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Original article

In Silico exploration of β-Glucogallin from Malacca Fruit (*Phyllanthus emblica*) as a dual inhibitor of ACE and AT1R in Chronic Kidney Disease

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Abstract

Chronic Kidney Disease (CKD) is one of the leading causes of death worldwide, with hypertension as its primary risk factor. Activation of the *renin-angiotensin system* (RAS) contributes to CKD complications, including hypertension and renal fibrosis. β-Glucogallin, a bioactive compound isolated from Malacca fruit (*Phyllanthus emblica*), exhibits therapeutic potential in RAS modulation. This study aims to identify and evaluate the potential of β-Glucogallin as an *angiotensin-converting enzyme* (ACE) and *angiotensin II type 1 receptor* (AT1R) inhibitor through an *in silico* approach, thereby providing new insights for CKD treatment. The methods used include ligand and protein preparation, bioavailability and toxicity prediction, molecular docking, and molecular dynamics simulation. The result showed β-Glucogallin exhibits strong binding affinity and stable interactions with key residues of ACE (Gln281, His353, His383, and Tyr523), and AT1R (Trp84 and Lys199), with binding affinity values of -8,0 Kcal/mol for ACE and -7,4 Kcal/mol for AT1R. Molecular dynamics simulations demonstrated good stability of the ligand-protein complex, with RSMD and RMSF values below 3Å, indicating stable interactions during simulations and suggesting its potential as an effective inhibitor. Despite slight deviations, β-Glucogallin meets Lipinski criteria as a viable drug candidate for CKD treatment through RAS inhibition. The *in silico* approach facilitates the rapid identification of active compounds. These findings confirm that β-Glucogallin warrants further investigation as a drug candidate.

Keywords: ACE, AT1R, β-Glucogallin, *Phyllanthus emblica*, molecular docking, molecular dynamics
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Introduction

Chronic kidney disease (CKD) is a major global health concern and ranks as the 10th leading cause of death worldwide, with an estimated prevalence of 8-16% (Rapa et al., 2020; Guo et al., 2025). Epidemiological evidence indicates that diabetes, glomerulonephritis, and hypertension are the primary causes of CKD globally. In Indonesia, hypertension is the main risk factor, accounting for 34.1% of CKD cases (Guo et al., 2025). Chronic kidney disease often results from untreated hypertension, which can lead to structural and functional kidney damage (Cahyani et al., 2022). The disease is characterized by decreased renal function, tubular injury, and oxidative stress, and it progresses slowly over time (Reiss et al., 2024). The pathophysiological mechanism of hypertension in CKD involves excessive activation of the *renin-angiotensin system* (RAS), a hormonal pathway that regulates blood pressure by modulating blood volume, electrolyte balance, systemic vascular resistance, arteriolar constriction, and renal function (Ksiazek et al., 2024). RAS hyperactivation elevates glomerular pressure by inducing renal blood vessel constriction and elevating vascular resistance, leading to progressive kidney damage. Therefore, targeting this pathway remains crucial for slowing CKD progression.

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At the molecular level, the *renin-angiotensin system* (RAS) converts angiotensin I (Ang I) into the vasoconstrictor peptide angiotensin II (Ang II) through the action of *angiotensin-converting enzyme* (ACE), a zinc metallopeptidase (Bitker & Burrell, 2019; Riyadi, 2018). ACE cleaves amino acids from Ang I to form Ang II, which binds to the angiotensin receptor (AT1R), a G protein-coupled receptor (GPCR) on the cell membrane, initiating an intracellular signaling cascade (Kadir, 2018). This binding activates the second messengers, resulting in potent vasoconstriction and elevated blood pressure.

Recommended treatments for CKD with hypertension include the use of ACE inhibitors (*ACEi*) or angiotensin receptor blockers (ARBs), such as lisinopril or candesartan, to preserve kidney function and prevent disease progression by controlling blood pressure. However, prolonged use may cause side effects such as headache, dry cough, and impaired kidney function (Bitker & Burrell, 2019). In contrast, natural plant compounds typically produce fewer side effects on normal metabolism and offer additional benefits through antioxidant and anti-inflammatory properties (Chakraborty & Roy, 2021). Therefore, it is essential to explore botanicals as safer and more effective complementary therapies, with an emphasis on identifying active compounds and specific molecular targets.

Malacca (*Phyllanthus emblica*) is a lesser-known Indonesian plant found in Java, Sumatra, Kalimantan, Maluku, Ternate, and Nusa Tenggara (Prananda et al., 2023). The fruit is the most commonly used part, as it contains flavonoids, alkaloids, tannins, vitamins, and phenolics, which have been shown to effectively treat

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various diseases (Gul et al., 2022). According to (Kusirisin et al., 2009; Asih et al., 2022), the primary chemical component of Malacca is phenolic compounds, including gallic acid, which contributes to its strong DPPH antioxidant properties (Jhaumeer et al., 2018). Interestingly, a unique compound derived from gallic acid glycoconjugates, known as gallic acid glucosyl esters or β-Glucogallin, has been identified with an HPLC analysis, with a measured content of 1,46% (Middha et al., 2015).

β-Glucogallin, a major bioactive constituent found in Malacca fruit, belongs to the hydrolyzed tannin class. It has antioxidant and antiinflamantory properties and the ability to modulate blood pressure related pathways (Shanmugarajan et al., 2021). Although there is no explicit statement that β-Glucogallin can directly lower blood pressure, the compound is known to have potent antioxidant properties. These properties are relevant because they could potentially contribute indirectly to vascular health and support blood pressure regulation.Previous studies have demonstrated the effectiveness of *Phyllanthus emblica* in controlling hypertension (Shanmugarajan et al., 2021). However, no studies have specifically identified the molecular interactions of β-Glucogallin and key blood pressure regulators such as ACE and AT1R. Modulating ACE and AT1R can suppress RAS overactivation, thereby reducing hypertension-induced renal damage, a critical factor in CKD progression. The study aims to analyze the potential of β-Glucogallin compounds from Malacca fruit as natural inhibitors of ACE and AT1R through an *in silico* approach.

Materials and Methods

Bioavailability, toxicity, and bioactivity prediction

Pharmacokinetic and toxicity predictions were performed online using the Swiss ADME web tool (http://www.swissadme.ch/) and ProTox 3.0 (https://tox.charite.de/protox3/), to predict the druglikeness and ADMET profile of β-Glucogallin, adhering to *Lipinski's rule of 5* (Szklarczyk et al., 2019). Predictions were based on its *Simplified Molecular Input Line Entry System* (SMILES) code retrieved from PubChem. Control ligands (lisinopril and candesartan) were included for comparison. Predicted toxicities included hepatotoxicity, neurotoxicity, carcinogenicity, mutagenicity, immunotoxicity, and cytotoxicity (Banerjee et al., 2024). The *Lethal Dose* 50% (LD50) parameter was used as an indicator of the toxicity (Kumar et al., 2019). Compound bioactivity was predicted using the *Prediction of Activity Spectra for Substances* (PASS) Online tool (https://www.way2drug.com/passonline/), which evaluates the correlation between molecular structure and biological activity, thereby streamlining drug discovery by efficiently identifying potential therapeutic effects (Goel et al., 2011). In *PASS Online*, the *canonical SMILES* of compounds are entered to generate bioactivity predictions. The Pa (*Potential activity*) value serves as a key indicator; compounds with Pa >0.7 are considered to be highly biologically active. Such predictions strongly correlate with experimental laboratory results (Malikhana et al., 2021; Yasmin et al., 2022).

Ligand and protein preparation

The 3D structures of the receptors (ACE ID: 1O86 and AT1R ID: 8TH4) were downloaded from the RSCB PDB database (https://www.rcsb.org/pdb/) in PDB format. Receptor preparation was performed using Biovia Discovery Studio 2021 by removing water molecules and non-essential residues, while Cl and Zn ions were retained due to their function as protein cofactors (Qian et al., 2019). The 3D structures of the test ligand (β-Glucogallin) and control ligands (lisinopril and candesartan) were obtained from the PubChem database (https://www.pubchem.ncbi.nlm.nih.gov) in SDF format. Ligand energy minimization was performed using the Open Babel Tool in PyRx 0.8, and the structures were converted to PDB format for molecular docking analysis.

Table 1. The active site of the protein

Protein	PDB ID	Active site
ACE	1O86	Glu162, Gln281, His353, Ala354, Glu384, Lys511, His513, Tyr520, Tyr523 (Looi et al., 2021)
AT1R	8TH4	Trp84, Lys199, Arg167, Asp263, Asp281 (Georgiou et al., 2024)

Molecular docking

Ligand and protein docking were performed using AutoDock vina in PyRx 0.8 software. The initial stage involved docking the β-Glucogallin and control ligands (lisinopril and candesartan) to their respective receptors. In addition, ligand-receptor interactions were visualized and analyzed using PyMOL 2.5.4. The resulting protein-ligand complexes were visualized and analyzed to determine binding affinity, interaction types, and similarities in amino acid residues between the test compound and controls. Hydrogen and hydrophobic bonds were identified to evaluate binding stability and potential inhibitory effects using Biovia Discovery Studio 2021 (Haque et al., 2022). Docking validation (redocking with native ligands) was previously performed for each protein, both in the presence and absence of inhibitors, to confirm the accuracy of virtual inhibition.

Molecular dynamics simulation

Molecular dynamics simulations were performed to evaluate the stability of ligand and protein interactions over time, using *Yet Another Scientific Artificial Reality Application* (YASARA) software. The simulations were run for 40 ns at 37˚C, pH 7.4, and 0.9% NaCl concentration (Grahadi et al., 2022). Assessed parameters included root-mean-square deviation (RMSD) of the backbone, hydrogen bonding, and root-mean-square fluctuation (RMSF).

Results

The profile bioavailability, toxicity, and bioactivity of β-Glucogallin from Malacca fruit (*Phylanthus emblica*)

Lipinski's Rules of Five is a widely used guideline for predicting the absorption and distribution of compounds in the human body. The main criteria included a molecular weight (MW) ≤500 Da, log P ≤5, hydrogen bond donors (HD) ≤5, and hydrogen bond acceptors (HA) ≤10. The Assessment of Absorption, Distribution, Metabolism, and Excretion (ADME) indicated that most compounds met Lipinski's criteria (Table 2).

β-Glucogallin from Malacca fruit met Lipinski criteria, despite a slight deviation in the number of hydrogen bond donors (HD = 7) (Table 2). The compound demonstrated good absorption and permeability potential due to its low MW (332.26 Da), Log P (-2.29), and HA (10). The negative Log P value indicates strong hydrophilic properties, while the small molecular size enhances interaction with the target protein. Although the HD value exceeds Lipinski's threshold, the compound's overall properties support its potential bioavailability.

Toxicity prediction results indicate that β-Glucogallin is non-toxic and non-carcinogenic to humans, based on its LD50 value and toxicity classification (Figure 1A and 1B). The LD50 β-Glucogallin (2260 mg/kg) suggests that a high dose is required to induce lethality in 50% of the tested animal population, indicating low toxicity. β-Glucogallin is classified as class 5, which denotes a low-toxicity category. In comparison, standard CKD drugs such as lisinopril exhibit the highest safety margin (LD50: 8500 mg/kg; ACE inhibitor), whereas candesartan shows intermediate toxicity (LD50: 1075 mg/kg; ARB). Mechanistically, both lisinopril and candesartan preserve renal function by modulating the RAS system. In contrast, β-Glucogallin is predicted to have potential through alternative pathways involving antioxidant and anti-inflammatory activities, which may help reduce the progression of CKD. Bioactivity predictions (Figure 1C) indicate that β-Glucogallin has therapeutic potential relevant to CKD management, including antioxidant activity (Pa = 0.8), vasoprotector effects (Pa = 0.8), anti-inflammatory activity (Pa = 0.7), peripheral vasodilation (Pa = 0.7), and antiuremic effects (Pa = 0.6). These findings suggest that β-Glucogallin may regulate blood pressure and renal protection in CKD through mechanisms distinct from conventional RAS inhibitors.

Table 2. Lipinski properties of β-Glucogallin compounds from Malacca fruit, lisinopril, and candesartan were analyzed with Swiss ADME

CID	Compound	MW (<500)	HA (<10)	HD (<5)	LogP (<5)	Lipinski violations
124021	β-Glucogallin	332.26	10	7	-2.29	1
5362119	Lisinopril	405.49	7	4	-1.46	0
2541	Candesartan	440.45	7	2	3.51	0

Note: CID = compound ID; MW = molecular weight; HA = hydrogen bond acceptor; HD = hydrogen bond donor

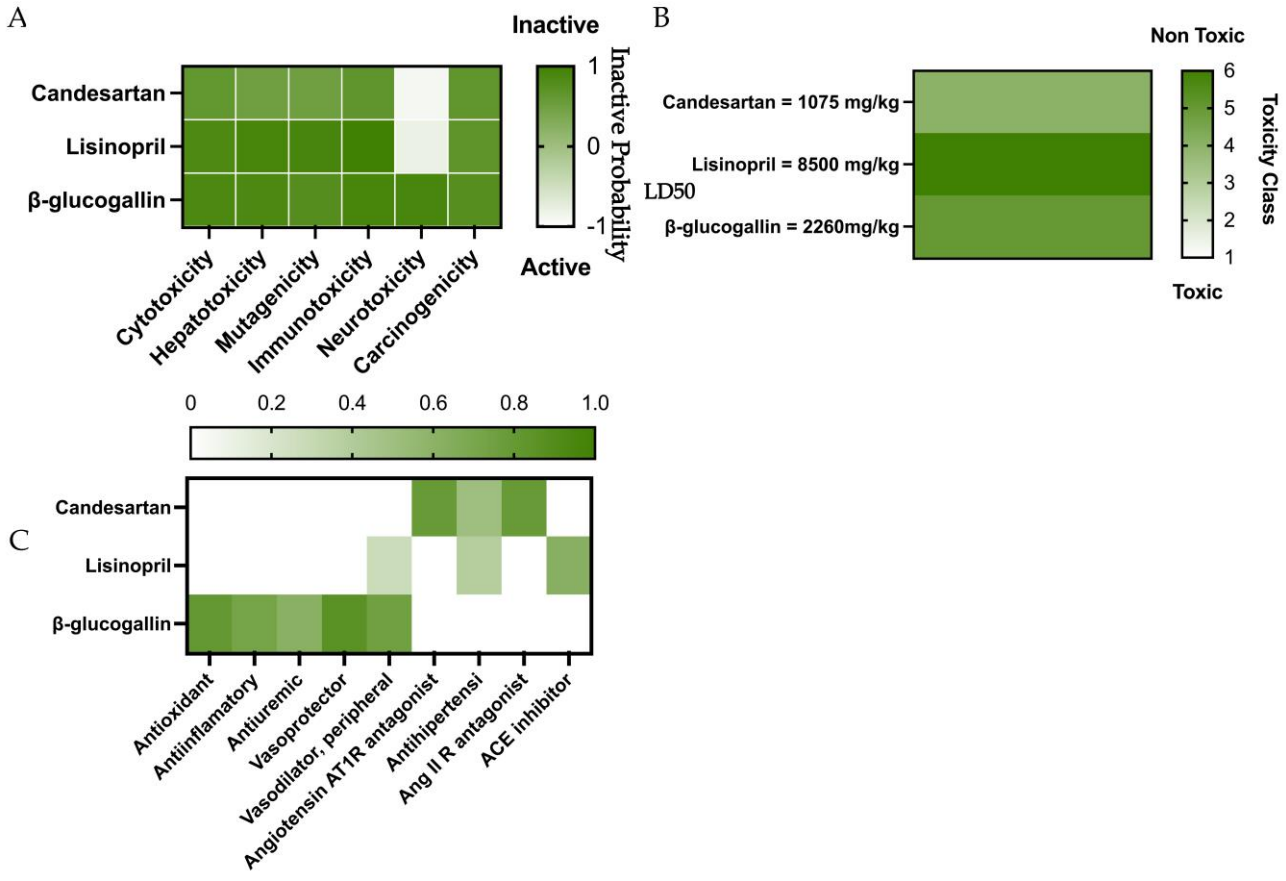

Figure 1. Prediction of bioavailability, toxicity, and bioactivity screening of β-Glucogallin. A) organ toxicity, B) LD50 and class toxicity, and C) bioactivity related to CKD

The docking result of β-Glucogallin from Malacca fruit toward ACE and AT1R

Based on the docking results, both lisinopril and β-Glucogallin targeted the ACE active site (Figure 2A-B and Table 3). Lisinopril formed hydrogen bonds with key ACE active site residues Glu162, Ala354, Glu384, and Tyr523, as well as hydrophobic interactions with His353, consistent with its clinically established mechanism of ACE inhibition. Notably, β-Glucogallin interacts via hydrogen bonds with residues Gln281, His353, Tyr520, and Tyr523, along with a hydrophobic interaction with His383. Importantly, β-Glucogallin demonstrated a higher binding affinity (-8.0 Kcal/mol) compared to the control ligand (-7.4 Kcal/mol), suggesting a more stable interaction with ACE. These interactions with critical ACE residues indicate a strong potential for ACE inhibition.

The docking results indicate that both candesartan (ligand control) and β-Glucogallin effectively target the AT1R binding site (Figure 2C-D and Table 3), with binding affinities of -9,8 Kcal/mol and -7,4 Kcal/mol, respectively. Although candesartan showed stronger binding, β-Glucogallin presents a significantly more favorable toxicity profile as a natural compound. Candesartan forms hydrogen bonds with the active site residue Arg167, an electrostatic bond with Lys199, and a hydrophobic interaction with Trp84. Similarly, β-Glucogallin also binds the same two key residues as the control ligand, forming a hydrogen bond with Lys199 and a hydrophobic interaction with Trp84.

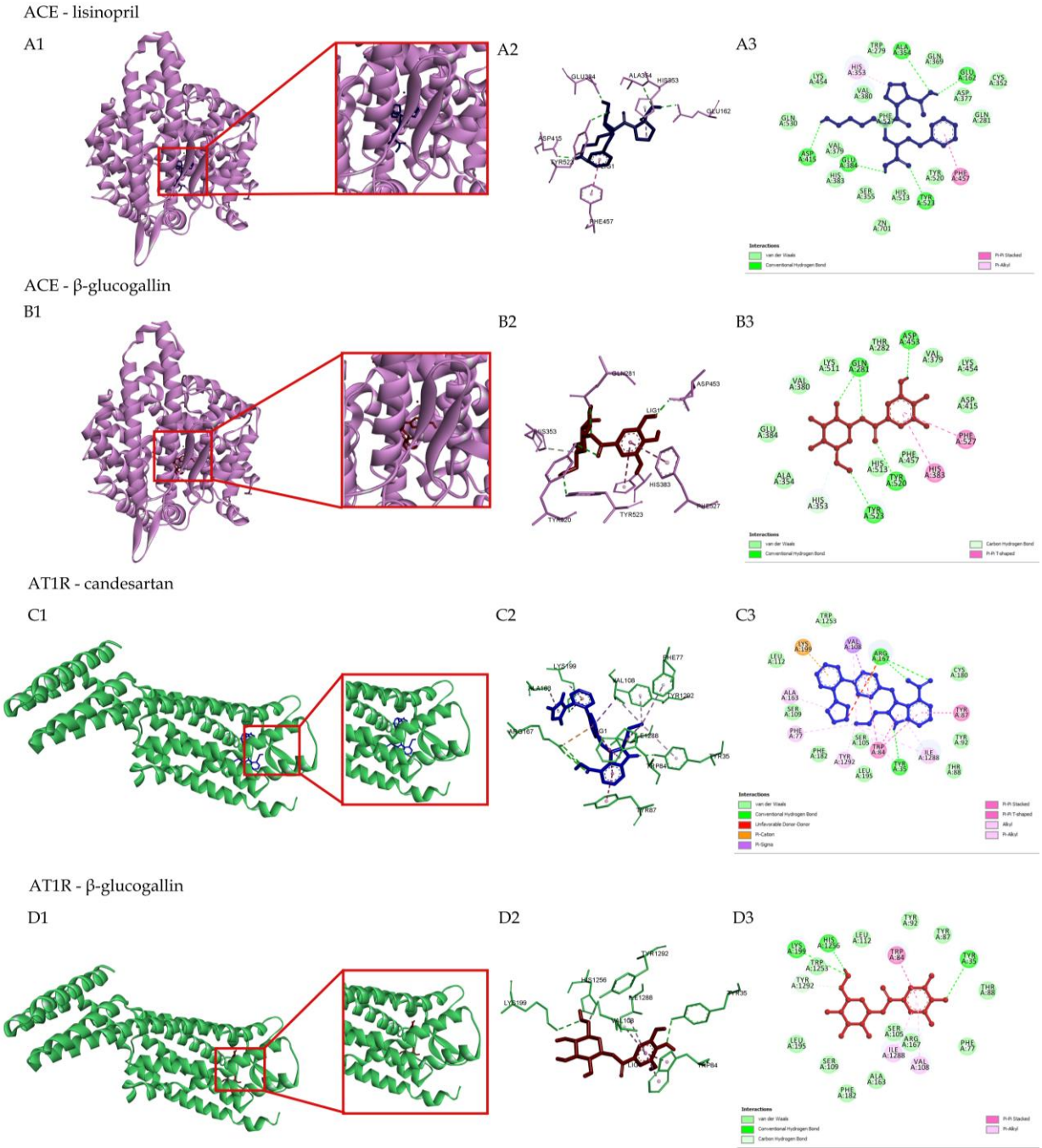

Figure 2. Molecular interaction between protein - ligand. The 3D structure of the complex is shown in the left panel inside the box. The 2D structure in the right panel shows the type of amino acid residues. A) ACE-lisinopril, B) ACE-β-Glucogallin, C) AT1R-candesartan, and D) AT1R-β-Glucogallin.

Table 3. List of binding interactions between β-Glucogallin compounds and control drugs with ACE and AT1R

Protein	Ligand	Binding affinity (Kcal/mol)	Residue	Category	Type
ACE	Lisinopril	-7,8	**Glu162**, **Ala354**, **Glu384**, Asp415, **Tyr523**	Hydrogen	Conventional hydrogen bond
			Phe457	Hydrophobic	Pi-Pi Stacked
			His353	Hydrophobic	Pi-Alkyl
	β-Glucogallin	-8,0	**Gln281**, Asp453, **Tyr520**, **Tyr523**	Hydrogen	Conventional hydrogen bond
			His353	Hydrogen	Carbon-hydrogen bond
			His383, Phe527	Hydrophobic	Pi-Pi T-Shaped
AT1R	Candesartan	-9,8	Tyr35, **Arg167**	Hydrogen	Conventional hydrogen bond
			Lys199	Electrostatic	Pi-cation
			Val108	Hydrophobic	Pi-Sigma
			Trp84	Hydrophobic	Pi-Pi Stacked
			Trp87	Hydrophobic	Pi-Pi T-Shaped
			Ile1288, Ala163, Phe77, Tyr1292	Hydrophobic	Pi-Alkyl
	β-Glucogallin	-7,4	His1256, Tyr35, **Lys199**	Hydrogen	Conventional hydrogen bond
			Tyr1292	Hydrogen	Carbon-hydrogen bond
			Trp84	Hydrophobic	Pi-Pi Stacked
			Val108, Ile1288	Hydrophobic	Pi-Alkyl

Note: The bold represents the same binding site as the control drug and the active site.

Molecular dynamic result of β-Glucogallin from Malacca fruit toward ACE and AT1R

The molecular dynamic simulations were used to evaluate the structural stability of the protein-ligand complex based on the RMSD of the backbone, hydrogen bonds, and RMSF value. The simulation result showed that the ACE and β-Glucogallin complex maintained structural stability, with a RMSD *backbone* value of less than 3Å (Figure 3A). This stability is supported by the formation of hydrogen bonds between ACE and β-Glucogallin, which are comparable to those observed in the ACE-lisinopril complex (Figure 3B). The RMSF values indicate that most amino acid residues exhibited minimal fluctuations during the simulation, with the majority remaining below 3Å (Figure 3C). The stable interaction between ACE and β-Glucogallin throughout the 0–40 ns simulation period further supports the structural integrity of the complex, suggesting its potential as an ACE pathway inhibitor in hypertension-induced CKD.

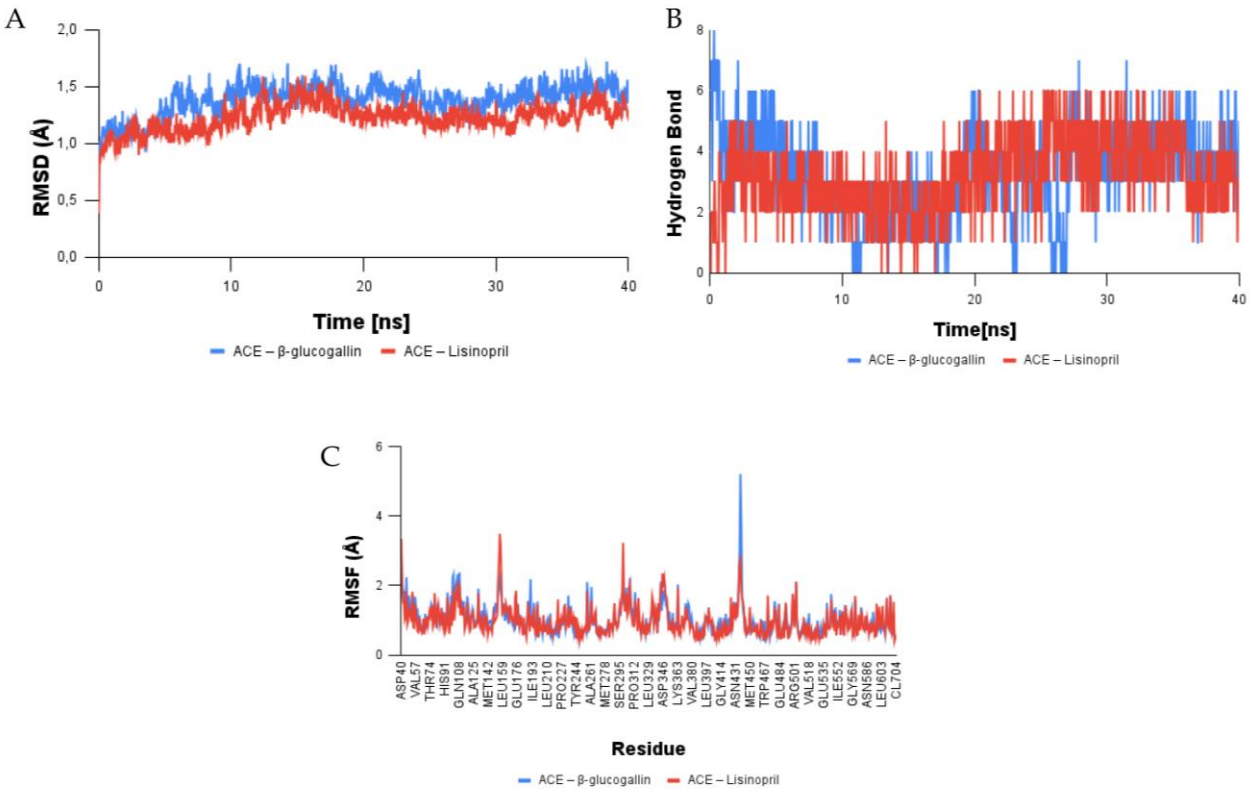

Figure 3. Molecular dynamics simulation result of ACE and β-Glucogallin. A) RMSD backbone, B) H-bonds, and C) RMSF value

The molecular dynamic simulation of the AT1R and β-Glucogallin complex demonstrated stable movement, with RMSD *backbone* values remaining below 3Å and minimal fluctuations up to 25 ns. This was followed by increased fluctuation between 26-33 ns (Figure 4A). However, stability was regained after 34 ns, indicating that β-Glucogallin remained bound with some flexibility. The number of hydrogen bonds in the complex of AT1R and β-Glucogallin was also similar to that in the AT1R and candesartan complex, and the RMSF values mostly remained below 3Å (Figure 4B and 4C), indicating strong ligand binding and minimal residue fluctuation. These results suggest that β-Glucogallin has the potential to inhibit enzyme activity for a period of time, making it a promising candidate for the development of new anti-hypertensive agents with reno-protective effects. Molecular dynamics simulation showed that the protein structures of ACE and AT1R remained relatively stable after interacting with β-Glucogallin, as indicated by the RMSD *backbone* values, the number of hydrogen bonds, and RMSF measurements.

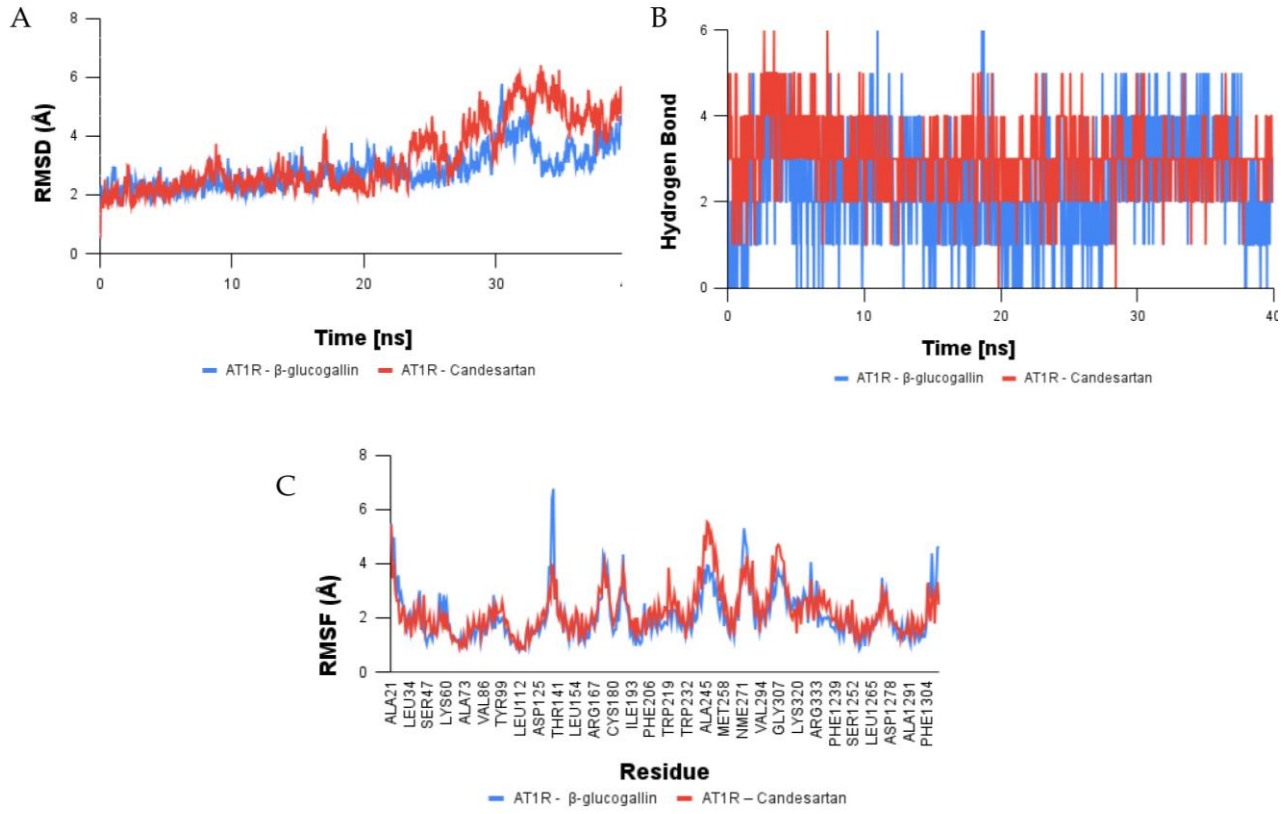

Figure 4. Molecular dynamics simulation result of AT1R and β-Glucogallin. A) RMSD backbone, B) H-bonds, and C) RMSF value

Discussion

The renin-angiotensin system (RAS) is a primary hormonal cascade responsible for regulating blood pressure through vasoconstriction and regulates circulating blood volume. RAS dysregulation can cause several pathological conditions, particularly hypertension (Fajloun & Sabatier, 2024). The kidney plays an important role in developing hypertension, mainly through increasing renal circulatory resistance and decreasing glomerular capillary function (Made Maharianingsih et al., 2024). In the RAS cascade, ACE plays a pivotal role by catalyzing the conversion of Ang I to Ang II, a vasoactive peptide that strongly increases blood pressure. In addition, Ang II contributes to key pathological processes, such as renal fibrosis, inflammation, and hypertension, which are the hallmarks of CKD. ACE inhibition is among the most widely observed mechanisms by which herbal compounds exert antihypertensive effects (Chakraborty & Roy, 2021).

This study employed an *in silico* approach to investigate the potential of the β-Glucogallin, a compound found in Malacca fruit, to inhibit the target proteins *Angiotensin-Converting Enzyme* (ACE) and *Angiotensin Type I Receptor* (AT1R), both of which play important role in blood pressure regulation and impaired renal function. *In silico* methods efficiently identify bioactive compounds and enable accurate prediction of molecular interactions before advancing to *in vitro* and *in vivo* validation (Ugurlu et al., 2024). Predicting bioavailability is essential in drug discovery, as it determines the therapeutic potential and pharmacokinetic profile of candidate compounds (Cascone et al., 2016). The Lipinski Rule of 5 serves as a fundamental parameter for assessing a compound's drug-likeness, focusing on oral bioavailability and therapeutic potential. These criteria evaluate fundamental molecular properties that determine pharmaco-

logical suitability against established drug standards (Chagas et al., 2018; Aziz & Purnama, 2024). Molecular docking analyzes the structural binding of ligands to proteins and estimates binding affinity (Tanchuk et al., 2016). The docking results are then refined through molecular dynamics simulations, incorporating biomolecular force fields, water molecules, ions, and other components that replicate *in vivo* conditions (Nair & Miners, 2014).

The drug-likeness prediction revealed that β-Glucogallin in Malacca fruit (*Phyllanthus emblica*) violates one of Lipinski's rules due to its high hydrogen bond donor (HD = 7), exceeding the threshold of ≥5 (Table 2). Previous studies reported that a high HD value may limit the compound's ability to penetrate lipophilic cell membranes, as it increases the tendency to form hydrogen bonds with polar solvents (Ivanović et al., 2020). Despite this violation, β-Glucogallin's overall adherence to Lipinski criteria comprising only a single exception supports its potential as an orally bioavailable drug for managing hypertension in CKD. Since molecules with more than two violations often exhibit poor absorption and are predicted to be inactive when orally consumed (Chagas et al., 2018; Shawky, 2019), the single violation in β-Glucogallin suggests a favorable pharmacokinetic profile.

In addition to this, β-Glucogallin exhibits low toxicity risk (LD50: toxicity class 5), and shows no evidence of organ-specific toxicity (e.g., hepatotoxicity, mutagenicity, or carcinogenicity) (Figure 1A and 1B). These findings indicate that β-Glucogallin meets the safety prerequisites for oral drug development. Toxicity evaluation is a crucial step in drug design, as it assesses the potential adverse effects of chemical compounds on the organ system before proceeding to humans or animal studies (Ahammad et al., 2021). PASS Online predicted that β-Glucogallin exhibits multi-target bioactivity, including antioxidant (Pa = 0.8), vasoprotector (Pa = 0.8), anti-inflammatory (Pa = 0.7), and vasodilator peripheral (Pa = 0.7), and antiuremic (Pa = 0.6) properties. These probabilities align with QSAR principle (Pa > 0.3 for all key activities) (Figure 1C). Given its combined effects, β-Glucogallin likely contributes to blood pressure reduction and may mitigate the progression of hypertensive chronic kidney disease (CKD).

The accuracy of molecular docking depends on how suitable the test ligand binding interaction with the macromolecule matches those native ligand. The *active site* of angiotensin-converting enzyme (ACE) contains a zinc ion coordinated by three residues (His383, His387, and Glu411) and three distinct binding pockets (S1, S1', and S2). The S1 pocket (Ala354, Glu384, and Tyr523), S1' pocket (Glu162), and S2 pocket (Gln281, His353, Lys511, His513, Tyr520) (Table 1).

This study performed molecular docking of lisinopril and β-Glucogallin with ACE. The results indicated that both compounds target the ACE active site (Figure 2A and 2B). The β-Glucogallin compound from Malacca fruit is predicted to function as a competitive inhibitor at the ACE active site, preventing Ang I from interacting with the active site. β-Glucogallin likely inhibits enzyme activity by a similar mechanism to the control drug, as both compounds share an identical number of amino acid residue interactions (Table 3) (Arwansyah et al., 2014). According to Schön et al. (2011), although the compound does not completely block the substrate from binding to the target protein due to the substrate's significantly larger size, its binding at key points in the protein's active site can affect the enzymatic activity. This occurs because the compounds can shift the ligand interaction site, thereby reducing its affinity for the protein.

Lisinopril formed five hydrogen bonds (Glu162, Ala354, Glu384, Asp415, and Tyr523), four of which were part of the ACE binding pocket (except Asp415), along with two hydrophobic interactions. One hydrophobic bond involved the binding pocket residue His353, while the other occurred with Phe457. In contrast, β-glucogallin established four hydrogen bonds (Gln281, His353, Tyr520, and Tyr523) and one hydrophobic interaction (His383), which is located at the zinc-binding region. Zinc ions are critical for ACE catalytic activity, as they coordinate the active site and facilitate nucleophilic attack on the peptide bond between the two C-terminal amino acids of Angiotensin II (Ang II), thereby enabling its cleavage. This zinc ion is indispensable for ACE's enzymatic function, as its absence disrupts the structural and electrostatic environment required for efficient catalysis (Guang et al., 2012; Irsal et al., 2022). Furthermore, hydrogen bond interactions with Gln281 and Tyr523 stabilize the inactive conformation of ACE. Gln281 is involved in substrate binding, while Tyr523 mediates the conformational changes required for enzyme activation. Inhibitors that stabilize the inactive conformation of ACE effectively prevent its transition to the active form, thereby blocking the conversion of Ang I to Ang II (Natesh et al., 2003).

Notably, β-Glucogallin exhibited binding interactions to lisinopril, particularly at Tyr523 and His353, suggesting the same inhibitory mechanism. Based on binding affinity values, β-Glucogallin exhibits a higher binding affinity (-8.0 kcal/mol) compared to the control ligand lisinopril (-7.8 kcal/mol), suggesting stronger ligand-receptor interactions (Widyananda et al., 2021). The RMSD analysis confirmed stable ACE inhibition (Figure 3A), with the docking method being declared valid when the resulting RMSD value was ≤ 3Å (Martínez, 2015). Hydrogen bond formation directly correlates with binding stability (Figure 3B), with a higher numbers of bonds indicating stronger interactions and improved ligand-receptor affinity (Tallei et al., 2020; Ya'u Ibrahim et al., 2020). These bonds are critical for maintaining protein secondary structure, ensuring functional integrity during molecular inhibition (Pace et al., 2014). Furthermore, hydrogen bonds govern molecular recognition, protein folding stabilization, and the formation and stability of protein-ligand complexes (Ferreira De Freitas & Schapira, 2017). RMSF analysis showed that β-Glucogallin consistently interacts with key ACE residues (fluctuations ≤ 3Å), while the protein structure exhibits minimal deviation. The consistent RMSD and RMSF values below 3Å confirm overall stability (Fonseca et al., 2024).

The molecular docking result shows that both candesartan and β-Glucogallin docked into the AT1R bind-

ing site at Trp84 and Lys199, exhibiting binding affinities of -9,8 kcal/mol and -7,4 kcal/mol, respectively. However, candesartan exhibits higher binding affinity, whereas β-Glucogallin is pharmacologically advantageous due to its naturally derived, low toxicity profile. The β-Glucogallin compound binds to Trp84 and Lys199 residues on AT1R, a key receptor mediating vasoconstriction, blood pressure regulation, and renal function. The positively charged Lys199 ligand-receptor binding site interacts electrostatically with negatively charged ligand groups, reinforcing complex stability (Zhang et al., 2015).

This study found that both candesartan and β-Glucogallin bind competitively to the AT1R active site, specifically targeting two key residues critical for the receptor's structural integrity for ligand binding (Vauquelin et al., 2001). Molecular dynamics simulations revealed that the AT1R and β-Glucogallin complex exhibits high stability, with an RMSD value ≤3Å and low RMSF fluctuations (≤3Å) at the binding residues. The protein structure is considered stable if the majority of amino acids shown an RMSF value ≤3Å (Schön et al., 2011). Although docking methods and molecular dynamics simulations offer valuable initial insight, these results must be validated through in vitro and in vivo experiments to ensure biological and clinical relevance.

Phyllanthus emblica has previously been reported as an antihypertensive (Shanmugarajan et al., 2021), but its specific inhibitory mechanism remains unexplored.Our findings demonstrate that β-Glucogallin functions as a multi-target compound, exhibiting dual inhibitory activity against both ACE inhibitors and a potentially acting as an AT1R antagonist. This was supported by strong, specific binding observed in molecular docking and molecular dynamics simulations. This dual blockade could produce synergistic effects in lowering blood pressure and slowing CKD progression. Compared to conventional ACE inhibitors or ARBs, Malacca fruit is predicted to have a safer pharmacological profile with favorable antihypertensive potential. Additionally, it offers benefits such as antioxidant, anti-inflammatory, and cardioprotective effects (Jain et al., 2016; Yan et al., 2022).

Thus, the *in silico* analysis of this study represents a critical first step before experimental validation to confirm the efficacy and mechanism of action of these compounds under study. The *in silico* method is an effective solution in the early stages of drug discovery and design because it enables researchers to quickly and efficiently model and simulate molecular interactions (Ugurlu et al., 2024). Additionally, this approach increases the likelihood of successful *in vivo* and *in vitro* studies by providing early insight into the potential biological activity of the compounds being studied (Bai et al., 2018).

Conclusion

This study demonstrates that β-Glucogallin from Malacca fruit shows therapeutic potential as an antihypertensive agent by modulating the RAS pathway. The mechanism of action involves inhibition of ACE activity and AT1R, achieved by binding to key residues in their active sites, thereby preventing the conversion of Ang I to Ang II. *In silico* approaches, including ADMET prediction, molecular docking, and molecular dynamics simulation, were employed to determine the ability of these compounds to bind to ACE and AT1R, molecular targets that play an important role in the pathogenesis of CKD. The results indicated that β-Glucogallin, derived from a natural compounds, meets the criteria of a potential drug candidate in the terms of good pharmacokinetic profile and strong binding affinity. Further *in vitro* and *in vivo* studies are necessary to confirm the inhibition mechanism and pharmacological efficacy of β-Glucogallin.

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