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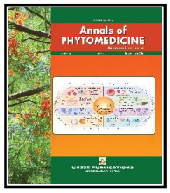

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Elucidation of nutritional and bioactive phytochemical profiling in elite Brahmi (*Bacopa monnieri* (L.) Wettst.) genotypes

C.S. Karthik*, K. Hima Bindu*◆, K.S. Divya*, V.K. Rao, B. Sreenu**, Shivanand D. Ainapur* and V. Sreenivasa***

**Division of Flower and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru-560 089, Karnataka, India*
***Division of Basic Sciences, ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru-560 089, Karnataka, India*

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Abstract

Bacopa monnieri (L.) Wettst. is a medicinal herb known for enhancing memory, mental alertness and reading skills earning it the title of "memory booster" or "thinking person's herb". The experiment was conducted to assess the phytochemical constituents, antioxidant activity, saponin content and essential macro and microelements, and to elucidate their associations with bioactive traits among 11 diverse brahmi genotypes, *viz*., IIHR-BM-06, IIHR-BM-82, IIHR-BM-Sel.08, IIHR-BM-101, IIHR-BM-Sel.07, IIHR-BM-99, IIHR-BM-Sel.03, IIHR-BM-Sel.02, IIHR-BM-Sel.05 and IIHR-BM-Sel.09 collected from diverse agro-ecological regions of India, with a check variety CIM-Jagriti. The outcomes depicted that the total phenols (36.39 mg/g), tannins (15.32 mg/g) and flavonoids (20.94 mg/g) were higher in IIHR-BM-Sel.02. The maximum content of DPPH activity was exhibited in IIHR-BM-Sel.07 (16.90 mg/g AAE) and highest FRAP activity in IIHR-BM-82 (44.80 mg/g). Similarly, among saponins, highest content of bacoside A3 (1.08%), jujubogenin (0.73%), bacopasaponin C (0.66%) and total bacoside A (3.27%) were observed in IIHR-BM-Sel.08. With respect to elemental analysis, the nitrogen content has varied from 1.84 to 3.42 per cent; however, phosphorous and potassium content ranged from 0.25 to 0.42 and 0.64 to 0.98 per cent. The microelements differed prominently amid genotypes, where IIHR-BM-Sel.08 recorded higher copper (26.51 ppm), CIM-Jagriti (Check) recorded higher zinc (77.21 ppm) while manganese (69.27 ppm) and iron (2321.27 ppm) in IIHR-BM-101. Phenols displayed a strong positive correlation with both tannins and flavonoids. DPPH activity was positively correlated with phenols and flavonoids, suggesting their major contribution to antioxidant capacity. Among saponin components, bacoside A3 showed strong positive relationships with jujubogenin and total bacoside A, whereas bacopaside II exhibited a negative correlation indicating coordinated saponin biosynthesis. Jujubogenin also exhibited positive associations with total bacoside A and bacosaponin C. The results clearly indicate that the evaluated brahmi genotypes exhibit strong potential based on their phytochemical, antioxidant, saponin compounds and elemental composition, highlighting them as a promising, nutritionally rich leafy medicinal crop and a viable alternative to conventional leafy vegetables to convene the growing requirements of the universal society and pharmaceutical sector.

1. Introduction

B. monnieri, belonging to the family Plantaginaceae, is generally recognized as 'Brahmi', 'Neeru-Brahmi' or 'Jalanimba'. It is a glabrous, perennial, creeping herb, succulent that branches profusely and roots at the nodes. India is one of the centers of origin of brahmi, and the species is widely distributed across the Indian subcontinent (Karthikeyan *et al*., 2011). It is also considered as a rejuvenating plant because of its medicinal properties and is a chief component in many Ayurvedic therapies (Kapil and Sharma, 2014). Since ancient times, Indians have greatly relied on several medicinal plants due to their numerous uses found in many parts. Ayurveda, Unani and Siddha are indigenous systems of medicine in which the use of plants for promoting individual's health is very prevalent. This herb finds essentially its mention in the "Charaka Samhita" during 6th Century AD which was recommended for poor cognition, anxiety, lack of concentration, memory enhancement, *etc*., further it aids as a diuretic, aphrodisiac and in the healing of Alzheimer's sickness and further psychological ailments, thereby underscoring its longstanding ethnopharmacological relevance (Devendra *et al*., 2018).

In plant materials, the most essential regular metabolic activities are governed by phytochemical constituents, which are biologically active chemical compounds. Furthermore, phytochemicals are categorized into two groups as primary and secondary metabolites based on plant growth, development and life cycle. Primary metabolites such as proteins, carbohydrates and lipids are crucial for plant function. Secondary metabolites, which are not vital for basic functions, but are formed as by-products in biochemical pathways. These contains a diverse range of bioactive compounds such as alkaloids, terpenoids, saponins, anthocyanins and phenolic compounds like flavonoids, tannins, *etc*. (Krishnaiah *et al*., 2009; Shivanand *et al*., 2025). Phenols, flavonoids, tannins, carotenoids, vitamins and anthocyanin are some of the important natural antioxidants essentially required for the functioning of human bodies, and therefore, plants are the richest source of biologically active

Corresponding author: Dr. K. Hima Bindu
Principal Scientist, Division of Flower and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru-560 089, Karnataka, India
E-mail: kaipa.himabindu@gmail.com
Tel.: +91-9449040463

components (Gayathri *et al*., 2021). Brahmi consists of several kind of compounds like alkaloids (brahmine, nicotine and herpestine), sterols (stigmasterol, stigmastano and β-sitosterol), flavonoids (apigenin and luteolin) and saponins (hersaponin, monnierin, bacopaside I, bacoside A and bacoside B) (Saha *et al*., 2020). The chief bioactive constituents of this herb are tetracyclic triterpenoid saponins known as bacosides A and B, with bacoside A being the predominant component, responsible for its broad pharmacological activities like anti-inflammatory, antioxidant, antiulcer, antipyretic, laxative, cooling, cardioprotective and adsorptive properties (Jain *et al*., 2016). In addition, brahmi is a wealthy origin of natural antioxidants such as phenolics, flavonoids, carotenoids and vitamins, which play a crucial role in mitigating oxidative stress and protecting biomolecules from free radical-induced damage (Phang *et al*., 2013 and Sharad and Radhika, 2014). Beside its immense medicinal principles, it is also in use as a leafy vegetable in certain regions, contributing essential nutrients, including proteins, vitamins, dietary fibres and macro (N, P, K, Ca, Mg, S) and micro (Cu, Zn, Mn, Fe) minerals, thereby highlighting its dual role as a therapeutic and nutritional resource (Genders, 2007; Nath *et al*., 2004).

According to the National Medicinal Plants Board (NMPB), Ministry of AYUSH, Government of India and the Technology Information, Forecasting and Assessment Council (TIFAC), New Delhi, brahmi is one amidst the seven prioritized medicinal plants comprehensible for research and cultivation. It is also classified as a highly endangered species and listed among 178 medicinal plant species traded in high volumes, ranging from 2000 to 5000 metric tonnes annually (Sudhakaran, 2020). Owing to its long recognized therapeutic properties, particularly as a memory enhancer and vitality promoter, it is often referred to as a "Celestial drug". Despite its immense medicinal and nutritional importance, *B. monnieri* faces increasing demand due to commercial usage. Although, several studies have documented its phytochemical composition and therapeutic potential, systematic investigations integrating biochemical, nutrient and bioactive compound profiling across diverse genotypes remain limited. More importantly, there is a lack of comprehensive studies that establish relationships among these traits and their utility in guiding selection strategies for genetic improvement. Such information is crucial in identifying the elite genotypes with superior biochemical and nutritional attributes, which can be effectively utilized in breeding programs and thereby supporting both conservation and commercial cultivation efforts. In this context, the present study was undertaken with an objective to assess the biochemical, nutritional profiling and to examine the interrelationships among traits through correlation analysis in elite brahmi genotypes.

2. Materials and Methods

2.1 Experimental site

The investigation was conducted at the Division of Flower and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru, Karnataka, India during 2024-25, located at an elevation of 890 m above mean sea level (MSL), at 13.58° N latitude and 78.45° E longitude. Eleven *B. monnieri* genotypes (Herbarium No. 6965) were authenticated by Dr. Noorunnisa Begum, Professor, Conservation of Natural Resources, Foundation for Revitalisation of Local Health Traditions (FRLHT), The University of Trans-Disciplinary Health Sciences and Technology (TDU), Yelahanka, Bengaluru. They were collected from diverse agro-ecological regions of India and details of collection sources are presented in Table 1. After collection, rooted cuttings of the genotypes were multiplied and maintained in the Medicinal Crops Field Gene Bank established at ICAR-IIHR, Hesaraghatta, Bengaluru.

Table 1: Details of the *B. monnieri* genotypes considered in the present study

S. No.	Genotype number/name	Indigenous collection number	Source of collection	Latitude (N)	Longitude(E)	Altitude (m)
1.	IIHR-BM-06	IC 653636	West Bengal	22.11°	88.58°	7
2.	IIHR-BM-82	IC 342108	Gujarat	23.02°	72.57°	55
3.	IIHR-BM-Sel.08	IC 321278	Himachal Pradesh	31.01°	77.49°	3647
4.	IIHR-BM-101	IC 565499	Kerala	8.89°	76.61°	7
5.	IIHR-BM-Sel.07	IC 658763	Kerala	10.64°	76.54°	57
6.	IIHR-BM-99	IC 256496	Kerala	10.53°	76.22°	3
7.	IIHR-BM-Sel.03	IC 353203	Delhi	28.39°	77.07°	248
8.	IIHR-BM-Sel.02	IC 392842	Odisha	21.56°	86.44°	684
9.	CIM-Jagriti (Check)	CIM-Jagriti	Uttar Pradesh	26.85°	80.95°	126
10.	IIHR-BM-Sel.05	IC 658761	West Bengal	22.50°	88.39°	6
11.	IIHR-BM-Sel.09	IC 565499	Kerala	8.89°	76.59°	5

2.2 Quantitative analysis of phytochemicals

All experiments, including phytochemical constituents, antioxidant activity and saponin components were performed at the Medicinal Crops Laboratory, ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru, Karnataka, India.

2.2.1 Chemicals and reagents

Methanol (CH_3OH; 80%; extrapure AR grade, 99.8% purity) and sodium carbonate (Na_2CO_3; 20%; extrapure AR grade, 99.9% purity) were bought from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Folin-Ciocalteu reagent (FCR) was sourced from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India. While gallic acid ($C_7H_6O_5$;

AR grade, 98% purity), Insoluble polyvinyl polypyrrolidone (PVPP K-30 $(C_6H_9NO)n$; 99.9% purity) and 4N sodium hydroxide (NaOH; 98.5% purity) were procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Aluminium chloride ($AlCl_3$; 10%) and sodium nitrite ($NaNO_2$; 5%) of AR grade (98% purity) were purchased from SD Fine-Chem. Ltd., Mumbai, India. Standard catechin ($C_{15}H_{14}O_6$; 100 µg/ml; analytical grade, 98% purity), 2,2-diphenyl-1-picrylhydrazyl ($C_{18}H_{12}N_5O_6$; DPPH), and 2,4,6-tris(2-pyridyl)-s-triazine ($C_{18}H_{12}N_6$; TPTZ) (extrapure AR grade, 99.9%, purity) were obtained from Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru, India.

2.2.2 Extract preparation

Herbal material from eleven brahmi genotypes was obtained by harvesting five randomly selected plants under each genotype, which were subsequently pooled for extract preparation. The freshly collected plant material was thoroughly rinsed with distilled water, air-dried under shade for 48 h and further dried in a hot air oven at 60°C till constant weight is attained (6 h). The dried samples were turned into a fine powder and passed through a sieve to ensure uniform particle size. For extraction, 0.2 g of the powdered material was soaked in 10 ml of 80% methanol and incubated overnight. The resulting extracts were filtered and stored at 4°C until further analysis of total phenols, flavonoids and tannins content.

2.2.3 Determination of total phenolic content (TPC)

Total phenol content was quantified using Folin-Ciocalteau method illustrated by Slinkard *et al*. (1999). A known volume of 0.2 ml aliquot was thoroughly mixed with 0.5 ml FCR and 7.3 ml of double-distilled water. After 10 min, 2 ml of 20% sodium carbonate solution was added, and the mixture was incubated in the dark for 1 h. The resulting blue color was observed at 660 nm using a UV-Visible spectrophotometer (Systronics, India, ver. 119). Gallic acid was utilized as a standard and results were expressed as (mg/g) gallic acid equivalents (GAE).

2.2.4 Determination of total flavonoid content (TFC)

Total flavonoid content was estimated deploying aluminum chloride colorimetric method defined by Chang *et al*. (2002). A 0.5 ml aliquot of the extract was diluted with 4.5 ml of double-distilled water, followed by the addition of 0.3 ml of 5% sodium nitrite. After 5 min, 0.3 ml of 10% aluminum chloride was added. Subsequently, 3.4 ml of 4N sodium hydroxide and 1 ml of double-distilled water were added to the mixture. The absorbance was noted at 510 nm using a UV-Visible spectrophotometer (Systronics, India, ver. 119). Catechin was used as the reference standard and flavonoid content was expressed as mg/g.

2.2.5 Determination of total tannin content (TTC)

Total tannin content was resolved following the Folin-Ciocalteu method with polyvinyl polypyrrolidone (PVPP) as described by Makkar (2003). An aliquot of 0.2 ml extract was mixed with 40 mg PVPP and 7.3 ml double-distilled water. Subsequently, 0.5 ml of Folin-Ciocalteau reagent (FCR) was added, vortexed and allowed to react for 10-15 min. This was, followed by adding 2 ml of 20% sodium carbonate and the mixture was incubated in the dark for 1 h. Absorbance was monitored at 660 nm using a UV-Visible spectrophotometer (Systronics, India, ver. 119). Non-tannin phenolics were quantified and tannin content was calculated by subtracting non-tannin phenols from total phenols. Results were expressed as gallic acid equivalents (GAE) (mg/g).

2.3 Determination of antioxidant activity

2.3.1 Extract preparation

A 0.1 g of finely ground material was extracted with 15 ml of 80% methanol and kept overnight at 4°C. Methanolic extracts were separated and utilized to assess antioxidant capacity through DPPH and FRAP assays. Antioxidant activity was expressed as milligrams of ascorbic acid equivalents per gram of dry weight (mg/g AAE).

2.3.2 2,2-diphenyl-1 picryl hydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity was estimated using the DPPH method as interpreted by Sundang *et al*. (2012) with minor alterations. 0.1 ml of the extract was combined with 6 ml of DPPH solution and incubated at 37^{O}C for about 30 min. The reduction in absorbance was recorded at 517 nm against a methanol blank using a UV-Visible spectrophotometer ver. 119.

2.3.3 Ferric reducing antioxidant potential (FRAP) assay

The ferric reducing ability of the extracts was outlined following the procedure of Benzie and Strain (1996). The stock solution of 5 ml acetate buffer, 0.5 ml each of TPTZ and ferric chloride was prepared. Then plant extract of 0.1 ml was added to 6 ml of FRAP reagent and incubated at 37^{O}C for 1 h. Absorbance at 593 nm was recorded using a UV-Visible spectrophotometer ver. 119 against a methanol blank.

2.4 Estimation of saponins by reverse-phase high performance liquid chromatography (RP-HPLC)

Bacoside A, a major secondary metabolite of *B. monnieri*, was estimated based on the procedure reported by Smitha *et al*. (2021) with minor adjustments. The bioactive estimation for analysing the mixture of total bacoside A components like bacoside A3, bacopaside II, jujubogenin and bacopasaponin C was done using Nexera X2 HPLC system (Shimadzu, Japan) equipped with an LC-30AD pump and an SPD-M20A photodiode array detector (PDA), operated with Lab Solutions Chromatography software (version 5.54 SP5).

2.4.1 Standards and reagents used

Anhydrous potassium dihydrogen phosphate (KH_2PO_4; ACS grade, 99% purity) and orthophosphoric acid (H_3PO_4; HPLC grade, 99% purity), along with methanol (CH_3OH; HPLC grade) and acetonitrile (CH_3CN; gradient grade, 99.9% purity), were obtained from Merck Life Science Pvt. Ltd., Mumbai, India. The bacoside A reference standard (>95% purity), comprising bacoside A3, bacopaside II, jujubogenin and bacopasaponin C was bought from Natural Remedies Pvt. Ltd., Bengaluru, India.

2.4.2 Sample preparation

Following the procedure used for phytochemical quantification, powdered samples were dried in a hot air oven at 50°C for 30 min to discard residual moisture and admitted to cool to ambient temperature. Precisely weighed 1.5 g of the dried powder was transferred to a 100 ml beaker, mixed with 40 ml of HPLC grade methanol and heated on a water bath supported at 90°C for 20 min with constant stirring. After cooling, the extract was shifted to a 50 ml volumetric flask. The remaining residue was subjected to sequential re-extraction with 30 ml and 20 ml of methanol for 10 min each under identical conditions. The pooled extracts were corrected to 50 ml volume with methanol, blended intensively, allowed to settle and filtered over a 0.2 µm nylon membrane filter prior to HPLC analysis.

2.4.3 Standard preparation

A stock standard solution was qualified by dissolving 5.4 mg of bacoside A in HPLC grade methanol to a 10 ml volumetric flask, assisted by sonication for 5 min. The final volume was modified with 10 ml methanol and blended intensively. A series of injections was made from lower to higher combinations of the standards. For calibration, peaks with a retention time of 16.946 min for bacoside A3, 17.530 min for bacopaside II, 19.110 min for jujubogenin and 20.003 min for bacopasaponin C were recognized as the standard concentration peaks were depicted in Figure 1. The peak at the definite retention time was achieved by replicated injections of the standard.

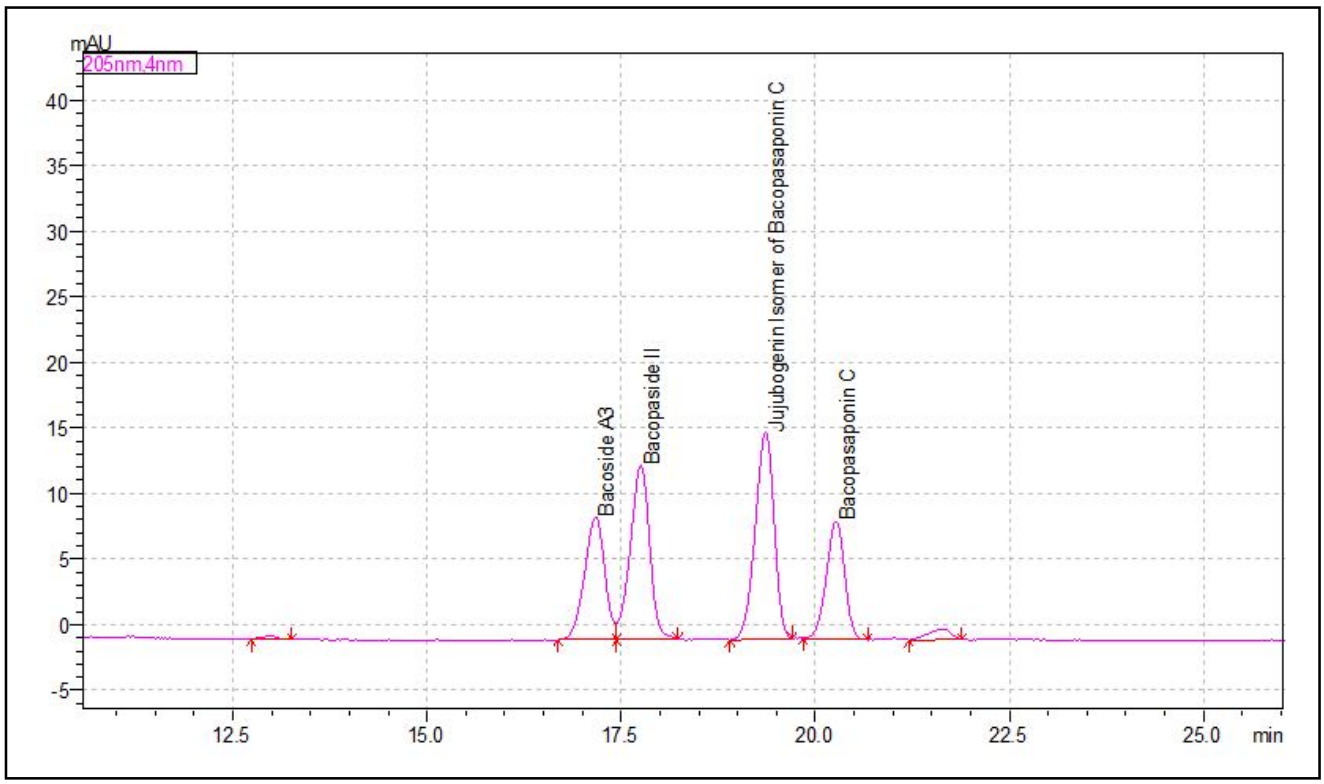

Figure 1: Chromatogram of *B. monnieri* bioactive standards.

2.4.4 Chromatographic conditions

Chromatographic separation was performed using a binary mobile phase system comprising potassium dihydrogen orthophosphate buffer (Solvent A) and acetonitrile (Solvent B), conveyed at a constant flow rate of 1.5 ml/min. Prior to analysis, both solvents were allowed over a 0.45 µm nylon membrane filter and degassed using a sonicator. Partition was accomplished on an ID CAPCELL PAK C18 column (Shiseido; 250 mm × 4.6 mm, 5 µm particle size) maintained at 27°C. Detection was prescribed out at 205 nm with an injection volume of 20 µl, and the complete run time for each analysis was 55 min. The gradient program employed for RP-HPLC analysis is detailed in Table 2.

Table 2: Gradient elution program for HPLC analysis

Time (in min)	Mobile phase A (%)	Mobile phase B (%)
0.01 - 25	70 → 60	30 → 40
25 - 30	60 → 35	40 → 65
30 - 35	35 → 15	65 → 85
35 - 40	15 → 10	85 → 90
40 - 45	10	90
45 - 50	10 → 70	90 → 30
50 - 55	70	30

2.4.5 Calculations

The bacoside concentrations in the samples were quantified using peak area comparisons with the external standard. The individual concentrations of bacoside A3, bacopaside II, jujubogenin and bacopasaponin C were calculated independently and their cumulative sum was expressed as total bacoside A content (w/w%) according to the formula:

$$\text{Bacoside A (\%)} = \frac{\text{Sample area} \times \text{Standard weight (mg)} \times \text{Sample dilution} \times \text{Purity of the standard}}{\text{Standard area} \times \text{Sample weight (mg)} \times \text{Standard dilution}}$$

2.5 Elemental analysis

The finely ground plant samples of different brahmi genotypes were analyzed at Bio Centre, Hulimavu, Bengaluru, State Department of Horticulture, Karnataka, India. Samples were digested using a conc. Nitric acid (HNO_3) and Perchloric acid ($HClO_4$). The resulting digests were analyzed for mineral elements employing Atomic Absorption Spectroscopy (AAS) (Perkin Elmer, USA) following established protocols (Prieto *et al*., 1999; Ozcan, 2004).

2.6 Statistical analysis

Experiment was statistically laid out using a Randomized Complete Block Design and the results were analyzed using SPSS version 31.0.2.0 and Microsoft Excel software packages. Treatment means were expressed including the standard error of the mean (SE) and the outcome was conferred at 5% level of significance *(p= 0.05)*. The critical difference (CD) values were estimated to facilitate comparison among treatment method. Associations among phytochemical constituents, antioxidant capacity and saponin components were examined adopting Pearson's correlation coefficient (r).

3. Results

3.1 Phytochemical analysis

A comprehensive quantitative assessment of methanolic extracts from powdered brahmi samples was carried out to estimate major phytochemical constituents like total phenols, flavonoids, tannins and antioxidant activity measured through DPPH and FRAP assays are depicted in Table 3.

3.1.1 Quantification of total phenolic content (TPC)

Phenolic compounds are broadly acknowledged for their health promoting properties and their role in disease prevention through dietary intake. The total phenolic content (TPC) among brahmi genotypes was quantified adopting the Folin-Ciocalteau method with gallic acid as the reference standard as shown in Figure 2. Total phenol content in brahmi genotypes ranged from 20.29 to 36.39 mg/g DW, IIHR-BM-Sel.02 recorded the significantly highest content, followed by IIHR-BM-Sel.09 (33.36 mg/g DW) and CIM-Jagriti (Check) (33.21 mg/g DW), while IIHR-BM-99 exhibited the lowest TPC content.

Table 3: Phytochemical constituents in different genotypes of *B. monnieri*

Genotypes	Total phenols (mg/g)	Total tannins (mg/g)	Total flavonoids (mg/g)	DPPH assay (mg/g)	FRAP assay (mg/g)
IIHR-BM-06	32.68	12.18	19.69	11.95	18.27
IIHR-BM-82	29.61	12.00	17.25	10.80	44.80
IIHR-BM-Sel.08	24.39	5.54	17.91	8.40	31.89
IIHR-BM-101	24.89	7.96	14.97	5.30	30.57
IIHR-BM-Sel.07	26.00	7.71	19.06	16.90	33.29
IIHR-BM-99	20.29	2.50	11.78	4.90	26.39
IIHR-BM-Sel.03	28.57	12.18	15.56	8.10	35.19
IIHR-BM-Sel.02	36.39	15.32	20.94	14.80	42.78
CIM-Jagriti	33.21	12.39	20.06	9.05	38.74
IIHR-BM-Sel.05	23.68	4.04	13.59	7.70	18.23
IIHR-BM-Sel.09	33.36	12.43	20.34	12.85	41.12
SE	0.438	0.179	0.262	0.172	0.569
CD *(p=0.05)*	1.302	0.531	0.778	0.512	1.689

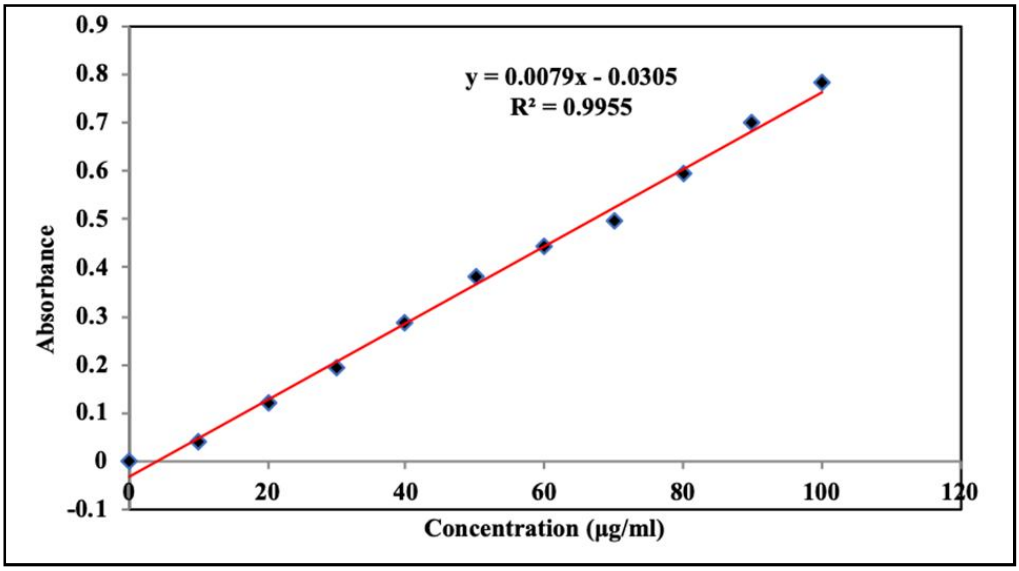

Figure 2: Standard calibration curve of gallic acid for total phenol content.

3.1.2 Quantification of total flavonoid content (TFC)

Flavonoid content was quantified based on catechin standard curve are presented in Figure 3. Total flavonoid content in brahmi genotypes ranged from 11.78 to 20.94 mg/g DW, where IIHR-BM-Sel.02 recorded the highest TFC, followed by IIHR-BM-Sel.09 (20.34 mg/g DW) and CIM-Jagriti (Check) (20.06 mg/g DW). While lowest TFC were recorded in IIHR-BM-99 and IIHR-BM-Sel.05 (13.59 mg/g DW) and IIHR-BM-101 (14.97 mg/g DW).

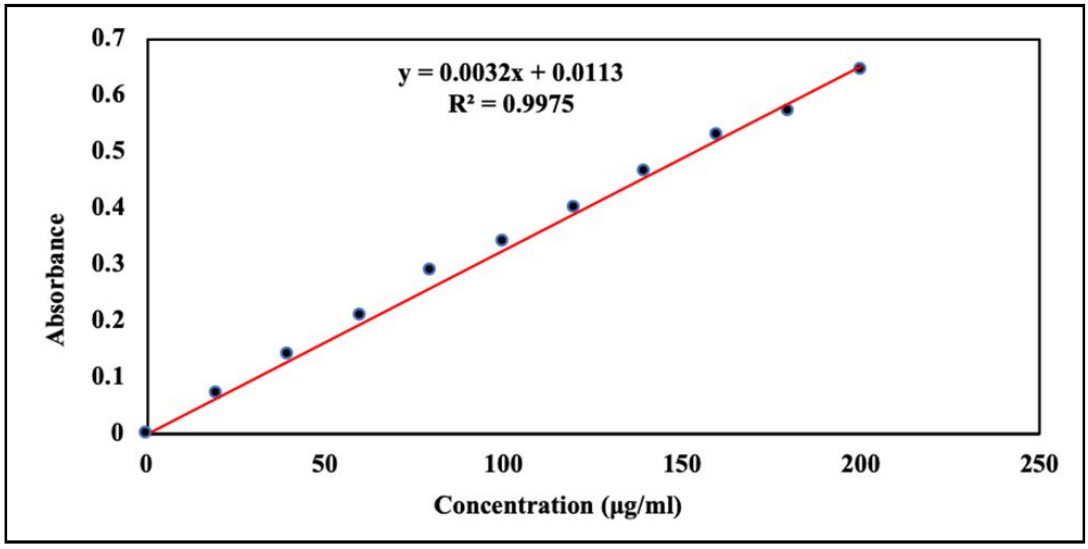

Figure 3: Standard calibration curve of catechin for total flavonoid content.

3.1.3 Quantification of total tannin content (TTC)

Total tannin content was estimated by utilizing the Folin-Ciocalteau method exhibited significant different among the genotypes which ranged from 2.50 to15.32 mg/g DW, where IIHR-BM-Sel.02 significantly highest TTC, followed by IIHR-BM-Sel.09 (12.43 mg/g). Whereas, the minimum TTC was found in IIHR-BM-99, followed by IIHR-BM-Sel.05 (4.04 mg/g) and IIHR-BM-Sel.08 (5.54 mg/g).

3.2 Quantification of antioxidant activity

3.2.1 Determination of DPPH radical scavenging assay

Antioxidant activity calculated by DPPH radical scavenging assay revealed significant variations among the genotypes were represented in Figure 4. It is evident from the outcome that the maximum DPPH activity was documented in IIHR-BM-Sel.07 (16.90 mg/g AAE), followed by IIHR-BM-Sel.02 (14.80 mg/g AAE) and IIHR-BM-Sel.09 (12.85 mg/g) of ascorbic acid equivalent. Whereas, the genotype IIHR-BM-99 (4.90 mg/g AAE), IIHR-BM-101(5.30 mg/g AAE) and IIHR-BM-Sel.05 (7.70 mg/g AAE) recorded the lowest.

3.2.2 Determination of FRAP assay

The FRAP assay further confirmed significant variation in antioxidant potential among genotypes is depicted in Figure 5. IIHR-BM-82 recorded the highest ferric reducing capacity (44.80 mg/g), followed by IIHR-BM-Sel.02 (42.78 mg/g) and IIHR-BM-Sel.09 (41.12 mg/g). Further, the lowest FRAP value was noted in IIHR-BM-Sel.05 (18.23 mg/g).

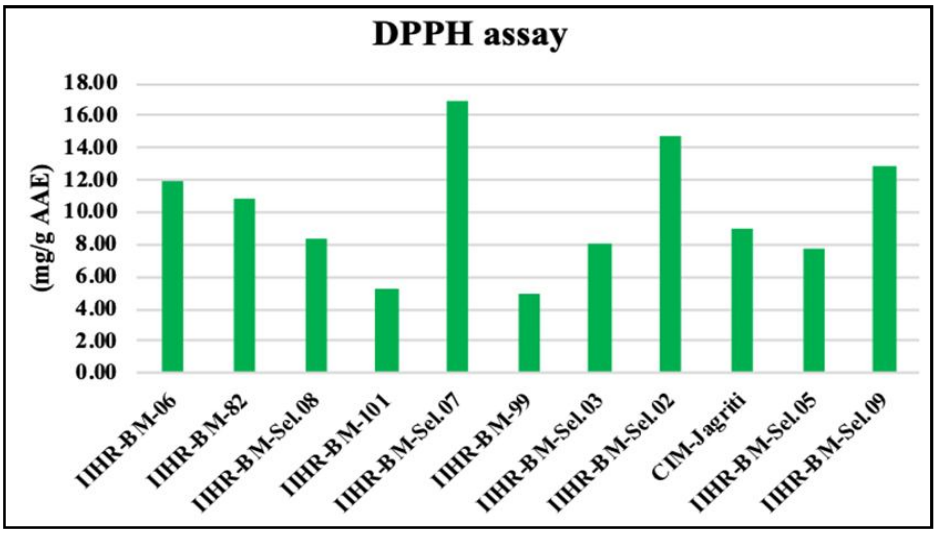

Figure 4: Graphical representation of DPPH assay of different brahmi genotypes.

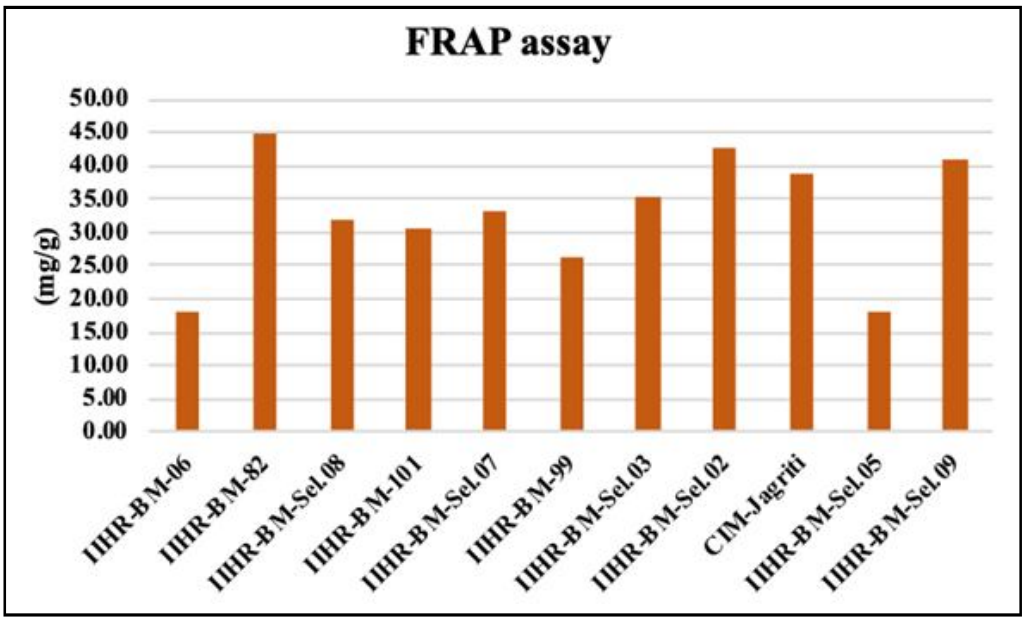

Figure 5: Graphical representation of FRAP assay of different brahmi genotypes.

3.3 Quantification of saponin components

Quantitative profiling of saponin components among eleven *B. monnieri* genotypes is listed in Table 4 and presented in Figures 6 and 7. Among the different genotypes, IIHR-BM-Sel.08 exhibited the highest concentrations of bacoside A3, jujubogenin, bacopasaponin C and total bacoside A (1.08%, 0.73%, 0.66% and 3.27%, respectively), followed by IIHR-BM-82 (2.98%) and IIHR-BM-06 (2.95%). Similarly, lowest bacopasaponin C (0.27%) and total Bacoside A (2.05%) concentrations were recorded with IIHR-BM-99 and IIHR-BM-Sel.09. Whereas, CIM-Jagriti (Check) (2.47%), followed by IIHR-BM-99 (2.54%) and IIHR-BM-Sel.03 (2.61%) also recorded lowest total bacoside A (%).

Table 4: Saponin components in different genotypes of *B. monnieri*

Genotypes	Bacoside A3 (%)	Bacopaside II (%)	Jujubogenin (%)	Bacopasaponin C (%)	Total bacoside A (%) (w/w)
IIHR-BM-06	0.89	0.86	0.65	0.56	2.95
IIHR-BM-82	0.99	0.77	0.63	0.59	2.98
IIHR-BM-Sel.08	1.08	0.80	0.73	0.66	3.27
IIHR-BM-101	0.81	1.11	0.55	0.48	2.94
IIHR-BM-Sel.07	0.54	1.28	0.44	0.56	2.83
IIHR-BM-99	0.78	1.13	0.37	0.27	2.54
IIHR-BM-Sel.03	0.70	0.98	0.52	0.41	2.61
IIHR-BM-Sel.02	0.94	0.75	0.63	0.61	2.94
CIM-Jagriti	0.49	1.23	0.26	0.49	2.47
IIHR-BM-Sel.05	0.96	0.83	0.59	0.53	2.91
IIHR-BM-Sel.09	0.49	0.89	0.34	0.34	2.05
SE	0.014	0.013	0.009	0.009	0.043
CD *(p=0.05)*	0.040	0.040	0.027	0.026	0.127

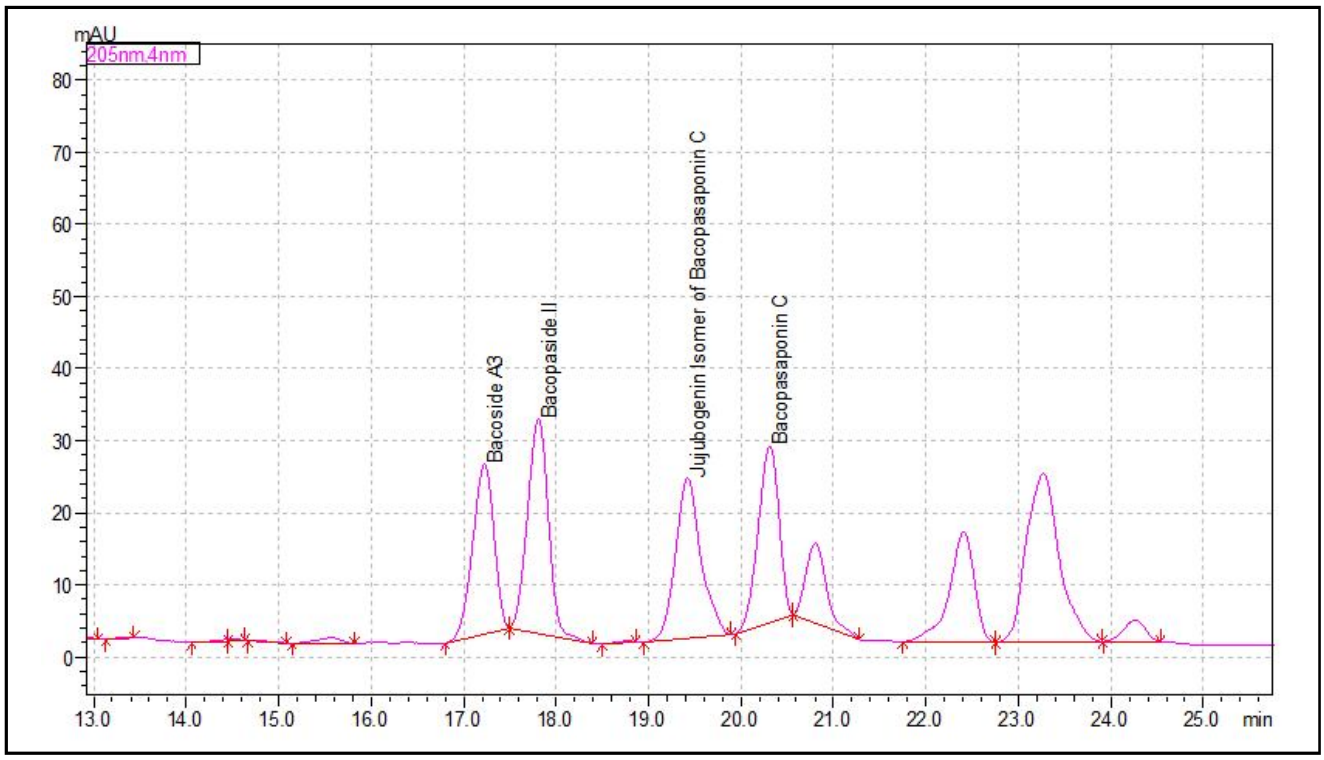

Figure 6: Chromatogram of total bacoside A in IIHR-BM-Sel.08 (highest) the y-axis is the measure of the intensity of absorbance (in milli absorbance units) and the x-axis is the retention time (in min) for each peak.

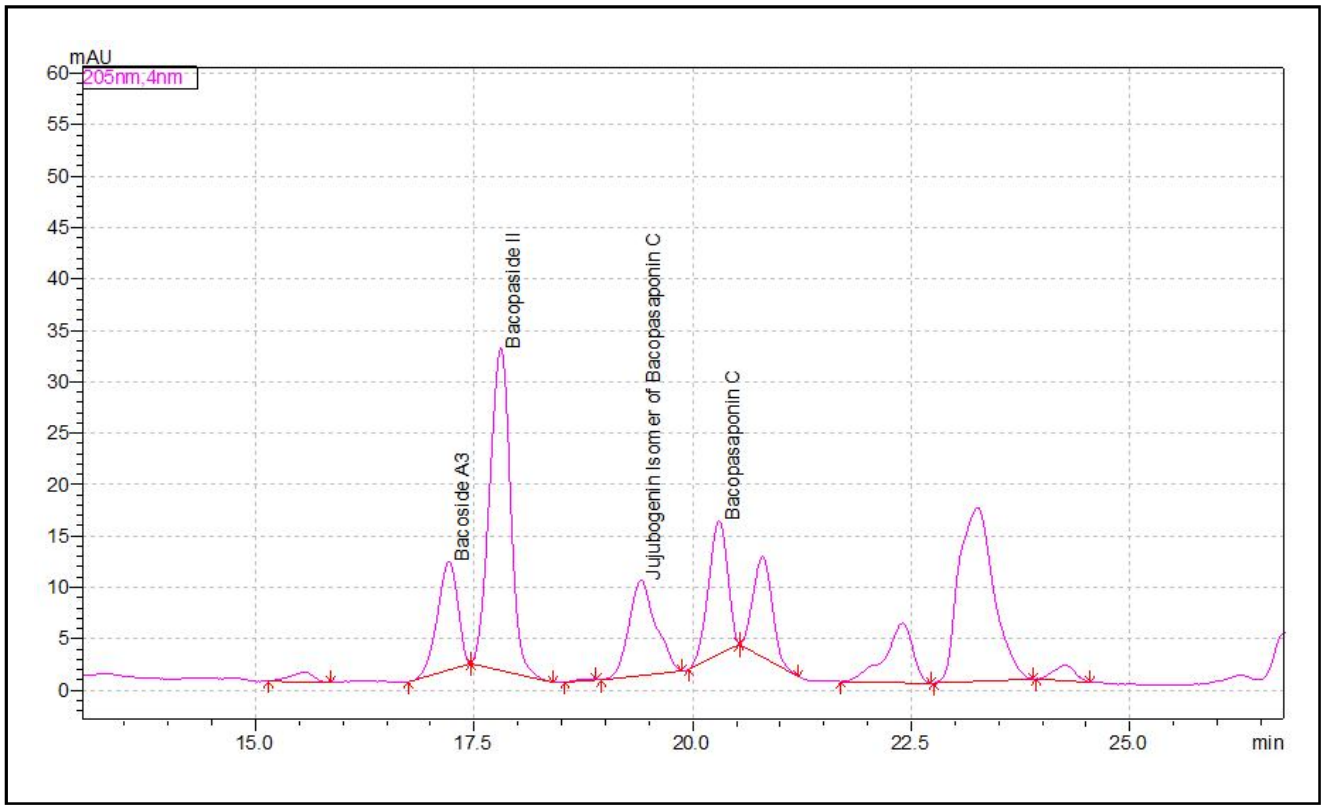

Figure 7: Chromatogram of total bacoside A in IIHR-BM-Sel.09 (lowest) the y-axis is the measure of the intensity of absorbance (in milli absorbance units) and the x-axis is the retention time (in min) for each peak.

3.4 Quantification of elemental composition

Macro elements (N, P, K, Ca, Mg and S) considered as essential nutrient elements, are required in larger quantities for plant growth and development, whereas micro elements (Cu, Zn, Mn and Fe) are needed in much smaller quantities. The mentioned macro and micro elements analyzed in the present study with their corresponding outcomes are accessible in Tables 5 and 6.

The nitrogen content in brahmi genotypes varied from 1.84 to 3.42%, with the highest concentration in IIHR-BM-82 and was remarkably lower in IIHR-BM-Sel.05 and IIHR-BM-Sel.02. Phosphorous content was found to be highest in genotypes IIHR-BM-06 (0.42%) and IIHR-BM-Sel.07 (0.34%), IIHR-BM-99 (0.32%), IIHR-BM-82 and IIHR-BM-Sel.08 (0.31%), IIHR-BM-Sel.03 and IIHR-BM-Sel.02 (0.29%). In contrast, the lowest phosphorous content was recorded in CIM-Jagriti (Check) (0.25%). Potassium content varied in the range of 0.64-0.98%, with IIHR-BM-Sel.08 (0.98%) and CIM-Jagriti (Check) (0.64%) recorded as the highest and lowest, respectively.

In addition, calcium content showed the highest in IIHR-BM-99 (0.59 ppm), IIHR-BM-Sel.05 (0.56 ppm). Higher magnesium content was exhibited in IIHR-BM-82 (0.35 ppm), which was at par with IIHR-BM-Sel.08, IIHR-BM-Sel.07, I9IHR-BM-99 and IIHR-BM-Sel.09 (0.34 ppm), whereas the lowest was found in IIHR-BM-Sel.02 (0.28 ppm). The sulphur content in the brahmi genotypes varied in the range of 0.44 to 0.72 ppm.

With reference to the micro elements, the genotypes revealed substantial variations from one another. Copper content was varied from 17.43-26.51 ppm, with the highest in IIHR-BM-Sel.08, followed by CIM-Jagriti (Check) (24.80 ppm) and IIHR-BM-Sel.03 (24.11 ppm). Zinc exhibited a range between 17.45-77.21ppm CIM-Jagriti (Check) and IIHR-BM-Sel.05. Whereas, manganese content was recorded highest with IIHR-BM-101 (69.27 ppm) and lowest with IIHR-BM-Sel.02 (27.52 ppm). Similarly, IIHR-BM-101 (2321.27 ppm) and IIHR-BM-06 (483.94 ppm) recorded highest and lowest iron content among the rest of the brahmi genotypes.

Table 5: Macroelemental composition in different genotypes of *B. monnieri*

Genotypes	N (%)	P (%)	K (%)	Ca (ppm)	Mg (ppm)	S (ppm)
IIHR-BM-06	2.12	0.42	0.80	0.40	0.30	0.72
IIHR-BM-82	3.42	0.31	0.88	0.47	0.35	0.49
IIHR-BM-Sel.08	2.20	0.31	0.98	0.41	0.34	0.54
IIHR-BM-101	2.02	0.28	0.86	0.42	0.30	0.50
IIHR-BM-Sel.07	1.96	0.34	0.95	0.44	0.34	0.48
IIHR-BM-99	2.34	0.32	0.86	0.59	0.34	0.59
IIHR-BM-Sel.03	3.13	0.29	0.81	0.44	0.32	0.54
IIHR-BM-Sel.02	1.84	0.29	0.68	0.44	0.28	0.44
CIM-Jagriti	1.90	0.25	0.64	0.46	0.33	0.58
IIHR-BM-Sel.05	1.84	0.28	0.79	0.56	0.29	0.57
IIHR-BM-Sel.09	2.69	0.28	0.93	0.42	0.34	0.57
SE	0.041	0.005	0.013	0.006	0.005	0.007
CD *(p=0.05)*	0.123	0.015	0.038	0.019	0.014	0.022

Table 6: Microelemental composition in different genotypes of *B. monnieri*

Genotypes	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)
IIHR-BM-06	22.00	23.49	35.77	483.94
IIHR-BM-82	23.22	23.33	37.84	1381.09
IIHR-BM-Sel.08	26.51	26.85	53.15	1271.70
IIHR-BM-101	21.04	21.96	69.27	2321.27
IIHR-BM-Sel.07	23.48	24.24	33.98	1172.25
IIHR-BM-99	18.03	22.85	49.16	1570.93
IIHR-BM-Sel.03	24.11	20.12	44.69	1555.78
IIHR-BM-Sel.02	17.43	28.43	27.52	689.92
CIM-Jagriti	24.80	77.21	34.97	1142.05
IIHR-BM-Sel.05	23.50	17.45	39.90	1081.57
IIHR-BM-Sel.09	23.07	25.92	36.99	890.90
SE	0.335	0.506	0.623	20.871
CD *(p=0.05)*	0.994	1.502	1.850	62.002

3.5 Correlation analysis among phytochemicals, antioxidant activity and bacoside components

The Pearson correlation analysis among the phytochemicals, antioxidant activity and saponin components is presented in Table 7. The findings indicated that multiple significant associations exhibited valuable insights for breeding applications and functional characterization. Phenols displayed a highly significant positive correlation with both tannins (r = 0.950) and flavonoids (r = 0.858), suggesting coordinated accumulation of these compounds in genotypes with superior phenolic content. DPPH activity, an indicator of antioxidant capacity, was positively correlated with phenols (r = 0.605) and flavonoids (r = 0.785), implying that these constituents contribute substantially to total radical scavenging potential. Among saponin components, bacoside A3 showed highly significant strong positive relationships with jujubogenin (r = 0.898) and total bacoside A (r = 0.818), while displaying a significant negative association with bacopaside II (r = -0.723). Moreover, jujubogenin was also positively correlated with total bacoside A (r = 0.861) and bacopasaponin C (r = 0.716), highlighting the interdependence of saponin components in brahmi. The largely non-significant correlations between antioxidant assays (DPPH and FRAP) and saponin components indicate that saponins may not directly contribute to antioxidant activity in these genotypes.

Table 7: Pearson correlation matrix indicating associations among phytochemicals, antioxidant activity and bacoside components

Characters	Phenols	Tannins	Flavonoids	DPPH activity	FRAP activity	Bacoside A3	Bacopaside II	Jujubogenin	Bacosaponin	Total bacoside A
Phenols	1.000	**0.950****	**0.858****	**0.605***	0.510^{NS}	-0.231^{NS}	-0.292^{NS}	-0.059^{NS}	0.233^{NS}	-0.252^{NS}
Tannins	0.950**	1.000	**0.770****	0.543^{NS}	**0.606***	-0.224^{NS}	-0.251^{NS}	-0.013^{NS}	0.208^{NS}	-0.213^{NS}
Flavonoids	0.858**	0.770**	1.000	**0.785****	0.485^{NS}	-0.267^{NS}	-0.145^{NS}	-0.016^{NS}	0.442^{NS}	-0.093^{NS}
DPPH activity	0.605*	0.543^{NS}	0.785**	1.000	0.361^{NS}	-0.246^{NS}	-0.092^{NS}	0.032^{NS}	0.399^{NS}	-0.037^{NS}
FRAP activity	0.510^{NS}	0.606*	0.485^{NS}	0.361^{NS}	1.000	-0.258^{NS}	-0.063^{NS}	-0.213^{NS}	0.062^{NS}	-0.264^{NS}
Bacoside A3	-0.231^{NS}	-0.224^{NS}	-0.267^{NS}	-0.246^{NS}	-0.258^{NS}	1.000	**-0.723***	**0.898****	0.556^{NS}	**0.818****
Bacopaside II	-0.292^{NS}	-0.251^{NS}	-0.145^{NS}	-0.092^{NS}	-0.063^{NS}	-0.723*	1.000	-0.720*	-0.406^{NS}	-0.349^{NS}
Jujubogenin	-0.059^{NS}	-0.013^{NS}	-0.016^{NS}	0.032^{NS}	-0.213^{NS}	0.898**	**-0.720***	1.000	**0.716***	**0.861****
Bacosaponin C	0.233^{NS}	0.208^{NS}	0.442^{NS}	0.399^{NS}	0.062^{NS}	0.556^{NS}	-0.406^{NS}	0.716*	1.000	**0.810****
Total bacoside A	-0.252^{NS}	-0.213^{NS}	-0.093^{NS}	-0.037^{NS}	-0.264^{NS}	0.818**	-0.349^{NS}	0.861**	0.810**	1.000

*Significant at 5% level, **Significant at 1% level.

4. Discussion

Brahmi has traditionally been utilized in Ayurvedic medication to enhance memory, support cognitive function, reduce anxiety and treat neurological conditions. It contains active compounds like phenols, flavonoids, tannins and saponins which contribute to its neuroprotective and antioxidant effects. Research elucidates its aspect as a natural nootropic, improving learning and memory while also alleviating stress and anxiety (Kongkeaw *et al*., 2014; Watcharatanon *et al*., 2019). The present study on phytochemical evaluation of brahmi genotypes revealed marked variation in secondary metabolite composition and antioxidant capacity, highlighting the influence of genetic diversity on phytochemical traits. The TPC appeared significantly higher in IIHR-BM-Sel.02 (36.39 mg/g), closely, followed by IIHR-BM-Sel.09 and CIM-Jagriti (Check). This underscores the prominence of IIHR-BM-Sel.02 as a rich source of phenolic compounds, which are well-recognized for its antioxidant and disease-preventing effects. Such variations arise from inherent genetic differences that modulate polyphenol biosynthesis, which are the key components in plant defense and stress adaptation (Martinez *et al*., 2023). Flavonoid content further reinforced this variability, with IIHR-BM-Sel.02 having the highest accumulation (20.94 mg/g), followed by IIHR-BM-Sel.09 and CIM-Jagriti (Check). Genotypes with higher flavonoid concentrations are significant from a phytopharmaceutical standpoint, as flavonoids play vital roles in antioxidant defense mechanisms and combat oxidative stress. Furthermore, these bioactive constituents are also linked to cognitive enhancement and cardiovascular protection, aligning with the traditional therapeutic use of brahmi (Otari and Ghane, 2024; Martinez *et al*., 2023). A comparable pattern was noticed in total tannin content, wherein IIHR-BM-Sel.02 and IIHR-BM-Sel.09 exhibited elevated levels compared to genotypes like IIHR-BM-99 with minimal tannin presence. Tannins, considered polyphenolics, contribute antimicrobial and anti-inflammatory benefits, enhancing the medicinal and adaptive value of these genotypes (Otari and Ghane, 2024). These differences can be attributed to unique biosynthetic capacities governed by genetic and environmental factors.

Antioxidant activity evaluated through DPPH and FRAP assays confirmed genotype-dependent differences. IIHR-BM-Sel.07 displayed the dominant free radical scavenging activity in the DPPH assay, indicating a robust capacity to nullify reactive oxygen species, which is an essential attribute for neuroprotection and anti-aging effects. Meanwhile, IIHR-BM-82 exhibited the greatest ferric ion-reducing power in the FRAP assay, showcasing its potent antioxidant potential *via* electron donation. These observations affirm that antioxidant efficacy in brahmi is a multifaceted trait, influenced by interplay among phenolics, flavonoids and other bioactive molecules (Nopparat *et al*., 2024). Saponin content analysis highlighted IIHR-BM-Sel.08 as the richest source of bacoside A3, bacopasaponin C and total bacoside A (3.27%), key bioactive constituents that underpin brahmi's cognitive-enhancing and neuroprotective effects. The considerable genotype-driven variability in total bacoside levels suggests promising avenues for breeding strategies aimed at improving pharmacological efficacy (Otari and Ghane, 2024).

Similar to phytochemical, antioxidant, saponin and elemental analysis of brahmi genotypes revealed notable variations in macro and micro

element concentrations are essential for plant growth and quality. Among macro elements, nitrogen content ranged widely, with IIHR-BM-82 showing the highest percentage (3.42%) that supports protein synthesis and biomass accumulation, while IIHR-BM-Sel.05 and IIHR-BM-Sel.02 contained considerably lower nitrogen. Phosphorus was abundant in genotypes IIHR-BM-06 and IIHR-BM-Sel.07, facilitating energy transfer and root development, whereas potassium ranged from 0.64% to 0.98%, peaking in IIHR-BM-Sel.08, which acts as an integral part in enzyme stimulation and stress tolerance (Tozihi *et al*., 2023). Calcium and magnesium, essential to cell wall structure and photosynthesis, were comparatively high in IIHR-BM-99, IIHR-BM- 05 and IIHR-BM-82 genotypes. Sulphur varied between 0.44 to 0.72 ppm, integral for amino acid and protein biosynthesis (Otari and Ghane, 2024). Micro elemental analysis revealed copper levels from 17.43 to 26.51 ppm, with IIHR-BM-Sel.08 having the greatest concentration, vital for enzymatic and antioxidant functions. Zinc content exhibited variation between 17.45 and 77.21 ppm, significant for hormone synthesis and growth. Manganese peaked at 69.27 ppm in IIHR-BM-101, essential for photosynthetic activity and enzyme cofactors, with iron showing the highest concentration in the same genotype IIHR-BM-101 (up to 2321.27 ppm), supporting chlorophyll synthesis and electron transport processes (Tozihi *et al*., 2023; Otari and Ghane, 2024).

The correlation scrutiny disclosed strong positive relationships between phytochemicals, antioxidant activity and saponin components reflecting coordinated biosynthesis of these metabolites. Phenolic compounds were positively associated with DPPH antioxidant activity, suggesting their vital role in radical scavenging mechanisms. A strong positive relationship was observed between bacoside A3 and jujubogenin with total bacoside A, indicating joint accumulation of key saponins and related aglycones. Conversely, bacopaside II demonstrated a negative correlation with bacoside A3 and jujubogenin, pointing to possible metabolic divergence within saponin biosynthesis. The results point to key traits that may serve as valuable selection criteria for breeding initiatives strengthening enhanced antioxidant and therapeutic efficacy in brahmi (Sarkar *et al*., 2022; Gemede *et al*., 2021).

This research initiates that brahmi genotypes differ significantly in their phytochemical makeup, antioxidant activity, saponin and element content. IIHR-BM-Sel.02 had the highest levels of phenolics and flavonoids compound that benefit to safeguard the body from injury instigated by destructive molecules. On the other hand, IIHR-BM-Sel.08 had the most saponins, which are linked to improved memory and brain function. Different genotypes showed varying abilities to fight oxidative stress, with some better at neutralizing free radicals. Elemental levels like nitrogen, potassium and other minerals also varied, especially in genotypes IIHR-BM-82 and IIHR-BM-Sel.08, supporting both plant growth and medicinal compounds. Phytochemical constituents and antioxidant assays (DPPH) have exhibited strong positive associations, highlighting their key contribution. Among saponin components, bacoside A3 and jujubogenin were positively associated with total bacoside A, whereas bacopaside II showed a negative correlation, indicating coordinated saponin biosynthesis. These differences highlight the need to choose specific genotypes for growing brahmi to get the best health benefits and yield, helping in better breeding and quality control.

5. Conclusion

The present investigation highlights the variation in bioactive phytochemical, saponins and elemental compositions among the *B. monnieri* genotypes. This plant is commercially valuable for its medicinal and nutritional properties, although awareness of its importance is declining among younger generations. The phytochemicals observed are essential in neutralizing free radicals responsible for inducing oxidative stress. The superior genotypes recorded higher levels of total phenols (36.39 mg/g), tannins (15.32 mg/g), flavonoids (20.94 mg/g), DPPH (16.90 mg/g AAE), and FRAP (44.80 mg/g). The highest total bacoside A (3.27%) content was also observed. Additionally, genotypes with higher macroelements (nitrogen-3.42%, phosphorous-0.42%, potassium-0.98%, calcium-0.59 ppm, magnesium-0.35 ppm and sulphur-0.72 ppm) and micro elements (copper-26.51 ppm, zinc-77.21 ppm, manganese-69.27 ppm and iron-2321.27 ppm) content indicate promising potential as nutritionally rich genotypes for leafy vegetable use. Total phenols exhibited strong positive associations with tannins (r = 0.950) and flavonoids (r = 0.858), and DPPH activity was positively correlated with phenols (r = 0.605) and flavonoids (r = 0.785), suggesting their major contribution to antioxidant capacity. Among saponin components, bacoside A3 and jujubogenin (r = 0.898) showed strong positive relationships with total bacoside A (r = 0.818), whereas bacopaside II (r = -0.723) displayed a negative correlation, indicating coordinated saponin biosynthesis. jujubogenin was also positively associated with total bacoside A (r = 0.861) and bacosaponin C (r = 0.716). Overall, the results demonstrate that the evaluated brahmi genotypes possess strong potential for commercial utilization based on their phytochemical, saponin and elemental composition. Moreover, the understanding of trait associations provided valuable guidance for crop improvement programs aimed at enhancing multiple desirable traits, while highlighting brahmi as a promising nutritionally rich leafy medicinal crop and a potential alternative to conventional leafy vegetables to convene the growing requirements of the universal society and pharmaceutical sector.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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