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Original Article

Virtual screening of *Zanthoxylum acanthopodium* DC. Fruit bioactive compounds as natural angiotensin-converting enzyme inhibitors

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Abstract

The angiotensin-converting enzyme inhibitors (ACEi) are widely prescribed anti-hypertensive drugs that target the renin-angiotensin system (RAS). Yet, these drugs have limitations due to their side effects. This study aims to explore the potential of bioactive compounds from andaliman fruit (Zanthoxylum acanthopodium DC.) as safer natural ACE inhibitors for hypertension treatment using an in-silico approach. Phytoconstituents from Z. acanthopodium fruits were screened using the SwissADME web server to assess their bioavailability, while their bioactivity was predicted by PASS online web server. Selected compounds were then docked to human ACE (PDB ID: 1O86) by site-specific docking to the ACE binding pocket. The stability of protein-ligand interactions was evaluated using molecular dynamics simulations in YASARA software. The possible toxicity of the selected compounds then evaluated using protox web server. Out of 39 compounds tested, we predicted that five compounds, namely kaempferol, quercetin, abscisic acid, benzophenone, and cis-3,5,3',4'-tetrahydroxystilbene could bind to ACE with good affinity and formed interactions with key residues. The presence of these compounds might reduce the binding affinity of angiotensin I toward ACE and altered the interaction site. Molecular dynamics simulations demonstrate that these compounds exhibit good interaction stability with ACE and remained in the active site throughout the simulation. This study showed that five bioactive compounds from andaliman fruit act potentially as natural ACE inhibitor agents.

Keywords: ACE-inhibitor, Andaliman fruit, hypertension, renin-angiotensin system, *Zanthoxylum acanthopodium*
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Introduction

Hypertension is defined as an increase in systolic blood pressure (SBP) that exceeds 130 mmHg and diastolic blood pressure (DBP) that exceeds 90 mmHg, being a major risk factor for cardiovascular disease (Vidal-Petiot, 2022). It is estimated that 20% of global mortalities are related to hypertension (Parati et al., 2023). Despite the complex etiology of hypertension, the renin-angiotensin system (RAS) undoubtedly plays an important role in the pathogenesis of hypertension. This system consists of an enzymatic cascade that regulates several activities to increase blood pressure (Savoia et al., 2021). The main axis of this system involves the formation of the vasoconstrictive peptide angiotensin II (Ang II) from Angiotensin I (Ang I) by angiotensin-converting enzyme (ACE). The binding of Ang II to the angiotensin II type 1 receptor (AT1R) activates intracellular secondary messengers that lead to vasoconstriction, inflammation, and aldosterone secretion that collectively contribute to increased blood pressure (Bitker & Burrell, 2019). Several studies have reported the increased expression of RAS component in hypertension (Hsu & Tain, 2021). This has led to the RAS system becoming one of the main targets for the management of hypertension (Ahmad et al., 2019).

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The ACE is a zinc metallopeptidase enzyme that responsible to form the Ang II by the removal of 2 amino acid residue from the C terminal of Ang I. Alongside with Ang I, the vasodilator peptide bradykinin is another substrate for ACE. Degradation of bradykinin by ACE will prevent its vasodilatory function. The angiotensin-converting enzyme inhibitors (ACEi), is a group of widely prescribed anti-hypertensive drugs that exert their blood pressure-lowering effects through the inhibition of ACE, thereby preventing the formation of Ang II. (Messerli et al., 2018). ACEi administration have been consistently lowers blood pressure and provides cardioprotective effects, positioning them as recommended first-line drugs in hypertension management (Denker & Cohen, 2015). However, commercially available synthetic ACEi such as lisinopril and captopril are associated with undesirable side effects including dry cough, angioedema, and hypotension (Qian et al., 2019). In contrast, natural ACEi derived from plants appear to be harmless and show no significant side effects on normal metabolism. In addition, the use of herbal remedies also offers other beneficial properties such as antioxidant effects (Chakraborty & Roy, 2021).

Andaliman (Zanthoxylum acanthopodium DC) also known as Batak cuisine, is a native plant species originating from North Sumatra. The andaliman fruit is mainly used as a food ingredient, but it has gained attention due to the various biological activities exhibited with it, including antioxidant, antidiabetic, anti-inflammatory, antibacterial, and anticancer. These activities are manifested by the bioactive compounds present in the fruit, which include terpenoids, alkaloids,

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flavonoids, phenolics, and various other bioactive compounds (Wijaya et al., 2019). Andaliman fruit is often used in meat dishes based on local beliefs that it can prevent hypertension. However, data regarding its anti-hypertensive activity is scarce. It is proposed that bioactive compounds in andaliman fruit, particularly the flavonoids are potential agents for inhibiting ACE activity (Silalahi & Megaputri, 2019). Recently, reports of andaliman's anti-hypertensive activity are limited to studies in a rat model of preeclampsia, where administration of andaliman led to a reduction in blood pressure (Situmorang et al., 2021). However, there is no clear information regarding the anti-hypertensive activity of the bioactive compounds of andaliman fruit. This study's purpose is to identify the potential of andaliman bioactive compounds as natural ACE inhibitors through an in-silico approach.

Methods

Bioavailability prediction

To select and predict the potential bioactive compounds from andaliman fruit, started from the selection of phytochemical content. The phytochemical content of andaliman was obtained from available references, including 29 compounds from GC-MS analysis of the fruit essential oil (Moektiwardoyo et al., 2014) and 10 compounds from LC-MS analysis of the fruit ethanol extract (Rienoviar et al., 2019). To assess the bioavailability of each compound, the SwissAdme web server (http://www.swissadme.ch/) was used to calculate the Lipinski rule of 5 and the gastrointestinal absorption (GIA) rate. Compounds that violated more than 2 Lipinski parameters or showed low GIA were eliminated from the docking analysis.

Ligand and protein preparation

The 3D structure of selected compounds was then downloaded through the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in structure data format (SDF). Energy minimization of the ligands was carried out using Open Babel Tool in PyRx 0.8 software and converted to Protein Data Bank (PDB) format for docking analysis. Angiotensin-converting enzyme (ACE) in complex with lisinopril (PDB ID: 1O86) downloaded from RCSB PDB database (https://www.rcsb.org/) in pdb Format. Protein preparation was performed using Biovia Discovery Studio 2019. Water molecules were cleaned from the complex, while the lisinopril molecule was separated and saved as a control ligand. One zinc ion and two chloride ions were kept as they act as cofactors (Qian et al., 2019). Cleaned protein structure then saved in pdb format.

QSAR analysis

PASS online web server (http://www.way2drug.com/passonline/) was used for QSAR analysis to predicts the bioactivity of the compounds. The related activities included vasodilator, vasoprotector, and cardioprotector. Potentially active compounds were determined based on the Pa value (Sjakoer et al., 2021).

Molecular docking

Molecular interaction between selected compounds and ACE was predicted by docking analysis using Vina Wizzard in PyRx 0.8 software. The grid box position was centered on the Zn atom (Sharifi et al., 2013) at the following coordinates x = 43.918418253, y = 38.1847170676, z = 46.7154768072, with a size of x = 25.0, y = 25.0, z = 25.0. The Ligand pose with the lowest biding score was then selected and merged with the ACE structure using PyMol 2.5.4 software. The native substrate angiotensin I then docked to empty ACE and ACE-compound complexes with maximum grid box size. Visualization and analysis of the interaction were then carried out using Biovia Discovery Studio Studio 2019.

Molecular dynamics simulation

Molecular dynamics simulation was performed to evaluate the interaction stability of the ligand and protein over a specific time. YASARA software was used to run the simulation using the YASARA 2 forcefield. The period was set to 50 ns with the following conditions, pH 7.4, 0.9% NaCl ion concentration, 310^{o}K, and 0.997 water density (Grahadi et al., 2022).

Toxicity predictions

The toxicity of the potential compounds then evaluated using ProTox 3.0 web server (https://comptox.charite.de/protox3/). The predicted toxicity including hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, cardiotoxicity, and carcinogenicity (Banerjee et al., 2018).

Results

Initially, the 39 compounds were screened based on Lipinski's rule of five and the gastrointestinal absorption (GIA) rate. The Lipinski parameter predicts the potential of a molecule to exert biological activity when consumed by oral administration. The molecule should not violate more than 2 rules including molecular weight (MW) 500, hydrogen bond acceptor (HA) 10, hydrogen bond donor (HD) 5, LogP 5, TPSA 140, and rotatable bonds 10 (Chagas et al., 2018). The GIA is one crucial pharmacokinetics properties that determines the possibility of a drug to reach the bloodstream after oral administration (Daina & Zoete, 2016). Based on the screening result, 29 out of 39 compounds fulfilled the bioavailability parameters. In the QSAR analysis, Pa > 0.7 indicates high probability for the compound to exhibit the activity, while Pa value > 0.3 indicates moderate probability (Sjakoer et al., 2021; Daniel et al., 2023). The compounds with the good bioavailability and showed related activity then continued to the further analysis. Only the screening result of 5 selected compounds is shown in Table 1 and Table 2.

Table 1. Bioavailability prediction result of phytoconstituent in andaliman fruit using SwissADME.

A. Canonical SMILE and Gastrointestinal Absorption			
CID	Compound	Canonical SMILE	GIA
5280863	Kaempferol	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O	High
5280343	Quercetin	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O	High
5375199	Abscisic acid	CC1=CC(=O)CC(C1(C=CC(=CC(=O)O)C)O)(C)C	High
8571	Benzophenone	C1=CC(=C(C=C1O)O)C(=O)C2=C(C=C(C=C2)O)O	High
6603962	cis-3,5,3',4'-Tetrahydroxystilbene	C1=CC(=C(C=C1C=CC2=CC(=CC(=C2)O)O)O)O	High

B. Lipinski rule of 5 analysis								
CID	Compound	TPSA (<140)	Rotatable bonds (<10)	MW (<500)	HA (<10)	HD (<5)	LogP (<5)	Lipinski violations
280863	Kaempferol	111.13	1	286.24	6	4	1.58	0
280343	Quercetin	131.36	1	302.24	7	5	1.23	0
375199	Abscisic acid	74.6	3	264.32	4	2	1.96	0
8571	Benzophenone-2	97.99	2	246.22	5	4	1.5	0
6603962	cis-3,5,3',4'-Tetrahydroxystilbene	80.92	2	244.24	4	4	2.14	0

Note: CID: compound ID; GIA: gastrointestinal absorption; HA: hydrogen bond acceptor; HD: hydrogen bond donor; MW: molecular weight; TPSA: total polar surface area.

Table 2. Bioactivity prediction result of phytoconstituent in andaliman fruit using PASS online web server.

Compound	Activity	Pa	Pi
Kaempferol	vasoprotector	0,807	0,005
	vasodilator	0,502	0,021
	Vasodilator, coronary	0,483	0,028
	Vasodilator, peripheral	0,399	0,116
	Cardioprotectant	0,814	0,003
Quercetin	vasoprotector	0,842	0,004
	Vasodilator, coronary	0,486	0,028
	vasodilator	0,472	0,025
	Vasodilator, peripheral	0,351	0,109
	cardioprotectant	0,833	0,003
Abscisic acid	vasoprotector	0,336	0,120
Benzophenone	Vasoprotector	0,566	0,025
	Vasodilator, coronary	0,385	0,064
	Vasodilator, peripheral	0,362	0,103
	vasodilator	0,222	0,126
	cardioprotectant	0,433	0,018
Cis-3,5,3',4'-tetrahydroxystilbene	vasoprotector	0,776	0,006
	Vasodilator, peripheral	0,492	0,046
	Vasodilator, coronary	0,446	0,037
	vasodilator	0,374	0,046
	cardioprotectant	0,499	0,010

Molecular docking study was used to predict the molecular interaction between phytoconstituents of andaliman and ACE. The docking area was focused on the active site of ACE by site-specific docking in PyRx 8.0 software. According to (Fadahunsi et al., 2022) the active site of ACE contains a zinc ion and groups of binding pockets. The zinc ion is coordinated by HIS383, HIS387, and GLU411 residues (Natesh et al., 2003), having an important role in cleaving 2 amino acid residues from the carboxyl-terminal of Angiotensin II by nucleophilic attack (Guang et al., 2012). The binding pockets act to stabilize ACE-substrate interaction during the cleavage. These pockets are devided to S1 pocket (of ALA354, GLU384, and TYR523), S1' pocket (GLU162), and S2 pocket (GLN 281, HIS353, LYS511, HIS513, TYR520). The binding of inhibitors to these residues can inhibit the enzymatic activity (Looi et al., 2021).

As previously demonstrated by (Fatchiyah et al., 2015), we docked the native substrate to the ACE with and without the presence of an inhibitor to demonstrate the inhibition virtually. The native substrate Ang I was docked to empty ACE and ACE-compound complexes (Figure 2). The initial binding score of Ang I was -11.7 Kcal/mol in empty ACE. A total of 39 interactions were formed between Ang I and ACE, consisting of 22 hydrogen bonds, 14 hydrophobic bonds, 1 salt bridge, and 2 electrostatic interactions. Six out of the 22 hydrogen bonds were formed with binding pocket residues, namely residues GLU162, GLU162, GLU162, GLU384, HIS353, and HIS513. Additionally, four out of the 14 hydrophobic bonds are formed with binding pocket residues HIS353, HIS353, HIS353, and TYR523. Only 1 out of the 3 electrostatic interactions is formed with binding pocket residue GLU162. Among the five binding pocket residues that interact with Ang I, GLU384 and TYR523 represented the S1 pocket, GLU162 represented S1' pocket, then HIS353 and HIS353 represented the S2 pocket. In the scheme illustrated by (Bernstein et al., 2013), the part of Ang I that interacts with the binding pocket is the carboxyl-terminal residues that will be cleaved, where the first, second, and third amino acids from the carboxyl end sequentially bind to the S2, S1', and S1 binding pockets. However, in our docking results, the part of Ang I that was interacting with the binding pocket is not the carboxyl end.

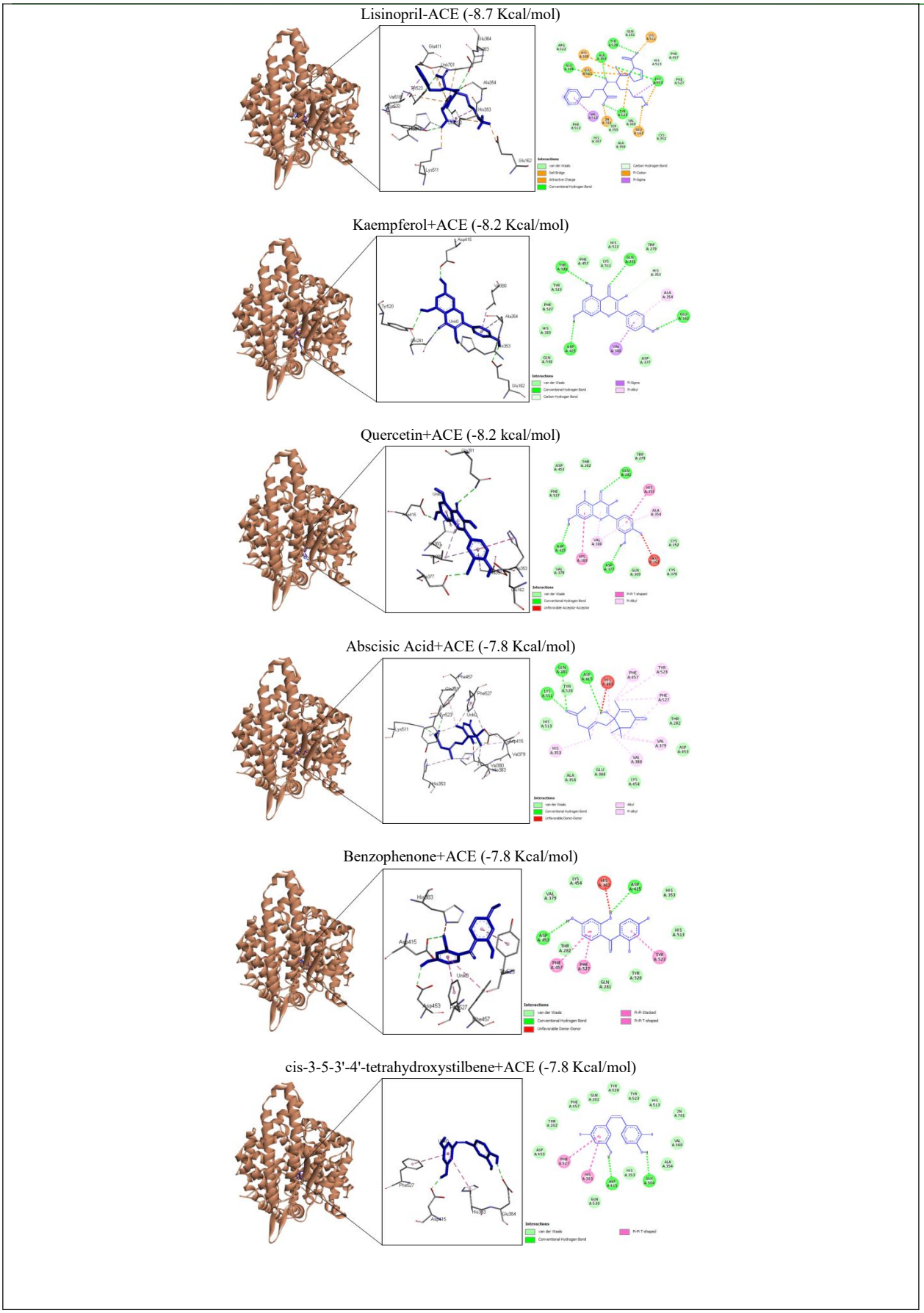
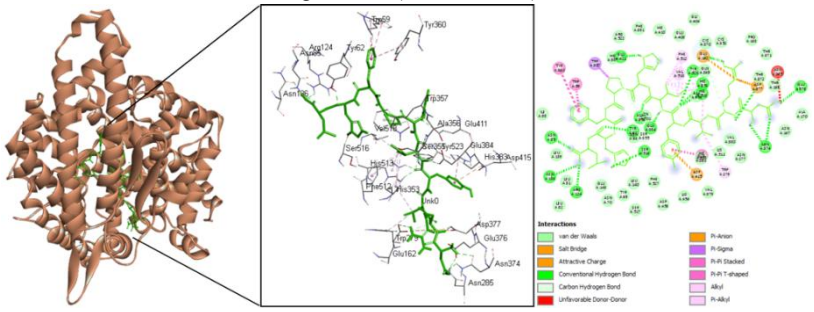
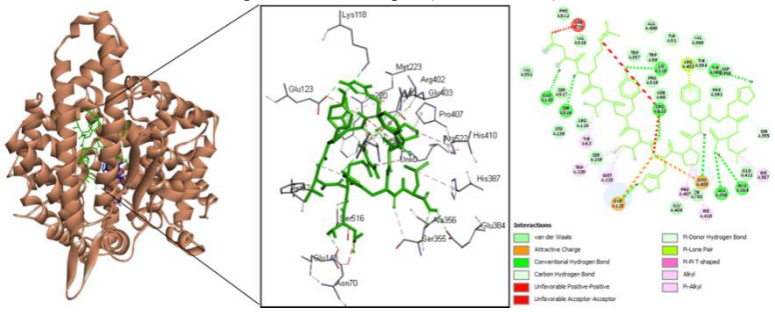


Figure 1 The molecular interaction between phytoconstituents of Andaliman and ACE. The 3D structure of the complexes is shown in the left panel and specified in the box panel. Receptor and ligand molecules were colored in brown and blue respectively. The 2D structure in the right panel showed the type of interactions

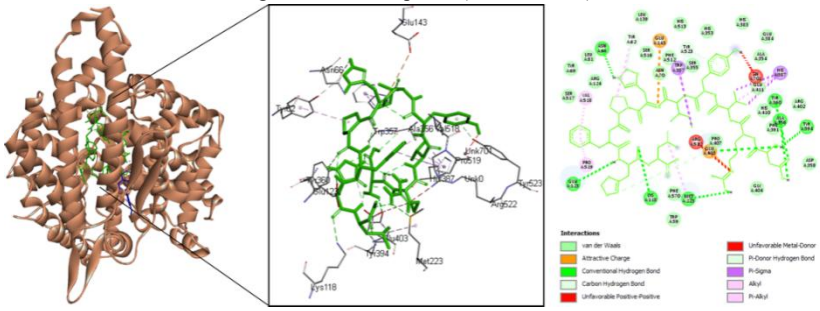
Ang I + ACE (-11.7 Kcal/mol)



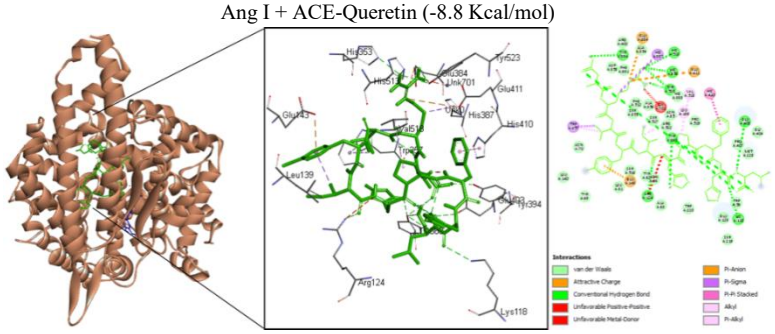
Ang I + ACE-Lisinopril (-5.8 Kcal/mol)



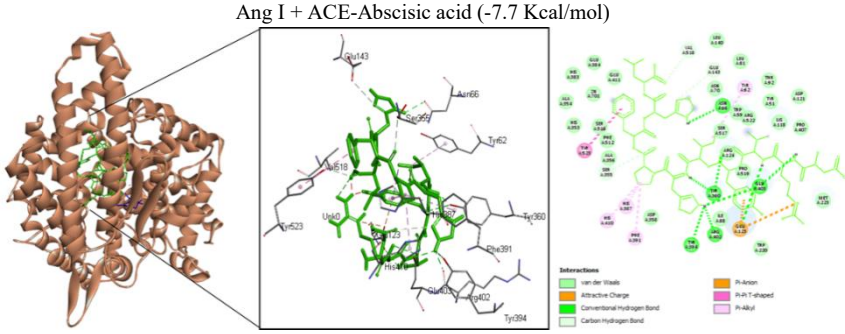
Ang I + ACE-Kaempferol (-8.0 Kcal/mol)



Ang I + ACE-Queretin (-8.8 Kcal/mol)



Ang I + ACE-Abscisic acid (-7.7 Kcal/mol)



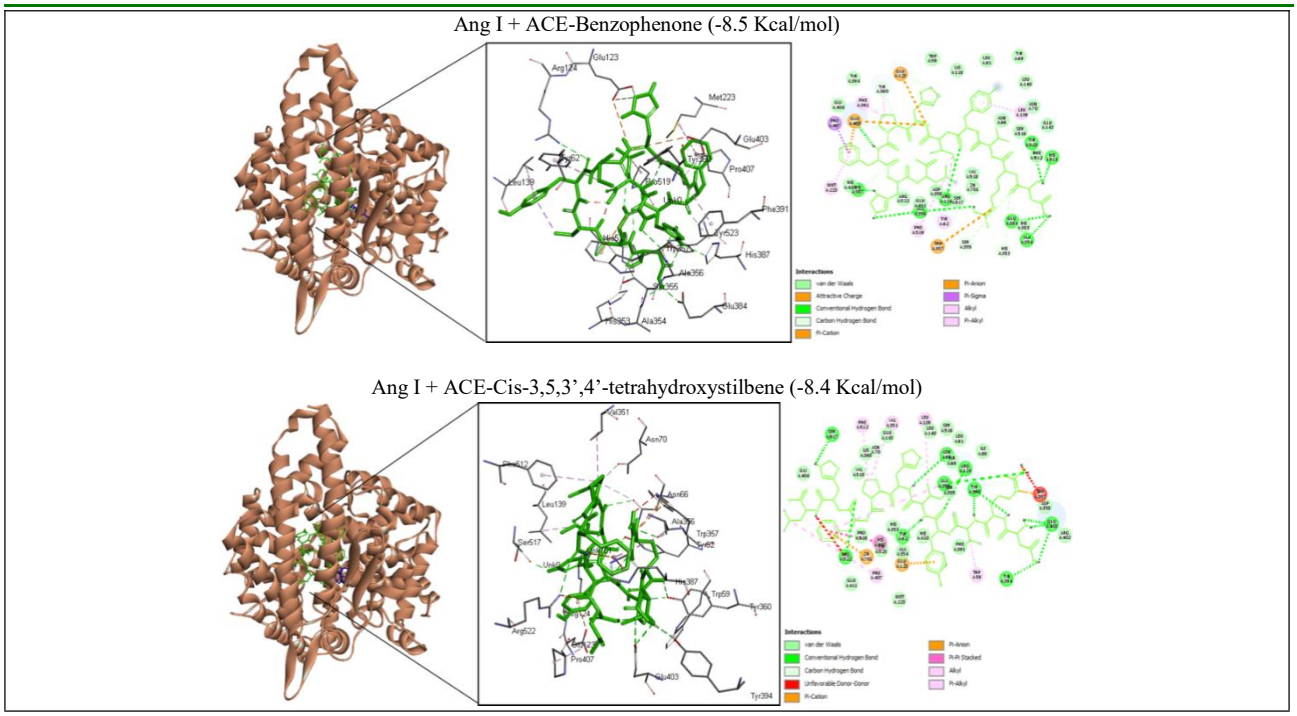


Figure 2. The molecular interaction between Angiotensin I (Ang I) and ACE/ACE-inhibitor complexes. The 3D structure of the complexes showed in the left panel and specified in the box panel. ACE, inhibitor, and Ang I molecules were colored in brown, blue, and green respectively. The 2D structure in the right panel showed the types of interactions.

The compounds from andaliman fruit were expected to act as a competitive inhibitor at the active site of ACE, preventing Ang I from interacting with the active site. According to (Schön et al., 2011) , although not completely obstructing the substrate from binding to the target protein due to the significantly larger size of the substrate, the binding of the compound at crucial points in the protein's active site can influence protein activity. This is caused by the presence of compounds capable of displacing ligand interaction sites and reducing their affinity for binding to the protein. In the presence of an inhibitor, we observed that Ang I binding affinity toward ACE was decreased. The most significant binding affinity decrease was exhibited by lisinopril (-5.8 Kcal/mol), followed by abscisic acid (-7,7 Kcal/mol), kaempferol (-8.0 Kcal/mol), Cis-3,5,3',4'-tetrahydroxystilbene (-8.4 Kcal/mol), benzophenone (-8.5 Kcal/mol), and quercetin (-8.8 Kcal/mol). The presence of these inhibitors was also managed to alter the Ang I interaction site inside ACE.

Cis-3,5,3',4'-tetrahydroxystilbene were found to completely prevent Ang I from interacting with the binding pocket. Notably, the key residues interacting with this compound were only 1 binding pocket residue and 1 zinc coordinating residue. In the presence of lisinopril, abscisic acid, and kaempferol, only 1 binding pocket residue was observed to interact with Ang I, but the affinity of Ang I toward the complexes was different. In previous study by (Zarei et al., 2019), the number of interactions between a compound with ACE was in correlation with the inhibitory activity level. Additionally, interaction with the zinc ion and its coordinating residue can give higher inhibition activity (Selmi et al., 2021). Lisinopril which formed interactions with 12 residues, including the zinc ion and the coordinating residues, exhibited the most significant decrease in Ang I binding affinity toward ACE (-5.8 Kcal/mol). Abscisic acid which formed interaction with 10 residues, including interaction with the zinc coordinating residue, reduced the affinity of Ang I closest to lisinopril (-7.7 Kcal/mol). Kaempferol which formed interaction with 7 residues without any interaction with the zinc coordinating residue or the zinc ion, showed a lower affinity decrease of Ang 1 (-8.0 Kcal/mol). In the ACE-quercetin complex, despite quercetin formed bonds with 3 binding pockets and 1 bond with the zinc coordinating residue, Ang I still managed interacts with 4 binding pocket residues GLU384, HIS513, HIS353, and TYR523. In the ACE-benzophenone complex, Ang I still interact with 5 binding pocket residues TYR523, HIS513, GLU384, ALA354, and HIS353. However, the S1' pocket which is only composed by GLU162 residue was still inaccessible. This pocket is responsible in binding the second amino acid residue from the carboxyl end of the substrate (Bernstein et al., 2013). Previously in the absence of the inhibitors, Ang I formed 4 bonds with the GLU162 residue.

Molecular dynamic simulation was carried out to evaluate the ACE-compound interactions stability. The complex of ACE with lisinopril as the control ligand, and complexes of ACE with five selected compounds were simulated for a 50 ns period. The root-mean-square deviation (RMSD) all value represents the structural similarity of all atoms in the complex at a specific time interval compared to the initial structure. A lower RMSD all value indicates a more stable structure with minimal conformational changes (Maruyama et al., 2023). The simulation result in Figure 3A showed all complexes have RMSD all values below 3 Å, indicating the structures are

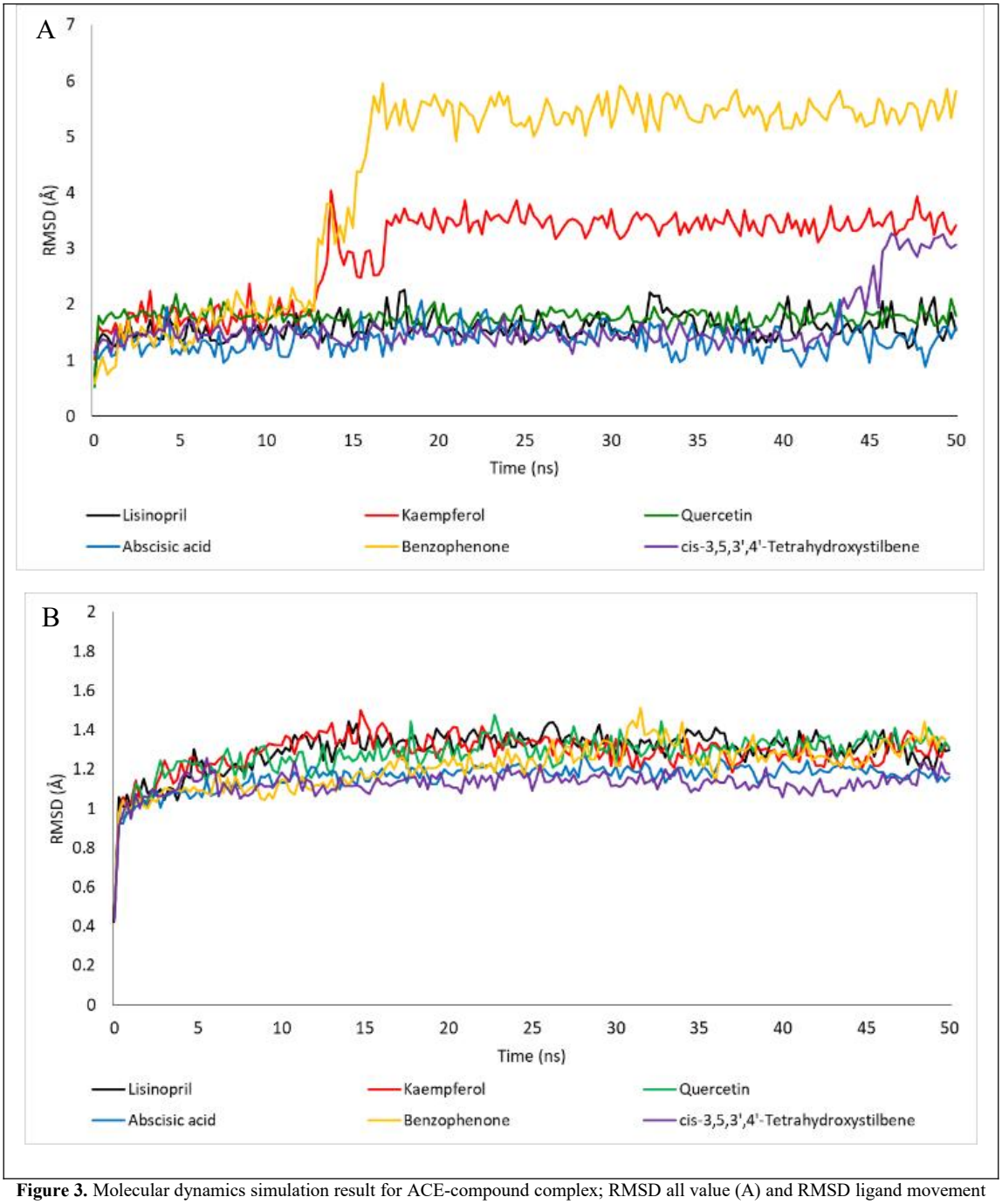


Figure 3. Molecular dynamics simulation result for ACE-compound complex; RMSD all value (A) and RMSD ligand movement value (B).

stable (Widyananda et al., 2023). The ligand movement value in Figure 3B represents the movement of the ligand inside the binding pocket of the protein (Istyastono & Riswanto, 2022). A stable ligand movement value indicating the ligand doesn't move much inside the protein (Widyananda et al., 2022). Lisinopril, abscisic acid, and quercetin showed stable ligand movement starting from the early period until the end of the simulation. The ligand movement value of cis-3,5,3',4'-tetrahydroxystilbene was stable in the early to the mid period of simulation but began to rise at 43 ns. Kaempferol and benzophenone showed a rise in ligan movement value at 13 ns and remained stable after 16 ns until the end of the simulation. This indicates the two compounds explored the binding pockets and changed their orientation until they reached the stable conformation (Kaye et al., 2006).

The compound's final conformation after the simulation was then visualized to confirm their position inside ACE (Nafisah et al., 2022). Overall, after the simulation, all compounds were remained in the active site area of ACE as they kept interacted with the binding pocket residue (Figure 4). Kaempferol formed an additional hydrogen bond to the zinc coordinating residue HIS383 after the simulation. Quercetin, abscisic acid, and benzophenone, which previously showed unfavorable

bonds, no longer showed this interaction after simulation. For abscisic acid, new hydrogen bonds were formed with 2 other binding pocket residues TYR520 and HIS513. The hydrogen bond with residue LYS511 was also changed to a salt bridge. Benzophenone which previously only interacted with 1 binding pocket residue, after simulation found to interact with 2 binding pocket residues and formed a hydrophobic bond with the zinc coordinating residue HIS383. The cis-3,5,3',4'-tetrahydroxystilbene before the simulation was interacted with only 1 binding pocket residue, and after the simulation found to interact with 3 binding pocket residues.

The protox web server was used to assess possible organ toxicity associated with the compounds, including hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, cardiotoxicity, and carcinogenicity. Abscisic acid was the only compound predicted not to be toxic, whereas the other four selected compounds were determined to potentially induce at least two types of toxicity (Table 3). Therefore, further *in vitro* and *in vitro* test are necessary to validate their toxicological effect and determine the safe dose for hypertension treatment.

Table 3. Toxicity prediction result of phytoconstituents of andaliman fruit using protox web server.

Compound	Hepato-toxicity	Neuro-toxicity	Nephro-toxicity	Respiratory toxicity	Cardio-toxicity	Carcino-genicity
Kaempferol	Inactive	Inactive	Active	Active	Inactive	Inactive
Quercetin	Inactive	Inactive	Active	Active	Inactive	Active
Abscisic acid	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Benzophenone	Inactive	Inactive	Active	Active	Inactive	Inactive
Cis-3,5,3',4'-tetrahydroxystilbene	Inactive	Inactive	Active	Inactive	Inactive	Active

Discussion

The Renin-Angiotensin System (RAS) is a primary hormonal cascade that regulates blood pressure by controlling vascular constriction and circulating blood volume throughout the body. Dysregulation of the RAS can lead to several pathological conditions, especially hypertension (Fajloun & Sabatier, 2024). Previous studies have highlighted ACE inhibition as one of the most reported mechanisms from medicinal plants to stabilize blood pressure (Chakraborty & Roy, 2021). By inhibiting ACE activity, the formation of Ang II is hindered, thereby preventing the activation of AT1R. This inhibition helps to prevent the downstream effects of RAS such as vasoconstriction and water retention, which could otherwise increase blood pressure (Khan & Kumar, 2019).

In this study, *in silico* methods were utilized to investigate the presence of bioactive compounds in andaliman fruits that capable to inhibit ACE. The bioavailability screening of the compounds is an important step during drug discovery (Cascone et al., 2016). The Lipinski's rule of 5 evaluated whether a compound may exhibit therapeutic effect after oral administration (Chagas et al., 2018). Additionally, these compounds should be adequately absorbed by the gastrointestinal tract to reach the circulation and find its target (Cascone et al., 2016). The QSAR analysis was utilized to determine each compound possibility to exhibit the desired bioactivity (Sjakoer et al., 2021). Molecular docking method then employed to visualize how the compounds interact with the target protein and estimated the binding affinity (Tanchuk et al., 2016). As many aspects were neglected in molecular docking, the docking result then refined through molecular dynamics simulation, by applying biomolecular forcefield, water molecules, ions, and other components that mimic the *in vivo* condition (Nairs & Miners, 2014).

Here, we identified five compounds from andaliman fruit, namely kaempferol, quercetin, abscisic acid, benzophenone, and cis-3,5,3',4'-tetrahydroxystilbene as potential natural ACE inhibitors. These compounds can interact with binding pocket residues or ion-coordinating residues in ACE active site, preventing Ang I from accessing the active site. However, none of the compounds managed to interact with the zinc ion as lisinopril does. While these compounds did not completely block Ang I from interacting with ACE, they managed to reduce Ang I's binding affinity toward ACE and altered the interaction sites. We observed that one residue, ASP415, which is not part of the binding pockets, formed a hydrogen bond with the five compounds. This residue is also reported to interact with some anti-hypertensive bioactive compounds and peptides in several studies (Liu et al., 2018; Ma et al, 2023; Oke et al., 2022).

Currently, precise data regarding the full orientation of the 10 amino acid residues from Ang I inside ACE is not available. As shown by Bernstein (2018), the three binding pockets of ACE are known to bind the last three amino acid residues from the C-terminal of Ang I, while information for the remaining amino acid residues is lacking. In our docking results, we observed several non-binding pocket residues that also interacted with Ang I, TRP59, TYR62, ASN85, ARG124, ASN136, TRP279, ASN374, GLU376, ASP377, SER355, ALA356, TRP357, TYR360, ASP415, SER516, PHE512, and VAL518 (Figure 2). These residues might contribute to stabilizing the receptor-ligand interaction by binding to the remaining amino acid residues outside the three residues from the C-terminal.

Among the five compounds, kaempferol and quercetin are two compounds that have been reported to have ACE inhibition activity. A study by (Olszanecki et al., 2008) revealed that kaempferol can inhibit the formation of Ang II in rat aortas. An *in vitro* study on *Ailanthus excelsa* showed that kaempferol-3-O-β-galactopyranoside is the flavonoid compound with the highest ACE inhibition

activity among other compounds present in the plant, with an IC50 value of 260 µM (Loizzo et al., 2007). In our study, collectively based on the interaction sites, affinity towards ACE, and molecular dynamics simulation, we suggest that kaempferol is one of the compounds that may have the best inhibitory activity. Quercetin also has been reported to exhibit ACE inhibition activity both *in vivo* and *in vitro*. Oral and intravenous administration of quercetin to Wistar rats can inhibit ACE activity, as indicated by a decrease in the hypertensive effect of Ang I injection and an increase in the hypotensive effect of bradykinin injection (Häckl et al., 2002). The study by (Loizzo et al., 2007) also showed that Quercetin-3-O-α-arabinopyranoside from *Ailanthus excelsa* has an IC50 value of 310 µM. The abscisic acid has previously been reported to lower blood pressure in hypertensive rats due to atherosclerosis (Amir et al., 2010), but there is no information on its effect on ACE activity. The other 2 compounds, benzophenone and cis-3,5,3',4'tetrahydroxystilbene, have not been reported to have ACE inhibition or antihypertensive activities.

Compared to synthetic ACE inhibitors (ACEi) drugs, andaliman fruit may offer several advantages. In addition to their anti-hypertensive activity, andaliman fruit provides other beneficial biological activities such as antioxidant, anti-inflammatory, and cardioprotective effects (Adrian et al., 2023). Despite this, their dosage and toxicity in treating hypertension still need to be evaluated.

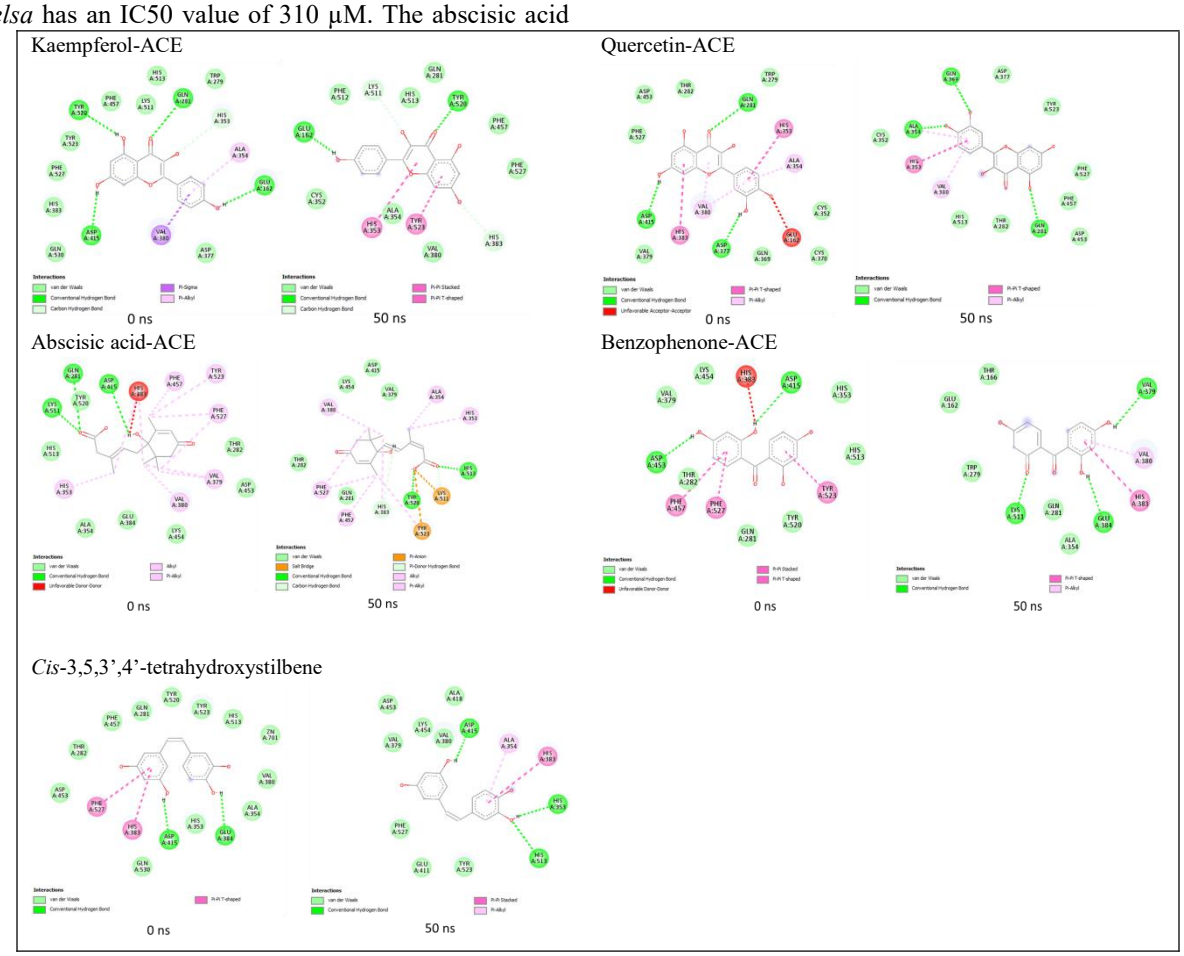


Figure 4. 2D diagram of interaction of the selected compounds toward ACE before molecular dynamic simulation (0 ns) and after simulation (50 ns).

Conclusion

This study concluded that the five bioactive compounds from Andaliman fruit namely kaempferol, quercetin, abscisic acid, benzophenone, and cis-3,5,3',4'-tetrahydroxystilben were identified as potential natural ACE inhibitors compounds from Andaliman fruit. Based on *in silico* analysis, these compounds were able to reduce the affinity of Ang I to bind to ACE and prevent it from accessing the ACE active site.

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References

Abdelhedi, O., Nasri, R., Jridi, M., Mora, L., Oseguera-Toledo, M. E., Aristoy, M. C., Amara, I. Ben, Toldrá, F., & Nasri, M. (2017). In silico analysis and antihypertensive effect of ACE-inhibitory peptides from smooth-hound viscera protein hydrolysate: Enzyme-peptide interaction study using molecular docking simulation. *Process Biochemistry*, *58*, 145–159. https://doi.org/10.1016/j.procbio.2017.04.032

Adrian, Syahputra, R. A., Juwita, N. A., Astyka, R., & Lubis, M. F. (2023). Andaliman (Zanthoxylum acanthopodium DC.) a herbal medicine from North Sumatera, Indonesia: Phytochemical and pharmacological review. In *Heliyon* (Vol. 9, Issue 5). Elsevier Ltd. https://doi.org/10.1016/j.heliyon.2023.e16159

Ahmad, I., Ambarwati, N., Elya, B., Omar, H., Mulia, K., Yanuar, A., Negishi, O., & Muńim, A. (2019). A new angiotensin-converting enzyme inhibitor from Peperomia pellucida (L.) Kunth. *Asian Pacific Journal of Tropical Biomedicine*, *9*(6), 257–262. https://doi.org/10.4103/2221-1691.260398

Amir J. Guri, Sarah A. Misyak, Raquel Hontecillas, Alyssa Hasty, Dongmin Liu, Hongwei Si, and J. B.-R. (2010). 基因的改变NIH Public Access. *The Journal of Nutritional Biochemistry*, *21*(12), 1178–1185. https://doi.org/10.1016/j.jnutbio.2009.10.003.Abscisic

Banerjee, P., Eckert, A. O., Schrey, A. K., & Preissner, R. (2018). ProTox-II: a webserver for the prediction of toxicity of chemicals. *Nucleic acids research*, *46*(1), 257-263. https://doi.org/10.1093/nar/gky318

Bernstein, K. E., Ong, F. S., Blackwell, W. L. B., Shah, K. H., Giani, J. F., Gonzalez-Villalobos, R. A., Shen, X. Z., & Fuchs, S. (2013). A modern understanding of the traditional and nontraditional biological functions of angiotensin-converting enzyme. *Pharmacological Reviews*, *65*(1), 1–46. https://doi.org/10.1124/pr.112.006809

Bitker, L., & Burrell, L. M. (2019). Classic and Nonclassic Renin-Angiotensin Systems in the Critically Ill. In *Critical Care Clinics* (Vol. 35, Issue 2, pp. 213–227). W.B. Saunders. https://doi.org/10.1016/j.ccc.2018.11.002

Cascone, S., Lamberti, G., Marra, F., Titomanlio, G., d'Amore, M., & Barba, A. A. (2016). Gastrointestinal behavior and ADME phenomena: I. In vitro simulation. In *Journal of Drug Delivery Science and Technology* (Vol. 35, pp. 272–283). Editions de Sante. https://doi.org/10.1016/j.jddst.2016.08.002

Chagas, C. M., Moss, S., & Alisaraie, L. (2018). Drug metabolites and their effects on the development of adverse reactions: Revisiting Lipinski's Rule of Five. *International Journal of Pharmaceutics*, *549*(1–2), 133–149. https://doi.org/10.1016/j.ijpharm.2018.07.046

Chakraborty, R., & Roy, S. (2021). Angiotensin-converting enzyme inhibitors from plants: A review of their diversity, modes of action, prospects, and concerns in the management of diabetes-centric complications. *Journal of Integrative Medicine*, *19*(6), 478–492. https://doi.org/10.1016/j.joim.2021.09.006

Daina, A., & Zoete, V. (2016). A BOILED-Egg To Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules. *ChemMedChem*, 1117–1121. https://doi.org/10.1002/cmdc.201600182

Daniel, N., Ferdinand, F., & Aditya, P. A. (2023). In silico targeting CYP51 of Naegleria fowleri using bioactive compounds from Indonesian plants. *Journal of Pharmacy & Pharmacognosy Research*, *11*(5), 841-862. DOI: https://doi.org/10.56499/jppres23.1693_11.5.841

Denker, M. G., & Cohen, D. L. (2015). Antihypertensive Medications in End-Stage Renal Disease. *Seminars in Dialysis*, *28*(4), 330–336. https://doi.org/10.1111/sdi.12369

Fadahunsi, O. S., Olorunnisola, O. S., Adegbola, P. I., Subair, T. I., & Elegbeleye, O. E. (2022). Angiotensin converting enzyme inhibitors from medicinal plants: a molecular docking and dynamic simulation approach. *In Silico Pharmacology*, *10*(1). https://doi.org/10.1007/s40203-022-00135-z

Fajloun, Z., & Sabatier, J. M. (2024). The Unsuspected Role of the Renin-Angiotensin System (RAS): Could its Dysregulation be at the Root of All Non-Genetic Human Diseases? In *Infectious Disorders - Drug Targets* (Vol. 24, Issue 1, pp. 69–72). Bentham Science Publishers. https://doi.org/10.2174/1871526524666230914114524

Fatchiyah, F., Hardiyanti, F., & Widodo, N. (2015). Selective inhibition on RAGE-binding AGEs required by bioactive peptide alpha-S2 case in protein from goat Ethawah breed milk: Study of biological modeling. *Acta Informatica Medica*, *23*(2), 90–96. https://doi.org/10.5455/aim.2015.23.90-96

Grahadi, R., Fatchiyah, F., & Kurniawan, N. (2022). Virtual prediction of potential immunogenic epitope of candoxin protein from Malayan krait (Bungarus candidus) venom. *Journal of Pharmacy and Pharmacognosy Research*, *10*(6), 1046–1057. https://doi.org/10.56499/jppres22.1469_10.6.1046

Guang, C., Phillips, R. D., Jiang, B., & Milani, F. (2012). Three key proteases - Angiotensin-I-converting enzyme (ACE), ACE2 and renin - Within and beyond the renin-angiotensin system. *Archives of Cardiovascular Diseases*, *105*(6–7), 373–385. https://doi.org/10.1016/j.acvd.2012.02.010

Häckl, L. P. N., Cuttle, G., Sanches Dovichi, S., Lima-Landman, M. T., & Nicolau, M. (2002). Inhibition of angiotensin-converting enzyme by quercetin alters the vascular response to bradykinin and angiotensin I. *Pharmacology*, *65*(4), 182–186. https://doi.org/10.1159/000064341

Hsu, C. N., & Tain, Y. L. (2021). Targeting the renin–angiotensin–aldosterone system to prevent hypertension and kidney disease of developmental origins. *International Journal of Molecular Sciences*, *22*(5), 1–23. https://doi.org/10.3390/ijms22052298

Istyastono, E. P., & Riswanto, F. D. O. (2022). Molecular Dynamics Simulations of the Caffeic Acid Interactions To Dipeptidyl Peptidase Iv. *International Journal of Applied Pharmaceutics*, *14*(4), 274–278. https://doi.org/10.22159/ijap.2022v14i4.44631

Kaye, S. L., Sansom, M. S. P., & Biggin, P. C. (2006). Molecular dynamics simulations of the ligand-binding domain of an N-methyl-D-aspartate receptor. *Journal of Biological Chemistry*, *281*(18), 12736–12742. https://doi.org/10.1074/jbc.M512728200

Khan, M. Y., & Kumar, V. (2019). Mechanism & inhibition kinetics of bioassay-guided fractions of Indian medicinal plants and foods as ACE inhibitors. *Journal of Traditional and Complementary Medicine*, *9*(1), 73–84. https://doi.org/10.1016/j.jtcme.2018.02.001

Li, P., Jia, J., Fang, M., Zhang, L., Guo, M., Xie, J., Xia, Y., Zhou, L., & Wei, D. (2014). In vitro and in vivo ACE inhibitory of pistachio hydrolysates and in silico mechanism of identified peptide binding with ACE. *Process Biochemistry*, *49*(5), 898–904. https://doi.org/10.1016/j.procbio.2014.02.007

Liu, C., Fang, L., Min, W., Liu, J., & Li, H. (2018). Exploration of the molecular interactions between angiotensin-I-converting enzyme (ACE) and the inhibitory peptides derived from hazelnut (Corylus heterophylla Fisch.). *Food Chemistry*, *245*, 471–480. https://doi.org/10.1016/j.foodchem.2017.10.095

Loizzo, M. R., Said, A., Tundis, R., Rashed, K., Statti, G. A., Hufner, A., Menichini, F., & Loizzo, M. R. (2007). Inhibition of Angiotensin Converting Enzyme (ACE) by Flavonoids isolated from Ailanthus excelsa (Roxb) (Simaroubaceae). *Phytother. Res*, *21*(27), 32–36. https://doi.org/10.1002/ptr

Loo, R. R. O., & Loo, J. A. (2016). Salt Bridge Rearrangement (SaBRe) Explains the Dissociation Behavior of Noncovalent Complexes. *Journal of the American Society for Mass Spectrometry*, *27*(6), 975–990. https://doi.org/10.1007/s13361-016-1375-3

Looi, D., Goh, B. H., Khan, S. U., Ahemad, N., & Palanisamy, U. D. (2021). Metabolites of the ellagitannin, geraniin inhibit human ACE; in vitro and in silico evidence. *International Journal of Food Sciences and Nutrition*, *72*(4), 470–477. https://doi.org/10.1080/09637486.2020.1830263

Ma, Z., Zheng, M., Liu, Z., Lu, H., Liu, Y., Yang, Y., Fang, Z. & Lu, S., (2023). Identification of potential angiotensin-I-converting enzyme inhibitory components in celery seed using UHPLC-MS and molecular docking. *Process Biochemistry*, *128*, 98-105. https://doi.org/10.1016/j.procbio.2023.02.025

Maruyama, Y., Igarashi, R., Ushiku, Y., & Mitsutake, A. (2023). Analysis of Protein Folding Simulation with Moving Root Mean Square Deviation. *Journal of Chemical Information and Modeling*, *63*(5), 1529–1541. https://doi.org/10.1021/acs.jcim.2c01444

Messerli, F. H., Bangalore, S., Bavishi, C., & Rimoldi, S. F. (2018). Angiotensin-Converting Enzyme Inhibitors in Hypertension: To Use or Not to Use? *Journal of the American College of Cardiology*, *71*(13), 1474–1482. https://doi.org/10.1016/j.jacc.2018.01.058

Nafisah, W., Fatchiyah, F., Widyananda, M. H., Christina, Y. I., Rifa'I, M., Widodo, N., & Djati, M. S. (2022). Potential of bioactive compound of Cyperus rotundus L. rhizome extract as inhibitor of PD-L1/PD-1 interaction: An in silico study. *Agriculture and Natural Resources*, *56*(4), 751–760. https://doi.org/10.34044/J.ANRES.2022.56.4.09

Nair, P. C., & Miners, J. O. (2014). Molecular dynamics simulations: from structure function relationships to drug discovery. *In silico pharmacology*, *2*, 1-4. doi:10.1186/s40203-014-0004-8

Natesh, R., Schwager, S. L. U., Sturrock, E. D., & Acharya, K. R. (2003). Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. *Nature*, *421*(6922), 551–554. https://doi.org/10.1038/nature01370

Odhar, H. A., Hashim, A. F., & Humad, S. S. (2022). Molecular docking analysis and dynamics simulation of salbutamol with the monoamine oxidase B (MAO-B) enzyme. *Bioinformation*, *18*(3), 304–309. https://doi.org/10.6026/97320630018304

Oke, A. M., Adelakun, A. O., Akintelu, S. A., Soetan, E. A., Oyebamiji, A. K., & Ewemoje, T. A. (2022). Inhibition of angiotensin converting enzyme by phytochemicals in Cucurbita pepo L.: In silico Approach. *Pharmacological Research - Modern Chinese Medicine*, *4*. https://doi.org/10.1016/j.prmcm.2022.100142

Olszanecki, R., Bujak-Gizycka, B., Madej, J., Suski, M., Wołkow, P. P., Jawień, J., & Korbut, R. (2008). Kaempferol, but not resveratrol inhibits angiotensin converting enzyme. *Journal of Physiology and Pharmacology*, *59*(2), 387–392.

Ompusunggu, N. P., & Irawati, W. (2021). Andaliman (Zanthoxylum Acanthopodium DC.), a Rare Endemic Plant from North Sumatra that Rich in Essential Oils and Potentially as Antioxidant and Antibacterial. *Jurnal Biologi Tropis*, *21*(3), 1063–1072. https://doi.org/10.29303/jbt.v21i3.2961

Parati, G., Goncalves, A., Soergel, D., Bruno, R. M., Caiani, E. G., Gerdts, E., Mahfoud, F., Mantovani, L., Mcmanus, R. J., Santalucia, P., & Kahan, T. (2023). New perspectives for hypertension management: progress in methodological and technological developments. *European Journal of Preventive Cardiology*, *30*(1), 48–60. https://doi.org/10.1093/eurjpc/zwac203

Pasqualoto, K. F. M., Silva, B. A. V. G., & Kodama, R. T. (2018). Exploring the C-domain inhibition of angiotensin converting enzyme through novel bradykinin potentiating peptides. *Pharmacy & Pharmacology International Journal*, *6*(6). https://doi.org/10.15406/ppij.2018.06.00210

Qian, B., Tian, C., Huo, J., Ding, Z., Xu, R., Zhu, J., Yu, L., & Villarreal, O. D. (2019). Design and evaluation of four novel tripeptides as potent angiotensin converting enzyme (ACE) inhibitors with anti-hypertension activity. *Peptides*, *122*, 170171. https://doi.org/10.1016/j.peptides.2019.170171

Rienoviar, R., & Setyaningsih, D. (2019). Studi Senyawa Aroma Ekstrak Andaliman (Zanthoxylum acanthopodium) dari Beberapa Pelarut Menggunakan Gas Chromatography - Mass Spectra (GC-MS). *Warta Industri Hasil Pertanian*, *35*(2), 85. https://doi.org/10.32765/wartaihp.v35i2.4292

Savoia, C., Volpe, M., & Kreutz, R. (2021). Hypertension, a moving target in COVID-19: Current views and perspectives. *Circulation Research*, *128*(7), 1062–1079. https://doi.org/10.1161/CIRCRESAHA.121.318054

Schön, A., Lam, S. Y., & Freire, E. (2011). Thermodynamics-based drug design: Strategies for inhibiting protein-protein interactions. *Future Medicinal Chemistry*, *3*(9), 1129–1137. https://doi.org/10.4155/fmc.11.81

Selmi, A., Aydi, R., Kammoun, O., Bougatef, H., Bougatef, A., Miled, N., Alghamdi, O. A., & Kammoun, M. (2021). Synthesis, crystal structure, molecular docking studies and biological evaluation of aryl substituted dihydroisoquinoline imines as a potent angiotensin converting enzyme inhibitor. *Journal of Molecular Structure*, *1235*, 130230. https://doi.org/10.1016/j.molstruc.2021.130230

Sharifi, N., Souri, E., Ziai, S. A., Amin, G., Amini, M., & Amanlou, M. (2013). Isolation, identification and molecular docking studies of a new isolated compound, from Onopordon acanthium: A novel Angiotensin Converting Enzyme (ACE) inhibitor. *Journal of Ethnopharmacology*, *148*(3), 934–939. https://doi.org/10.1016/j.jep.2013.05.046

Silalahi, S. & Megaputri, T. R. (2019). Effect of extraction solvent on total flavonoid content of andaliman fruit (Zanthoxylum acanthopodium DC). *Pro Food (Jurnal Ilmu Dan Teknologi Pangan)*, *5*(2), 540–543.

Situmorang, P. C., Ilyas, S., Hutahaean, S., & Rosidah, R. (2021). Histological changes in placental rat apoptosis via FasL and cytochrome c by the nano-herbal Zanthoxylum acanthopodium. *Saudi Journal of Biological Sciences*, *28*(5), 3060–3068. https://doi.org/10.1016/j.sjbs.2021.02.047

Sjakoer, N., Mubarakati, N., & Taufiq, A. (2021). Investigation of Excellent ACE Inhibitor Agents from Scurrula atropurpure and Dendrophthoe pentandra for Anti-Hypertension. *Chiang Mai University Joural of Medical Science*, *20*(3), 1-17. https://doi.org/10.12982/CMUJNS.2021.068

Tanchuk, V. Y., Tanin, V. O., Vovk, A. I., & Poda, G. (2016). A New, Improved Hybrid Scoring Function for Molecular Docking and Scoring Based on AutoDock and AutoDock Vina. *Chemical Biology and Drug Design*, *87*(4), 618–625. https://doi.org/10.1111/cbdd.12697

Vidal-Petiot, E. (2022). Thresholds for Hypertension Definition, Treatment Initiation, and Treatment Targets: Recent Guidelines at a Glance. *Circulation*, *146*(11), 805–807. https://doi.org/10.1161/CIRCULATIONAHA.121.055177

Widyananda, M. H., Fatchiyah, F., Muflikhah, L., Ulfa, S. M., & Widodo, N. (2023). Computational examination to reveal Kaempferol as the most potent active compound from Euphorbia hirta against breast cancer by targeting AKT1 and ERα. *Egyptian Journal of Basic and Applied Sciences*, *10*(1), 753–767. https://doi.org/10.1080/2314808X.2023.2272385

Widyananda, M. H., Pratama, S. K., Samoedra, R. S., Sari, F. N., Kharisma, V. D., Ansori, A. N. M., & Antonius, Y. (2021). Molecular docking study of sea urchin (Arbacia lixula) peptides as multi-target inhibitor for non-small cell lung cancer (NSCLC) associated proteins. *Journal of Pharmacy and Pharmacognosy Research*, *9*(4), 484–496. https://doi.org/10.56499/jppres21.1047_9.4.484

Widyananda, M. H., Wicaksono, S. T., Rahmawati, K., Puspitarini, S., Ulfa, S. M., Jatmiko, Y. D., Masruri, M., & Widodo, N. (2022). A Potential Anticancer Mechanism of Finger Root (Boesenbergia rotunda) Extracts against a Breast Cancer Cell Line. *Scientifica*, *2022*. https://doi.org/10.1155/2022/9130252

Wijaya, C. H., Napitupulu, F. I., Karnady, V., & Indariani, S. (2019). A review of the bioactivity and flavor properties of the exotic spice "andaliman" (Zanthoxylum acanthopodium DC.). In *Food Reviews International* (Vol. 35, Issue 1, pp. 1–19). Taylor and Francis Inc. https://doi.org/10.1080/87559129.2018.1438470

Zarei, M., Abidin, N. B. Z., Auwal, S. M., Chay, S. Y., Haiyee, Z. A., Sikin, A. M., & Saari, N. (2019). Angiotensin converting enzyme (ACE)-peptide interactions: Inhibition kinetics, in silico molecular docking and stability study of three novel peptides generated from palm kernel cake proteins. *Biomolecules*, *9*(10). https://doi.org/10.3390/biom91005