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Molecular docking of piperine, limonene, and eugenol compounds in black pepper (*Piper nigrum* L.) as an anti-stroke

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Abstract: Stroke remains a leading cause of death and disability worldwide, necessitating the development of effective therapeutic agents. This study explores the potential of bioactive compounds from Piper nigrum L. (black pepper) as anti-stroke candidates targeting the cGMP-specific phosphodiesterase 5A (PDE5A) receptor (PDB ID: 3BJC). The evaluation employed molecular docking and drug-likeness assessment methodologies. Piperine, limonene, and eugenol were assessed using Lipinski's Rule of Five (RO5) to predict oral bioavailability. All compounds met the RO5 criteria, indicating favorable drug-likeness characteristics. Molecular docking was validated through re-docking of the native ligand WAN, yielding an RMSD ≤ 2 Å, confirming the accuracy of the docking protocol. Following validation, molecular docking analysis revealed that piperine demonstrated the lowest binding energy (-7.71 kcal/mol), followed by limonene (-5.28 kcal/mol) and eugenol (-4.86 kcal/mol). Visualization results revealed that piperine shared key interaction motifs and amino acid residues with the native ligand, including hydrogen bonding and hydrophobic interactions, indicating strong receptor affinity and molecular stability. Additionally, the ligand-receptor interaction distance of piperine (2.21 Å) closely resembled the native ligand (2.15 Å), further supporting its structural and functional similarity. These findings highlighted piperine as the most promising anti-stroke candidate among the tested compounds. Further in vitro and in vivo studies are recommended to validate its pharmacological efficacy and safety.

Keywords: eugenol, limonene, molecular docking, piperine, stroke

Introduction

Stroke is a major global health challenge with a significant epidemiological impact. As a leading cause of disability and mortality worldwide, it is a substantial contributor to the global burden of disease [1]. The prevalence of stroke is increasing, with Indonesia experiencing a notable rise. National data indicate the incidence of stroke in Indonesia increased from 7.0% in 2013 to 10.9% in more recent years. A similar upward trend has been observed in Lampung Province, where reported cases rose from 4.0% in 2013 to 8.0% in 2018 [2].

Stroke is a cerebrovascular event characterized by the disruption of cerebral blood flow, leading to the acute onset of neurological dysfunction [3]. The underlying pathophysiology involves complex cascades of cellular damage that can result in severe impairment of motor, sensory, and cognitive functions. Due to the brain's integral role in coordinating physiological processes, stroke-induced damage can significantly disrupt motor system integrity and overall neurological function [4].

Current clinical management for stroke recovery emphasizes rehabilitation strategies aimed at restoring lost function. Hemiparesis, a weakness affecting one side of the body, is among the most common and debilitating sequelae of stroke, indicative of upper motor neuron damage. Standard rehabilitation often includes targeted interventions such as circle grip training, a functional exercise designed to promote upper limb recovery and motor relearning through the grasping of spherical objects [5].

Despite established rehabilitation protocols, outcomes in functional recovery are often suboptimal. This limitation has prompted investigation into complementary therapeutic strategies, including the use of natural compounds with potential neuroprotective properties. *Piper nigrum* (black pepper) contains several bioactive compounds, such as piperine, which have been

reported to possess anti-inflammatory, antioxidant, and neuroregenerative characteristics *in vitro* and in animal models. However, the specific molecular interactions through which these compounds might exert therapeutic effects on stroke-related targets remain insufficiently elucidated.

To address this knowledge gap, this study employs an *in silico* approach. Using molecular docking simulations, we systematically evaluate the binding affinities and interaction patterns of three key bioactive compounds from *Piper nigrum*—piperine, limonene, and eugenol—with specific protein targets implicated in stroke pathology. The aim is to provide a theoretical foundation for their potential development as anti-stroke therapeutic agents.

Methods

Materials

This study utilized the three-dimensional (3D) structure of cGMP-specific phosphodiesterase 5A (PDE5A) receptor, which was retrieved from the Protein Data Bank with PDB ID: 3BJC, the 3D structure of the native ligand, and the 3D structures of tested compounds from *Piper nigrum* L., including piperine, limonene, and eugenol.

Software and websites

Several computational tools and databases were employed in this research. PubChem (https://pubchem.ncbi.nlm.nih.gov/) was used to obtain the 3D structures of the tested compounds. SwissADME (http://www.swissadme.ch/) was employed to predict pharmacokinetic properties based on Lipinski's Rule of Five. RCSB Protein Data Bank (https://www.rcsb.org/) served as the source for the 3D structure of the target receptors. AutoDockTools v.1.5.7 was utilized to prepare receptor structures, native ligand validation, and molecular docking studies. Molview (https://molview.org/) assisted in redrawing the molecular structures. BIOVIA Discovery Studio Visualizer 2021 was used for analyzing and visualizing protein-ligand interactions.

Lipinski's rule of five prediction

The structures of piperine, limonene, and eugenol compounds were uploaded to the SwissADME website (http://www.swissadme.ch/). This analysis evaluated parameters specified in Lipinski's rules: molecular mass less than 500 Da, hydrogen bond acceptors fewer than 10, hydrogen bond donors fewer than 5, Log P less than 5, and Topological Polar Surface Area less than 140 $Å^2$ [6]. This assessment determined their potential as oral bioavailable drug candidates by evaluating their compliance with criteria predictive of good absorption and permeability.

Protein preparation

The target protein was retrieved from the RCSB Protein Data Bank with PDB ID: 3BJC. Protein preparation was performed using AutoDockTools v.1.5.7 software. The receptor and native ligand were separated, and water molecules were removed to eliminate potential interference. The refined structure was then saved in PDB format for further analysis.

Validation

The validation process involved re-docking the native ligand to the target protein using AutoDockTools v.1.5.7. The grid box dimensions were carefully adjusted to encompass the native ligand's binding site, ensuring accurate docking simulations. This step was crucial for validating the docking protocol, with results assessed based on the Root Mean Square Deviation (RMSD) between the docked and crystallized ligand poses. An RMSD value of less than 2.0 Å was considered acceptable, confirming the reliability of the docking parameters [7].

Molecular docking

Molecular docking was performed using a grid box centered on the native ligand's binding site to ensure accurate ligand positioning. The prepared ligands, converted into PDB format, were processed using AutoDockTools v.1.5.7, where each ligand was assigned a grid box with dimensions established during validation. The docking simulations generated an output parameter of binding affinity expressed in kcal/mol, which was used to evaluate the strength and potential efficacy of the ligand-protein interactions.

Protein-ligand interaction

The hydrogen bond interactions between the ligand and receptor were analyzed using the best docking conformation of each compound, visualized in two-dimensional format using BIOVIA Discovery Studio Visualizer 2021. This visualization revealed

Table 1. Lipinski's Rule of Five prediction results

Compound	Molecular mass (<500 Da)	Hydrogen bond acceptors (<10)	Hydrogen bond donors (<5)	Log P (<5)	Topological polar surface area (<140Å^2)	Lipinski's rule
Piperine	285.34	3	0	2.39	38.77	Yes
Limonene	136.23	0	0	3.27	0.00	Yes
Eugenol	164.20	2	1	2.01	29.46	Yes

key binding interactions, including specific amino acid residues involved in hydrogen bonding and the distances between the ligand and the receptor's active site residues. Additionally, the spatial proximity between the docked compound and the native ligand was assessed to evaluate binding pose accuracy.

Results

Lipinski's rule of five prediction

The Lipinski's Rule of Five (RO5) prediction analysis confirmed that all tested compounds—piperine, limonene, and eugenol—fully comply with the established criteria. These compounds exhibited no violations in key parameters, including molecular weight, lipophilicity (LogP), hydrogen bond donors, hydrogen bond acceptors, and topological polar surface area, indicating favorable drug-like properties (Table 1).

Validation results

The validation procedure was conducted by re-docking the native ligand into the protein. The grid box was centered at coordinates X = 43.78, Y = 14.58, and Z = 46.35, with a box size of 80 × 80 × 106. The RMSD value was 1.26 Å, indicating reliable alignment between the docked and reference conformations. This validated grid box served as the reference for subsequent molecular docking processes with the tested compounds.

Molecular docking analysis

The molecular docking analysis reveals significant differences in binding affinity among the tested compounds (Table 2). Piperine demonstrates the lowest binding free energy (ΔG = -7.71 kcal/mol), indicating superior binding affinity compared to limonene (ΔG = -5.28 kcal/mol) and eugenol (ΔG = -4.86 kcal/mol). The binding free energy value (ΔG) indicates conformational stability between the ligand and enzyme, with lower values representing more stable interactions [8,9].

Piperine exhibits particularly noteworthy binding characteristics, interacting with eight amino acid residues and closely approximating the native ligand's interaction with ten residues. In contrast, both limonene and eugenol engage with seven residues each. This structural similarity suggests that piperine shares analogous binding interactions with the target protein, mirroring the native ligand's activity. The convergence in binding patterns positions piperine as the most promising candidate for anti-stroke activity among the compounds evaluated, as molecular similarity in residue interaction patterns may predict pharmacological efficacy relative to native ligands [10].

Protein-ligand interaction analysis

Analysis of the protein-ligand interactions reveals distinct binding patterns among the tested compounds (Figure 1). The native ligand formed conventional hydrogen bonds with Phe820 and Gln817, accompanied by carbon-hydrogen, pi-sigma, alkyl, and pi-alkyl interactions. Piperine demonstrated a hydrogen bond with His613 and exhibited similar hydrophobic interactions. Limonene lacked hydrogen bonding capacity but engaged in alkyl and pi-alkyl interactions. Eugenol similarly showed no hydrogen bonds but exhibited a pi-sigma interaction with Val782 and Phe820.

Amino acid residue binding profile

Detailed analysis of amino acid residue interactions using BIOVIA Discovery Studio 2021 revealed distinct binding profiles for each compound (Table 3). The native ligand (WAN) demonstrated the most extensive binding network, interacting with ten residues: Phe820, Gln817, Ala767, Val782, Tyr612, Phe787, Met816, Ala783, Phe786, and His613.

Table 2. Molecular docking results of native ligand and test ligand on 3BJC protein.

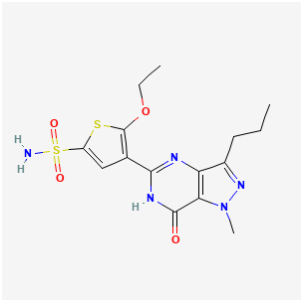
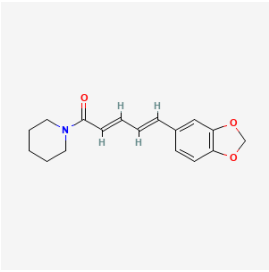
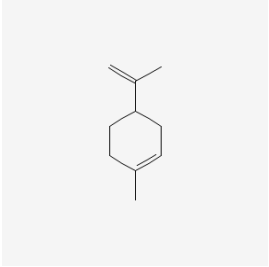
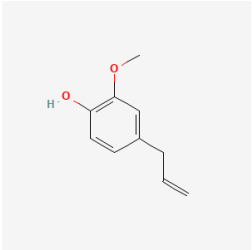
Ligand	ΔG (kkal/mol)	Amino acid residues
 **WAN (native ligand)**	-8.84	10
 **Piperine**	-7.71	8
 **Limonen**	-5.28	7
 **Eugenol**	-4.86	7

Table 3. Amino acid residues bound to ligands

Ligand	Amino Acid Residues									
	Phe820	Gln817	Ala767	Val782	Tyr612	Phe787	Met816	Ala783	Phe786	His613
WAN	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Piperine	✓			✓			✓	✓	✓	✓
Limonen	✓		✓	✓	✓					
Eugenol	✓		✓	✓	✓					

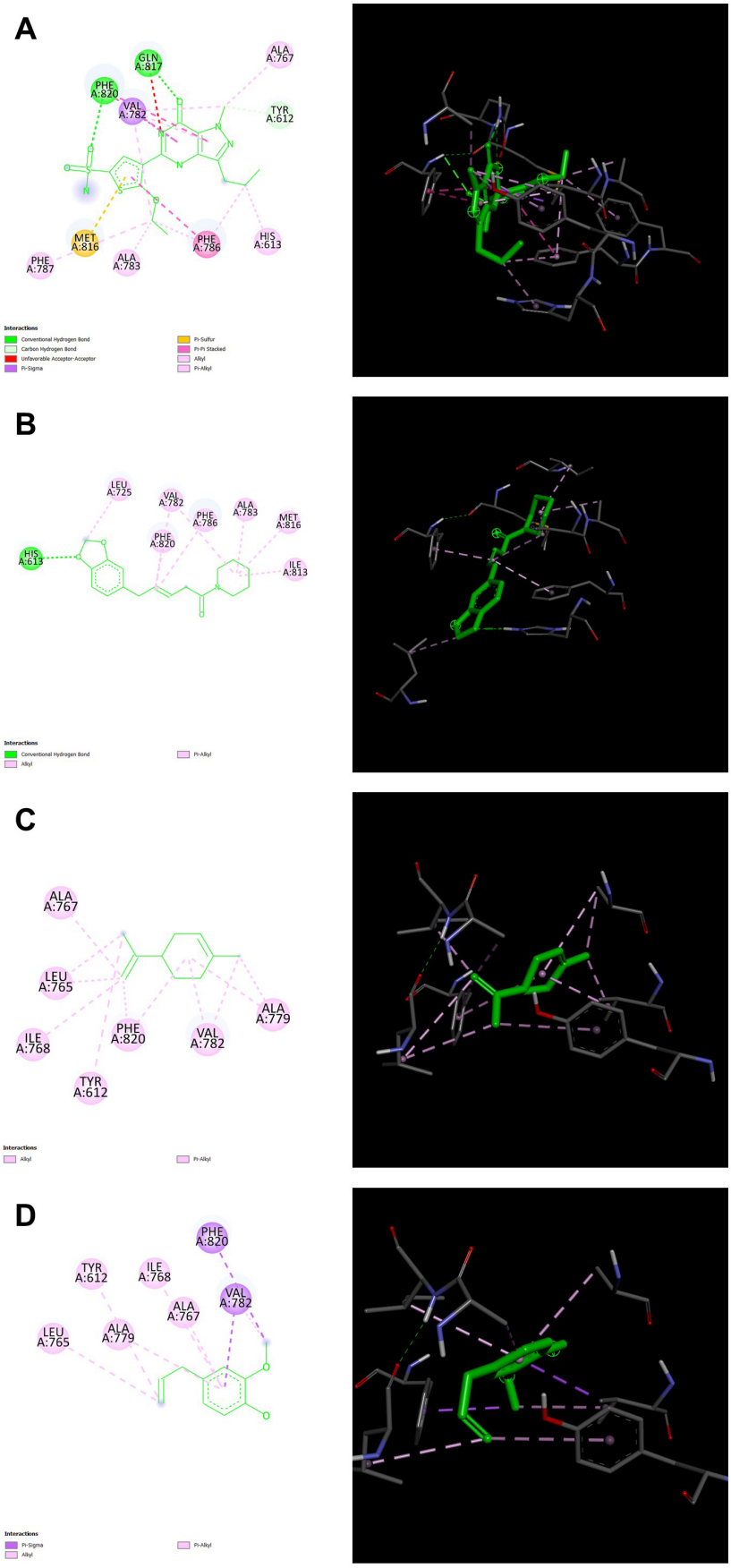


Figure 1. 2D and 3D visualization of protein-ligand interactions. (A) Native ligand, (B) piperine, (C) limonene, and (D) eugenol

Table 4. Types of bonds and distances

Ligand	Hydrogen bonds	Hydrophobic bonds	Other bonds	Distance (Å)
WAN (native)	3	14	1	2.15334
Piperine	1	8	–	2.21297
Limonen	–	11	–	3.97791
Eugenol	–	8	–	3.72258

Piperine exhibited the highest degree of overlap with the native ligand, sharing six common binding residues (Phe820, Val782, Phe787, Met816, Ala783, Phe786, and His613). Both limonene and eugenol demonstrated more limited binding profiles, each interacting with four residues (Phe820, Ala767, Val782, and Tyr612). The significant residue similarity between piperine and the native ligand suggests potential to mimic WAN's inhibitory activity through analogous binding mechanisms.

Interaction characterization and distance analysis

Comprehensive interaction analysis reveals substantial differences in interaction patterns among the tested compounds (Table 5). The native ligand established the most complex binding network with three hydrogen bonds, fourteen hydrophobic bonds, and one additional bond type, maintaining an average distance of 2.15 Å.

Piperine demonstrated moderate binding complexity with one hydrogen bond and eight hydrophobic bonds at a distance of 2.21 Å, maintaining close proximity to the native ligand's binding characteristics. Limonene and eugenol showed exclusively hydrophobic interactions with eleven and eight bonds respectively, but operated at considerably greater distances (3.98 Å and 3.72 Å). These distance measurements exceed the 3.9 Å criterion typically considered optimal for hydrogen donor-acceptor interactions, highlighting the superior binding geometry achieved by the native ligand and piperine compared to the other tested compounds.

Discussion

Molecular docking represents a computational chemistry approach used to identify novel candidate compounds that function as therapeutic agents through their interactions with biological targets such as proteins, DNA, or RNA via specific ligands. This study aimed to identify new compound candidates from *Piper nigrum* that demonstrate oral bioavailability and target the 3BJC receptor as potential anti-stroke therapeutics.

Lipinski's Rule of Five (RO5) serves as a computational guideline in drug discovery to prioritize compounds with potential for effective oral absorption. According to RO5 criteria, compounds suitable for oral administration should not violate more than one of the following parameters: molecular weight less than 500 Da, hydrogen bond donors fewer than five, hydrogen bond acceptors fewer than ten, and LogP value less than five [6]. The LogP parameter relates to compound lipophilicity and proves useful for predicting drug absorption through intestinal epithelium [11]. Hydrogen bonds remain essential for drug partitioning and permeability, though excessive hydrogen bond donors or acceptors can reduce membrane partitioning capabilities. Polar groups may decrease affinity to hydrophobic membrane areas and increase water desolvation penalties during drug penetration. Increased molecular weight correlates with decreased permeation through lipid bilayers, as compounds with larger molecular weights typically demonstrate reduced oral activity compared to smaller molecular weight compounds. Analysis of the three compounds from *Piper nigrum* L. revealed complete compliance with all RO5 criteria, suggesting favorable potential as oral pharmaceutical preparations.

Molecular docking of the test compounds was performed following validation through re-docking of the native ligand WAN with the 3BJC protein. This validation step confirmed that the grid box parameters produced acceptable RMSD values. RMSD represents the deviation in ligand conformation due to structural changes before and after docking, with acceptable values of RMSD ≤ 2 Å [12]. Low RMSD values indicate that the docked ligand conformation closely resembles the original structure, confirming that the docking model accurately predicts ligand-receptor interactions. This

validation process remains critical for ensuring reliable results in drug development applications [13].

Analysis of amino acid residue interactions for piperine, limonene, and eugenol in *Piper nigrum* L. aimed to identify binding patterns and potential interactions contributing to the pharmacological effects of these compounds. Hydrogen bonds, hydrophobic interactions, and other molecular forces represent key binding mechanisms in ligand-receptor interactions. Hydrogen bonds constitute the most essential specific interactions in ligand-receptor binding processes, as target protein molecules that establish electrostatic interactions through hydrogen donors and acceptors significantly influence binding affinity [14].

Visualization results demonstrated that all piperine compound residue interactions exhibit similarities with the native ligand. Therefore, piperine is expected to demonstrate superior anti-stroke activity compared to other test compounds [15]. Molecular docking results further validate piperine's potential through its favorable ΔG value, which approaches that of the native ligand WAN, reflecting stronger binding affinity. Hydrogen bond interactions and shared amino acid residues with WAN suggest comparable molecular stability and specificity. These parallels in ΔG values, binding motifs, and interaction profiles indicate that piperine likely mimics WAN's bioactivity, positioning it as the most promising anti-stroke candidate among the tested compounds.

Interaction distances between ligands and the 3BJC receptor provide additional support for these findings. The native ligand WAN exhibited a distance of 2.15 Å, while piperine, limonene, and eugenol demonstrated distances of 2.21 Å, 3.98 Å, and 3.72 Å, respectively. Optimal ligand-receptor interaction stability typically occurs within a 2.5–3.5 Å range. Although piperine's distance of 2.21 Å falls slightly below this range, its proximity to the native ligand's distance suggests structural and functional similarity. Combined with its residue overlap with WAN, piperine emerges as the most promising anti-stroke candidate, likely mirroring the native ligand's bioactivity. In contrast, the larger distances observed for limonene and eugenol imply weaker binding interactions and reduced therapeutic efficacy [15]. This finding demonstrated that piperine exhibits the closest similarity to the native ligand and possesses the greatest potential as an anti-stroke agent compared to other tested compounds.

Conclusion

The molecular docking results demonstrated that piperine exhibits the lowest binding energy value among the tested compounds. Visualization analysis further supports this finding, revealing interaction patterns similar to the native ligand WAN. Binding energy analysis ranked the compounds in order of affinity: piperine (-7.71 kcal/mol), limonene (-5.28 kcal/mol), and eugenol (-4.86 kcal/mol). All compounds satisfied Lipinski's Rule of Five criteria, suggesting favorable drug-likeness characteristics and oral bioavailability potential. Collectively, piperine emerged as the most promising candidate due to its strong binding affinity and demonstrated ability to mimic WAN's interaction profile. Further in vitro and in vivo studies are necessary to validate piperine's therapeutic potential for stroke treatment applications.

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Declaration of interest

None.

Author contributions

Conceptualization, AHS, FA, WNA; Methodology, EGON, NC, FAS, MMA, WNA; Investigation, WNA, AHS; Writing – Original Draft, FA, EGON, NC, FAS, MMA, WNA, AHS; Writing – Review & Editing, AHS.

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