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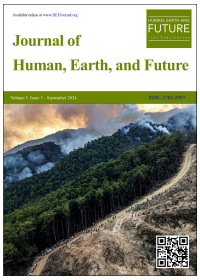

Investigating Ethanolic Extract from Acehnese Lime (*Citrus aurantifolia*) Peel as Potential Anti-Hypercholesterolemia Agent

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Abstract

Lime peels are rich in flavonoids, alkaloids, phenols, saponins, and tannins, exhibiting antibacterial, antioxidant, anti-inflammatory, anti-hypertensive, and anti-hypercholesterolemia properties. However, the specific active constituents of Acehnese lime peels and their impact on anti-hypercholesterolemia effects remain undisclosed. This present investigation aims to identify the active compounds in the ethanolic extract of locally obtained Acehnese lime (*Citrus aurantifolia* (Christm.) Swingle) peels and assess their potential as inhibitors of Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) and 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG Co-A) reductase using *in-silico* approach. The composition of the ethanolic extract Acehnese lime peel was determined through phytochemical analysis, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, gas chromatography-mass spectrometry (GC-MS) analysis, and predictions of biological activity, while the biological activities of their compounds were evaluated through molecular docking. Phytochemicals revealed the presence of phenolics, flavonoids, tannins, saponins, and terpenoids in the ethanolic extract of local Aceh lime peel. The total phenolic and flavonoid contents were 29.992 ± 0.274 mg gallic acid equivalents (GAE)/g extract and 5.983 ± 0.017 mg quercetin equivalents (QE)/g extract, respectively. The anti-oxidant activity was notably strong with an IC_{50} value of 49.51 ppm. GC-MS analysis identified 6-methoxychroman-2-one (27.64%) as the primary component in ethanolic extract Acehnese lime peel, along with neric acid ($C_{13}H_{22}O_2$) known as a regulator of lipid metabolism (Pa: 0.941). *In-silico* investigations indicated that pterin-6-carboxylic acid (-7.8 kcal/mol) exhibited a higher binding free energy for the PCSK9 receptor compared to simvastatin (-7.6 kcal/mol), whereas the active compound (R)-9-(2,3-dihydroxy-3-methylbutoxy)-4- exhibited the highest binding capacity for HMG Co-A reductase (-6.9 kcal/mol) compared to other compounds. These findings suggest that the ethanolic extract of Acehnese lime peels could serve as an effective inhibitor of PCSK9 and HMG Co-A reductase, highlighting its potential as a novel anti-hypercholesterolemia agent.

Keywords: Citrus aurantifolia; Aceh Lime Peel; Anti-Hypercholesterolemia Agent; PCSK9; HMG-Coa Reductase.

1. Introduction

Hypercholesterolemia stands as a significant contributor to cardiovascular disease [1], representing a leading cause of mortality worldwide, accounting for approximately 17.9 million deaths annually [2]. Elevated levels of low-density lipoprotein (LDL) and total cholesterol in the bloodstream characterize hypercholesterolemia [3]. Lifestyle and stress

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influence patients' cholesterol levels, affecting lipid metabolism and adaptive responses to pathophysiological changes. Although elevated blood lipids are necessary for people to survive and adapt to stressors, long-term changes in lipid metabolism due to chronic stress can lead to atherosclerosis, coronary heart disease, and stroke [4]. Atherosclerotic lesions typically exhibit an abundant number of monocytes, macrophages, lipoproteins, and LDL, manifesting through a degenerative process unfolding in multiple stages. This accumulation of lipids, calcium, platelets, and other blood components leads to damage to the blood vessel walls [5]. Statins are commonly employed in the management of hypercholesterolemia. Within the liver, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase plays a pivotal role in cholesterol synthesis. This enzyme catalyzes the initial step of cholesterol biosynthesis, converting HMG-CoA to mevalonic acid [6].

Statins are potent HMG-CoA inhibitors that show highly effective cholesterol reduction; however, the residual risk of cardiovascular disease remains high despite optimal therapy [7]. Moreover, statins have been reportedly intolerable for over 30% of individuals with hypercholesterolemia [8]. Thus, there is a need for a more effective LDL-lowering agent, with a PCSK9 inhibitor emerging as a potential candidate [9]. Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) inhibitors offer promising responses to hypercholesterolemia, as this molecule is responsible for the occurrence of familial hypercholesterolemia [10], regulates apoB lipoprotein degradation and cholesterol metabolism [11], and reduces blood LDL particle concentration [12]. For patients intolerant to statins, PCSK9 inhibition presents a significant therapeutic advancement in managing lipid disorders and reducing the risk of cardiovascular disease [13]. When maximally tolerated statin doses are inadequate to control chronically elevated LDL levels, the use of PCSK9 inhibitors is advised [14].

To provide another treatment option for hypercholesterolemia, scientists have explored the use of natural compounds. Approximately 80% of people have utilized herbal plants as a kind of treatment for various illnesses [15]. Due to their numerous health benefits, including lowering levels of inflammation, LDL cholesterol, and oxidative stress, many different types of plants are used as medication [16]. Plants, rich in secondary metabolites, are increasingly employed in both medicine and diet [17]. Many phytochemical compounds, also known as secondary metabolites, are produced by plants that are advantageous to health [18]. Plant-derived flavonoid compounds play crucial roles in balancing diet and preventing diseases such as obesity, diabetes mellitus (DM), lowering blood fat levels, cardiovascular disease, and several types of cancer [19]. Among herbal plants, lime (*Citrus aurantifolia*) has often been used as medicine due to its various biological properties, including anti-obesity [20], spasmolytic agent [21], anti-oxidant [22], anti-inflammatory [23], anti-bacterial, and anti-fungal activities [24]. Lime peel is difficult to process, so it is frequently discarded despite its numerous benefits. Previous studies discovered that lime peels contain antibacterial compounds [25] and can lower the levels of aspartate transaminase, triglycerides, LDL, alanine aminotransferase, and total cholesterol in hyperlipidemic mice [26]. Limonene, linalool, and 4-terpineol extracted from lime peels can help reduce cardiovascular disease and hypertension [22].

Previous studies showed that *Tacca leontopetaloides* from Aceh Province can be used as inhibitors of HMG-CoA [26]. However, studies on the potential of Acehnese lime peels (*C. aurantiifolia* (Christm.) Swingle) in the treatment of hypercholesterolemia and its inhibitory effects on PCSK9, especially, and HMG Co-A reductase are still limited. Therefore, this study aimed to investigate active compounds contained in the ethanol extract of Acehnese lime peel as well as identify its *in-silico* potential as a PCSK9 and HMG Co-A reductase inhibitor using six different analyses. This research not only will show an active compound from Acehnese lime but can be a milestone in the exploration of anti-hypercholesterolemia agents.

2. Materials and Methods

2.1. Plant Material

The plant sample used in this study was lime peels, cultivated by the community in Suka Damai village, Lembah Seulawah District, Aceh Besar Regency, Aceh, Indonesia, with coordinates 5° 25’ 37’’ East longitude, 95° 42’ 49’’ South latitude, and an altitude of 421 meters above sea level (Figure 1).

2.2. Plant Identification

The plant was identified at the National Research and Innovation Agency (BRIN) Laboratory, Indonesia.

2.3. Sample Preparation and Extraction

In total, 20 kg of fresh lime fruit was washed using tap water to remove dirt from the peel. The peel was then separated from the fruit, thinly sliced to obtain 2000 g, and dried at room temperature for 14 days until it became thin, dry, and dark brown. The dried lime peels were weighed, yielding 444 g, and then ground using a blender. The obtained dried leaf samples were extracted using a 3×24-hour maceration method using 96% ethanol solvent with a ratio of 1:10 [27].

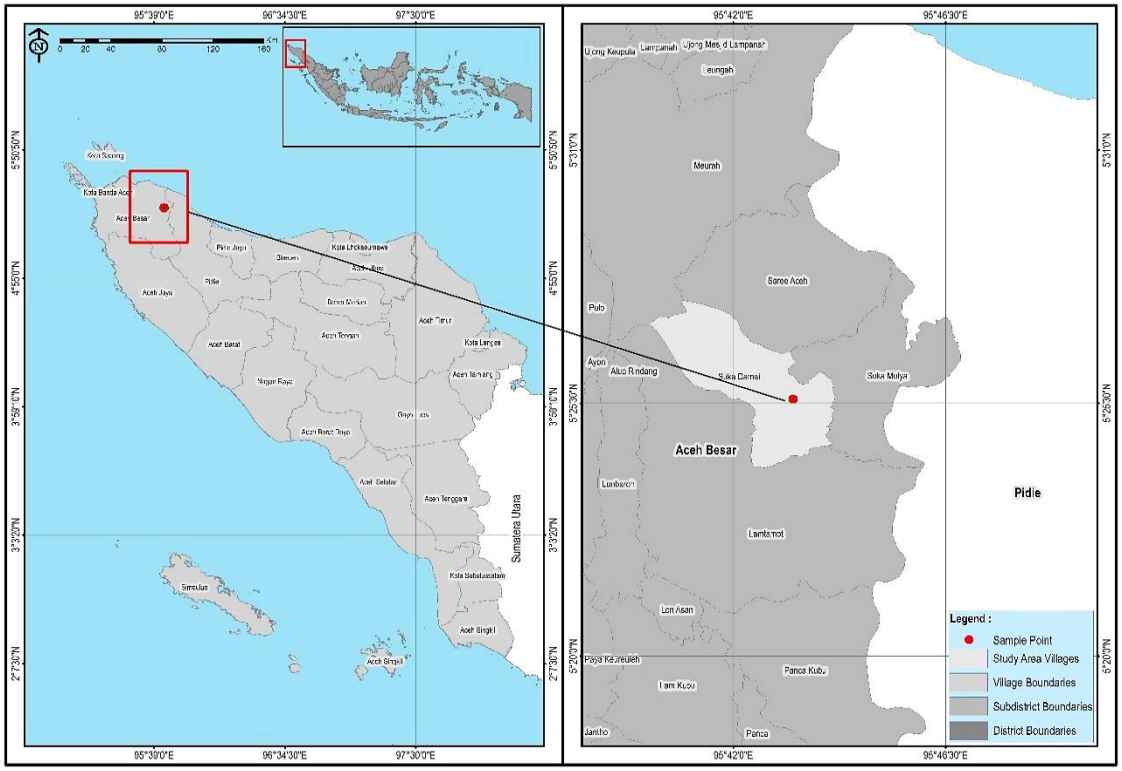


Figure 1. Sampling Location (Suka Damai village, Lembah Seulawah District, Aceh Besar Regency, Aceh, Indonesia)

2.4. Total Phenolics and Total Flavonoids Analysis

The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were measured using the Dowd method using Spectrophotometry (Thermo Fisher Scientific Genesys Model 10UV, CAT 335906-02, SN 2L7N006001). TFC was expressed as milligrams of quercetin per gram of sample (mgQE/g sample) with concentrations ranging from 0-100 g/mL. A 1 mL aliquot of the sample solution at a concentration of 10 mg/mL was mixed with 0.2 mL of 10% (w/v) $AlCl_3$ solution in methanol. Afterward, the sample solution was mixed with 0.2 mL of 1 M CH_3COOK and 5.6 mL of distilled water, homogenized, and allowed to incubate for 30 minutes. Following incubation, the absorbance of the sample solution was measured using a spectrophotometer at a wavelength of 415 nm [28].

The Total Phenolic Content (TPC) was quantified in milligrams of gallic acid equivalents per gram of sample (mgGAE/g sample) within concentrations of 0-200 µg/mL. A 0.2 mL aliquot of the sample (at 10 mg/mL) was mixed with 1.8 mL of distilled water and 0.2 mL of Folin-Ciocalteu reagent. This mixture was thoroughly mixed and allowed to incubate for 6 minutes. Subsequently, the sample mixture was supplemented with 2 mL of a 7% (w/v) Na_2CO_3 solution, thoroughly mixed again, and then allowed to incubate for 90 minutes. Finally, the absorbance of the resulting solution was measured at a wavelength of 750 nm using a spectrophotometer [29].

2.5. 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay

The ethanol extract of lime peels was diluted with methanol to obtain concentrations of 6.25, 12.5, 25, 50, and 100 parts per million (ppm). In the reaction tube containing the dissolved sample (5 mL), one milliliter (1 mL) of 0.4 mM DPPH was added. The concoction was then mixed and homogenized using a vortex and incubated for 30 minutes at 37°C. A mini-1240 ultraviolet-visible spectrophotometer (Kyoto, Japan) was used to measure the lower concentration at $\lambda = 517$ nm. The minimum concentration required to reduce the reagent concentration by 50% (IC_{50}) was previously calculated using a linear curve equation. Ascorbic acid (3–9 ppm) was shown to have an IC_{50} using the same method and was used in a previous study [30].

2.6. Gas Chromatography-Mass Spectrometry (GC-MS) for Active Compound Identification

The ethanol extract of lime peels was analyzed using an iSQ 7000 Single Quadrupole GC-MS System (Thermo Fisher Scientific Inc.) with TraceGOLD™ 1300/1310 GC TG-5MS column (column length: 30 m × 0.25 mm ID × 0.25 µm and column mode: Flow (constant). The carrier gas used was helium (1.2 mL/min). Oven temperature: Initial temperature 35°C (0 min), further increased 18°C/min to 100°C (2 min) and 20°C/min to 250°C (1.0 min). Injection site temperature: 250°C. Ion source temperature: 300°C. Ionization mode: EI. Injection volume: 5 µL. Method data: Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) 7.2.

2.7. Biological Activity Predictions

The prediction of activity spectra for substances (PASS) method is used in further analysis to identify the most active phytochemical constituent in the ethanol extract of lime peels [31, 32]. The examination results were represented as Pa (probability of activity) and Pi (probability of inactivity), where the values of Pa and Pi could range from 0.000 to 1.000. A molecule's bioactivity is determined by Pa values surpassing Pi and exceeding 0.700 [33].

2.8. Molecular Docking (*In-silico*)

The *in-silico* test was conducted in several steps, including protein preparation, ligand preparation, active site determination, molecular docking, and analysis [27, 34, 35]. A molecular analysis was conducted on the active compounds from the ethanol extract of orange peel identified through GC-MS analysis. These active compounds were used as ligands, and PCSK9 and HMG-CoA, which could be accessed and downloaded from the Protein Data Bank (PDB), as of August 20, 2023, were used as the protein targets. The protein targets were prepared using *BIOVIA Discovery Studio* 2021 software. The ligands were obtained from the PubChem database and then prepared with *OPEN BABEL Sketch*. Subsequently, the ligands were docked onto the protein targets and compared with simvastatin as the control ligand. Simvastatin is a standard drug known to inhibit the activity of PCSK9 and HMG-CoA reductase proteins. The docking procedure was performed using *Autodock Vina software* with *PyRx*. During this process, the ligand molecules would engage with the active site of the receptor, potentially leading to the inhibition of receptor function, thus serving as a prospective drug. The flowchart of the research methodology can be seen in Figure 2.

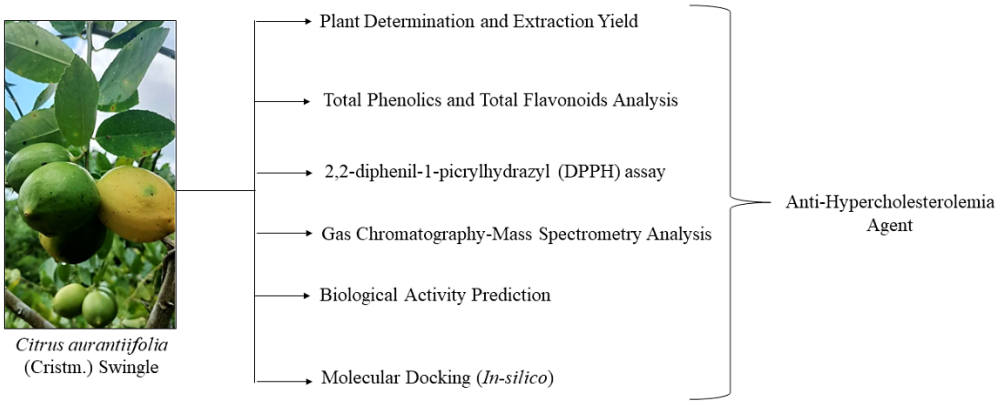


Figure 2. A flowchart

3. Results and Discussion

3.1. Plant Determination and Extraction Yield

The plant was identified (Number: B-2035/II.6.2/R.01.02/8/2023) as *Citrus aurantiifolia* (Cristm.) Swingle, a species of the *Rutaceae* family (Figure 3). The thick lime peel extract used was 11.38 grams, and the yield percentage value was 2.49%.

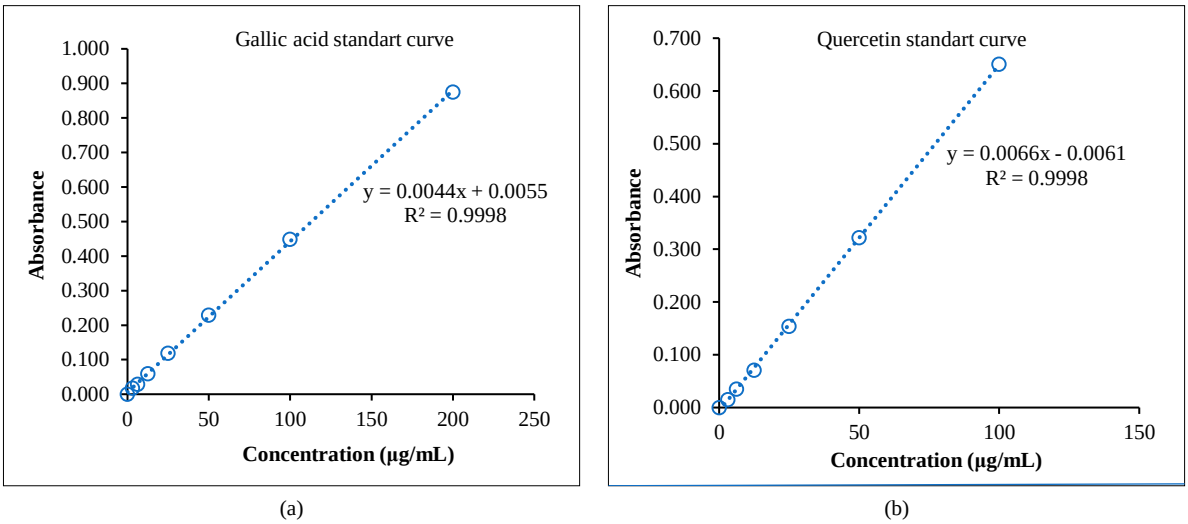
Figure 3. Local Aceh *C. aurantifolia* plants

3.2. Total Phenolics and Total Flavonoids Analysis

Qualitative phytochemical screening revealed the presence of tannins, phenolics, flavonoids, saponins, and terpenoids in the ethanol extract of Acehnese lime peels. Meanwhile, alkaloid and steroid compounds were negative. This finding was slightly different from that reported in a previous study, suggesting the presence of alkaloids, phenols, flavonoids, saponins, and tannins in lime peels [36]. C. aurantifolia peels contain various bioactive substances, including terpenoids, flavonoids, phenolics, alkaloids, and essential oils [37]. Environmental conditions, especially altitude, exert a notable influence on plant growth and development, including secondary metabolites [38]. Climate, geographical conditions, genetic variations, agronomy, and plant storage [39, 40], as well as the plant parts used [41], are other factors contributing to secondary metabolites contained in plants [42]. Table 1 shows the results of total phenolics and total flavonoids analysis, and Figure 4 shows the standard curves for gallic acid (Figure 4-a) and quercetin standard curve (Figure 4-b).

Table 1. Total phenolics and total flavonoids

Phytochemicals	Results
Total phenolic	29.99±0.27 mg gallic acid equivalents g^{-1} extract
Total flavonoid	5.98±0.02 mg quercetin equivalents g^{-1} extract



(a) (b)

Figure 4. (a) Gallic acid standard curve, and Figure (b) Quercetin standard curve

The total phenolic content in the ethanol extract of lime peels was 29.99 ± 0.27 mg GAE/g extract, while the total flavonoid content was 5.98 ± 0.02 mg QE/g extract. Previous research indicated that *C. australasica* peels contain a higher concentration of phenolic compounds, whereas the fruit exhibits higher levels of flavonoids [43]. The degree of fruit development affects the number of phenolic compounds in lime fruit, in which young fruit has the highest phenolic content [44].

Alkaloids, phenolics, terpenoids, and tannins play a significant role in disease prevention [45]. Several studies on plant phytochemicals that reduce blood cholesterol levels in hypercholesterolemia along with various heart-related conditions have been carried out [46], specifically polyphenols [47] and flavonoids [48]. Polyphenols serve as anti-oxidants that may decrease the levels of cholesterol by preventing the production of mevalonate through gene regulation [49]. On the other hand, flavonoids and saponin function as anti-oxidants as they can prevent reactive oxygen species (ROS)-associated cell damage [50]. Phenolics also play crucial roles in plant defense mechanisms against a wide range of bacterial, viral, and fungal infections [51], thus making them potential anti-oxidant compounds [52].

Many plants and fruits contain secondary metabolites, which are useful for the prevention and treatment of various diseases in humans [53]. Plant-based secondary metabolites are powerful biological process effectors that can lower the risk of illness through complementary pathways. Additionally, an in-vitro study showed that plant extracts' bioactive components have an anti-hypercholesterolemia effect [54] and could cure a large number of chronic diseases, including diabetes mellitus [55], cancer [56], and cardiovascular [57]. Plant secondary metabolites can reduce cholesterol levels significantly. Alkaloids from the rhizome root extract can reduce total cholesterol, triglyceride (TG), and LDL levels [45].

3.3. 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay

Anti-oxidant activities were tested at various concentrations of 6.25, 12.5, 25, 50, and 100 ppm. Absorbance measurements were conducted at 517 nm using a UV-Vis spectrophotometer, and the results were reported as an IC_{50} value. As presented in Table 2, the anti-oxidant activity of lime peels was categorized as very strong, with an IC_{50} value of 49.51 ppm (<50 ppm). Anti-oxidant activity is considered very strong if a compound's IC_{50} value is <50 ppm; strong

(50 - 100 ppm); moderate (100 - 150 ppm); weak (150 - 200 ppm); and very weak >200 ppm [58]. Many factors affect a plant's anti-oxidant activity and strength, such as the process by which plants and their derivative chemicals are screened for anti-oxidant qualities [59], as well as the extraction solvent [60]. The polarity of the extraction solvent can have an impact on the phytochemical and antioxidant constituents of plants [61]. Ethanol, being a safe solvent, is suitable for extracting a wide range of bioactive compounds with different polarities [62]. Among extraction solvents, ethanol is deemed the most effective, and it can also impact a plant's inherent anti-oxidant properties [63]. The ethanol extract of lime peels in this study contains beneficial chemical compounds, including flavonoid and phenolic compounds. Flavonoids have the ability to scavenge free radicals, thereby preventing liver damage [64, 65]. The results of a study carried out by Kumar et al. (2014) showed a relationship between total phenolic contents to suppress lipid peroxidation and anti-oxidant capability [66].

Table 2. Results of testing the anti-oxidant activity of lime peel ethanol extract using DPPH

Concentration (ppm)	Absorbance Average	Inhibition (%)	IC_{50} (ppm)
6.25	0.69	17.44	
12.5	0.68	18.64	
25	0.56	33.09	49.51
50	0.35	58.42	
100	0.14	83.15	

3.4. Gas Chromatography-Mass Spectrometry Analysis

The GC-MS analysis is able to analyze molecules at the lowest concentration and identify secondary metabolites in plants using information from mass spectra and chromatograms [67]. The GC-MS chromatogram of the ethanol extract of lime peels showed the presence of 22 peaks (Figure 4). However, due to the repetition of 1 compound, 21 active compounds were found (Table 3), comprising 5 compounds with a percent area of >5% and 16 compounds with a percent area of <5%. Compounds with a percentage of more than 5% included m-toluic acid, 2-ethylcyclohexyl ester (12.34%), neric acid (7.19%), 6-methoxychroman-2-one (27.64%), *n*-hexadecanoic acid (5.79%), and 1H-inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl- (10.07%) (Figure 5).

Table 3. Data on compound components of ethanolic extract lime peel

Retention Time	Compound Name	X Molecular Formula	Area (%)	Similarity Index (%)
11.09	2-furanmethanol,5-ethenyltetrahydro-a,a,5-trimethyl-, *cis*-	$C_{14}H_{22}O_2$	1.44	80.2
11.54	a-methyl-a-[4-methyl-3pentenyl] oxirane methanol	$C_{12}H_{22}O_2$	1.27	71.3
15.52	m-toluic acid, 2-ethyl cyclohexyl ester	$C_{20}H_{30}O_2$	12.34	62.9
16.31	2-methyl-oct-2-enedial	$C_9H_{14}O_2$	2.66	67.2
17.94	Phenol, 5-ethenyl-2-methoxy-	$C_{10}H_{10}O_2$	2.72	81.9
18.16	Pterin-6-carboxylic acid	$C_7H_5N5O_4$	0.40	67.2
19.59	Neric acid	$C_{13}H_{22}O_2$	7.19	78.9
21.83	E-8-methyl-7-dodecen-1-ol acetate	$C_{16}H_{30}O_2$	1.00	66.2
23.75	2h-oxecin-2-one, 3,4,7,8,9,10-hexahydro-4-hydroxy-10-methyl-,[4S-(4R*,5E,10S*)]-	$C_{15}H_{24}O_3$	1.23	68.7
24.31	1,2,4 cyclopentanetrione, 3-(2-pentenyl)-	$C_{11}H_{14}O_3$	1.51	61.5
26.18	6-methoxychroman-2-one	$C_{10}H_8O_3$	27.64	69.4
27.99	2h-1-benzopyran-2-one, 7-methoxy-	$C_{10}H_8O_3$	3.86	68.4
28.65	(2,4,6-trimethyl-5-oxocyclohex-3-enyl) acetic acid, methyl ester	$C_{14}H_{20}O_3$	3.85	61.2
31.88	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	1.45	71.5
32.75	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	5.79	80.0
33.10	1h-inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl-	$C_{16}H_{18}O_4$	10.07	76.0
35.87	[1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{24}H_{42}O_2$	1.82	80.7
35.99	*Cis*-vaccenic acid	$C_{18}H_{34}O_2$	3.80	84.7
44.51	3-(octanoyloxy)propane-1,2-diyl bis (decanoate)	$C_{40}H_{76}O_8$	3.84	67.5
45.16	Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{16}H_{32}O_4$	2.68	65.0
47.40	(R)-9-(2,3-dihydroxy-3-methylbutoxy)-4-methoxy-7h-furo(3,2-g)(1)benzopyran-7-one	$C_{22}H_{24}O_8$	2.42	64.5

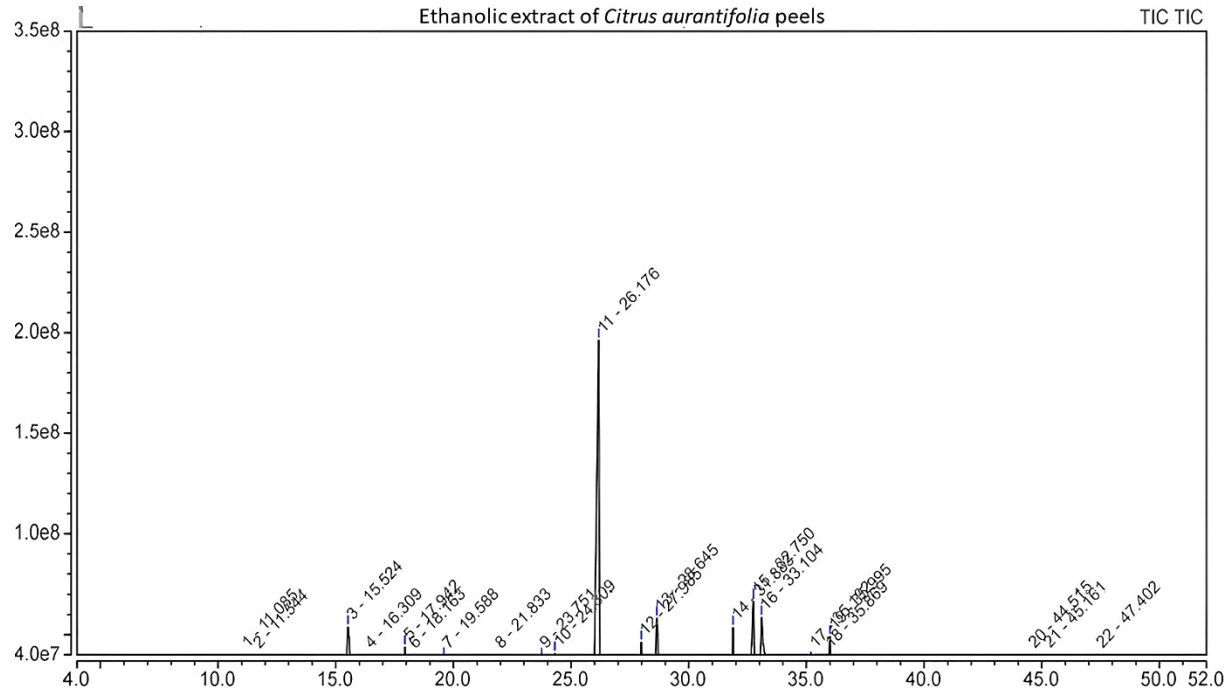


Figure 5. Chromatogram of ethanolic extract *C. aurantifolia* peel

Among the total 21 compounds obtained in the lime peel ethanol extract, 6-methoxychroman-2-one (27.64%) was found as the most dominant compound, followed by m-toluic acid, 2-ethylcyclohexyl ester (12.34%), 1H-inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl- (10.07%), neric acid (7.19%), and *n*-hexadecanoic acid (5.79%). 6-Methoxychroman-2-one, a coumarin compound, has been reported to possess various biological activities. Previous studies showed that coumarin and its derivatives can reduce lipid levels [68, 69] and exhibited anti-inflammatory and anti-nociceptive effects [70]. Another study reported the presence of 62 compounds in the ethanol extract of lime peels, which belonged to the groups of alkaloids, glycosides, flavonoids, saccharides, furanocoumarins, amino acids, terpenoids, organic acids, and glycosides [71]. Based on this, Seulawah Valley, Aceh Province, Indonesia, influenced the phytochemical components contained in lime peels, including a unique class of secondary metabolite compounds, which is considerably important to be analyzed using molecular docking.

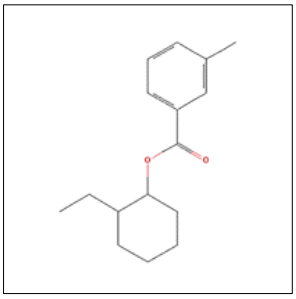
3.5. Biological Activity Prediction

Table 4 presents the biological activity predictions of compounds found in *C. aurantifolia* peels. The results of predicting activity spectra for substances are reported as active (Pa) and inactive (Pi) outputs, with probability ranging from 0 to 1, with values closer to 1 indicating higher activity of the compound [72]. Based on the PASS online test, secondary metabolites from ethanolic extracts of lime peels showed numerous activities as an inhibitor of human diseases. m-toluic acid, 2-ethylcyclohexyl ester (Figure 6-a) served as CYP2H substrate (Pa: 0.956), neric acid (Figure 6-b) as muco-membranous protector (Pa: 0.971), 6-methoxychroman-2-one (Figure 6-c) as aspulvinone dimethylallyl transferase inhibitor (Pa: 0.911), *n*-hexadecanoic acid (Figure 6-d) as acylcarnitine hydrolase inhibitor (Pa: 0.973), and 1h-inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl- (Figure 6-e) as cholinergic (Pa: 0.788). Neric acid ($C_{13}H_{22}O_2$) was found to regulate lipid metabolism (Pa: 0.941). Numerous metabolic diseases, including diabetes, the metabolic syndrome, and cancer, are largely influenced by lipid metabolism [73, 74]. Metabolic disorders are those involving lipid metabolism. Inflammatory and immunological responses are linked to lipid metabolism [75]. The results of previous research on the screened compounds from non-oilseed legumes using PASS found compounds that have functioned as anti-infective, muco-membranous protective, anti-eczematic, anti-septic, anti-mutagenic, fibrinolytic, anti-carcinogenic, cardio-protective, hepato-protective, anti-oxidant, and astringent effects [76].

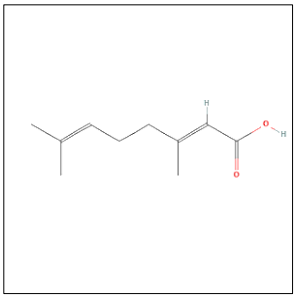
Table 4. Biological activity of metabolite compounds

m-toluic acid, 2-ethylcyclohexyl ester		
Pa	Pi	Activity name
0.956	0.003	CYP2H substrate
0.879	0.009	Testosterone 17β-dehydrogenase (NADP+) inhibitor
0.819	0.015	Alkenylglycerophosphocholine hydrolase inhibitor
0.817	0.017	CYP2J substrate
0.815	0.015	Antieczematic

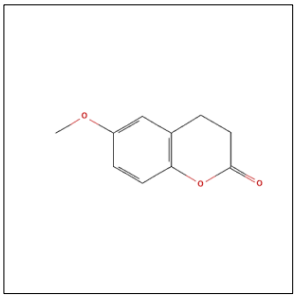
Neric acid		
Pa	Pi	Activity name
0.971	0.002	Muco-membranous protector
0.948	0.000	BRAF expression inhibitor
0.941	0.003	Lipid metabolism regulator
0.915	0.004	CYP2J substrate
0.900	0.002	Undecaprenyl-phosphate mannosyltransferase inhibitor
6-methoxychroman-2-one		
Pa	Pi	Activity name
0.911	0.006	Aspulvinone dimethylallyltransferase inhibitor
0.907	0.010	CYP2C12 substrate
0.852	0.003	CYP2B5 substrate
0.848	0.003	4-Nitrophenol 2-monooxygenase inhibitor
0.853	0.011	Chlordecone reductase inhibitor
n-hexadecenoic acid		
Pa	Pi	Activity name
0.973	0.001	Acylcarnitine hydrolase inhibitor
0.966	0.001	Alkylacetylglycerophosphatase inhibitor
0.963	0.002	Alkenylglycerophosphocholine hydrolase inhibitor
0.962	0.002	CYP2J substrate
0.961	0.001	CYP2J2 substrate
1H-inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl-		
Pa	Pi	Activity name
0.788	0.003	Cholinergic
0.705	0.054	Gluconate 2-dehydrogenase (acceptor) inhibitor
0.675	0.029	5 Hydroxytryptamine release stimulant
0.651	0.019	Neurotransmitter uptake inhibitor
0.638	0.011	MAP kinase stimulant



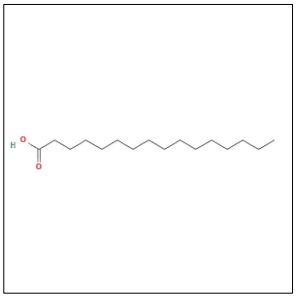
(a) m-toluic acid, 2-ethylcyclohexyl ester



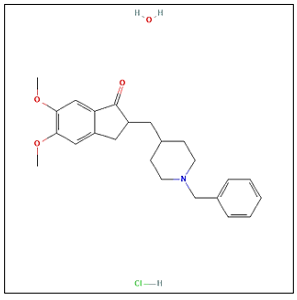
(b) Neric acid



(c) 6-methoxychroman-2-one



(d) *n*-hexadecanoic acid



(e) 1h-inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl-

Figure 6. Metabolite active from ethanolic extract of lime peel from *C. aurantifolia*

The compound m-toluic acid, 2-ethylcyclohexyl ester, acted as an inhibitor of testosterone 17β-dehydrogenase (NADP+) with Pa = 0.879. Testosterone hormone functions distinctly from estrogen, primarily stimulating de novo

lipogenesis (DNL), whereas estrogen can reduce plasma triglyceride and LDL cholesterol levels. Treatment with dihydrotestosterone (DHT) has been shown to increase triglyceride formation, resulting in increased body weight and visceral fat mass. Treatment with DHT reduces phosphorylation that inactivates Acetyl CoA carboxylase (ACC) and increases the production of fatty acid synthase (FAS) and sterol regulatory element binding transcription factor 2 (SREBP2) [77]. The intricate process known as de novo lipogenesis transforms circulating carbohydrates into fatty acids, which are then utilized to create triglycerides and other lipid molecules. Fat formation is determined by the equilibrium between TG production and breakdown [78]. Lipid oxidation is stimulated by testosterone as one of the mechanisms involved in controlling fat mass [79]. Transdermal testosterone treatment improves body fat oxidation [80] and growth hormone (GH) secretion [79, 81]. Hormonal therapy has the potential as a new therapy for treating obesity and complications such as morbidity and mortality [82].

3.6. Molecular Docking (*In-silico*)

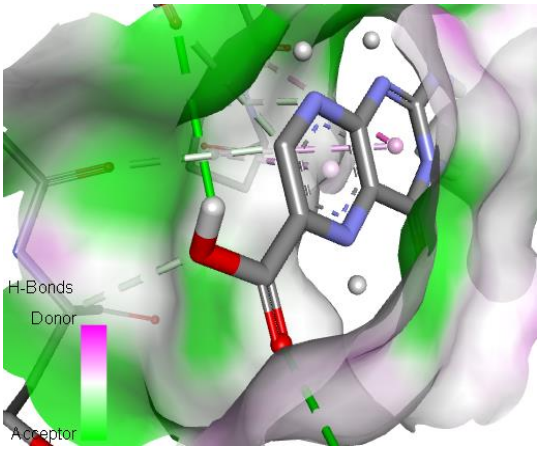
In-silico is a method for identifying interactions between active ligands and target proteins using computers [83]. Molecular docking studies offer a cost-effective and efficient means of discovering new drug candidates [84]. A study by El Fadili et al. (2023) suggested that C19 ligands have the potential to treat schizophrenia and other disorders caused by glutaminergic N-methyl-D-aspartate (NMDA) receptor hypofunction [84]. Another docking study on the methanol extract of *Polygonatum odoratum* (Mill.) Druce found five main compounds, namely, 9-aminocamptothecin, 9-methoxycampothecin, 5,7,30-tri hydroxy-6,40,50-trimethoxyflavone, diacerein, and 5-hydroxy-1- tetralin had potential as an anti-diabetic type 2 [85]. In the present study, molecular docking was performed by docking 21 active compounds contained in the ethanol extract of lime peels as test ligands and simvastatin as a control ligand against the target proteins PCSK9 and HMG-CoA reductase. The PCSK9 and HMG-CoA reductase enzyme 3D structures were obtained from the Protein Data Bank (PDB). The PCSK9 receptor's molecular structure is PDB ID: 6U26, while the HMG-CoA reductase receptor's is PDB ID: 1HW9. Molecular docking results of active compounds of ethanol extract lime peels and simvastatin against PCSK9 and HMG-CoA are shown in Table 5. Determination of the docking score is carried out by selecting the protein ligand that has the highest binding free energy (BFE) [86].

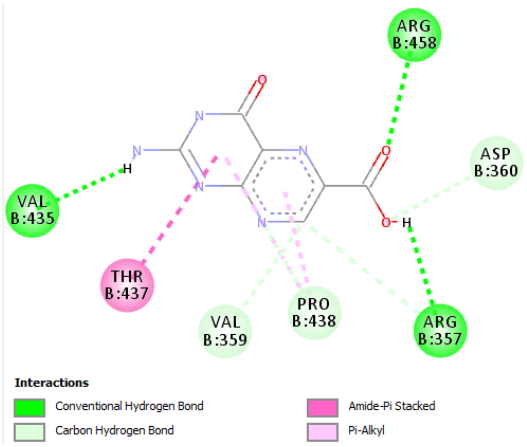
Table 5. Molecular docking results of active compounds of ethanol extract lime peels and simvastatin against PCSK9 and HMG-CoA

Ligand	Binding Free Energy (Kcal/mol)	
	PCSK9	HMG-CoA
Pterin-6- carboxylic acid	-7.8	-6.5
Simvastatin	-7.6	-7.6
(2,4,6-trimethyl-5-oxocyclohex-3-enyl)acetic	-7.2	-5.5
2h-oxecin-2-one_3 4 7 8 9 10-hexahydro-4-	-7.1	-5.5
1h-inden-1-one_2,3-dihydro-5,6-dimethoxy-3 methyl-	-7	-5.4
E-8-methyl-7-dodecen-1-ol acetate	-7	-4.7
[1,1_-bicyclopropyl]-2-octanoic_acid_2_-hexyl-	-6.9	-4.7
(R)-9-(2,3-dihydroxy-3-methylbutoxy) 4-	-6.8	-6.9
2H-1-benzopyran-2-one_7-methoxy-	-6.8	-5.8
6-methoxychroman-2-one	-6.7	-5.5
m-toluic_acid_2-ethylcyclohexyl_ester	-6.4	-6.4
1,2,4_cyclopentanetrione 3-(2-pentenyl)-	-6.2	-4.9
Neric_acid	-6.1	-4.9
Hexadecanoic_acid_methyl_ester	-5.9	-4.1
a-Methyl-a-[4-methyl-3pentenyl] oxiranemethanol	-5.8	-4.8
Hexadecanoic acid	-5.8	-4.4
Phenol_5-ethenyl-2-methoxy	-5.7	-4.9
2-furanmethanol 5-ethenyltetrahydro 5-trimethyl-*cis*-	-5.4	-4.9
Dodecanoic acid, 1-(hydroxymethyl)-1,2-	-5.4	-4.6
2-methyl-oct-2-enedial	-5.3	-4.3
3-(octanoyloxy)propane-1 2-diyl_bis_(decanoate)	-5.3	-4.3
Cis-vaccenic_acid	-4.7	-4.6

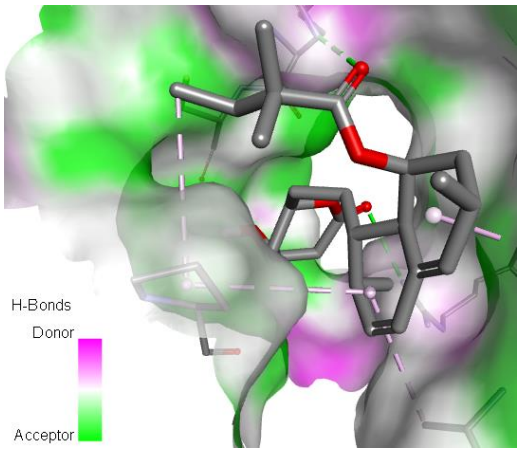
The findings of the molecular docking test in this study indicated that pterin-6- carboxylic acid compound had a lower free binding energy compared to simvastatin, indicating that pterin-6-carboxylic acid (-7.8 kcal/mol) in the ethanol extract of lime peels exhibited higher binding affinity against the PCSK9 receptor compared to simvastatin (-7.6 kcal/mol). These results prove that pterin-6- carboxylic acid has more potential to be a PCSK9 inhibitor. Pterin-6-carboxylic acid ($C_7H_5N_5O_4$) is a derivative of pterin that plays a role in maintaining biochemical balance and physiological functions in living organisms [87]. The interaction between the pterin-6- carboxylic acid molecule and PCSK9 as a ligand has the maximum binding value (Figure 7), with eight amino acid residues and six hydrogen bonds, as shown in Table 6. Hydrogen bonds are particularly significant in medications because they mediate drug interaction with receptors and alter physicochemical qualities such as solubility and absorption [87, 88]. Previous clinical trial results suggest that PCSK9 inhibitors are potential agents representing statins [89].

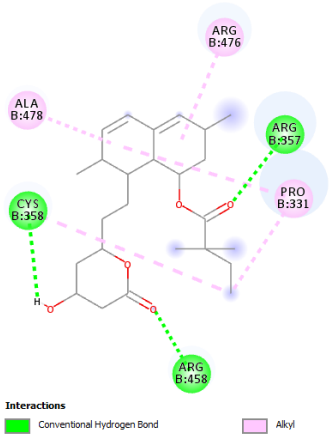
2D Interactions **3D Interactions**



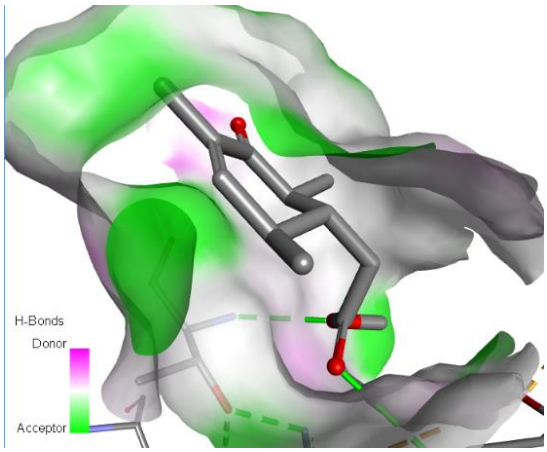


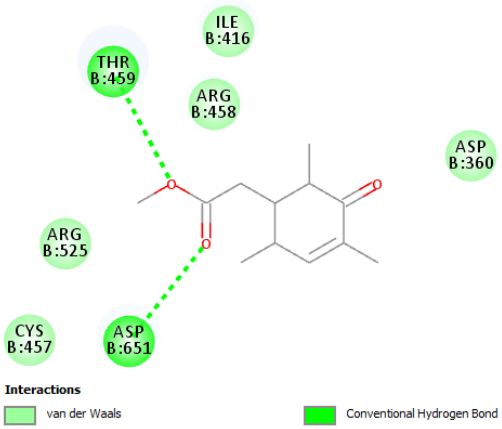
Pterin-6-carboxylic acid Pterin-6-carboxylic acid





Simvastatin Simvastatin





(2,4,6-Trimethyl-5-oxocyclohex-3-enyl) acetic (2,4,6-Trimethyl-5-oxocyclohex-3-enyl) acetic

Figure 7. PCSK9 receptor docking visualization (PDB ID: 6U26) with Pterin-6-carboxylic acid, simvastatin and (2,4,6-Trimethyl-5-oxocyclohex-3-enyl) acetic

Table 6. Description of PCSK9 amino acid residues

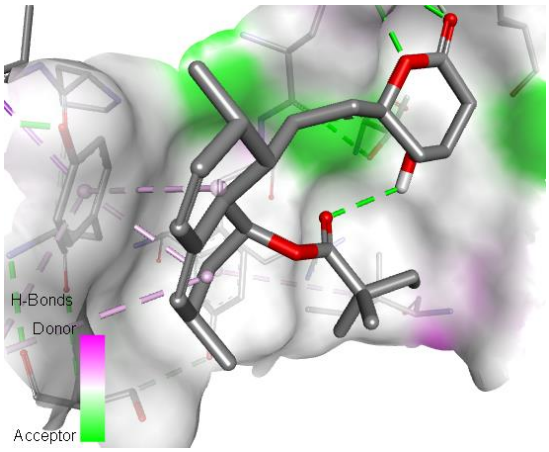
Ligand	Amino Acid Residue
Pterin-6- carboxylic acid	Conventional Hydrogen Bond: ARG B:357, VAL B:435, ARG B:458 Carbon Hydrogen Bond: ARG B:357, VAL B:359, ASP B:360 Amide Pi Stacked: THR B:437 Pi-Alkyl: PRO B:438
Simvastatin	Conventional Hydrogen Bond: ARG B:357, CYS B:358, ARG B:458 Pi-Alkyl: PRO B:331, ARG B: 476, ALA B:478
(2,4,6-Trimethyl-5-oxocyclohex-3-enyl) acetic	van der Waals: ASP B:360, ILE B:416, CYS B:457, ARG B:458, ARG B:525 Conventional Hydrogen Bond: THR B:459, ASP B:651

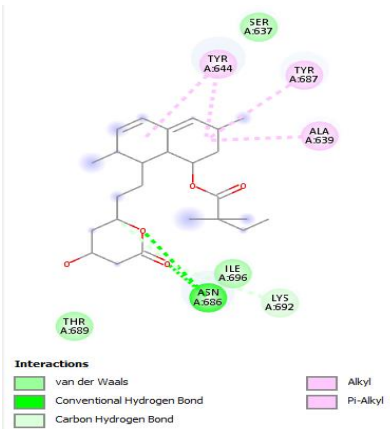
The outcomes of molecular docking of the active components in the lime peel ethanol extract against the HMG-CoA receptor showed that simvastatin had a lower free binding energy than its active ligand, indicating that simvastatin had a higher binding affinity towards the HMG-CoA reductase receptor (Figure 8) with two Van der Waals bonds, one hydrogen bond, one hydrocarbon bond, one amide bond, and one alkyl bond (Table 7). This docking examination also identified the active component with the highest binding affinity in the ethanol extract of lime peels, namely (R)-9-(2,3-dihydroxy-3-methylbutoxy)-4 at 6.9 kcal/mol, which is a furanocoumarin whose molecular structure is equivalent to coumarin [90], having a furan ring structure connected to carbon 6 and 7 or 7 and benzo-a-pyrone coumarin [91]. Many plants, especially members of the Rutaceae and Apiaceae families, contain these chemicals. This metabolite is known to exhibit anticancer [92, 93], anti-oxidant, anti-inflammatory, anti-cancer, and bone health-promoting activities [91]. In this present study, it is known that Acehnese lime (*C. aurantifolia*) can gain high attention to be one of the natural products from Aceh Province, Indonesia, as anti-hypercholesterolemia agents. The two main compounds, namely pterin-6-carboxylic acid and (R)-9-(2,3-dihydroxy-3-methylbutoxy)-4, can be used as promising candidates from *C. aurantifolia.*

Table 7. Description of HMG-CoA amino acid residues

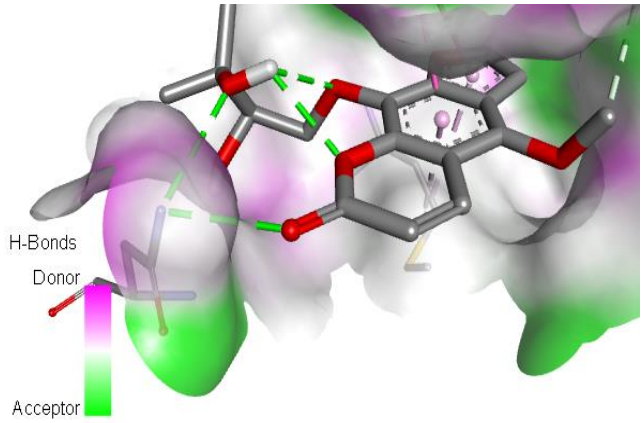
Ligand	Amino Acid Residue
Simvastatin	Van der waals: THR A:689, ILE A:696 Conventional hydrogen bond: ASN A: 658 Carbon hydrogen bond: ASN A: 686, LYS A:692 Amide pi stacked: TYR A:644, TYR A:687 Pi-alkyl: ALA A:639
(R)-9-(2,3-dihydroxy-3-methylbutoxy)-4-	Van der waals: GLY A:807 Conventional hydrogen bond: ASN A: 658 Carbon hydrogen bond: GLY A:765 Unforable donor-donor: ASN A: 658 Amide pi stacked: GLY A:806 Pi-alkyl: MET A:655
Pterin-6-carboxylic acid	Van der waals: ASN A:658, GLY A:808 Conventional hydrogen bond: MET A: 655, GLY A:656, GLY A:765 Unforable donor-donor: MET A: 657 Pi donor hydrogen bond: GLY A:808 Pi-sigma: GLY A:807 Pi-alkyl: MET A:655

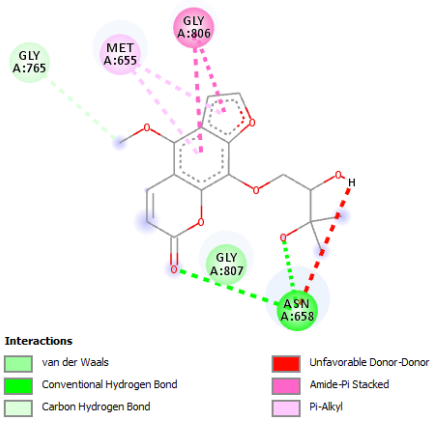
3D Interactions **2D Interactions**



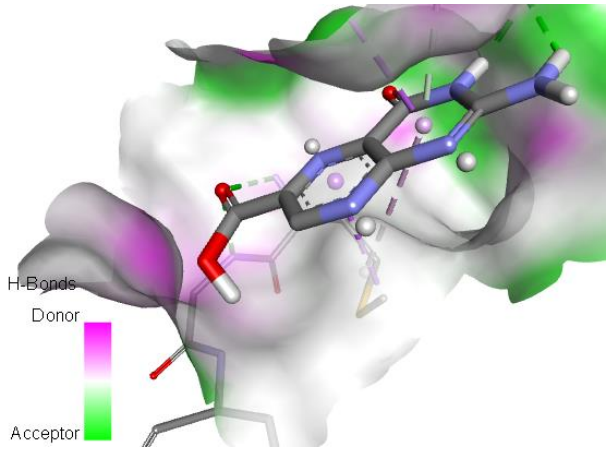


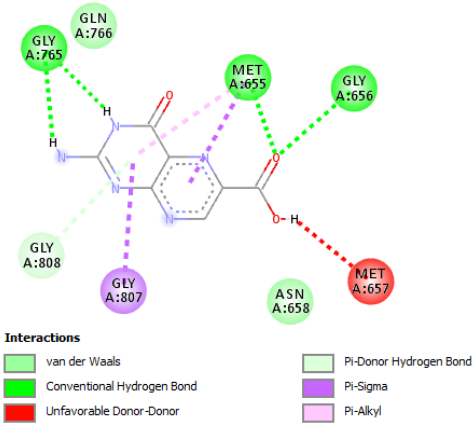
Simvastatin Simvastatin





(R)-9-(2,3-dihydroxy-3-methylbutoxy)-4- (R)-9-(2,3-dihydroxy-3-methylbutoxy)-4-





Pterin-6-carboxilic acid Pterin-6-carboxilic acid

Figure 8. HMG-CoA receptor docking visualization (PDB ID: 1HW9) with simvastatin, (R)-9-(2,3-dihydroxy-3-methylbutoxy)-4, and pterin-6-carboxylic acid

4. Conclusion

This study highlights the potential of the ethanol extract of Acehnese lime peels as a natural anti-hypercholesterolemia agent, attributed to the ability of its active chemical constituents to inhibit PCSK9 and HMG Co-A reductase. Using six different analyses, namely plant determination and extraction yield, total phenolics and total flavonoids analysis, 2,2-diphenil-1-picrylhydrazyl (DPPH) assay, gas chromatography-mass spectrometry analysis, biological activity predictions, and *in-silico* approach using molecular docking, it is known that the ethanolic extract of Acehnese lime peels meet the criteria as a candidate for anti-hypercholesterolemia. The ethanol extract of lime peels exhibited various phytochemicals, including tannins, phenolics, flavonoids, saponins, and terpenoids. Next, the total phenolic and flavonoid content found in the extract were 29.99 ± 0.27 mg GAE/g extract and 5.98 ± 0.02 mg QE/g

extract, respectively, along with a high anti-oxidant activity using DPPH assays (49.51 ppm). The biological activity prediction using the PASS online test indicated that several compounds in the lime peel extract, such as m-toluic acid, 2-ethylcyclohexyl ester, nerat acid, 6-methoxychroman-2-one, *n*-hexadecanoic acid, and 1H-inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl, exhibited a promising anti-hypercholesterolemia activity and other relative to human diseases. Molecular docking analysis confirmed that there are interactions between the active compounds from the extract and the target proteins PCSK9 and HMG-CoA reductase, with the compound pterin-6-carboxylic acid showing higher binding free energy to the PCSK9 receptor compared to simvastatin (-7.8 kcal/mol > -7.6 kcal/mol), thus potentially acting as a PCSK9 inhibitor, and the active molecule (R)-9-(2,3-dihydroxy-3-methylbutoxy)-4- showing the highest binding affinity to HMG Co-A reductase (-6.9 kcal/mol) compared to other components in the lime peel extract. This study not only showed the activity of Acehnese lime but also confirmed that it is one of the natural medicinal plants from Aceh as anti-hypercholesterolemia.

5. Declarations

5.1. Author Contributions

Conceptualization, R.S., M.A., and G.G.; methodology, R.S. and M.A.; software, G.G.; validation, M.A., M.M., and G.G.; formal analysis, M.A., M.M., and G.G.; investigation, R.S. and M.M.; resources, G.G.; data curation, R.S., M.A., and G.G.; writing—original draft preparation, R.S., M.A., M.M., and G.G.; writing—review and editing, M.A. and G.G.; visualization, M.M.; supervision, M.A., M.M., and G.G.; project administration, R.S. and M.A.; funding acquisition, R.S. All authors have read and agreed to the published version of the manuscript.

5.2. Data Availability Statement

The data presented in this study are available in the article.

5.3. Funding

This research was supported by the Ministry of Health of the Republic of Indonesia.

5.4. Acknowledgements

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5.5. Institutional Review Board Statement

Not applicable.

5.6. Informed Consent Statement

Not applicable.

5.7. Declaration of Competing Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

6. References

[1] Rivera, D., Bermúdez, A., Obón, C., Alcaraz, F., Ríos, S., Sánchez-Balibrea, J., Pablo Ferrer-Gallego, P., & Krueger, R. (2022). Analysis of 'Marrakesh limetta' (Citrus × limon var. limetta (Risso) Ollitrault, Curk & R.Krueger) horticultural history and relationships with limes and lemons. Scientia Horticulturae, 293, 110688. doi:10.1016/j.scienta.2021.110688.

[2] WHO. (2023). World Heart Report 2023-Confronting the World's Number One Killer. World Health Organization (WHO), Geneva, Switzerland.

[3] Johnston, T. P., Korolenko, T. A., Pirro, M., & Sahebkar, A. (2017). Preventing cardiovascular heart disease: Promising nutraceutical and non-nutraceutical treatments for cholesterol management. Pharmacological Research, 120, 219–225. doi:10.1016/j.phrs.2017.04.008.

[4] Klein, F., das Neves, V. J., Costa, R., Sanches, A., Sousa, T., Sampaio Moura, M. J. C., Paula, A., & Elena, D. (2012). Dyslipidemia Induced by Stress. Dyslipidemia - From Prevention to Treatment. doi:10.5772/28163.

[5] Oppi, S., Lüscher, T. F., & Stein, S. (2019). Mouse Models for Atherosclerosis Research—Which Is My Line? Frontiers in Cardiovascular Medicine, 6. doi:10.3389/fcvm.2019.00046.

[6] Gesto, D. S., Pereira, C. M. S., Cerqueira, N. M. F. S., & Sousa, S. F. (2020). An atomic-level perspective of HMG-CoA-reductase: The target enzyme to treat hypercholesterolemia. Molecules, 25(17), 3891. doi:10.3390/molecules25173891.

[7] Page, M. M., & Watts, G. F. (2017). PCSK9 in context: contemporary review of an important biological target for the prevention and treatment of atherosclerotic cardiovascular disease. Diabetes, Obesity and Metabolism, 20(2), 270–282.. doi:10.1111/dom.13070.

[8] Al-Mohaissen, M. A., Ignaszewski, M. J., Frohlich, J., & Ignaszewski, A. P. (2016). Statin-associated muscle adverse events: Update for clinicians. Sultan Qaboos University Medical Journal, 16(4), e406–e415. doi:10.18295/squmj.2016.16.04.002.

[9] Tavori, H., Fan, D., Blakemore, J. L., Yancey, P. G., Ding, L., Linton, M. F., & Fazio, S. (2013). Serum proprotein convertase subtilisin/kexin type 9 and cell surface low-density lipoprotein receptor evidence for a reciprocal regulation. Circulation, 127(24), 2403–2413. doi:10.1161/CIRCULATIONAHA.113.001592.

[10] Anderson, T. J., Grégoire, J., Pearson, G. J., Barry, A. R., Couture, P., Dawes, M., Francis, G. A., Genest, J., Grover, S., Gupta, M., Hegele, R. A., Lau, D. C., Leiter, L. A., Lonn, E., Mancini, G. B. J., McPherson, R., Ngui, D., Poirier, P., Sievenpiper, J. L., … Ward, R. (2016). 2016 Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in the Adult. Canadian Journal of Cardiology, 32(11), 1263–1282. doi:10.1016/j.cjca.2016.07.510.

[11] Guo, Y. L., Zhang, W., & Li, J. J. (2014). PCSK9 and lipid lowering drugs. Clinica Chimica Acta, 437, 66–71. doi:10.1016/j.cca.2014.07.008.

[12] Gearing, M. E., & Seim, K. (2015). Science in the News; A potential new weapon against heart disease: PCSK9 inhibitors. Available online: http://sitn.hms.harvard.edu/flash/2015/a-potential-new-weapon-against-heart-disease-pcsk9-inhibitors/ (accessed on May 2024).

[13] Lin, X. L., Xiao, L. Le, Tang, Z. H., Jiang, Z. S., & Liu, M. H. (2018). Role of PCSK9 in lipid metabolism and atherosclerosis. Biomedicine and Pharmacotherapy, 104(December 2017), 36–44. doi:10.1016/j.biopha.2018.05.024.

[14] Catapano, A. L., Graham, I., De Backer, G., Wiklund, O., John Chapman, M., Drexel, H., Hoes, A. W., Jennings, C. S., Landmesser, U., Pedersen, T. R., Reiner, Ž., Riccardi, G., Taskinen, M. R., Tokgozoglu, L., Monique Verschuren, W. M., Vlachopoulos, C., Wood, D. A., Zamorano, J. L., Badimon, L., … Wald, D. (2016). 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias. European Heart Journal, 37(39), 2999-3058l. doi:10.1093/eurheartj/ehw272.

[15] Tugume, P., & Nyakoojo, C. (2019). Ethno-pharmacological survey of herbal remedies used in the treatment of paediatric diseases in Buhunga parish, Rukungiri District, Uganda. BMC Complementary and Alternative Medicine, 19(1), 1–10. doi:10.1186/s12906-019-2763-6.

[16] Scolaro, B., Soo Jin Kim, H., & de Castro, I. A. (2018). Bioactive compounds as an alternative for drug co-therapy: Overcoming challenges in cardiovascular disease prevention. Critical Reviews in Food Science and Nutrition, 58(6), 958–971. doi:10.1080/10408398.2016.1235546.

[17] Yang, L., Wen, K. S., Ruan, X., Zhao, Y. X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. Molecules, 23(4), 1–26. doi:10.3390/molecules23040762.

[18] Badawi, A. N. (2012). Medicinal & Aromatic Plants. Medicinal & Aromatic Plants, 1(02). doi:10.4172/2167-0412.1000e109.

[19] González-Molina, E., Domínguez-Perles, R., Moreno, D. A., & García-Viguera, C. (2010). Natural bioactive compounds of Citrus limon for food and health. Journal of Pharmaceutical and Biomedical Analysis, 51(2), 327–345. doi:10.1016/j.jpba.2009.07.027.

[20] Shafreen, R. B., Lubinska-Szczygeł, M., Różańska, A., Dymerski, T., Namieśnik, J., Katrich, E., & Gorinstein, S. (2018). Human serum interactions with phenolic and aroma substances of Kaffir (Citrus hystrix) and Key lime (Citrus aurantifolia) juices. Journal of Luminescence, 201(March), 115–122. doi:10.1016/j.jlumin.2018.04.010.

[21] Spadaro, F., Costa, R., Circosta, C., & Occhiuto, F. (2012). Volatile composition and biological activity of key lime citrus aurantifolia essential oil. Natural Product Communications, 7(11), 1523–1526. doi:10.1177/1934578x1200701128.

[22] Tundis, R., Loizzo, M. R., Bonesi, M., Menichini, F., Mastellone, V., Colica, C., & Menichini, F. (2012). Comparative study on the antioxidant capacity and cholinesterase inhibitory activity of Citrus aurantifolia Swingle, C. aurantium L., and C. bergamia Risso and Poit. peel essential oils. Journal of Food Science, 77(1), 40–46. doi:10.1111/j.1750-3841.2011.02511.x.

[23] Amorim, J. L., Simas, D. L. R., Pinheiro, M. M. G., Moreno, D. S. A., Alviano, C. S., Da Silva, A. J. R., & Fernandes, P. D. (2016). Anti-inflammatory properties and chemical characterization of the essential oils of four Citrus species. PLoS ONE, 11(4). doi:10.1371/journal.pone.0153643.

[24] Fagodia, S. K., Singh, H. P., Batish, D. R., & Kohli, R. K. (2017). Phytotoxicity and cytotoxicity of Citrus aurantiifolia essential oil and its major constituents: Limonene and citral. Industrial Crops and Products, 108(May), 708–715. doi:10.1016/j.indcrop.2017.07.005.

[25] Lin, L.-Y., Chuang, C.-H., Chen, H.-C., & Yang, K.-M. (2019). Lime (Citrus aurantifolia (Christm.) Swingle) Essential Oils: Volatile Compounds, Antioxidant Capacity, and Hypolipidemic Effect. Foods, 8(9), 398. doi:10.3390/foods8090398.

[26] Lin, L., Chuang, C., & Chen, H. (n.d.). Lime (Citrus aurantifolia (Christm.) Swingle) Essential Oils: Volatile Compounds, Antioxidant Capacity, and Hypolipidemic Effect. Foods, 8(398), 1–11.

[27] Rachmawati, R., Idroes, R., Suhartono, E., Maulydia, N. B., & Darusman, D. (2022). In Silico and In Vitro Analysis of Tacca Tubers (Tacca leontopetaloides) from Banyak Island, Aceh Singkil Regency, Indonesia, as Antihypercholesterolemia Agents. Molecules, 27(23), 1–14. doi:10.3390/molecules27238605.

[28] Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total Phenolic content, Flavonoid content and antioxidant potential of wild vegetables from western Nepal. Plants, 8(4), 1–12. doi:10.3390/plants8040096.

[29] Maeng, J. H., Muhammad Shahbaz, H., Ameer, K., Jo, Y., & Kwon, J. H. (2017). Optimization of Microwave-Assisted Extraction of Bioactive Compounds from Coriolus versicolor Mushroom Using Response Surface Methodology. Journal of Food Process Engineering, 40(2), 12421. doi:10.1111/jfpe.12421.

[30] Ginting, B., Maulana, I., Yahya, M., Saidi, N., Murniana, M., Hasballah, K., Maulidna, M., & Rawati, S. (2022). Antioxidant and antiproliferative activities of n-hexane extract and its fractions from Blumea balsamifera L. leaves. Journal of Advanced Pharmaceutical Technology and Research, 13(3), 216–220. doi:10.4103/japtr.japtr_105_22.

[31] Lagunin, A., Stepanchikova, A., Filimonov, D., & Poroikov, V. (2000). PASS: Prediction of activity spectra for biologically active substances. Bioinformatics, 16(8), 747–748. doi:10.1093/bioinformatics/16.8.747.

[32] Maulydia, N. B., Khairan, K., Tallei, T. E., Estevam, E. C., Patwekar, M., Mohd Fauzi, F., & Idroes, R. (2023). GC-MS Analysis Reveals Unique Chemical Composition of Blumea balsamifera (L.) DC in Ie-Jue Geothermal Area. Grimsa Journal of Science Engineering and Technology, 1(1), 9–16. doi:10.61975/gjset.v1i1.6.

[33] Zeb, M. A., Rahman, T. U., Sajid, M., Xiao, W., Musharraf, S. G., Bibi, S., Akitsu, T., & Liaqat, W. (2021). GC-MS Analysis and In Silico Approaches of Indigofera heterantha Root Oil Chemical Constituents. Compounds, 1(3), 116–124. doi:10.3390/compounds1030010.

[34] Jasmine, J. M., & Vanaja, R. (2013). In silico analysis of phytochemical compounds for optimizing the inhibitors of HMG CoA reductase. Journal of Applied Pharmaceutical Science, 3(9), 43–47. doi:10.7324/JAPS.2013.3908.

[35] Maulydia, N. B., Tallei, T. E., Ginting, B., Idroes, R., Illian, D. N., & Faradilla, M. (2022). Analysis of flavonoid compounds of Orange (Citrus sp.) peel as anti-main protease of SARS-CoV-2: A molecular docking study. IOP Conference Series: Earth and Environmental Science, 951(1), 1–8. doi:10.1088/1755-1315/951/1/012078.

[36] Okwu, D. E. (2008). Citrus fruits: A rich source of phytochemicals and their roles in human health. International Journal of Chemical Sciences, 6(2), 451–471.

[37] Indriyani, N. N., Anshori, J. Al, Permadi, N., Nurjanah, S., & Julaeha, E. (2023). Bioactive Components and Their Activities from Different Parts of Citrus aurantifolia (Christm.) Swingle for Food Development. Foods, 12(10), 1–23. doi:10.3390/foods12102036.

[38] Herlina, Aziz, S. A., Kurniawati, A., & Faridah, D. N. (2017). Changes of Thymoquinone, Thymol, and Malondialdehyde Content of Black Cumin (Nigella sativa L.) in Response to Indonesia Tropical Altitude Variation. HAYATI Journal of Biosciences, 24(3), 156–161. doi:10.1016/j.hjb.2017.08.004.

[39] Gahukar, R. T. (2014). Factors affecting content and bioefficacy of neem (Azadirachta indica A. Juss.) phytochemicals used in agricultural pest control: A review. Crop Protection, 62, 93–99. doi:10.1016/j.cropro.2014.04.014.

[40] Tangpao, T., Chung, H. H., & Sommano, S. R. (2018). Aromatic profiles of essential oils from five commonly used Thai basils. Foods, 7(11), 1–13. doi:10.3390/foods7110175.

[41] Czech, A., Malik, A., Sosnowska, B., & Domaradzki, P. (2021). Bioactive Substances, Heavy Metals, and Antioxidant Activity in Whole Fruit, Peel, and Pulp of Citrus Fruits. International Journal of Food Science, 2021, 1–14. doi:10.1155/2021/6662259.

[42] Liu, W., Wang, D., Hou, X., Yang, Y., Xue, X., Jia, Q., Zhang, L., Zhao, W., & Yin, D. (2018). Effects of Growing Location on the Contents of Main Active Components and Antioxidant Activity of Dasiphora fruticosa (L.) Rydb. by Chemometric Methods. Chemistry and Biodiversity, 15(7), 1–42. doi:10.1002/cbdv.201800114.

[43] Johnson, J. B., Batley, R., Manson, D., White, S., & Naiker, M. (2022). Volatile compounds, phenolic acid profiles and phytochemical content of five Australian finger lime (Citrus australasica) cultivars. LWT, 154(2022), 1–11. doi:10.1016/j.lwt.2021.112640.

[44] Ogundele, O. O., & Bolade, M. K. (2021). Biochemical Characteristics and Antioxidant Properties of Citrus Juice from Lemon (Citrus limon), Lime (Citrus aurantifolia) and Grapefruit (Citrus paradisi) as Influenced by Degree of Ripening. Asian Food Science Journal, 40–51. doi:10.9734/afsj/2021/v20i330277.

[45] Cao, Y., Bei, W., Hu, Y., Cao, L., Huang, L., Wang, L., Luo, D., Chen, Y., Yao, X., He, W., Liu, X., & Guo, J. (2012). Hypocholesterolemia of Rhizoma Coptidis alkaloids is related to the bile acid by up-regulated CYP7A1 in hyperlipidemic rats. Phytomedicine, 19(8–9), 686–692. doi:10.1016/j.phymed.2012.03.011.

[46] Castro-Torres, I. G., De La O-Arciniega, M., Gallegos-Estudillo, J., Naranjo-Rodríguez, E. B., & Domínguez-Ortíz, M. Á. (2014). Raphanus sativus L. var niger as a source of Phytochemicals for the prevention of cholesterol gallstones. Phytotherapy Research, 28(2), 167–171. doi:10.1002/ptr.4964.

[47] Millar, C. L., Duclos, Q., & Blesso, C. N. (2017). Effects of dietary flavonoids on reverse cholesterol transport, HDL metabolism, and HDL function. Advances in Nutrition, 8(2), 226–239. doi:10.3945/an.116.014050.

[48] Leng, E., Xiao, Y., Mo, Z., Li, Y., Zhang, Y., Deng, X., Zhou, M., Zhou, C., He, Z., He, J., Xiao, L., Li, J., & Li, W. (2018). Synergistic effect of phytochemicals on cholesterol metabolism and lipid accumulation in HepG2 cells. BMC Complementary and Alternative Medicine, 18(1), 1–10. doi:10.1186/s12906-018-2189-6.

[49] Li, Y., Xu, S., Mihaylova, M. M., Zheng, B., Hou, X., Jiang, B., Park, O., Luo, Z., Lefai, E., Shyy, J. Y.-J., Gao, B., Wierzbicki, M., Verbeuren, T. J., Shaw, R. J., Cohen, R. A., & Zang, M. (2011). AMPK Phosphorylates and Inhibits SREBP Activity to Attenuate Hepatic Steatosis and Atherosclerosis in Diet-Induced Insulin-Resistant Mice. Cell Metabolism, 13(4), 376–388. doi:10.1016/j.cmet.2011.03.009.

[50] Ubwa, S. T., Anhwange, B. A., & Chia, J. T. (2011). Chemical Analysis of Tacca leontopetaloides Peels. American Journal of Food Technology, 6(10), 932–938. doi:10.3923/ajft.2011.932.938.

[51] Singh, A., Maurya, S., Singh, U. P., & Singh, K. P. (2014). Chromatographic Analysis of Phenolic Acids in the Fruit Pulp of Some Citrus Varieties and Their Therapeutic Importance in Human Health. International Journal of Applied Science-Research and Review, 1(3), 150–154.

[52] Kim, J. W., Ko, H. C., Jang, M. G., Han, S. H., Kim, H. J., & Kim, S. J. (2023). Phytochemical content and antioxidant activity in eight citrus cultivars grown in Jeju Island according to harvest time. International Journal of Food Properties, 26(1), 14–23. doi:10.1080/10942912.2022.2151620.

[53] Leitzmann, C. (2016). Characteristics and Health Benefits of Phytochemicals. Forschende Komplementarmedizin, 23(2), 69–74. doi:10.1159/000444063.

[54] Baskaran, G., Salvamani, S., Ahmad, S. A., Shaharuddin, N. A., Pattiram, P. D., & Shukor, M. Y. (2015). HMG-CoA reductase inhibitory activity and phytocomponent investigation of Basella alba leaf extract as a treatment for hypercholesterolemia. Drug Design, Development and Therapy, 9, 509–517. doi:10.2147/DDDT.S75056.

[55] Sluijs, I., Cadier, E., Beulens, J. W. J., van der A, D. L., Spijkerman, A. M. W., & van der Schouw, Y. T. (2015). Dietary intake of carotenoids and risk of type 2 diabetes. Nutrition, Metabolism and Cardiovascular Diseases, 25(4), 376–381. doi:10.1016/j.numecd.2014.12.008.

[56] Leenders, M., Leufkens, A. M., Siersema, P. D., Van Duijnhoven, F. J. B., Vrieling, A., Hulshof, P. J. M., Van Gils, C. H., Overvad, K., Roswall, N., Kyrø, C., Boutron-Ruault, M. C., Fagerhazzi, G., Cadeau, C., … Bueno-de-Mesquita, H. B. (2014). Plasma and dietary carotenoids and vitamins A, C and e and risk of colon and rectal cancer in the European Prospective Investigation into Cancer and Nutrition. International Journal of Cancer, 135(12), 2930–2939. doi:10.1002/ijc.28938.

[57] Blekkenhorst, L. C., Sim, M., Bondonno, C. P., Bondonno, N. P., Ward, N. C., Prince, R. L., Devine, A., Lewis, J. R., & Hodgson, J. M. (2018). Cardiovascular health benefits of specific vegetable types: A narrative review. Nutrients, 10(5), 1–24. doi:10.3390/nu10050595.

[58] Badarinath, A. V., Mallikarjuna Rao, K., Madhu Sudhana Chetty, C., Ramkanth, S., Rajan, T. V. S., & Gnanaprakash, K. (2010). A review on In-vitro antioxidant methods: Comparisions, correlations and considerations. International Journal of PharmTech Research, 2(2), 1276–1285.

[59] Gulcin, İ. (2020). Antioxidants and antioxidant methods: an updated overview. Archives of Toxicology, 94(3), 651–715. doi:10.1007/s00204-020-02689-3.

[60] Saeed, N., Khan, M. R., & Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complementary and Alternative Medicine, 12(221), 1–12. doi:10.1186/1472-6882-12-221.

[61] Nawaz, H., Shad, M. A., Rehman, N., Andaleeb, H., & Ullah, N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds. Brazilian Journal of Pharmaceutical Sciences, 56(e17129), 1–9. doi:10.1590/s2175-97902019000417129.

[62] Sun, S. X., Xie, R., Wang, X. X., Wen, G. Q., Liu, Z., Wang, W., Ju, X. J., & Chu, L. Y. (2015). Fabrication of nanofibers with phase-change core and hydrophobic shell, via coaxial electrospinning using nontoxic solvent. Journal of Materials Science, 50(17), 5729–5738. doi:10.1007/s10853-015-9118-6.

[63] Lim, S., Choi, A. H., Kwon, M., Joung, E. J., Shin, T., Lee, S. G., Kim, N. G., & Kim, H. R. (2019). Evaluation of antioxidant activities of various solvent extract from Sargassum serratifolium and its major antioxidant components. Food Chemistry, 278, 178–184. doi:10.1016/j.foodchem.2018.11.058.

[64] Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. Journal of Nutritional Biochemistry, 13(10), 572–584. doi:10.1016/S0955-2863(02)00208-5.

[65] Kim, S. Y., Kim, H. J., Lee, M. K., Jeon, S. M., Do, G. M., Kwon, E. Y., Cho, Y. Y., Kim, D. J., Jeong, K. S., Park, Y. B., Ha, T. Y., & Choi, M. S. (2006). Naringin time-dependently lowers hepatic cholesterol biosynthesis and plasma cholesterol in rats fed high-fat and high-cholesterol diet. Journal of Medicinal Food, 9(4), 582–586. doi:10.1089/jmf.2006.9.582.

[66] Kumar, S., Sandhir, R., & Ojha, S. (2014). Evaluation of antioxidant activity and total phenol in different varieties of Lantana camara leaves. BMC Research Notes, 7(1), 1–9. doi:10.1186/1756-0500-7-560.

[67] Al-Rubaye, A. F., Hameed, I. H., & Kadhim, M. J. (2017). A Review: Uses of Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Natural Compounds of Some Plants. International Journal of Toxicological and Pharmacological Research, 9(01), 81–85. doi:10.25258/ijtpr.v9i01.9042.

[68] Madhavan, G. R., Balraju, V., Mallesham, B., Chakrabarti, R., & Lohray, V. B. (2003). Novel coumarin derivatives of heterocyclic compounds as lipid-lowering agents. Bioorganic and Medicinal Chemistry Letters, 13(15), 2547–2551. doi:10.1016/S0960-894X(03)00490-6.

[69] Taşdemir, E., Atmaca, M., Yildirim, Y., Bilgin, H. M., Demirtaş, B., Obay, B. D., Kelle, M., & Oflazoğlu, H. D. (2017). Influence of coumarin and some coumarin derivatives on serum lipid profiles in carbontetrachloride-exposed rats. Human and Experimental Toxicology, 36(3), 295–301. doi:10.1177/0960327116649675.

[70] Tosun, A., Akkol, E. K., & Yeşilada, E. (2009). Anti-inflammatory and antinociceptive activity of coumarins from Seseli gummiferum subsp. corymbosum (Apiaceae). Zeitschrift Fur Naturforschung - Section C Journal of Biosciences, 64(1–2), 56–62. doi:10.1515/znc-2009-1-210.

[71] Phucharoenrak, P., Muangnoi, C., & Trachootham, D. (2023). Metabolomic Analysis of Phytochemical Compounds from Ethanolic Extract of Lime (Citrus aurantifolia) Peel and Its Anti-Cancer Effects against Human Hepatocellular Carcinoma Cells. Molecules, 28(7), 2965. doi:10.3390/molecules28072965.

[72] Filimonov, D. A., Lagunin, A. A., Gloriozova, T. A., Rudik, A. V., Druzhilovskii, D. S., Pogodin, P. V., & Poroikov, V. V. (2014). Prediction of the biological activity spectra of organic compounds using the pass online web resource. Chemistry of Heterocyclic Compounds, 50(3), 444–457. doi:10.1007/s10593-014-1496-1.

[73] Zhao, Y. Y., Cheng, X. L., Lin, R. C., & Wei, F. (2015). Lipidomics applications for disease biomarker discovery in mammal models. Biomarkers in Medicine, 9(2), 153–168. doi:10.2217/BMM.14.81.

[74] Perrotti, F., Rosa, C., Cicalini, I., Sacchetta, P., Del Boccio, P., Genovesi, D., & Pieragostino, D. (2016). Advances in lipidomics for cancer biomarkers discovery. International Journal of Molecular Sciences, 17(12), 1–26. doi:10.3390/ijms17121992.

[75] Grigoletto, L., Ferraz, J. B. S., Oliveira, H. R., Eler, J. P., Bussiman, F. O., Abreu Silva, B. C., Baldi, F., & Brito, L. F. (2020). Genetic Architecture of Carcass and Meat Quality Traits in Montana Tropical® Composite Beef Cattle. Frontiers in Genetics, 11(February), 1–13. doi:10.3389/fgene.2020.00123.

[76] Diniyah, N., Alam, M. B., Javed, A., Alshammari, F. H., Choi, H. J., & Lee, S. H. (2023). In silico and docking studies on the binding activities of Keap1 of antioxidant compounds in non-oilseed legumes. Arabian Journal of Chemistry, 16(1), 1–8. doi:10.1016/j.arabjc.2022.104414.

[77] McInnes, K. J., Corbould, A., Simpson, E. R., & Jones, M. E. (2006). Regulation of adenosine 5,monophosphate-activated protein kinase and lipogenesis by androgens contributes to visceral obesity in an estrogen-deficient state. Endocrinology, 147(12), 5907–5913. doi:10.1210/en.2006-0879.

[78] Song, Z., Xiaoli, A. M., & Yang, F. (2018). Regulation and metabolic significance of De Novo lipogenesis in adipose tissues. Nutrients, 10(10), 1–22. doi:10.3390/nu10101383.

[79] Gibney, J., Wolthers, T., Johannsson, G., Umpleby, A. M., & Ho, K. K. Y. (2005). Growth hormone and testosterone interact positively to enhance protein and energy metabolism in hypopituitary men. American Journal of Physiology-Endocrinology and Metabolism, 289(2), E266–E271. doi:10.1152/ajpendo.00483.2004.

[80] Xu, X., De Pergola, G., & Björntorp, P. (1991). Testosterone increases lipolysis and the number of β-adrenoceptors in male rat adipocytes. Endocrinology, 128(1), 379–382. doi:10.1210/endo-128-1-379.

[81] Eakman, G. D., Dallas, J. S., Ponder, S. W., & Keenan, B. S. (1996). The effects of testosterone and dihydrotestosterone on hypothalamic regulation of growth hormone secretion. The Journal of Clinical Endocrinology & Metabolism, 81(3), 1217–1223. doi:10.1210/jcem.81.3.8772602.

[82] Zhang, D., Wei, Y., Huang, Q., Chen, Y., Zeng, K., Yang, W., Chen, J., & Chen, J. (2022). Important Hormones Regulating Lipid Metabolism. Molecules, 27(20), 1–20. doi:10.3390/molecules27207052.

[83] Morris, G. M., & Lim-Wilby, M. (2008). Molecular docking. Methods in Molecular Biology, 443, 365–382. doi:10.1007/978-1-59745-177-2_19.

[84] El fadili, M., Er-rajy, M., Ali Eltayb, W., Kara, M., Assouguem, A., Saleh, A., Al Kamaly, O., Zarougui, S., & Elhallaoui, M. (2023). In-silico screening based on molecular simulations of 3,4-disubstituted pyrrolidine sulfonamides as selective and competitive GlyT1 inhibitors. Arabian Journal of Chemistry, 16(10), 1–15. doi:10.1016/j.arabjc.2023.105105.

[85] Liu, Q. H., Li, J. Q., Tang, J. W., Zhang, Y. D., Zhou, M. Y., Zhang, W., & Wang, L. (2023). Identification of antidiabetic constituents in Polygonatum odoratum (Mill.) Druce by UPLC-Orbitrap-MS, network pharmacology and molecular docking. Arabian Journal of Chemistry, 16(9), 1–12. doi:10.1016/j.arabjc.2023.105032.

[86] Tallei, T. E., Tumilaar, S. G., Niode, N. J., Fatimawali, Kepel, B. J., Idroes, R., Effendi, Y., Sakib, S. A., & Emran, T. B. (2020). Potential of Plant Bioactive Compounds as SARS-CoV-2 Main Protease (Mpro) and Spike (S) Glycoprotein Inhibitors: A Molecular Docking Study. Scientifica, 2020, 1–18. doi:10.1155/2020/6307457.

[87] Bulusu, G., & Desiraju, G. R. (2020). Strong and Weak Hydrogen Bonds in Protein–Ligand Recognition. Journal of the Indian Institute of Science, 100(1), 31–41. doi:10.1007/s41745-019-00141-9.

[88] Sahil, M., Sarkar, S., & Mondal, J. (2023). Long-time-step molecular dynamics can retard simulation of protein-ligand recognition process. Biophysical Journal, 122(5), 802-816. doi:10.1016/j.bpj.2023.01.036.

[89] Seidah, N. G. (2013). Proprotein Convertase Subtilisin Kexin 9 (PCSK9) Inhibitors in the Treatment of Hypercholesterolemia and other Pathologies. Current Pharmaceutical Design, 19(17), 3161–3172. doi:10.2174/13816128113199990313.

[90] Delgoda, R. (2016). Pharmacognosy: Fundamentals, applications and strategies. Academic Press, Cambridge, United States.

[91] Hung, W. L., Suh, J. H., & Wang, Y. (2017). Chemistry and health effects of furanocoumarins in grapefruit. Journal of Food and Drug Analysis, 25(1), 71–83. doi:10.1016/j.jfda.2016.11.008.

[92] Sumorek-Wiadro, J., Zając, A., Maciejczyk, A., & Jakubowicz-Gil, J. (2020). Furanocoumarins in anticancer therapy – For and against. Fitoterapia, 142(2020), 1–8. doi:10.1016/j.fitote.2020.104492.

[93] Zhang, Y. Y., Zhang, Q. Q., Song, J. L., Zhang, L., Jiang, C. S., & Zhang, H. (2018). Design, synthesis, and antiproliferative evaluation of novel coumarin/2-cyanoacryloyl hybrids as apoptosis inducing agents by activation of caspase-dependent pathway. Molecules, 23(8), 1–19. doi:10.3390/molecules23081972.