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Bacopa monnieri (Brahmi): Pharmacognostical, Phytochemical, Spectroscopic, and *In-Silico* Investigations for its potential role in Hypertension

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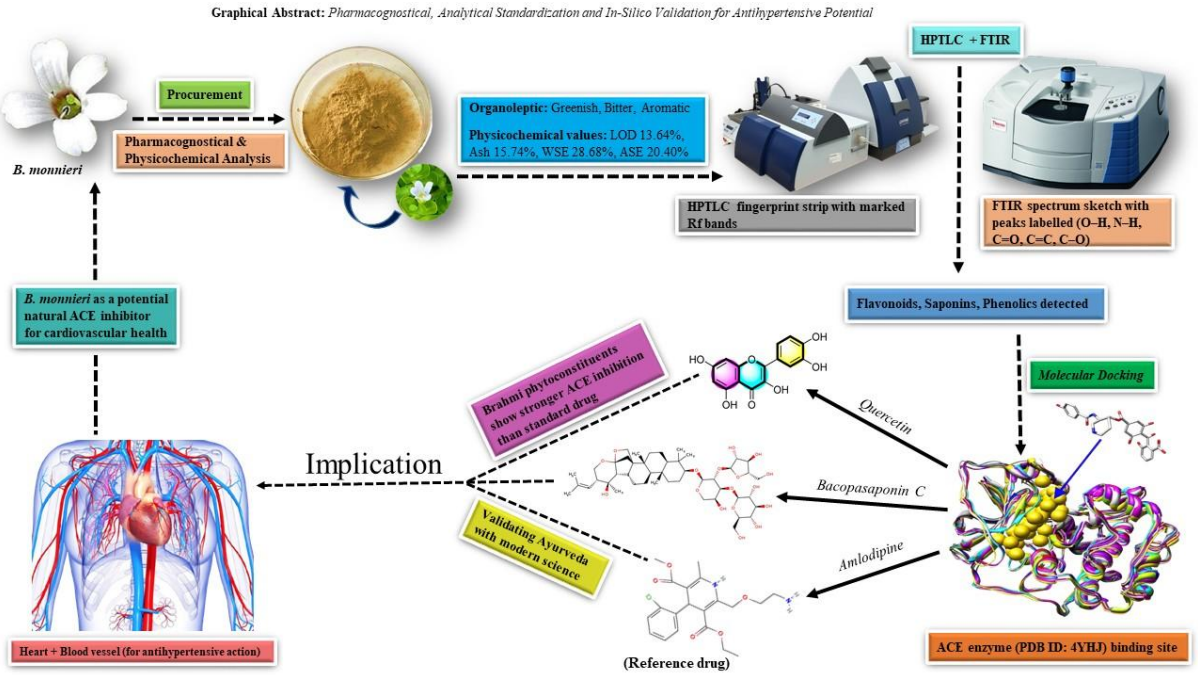
ABSTRACT

Background: *Bacopa monnieri (L.) Wettst. (Brahmi)* is recognized in Ayurveda as a Medhya Rasayana, traditionally employed to improve cognitive function, provide neuroprotection, and promote overall systemic health. In addition to its nootropic function, recent studies indicate potential antihypertensive effects via antioxidant, vasodilatory, and ACE-inhibitory mechanisms. Nonetheless, the integration of comprehensive analytical standardization with *In-Silico* validation is still constrained.
Objective: The objective is to authenticate, standardize, and characterize B. monnieri through pharmacognostical, physicochemical, chromatographic, spectroscopic, and molecular docking methods, focusing on its antihypertensive potential.
Methods: Authentic plant material was gathered, processed into powder, and pharmacognostically verified at RRDR, AIIA, New Delhi. Organoleptic and physicochemical characteristics were assessed according to the Ayurvedic Pharmacopoeia of India. HPTLC was used for phytochemical profiling and FTIR for functional group identification. Methanolic extracts were characterized for standardization. Bioactive phytoconstituents (*quercetin*, *bacosides*, *cucurbitacin B*, *stigmasterol*, etc.) underwent molecular docking analysis against angiotensin-converting enzyme (ACE, PDB ID: 4YHJ) and were compared to amlodipine. **Results:** The organoleptic assessment verified a refined greenish powder possessing a bitter taste and distinctive aroma. The physicochemical characteristics (loss on drying: 13.64%, total ash: 15.74%, water-soluble extractive: 28.68%, alcohol-soluble extractive: 20.40%) conformed to pharmacopeial criteria. HPTLC demonstrated specific Rf

values associated with flavonoids, saponins, and phenolics. FTIR spectra exhibited peaks corresponding to O–H, N–H, C=O, C=C, and C–O groups, aligning with secondary metabolites. Docking studies demonstrated that *quercetin* (–8.93 kcal/mol) and *bacopasaponin C* (–6.60 kcal/mol) possess greater binding affinities to ACE compared to amlodipine (–5.27 kcal/mol), indicating possible natural ACE inhibition.

Conclusion: Comprehensive pharmacognostical, phytochemical, spectroscopic, and *In-Silico* analyses validate the authenticity, chemical diversity, and therapeutic potential of B. monnieri. The results confirm its antihypertensive potential through ACE inhibition, reinforcing traditional assertions and emphasizing its applicability in cardiovascular drug development.



INTRODUCTION:

Essential hypertension, also referred to as primary or idiopathic hypertension, is characterized by consistently elevated blood pressure in the absence of any identifiable underlying cause. It is indicated by a systolic blood pressure (SBP) of ≥140 mm Hg, a diastolic blood pressure (DBP) of ≥90 mm Hg, or both, measured multiple times.[i] This issue constitutes a significant global health challenge, serving as the primary preventable risk factor for cardiovascular diseases and a substantial contributor to morbidity and mortality rates. The prevalence of hypertension rose significantly, from 594 million individuals in 1975 to about 1.13 billion in 2015, with projections suggesting over 1.6 billion cases by 2025.[ii] Hypertension represents a significant independent risk factor for cardiovascular diseases, responsible for around 10.7 million deaths and 212 million disability-adjusted life years (DALYs) worldwide in 2015.[iii,iv] The hypertension control rate in India is notably low, with uncontrolled blood pressure serving as a significant risk factor for microvascular and macrovascular complications, including stroke, coronary heart disease, chronic kidney disease, and retinopathy.[v,vi] Despite the availability of numerous antihypertensive therapies, global treatment success remains suboptimal, with only 37.1% of treated individuals achieving target blood pressure levels[vii]. Current pharmacological strategies face several challenges: polypharmacy often leads to complex regimens that compromise adherence;[viii] adverse effects of conventional drugs such as diuretics and beta-blockers—including electrolyte imbalances and fatigue—discourage sustained use;[ix] and the widely used step-care approach has been criticized for its unfavourable metabolic effects and limited efficacy in reducing coronary artery disease morbidity.[x] Furthermore, while newer agents show promise, they have yet to provide conclusive evidence of superior outcomes in terms of cardiovascular event reduction[xi]. Another challenge is the limited implementation of

individualized treatment strategies based on hemodynamic profiles, largely due to the unavailability of advanced diagnostic technologies and trained healthcare professionals in resource-limited settings[xii]. These challenges underscore the need for continued research into more effective, accessible, and personalized hypertension management strategies that can improve both clinical outcomes and patient adherence globally. Bacopa monnieri, or Brahmi, has been widely utilized in traditional Ayurvedic medicine for its various therapeutic properties, including a possible role in managing essential hypertension. Traditionally recognized as a cardiotonic, Bacopa was believed to support heart function and circulation[xiii]. Its use as a natural tranquilizer and sedative also highlights its role in stress reduction, a critical factor in the development and progression of hypertension[xiv]. Moreover, its cognitive-enhancing effects, which contribute to improved mental well-being, may indirectly benefit cardiovascular health by mitigating stress-induced hypertensive responses[xv]. Recent pharmacological investigations support these traditional uses, identifying Bacopa monnieri's antihypertensive potential through mechanisms such as vasodilation and modulation of vascular resistance[xvi]. The strong antioxidant properties, including free radical scavenging activity, are recognized for their role in reducing oxidative stress, a key factor in vascular dysfunction and increased blood pressure. Additionally, its neuroprotective effects, mediated through the regulation of neurotransmitter signalling, further aid in reducing anxiety and stress-related hypertension[xvii]. While Bacopa monnieri shows promise as a supportive intervention in managing essential hypertension, experts emphasize the importance of integrating such herbal therapies with lifestyle modifications and conventional pharmacological treatments to ensure comprehensive and evidence-based hypertension management. Integrating analytical and *In-Silico* approaches is increasingly recognized as an essential strategy in contemporary biomedical research, predominantly in the domains of drug discovery and disease understanding. This integration enables a more comprehensive analysis of complex biological systems by combining experimental data with computational modelling to yield systems-level insights, which are essential for decoding intricate molecular interactions[xviii]. Given this background, the present study aimed to (i) perform a detailed analytical characterization of *Bacopa monnieri* powder to confirm its authenticity, quality, and phytochemical profile, (ii) evaluate its antihypertensive potential through target-based *In-Silico* docking studies against key molecular targets involved in blood pressure regulation. This integrative approach seeks to provide a scientifically robust foundation for the rational development of *Bacopa monnieri*-based interventions for hypertension management.

MATERIALS AND METHODS:

Procurement and preparation of the drug:

Collection and Authentication of *Bacopa monnieri:* The raw plant material of *Bacopa monnieri* (commonly known as Brahmi in Ayurveda) was procured from an authenticated herbal vendor based in Sambhajinagar district in Maharashtra, India. The identity of the drug was confirmed and authenticated through morphological and pharmacognostical evaluation conducted by the Pharmacognosy Laboratory, Regional Raw Drug Repository (RRDR) with accession no. (RRDR/AIIA/472), Department of Dravyaguna, All India Institute of Ayurveda, New Delhi. Drying, Powdering, and Storage: The authenticated plant material was shade-dried at room temperature to retain its phytochemical integrity. The dried material was pulverized and subsequently passed through sieve no. 80 to achieve a fine and uniform powder. The final powder was stored in an airtight container in a cool, dry environment under room temperature to prevent degradation of active constituents.

Analysis of the drug

Organoleptic and physicochemical parameters were evaluated at the Quality Control Laboratory of the All India Institute of Ayurveda (AIIA), New Delhi, in accordance with the standard protocols outlined in the Ayurvedic Pharmacopoeia of India (API), having Certificate No. (AIIA/QC/KS/2024/01).

Organoleptic Characteristics: A crucial step in identifying any adulterant in medicinal plants. This pertains to sensory evaluation, wherein characteristics such as color, odor, taste, and appearance are assessed.[xix] (Table 1)

Table 1 Organoleptic parameters of BM

Test	Result
Appearance	Fine powder
Color	Greenish
Odor	Characteristic
Taste	Bitter

Physicochemical Parameters: The powdered *Bacopa monnieri* was subjected to standard physicochemical tests to determine its quality and purity. The following parameters were assessed:

Loss on Drying at 105°C (% w/w): Loss-on-drying (LOD) is defined as the mass of water lost during the drying

process divided by the total sample mass.[xx]
Total Ash Value (% w/w): Total Ash Value is defined as the weight of inorganic residue after combustion, indicating the mineral content of a sample[xxi].
Acid-Insoluble Ash (% w/w): It is typically determined through a process involving the ashing of organic matter, followed by treatment with hydrochloric acid and subsequent re-ashing.[xxii]
Water-Soluble Ash (% w/w): Reflects the portion of ash soluble in water, indicating water-soluble inorganic constituents. It can enhance the solubility of poorly soluble drugs, facilitating better absorption in the digestive tract[xxiii].
Water-Soluble Extractive (% w/w): The water-soluble extractive value plays an important role in evaluating the quality of crude drugs. A low extractive value may indicate the presence of exhausted material, adulteration, or errors in processing such as improper drying, storage, or formulation.[xxiv]
Alcohol-Soluble Extractive (% w/w): A high alcohol-soluble extractive value suggests the presence of polar constituents, including steroids, phenolics, flavonoids, and glycosides.[xxv]
All parameters were analysed in triplicate, and mean values were calculated. The results were compared with the standard limits prescribed in the API monograph of *Bacopa monnieri*.
Physicochemical analysis revealed values within the acceptable limits prescribed in the Ayurvedic Pharmacopoeia of India. (Table 2).

Table 2 Physicochemical Parameters of Bacopa monnieri Powder

Parameter	Specification	Result	API Standard
Loss on drying (%)	-	13.64	-
Total ash (%)	NMT 18%	15.74	Max. 18
Acid-insoluble ash (%)	NMT 6%	0	Max. 6
Water-soluble ash (%)	-	12.49	-
Water-soluble extractive (%)	NLT 15%	28.68	Min. 15
Alcohol-soluble extractive (%)	NLT 6%	20.40	Min. 6

HPTLC Profiling

HPTLC was performed to identify the presence of bioactive compounds, including flavonoids, terpenoids, and phenolic compounds, in plant extracts.[xxvi]
Sample Preparation: Five grams of the powdered drug were extracted with methanol via Soxhlet extraction. The extract underwent filtration and concentration prior to analysis.
Chromatographic Conditions: HPTLC analysis was carried out using a CAMAG system under the following conditions:
Stationary phase: TLC precoated plate silica gel 60 F_{254}, 0.2 mm thickness (Merck, Darmstadt, Germany)
Mobile phase: Toluene: Ethyl acetate: Formic acid (7:3:0.1, v/v/v)
Development chamber: CAMAG twin-trough chamber
Sample application volumes: 2.0, 4.0, 6.0, and 8.0 µL of methanolic extract
Detection: UV at 254 nm and 366 nm before derivatization.
Derivatization: Spraying with anisaldehyde–sulphuric acid reagent followed by heating at 105 °C
Post-derivatization detection: White light scanning at 540 nm
Retention factor (Rf) values were recorded, and photographic documentation of plates was performed under UV at 254 nm, 366 nm, and after derivatization. (Table 3, Figure 1.1 to Figure 1.3).

Table 3 Rf values of bands observed in methanolic extract of Bacopa monnieri.

Results:	
Sample name	**Rf (8-microliter)**
At 240nm	**0.13, 0.18, 0.78**
At 366nm	**0.21, 0.41, 0.53, 0.57, 0.62, 0.67, 0.71, 0.78**
After derivation	**0.25, 0.54, 0.78, 0.89**

Distinct bands with varying intensities and colors were noted, suggesting the presence of diverse secondary metabolites, including flavonoids, triterpenoid saponins, and phenolic compounds. The obtained fingerprint profile may serve as a reference for authentication and standardization of *Bacopa monnieri* raw material.

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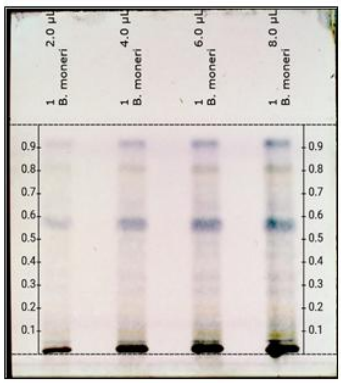


Figure 1.1 After derivation at 540nm

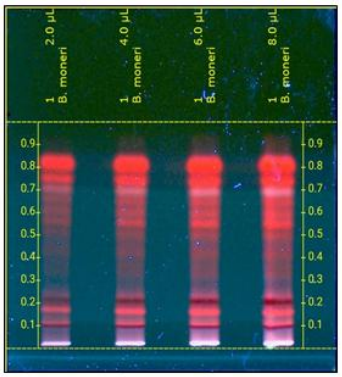


Figure 1.2 Under UV light at 366nm

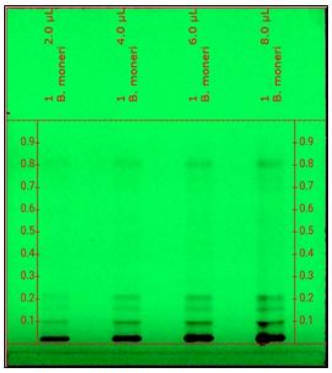


Figure 1.3 Under UV light at 254nm

Fourier-transform infrared (FTIR) analysis

FTIR spectroscopy was employed to identify and characterize the functional groups in the BM sample. A finely ground powder of BM (less than 1 g) was placed into the sample holder of a PerkinElmer UATR, located in Waltham, MA, USA. The sample was subsequently scanned over a mid-infrared (mid-IR) range of 4000–400 cm−1 utilizing a deuterated triglycine sulfate detector to ensure optimal signal acquisition.[xxvii] The FTIR spectrum of *Bacopa monnieri* methanolic extract exhibited eleven distinct absorption peaks, indicating the presence of diverse functional groups (Table 4, Figure 2). These findings confirm the presence of multiple phytochemical classes in *Bacopa monnieri*, including phenolic compounds, flavonoids, alkaloids, fatty acids, and other secondary metabolites.

Table 4 FTIR peak assignments for Bacopa monnieri methanolic extract.

Peak No.	Wavenumber (cm^{-1})	Type of Stretching	Possible Functional Group / Bond
1	3272.67	O–H / N–H stretching (broad)	Alcohols, Phenols, Amines (–OH, –NH)
2	2918.56	C–H stretching (asymmetric)	Alkanes, Methyl/Methylene groups (–CH_3, –CH_2–)
3	1734.65	C=O stretching (sharp)	Ester, Aldehyde, or Carboxylic Acid carbonyl group
4	1614.51	C=C stretching (aromatic ring) / N–H bend	Aromatic rings, Secondary amines
5	1415.62	C–H bending (deformation)	Alkanes, Methyl groups
6	1372.55	Symmetric bending of CH_3	Alkanes, Methoxy or methyl groups
7	1321.10	C–N or O–H bending	Phenols, Amines
8	1246.38	C–O stretching	Esters, Ethers, Phenols
9	1022.26	C–O or C–N stretching	Alcohols, Ethers, Amines
10	930.94	=C–H bending (out-of-plane)	Alkenes or aromatic rings
11	890.02	=C–H bending (aromatic)	Aromatic C–H bending

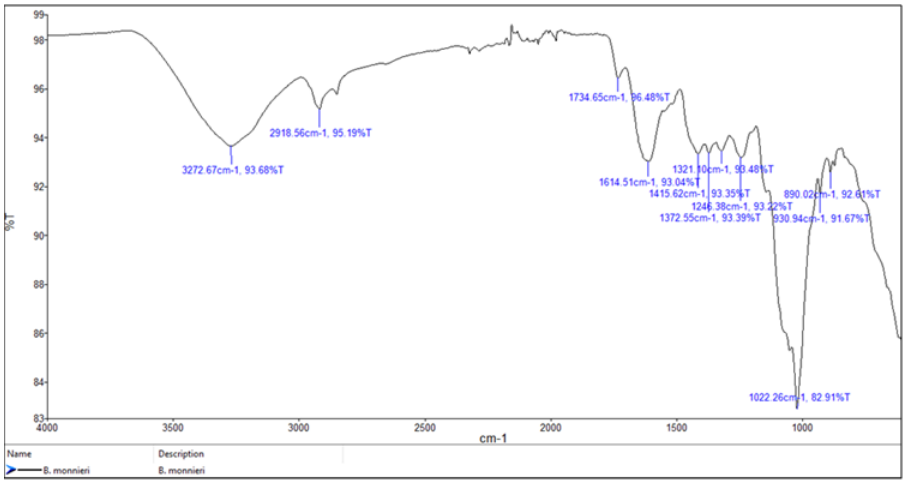


Figure 2 FT-IR spectrum representing potential bands

***In Silico* Analysis of Antihypertensive Activity**

Protein Preparation

The target protein (PDB ID: 4YHJ, 3D structure illustrated in Fig. 3) was obtained from the Protein Data Bank (PDB). Since raw PDB files typically contain water molecules, metal ions, cofactors, and co-crystallized ligands that may interfere with docking accuracy, the structure was pre-processed using the Protein Preparation Wizard in Schrödinger Maestro (version 12.8). Pre-processing steps included removal of non-essential molecules, addition of hydrogen atoms, optimization of bond orders, and energy minimization to obtain a stable, low-energy conformation of the protein[xxviii]. The refined protein structure was then employed for grid generation and subsequent docking studies.

Ligand Preparation

Twelve phytochemicals with reported pharmacological relevance were docked utilizing blind docking with *Amlodipine* as the standard hypertension medication after a comprehensive literature analysis. Maestro's LigPrep module optimized hydrogen addition, energy minimization, stereochemical refinement, charge assignment, and solvation energy computation to produce the ligand structures[xxix]. The optimized ligands were subsequently converted into 3D structures suitable for docking simulations.

Molecular Docking

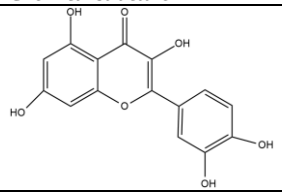
Docking simulations were carried out using Glide (Schrödinger Maestro 12.8) with an automated grid mapping approach to define the binding pocket. Sampling was performed to predict the most favourable binding poses of ligands within the target receptor. More negative docking scores (kcal/mol) indicate greater binding affinities.

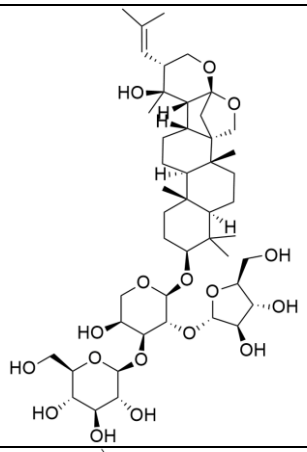
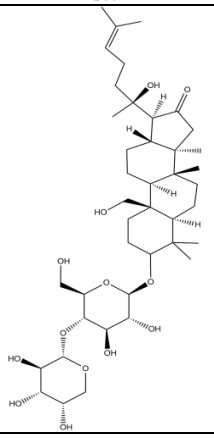
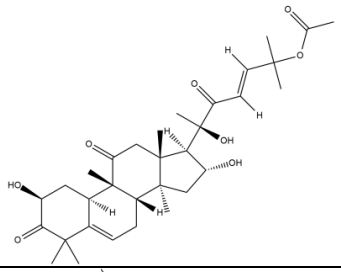
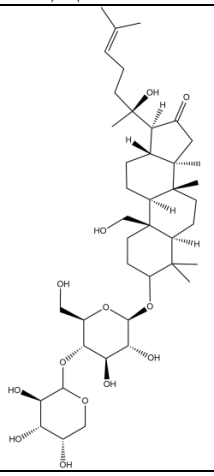
Docking Results

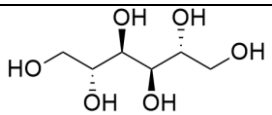
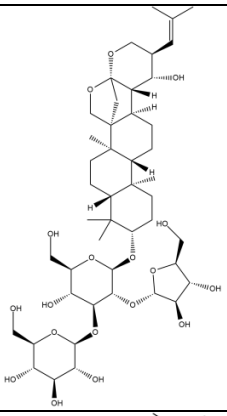
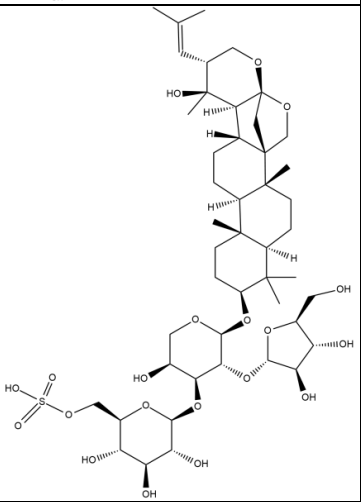
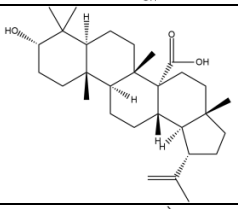
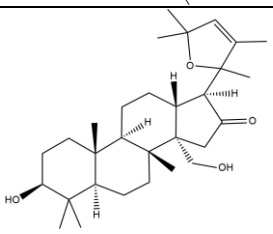
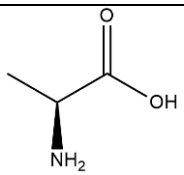
Among the phytochemicals, Quercetin exhibited the highest binding affinity (–8.93 kcal/mol), surpassing the standard drug *Amlodipine* as well as all other tested phytochemicals. Comparative docking scores are summarized in Table 5 and Table 6. Interestingly, most phytochemicals demonstrated higher binding affinities compared to *Amlodipine*, indicating their potential as calcium channel modulators.

The docking study revealed that several phytochemicals, particularly *Quercetin*, exhibited strong binding interactions with the target receptor, suggesting their potential as antihypertensive agents. These results are consistent with previous L-type calcium channel docking studies conducted using Maestro 12.8, further supporting the hypothesis that these compounds may function as calcium channel inhibitors. The combination of protein refinement, ligand optimization, and advanced scoring functions in Maestro 12.8 contributed to the improved accuracy of the docking results. The 2D interaction diagrams of the docked conformations of the phytochemicals are presented in Figures 4.1–4.6. The findings suggest plant-derived phytochemicals may be attractive antihypertensive therapeutic leads, requiring in vitro and in vivo validation.

Table 5 *In-Silico* screening of *Bacopa Monnieri* Phytoconstituents derivatives

S.No.	Name of phytoconstituents	Chemical structure	Docking score	Glide energy
	Quercitin		-8.93	-43.01

	Bacopasaponin C		-6.61	-50.10
	Bacoside A		-6.58	-47.05
	Cucurbitacin B		-6.09	-54.57
	Bacoside B		-5.27	-48.95

	D mannitol		-5.11	-27.44
	Bacopaside 2		-5.10	-33.75
	Bacopaside 1		-4.77	-44.93
	Bacosine		-4.57	-31.97
	Bacogenin A		-4.39	-35.67
	Alanine		-3.55	-19.18

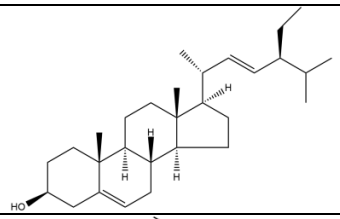
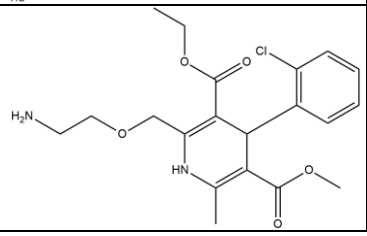
	Stigmasterol		-2.84	-32.80
	Amlodipine		-5.27	-42.28

Table 6 Comparative analysis with standard drugs

S.no.	Compounds	Docking score (PDB ID 4YHJ)
	Quercitin	-8.93
	Bacopasaponin C	-6.60
	Bacoside A	-6.58
	Cucurbitacin B	-6.09
	Bacoside B	-5.27
	D mannitol	-5.10
	Bacopaside 2	-5.10
	Bacopaside 1	-4.77
	Bacosine	-4.56
	Bacogenin A	-4.39
	Alanine	-3.55
	Stigmasterol	-2.84
	Standard drug	*Amlodipine* (Anti-hypertensive) -5.27

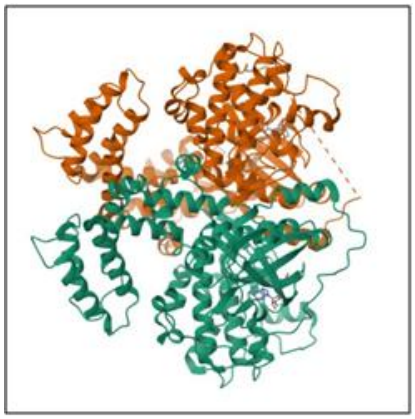


Figure 3 3D-Structure of protein 4YHJ

Docking Results:

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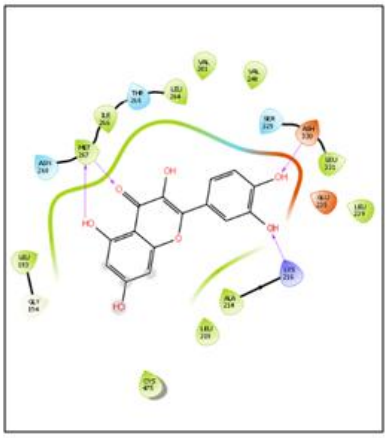

Figure 4.1 *Quercitin* 2D diagram of docked confirmation compound

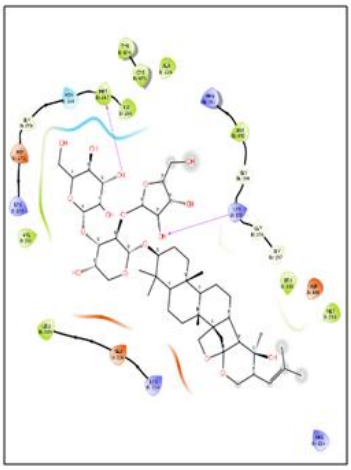

Figure 4.2 *Bacopasaponin C* 2D diagram of docked confirmation compound

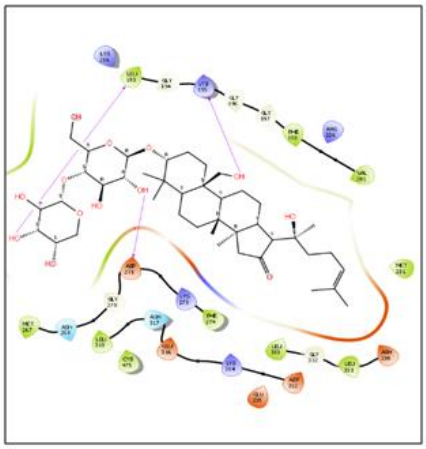

Figure 4.3 *Bacoside A* 2D diagram of docked confirmation compound

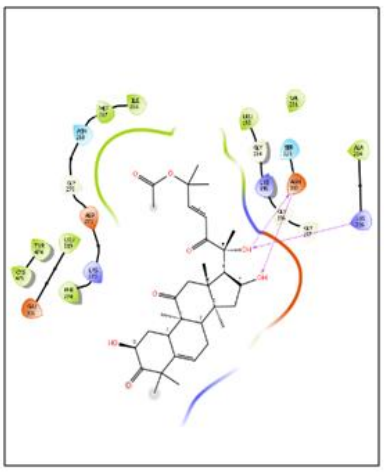

Figure 4.4 *Cucurbitacin B* 2D diagram of docked confirmation compound

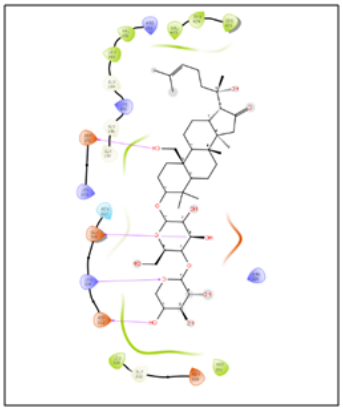

Figure 4.5 *Bacoside B* 2D diagram of docked confirmation compound

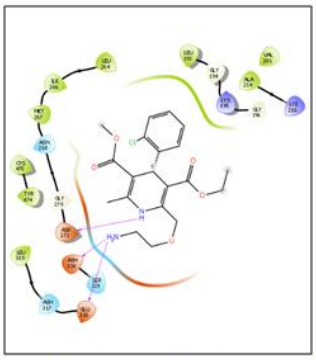

Figure 4.6 *Amlodipine* 2D diagram of docked confirmation compound

RESULT AND DISCUSSION:

The raw material of *Bacopa monnieri* was sourced from Sambhajinagar, Maharashtra and verified at RRDR, AIIA, New Delhi, adhering to pharmacognostical and processing protocols to ensure the sample's integrity for further analysis. The organoleptic assessment revealed that the powder is fine, greenish, exhibits a bitter taste, and possesses a characteristic odor, consistent with Ayurvedic descriptions. The measured physicochemical parameters, including loss on drying (13.64%), total ash (15.74%), and acid-insoluble ash (0%), were within acceptable limits. The values for water-soluble extractive (28.68%) and alcohol-soluble extractive (20.40%) exceeded API standards, suggesting a significant concentration of phytoconstituents and confirming the absence

of adulteration. The HPTLC fingerprinting of the methanolic extract (8 µL) demonstrated multiple Rf values—0.13, 0.18, and 0.78 at 240 nm; various bands at 366 nm; and post-derivatization bands at 0.25, 0.54, 0.78, and 0.89—signifying the presence of flavonoids, saponins, and phenolic compounds, thus offering a dependable fingerprint for authentication and standardization. FTIR spectroscopic analysis revealed eleven distinct peaks, including a broad band at 3272 cm^{-1} (O–H/N–H groups), sharp absorptions at 1734 cm^{-1} (C=O) and 1614 cm^{-1} (aromatic C=C), along with bands between 1246–1022 cm^{-1} linked to C–O and C–N stretching, thereby confirming the presence of phenolics, flavonoids, saponins, and alkaloids. Molecular docking studies revealed significant bioactivity, with *quercetin* attaining the highest binding score of –8.93 kcal/mol, surpassing the standard drug *amlodipine*, which scored –5.27 kcal/mol. *Bacopasaponin* C (–6.60 kcal/mol), bacoside A (–6.58 kcal/mol), and cucurbitacin B (–6.09 kcal/mol) exhibited significant ACE binding affinity. The pharmacognostical, physicochemical, HPTLC, and FTIR results collectively confirm the authenticity and comprehensive phytochemical profile of B. monnieri. Molecular docking provides mechanistic insights into its antihypertensive potential via ACE inhibition, supporting its classification as a "*medhya rasayana*" (nootropic) and expanding its therapeutic implications for cardiovascular health.

CONCLUSION:

This study on *Bacopa monnieri* (*Brahmi*) encompassed collection, authentication, and processing, succeeded by comprehensive pharmacognostical and organoleptic evaluations in alignment with Ayurvedic Pharmacopoeia standards. The physicochemical analyses confirmed compliance with established quality parameters, ensuring authenticity and purity. The chromatographic profiling of the methanolic extract via HPTLC revealed a range of phytoconstituents, while FTIR spectroscopy provided insights into the functional groups associated with its pharmacological activities. Molecular docking studies demonstrated the binding efficiency of bioactive phytoconstituents against angiotensin-converting enzyme (PDB ID: 4YHJ), suggesting a potential comparative efficacy with *Amlodipine*. This approach validates existing claims, establishes a scientific framework for standardization, and highlights *Bacopa monnieri* as a promising candidate for future drug development, particularly in cardiovascular and neurological therapies.

Limitations of the Study:

This study was limited to pharmacognostical, physicochemical, spectroscopic, and in-silico evaluations, without in-vivo or clinical validation. Molecular docking, though predictive, does not account for confirmation of the bioavailability, metabolism, or systemic interactions. Additionally, variations in cultivation and sourcing may affect reproducibility. Future studies should include preclinical and clinical investigations to substantiate these findings.

Declarations:

Ethical Approval and Consent to Participate:

Not applicable. This study is a original work article and does not involve any new data collection from human or animal subjects.

Human Ethics:

Not applicable. The study does not involve human participants or the use of human data or tissue.

Consent for Publication:

Not applicable. No individual person's data in any form (including individual details, images, or videos) is included in this review.

Competing Interests:

The authors declare that they have no competing interests.

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Authors' Contributions:

NG: Conceptualization, Data curation, Methodology, Writing – original draft, Writing – review & editing, Investigation, Project administration. **SKJ:** Writing – review & editing, Investigation, Project administration.

MKB: Supervision, Formal analysis, Visualization, Resources, Funding acquisition. **LG:** Data acquisition.

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