



IN SILICO MOLECULAR DOCKING STUDY OF PHYTOCONSTITUENTS OF *BACOPA MONNIERI* AGAINST GABA-A RECEPTOR FOR POTENTIAL ANXIOLYTIC ACTIVITY

Pushpraj Singh Chouhan, Muskan Tomar*, Manoj Goyal

Department of Pharmacology Indore Mahavidyalaya, Indore, Madhya Pradesh.

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*Corresponding Author

Muskan Tomar

Department of Pharmacology Indore
Mahavidyalaya, Indore, Madhya
Pradesh.



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ABSTRACT

Anxiety disorders are among the most prevalent neuropsychiatric conditions worldwide and are closely associated with dysfunction of the gamma-aminobutyric acid (GABA) neurotransmitter system. The GABA-A receptor plays a crucial role in regulating neuronal excitability and emotional responses, making it an important therapeutic target for anxiolytic drug development. *Bacopa monnieri*, a well-known medicinal plant in traditional medicine, possesses several neuroprotective and anxiolytic properties attributed to its diverse phytoconstituents. The present study aimed to evaluate the interaction of selected phytoconstituents of *Bacopa monnieri* with the GABA-A receptor using molecular docking analysis. The three-dimensional structure of the human GABA-A receptor (PDB ID: 6HUO) was retrieved from the Protein Data Bank and prepared for docking studies. Major

phytoconstituents including Bacoside A, Bacoside I, BacosideII, and Luteolin were selected as ligands, while diazepam was used as the standard reference drug. Molecular docking simulations were performed using Molegro Virtual Docker to predict binding affinity and interaction patterns within the receptor binding site. The docking results revealed that several phytoconstituents exhibited strong binding affinity towards the GABA-A receptor. Among the tested compounds, Bacoside II and Bacoside I demonstrated favorable MolDock scores and significant interactions with key amino acid residues in the active binding cavity. These findings suggest that phytoconstituents of *Bacopa monnieri* may interact effectively with the

GABAergic system and could contribute to its anxiolytic activity. The study highlights the potential of plant-derived compounds as natural therapeutic candidates for the management of anxiety disorders; however, further experimental validation through in vitro and in vivo studies is required to confirm these computational findings.

KEYWORDS: Bacopa monnieri, Molecular Docking, GABA-A Receptor, Anxiety Disorder, Phytoconstituents, Anxiolytic Activity.

1. INTRODUCTION

Neuropsychiatric disorders represent a major global health concern and significantly affect the quality of life of millions of people worldwide. Anxiety disorder is the most prevalent and disabling mental disorder. Excessive fear, anxiety, and a range of autonomic interferences are some of the many symptoms that occur as a result of having anxiety disorder.^[1] These symptoms can inhibit the person from being able to function in social settings, be employed or engage in other activities of daily living. Numerous research studies provide evidence that deficiencies in the inhibitory neurotransmitter system, for example gamma-aminobutyric acid (GABA), contribute significantly to the development of anxiety symptoms.^[2] While there are a number of pharmaceutical agents off-label to treat anxiety, long term use is limited due to side effects and/or possible dependency. Therefore, there is an increased interest in finding plant derived bio-active compounds to serve as safe alternatives to pharmaceutical agents.^[3] One such plant that is receiving a lot of attention is Bacopa monnieri because it is traditionally used as a natural medicine for neuroprotection and anxiolysis. The objective of this current study is to examine the interaction of phytochemical constituents of B. monnieri with the GABA-A receptor through molecular docking methodology to provide possible mechanistic understanding.^[4]

1.1 Anxiety disorders: Pathophysiology and Global Burden

Anxiety disorders are one of the most frequently diagnosed mental health disorders around the globe and pose a significant concern to society at large. Patients suffer from continuous and overwhelming amounts of fear, sense of impending doom and behavioral problems that disrupt their normal ability to function as an individual in society.^[5] The exact cause(s) of anxiety disorders are not fully understood. However, it is known that complex interactions among genetic predispositions, environmental triggers (including life events) and physiologic factors (or biological bases) can lead to anxiety-related illnesses. One of the primary contributing factors to the development of anxiety disorders includes a disruption of the

brain's neuro-devices.^[6] The brain's neurochemical systems specifically, the γ -amino busieric (γ -A) acid, serotonin, dopamine, and norepinephrine systems are thought to be highly important in the progression of anxiety disorders. There are various types of functional and structural brain abnormalities associated with each of the following regions within the human brain: amygdala, hippocampus, and prefrontal cortex which are responsible for controlling many behaviours that are associated with anxiety.^[7] Impairment of inhibitory neurotransmission that is related to decreased amount of GABA's activity is believed to be one of the major causes of neuronal excitability related to anxiety disorders. The rising rate of occurrence of these illnesses, their chronic nature and their resulting economic impact necessitate that we seek safer and more effective methods for treating anxiety-related illnesses.^[8]

1.2 Role of GABA-A Receptor in Anxiety Modulation - GABA (gamma-aminobutyric acid) is an important inhibitory neurotransmitter in the CNS (central nervous system); it is thought to exert its effects through the GABA-A receptor (GABAAR), a chloride-gated ligand-protein structure formed by various subunits.^[9] When GABAAR is activated, it increases the amount of chlorine flowing into nerve cells (neurons); this leads to the hyperpolarization of the cell membrane and reduced excitability of the neuron. The GABAAR is very important in controlling anxiety, reactions to stress, sleep patterns, and emotional stability. When the GABAAR does not work properly or works less well, there is a high chance that the person will be anxious.^[10] As such, pharmacological agents that enhance GABAAR activity have been reported as successful for treating anxiety. The GABAAR has been validated as an important molecular target for researching anxiolytic (anti-anxiety) drugs because of its significant role in providing inhibitory neurotransmission in the CNS.^[11]

1.3 Limitations of Current Anxiolytic Drugs - Conventional anxiolytic agents include both benzodiazepines and some types of antidepressants that have therapeutic effects by modulation of GABAergic and/or serotonergic pathways. While they can quickly relieve symptoms, long-term use carries a high risk of several side effects including sedation, cognitive impairment, tolerance, dependence, withdrawal symptoms, and psychomotor dysfunction.^[12] Caution is also advised when using SSRIs, which are often prescribed for the treatment of anxiety disorders; among their most common side effects directly related to treatment are: delayed onset of action, gastrointestinal problems, sexual dysfunction, and insomnia. As a result of this, many patients will experience lack of compliance to their

treatment over time and therefore receive poor therapeutic responses. As a result, elements of the natural product marketplace have gained great attention for these patients as they are searching for more natural alternatives to traditional treatment and as a means to achieve fewer side effects and improved tolerability.^[13,14]

1.4 Therapeutic Potential of *Bacopa monnieri* - Brahmi (*Bacopa monnieri*) is a renowned health-supporting agent that has been historically recognized as a "Medhya Rasayana" for improving cognitive function and mental health in Ayurveda. Research into the bioactive constituents of *Bacopa monnieri* has unveiled various compounds with potential therapeutic properties, including alkaloids, flavonoids, saponins, bacosides, and bacopaside.^[15] *Bacopa monnieri* has been studied for its neuroprotective properties as well as its adaptogenic and antioxidant effects. *Bacopa monnieri*'s potential as an anxiolytic agent may also be due to its ability to modulate the activity of neurotransmitter systems (e.g., the GABAergic system). Certain bacosides have been shown to impact synaptic transmission and neuronal signaling, and it is believed that these bacosides are interacting with specific subunits of the GABA-A receptor. Therefore, *Bacopa monnieri* may be a favorable choice for treating anxiety naturally.^[16]

1.5 Rationale of the Study - Although there is both traditional and experimental data that support the neuroprotective and anxiolytic effects of *Bacopa monnieri*, there is still a lack of exploration into the molecular mechanisms by which the phytoconstituents of *Bacopa monnieri* interact with the GABA-A receptor. Understanding these mechanisms of ligand-receptor binding will help identify any bioactive compounds that may be responsible for the therapeutic activity of these compounds.^[17] Molecular docking is a computer-based technique that predicts how well small molecules will bind to their target proteins by estimating their binding affinity and interaction patterns. This in-silico analysis is a cheaper and faster method than conducting experimental validation of the phytoconstituents first. Therefore, the goal of this study is to evaluate the interaction profiles of specific phytoconstituents of *Bacopa monnieri* with the GABA-A receptor using molecular docking analysis. It is anticipated that the results of this study will provide additional mechanistic information associated with the potential anxiolytic activity of *Bacopa monnieri* and provide support for the development of plant-based therapeutic alternatives.^[18]

2. MATERIALS AND METHODS

2.1 Selection of Target Protein

2.1.1 Retrieval of GABA-A Receptor Structure from Protein Data Bank

The three-dimensional crystal structure of the human GABA-A receptor was retrieved from the Protein Data Bank (RCSB PDB). The structure with PDB ID **6HUO** was selected for molecular docking analysis based on its human origin, high structural resolution, and availability of ligand-binding site information.^[19]

2.1.2 Protein Structure Details (PDB ID, Resolution, Chain Selection)

The selected protein structure (PDB ID: 6HUO) has a resolution of approximately **3.2 Å**. For docking analysis, **Chain A** was selected based on structural integrity and suitability for ligand interaction studies. The selected chain was further processed for computational analysis.^[20]

2.2 Protein Preparation

Protein preparation was performed using Molegro Virtual Docker (MVD).

2.2.1 Removal of Water Molecules

All crystallographic water molecules and irrelevant heteroatoms were removed from the protein structure to prevent interference during docking.^[21]

2.2.2 Addition of Hydrogen Atoms

Polar hydrogen atoms were added to ensure correct protonation states and proper hydrogen bonding interactions during docking simulation.

2.2.3 Binding Site Identification

The active binding cavity was identified using the cavity detection tool available in Molegro Virtual Docker. The cavity corresponding to the benzodiazepine binding region was selected based on volume and spatial orientation. The grid resolution was set to **0.30 Å** for docking calculations.^[22]

2.3 Ligand Selection and Preparation

2.3.1 Selection of Phytoconstituents of *Bacopa monnieri*

Major phytoconstituents of *Bacopa monnieri*, including bacosides and related compounds, were selected based on literature reports indicating their neuroprotective and CNS-modulating properties.

2.3.2 Retrieval of Ligand Structures from PubChem: Three-dimensional structures of selected phytoconstituents and the standard drug diazepam were retrieved from the PubChem database in SDF format and converted into suitable format for docking analysis. The overall structure of the receptor is shown in Figure 1.^[23]



Figure 1: Three-dimensional structure of GABA-A receptor retrieved from Protein Data Bank (PDB ID: 6HUO).

2.3.3 Energy Minimization

Energy minimization of ligands was performed within Molegro Virtual Docker using default force field parameters to obtain stable conformations prior to docking.

2.4 Molecular Docking Procedure

2.4.1 Docking Software

Docking simulations were carried out using Molegro Virtual Docker (MVD), which employs a differential evolution algorithm for flexible ligand docking.

2.4.2 Docking Parameters

The following parameters were used:

- Grid resolution: 0.30 Å
- Maximum iterations: 1500
- Population size: 50
- Number of runs: 10
- Maximum poses stored: 5

The MolDock scoring function was used to evaluate binding affinity.

2.4.3 Docking of Standard Drug (Diazepam)

Diazepam was docked under identical parameters to serve as a reference standard for comparison of binding affinity and interaction profile.

2.4.4 Validation by RMSD Calculation

Docking accuracy was validated by calculating RMSD values between predicted and reference poses. RMSD values below 2.0 Å were considered acceptable.

3. RESULTS

The molecular docking analysis was performed to evaluate the binding affinity of selected phytoconstituents of *Bacopa monnieri* against the GABA-A receptor. The docking simulations generated multiple binding poses for each ligand, and the best pose was selected based on the lowest MolDock score and favorable interaction with active site residues. The docking results indicated that several phytoconstituents exhibited significant binding affinity comparable to the reference drug diazepam.

3.1 Docking Score Analysis

Table 1: Molecular docking scores of *Bacopa monnieri* phytoconstituents against GABA-A receptor.

Compound	MolDock Score	Rerank Score	H-Bond Energy	Key Residues
Bacoside A	1872.81	-46.7888	-5.55465	ASN 275(A),HIS 267(E)
Bacoside I	-181.887	-98.1796	-9.41424	Ser51 (E), Val50 (E), Lys279 (A), and Thr271 (E)
Bacoside II	-157.448	-40.6884	-14.3721	Val50(E), Gln185 (E), Glu52 (E), Lys279 (A), His56 (A), Asp57 (A), Lys274 (B), Ser276 (A).
Diazepam (Standard)	-90.9463	-69.0425	-3.09096	Val50(E), Gln185 (E),
Luteolin	-89.4522	-71.966	-7.37482	Gln185 (E), Glu52 (E), Val53 (E), Ser51 (E)

Among the tested compounds, **Bacoside I and Bacoside II showed the lowest MolDock scores**, indicating stronger binding affinity with the active site of the GABA-A receptor. The unusually high MolDock score obtained for Bacoside A may be attributed to its large molecular size and complex structure, which can sometimes lead to exaggerated scoring values during docking simulations.

3.2 Interaction Analysis - Important residues involved in ligand interaction included **Ser51, Val50, Lys279, Gln185, Glu52, His56, Asp57, and Ser276**, which are located within the active binding pocket of the GABA-A receptor. These interactions contribute to the stabilization of ligand-receptor complexes and may enhance the inhibitory activity of the receptor. Detailed interaction analysis revealed that the selected phytoconstituents formed multiple hydrogen bonds and hydrophobic interactions with key amino acid residues present in the active binding site of the receptor. The interaction profile of Bacoside A with the GABA-A receptor is illustrated in **Figure 2**. The docking analysis revealed that Bacoside I formed interactions with several amino acid residues including Ser51, Val50, Lys279, and Thr271 within the active binding site of the GABA-A receptor. The interaction pattern is shown in **Figure 3**. Bacoside II exhibited multiple interactions with amino acid residues including Val50, Gln185, Glu52, Lys279, His56, Asp57, Lys274, and Ser276 within the active binding site of the GABA-A receptor. The docking interaction pattern is illustrated in **Figure 4**. The docking interaction of the standard drug diazepam with the GABA-A receptor showed binding with amino acid residues Val50 and Gln185 within the receptor binding site. The interaction profile is illustrated in **Figure 5**. The docking interaction of Luteolin with the GABA-A receptor showed binding with amino acid residues Gln185, Glu52, Val53, and Ser51 within the active binding site, as illustrated in **Figure 6**.

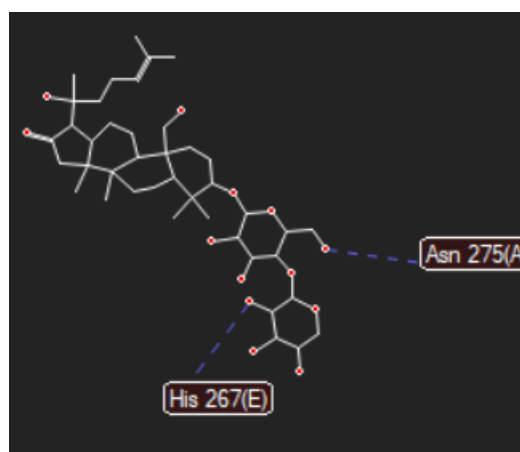


Figure 2: Molecular docking interaction of Bacoside A with the GABA-A receptor showing interactions with amino acid residues Asn275 (A) and His267 (E).

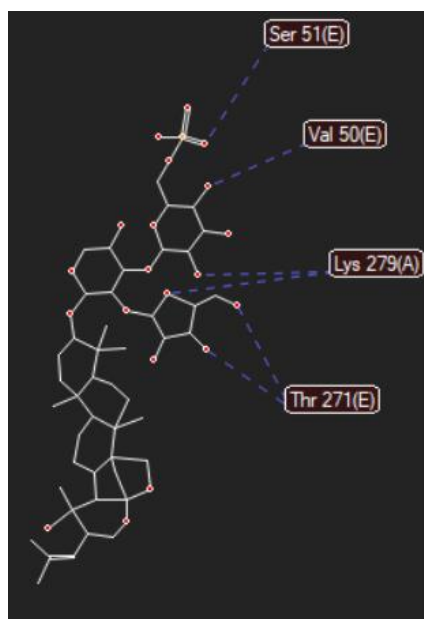


Figure 3: Molecular docking interaction of Bacoside I with the GABA-A receptor showing interactions with amino acid residues Ser51 (E), Val50 (E), Lys279 (A), and Thr271 (E).

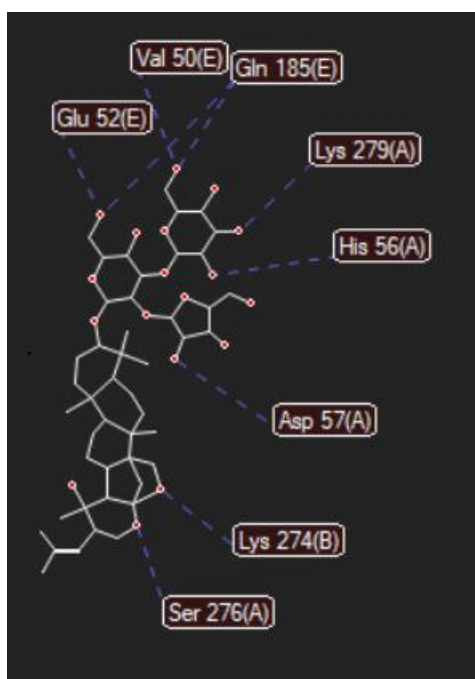


Figure 4: Molecular docking interaction of Bacoside II with the GABA-A receptor showing interactions with amino acid residues Val50 (E), Gln185 (E), Glu52 (E), Lys279 (A), His56 (A), Asp57 (A), Lys274 (B), and Ser276 (A).

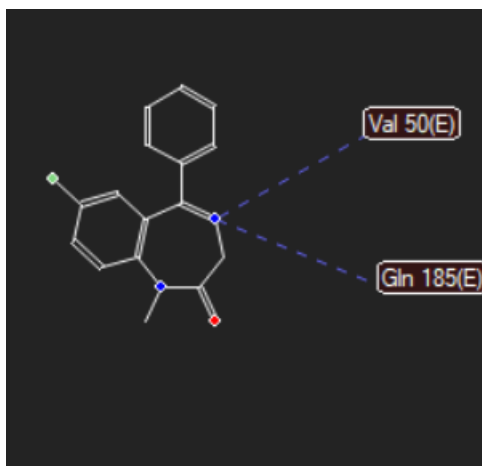


Figure 5: Molecular docking interaction of Diazepam (standard drug) with the GABA-A receptor showing interactions with amino acid residues Val50 (E) and Gln185 (E).

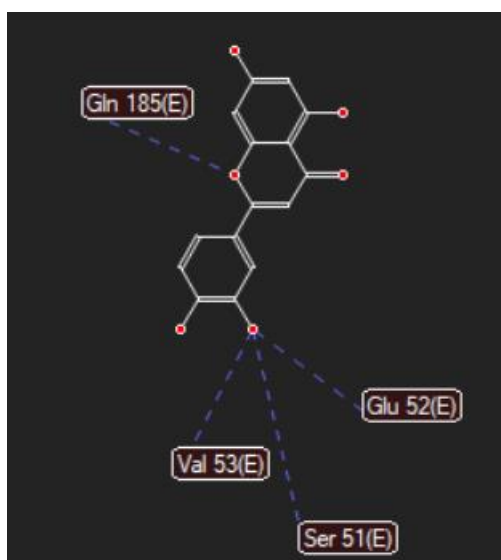


Figure 6: Molecular docking interaction of Luteolin with the GABA-A receptor showing interactions with amino acid residues Gln185 (E), Glu52 (E), Val53 (E), and Ser51 (E).

3.3 Binding Pose Visualization

The docking poses of the best-scoring compounds were visualized using molecular visualization tools integrated within the docking software. The compounds were found to fit well within the benzodiazepine binding cavity of the receptor. The orientation of the ligands indicated favorable interactions with surrounding amino acid residues, supporting their potential to modulate receptor activity.

4. DISCUSSION

The present study investigated the binding interaction of major phytoconstituents of *Bacopa monnieri* with the GABA-A receptor using molecular docking analysis. The docking results demonstrated that several compounds exhibited considerable binding affinity within the active binding cavity of the receptor. Among the evaluated ligands, Bacoside I and Bacoside II showed more favorable MolDock scores compared to other phytoconstituents, indicating stronger interaction with the receptor binding site. These findings suggest that bacoside derivatives may play a significant role in the modulation of GABAergic neurotransmission.^[24] Detailed interaction analysis revealed that the ligands formed interactions with important amino acid residues such as Ser51, Val50, Lys279, Gln185, Glu52, His56, and Asp57, which are located within the active binding pocket of the GABA-A receptor. These interactions may contribute to the stabilization of the ligand–receptor complex and enhance receptor modulation. The presence of hydrogen bonding and hydrophobic interactions further supports the potential of these phytoconstituents to interact effectively with the receptor. The docking results were also compared with the standard anxiolytic drug diazepam, which is widely known for its ability to enhance GABAergic neurotransmission through the benzodiazepine binding site of the GABA-A receptor. Interestingly, some of the phytoconstituents of *Bacopa monnieri* exhibited comparable binding affinity and interaction patterns with the receptor, suggesting that these natural compounds may act through mechanisms similar to benzodiazepines.^[25] Previous pharmacological studies have reported that *Bacopa monnieri* possesses neuroprotective, antioxidant, and anxiolytic properties. The bacosides present in this plant have been shown to influence synaptic transmission and neuronal signaling pathways. The present docking study provides a molecular-level explanation for these pharmacological effects by demonstrating the potential interaction of bacosides with the GABA-A receptor. Overall, the findings of this study support the hypothesis that bioactive constituents of *Bacopa monnieri* may contribute to anxiolytic activity through modulation of the GABAergic system. However, molecular docking provides only predictive information regarding ligand–receptor interactions. Therefore, further *in vitro* and *in vivo* pharmacological studies are required to validate these computational results and to explore the therapeutic potential of these phytoconstituents for the management of anxiety disorders.^[26] These findings highlight the importance of plant-derived phytoconstituents as potential lead molecules for the development of safer anxiolytic drugs targeting the GABAergic system.

5. CONCLUSION

The present molecular docking study demonstrated that phytoconstituents of *Bacopa monnieri* possess significant binding affinity towards the GABA-A receptor. Among the tested compounds, certain bacosides showed promising interaction with the receptor binding site, suggesting their potential as natural anxiolytic agents. These findings support the traditional use of *Bacopa monnieri* in neurological disorders and highlight its potential for the development of plant-based therapeutic alternatives. However, further in vitro and in vivo studies are required to validate these computational predictions.^[27,28,29]

6. Future Scope: Future studies should focus on experimental validation of these phytoconstituents through in vitro receptor binding assays and in vivo pharmacological studies to confirm their anxiolytic activity and therapeutic potential.^[30]

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