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Effect of Plant Growth Regulators on *in vitro* Micro Propagation of *Bacopa monnieri* and Expression of Secondary Metabolites

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ABSTRACT

Bacopa monnieri (L.), commonly known as “Brahmi”, is an important medicinal herb from the Scrophulariaceae family, valued as a tonic for nervous disorders and mental illnesses. This study examined the impact of various plant growth regulators on the *in vitro* multiplication and survival of *B. monnieri* plants. Cultures were grown on Murashige and Skoog (MS) medium supplemented with different growth regulators–Benzylaminopurine (BAP), Kinetin (KIN), Indole-3-acetic acid (IAA) and Naphthaleneacetic acid (NAA) used individually and in combinations. When used alone, the highest shoot numbers were observed on the 28th day with MS+BAP (0.5 mg/l) producing 6.67±0.69 shoots, MS+KIN (0.75 mg/l) with 6.33±0.58 shoots, MS+NAA (0.25 mg/l) with 5.89±0.29 shoots, and MS+IAA (1.25 mg/l) with 5.11±0.22 shoots. Increasing the concentrations of these plant growth regulators did not enhance shoot numbers. Among combinations, the best results were obtained with BAP (0.50 mg/l) + KIN (0.75 mg/l) + IAA (1.50 mg/l), yielding 8.11±0.29 shoots, followed by BAP (0.50 mg/l) + KIN (0.75 mg/l) + IAA (0.50 mg/l) with 7.22±0.11 shoots. Rooting was most effective using half-strength basal media. Well-rooted plantlets were transplanted into pots with various soil mixtures, achieving a 100% survival rate in several combinations, including sand: soil: FYM, sand: soil: vermin compost, and soil: FYM: vermin compost and coco peat: vermin compost: perlite under greenhouse conditions. Phytochemical screening of ethanolic and aqueous extracts revealed the presence of carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, alkaloids and tannins. This study enhanced the propagation efficiency of *B. monnieri* and laid the groundwork for further research in controlled and field environments.

Key words: Micro propagation, secondary metabolites, *Bacopa monnieri*, plant growth regulators, shoot multiplication, hardening

INTRODUCTION

Medicinal plants have been used in healthcare since ancient times, particularly in Ayurvedic, Unani, and traditional folk medicine, due to their therapeutic properties (Islam *et al*., 2017). In a comprehensive review, Fatima *et al*. (2022) highlighted the neuroprotective effects of *Bacopa monnieri*, detailing its rich phytochemical profile, including saponins, steroids, alkaloids, glycosides, flavonoids and cucurbitacins. Its extracts also contain specific amino acids like brahmin, hydrocotyline, nicotine, herpestine, D-mannitol, stigmasterol, glutamic acid, aspartic acid, alanine and serine, contributing to its anticancer, antioxidant, memory-enhancing and cardiotonic properties. Further, *B. monnieri* may modulate immune function by suppressing the production of certain pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) from activated microglial cells (Nemetchek *et al*., 2016).

Despite its medicinal value, *B. monnieri* faces a decline in the wild, leading to its classification as a threatened species by the IUCN. Traditional propagation methods are limited by slow growth, low seed viability and high seedling mortality. To meet the growing demand for high-quality *Bacopa* material, micro propagation has emerged as an effective method for producing large quantities of genetically uniform, disease-free plants (Sharma *et al*., 2016; Bansal *et al*., 2017; Zote *et al*., 2018).

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The success of micro propagation largely depends on the use of plant growth regulators (PGRs), which influence key physiological processes like cell division and morphogenesis. By optimizing PGR types and concentrations, researchers have significantly improved shoot proliferation and root development in *B. monnieri* (Pothiaraj *et al*., 2016; Faisal *et al*., 2018; Chauhan and Shirkot, 2020; Rahe *et al*., 2020). This study aimed at examining the effects of different PGRs on the micro propagation of *B. monnieri*, with the goal of enhancing sustainable and efficient cultivation methods. It also included a qualitative analysis of primary metabolites using aqueous and ethanol solvents.

MATERIALS AND METHODS

The *Bacopa monnieri* plant material was obtained from the nursery of the erstwhile Centre for Plant Biotechnology at CCS Haryana Agricultural University, Hisar, Haryana. For initiating *in vitro* cultures, shoot tips of approximately 2-3 cm in length were chosen as explants. These shoot cultures were subsequently placed on Murashige and Skoog (MS) medium, supplemented with sucrose as an energy source and agar as a gelling agent. MS medium was prepared with 3% (w/v) sucrose and 0.8% (w/v) agar. Stock solutions of high-purity chemicals (macro and microelements, vitamins and plant growth regulators) were made in double-distilled water and stored at 4°C. The pH was adjusted to 5.8 using 0.1N NaOH or HCl. Agar was dissolved in the medium with constant stirring until transparent, ensuring even mixing of the gelling agent. The medium was poured into culture bottles, sealed with plastic caps and autoclaved at 121°C for 20 min under 15 psi pressure.

Healthy vegetative shoots were excised, rinsed under running tap water, and stripped of leaves. Shoot tip explants (2-3 cm) were then prepared. The explants were treated with liquid detergent (Teepol), washed with running water, and rinsed with double-distilled water. To ensure sterility, the explants were surface-sterilized with 0.1% HgCl, for 4-5 min, followed by multiple washes with sterilized double-distilled water to remove any residue.

Sterilized explants were inoculated onto MS medium supplemented with varying concentrations of BAP (0.25-1.0 mg/l), alone or in combination with fixed concentrations of NAA (0.20 mg/l) and KIN (0.50 mg/l). The cultures were incubated in the culture room at 25±2°C under a 16-hour light/8-hour dark photoperiod, with cool white fluorescent light at an average intensity of 2500 lux.

Healthy shootlets from the basal medium were transferred to a multiplication medium with varying concentrations of BAP, Kinetin, NAA and IAA, either alone or in combinations. After four weeks, the number of shoots and their lengths (in cm) were recorded for each plant growth regulator combination.

Shoots (3-4 cm) were transferred to MS medium supplemented with NAA (0.5-2.0 mg/l) and IBA (0.5-2.0 mg/l) to induce root formation. The number of days for visible root formation and the number of roots per shoot were recorded on the 28th day.

The survival and establishment of *in vitro* plantlets were evaluated after transplanting into various potting media: (1) Sand, (2) Sand + Soil (1:1), 3) Sand: Soil: FYM (1:1:1), (4) Sand: Soil: Vermicompost (1:1:1) and (5) Coco peat: Vermicompost : Perlite (3:1:1). Survival rates of the transplanted plantlets were monitored over a 4-week period.

The extracts of *B. monnieri* were prepared by Soxhlet extraction method placing the dried powder of *in vitro* raised plants (5 g) inside porous bag (thimble) and using the extraction solvents (petroleum ether, n-hexane, ethanol and water). The extracts were kept cold, at 4°C, until additional testing was done.

The prepared extracts were dried properly and weighed accurately. This weight was divided by total weight of plant material taken for extraction. The percentage yield was calculated using the following formula:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material taken}} \times 100$$

Preliminary phytochemical analysis was carried out for the all extracts as per standard methods.

The solvent-free extract was mixed with 10 ml of dilute hydrochloric acid and then filtered. The resulting filtrate was tested using the following alkaloidal reagents:

Mayer's test: The filtrates were reacted with Mayer's reagent, and the appearance of a

yellowish-cream precipitate confirmed the presence of alkaloids.

Wagner's test: Few drops of Wagner's reagent were added to 2 ml of filtrate by the side of the test tube. A reddish-brown precipitate confirmed the presence of alkaloids.

Alkaline reagent test: Addition of increasing amount of sodium hydroxide to the extract showed deep yellow colouration. This deep yellow colour gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.

Litmus test: Red litmus paper turned blue while blue litmus paper remained unchanged in the presence of a base. Phenol turned blue litmus paper red. This showed that phenol was acidic in nature.

Ellagic acid test: Few drops of 5% glacial acetic acid were added to 0.5 ml of plant extract samples followed by addition of few drops of 5% $NaNO_2$ solution. Muddy brown colour formation revealed the existence of phenols in the test samples.

Foam test: Five millilitres of distilled water were used to shake about half a millilitre of the extracts. The formation of creamy, tiny bubbles, or foaming, indicated the presence of saponins.

Alkaline reagent test: About 2 ml of 1N of NaOH was added to 2 ml of plant extract samples. Appearance of yellow to red colour revealed the existence of tannins.

Each 0.5 ml extract was diluted in 5 ml of distilled water and filtered. Carbohydrate content was examined in the filtrate.

Fehlings test: To 1 ml of extract,1 ml Fehling's A solution and 1 ml of Fehling's B solution were mixed and heated in boiling water bath for 5-10 min. First a yellow, then brick red precipitate confirmed the presence of carbohydrates.

To the alcoholic solution of extract few drops of NaOH followed by 2% solution of 3, 5-dinitro benzoic acid was added. Formation of pink colour indicated presence of cardiac glycosides.

Leibermann-Burchard reaction: Three ml of the extract were mixed with 10 ml of chloroform, followed by addition of 2 ml of acetic anhydride. Subsequently, two drops of concentrated sulphuric acid were carefully added along the side of the test tube. The development of a blue-green colour signalled the presence of steroids.

Biuret test: Addition of 0.5 ml of 40% NaOH solution and two drops of 1% copper sulphate solution to 0.5 ml of extract, resulted in the emergence of violet colour, thus confirming the presence of proteins.

Ninhydrin test: Two drops of freshly made 0.2% Ninhydrin reagent were added to around 0.5 ml of extract, which were then boiled. Proteins, peptides, or amino acids were indicated by the appearance of pink or purple colour.

All the experiments were carried out under completely randomized design (CRD) using R software for data analysis.

RESULTS AND DISCUSSION

The effects of various plant growth regulators (PGRs) on the *in vitro* multiplication of *Bacopa monnieri* were assessed by inoculating surface-sterilized apical shoots onto MS medium supplemented with different concentrations of BAP (6-Benzylaminopurine), KIN (Kinetin), NAA (á-Naphthaleneacetic acid) and IAA (Indole-3-acetic acid), either alone or in combinations. The study focused on the number of shoots per explant and shoot length after four weeks of culture. Among the PGRs tested, BAP at 0.5 mg/l (M_2) was the most effective for shoot multiplication, resulting in an average of 6.67 ± 0.69 shoots per explant, while KIN at 0.75 mg/l (M_{11}) produced 6.33 ± 0.58 shoots. Shoot lengths were also longest under BAP (4.69 ± 0.17 cm) and KIN (3.92 ± 0.21 cm). The lowest number of shoots, 2.44 ± 0.11, was observed with 2.0 mg/l IAA (M_{32}). Perusal of data in Table 1 clearly show that increasing the concentrations of these growth regulators did not lead to an enhancement in the number of shoots or shoot length when applied alone. Earlier studies conducted by Bagari and Sharma (2018) have shown that BAP at its 1.0 mg/l concentration evoked best response in shoot proliferation. The results reported in this study are also in line with the results reported by Kumar (2022). The use of KIN alone was reported for shoot multiplication in *B. monnieri* (Wangdi and Sarethy, 2016; Bagri and Sharma, 2018). Kumari *et al*. (2014) demonstrated that 0.5 mg/l KIN induced the highest shoot proliferation in nodal explants, with up to 12.4 shoots per explant. However, contradictory results were noted in studies by Talekar *et al.* (2020) reporting that higher concentrations of KIN (2 mg/l) were more effective in promoting shoot regeneration.

Table 1. Effect of different concentrations of BAP, KIN, NAA and IAA alone on average number of shoot(s) and shoot length after 28 days

Media code	Growth hormone conc. (mg/l)				No. of shoots	Shoot length (cm)
	BAP	KIN	NAA	IAA		
M_0	-	-	-	-	1.67±0.33	0.7±0.35
M_1	0.25	-	-	-	6.45±0.69	4.01±0.38
M_2	0.50	-	-	-	6.67+0.78	4.69±0.17
M_3	0.75	-	-	-	5.33±0.19	4.43±0.05
M_4	1.00	-	-	-	6.56±0.29	4.29±0.29
M_5	1.25	-	-	-	6.22±0.29	3.81±0.20
M_6	1.50	-	-	-	5.22±0.40	3.50±0.35
M_7	1.75	-	-	-	4.89±0.29	4.08±0.17
M_8	2.00	-	-	-	4.67±0.19	3.51±0.33
M_9	-	0.25	-	-	5.89±0.11	4.08+0.21
M_{10}	-	0.50	-	-	5.67±0.58	4.45+0.12
M_{11}	-	0.75	-	-	6.33±0.58	3.92+0.21
M_{12}	-	1.00	-	-	5.78±0.55	3.32+0.07
M_{13}		1.25		-	5.44±0.29	4.03±0.35
M_{14}	-	1.50	-	-	5,33±0.58	3.47±0.34
M_{15}		1.75	-	-	5.22±0.29	4.97±0.32
M_{16}	-	2.00	-	-	5.11±0.29	4.11+0.24
M_{17}	-	-	0.25	-	5.89±0.29	4.20±0.24
M_{18}	-	-	0.50	-	4.44±0.40	4.10±0.16
M_{19}	-	-	0.75	-	3.89±0.22	3.86±0.21
M_{20}	-	-	1.00	-	3.89±0.29	3.64±0.04
M_{21}			1.25		5.11±0.62	4.17±0.18
M_{22}	-	-	1.50	-	4.44±0.78	4.61±0.20
M_{23}	-	-	1.75	-	4.89±0.62	4.39±0.11
M_{24}	-	-	2.00	-	2.78±0.59	3.92±0.23
M_{25}	-	-	-	0.25	4.11±0.11	3.57±0.11
M_{26}	-	-	-	0.50	3.67±0.00	3.44±0.12
M_{27}	-	-	-	0.75	4.33±0.19	3.90±0.07
M_{28}	-	-	-	1.00	3.44±0.48	4.03±0.05
M_{29}				1.25	5.11±0.22	3.96±0.20
M_{30}	-	-	-	1.50	3.55±0.40	3.92±0.10
M_{31}	-	-	-	1.75	4.22±0.59	3.96±0.08
M_{32}	-	-	-	2.00	2.44±0.11	3.74±0.14
	C. D.				1.246	0.607
	S. E. (Mean)				0.441	0.214

In addition to individual PGRs, combinations of BAP, KIN and auxins (IAA, NAA) were tested. The most effective combination (Table 2) was BAP (0.5 mg/l), KIN (0.75 mg/l) and IAA (1.5 mg/l), which resulted in 8.11 ± 0.29 shoots per explant and a shoot length of 4.77 ± 0.15 cm. This combination was followed by BAP (0.5 mg/l), KIN (0.75 mg/l) and IAA (0.5 mg/l), producing 7.22 ± 0.11 shoots per explant. Many of the previous reports on *B. monnieri* micro propagation used different combinations of cytokinins and auxins for maximum shoot induction. These combinations included BAP + IAA (Narwal, 2016; Ranjan and Kumar, 2018), BAP + NAA (Rency *et al.*, 2016; Ranjan *et al.*, 2018), BAP + Kn (Chauhan and Shirkot, 2020). Aggarwal *et al.* (2020) reported that on MS medium containing 5.0 µM of BAP and 1.0 iM NAA, the highest shoot regeneration frequency was seen. The optimal shoot multiplication observed in this study can be attributed to the synergistic effects of cytokinins (BAP, KIN) and auxins (IAA, NAA). Cytokinins promoted cell division and shoot initiation, while auxins regulated cell elongation and root development. The combination of these hormones created an ideal *in vitro* environment for shoot proliferation, as confirmed by similar findings in other studies on *Bacopa* and related species (Aggarwal *et al.*, 2020).

The most favourable results were obtained with RM_4 (half-strength MS medium + 2.0 mg/

Table 2. Effect of different combinations of growth regulators on average number of shoot(s) multiplication after 28 days

Media code	Growth hormone conc. (mg/l)				No. of shoots	Shoot length (cm)
	BAP	KIN	NAA	IAA		
MC_1	0.50	-	0.50	-	6.11±0.11	3.56±0.29
MC_2	0.50	-	1.00	-	5.33±0.29	4.77±0.15
MC_3	0.50	-	1.50	-	6.67±0.33	3.22±0.40
MC_4	0.50	-	2.00	-	6.22±0.11	3.44±0.29
MC_5	0.50	-	-	0.50	5.89±0.40	3.61±0.46
MC_6	0.50	-	-	1.00	6.67±0.29	2.31±0.18
MC_7	0.50	-	-	1.50	5.22±0.48	3.78±0.22
MC_8	0.50	-	-	2.00	5.78±0.88	3.72±0.43
MC_9	-	0.75	0.50	-	5.44±0.11	3.44±0.11
MC_{10}	-	0.75	1.00	-	5.78±0.29	4.33±0.38
MC_{11}	-	0.75	1.50	-	5.22±0.33	3.33±0.00
MC_{12}	-	0.75	2.00	-	5.67±0.33	3.34±0.33
MC_{13}	-	0.75	-	0.50	6.10±0.11	3.55±0.22
MC_{14}	-	0.75	-	1.00	7.10±0.29	2.67±0.19
MC_{15}	-	0.75	-	1.50	6.22±0.11	4.70±0.15
MC_{16}	-	0.75	-	2.00	5.89±0.13	3.68±0.19
MC_{17}	0.50	0.75	0.50	-	7.10±0.22	4.11±0.49
MC_{18}	0.50	0.75	1.00	-	6.10±0.29	3.67±0.33
MC_{19}	0.50	0.75	1.50	-	6.03±0.11	3.73±0.54
MC_{20}	0.50	0.75	2.00	-	6.67±0.33	3.94±0.34
MC_{21}	0.50	0.75	-	0.50	7.22±0.11	4.39±0.39
MC_{22}	0.50	0.75	-	1.00	6.22±0.11	4.33±0.38
MC_{23}	0.50	0.75	-	1.50	8.11±0.29	4.77±0.15
MC_{24}	0.50	0.75	-	2.00	7.11±0.11	3.56±0.29
	C. D.				1.173	0.900
	S. E. (Mean)				0.412	0.316

Table 3. Effect of different growth regulators on number of days taken for visible root formation from regenerated shoots

Media code	No. of days for root(s) initiation	No. of roots/ shoot on 28th day
RM_0 (control)	14.7±0.19	2.00±0.19
RM_1 (IBA 0.5 mg/l)	11.8±0.62	3.89±0.29
RM_2 (IBA 1.0 mg/l)	10.9±0.40	3.78±0.59
RM_3 (IBA 1.5 mg/l)	10.0± 0.19	4.78±0.48
RM_4 (IBA 2.0 mg/l)	9.4±0.11	5.67±0.38
RM_5 (NAA 0.5 mg/l)	11.6±0.30	3.78±0.40
RM_6 (NAA 1.0 mg/l)	13.8±0.29	4.22±0.29
RM_7 (NAA 1.5 mg/l)	13.7±0.19	4.11±0.44
RM_8 (NAA 2.0 mg/l)	13.5±0.15	3.33±0.33

l IBA), which showed an average of 9.4 ± 0.11 days for root initiation, with 5.67 ± 0.38 roots per shoot after 28 days (Table 3). This was followed by RM_3 (half-strength MS medium + 1.5 mg/l IBA). In contrast, roots cultured in hormone-free medium exhibited poor development indicating that the presence of growth regulators was essential for successful rooting. The application of auxins such as IBA, IAA and NAA played a crucial role in root induction, as evidenced by their ability to promote rootlet formation and subsequent plantlet development. For instance, Chauhan and Shirkot (2020) reported that 0.4 mg/l IAA was most effective in promoting a high root formation rate of 93.33%, whereas Rajendra Prasad *et al*. (2018) obtained 70% *in vitro* rooting after 25 days when shoot clusters were cultured on MS medium supplemented with 0.5-1.0 mg/ LIBA and 0.5-1.0 mg/L IAA. Furthermore, Rahe *et al*. (2020) investigated both full- and half-strength MS media with varying IBA concentrations (0.1–0.5 mg/l) and determined that half-strength MS with 0.25 mg/l IBA was the most effective for initiating roots from *in vitro Bacopa* shoots.

The acclimatization phase is critical for the survival of micro propagated plantlets, often leading to high mortality rates. In this study, plantlets of *B. monnieri* with well-formed shoots and roots achieved a 100% survival rate when acclimatized in pots containing autoclaved

Table 4. Effect of different types of potting mixtures on the survival rate of raised plants in green house

S. No.	Potting mixtures	Survival percentage on 28th day
PM_1	Sand (1)	80
PM_2	Sand:Soil (1:1)	90
PM_3	Sand:Soil: FYM (1:1:1)	100
PM_4	Sand:Soil:Vermicompost (1:1:1)	100
PM_5	Cocopea:Vermicompost: Perlite (3:1:1)	100

mixtures of sand, soil and farmyard manure (FYM); sand, soil and vermicompost; coco peat, vermicompost and perlite (Table 4). In contrast, a potting mixture with only sand resulted in an 80% survival rate. The organic matter from FYM and vermicompost likely provided essential nutrients, enhancing plantlet survival in greenhouse conditions. Similar acclimatization success was reported by Kumar (2022) in *B. monnieri*. Also, Mehta (2017) observed 100% survival of *in vitro Bacopa* plantlets when acclimatized in sterile coco peat. Differences in greenhouse conditions, as well as variations in the preparation of vermicompost and FYM, could account for the observed variations in survival rates.

In the present study, the percentage yield of *B. monnieri* in Petroleum ether, N-Hexane, ethanol and aqueous extract was determined and presented in Fig.1. The percentage yield was maximum in ethanolic extract (11.15%) as compared to other extracts. The extractive value of *B. monnieri* in aqueous extract was 8.85%. The petroleum ether and N-Hexane extract showed very less extractive value. Phytochemical screening is a vital tool in analyzing bioactive compounds within plants, offering a quick, cost-effective and straight

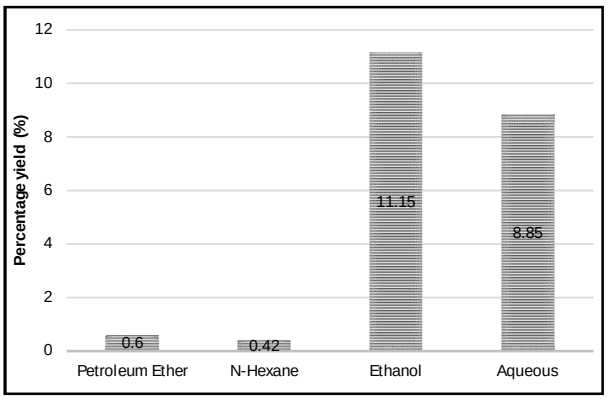

Fig. 1. Percentage yield of different extracts of *Bacopa monnieri.*

forward method to identify various phytochemicals present. Pawar *et al*. (2016) evaluated the extractive values of *B. monnieri* using different solvents. The findings revealed that the methanolic extract had the highest yield (10.1%), followed by the ethanolic extract (8.6%), and the aqueous extract (7.6%). Other solvents like chloroform, acetone, dichloromethane, ethyl acetate and petroleum ether resulted in significantly lower yields, ranging from 0.5 to 2%. These results suggest that methanol and ethanol are the most effective solvents for extracting phytochemicals from *B. monnieri*. Perusal of data in Table 5 reveals that alkaloids, flavonoids, phenols, saponins, tannins, carbohydrates and steroids were detected in both ethanolic and aqueous extracts. However, proteins/amino acids were absent in the ethanolic extract, and glycosides were not found in either extract. Indoliya *et al*. (2022) reported the presence of carbohydrates, proteins, amino acids, steroids, glycosides, cardiac glycosides, anthraquinone glycosides,

Table 5. Phytochemical screening of ethanolic and aqueous extracts of *Bacopa monnieri*

S. No.	Secondary metabolites	Phytochemical tests	Ethanol	Aqueous
1.	Alkaloids	Mayer's test	++	++
		Wagner's test	++	++
2.	Flavonoids	Alkaline reagent test	++	++
3.	Phenol	Litmus test	++	++
		Ellagic acid test	++	++
4.	Saponins	Foam test	++	++
5.	Tannins	Alkaline reagent test	++	++
6.	Carbohydrates	*Fehlings* test	++	++
7.	Glycosides	Borntrager's test	—	—
8.	Steroids	Leibermann-Burchard reaction	++	++
9.	Protein/Amino acids	Ninhydrin test	—	++

++ = Present and — = Absent.

saponin glycosides, flavonoids, alkaloids and tannins in both ethanolic and aqueous extracts of *B. monnieri*. Gayathri *et al*. (2021) analyzed the phytochemical compounds of *B. monnieri* using different solvents like aqueous, ethanol and methanol and reported the presence of saponnins, tannins, phenolic compounds, steroids, alkaloids and flavonoids, while reducing sugars, terpenoids and anthraquinone were absent in both aqueous and ethanolic extracts.

CONCLUSION

In conclusion, this study established an efficient micro propagation protocol for *Bacopa monnieri*, showing that BAP, either alone or in combination with KIN and IAA, effectively promoted shoot multiplication. Optimizing auxin concentrations, particularly IBA, was crucial for healthy root development. These findings enhanced micro propagation techniques, facilitating large-scale propagation and conservation of this threatened medicinal plant. The results highlighted the essential roles of cytokinins and auxins in optimizing shoot and root growth, supporting sustainable *B. monnieri* production. Additionally, the phytochemical screening confirmed the presence of bioactive compounds, contributing to the potential development of natural drugs.

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