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ORIGINAL ARTICLE

Quantitative Phytochemical Profiling of *In Vitro*–Raised *Bacopa Monnieri* (L.) Pennell

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ABSTRACT
Bacopa monnieri (L.) Pennell is an important medicinal plant extensively used in traditional Indian systems of medicine for enhancing memory and cognitive functions. The present investigation was undertaken to quantitatively analyse major primary and secondary metabolites in in vitro–raised plantlets of B. monnieri to evaluate their biochemical and medicinal potential. In vitro–propagated plant material was harvested at an appropriate growth stage and subjected to standard biochemical assays for the estimation of tannins, saponins, total phenolics, total flavonoids, crude fat, crude protein, and total carbohydrates. The results revealed that in vitro–raised plantlets contained appreciable quantities of bioactive compounds, including total phenolics (14.19 ± 0.005 mg GAE/g), flavonoids (22.15 ± 0.019 mg QE/g), saponins (14.64 ± 1.04 g/100 g), and tannins (0.104 ± 0.0001 mg/mL). Significant levels of proteins, fats, and carbohydrates were also recorded, indicating good nutritional value. Low standard deviation values confirmed the reproducibility and reliability of the analytical methods. The study demonstrates that in vitro culture techniques are effective for producing phytochemically rich B. monnieri plantlets, supporting their potential use in pharmaceutical and nutraceutical applications.
Keywords: *Bacopa monnieri, in vitro culture, phytochemical analysis, phenolics, flavonoids, saponins*

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INTRODUCTION

The genus *Bacopa* comprises several aquatic and semi-aquatic plant species predominantly distributed in tropical and subtropical regions worldwide. Among the species reported from India, *Bacopa monnieri* (L.) Pennell has received considerable attention due to its extensive medicinal importance [27]. The plant belongs to the family Scrophulariaceae, which has been reclassified under Plantaginaceae, and occupies a significant place in traditional Indian systems of medicine [1-3]. *Bacopa monnieri*, commonly known as Brahmi, is also referred to by regional names such as Neerbrahmi, Jalanimba, Safed Chamani, and Kiru-Brahmi. In English literature, it is known as water hyssop or thyme-leaved gratiola [2]. The plant naturally thrives in moist, marshy, and waterlogged habitats and is widely distributed in different regions of India, including West Bengal, Punjab, Haryana, and Himachal Pradesh. In Himachal Pradesh, it occurs in low-lying wet areas of districts such as Una, Hamirpur, Kangra, Bilaspur, Solan, and Sirmaur up to an altitude of approximately 1000 m under subtropical climatic conditions [4, 8, 9, 22, 15]. The flowering period of the plant generally extends from July to January.

The entire plant of *B. monnieri* is used therapeutically in Ayurvedic, Unani, and Siddha systems of medicine. It is described as bitter and astringent in taste, cooling in nature, and rich in vitamin C. The medicinal properties of *B. monnieri* are attributed to a wide range of bioactive constituents, particularly triterpenoid saponins, alkaloids, sterols, and flavonoids [24-26]. Among these compounds, bacoside A and

bacoside B are considered the principal active constituents. Several other compounds, including bacopasaponins (A–G), bacopasides I and II, betulinic acid, D-mannitol, herpaponin, brahmine, herpestine, phytosterols, and flavonoids, have also been reported [3, 4, 13, 23, 27, 28].

In ancient Indian literature, *B. monnieri* is classified as a "Medhyarasayana," a group of drugs known for promoting intellect, memory, and mental clarity. The name Brahmi is derived from "Brahma," symbolising knowledge and creation, reflecting the plant's long-standing association with cognitive enhancement [24-26, 19, 20, 11]. Classical Ayurvedic texts such as the *Charaka Samhita* recommend Brahmi for the management of neurological disorders, anxiety, epilepsy, impaired cognition, and lack of concentration, as well as for strengthening the nervous and cardiovascular systems [10, 11, 21, 17, 25, 29].

Modern scientific investigations have validated many of these traditional claims. Several studies have demonstrated the nootropic potential of *B. monnieri*, reporting improvements in memory retention, learning ability, and information processing speed [30, 31, 22]. The neuroprotective activity of the plant is largely attributed to bacosides, which are known to enhance neuronal repair, improve synaptic transmission, and support nerve impulse conduction [29]. In addition to its cognitive benefits, *B. monnieri* exhibits a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, antidepressant, and gastroprotective effects [5-7, 14, 20, 18].

Due to increasing demand for standardised medicinal plant material, *in vitro* culture techniques offer an effective alternative for producing uniform and phytochemically consistent plant material. The present study was therefore designed to quantitatively evaluate major phytochemical constituents in *in vitro*–raised *Bacopa monnieri* plantlets.

MATERIAL AND METHODS

Plant Material: The experimental material used in the present study was *Bacopa monnieri* (L.) Pennell, commonly known as Brahmi. *In vivo*–raised plantlets were obtained and maintained under controlled laboratory conditions. Plant material was collected from the campus of Maratha Vidya Prasarak Samaj's KRT Arts, BH Commerce and AM Science College (KTHM), Nashik, India.

Fig 1: *Bacopa monnieri* (L.) Pennell

Preparation of Plant Samples

Plantlets were harvested at an appropriate growth stage and washed thoroughly with distilled water to remove culture medium and surface contaminants. The samples were dried in a hot air oven at 60 °C until constant weight was achieved. Dry weights of plantlets were recorded using a digital balance. The dried plant material was finely powdered and stored for further phytochemical analysis.

Phytochemical Analysis [15, 16. 12, 30-33]

Determination of Tannin Content

Tannin content was estimated using the Folin–Ciocalteu colorimetric method. Dried powdered plant material (5 g) was extracted with methanol and processed to obtain a stock solution (1 mg/mL). The reaction mixture was developed using Folin–Ciocalteu reagent and sodium carbonate, and absorbance was measured at 748.5 nm. Tannin content was calculated using a gallic acid standard curve and expressed as mg gallic acid equivalents per gram of extract.

Determination of Saponin Content

Saponin content was estimated using a gravimetric method involving sequential solvent extraction and precipitation. The dried saponin fraction obtained after evaporation and precipitation was weighed, and saponin content was expressed as g/100 g of extract.

Determination of Total Phenolic Content

Total phenolic content was determined using the Folin–Ciocalteu method with gallic acid as the standard. Absorbance was recorded at 765 nm, and results were expressed as mg gallic acid equivalents per gram of sample.

Determination of Total Flavonoid Content

Total flavonoid content was estimated using the aluminum chloride colorimetric method. Quercetin was used as the reference standard, and absorbance was measured at 415 nm. Flavonoid content was expressed as mg quercetin equivalents per gram of sample.

Determination of Crude Fat Content

Crude fat content was determined using the Soxhlet extraction method with hexane as the solvent. Extracted fat was weighed after solvent evaporation, and fat content was expressed as g/100 g of sample.

Determination of Crude Protein Content

Protein content was estimated using the Kjeldahl method. Nitrogen content was determined and multiplied by a conversion factor (6.25) to obtain crude protein content, expressed as g/100 g of sample.

Determination of Total Carbohydrate Content

Total carbohydrate content was estimated using the Anthrone method with glucose as the standard. Absorbance was measured at 620 nm, and carbohydrate content was expressed as mg/100 g of dry sample.

RESULT

Quantitative Estimation of Tannin

The total tannin content in *in vitro*–raised *Bacopa monnieri* plantlets was estimated using the standard colorimetric method with gallic acid as the reference compound. The absorbance of the reaction mixture was measured at 748–748.5 nm, and the tannin content of the sample was calculated by comparing it with the standard calibration curve.

Preparation of Standard Curve: A set of gallic acid standard solutions ranging from 5 to 80 µg/mL was prepared, and their absorbance values were measured at 748 nm. A consistent increase in absorbance was observed with rising concentrations of gallic acid (Table 1), demonstrating a direct linear correlation between concentration and absorbance. This linearity was used to generate a standard calibration curve (Fig. 2), which was subsequently applied for the quantitative estimation of tannin content in the experimental samples.

Table 1. Observed absorbance of standard gallic acid for tannin estimation

Sr. No	Code	Concentration (µg/ml)	Absorbance (748 nm)
0	Blank	0	0
1	Std 1	5	0.08
2	Std 2	10	0.21
3	Std 3	20	0.53
4	Std 4	40	1.01
5	Std 5	80	1.64

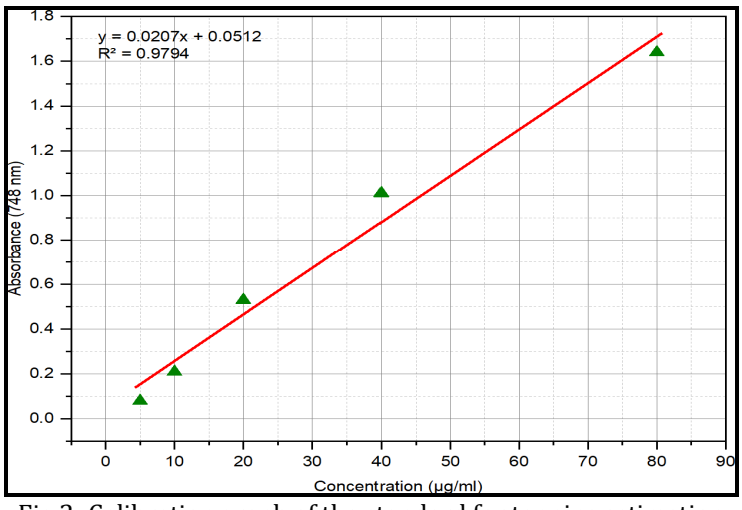

Fig 2- Calibration graph of the standard for tannins estimation

The calibration curve obtained from the gallic acid standards showed good linearity, confirming the suitability of the method for tannin estimation.

Estimation of Tannin Content in Sample BMC

The powdered sample of *in vitro*–raised *Bacopa monnieri* plantlets (sample code: BMC) was analysed in three replicates. The absorbance values of the sample extract were recorded at 748.5 nm, and the corresponding tannin concentration (µg/ml) was calculated using the standard calibration curve. The calculated values were then converted into mg/ml.

Table 2. Final calculation of total tannin content in sample BMC

Sr. No.	Sample Code	Replicate	Absorbance (748.5 nm)	Concentration of tannins (x) (µg/ml)	Concentration of Tannins mg/ml	Tannin content Mean	SD
1	BMC	1	2.225	105.014	0.105	0.105	0.0001
		2	2.223	104.918	0.105		
		3	2.220	104.773	0.105		

The absorbance values for the three replicates ranged from 2.220 to 2.225, corresponding to tannin concentrations between 104.773 and 105.014 µg/ml. The mean tannin content of the sample was calculated as 0.105 mg/ml, with a very low standard deviation (± 0.0001), indicating high precision and reproducibility of the results.

Estimation of Saponin

Saponin content in *in vitro*–raised *Bacopa monnieri* plantlets was estimated using a gravimetric technique. In this method, saponins were extracted from the dried powdered plant material, after which the solvent was removed, and the remaining saponin residue was weighed. All measurements were performed in triplicate to ensure the reliability and reproducibility of the results.

Estimation of Saponin Content in Sample BMC

The dried powdered plant material (sample code: BMC) was precisely weighed and processed for saponin extraction. Following completion of the extraction procedure, the solvent was removed, and the mass of the isolated saponin residue was determined. The saponin content was then expressed as a percentage by calculating the ratio of the recovered saponin weight to the original sample weight.

Table 3. Observed saponin content in the sample code BMC

S. N.	Sample Code	Replicate	Weight of sample (g)	Weight of extract (g)	Weight of saponin (g)	Saponin content %	Mean Saponin content%	SD
1	BMC	R1	1.012	0.611	0.093	15.221	14.620	1.020
		R2	1.013	0.610	0.082	13.443		
		R3	1.014	0.612	0.093	15.196		

The results revealed that the saponin content of the sample varied slightly among replicates. The percentage saponin content was recorded as 15.221% in replicate 1, 13.443% in replicate 2, and 15.196% in replicate 3. The mean saponin content of the sample was calculated as **14.620%**, with a standard deviation of **± 1.020**, indicating minor variation among replicates.

Estimation of Total Phenolic Content

The total phenolic content of *in vitro*–raised *Bacopa monnieri* plantlets was determined using the Folin–Ciocalteu colorimetric assay, with gallic acid serving as the reference standard. The blue-colored reaction complex was measured at 765 nm using a spectrophotometer, and phenolic concentration in the samples was calculated from the gallic acid standard calibration curve.

Preparation of Standard Curve

Gallic acid standard solutions in the concentration range of 10–100 µg/mL were prepared, and their absorbance was measured at 765 nm (Table 4). A proportional increase in absorbance was observed with increasing gallic acid concentration, demonstrating a strong linear relationship between concentration and absorbance. This linear response was used to generate a standard calibration curve (Fig. 3), which served as the basis for determining the total phenolic content of the test samples.

Table 4. Observed absorbance of standard gallic acid for total phenolic content estimation

Sr. No.	Code	Concentrations of Gallic Acid (µg/ml)	Absorbance at 765nm
1	Blank	0	0
2	Std 1	10	0.107
3	Std 2	20	0.126
4	Std 3	40	0.155
5	Std 4	80	0.225
6	Std 5	100	0.291

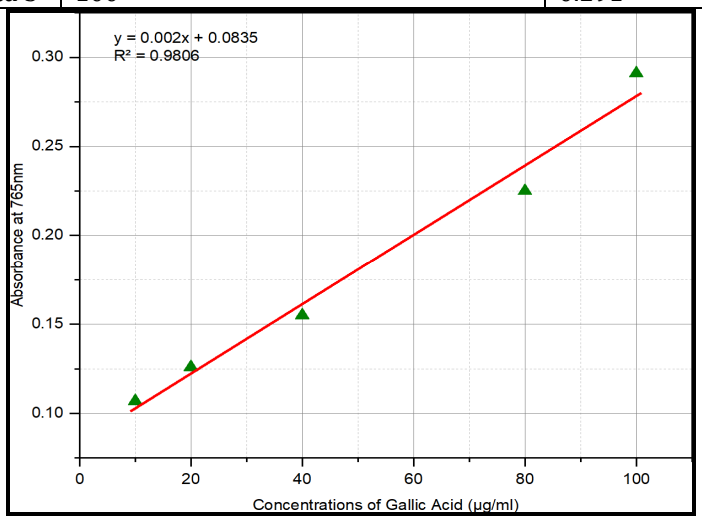

Fig 3- Calibration graph of the standard for total Phenol estimation

The linearity of the standard curve confirmed the reliability of the method for phenolic quantification.

Estimation of Total Phenolic Content in Sample BMC

The sample extract (code BMC) was evaluated in triplicate. Absorbance readings for each replicate were recorded at 765 nm, and the corresponding phenolic concentrations (µg/mL) were determined using the gallic acid standard curve. The results were subsequently expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/g).

Table 5. Final calculation of total phenolic content in the sample BMC

Sample Code	Replicate	Absorbance (y)	Concentration of phenolic (x)(µg/ml)	TPC (mg GAE/g)	Mean	SD
1	R1	2.022	1419.25	14.192	14.19	0.005
	R2	2.021	1418.75	14.187		
	R3	2.023	1419.75	14.197		

The absorbance values ranged from 2.021 to 2.023, corresponding to phenolic concentrations between 1418.75 and 1419.75 µg/ml. The calculated total phenolic content of the sample was **14.19 mg GAE/g**, with a very low standard deviation (**± 0.005**), indicating high consistency among replicates.

Estimation of Total Flavonoid Content

The total flavonoid content of *in vitro*–raised *Bacopa monnieri* plantlets was estimated using a colorimetric assay with quercetin as the reference standard. The intensity of the yellow-colored complex formed during the reaction was measured at 415 nm using a spectrophotometer, and flavonoid concentration was determined from the quercetin calibration curve.

Preparation of Standard Curve

Quercetin standard solutions in the concentration range of 10–100 µg/mL were prepared, and their absorbance was measured at 415 nm (Table 6). A steady increase in absorbance was observed with increasing quercetin concentration, indicating a linear correlation between concentration and absorbance. This linear relationship was used to construct a standard calibration curve (Fig. 4), which was subsequently applied for the quantitative estimation of flavonoids in the sample.

Table 6. Observed absorbance of standard quercetin for total flavonoid estimation

Sr no.	Code	Concentrations of Quercetin (µg/ml)	Absorbance at 415nm
1	Blank	0	0
2	Std 1	10	0.149
3	Std 2	20	0.211
4	Std 3	40	0.283
5	Std 4	80	0.466
6	Std 5	100	0.698

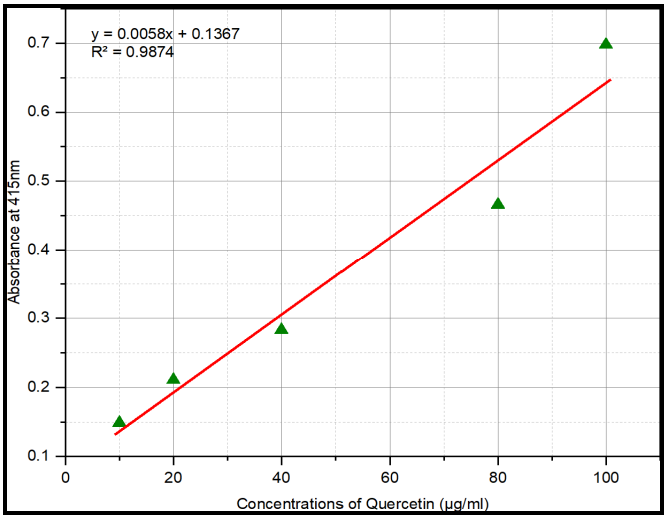

Fig. 4- Calibration graph of the standard for total flavonoid estimation

The linearity of the calibration curve confirmed the suitability of the method for flavonoid quantification.

Estimation of Total Flavonoid Content in Sample BMC

The extract of the sample BMC was examined in three replicates. Absorbance values were recorded at 415 nm for each replicate, and the corresponding flavonoid concentrations (µg/mL) were calculated using the quercetin standard calibration curve. The flavonoid content was finally expressed as milligrams of quercetin equivalents per gram of sample (mg QE/g).

Table 7. Final calculation of total flavonoid content in the sample BMC

Sample code	Replicate	absorbance (y)	Concentration of flavonoid (x)(µg/ml)	TFC (mg QE/g)	Mean	SD
1	R1	1.149	221.672	22.167	22.156	0.0199
	R2	1.147	221.327	22.133		
	R3	1.149	221.672	22.167		

The absorbance values ranged from 1.147 to 1.149, corresponding to flavonoid concentrations between 221.327 and 221.672 µg/ml. The mean total flavonoid content of the sample was calculated as **22.156 mg QE/g**, with a low standard deviation (**± 0.0199**), indicating high reproducibility of the results.

Total Fat Content Determination

The total fat content of *in vitro*–raised *Bacopa monnieri* plantlets was estimated using a gravimetric technique. In this procedure, lipids were extracted from the dried powdered plant material, the solvent was removed, and the remaining fat was weighed. All estimations were performed in triplicate to ensure accuracy and reproducibility of the results.

Estimation of Total Fat Content in Sample BMC

The dried powder (sample code BMC) was precisely weighed and processed for lipid extraction. The extracted fat was quantified and expressed as grams per 100 g of sample, and the corresponding results are summarised in Table 8.

Table 8. Observation of total fat content in the sample code BMC

Sr No.	Sample Code	Replicate	Sample weight	Fat g/100g	Mean	SD
1	BMC	R1	0.5121	15.622	15.615	0.006
		R2	0.5125	15.610		
		R3	0.5124	15.613		

The total fat content recorded for the three replicates was 15.622, 15.610, and 15.613 g/100 g, respectively. The mean total fat content of the sample was calculated as 15.615 g/100 g, with a very low standard deviation (**± 0.006**), indicating high precision and consistency among replicates.

Total Protein Content Determination

The total protein content of *in vitro*–raised *Bacopa monnieri* plantlets was determined using a nitrogen-based analytical method. The nitrogen percentage in the sample was measured and converted to protein content using a suitable conversion factor. All estimations were performed in triplicate to ensure accuracy and reproducibility of the results.

Estimation of Total Protein Content in Sample BMC

The dried powder (sample code BMC) was accurately weighed and analysed to determine its nitrogen content. The nitrogen values obtained were used to calculate the protein concentration, which was expressed as grams per 100 g of sample. The results of the total protein estimation are shown in Table 9.

Table 9. Observation of total protein content in the sample code BMC

Sr No.	Sample code	Replicate	Sample weight	% N	Protein g/100gm	Mean	SD
1	BMC	R1	0.2101	2.4667	15.4170	14.993	0.727
		R2	0.2102	2.4656	15.4097		
		R3	0.2103	2.2646	14.1535		

The protein content of the three replicates ranged from 14.1535 to 15.4170 g/100 g. The mean protein content of the sample was calculated as 14.993 g/100 g, with a standard deviation of **± 0.727**, indicating moderate variation among replicates.

Estimation of Total Carbohydrate Content

The total carbohydrate content of *in vitro*–raised *Bacopa monnieri* plantlets was determined using a colorimetric assay with glucose as the reference standard. The intensity of the colored reaction complex was measured at 620 nm using a spectrophotometer, and carbohydrate concentration was calculated by comparison with the glucose standard calibration curve.

Preparation of Standard Curve

Glucose standard solutions with concentrations ranging from 6.25 to 100 µg/mL were prepared, and their absorbance was measured at 620 nm (Table 10). A gradual increase in absorbance was observed with increasing glucose concentration, indicating a positive linear relationship between concentration and absorbance. This relationship was used to generate a standard calibration curve for the estimation of total carbohydrate content.

Table 10. Observed absorbance of glucose standards for carbohydrate estimation

Sr. no.	Code	Concentrations of Glucose (µg/ml)	Absorbance at 620 nm
1	Blank	0	0
2	Std 1	6.25	0.128
3	Std 2	12.5	0.129
4	Std 3	25	0.226
5	Std 4	50	0.415
6	Std 5	100	0.631

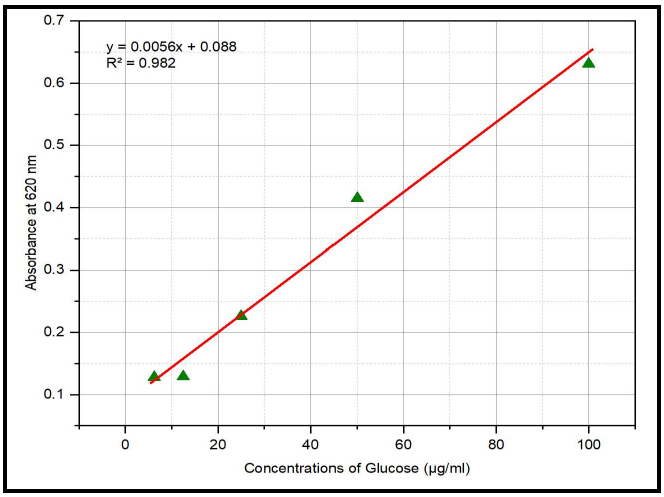

Fig. 5- Calibration graph of the standard for total flavonoid estimation

The standard curve thus obtained was used for calculating the carbohydrate concentration in the test sample.

Estimation of Carbohydrate Content in Sample BMC

The extract of the sample BMC was analysed in three replicates. Absorbance readings for each replicate were recorded at 620 nm, and the corresponding carbohydrate concentrations (µg/mL) were determined using the glucose standard calibration curve. The calculated values were subsequently converted and expressed as milligrams per 100 g of sample.

Table 11. Calculation of carbohydrate content in the sample BMC

Sr. No.	Sample Code	Replicate	Absorbance	Concentration (µg/ml)	Concentration (mg/100g)	Concentration (mg/100g)	Mean	SD
1	BMC	R1	0.0985	1.875	0.2625	26.25	26.50	0.250
		R2	0.0987	1.911	0.2675	26.75		
		R3	0.0986	1.893	0.265	26.5		

The absorbance values ranged from 0.0985 to 0.0987, corresponding to carbohydrate concentrations between 26.25 and 26.75 mg/100 g. The mean carbohydrate content of the sample was calculated as 26.50 mg/100 g, with a standard deviation of ± 0.250, indicating good reproducibility among replicates.

DISCUSSION

Quantitative phytochemical analysis revealed that *in vitro*–raised *Bacopa monnieri* plantlets accumulated significant levels of both primary and secondary metabolites. Tannin estimation showed a mean value of 0.104 ± 0.0001 mg/mL, with minimal variation among replicates, indicating high analytical precision. The saponin content ranged between 13.44% and 15.22%, with a mean value of 14.62 ± 1.02%. The presence of substantial saponin levels confirms the retention of important bioactive constituents under *in vitro* conditions. Total phenolic content was recorded as 14.19 ± 0.005 mg GAE/g, reflecting strong antioxidant potential. Similarly, total flavonoid content was found to be 22.15 ± 0.019 mg QE/g, indicating a high concentration of flavonoid compounds known for their pharmacological significance. The nutritional profile of the plantlets was supported by appreciable levels of primary metabolites. Total fat content was recorded as 15.61 ± 0.006 g/100 g, while protein content averaged 14.99 ± 0.727 g/100 g. Total carbohydrate content was measured as 26.50 ± 0.250 mg/100 g. The low standard deviation values observed across most parameters confirm the reproducibility of the analytical methods and the biochemical stability of *in vitro*–raised plant material.

These findings collectively demonstrate that tissue-cultured *B. monnieri* plantlets retain their phytochemical integrity and medicinal value, supporting earlier reports on the suitability of *in vitro* culture techniques for medicinal plant propagation.

CONCLUSION

The present study provides a comprehensive quantitative evaluation of major phytochemical constituents in *in vitro*–raised *Bacopa monnieri* (L.) Pennell plantlets. Significant levels of tannins, phenolics, flavonoids, saponins, proteins, fats, and carbohydrates were recorded, indicating strong biochemical and

medicinal potential. The results confirm that *in vitro* culture techniques are effective in producing phytochemically rich and nutritionally valuable plant material. Such plantlets can serve as a reliable source of bioactive compounds for pharmaceutical and nutraceutical applications, thereby reducing pressure on natural populations of this valuable medicinal plant.

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