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The Potency of Wax Apple Leaves (*Syzygium samarangense*) as Angiotensin-Converting Enzyme Inhibitors: Computational Approach

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ABSTRACT: Hypertension is a chronic disease that significantly contributes to global mortality, often presenting without symptoms but potentially leading to serious complications. Angiotensin-converting enzyme (ACE) inhibitors are one of the first-line therapies for hypertension, working by reducing the formation of angiotensin II. Wax apple (*Syzygium samarangense*) leaves have been studied for their potential pharmacological activities, including in maintaining blood pressure. This study aims to evaluate the potential of secondary metabolites from wax apple leaves as ACE inhibitor candidates using an in silico approach, including Lipinski's Rule of Five (Ro5) screening, ADMET prediction, pharmacophore modeling, and molecular docking. A total of 22 compounds were tested, with β-sitosteryl stearate showing the highest binding affinity to the ACE receptor, indicated by its low binding energy and strong interactions with key residues at the active site. Meanwhile, gallic acid was identified as a hit compound through pharmacophore modeling and demonstrated favorable ADMET properties. These findings support the potential of *Syzygium samarangense* as a natural source of antihypertensive compounds and provide a foundation for further research in the development of computational-based herbal medicines.

Keywords: ACE inhibitors; antihypertensive; computer-aided drug design; wax apple

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INTRODUCTION

Hypertension is a chronic disease characterized by elevated arterial blood pressure, driven by the force of the heart when pumping blood (Lukitaningtyas & Cahyono, 2023). According to the World Health Organization (2022), about 34.11% of the population in Indonesia aged ≥18 years suffers from hypertension, with the prevalence of hypertension in the world being 22% of the global population, and it is estimated to cause 7.5 million deaths, about 12.8% of all deaths (Moonti et al., 2023). Hypertension does not cause significant symptoms, but if left untreated, it can lead to serious complications in various organs, including the heart, kidneys, and brain. Over a long period, high blood pressure can result in organ dysfunction and even death (Silvianah & Indrawati, 2024). In the treatment of hypertension, angiotensin-converting enzyme (ACE) inhibitors are regarded as first-line therapy, along with calcium channel blockers (CCBs), thiazide diuretics, and ARBs. ACE inhibitors work in the renin-angiotensin system (RAS) by inhibiting ACE, which is an enzyme that converts angiotensin I to angiotensin II. A reduction in angiotensin II synthesis leads to natriuresis, arterial and venous vasodilation, and decreased sympathetic activity (Cutrell et al., 2023). In numerous randomized controlled trials, ACE inhibitors have been evaluated as antihypertensive medications and have been shown to reduce mean arterial blood pressure, as well as systolic and diastolic blood pressure, in hypertensive subjects (Herman et al., 2023).

Several compounds and plant extracts have been shown to have biological activity in lowering blood pressure. Plant extract-derived compounds, especially flavonols, have ACE inhibitory action, which helps lower blood pressure and prevent left ventricular hypertrophy. In addition to their antihypertensive effects, ACE inhibitors protect blood vessels by inhibiting carotid atherogenesis and thrombogenesis. This compound is frequently utilized during the early stages of myocardial infarction to decrease mortality risk, improve heart conditions, and reduce the likelihood of left ventricular failure. Additionally, by reducing pressure on the heart walls, ACE inhibitors contribute to post-infarction remodeling, making them valuable in managing cardiovascular disease (Pintaningrum et al., 2023). Testing biological activity in a substance that could be effective in treating a disease in test cells or animals still faces several challenges, despite the numerous studies that have been carried out. Therefore, the in silico method, which involves modeling or simulation with computational support, is highly effective in the discovery of new drugs. The advantage of the in silico method is that it can predict the physicochemical and pharmacokinetic properties of a compound that align with its activity tests (Sab'anah et al., 2024).

The leaves of wax apple (*Syzygium samarangense*) have been used in traditional medicine in several Asian countries, including for the treatment of colds, itches, and waist pain (Shu & Shelomi, 2020). In addition to its traditional applications, the phytochemical compounds found in wax apples such as flavonoids, phenolic compounds, tannins, terpenoids, and sterols have been reported to exhibit various pharmacological effects with potential therapeutic benefits, including antioxidant, antimicrobial (particularly against the herpes virus), analgesic, anti-inflammatory, antihypertensive, antihyperglycemic, anticonvulsant, and other bioactivities (Tarigan et al., 2022). A previous study shows that the antihypertensive activity of wax apple extract (10-120 g/mL) exhibits concentration-dependent vasodilation in rat aortic rings pre-constricted with phenylephrine (PE) (Hassan et al., 2022). The results of this study are expected to contribute additional scientific evidence regarding the potential of wax apples for future research as a candidate for plant-based antihypertensive agents. This study aims to identify and evaluate active compounds

in wax apple leaves that have the potential to inhibit the Angiotensin-Converting Enzyme (ACE) through a computational approach, to support the development of natural ingredient-based antihypertensive therapy.

METHODS

The research will be conducted using a computational approach, involving screening of physicochemical properties by analyzing pharmacokinetic profiles, evaluating Lipinski's Rule of Fiv (Ro5) e, assessing ADME parameters (absorption, distribution, metabolism, and excretion), examining potential toxicity, and performing molecular docking and visualization. A literature review by Tarigan et al. (2022), was performed to identify the secondary metabolite activities contained in the wax apple leaves. Several compounds that were used in this study included quercetin, jasmonic acid, ellagic acid, β-sitosterol, β-sitosteryl stearate, daucosterol, salicylic acid, cyanidin-3-glucoside, myricetin 3'-rhamnoside, myrigalone H, ursane, oleanane, epigallocatechin, epigallocatechin gallate, betulinic acid, epibetulinic acid, lupeol, carotene, prunasin, and strobopinin. Several software tools were applied in this study, including ChemDraw Ultra 12.0 and Chem3D Pro 12.0 for structure modeling, AutoDock 4.2.7 for docking analysis, BIOVIA Discovery Studio 2020 for preparation and visualization, and LigandScout 4.4.5 for pharmacophore modeling. Online databases such as the Protein Data Bank (https://www.rcsb.org/), PubChem (https://pubchem.ncbi.nlm.nih.gov/), and PreADMET (https://preadmet.bmdrc.kr/) were also used for protein structures, compound data, and ADMET predictions.

Lipinski Rule of Five Prediction

Lipinski rule of five (Ro5) was determined by entering the SMILES code of the compound retrieved from the PubChem database into the mcule website; the system will initiate the calculation process. The output provided the physicochemical characteristics of the secondary metabolites based on Lipinski's criteria, including molecular weight, Log P value, number of hydrogen bond donors, and number of hydrogen bond acceptors. Lipinski's Rule serves to evaluate the physicochemical properties of compounds, particularly their similarity to those of orally active drugs (Ruswanto et al., 2022).

ADMETOX Assessment

PreADMET platform (https://preadmet.webservice.bmdrc.org/) was used to evaluate ADME (absorption, distribution, metabolism, and excretion) and toxicity profiles. SDF files obtained from PubChem were uploaded to the site, which then generated data such as HIA, Caco-2 permeability, plasma protein binding (PPB), blood-brain barrier (BBB) penetration, and mutagenic or carcinogenic potential. This computational analysis helps evaluate the safety and pharmacokinetic properties of each compound. The analysis was used for filtering out compounds with high mutagenic risk and poor bioavailability, while retaining those with better safety and absorption profiles (Zhafira et al., 2024).

Pharmacophore Screening

Pharmacophore screening was used to identify hit compounds with structural features similar to known pharmacophores and potential pharmacological activity. This process was conducted using LigandScout 4.4.5. The target receptor ACE (PDB ID: 3BKL) was downloaded from https://dude.docking.org/target, along with active and decoy compounds. Test compounds were converted to .ldb format before screening. Ten pharmacophore models were tested, and validation was done by evaluating ROC curves.

The best model was selected based on the highest AUC_{100} value, with values above 0.70 considered valid (Hess et al., 2024).

Geometry Optimization

Geometry optimization is needed in the preparation of ligands from test compounds to continue the molecular docking process. Geometry optimization was carried out using Chem3D Pro 12.0 software with MM2 molecular mechanics method. By entering the SMILES code of the compound found on the site (https://pubchem.ncbi.nlm.nih.gov/) in the software, then optimizing it by clicking minimize energy on the Calculations tab. In the MM2 (molecular mechanics) optimization, the minimum RMS gradient was set to 0.010, then run until the calculation was completed. The structure of the optimized compound was saved in .mol, .sdf, and .pdb formats.

Molecular Docking

Molecular docking was performed using BIOVIA Discovery Studio 2020 and AutoDock 4.2.7. The ACE receptor structure with PDB ID 3bkl can be downloaded from the site https://www.rcsb.org/ in .pdb format. The native ligand and target protein need to underwent a dehydrogenation process and then be separated from unnecessary molecules using the BIOVIA Discovery Studio 2020. Preparation of the native ligand and the specified target receptor was carried out using the AutoDock 4.2.7 software, resulting in ligand and receptor files in .pdbqt format. To perform the docking process, a working folder with autogrid4.exe and autodock4.exe is required. Determining the size and position of the grid box can affect the RMSD results, so adjustments are necessary to achieve the best outcome. Docking setup involved defining a grid box (X = 40, Y = 40, Z = 40) with coordinates (X = 45.053; Y = 44.587; Z = 44.978) and spacing of 0.375 Å. The Lamarckian Genetic Algorithm was set to 100, and the configuration was saved in .gpf format. Docking was run via command prompt, producing binding energy and inhibition constant values. Visualization of ligand–amino acid interactions was done in 2D and 3D using BIOVIA Discovery Studio. An RMSD value ≤2 Å was used as the validity reference (Salsabila et al., 2024).

RESULT AND DISCUSSION

Lipinski's Rule of Five is used to predict the physicochemical properties of test compounds by comparing their data with established parameters to evaluate their similarity to orally administered drugs. A compound is considered to meet the criteria of Lipinski's Rule of Five if it does not violate more than two of the rules(Lipinski, 2001). The results of the Lipinski Rule of Five prediction are presented in table 1, which demonstrates that compounds such as gallic acid, ellagic acid, jasmonic acid, salicylic acid, myrigalone H, and strobopinin fully comply with all parameters, suggesting good potential for oral administration. Components that have a molecular weight within the acceptable range will be more easily absorbed into the cell membrane, the log P results of the six compounds show that these compounds have good potential for distribution and absorption in the body, and the hydrogen bonds (HBD and HBA) result of the six compounds show that the compounds have high potential in forming interactions with drug targets. We noticed that several compounds, including β-sitosterol, β-sitosteryl stearate, cyanidin-3-glucoside, myricetin-3′-rhamnoside, epigallocatechin gallate, and β-carotene, violated the Ro5. These violations were mainly due to their high molecular weights, which consequently increase the number of hydrogen bond donors and/or acceptors. However, it should be noted that these compounds were detected in complex with other macromolecules (e.g., sugars), which contributed to their failure to meet the Ro5 criteria. In reality, these compounds are

likely metabolized in the body and converted into their unbound forms before reaching the target protein (Bilia et al., 2019).

Table 1. Lipinski's rule of five prediction results of *Syzygium samarangense* leaves bioactive compounds

No.	Compound Name	Molecular Weight (Da)	Log P	Hydrogen Bond		Lipinski Violations
				Donor	Acceptor	
1.	Quercetin	456.699	7.089	2	3	1
2.	Ellagic Acid	302.191	1.312	4	8	0
3.	Jasmonic Acid	210.269	2.412	1	3	0
4.	Gallic Acid	170.119	0.501	4	5	0
5.	β-Sitosterol	414.705	8.024	1	1	1
6.	β-Sitosteryl Stearate	681.166	14.837	0	2	2
7.	Daucosterol	414.705	8.024	1	1	1
8.	Salicylic Acid	138.120	1.090	2	3	0
9.	Cyanidin-3-glucoside	484.835	-2.614	8	11	3
10.	Myricetin 3'-rhamnoside	464.374	0.194	8	12	2
11.	Myrigalone H	286.321	3.230	2	4	0
12.	Ursane	412.732	9.133	0	0	1
13.	Oleanane	412.732	9.278	0	0	1
14.	Epigallocatechin	306.266	1.251	6	7	1
15.	Epigallocatechin Gallate	458.370	2.233	8	11	2
16.	Betulinic acid	456.699	7.089	2	3	1
17.	Epibetulinic acid	456.699	7.089	2	3	1
18.	Ursolic acid	456.699	7.089	2	3	1
19.	Lupeol	426.716	8.024	1	1	1
20.	β-carotene	536.870	12.605	0	0	2
21.	Prunasin	295.287	-0.932	4	7	1
22.	Strobopinin	270.278	3.112	2	4	0

ADME and toxicity predictions are performed to ensure the safety and bioavailability levels of the tested compounds. Compounds with high toxicity and low bioavailability are typically eliminated, leaving only the most promising candidates for further development. One of the assessed parameters is Human Intestinal Absorption (HIA), which is categorized into three ranges: 0%–20% (poor), 20%–70% (moderate), and 70%–100% (good). Another parameter is Caco-2 permeability, which indicates cell permeability, where values below 4 signify low permeability, 4–70 moderate, and above 70 high. Additionally, the plasma protein binding (PPB) parameter is used; values above 90% indicate high protein-binding affinity, while values below 80% indicate lower affinity. The blood-brain barrier (BBB) parameter is also assessed to indicate a compound's ability to cross the brain barrier, interpreted as < 0.1 (low), 0.1–2 (moderate), and > 2 (high). The final parameter is toxicity, evaluated through mutagenic and carcinogenic properties (Hess et al., 2024). Based on the data presented in table 2, the majority of the tested compounds exhibit favorable absorption, distribution, and safety profiles, supporting their potential as orally active drug candidates.

Table 2. ADMETox results of *Syzygium samarangense* leaves bioactive compounds

No.	Compound Name	Absorption		Distribution		Toxicity	
		HIA (%)	Caco-2 (nm/sec)	PPB (%)	BBB	Mutagen	Carsinogen
1.	Quercetin	63.485	3.412	93.236	0.172	Mutagen	Mouse (-) Rat (+)
2.	Ellagic Acid	61.395	20.488	88.402	0.321	Mutagen	Mouse (-) Rat (+)
3.	Jasmonic Acid	93.943	15.029	73.698	1.551	Mutagen	Mouse (+) Rat (+)
4.	Gallic Acid	90.027	25.233	100.000	5.303	Non-Mutagen	Mouse (+) Rat (-)
5.	β-Sitosterol	100.000	52.373	100.000	19.888	Non-Mutagen	Mouse (+) Rat (-)
6.	β-Sitosteryl Stearate	100.000	57.486	100.000	25.073	Non-Mutagen	Mouse (+) Rat (+)
7.	Daucosterol	90.027	25.233	100.000	5.303	Non-Mutagen	Mouse (+) Rat (-)
8.	Salicylic Acid	88.138	20.314	8.772	0.643	Mutagen	Mouse (-) Rat (-)
9.	Cyanidin-3-glucoside	19.721	5.922	79.610	0.035	Non-Mutagen	Mouse (-) Rat (-)
10.	Myricetin 3'-rhamnoside	11.646	6.140	65.378	0.033	Non-Mutagen	Mouse (-) Rat (-)
11.	Myrigalone H	92.766	18.491	96.084	0.965	Mutagen	Mouse (-) Rat(-)
12.	Ursane	100.000	22.201	100.000	23.631	Non-Mutagen	Mouse (-) Rat (+)
13.	Oleanane	100.000	22.201	100.000	26.637	Non-Mutagen	Mouse (-) Rat (+)
14.	Epigallocatechin	45.952	0.377	100.000	0.222	Mutagen	Mouse (-) Rat (-)
15.	Epigallocatechin Gallate	20.712	12.042	100.000	0.087	Non-Mutagen	Mouse (-) Rat (+)
16.	Betulinic acid	95.996	21.861	100.000	8.197	Mutagen	Mouse (-) Rat (+)
17.	Epibetulinic acid	95.996	21.861	100.000	8.197	Mutagen	Mouse (-) Rat (+)
18.	Ursolic acid	95.996	21.861	100.000	8.007	Non-Mutagen	Mouse (+) Rat (+)
19.	Lupeol	100.000	22.711	100.000	22.711	Mutagen	Mouse (-) Rat (+)
20.	β-carotene	100.000	23.225	25.994	25.994	Non-Mutagen	Mouse (+) Rat (+)
21.	Prunasin	53.324	3.172	38.340	0.115	Mutagen	Mouse (-) Rat (-)
22.	Strobopinin	92.570	7.143	97.552	.383	Mutagen	Mouse (-) Rat (-)

Six compounds show high human intestinal absorption (HIA), reaching 100%, such as β-sitosterol, β-sitosterol stearate, ursane, oleanane, lupeol, and β-carotene, indicating efficient uptake in the gastrointestinal tract. In terms of distribution, 12 out of 22 compounds display high plasma protein binding (PPB), such as gallic acid, β-sitosterol, β-sitosteryl stearate, daucosterol, ursane, oleanane, epigallocatechin, epigallocatechin gallate, betulinic acid, epibetulinic acid, ursolic acid, and lupeol, indicates the extent to

which a drug candidate binds to proteins in the blood plasma (primarily albumin). Furthermore, 11 out of 22 compounds show high blood-brain barrier (BBB) permeability, suggesting potential CNS activity. Regarding safety, the mutagenicity and carcinogenicity assessments indicate that 2 compounds are non-mutagenic and non-carcinogenic in mice or rats, namely cyanidin-3-glucoside and myricetin 3'-rhamnoside. Even though both compounds are non-mutagenic and non-carcinogenic, their absorption in the intestine (HIA) is small because of their large molecular size and binding to glycoside groups, thus they should be metabolized first before being absorbed into the intestine.

Pharmacophore screening was conducted to evaluate the model's ability to identify test compounds based on their structural features' compatibility with the biological target. The results of the pharmacophore screening produced a ROC (Receiver Operating Characteristic) curve, which plots the true positive rate against the false positive rate, and yields a corresponding AUC (area under curve) value. In this pharmacophore screening, 10 pharmacophore models were tested. Model 2 was identified as the most suitable for pharmacophore screening of *Syzygium samarangense* compounds (table 3).

Table 3. Pharmacophore screening validation results from *Syzygium samarangense* leaves bioactive compounds

Pharmacophore Model	Hits	True Positive	False Positive	AUC 100%
1	903	196	707	0.69
2	778	196	582	0.77
3	904	196	708	0.70
4	904	196	708	0.72
5	899	196	703	0.67
6	900	196	704	0.68
7	900	196	704	0.68
8	910	196	714	0.72
9	902	196	706	0.69
10	903	196	707	0.67

Model 2 showed the best performance, with the highest AUC (0.77, 77%) and the fewest false positives (582) among the models (figure 1A). These results indicate that Model 2 has a strong ability to differentiate between active and inactive compounds accurately. Therefore, Model 2 is considered the most suitable pharmacophore model for screening compounds from *Syzygium samarangense*. Gallic Acid was identified as a hit compound; this compound showed compatibility with Pharmacophore Model 2, as evidenced by a pharmacophore fit score of 34.72 and successful arrangement of interaction features such as hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) (figures 1B and 1C).

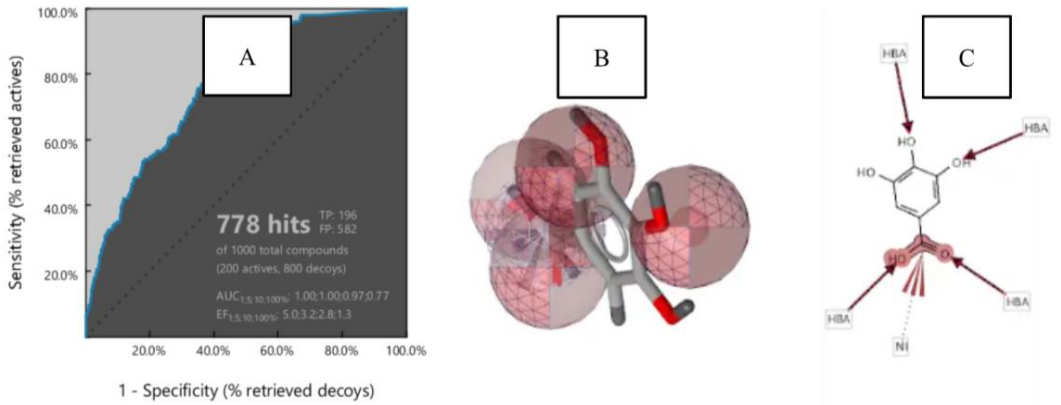


Figure 1. Pharmacophore screening validation curve (ROC curve model 2) (a); 3D visualization of pharmacophore (b); and 2D visualization of pharmacophore (c)

Molecular docking was performed to validate the interactions between the active compounds and the target protein involved in a specific biological mechanism. The key parameters used to evaluate a compound's inhibitory activity are Binding Energy (ΔG) and Inhibition Constant (Ki); the more negative the ΔG value, the lower the resulting Ki, indicating greater binding affinity and inhibitory potential toward the target protein.

The molecular docking results in table 4 show that β-sitosteryl stearate has the strongest affinity for the target protein among the bioactive constituents of *Syzygium semarangense*. Although its binding energy is not stronger than that of the native ligand (ΔG -10.77 kcal/mol; Ki 12.8 μM), the binding energy of β-sitosteryl stearate is stronger than that of lisinopril as a reference drug (ΔG -9.6 kcal/mol; Ki 92.07 μM), therefore indicating a more effective inhibitory potential than lisinopril. The high affinity of β-sitosteryl stearate is supported by interactions with several key residues in the active site of the protein as found in native ligand and lisinopril, including HIS A:353, TYR A:523, HIS A:387, HIS A:410, and VAL A:518. According to research by Irsa et al. (2022), the key interactions between the test compound and the ACE receptor that contribute to receptor inhibition involve the amino acid residues HIS383, HIS387, GLU411, and GLU384.

These residues are also involved in interactions with the native ligand. The native ligands are known to form hydrogen bonds and π interactions with residues such as TYR A:520, GLN A:281, GLU A:411, TYR A:523, ALA A:356, HIS A:383, and GLU A:384, as well as aromatic interactions with PHE A:427, PHE A:527, HIS A:387, HIS A:410, and VAL A:518. Meanwhile, lisinopril interacts through hydrogen bonds with LYS A:511, TYR A:520, GLN A:281, HIS A:353, ALA A:354, and GLU A:384, as well as hydrophobic interactions with TYR A:523 and PHE A:527. β-sitosteryl stearate has a similar interaction pattern, especially with HIS A:353, TYR A:523, and ALA A:354, indicating that these compounds occupy the same binding site.

Table 4. Docking results of *Syzygium samarangense* leaves bioactive compounds

No.	Compound Name	Binding Energy (kcal/ mol)	Ki (μM)	Amino Acid Interactions	
				Hydrogen Bond	Various
	N-{(5S)-4,4-dihydroxy-6-phenyl-5-[(phenylcarbonyl)amino]hexanoyl}-L-tryptophan (Native Ligand)	-10.77	12.8	Hydrogen Bond Conventional : TYR A:520 ; GLN A:281 ; GLU A: 411 ; TYR A:523 ; ALA A: 356 ; HIS A:383 ; GLU A:384	Pi-pi : PHE A:427 ; PHE A:527 ; HIS A:387 ; HIS A:410 Pi-sigma : VAL:518
1.	Lisinopril (Reference drug)	-9.6	92.07	Hydrogen Bond Conventional : LYS A:511 ; TYR A:520 ; GLN A:281 ; HIS A:353 ; ALA A:354 ; GLU A:384 Hydrogen Bond Carbon : HIS A:513 ; HIS A:383	Pi-Alkyl : TYR A:523 ; PHE A:527
2.	Quercetin	-5.93	23.43	Hydrogen Bond Conventional : HIS A:513 ; **ALA A:356**	Pi-pi Stacked : HIS A:383 Pi-alkyl : VAL A:380 ; ALA A:354 Pi-anion : GLU A:411
3.	Ellagic Acid	-6.85	9.58	Hydrogen Bond Conventional : **GLU A:384** ; HIS A:513 ; TYR A:520 ; LYS A:511 ; GLN A:281	Pi-pi T-shaped : HIS A:383 Pi-alkyl : ALA A:354 ; VAL A:380
4.	Jasmonic Acid	-6.27	25.49	Hydrogen Bond Conventional : LYS A:511 ; **GLN A:281**	Pi-alkyl : PHE A:527 ; HIS A:383
5.	Gallic Acid	-4.45	543.47	Hydrogen Bond Conventional : **TYR A:520** ; HIS A:513 ; **TYR A:523** ; **GLN A:281**	Pi-Donor : HIS A:383
6.	β-Sitosterol	-8.96	0.273	-	Alkyl & Pi-alkyl : PHE A:512 ; ALA A:354 ; HIS A:353 ; HIS A:513 ; HIS A:420 ; ALA A:356 ; PHE A:391 Pi-sigma : HIS A:383 ; HIS A:387 ; TYR A:523
7.	β-Sitosteryl Stearate	-10.2	0.034	Hydrogen Bond Conventional : HIS A:353	Alkyl & Pi-Alkyl : PHE A:391 ; HIS A:387 ; HIS A:410 ; VAL A:518 ; PRO A:407 ; HIS A:382 ; TYR:523 ; PHE A:457 ; VAL A:380 ; ALA A:354
8.	Daucosterol	-9.18	185.57	Hydrogen Bond Conventional : **GLN A:281** ; GLU A:162	Alkyl & Pi-alkyl : HIS A:387 ; HIS A:383 ; ALA A:354 ; HIS A:353 ; VAL A:380 ; VAL A:518 ; TYR A:523 ; PHE A:512

9.	Salicylic Acid	-4.79	309.47	Hydrogen Bond Conventional : **GLN A:281** HIS A:513 Carbon : HIS A:353 ;	Pi-pi T-shaped : HIS A:383
10.	Cyanidine-3-glucoside	-6.61	14.36	Hydrogen Bond Conventional : ALA A:354 ; HIS A:353 ; **GLN A:281** ; **GLU A:411** ; **HIS A:383**	Pi-alkyl : VAL A:380 Pi-Lone Pair : TYR A:523 Pi-cation & pi-anion : GLU A:384 ; HIS A :513
11.	Myricetin-3'-Rhamnoside	-7.18	5.46	Hydrogen Bond Conventional : **ALA A:356** ; HIS A:353 ; **GLN A:281** Hydrogen Bond Carbon : HIS A:387 ; **HIS A:383**	Pi-alkyl : PHE A:457 ; ALA A:354 ; VAL A:380
12.	Myrigalone H	-7.21	5.2	Hydrogen Bond Conventional : **GLN A:281** ; **TYR A:520** ; LYS A:511 ; TYR A:523 ; GLU A:384 Hydrogen Bond Carbon : HIS A:513	Alkyl & pi-alkyl : VAL A:380 ; ALA A:354 Pi-pi T-shaped : PHE A:457 ; PHE A:527 ; HIS A:383 Pi-cation : HIS A:353
13.	Ursane	-4.98	223.17	-	Alkyl & Pi-alkyl : TYR A:523 ; HIS A:513 ; VAL A:380 ; HIS A:383 ; ALA A:354 ; HIS A:353 ; VAL A:518 Pi-sigma : HIS A:387
14.	Oleanane	-7.18	1.87	-	Alkyl & pi-alkyl : PHE A:457 ; TYR A:523 ; HIS A:387 ; VAL A:380 ; ALA A:354 ; HIS A:353 ; VAL A:518 Pi-sigma : HIS A:383
15.	Epigallocatechin	-6.87	9.14	Hydrogen Bond Carbon : HIS A:353 ; HIS A:513 ; **GLU A:384**	Alkyl : TYR A:523 Pi-pi T-Shaped : HIS A:383
16.	Epigallocatechin Gallate	-6.95	7.99	Hydrogen Bond Conventional: **HIS A:383** ; LYS A:511 ; **GLN A:281** ; HIS A:513 ; **GLU A:384**	Alkyl & Pi-alkyl : ALA:354 Pi-pi T-Shaped : TYR A:523 Pi-Cation & Pi-Anion : HIS A:353 ; GLU A:411
17.	Betulinic Acid	-3.88	1.42	Hydrogen Bond Conventional : **GLN A:281**	Alkyl & Pi-Alkyl : PHE A:527 ; VAL A:380 ; HIS A:383 ; VAL A:379 ; HIS A:353 ; HIS A:513 ; HIS A:387 ; VAL A:518 Pi-sigma : TYR A : 523

18.	Epibetulinic Acid	-4.34	656.83	Hydrogen Bond Conventional : **GLN A:281** ; **GLU A:411**	Alkyl & Pi-Alkyl : VAL A:379 ; PHE A:527 ; VAL A:380 ; HIS A:387 ; HIS A:353 ; HIS A:513 ; VAL A:518 Pi-sigma : TYR A:523 Unfavorable Donor-donor : HIS A:383
19.	Ursolic Acid	-0.89	221.96	-	Alkyl & Pi-alkyl : PHE A:457 ; PHE A:527 ; HIS A:383 ; TYR A:523 ; HIS A:387 ; HIS A:353 ; HIS A:513 ; VAL A:518
20.	Lupeol	-4.91	250.75	-	Alkyl & Pi-alkyl : VAL A:518 ; HIS A:513 ; HIS A:387 ; HIS A:353 ; TYR A:523 ; VAL A:379 ; VAL A:380
21.	β-Carotene	+155000	Unavailable	-	Pi-alkyl & alkyl : TRP A:279 ; VAL A:380 ; ALA A:354 ; TYR A:523 ; HIS A : 383 ; HIS A:519 ; HIS A:387 ; MET A: 223 ; PHE:391 ; PRO A:407 Pi-sigma : HIS A:353 ; HIS A:410
22.	Prunasin	-7.02	7.11	Hydrogen Bond Conventional : **TYR A:520** ; LYS A:511 ; HIS A:513 ; **GLU A :384**	Pi-Alkyl : VAL A:380 Pi-cation : HIS A:383 Unfavorable Donor-donor : TYR A:523 ; ALA A:356
23.	Strobopinin	-7.11	6.18	Hydrogen Bond Conventional : HIS A:353 **TYR:523**	Pi-cation : HIS A:383 Alkyl & Pi-Alkyl : ALA A:354

The similarity of the interacting residues, combined with the much lower Ki (μM) value, suggests that β-sitosteryl stearate not only mimics the binding mechanism of the native ligand and the reference drug but also binds more strongly and efficiently. Therefore, β-sitosteryl stearate has the potential to be a pharmacological inhibitor candidate compared to lisinopril, while also showing promise as a promising alternative inhibitory agent. Despite its strong binding affinity shown in molecular docking, β-sitosteryl stearate was not identified as a hit compound in the pharmacophore screening. This indicates a difference between the results of pharmacophore screening and molecular docking. It is because the pharmacophore approach focuses on the match between chemical features and the spatial model of the reference ligand, while docking evaluates the stability of ligand-protein interactions based on the binding energy formed (Chan et al., 2024; Pawar et al., 2023).

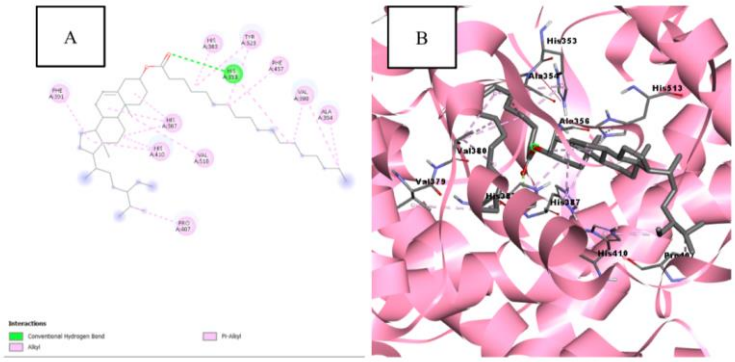


Figure 2. Molecular docking 2D visualization (a) and 3D visualization (b) of β-Sitosterol Stearate

The difference between the pharmacophore screening and docking results may stem from the fact that the pharmacophore model emphasizes hydrogen-bonding features, whereas β-sitosteryl stearate mainly interacts through hydrophobic interactions, driven by its high log P. Meanwhile, gallic acid fulfills the pharmacophore hydrogen bond features but exhibits weak docking affinity due to its small size and lack of hydrophobic bonds. Further research is needed to validate this observation.

Based on its structure, β-sitosteryl stearate is a lipophilic ester compound with a long C18 carbon chain, resulting in a high Log P value. Then, once the ester compound enters the body, lipase enzymes and bile micelles are involved in the hydrolysis of the ester (Shen et al., 2024). The presence of lipase in the intestine can hydrolyze β-sitosteryl stearate and convert it into β-sitosterol, which is then absorbed by bile micelles, allowing β-sitosterol to be absorbed in the intestine (HIA 100%). Therefore, even though β-sitosteryl stearate has a high Log P value, the compound can still be well absorbed in the intestine due to the assistance of lipase.

The toxicity profile of β-sitosterol indicates that it has no mutagenic potential but does exhibit carcinogenic activity in rats. However, recent epidemiological studies show that it has anticancer effects based on cell signaling pathways in cancers such as prostate, leukemia, breast, lung, stomach, and colon cancer (Gu et al., 2023; Shen et al., 2024). β-sitosterol also exhibits strong binding affinity (ΔG -8.96 kcal/mol; Ki 0.273 μM), supported by hydrophobic interactions with several key residues in the active site of the protein, including HIS A:383 and HIS A:387 (table 4), though we also realized that the long carbon chain in β-sitosteryl stearate bound to ACE2 through hydrogen binding at HIS A:353, which also seen in lisinopril-ACE2 molecular interaction. This phenomenon is likely to cause the stronger binding of the ester β-sitosteryl stearate compared to β-sitosterol. Therefore, additional experimental evidence is needed to fully confirm the safety of this compound as an oral drug. This difference emphasizes the importance of combining the three approaches: pharmacophore modeling, ADMET prediction, and molecular docking to gain a comprehensive understanding of a compound's potential activity as an ACE inhibitor.

CONCLUSION

These computational studies confirm the potential of wax apple leaves as a natural source of antihypertensive compounds, with β-sitosterol identified as a promising compound. Based on the evaluation of physicochemical properties using Lipinski's Rule, ADMET profile prediction, and molecular docking analysis of 22 compounds from wax apple leaves,

β-sitosterol was found to have potential activity against the ACE. The β-sitosteryl stearate showed strongest potential due to its strong binding affinity and key interactions with active site residues of ACEi. Therefore, β-sitosteryl stearate (with β-sitosterol as the parent compound) could serve as a lead compound for further antihypertensive drug development, including structural modification.

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AUTHOR CONTRIBUTION
SF: Investigation, visualization, formal analysis, and writing-original draft.
AAP: Investigation, visualization, formal analysis, and writing-original draft
NRN: Investigation, visualization, formal analysis, and writing-original draft
FZZ: Investigation, visualization, formal analysis, and writing-original draft
DPFI: Methodology, conceptualization, writing-review, and editing
BSM: Methodology, conceptualization, writing-review, and editing
DN: Methodology, conceptualization, supervision, writing-review, and editing

CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest related to this article.

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