

Potential of bioactive compounds *Piper betle* L. as angiotensin converting enzyme inhibitors: molecular docking study and in silico analysis evaluation of physicochemical properties, drug-likeness, and toxicity

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ABSTRACT: Hypertension remains a major global health burden, with angiotensin-converting enzyme (ACE) representing a central therapeutic target in blood pressure regulation via the renin-angiotensin-aldosterone system (RAAS). Although synthetic ACE inhibitors are clinically effective, their long-term use is frequently associated with adverse effects, motivating the search for safer alternative agents to treat hypertension. *Piper betle* L. is a medicinal plant rich in diverse bioactive phytochemicals with reported cardiovascular benefits. This study aimed to systematically evaluate the ACE inhibitory potential of *Piper betle* leaf constituents using an integrated, in silico approach. A total of 43 compounds were docked against human ACE (PDB ID: 1UZF) using the PLANTS algorithm, with protocol validation performed by captopril redocking. Binding affinities and key interactions were analyzed, followed by the prediction of physicochemical properties, drug-likeness, and toxicity using ADMETlab 3.0 and ProTox-3.0. Docking results revealed that several compounds, including cerebroside, lignans, and long-chain esterified phenolics, exhibited strong predicted binding to ACE. However, many high-affinity ligands exhibit unfavorable physicochemical characteristics, low quantitative estimates of drug-likeness, and multiple medicinal chemistry rule violations. In contrast, Piperenamide A and Piperenamide B demonstrated a balanced profile, combining stable ACE binding with favorable physicochemical properties, compliance with Lipinski's Rule of Five, and low predicted toxicity. Simple phenolic compounds exhibited good safety profiles despite their moderate binding affinity. Overall, this integrative in silico analysis highlights the potential of compounds derived from *Piper betle* L. leaves as promising natural sources of ACE-inhibitory scaffolds. Furthermore, it provides a rational framework for subsequent experimental validation and lead optimization in antihypertensive drug discovery.

KEYWORDS: ACE inhibitors; drug-likeness; hypertension; in silico; *Piper betle* L.

INTRODUCTION

Hypertension is a major public health challenge and a leading contributor to global cardiovascular morbidity and mortality [1]. Persistent elevation of blood pressure promotes endothelial injury, accelerates atherosclerosis, and increases the risk of myocardial infarction and stroke [2]. More than one billion individuals worldwide are affected, and hypertension remains one of the top causes of premature death globally, including in Indonesia [3]. Clinically, hypertension is defined as a systolic blood pressure of ≥ 140 mmHg and/or diastolic blood pressure of ≥ 90 mmHg [4].

The renin-angiotensin-aldosterone system (RAAS) plays a central role in regulating vascular tone and blood pressure. In the classical RAAS pathway, angiotensin-converting enzyme (ACE) catalyzes the conversion of angiotensin I into angiotensin II, a potent vasoactive peptide that induces vasoconstriction via

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angiotensin type 1 receptors and stimulates aldosterone secretion, thereby increasing peripheral vascular resistance, intravascular volume, and arterial pressure [5]. Angiotensin II further drives oxidative stress, endothelial dysfunction, inflammation, vascular hypertrophy, and fibrosis, thereby contributing to long-term cardiovascular and renal damage [6]. Excessive ACE activity is strongly linked to the pathogenesis of hypertension and its related complications, making ACE inhibition a well-established therapeutic strategy [7].

Clinically used synthetic ACE inhibitors, such as captopril, enalapril, and lisinopril, are effective but may cause adverse effects, including dry cough, hyperkalemia, renal impairment, and angioedema [9]. These limitations highlight the need for safer alternatives to these agents. Natural products remain an important source of pharmacologically active scaffolds, with more than half of the current drugs derived from or inspired by natural compounds [10]. Various plant-derived phenolics, flavonoids, alkaloids, terpenoids, and lignans have demonstrated ACE-inhibitory activity in biochemical and computational studies [11].

Previous ethnobotanical studies in Indonesia have reported that *Piper betle* is frequently used by local communities in Sumatra and Java as a traditional remedy for hypertension [13]–[15]. This traditional use is supported by its rich phytochemical composition. *Piper betle*, an ethnomedicinal plant widely utilized in Southeast Asia, contains diverse bioactive metabolites, including eugenol, hydroxychavicol, chavibetol, phenylpropanoids, allylpyrocatechol derivatives, terpenoids, and amide compounds [16]. Many of these constituents exhibit antioxidant, anti-inflammatory, vasorelaxant, and cardioprotective activities [17], suggesting their potential relevance in modulating the renin–angiotensin–aldosterone system (RAAS). Furthermore, an *in vitro* study by Somanadhan et al. (1999) demonstrated that aqueous and ethanolic extracts of *Piper betle* exhibit angiotensin-converting enzyme (ACE) inhibitory activity, indicating that ACE inhibition may represent an additional mechanism underlying its hypotensive effects [18]. Despite this extensive phytochemical diversity and preliminary experimental evidence, the capacity of individual *P. betle* metabolites to inhibit ACE has not been comprehensively evaluated using structure-based approaches.

Given the pivotal role of ACE in the pathogenesis of hypertension, the identification of novel ACE inhibitors remains a key strategy for antihypertensive drug discovery. *Piper betle* L. leaves, which are rich in diverse bioactive constituents, represent a promising source of natural compounds. In this study, the chemical structures of the evaluated compounds were obtained from previously reported literature and from publicly available databases. An integrated *in silico* workflow was applied, encompassing molecular docking to assess the binding interactions with ACE, followed by the evaluation of physicochemical properties, drug-likeness, and toxicity profiles. This comprehensive computational approach aims to identify promising natural ACE inhibitor candidates with favorable efficacy, safety, and developability profiles for further investigation.

▪ MATERIALS AND METHODS

Materials

Molecular docking and *in silico* analyses were performed using the YASARA Structure integrated with the Protein-Ligand ANT System (PLANTS). Ligand structures were prepared using MarvinSketch and PerkinElmer ChemOffice Professional 17.1 (ChemDraw and Chem3D), and protein-ligand interactions were visualized using BIOVA Discovery Studio 2024 Client. The three-dimensional structure of angiotensin-converting enzyme (ACE) was obtained from the Protein Data Bank (RCSB PDB; www.rcsb.org) using PDB ID 1UZF. Ligand structures were derived from literature-reported natural compounds and relevant chemical databases. The physicochemical properties, drug-likeness, and ADME parameters were predicted using ADMETlab 3.0, and toxicity profiles were evaluated using ADMETlab 3.0 and ProTox-3.0. All computations were conducted on an HP Laptop 14s-dq2xxx equipped with an 11th Gen Intel® Core™ i7-1165G7 processor, 16 GB RAM, Intel Iris Xe Graphics, and Windows 11 Home 64-bit operating system.

Preparation of target protein and ligands

The three-dimensional structure of angiotensin-converting enzyme (ACE) was retrieved from the Protein Data Bank (RCSB PDB) under the accession code 1UZF, which contains the native inhibitor captopril (1-(3-mercapto-2-methylpropionyl)-pyrrolidine-2-carboxylic acid). Protein preparation was performed using the YASARA Structure, in which the native ligand and non-essential residues were separated from the target protein. Prior to the docking process, the protein structure was optimized by removing crystallographic water

molecules, polar hydrogen atoms were added, and the prepared structure was saved in the MOL2 (SYBYL Mol2) format for subsequent docking analysis.

The native ligand (captopril) was isolated from the protein complex and saved in the mol2 - SYBYL Mol2 format. Ligand preparation included structural optimization using the *Clean 2D* function, followed by protonation state adjustment at a physiological pH (7.4). Subsequently, 20 ligand conformations were generated and stored in the Tripos Mol2 (mol2) format.

Sample ligands obtained from relevant literature sources were initially constructed as two-dimensional structures using ChemDraw Professional 17.1 and converted into three-dimensional structures using Chem3D 17.1. Energy minimization was performed using the MMFF94 force field through the calculations module to obtain stable conformations. The optimized ligand structures were saved in SYBYL Mol2 format, protonated at pH 7.4, and subjected to conformational analysis by generating 20 conformers for each ligand, which were subsequently saved in Tripos Mol2 (mol2) format.

Method validation and molecular docking

Molecular docking simulations were performed using the Protein-Ligand ANT System (PLANTS), which employs an ant colony optimization (ACO) algorithm to explore the ligand conformational space and predict optimal binding modes. Docking calculations were executed via the command-line interface (CMD). The binding site of angiotensin-converting enzyme (ACE) was defined based on the coordinates of the native ligand and was determined at $x = 41.3709$, $y = 34.5804$, and $z = 43.8915$, with a binding radius of 9.92956 \AA . These parameters were stored in a command file (.cmd).cmd.txt file and was used consistently for all docking simulations.

Method validation was conducted by redocking the native ligand (captopril) into the prepared ACE binding site. The protein target and native ligand, represented by 20 generated conformations, were docked using PLANTS. The best-ranked docked pose was compared with the original crystallographic conformation of captopril prior to docking. The accuracy of the docking protocol was evaluated by calculating the root mean square deviation (RMSD) between the docked and crystallographic ligand poses.

A docking protocol was considered valid when the RMSD value was $\leq 2.0 \text{ \AA}$ [19], indicating a reliable reproduction of the experimental binding mode. Upon successful validation, the defined binding site and docking parameters were applied to the molecular docking of 43 isolated compounds from green betel (*Piper betle* L.) leaves to evaluate their binding affinities and interaction profiles with ACE.

Visualization and analysis of ligand-protein interactions

The molecular docking results were visualized using BIOVA Discovery Studio 2024. Prior to visualization, the top-ranked ligand conformations were merged with the target protein structure in YASARA and subsequently represented in 2D using BIOVA Discovery Studio 2024. The docking results were interpreted by ranking the ligands based on the predicted binding affinity and examining the key non-covalent interactions within the protein active site. The docking score, which approximates the binding free energy, was used to select the most favorable ligand poses, with more negative scores indicating a stronger predicted binding affinity. Interaction profiling focused on hydrogen bonding, hydrophobic contacts, and electrostatic interactions to elucidate molecular recognition patterns that complement the receptor-binding pocket. This combined scoring and interaction analysis supports an objective comparison of ligand-binding modes in structure-based design workflows [20].

In silico drug-likeness, physicochemical, and toxicity prediction

The SMILES representations of the studied compounds were generated using MarvinSketch (ChemAxon, <https://chemaxon.com/products/marvin>) from the pre-existing chemical structures. These SMILES strings were subsequently submitted for computational evaluation using ADMETlab 3.0 (<https://admetlab3.scbdd.com/>) and ProTox-3.0 (https://tox-new.charite.de/protox_III/) to comprehensively predict drug-likeness, physicochemical properties, and toxicity profiles.

Drug-likeness, physicochemical, and toxicity properties were evaluated using integrated in silico approaches. Drug-likeness was assessed using QED, SA score, Fsp³, MCE-18, and structural alert filters (PAINS, Alarm NMR, BMS, and chelating rules), together with established criteria including Lipinski's Rule

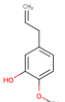
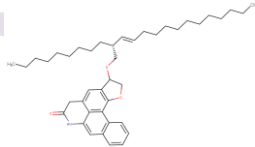
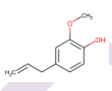
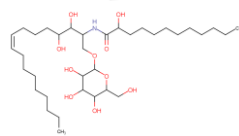
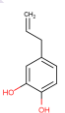
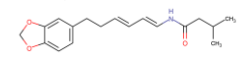
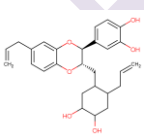
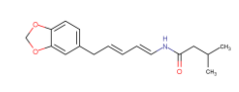
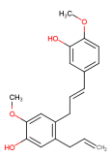
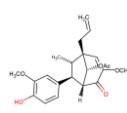
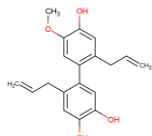
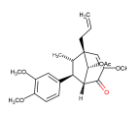
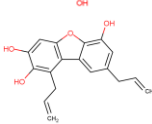
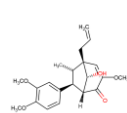
of Five, the Pfizer and GSK rules, and the Golden Triangle to define optimal drug-like chemical space. The pharmacokinetic (ADME) profiles were predicted based on key physicochemical descriptors, including molecular weight (MW), hydrogen bond acceptors and donors (nHA and nHD), topological polar surface area (TPSA), solubility (logS), lipophilicity (logP and logD), number of rotatable bonds (nRot), rings (nRing), and heteroatoms (nHet). Toxicity was evaluated by predicting hepatotoxicity, mutagenicity (AMES), carcinogenicity, hERG channel inhibition, median lethal dose (LD₅₀), toxicity class, and potential immunotoxic, mutagenic, carcinogenic, and cytotoxic effects.

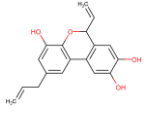
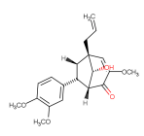
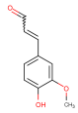
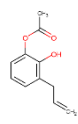
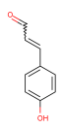

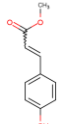
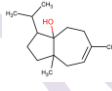

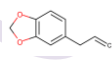
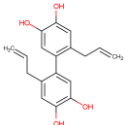

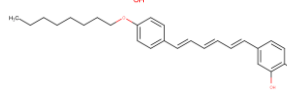
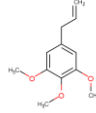
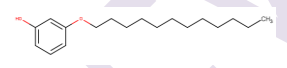
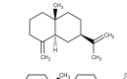
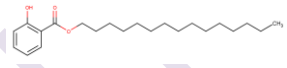
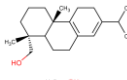
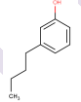

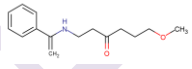

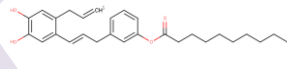

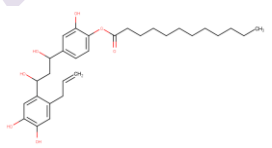
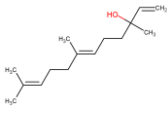
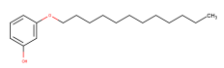
RESULTS

Chemical structures of compounds from *Piper betle* L. leaves

The compounds identified in *Piper betle* L. (green betel leaf) comprise a chemically diverse set of secondary metabolites, including phenylpropanoids and phenolic derivatives, lignans and neolignans, amides, terpenoids, and long-chain alkyl phenols and esters. As shown in **Table 1**, the two-dimensional (2D) chemical structures of these compounds were collected from various literature and database sources, standardized, and subsequently employed as representative ligand samples for molecular docking against angiotensin-converting enzyme (ACE). This structural diversity provides a robust chemical basis for subsequent structure-based and in silico pharmacological analyses of these compounds.

Table 1. Chemical structures of the *Piper betle* L. leaf constituents used for molecular docking.

Compounds	Structure	Ref.	Compounds	Structure	Ref.
Chavibetol (C ₁₀ H ₁₂ O ₂)		[21]	Desmethylenesqualenyl deoxycepharadione A (C ₄₂ H ₅₉ NO ₃)		[22]
Eugenol (C ₁₀ H ₁₂ O ₂)		[21] / [23]	Pipercerebroside A (C ₄₁ H ₇₉ NO ₄)		[24]
Hydroxychavicol (C ₉ H ₁₂ O ₂)		[21] / [23] / [25] / [26]	Piperenamide A (C ₁₈ H ₂₃ NO ₃)		[27]
Piperneolignan A (C ₂₇ H ₃₂ O ₆)		[23]	Piperenamide B (C ₁₇ H ₂₁ NO ₃)		[27]
Piperneolignan B (C ₂₀ H ₂₂ O ₄)		[23]	Piperbetol (C ₂₂ H ₂₆ O ₆)		[28]
Piperneolignan C (C ₁₉ H ₂₀ O ₄)		[23]	Methylpiperbetol (C ₂₃ H ₂₈ O ₆)		[28]
Piperneolignan D (C ₁₈ H ₁₆ O ₄)		[23]	Piperol A (C ₂₁ H ₂₆ O ₅)		[28]

Compounds	Structure	Ref.	Compounds	Structure	Ref.
Piperneolignan E (C ₁₈ H ₁₆ O ₄)		[23]	Piperol B (C ₂₀ H ₂₄ O ₅)		[28]
Coniferaldehyde (C ₁₀ H ₁₀ O ₃)		[23]	Allylpyrocatechol monoacetate (C ₁₁ H ₁₂ O ₃)		[29]
<i>p</i> -Hydroxycinnamaldehyde (C ₉ H ₈ O ₂)		[23]	Camphene (C ₁₀ H ₁₆)		[29]
Methyl <i>p</i> -coumarate (C ₁₀ H ₁₀ O ₃)		[23]	Carotol (C ₁₅ H ₂₆ O)		[29]
Vanillin (C ₈ H ₈ O ₃)		[23]	Safrole (C ₁₀ H ₁₀ O ₂)		[29]
Diallylcatechol (C ₁₈ H ₁₈ O ₄)		[23]	1-Phenylpropene-3,3-diol diacetate (C ₁₃ H ₁₄ O ₄)		[29]
4-((1 <i>E</i> ,3 <i>E</i> ,5 <i>E</i>)-6-(4-(Octyloxy)phenyl)hexa-1,3,5-trien-1-yl)benzene-1,2-diol (C ₂₆ H ₃₂ O ₃)		[30]	Elemicin (C ₁₂ H ₁₆ O ₃)		[29]
3-(<i>n</i> -Dodecyloxy)phenol (C ₁₈ H ₃₀ O ₂)		[30]	β-Selinene (C ₁₅ H ₂₄)		[29]
1- <i>n</i> -Decanoyl phenol (C ₂₂ H ₃₆ O ₃)		[31]	Palustrol (C ₂₀ H ₃₂ O)		[29]
3-Butylphenol (C ₁₀ H ₁₄ O)		[31]	<i>p</i> -Menthane-1,3-diol (C ₁₀ H ₂₀ O ₂)		[32]
3-Benzamide-2'-methoxy-1-ethylpropanoate (C ₁₅ H ₂₁ NO ₂)		[33]	Allo-aromadendrene (C ₁₅ H ₂₄)		[34]
Bis-chavicol dodecanoyl ester (C ₃₀ H ₄₀ O ₄)		[35]	Piperitone (C ₁₀ H ₁₆ O)		[32]
Bis-hydroxychavicol dodecanoyl ester (C ₃₀ H ₄₂ O ₇)		[35]	Nerolidol (C ₁₅ H ₂₆ O)		[32] [34]
1- <i>n</i> -Dodecanyloxy resorcinol (C ₁₈ H ₃₀ O ₂)		[22]			

Docking method validation

The docking protocol was validated using the crystal structure of the angiotensin-converting enzyme (ACE) complexed with its native ligand, captopril (PDB ID: 1UZF). Redocking of captopril into the ACE active site yielded a root-mean-square deviation (RMSD) of 1.526 Å, which is within the acceptable threshold (≤ 2.0 Å).

Å), indicating the reliability of the docking method. The native ligand exhibited a docking score of -73.74 and formed key interactions with catalytically relevant residues, including Zn^{2+} , His353, His513, His383, Ala354, Tyr523, Lys511, Gln281, and Phe457. **Figure 1** illustrates the superimposition of the original crystallographic pose and the redocked conformation, demonstrating a high degree of spatial overlap, where the red structure represents the crystallographic ligand and the purple structure corresponds to the docking results. This close alignment confirmed the accuracy of the docking protocol in reproducing the experimentally observed binding mode.

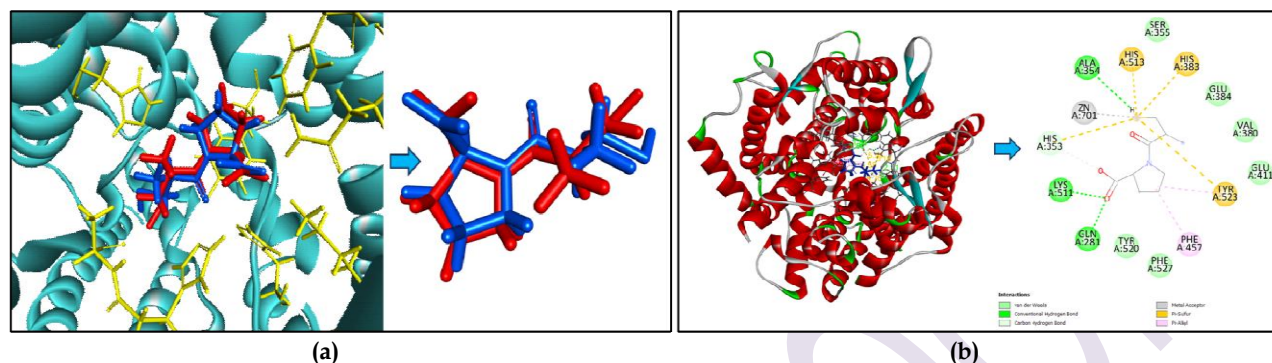


Figure 1. Redocking validation of ACE protein with its native ligand. (a) Visualization of the binding position comparison between the native ligand before docking and the redocked pose (red). (b) Interactions of redocked captopril with amino acid residues in the ACE crystal structure.

Molecular docking

As shown in **Table 2**, the docking results revealed that *Piper betle* leaf compounds belonging to different chemical classes exhibited distinct binding affinities toward ACE activity. Glycosides and complex lipophilic derivatives, represented by Piperocerebrosides A and long-chain esterified phenolics, exhibited the strongest binding, with docking scores ranging from -108.74 to -125.96 , indicating extensive interactions within the ACE active site. Lignan derivatives, including piperneolignans (A-E), displayed consistently high affinities with docking scores between -71.80 and -86.53 , reflecting stable binding driven by aromatic stacking and hydrophobic contacts. In contrast, simple phenylpropanoids and phenolic monomers, such as chavibetol, eugenol, hydroxychavicol, and vanillin, exhibited moderate binding energies in the range of -59.67 to -70.37 kcal/mol. In contrast, terpenoids and small volatile compounds exhibited comparatively weaker interactions, with docking scores ranging from -41.79 to -75.91 . Overall, these results indicate that increased molecular complexity and lipophilicity enhance the ACE-binding affinity of *Piper betle* phytochemicals.

Table 2. Binding energy scores and interactions between green betel leaf compounds and ACE amino acid residues.

No.	Compounds	Docking Score	Interaction of ACE binding site								Other interaction residues	
			Zn ²⁺	His 353	His 513	His 383	Tyr 520	Tyr 523	Lys 511	Gln 281		Phe 457
1	Captopril (Native)	-	√	√	√	√	√	√	√	√	√	-
2	Captopril (Redocking)	-73.74	√	√	√	√	-	√	√	√	√	Ala354
3	Pipercerebroside A	-125.96	√	√	√	√	√	√	-	√	√	Leu161, Trp279, Val380, Glu384, Glu411, Ala354, Arg522, Phe527, Val379, Glu411, Phe527, Val380, Asp453, Val379, Leu1161, Trp279, Thr282, Glu384
4	Bis-chavicol dodecanoyl ester	-111.90	√	√	-	√	-	√	-	√	-	Ala354, Thr282, Asn277, Val380, Glu384, Phe527, Val379, Glu411
5	Bis-hydroxychavicol dodecanoyl ester	-108.74	√	√	-	√	√	√	√	√	√	Val518, Thr282, Gln369, Glu162, Leu161, Val380, Val379, His383
6	Desmethylenesqualenyl deoxycepharadione A	-105.86	-	√	√	-	√	√	-	-	√	

No.	Compounds	Docking Score	Interaction of ACE binding site								Other interaction residues	
			Zn ²⁺	His 353	His 513	His 383	Tyr 520	Tyr 523	Lys 511	Gln 281		Phe 457
7	1- <i>n</i> -Decanoyl phenol	-98.32	√	√	-	√	√	√	-	-	√	Val380, Val379, Phe527, His387, Val518
8	Piperneolignan A	-86.53	√	√	-	√	-	√	-	-	-	Asp453, Tyr146, Leu161, Trp279, Glu384, Val380, Val379
9	Piperenamamide A	-85.39	√	√	√	√	√	√	-	-	√	Phe512, Ala354, Glu384, Val380,
10	3-(<i>n</i> -Dodecyloxy)phenol	-83.64	√	√	-	√	√	√	-	-	√	Val380, Val379, Phe527, His387, Val518
11	1- <i>n</i> -Dodecanyloxy resorcinol	-83.43	-	-	-	√	-	√	√	√	-	Val380, Val379, Phe527
12	3-Benzamide-2'-methoxy-1-ethylpropanoate	-82.97	-	√	-	-	-	√	√	√	√	Asp415, Val380, Ala354, Phe527
13	Diallylcatechol	-82.95	-	√	√	√	√	√	√	-	-	Phe527, Val380, Asp415
14	Piperneolignan E	-82.86	-	√	-	√	-	√	-	-	√	Glu384, Phe527, Asp415, Lys454, Val380
15	Piperenamamide B	-82.71	√	√	√	√	-	√	-	-	-	Phe512, Phe527, Ala354
16	1-Phenylpropene-3,3-diol diacetate	-82.23	√	-	-	√	-	-	√	√	-	Val380, Asp415
17	4-((1E,3E,5E)-6-(4-(Octyloxy)phenyl)hexa-1,3,5-trien-1-yl)benzene-1,2-diol	-79.23	-	√	√	√	-	√	-	-	-	Ala356, Asp415, Val379, Val380, Thr282, Asp453
18	Piperneolignan D	-79.13	-	√	-	√	√	√	√	√	-	Val380, Val379, Lys454, Asp453, 2Phe527, Ala354
19	Piperneolignan C	-77.92	-	√	√	√	-	√	√	-	-	Val380, Asp415, Phe527, Ala354
20	Piperbetol	-76.29	-	√	√	√	-	-	√	√	-	Ala354, Val380, Lys454, Val379,
21	Nerolidol	-75.91	-	-	√	√	√	√	√	√	√	Phe527, Val379, Val380, Ala354
22	Palustrol	-74.26	-	√	√	√	-	√	√	√	√	Val380, Val379,
23	Piperneolignan B	-71.80	-	√	√	√	-	√	-	-	-	Phe457, Asp415, Val379, Thr282, Val380, Cys370, Asp377, Ala354
24	Hydroxychavicol	-70.37	√	√	-	-	-	√	-	-	√	Phe527
25	3-Butylphenol	-69.06	-	-	-	√	-	√	√	√	-	-
26	Methylpiperbetol	-67.92	-	√	-	√	√	-	√	√	-	Phe527, Val380, Ala354, Gln369, Cys370, Glu162, Glu376, Thr282
27	Allo-aromadendrene	-67.44	-	√	-	√	-	√	-	-	√	Val380, Phe527
28	β-Selinene	-66.86	-	-	-	√	-	√	-	-	√	Val380, Val379, Phe527
29	Allylpyrocatechol monoacetate	-65.96	-	-	√	√	-	-	√	√	-	Phe527, Val379, Val380
30	Piperol A	-65.08	-	√	√	√	-	-	√	√	-	Val380, Ala354, Cys370, Glu162
31	Coniferyl alcohol	-65.01	√	√	-	√	√	√	-	-	-	Asp415,
32	Carotol	-64.93	-	√	√	√	-	√	-	-	-	Val379,
33	<i>p</i> -Hydroxycinnamaldehyde	-64.39	-	-	-	-	-	-	√	√	-	Ser526, Asp415
34	Safrole	-63.58	√	√	-	√	-	√	-	-	√	Phe527
35	Methyl <i>p</i> -coumarate	-63.46	√	√	-	√	-	√	-	-	-	Ala354, Glu411, Val380, Asp453, Val379, Phe527
36	Piperitone	-63.31	-	√	√	√	-	√	√	√	√	Phe527
37	Coniferaldehyde	-62.87	√	√	-	√	√	√	-	-	√	-
38	Chavibetol	-62.84	-	√	√	√	√	√	√	√	√	Ala354, Phe527,
39	Eugenol	-61.97	-	√	√	√	-	√	-	√	√	Phe527
40	<i>p</i> -Menthane-1,3-diol	-61.81	-	√	√	√	√	√	-	-	-	Val380, Ala354, Glu384,

No.	Compounds	Docking Score	Interaction of ACE binding site					Other interaction residues				
			Zn ²⁺	His 353	His 513	His 383	Tyr 520	Tyr 523	Lys 511	Gln 281	Phe 457	
41	Piperol B	-61.79	-	√	-	√	-	√	-	-	√	Ala354, Asp415, Phe527, Val380
42	Acetyeugenol	-60.78	√	√	√	√	-	√	-	-	√	Phe527
43	Vanillin	-59.67	-	-	-	√	-	-	√	√	√	
44	Camphene	-53.18	-	-	√	√	√	√	-	-	√	Phe527
45	Elemicin	-41.79	-	√	√	√	-	√	-	√	√	Val380, Val379, Phe527

Physicochemical properties

Table 3 presents the physicochemical and molecular descriptors of the *Piper betle* leaf compounds predicted through in silico ADMET analysis. The table provides a comprehensive overview of key molecular parameters, including molecular weight (MW), hydrogen bond acceptors (nHA) and donors (nHD), topological polar surface area (TPSA), solubility (logS), lipophilicity (logP), distribution coefficient (logD), rotatable bond count (nRot), ring count (nRing), and heteroatom content (nHet). These data collectively describe the physicochemical profiles of the investigated compounds and serve as a basis for evaluating their potential drug-like properties.

Table 3. Predicted physicochemical properties of *Piper betle* leaf compounds based on ADMETlab 3.0.

No.	Compounds	MW (Da)	nHA	nHD	TPSA (Å ²)	logS	logP	logD	nRot	nRing	nHet
1	1-n-Decanoyl phenol	348.27	3	1	46.5	-6.993	8.022	4.130	16	1	3
2	1-n-Dodecanoyloxy resorcinol	625.45	4	1	47.5	-8.643	11.526	6.126	12	1	2
3	1-Phenylpropene-3,3-diol diacetate	208.11	3	0	27.6	-2.576	2.409	2.412	6	1	4
4	3-(n-Dodecyloxy)phenol	278.22	2	1	29.4	-5.665	6.948	4.289	12	1	2
5	3-Benzamide-2'-methoxy-1-ethylpropanoate	247.16	3	1	38.3	-2.084	2.001	1.886	9	1	3
6	3-Butylphenol	150.10	1	1	20.2	-2.280	3.393	3.094	3	1	1
7	4-((1E,3E,5E)-6-(4-(Octyloxy)phenyl)hexa-1,3,5-trien-1-yl)benzene-1,2-diol	392.24	3	2	49.6	-4.653	5.470	3.416	12	2	3
8	Acetyeugenol	136.13	0	0	0.0	-3.884	3.751	3.160	5	1	3
9	Allo-aromadendrene	164.08	2	1	29.4	-1.945	2.421	2.188	0	3	0
10	Allylpyrocatechol monoacetate	206.09	3	0	35.5	-2.644	2.486	2.161	4	1	3
11	Bis-chavicol dodecanoyl ester	464.29	4	2	66.7	-5.591	7.866	4.585	17	2	4
12	Bis-hydroxychavicol dodecanoyl ester	514.29	7	5	127.4	-3.562	5.140	3.263	18	2	7
13	Camphene	222.20	1	1	20.2	-3.130	3.689	3.147	0	2	0
14	Carotol	162.07	2	0	18.4	-2.780	2.794	2.484	1	2	1
15	Chavibetol	164.08	2	1	29.4	-1.945	2.421	2.188	1	6	2
16	Coniferaldehyde	178.06	3	1	46.5	-2.251	1.885	1.843	3	1	3
17	Coniferyl alcohol	180.08	3	2	49.6	-1.737	1.344	1.539	3	1	3
18	Desmethylenesqualenyl deoxycepharadione A	661.48	11	8	189.1	-4.942	4.331	4.331	22	5	4
19	Diallylcatechol	298.12	4	4	80.9	-2.579	3.243	3.156	5	2	4
20	Elemicin	204.19	0	0	0.0	-4.044	4.183	3.349	5	1	3
21	Eugenol	164.08	2	1	29.4	-1.891	2.321	2.146	3	1	2
22	Hydroxychavicol	150.07	2	2	40.4	-0.846	2.183	1.865	2	1	2
23	Methyl p-coumarate	178.06	3	1	46.5	-2.623	2.351	2.359	3	1	3
24	Methylpiperbetol	358.18	5	1	64.9	-3.777	2.619	2.743	8	3	6
25	Nerolidol	150.07	2	2	40.4	-0.846	2.183	1.865	7	0	1
26	Palustrol	172.15	2	2	40.4	-1.903	2.319	2.181	2	3	1
27	p-Hydroxycinnamaldehyde	148.05	2	1	37.3	-2.117	1.871	1.857	2	1	2
28	Piperbetol	400.19	6	0	71.0	-3.899	2.715	2.928	7	3	6
29	Pipercerebroside A	301.17	4	1	47.5	-4.626	3.938	3.601	29	1	11
30	Piperenamamide A	287.15	4	1	47.5	-4.449	3.577	3.438	8	2	4
31	Piperenamamide B	386.17	6	1	82.0	-4.058	2.469	2.589	7	2	4
32	Piperitone	164.08	2	1	29.4	-1.891	2.321	2.146	1	1	1

No.	Compounds	MW (Da)	nHA	nHD	TPSA (Å ²)	logS	logP	logD	nRot	nRing	nHet
33	Piperneolignan A	452.22	6	4	99.3	-4.435	3.547	3.361	7	4	6
34	Piperneolignan B	326.15	4	2	58.9	-4.482	4.273	3.344	7	2	4
35	Piperneolignan C	312.14	4	3	69.9	-3.617	3.689	3.386	6	2	4
36	Piperneolignan D	296.10	4	3	73.8	-4.338	3.680	3.134	4	3	4
37	Piperneolignan E	296.10	4	3	69.9	-3.591	3.510	3.124	3	3	4
38	Piperol A	344.16	5	1	64.9	-3.823	2.352	2.482	6	3	5
39	Piperol B	192.08	3	1	46.5	-2.582	2.528	2.221	6	3	5
40	p-Menthane-1,3-diol	204.19	0	0	0.0	-5.675	5.282	4.072	1	1	2
41	Safrole	234.09	4	0	52.6	-2.517	2.269	2.201	2	2	2
42	Vanillin	152.05	3	1	46.5	-1.773	1.124	1.265	2	1	3
43	β-Selinene	288.25	1	1	20.2	-4.248	4.046	3.514	1	2	0

Drug-Likeness profile

As shown in **Table 4**, the drug-likeness prediction of 43 *Piper betle* leaf compounds using ADMETlab3 revealed marked differences in their medicinal chemistry profiles. Several compounds exhibited favorable characteristics, including high quantitative estimates of drug likeness (QED) values, acceptable synthetic accessibility scores, absence of PAINS alerts, and compliance with key drug-likeness rules. Among the evaluated compounds, Piperol B, Piperol A, and Piperenamamide B exhibited the most optimal overall profiles, with QED values ≥ 0.79 , low structural alert counts, and full compliance with the Lipinski Rule of Five and Golden Triangle criteria. In contrast, several compounds exhibited less favorable profiles, including Piperocerebroside A, Desmethylenesqualenyl deoxycepharadione A, Bis-hydroxychavicol dodecanoyl ester, Bis-chavicol dodecanoyl ester, and 4-((1E,3E,5E)-6-(4-(Octyloxy)phenyl)hexa-1,3,5-trien-1-yl)benzene-1,2-diol, which showed low QED values, increased structural alerts, and multiple violations of drug-likeness filters, suggesting their limited suitability for further drug development.

Table 4. Drug-likeness properties and medicinal chemistry friendliness of the evaluated compounds were predicted using ADMETlab3.

No.	Compounds	QED	SA Score	Fsp3	MCE-18	PAINS	Alarm NMR rule	BMS rule	CR	LRF	Pfizer Rule	GSK Rule	GT
1	Piperol B	0.804	4.0	0.450	66.897	0	2	0	0	✓	✓	✓	✓
2	Piperol A	0.792	4.0	0.476	69.774	0	2	0	0	✓	✓	✓	✓
3	Piperenamamide B	0.817	2.0	0.353	22.957	0	0	0	0	✓	×	✓	✓
4	Piperenamamide A	0.783	2.0	0.389	22.880	0	0	0	0	✓	×	✓	✓
5	Piperbetol	0.596	5.0	0.455	72.875	0	3	0	1	✓	✓	✓	✓
6	Carotol	0.667	4.0	0.867	38.571	0	0	0	0	✓	×	✓	✓
7	Elemicin	0.695	1.0	0.333	7.000	0	1	0	0	✓	✓	✓	✓
8	Acetyeugenol	0.431	1.0	0.250	7.000	0	1	0	0	✓	✓	✓	✓
9	Diallylcatechol	0.502	2.0	0.111	14.000	1	2	0	1	✓	✓	✓	✓
10	3-Benzamide-2'-methoxy-1-ethylpropanoate	0.682	2.0	0.400	6.000	0	0	0	0	✓	✓	✓	✓
11	Methylpiperbetol	0.514	4.0	0.478	72.471	0	2	0	0	✓	✓	×	✓
12	Eugenol	0.693	1.0	0.200	6.000	0	2	0	1	✓	✓	✓	×
13	Chavibetol	0.693	2.0	0.200	6.000	0	2	0	1	✓	✓	✓	×
14	Coniferyl alcohol	0.739	2.0	0.200	6.000	0	2	0	1	✓	✓	✓	×
15	Vanillin	0.648	1.0	0.125	6.000	0	4	1	1	✓	✓	✓	×
16	Safrole	0.620	2.0	0.200	19.500	0	0	0	0	✓	✓	✓	×
17	p-Menthane-1,3-diol	0.630	3.0	1.000	23.400	0	0	0	0	✓	✓	✓	×
18	Piperitone	0.564	3.0	0.700	16.471	0	1	0	0	✓	✓	✓	×
19	Nerolidol	0.633	3.0	0.600	5.000	0	0	0	0	✓	×	×	✓
20	Methyl p-coumarate	0.552	1.0	0.100	6.000	0	2	0	0	✓	✓	✓	×
21	p-Hydroxycinnamaldehyde	0.511	2.0	0.000	5.000	0	3	1	0	✓	✓	✓	×
22	Coniferaldehyde	0.565	2.0	0.100	6.000	0	4	1	1	✓	✓	✓	×
23	Hydroxychavicol	0.498	2.0	0.111	6.000	1	2	0	1	✓	✓	✓	×

No.	Compounds	QED	SA Score	Fsp ³	MCE-18	PAINS	Alarm NMR rule	BMS rule	CR	LRF	Pfizer Rule	GSK Rule	GT
24	Allylpyrocatechol monoacetate	0.453	2.0	0.182	7.000	0	2	0	0	✓	✓	✓	×
25	3-Butylphenol	0.702	1.0	0.400	5.000	0	1	0	0	✓	×	✓	×
26	Piperneolignan B	0.752	2.0	0.200	13.000	0	2	0	1	✓	×	×	✓
27	Piperneolignan C	0.558	2.0	0.158	14.000	1	2	0	2	✓	×	✓	✓
28	Piperneolignan D	0.499	3.0	0.111	18.000	1	1	0	1	✓	×	✓	✓
29	Piperneolignan E	0.595	3.0	0.111	55.800	1	1	0	2	✓	×	✓	✓
30	Piperneolignan A	0.367	4.0	0.407	82.105	1	2	0	1	✓	✓	×	✓
31	Camphene	0.449	4.0	0.800	32.000	0	0	0	0	✓	×	✓	×
32	β-Selinene	0.542	3.0	0.733	35.000	0	0	0	0	✓	×	×	✓
33	Palustrol	0.735	4.0	0.800	51.111	0	0	0	0	✓	×	×	✓
34	Allo-aromadendrene	0.517	4.0	0.867	48.857	0	0	0	0	✓	×	×	✓
35	3-(n-Dodecyloxy)phenol	0.497	1.0	0.667	5.000	0	1	1	0	✓	×	×	✓
36	1-n-Dodecanyloxy resorcinol	0.497	1.0	0.667	5.000	0	1	1	0	✓	×	×	✓
37	1-n-Decanoyl phenol	0.285	1.0	0.682	6.000	0	1	1	0	✓	×	×	✓
38	1-Phenylpropene-3,3-diol diacetate	0.591	2.0	0.231	7.000	0	0	1	0	✓	✓	✓	✓
39	Bis-chavicol dodecanoyl ester	0.087	2.0	0.433	13.000	1	2	1	1	✓	×	×	✓
40	4-((1E,3E,5E)-6-(4-(Octyloxy)phenyl)hexa-1,3,5-trien-1-yl)benzene-1,2-diol	0.230	2.0	0.308	11.000	1	2	1	1	✓	×	×	✓
41	Piperocerebroside A	0.046	4.0	0.914	27.940	0	0	2	0	×	✓	×	×
42	Desmethylenesqualenyl deoxycepharadione A	0.069	4.0	0.595	80.328	0	2	1	0	×	×	×	×
43	Bis-hydroxychavicol dodecanoyl ester	0.053	3.0	0.500	32.000	1	2	1	1	×	✓	×	×

QED: Quantitative Estimate of Drug-likeness; SA Score: Synthetic Accessibility Score; Fsp³: Fraction of sp³-hybridized carbons; MCE-18: Medicinal Chemistry Evolution score; PAINS: Pan-Assay Interference Compounds alerts; NMR Alert Rule: Nuclear Magnetic Resonance alert rule; BMS Rule: Bristol-Myers Squibb rule; CR: Chelator Rule; LRF: Lipinski's Rule of Five; GT: Golden Triangle; ✓: indicates compliance; ×: indicates non-compliance.

Toxicity predictions

As shown in Table 5, ten compounds among the 43 identified *Piper betle* leaf constituents were selected as having the most favorable predicted safety profiles based on ADMETlab 3.0 and ProTox-3.0 analyses. These compounds generally exhibited low predicted risks of hERG inhibition, drug-induced liver injury (DILI), AMES mutagenicity, respiratory toxicity, and human hepatotoxicity, along with relatively high LD₅₀ values ranging from 2000 to 5000 mg/kg, indicating low acute toxicity. In addition, all compounds showed very low probabilities of eye corrosion and eye irritation, whereas the skin sensitization values remained within acceptable limits. The compounds were further arranged according to a weighted prioritization of the most critical toxicity endpoints, with primary emphasis on hERG inhibition, DILI, AMES mutagenicity, and human hepatotoxicity, followed by respiratory toxicity and acute toxicity indicators (LD₅₀ and toxicity class). Local toxicity parameters, such as the FDA MDD, skin sensitization, eye corrosion, and eye irritation, were considered supportive factors. Based on this prioritization, 1-n-decanoyl phenol, piperenamide A, and 1-n-dodecanyloxy resorcinol demonstrated the most balanced safety profiles, characterized by consistently low toxicity predictions across multiple high-priority endpoints and the highest LD₅₀ values.

Tabel 5. Predicted toxicity profiles of *Piper betle* leaf compounds based on ADMETlab 3.0 and ProTox-3.0 analyses.

No.	Compounds	hERG blocker	DILI	AMES tox.	FDA MDD	Skin sensitization	Eye corrosion	Eye irritation	Respiratory tox.	Human hepato tox.	LD ₅₀ (mg/Kg)	Tox. Class
1	1-n-Decanoyl phenol	0.071	0.048	0.215	0.089	0.882	0.999	0.999	0.693	0.204	5000	6
2	Piperenamamide A	0.084	0.061	0.186	0.103	0.870	0.999	0.999	0.695	0.214	5000	4
3	1-n-Dodecanoyloxy resorcinol	0.089	0.072	0.198	0.112	0.874	0.999	0.999	0.702	0.219	5000	5
4	Pipercerebroside A	0.092	0.083	0.254	0.143	0.905	0.998	0.999	0.711	0.243	3000	5
5	Desmethylenesqualenyl deoxycepharadione A	0.101	0.131	0.316	0.168	0.889	0.998	0.999	0.724	0.264	2500	5
6	3-Butylphenol	0.109	0.126	0.342	0.174	0.901	0.998	0.999	0.728	0.281	2500	5
7	Piperenamamide B	0.116	0.102	0.318	0.187	0.912	0.998	0.999	0.739	0.279	2000	4
8	Hydroxychavicol	0.121	0.134	0.431	0.367	0.914	0.998	0.999	0.680	0.231	2000	4
9	Piperbetol	0.133	0.149	0.372	0.216	0.927	0.997	0.999	0.761	0.301	3000	4
10	Coniferyl alcohol	0.151	0.118	0.453	0.509	0.915	0.996	0.999	0.731	0.276	3000	5

DISCUSSION

Molecular docking analysis demonstrated that several *Piper betle* constituents exhibited strong binding affinities toward the ACE active site, with some compounds showing docking scores comparable to or higher than those of the reference inhibitor captopril. These results indicate a favorable interaction between *Piper betle* phytochemicals and key residues within the ACE catalytic pocket, supporting the potential of this plant as a natural source of ACE-inhibitory compounds. Given the central role of ACE as a zinc-dependent metalloprotease in regulating blood pressure via the renin-angiotensin-aldosterone system (RAAS), inhibition of this enzyme reduces the formation of the potent vasoconstrictor angiotensin II while enhancing bradykinin availability, ultimately leading to vasodilation and antihypertensive effects [36], [37]. The observed docking performance provides molecular-level evidence supporting the reported antihypertensive and cardiovascular protective properties of *Piper betle*.

The validation of the molecular docking protocol is a critical step in structure-based drug discovery to ensure the accurate reproduction of experimentally determined ligand-protein binding modes. In this study, the RMSD value of 1.526 Å, which is well below the accepted 2.0 Å threshold, confirms the reliability and robustness of the docking methodology, as reported in recent ACE-focused computational studies. The close spatial overlap between the crystallographic and redocked conformations further supports the accuracy of this protocol. Importantly, redocking preserved the key molecular interactions essential for ACE inhibition, including coordination with the catalytic Zn²⁺ ion and stabilizing interactions with critical active-site residues, such as His353, Ala354, Tyr523, and Lys511. Given the zinc-dependent metalloprotease nature of ACE, the accurate reproduction of metal coordination and associated interactions indicates that the docking protocol effectively captures the fundamental physicochemical determinants governing ACE-ligand recognition [38].

Among the 43 phytochemicals screened against ACE, several compounds demonstrated strong inhibitory potential, as indicated by favorable docking scores and key interactions with the active site residues. Highly negative binding energies, Zn²⁺ coordination, and engagement of the S1 and S2 subsites are critical determinants of ACE inhibition. Pipercerebroside A (-125.96) exhibited the strongest binding affinity, characterized by extensive interactions with catalytic residues and effective coordination with Zn²⁺ ions, consistent with the binding patterns of clinically established ACE inhibitors. Bis-chavicol dodecanoyl ester (-111.90) and bis-hydroxychavicol dodecanoyl ester (-108.74) also exhibited high binding affinities, retained Zn²⁺ coordination, and engaged multiple active-site residues, supporting the role of lipophilic natural derivatives in enhancing ACE inhibition. Notably, desmethylenesqualenyl deoxycepharadione A demonstrated strong binding despite the absence of direct Zn²⁺ coordination, indicating that alternative non-covalent interactions can substantially contribute to ACE binding. Collectively, these results underscore the potential of selected *Piper betle* constituents as promising leads for the development of natural ACE inhibitors.

Physicochemical and ADMET profiling of the *Piper betle* leaf compounds was performed to evaluate their suitability as oral ACE inhibitors. Low-molecular-weight phenolics, such as eugenol, hydroxychavicol, and chavibetol, exhibit favorable absorption-related properties, characterized by an MW of < 200 Da, TPSA of < 50

Å², and moderate lipophilicity (logP ~1–2.5). These features are consistent with good intestinal permeability; however, their relatively simple scaffolds may limit sustained interactions within the ACE catalytic pocket, as reflected by the weaker docking affinities reported in similar studies [39]. A subset of medium-sized compounds including piperneolignans (A–E), piperenamides (A–B), piperbetol derivatives, and Piperocerebroside A displayed a balanced physicochemical profile. Most of these compounds comply with Lipinski's rule of five and Veber's criteria (TPSA < 90 Å², moderate nRot) while maintaining sufficient lipophilicity (logP 2–4) to support membrane permeability. Notably, Piperocerebroside A has a moderate MW (301.17 Da), acceptable TPSA (47.56 Å²), and strong docking performance, highlighting it as the most promising lead when physicochemical and binding data are jointly considered. In contrast, highly lipophilic long-chain derivatives (e.g., bis-chavicol esters and long alkyl phenols) exhibit excellent binding affinity but poor predicted solubility (logS < -5) and excessive lipophilicity, suggesting potential limitations for oral delivery without structural optimization.

Several *Piper betle*-derived compounds exhibited favorable drug-like profiles, characterized by moderate-to-high QED values (≥0.6), good synthetic accessibility (SA ≤3), absence of PAINS alerts, and compliance with Lipinski's Rule of Five. Notably, Piperenamide A and B, Piperol A and B, Piperbetol, Carotol, Elemicin, Acetyeugenol, Diallylcatechol, and 3-Benzamide-2'-methoxy-1-ethylpropanoate satisfied most developability criteria, displaying balanced Fsp³ values and low chelator risk, which are associated with favorable oral bioavailability and reduced assay interference. Although Piperol A and B showed only moderate ACE-binding affinities, their overall physicochemical balance and chemical tractability support their suitability as lead scaffolds for further optimization. In contrast, several top-ranked docking hits, including Piperocerebroside A, desmethylenesqualenyl deoxycepharadione A, and bis-chavicol derivatives, exhibited low QED scores, high molecular complexity, and multiple violations of key medicinal chemistry rules, underscoring a common limitation of natural products wherein high target affinity is often accompanied by suboptimal pharmacokinetic and developability properties [40].

The predicted toxicity profiles indicate that several *Piper betle* phytochemicals exhibit favorable safety characteristics. Among the evaluated compounds, 1-n-decanoyl phenol, piperenamide A, and 1-n-dodecanyloxy resorcinol showed the most favorable overall profiles, characterized by low predicted hERG inhibition (0.071–0.089), low DILI risk (0.048–0.072), low-to-moderate AMES mutagenicity (0.186–0.215), and high LD₅₀ values (5000 mg/kg), indicating relatively low acute toxicity and low genotoxic risks consistent with their traditional safety profile [41]. Other compounds, such as piperocerebroside A, desmethylenesqualenyl deoxycepharadione A, and 3-butylphenol, also demonstrated acceptable safety profiles, although with slightly higher predicted cardiotoxicity, hepatotoxicity, and mutagenicity risks, accompanied by lower LD₅₀ values (2500–3000 mg/kg). Piperenamide B and hydroxychavicol exhibited moderate toxicity probabilities and lower LD₅₀ values (2000 mg/kg), suggesting comparatively reduced safety margins. Piperbetol and coniferyl alcohol showed relatively higher predicted hERG inhibition and AMES toxicity among the evaluated compounds, despite maintaining moderate LD₅₀ values (3000 mg/kg). Overall, these results suggest that amide-type and long-chain phenolic compounds demonstrate the most favorable predicted safety profiles, whereas phenylpropanoid derivatives and several structurally complex constituents exhibit comparatively higher toxicity risks and may require further evaluation of their safety.

CONCLUSION

Based on an integrated evaluation of docking affinity, physicochemical properties, drug-likeness, and predicted toxicity, Piperenamide A and Piperenamide B emerged as the most promising drug candidates among *Piper betle* leaf compounds. Despite showing only moderate docking scores, both compounds formed stable interactions within the ACE active site and met essential developability criteria, including high QED values, compliance with Lipinski's Rule of Five, acceptable synthetic accessibility, and low predicted toxicity. Several simple phenolic compounds, including chavibetol, eugenol, vanillin, elemicin, and coniferyl alcohol, were identified as safe and drug-like secondary candidates with favorable physicochemical profiles, despite their moderate ACE-binding affinities. In contrast, compounds with the strongest docking scores, such as Piperocerebroside A bis-chavicol derivatives, exhibited poor drug-likeness and multiple rule violations, suggesting their potential utility primarily as lead optimization scaffolds rather than direct drug candidates.

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REFERENCES

- [1] K. T. Mills, A. Stefanescu, and J. He, "The global epidemiology of hypertension," *Nat. Rev. Nephrol.*, vol. 16, pp. 223–237, 2020.
- [2] A. Durante, A. Mazzapicchi, and M. B. Redaelli, "Systemic and Cardiac Microvascular Dysfunction in Hypertension," *Int. J. Mol. Sci.*, vol. 25, no. 24, pp. 1–16, 2024.
- [3] X. Y. Zhu, M. Q. Shi, Z. M. Jiang, X. Li, J. W. Tian, and F. F. Su, "Global, regional, and national burden of cardiovascular diseases attributable to metabolic risks across all age groups from 1990 to 2021: an analysis of the 2021 global burden of disease study data," *BMC iPublic Heal.*, vol. 25, no. 1704, pp. 1–21, 2025, doi: 10.1186/s12889-025-22702-7.
- [4] C. P. McCarthy, R. M. Bruno, K. Rahimi, R. M. Touyz, and J. W. Mcevoy, "What Is New and Different in the 2024 European Society of Cardiology Guidelines for the Management of Elevated Blood Pressure and Hypertension?," *Hypertension*, vol. 82, no. 3, pp. 432–444, 2025, doi: 10.1161/HYPERTENSIONAHA.124.24173.
- [5] H. Triebel and H. Castrop, "The renin angiotensin aldosterone system," *Pflügers Arch. - J. Physiol.*, vol. 476, no. 5, pp. 705–713, 2024, doi: 10.1007/s00424-024-02908-1.
- [6] F. Bioletto, M. Bollati, C. Lopez, S. Arata, M. Procopio, and F. Ponzetto, "Primary Aldosteronism and Resistant Hypertension: A Pathophysiological Insight," *Int. J. Mol. Sci.*, vol. 23, no. 4803, pp. 1–16, 2022.
- [7] S. Cutrell, I. S. Alhomoud, A. Mehta, A. H. Talasaz, and B. Van Tassell, "ACE - Inhibitors in Hypertension: A Historical Perspective and Current Insights," *Curr. Hypertens. Rep.*, vol. 25, no. 9, pp. 243–250, 2023, doi: 10.1007/s11906-023-01248-2.
- [8] H. Ahmad, H. Khan, S. Haque, S. Ahmad, N. Srivastava, and A. Khan, "Angiotensin-Converting Enzyme and Hypertension: A Systemic Analysis of Various ACE Inhibitors, Their Side Effects, and Bioactive Peptides as a Putative Therapy for Hypertension," *J. Renin-Angiotensin-Aldosterone Syst.*, no. 7890188, pp. 1–9, 2023.
- [9] M. N. Takuathung et al., "Adverse Effects of Angiotensin-Converting Enzyme Inhibitors in Humans: A Systematic Review and Meta-Analysis of 378 Randomized Controlled Trials," *Int. J. Environ. Res. Public Heal.*, vol. 19, no. 14, pp. 1–13, 2022.
- [10] D. J. Newman and G. M. Cragg, "Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019," *J. Nat. Prod.*, vol. 83, no. 3, pp. 770–803, 2020, doi: 10.1021/acs.jnatprod.9b01285.
- [11] R. Chakraborty and S. Roy, "Angiotensin-converting enzyme inhibitors from plants: A review of their diversity, modes of action, prospects, and concerns in the management of diabetes-centric complications," *J. Integr. Med.*, vol. 19, no. 6, pp. 478–492, 2021, doi: 10.1016/j.joim.2021.09.006.
- [12] T. A. F. T. Anuar and A. Ismail, "Southeast Asian Medicinal Plants with Angiotensin Converting Enzyme (ACE) Inhibition Properties," *Pharmacogn J.*, vol. 12, no. 6, pp. 1429–1439, 2020.
- [13] A. Riany, A. P. Permatasari, N. Salsabila, N. Rahayu, S. Endarti, and M. Marcelina, "Ethnobotanical Study of Medicinal Plants in Bangbayang Village, Sumedang, West Java," *J. Trop. Biodivers.*, vol. 4, no. 2, pp. 89–111, 2024.
- [14] E. Singarimbun, E. Elfrida, and I. Indriaty, "Indigenous Knowledge and Herbal Medicine: Exploring the Ethnobotany of the Karo Tiganderket Tribe in Indonesia," *Heca J. Appl. Sci.*, vol. 2, no. 2, pp. 74–86, 2024, doi: 10.60084/hjas.v2i2.208.
- [15] C. A. I. Nufus et al., "Ethnobotanical Study and Medicinal Plant Bioprospecting in Tamiang Tribal Community, Aceh," *J. Trop. Ethnobiol.*, vol. 7, no. 1, pp. 1–20, 2024.

- [16] P. Biswas *et al.*, "Betelvine (Piper betle L.): A comprehensive insight into its ethnopharmacology , phytochemistry , and pharmacological , biomedical and therapeutic attributes," *J Cell Mol Med.*, vol. 26, pp. 3083–3119, 2022, doi: 10.1111/jcmm.17323.
- [17] J. Seo *et al.*, "Anti-inflammatory and antioxidant activities of methanol extract of Piper betle Linn. (Piper betle L.) leaves and stems by inhibiting NF- κ B/MAPK/Nrf2 signaling pathways in RAW 264.7 macrophages," *Biomed. Pharmacother.*, vol. 155, p. 113734, 2022, doi: 10.1016/j.biopha.2022.113734.
- [18] B. Somanadhan *et al.*, "An ethnopharmacological survey for potential angiotensin converting enzyme inhibitors from Indian medicinal plants," *J. Ethnopharmacol.*, vol. 65, no. 2, pp. 103–112, 1999, doi: 10.1016/S0378-8741(98)00201-3.
- [19] S. S. Cınaroğlu and E. Timuçin, "Comparative Assessment of Seven Docking Programs on a Nonredundant Metalloprotein Subset of the PDBbind Refined," *J. Chem. Inf. Model.*, vol. 59, no. 9, p. 3846–3859, 2019, doi: 10.1021/acs.jcim.9b00346.
- [20] J. Li, A. Fu, and L. Zhang, "An Overview of Scoring Functions Used for Protein – Ligand Interactions in Molecular Docking," *Interdiscip. Sci. Comput. Life Sci.*, vol. 11, no. 2, pp. 320–328, 2019, doi: 10.1007/s12539-019-00327-w.
- [21] N. Patel and J. Mohan, "Isolation and characterization of potential bioactive compounds from Piper betle varieties Banarasi and Bengali leaf extract," *Int. J. Herb. Med.*, vol. 5, no. 5, pp. 182–191, 2017.
- [22] A. Atiya, B. N. Sinha, and U. R. Lal, "New chemical constituents from the Piper betle Linn. (Piperaceae)," *Nat. Prod. Res.*, pp. 1–9, 2017, doi: 10.1080/14786419.2017.1380018.
- [23] T. T. San *et al.*, "A new sesquieolignan and four new neolignans isolated from the leaves of Piper betle, a traditional medicinal plant in Myanmar," *Bioorganic Med. Chem. Lett.*, vol. 31, pp. 1–19, 2021, doi: 10.1016/j.bmcl.2020.127682.
- [24] D. Z. Chen, H. Bin Xiong, K. Tian, J. M. Guo, X. Z. Huang, and Z. Y. Jiang, "Two new sphingolipids from the leaves of Piper betle L.," *Molecules*, vol. 18, no. 9, pp. 11241–11249, 2013, doi: 10.3390/molecules180911241.
- [25] I. Ali *et al.*, "In vitro antifungal activity of hydroxychavicol isolated from Piper betle L.," *Ann. Clin. Microbiol. Antimicrob.*, vol. 9, no. 7, pp. 1–9, 2010.
- [26] M. Nagabhushan, A. J. Amonkar, U. J. Nair, A. V. D'souza, and S. V. Bhide, "Hydroxychavicol: A new anti-nitrosating phenolic compound from betel leaf," *Mutagenesis*, vol. 4, no. 3, pp. 200–204, 1989, doi: 10.1093/mutage/4.3.200.
- [27] F. Prasetya *et al.*, "Novel amides derivative with antimicrobial activity of Piper betle var. nigra leaves from Indonesia," *Molecules*, vol. 26, no. 2, pp. 1–8, 2021, doi: 10.3390/molecules26020335.
- [28] H. Zeng, Y.-Y. Jiang, D. Cai, K. Long, and Z. Chen, "Piperbetol, Methylpiperbetol, Piperol A and Piperol B: A New Series of Highly Specific PAF Receptor Antagonists from Piper betle," *Planta Med.*, vol. 63, pp. 296–298, 1997.
- [29] M. Madhumita, P. Guha, and A. Nag, "Bio-actives of betel leaf (Piper betle L.): A comprehensive review on extraction, isolation, characterization, and biological activity," *Phyther. Res.*, vol. 34, no. 10, pp. 2609–2627, 2020, doi: 10.1002/ptr.6715.
- [30] T. Noshita, T. Sato, T. Iwayama, Y. Yamada, and H. Ouchi, "The proposed structures of phenolic compounds isolated from Piper betle L. differ from those of the compounds obtained by total synthesis," *Nat. Prod. Res.*, vol. 35, no. 21, pp. 3787–3793, 2021, doi: 10.1080/14786419.2020.1739038.
- [31] A. Atiya, M. A. Salim, B. N. Sinha, and U. R. Lal, "Two new anticancer phenolic derivatives from leaves of Piper betle Linn.," *Nat. Prod. Res.*, vol. 36, no. 16, pp. 5021–5029, 2020, doi: 10.1080/14786419.2020.1762186.
- [32] I. Bin Jantan, A. R. Ahmad, A. S. Ahmad, and N. A. M. Ali, "A comparative study of the essential oils of five piper species from Peninsular Malaysia," *Flavour Fragr. J.*, vol. 9, no. 6, pp. 339–342, 1994, doi: 10.1002/ffj.2730090611.
- [33] N. A. Bhat, B. K. Tiwari, and A. Bhardwaj, "Isolation and identification of an aromatic compound from the Alcoholic extract of Piper betle Linn. (Leaf stalk)," *J. Xi'an Univ. Archit. Technol.*, vol. 12, no. 6, pp. 1145–1151, 2020.
- [34] D. Suryasnata, I. S. Sandeep, R. Parida, S. Nayak, and S. Mohanty, "Variation in Volatile Constituents and Eugenol Content of Five Important Betelvine (Piper betle L.) Landraces Exported from Eastern India," *J. Essent. Oil-Bearing Plants*, vol. 19, no. 7, pp. 1788–1793, 2016, doi: 10.1080/0972060X.2016.1179131.
- [35] A. Atiya, B. N. Sinha, and U. R. Lal, "Bioactive phenylpropanoid analogues from Piper betle L. var. birkoli leaves," *Nat. Prod. Res.*, vol. 31, no. 22, pp. 2604–2611, 2017, doi: 10.1080/14786419.2017.1285297.
- [36] L. te Riet, J. H. M. van Esch, A. J. M. Roks, A. H. van den Meiracker, and A. H. J. Danser, "Hypertension: Renin-Angiotensin-Aldosterone System Alterations," *Circ. Res.*, vol. 116, no. 6, pp. 960–975, 2015, doi:

10.1161/CIRCRESAHA.116.303587.

- [37] A. Valentini, R. M. Heilmann, A. Kühne, L. Biagini, D. De Bellis, and G. Rossi, "The Renin - Angiotensin - Aldosterone System (RAAS : Beyond Cardiovascular Regulation," *Vet. Sci.*, vol. 12, no. 8, pp. 1-18, 2025.
- [38] J. Song, J. Ha, J. Lee, J. Ko, and W. Shin, "Improving docking and virtual screening performance using AlphaFold2 multi-state modeling for kinases," *Sci. Rep.*, vol. 14, no. 25167, pp. 1-13, 2024.
- [39] J. H. Tina *et al.*, "Molecular docking and ADMET profiling of xanthenes from *Securidaca longepedunculata* targeting oxidative stress in hypertension," *Discov. Chem.*, vol. 3, no. 17, pp. 1-16, 2026.
- [40] E. Mavridis and D. Hadjipavlou-litina, "Using a Novel Consensus-Based Chemoinformatics Approach to Predict ADMET Properties and Druglikeness of Tyrosine Kinase Inhibitors," *Int. J. Mol. Sci.*, vol. 26, no. 20, p. 10207, 2025.
- [41] S. Gould and M. V Templin, "Off target toxicities and links with physicochemical properties of medicinal products , including antibiotics, oligonucleotides , lipid nanoparticles (with cationic and/or anionic charges). Data review suggests an emerging pattern," *Toxicol. Lett.*, vol. 384, pp. 14-29, 2023.

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