside A compound was prepared at a concentration of 2.0 mg/25 mL in methanol. From which 20 µL were injected in HPLC system for making standard curve.

Sample Preparation: Dried and finely milled plant materials (5.0 g) of *B. monnieri* were extracted with the aid of reflux and sonication in 50 mL methanol. The supernatant was transferred to a flask. The procedure was repeated thrice, and pooled extract was concentrated under vacuum and volume adjusted to 50.0 mL with methanol. Aliquots were filtered through 0.45 μm membrane filter before analysis. Similarly, 100 mg of dry extracts were dissolved in 25 ml methanol (HPLC grade) to get 1 mg/ml solution, filtered through 0.45 µm membrane filter and injected to waters HPLC system.

HPLC Instrumentation and Conditions

HPLC-UV/DAD analysis was performed on a Waters Alliance 2695 (Millford, MA, USA) system connected to Waters 2996 photodiode array detector (DAD). The chromatographic separation was performed using a Hyperclone BDS C18 LC column (250 × 4.6 mm, 5 μm, Phenomenex, USA) at 250C. The mobile phase comprised a gradient with two solvents – Solvent A and Solvent B.

HPLC mobile phase preparation: Solution A was made by dissolving 0.136 g of potassium dihydrogen orthophosphate in 900 mL of HPLC-grade water, adding 0.5 mL of o-phosphoric acid, and then bringing the total volume up to 1000 mL with water. The solution was filtered through a 0.45 µm membrane filter and degassed in a sonicator for 3 minutes. Solution B consisted of acetonitrile. The mobile phase was run with gradient elution as follows: starting at 30% Solution B, increasing to 40% over 25 minutes, then to 65% over the next 5 minutes, to 85% over the next 5 minutes, maintained at 90% for 5 minutes, increased to 95% over the next 5 minutes, then brought back down to 30% over the next 5 minutes, followed by a 5-minute equilibration period. The flow rate was set at 1.5 mL/min, and the injection volume was 20 µL. Detection and analysis of the eluents were performed at 205 nm.

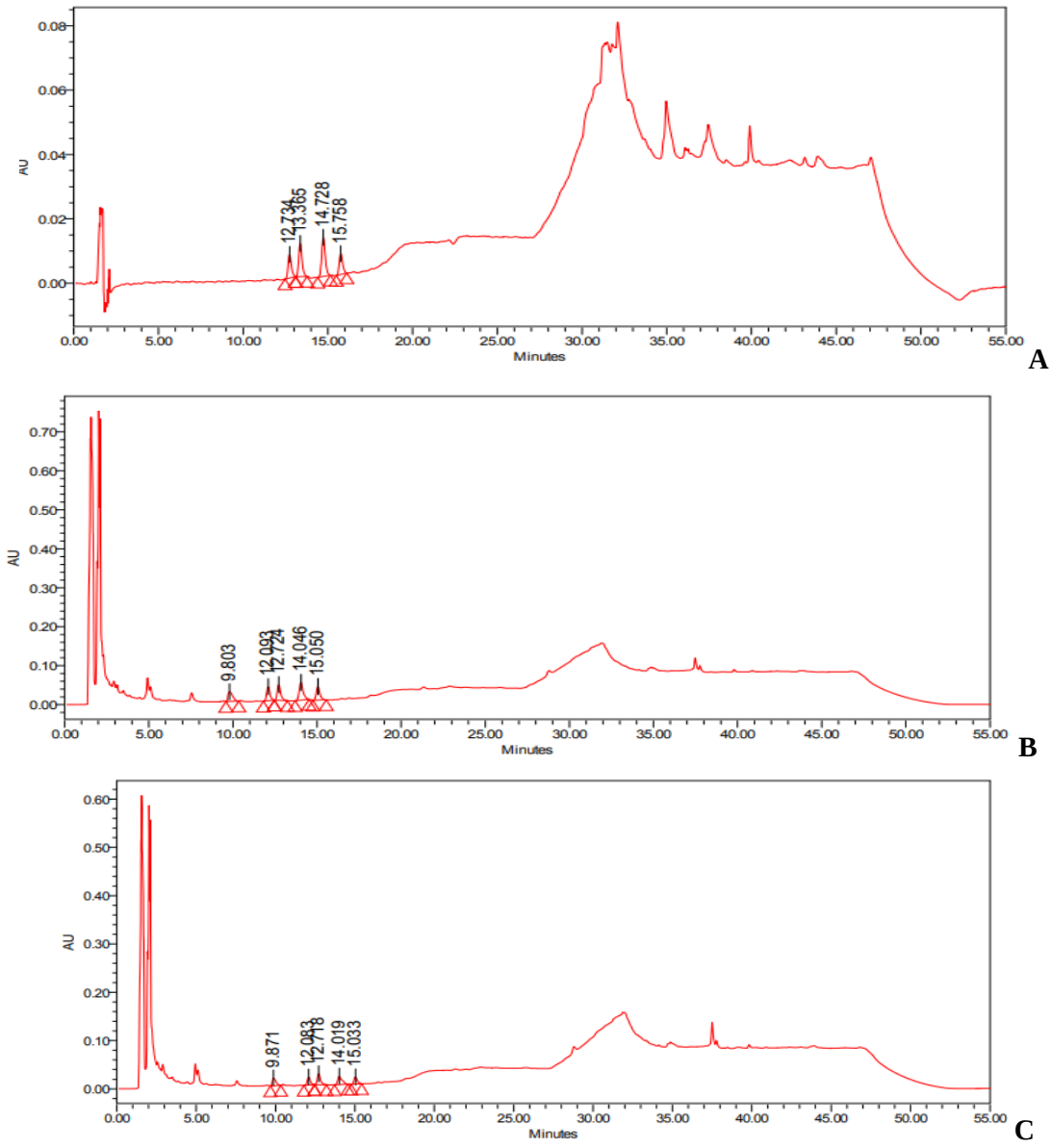

Figure 4. HPLC chromatogram of *B. monnieri*. (A) Std Bacosides A. (B) alcoholic extracts of samples collected from hydroponic system extract; (C) alcoholic extracts of samples collected from Chywan vatika.

Results and discussion

Extract yield: Alcohol extract of *B. monnieri* whole plant shows higher percentage of yield (Table 1). In this study, enriched ethanol extract yield found in the range of 11.83 to 12.83.

HPLC Analysis: Initially various mobile phases were tried in attempts to obtain the best separation and resolution of bacosides in extracts. The mobile phase consisting of gradient elution of potassium dihydrogen orthophosphate (0.136 g) in water with 0.5 mL of o-phosphoric acid (solvent A) and acetonitrile (solvent B) was found to be an appropriate mobile phase allowing adequate separation of bacosides using Hyperclone BDS C18 LC column column at a flow rate of 1.5 ml/min. Under this system, the chromatogram of Bacoside A and extracts of *B. monnieri* is shown in Fig. 4. The injection volume was 20 μL. Signal was monitored at 205 nm.

Quantitative estimation of Bacoside in different extracts of *B. monnieri* by HPLC

Bacoside A reference standard and extracts sample solutions were run in gradient mobile phase systems mentioned in HPLC methodology section. Highest bacoside content (19.93%) found in ethanol extract of *B. monnieri* collected from hydroponic system in comparison with another sample (wild) which was collected from Chywan vatika. The highest total bacosides content (1.78%) found in raw material of *B. monnieri* collected from hydroponic system (Table 2).

Table 1. Percentage of yield of different extracts of *B. monnieri*

Plant name	Part used	Collection region	Name of Solvent	Weight of plant material (g)	Weight of dried extract (g)	Extractive value (%)
B. monnieri	Whole plant	Hydroponic	Ethanol	100	12.83	12.83
		Chywan vatika	Ethanol	100	11.83	11.83

Table 2. Bacosides content in raw materials and extracts of *B. monnieri* collected from hydroponic system and Chywan vatika

Plant Name	Sample	Region	%Results (w/w)[a]
B. monnieri (Whole plant)	Dried whole plant	Hydroponic	1.78
	Dried whole plant	Chywan vatika	0.74
	Alcohol extract	Hydroponic	19.93
	Alcohol extract	Chywan vatika	9.87

[a] the results (%w/w) presented in the above table are the average values of 'n' concentrations of sample solutions (n =2).

Conclusion

This study presents a comparative analysis of *B. monnier*i for secondary metabolite production between hydroponic and conventional cultivation methods. Effective nutrient management in hydroponic systems is essential for maintaining optimal medicinal herb characteristics, physiological health, productivity, and the phytochemical properties of therapeutic plants. The ethanolic extracts of B. monnieri grown hydroponically exhibited high levels of secondary metabolites, particularly bacosides. Our findings demonstrate that *B. monnieri* grown hydroponically for a short period (three weeks) produced higher and more diverse bacoside content compared to those grown conventionally over a longer duration. These results suggest that hydroponic cultivation can significantly enhance the quality of *B. monnieri*, making it a promising method for the pharmaceutical and nutraceutical industries.

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