Pharmacognostic Standardization, HPTLC-Based Phytochemical Profiling, Behavioral Assessment, Acetylcholinesterase Inhibition, Oxidative Stress Marker Estimation, and Histopathological Evaluation of Bacopa monnieri Extract Against Scopolamine-Induced Memory Impairment in Wistar Rats

Rajdeo Archana Vitthal[1], Somesh Thapliyal[2], Bhanita Das[3], Shubham Ghosh[4], Snehal Tukaram Hase[5], Sanket Mukesh Kalmegh[6], Devendra Singh[7], Palvi Kumari[8], Mahalakshmi Kodadi[9*]

[1]Assistant Professor, Department of Pharmaceutics, Pravara Rural Education Society's Institute of Pharmacy, Loni A/P-Loni, Maharashtra, India

[2]Assistant Professor, Department of Pharmaceutical Sciences, HNBGU Srinagar Garhwal, Uttarakhand, India

[3]Assistant Professor, Department of Pharmaceutical Chemistry, University of Science and Technology Meghalaya (USTM), Techno City, Kling Road, Baridua, Ri-Bhoi District – 793101, Meghalaya, India

[4]Assistant Professor (Pharmacognosy), School of Pharmacy, Arka Jain University, Mohanpur, Gamharia, Seraikela Kharsawan, Jharkhand - 832108

[5]Assistant Professor, Department of Pharmaceutics, School of Pharmacy, Vishwakarma University, Pune, Maharashtra, India

[6]Assistant Professor, Department of Pharmaceutics, Amrutvahini College of Pharmacy, Sangamner, Ahmednagar, Maharashtra, India

[7]Associate Professor, Department of Pharmacology, School of Pharmacy, Mangalayatan University, Beswan, Aligarh, Uttar Pradesh, India

[8]Assistant Professor, Department of Allied Health Sciences, Saraswati College of Pharmacy, SGC Group, Gharuan, Mohali, Punjab, India

[9*]Professor & Deputy Director, Amity Institute of Pharmacy, Amity University, Math, Kharora, SH-9, Raipur, Baloda Bazar, Chhattisgarh, India (Corresponding Author).
Email: mkodadi@rpr.amity.edu

ABSTRACT

The present study was designed to investigate the pharmacognostic standardization, HPTLC-based phytochemical profiling, and neuroprotective efficacy of Bacopa monnieri extract against scopolamine-induced memory impairment in Wistar rats. The whole plant material was collected, authenticated, and subjected to hydroalcoholic extraction using a Soxhlet apparatus. Pharmacognostic evaluation included macroscopic, microscopic, powder analytical, and physicochemical studies for authentication and quality assessment of the crude drug. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, phenolics, and terpenoids. HPTLC fingerprint profiling confirmed the presence of characteristic marker

compounds including bacoside A and bacoside B. Neuroprotective activity was evaluated using a scopolamine-induced amnesia model through behavioral paradigms such as Morris water maze, elevated plus maze, and passive avoidance tests. Biochemical estimations included acetylcholinesterase activity and oxidative stress markers including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). Histopathological examination of hippocampal regions was performed to assess neuronal integrity and neurodegenerative alterations. Results demonstrated that treatment with Bacopa monnieri extract significantly improved learning and memory performance by reducing escape latency and enhancing retention memory in experimental animals. The extract also exhibited significant acetylcholinesterase inhibitory activity and restored endogenous antioxidant enzyme levels while reducing lipid peroxidation. Histopathological studies revealed substantial protection against scopolamine-induced neuronal degeneration and preservation of hippocampal architecture. The neuroprotective effects observed may be attributed to the synergistic antioxidant and cholinergic modulatory activities of bacosides and other phytoconstituents present in the extract. The findings of the present investigation scientifically validate the traditional use of Bacopa monnieri as a memory-enhancing and neuroprotective medicinal plant. The study further highlights the importance of pharmacognostic standardization and HPTLC fingerprinting in quality control and authentication of herbal drugs. Overall, the extract demonstrated promising anti-amnesic and neuroprotective potential, suggesting its possible therapeutic application in the management of cognitive dysfunction and neurodegenerative disorders.

Keywords: Bacopa monnieri, Scopolamine-induced amnesia, Neuroprotection, Acetylcholinesterase inhibition, Oxidative stress, HPTLC profiling, Pharmacognostic standardization, Cognitive enhancement, Antioxidant activity, Hippocampal neurodegeneration.

How to cite this article: Vitthal RA, Thapliyal S, Das B, Ghosh S, Hase ST, Kalmegh SM, Singh D, Kumari P, Kodadi M. Pharmacognostic Standardization, HPTLC-Based Phytochemical Profiling, Behavioral Assessment, Acetylcholinesterase Inhibition, Oxidative Stress Marker Estimation, and Histopathological Evaluation of Bacopa monnieri Extract Against Scopolamine-Induced Memory Impairment in Wistar Rats. Int J Drug Deliv Technol. 2026;16(48s): 951-967. DOI: 10.25258/ijddt.16.48s.89

Source of support: Nil.
Conflict of interest: None.

1. Introduction

Neurodegenerative disorders are progressive and debilitating diseases characterized by gradual neuronal dysfunction, synaptic degeneration, and cognitive decline. Among these disorders, Alzheimer's disease, Parkinson's disease, Huntington's disease, and vascular dementia are the most prevalent causes of memory impairment and reduced

quality of life worldwide. Cognitive dysfunction associated with these disorders is primarily manifested through deficits in learning, memory retention, attention, and executive functioning. The increasing prevalence of neurodegenerative diseases in aging populations has created a major global healthcare burden, necessitating the exploration of safer and more effective therapeutic strategies (Kumar & Singh, 2015). Current synthetic drugs used for cognitive enhancement, including cholinesterase inhibitors and NMDA receptor antagonists, often produce adverse effects and provide only symptomatic relief rather than complete neuroprotection. Consequently, growing scientific interest has focused on medicinal plants possessing neuroprotective and memory-enhancing activities.

One of the major pathological mechanisms involved in memory impairment is cholinergic dysfunction. The cholinergic hypothesis proposes that reduced acetylcholine levels and degeneration of cholinergic neurons significantly contribute to cognitive decline and dementia. Acetylcholine plays a critical role in synaptic plasticity, learning, and memory consolidation within the hippocampus and cerebral cortex. Excessive activity of acetylcholinesterase (AChE), the enzyme responsible for acetylcholine degradation, leads to decreased cholinergic neurotransmission and impaired cognitive performance (Terry & Buccafusco, 2003). In addition to cholinergic deficits, oxidative stress is another crucial factor implicated in neurodegeneration. Oxidative stress results from an imbalance between reactive oxygen species (ROS) generation and endogenous antioxidant defense systems. Elevated ROS levels induce lipid peroxidation, mitochondrial dysfunction, DNA damage, neuronal apoptosis, and neuroinflammation, ultimately contributing to neuronal degeneration and memory loss (Butterfield et al., 2013). Therefore, agents possessing both acetylcholinesterase inhibitory and antioxidant properties are considered promising candidates for the management of cognitive disorders.

Experimental animal models are extensively utilized to investigate the pathophysiology of memory impairment and evaluate potential neuroprotective agents. Scopolamine-induced amnesia in Wistar Rat is one of the most widely accepted pharmacological models for studying cognitive dysfunction. Scopolamine, a non-selective muscarinic receptor antagonist, disrupts cholinergic neurotransmission by blocking central muscarinic receptors, thereby producing temporary learning and memory deficits resembling those observed in Alzheimer's disease (Ebert & Kirch, 1998). Administration of scopolamine also promotes oxidative stress, neuronal damage, and hippocampal dysfunction, making this model suitable for assessing both anti-amnesic and neuroprotective effects of herbal formulations. Behavioral paradigms such as the Morris water maze, elevated plus maze, and passive avoidance test are commonly employed in this model to evaluate learning and memory performance.

Among medicinal plants traditionally used for neurological disorders, Bacopa monnieri has gained considerable scientific attention due to its nootropic and neuroprotective properties. Commonly known as Brahmi, this medicinal herb has been extensively utilized in Ayurvedic medicine as a memory enhancer, nervine tonic, and rejuvenating agent. The pharmacological activities of *Bacopa monnieri* are mainly attributed to bioactive constituents such as bacosides, alkaloids, flavonoids, saponins, and triterpenoids. These phytoconstituents have demonstrated antioxidant, anti-inflammatory, anti-apoptotic, and cholinergic modulatory effects that collectively contribute to cognitive enhancement (Russo & Borrelli, 2005). Experimental studies have shown that *Bacopa monnieri* improves synaptic communication, enhances neuronal regeneration, reduces β-amyloid toxicity, and protects against oxidative neuronal damage. Furthermore, medicinal plants of Indian origin possessing neuropharmacological activities have been comprehensively discussed by S. K. Jain, S. Parihar, and Niraj Pandey, who highlighted the therapeutic significance of traditional herbal medicines in neurological disorders (Jain et al., 2014).

Despite increasing interest in herbal neurotherapeutics, standardization and quality control of medicinal plants remain major

challenges. Variability in phytochemical composition due to geographical conditions, harvesting time, extraction procedures, and adulteration can significantly affect therapeutic efficacy. Pharmacognostic standardization therefore plays a vital role in ensuring the identity, purity, and quality of herbal drugs through macroscopic, microscopic, and physicochemical evaluation. In addition, High-Performance Thin Layer Chromatography (HPTLC) serves as a reliable analytical technique for phytochemical fingerprinting and marker compound identification. HPTLC profiling enables authentication of herbal extracts and assists in correlating phytochemical composition with biological activity (Mukherjee, 2019). Establishing standardized phytochemical profiles is essential for the development of reproducible and scientifically validated herbal formulations.

Although several studies have reported the neuroprotective potential of *Bacopa monnieri*, comprehensive investigations integrating pharmacognostic standardization, HPTLC-based phytochemical profiling, behavioral assessment, acetylcholinesterase inhibition, oxidative stress marker estimation, and histopathological evaluation in a single experimental design remain limited. Moreover, systematic correlation between phytochemical constituents and neuroprotective mechanisms against scopolamine-induced cognitive dysfunction requires further scientific validation. Therefore, the present study was designed to evaluate the anti-amnesic and neuroprotective potential of *Bacopa monnieri* extract using a multidimensional experimental approach in scopolamine-induced memory-impaired Wistar Rat models.

The primary aim of the present investigation was to perform pharmacognostic standardization and HPTLC-based phytochemical profiling of Bacopa monnieri extract and to evaluate its neuroprotective efficacy against scopolamine-induced memory impairment in experimental animals. The objectives included assessment of learning and memory behavior, estimation of acetylcholinesterase inhibitory activity, evaluation of oxidative stress biomarkers such as superoxide dismutase, catalase, reduced glutathione, and lipid peroxidation, along with histopathological examination of brain tissue to determine neuronal protection and restoration of hippocampal architecture.

2. Materials and Methods

2.1 Plant Material and Extraction

Collection, Authentication, and Processing of Plant Material

Fresh whole plant material of Bacopa monnieri was collected during the flowering season from the herbal garden and surrounding marshy regions of Lucknow, Uttar Pradesh, India. The collected plant specimen was authenticated by a qualified taxonomist from the Department of Botany, and a voucher specimen was deposited in the departmental herbarium for future reference. Foreign matter, soil particles, and extraneous contaminants were manually removed, followed by washing with distilled water to ensure cleanliness. The plant material was shade dried at room temperature (25–30°C) for approximately 10–15 days to prevent degradation of thermolabile phytoconstituents. The dried material was coarsely powdered using a mechanical grinder and passed through sieve No. 40 to obtain a uniform powder suitable for extraction and pharmacognostic studies.

Preparation of Extract Using Suitable Extraction Method

The powdered plant material was subjected to hydroalcoholic extraction using the Soxhlet apparatus. Approximately 500 g of coarse powder was packed in a thimble and extracted with ethanol:water (70:30 v/v) for 48 hours until complete exhaustion of phytoconstituents was achieved. The extract obtained was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary vacuum evaporator at controlled temperature below 45°C. The concentrated extract was further dried in a desiccator to obtain a semisolid mass and stored in an airtight container at 4°C until further use.

Percentage Yield Determination

The percentage yield of the extract was calculated based on the weight of dried extract obtained relative to the initial weight of powdered crude drug using the following formula:

$$\% \, Yield = \frac{Weight \, of \, dried \, extract}{Weight \, of \, crude \, drug} \times 100$$

The percentage yield was recorded and expressed as mean percentage w/w of dried plant material.

2.

2 Pharmacognostic and Phytochemical Evaluation

Macroscopic and Microscopic Characterization

Macroscopic evaluation of Bacopa monnieri was performed by examining morphological characteristics including color, odor, taste, texture, leaf arrangement, stem morphology, and overall appearance according to standard pharmacognostic procedures. Microscopic analysis was carried out using transverse sections of leaf and stem prepared by free-hand sectioning technique. Sections were stained with phloroglucinol and hydrochloric acid for lignified tissues and mounted in glycerin for microscopic observation under a trinocular microscope. Diagnostic features such as epidermal cells, stomata, vascular bundles, xylem vessels, trichomes, and parenchymatous tissues were examined and documented.

Powder Analysis and Physicochemical Parameters

Powder microscopy was conducted using powdered crude drug treated with chloral hydrate and specific staining reagents to identify diagnostic cellular components including fibers, starch grains, calcium oxalate crystals, vessels, and epidermal fragments. Physicochemical parameters such as total ash value, acid-insoluble ash, water-soluble ash, loss on drying, alcohol-soluble extractive value, and water-soluble extractive value were determined according to WHO guidelines for herbal drug standardization (WHO, 2011).

Preliminary Phytochemical Screening

Preliminary phytochemical investigations of the hydroalcoholic extract were carried out using standard qualitative chemical tests for detection of alkaloids, glycosides, flavonoids, saponins, tannins, phenolics, steroids, terpenoids, carbohydrates, proteins, and amino acids. Formation of characteristic color changes or precipitates was considered indicative of the presence of corresponding phytoconstituents.

HPTLC Fingerprint Profiling and Marker Compound Identification

High-Performance Thin Layer Chromatography (HPTLC) analysis was performed for phytochemical fingerprinting and identification of characteristic marker compounds. Precoated silica gel 60 F254 aluminum plates were used as stationary phase. The extract and standard bacoside solution were applied using an automatic sample applicator. The chromatographic plates were developed in a suitable solvent system consisting of toluene:ethyl acetate:methanol in optimized proportion inside a CAMAG twin-trough chamber. After development, plates were dried and scanned using CAMAG TLC scanner at suitable wavelength under UV light at 254 nm and 366 nm. Retardation factor (Rf) values, peak area, and densitometric profiles were recorded for characterization of phytoconstituents.

2.3 Experimental Animals and Study Design

Procurement and Maintenance of Wistar Rat

Healthy adult male Wistar Rat weighing between 180–220 g were procured from a CPCSEA-registered animal house facility. Animals were housed in polypropylene cages under standard laboratory conditions of temperature (25 ± 2°C), relative humidity (55 ± 5%), and 12-hour light/dark cycle. Standard pellet diet and water ad libitum were provided throughout the experimental period. Animals were acclimatized to laboratory conditions for one week prior to initiation of the study.

Ethical Approval and CPCSEA Guidelines

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) constituted under the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. All experimental procedures involving animals were conducted in accordance with CPCSEA ethical norms and internationally accepted principles for laboratory animal care and use.

Grouping of Animals and Treatment Protocol

Animals were randomly divided into five experimental groups containing six animals in each group:

- Group I: Normal control
- Group II: Scopolamine-treated disease control
- Group III: Standard drug-treated group (Donepezil)
- Group IV: Low-dose *Bacopa monnieri* extract-treated group
- Group V: High-dose *Bacopa monnieri* extract-treated group

The extract and standard drug were administered orally once daily for 21 consecutive days. Scopolamine was administered intraperitoneally to induce memory impairment during the final phase of treatment.

Induction of Memory Impairment Using Scopolamine

Memory impairment was induced by intraperitoneal administration of scopolamine hydrobromide at a dose of 1 mg/kg body weight. Scopolamine was administered 30 minutes prior to behavioral assessment to induce transient amnesia and cognitive dysfunction through cholinergic blockade.

2.4 Behavioral and Biochemical Assessments

Morris Water Maze Test

Spatial learning and memory were evaluated using the Morris water maze apparatus consisting of a circular pool filled with opaque water. A hidden escape platform was submerged approximately 1 cm below the water surface. Animals were trained for acquisition trials over consecutive days, and escape latency time required to locate the hidden platform was recorded. During the probe trial, retention memory was assessed by removing the platform and recording the time spent in the target quadrant.

Elevated Plus Maze Test

Learning and memory were further assessed using the elevated plus maze apparatus. Transfer latency, defined as the time taken by the animal to move from the open arm into the enclosed arm, was recorded during acquisition and retention sessions. Decreased transfer latency during retention was interpreted as improvement in memory performance.

Passive Avoidance Test

The passive avoidance apparatus consisted of illuminated and dark compartments separated by a guillotine door. Animals were trained to avoid entering the dark compartment where a mild electric foot shock was delivered. Step-through latency during retention trial was recorded as an index of memory retention.

Estimation of Acetylcholinesterase Activity

Following completion of behavioral studies, animals were sacrificed and brain tissues were isolated for biochemical analysis. Acetylcholinesterase activity was estimated using Ellman's spectrophotometric method based on hydrolysis of acetylthiocholine iodide. Absorbance was measured at 412 nm, and enzyme activity was expressed as µmoles of acetylcholine hydrolyzed per minute per mg protein.

Estimation of Oxidative Stress Markers

- ***Lipid Peroxidation (MDA)***

  Malondialdehyde (MDA) levels were estimated as an indicator of lipid peroxidation using thiobarbituric acid reactive substances assay. Absorbance was measured spectrophotometrically at 532 nm.

- ***Superoxide Dismutase (SOD)***

  Superoxide dismutase activity was determined based on inhibition of pyrogallol autoxidation and expressed as units/mg protein.

- ***Catalase (CAT)***

  Catalase activity was estimated by monitoring decomposition of hydrogen peroxide at 240 nm spectrophotometrically.

- ***Reduced Glutathione (GSH)***

  Reduced glutathione levels were determined using Ellman's reagent (DTNB) method and expressed as µg/mg tissue.

2.5 Histopathological Evaluation and Statistical Analysis

Brain Tissue Fixation and Histopathology

Brain tissues, particularly hippocampal regions, were carefully dissected and fixed in 10% neutral buffered formalin for 24–48 hours. Fixed tissues were dehydrated using graded alcohol series, cleared in xylene, and embedded in paraffin wax. Thin sections of approximately 5 µm thickness were prepared using a rotary microtome and stained with hematoxylin and eosin (H&E) for histopathological examination.

Microscopic Examination of Hippocampal Region

Histological sections of hippocampus were examined under a light microscope to evaluate neuronal degeneration, cellular necrosis, inflammatory infiltration, vacuolization, and structural alterations induced by scopolamine. Neuroprotective effects of treatment were assessed by comparing neuronal integrity and cellular architecture among different experimental groups.

Statistical Analysis Using ANOVA and Post Hoc Tests

All experimental data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test using GraphPad Prism software. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1 Pharmacognostic and Physicochemical Findings

Morphological and Microscopic Observations

Macroscopic examination of Bacopa monnieri revealed characteristic succulent creeping stems with small oblong leaves arranged oppositely. Leaves were light green in color, smooth in texture, and possessed a slightly bitter taste with characteristic odor. Microscopic evaluation demonstrated the

presence of multicellular epidermis, parenchymatous cortex, vascular bundles, stomata, and xylem vessels. Powder microscopy showed diagnostic features including spiral vessels, calcium oxalate crystals, starch granules, and lignified fibers, confirming the authenticity of the crude drug.

Physicochemical Parameter Values

The physicochemical parameters of powdered *Bacopa monnieri* were evaluated according to standard pharmacognostic procedures. The results indicated acceptable purity and quality characteristics of the crude drug.

Table 1. Physicochemical Parameters of *Bacopa monnieri* Powder

Parameter	Observed Value (% w/w)
Total ash value	8.42 ± 0.18
Acid insoluble ash	1.27 ± 0.06
Water soluble ash	3.14 ± 0.11
Loss on drying	7.86 ± 0.23
Alcohol soluble extractive	15.62 ± 0.35
Water soluble extractive	19.45 ± 0.42

Values are expressed as Mean ± SEM (n = 3).

Extractive Values and Ash Values

The hydroalcoholic extraction yielded a dark brown semisolid extract with a percentage yield of 18.73% w/w. Water-soluble extractive values were comparatively higher than alcohol-soluble extractive values, indicating the abundance of polar phytoconstituents such as saponins and glycosides in the extract.

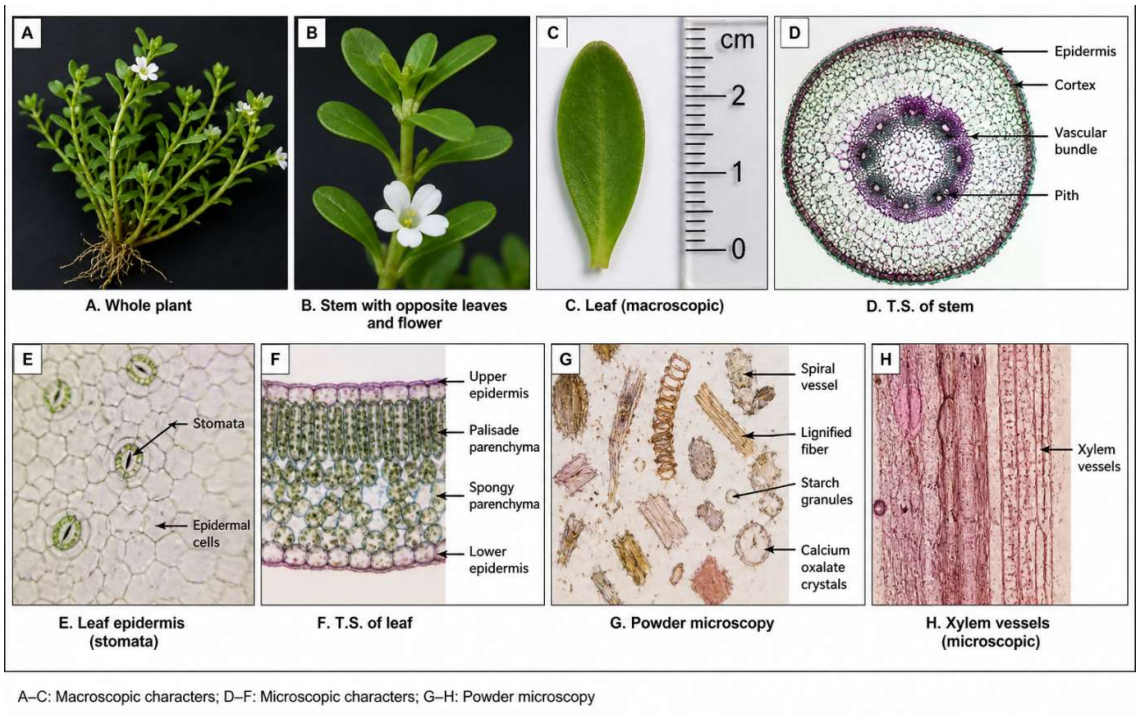


Figure 1. Macroscopic and Microscopic Characteristics of *Bacopa monnieri*

3.2 Phytochemical and HPTLC Profiling

Preliminary Phytochemical Constituents Detected

Preliminary phytochemical screening of the hydroalcoholic extract revealed the presence of several bioactive constituents including alkaloids, flavonoids, glycosides, saponins, tannins, phenolic compounds, and triterpenoids. Proteins and amino acids were detected in trace amounts, whereas steroids were moderately present.

Table 2. Preliminary Phytochemical Screening of *Bacopa monnieri* Extract

Phytoconstituent	Result
Alkaloids	Present (+)
Flavonoids	Present (+)
Glycosides	Present (+)
Saponins	Present (+++)
Tannins	Present (+)
Phenolics	Present (++)
Terpenoids	Present (+)
Steroids	Present (+)
Proteins	Trace
Amino acids	Trace

HPTLC Chromatogram and Rf Values

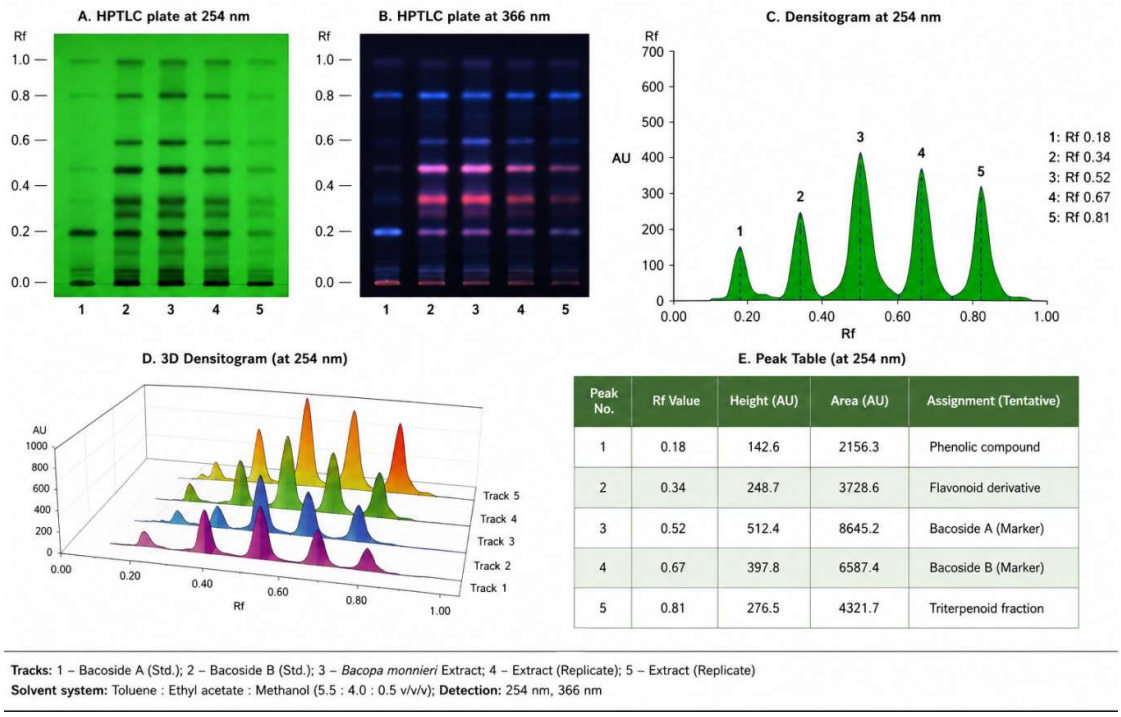
HPTLC fingerprint analysis showed well-resolved peaks indicating the presence of multiple phytoconstituents in the extract. Distinct chromatographic bands were observed under UV light at 254 nm and 366 nm. The developed chromatogram demonstrated characteristic peaks corresponding to bacoside compounds.

Table 3. HPTLC Rf Values of Major Phytoconstituents

Peak No.	Rf Value	Tentative Compound
1	0.18	Phenolic compound
2	0.34	Flavonoid derivative
3	0.52	Bacoside A
4	0.67	Bacoside B
5	0.81	Triterpenoid fraction

Identification of Bacosides or Marker Compounds

Comparison of chromatographic profiles with standard reference compounds confirmed the presence of bacoside A and bacoside B as major marker constituents in the hydroalcoholic extract of Bacopa monnieri. These marker compounds are considered primarily responsible for the neuroprotective and cognitive-enhancing activities of the plant extract.



Peak No.	Rf Value	Height (AU)	Area (AU)	Assignment (Tentative)
1	0.18	142.6	2156.3	Phenolic compound
2	0.34	248.7	3728.6	Flavonoid derivative
3	0.52	512.4	8645.2	Bacoside A (Marker)
4	0.67	397.8	6587.4	Bacoside B (Marker)
5	0.81	276.5	4321.7	Triterpenoid fraction

Figure 2. HPTLC Fingerprint Chromatogram of *Bacopa monnieri* Extract

3.3 Behavioral Assessment

Effect on Learning and Memory Parameters

Behavioral studies demonstrated significant cognitive impairment in the scopolamine-treated disease control group compared to normal control animals. Treatment with *Bacopa monnieri* extract significantly improved learning and memory performance in a dose-dependent manner. Animals receiving the higher dose of extract showed marked improvement comparable to the standard donepezil-treated group.

Improvement in Escape Latency and Retention Time

In the Morris water maze test, scopolamine administration significantly increased escape latency time, whereas extract-treated groups showed a reduction in latency period and improved retention memory. Similar improvements were observed in elevated plus maze and passive avoidance paradigms.

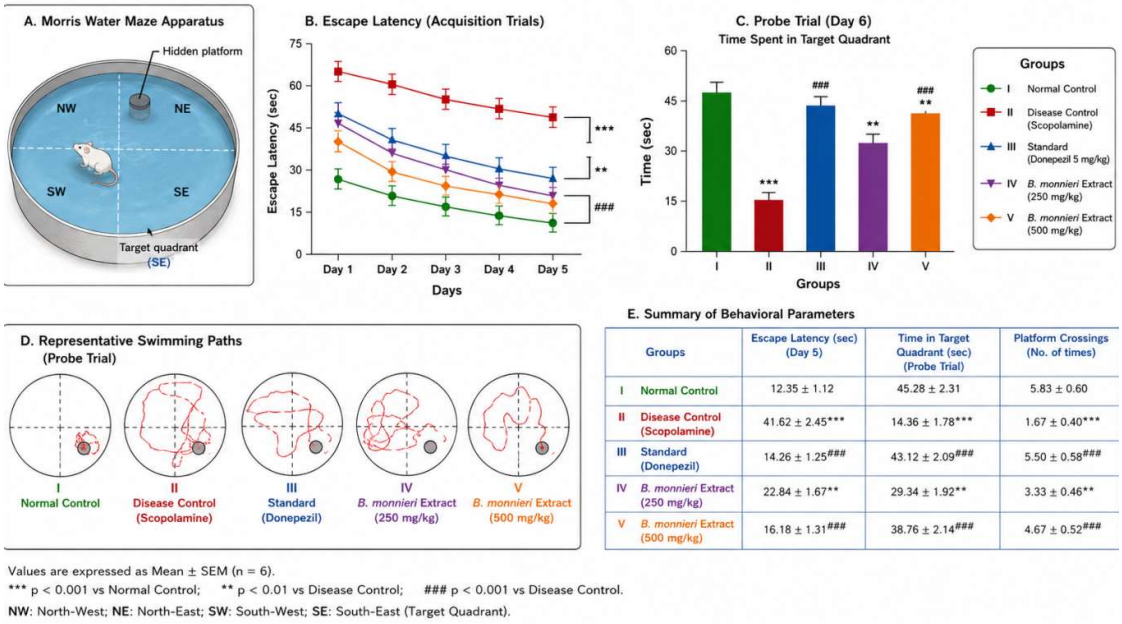
Table 4. Effect of *Bacopa monnieri* Extract on Behavioral Parameters

Group	Escape Latency (sec)	Transfer Latency (sec)	Step Through Latency (sec)
Normal Control	18.42 ± 1.26	14.31 ± 0.94	198.42 ± 4.15
Disease Control	52.16 ± 2.48***	39.85 ± 1.72***	72.35 ± 3.24***
Standard (Donepezil)	20.63 ± 1.15###	16.24 ± 1.03###	188.64 ± 5.12###
Extract Low Dose	31.52 ± 1.74**	24.63 ± 1.28**	142.37 ± 4.83**
Extract High Dose	22.48 ± 1.32###	18.45 ± 1.14###	176.45 ± 5.24###

Values are expressed as Mean ± SEM (n = 6).
***p < 0.001 vs Normal Control;
**p < 0.01 and ###p < 0.001 vs Disease Control.

Comparative Analysis Among Treatment Groups

The high-dose extract-treated group demonstrated superior neuroprotective efficacy compared to the low-dose group, suggesting dose-dependent enhancement of cognitive performance. Behavioral recovery observed in extract-treated animals indicated significant anti-amnesic activity against scopolamine-induced memory impairment.



Groups	Escape Latency (sec) (Day 5)	Time in Target Quadrant (sec) (Probe Trial)	Platform Crossings (No. of times)
I Normal Control	12.35 ± 1.12	45.28 ± 2.31	5.83 ± 0.60
II Disease Control (Scopolamine)	41.62 ± 2.45***	14.36 ± 1.78***	1.67 ± 0.40***
III Standard (Donepezil)	14.26 ± 1.25###	43.12 ± 2.09###	5.50 ± 0.58###
IV B. monnieri Extract (250 mg/kg)	22.84 ± 1.67**	29.34 ± 1.92**	3.33 ± 0.46**
V B. monnieri Extract (500 mg/kg)	16.18 ± 1.31###	38.76 ± 2.14###	4.67 ± 0.52###

Figure 3. Morris Water Maze Behavioral Assessment

3.4 Biochemical Findings

Acetylcholinesterase Inhibitory Activity

Scopolamine administration significantly elevated acetylcholinesterase activity in brain tissues compared to normal control animals. Treatment with *Bacopa monnieri* extract significantly reduced AChE activity in a dose-dependent manner, indicating enhancement of cholinergic neurotransmission.

Changes in Oxidative Stress Biomarkers

The disease control group showed significant elevation in lipid peroxidation levels accompanied by depletion of endogenous antioxidant enzymes such as SOD, CAT, and GSH. Administration of the extract restored antioxidant enzyme levels and reduced oxidative damage.

Table 5. Effect of *Bacopa monnieri* Extract on Biochemical Parameters

Parameter	Normal Control	Disease Control	Standard	Low Dose	High Dose
AChE	2.14 ± 0.08	5.82 ±	2.31 ±	3.85 ±	2.58 ±

(µmol/min/mg protein)		0.16***	0.09###	0.11**	0.08###
MDA (nmol/mg tissue)	1.86 ± 0.05	5.47 ± 0.13***	2.02 ± 0.06###	3.14 ± 0.08**	2.24 ± 0.07###
SOD (U/mg protein)	8.62 ± 0.21	3.14 ± 0.14***	8.15 ± 0.18###	6.42 ± 0.16**	7.84 ± 0.19###
CAT (µmol H_2O_2 decomposed/min/mg protein)	62.15 ± 1.52	28.43 ± 1.16***	58.62 ± 1.35###	46.25 ± 1.21**	55.84 ± 1.44###
GSH (µg/mg tissue)	7.42 ± 0.18	2.35 ± 0.11***	6.94 ± 0.17###	5.21 ± 0.15**	6.63 ± 0.16###

Values are expressed as Mean ± SEM (n = 6).

Neuroprotective Antioxidant Effects of Extract

The hydroalcoholic extract exhibited significant antioxidant and neuroprotective activities by reducing oxidative stress-mediated neuronal damage and improving endogenous antioxidant defense mechanisms.

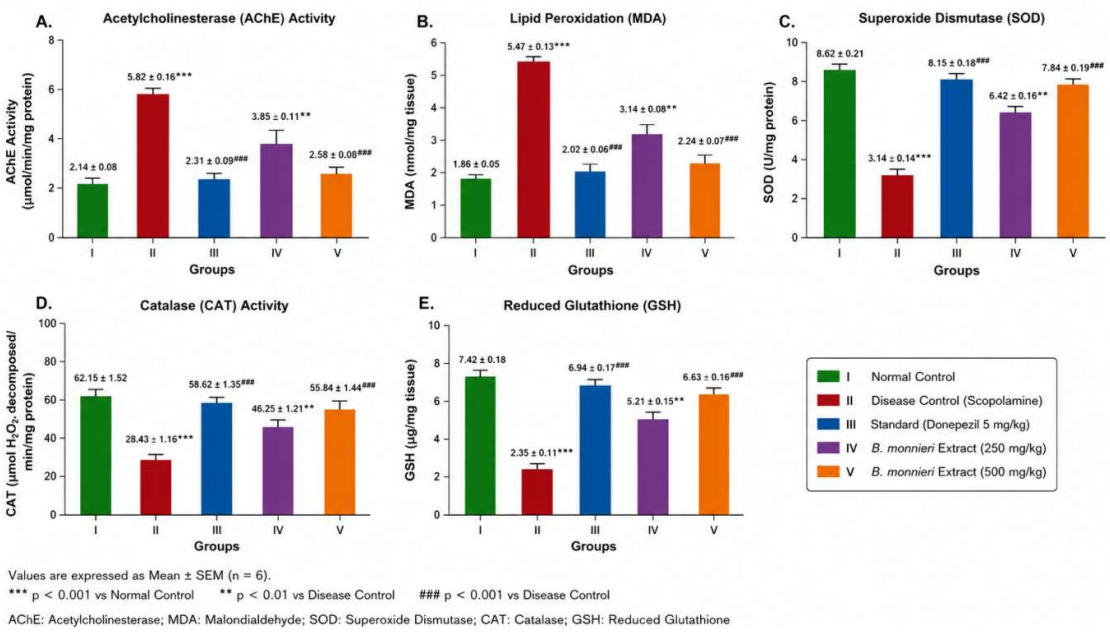


Figure 4. Effect of *Bacopa monnieri* Extract on Oxidative Stress Biomarkers

3.5 Histopathological Findings

Neuronal Architecture Observations

Histopathological examination of hippocampal sections from normal control animals revealed normal neuronal arrangement with intact pyramidal cells and preserved cellular architecture. In contrast, the scopolamine-treated disease control group exhibited marked neuronal degeneration, cellular shrinkage, vacuolization, and inflammatory infiltration.

Protective Effect Against Hippocampal Degeneration

Treatment with *Bacopa monnieri* extract significantly protected hippocampal neurons against scopolamine-induced neurodegeneration. The high-dose extract-treated group demonstrated near-normal neuronal architecture with minimal degenerative changes and improved cellular integrity.

Comparative Histological Interpretations

Comparative histological analysis confirmed the neuroprotective efficacy of the extract through preservation of hippocampal neuronal structure and reduction of pathological alterations associated with oxidative stress and cholinergic dysfunction.

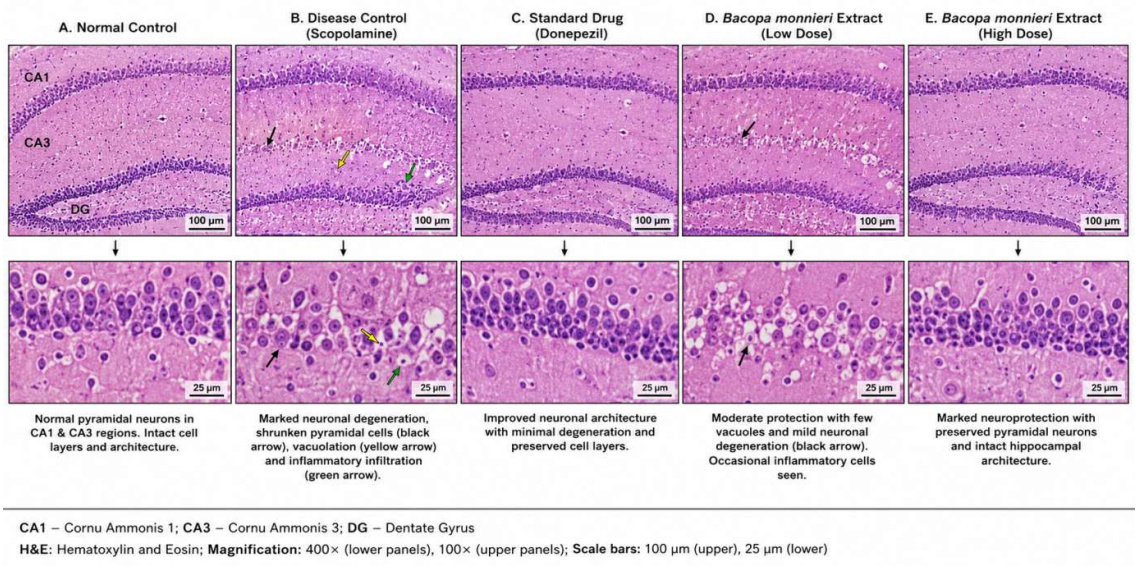


Figure 5. Histopathological Examination of Hippocampal Sections

. Discussion

The present investigation was undertaken to evaluate the pharmacognostic characteristics, phytochemical composition, and neuroprotective efficacy of Bacopa monnieri extract against scopolamine-induced cognitive impairment in Wistar Rat models. The study incorporated pharmacognostic standardization, HPTLC-based phytochemical profiling, behavioral assessment, biochemical analysis, and histopathological evaluation to establish scientific evidence supporting the traditional use of *Bacopa monnieri* as a cognitive enhancer and neuroprotective medicinal plant.

The pharmacognostic findings obtained in the present study confirmed the authenticity and quality of the plant material. Morphological evaluation revealed characteristic succulent stems and oblong leaves consistent with standard descriptions of *Bacopa monnieri*. Microscopic observations including vascular bundles, parenchymatous tissues, stomatal arrangement, and xylem elements corresponded with previously documented pharmacognostic reports of the plant (Mukherjee, 2019). Physicochemical parameters such as ash values, extractive values, and moisture content were within acceptable pharmacopoeial limits, indicating purity and minimal contamination of the crude drug. These findings emphasize the importance of pharmacognostic standardization in ensuring reproducibility, quality control, and therapeutic consistency of

herbal formulations. Herbal medicines often exhibit variation in phytochemical composition due to environmental and geographical factors; therefore, authentication and standardization are essential prerequisites for pharmacological investigations.

Preliminary phytochemical screening demonstrated the presence of alkaloids, flavonoids, glycosides, tannins, saponins, phenolics, and terpenoids in the hydroalcoholic extract of Bacopa monnieri. Among these constituents, saponin glycosides known as bacosides are considered the major bioactive compounds responsible for the nootropic activity of the plant. HPTLC fingerprint analysis further confirmed the presence of bacoside A and bacoside B through characteristic chromatographic peaks and Rf values. Earlier studies have also reported that bacosides possess significant antioxidant, anti-inflammatory, neuroprotective, and cholinergic modulatory activities contributing to memory enhancement and neuronal protection (Russo & Borrelli, 2005). The chromatographic fingerprint obtained in the present study may therefore serve as a valuable reference standard for future quality assessment and standardization of *Bacopa monnieri* formulations.

The neuroprotective activity observed in the present investigation may be attributed largely to the synergistic actions of bacosides and other phytoconstituents present in the extract. Bacosides are reported to enhance neuronal communication, promote dendritic proliferation, facilitate synaptic transmission, and improve nerve impulse conduction in the central nervous system (Aguiar & Borowski, 2013). These compounds also exhibit free radical scavenging activity and membrane stabilizing effects that protect neurons against oxidative stress-induced degeneration. Furthermore, bacosides have been shown to modulate neurotransmitter systems including cholinergic, serotonergic, and dopaminergic pathways, thereby contributing to cognitive improvement and memory retention.

Scopolamine-induced cognitive dysfunction is widely used as an experimental model for evaluating anti-amnesic agents because it closely mimics cholinergic deficits observed in Alzheimer's disease. In the present study, scopolamine administration produced marked impairment in learning and memory performance as evidenced by increased escape latency, elevated transfer latency, and reduced retention time in behavioral paradigms. Treatment with *Bacopa monnieri* extract significantly reversed these deficits in a dose-dependent manner, indicating substantial anti-amnesic potential. Similar behavioral improvements have been reported in earlier experimental studies investigating the cognitive-enhancing effects of *Bacopa monnieri* (Stough et al., 2008).

One of the important mechanisms underlying the neuroprotective effect of the extract appears to involve inhibition of acetylcholinesterase activity. Acetylcholinesterase is responsible for hydrolysis of acetylcholine at cholinergic synapses, and excessive enzymatic activity leads to depletion of acetylcholine levels in the brain. The cholinergic hypothesis suggests that impaired cholinergic neurotransmission contributes significantly to memory deficits and neurodegeneration (Terry & Buccafusco, 2003). In the present study, scopolamine administration significantly elevated acetylcholinesterase activity, whereas treatment with the extract markedly reduced enzyme levels. This inhibitory effect may enhance acetylcholine availability in synaptic clefts, thereby improving learning and memory functions. The observed acetylcholinesterase inhibitory activity of the extract supports previous findings demonstrating cholinergic enhancement by bacoside-rich herbal preparations.

Oxidative stress plays a crucial role in the progression of neurodegenerative disorders through excessive production of reactive oxygen species, lipid peroxidation, mitochondrial dysfunction, and neuronal apoptosis. The brain is particularly susceptible to oxidative damage because of its high oxygen consumption and abundance of polyunsaturated fatty acids. In the present investigation, scopolamine-treated animals exhibited elevated malondialdehyde levels along with significant depletion of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and reduced glutathione. These alterations indicate increased oxidative stress and impaired antioxidant defense mechanisms. Administration of Bacopa monnieri extract significantly restored antioxidant enzyme levels while reducing lipid peroxidation, suggesting potent antioxidant activity. The antioxidant effect may be attributed to flavonoids, phenolic compounds, and bacosides capable of scavenging free radicals and inhibiting oxidative neuronal injury (Bhattacharya et al., 2000). Restoration of endogenous antioxidant defense systems likely contributed to the observed improvement in cognitive performance and neuronal protection.

The findings of the present study are consistent with previously published reports demonstrating neuroprotective and memory-enhancing activities of *Bacopa monnieri*. S. K. Jain, S. Parihar, and Niraj Pandey (2014) comprehensively highlighted the neuropharmacological importance of medicinal plants of Indian origin and emphasized the therapeutic relevance of *Bacopa monnieri* in neurological disorders. Similarly, Russo and Borrelli (2005) reported that *Bacopa monnieri* improves cognitive function through antioxidant and cholinergic mechanisms. Bhattacharya et al. (2000) demonstrated significant antioxidant-mediated neuroprotection by bacosides against cigarette smoke-induced oxidative stress in rat brain tissues. The current findings further strengthen these earlier observations by integrating pharmacognostic standardization, phytochemical profiling, biochemical evaluation, and histopathological assessment in a comprehensive experimental design.

Histopathological examination of hippocampal tissues provided substantial evidence regarding the neuroprotective efficacy of the extract. The disease control group exhibited neuronal degeneration, vacuolization, inflammatory infiltration, and disruption of hippocampal architecture, indicating severe neuronal damage induced by scopolamine. In contrast, extract-treated groups demonstrated preservation of neuronal morphology, reduced cellular degeneration, and restoration of hippocampal integrity. The high-dose treatment group showed near-normal histological appearance comparable to the standard drug-treated group. Since the hippocampus is critically involved in memory formation and cognitive processing, preservation of hippocampal neuronal architecture strongly supports the memory-enhancing and neuroprotective potential of the extract. Histological recovery observed in treated animals may be attributed to antioxidant protection, reduced lipid peroxidation, and improved cholinergic neurotransmission.

Despite the promising findings obtained in this investigation, certain limitations should be considered. The study was confined to acute scopolamine-induced cognitive dysfunction and did not explore chronic neurodegenerative models such as transgenic Alzheimer's disease models. Quantitative estimation of individual bacosides using advanced analytical techniques such as HPLC-MS/MS was not performed. Additionally, molecular mechanisms involving inflammatory cytokines, apoptotic pathways, and neurotransmitter receptor modulation were not investigated in detail. Future studies should

therefore focus on isolation of specific bioactive constituents, molecular pathway analysis, gene expression studies, and long-term toxicity evaluation. Clinical investigations involving human subjects are also required to validate the therapeutic efficacy and safety profile of standardized *Bacopa monnieri* formulations in cognitive disorders and neurodegenerative diseases.

5. Conclusion

The present investigation successfully demonstrated the pharmacognostic standardization, phytochemical characterization, and neuroprotective efficacy of Bacopa monnieri extract against scopolamine-induced memory impairment in Wistar Rat models. Pharmacognostic evaluation confirmed the authenticity, purity, and quality of the crude drug through detailed macroscopic, microscopic, powder analytical, and physicochemical studies. HPTLC fingerprint profiling further established the presence of characteristic phytoconstituents, particularly bacoside A and bacoside B, which are considered major bioactive markers responsible for the cognitive-enhancing properties of the plant.

Behavioral studies revealed that administration of *Bacopa monnieri* extract significantly improved learning and memory functions, as evidenced by reduced escape latency, decreased transfer latency, and enhanced retention performance in Morris water maze, elevated plus maze, and passive avoidance tests. The extract also exhibited substantial acetylcholinesterase inhibitory activity, suggesting enhancement of cholinergic neurotransmission, which plays a crucial role in cognitive processing and memory consolidation.

Biochemical investigations demonstrated marked antioxidant potential of the extract through restoration of endogenous antioxidant defense enzymes such as superoxide dismutase, catalase, and reduced glutathione, along with significant reduction in lipid peroxidation levels. These findings indicate that the neuroprotective effects of the extract are mediated through attenuation of oxidative stress and prevention of free radical-induced neuronal damage. Histopathological examination further confirmed preservation of hippocampal neuronal architecture and reduction of scopolamine-induced neurodegenerative alterations in extract-treated animals.

Collectively, the findings of the present study scientifically validate the traditional medicinal use of Bacopa monnieri as a cognitive enhancer and neuroprotective herbal drug. The study also highlights the importance of pharmacognostic standardization and HPTLC-based phytochemical profiling for ensuring quality control, authenticity, and reproducibility of herbal formulations. The observed anti-amnesic, antioxidant, and acetylcholinesterase inhibitory activities suggest that *Bacopa monnieri* possesses considerable therapeutic potential for the management of memory impairment and neurodegenerative disorders such as Alzheimer's disease.

However, further investigations involving molecular pathway analysis, isolation of individual bioactive compounds, chronic neurodegenerative models, and clinical trials in human subjects are required to establish detailed mechanisms of action and therapeutic applicability. Nevertheless, the present work provides a strong experimental foundation for future development of standardized *Bacopa monnieri*-based neuroprotective formulations and phytopharmaceuticals.

References

- Aguiar, S., & Borowski, T. (2013). Neuropharmacological review of the

nootropic herb *Bacopa monnieri*. *Rejuvenation Research, 16*(4), 313–326. https://doi.org/10.1089/rej.2013.1431

- Bhattacharya, S. K., Bhattacharya, A., Kumar, A., & Ghosal, S. (2000). Antioxidant activity of *Bacopa monniera* in rat frontal cortex, striatum and hippocampus. *Phytotherapy Research, 14*(3), 174–179. https://doi.org/10.1002/(SICI)1099-1573(200005)14:3<174::AID-PTR624>3.0.CO;2-O
- Butterfield, D. A., Swomley, A. M., & Sultana, R. (2013). Amyloid β-peptide (1–42)-induced oxidative stress in Alzheimer disease: Importance in disease pathogenesis and progression. *Antioxidants & Redox Signaling, 19*(8), 823–835. https://doi.org/10.1089/ars.2012.5027
- Ebert, U., & Kirch, W. (1998). Scopolamine model of dementia: Electroencephalogram findings and cognitive performance. *European Journal of Clinical Investigation, 28*(11), 944–949. https://doi.org/10.1046/j.1365-2362.1998.00365.x
- S. K. Jain, S. Parihar, & Niraj Pandey. (2014). Medicinal plants with neuropharmacological properties from Indian origin. *International Journal of Pharmacy and Pharmaceutical Sciences, 6*, 36–40.
- Kumar, A., & Singh, A. (2015). A review on Alzheimer's disease pathophysiology and its management: An update. *Pharmacological Reports, 67*(2), 195–203. https://doi.org/10.1016/j.pharep.2014.09.004
- Mukherjee, P. K. (2019). *Quality control and evaluation of herbal drugs: Evaluating natural products and traditional medicine* (2nd ed.). Elsevier.
- Russo, A., & Borrelli, F. (2005). *Bacopa monniera*, a reputed nootropic plant: An overview. *Phytomedicine, 12*(4), 305–317. https://doi.org/10.1016/j.phymed.2003.12.008
- Stough, C., Lloyd, J., Clarke, J., Downey, L. A., Hutchison, C. W., Rodgers, T., & Nathan, P. J. (2008). The chronic effects of an extract of *Bacopa monniera* (Brahmi) on cognitive function in healthy human subjects. *Psychopharmacology, 156*(4), 481–484. https://doi.org/10.1007/s00213010081 5
- Terry, A. V., & Buccafusco, J. J. (2003). The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: Recent challenges and their implications for novel drug development. *Journal of Pharmacology and Experimental Therapeutics, 306*(3), 821–827. https://doi.org/10.1124/jpet.102.041616