International Journal Of Health, Engineering And Technology (IJHET) *E-ISSN 2829 - 8683*
Volume 4, Number 6, March 2026, *Page. 2273 - 2288*
Email : editorijhess@gmail.com

Test In Silico Compounds Of Lime Leaves (Citrus Amblycarpa (Hassk.) Ochse) As Inhibitors Of Angiotensin Converting Enzyme (ACE) Causes Hypertension

Fitrohrezky Akbar Maulana[1)*], Tiara Ajeng Listyani[2)], Anna Fitriawati[3)]
[1,2,3)] Duta Bangsa University, Surakarta

*Corresponding Author
Email : fitrohrezkya@gmail.com

Abstract

Hypertension is a global health problem with high prevalence and is a major risk factor for cardiovascular disease. One of the main mechanisms of hypertension is increased Angiotensin Converting Enzyme (ACE) activity. The use of synthetic ACE inhibitors often causes side effects, such as nausea, hyperkalaemia, headaches, swelling of the lower skin, coughing, taste disturbances, and angioneurotic edema. Therefore, natural-based alternatives are needed. Lime leaves (Citrus amblycarpa) are known to contain various bioactive compounds that have the potential to act as ACE inhibitors. This study aims to analyze the interaction of active compounds in lime leaves with ACE through molecular docking, as well as to predict their ADME profile and toxicity. The study was conducted in silico using PyRx–AutoDock Vina for molecular docking, PyMOL and BIOVIA Discovery Studio for interaction visualization, SwissADME for ADME prediction, and ToxTree for toxicity analysis. Method validation was performed based on an RMSD value < 2Å. The results of the study indicate that 1,3-benzenedicarboxamide is the best compound with an RMSD value of 1.998 Å and a bond free energy (ΔG) of −6.4 kcal/mol, forming specific amino acid residue bonds similar to the native captopril ligand. ADME predictions meet Lipinski's Rule of Five criteria and fall into the low toxicity category (Class I) based on Cramer's rules. The compound 1,3-benzenedicarboxamide has the potential to be developed as a candidate ACE inhibitor for hypertension therapy.

Keywords: ACE, ADME, Docking, Lime, Toxicity.

INTRODUCTION

Hypertension is a common blood vessel disease and is classified as a degenerative disease. The American Heart Association (AHA) defines hypertension as having a systolic blood pressure of 140 mmHg or higher or a diastolic blood pressure of 90 mmHg or higher.(Pratama et al., 2024). According to the World Health Organization (WHO) (WHO, 2023), the number of hypertension sufferers aged 30-79 years has increased from 650 million to 1.28 billion in the last thirty years.(Sikala et al., 2025). According to the Indonesian Ministry of Health in 2020, hypertension ranked fifth out of the ten highest causes of death in the country, causing approximately 41,590 deaths from January to December 2020.(Rifki & Indawati, 2024)Based on WHO predictions, in 2025, around 29% of the world's adults will suffer from hypertension.(Novitri et al., 2021). Angiotensin Converting Enzyme (ACE) is a zinc (Zn2+) metallopeptidase that converts angiotensin I (an inactive decapeptide) into angiotensin II, a powerful vasoconstrictor that causes hypertension.(Kurniawan et al., 2022)Commercially available synthetic ACE inhibitors (captopril, enalapril, lisinopril, etc.) cause side effects such as nausea, hyperkalemia, headache, subcutaneous swelling, cough, taste disturbances, and angioneurotic edema. Various plants have been reported to possess potential ACE inhibitor properties. Plant-based bioactive compounds are a better alternative to synthetic drugs because they are non-toxic, readily available, and have no side effects. Therefore, it is necessary to shift and search for alternative natural resources such as medicinal plants that have promising health benefits without side effects.(Bari et al., 2023)Citrus amblycarpa leaves have been used in traditional medicine to treat various ailments for thousands of years. In Indonesia, Citrus amblycarpa leaves are often used to treat fever, coughs, and colds. Furthermore, Citrus amblycarpa leaves can also treat digestive problems such as bloating, gas, and nausea. Citrus amblycarpa leaves are believed to have a cooling effect on the body, making them useful in treating fever and other inflammatory disorders.(Arsana, Ketut, Juliasih, Disebut, et al., 2024). In previous research to find ACE inhibitor activity in vitro and bioactive compounds of Citrus amblycarpa conducted by (Ayu et al., 2021)Citrus amblycarpa contains

compounds that inhibit the conversion of angiotensin-converting enzyme 1 (ACE 1) to angiotensin-converting enzyme 2 (ACE 2) in hypertension. Compounds derived from citrus plants have the potential to inhibit ACE. Research that can be conducted to strengthen previous research is molecular docking, which is a prediction of the occurrence of bond conformation in the form of position, type, and affinity based on the bond energy between the ligand (active compound) and macromolecule (target protein) efficiently using computational techniques.(Kurniawan et al., 2022)In silico approaches such as molecular docking, a computational method widely used in early drug development, evaluate the affinity, stability, and pharmacokinetic potential of compounds for protein targets. This method can be used after or in conjunction with in vitro assays to predict biological activity.(Isa et al., 2025). This study aims to determine the interaction pattern of citrus amblycarpa leaf ligands on ACE protein. This method was chosen to provide further knowledge after in vitro research to determine the type of compound binding in citrus amblycarpa leaf content as an ACE inhibitor without causing adverse and toxic biological effects, to determine the prediction of absorption, distribution, metabolism, and excretion (ADME), to find new drug candidates that are more potential and have the opportunity for safe and effective oral preparations.

To determine the interaction pattern between active compounds in lime leaves (Citrus amblycarpa (Hassk.) Ochse) with ACE protein based on molecular docking analysis. which is seen from the interaction of amino acids involved, the value of free binding energy (ΔG) and the Root Mean Square Deviation (RMSD) value. To determine the prediction of absorption, distribution, metabolism, and excretion (ADME) of lime leaves (Citrus amblycarpa (Hassk.) Ochse). To determine the prediction of toxicity parameters of lime leaves (Citrus amblycarpa (Hassk.) Ochse). To determine the best active compounds in lime leaves (Citrus amblycarpa (Hassk.) Ochse) which have the potential as antihypertensive candidates based on the results of molecular docking, ADME prediction, and toxicity parameters in silico. For Researchers the results of this study are expected to increase the author's insight, knowledge, and skills in the fields of computational chemistry and bioinformatics, especially regarding the application of molecular docking, ADME prediction, and toxicity. This research also trains researchers in analyzing the interaction of bioactive compounds of lime leaves (Citrus amblycarpa (Hassk.) Ochse) against Angiotensin converting enzyme (ACE) protein as a target for hypertension therapy, thereby improving scientific capabilities in the development of natural-based drug candidates. For Duta Bangsa University Surakarta This research is expected to add scientific references and enrich library sources in the fields of computational pharmacy, medicinal chemistry, and natural-based drug development. In addition, the results of this study can be a reference for other researchers in developing further research on the potential of local medicinal plants, especially lime leaves (Citrus amblycarpa) as ACE inhibitor candidates in hypertension therapy. For the Community The results of this study are expected to provide scientific information on the potential of lime leaves (Citrus amblycarpa) as a source of natural bioactive compounds that have the potential to inhibit ACE protein as one of the causes of hypertension. Thus, this research can be a scientific basis in the development of herbal medicines or health supplements that are safer, more effective, and more affordable, as well as increasing public awareness of the use of local plants as an alternative supportive therapy for hypertension.

RESEARCH METHODS

Types and Methods of Research

This study uses a quantitative research type with a computer-based pre-experimental approach carried out in silico on citrus amblycarpa leaves against ACE protein using ChemDraw, Chem3D, Discovery Studio Visualizer, VegaZZ, PyRx-Python, AutoDock Vina, PyMOL, Toxtree, and SwissADME software.

Data Analysis Instruments and Techniques

The tools used are a set of laptop hardware with specifications of AMD Ryzen 5 5600H Processor with Radeon Graphics, 16GB RAM, 477GB system storage. The software used is the Windows 11 Home operating system, ChemDraw, Chem3D, Discovery Studio Visualizer, VegaZZ, PyRx-Python AutoDock Vina, Swiss ADME, Toxtree, and PyMOL. The material used in this study is the 3D structure of the ACE protein downloaded from the Protein Data Bank (RCSB PDB) with the identity code PDBID: 4C2P stored in .pdb format. This structure was prepared by a series of water molecule removal, structure repair, removal of native ligands, and search for the active site of the protein as well as the design of the three-dimensional structure of 35 compounds from citrus amblycarpa leaves.

Research Procedures

ACE protein download

The ACE protein downloaded from the Protein Data Bank (RCSB PDB) with the identity code PDBID: 4C2P(RCSB) is saved in .pdb format.

Separation of macromolecular chains

Separation of macromolecular structures from their ligands is performed using Discovery Studio Visualizer. The procedure begins by opening the application via the Discovery Studio Visualizer icon, then selecting File → Open to browse to the folder containing the macromolecule file to be processed. Once the target protein structure is displayed in three dimensions, unnecessary components, such as molecular chains, ligands, and specific residues, are removed. Deletion is performed via the Script → Selection menu by selecting Select Water Molecules / Ligand / Protein Chains, followed by the Edit → Delete command. The cleaned macromolecule is then saved in pdb format.(Listyani et al., 2022).

Three-dimensional structure of the test ligand

The two-dimensional structure of the test ligand compound was created using the ChemDraw application, then converted into a three-dimensional model using Chem3D by selecting the Edit → Get 3D Model menu. The three-dimensional structure was then saved in .pdb format. The next step was structure optimization using VegaZZ (Freeware) by selecting the Calculate → Ammp → Minimization menu. The optimization results were then saved as a .mol file for ADME and toxicity prediction purposes, and in .pdb format for the molecular docking process.(Listyani et al., 2022).

Geometry optimization of test compound structures

The two-dimensional structure of the compound to be added is opened using the VegaZZ program and then displayed in three dimensions. At this stage, hydrogen atoms are added and the compound's charge is corrected by applying Gasteiger partial charges, then adjusted using the Autodock force field. Next, an energy minimization process of 3000 steps is performed to obtain the most stable conformation. This optimization aims to produce the lowest molecular energy. Each optimized compound is then saved in .mol format.(Listyani et al., 2022).

RESULTS AND DISCUSSION

ACE Protein Download

ACE as a protein or macromolecule in this study was obtained through the Protein Data Bank (PDB) website with ID: 4C2P (source:www.rcsb.org) shown in Figure 1. ACE was chosen because it has a direct bond with captopril as a native ligand. Another criterion is that ACE data is part of the transport protein in humans (Homo sapiens) which is in accordance with this study.

Figure 1. 4C2P (RCSB: GDP, 2025)

Preparation of 3D Structure of ACE Protein

The protein structure downloaded from PDB is still bound to the native ligand, so it must be removed because it can interfere with the docking process.

Figure 2. ACE Protein Before Preparation

Figure 3. ACE Protein After Preparation

Creation of 2D and 3D Structures of Test Ligands

Creation of 35 test ligand structures in 2D format using the ChemDraw Professional 15.0 application.

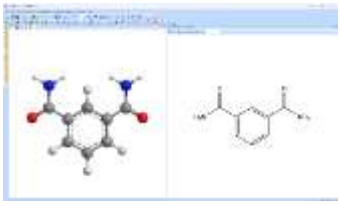

Figure 4. 2D and 3D Structures of Test Ligands

Test Ligand Optimization

Test ligand optimization was performed using the VegaZZ application. Geometry optimization was performed to produce the lowest molecular energy that exhibits the best stability in structures with folds different from the initial structure (Pratama et al., 2021).

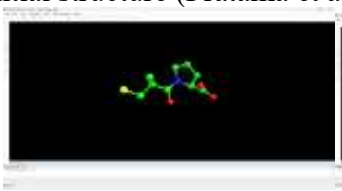

Figure 5. Test Ligand Before Optimization

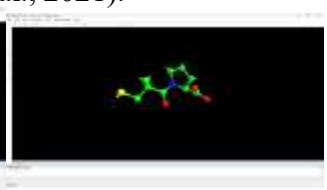

Figure 6. Test Ligand After Optimization

Validation of Molecular Docking Method

Validation of the docking method against native ligands was performed to find the conformation of the native ligand. The resulting docking conformation was then compared with the native ligand conformation from crystallography expressed in RMSD values. Gridbox settings during the redocking process were used to determine the ligand binding area to be tested for docking. Validation was performed using PyMOL and the BIOVIA application.

Figure 7. Size*Gridbox*(PyRX)

Figure 8. Results of ligand docking using the docking method with (PyRX)

Figure 9. Validation results of the docking method with PyMOL

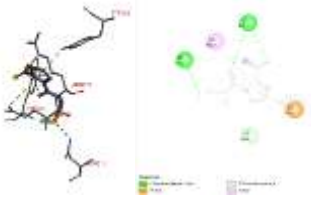

Figure 10. Visualization of the Interaction Results of Captopril and ACE

Molecular Docking Results

The analysis of docking results in this study focuses on the binding free energy (ΔG) value, RMSD value, hydrogen bond characteristics, and the interaction pattern of the ligand with amino acid residues in the binding site of the target protein. These parameters are used to assess the stability of the ligand and receptor complex, the suitability of the binding pose generated by the docking algorithm, and the potential affinity of the ligand as a drug candidate (Halder et al., 2023). The ΔG binding value indicates the strength and stability of the ligand-receptor interaction, where a more negative value generally indicates a more stable binding affinity, while a less negative ΔG binding value or close to zero indicates a less stable complex (Listyani and Azizah, 2025). Furthermore, RMSD is used to assess the accuracy of the docking pose, with a low RMSD value describing a more reliable conformation. In addition, hydrogen bonds and non-covalent interactions were analyzed to identify amino acid residues between the ligand and receptor which are supporting indicators in explaining the ΔG value and affinity profile between the ligands tested (Terefe and Gosh, 2022).

Table 1. Molecular Docking Results of Test Compounds

Test compound	ΔGBinding value (kcal/mol)	RMSD value
Native Ligand captopril	-5.5	1,258
Paracetamol Negative Control	-5.2	1,902
Taraxasterol	-9.7	1,375
Benzo[b]naphtho[2,3-d]furan	-8.0	1,072
Phytol isomer	-6.1	1,202
Linolenic acid	-5.3	1,394
Squalene	-5.9	1,306
Beta-sitosterol	-7.8	1,583
Nonanoic acid	-4.7	1,503
Gamma-Tocopherol	-7.3	1,238
Neophytadiene	-5.4	1,183
Dihydrolanosterol	-8.6	1,422
2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	-5.6	1,961
Methyl linolenate	-5.1	1,304
1-[2-methyl-2-(4-methyl-3-pentenyl) cyclopropyl] ethanol	-5.0	1,573
(+)-1-menthene	-5.1	1.02
Butyric acid, ester with citronellol	-5.2	1,704
Mehp	-6.1	1,517
Beta amyrin	-8.3	1,417
Alpha-terpinene	-5.5	1,042
Beta-Element	-6.1	1.21
Germacrene-B	-6.8	1,232
Germacrene-D	-7.1	1,476
Lepidozene	-6.9	1,816
Beta-cadinene	-7.0	1,592
Alpha-Gurjunene	-7.0	1,282
4-Hydrazinopyrazino [3,2-D]	-5.4	1,231
Palmitic acid, n-Hexadecanoic acid	-5.1	1,343
9-Octadecenoic acid	-5.6	1,523
Dioctyl ester, 1-2	-6.1	1,537
Cinnamamide	-5.5	1,826
Dihydrofarnesol	-5.3	1,478
Stigmasterol	-7.9	1,527
Aristolone	-6.9	1,611
Maragenin I	-9.9	1,577
2,3,8-Trioxocephalotaxane	-8.3	1,718
1,3-Benzenedicarboxamide	-6.4	1,998

Interaction of Amino Acids of Test Ligands with Receptors

From the results of the interaction of 35 compounds from lime leaves (Citrus Amblycarpa), there are 2 compounds that have the same amino acid residue interactions with native ligands in the hydrogen bonds of the Discovery Studio Visualizer application.

Table 2.Interaction of Amino Acids of Test Ligands with Receptors

Test Compound	ΔGBinding value (kcal/mol)	*Category*	*Chemical Bonding*	*Amino Acid Residue and Boud Distance*
Native Ligand captopril	-5.5	*Hydrogen Bonding*	*Conventional Hydrogen Bond*	**TRP A: 220 ASN A:211**
			Pi-Donor Hydrogen	TYR A:135
		Electrostatic	*Pi-Anion*	GLU A:123
		Hydrophobic	*Pi-Alkyl*	ARG A:124
Paracetamol Negative Control	-5.8	*Hydrophobic*	*Pi-Pi Stacked*	TYR A: 523
Taraxasterol	-9.7	*Hydrophobic*	*Pi-Alkyl*	VAL A:518

Test Compound	ΔGBinding value (kcal/mol)	Category	Chemical Bonding	Amino Acid Residue and Boud Distance
Benzo[b]naphtho[2,3-d]furan	-8.0	Hydrogen Bonding	Pi-Donor Hydrogen	SER A:298
		Electrostatic	Pi-Cation	LYS A:449
			Pi-Anion	ASP A:300
		Hydrophobic	Pi-Pi T-shaped	TYR A: 287
			Pi-Alkyl	LEU A: 433
Phytol isomer	-6.1	Hydrophobic	Pi-Sigma	TRP A:357
			Pi-Alkyl	TRP A: 59 TYR A:62 TYR A:360
			Alkyl	ILE A:88 ALA A:63
Linolenic acid	-5.3	Hydrogen Bonding	Conventional Hydrogen Bond	TYR A:523
		Unfavorable	Unfavorable Acceptor-acceptor	HIS A:383
		Hydrophobic	Pi-Alkyl	VAL A:380 PHE A:527 PHE A:457 HIS A:513 HIS A: 353
			Alkyl	ALA A: 354
Squalene	-5.9	Hydrophobic	Pi-Sigma	HIS A:383 TYR A:523
			Alkyl	VAL A: 380
			Pi-Alkyl	PHE A:457
Beta-sitosterol	-7.8	Hydrophobic	Pi-Sigma	TRP A:357
			Alkyl	LEU A:139
Nonanoic acid	-4.7	Hydrogen Bonding	Conventional Hydrogen Bond	TYR A:523
		Hydrophobic	Pi-Alkyl	PHE A:457 PHE A:527
Gamma-Tocopherol	-7.3	Hydrogen Bonding	Conventional Hydrogen Bond	GLU A:162 HIS A:353
		Hydrophobic	Pi-Sigma	TYR A:523
			Pi-Alkyl	PHE A:457
			Alkyl	VAL A:380
Neophytadiene	-5.4	Hydrophobic	Pi-Sigma	TYR A:523
			Pi-Alkyl	HIS A:383
			Alkyl	VAL A:380
Dihydrolanosterol	-8.6	Hydrophobic	Alkyl	LEU A:139
2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	-5.6	Hydrogen Bonding	Conventional Hydrogen Bond	GLN A:281
			Carbon Hydrogen Bond	THR A:282
		Hydrophobic	Pi-Sigma	TYR A:523
			Pi-Alkyl	PHE A:457
Methyl linolenate	-5.1	Hydrogen Bonding	Carbon Hydrogen Bond	GLU A:384 TYR A:523
		Hydrophobic	Alkyl	VAL A:380 VAL A:329
			Pi-Alkyl	PHE A:457 PHE A:527
1-[2-methyl-2-(4-methyl-3-pentenyl) cyclopropyl] ethanol	-5.0	Hydrogen Bonding	Conventional Hydrogen Bond	THR A:301
		Unfavorable	Unfavorable Acceptor-acceptor	ASP A:300
		Hydrophobic	Alkyl	LYS A:449 PRO A:424 LEU A:433
			Pi-Alkyl	TYR A:287 PHE A446
(+)-1-menthene	-5.1	Hydrophobic	Alkyl	ALA A:207 ALA A:208
Butyric acid, ester with citronellol	-5.2	Hydrophobic	Pi-Sigma	TYR A:523
			Pi-Alkyl	PHE A:457 PHE A:527 HIS A:383
Mehp	-6.1	Hydrophobic	Pi-Pi Stacked	TYR A:523
			Alkyl	VAL A:380
Beta amyrin	-8.3	Hydrogen Bonding	Conventional Hydrogen Bond	GLU A:123
		Hydrophobic	Pi-Alkyl	TRP A:357
Alpha-terpinene	-5.5	Hydrophobic	Pi-Alkyl	LEU A:140

Test Compound	ΔGBinding value (kcal/mol)	Category	Chemical Bonding	Amino Acid Residue and Boud Distance
Beta-Element	-6.1	Hydrophobic		LEU A:82 LEU A:81
			Alkyl	TYR A:69
			Pi-Sigma	TYR A:523 PHE A:457
			Pi-Alkyl	HIS A:383
Germacrene-B	-6.8	Hydrophobic	Pi-Sigma	TYR A:523
			Alkyl	ALA A:354 VAL A:380
			Pi-Alkyl	HIS A:383 TYR A:520 PHE A:457
Germacrene-D	-7.1	Hydrophobic	Alkyl	LEU A:139 LEU A:82 LEU A:140 LEU A:81
Lepidozene	-6.9	Hydrophobic	Pi-Sigma	HIS A:383
			Alkyl	VAL A:379 VAL A:380
Beta-cadinene	-7.0	Hydrophobic	Pi-Alkyl	TRP A: 59 TYR A:62
			Alkyl	ILE A:88
Alpha-Gurjunene	-7.0	Hydrophobic	Pi-Sigma	TYR A:523
			Pi-Alkyl	PHE A:527 PHE A:457 HIS A: 383
			Alkyl	VAL A:380 VAL A:379
4-Hydrazinopyrazino [3,2-D]	-5.4	Hydrogen Bonding	Conventional Hydrogen Bond	SER A:219
		Hydrophobic	Amide-Pi Stacked	GLU A: 123
			Alkyl	ALA A:207 ARG A:124 ILE A:204
			Pi-Alkyl	TRP A: 220 TYR A:123
Palmitic acid, n-Hexadecanoic acid	-5.1	Hydrogen Bonding	Conventional Hydrogen Bond	GLN A:281 TYR A:520
			Carbon Hydrogen Bond	HIS A:513
		Electrostatic	Salt Bridge	LYS A:511
		Hydrophobic	Alkyl	VAL A:380
			Pi-Alkyl	TYR A:523 HIS A:383 PHE A:457
9-Octadecenoic acid	-5.6	Hydrogen Bonding	Conventional Hydrogen Bond	THR A:92
		Hydrophobic	Alkyl	ALA A:63 ILE A:88
			Pi-Alkyl	TRP A: 59 TRP A:357 TYR A:360 TYR A:62 PHE A:391 HIS A:410
Dioctyl ester, 1-2	-6.1	Hydrogen Bonding	Conventional Hydrogen Bond	LYS A:449 THR A:302 THR A:301
			Carbon Hydrogen Bond	ASN A:285
		Electrostatic	Pi-Anion	ASP A:300
		Hydrophobic	Alkyl	LEU A:433 LEU A:427
			Pi-Alkyl	TYR A:287
Cinnamamide	-5.5	Hydrogen Bonding	Conventional Hydrogen Bond	TYR A:523 GLU A:384
Dihydrofarnesol	-5.3	Hydrogen Bonding	Conventional Hydrogen Bond	ALA A:354
			Carbon Hydrogen Bond	TYR A:523 HIS A:353
		Hydrophobic	Alkyl	VAL A:379
			Pi-Alkyl	PHE A:457 PHE A:527

Test Compound	ΔGBinding value (kcal/mol)	Category	Chemical Bonding	Amino Acid Residue and Boud Distance
Stigmasterol	-7.9	*Hydrogen Bonding*	*Conventional Hydrogen Bond*	GLU A:411
		Hydrophobic	*Alkyl*	VAL A:518 LEU A:139 LEU A:140
Aristolone	-6.9	*Hydrogen Bonding*	*Conventional Hydrogen Bond*	**ASN A:85** ARG A:124 TYR A:62
Maragenin I	-9.9	*Hydrogen Bonding*	*Conventional Hydrogen Bond*	ARG A:124 ASP A:358
		Hydrophobic	*Pi-Sigma*	TRP A:357
2,3,8-Trioxocephalotaxane	-8.3	*Hydrogen Bonding*	*Conventional Hydrogen Bond*	TYR A:520
			Carbon Hydrogen Bond	ASP A:377
		Hydrophobic	*Pi-Alkyl*	ALA A:354 VAL A:380
1,3-Benzenedicarboxamide	-6.4	*Hydrogen Bonding*	*Conventional Hydrogen Bond*	**ASN A:211** ASP A:121 GLU A:123 **TRP A: 220**
			Carbon Hydrogen Bond	ARG A:124
		Hydrophobic	*Pi-Alkyl*	ILE A:204 ALA A:207

ADME Parameter Prediction

Pharmacokinetic testing, or ADME prediction, is performed to assess the likelihood of a compound being an oral drug or being similar to an oral drug. The SwissADME website displays pharmacokinetic test results using several parameters, one of which is Lipinski's rule of five.

Table 3. ADME test results of test compounds with Lipinski parameters

Test compound	Formula	Molecular Weight (g/mol)	*H-Bond acceptors*	*H-Bond donors*	LogP	Solubility in water
Taraxasterol	C30H50O	426.72	1	1	7.12	Slightly soluble
Benzo[b]naphtho[2,3-d]furan	C16H18O	226.31	1	0	2.25	Slightly Dissolved
Phytol isomer	C20H40O	296.53	1	1	6.25	Slightly Dissolved
Linolenic acid	C18H36O2	284.48	2	1	5.93	Slightly Dissolved
Squalene	C30H50	410.72	0	0	9.38	Slightly soluble
Beta-sitosterol	C29H50O	414.71	1	1	7.25	Slightly soluble
Nonanoic acid	C9H18O2	158.24	2	1	2.60	Late
Gamma-Tocopherol	C28H4802	416.68	2	1	7.98	Slightly soluble
Neophytadiene	C20H38	278.52	0	0	6.97	Slightly soluble
Dihydrolanosterol	C30H52O	428.73	1	1	7.59	Slightly soluble
2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	C15H30O	226.40	1	0	4.46	Slightly Dissolved
Methyl linolenate	C19H38O2	298.50	2	0	6.24	Slightly Dissolved
1-[2-methyl-2-(4-methyl-3-pentenyl) cyclopropyl] ethanol	C12H24O	184.32	1	1	3.31	Late
1-menthene	C10H20	140.27	0	0	3.77	Late
Butyric acid, ester with citronellol	C14H28O2	228.37	2	0	4.12	Late
Mehp	C16H21O4	152.23	1	0	2.66	Late
Beta amyrin	C30H50O	426.72	1	1	7.17	Slightly soluble
Alpha-terpinene	C10H16	136.23	0	0	3.30	Late
Beta-Element	C15H24	204.35	0	0	4.63	Slightly Dissolved
Germacrene-B	C15H24	204.35	0	0	4.63	Slightly Dissolved
Germacrene-D	C15H24	204.35	0	0	4.30	Slightly Dissolved
Lepidozene	C15H24	204.35	0	0	4.15	Late
Beta-cadinene	C15H24	204.35	0	0	4.14	Late

Alpha-Gurjunene	C15H24	204.35	0	0	4.27	Late
4-Hydrazinopyrazino [3,2-D]	C9H12O	136.19	1	1	2.43	Late
Test compound	Formula	Molecular Weight (g/mol)	*H-Bond acceptors*	*H-Bond donors*	LogP	Solubility in water
Palmitic acid, n-Hexadecanoic acid	C16H3O2	255.42	2	1	4.94	Slightly Dissolved
9-Octadecenoic acid	C18H33O2	281.45	2	0	5.44	Slightly Dissolved
Dioctyl ester, 1-2	C34H58O4	530.82	4	0	9.91	Insoluble
Cinnamamide	C9H9NO	147.17	1	1	1.36	Very Soluble
Dihydrofarnesol	C15H28O	224.38	1	1	4.46	A little Late
Stigmasterol	C29H48O	412.69	1	1	6.98	Difficult Late
Aristolone	C15H22O	218.33	1	0	3.42	Late
Maragenin I	C29H46O2	426.67	2	1	5.96	Slightly soluble
2,3,8-Trioxocephalotaxane	C17H15NO5	313.30	5	0	1.09	Late
1,3-Benzenedicarboxamide	C8H8N2O2	164.16	2	2	0.13	Very Soluble

Table 4. Pharmacokinetic Results of Test Compounds

Test compound	**GI absorption**	**BBB Permeant**	**P-GP Substrate**	**CYP1A2 inhibitor**	**CYP2C19 Inhibitor**	**CYP2C9 Inhibitor**	**CYP2D6 Inhibitor**	**CYP3A4 Inhibitor**	***Bioavailability Score***
Taraxasterol	*Low*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	0.55
Benzo[b]naphtho[2,3-d]furan	*High*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	0.55
Phytol isomer	*Low*	*No*	*Yes*	*No*	*No*	*Yes*	*No*	*No*	0.55
Linolenic acid	*Low*	*Yes*	*No*	*No*	*No*	*No*	*No*	*No*	0.85
Squalene	*Low*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	0.55
Beta-sitosterol	*Low*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	0.55
Nonanoic acid	*High*	*Yes*	*No*	*No*	*No*	*No*	*No*	*No*	0.85
Gamma-Tocopherol	*Low*	*No*	*Yes*	*No*	*No*	*No*	*No*	*No*	0.55
Neophytadiene	*Low*	*No*	*Yes*	*No*	*No*	*Yes*	*No*	*No*	0.55
Dihydrolanosterol	*Low*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	0.55
2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	*High*	*Yes*	*No*	*No*	*No*	*No*	*No*	*No*	0.55
Methyl linolenate	*High*	*No*	*No*	*Yes*	*No*	*No*	*No*	*No*	0.55
1-[2-methyl-2-(4-methyl-3-pentenyl) cyclopropyl] ethanol	*High*	*Yes*	*No*	*No*	*No*	*No*	*No*	*No*	0.55
1-menthene	*Low*	*Yes*	*No*	*No*	*No*	*Yes*	*No*	*No*	0.55
Butyric acid, ester with citronellol	*High*	*Yes*	*No*	*No*	*No*	*No*	*No*	*No*	0.55
Mehp	*High*	*Yes*	*No*	*Yes*	*Yes*	*No*	*No*	*No*	0.85
Beta amyrin	*Low*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	0.55
Alpha-terpinene	*Low*	*Yes*	*No*	*No*	*No*	*No*	*No*	*No*	0.55
Beta-Element	*Low*	*No*	*No*	*No*	*Yes*	*Yes*	*No*	*No*	0.55
Germacrene-B	*Low*	*No*	*No*	*No*	*Yes*	*Yes*	*No*	*No*	0.55
Germacrene-D	*Low*	*No*	*No*	*No*	*No*	*Yes*	*No*	*No*	0.55
Lepidozene	*Low*	*No*	*No*	*No*	*Yes*	*Yes*	*No*	*No*	0.55
beta-cadinene	*Low*	*No*	*No*	*No*	*Yes*	*Yes*	*No*	*No*	0.55
Alpha-Gurjunene,.	*Low*	*No*	*No*	*No*	*Yes*	*Yes*	*No*	*No*	0.55
4-Hydrazinopyrazino [3,2-D]	*High*	*Yes*	*No*	*Yes*	*No*	*No*	*No*	*No*	0.55
Palmitic acid, n-Hexadecanoic acid	*High*	*Yes*	*No*	*Yes*	*No*	*No*	*No*	*No*	0.85
9-Octadecenoic acid	*High*	*Yes*	*No*	*Yes*	*No*	*No*	*No*	*No*	0.85
Dioctyl ester, 1-2	*Low*	*No*	*Yes*	*No*	*No*	*No*	*No*	*No*	0.17
Cinnamamide	*High*	*Yes*	*No*	*No*	*No*	*No*	*No*	*No*	0.55
Dihydrofarnesol	*High*	*Yes*	*No*	*Yes*	*No*	*Yes*	*No*	*No*	0.55
Stigmasterol	*Low*	*No*	*No*	*No*	*No*	*Yes*	*No*	*No*	0.55
Aristolone	*High*	*Yes*	*No*	*No*	*Yes*	*Yes*	*No*	*No*	0.55
Maragenin I	*Low*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	0.55
2,3,8-Trioxocephalotaxane	*High*	*No*	*Yes*	*No*	*No*	*No*	*No*	*No*	0.55
1,3-Benzenedicarboxamide	*High*	*No*	*No*	*No*	*No*	*No*	*No*	*No*	0.55

Toxicity Parameters

Toxicity tests were conducted on 35 compounds from lime leaves (Citrus amblicarpa) using the Toxtree application. The parameters observed in the toxicity test were Cramer's Rules, Carcinogenicity (genotox and nongenotox) and mutagenicity rulebase by ISS, and in Vitro Mutagenicity (Ames test) alert by ISS. The results of the toxicity test of the test compounds can be seen in the following table.

Table 5.Toxicity Test Results of Test Compounds with Cramer Rules Parameters

Test Compound	Parameter		
	Cramer Rules	***Carcinogenicity and Mutagenicity***	***In Vitro Mutagenicity (Ames Test)***
Taraxasterol	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Benzo[b]naphtho[2,3-d]furan	*High*(Class III)	*Structural Alert for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*Structural Alert for S. typhimurium mutagenicity*
Phytol isomer	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Linolenic acid	*High*(Class III)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Squalene	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Beta-sitosterol	*High*(Class III)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Nonanoic acid	*High*(Class III)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Gamma-Tocopherol	*High*(Class III)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Neophytadiene	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Dihydrolanosterol	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	*High*(Class III)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*Structural Alert for S. typhimurium mutagenicity*
Methyl linolenate	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
1-[2-methyl-2-(4-methyl-3-pentenyl) cyclopropyl] ethanol	*Intermediate* (Class II)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
-1-menthene	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Butyric acid, ester with citronellol	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Mehp	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Structural Alert for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Beta amyrin	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Alpha-terpinene	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Beta-Elements -	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Germacrene-B	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Germacrene-D	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Lepidozene	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Beta-cadinene	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Alpha-Gurjunene	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
4-Hydrazinopyrazino [3,2-D]	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Palmitic acid, n-Hexadecanoic acid	*High*(Class III)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
9-Octadecenoic acid	*High*(Class III)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Dioctyl ester, 1-2	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Structural Alert for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Cinnamamide	*Low*(Class I)	*Negative Alert for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Dihydrofarnesol	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
Stigmasterol	*High*(Class	*Negative for genotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*

Test Compound	Parameter		
	Cramer Rules	*Carcinogenicity and Mutagenicity*	*In Vitro Mutagenicity (Ames Test)*
	III)	*Negative for nongenotoxic carcinogenicity*	
Aristolone	*High*(Class III)	*Structural Alert for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*Structural Alert for S. typhimurium mutagenicity*
Maragenin I	*High*(Class III)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
2,3,8-Trioxocephalotaxane	*High*(Class III)	*Negative for genotoxic carcinogenicity* *Structural Alert for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*
1,3-Benzenedicarboxamide	*Low*(Class I)	*Negative for genotoxic carcinogenicity* *Negative for nongenotoxic carcinogenicity*	*No alert for S. typhimurium mutagenicity*

DISCUSSION

ACE Protein Download

The protein I used is the Identity Code 4C2P, Macromolecular Angiotensin-Converting Enzyme of Homo sapiens Organism Resolution 1.99 Å. The selection of specific samples in humans (Homo sapiens) is based on the desired effect of the compound. This study is expected to explain the effect of lime leaf compounds as ACE inhibitors in humans. The structural analysis method chosen uses electron microscopy and has a resolution of 2Å. In general, structures with small resolution values (e.g. 1.5 Å) have better quality compared to structures with higher resolutions such as 3 Å. However, structures with high resolutions of around 3 Å still provide sufficient information about the binding site for use in molecular docking (Lee et al., 2025).

Preparation of 3D Structure of ACE Protein

The protein structure downloaded from PDB is still bound to the native ligand, so it must be removed because it can interfere with the docking process.-an. The presence of a ligand bound to the active site of a macromolecule will hinder the interaction of the ligand added during the docking process (Listyani and Azizah, 2025). The ACE protein that has not been prepared using the BIOVIA application in Figure 2. The macromolecule that has been separated from the residue and the native ligand is ready for the docking process, so that the results of the molecular docking are better because there are no obstacles during the docking process with the test ligand (Listyani and Azizah, 2025). The optimized 3D structure of the ACE protein is then saved in .pdb format in Figure 3.

Validation of Molecular Docking Method

Validation of the docking method against native ligands was performed to determine the conformation of the native ligand. The resulting docking conformation was then compared with the crystallographic conformation of the native ligand, expressed in RMSD values. Gridbox settings during the redocking process were used to determine the ligand binding area to be docked. The gridbox was adjusted based on the native ligand already bound to the protein macromolecule when downloaded. Docking method validation was performed on native ligands extracted from the macromolecule and optimized by VegaZZ software (Pratama et al., 2021). The saved macromolecule was then subjected to preliminary testing and method validation using the PyRx application. Macromolecular settings were performed by specifying the gridbox. Gridbox settings include center_x, center_y, and center_z to determine the position of the parameter box on the macromolecule, and size_x, size_y, and size_z to determine the gridbox size to be used for the ligand binding space of the test results. An example is in Figure 7.

Molecular Docking Results

Based on the results of the molecular docking process of the test compounds against the ACE protein, it shows that 35 test ligands and 2 reference ligands produce the best (lowest) RMSD and ΔG Binding values. Docking data obtained binding energy values range from -5.0 kcal/mol to -9.9 kcal/mol and RMSD values range from 1.02 to 1.998. The results of the RMSD and ΔG Binding values are in accordance with the literature, namely an RMSD value of less than 3 for structural conformational alignment is still acceptable but the most optimal value is less than 2. The smaller the ΔG Binding value, the better the interaction with the reference compound (Listyani et al., 2022).

Interaction of Amino Acids of Test Ligands with Receptors

From the results of the interaction of 35 compounds of lime leaves (Citrus Amblycarpa), there are 2 compounds that have the same amino acid residue interactions with native ligands in hydrogen bonds. Although it has been mentioned that in terms of ΔG binding value Maragenin I has the lowest value among 36 test compounds, namely -9.9 with a RMSD value of 1.577 Å, but no amino acid residues were found that interact in terms of hydrogen bonds, namely TRP A: 220 and ASN A: 211 are the same bonds with native ligands, but there are also two compounds that have the same amino acid activity as the native ligand captopril, namely, Aristolone and 1,3-Benzenedicarboxamide, where each of these compounds has its own bond that is similar to the native ligand has a TRP A bond, and ASN A, but its ΔG binding is not as low as Maragenin I. The results of amino acid binding of native ligands, negative controls and test compounds. Based on the interaction pattern of amino acid residues, Captopril, as a positive control, is an ACE inhibitor that works by binding directly to the active site of the enzyme. The thiol (–SH) group in captopril is able to coordinate with the Zn^{2+} ion found in the active site of ACE, thereby inhibiting the enzyme's catalytic activity and preventing the conversion of angiotensin I to angiotensin II. This inhibition causes decreased vasoconstriction and aldosterone secretion, thereby lowering blood pressure.(Jiao et al., 2025). a number of test compounds showed compatibility of conventional hydrogen bonding with captopril as a native ligand, namely Tryptophan (TRP), Asparagine (ASN). Thus indicating the ability to mimic some of the standard ligand binding models in the ACE binding site and potentially produce similar biological activity. Compounds that have specific amino bond residues with the native ligand captopril test ligand include Aristolone and 1,3-Benzenedicarboxamide. This similarity supports the interpretation that these ligands have the potential to access the same active site and utilize similar residues as captopril in the binding process, as the concept that the more amino acid residues in common with the native ligand, the greater the chance that the test ligand has similar activity (Baroroh et al., 2023).

ADME Parameter Prediction

Pharmacokinetic testing, or ADME prediction, is performed to assess the potential of a compound to be an oral drug or a drug-like agent. The SwissADME website displays pharmacokinetic test results using several parameters, one of which is Lipinski's rule of five. Lipinski's rule of five is a guideline for categorizing oral drug compounds from non-drug compounds based on their chemical structure, proposed by Lipinski and his team. This rule can predict the likelihood of a compound being an oral drug, as drug-like compounds tend to meet two or more of these criteria. Lipinski's rule can identify a compound's physicochemical properties to determine its solubility in oil or water so that it can passively diffuse through cell membranes. Lipinski's rule states that the molecular weight must not exceed 500 g/mol, the partition coefficient (logP, related to solubility in oil or water) must be less than 5, the number of hydrogen bond acceptors must be less than 10, and the number of hydrogen bond donors must be less than 5 (Listyani et al., 2022). The following are the predicted results of the ADME Lipinski's Rule activity test of 35 test ligand compounds:

Ligands with a molecular weight of more than 500 g/mol cannot penetrate the cell membrane.(Yuliana et al., 2022)so that the activity of the molecule is less influential. In this study, it was found that the ligand with a molecular weight greater than 500 g/mol is Dioctyl ester, 1-2. This compound does not meet the molecular weight criteria requirements in Lipinski's law and is predicted to have lower membrane permeability. The higher the logP value, the more hydrophobic the molecule is and tends to partition more into the lipid phase than the aqueous phase. Highly hydrophobic molecules generally exhibit higher toxicity because they will be distributed more widely in body tissues. The molecules will be retained longer in the fat layer, thus extending the residence time in the body and potentially increasing non-selective interactions with various biological macromolecules, obtained logP that meets the requirements of Benzo[b]naphtho[2,3-d]furan, Nonanoic acid, 2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane, 1-menthene, Butyric acid, ester with citronellol, Mehp, Alpha-terpinene, Beta-Elemene, Germacrene-B, Germacrene-D, Lepidozene, beta.-cadinene, Alpha-

CONCLUSION

There is an interaction between compounds contained in lime leaves (Citrus amblicarpa) with amino acid residues of amino acid proteins. Compounds that have a specific interaction pattern with captopril as a native ligand are Aristolone and 1,3-Benzenedicarboxamide. All test compounds have a fairly stable binding affinity value to the ACE protein. Of the two compounds that bind to amino acid residues, ADME parameter predictions using SwissADME show that the 1,3-Benzenedicarboxamide compound of lime leaves meets the druglikeness criteria and Lipinski's five laws, with a molecular weight profile, the number of hydrogen bond donors and acceptors, and lipophilicity that are still within acceptable limits. The compound also has a good gastrointestinal absorption prediction and a sufficient bioavailability score. The results of the toxicity prediction of the 1,3-Benzenedicarboxamide compound in lime leaves with toxtree have a low toxicity category (Class I) based on Cramer's rules. The best compound obtained was 1,3-Benzenedicarboxamide (RMSD 1.998 and ΔG −6.4), specific amino acid residue bonds such as captopril, fulfilling Lipinski's Rule of Five criteria, and in the low toxicity category (class I) based on Cramer's rules.

REFERENCES

Ancuceanu, R., Lascu, B.E., & Dinu, M. (2025). In Silico ADME Methods Used in the Evaluation of Natural Products. 1–58.

Arsana, IN, Ketut, N., Juliasih, A., Disebut, AAA, & Widyantari, S. (2024). GCÿMS Analysis of Bioactive Compounds in Ethanol Extract of Lime Leaves (Citrus amblycarpa (Hassk.) Ochse), and Its Potential as a Traditional Medicinal Agent. April. https://doi.org/10.29303/jppipa.v10i4.3735

Arsana, IN, Ketut, N., Juliasih, A., & Widyantari, AAASS (2024). GC – MS Analysis of Bioactive Compounds in Lime Leaf Ethanol Extract (Citrus amblycarpa (Hask.) Ochse), and Its Potential as a Traditional Medicine Agents. 10(4), 1994–2006. https://doi.org/10.29303/jppipa.v10i4.3735

Ayu, IG, Kusumawati, W., Wisnu, IM, Putra, A., Bagus, I., Yogeswara, A., Gizi, I., Kesehatan, F., Pura, UD, Kesehatan, F., & Pura, UD (2021). In Vitro ACE Inhibitory Activity and Bioactive Compounds from Citrus amblycarpa Water Extract. 26(2), 117–122. https://doi.org/10.22146/mot.65028

Bachtiar, KR, Susanti, S., Mardianingrum, R., & Tasikmalaya, UP (2021). Anti-inflammatory activity test of compounds in essential oil of bangle rhizome (. 4(1), 36–43.

Bagus Dermawan, TD (2024). The Use of Sublingual Captopril as an Effective Method in Lowering Blood Pressure Before Dental Procedures: Analysis Through Case Studies. 6(1), 111–115.

Bari, U., Moro, A., Caponio, G.R., Bari, U., Moro, A., Centrone, M., Bari, U., & Moro, A. (2023). Machine Translated by Google In silico analysis of angiotensin-converting enzyme inhibitor compounds obtained from soybean [Glycine max (L.)]. https://doi.org/10.3389/fphys.2023.1172684

Baroroh, U., Muscifa, ZS, Destiarani, W., Rohmatulloh, FG, & Yusuf, M. (2023). Molecular interaction analysis and visualization of protein-ligand docking using Biovia Discovery Studio Visualizer. 2(1), 22–30.

Bahi, RRR, Herowati, R. and Harmastuti, N., 2020. Biochemoinformatics study of the chemical content of sambiloto leaves (Andrographis paniculata (Burm. f.) Nees) as antihyperglycemia and prediction of pharmacokinetic and toxicity parameters. PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia), pp.466-477.

Budiarto, R., Poerwanto, R., Santosa, E., & Efendi, D. (2017). The Potentials of Lime (Citrus amblycarpa Hassk. Ochse) as A Functional Food and Ornamental Mini Tree Based on Metabolomic and Morphological Approaches. Journal of Tropical Crop Science, 4(2), 49–57. https://doi.org/10.29244/jtcs.4.2.49-57

Cornelissen, FM, Markert, G., Deutsch, G., Antonara, M., Faaij, N., Bartelink, I., Noske, D., Vandertop, WP, Bender, A. and Westerman, B.A., 2023. Explaining blood–brain barrier permeability of small molecules by integrated analysis of different transport mechanisms. Journal of Medicinal Chemistry, 66(11), pp.7253-7267

Chen, G., Seukep, AJ, & Guo, M. (2020). Recent advances in molecular docking for the research and discovery of potential marine drugs. Marine Drugs, 18(11), 545. https://doi.org/10.3390/md18110545

Ekins, S., Lane, T.R., Urbina, F., & Puhl, A.C. (2025). In Silico ADME/Tox Comes of Age: Twenty Years Later. 54(7), 352–358. https://doi.org/10.1080/00498254.2023.2245049.In

Ghahremanian, S., Mehdi, M., Raeisi, K., & Toghraie, D. (2022). Molecular dynamics simulation approach for discovering potential inhibitors against SARS-CoV-2: A structural review of information. January.

Gupta, P.K., Pal, Y., Kumar, P., Gupta, S., Singh, S.D., & Tiwari, S.B. (2024). A Critical Review on Computational Techniques through in silico Assisted Drug Design. 14(4), 1035–1041. https://doi.org/10.5530/ijpi.14.4.113

Halder, SK, Sultana, I., Shuvo, MN, Shil, A., Himel, MK, Hasan, MA and Shawan, MMAK, 2023. In Silico Identification and Analysis of Potentially Bioactive Antiviral Phytochemicals against SARS-CoV-2: A Molecular Docking and Dynamics Simulation Approach. BioMed Research International, 2023(1), p.5469258.

Herline, F., Susilowati, SS, & Kurniawan, DW (2020). Quality Evaluation of Generic Paracetamol Tablets Circulating in the Purwokerto Region. Research Article. 12, 38–47.

Hess, AY, Ramadhani, SZ, Andhryanti, RN, & Zhafirah, N. (2024). Indonesian Journal of Chemical Science In Silico Study of Bioactive Compounds in Guava Leaves (Psidium guajava L.) toward Angiotensin Converting Enzyme (ACE) as target for hypertension. 13(3).

Huang, Y., Wu, P., Ying, J., Dong, Z. and Chen, XD, 2021. Mechanistic study on inhibition of porcine pancreatic α-amylase using the flavonoids from dandelion. Food Chemistry, 344, p.128610.

Hussain, M., Khan, K.M., Ahmad, A., Rasheed, S., & Taha, M. (2022). Amide derivatives as potential therapeutic agents: Synthesis, biological evaluation, and ADME prediction. ACS Omega, 7, 42835-42846. https://doi.org/10.1021/acsomega.2c05550

Irmayanti, S., Dewi, C., & Natsir, N. (2025). Acute Toxicity Test of Ethanol Extract of Suruhan Leaves (Peperomia pellucida L. Kunth) Cu te T oxici ty T es t in g O f E thano l E xt rac t O f S e ruha n L e av es (Pi p eromi a pelluci da L. Kun th). 4(5).

Jiao, F., Yang, J., & Wang, F. (2025). Discovery of novel angiotensin-converting enzyme inhibitory peptides by in silico and in vitro studies. 39885–39897. https://doi.org/10.1039/d5ra06104k

Kasman, K., Ishak, NI, Hastutiek, P., Suprihati, E., & Mallongi, A. (2020). Identification of Active Compounds of Ethanol Extract of Citrus amblycarpa leaves by Analysis of Thin-layer Chromatography and Gas Chromatography-Mass Spectrometry as Bioinsecticide Candidates for Mosquitoes. 8, 1–6.

Khoirunnisaa, DM (2022). Thesis: The Effect of Giving 70% Ethanol Extract of Lime Peel (Citrus Amblycarpa) Through Malondialdehyde Test and Cardiac Histopathy.

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B.A., Thiessen, P.A., Yu, B., Zaslavsky, L., Zhang, J., & Bolton, E.E. (2021). PubChem in 2021 : new data content and improved web interfaces. 49(November 2020), 1388–1395. https://doi.org/10.1093/nar/gkaa971

Klomp, F., Wenzel, C., Drozdzik, M., & Oswald, S. (2020). Drug – Drug Interactions Involving Intestinal and Hepatic CYP1A Enzymes.

Conversion, A., Human, E., & Ace, C. A. (2021). Simulation Docking Active Compounds Lab As. 12, 159–166.

Kurniawan, A., Mumpuni, E., Abdillah, S., Farmasi, PS, Gunadarma, U., Doktor, P., Farmasi, I., Farmasi, F., Pancasila, U., Farmasi, DK, Farmasi, F., Airlangga, U., Farmasi, DK, Farmasi, F., Pancasila, U., Pharmacology, D., Farmasi, F., & Pancasila, U. (2022). In Silico Molecular

Docking and Toxicity Studies of Bioactive Fucoidan Compound from Brown Seaweed as Potential of Antihypertensive. 19(1), 1–9.

Larisa, I., & Karelson, M. (2022). The Impact of Software Used and the Type of Target Protein on Molecular Docking Accuracy.

Latif, Multiani S; Rusdiana, Taofik; Gozali, Dolih (2023). The Effect of P-Glycoprotein (P-Gp) on Drug Bioavailability: A Review. Farmaka, [SL], V. 15, N. 3, P. 1-6, Feb. 2018. Issn 2716-3075.

Lee, DH, Kim, JW, Cong, R., Park, JS, Nguyen, CHB, Park, K., Kang, K. and Shim, SM, 2025. Exploring absorption indices for a variety of polyphenols through Caco-2 cell model: insights from permeability studies and principal component analysis. Journal of the Science of Food and Agriculture

Listyani, TA and Azizah, D., 2025. Molecular Docking Analysis and Prediction of Adme Flavonoid Derivatives of Chemical Composition Compounds of Roselle Flower Extract (Hibiscus Sabdariffa L.) as Antiobesity. Duta Pharma Journal, 5(2), Pp.141-158.

Listyani, TA, Fauzi, F., Siska Wardani, T., Selangor Malaysia, M., Ilmu, J., Alam, S., Program, S., & Tinggi Ilmu Kesehatan Kendal, S. (2022). In Silico ADME AND Toxicity Studies Of Derivate Phthalimide Compounds As Non-Nucleuside HIV-1 Reverse Transcriptase Inhibitor. Global Health ScienceGroup, 3(1), 17–26.

Mayasari, S. (2020). Analysis of Captopril Drug Use with Blood Pressure in Hypertensive Patients. 8(2), 123–127. https://Doi.Org/10.36858/Jkds.V8i2.225mazzolari, A., Scaccabarozzi, A., Vistoli, G., & Pedretti, A. (2021). Metaclass, A Comprehensive Classification System For Predicting The Occurrence Of Metabolic Reactions Based On The Metaqsar Database.

Moshawih, S., Bu, Z. H., Goh, H. P., Kifli, N., Lee, L. H., & Goh, K. W. (2024). Consensus holistic virtual screening for drug discovery : a novel machine learning model approach. Journal of Cheminformatics, 8, 1–27. https://doi.org/10.1186/s13321-024-00855-8

Mumpuni, M., Zakiyyah, HN, & Manurung, S. (2023). A Comparative Study of Employment Status in Counseling on Medication Adherence in Hypertension Patients at Community Health Centers. Quality: Health Journal, 17(2), 96–104. https://doi.org/10.36082/qjk.v17i2.1206

Nahid, N. A., & Johnson, J. A. (2024). CYP2D6 pharmacogenetics and phenoconversion in personalized medicine. https://doi.org/10.1080/17425255.2022.2160317.CYP2D6

Niu, Y., Liu, R., Guan, C., Zhang, Y., Chen, Z., Hoerer, S., Nar, H. and Chen, L., 2022. Structural basis of Inhibition of the Human SGLT2–MAP17 Glucose Transporter. Nature, 601(7892), pp. 280-284.

Novitri, SA, Saibi, Y., & Muhtaromah, M. (2021). Study of Compliance-Enhancing Methods in Hypertension Patients: A Systematic Literature Review. Pharmaceutical and Biomedical Sciences Journal (PBSJ), 3(1), 17–30. https://doi.org/10.15408/pbsj.v3i1.20357

Nurfadhila, L., Rahmawati, M., Komala Fitri, N., Granadha Nibullah, S., & Windari, W. (2023). Analysis of acetaminophen compounds in biological samples using various methods. 6(3), 1221–1237.

Ochse, H., Potential, I., Medicine, T., Arsana, IN, Ketut, N., Juliasih, A., & Widyantari, AAASS (2024). GC-MS Analysis of Bioactive Compounds in Lime Leaf Ethanol Extract (Citrus GC – MS Analysis of Bioactive Compounds in Lime Leaf Ethanol Extract (Citrus amblycarpa (Hask.) Ochse), and Its Potential as a Traditional Medicine Agents. April. https://doi.org/10.29303/jppipa.v10i4.3735

Octavianie, G., Nina, Pakpahan, J., Maspupah, T., & Debora, T. (2022). Health Promotion of Hypertension in Productive Age to Elderly in Lulut Village Area RT 04 RW 02 Klapanunggal District, Bogor Regency. 01(02).

Pablo, J., Arango, B., Yuliana, D., Rodriguez, M., & Ocampo, G. T. (2025). In silico evaluation of pharmacokinetic properties and molecular docking for the identification of potential anticancer compounds. 120(May 2025). https://doi.org/10.1016/j.compbiolchem.2025.108626

Panghiyangani, R., Utami, Putri, J., Baitullah, Akbar, M., Maulida, D, N., & Wi. (2023). Molecular

Docking of Citrus amblycarpa Active Compounds against FTO, Leptin, and Resistin Protein. 7(1), 38–46. https://doi.org/10.21705/mcbs.v7i1.295

Perdana, AT, Permana, AA, Biology, PS, Science, F., Indonesia, UA, Baru, K., Selatan, J., Studi, P., Informatics, T., Engineering, F., & Tangerang, UM (2021). MOLECULAR DOCKING OF POTENTIAL ANTICOVID-19 COMPOUNDS IN SILICO Analekta. 2019, 159–166.

Pratama, KJ, Fitriawati, A., & Hendra, ND (2024). Treatment compliance of hypertension patients at the Kanegaran Community Health Center, Probolinggo City, East Java 1,. 318–324.

Pratama, AB, Herowati, R. and Ansory, HM, 2021. Molecular Docking Study of Compounds in Nutmeg Essential Oil (Myristica fragrans H.) and Myristicin Derivative Compounds Against Skin Cancer Therapy Targets. Pharmaceutical Magazine, 17(2), pp.233-242.

Putri, SA, Ramdini, DA, Wardhana, MF, Pharmacy, PS, Medicine, F., & Lampung, U. (2023). Literature Review: Side Effects of Using Hypertension Drugs. 13(April), 583–589.

Rifki, R., & Indawati, E. (2024). The Effectiveness of Counseling on Medication Compliance in Hypertension Patients at Bahagia Community Health Center. Malahayati Nursing Journal, 6(2), 516–531. https://doi.org/10.33024/mnj.v6i2.10759

Riyadi, PH, Sari, ID, Kurniasih, RA, Agustini, TW, Swastawati, F., Herawati, VE and Tanod, WA, 2021. SwissADME predictions of pharmacokinetics and drug-likeness properties of small molecules present in Spirulina platensis. In IOP conference series: earth and environmental science (Vol. 890, No. 1, p. 012021). IOP Publishing.

Samuel, O., Olubukola, F., Olorunnisola, S., Ifeoluwa, P., Subair, T.I., & Emmanuel, O. (2022). Angiotensin-converting enzyme inhibitors from medicinal plants: a dynamic simulation and molecular docking approach. 1–17.

Santoso, R., Rahman, MF, Nurakillah, H., & Herawati, AT (2022). Overcoming and Preventing Hypertension by Recognizing Hypertension for a Healthy Lifestyle in Cipadung Wetan Village, Bandung City. 1(3), 221–228.

Sida, NA, & Kasmawati, H. (2025). Sansevieria trifasciata Prain.: Antibacterial Activity, Toxicity, and ADME Prediction of In silico Compounds. 14(2), 165–176.

Sikala, S., Distinarista, H., & Rahayu, T. (2025). Factors Affecting the Incidence of Hypertension in the Elderly at Bhayangkara Hospital Level II Jayapura Sultan Agung Islamic University, Indonesia coronary heart disease, stroke and myocardial infarction. Hypertension causes heart conditions. 3, 15–18.

Terefe, EM and Ghosh, A., 2022. Molecular docking, validation, dynamics simulations, and pharmacokinetic prediction of phytochemicals isolated from Croton dichogamus against the HIV-1 reverse transcriptase. Bioinformatics and biological insights, 16, p.11779322221125605.

Tiwari, P., & Sumitha, A. (2020). Molecular docking, validation, dynamics simulations, and pharmacokinetic prediction of natural compounds against the SARS-CoV-2 main-protease. Journal of Biomolecular Structure and Dynamics, 0(0), 1–27. https://doi.org/10.1080/07391102.2020.1815584

Yuliana, A., Wulandari, WT, & Ratnasari, I. (2022). Antiviral Activity Test of Catechin Derivative Compounds against SARS-CoV-2 M-Protease in Silico. 2, 407–420.

Zhang, H., Zhou, Y., Liu, D., & Chen, J. (2022). Molecular docking and ADMET evaluation of flavonoids as potential antidiabetic agents. Molecules, 27(18), 6041.