



# A COMPARATIVE ANALYTICAL STUDY OF BRAHMI (*Bacopa monnieri* Linn.) CULTIVATED BY VRIKSHAYURVEDA AND CONVENTIONAL METHOD WITH SPECIAL REFERENCE TO YIELD AND QUALITY

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## ABSTRACT

*Bacopa monnieri* (Brahmi), an important *Medhya Rasayana* drug in *Ayurveda*, is extensively valued for its neuroprotective, adaptogenic, and antioxidant activities. With the growing global demand for *Brahmi*, conventional cultivation practices increasingly rely on chemical fertilizers, which raise concerns regarding environmental sustainability and medicinal quality. *Vrikshayurveda*, the ancient Indian system of plant science, advocates organic and sustainable cultivation methods, particularly the use of *Kunapajala*, a fermented, nutrient-rich liquid manure.

The present study aimed to compare the yield and quality of *Bacopa monnieri* cultivated using *Vrikshayurveda* and conventional farming methods, and to scientifically validate the role of *Kunapajala* in enhancing plant biomass and active phytochemical content. The experiment was conducted on three groups: *Vrikshayurveda* (*Kunapajala*-treated), Conventional (chemical fertilizers), and Control (without manure). *Kunapajala* was prepared according to classical texts and sprinkled every 15 days in a 1:30 dilution over a five-month period. Growth parameters, biomass yield, physicochemical properties, and phytochemical profiles were assessed. Quantification of the active constituents, Bacopasides, was performed using High Performance Liquid Chromatography (HPLC).

Results indicated that the *Vrikshayurveda* group exhibited superior growth and biomass yield (185.36 q/ha) compared to the Conventional (158.4 q/ha) and Control (82.05 q/ha) groups. Physicochemical analysis showed lower total and acid-insoluble ash values and higher water- and alcohol-soluble extractives in the *Vrikshayurveda* group, suggesting improved purity and active content. HPLC analysis confirmed a higher concentration of total Bacopasides (6.03 mg/dl) in the *Vrikshayurveda* group than in the Conventional (2.44 mg/dl) and Control (2.63 mg/dl) groups.

The findings validate that *Vrikshayurveda* method of cultivation using *Kunapajala* enhances soil fertility, plant vigor, yield, and phytochemical quality, highlighting its potential as a sustainable and eco-friendly approach for medicinal plant cultivation.

**Keywords:** *Vrikshayurveda*; *Kunapajala*; *Vrikshayurveda*; *Bacopa monnieri*. Linn.

## INTRODUCTION

Over recent decades, medicinal plants have gained renewed global importance owing to their therapeutic efficacy, safety, and minimal adverse effects. The herbal industry is expected to reach nearly US \$5 trillion by 2050, indicating rising global demand.<sup>1</sup> In India, about 95% of herbal raw materials are sourced from the wild; however, overharvesting, habitat loss, and climate change have led to species depletion and biodiversity decline. Cultivation of medicinal plants has therefore become essential for sustainable and quality raw material production.<sup>2</sup>

In Ayurveda, Dravya is a key component of Chikitsa *Chatushpada*, crucial for health preservation and disease management. Its ideal qualities—*Bahukalpam*, *Bahugunam*, *Sampannam*, and *Yogya*—depend on proper cultivation.<sup>3</sup> Excessive use of chemical inputs has degraded soil health, highlighting the need for eco-friendly practices. *Bacopa monnieri* (*Brahmi*), an important Medhya Rasayana<sup>4</sup>, is endangered due to over exploitation. *Vrikshayurveda* advocates organic methods such as use of *Kunapajala*. This study compares *Vrikshayurveda* and conventional cultivation methods of *Brahmi* with respect to yield, physicochemical, and phytochemical parameters to validate traditional practices.

## OBJECTIVES:

1. To cultivate *Brahmi* (*Bacopa monnieri*. Linn) by *Poshana vidhi* according to *Vrikshayurveda*
2. To cultivate *Brahmi* (*Bacopa monnieri*. Linn) by Conventional method.
3. To cultivate *Brahmi* (*Bacopa monnieri*. Linn) by Control method
4. To observe and compare the effect of various cultivation methods of *Brahmi* (*Bacopa monnieri*. Linn) w.s.r to growth and biomass at the end of harvest.
5. To observe and compare the outcome of both *Vrikshayurveda* and Conventional methods of cultivation within and with that of other groups in terms of quantity

and quality of some phytochemical standards along with marker compound.

## MATERIALS AND METHODS

*Brahmi* (*Bacopa monnieri*. Linn) was collected from Dhanwantari Vana, Jnana Jyothi Nagar, Gnana Bharathi, Bengaluru, Karnataka. The genuinity of the cultivational drug was confirmed and authenticated as *Brahmi* (*Bacopa monnieri*. Linn) at F.R.L.H.T, Yelahanka Bengaluru.

## KUNAPAJALA PREPARATION

Table no.1: Ingredients of Kunapajala

Sl. No	Ingredients	Quantity
1.	<i>Matsya mamsa</i> (mackerel) (procured from local vendor)	3kg
2.	<i>Masha</i> ( <i>Vigna mungo</i> ) soaked in water for overnight. (procured from local vendor)	500gms
3.	<i>Madhu</i> (lions brand honey)	2.5kgs
4.	<i>Go-ksheera</i> (Nandini milk)	6 liters
5.	<i>Tila choorna</i> (procured from local vendor)	500gms
6.	<i>Ghritha</i> (Nandini brand)	500gms
7.	<i>Jala</i>	12 Litres

## PREPARATION OF KUNAPAJALA<sup>5</sup>:

*Masha* was soaked overnight, and *tila pisti* was prepared using a mixer grinder. For the main procedure, whole fish along with the soaked *masha* were boiled in four parts (approximately 12 liters) of water on *madhyamagni* (moderate heat) until the volume was reduced to half, about 6 liters. The vessel was then removed from heat, allowed to cool, and the mixture was filtered first through a mesh and then through a cloth. The filtrate was collected in a plastic container, after which *tila pisti*, *ksheera*, *ghruta*, and *madhu* were added sequentially and mixed thoroughly. The container was covered with a cloth, tied securely with a rope, and kept in a warm environment at around 37°C for 15 days to allow fermentation. After the fermentation period, the container was opened, and the liquid was filtered again through a clean cloth and stored in an airtight plastic container. Finally, the prepared *kunapajala* sample was sent to a bio-centre laboratory for analysis and was also used for irrigation in the *Vrikshayurveda* plot.

## KUNAPAJALA, WATER AND SOIL ANALYSIS

*Kunapajala*, Soil and water of the cultivational plot was analysed for Electrical conductivity, Macronutrients, Micronutrients and Heavy metals at Bio-centre, Bengaluru.

## CULTIVATION OF *BRAHMI*

Table no.2: Techniques involved in Cultivation

Sr no	Technique	<i>Vrikshayurveda</i>	conventional	Control
1	<b>Ploughing</b>	yes	yes	yes
2	<b>Harrowing</b>	yes	yes	yes
4	<b>Farm yard manure at the rate of 10t/ha</b>	yes	yes	yes
5	<b>NPK fertilizers 5:3:3kg/NPK/ha</b>	no	yes	no

**Spacing:** between samplings was 10cm x10cm according to National Medicinal Plants Board.<sup>6</sup>

### Planting:

Table no.3: Number of plant cutting of *brahmi*

	<i>Vrikshayurveda</i>	Conventional	Control
No of cloning	3	3	3
No of plant cutting	50	50	50
Total no of plants in group	150	150	150

**Irrigation:** Frequency of irrigation was daily/ as per need of the plant.

**Manuring:** Nitrogen(N)(5kgs), Phosphorous(P)(3kgs), and Potassium(3kgs) fertilizers are commonly used for growing Brahmi, with application ratio of 5:3:3<sup>7</sup>, *Kunapajala* was sprinkled with water in the ratio of 1:30 liters. *Kunapajala* is classically indicated at every 15 days. NPK is normally applied 30 days once.

## VEGETATIVE GROWTH ANALYSIS

Growth parameters like Root length, Stem length, Number of nodes, Leaf length, Leaf width, Leaf area were recorded from randomly selected ten plants in each group the data was statistically analyzed.

## ANALYTICAL EVALUATION

Analytical evaluation of the trial drug *Brahmi* (*Bacopa monnieri* Linn.) was carried out through macroscopic, microscopic, physico-chemical, phytochemical, inorganic, and chromatographic analyses as per standard ICMR guidelines. Chromatographic analysis was performed using RP-HPLC to quantitatively estimate Bacoside-A content in three groups of *Brahmi churna*, employing a C18 column with acetonitrile and water (7:3) as the mobile phase, UV detection, and calibration against standard Bacoside-A. The results obtained from these analyses provided comprehensive quality control and standardization data for the trial drug.

## STATISTICAL ANALYSIS

All the data were compiled using one way ANOVA. P value <0.05 were considered as statistically significant. Mean values and standard error of mean were calculated and all the values were expressed as Mean  $\pm$  SD.

## OBSERVATIONS AND RESULTS –

Fig no.1: 30<sup>th</sup> day of cultivation

<p><i>Vrikshayurveda</i> group</p>	
<p>Conventional group</p>	
<p>Control group</p>	

Fig no.2: 100<sup>th</sup> day of cultivation

<p><i>Vrikshayurveda</i> group</p>	
<p>Conventional group</p>	
<p>Control group</p>	

## VEGETATIVE GROWTH PARAMETERS

After 170 days, the vegetative growth parameters of *Brahmi* showed statistically significant differences among the *Vrikshayurveda*, Conventional, and Control groups. Root length was highest in the *Vrikshayurveda* group ( $4.20 \pm 0.238$  cm), followed by the Conventional group ( $4.12 \pm 0.238$  cm), while the Control group showed the least root length ( $2.78 \pm 0.238$  cm), with differences being highly significant ( $p < 0.001$ ). Stem length also followed a similar trend, with the *Vrikshayurveda* group recording the maximum mean length ( $11.96 \pm 0.784$  cm), Conventional group moderate growth ( $10.16 \pm 0.784$  cm), and Control group the least ( $6.65 \pm 0.784$  cm). The number of nodes was significantly higher in the *Vrikshayurveda* group ( $7.26 \pm 0.229$ ) compared to the Conventional ( $6.13 \pm 0.229$ ) and Control groups ( $6.00 \pm 0.229$ ). Leaf length, leaf width, and leaf area were all significantly greater in the *Vrikshayurveda* group, with mean values of  $1.92 \pm 0.155$  cm,  $0.55 \pm 0.042$  cm, and  $1.04 \pm 0.119$  cm<sup>2</sup> respectively, whereas the Control group consistently showed the lowest values. All vegetative parameters across treatments remained within normal standard limits. The fresh weight of the

whole plant harvest was highest under *Vrikshayurveda* treatment (25.81 kg/150 sq ft; 185.36 q/ha), which was markedly higher than the Conventional and Control groups. Similarly, the air-dried harvest weight was maximum in the *Vrikshayurveda* group (6.53 kg/150 sq ft; 46.87 q/ha), followed by the Conventional group and then the Control group. Overall, both fresh and dry biomass production were significantly greater in the *Vrikshayurveda* group, indicating superior growth performance compared to Conventional and Control treatments.

## PHYSICO-CHEMICAL ANALYSIS

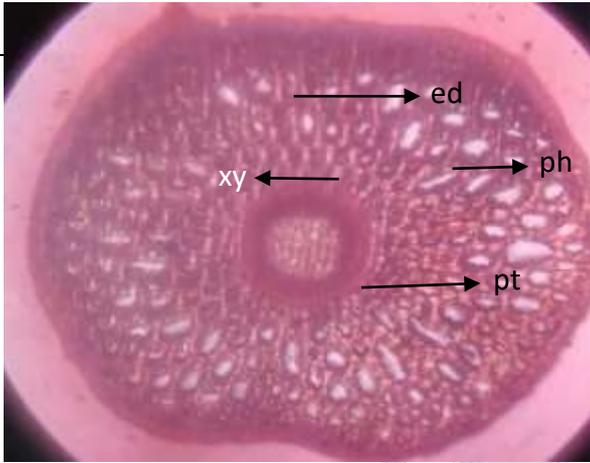
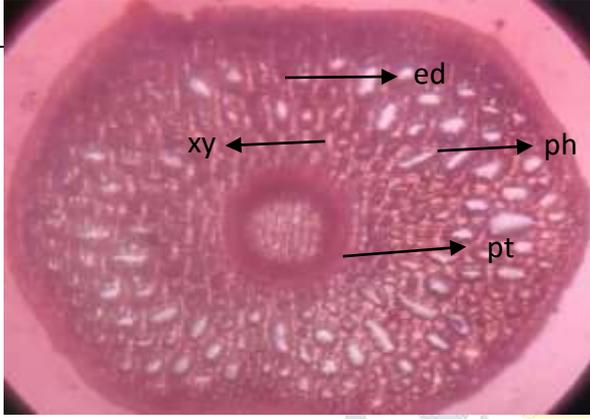
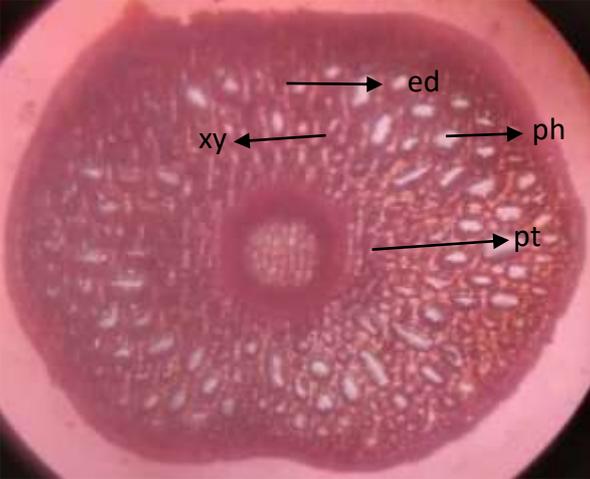
The loss on drying was highest in the conventional group (5.12%) compared to the *Vrikshayurveda* (3.61%) and control groups (2.83%), with the control group showing the least moisture content. All *Bacopa monnieri* samples complied with API limits for ash values; however, the conventional group showed higher total ash (17.9448%) and acid-insoluble ash (3.63%) than the *Vrikshayurveda* group, which exhibited the lowest total ash (16.3093%) and lower acid-insoluble ash (2.247%). Water-soluble extractive values exceeded API standards in both the *Vrikshayurveda* and conventional groups, with the highest value observed in the *Vrikshayurveda* group (17.457%), followed by the conventional (16.66%) and control groups (15.66%). Alcohol-soluble extractive values of all samples were within API limits, with the *Vrikshayurveda* group (9.533%) showing higher values than the conventional (8.582%) and control groups (7.561%). The pH values of all three groups were nearly identical, ranging from 6.26 to 6.28, indicating similar acidity profiles.

## ANALYTICAL EVALUATION

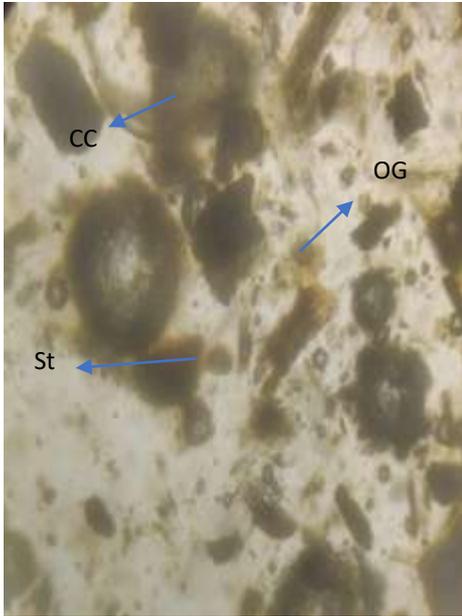
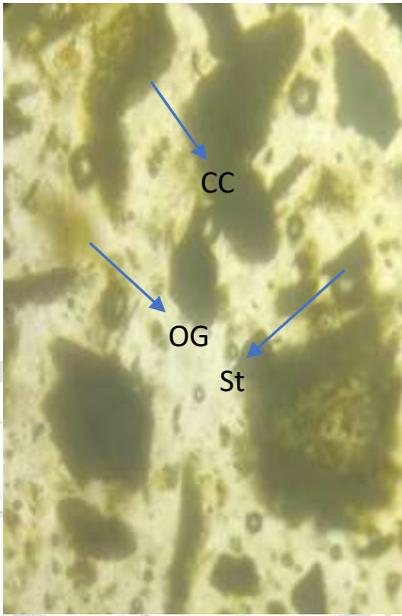
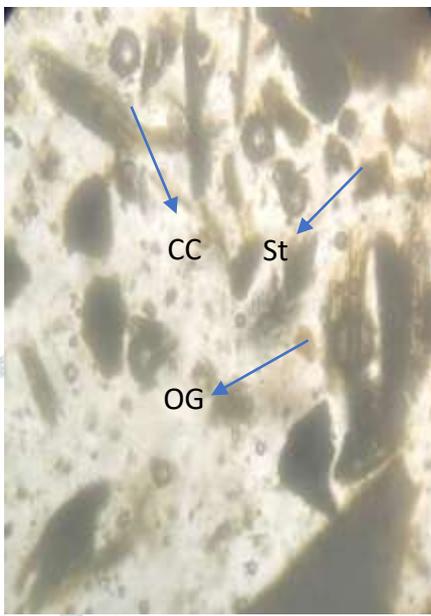
The organoleptic evaluation of fresh *Brahmi* (*Bacopa monnieri* Linn.) samples from the *Vrikshayurveda*, conventional, and control groups showed no noticeable differences, as all exhibited a smooth and fleshy touch, light greenish color, bitter taste ending in mucilage, and a specific characteristic odour. Similarly, the dried *Brahmi* samples from all three groups demonstrated comparable organoleptic characters, with bitter nature, light green to yellowish-green coloration, bitter and slightly astringent taste, and a specific odour. Overall, both fresh and dried samples across all groups displayed uniform organoleptic properties, indicating consistency in sensory characteristics irrespective of the cultivation method.

**T.S OF STEM OF ALL THREE GROUPS OF *BRAHMI***

Fig no.3: T.S of Stem of *Brahmi*

	<p><b><i>Vrikshayurveda</i></b></p> <p>ED-endodermis, ph- Phloem, Xy- xylem, pt-pith</p>
	<p><b>Conventional</b></p> <p>ED-endodermis, ph- Phloem, Xy- xylem, pt-pith</p>
	<p><b>Control</b></p> <p>ED-endodermis, ph- Phloem, Xy- xylem, pt-pith</p>

**POWDER MICROSCOPY OF ALL THREE GROUPS OF *BRAHMI***Fig no.4: Powder microscopy of *brahmi*

<i>Vrikshayurveda</i>	Conventional	Control
		
<b>CC- Chlorophyll content,</b> <b>St- Starch,</b> <b>OG- Oil globule</b>	<b>CC-Chlorophyll content,</b> <b>St- Starch,</b> <b>OG-Oil globule</b>	<b>CC-Chlorophyll content,</b> <b>St- Starch,</b> <b>OG-Oil globule</b>

**PHYTOCHEMICAL ANALYSIS**

Phytochemical analysis of *Brahmi* revealed a similar profile across the *Vrikshayurveda*, conventional, and control groups. The organic constituent analysis showed the presence of alkaloids, flavonoids, triterpenoids, saponins, tannins, and phenolic compounds in all three groups, while glycosides, steroids, resins, carbohydrates, starch, and proteins were absent uniformly. Inorganic constituent analysis further demonstrated the presence of essential minerals such as calcium, magnesium, sodium, potassium, iron, sulphate, phosphate, and chloride in all samples, whereas carbonate and nitrates were absent in every group. Overall, the phytochemical composition of *Brahmi* remained consistent irrespective of the cultivation method.

## HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

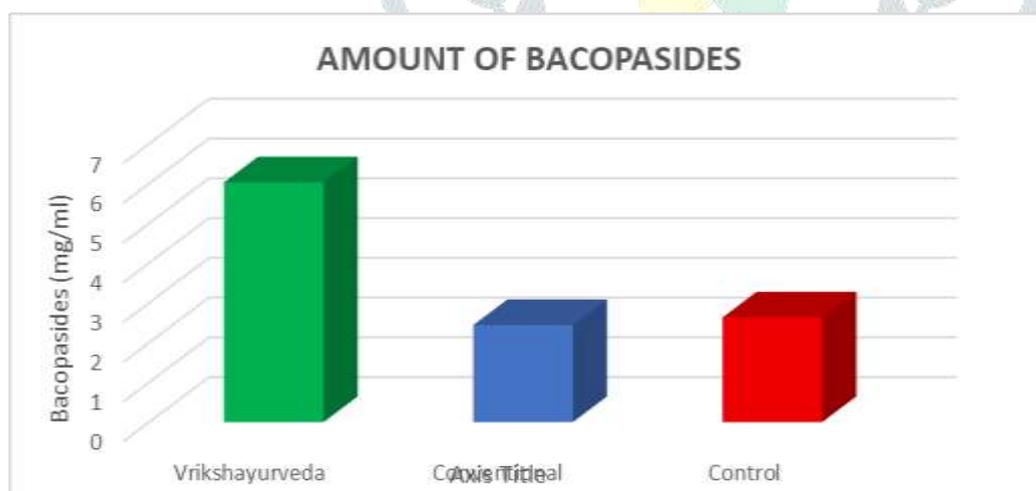
The HPLC profile of standard bacopasides at 205 nm showed four characteristic peaks corresponding to Bacoside A3, Bacopaside II, Bacopaside X, and Bacosaponin C with retention times of 23.726, 26.519, 30.289, and 35.257 minutes respectively, confirming the standard reference pattern. The Vrikshayurveda group exhibited all four bacopasides with comparable retention times, where Bacopaside II was the major constituent (RT 26.409 min; area 17282 mV·s), followed by Bacoside A3, Bacosaponin C, and Bacopaside X. Similarly, the conventional group showed retention times closely matching the standard, with Bacopaside II again predominating (RT 26.509 min; area 8896 mV·s), while other bacopasides were present in lower amounts. In the control group, Bacopaside II was the dominant peak (RT 26.446 min; area 11449 mV·s), along with Bacopaside X and Bacosaponin C, whereas Bacoside A3 was not detected. Overall, the HPLC analysis demonstrated comparable qualitative profiles across all groups, with variations observed mainly in the relative abundance of individual bacopasides.

### Total Bacopaside quantification:

Tab no.4: Bacopaside quantification

Parameters	<i>Vrikshayurveda</i> (mg/mL)	Conventional(mg/mL)	Control(mg/mL)
Total bacopasides	6.03	2.44	2.63

### QUANTIFICATION OF BACOPASIDE:



The analysis revealed that the *Vrikshayurveda Brahmi swarasa* contained the highest total bacopaside concentration (6.03 mg/mL), whereas the conventional group showed the lowest level (2.44 mg/mL). The bacopaside content in the *Vrikshayurveda* group was markedly higher than in the other groups, exceeding the control group by 3.4 mg/mL and showing a substantial increase when compared to the conventional group. The control group demonstrated intermediate bacopaside levels, with a concentration 2.63 mg/mL higher than that of the

conventional group. Overall, the findings indicate superior bacopaside content in the *Vrikshayurveda* group.

## DICUSSION

The present study demonstrates that the *Vrikshayurveda* method of cultivation using *Kunapajala* has a significant positive influence on the growth, yield, and quality of *Bacopa monnieri* when compared to conventional cultivation practices. *Kunapajala*, a fermented organic liquid manure, supplies readily available nutrients due to the breakdown of complex proteins, fats, and carbohydrates into simpler forms, thereby enhancing nutrient absorption and bioavailability. Its preparation process, comparable to *Sandhana Kalpana* in Ayurveda, ensures rapid nutrient uptake because of its *teekshna*, *sukshma*, and *ashukari* properties, facilitating efficient penetration into soil and plant tissues.<sup>8</sup> Unlike inorganic NPK fertilizers, which mainly support primary growth functions such as chlorophyll formation, root development, and disease resistance, *Kunapajala* provides a holistic nutrient profile along with bioactive compounds, vitamins, minerals, and essential fatty acids. The predominance of *madhura rasa*, *guru* and *snigdha guna*, and *prithvi-jala mahabhuta* attributes of *Kunapajala* ingredients contributes to *brimhana*, *tarpana*, and *balya* effects, leading to enhanced root length, stem growth, node formation, leaf expansion, and overall biomass. The significantly higher fresh biomass observed in the *Vrikshayurveda* group confirms its superior yield potential. Improved physicochemical parameters, such as lower ash values and higher extractive values, further indicate better purity and quality of the drug. Phytochemical analysis revealed the presence of essential secondary metabolites across all groups, while HPLC analysis showed markedly higher bacopaside content in the *Vrikshayurveda* group, establishing its superior therapeutic quality. Additionally, *Kunapajala* improves soil texture, moisture retention, and microbial activity while minimizing the risk of soil-borne pathogens. Overall, the study conclusively indicates that *Kunapajala* not only enhances plant growth and yield but also significantly increases secondary metabolite production, validating the effectiveness of the *Vrikshayurveda* method for sustainable and high-quality medicinal plant cultivation.

## CONCLUSION

The results clearly indicate that the *Vrikshayurveda* method was superior in enhancing both the yield and quality of *Brahmi*. Plants cultivated under this traditional approach exhibited improved vegetative growth, as reflected by increased root and stem length, a higher number of nodes, and larger leaf area, suggesting better nutrient absorption and overall plant vigor. Furthermore, the fresh biomass yield was markedly higher in the *Vrikshayurveda*-treated plants (185.36 q/ha) compared to those grown using conventional methods (158.4 q/ha) and the control group (82.05 q/ha), highlighting the significant growth-promoting potential of *Kunapajala*.

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