

Antihypertensive Activity of Arabica Coffee Husks (Cascara) using Metabolomic Profiling and Molecular Docking

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ABSTRACT

Green coffee beans have been reported to lower blood pressure, and compounds derived from cascara arabica have shown potential anti-hypertensive activity. In this study, we investigated the angiotensin-converting enzyme (ACE) inhibitory activity of these compounds and identified potential ACE inhibitors through metabolomic profiling and molecular docking analysis. Metabolomic data were processed using chemometric analysis, followed by molecular docking to assess the binding interactions of the compounds with the active site residues of the ACE and AGTR1 enzymes. LC-HRMS revealed that cascara arabica contained primarily caffeine, neochlorogenic acid, protocatechuic acid, caffeic acid, trans-caffeic acid, chlorogenic acid, and gallic acid. The docking results revealed the strongest binding site residues for quercetin-3 β -D-glucoside, as well as for other docked compounds, including kaempferol, chlorogenic acid (5-CQA), neochlorogenic acid, gallic acid, and caffeic acid, which all exhibited good binding affinities with proteins 1O86 and 4ZUD. The activity of compounds was better than that of captopril, which was used as a positive control. The validation method of molecular docking was based on an RMSD value <2.00 Å for native ligand redocking, with RMSD values of 0.45 Å for the lisinopril ligand and 1.92 Å for the olmesartan ligand. Binding affinity scores for ACE protein controls were captopril at -5.50 Å and lisinopril at -8.10 Å, whereas for AGTR1 protein controls, candesartan was -8.90 Å and olmesartan was -9.80 Å. Results showed that the compounds from cascara arabica could be serve as an initial benchmark in determining potential drug compounds.

Key words: Angiotensin; agtr1; captopril; chlorogenic acid; caffeic acid.

1 INTRODUCTION

Hypertension is often referred to as the “silent killer” owing to its status as a leading cause of mortality, largely because many cases are undiagnosed until advanced stages. Conventional anti-hypertensive treatments are associated with adverse effects in certain individuals and are often less accessible to populations in rural areas, leading to inadequate blood pressure control (World Health Organization - WHO, 2023). Herbal medicines are used by 80% of the global population, for hypertension management, largely due to their affordability and minimal side effects (Tabassum & Ahmad, 2011).

Plant-derived metabolites have shown potential anti-hypertensive properties (Kamyab et al., 2021). Among these, chlorogenic acids are the primary bioactive compounds identified in various parts of the *Coffea arabica* plant, including the husks of both arabica and robusta varieties, silverskins, pulp or parchment, and other coffee by-products (Ali et al., 2022; Cangeloni et al., 2022; Maliza et al., 2021; Sirotkin & Kolesarova, 2021). Ethanol extracts of arabica coffee peels contain three major compounds: gallic acid, chlorogenic acid, and caffeic acid (Silva et al., 2021). Notably, chlorogenic acid has demonstrated inhibitory activity against components of the renin-angiotensin-aldosterone system (RAAS), particularly angiotensin-converting enzyme (ACE).

However, the ACE inhibitory potential of compounds present in cascara arabica has not yet been empirically tested. This study therefore investigated the ACE inhibition activity of cascara arabica constituents through metabolomic analysis and molecular docking.

Metabolomics is one of the analysis methods used to study biological systems in plants. Metabolomic analysis in phytochemical studies can be applied to identify active compounds and evaluate the correlation between their activity and metabolite compounds (Waris et al., 2022). The multivariate analysis methods, such as principal component analysis (PCA) and partial least squares - discriminant analysis (PLS-DA,) used in metabolomics analysis reduce the complexity of the data from metabolite profiling and facilitate identification (Lee et al., 2017). Molecular docking is used to rationalize the activity of ligands against target proteins and to identify a ligand that binds to a specific target (Pinzi & Rastelli, 2019).

The high number of coffee producers has led to an increasing amount of coffee waste, which is currently underutilized, and is primarily limited to fertilizer and animal feed. Recently, the use of coffee cherry pulp waste as a component in cascara tea has garnered interest among some consumers. Research into its health benefits, including its impact on human blood pressure, is underway, particularly since coffee is known to potentially contribute to high blood pressure.

2 MATERIAL AND METHODS

2.1 Experimental details and treatments

Plant material: Arabica coffee husks were obtained from coffee farmers in Gayo City, Aceh, Indonesia. The species at Herbarium Depokensis (UIDEP), UI Biota Collection Room, Department of Biology, Universitas Indonesia, was then determined.

Chemical: Ethanol, ethyl acetate, hexane, and aquadest were purchased from Brataco, Indonesia, and methanol for analysis was purchased from Merck.

Treatments: The dried cascara arabica was sorted and ground using a grinder and then sifted through mesh. Extraction was performed using ultrasound assisted extraction (UAE) (Branson, 3800) adapted from Silva *et al.* (2021) with minor modifications. Extraction was carried out for 1 hour with 70% ethanol 1:10 (w/v) at 40 °C. The filtrate was subsequently collected and filtered with Whatman 42 filter paper. The extraction process was repeated three times, and the products were evaporated using a rotary vacuum evaporator. After that, the extract was diluted in warm water at a 1:10 (w/v) ratio. Fractionation was carried out using a separate funnel, and was repeated 3 times. After the extract was diluted, it was partitioned using hexane at a ratio of 1:1, shaken for about 3 min, and then left to sit until 2 layers formed. The filtrates were subsequently combined and evaporated.

2.2 Metabolomic Profiling

Samples used for metabolomic profiling were ethanol extract, hexane fraction, ethyl acetate fraction, and water fraction. Ethanol extract and water fraction were subsequently dissolved in water, and ethyl acetate fraction and hexane fraction were subsequently dissolved in methanol. Metabolite analysis was performed using LC-HRMS with a method adapted from Windarsih *et al.* (2022).

2.3 Chemometric Analysis

Principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) were used for chemometric analysis via MetaboAnalyst 6.0 which was accessed online through the following website: <https://www.metaboanalyst.ca/MetaboAnalyst/ModuleView.xhtml>.

2.4 Molecular Docking

The active compounds obtained from the metabolomic profiling results were docked using the following processor: 12th generation Intel Core i5-1235U 16 GB RAM Intel Iris Xe Graphics Card. Metabolite compounds were selected on the basis of the gene expression of both ACE (Angiotensin Converting Enzyme) and AGTR1 (Angiotensin 1 Receptor

Blocker) proteins identified using the GeneCards (<https://www.genecards.org/>) website and compliance with at least 4 of Lipinski's Rule of Five rules identified online using Lipinski's Rule of Five (<https://scfbio-iitd.res.in/software/drugdesign/lipinski.jsp#anchortag>) website.

The metabolite structure was obtained from the PubChem website (<https://pubchem.ncbi.nlm.nih.gov/>) in 3D form with SDF format, and then the file was converted into PDB format using PyMOL software. Structures that were already in the PDB format were imported into PyRx 8.0 via the Babel tool for energy minimization and then converted to the ligand AutoDock (PDBQT) format.

The target proteins ACE (PDB ID: 1O86) and AGTR1 (PDB ID: 4ZUD) were retrieved from the RCSB Protein Data Bank (<https://www.rcsb.org/>). Ligands and water molecules were separated from protein structures using PyMOL (trial version), and the resulting purified proteins were saved in PDB format. The protein structures were subsequently optimized and converted into PDBQT format using PyRx. The native ligands separated during preprocessing were preserved in PDB format to serve as control ligands.

Molecular docking studies involving selected ligands and target metabolites were performed using AutoDock integrated within the PyRx 0.8 virtual screening platform. Grid box parameters were defined based on the binding site coordinates of the native ligand, and docking simulations were conducted with an exhaustiveness setting of 32. The docking results were combined with the purified proteins and visualized via BIOVIA Discovery Studio Client 2025 software.

Molecular docking was validated on the basis of the value of the root mean square deviation (RMSD) between the docked ligand and the pure ligand derived from the protein crystal structure. The RMSD value, used as a validation parameter was categorized into three classifications: good solution (RMSD $\leq$ 2.00 Å), acceptable solution (RMSD $\geq$ 2.00 Å), and poor solution (RMSD $\geq$ 3.00 Å) (Ramírez & Caballero, 2018).

3 RESULTS

3.1 Metabolomic Profiling

There were 193 total metabolites in the ethanol extract, 782 in the hexane fraction, 946 in the ethyl acetate fraction, and 367 in the water fraction. The metabolites were selected based on the mz-Cloud best match range of 91-100. Therefore, the sample contained 33 metabolites in the extract, 74 in the hexane fraction, 104 in the ethyl acetate fraction, and 42 metabolites in the water fraction, as shown in Table 1. The sample was divided based on the level of polarity of the compounds, namely, n-hexane, ethyl acetate, and water, as well as the crude 70% ethanol extract.

Table 1: Metabolite profile of cascara arabica using LC-HRMS.

Name Compounds	Molecule Formula	m/z	RT (minute)				% Peak Area			
			Ethanol extract	Hexane fraction	Ethyl acetate fraction	Water fraction	Ethanol extract	Hexane fraction	Ethyl acetate fraction	Water fraction
Hydroxycinnamic acid										
Chlorogenic acid	C16 H18 O9	354.095	3.71	-	3.84	3.72	0.82	-	0.98	1.63
Neochlorogenic acid	C16 H18 O9	354.095	2.13	-	6.36	2.12	0.03	-	0.01	0.12
3-Feruloylquinic acid	C17 H20 O9	368.111	5.07	-	5.05	5.06	0.12	-	0.44	0.14
Caffeic acid	C9 H8 O4	180.042	-	-	4.05	-	-	-	0.14	-
trans-caffeic acid	C9 H8 O4	180.042	-	-	3.64	-	-	-	0.015	-
4,5-Dicaffeoylquinic acid	C25 H24 O12	516.127	-	-	6.34	-	-	-	0.38	-
4-Coumaric acid	C9 H8 O3	164.048	-	-	5.28	-	-	-	0.03	-
Phenolic acid										
Gallic acid	C7 H6 O5	170.021	1.25	-	-	1.25	0.19	-	-	0.06
Protocatechuic acid	C7 H6 O4	154.027	2.01	-	1.87	-	0.48	-	0.96	-
2-Furoic acid	C5 H4 O3	112.016	1.26	-	1.24	1.27	0.25	-	0.69	0.16
4-Amino-3-hydroxybenzoic acid	C7 H7 N O3	153.043	1.44	-	-	-	0.03	-	-	-
Vanillic acid	C8 H8 O4	168.042	-	-	5.15	-	-	-	0.02	-
Flavonoids										-
Quercetin	C15 H10 O7	302.043	-	7.49	7.73	-	-	0.03	0.02	
Kaempferol-3-O-rutinoside	C27 H30 O15	594.158	5.98	-	-	5.98	0.01	-	-	0.08
Kaempferol	C15 H10 O6	286.048	-	8.39	8.39	5.48	-	0.05	0.16	0.01
Quercetin-3β-D-glucoside	C21 H20 O12	464.096	-	-	5.72	5.74	-	-	0.13	0.02
Quercetin 3-O-rhamnoside-7-O-glucoside	C27 H30 O16	610.153	-	-	5.52	5.54	-	-	0.02	0.17
Pinocembrin	C15 H12 O4	256.074	7.49	7.48	10.28	-	0.01	0.01	0.04	-
Xanthines										
Caffeine	C8 H10 N4 O2	194.080	4.06	4.03	4.00	4.07	44.85	7.64	40.45	28.95
Theobromine	C7 H8 N4 O2	180.065	1.92	-	1.80	1.92	0.34	-	0.37	0.80
Theophylline	C7 H8 N4 O2	180.065	2.49	-	2.41	2.49	0.48	-	0.62	0.60
7-Methylxanthine	C6 H6 N4 O2	166.049	1.31	-	1.29	1.32	0.18	-	0.18	0.24
Pyridines										
Methyl isonicotinate	C7 H7 N O2	137.048	0.84	0.80	-	0.87	28.16	0.92	-	24.73
Nicotinic acid	C6 H5 N O2	123.032	-	-	-	0.99	-	-	-	0.13
Nicotinamide	C6 H6 N2 O	122.048	-	-	-	-	-	-	-	-
3-Hydroxypyridine	C5 H5 N O	95.037	-	-	0.82	-	-	-	0.05	-

The total ion chromatogram (TIC) of the extract revealed that more compounds were present at retention times of 0.50 – 2.00 min and at 4.00 – 5.00 min, in the hexane fraction at 10.00 – 25.00 min, and in the ethyl acetate fraction and the water fraction many compounds were found at 4.00-5.00 min. The TIC in the extract showed that the highest chromatogram was that of methyl isonicotinate compounds, followed by the

caffeine chromatogram. Similarly, in the water fraction, the compound with the highest chromatographic peak was caffeine, followed by methyl isonicotinate. In contrast, the hexane fraction also presented caffeine as the dominant peak, followed by methyl isonicotinate. Moreover, the ethyl acetate fraction contained the most prominent compound, caffeic acid, followed by caffeine and chlorogenic acid compounds.

The dominant compound contained in the extract and fraction was caffeine. In addition to caffeine, several polyphenol compounds, such as chlorogenic acid (5-CQA), gallic acid, caffeic acid, neochlorogenic acid, 4,5-dicaffeoylquinic acid, *trans*-caffeic acid, and protocatechuic acid, were present in the extract, ethyl acetate fraction, and water fraction. Several flavonoid compounds, such as quercetin, quercetin-3 β -D-glucoside, quercetin 3-O-rhamnoside-7-O-glucoside, and kaempferol, as well as several other compounds, such as theobromine, nicotinic acid, and methyl isonicotinate, were also present in the ethyl acetate, extract, and water fraction. In the hexane fraction, the dominant compounds were nonpolar compounds, including octadecadienoic acid, α -elostearic acid, and α -linolenic acid.

3.2 Chemometric Analysis

Chemometric analysis via PCA was used to separate each obtained sample based on polarity, with a value of principal component 1 (PC1) of 90.9% and a value of principal component 2 (PC2) of 9%. The position of each compound (Figure 1) was as follows: quadrant I contained the ethyl acetate fraction, which indicated polyphenol compounds, quadrant II contained the hexane fractions, quadrant III

contained the extract and water fractions, which indicated the presence of gallic acid compounds, which were more dominant at the extraction point, and quadrant IV was absence of sample points, with some compounds present in quadrant IV, such as neochlorogenic acid.

The classification based on compound polarity was analyzed using PLS-DA. Metabolites were significantly separated according to their polarity, with a p-value < 0.05. The VIP scores from the PLS-DA analysis indicated that several target compounds presented relatively high intensities across different extracts and fractions. Chlorogenic acid (5-CQA), caffeic acid, and protocatechuic acid showed high intensities in the ethyl acetate fraction. In contrast, gallic acid was most abundant in the crude extract, and neochlorogenic acid was predominantly found in the water fraction (Figure 2). Most target compounds were concentrated in the ethyl acetate fraction, with VIP scores less than 2.00. Compounds with VIP scores greater than two included methyl isonicotinate, which exhibited high intensity in the water fraction; bis(2-ethylhexyl) phthalate, which showed high intensity in the ethyl acetate fraction; and 9-oxo-10 (E), 12(E)-octadecadienoic acid, which was prominent in the hexane fraction.

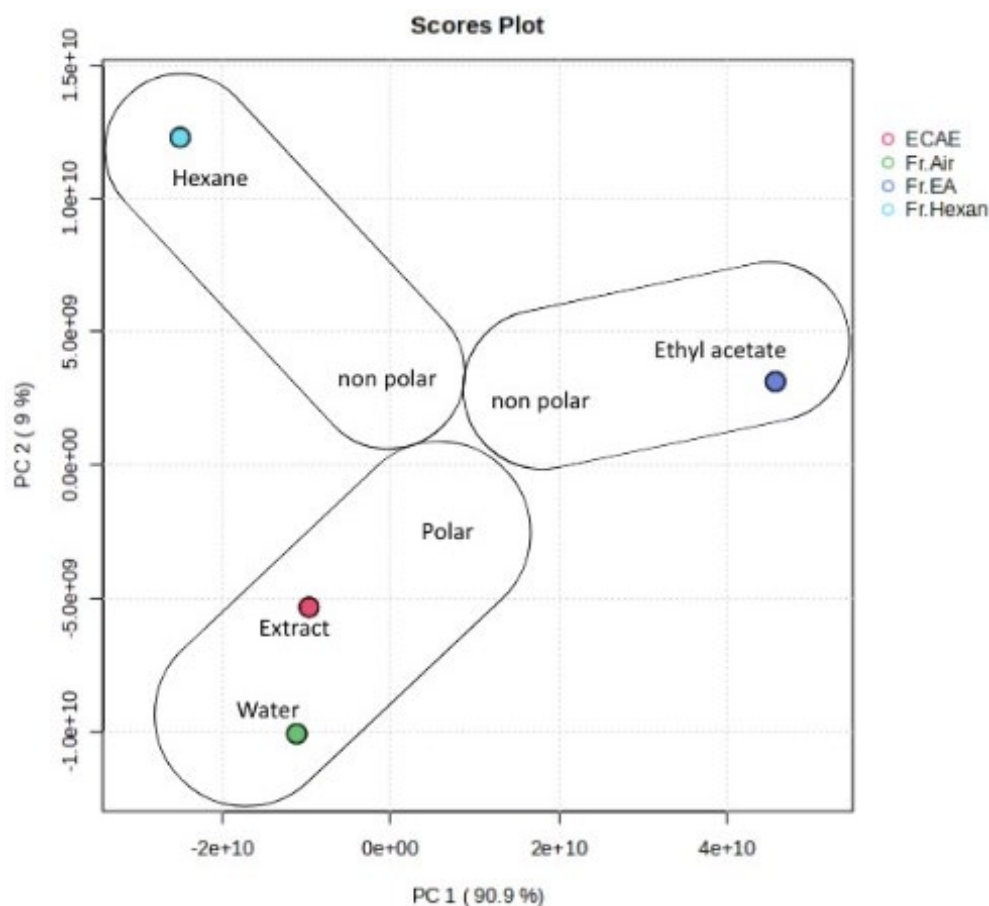


Figure 1: PCA Score Plot. Showed the separation based on polarity.

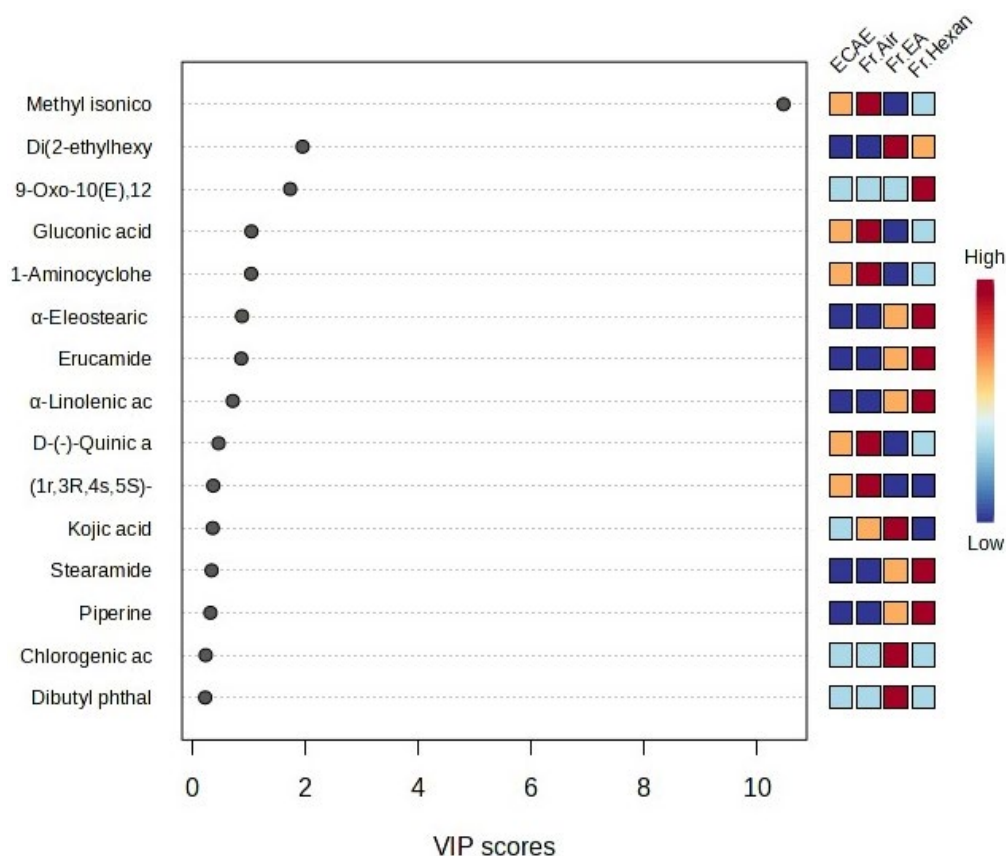


Figure 2: PLS-DA VIP Score top 15.

The presence of chlorogenic acid compounds was high in the ethyl acetate fraction, low in the extract and water fractions, and absent in the hexane fraction. The VIP score of chlorogenic acid (5-CQA) ranged from 0.20 – 0.30, while that of methyl isonicotinate was 10.00, and that of quinic acid was 0.40 – 0.90. The VIP score reflected the relative dominance of compounds within the sample; for example, methyl isonicotinate was predominant in the water fraction, whereas quinic acid was most abundant in the ethanol extract.

3.3 Molecular Docking

The compounds selected as target ligands were derived from metabolomic profiling, where those with the ten largest peak areas and a cloud-based best match m/z value of ≥ 99.00 , were considered. Additionally, marker compounds previously reported in the literature were included as target ligands. Each of the 62 selected compounds were tested for gene expression using GeneCards and only the compounds that showed angiotensin gene expression (ACE/ACE2/AGTR1/AGTR2) were considered at the next stage. A total of 22 compounds associated with the expression of the angiotensin gene were initially identified and subsequently evaluated using Lipinski's Rule to Five. Of

these, 20 secondary metabolite compounds met the criteria and were selected as target ligands. In addition, two control compounds were used for each target protein. For the ACE protein (PDB ID: 1O86), captopril and lisinopril, which are commonly used angiotensin-converting enzyme inhibitors (ACEIs), served as controls. For the AGTR1 protein (PDB ID: 4ZUD), candesartan and olmesartan, which are widely used angiotensin receptor blockers (ARBs), were used as comparators. The list of metabolite compounds selected for molecular docking is presented in Table 2.

The control ligands used were pure ligands that were complexed with proteins, namely, LPR ligands (lisinopril) of the 1O86 protein complex, and OLM ligand (olmesartan) complexes of the 4ZUD proteins. The results of the re-docking of the native ligands revealed that the RMSD value for the native ligand LPR was 0.45 Å and that for the native ligand OLM was 1.92 Å. Difference in the ligand's position before and after docking is evident in Figures 3a and 3b.

Ligands and receptors were optimized using the Open Babel-based PyRx Virtual Screening Tool software. Before the docking process was carried out, the position of the native ligand on the grid box was adjusted. A total of 20 metabolite compounds were docked together with native ligands and control ligands with proteins in the

grid box position with the center coordinate point at x: 41.2052; and: 31.9472; z: 47.2328 and dimensions at x: 23.7844; y: 23.3749, z: 25.5171 (LPR control ligand), whereas in the OLM control ligand, it was specified at the center coordinate point at x: -40.6644; and y: 63.3323; z: 28.3292 and dimensions at x: 27.7827; and y: 20.3008; and z: 21.8328.

The docking process was carried out using Vina Wizard-based PyRx Virtual Screening Tool software, which utilizes Gibbs free energy (ΔG) or binding affinity (kcal/mol) parameters. Binding affinity values were calculated for 20 metabolite compounds, 4 controls, and native ligands, as shown in Table 3 below.

As shown in Table 3, the docking score value revealed that the quercetin-3 β -D-glucoside compound had a binding affinity score of -9.30 kcal/mol for the ACE protein receptor and was smaller than the native ligand (-8.90 kcal/mol) as well as the control compounds captopril (-5.50 kcal/mol) and lisinopril (-8.10 kcal/mol). For the AGTR1 protein, the

best binding affinity score was for the olmesartan control/comparator compound, with a score of -9.80 kcal/mol, which was lower than that of the native ligand (-9.00 kcal/mol) and another comparator compound, namely, candesartan (-8.90 kcal/mol). Cascara arabica marker metabolite compounds such as chlorogenic acid (5-CQA), neochlorogenic acid, caffeic acid, and gallic acid have binding affinity scores of -8.60 kcal/mol, -8.30 kcal/mol, -6.40 kcal/mol, and -6.50 kcal/mol (interaction of compounds with ACE protein receptors) and -7.60 kcal/mol, -7.50 kcal/mol, -6.00 kcal/mol, and -5.50 kcal/mol (interaction of compounds with AGTR1 proteins), respectively.

The docking results were then combined with the purified ligands using PyMOL software, as shown in Figure 4, and then visualized via BIOVIA Discovery Studio 2025 software. Visualization was performed on native ligands, controls, metabolite marker compounds, and compounds with the highest binding affinity. The visualization results showed hydrogen bonds with protein residues (Table 4).

Table 2: Target ligand compounds.

Name Compound	PubChem ID	Molecule Formula	Mass Molecule	H Bond Donor	H Bond Acceptor	Log P
2,3-Dihydro-1-benzofuran-2-carboxylic acid	2776555	C ₉ H ₈ O ₃	164	1	3	1.07
Adenine	190	C ₅ H ₅ N ₅	135	3	4	-0.06
Caffeine	2519	C ₈ H ₁₀ N ₄ O ₂	194	0	5	0.06
Chlorogenic acid	1794427	C ₁₆ H ₁₈ O ₉	354	6	9	-0.65
Choline	305	C ₅ H ₁₄ NO ⁺	104	1	1	-0.32
D-(-)-Quinic acid	6508	C ₇ H ₁₂ O ₆	192	5	6	-2.32
Dodecyl sulfate	8778	C ₁₂ H ₂₆ O ₄ S	266	1	4	4.81
Gallic acid	370	C ₇ H ₆ O ₅	170	4	5	0.50
Gluconic acid	10690	C ₆ H ₁₂ O ₇	196	6	7	-3.49
Kaempferol	5280863	C ₁₅ H ₁₀ O ₆	286	4	6	2.31
Kynurenic acid	3845	C ₁₀ H ₇ NO ₃	189	2	4	1.26
Linoleic acid	5280450	C ₁₈ H ₃₂ O ₂	280	1	2	5.88
Neochlorogenic acid	5280633	C ₁₆ H ₁₈ O ₉	354	6	9	-0.65
Nicotinamide	936	C ₆ H ₆ N ₂ O	122	2	3	0.18
Nicotinic acid	938	C ₆ H ₅ NO ₂	123	1	3	0.78
Oleic Acid	445639	C ₁₈ H ₃₄ O ₂	282	1	2	6.11
Quercetin-3 β -D-glucoside	44259136	C ₂₁ H ₂₀ O ₁₂	312	5	6	-0.05
Theophylline	2153	C ₇ H ₈ N ₄ O ₂	180	1	5	0.05
Caffeic acid	689043	C ₉ H ₈ O ₄	180	3	4	1.20
3-Hydroxypyridine	7971	C ₅ H ₅ NO	95	1	2	0.79
Captopril	44093	C ₉ H ₁₅ NO ₃ S	217	1	4	0.67
Lisinopril	5362119	C ₂₁ H ₃₁ N ₃ O ₅	405	5	8	1.23
Candesartan	2541	C ₂₄ H ₂₀ N ₆ O ₃	440	2	7	4.03
Olmesartan	158781	C ₂₄ H ₂₆ N ₆ O ₃	446	3	7	3.66

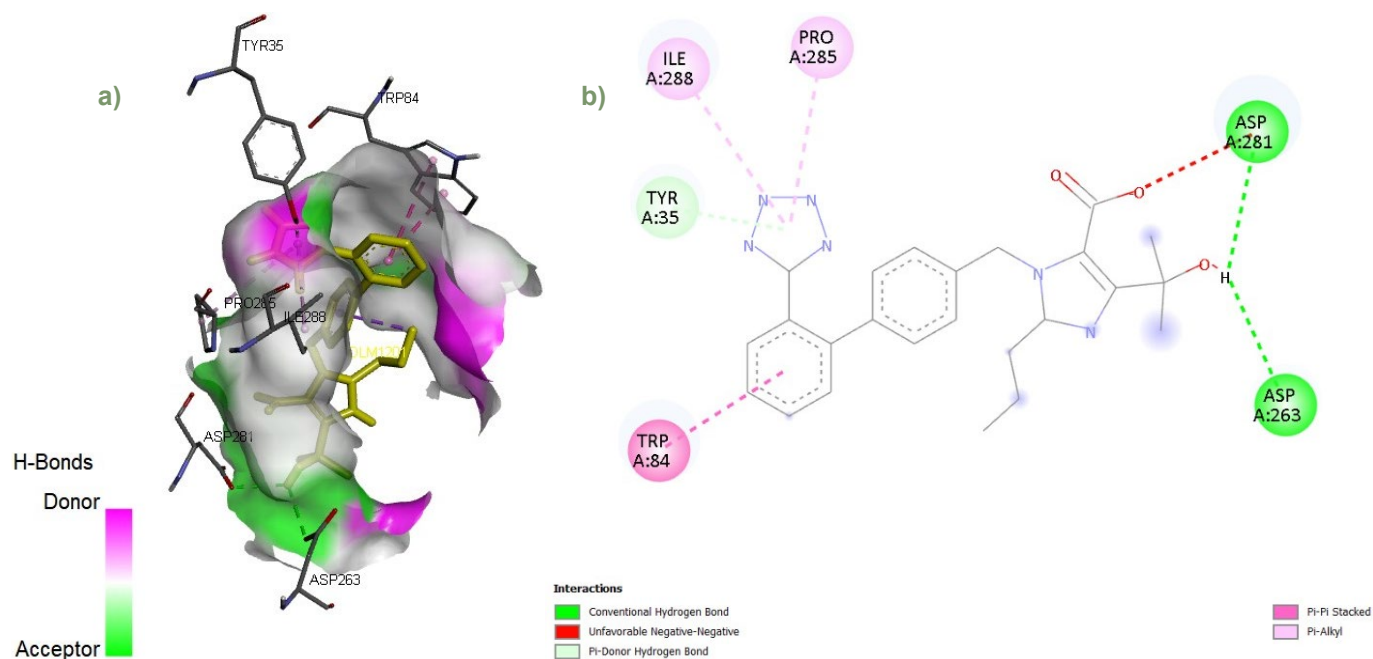


Figure 3: Visualization of OLM's native ligand-protein interaction with 4ZUD protein, (a) ligand-protein interaction in H-Bond surface, (b) 2-dimensional ligand-protein interaction

Table 3: Binding affinity value of molecular docking results.

Compound Name	Protein 1O86 (kcal/mol)	Protein 4ZUD (kcal/mol)
Native Ligand	-8.9	-9.0
Lisinopril	-8.1	-
Captopril	-5.5	-
Candesartan	-	-8.9
Olmesartan	-	-9.8
Chlorogenic Acid	-8.6	-7.6
Neochlorogenic Acid	-8.3	-7.5
Gallic Acid	-6.5	-5.5
Caffeic Acid	-6.4	-6.0
Quercetin-3 β -D-glucoside	-9.3	-7.9
Kaempferol	-8.1	-8.2
Kyuronic Acid	-7.2	-7.0
2,3-Dihydro-1-benzofuran-2-carboxylic_acid	-6.5	-6.5
Gluconic Acid	-6.2	-4.3
Linoleic Acid	-6.1	-6.9
Quinic Acid	-6.1	-5.3
Theophylline	-6.1	-6.1
Caffeine	-6.0	-5.8
Docecyl Sulfate	-5.8	-6.7
Nicotinamide	-5.8	-5.0
Nicotinic Acid	-5.8	-5.0
Oleic Acid	-5.7	-6.5
Adenine	-5.6	-4.7
3-Hydroxypyridine	-4.9	-3.9
Choline	-4.0	-3.0

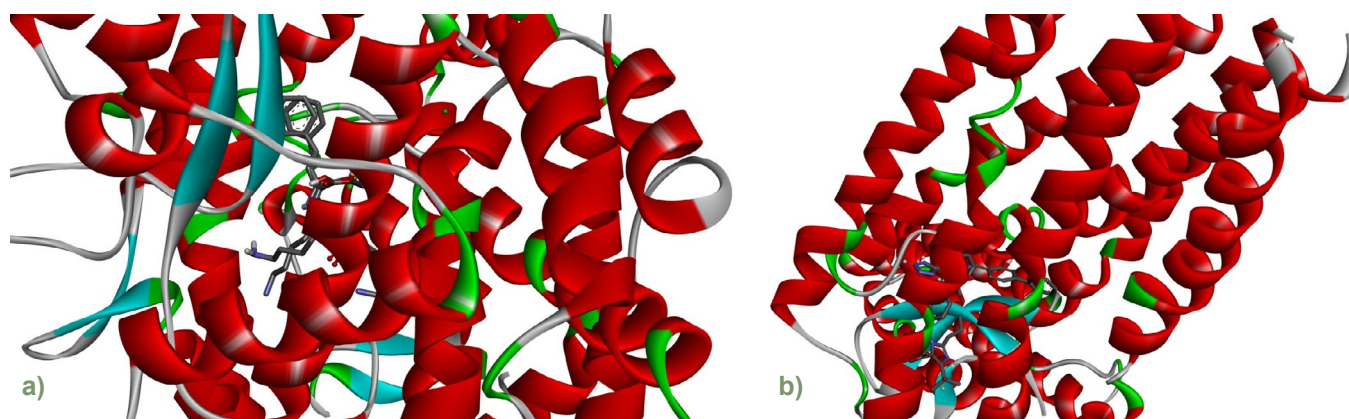


Figure 4: The interaction position of the docking native ligand with the pure protein (a) the docking LPR ligand with the pure 1086 protein, (b) the docking OLM ligand with the pure 4ZUD protein.

Table 4: Interaction of ligands with amino acid residues.

Receptor	Name Compound	Hydrogen bond residues (<2.5 Å)	Residu Other bonds
ACE (1086)	Native Ligand (LPR)	A:LYS511 (2.09 Å); A:HIS353 (2.17 Å); A:GLU384 (2.18 Å);	A:HIS513 (4.09 Å); A:VAL518 (3.55 Å); A:ALA354 (4.06 Å);
	Lisinopril (Control)	A:ALA354 (2.14 Å); A:ALA354 (2.05 Å); A:HIS513 (2.27 Å); A:ALA354 (2.12 Å)	A: PHE457 (4.55 Å); A:LPR702 (3.47 Å); A:HIS383 (4.36 Å)
	Captopril (Kontrol)	A:HIS353 (2.08 Å); A:HIS513 (2.25 Å) A:LPR702 (1.75 Å)	A:VAL380 (4.38 Å); A:HIS383 (4.53 Å); A:HIS383 (4.25 Å)
ACE (1086)	Quercetin-3β-D-glucoside	A:LYS511 (2.09 Å); A:HIS353 (2.17 Å); A:GLU384 (2.18 Å); A:LPR702 (2.29 Å)	A:VAL518 (3.55 Å); A:LPR702 (2.90 Å); A:ALA354 (4.06 Å)
	Chlorogenic Acid	A:HIS353 (2.11 Å); A:HIS353 (2.49 Å); A:ALA356 (1.78 Å); A:HIS513 (2.35 Å); A:TYR523 (2.31 Å); A:HIS387 (2.31 Å)	A:HIS383 (3.75 Å); A:HIS387 (5.26 Å); A:LPR702 (5.13 Å)
	Neochlorogenic Acid	A:LYS511 (2.09 Å); A:HIS353 (2.17 Å); A:LPR702 (2.18 Å);	A:VAL518 (3.55 Å); A:ALA354 (4.06 Å); A:LPR702 (3.77 Å);
	Gallic Acid	A:LEU161 (2.42 Å); A:HIS353 (2.49 Å); A:GLU162 (2.08 Å)	A:LEU161 (4.46 Å); A:CYS352 (4.96 Å)
	Caffeic Acid	A:ARG124 (2.04 Å); A:TYR62 (2.26 Å); A:ASN85 (2.26 Å)	A:LEU81 (5.11 Å); A:LEU139 (5.31 Å); A:LEU140 (5.42 Å)
	Kaempferol	A:GLN281 (2.32 Å); A:ASP415 (2.32 Å)	A:VAL380 (3.63 Å); A:LPR702 (2.87 Å); A:LPR702 (4.74 Å);
	Native Ligand (OLM)	A:ASP281 (2.46 Å); A:TYR35 (1.95 Å)	A:OLM1201 (3.64 Å); A:TRP84 (4.49 Å); A:TRP84 (3.94 Å)
AGTR1 (4ZUD)	Candesartan	A:ARG167 (2.55 Å); A:TYR87 (2.27 Å)	A:VAL108 (3.99 Å); A:TRP84 (4.50 Å); A:OLM1201 (4.24 Å)
	Olmesartan	A:ASP281 (2.47 Å); A:TYR35 (1.95 Å)	A:OLM1201 (3.64 Å); A:TRP84 (4.49 Å); A:TRP84 (3.94 Å)
	Quercetin-3β-D-glucoside	A:ASP281 (2.47 Å); A:TYR35 (1.95 Å)	A:OLM1201 (3.64 Å); A:TRP84 (4.49 Å); A:TRP84 (3.94 Å);
AGTR1 (4ZUD)	Chlorogenic Acid	A:TYR35 (2.05 Å); A:ARG167 (2.42 Å); A:ASP281 (2.45 Å)	A:TYR92 (4.02 Å); A:OLM1201 (5.32 Å)
	Neochlorogenic Acid	A:ASP263 (2.66 Å); A:ASP281 (2.47 Å); A:TYR35 (1.95 Å)	A:OLM1201 (3.64 Å); A:TPR84 (4.49 Å); A:TPR84 (3.94 Å);
	Gallic Acid	A:TYR35 (2.07 Å); A:ARG167 (2.34 Å)	A:TPR84 (4.27 Å); A:TPR84 (3.71 Å)
	Caffeic Acid	A:TYR35 (2.17 Å)	A:TPR84 (4.43 Å); A:TPR84 (3.69 Å)
	Kaempferol	A:TYR35 (1.81 Å)	A:TRP843 (3.80 Å); A:TYR92 (3.78 Å); A:OLM1201 (4.43 Å)

In addition to hydrogen bonds, other types of bonds, such as hydrophobic bonds and electrostatic bonds, were observed (Figures 4 and 5).

Both the target compounds and native ligands interact with amino acid residues through non-hydrogen bonds, often characterized by relatively long bond distances (weaker bond strength). For example, the native ligand lisinopril interacted with several residues of ACE, including A:HIS353 (5.18 Å), A:HIS513 (4.09 Å), A:HIS383 (4.90 Å), A:TYR523 (4.34 Å), A:VAL518 (3.55 Å), A:ALA354 (4.06 Å), A:VAL380 (4.34 Å), and again A:HIS353 (4.59 Å). Similarly, the native ligand olmesartan formed non-hydrogen bonding interactions with residues such as A:OLM1201 (3.64 Å), A:TRP84 (4.49 Å, and 3.94 Å), A:PRO285 (5.13 Å), and A:ILE288 (5.04 Å). In addition to hydrogen bonds, native ligand interactions also involved other bonding types, including electrostatic interactions such as attractive charges and pi-cation bonds, as well as hydrophobic interactions, including alkyl, pi-alkyl, and pi-sigma bonds.

4 DISCUSSION

4.1 Metabolomic profiling

Metabolomics is the study of small molecules known as metabolites, which are present in cells, tissues, and organisms. These metabolites are collectively referred to as metabolome (Roberts et al., 2012). The metabolite profiles obtained from LC-HRMS analysis of four different samples revealed caffeine as the primary compound in each sample. This finding is consistent with the research of Machado et al. (2023), which demonstrated that caffeine is present throughout all parts of coffee, including pulp, husk, parchment, silverskin, and other coffee by-products. Caffeine is an alkaloid that is the main active compound in coffee and acts as an antagonist of adenosine receptors (Burdan, 2015). The dominant compounds present in cascara arabica were polyphenols such as chlorogenic acid compounds, caffeic acid, gallic acid, neochlorogenic acid, 4,5-dicaffeoylquinic acid, and quinic acid, these findings are consistent with Ali et al. (2022). Polyphenolic compounds play important roles in human health, offering various health benefits, including anti-inflammatory and antioxidant effects (Sun et al., 2024). The ethyl acetate fraction contained trans-caffeic acid compounds, which are isomers of caffeic acid formed due to thermal processes, including exposure to ultraviolet radiation (IARC, 1993). These compounds were first reported in cascara arabica. Additionally, vanillic acid compounds were detected in the ethyl acetate fraction. Vanillic acid, a derivative of benzoic acid, contributes to the aroma of food and had both

antioxidant and anti-inflammatory effects (Magiera et al., 2025). Some xanthine compounds, such as theobromin and theophylline, have also been found in cascara arabica. Caffeic acid was identified in the ethyl acetate fraction, and demonstrated a reduction in hypertension in a mouse model (Wu et al., 2021). Chlorogenic acid is a potential ACEI (Zhao et al., 2012). Metabolomic profiling revealed that ACEI was predominantly present in the ethyl acetate fraction.

Metabolomic profiling was conducted using chemometric analysis, including PCA and PLS-DA. PCA results, demonstrated clear separation of samples (Figure 6).

The score plot indicated distinct separation between samples, primarily based on polarity differences. The biplot, further illustrated the grouping of compounds according to their affinity to solvents. Chemometric analysis confirmed that compounds in the sample can be effectively separated based on polarity. Many compounds are more attracted to ethyl acetate than to water or hexane, which indicated that compounds in extracts are indirectly concentrated in the presence of ethyl acetate. Chemometric analysis, allowed the identification of polar and apolar compounds based on their partitioning into different fractions. This study revealed that the predominant compounds in cascara arabica were polar compounds that functioned as ACEIs. The partitioning of compounds into the apolar-phase hexane fraction showed only a minor abundance. Gallic acid was detected exclusively in the ethanol extract and water fraction. However, in the ethyl acetate fraction, it was not detected because the concentration of the compounds drawn into the solvent was insufficient. In contrast, chlorogenic acid (5-CQA) and neochlorogenic acid were not found in the hexane fraction because of their polarity.

PLS-DA indicated the intensity of compounds in a given sample, and the VIP score value obtained from PLS-DA could be used to select compounds as markers for sampling. The assessment of VIP score is a crucial parameter in PLS-DA, with a VIP score greater than 1 considered significant (Rueangsri et al., 2025). A high VIP score indicates that the variable plays a significant role in differentiating the properties of compounds between sample groups (Zhu et al., 2022). Methyl isonicotinate, with a VIP score of 10 and high intensity in the water fraction, could thus be used as a marker for the water fraction of cascara arabica.

4.2 Molecular Docking

The results of re-docking the native ligands were expressed as the root mean square deviation (RMSD), which served as a validation parameter for the docking by reflecting the difference in ligand orientation before and

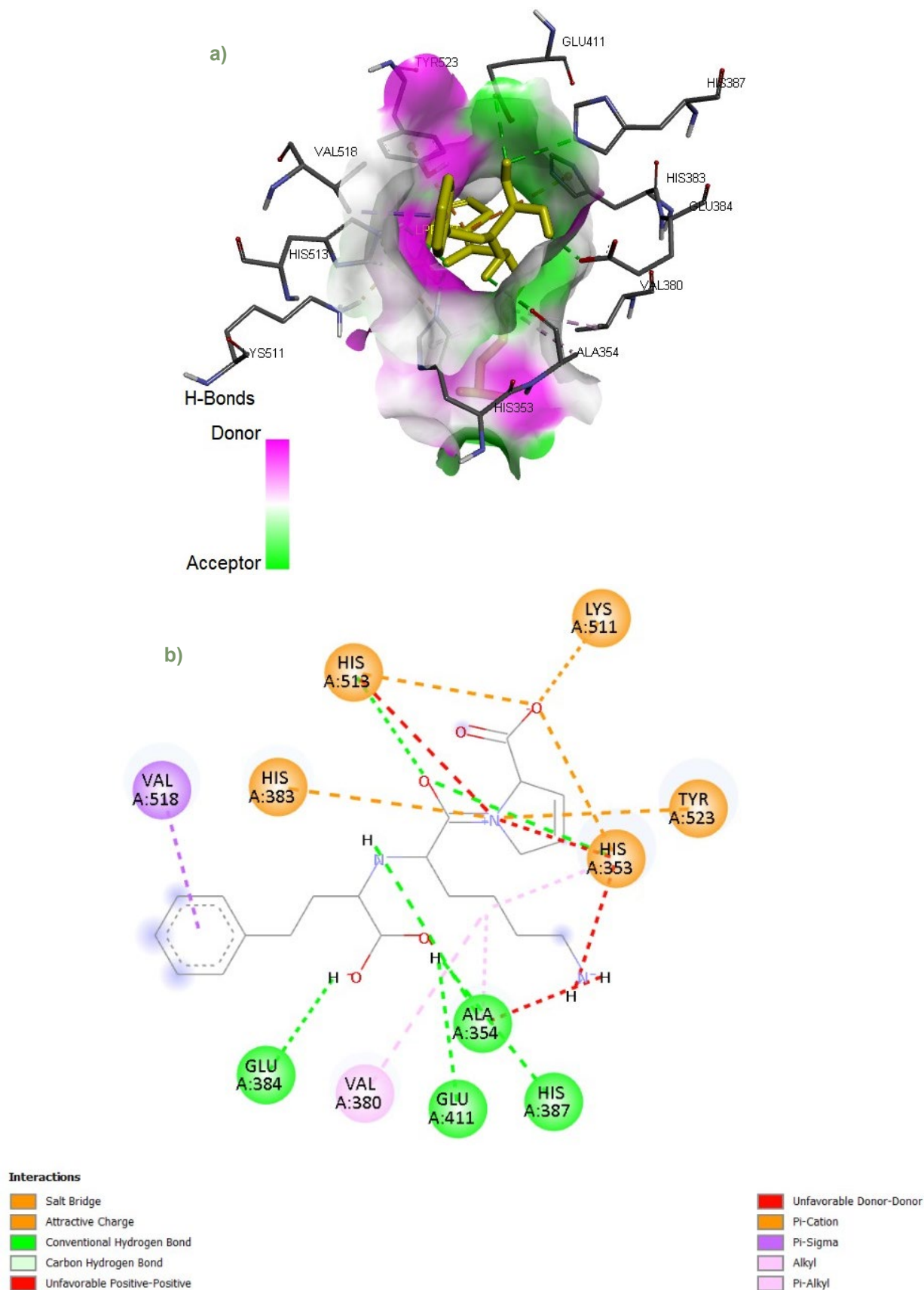


Figure 5: Visualization of the interaction of the native ligand of LPR with the 1086 protein, (a) the interaction of the ligand-protein in the surface of the H-Bond, (b) the 2-dimensional ligand-protein interaction.

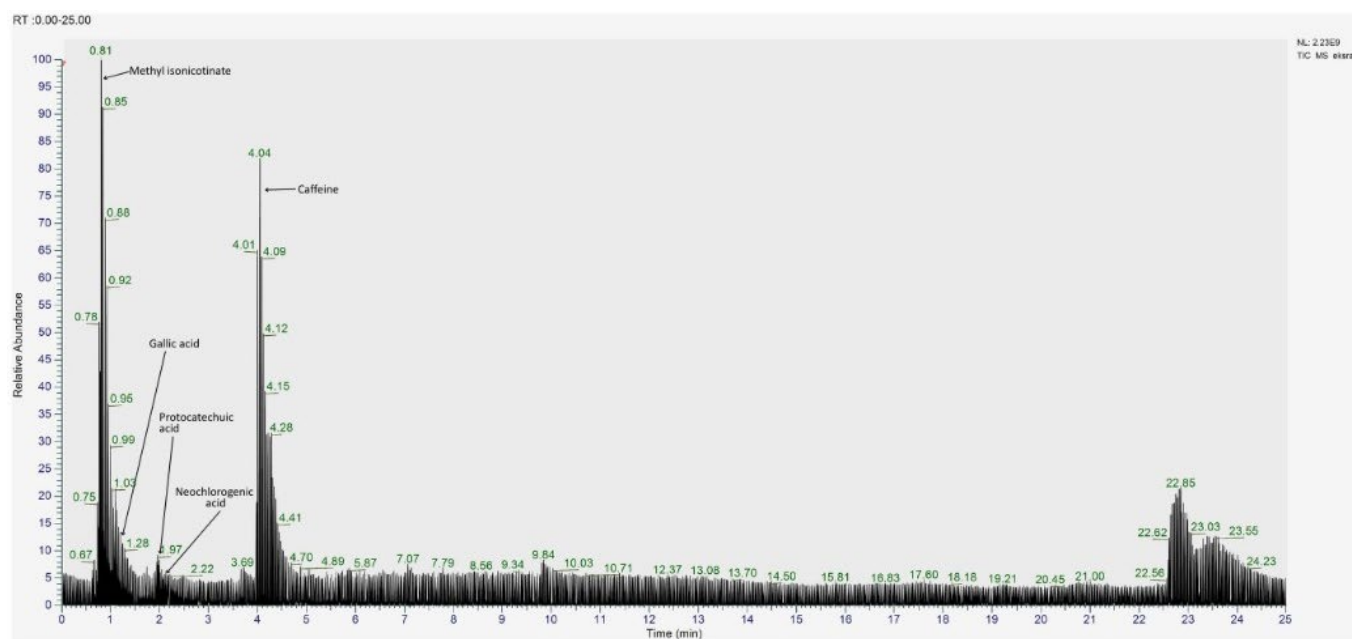


Figure 6: Total ion chromatogram (TIC) extract.

after docking. A smaller RMSD value indicated minimal change, with an RMSD value of ≤ 2.00 Å considered acceptable (Ramírez & Caballero, 2018). The RMSD value indicated that the docking method, including the grid box position, exhaustiveness value, and geometry optimization, was appropriate for the ligand–protein binding mode in the docking process (Gohlke et al., 2000). The ligand pose within the grid box was approximately three times larger than the size of the ligand. The minimum and maximum ligand poses were obtained using the “get_extent” command in PyMOL, allowing for the calculation of the grid box size based on the ligand dimensions. Determining the grid box size is a critical step in the docking process, as it defines the location of the ligand’s active binding residues (Budiarto et al., 2023). Optimal ligand binding occurs when the grid box space is 2.9 times larger than the size or radius of the ligand’s gyration (Feinstein & Brylinski, 2015). The positions of the ligands before and after docking, shown in Figure 7, revealed no significant differences in their placement.

The ligand–to–receptor binding score was expressed as the binding affinity score which is an important parameter in docking for evaluating the binding affinity and potential inhibitory activity of a ligand (Agu et al., 2023). The binding strength of the ligand to the receptor was assessed based on binding affinity score values, the lower the binding affinity, the stronger the ligand–to–receptor binding, and the greater the effect on the physiological function of the protein (Budiarto et al., 2023; Seo et al., 2021). The highest binding affinity score value of the target ligand to the target protein was for the compound quercetin–3β–D–glucoside with scores of -9.30 kcal/mol for ACE and -7.90 kcal/mol

for AGTR1. Folding docking affinity (FDA)–approved binding affinities ranged from -5.63 kcal/mol to -6.85 kcal/mol, thus, docking results of target compounds were largely superior to FDA–approved binding affinity results (Terefe & Ghosh, 2022). Compounds from cascara arabica that showed good binding affinity constituted most of the ethyl acetate fraction. In the future, ethyl acetate fraction of cascara arabica should be further studied to investigate its ACEI activity.

Generally, the types of bonds that occur in ligand–protein interactions are hydrogen, van der Waals, hydrophobic (Seo et al., 2021), and electrostatic bonds (Agu et al., 2023). Hydrogen bonds play a crucial role in docking, establishing the occurrence of ligand–protein interactions (Owoloye et al., 2022), and serve as a key parameter for selecting the best ligand conformation (Frimayanti et al., 2021). Docking results of target compounds revealed a sufficiently strong hydrogen bond with a bond distance of ± 2.00 Å, which can influence the effectiveness of a compound (Budiarto et al., 2023). In the interaction between the ACE and AGTR1 proteins, there are other types of bonds, such as hydrophobic, and electrostatic bonds, in addition to hydrogen bonds. However, these bonds tend to be weak, with distances greater than 3.50 Å. While hydrogen bonds play a crucial role in stabilizing the complex, the presence of other bonds, such as hydrophobic interactions, can reduce the binding affinity due to their potential to interfere with hydrogen bond interactions in certain conformers (Vaidyanathan et al., 2023). Hydrophobic and electrostatic bonds are not expected to significantly affect hydrogen bonds, as their distances are relatively large, indicating their weaker nature.

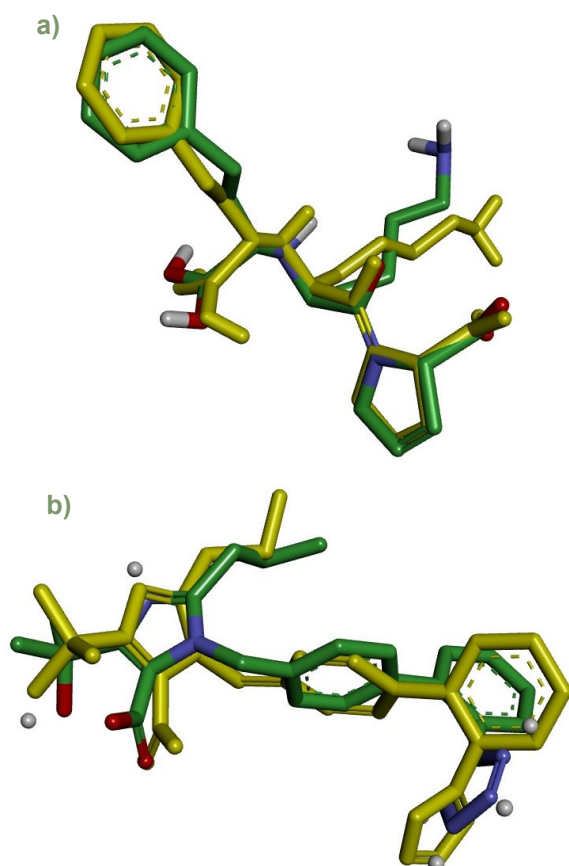


Figure 7: (a) Native ligand lisinopril; (b) Native ligand olmesartan. Native ligand position before docking (yellow) and after docking (green).

5 CONCLUSIONS

The main compound in cascara arabica is the alkaloid caffeine and the dominant compound is a polyphenolic compound, such as chlorogenic acid compounds. Hence, the greatest potential for activity was likely in the ethyl acetate fraction. The binding affinity provided valuable insights that could serve as an initial benchmark for identifying potential drug compounds in the RAAS. The use of herbs as a treatment could offer multitarget and multi-compound benefits, promoting synergistic effects on the target receptor.

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7 AUTHOR CONTRIBUTIONS

Conceptual Idea: Abdul, M.; Rissyelly; Methodology design: Abdul, M.; Rissyelly.; Syiria, S.; Data collection: Syiria, S.; Marissa, A.; Data analysis and interpretation: Abdul, M.; Rissyelly.; Marissa, A.; Syiria, S.; and Writing and editing: Abdul, M.; Rissyelly.; Syiria, S.

8 DATA AVAILABILITY STATEMENTData

Available upon Request to Authors.

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