TiS Trends in Sciences
TRENDS IN SCIENCES 2027; 24(2): 14200
SPRINT Scholarly Publishing and Repository Integration Center
https://doi.org/10.48048/tis.2027.14200
RESEARCH ARTICLE

Antihypercholesterolemic Activity of Flavonoid Compounds from Sea Apple Leaves (*Syzygium grande* L.) through HMG-CoA Reductase Inhibition

Alifa Husnun Kholieqoh[1], Yosie Andriani[1,*], Wahdini Hanifah[1], Kurnia Nastira Ningsih[2,3], Sura Menda Ginting[4], Nurhamidah Nurhamidah[4], Hermansyah Amir[4], Ariyanti Suhita Dewi[5], Tengku Sifzizul Tengku Muhammad[1] and Dwi Hudiyanti[6]

[1]*Higher Institution Centre of Excellence, Institute of Climate Adaptation and Marine Biotechnology, Universiti Malaysia Terengganu, Terengganu, Malaysia*
[2]*University Center of Excellence for Nutraceuticals, Biosciences, and Biotechnology Research Center, Bandung Institute of Technology, Bandung, West Java, Indonesia*
[3]*Markherb Laboratory, EBM Scitech Research Center, ITB Innovation Park Bandung Technopolis, Bandung, West Java, Indonesia*
[4]*Undergraduate School of Chemistry Education, University of Bengkulu, Bengkulu, Indonesia*
[5]*Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency, Bogor, West Java, Indonesia*
[6]*Department of Chemistry, Faculty of Science and Mathematics, Diponegoro University, Semarang, Indonesia*

([*]Corresponding author's e-mail: yosie.hs@umt.edu.my)

Received: 27 April 2026, Revised: 9 June 2026, Accepted: 20 June 2026, Published: 20 September 2026

Abstract

Hypercholesterolemia is a lipid disorder in which total cholesterol levels in the body exceed 200 mg/dL. This condition can lead to atherosclerosis and cardiovascular diseases. A common drug to treat this condition is statin, but it is associated with adverse side effects such as nausea, muscle pain, and abnormal liver function. Thus, alternative medicine using natural products from plants was needed as they have been recognized as medicinal agents, due to their safety and effectiveness. This study aims to examine the potency of a coastal plant, *Syzygium grande* L., to lower cholesterol levels by inhibiting HMG-CoA (3-hydroxyl-3-methylglutaryl coenzyme A) reductase (HMGCR) activity. The methanol extract of *S. grande* leaves (SGLM) was assessed for HMGCR inhibition activity *in vitro*. The extracts significantly reduced HMGCR activity. The presence of SGLM as inhibitor gave a strong inhibitory effect (0.1105 Units/mgP at 200 mg/mL) towards HMGCR enzyme activity (0.1933 Units/mgP at 200 mg/mL, $p < 0.05$). Interestingly, the HMGCR inhibitory effect of SGLM extracts was not significantly different ($p > 0.05$) compared to atorvastatin drug, indicating that the *S. grande* L. could be an alternative antihypercholesterolemia agent via the same mechanism as atorvastatin. Additionally, *S. grande* leaves were isolated to get the bioactive compounds. Molecular docking studies were carried out to understand the interaction between isolated compounds: myricetin, rutin, and luteolin, to HMGCR. The molecular docking analysis showed strong binding affinities between the HMGCR enzyme and luteolin, rutin, and myricetin with values of −9.0, −9.6, and −8.5 kcal/mol, respectively, which suggest a strong compound-protein interaction that might contribute in inhibiting HMGCR enzyme activity. These compounds also bind to a similar catalytic site of HMGCR enzyme for atorvastatin. These findings suggest that extracts from *S. grande* can potentially lower cholesterol production by inhibiting HMGCR, hence highlighting their usefulness as natural agents for managing cholesterol levels.

Keywords: HMGCR, Inhibitor, Antihypercholesterolemia, *Syzygium grande*, Luteolin, Rutin, Myricetin

Introduction

Hypercholesterolemia is a lipid disorder where total cholesterol levels surpass 200 mg/dL or low-density lipoprotein (LDL) levels go above 130 mg/dL [1]. This condition leads to atherosclerosis, a situation where lipid plaques build up in the arteries. This buildup blocks blood and oxygen flow to important organs, including the brain, and raises the risk of cardiovascular diseases [2]. Within the cholesterol biosynthesis pathway, HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase (HMGCR) plays an important role. It catalyzed the conversion of mevalonate into isoprenoids, sterols, and other lipids [3,4]. HMGCR, as a rate-limiting enzyme, becomes a prime candidate for many drugs that lower cholesterol by blocking its activity, thus helping to reduce the production of excess cholesterol [3-5]. Therefore, understanding the HMGCR mechanism in cholesterol metabolism is vital for creating effective strategies to treat this common condition.

Statins are currently used to treat hypercholesterolemia, effectively lower the risk of atherosclerosis, and prevent primary and secondary cardiovascular diseases. Nonetheless, they may induce negative adverse side effects in certain individuals, such as abdominal pain, diarrhea, headache, and muscle pain. A study revealed that rats treated with atorvastatin showed signs of significant liver damage, including decreased liver enzyme activity, increased bilirubin levels, and hepatocyte death by necrosis [6].

As an alternative treatment for hypercholesterolemia, natural products could be the best option. They have shown their potential for the prevention and treatment of many diseases. The compounds found in natural products can be alternative sources of medications and often have fewer side effects than synthetic drugs [7,8]. Coastal plants have significant habitats and ecosystem services in addition to long-term protection against erosion and wave energy [9]. Unlike terrestrial plants, many coastal plants are still underutilized. The extracts from these plants are vital because they produce a range of metabolites that can deal with the harsh climate. Syzygium, a genus of flowering plants, grows in tropical and subtropical regions, spanning from Africa to India. Indonesia and Malaysia are known for their variety of species [10]. Among them, *Syzygium grande* L., or sea apple, is one of the coastal plants that commonly grows as shade trees in Peninsular Malaysia. This species is part of a diverse and widespread group within the Syzygium genus. Research has shown that *S. grande* offers several beneficial properties, including antimicrobial [11,12], antioxidant [13], antibacterial [14], anti-inflammatory [15], tyrosinase-inhibiting [16], and antifungal activities [17]. These pharmacological effects are due to its rich mix of bioactive compounds, including phenolics, flavonoids, alkaloids, terpenoids, steroids, and saponins [14]. Given its promising potential for treatment, *S. grande* may offer a valuable resource for creating natural alternatives to standard medications, especially for managing various health conditions.

Despite many bioactivities of *S. grande,* to date, no studies have investigated the bioactive compounds from *S. grande* as potential agents for lowering cholesterol levels. Studies revealed that there were correlations between oxidative stress and inflammation in the progression of atherosclerotic plaques [18-20]. Hence, the reported antioxidant and anti-inflammatory properties of *S. grande* might also make this plant a potential medicine for antihypercholesterolemia. This study aimed to determine the anti-hypercholesterolemic activity of selected extracts of *S. grande* by inhibiting HMGCR enzyme activity, following structural analysis of the isolated compounds. Additionally, the molecular docking study was done to assess the compounds' interaction with the HMGCR protein through structure-based drug design. Through this study, the molecular basis for these compounds' potency as a treatment for hypercholesterolemia was investigated.

Materials and methods

Plant collection and extraction

The *Syzygium grande* L. sample was collected in June from Tanjung Gelam Beach, Kuala Terengganu, Malaysia. A voucher of specimen (No. COA0118003) has been deposited in the Natural Product Laboratory, Institute of Climate Adaptation and Marine Biotechnology, Universiti Malaysia Terengganu. The leaves of *S. grande* were cut into small pieces, dried using a freeze-dryer, and ground into powder. The powdered leaves (500 g) were extracted by the sequential extraction maceration method, starting from

hexane as a non-polar solvent, continuing with methanol [14,21]. The methanol extracts of *S. grande* leaves (SGLM) were used as the samples in this study, as they contained the most of secondary metabolites based on a previous study [14]. The SGLM was then evaporated under vacuum to afford crude extracts for further analysis.

Isolation of flavonoids from *Syzygium grande* L.

The SGLM was fractionated by radial chromatography (RC) using silica gel 60 PF254 containing gypsum (MERCK) as the stationary phase, and a gradient mobile phase system starting from ethyl acetate:acetone:methanol (6:4:1) to chloroform: methanol:water (9:0.5:0.5) and finally 100% methanol as the mobile phase and 8 fractions (F1-8) were obtained. The fractions were monitored by thin-layer chromatography (TLC) using silica gel 60 F254 aluminium sheets (MERCK) as the stationary phase and ethyl acetate:methanol:water (8:1.5:0.5) as the mobile phase. Then the visualization was performed under UV light at 254 and 366 nm, followed by staining with a 10% sulfuric acid in methanol as TLC spot visualization reagent, and subsequent re-examination under UV light at 366 nm. TLC profiling showed that Fraction 6 exhibited a single spot, indicating that it contained a pure compound (Compound 2). Fraction 5 was further purified using Radial Chromatography (RC) using silica gel 60 PF254 containing gypsum (MERCK) as the stationary phase, and a gradient solvent system composed of chloroform/ethyl acetate/methanol/water and finally 100% methanol as the mobile phase, resulting in 6 combined fractions (F5.1-5.6). The fractions were monitored by thin-layer chromatography (TLC) using silica gel 60 F254 aluminium sheets (MERCK) as the stationary phase and ethyl acetate:methanol:water (8:1.5:0.5) as the mobile phase. Visualization was carried out under UV light at 254 and 366 nm, followed by staining reagent and subsequent re-observation under UV light at 366 nm. Based on TLC profiling, fractions exhibiting spots with same Rf values were combined, yielding Compound 1 (F5.4 and F5.5) and Compound 3 (F5.1 and F5.2). Each combined fraction displayed a single spot on TLC, indicating the presence of a pure compound. The chemical structures of the isolated compounds (Compounds 1 - 3 are shown in **Figure 1**) were elucidated by NMR spectroscopy.

Compound 1, Luteolin: Obtained as a pale yellow solid.

^{1}H-NMR (DMSO, 500 MHz) δH (in ppm): 5.99 (1H, d, H-6), 6.24 (1H, d, H-8), 6.47 (1H, s, H-3), 6.74 (1H, d, *J* = 10 Hz, H-5'), 7.31 (2H, m, *J* = 6 Hz, H-2', 6'); *^{13}C-NMR* (DMSO, 125 MHz) δC (in ppm): 94.86 (C-8), 100.15 (C-6), 102.38 (C-5), 102.84 (C-3), 112.85 (C-2'), 116.37 (C-5'), 119.47 (C-6'), 120.44 (C-1'), 146.93 (C-9), 158.06 (C-10), 161.89 (C-2), 164.15 (C-7), 181.55 (C=O).

Compound 2, Rutin: Obtained as a yellow-greenish powder.

^{1}H-NMR (DMSO, 500 MHz) δH (in ppm): 1.01 (3H, d, *J* = 6.25 Hz, H-6"'), 3.08 (3H, m, H-4", 4"', 5"), 3.26 (3H, m, H-3", 5"', 2"), 3.71 (1H, d, *J* = 10.05 Hz, H-3"'), 4.37 (2H, d, *J* = 5.75 Hz, H-6"), 4.43 (1H, d, *J* = 5 Hz, H-2"'), 4.56 (1H, d, *J* = 5.45 Hz, H-1"'), 5.13 (1H, d, *J* = 3.95 Hz, H-1"), 5.34 (1H, d, *J* = 3.9 Hz, H-6), 6.29 (1H, d, *J* = 2.05 Hz, H-8), 6.39 (1H, d, *J* = 2.05 Hz, H-3'), 6.84 (1H, d, *J* = 8.1 Hz, H-2'), 7.55 (1H, d, *J* = 8.05 Hz, H-6'); *^{13}C-NMR* (DMSO, 125 MHz) δC (in ppm): 17.63 (C-6"'), 66.87 (C-6"), 68.13 (C-4"), 69.85 (C-3"'), 70.24 (C-4"'), 70.41 (C-2"'), 71.69 (C-5"'), 73.93 (C-2"), 75.76 (C-3"), 76.28 (C-5"), 93.46 (C-8), 98.54 (C-6), 100.63 (C-1"), 101.03 (C-1"'), 103.83 (C-10), 115.09 (C-6'), 116.13 (C-3'), 121.03 (C-2'), 121.46 (C-1'), 133.15 (C-3), 144.62 (C-5'), 148.28 (C-4'), 156.29 (C-2), 156.49 (C-9), 161.09 (C-5), 163.93 (C-7), 177.24 (C-4).

Compound 3, Myricetin: Obtained as a yellow powder.

^{1}H-NMR (MeOD, 500 MHz) δH (in ppm): 6.18 (1H, d, *J* = 2.05 Hz, H-6), 6.38 (1H, d, *J* = 2 Hz, H-8), 7.34 (2H, d, H-2', 6'); *^{13}C-NMR* (MeOD, 125 MHz) δC (in ppm): 94.41 (C-8), 99.23 (C-6), 104.48 (C-10), 108.52 (C-2', 6'), 123.05 (C-1'), 136.95 (C-4'), 137.36 (C-3), 146.7 (C-3', 5'), 148.03 (C-2), 158.15 (C-9), 162.41 (C-5), 165.54 (C-7), 177.29 (C-4).

***In vitro* HMG-CoA reductase (HMGCR) inhibition activity**

The SGLM were tested for their antihypercholesterolemia activity. Based on a previous study, these methanol crude extracts contained more secondary metabolites than their hexane crude extracts

[14]. The antihypercholesterolemic activity was assessed following a modified protocol derived from Abcam (ab204701, Boston, MA, USA), with slight modifications. The assay was conducted using a TECAN multiplate reader spectrophotometer in kinetic program at 37 °C. Measurements were taken at a wavelength of 340 nm. Atorvastatin (with a concentration of 10 µM) served as the positive control provided by the assay kit, while its commercial formulation was used as a reference for marketed drugs. To ensure consistency in comparative evaluations of their inhibitory effects on HMGCR enzyme activity, SGLM and the commercial atorvastatin drug were prepared at a concentration of 200 µg/mL as reported in previous study [5]. The reaction volume for 96 well plate samples are provided in the table below:

Table 1 Reaction volumes for 96 well plates sample.

Sample	1× Assay buffer (µL)	Standard or samples (µL)	NADPH (µL)	HMG-CoA (µL)	HMGCR (µL)
Blank	184	-	4	12	-
Activity[1]	182	-	4	12	2
Inhibition (sample)[2]	181	1	4	12	2

[1]activity = the absorbance value was used to calculate initial enzyme activity.
[2]inhibition (sample) = the absorbance value was used to calculate enzyme activity in the presence of inhibitors.

The HMGCR enzyme activity was calculated using the following equation:

$$Activity\ \left(Units/mgP\right) = \frac{\left(\Delta A_{340\ nm}/\Delta T_{min} Sample - \Delta A_{340\ nm}/\Delta T_{min} blank\right) \times 0.2}{12.44 \times V \times 0.6 \times 0.55}$$

where, 2.44 = the extinction coefficient for NADPH at 340 nm; 0.2 = total volume reaction in mL (0.2 mL for each sample); V = enzyme volume used in the reaction (mL); 0.6 = enzyme concentration (mgP/mL). 0.55 = light path in cm for 96 well plates.

Molecular docking study of isolated compounds

Protein processing

The Protein Data Bank (PDB) file for the targeted enzyme (1DQ9), HMGCR, was accessed from the Research Collaboratory for Structural Bioinformatics PDB website (www.rcsb.org). This structure was noted to have a resolution of approximately 2Å, indicating high quality and compliance with standard structural criteria. BIOVIA Discovery Studio Visualizer 2024 (Dassault Systemes, France) was used as the graphics tool to prepare the structure for further analysis. Using this software, cofactors, water molecules, and any unique ligands associated with the protein structure were cleared. Also, polar hydrogen atoms were added to the structure to ensure. This was to ensure that the protein would be properly represented for molecular docking studies. The modified protein structure was then saved in PDB format for subsequent analyses.

Ligand processing

The 3-dimensional structures of the flavonoids luteolin ($C_{15}H_{10}O_6$, PubChem compound CID: 5280445), rutin ($C_{27}H_{30}O_{16}$, PubChem compound CID: 5280805), and myricetin (C15H10O8, PubChem compound CID: 5281672), were obtained from the chemical database PubChem (National Center for Biotechnology Information, United States National Institutes of Health) in SDF format, as illustrated in **Figure 1**. To optimize the structures and minimize accumulated potential energy, the MM2minimize force field was applied using Chem3D 15.0 (PerkinElmer, Inc., Waltham, MA, USA). Following the optimization process, the ligands underwent conversion to PDB format to enable subsequent molecular docking studies and analyses of their interactions with the HMGCR enzyme.

Molecular docking

The AutoDock Vina facilitated molecular docking simulation of isolated compounds to HMGCR. In terms of reconstructing and estimating the drug-protein interactions, it is considered the most effective docking tool [22,23]. The protein in AutoDock Tool 1.5.6 was first given polar hydrogen. Then data were stored in the

PDB partial charge (Q) and atom type (T) (PDBQT) format. The protein had 4 possible binding sites, so docking was performed for each site with every isolated compound. The grid box dimensions in the x, y, and z directions were 30, 30 and 30 Å, respectively. The coordinate of grid box was *X* coordinate of the center = 24.252, *Y* coordinate of the center = −33.146, and *Z* coordinate of the center = 34.378. Isolated compounds were docked to HMG-CoA reductase and the results were stored for subsequent visual examinations.

Analysis and visualization of interactions

BIOVIA Discovery Studio Visualizer 2024 v24.1.0.23298 was used for analysis as well as visualization. The all-inclusive program can be used with micro- to macromolecules for any type of molecular interaction, including biological and antipathogenic properties [24]. The PDBQT formats for the ligand output and the protein structure were imported and defined within the software. After selecting the ligand-nonbond interactions, all interacting residues were labelled for clarity. Only the interactions corresponding to the protein-ligand complexes with the lowest root-mean-square deviation (RMSD) values plus binding energies remained for further analysis, ensuring that the most relevant interactions were focused upon in the study.

Assessment of drug-likeness of isolated compounds

Lipinski's rule of 5 was used to predict the drug-likeness properties of the compounds. In addition, target prediction was also carried out to investigate the potential adverse effects and bioactivities of medication candidates. The analysis utilized Simplified Molecular Input Line Entry System (SMILES) data of isolated compounds: Myricetin, rutin, and luteolin from PubChem (https://pubchem.ncbi.nlm.nih.gov/), which were submitted to the pkCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction/) and ProTox-II (https://tox.charite.de/protox3/) to investigate the *in-silico* bioactivity and toxicity study.

Results and discussion

Isolation of *S. grande* leaves methanolic extract (SGLM)

S. grande leaves have been reported to have antioxidant activity. The inhibitory activity is likely related to the phenolic compounds present in the extracts [14,17]. A study found new antioxidant compounds from *S. grande*, such as flavonol glycoside myricetin 4'-methyl ether 3-O-β-d-xylopyranoside and triterpene glycoside [17]. Our study was also successfully isolated and characterized flavonoid compounds in *S. grande* leaves, including luteolin, rutin, and myricetin (**Figure 1**). These 3 compounds were reported to have several bioactivities such as anticancer [25], antifungal [26], antidiabetic [27], anti-inflammatory [28], and antioxidant [29,30].

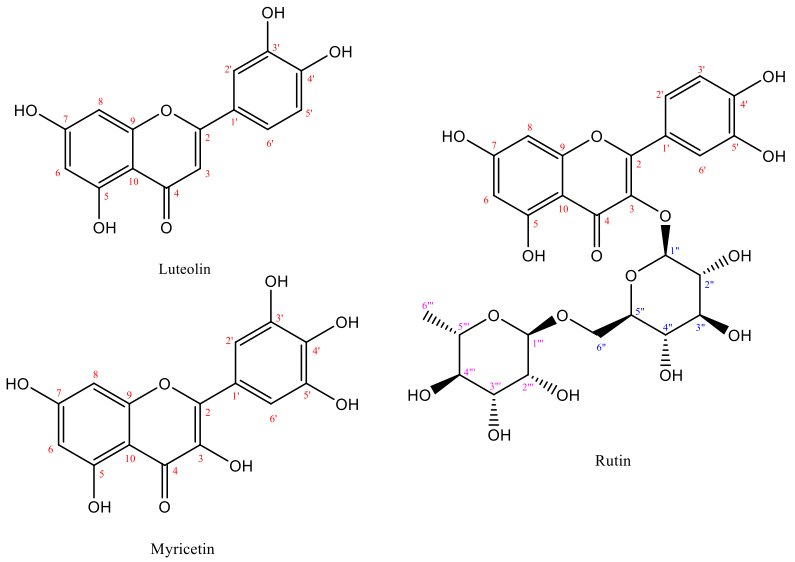


Figure 1 Structure of isolated flavonoid compounds from *Syzygium grande* leaves.

Compounds 1 (Luteolin) and Compounds 3 (Myricetin) are C15 flavonoid aglycones with Luteolin possesses a flavone backbone, whereas Myricetin is a flavonol backbone characterized by the presence of a hydroxyl (−OH) group at the C-3 position. Compound 1 (Luteolin) exhibits 6 aromatic methine protons at chemical shifts of δH (in ppm): 5.99 (H-6) & 6.24 (H-8) on ring A, 6.47 (H-3) on ring C, and 6.74 (H-5') & 7.31 (H-2' & H-6') on ring B. In contrast, Compound 3 (Myricetin) shows 4 aromatic methine protons at chemical shifts of δH (in ppm): 6.18 (H-6) & 6.38 (H-8) on ring A, and 7.34 (H-2' & H-6') on ring B.

Compound 2 (Rutin), formally known as quercetin-3-O-rutinoside, is a C27 flavonoid glycoside consisting of a quercetin aglycone linked to a disaccharide moiety (rutinose, composed of rhamnose and glucose) at the C-3 position of the flavonol skeleton. Compound 2 (Rutin) displays 5 aromatic methine protons at chemical shifts of δH (in ppm): 5.34 (H-6) & 6.29 (H-8) on ring A, and 6.39 (H-3'), 6.84 (H-2') & 7.55 (H-6') on ring B, corresponding to the aglycone moiety. Additionally, 12 aliphatic protons groups are observed at chemical shifts of δH (in ppm): 1.01 (H-6"', methyl group, $–CH_3$), 3.08 (H-4", H-4"', H-5"), 3.26 (H-3", H-5"', H-2"), 3.71 (H-3"'), 4.43 (H-2"') corresponding to oxygenated methine groups (–CH–O), 4.37 (H-6") from an oxygenated methylene group ($–CH_2–O$), and 4.56 (H-1"') & 5.13 (H-1") attributed to acetal-type protons or methine dioxy group (O–CH–O).

***In vitro* HMG-CoA reductase (HMGCR) inhibition activity**

The HMGCR, the rate-regulating enzyme of mevalonate pathway, catalyzes the cholesterol's hepatic synthesis and other isoprenoids. Numerous medications, particularly statins, inhibit HMGCR, thereby reducing cholesterol production in the liver. Recent evidence showed statins also regulate the Nrf2/HO-1 pathway, enhancing Nrf2's DNA-binding capacity and promoting the expression of target genes [31,32]. These genes are glutathione peroxidase (GPx) and HO-1, which shield cells from oxidative stress [32]. Statins are beneficial in lessening the risk of cardiovascular diseases, but their use is associated with several adverse effects, including headaches, diarrhoea, muscle pain, plus, in severe cases, liver cirrhosis [6].

Research on various medicinal plants has highlighted their advantages over statin medications, with numerous studies documenting their potential benefits. There is a growing interest in pharmacologically bioactive compounds derived from natural sources, particularly plants, as treatments for a wide range of diseases [15,16,33]. *S. grande* has been proven to have antioxidant activity [13], which may contribute to its antihypercholesterolemia effects. Oxidative stress becomes a great risk factor for atherosclerosis development and progression, as it promotes processes that exacerbate the condition [27-29]. When reactive oxygen species accumulate, they may result in endothelial cell dysfunction, increase inflammation, attract macrophage, and oxidize LDL [34-37].

The mechanism of *Syzygium grande* L. as an antihypercholesterolemia agent was hypothesised to be via the inhibition of HMGCR activity. Thus, the anti-hypercholesterolemia activity of SGLM was evaluated by measuring HMGCR activity. This assay's principle is a spectrophotometric measurement of the decrease in NADPH absorbance, which indicates the rate of NADPH oxidation.

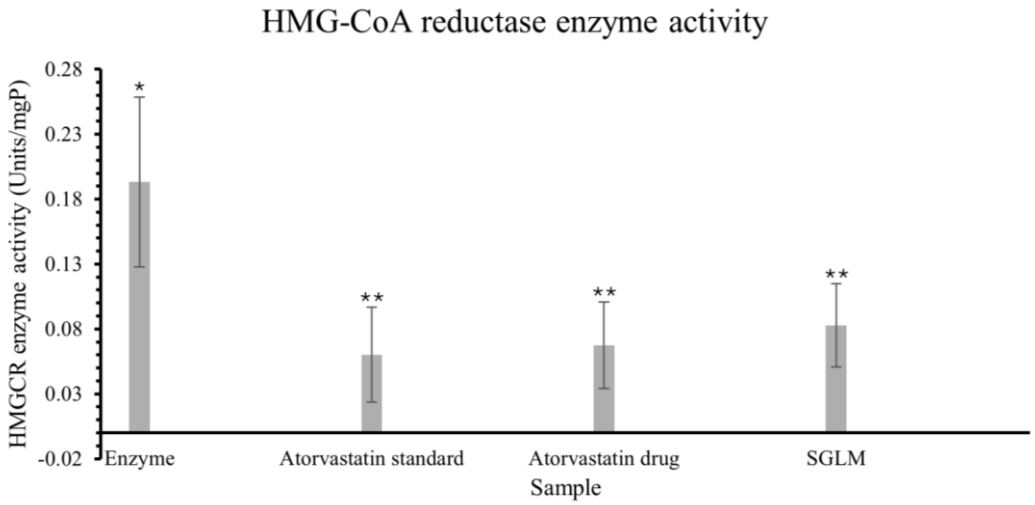


Figure 2 Inhibitory activity of *S. grande* leaves (SGLM) extracts towards HMG-CoA reductase enzyme activity (Units/mgP) compared to atorvastatin drug at the same concentration (200 µg/mL). Data expressed as mean ± SD. **Significantly different compared to normal enzyme (HMG-CoA reductase (HMGCR)) activity at $p < 0.05$.

The SGLM and atorvastatin drug were prepared at 200 µg/mL and compared with the Atorvastatin standard (10 mM) inhibitors from the kit. The HMGCR enzyme activity after treatment with the samples and standard is presented in **Figure 2**. As shown, all inhibitors can significantly inhibit HMGCR enzyme activity ($p < 0.05$). The initial HMGCR activity was 0.1933 ± 0.065 Units/mg protein. With the presence of its inhibitor, the atorvastatin standard (10 mM) and commercial atorvastatin (200 mg/mL), the HMGCR enzyme activity became 0.0601 ± 0.036 and 0.0674 ± 0.033 Units/mg protein, respectively. The inhibitors significantly inhibited HMGCR activity ($p < 0.05$). At the same dose with atorvastatin drug (200 mg/mL), SGLM extracts also significantly inhibited the enzyme, reducing enzyme activity to 0.0828 ± 0.032 Units/mg protein ($p < 0.05$). The results showed that the activity of SGLM extract was comparable to that of atorvastatin, as proven by the inhibitory activity, which was not significantly different ($p > 0.05$) compared to atorvastatin. This finding suggested that SGLM has a potential to be alternative herbal medicine to reduce the cholesterol levels by the same mechanism as atorvastatin via the inhibition of HMGCR enzyme. However, further *in vivo* study was needed to investigate the mechanism of action and efficacy of SGLM to be an alternative herbal medicine.

Molecular docking study of isolated compounds

The computational technique for molecular docking is needed in drug development. In this study, molecular docking was used to investigate how the isolated compound binds to the target. Positive results were obtained from the *in vitro* study of the antihypercholesterolemic activity. To support these results, a molecular docking study was performed. Docking analysis allows candidate drugs to be placed into the binding pockets of target proteins. It will make it easier to identify promising medications. In this case, compounds from *S. grande* leaves were tested against human HMGCR, which is an enzyme protein in humans [38]. Among the tested flavonoids, myricetin and luteolin were identified as flavonol aglycons. These flavonols are characterized by differing hydroxyl group distributions, in terms of position and number. Rutin represented the flavonol glycoside of quercetin. In addition, atorvastatin was also studied as one of the commercial lipid-lowering drugs.

Table 2 Molecular docking simulation of isolated compounds from *S. grande* leaves and atorvastatin drug.

Model	Interaction			Binding affinity (kcal/mol)
	Residue	Distance (Å)	Type	
Luteolin-HMGCR	Asn658, Ala751, Lys692, Asn686, Gly560	1.79, 2.22, (2.26, 2.59), 3.04, 2.43	H-bond	−9.0
	Leu862, Leu853	(4.53, 5.28), 4.75	Alkyl	
Rutin-HMGCR	Asp690, Asp767, Gly808, Asn658, Gly560, His752, Asn755	2.34, 3.01, 2.63, 2.37, 2.29, 3.20, 2.64	H-bond	−9.6
	Met655, Cys561	5.00, 4.08	Alkyl	
	Glu559	2.83	Unfavorable acceptor-acceptor	
Myricetin-HMGCR	Lys735, Lys692, Asp690, Asn658, Leu862	2.27, 2.68, 3.30, 2.36, 2.23	H-bond	−8.5
	Leu853	5.00	Alkyl	
	Glu559	1.29	Unfavorable donor-donor	
Atorvastatin-HMGCR	Glu559, Asp690, Ser684, Lys692	2.34, (2.60, 2.81), 2.77, 2.67	H-bond	−8.5
	Cys561	3.48	Halogen (F)	
	Met655, Cys561	5.49, (4.24, 4.89)	Alkyl	

The capabilities of isolated compounds to be HMGCR enzyme inhibitors have been demonstrated through molecular docking screening (**Table 2**). According to docking models, myricetin, luteolin, and rutin strongly bind to HMGCR, an essential enzyme in cholesterol synthesis. Luteolin-HMGCR, rutin-HMGCR, myricetin-HMGCR, and atorvastatin-HMGCR showed high binding strengths, with values of −9.0, −9.6, −8.5, and −8.5 kcal/mol, respectively. According to the findings, all isolated compounds showed a possible inhibitory effect due to low-energy binding. A low energy binding value corresponds to lower energy activation, so the compounds and receptor will interact and trigger a spontaneous reaction [39]. All compounds bound to the HMGCR protein exhibited negative binding energy in every interaction examined, predict the possibility of isolated compounds to affect the HMGCR activity due to the compound-protein binding in the active site.

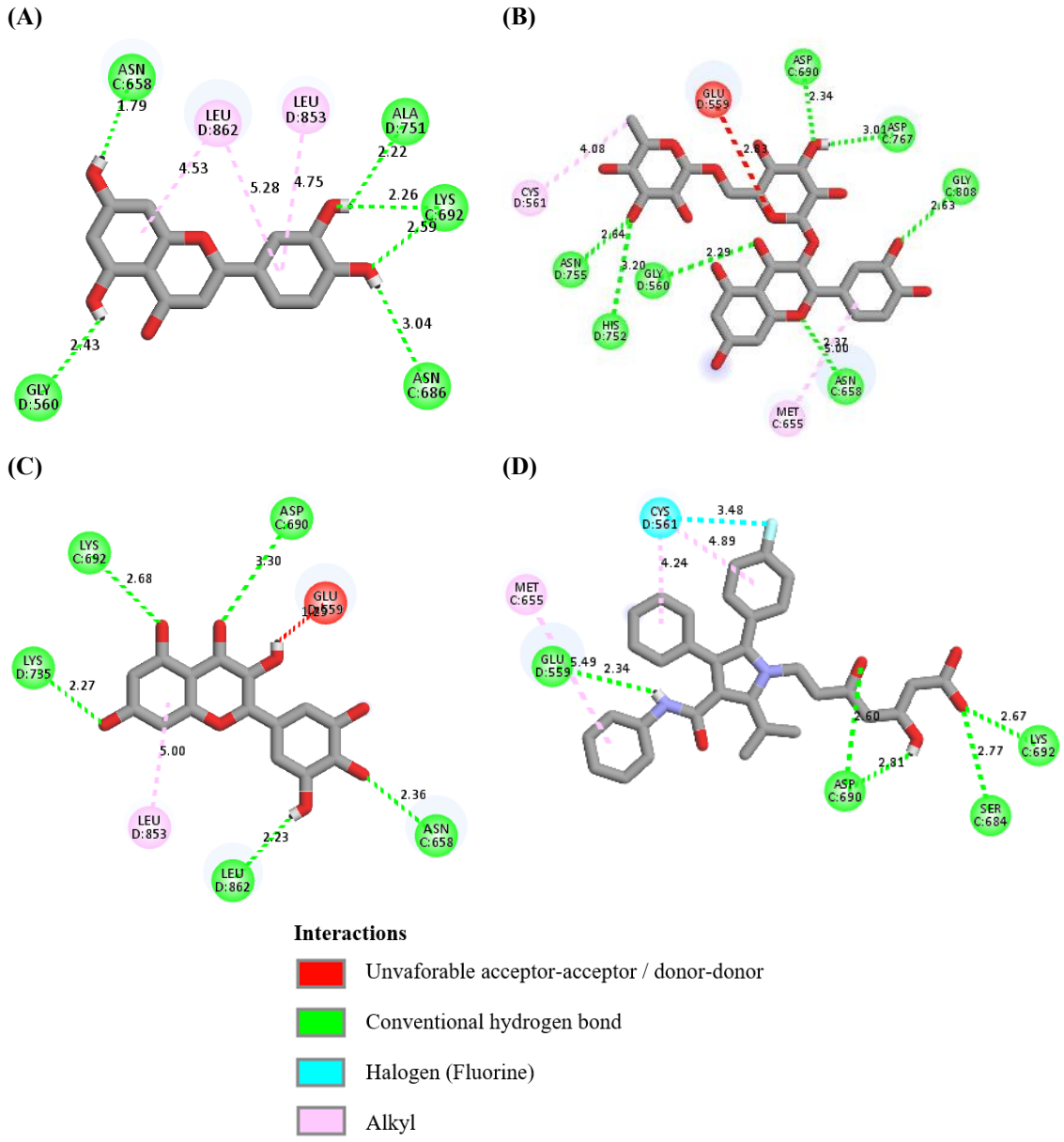


Figure 3 2D-map of protein binding pocket residues of HMG-CoA reductase subunit and the type of interactions with (A) Luteolin, (B) Rutin, (C) Myricetin, and (D) Atorvastatin.

The binding mode of luteolin, rutin, myricetin, and atorvastatin with HMGCR were examined through molecular docking studies to explore the key residues involved in the compounds-enzyme interactions. **Figure 3** shows the compounds' docking position amid the HMG-CoA reductase residues. The receptor interacted with both hydrogen and non-hydrogen bonds in various amino acid residues, as indicated by the binding energy values (**Table 2**).

Compared to other isolated compounds from *S. grande* in this study, rutin had the lowest binding affinity value, indicating the high possibility of rutin to inhibit enzyme activity due to the stronger binding to the active site of the enzyme. This may be related to the structure-activity relationship between compounds and enzymes, as determined by molecular docking simulations. Rutin is a glycosidic derivative of quercetin. The flexibility and improved hydrogen bonding caused by glycosidic linkages increase binding adaptability and a higher inhibitory effect. Nevertheless, longer branches may result in localised aggregation [40]. This may result in false positives due to ligand self-association into massive colloidal assemblies that nonspecifically block target proteins [41]. Generally, flavonoid aglycones work better as medicines than flavonoid glycosides [42]. The bulky size of the

glycoside structure can block the active hydroxyl group of the flavonoid aglycon from connecting with the enzyme's binding site.

Figure 3 shows the visualization of isolated compounds' binding sites on the receptor (HMGCR enzyme). The interaction strength decreases with increasing distance. The interaction distance between isolated compounds (luteolin, rutin, and myricetin) and protein was 1.79 - 5.49 Å. The type of interactions was non-covalent, namely hydrogen bonding, alkyl, halogen and donor-acceptor interactions. Thus, there were strong interactions between the compounds and the HMGCR enzyme, allowing them to possibly block HMGCR activity.

All compounds formed multiple stabilizing hydrogen-bonds, mainly through their abundant hydroxyl (−OH) groups, which is expected for polyhydroxylated flavonoids. Several similar key interacting residues form hydrogen bonds with each compound, including Lys692, Gly560, Asp690, Lys735, Ser684, and Asn659. Particularly, Asp690, Glu559, and Lys692 were responsible for the statin binding region and contributed to the catalytic activity of the HMGCR enzyme and substrate stabilization [43]. Lysine residue was important for catalytic stabilization. The binding pattern for all isolated compounds supported potential competitive inhibition of HMGCR. This also supports the findings of Istvan and Deisenhofer, who identified that amino acids 682 - 694 are included in the binding pocket of the HMGCR enzyme, which produces the cis loop, the enzyme's active site [44]. Rutin and myricetin formed unfavorable interaction with HMGCR that reduced their binding stability. However, since the total binding energy still −9.6 kcal/mol for rutin and −8.5 kcal/mol for myricetin, the unfavorable interaction was not dominant and did not weaken overall binding.

Luteolin had a significant overlap with the binding site of statin in HMGCR enzyme, showing that it might interact with the enzyme as an HMGCR inhibitor similar to statin drugs. Two H-bonds formed by Lys692 with the luteolin compound demonstrate this residue's high activity. It also had less steric hindrance than rutin and more drug-like than myricetin. The flavonoid mechanism appeared to be mostly hydrogen-bond driven rather than transition-state mimicking, as seen with atorvastatin, despite the fact that they overlapped with the statin-binding area. Thus, from a mechanistic molecular interaction standpoint, luteolin in *S.grande* leaves might be the most responsible compound in inhibiting HMGCR enzyme activity compared to rutin and myricetin.

Several studies have proven that the isolated compounds in this study effectively control blood cholesterol levels through *in vivo* studies. Myricetin administration in rabbits significantly improved oxidative stress, dyslipidemia indicators, and lipid profiles [38]. Rutin treatment could reduce total cholesterol, triglycerides, and LDL. Rutin protected hyperlipidemia in approximately 55.7% of cases, with the result not being significantly different from that of atorvastatin, which is 60.63% [45]. The treatment with luteolin increased the expression of SR-B1, ABCG1, and cholesterol 7α-hydroxylase (Cyp7α1) in the liver of obese mice, while reducing blood cholesterol and non-HDL cholesterol levels[46].

Assessment of drug-likeness of isolated compounds

Alongside strong efficacy, a satisfactory ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profile is crucial in drug discovery. Virtual screening is crucial for drug discovery since it significantly helps with drug repurposing through the identification of off-target effects and leads to compound optimization [47]. Thus, this study made predictions about the drug-likeness behaviour and ADME characteristics of luteolin, rutin, and myricetin, which are listed in **Table 3** and toxicity in **Table 4**.

Table 3 Drug-likeness and ADME characteristics of isolated compounds (luteolin, rutin, and myricetin) from *S. grande* leaves.

Parameter		Predicted value				Reference
		Atorvastatin	Luteolin	Rutin	Myricetin	
Drug-likeness	Molecular weight (g/mol)	558.65	286.24	610.52	318.24	-
	Lipinski's rule	2	4	1	3	≥ 3 passed
Absorption	Aqueous solubility (log mol/L)	−4.531	−3.094	−2.892	−2.915	< −4 insoluble > 0 soluble
	Intestinal absorption (%absorbed)	59.86	81.13	23.45	65.93	< 30% poor absorption
	Skin permeability (log Kp)	−2.735	−2.735	−2.735	−2.735	> −2.5 low permeability
Distribution	Blood-brain barrier permeability (log BB)	−1.162	−0.907	−1.899	−1.493	> 0.3 permeable < −1 impermeable
Metabolism	CYP3A4 inhibitor	No	No	No	No	Yes/No
	CYP3A4 substrate	Yes	No	No	No	Yes/No
Excretion	Total clearance (log mL/min/kg)	0.437	0.495	−0.369	0.422	> 1 high

ADME properties from online webserver (pkCSM) were presented in **Table 3**. The Lipinski's rule of 5 was used to evaluate the "drug-likeness" properties of isolated compounds. Oral-administered drugs should have no more than one violation of the rule [48]. Luteolin and myricetin passed Lipinski's rule, while rutin did not pass the rule. Rutin may have poor oral absorption and permeation. A glycoside usually broke down into the aglycon form by microbial β-glucosidase in the intestine. Absorption will be significantly slowed down or even decrease if there are insufficient enzyme or the glycosidic bond is extremely strong [49]. Other than that, larger, highly polar, or bulky glycosides like rutin frequently exhibit reduced capacity to enter lipid-rich intestinal epithelial cell membranes, despite having better water solubility than luteolin and myricetin [50,51].

In comparison, atorvastatin also violated Lipinski's rule yet effectively worked as a lipid-lowering agent. This is a great study of how Lipinski's rule is a recommendation rather than an absolute set of regulations and how other factors can influence a drug's bioavailability. All isolated compounds were insoluble in nature, but luteolin and myricetin have high intestinal absorption due to the small aglycon structure. These results are similar to those observed in atorvastatin. The physiological distribution properties of isolated compounds, predicted by the blood-brain barrier (BBB), showed no permeability by all compounds. Luteolin, rutin, and myricetin were not inhibitors for CYP3A4, which means that they might be metabolized by cytochrome P450 and do not adversely affect P450 pharmacokinetics.

Table 4 Toxicity study of isolated compounds by ProTox-II *in silico* study.

	Luteolin	Rutin	Myricetin	Atorvastatin
Hepatotoxicity	Inactive	Inactive	Inactive	Active
Neurotoxicity	Inactive	Inactive	Inactive	Active
Cardiotoxicity	Inactive	Inactive	Inactive	Inactive
Nephrotoxicity	Active	Active	Active	Active
Cytotoxicity	Inactive	Inactive	Inactive	Inactive
Predicted LD50	3,919 mg/kg	5,000 mg/kg	159 mg/kg	5,000 mg/kg
Predicted toxicity class	5	5	3	5

In silico toxicity study was investigated by ProTox-II. Related to the safety of antihypercholesterolemic drugs, atorvastatin, as a statin drug, had several adverse side effects such as significant liver damage, including decreased liver enzyme activity, increased bilirubin levels, and hepatocyte death by necrosis [6]. In contrast with atorvastatin, investigation of toxicity by ProTox-II showed that luteolin, rutin, and myricetin were not toxic to the liver. Along with hepatotoxicity, *in silico* model also forecast possible neurotoxic risk and CYP enzyme inhibition. Myricetin was predicted to be in class 3 toxicity (LD50 value between 50 - 300 mg/kg), indicating it could be toxic if swallowed in high dosage. However, research reported that in appropriate dosage and non-toxic quantities, myricetin could have strong hepatoprotective effects [52]. Flavonoids generally have low toxicity and a lower risk of severe adverse effects in the aglycon form [53].

Based on ProTox-II prediction, luteolin, rutin, myricetin, and atorvastatin might raise the possibility of nephrotoxicity. This could be caused of their strong affinity for renal regulatory enzyme such as CYP enzymes, structural alarms (such as catechol group), and quick accumulation in the kidneys [54]. In contrast, a study found that a flavonoid (6-hydroxyflavone) may have nephroprotective qualities and be utilized to treat renal problems brought on by chemotherapy [55]. Thus, the amount of flavonoid consumption should be in a safe and optimal dosage to prevent autoimmune reaction that results in acute nephritis.

Conclusions

The *in vitro* study found that extracts from *Syzygium grande* L. leaves offer an alternative option for treating and preventing hypercholesterolemia. They showed inhibition activity towards HMG-CoA reductase (HMGCR) similar to the commercial atorvastatin. Interestingly, flavonoid compounds isolated from *S. grande* leaves, namely luteolin, rutin, and myricetin, showed significant binding affinity HMGCR based on docking simulations. Although rutin showed the strongest expected binding affinity toward HMGCR, luteolin demonstrated a better balance between catalytic-site engagement and may possess more favorable pharmacological characteristics due to its smaller molecular weight and better drug-likeness prediction. These findings suggest that *S. grande* could be a valuable alternative herbal medicine with therapeutic potential. However, *in vivo* studies are needed to thoroughly investigate the ways these compounds affect cholesterol levels. In future research, there might be more focus on the efficacy of the extract and clarifying the involved pathways by *in vivo* study, like the inhibition of PCSK9 and the increase of SR-B1 gene expression.

Acknowledgements

The authors are grateful for the research support provided by Universiti Malaysia Terengganu (UMT) and Universitas Bengkulu (UNIB), Indonesia, through International Partnership Research Grant (IPRG), UMT/PPP/2-2/2/23 Jld.3 (127), with vote number 55572.

Declaration of generative AI in scientific writing

The authors stated the use of degenerative AI (QuillBot) to improve language, grammar, and readability of this manuscript. The AI tools did not create any content or analyze any data. The content and conclusions of this work are entirely the authors' responsibility.

CRediT author statement

Alifa Husnun Kholieqoh: Conceptualization, Methodology, Formal Analysis, Investigation, Writing - Original Draft, Visualization. **Yosie Andriani**: Conceptualization, Methodology, Writing - Review & Editing, Supervision, Funding Acquisition. **Wahdini Hanifah**: Formal Analysis, Investigation. **Kurnia Nastira Ningsih**: Formal Analysis, Investigation, Writing - Original Draft. **Sura Menda Ginting:** Writing - Review & Editing. **Hermansyah Amir:** Writing - Review & Editing. **Nurhamidah Nurhamidah:** Resources, Writing - Review & Editing. **Ariyanti Suhita Dewi:** Writing - Review & Editing. **Tengku Sifzizul Tengku Muhammad:** Supervision, Writing - Review & Editing. **Dwi Hudiyanti:** Writing - Review & Editing.

References

[1] Institute for Quality and Efficiency in Health Care. *High cholesterol: Overview*. National Library of Medicine, Maryland, United States, 2017.

[2] M Spacek, D Zemanek, M Hutyra, M Sluka and M Taborsky. Vulnerable atherosclerotic plaque - a review of current concepts and advanced imaging. *Biomedical Papers* 2018; **162(1)**, 10-17.

[3] JK Aronson. *HMG coenzyme-A reductase inhibitors. In*: JK Aronson (Ed.). Meyler's side effects of drugs (16^{th}). Elsevier, Amsterdam, Netherlands, 2016, p. 763-780.

[4] SY Jiang, H Li, JJ Tang, J Wang, J Luo, B Liu, JK Wang, XJ Shi, HW Cui, J Tang, F Yang, W Qi, WW Qiu and BL Song. Discovery of a potent HMG-CoA reductase degrader that eliminates statin-induced reductase accumulation and lowers cholesterol. *Nature Communications* 2018; **9(1)**, 5138.

[5] I Pangestika, E Oksal, TST Muhammad, H Amir, DF Syamsumir, MEA Wahid and Y Andriani. Inhibitory effects of tangeretin and trans-ethyl caffeate on the HMG-CoA reductase activity: Potential agents for reducing cholesterol levels. *Saudi Journal Bioogical Science* 2020; **27(8)**, 1947-1960

[6] H Zeng and Z Liu. Atorvastatin induces hepatotoxicity in diabetic rats via oxidative stress, inflammation, and anti-apoptotic pathway. *Medical Science Monitor* 2019; **25**, 6165-6173.

[7] CM Uritu, CT Mihai, GD Stanciu, G Dodi, TA Stratulat, A Luca, MML Constantin, R Stefanescu, V Bild, S Melnic and BI Tamba. Medicinal plants of the family lamiaceae in pain therapy: A review. *Pain Research and Management* 2018; **2018(1)**, 7801543.

[8] A Shang, R Gan, X Xu, Q Mao, P Zhang and HBL Li. Effects and mechanisms of edible and medicinal plants on obesity : an updated review. *Critical Reviews in Food Science and Nutrition* 2020; **61(12)**, 2061-2077.

[9] JA Burt and A Bartholomew. Towards more sustainable coastal development in the Arabian Gulf: Opportunities for ecological engineering in an urbanized seascape. *Marine Pollution Bulletin* 2019; **142**, 93-102.

[10] R Govaerts. *World checklist of myrtaceae*. Kew Publishing, London, 2008.

[11] KB Abdusalam, LS Yee, A Mediani, MT Akhtar, N Buzgaia, Y Rukayadi, IS Ismail and K Shaari. 1h nmr-based metabolomics profiling of *Syzygium grande* and *Oenanthe javanica* and relationship between their metabolite compositions and antimicrobial activity against Bacillus species. *Records of Natural Products* 2022; **16(2)**, 128-143.

[12] R Sarvesan, P Eganathan, J Saranya and P Sujanapal. Chemical composition and antimicrobial activity of leaf essential oil of *Syzygium grande* (Wight) Walp. *Journal of Essential Oil-Bearing Plants* 2015; **18(3)**, 642-646.

[13] S Jothiramshekar, P Eganathan and S Puthiyapurayil. Antioxidant activity of the leaf essential oil of *Syzygium calophyllifolium, Syzygium makul, Syzygium grande* and *Eugenia cotinifolia ssp. codyensis*. *Journal of Biologically Active Products from Nature* 2014; **4(1)**, 12-18.

[14] W hanifah, Y Andriani, AM Zin, D Ryandini, FAA Majid, B Subhan and TST Muhammad. Selected coastal plants as potential treatment for pneumonia disease: Determination of their phytochemicals and antibacterial activity against some pneumonia bacteria. *Oriental Journal of Chemistry* 2023; **39(6)**, 1479-1494.

[15] SV Kumar, JD Kingsley, S Dharani, N Narayanan and SM Thejan. Floristic analysis of medicinal plants from the Sholas of Nilgiris, Tamil Nadu, India. *Journal of Drug Delivery and Therapeutics* 2022; **12(6)**, 16-21.

[16] WY Lim, EWC Chan, CW Phan and CW Wong. Tyrosinase inhibiting extracts from coastal plants as potential additives in skin whitening formulations. *Current Journal of Applied Science and Technology* 2021; **21(3)**, 481-494.

[17] MN Samy, S Sugimoto, K Matsunami, H Otsuka and MS Kamel. One new flavonoid xyloside and one new natural triterpene rhamnoside from the leaves of *Syzygium grande*. *Phytochemistry Letters* 2014; **10(1)**, 86-90.

[18] M Koren-Gluzer, M Rosenblat and T Hayek. Paraoxonase 2 induces a phenotypic switch in macrophage polarization favoring an M2 anti-

inflammatory state. *International Journal of Endocrinology* 2015; **2015(1)**, 915243.

[19] LM Hulten, C Ullstrom, A Krettek, DV Reyk, SL Marklund, C Dahlgren and O Wiklund. Human macrophages limit oxidation products in low density lipoprotein. *Lipids in Health and Disease* 2005; **4(6)**, 6.

[20] J DeFuria, G Bennett, KJ Strissel, JW Perfield II, PE Milbury, AS Greenberg and MS Obin. Dietary blueberry attenuates whole-body insulin resistance in high fat-fed mice by reducing adipocyte death and its inflammatory sequelae. *The Journal of Nutrition* 2009; **139(8)**, 1510-1516.

[21] Y Andriani, W Hanifah, AH Kholieqoh, FAA Majid, H Hermansyah, H Amir and TST Muhammad. Antibacterial activity of hexane and methanol fractions of some selected plants against *Klebsiela pneumoniae. Journal of Advanced Pharmaceutical Technology & Research* 2023; **14(3)**, 220-225.

[22] S Tang, R Chen, M Lin, Q Lin, Y Zhu, J Ding, H Hu, M Ling and J Wu. Accelerating AutoDock Vina with GPUs. *Molecules* 2022; **27(9)**, 3041.

[23] O Trott and AJ Olson. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry* 2010; **31(2)**, 455-461.

[24] BL Jejurikar and SH Rohane. Drug designing in discovery studio. *Asian Journal of Research in Chem*istry 2021; **14(2)**, 135-138.

[25] S Akash, A Kumer, MM Rahman, TB Emran, R Sharma, RK Singla, FA Alhumayadhi, MU Khandaker, MN Park, AM Idris, P Wilairatana and B Kim. Development of new bioactive molecules to treat breast and lung cancer with natural myricetin and its derivatives: A computational and SAR approach. *Frontiers in Cellular and Infection Microbiology* 2022; **12**, 952297.

[26] X Cao, B He, F Liu, Y Zhang, L Xing, N Zhang, Y Zhou, C Gong and W Xue. Design, synthesis and bioactivity of myricetin derivatives for control of fungal disease and tobacco mosaic virus disease. *RSC Advances* 2023; **13(10)**, 6459-6465.

[27] J Qian, J Zhang, Y Chen, C Dai, J Fan and H Guo. Hypoglycemic activity and mechanisms of myricetin. *Natural Product Research* 2022; **36(23)**, 6177-6180.

[28] S Caporali, AD Stefano, C Calabrese, A Giovannelli, M Pieri, I Savini, M Tesauro, S Bernardini, M Minieri and A Terrinoni. Anti-inflammatory and active biological properties of the plant-derived bioactive compounds luteolin and luteolin 7-glucoside. *Nutrients* 2022; **14(6)**, 1155.

[29] FM Djeujo, V Stablum, E Pangrazzi, E Ragazzi and G Froldi. Luteolin and vernodalol as bioactive compounds of leaf and root *Vernonia amygdalina* extracts: Effects on α-glucosidase, glycation, ROS, cell viability, and *in silico* ADMET parameters. *Pharmaceutics* 2023; **15(5)**, 1541.

[30] BM Popović, D Uka, O Alioui, RZ Pavlović and Y Benguerba. Experimental and COSMO-RS theoretical exploration of rutin formulations in natural deep eutectic solvents: Solubility, stability, antioxidant activity, and bioaccessibility. *Journal of Molecular Liquids* 2022; **359**, 119266.

[31] YH Yeh, CT Kuo, GJ Chang, YH Chen, YJ Lai, ML Cheng and WJ Chen. Rosuvastatin suppresses atrial tachycardia-induced cellular remodeling via Akt/Nrf2/heme oxygenase-1 pathway. *Journal of Molecular and Cellular Cardiology* 2015; **82**, 84-92.

[32] A Mansouri, Z Reiner, M Ruscica, ET Reiner, S Radbakhsh, MB Ekta and A Sahebkar. Antioxidant effects of statins by modulating Nrf2 and Nrf2/HO-1 signaling in different diseases. *Journal of Clinical Medicine* 2022; **11(5)**, 1313.

[33] AH Kholieqoh, MNI Kassim, TST Muhammad, K Anam, YY Sung, H Amir, HN Praja and Y Andriani. SNEDDS to improve the bioactivities of *Pandanus tectorius* leaves: Optimization, antioxidant, and anticancer activities via apoptosis induction in human cervical cancer cell line. *Journal of Applied Pharmaceutical Science* 2024; **14(10)**, 175-189.

[34] M Batty, MR Bennett and E Yu. The role of oxidative stress in atherosclerosis. *Cells* 2022; **11(23)**, 3843.

[35] A Popolo, G Autore, A Pinto and S Marzocco. Oxidative stress in patients with cardiovascular disease and chronic renal failure. *Free Radical Research* 2013; **47(5)**, 346-356.

[36] F Ito, Y Sono and T Ito. Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: Oxidative stress in diabetes, atherosclerosis, and chronic inflammation. *Antioxidants* 2019; **8(3)**, 72.

[37] J Bore, MJ Chapman, RM Krauss, CJ Packard, JF Bentzon, CJ Binder, MJ Daeman, LL Demer, RA Hegele, SJ Nicholls, BG Nordestgaard, GF Watts, E Bruckert, S Fazio, BA Ference, A Graham, JD Horton, U Landmesser, U Laufs, …, HN Ginsberg. Low-density lipoproteins cause atherosclerotic cardiovascular disease: Pathophysiological, genetic, and therapeutic insights: A consensus statement from the European Atherosclerosis Society Consensus Panel. *European Heart Journal* 2020; **41(24)**, 2313-2330.

[38] P Riyad, A Purohit, K Sen, A Panwar and H Ram. HMG-CoA reductase inhibition mediated hypocholesterolemic potential of myricetin and quercetin: *In-silico* and *in-vivo* studies. *CYTA - Journal of Food* 2023; **21(1)**, 115-125.

[39] A Alaofi, E Farokhi, VD Prasasty, A Anbanandam, K Kuczera and TG Siahaan. Probing the interaction between cHAVc3 peptide and the EC1 domain of E-cadherin using NMR and molecular dynamics simulations. *Journal of Biomolecular Structure & Dynamics* 2017; **35(1)**, 92-104.

[40] R Huo, Wuhanqimuge, M Zhang, M Sun and Y Miao. Molecular dynamics modeling of different conformations of beta-glucan, molecular docking with dectin-1, and the effects on macrophages. *International Journal of Biological Macromolecules* 2025; **293**, 139382.

[41] S Boulton, R Selvaratnam, R Ahmed, K Van, X Cheng and G Melacini. Mechanisms of specific versus nonspecific interactions of aggregation-prone inhibitors and attenuators. *Journal of Medicinal Chemistry* 2019; **62(10)**, 5063-5079.

[42] J Xiao. Dietary flavonoid aglycones and their glycosides: Which show better biological significance? *Critical Reviews in Food Science and Nutrition* 2019; **57(9)**, 1874-1905.

[43] BE Haines, O Wiest and CV Stauffacher. The increasingly complex mechanism of HMG-CoA reductase. *Accounts of Chemical Research* 2013; **46(11)**, 2416-2426.

[44] ES Istvan and J Deisenhofer. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science* 2001; **292(5519)**, 1160-1164.

[45] L NR, AR Nair, S Senthilpandian and V Ravi. Hypolipidemic action of Rutin on Triton WR-1339 induced hyperlipidemia in rats. *Journal of Pre-Clinical and Clinical Research* 2021; **15(2)**, 200-208.

[46] HS Park, K Lee, SH Kim, MJ Hong, NJ Jeong and MS Kim. Luteolin improves hypercholesterolemia and glucose intolerance through LXRα-dependent pathway in diet-induced obese mice. *Journal of Food Biochemistry* 2020; **44(9)**, e13358.

[47] LRDZ Neto, JTM Filho, BJ Neves, RLBR Maidana, ACR Guimaraes, N Furnham, CH Andrade and FP Silva. *In silico* strategies to support fragment-to-lead optimization in drug discovery. *Frontiers in Chemistry* 2020; **8**, 93.

[48] CA Lipinski, F Lombardo, BW Dominy and PJ Feeney. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews* 2001; **46(1-3)**, 3-26.

[49] L Hu, Y Luo, J Yang and C Cheng. Botanical flavonoids: Efficacy, absorption, metabolism and advanced pharmaceutical technology for improving bioavailability. *Molecules* 2025; **30(5)**, 1184.

[50] D Tungmunnithum, A Thongboonyou, A Pholboon and A Yangsabai. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines* 2018; **5(3)**, 93.

[51] JX Wang, ZF Lin, XY Zheng, JL Zhou, JJ Huang and YJ Lu. Engineering galactoside acetyltransferase for enhanced hesperetin-7-O-glucoside bioavailability. *Applied Microbiology and Biotechnology* 2026; **110(1)**, 2.

[52] H Lv, B An, Q Yu, Y Cao, Y Liu and S Li. The hepatoprotective effect of myricetin against lipopolysaccharide and D-galactosamine-induced fulminant hepatitis. *International Journal of Biological Macromolecules* 2020; **155**, 1092-1104.

[53] WS Hashim, YS Yasin, AH Jumaa, MI Al-Zuhairi and AH Abdulkareem. Physiological scrutiny to appraise a flavonol versus statins. *Biomedical and Pharmacology Journal* 2023; **16(1)**, 289-293.

[54] Y Gong, D Teng, Y Wang, Y Gu, Z Wu, W Li, Y Tang and G Liu. *In silico* prediction of potential drug-induced nephrotoxicity with machine learning methods. *Journal of Applied Toxicology* 2022; **42(10)**, 1639-1650.

[55] ZU Din, SU Farooq, M Shahid, O Alghamdi, N Al-Hamoudi, F Vohra and T Abduljabbar. The flavonoid 6-hydroxyflavone prevention of cisplatin-induced nephrotoxicity. *Histology and Histopathology* 2020; **35(10)**, 1197-1209.