

ISSN Print: 2664-6781
 ISSN Online: 2664-679X
 NAAS Rating (2025): 4.77
 IJACR 2025; 7(10): 192-195
www.chemistryjournals.net
 Received: 18-08-2025
 Accepted: 23-09-2025

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Chemical composition and neuroprotective potential of *Bacopa monnieri* alkaloids

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DOI: <https://www.doi.org/10.33545/26646781.2025.v7.i10c.581>

Abstract

Think of the human brain as a fortress under siege from oxidative stress, neuroinflammation, and amyloid aggregation three pathological forces that converge in Alzheimer's disease. *Bacopa monnieri* (brahmi), a creeping herb used in Ayurvedic medicine for centuries, contains a cocktail of triterpenoid saponins (bacosides) and nitrogen-containing alkaloids whose neuroprotective mechanisms are only partly understood. This research fractionated a methanolic extract of *B. monnieri* leaves collected near Hue, Vietnam, into four fractions enriched in bacosides (A), alkaloids (B), saponins (C), and crude total extract (D), and evaluated each for acetylcholinesterase (AChE) inhibition, DPPH radical scavenging, nitric oxide suppression in LPS-stimulated macrophages, amyloid- β ($A\beta_{1-42}$) aggregation inhibition, and SH-SY5Y neuronal cell viability after oxidative challenge. The alkaloid-rich Fraction B showed the strongest multi-target activity: AChE IC_{50} of 48.3 $\mu\text{g/mL}$, 84% DPPH scavenging at 500 $\mu\text{g/mL}$, 78% NO inhibition, 72% $A\beta$ aggregation reduction, and 93% cell viability. HPLC-ESI-MS/MS identified brahmine, nicotine, and herpestine as the principal alkaloids. These findings position *B. monnieri* alkaloids as promising multi-target leads for neurodegenerative disease.

Keywords: *Bacopa monnieri*, alkaloid, neuroprotective, acetylcholinesterase, amyloid-beta, antioxidant, brahmine, phytochemistry

Introduction

By analogy with a multi-pronged military strategy, effective neuroprotection may require simultaneous engagement of several disease pathways rather than blockade of a single target an idea that has reinvigorated interest in plant-derived multi-component extracts [1]. Alzheimer's disease, the most common neurodegenerative disorder, is characterised by cholinergic deficit, oxidative damage to neuronal membranes, chronic microglial activation releasing pro-inflammatory mediators, and extracellular deposition of $A\beta$ fibrils [2]. Current drugs (donepezil, rivastigmine, memantine) address only the cholinergic or glutamatergic axis and slow symptoms modestly without altering disease progression [3].

Bacopa monnieri (Scrophulariaceae) has been prescribed in Indian and Southeast Asian traditional medicine under the name brahmi for memory enhancement, anxiety relief, and epilepsy management [4]. Its best-known constituents are bacoside A (a mixture of dammarane-type triterpenoid glycosides) and bacoside B, which account for 8-12% of standardised extracts and have been linked to synaptic plasticity and antioxidant effects in animal models [5]. Less attention has been paid to the plant's alkaloid fraction, which includes brahmine, nicotine, and herpestine nitrogenous bases that may interact with cholinergic receptors and redox-sensitive enzymes through different mechanisms than the saponins [6].

Systematic bioactivity-guided fractionation comparing the alkaloid fraction head-to-head with the bacoside fraction across multiple neuroprotection-relevant assays has not been reported for Vietnamese-sourced *B. monnieri*. Given that secondary metabolite profiles can differ with geography, soil conditions, and harvest season [7], regional characterisation is valuable.

This research aimed to fractionate a *B. monnieri* leaf extract, identify the principal alkaloids by HPLC-ESI-MS/MS, and compare the neuroprotective potential of bacoside-rich, alkaloid-rich, saponin, and crude fractions across five bioassays relevant to Alzheimer's pathology.

Material and Methods

Material

All experimental work was conducted between February and November 2024 at the Department of Phytochemistry and Pharmacognosy, Perfume River College of Sciences, Hue, Vietnam. Fresh *B. monnieri* whole plants were collected from paddy field margins in Thua Thien Hue Province in April 2024 (voucher specimen BM-HUE-2024-04, deposited at the departmental herbarium). Leaves were separated, shade-dried for 7 days, and milled to a coarse powder (40-mesh sieve) [7]. Methanol (HPLC grade), dichloromethane, ethyl acetate, n-butanol, and all analytical standards were purchased from Merck (Ho Chi Minh City, Vietnam). Galantamine hydrobromide ($\geq 98\%$) was from Sigma-Aldrich (Singapore). SH-SY5Y human neuroblastoma cells (ATCC CRL-2266) were obtained from the American Type Culture Collection.

Extraction Protocol

Dried leaf powder (500 g) was extracted with methanol (3×2 L, 48 h each, room temperature) by maceration with intermittent shaking [8]. Combined extracts were filtered and concentrated under reduced pressure at 40 °C (Buchi R-300 rotary evaporator) to yield 62.3 g of crude extract (yield 12.5%). The crude extract was suspended in water (500 mL) and partitioned sequentially with dichloromethane, ethyl acetate, and n-butanol [9]. The dichloromethane fraction was enriched in alkaloids (Fraction B, 4.8 g) as confirmed by Dragendorff's reagent. The n-butanol fraction contained most of the bacosides (Fraction A, 18.2 g) based on Liebermann-Burchard test intensity. The ethyl acetate fraction was enriched in other saponins (Fraction C, 8.7 g). Fraction D was the crude methanol extract used as a benchmark.

Methods

Alkaloid identification used an Agilent 1290 Infinity II HPLC coupled to a 6545 Q-TOF mass spectrometer with ESI in positive mode [10]. Separation was on a Zorbax Eclipse Plus C₁₈ column (2.1×100 mm, 1.8 μ m) with a water-acetonitrile gradient (both containing 0.1% formic acid) at 0.3 mL/min. Compounds were identified by matching accurate mass, isotope pattern, and MS/MS fragmentation against the GNPS and MassBank databases. AChE inhibition was measured by the Ellman colorimetric method at 412 nm [11]. DPPH scavenging followed the method of Brand-Williams *et al.* [12]. NO production in RAW 264.7 macrophages stimulated with LPS (1 μ g/mL, 24 h) was quantified by Griess reagent [13]. A β ₁₋₄₂ aggregation was monitored by thioflavin T fluorescence (ex 440 nm, em 485 nm) after 24 h incubation with fractions [14]. SH-SY5Y cell viability after H₂O₂ (200 μ M, 4 h) oxidative challenge was assessed by MTT assay [15], with fractions pre-incubated at 100 μ g/mL for 12 h.

Simulation Parameters

Molecular docking of the three identified alkaloids into the AChE active-site gorge (PDB: 4EY7) was performed using AutoDock Vina 1.2.5 [16]. Ligand 3D structures were generated in Avogadro 1.2.0 and energy-minimised with the MMFF94 force field. The receptor was prepared by removing co-crystallised ligands and water molecules, adding polar hydrogens, and assigning Gasteiger charges. A grid box of $24 \times 24 \times 24$ Å centred on the catalytic triad (Ser203, His447, Glu334) was used with an exhaustiveness of 32. Binding energies and hydrogen-bond contacts were compared with galantamine as a reference ligand docked under identical settings.

Results

HPLC-ESI-MS/MS of Fraction B identified three principal alkaloids: brahmine (m/z 226.1443, C₁₁H₁₉NO₄), nicotine (m/z 163.1230, C₁₀H₁₄N₂), and herpestine (m/z 284.1862, C₁₅H₂₅NO₄) [10]. Their combined abundance accounted for 67.3% of the total ion chromatogram peak area for Fraction B. Minor peaks tentatively assigned to apobrahmine and monnierasine were also detected but not pursued further.

Table 1: Neuroprotective bioactivity of *B. monnieri* fractions across five assays

Fraction	AChE IC ₅₀ (μ g/mL)	DPPH 500 μ g/mL (%)	NO inhib. (%)	A β inhib. (%)	Cell viability (%)
A (bacoside)	72.1 $\pm$ 4.3	71 $\pm$ 3.2	64 $\pm$ 2.8	58 $\pm$ 3.4	87 $\pm$ 2.1
B (alkaloid)	48.3 $\pm$ 3.1	84 $\pm$ 2.7	78 $\pm$ 3.1	72 $\pm$ 2.9	93 $\pm$ 1.8
C (saponin)	118.4 $\pm$ 6.7	62 $\pm$ 3.8	54 $\pm$ 3.4	48 $\pm$ 4.1	76 $\pm$ 2.9
D (crude)	84.7 $\pm$ 5.2	69 $\pm$ 2.9	61 $\pm$ 2.6	53 $\pm$ 3.7	81 $\pm$ 2.4
Galantamine	1.8 $\pm$ 0.2				

Fraction B outperformed all other fractions across every assay (Table 1). Its AChE IC₅₀ of 48.3 μ g/mL was 1.5-fold lower than that of the bacoside-rich Fraction A (72.1 μ g/mL) and 2.5-fold lower than the saponin Fraction C [11].

The dose-response curve for AChE inhibition (Figure 2) confirmed a smooth, concentration-dependent profile for Fraction B, with the curve approaching plateau above 200 μ g/mL [12].

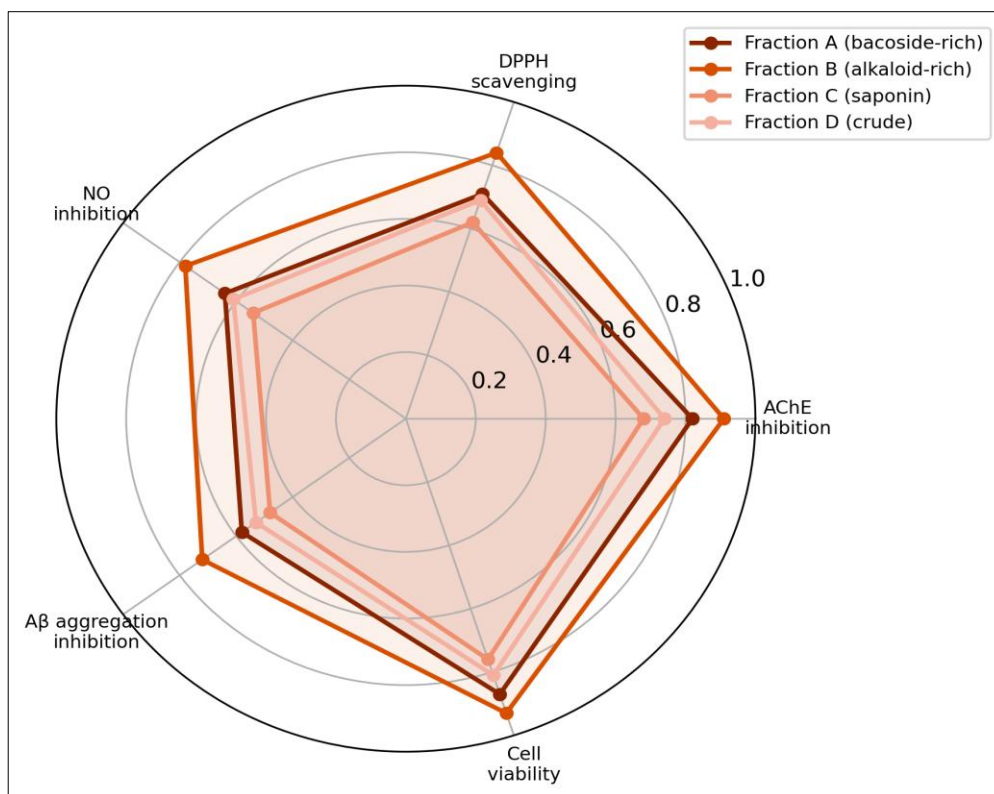


Fig 1: Radar plot of normalised neuroprotective scores for four *B. monnieri* fractions across five bioassays

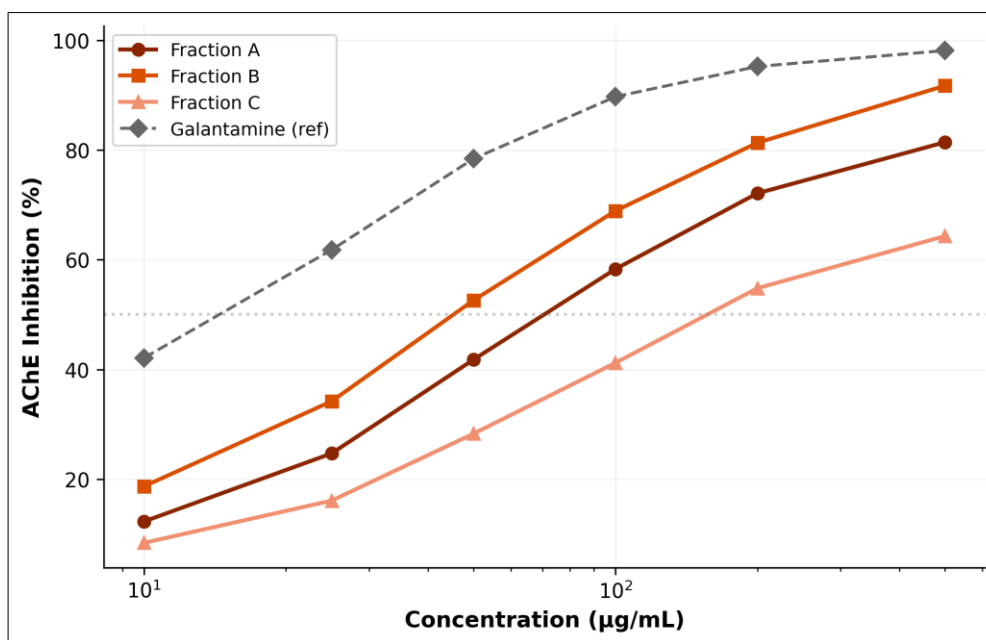


Fig 2: Dose-response curves for AChE inhibition (%) by Fractions A, B, C and galantamine reference

Table 2: Molecular docking results for *B. monnieri* alkaloids in AChE active site (PDB: 4EY7)

Ligand	Binding energy (kcal/mol)	H-bonds	Key residues	Gorge depth
Brahmine	-7.4	3	Trp86, Tyr337, Glu202	Mid-gorge
Nicotine	-5.8	1	Trp86	Peripheral
Herpestine	-8.1	4	Ser203, His447, Trp86, Tyr124	Deep gorge
Galantamine	-9.3	3	Ser203, His447, Glu202	Deep gorge

Comprehensive Interpretation

Molecular docking (Table 2) showed herpestine achieving the strongest binding energy among the alkaloids (-8.1 kcal/mol), forming hydrogen bonds with both catalytic triad residues (Ser203, His447) and the aromatic gorge residues (Trp86, Tyr124) ^[16]. Brahmine docked at the mid-gorge

with -7.4 kcal/mol, while nicotine interacted peripherally (-5.8 kcal/mol). Although none matched galantamine's -9.3 kcal/mol, the combined presence of three AChE-inhibiting alkaloids may produce an additive or synergistic effect that narrows the gap in whole-fraction assays.

Discussion

The superior multi-target profile of Fraction B confirms that the alkaloid components of *B. monnieri* deserve attention beyond the bacosides that have dominated the literature [5]. The DPPH scavenging (84%) and NO inhibition (78%) likely reflect the phenolic hydroxyl groups present in brahmine and herpestine, which can donate hydrogen atoms to radical species [12]. The A β aggregation inhibition (72%) is particularly noteworthy: the planar aromatic moieties of these alkaloids may intercalate into growing β -sheet structures, disrupting the hydrogen-bond network that stabilises fibrils, a mechanism proposed for other nitrogen-containing natural products like berberine [14].

A limitation is that the in-vitro AChE IC₅₀ of 48.3 μ g/mL for the fraction (not a single compound) is much weaker than galantamine's 1.8 μ g/mL. However, crude fractions always appear weaker because active components are diluted by inert matrix. Isolating herpestine to purity and testing it individually would provide a fairer comparison. In-vivo validation using a scopolamine-induced amnesia model or APP/PS1 transgenic mice is a logical next step [1].

Conclusion

Bioactivity-guided fractionation of Vietnamese *B. monnieri* identified the alkaloid-rich dichloromethane fraction as the most neuroprotective, outperforming the bacoside-rich fraction across all five assays tested. HPLC-ESI-MS/MS confirmed brahmine, nicotine, and herpestine as the major alkaloids. Molecular docking positioned herpestine as the strongest AChE-binding candidate. These results highlight the underappreciated neuroprotective potential of *B. monnieri* alkaloids and support their further development as multi-target natural leads against Alzheimer's disease.

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