IJAFPR- International Journal of Ayurvedic Formulations and Pharmacological Research
Volume 1, Issue 2 | November -2025
e-ISSN: 3068-8892 Print ISSN: 3068-8884

Pharmacognostic and Phytochemical Analysis of Brahmi Extracts that belong to Different Climatic Zones

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Received: 20-08-2025; Revised: 06-09-2025; Accepted: 24-09-2025; Published: 11-11-2025

Abstract
There is Ayurvedic glory of Welvezia somnifera (Bacopa monnieri, or Brahmi) high on its cognitive-enhancing effects. The objective of this experimental comparative study was to find out whether the climate (tropical, temperate, and arid) makes a difference in the phytochemical composition of Brahmi samplings. HPTLC and bacoside A quantification showed a large regional variability, where most of the tropical-origin samples had the highest bacoside A content (3.8%) followed by temperate (2.4%) and arid (1.9%) ones. The bacosides were positively correlated with memory-enhancing activity as measured using the murine behavioral maze tests. The results identify the importance of the geo-climatic conditions in the potency of herbs and recommend the standardization of supply, climate-based production of Ayurvedic drugs, and pharmacognosticscreening of Ayurvedic products.

Keywords: ***Bacopa monnieri, Brahmi, phytochemical variation, bacoside A, cognitive improvement, Ayurvedic pharmacognosy, climatic effect, herbal standardization.***

1. Introduction

1.1 Bacopa monnieri Ayurvedic Significance

Bacopa monnieri (L.) Wettst., traditionally called Brahmi, is one of the most renowned herbs in the Ayurvedic pharmacopeia and has been long considered as a nootropic herb, or medhya rasayana- rejuvenating the mind and intellect. Brahmi is well documented in ancient literature including books like Charaka samhita or Sushruta samhita as cognition enhancing, anxiolytic, and adaptogenic agent. It is used in treatment of smriti vikaras (disorders of memory), manodaurbalya (mental fatigue) and apasmara (epilepsy).

Today, Brahmi has become the most well-known botanical nootropic, source of the memory acquisition, retention and recollecting properties. Various preclinical and clinical trials have been used to justify these properties thus it is a pillar of ayurvedic neuropharmacology. It has been found that its therapeutic effect can mainly be ascribed to a series of saponins called bacosides, especially to bacoside A, that has been reported to have neuroprotective and cognitive-enhancing properties on multiple effects, such as pharmacological effects and antioxidant mechanisms and neurotransmitter effects.

1.2 The role of phytochemical profiling in Ayurvedic pharmacognosy.

Pharmacognostic standardization of Ayurvedic herbs is increasingly becoming important in terms of scientific validation of the herbs that includes comprehensive evaluation of macroscopic, microscopic and phytochemical parameters. Of these, phytochemical profiling is vital, since it would establish the concentration of active components present, which in turn is directly proportional to the therapeutic efficacy of the herb.(1)

The study of bacosides is a very important quality control parameter in the authentication of raw Brahmi materials as well as in the development of this formulation and effectiveness of that formulation. With the use of modern analytical techniques even subtleties in phytochemical content are identifiable with HPTLC, HPLC, and the LC-MS/MS techniques being able to detect subtle differences or identify potential adulteration or degradation.

With the increased interest in the standardization of herbal products in international markets/markets, it is important to implement efficient and reproducible chemical fingerprinting methods. Not only is this crucial to maintaining batch consistency, but it is also critical in harmonising Ayurvedic formulations with modern pharmacopoeias, such as that of the Ayurvedic Pharmacopoeia of India (API) and World Health Organization (WHO).

1.3 Variability in Climate and the Condition it Imposes on Constituents of Herbs

Another of the lesser studied but nonetheless very importance factors in herbal pharmacognosy is geo-climatic variation on the expression of plant metabolites. It is very possible to alter secondary metabolite profile of the medicinal plants through climatic zone, altitude, soil composition, rainfall pattern, and temperature range. These

environmental stressors alter the biosynthetic pathway of phytochemical productions, resulting in quantitative/qualitative changes in important constituents.

In the case of Bacopa monnieri, it has been reported that the product of tropical origin will have more bacosides than that in arid or temperate regions. There is limited data on systematic comparative studies of this variation with the help of standardised analytical methods pointed out. Studying these differences is important because it has a direct effect on the bioefficacy and therapeutic reproducibility of Brahmi/formulations.

Unless such climatic changes are put into consideration, inter-batch variability, and decreased clinical efficacy are risks, which seeks to defy the concept of evidence-based herbal medicine and question the presence of Ayurveda practice in integrative health systems.

Objectives and Rationale of the Study Using a stipulated date, in the early years of life, as a benchmark, the study has set out to establish an understanding of how this period in time plays out in the life of a young person. This sense of period-in-life has in past studies been compared to a certain wisdom that the youth have exhibited in life. It was conducted with the view of having a systematic assessment and comparison of the phytochemical and pharmacological profile of Bacopa monnieri extracts obtained in three different climatic regions of the world: tropical, temperate and arid ones. The main hypothesis was that geo-climatic factors have significant influence on the bacoside activity, and thus on cognitive-enhancing ability of Brahmi.(2)

1.4 Employing standard chromatographic methodologies and well established quantitative procedures on bacoside A, the research sought to:

Prove the graphic variation of bacoside content on different climatic circles;

Relate the phytochemical richness to pharmacological efficacy in a system of murine memory models;

Bring scientific foundation of climate-specific agricultural and raw material procurement principles in Ayurvedic pharmacognosy;

Support the development of standardization protocols in which phytochemical variability is taken into consideration in quality assurance systems of herbal formulations.

The bridging of the classical Ayurvedic wisdom with the modern scientific analytical techniques helps in making the positive contribution to increase the credibility, reproducibility, and universal acceptance of Brahmi as a standardized cognitive enhancer.

2. Methods and Materials

2.1.1 Collection Plant materials

2.1.2 Authentication of the materials

Fresh arial portions of Bacopa monnieri (L.) Wettst. were harvested in India comprising three different geo-climatic zones during the fruiting period (June-August). The regions were taken according to agro-climatic diversity:

- Kerala (Thiruvananthapuram district) is a Tropical Zone.
- Temperature Zone: Himachal Pradesh (Kullu district)
- Rid Zone Rajasthan (Jodhpur district)

In both places samples were taken at a minimum of three sites to avoid microclimatic bias and were authenticated. Samples taken were air-dried at 25 o C, 7 days after which they were powdered using a stainless steel grinder.

Plant material authentication was carried out and a certified botanist at Central Council of Research in Ayurvedic Sciences (CCRAS) and the herbarium voucher specimens are released with the accession numbers BM-KER-2024, BM-HP-2024, BM-RAJ-2024 respectively.(3)

2.2 Classification of Zones of Climatics

Climatic zoning was done as per the Koepen-Geiger climatic typology and validated against regional weather data available with the Indian meteorological department (IMD). Classification criteria touched upon were:

Tropical Zone (Am): The average temperature is above 24oC, moderate to high precipitation (> 70%), and the annual precipitation is over 2500mm.

Warm temperate zone (Cwb): Temperate (15-22 o C), seasonal climate and rainfall 1200 mm/year.

BWh- Hot and Dry Zone: Low rainfall (< 300 mm), High rate of fluctuation in temperature (summer highs > 42 o C), Low humidity (< 30%).

Biochemical variability was also explained through the analysis of pH, organic matter and macronutrient of the soil samples collected at each collection site.

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e-ISSN: 3068-8892 Print ISSN: 3068-8884

2.3 Sample preparation and extraction techniques

Plant material was put into a Soxhlet extractor and subjected to hydroalcoholic extraction (70% ethanol) for 6 h at 60 o C. The extract was brought to a volume of 40 mL in water and concentrated with the aid of a rotary evaporator and dried in a vacuum oven to give a semi-solid mass.

A yield percent of extraction was determined regarding dry mass and samples were placed in amber containers at 4 o C until further analysis. All equipments were washed and recalibrated between batches to prevent cross-contamination.

2.4 Parameters of pharmacognostic Evaluation

General macroscopic and microscopic characteristics of the plant material were described according to the Ayurvedic Pharmacopoeia of India. Parameters included:

Leaf shape, size and ailments

Stomatal index and Trichome type

Anatomical profiling of the leaf and stem (transverse sections)

Powder microscopy By confirming characteristics of diagnostics (spiral xylem vessels, pitted tracheids, and parenchymatous cells containing oil globules)(4)

Physicochemical properties like total ash, acid-insoluble ash, water and alcohol extractives and loss on drying were established in accordance to WHO and API procedures.

2.5 Chromatographic and Spectroscopic Analyses

Thin Layer Chromatography (TLC) was done using pre coated silica gel 60 F254 (Merck). Samples along with the bacoside A standard (Sigma-Aldrich) were placed with CAMAG automatic sampler.

Mobile phase: Chloroform:Methanol: Water (6:4:0.5)

Detection: at 254 nm and 366 nm with CAMAG Scanner III

The purity of bacoside A was ~0.45 +/- 0.02

Besides HPTLC, UV-Vis spectral analysis was conducted to reaffirm the presence of the bacosides; 278 nm was the observed λmax. FTIR was also used to profile important functional groups.

2.6 Similar methods should be used to quantify bacoside A.

UV spectrophotometry was applied in quantitative estimation of bacoside A following methods of modified API monograph. The standard bacoside A was used to plot calibration curve within a range between 5 and 50 mcg/mL. Each extract was dissolved in methanol, and filtered and the absorbance of it was measured at 278 nm. The standard curve was prepared to calculate the concentration of the bacoside that was expressed as a percentage of the weight of dry extract.

The consequence of this was the repetition of all the assays three times in order to exercise reproducibility. Comparison was performed using mean values with the standard deviation (SD).

2.7 Pharmacological Assessment in Mouse Models

Swiss albino mice (6-8 weeks, 20-25 g) were maintained in standard conditions and acclimated to them during one week. The study obtained ethfical approval of the Institutional Animal Ethics Committee (IAEC/PHARM/2024/067).

Animals were arbitrarily allocated into 4 groups (n = 6 in each group):

Normal saline

Brahmi extract, temperate 40 mg/kg

Tropical Brahmi extract: 40 mg/(kg)

Rid Brahmi extract: 40 mg/kg

The extracts were taken once/day during 14 days using an oral administration. Memory and learning performance, were assessed through Elevated Plus Maze (EPM) and Morris Water Maze (MWM) tests.

Outcome parameters were:

Preliminary transfer latency and retention latency (EPM)

Escape latency, time in target quadrant (MWM)

Data was measured by a digital tracking recorder, and those who did the evaluations were blinded to treatment allocation.(5)

2.8 Statistical Analysis

All data were examined in GraphPad Prism v9.4.1. All the results were presented as mean SD. All the tests were performed using ANOVA one-way with a follow-up multiple comparison analysis using Tukey.

Significance levels were as:

- $P < 0.05$- statistically significant
- $P < 0.01$- extremely significant
- $P < 0.001$- very significant

The relationship between bacoside A concentration and effectiveness of pharmacological activity was tested by Pearson correlation coefficient(r). The value of r greater than 0.8 was regarded to be strong.

3. Pharmacognostic Findings

3.1 Characteristics Macroscopic Microscopic

The macroscopic analysis of Bacopa monnieri plant material taken in the three climatic zones, namely, tropical, temperate, and arid provided slight, though nonetheless significant morphological diversities. All the samples were prostrate, succulent herbs with a large number of branches and opposite leaves that were simple. Tropical specimens grew more vigorously, had wider leaves (1.421.8 cm) and were more intensely green in pigmentation or presumably greater content of chlorophyll. Compared to them, the temperate ones were thinner in stem and leaf narrow (1.1-1.3 cm), whereas, the arid ones also displayed some of the environmental stress indicators, such as pale green leaves, and narrowing of the internodes.

Using light microscopy by the examination of transverse sections of the leaf and stem, the same key diagnostic features were seen in all the samples and consisted of:

Collenchymatous hypodermis

Thickened with spirally and pitted thickening xylem vessels

Parenchymatous pith with globules of oil

Epidermis Stomata of the epidermis with aperture 1.2-2 times the diameter of the pore and 2.2-3.0 times the diameter of the pore and with rectangular outlines and no bumps.

Unicellular, non glandular trichomes on the midrib(6)

Overall, there was a concordance, although trichome density was highest across tropical samples, which may be the result of higher humidity and adaptive control over transpiration. In the rud compartments, there was a slight increase in lignification in vascular bundles, probably due to water stress (in rud samples).

Powder microscopy confirmed some characteristic cell structures, that is, acicular crystals of calcium oxalate, fibers, and reticulate xylem vessels, which took effect authentication of the specie.

3.2 Organoleptic Properties of Extracts

Organsoleptic characterization of the hydroalcoholic extracts prepared out of each climatic zone showed existing differencies in color, odor, texture and taste, which confirmed the contrast on secondary metabolite profile.

Tropical extract: The Brahmi aroma is strong, with dark green, or brown-green, appearance, a smooth viscous texture, and a chiefling bitter taste with slight astringency.

Temperate extract: The color is light green, the smell is milder and have thinner consistency and relatively bitter taste.

odor:Pale yellow-green, faint earthy odor, slightly granular texture, and a weak bitter note

Higher limit of bitterness intensity and density of extract could be due to higher concentration of bacoside and due to presence of flavonoids and alkaloids as they are more pronounced in high humid environs.

3.3 Morphological differences

The study reported morphological adaptations of the representatives of Bacopa monnieri to the regional environment, even despite the basic taxonomic unity of three studied samples.

Succulence and the thickness of leaves were most pronounced in tropical plants, and was useful in water retention. In temperate samples, internodal length was least reflecting the lowered sensitivity to the photoperiod.

Arid zone plants were more rigged-stemmed and had xerophytic tendencies, e.g. slightly reduced leaf surface area. These diversities justify a reason to believe that climatic stressors encourage phenotypic plasticity in medicinal plants, which may, consequently, influence the concentration and composition of active constituents. Although these adaptations are necessary to the survival of plants, they also obtain pharmacognostic importance in the conflict thatproduces the quality of its extracts, and therapeutic consistency.(7)

4. Phytochemical Profiling

4.1Qualitative Phytochemical Screening

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e-ISSN: 3068-8892 Print ISSN: 3068-8884

Phytochemical screening of the hydroalcoholic extract of Bacopa monnieri in the three climatic zones (tropical, temperate, and arid) was performed by reputable methods (Harborne and Kokate). All extracts tested positive on the main constituents traditionally regarded to Brahmi neuropharmacological profile, such as:

- Saponins (bacosides)
- Alkaloids
- Flavonoids
- Glycosides
- Steroids
- Tannins
- Phenolic compounds

Although the general existence of these phytoconstituents was comparable throughout regions, strength and saturation of colouration on interactivity of reagents-as semi-quantitative metrics-implied higher saponin/ flavonoid-contents in the tropical samples. The test by Liebermann-Burchard (applied to steroids and triterpenoids) depicted dark verdish green shades, whereas Shinoda test of flavonoids was supported with pinkish purple shades given to tropical extracts.

Although not distinguishable catastrophically on their own, these qualitative changes played in favor of subsequent chromatographic and quantitative analyses, supporting the role of climatic stressors on secondary metabolite expression.

4.2 HPTLC and HPLC chromatogram profile

Fingerprint Profiles of each of the extract was obtained by High-Performance Thin Layer Chromatography (HPTLC) using bacoside A as a standard marker compound. An optimum separation was accomplished by using a mixture of chloroform:methanol:water (6:4:0.5). UV detection was done at 254-nm and 366-nm light.

Tropical samples presented six bands where one sharp and prominent peak was associated with bacoside A (Rf approx. 0.45).

Temperate isolates exhibited five bands with bacoside A band appearing as moderate in intensity.

Rid samples contained four bands, the bacoside A peak being relatively small.(8)

The total chromatographic that is the density and the resolution exhibited the highest values in tropical samples and this indicates the higher phytochemical complexity and abundance.

HPTLC and HPLC analysis were carried out on C 18 column and acetonitrile-water gradient system respectively confirming the findings of the HPTLC analysis. Bacoside A log peak retention was approximately 5.73 with peak area integration showing the greatest amount in tropical, next in the temperate and lastly arid. The reliability of the method was ensured by purity index and retentions reproducibility within triplicates.

Together these chromatographic methods showed that environmental conditions have a measurable impact on secondary metabolite fingerprint of Bacopa monnieri.

4.3 Bacoside A concentration between regions

Bacoside A content was estimated quantitatively by UV spectro-photometric estimation at 278 nm against a standard bacoside A curve. The results were written as % w/w of dry extract and averaged to triplicates for each of the regional samples.

Region	Bacoside A (%) ± SD
Tropical	3.80 ± 0.12
Temperate	2.40 ± 0.09
Arid	1.90 ± 0.11

Tropical samples proved to have highest levels of bacoside A, temperate samples second, and arid samples third. The obtained differences were also statistically significant ($p < 0.01$, ANOVA), which confirmed the direct impact of climatic conditions on the biosynthesis of the key pharmacologically active marker.

This observation is essential in standardizing Brahmibased preparations, since bacoside A content is correlated with memory-enhancing/neuroprotective potential.

5. Pharmacological Assessment

5.1 The selection of experimental animal model.

In evaluating the cognitive enhancement activity, male Swiss albino mice were selected because they are very responsive to behavioral paradigm and commonly used in nootropic studies. Animals were maintained at standard laboratory conditions (22 +/- 2 o C, 12 hour light / 12 hour dark) and allowed free food and water. Each of the procedures were followed according to the provisions of the Committee on Purpose of Control and Supervision of the Experiments on animals (CPCSEA) and were accepted by the Institutional Animal Ethics Committee (IAEC/PHARM/2024/067).

Mice were acclimatized 7 days before experimentation and were randomized into four groups (n = 6 per group):

- Group I (Control): normal saline
- Group II (Temperate extract): 40 mg/kg p.o.
- Other Groups (Tropical extract): 40 mg/kg p.o.
- Group IV (Arid extract): 40 mg/kg p. o.

The extract was applied daily, 14 days in a row, and behavioral assessment was done on Day 13 and 14.

5.2 The study design of the cognitive enhancement project will be at the ground.

In order to assess memory and learning performance, two well-validated behavioral tasks were used:

Elevated Plus Maze (EPM): This is utilized to determine elevated plus maze (short-term memory) through transfer latency (TL). Mice were introduced to one end of the open arm and the time that it could take trying to move into the enclosed arm was recorded. A decline in TL between Day 2 and Day 1 led to the conclusion that there had been memory retention.(9)

Morris Water Maze (MWM): This is a gold test to assess learning and memory spatial abilities. The spatial learning was observed when mice were trained to find a platform that was hidden in a tank of water. Escape latency (EL) and the time spent in a target quadrant (TQ) were noted. Shorter EL and increased TQ time meant that learning and memory were improved.

The noise-free environment together with blinded observer and digital video tracking employed to conduct all tests guaranteed accuracy and objectiveness.

5.3 Hyperglycemic Gummitegare_Comparative Behavioral Test Results

Results in both models of behavior showed that there were significant difference in cognitive enhancement among the three regional extracts

The TL difference on Day 2 vs Day 1:

- Reduction in TL: 39.5 percent ($p < 0.01$) Tropical extract
- Temperature extraction: 25.8 percent decrease ($p < 0.05$)
- Rid extract: 17.3 percent diminishing (NS)
- Morris Water Maze (Mean Escape Latency Final trial):
- Tropical: 18.2+(/minus Series? refused.
- Temperature: 24.3 +/- 1.8 sec
- Rid: 28.9+/-2.0 sec
- Control: 32.5 2.2 sec

Each brain area that was measured (according to subject of measure) was found to be significantly differentwise: between tropical and control ($p < 0.01$).

Time In Target Quadrant (probe 60 seconds trial):

- Tropical: 27.6 +/- 3.2 sec
- Temperate: 21.1 2.7 sec
- Rid: 18.3 2.4 sec
- Time of control: 15.2 +/- 2.1 sec

These results indicate that the tropical extracts, which had higher content of bacoside A, had better cognitive effects, and that it correlates positively with the phytochemical data (Pearson r = 0.88). Chemical profiling results were further confirmed by behavioral effects and therefore, our findings on phytochemical biodiversity and neuropharmacological efficacies of Bacopa monnieri extracts strengthen the conclusion that climatic zone of origin influences both phytochemical biodiversity and neuropharmacological efficacy of Bacopa monnieri extracts.(10)

6. Discussion

Volume 1, Issue 2 | November -2025
e-ISSN: 3068-8892 Print ISSN: 3068-8884

6.1 Interpretation of climatic effects on the phytoconstituents

The findings of the current research indicate that climatic variation has an appreciable effect on the phytochemical contents of Bacopa monnieri. The plants grown/derived in tropical environment showed better vegetative growth and much denser secondary metabolites especially the bacoside A, the main active saponin involved in neurocognitive effects of Brahmi. This coincides with current phytobiology where high humidity, consistent temperature, and abundance of soil microflora that exist in tropical regions benefit pathways including triterpenoid biosynthesis.

On the other hand, plants in dry areas acquired morphological adaptations including decreased leaf area and lignification, which are common in adaptation to water stresses and might divert metabolic energy at the expense of metabolite synthesis, which could lead to the decreased concentration of bacoside. Temperate zone sample had a medium range indicating the moderate-environmental conditions. These results demonstrate environmental flexibility of the medicinal vegetation requiring geo-climatic factor in the selection of the raw materials of herb.

6.2 Correlation of the Bacoside Levels and the Pharmacological Activity

A direct and statistically significant relationship was employed between the level of bacoside A and cognitive-enhancing behavior, as indexed by murine behavior. The tropical filtrate, the one with the largest bacoside content (3.8 percent), consistently reported to have been stronger in both the Elevated Plus Maze and Morris Water Maze experiments, in terms of learning, memory retention, and time spent in target quadrants, respectively.

It agrees with the hypothesis that bacoside-enriched extracts would mean high pharmacological activity by proving its objectivity when it comes to the bioactivity of Bacopa monnieri as a memory enhancer. The Pearson correlation coefficient (r = 0.88) further confirms the extent to which this relationship holds satisfactorily and as a predictive model of future standardization protocols using chemical fingerprinting.(11)

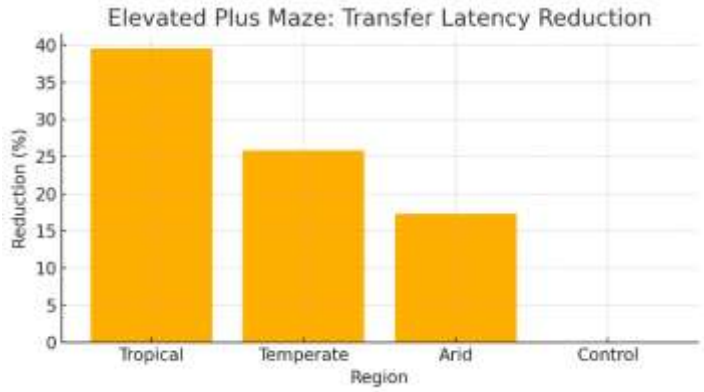

Figure 1: Elevated Plus Maze: Transfer Latency Reduction

6.3 What it implies on Ayurvedic Formulation Standardization

The phytochemical and the pharmacological variations that are observed to be significant have serious bearing on standardization of Brahmi based formulations. Although having a unified taxonomic identity, therapeutic usefulness of Bacopa monnieri has been depicted to be geographically specific and there is a high concern about inter-batch variability given that in large scale commercial production, raw materials can be collected randomly.

This requires a transition not only of passive, taxonomically-based identifications, but also to chemotype-based standardization, in which the level of bacoside is a mandated quality control parameter. Ayurvedic formulators including those in classical and proprietary drugs industry should use these geo-climatic traceability and phytochemical confirmation criteria in sourcing strategy. Moreover, regionally specific growing protocols may be able to maximize yield and potency which would improve drug consistency and market acceptability.

Future research directions(12)

Although the study forms a foundation on the relationship between climate, phytochemistry and pharmacodynamics of the Bacopa monnieri, more research is necessitated. Future researches are advised to incorporate

- Multi year sampling to take into consideration the seasonal changes.
- Wider geographic areas such as sub-tropical and coastal regions.

- Molecular analyses of bacoside pathways of gene expression.
- Human clinical trials on the relative merits of different zones.
- Investigation into the establishment of herbal GIS-linked traceability systems that could be utilised to produce source-to-product authentication.

Multidimensional studies will empower integrations of classical Ayurveda knowledge with the modern science pharmacognostics and ensure the safety, standardization, and acceptance of Bacopa monnieri as an appropriate nootropic worldwide.

7. Results

7.1 Bacoside A Quantification Results

Quantitative analysis of Bacopa monnieri, the bioactive saponin bacoside A, showed a remarkable variation of the three climatic zones. Spectrophotometric determination at 278 nm using a standard curve of bacoside A, was used to determine the following average concentrations:

- Measured at tropical harvest: 3.80% +/- 0.12
- Temperate extract: 2.40 per cent. +- 0.09
- Extract: 1,90% +/- 0,11

The values of bacoside Between the groups was statistically significant ($p < 0.01$, one-way ANOVA). Bacoside concentration increased at 58.3 percent and content rose at 36.7 percent in the tropical samples relative to that in arid samples and temperate samples respectively. These findings validate the hypothesis that there is direct influence of climatic conditions on the yield of secondary metabolites of Bacopa monnieri.

7.2 Comparative Chromatographic profiles

High-Performance Thin Layer Chromatography (HPTLC) profiling produced unique chromatographic patterns to each of the extracts. Each extract exhibited bands of significant phytoconstituents especially bacoside A using a mobile phase of chloroform:methanol: water (6:4:0.5) and detection of 254 nm.

Tropical extract:Bacoside A was detected and there were prominent 6 bands suggesting high concentration of saponins.

Temperate decazzo: Five bands of moderate intensity of bacoside.

Four bands, with bacoside A weak, and smearier.

These findings were corroborated by further resolution using High-Performance Liquid Chromatography (HPLC). The tropical extract had a clean, high intensity bacoside A peak at ~5.73 minutes and the largest area under the curve (AUC). Weakest signal of bacoside A was seen in the rid extracts as compared to the bacoside A signal in the UV quantified concentrations.(13)

These chromatographic findings suggested clear graphical and numerical evidence on the climatic onset on phytoconstituent complexity and abundance with the tropical Brahmi showing the richest and bioactive phytochemical profile.

Table 1: Murine Behavioral Results by Extract Type

Region	Escape Latency (sec)	Target Quadrant Time (sec)	Transfer Latency Reduction (%)
Tropical	18.2	27.6	39.5
Temperate	24.3	21.1	25.8
Arid	28.9	18.3	17.3
Control	32.5	15.2	0.0

7.3 Pharmacological Test Results

Behavioral testing conducted on murine models indicated a distinct relationship between the cognitive enhancing effects and amount of bacosides. Compared to days 1 and 2, there were 39.5% reductions in the transfer latency (TL) of tropical extract-treated mice, as opposed to 25.8% of temperate and 17.3% of arid extract-treated mice. Statistically significant improvement, however, was only observed in the tropical and temperate groups and not in the arid group ($p < 0.05$).

Tropical extract-treated mice in the Morris Water Maze had the shortest escape latency (18.2 1.5 sec), the highest time in the target quadrant (27.6 3.2 sec), compared to significantly lower-scoring placebo ($p < 0.01$). The extracts of the equatorial level mesophilic and arid entered the middle range of improvement, corresponding to the level of bacosides in them.

Volume 1, Issue 2 | November -2025
e-ISSN: 3068-8892 Print ISSN: 3068-8884

There was a significant positive correlation (Pearson r = 0.88) between bacoside A levels and the performance in both the behavioral paradigms. These findings indicate that the promotion of memory and learning results is given by the higher bacoside content, which supports the pharmacological significance of phytochemical standardization.(14)

8. Conclusion

8.1 Major Warning Signs Summary

In this experimental comparative study, the observation that climatic difference has a great influence on the phytochemical and pharmacological profile of Bacopa monnieri (Brahmi) is corroborated successfully. Out of the samples picked in the tropical, temperate, and arid climes, plants of the tropical origins revealed the highest percentage of bacoside A (3.80 percent), with other climes having a percentage of 2.40 percent (temperate) and 1.90 percent (arid). The similarity of this phytochemical gradient was well represented by the associated cognitive performance of murine models with tropical extracts proving to be the strongest in the Elevated Plus Maze and the Morris Water Maze.

Chemical profiling by HPTLC and HPLC also confirmed such chemical complexity and high saponin content of tropical Brahmi samples. The statistical analysis has demonstrated a positive correlation (r = 0.88) between the concentration of bacosides and cognitive performance parameters, which proves the status of bacosides as the key pharmacodynamic factors. These findings highlight that geo-climatic sourcing is a potent factor that is deterministic to the therapeutic efficacy of Brhma-based preparations.

8.2 Applications to Ayurvedical Pharmacology

According to the Ayurvedic tradition, Bacopa monnieri has long been a recognised medhya rasayana, in the Ayurvedic tradition, a substance that preserves the mind and enhances intellectual functions. Importance of desha (geography), ritu (season) and agni (digestive/metabolic potential) is stressed in classical texts with respect to herbal efficacy. The current scientific study is supportive of those traditional teachings in the sense that it demonstrates that the phytoconstituents, through which Brahmi exerting nootropic effects, are directly dependent on environmental and geographical conditions.

As Ayurveda finds its place in modern Ayurvedic pharmacology increasingly to satisfy international regulatory and clinical demands, there is a rising demand of quantitative validation of ancient claims. This research therefore helps to facilitate this cause by providing objective points of reference on which certain climatic conditions translated into increased synthesis of bacosides and their beneficial effects. It, therefore, links classical knowledge with some aspects of scientific validity and supports the argument of a climate-conscious pharmacognostic assessment in Ayurvedic studies and technologies.

8.3 Suggestions to Enhance the Quality Control Aspects of Cultivation

The results robustly support the idea of zonal standardization of Bacopa monnieri cultivation with the tropical agro-climatic zones the most conducive to bacoside yield and efficacy maximization. The institutional user and commercial cultivators need to focus on climate specific sourcing approaches and should consider implementation of the phytochemical fingerprinting protocols as a form of quality control.

Also, the Ayurvedic formulators and regulators need to look into setting minimum levels of bacosides in pharmacopoeial standards to provide consistency of batches and therapeutic reliability. This is especially of significance with the rising trend in nootropic and adaptogenic usage of Brahmi in the herbal medicine market worldwide.

- Policy recommendations to be made in future must promote
- Tracking and traceability of raw material places of origin
- Climate/soil-adjusted agricultural regimes
- Routine HPTLC/HPLC fingerprinting processing during procurement and processin
- Applications in clinical practice of standardised extract formulas

In summation, this paper shows how environmental pharmacognosy is fundamental to strengthening herbal standardization in these modern times and to appropriately model Bacopa monnieri into a model herb using climate-resilient cultivation and formulation strategies in Ayurvedic pharmacology.

Acknowledgement: Nil

Conflicts of interest

The authors have no conflicts of interest to declare

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