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Original article

Virtual selection of γ-oryzanol derivatives of brown rice (*Oryza sativa* L.) blocking HMG-CoA reductase in hypercholesterolemia disease

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Abstract

Simvastatin is an inhibitor that has been widely recommended as an anti-cholesterol drug that targets HMG-CoA reductase. Yet, these drugs have limitations due to drug resistance effects. This study aims to select the γ-oryzanol derivatives from brown rice as potential HMG-CoA reductase inhibitors using an *in silico* approach. Gene ontology and protein interaction of hypercholesterolemia were analyzed using ShinyGO and STRINGdb web server. γ-Oryzanol derivatives were screened using the SwissADME web server to assess their drug-likeness, while the PASS Online web server predicted their bioactivity. γ-oryzanol derivatives were then specifically docked at the site of the binding pocket of HMG-CoA reductase (PDB ID: 1DQ9). The stability interaction of ligan-protein was evaluated using YASARA. Cycloartenyl ferulate is predicted to have more potential anti-hypercholesterolemia bioactivity than other derivatives. γ-Oryzanol derivatives interacted with HMG-CoA reductase through hydrogen bonds in the ferulic component and hydrophobic bonds in the phytosterol component. Cycloartenyl ferulate interacted on key residues Asn658, Phe628, Met655, and Val805 in the NADPH binding domain with the strongest binding affinity (-9.2 Kcal/mol). Cycloartenyl ferulate binds to the NADPH binding domain, similarly to simvastatin, which might prevent the NADPH cofactor from helping enzyme catalyzing. The stability of the cycloartenyl ferulate molecular interaction was close to the simvastatin interaction on HMG-CoA reductase. This study showed that γ-oryzanol derivatives, particularly cycloartenyl ferulate, have strong potential as anti-hypercholesterolemia agents by blocking the NADPH binding domain of HMG-CoA reductase.

Keywords: γ-oryzanol derivatives, brown rice, cycloartenyl ferulate, HMG-CoA reductase, molecular docking
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Introduction

Hypercholesterolemia is an increase in cholesterol levels exceeding 200 mg/dL in human blood plasma, a major cardiovascular disease factor (Benito-Vicente et al., 2018). Elevated blood cholesterol levels, particularly low-density lipoprotein-cholesterol (LDL) have triggered the pathogenesis of atherosclerosis, stroke, and cardiovascular diseases that contribute to 4.5% of total mortality in the world (Abbasi et al., 2024). The World Health Organization (WHO) recorded the prevalence of hypercholesterolemia reached 30% in Southeast Asia, and up to 35% in Indonesia (Amirus et al., 2024). Although the disease can be caused by genetic inheritance such as mutations in the LDL receptor gene, the pathway of cholesterol biosynthesis undoubtedly plays an important role in the pathogenesis of hypercholesterolemia. Increased consumption of high-fat diets, particularly those rich in saturated and trans fats, stimulates hepatic de novo cholesterol synthesis by providing substrates for the mevalonate pathway (Zhao et al., 2019).

3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) (EC 1.1.1. 34) is the key enzyme that catalyzes the conversion of HMG-CoA to mevalonate, and its activity is tightly regulated to control the rate of cholesterol synthesis (Schumacher & DeBose-Boyd, 2021). Increased expression of HMG-CoA reductase triggers upregulated endogenous cholesterol production. Cholesterol-lowering drugs such as simvastatin have been widely prescribed to treat this disease (Crismaru et al., 2020). This active site of this protein divided into three active sites domain for HMG, CoA, and NADPH groups (Gesto et al., 2020). Simvastatin addresses this by competitively inhibiting the HMG-CoA reductase, reducing LDL-cholesterol levels (Hoyos et al., 2019). Simvastatin drugs bind to the CoA group, thus blocking HMG-CoA access to the binding site (Moore II & Cook, 2022). However, most hypercholesterolemia subjects do not reach the target of balanced cholesterol levels (Jukema et al., 2012). Simvastatin-induced cholesterol reduction gives the body feedback to increase HMG-CoA reductase protein levels in the liver (Jiang et al., 2018). The accumulation of HMG-CoA reductase can reduce the efficacy of simvastatin, leading to simvastatin resistance (Chen et al., 2014). The use of high doses of simvastatin, often required due to resistance, can raise the risk of adverse effects such as diabetes mellitus, muscle pain, and intolerance to the medication (Reiner, 2014). This underscores the need for alternative strategies to lower cholesterol levels that can improve the expression of cholesterol-regulating proteins and reduce side effects in the body.

γ-Oryzanol is one of the unique bioactive compounds of brown rice (*Oryza sativa* L.), which has been reported to have a wide safety profile with no major side effects

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observed in animal studies and human clinical trials (Szcześniak et al., 2016). γ-Oryzanol is composed of ferulic acid and phytosterol components that are similar in structure to cholesterol and have been found to reduce and maintain blood cholesterol levels (Lesma et al., 2018; Lemus et al., 2014). γ-Oryzanol has been revealed to have several useful pharmacological properties, such as antioxidant, anti-inflammatory, anti-diabetic, cholesterol-lowering, and improving plasma lipid patterns (Ramazani et al., 2021). Recently reports of γ-oryzanol anti-cholesterol activity can reduce cholesterol and LDL-cholesterol levels are limited to studies in hypercholesterolemia patients (Phunikhom et al., 2021). However, the precise mechanism underlying the lowering cholesterol activity of main γ-oryzanol derivatives inhibition remains unclear. γ-Oryzanol of brown rice with four main derivatives: cycloartenyl ferulate (CAF), 24-methylenecycloartanyl ferulate (24-MCF), campesteryl ferulate (CF), and β-sitosteryl ferulate (β-SF) can potentially lower cholesterol by inhibiting HMG-CoA reductase (Ravichanthiran et al., 2018). Therefore, this study's purpose it to analyze and select potent inhibition of γ-oryzanol derivatives on HMG-CoA reductase *in silico* approach.

Methods

Gene Functional Annotation

Hypercholesterolemia Gene Ontology (GO) in the Gene Set Enrichment Analysis database was used (https://www.gsea-msigdb.org/). GO genes categorize proteins into three domains: molecular function, cellular component, and biological process. ShinyGO 0.81 webserver categorizes target proteins based on their role in human cellular pathways (Ge et al., 2020). HMG-CoA Reductase was entered in STRINGdb webserver to analyze the network protein-protein interactions related to the function (Widyananda et al., 2023a; Szklarczyk et al., 2021).

Identification of Physical Chemistry, Drug-likeness, Bioactivity, and Toxicity

Canonical smiles of γ-oryzanol derivatives: CAF (CID 5282164), 24-MCF (CID 990169), CF (CID 15056832), β-SF (CID 9938436), and simvastatin (CID 54454) were copied from PubChem database web server. The canonical smiles were entered into the SwissADME webserver to screen physical chemistry and drug-likeness by Lipinski, Veber, and Egan rules (Widyananda et al., 2023b). The toxicity prediction of the compounds was conducted in the Toxtree web server (https://apps.ideaconsult.net/data/ui/toxtree). Additionally, PASSOnline way2drug webserver (https://www.way2drug.com/passonline/predict.php) to predict bioactivity-related anti-hypercholesterolemia.

Ligand Preparation

All of γ-oryzanol, and simvastatin (CID 54454) as drug ligand control were retrieved from PubChem database webserver (https://pubchem.ncbi.nlm.nih.gov/). The ligands were entered into Open Babel integrated with PyRx software version 0.8 to minimize all their energies and ensure stable conditions during docking simulations. All ligands were then converted as test ligands in the Vina Wizard of PyRx.

Protein Preparation

The target protein for HMG-CoA reductase in humans (PDB ID: 1DQ9) was retrieved from the RCSB PDB (Protein Data Bank) database (https://www.rcsb.org/). Water molecules and any ligand contaminants present in the protein structure were eliminated using BIOVIA Discovery Studio Visualizer 2024 software. The processed protein structure was saved in Protein Data Bank (PDB) file format.

Molecular docking

The HMG-CoA reductase was entered in Autodock Wizard of PyRx a macromolecule receptor. The specific docking was performed between γ-oryzanol derivatives with HMG-CoA reductase sequentially including cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, campesteryl ferulate, and β-sitosteryl ferulate, and simvastatin (control). The amino acid residues of the HMG-CoA reductase active site were marked, and the design grid of docking in Table 1 was focused on the marked active site region. The ligand-protein docking was run. The binding affinity scores and ligand docking results were saved in all file formats.

Table 1. The binding pocket of HMG-CoA reductase and Grid specific docking

Protein (PDB ID)	Domain	Binding Pocket	Ref	Grid	
				Center	Dimension (Å)
HMG-CoA reductase (1DQ9)	NADP	Ser626, Arg627, Arg871, Met657, Asn658, Val805, Asn870, Phe628, Asp653, Met655, Gly656	(Gesto et al., 2020)	x:5.2801 y:9.7685 z:8.6703	x:33.7370 y:32.1518 z: 31.0298
	HMG	Asp690, Lys735, Asn755, Asp767, Glu559, Leu853, Ser684, His866, Lys691, Lys692			
	CoA	Asn567, Ser865, His866, Tyr479, Ser565, Arg568, Lys722			

The fusion of ligand and protein was performed by inserting the docking result ligand and HMG-CoA reductase protein in PyMol software 3.0. The ligand and protein docking complexes were saved in PDB file format. Visualization of docking results and analysis of interacting residues were performed with Discovery Studio Visualizer 2024 software (Palis et al., 2023).

Molecular Dynamic Simulation

The derivate of γ-oryzanol-HMG-CoA reductase with the lowest affinity value from the docking assay was tested for molecular dynamics using YASARA software (Ozvoldik et al., 2023). The simulation accurately modeled protein-ligand interactions using the AMBER14 force field. The cellular environmental parameters were configured to mimic normal physiological conditions (a water density of 0.997 g/mL, a temperature of 37°C, a pressure of 1 atmosphere, a pH of 7.4, and a 0.9% NaCl). The simulation was conducted for 40 ns, with snapshots recorded every 25 ps (Widyananda et al., 2022). RMSD values of All and γ-oryzanol ligand movement were analyzed and compared with the molecular dynamics simulation results of simvastatin-HMG-CoA reductase to assess the stability of the interaction.

Results

Gene Ontology of Hypercholesterolemia

Gene ontology annotation analysis revealed the most significant biological pathways associated with hypercholesterolemia. Many biological pathways are involved in various processes of hypercholesterolemia development. The hypercholesterolemia pathway begins with molecular damage, such as the tumor necrosis factor (TNF) signaling pathway (Figure 1a). The inflammation-associated TNF signaling pathway plays an important role in developing atherosclerosis, which causes complications from hypercholesterolemia (Kong et al., 2022). Cellular components such as the insulin and glucagon signaling pathways are disrupted (Janus et al., 2016). The 'Cholesterol metabolism' pathway shows high fold enrichment values, indicating that perturbations in the pathway influence the development of hypercholesterolemia (Figure 1b). Disruption of molecular function and cellular components can disrupt biological processes in cholesterol metabolism. Disturbances in cholesterol metabolism pathways, such as increased expression of genes encoding HMG-CoA reductase enzymes, lead to increased LDL cholesterol (Benito-Vicente et al., 2018). HMG-CoA reductase showed a strong level of interaction with 10 cholesterol metabolism proteins (Figure 1c). According to Chou et al. (2013), more than 23 enzymes are involved in cholesterol synthesis, including HMG-CoA reductase. Inhibition of HMG-CoA reductase might directly affect the catalytic function of squalene epoxidase (SQLE).

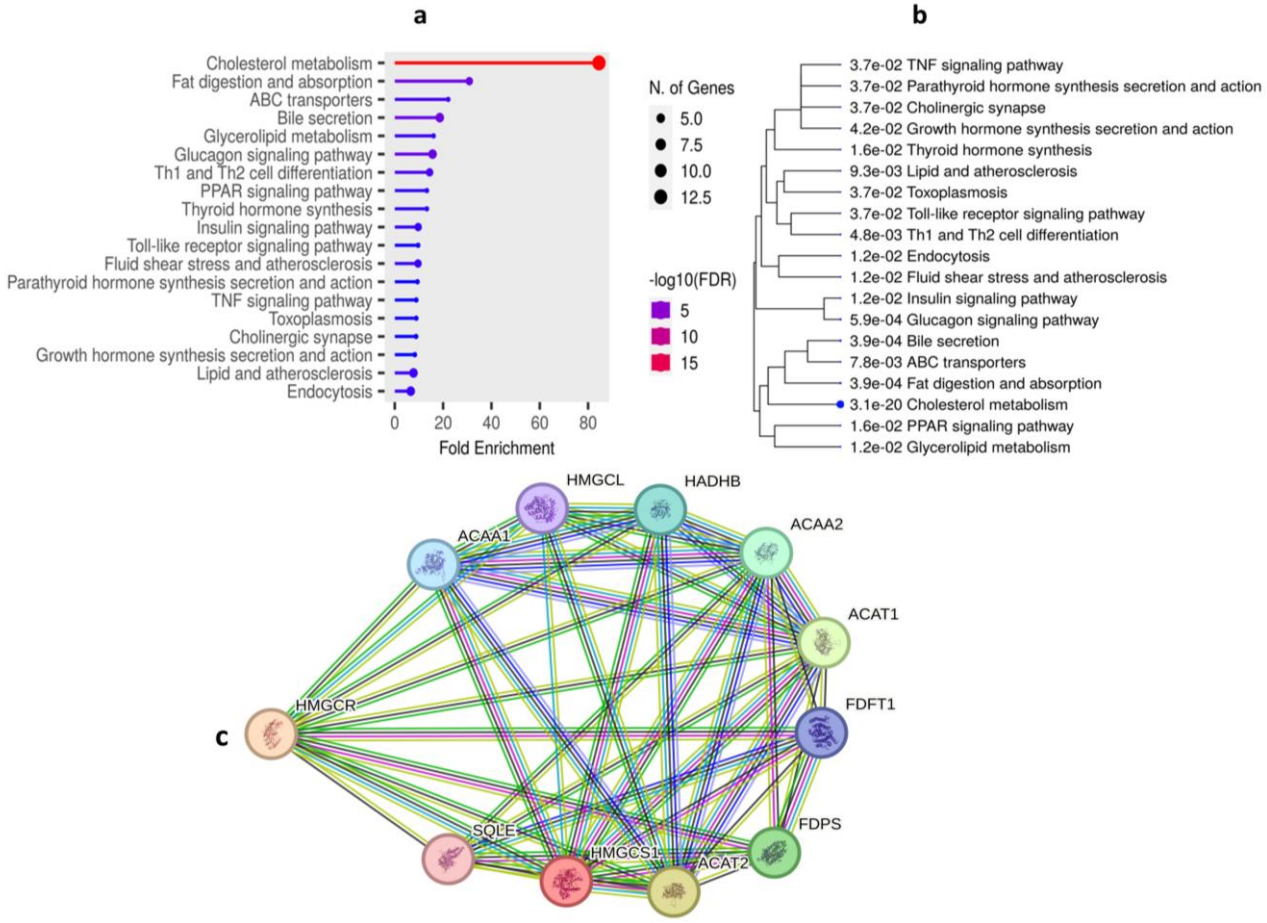

Figure 1. Gene ontology of hypercholesterolemia. a) gene ontology fold enrichment, b) gene ontology annotation, c) protein interaction of HMG-CoA reductase

Physicochemistry, drug-likeness, and bioactivity of γ-oryzanol derivatives

γ-oryzanol showed potential violations of Lipinski, Ghose, and Egan rule violation values, indicating challenges in developing this compound as a pharmacokinetic drug in (Figure 2a). γ-Oryzanol derivatives showed violations of the Lipinski rule, particularly in the molecular weight parameter (≥500g/mol) and MLogP (>4.15) parameters, while by Egan rules, they commonly violated WLogP parameter (≤5,88) (Ivanović et al., 2020). A compound that possesses molecular weight greater than 500g/mol indicates limit ability in absorption and affects metabolism in the body, while high MLogP value suggests that the compound is pore probability to be soluble in non-polar solvents (Valko et al., 2013). According to the Toxtree toxic hazard prediction, only simvastatin and CAF were available to be identified, while 24-MCF, CF, and β-SF were not available to be identified by Toxtree. The Cramer rules classified CAF as having intermediate toxicity (class II), while simvastatin classified high toxicity (class III). According to the Benigni/Bossa rules for carcinogenicity and mutagenicity, cycloartenyl ferulate demonstrated inactive genotoxicity, suggesting it is a safer compound for hypercholesterolemia, whereas simvastatin exhibited active genotoxicity, raising concerns about long-term use.

The present result showed that CAF had stronger anti-cholesterol activity than other oryzanol derivatives. The potential bioactivity of the compound is indicated by a high Pa value (Pa>0.6). ThisPa value suggests a probability that the compound belongs to a subclass of active compounds that excert certain bioactivity (Filimonov et al., 2014). CAF is a potential anti-cholesterol agent as a hypolipemic, cholesterol antagonist, hepatoprotection, cholesterol synthesis inhibitor, sterol 24-C methyltransferase inhibitor, and anti-hypercholesterolemia (Figure 2b). Meanwhile, simvastatin showed anti-cholesterol as a hypolipemic, cholesterol synthesis inhibitor, anti-hypercholesterolemia, and lipid metabolism regulator.

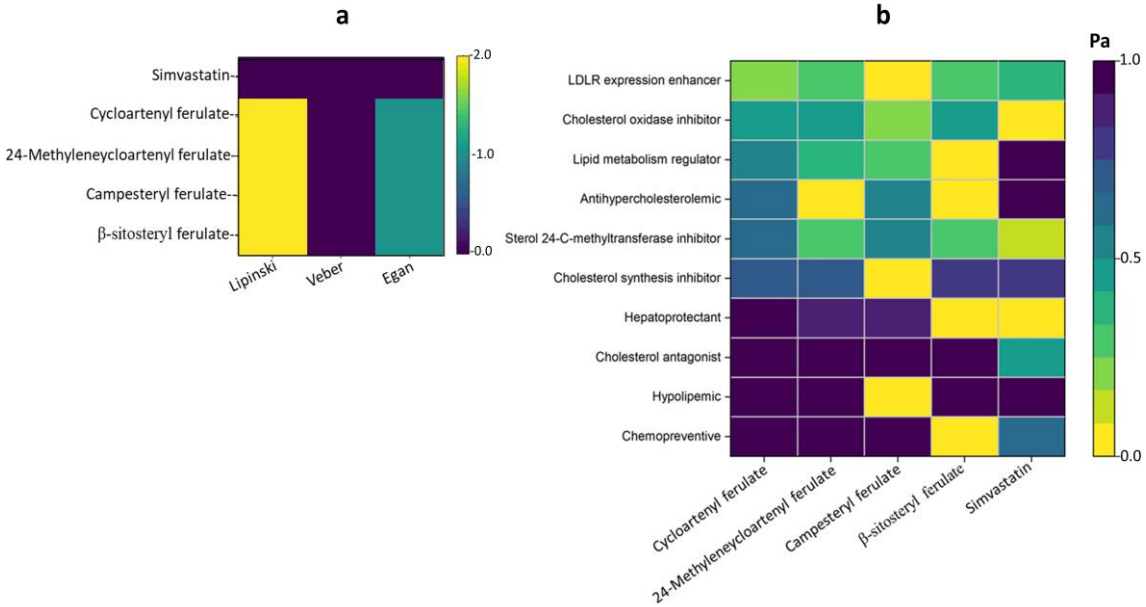

Figure 2. Identification of γ-oryzanol derivatives: cycloartenyl ferulate (CAF), 24-methylenecycloartenyl ferulate (24-MCF), campesterol ferulate (CF), β-sitosteryl ferulate (β-SF), simvastatin. A) Violation value, b) Bioactivity

Molecular Docking

Molecular docking has become an important tool for identifying potential drug candidates for specific target of disease (Luo et al., 2016). Molecular docking can predict the binding pose and conformation of ligand-protein interactions and help develop strategies for various diseases (Widyananda et al., 2023c). Based on docking assay result, 3 γ-oryzanol derivatives (CAF, 24-MCF, and CF) through the ferulic acid group, were found to interact via hydrogen bonds on HMG-CoA reductase. Meanwhile, all γ-oryzanol derivatives in the phytosterol groups were dominated by hydrophobic bonds on target protein (Figure 3 and Table 2). In total, CAF exhibited seven hydrophobic interactions and two hydrogen interactions. Meanwhile, 24-MCF interacts through four hydrogen interactions, seven hydrophobic interactions, one electrostatic interaction, and one other interaction (Pi-sulfur). In contrast, CF interacts through two hydrogen bonds and six hydrophobic bonds, while β-SF only interacts through five hydrophobic bonds. CAF binds to four important HMG-CoA reductase residues, namely Asn658, Phe628, Met655, and Val805. Residue Asn658 hydrogen interacts with CAF, while the other residues interact hydrophobic.

Residues engaged in the NADPH and HMG bindings on HMG-CoA reductase were previously identified. NADPH acts as an electron donor during the mevalonate catalysis reaction (Gesto et al., 2020). It is notable that among the interactions, CAF interacted with four known NADPH binding site residues within HMG-CoA reductase including Asn658 via hydrogen bond, and Phe628, Met655, and Val805 through hydrophobic interactions. In addition, 24-MCF interacted at six important residues of HMG-CoA reductase, namely Met657, Asn658,

Met655, Val805, Phe628, and Glu559. Residues Met657 and Asn658 interacted hydrogen with 24-MCF, while Met655, Val805, and Phe628 interacted via hydrophobic bond, and Glu559 interacts electrostatically on the 24-MCF ligand. The five important residues bound by 24-MCF belong to the NADPH binding pocket, while Glu559 as part of HMG binding site. HMG functions as a native ligand catalyzed into mevalonic acid as the final product in the HMG-CoA reductase enzymatic reaction. The CF bound four important residues of HMG-CoA reductase, namely Arg627, Met657, Met655, and Val805. Arg627 hydrogen interacts with CF, while the other three residues interact hydrophobically. The NADPH binding pocket includes the four residues that act as an electron reductant. Lastly, β-SF interacts with two important residues of HMG-CoA reductase, namely Val805 and Phe628. Both residues engaged in hydrophobic interaction with β-SF. Simvastatin, as a drug ligand, was to observed to bind with two important residues of NADPH-binding site, namely Asn658 and Met655. Asn658 interacted through hydrogen, while Met655 interacted through hydrophobic interaction with simvastatin (Figure 3 and Table 2).

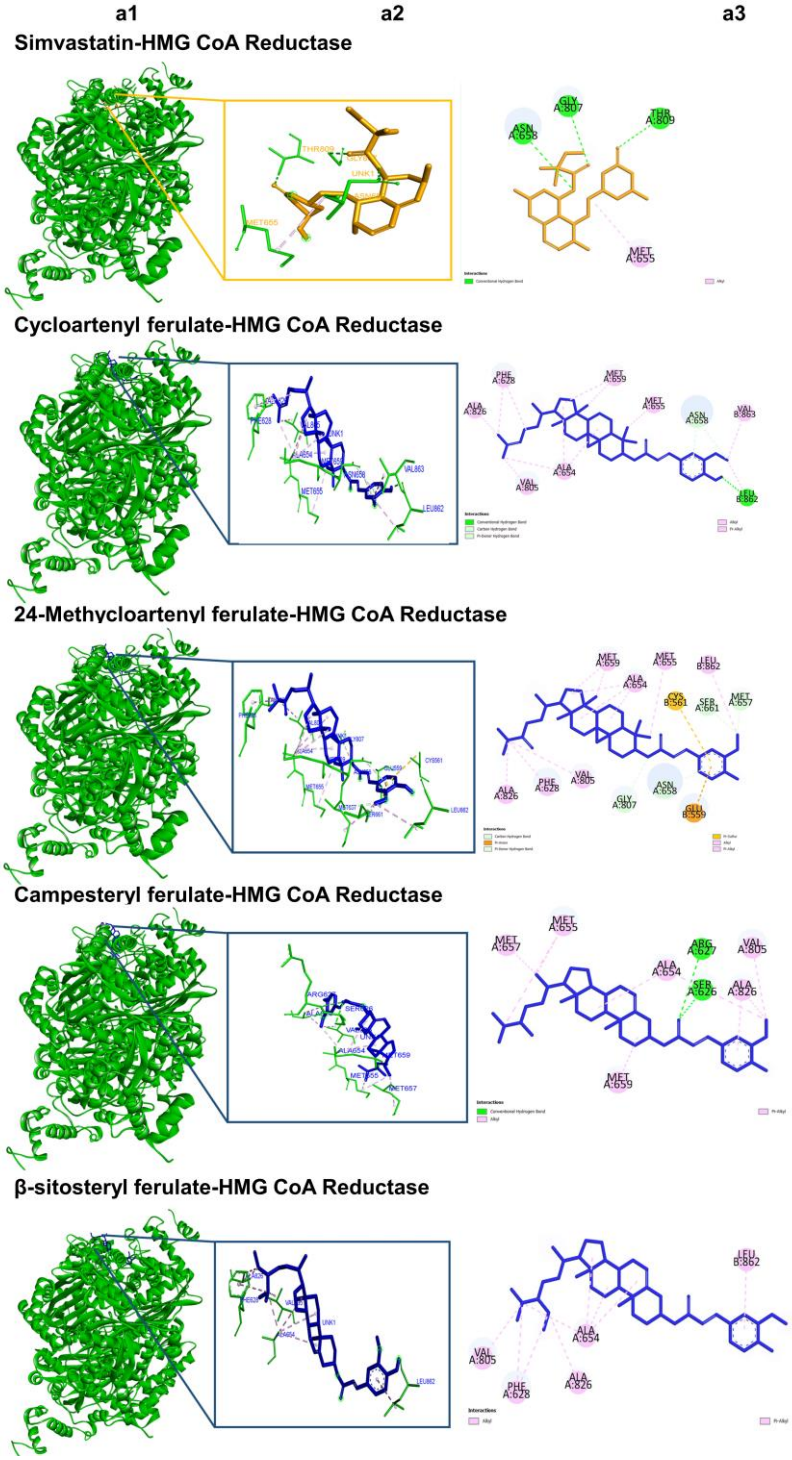

Figure 3. Docking interaction result of γ-oryzanol derivatives with HMG-CoA reductase (HMGR). The green structure is HMGR; the blue structure is γ-oryzanol derivative; the brown structure is simvastatin. a1: 3-dimensional interaction, a2: protein-ligand complex, a3: 2-dimensional

Table 2. Binding interaction of γ-oryzanol derivatives with HMG-CoA reductase

Complex (binding afinity)	Binding afinity (Kcal/mol)	Interaction	Amino Acid residue(s)
Simvastatin-HMG-CoA Reductase	-8,2	Conventional hydrogen bond	**Asn658**, Gly807, Thr809
Cycloartenyl ferulate-HMG-CoA Reductase	-9,2	Hydrophobic Conventional hydrogen bond Carbon hydrogen bond Hydrophobic	**Met655** Leu862 **Asn658** Ala826, **Phe628**, Met659, **Met655**, Val863, Ala654, **Val805**
24-*Metylenecycloartanyl feruleate*-HMG-CoA Reductase	-9,1	Carbon hydrogen bond Hydrophobic Electrostatic Other (Pi-sulfur)	**Met657**, Ser661, **Asn658**, Gly807 Met659, Ala654, **Met655**, Leu862, **Val805**, **Phe628**, Ala826 **Glu559** Cys561
Campesteryl ferulate-HMG-CoA Reductase	-8,5	Conventional hydrogen bond Hydropobic	**Arg627**, Ser26 **Met657**, **Met655**, Ala654, **Val805**, Ala826, Met659
β-Sitosteryl ferulate-HMG-CoA Reductase	-8,0	Hydrophobic	**Val805**, **Phe628**, Ala826, Ala654, Leu862

Note: Bolded residues show HMG-CoA reductase active site residues

In a perspective of residues bound by the four γ-oryzanol derivative all γ-oryzanol derivative ligands, Val805 became an important residue bound by the four derivatives. In addition, Met655 and Phe628 was bound by three γ-oryzanol derivatives (CAF, 24-MCF, β-SF), while Asn658 was bound by two γ-oryzanol derivatives (CAF, 24-MCF). All four γ-oryzanol derivatives hydrophobically bound Val805, while Asn658 hydrogen-interacted to CAF and 24-MCF. Simvastatin, as a drug ligand, binds to 2 important residues of HMG-CoA reductase, namely Asn658 and Met655. Asn658 interacts with hydrogen, and Met655 interacts hydrophobically on simvastatin (Figure 3 and Table 2). Asn658 and Met655 belong to the NADPH pocket binding site of the HMG-CoA reductase domain. Simvastatin interacted via hydrogen bond to HMG-CoA reductase for 3 interactions and hydrophobic for 1 interaction. Interestingly, CAF derivatives interacted with the strongest interaction (-9.2 kcal/mol) and slightly stronger compared to 24-MCF (-9.1 kcal/mol). The lower the binding affinity value indicates a closer, stronger, and more stable interaction, suggesting that CAF had the strongest binding to HMG-CoA reductase compared to other ligands.

Molecular Dynamic Simulation

The lower RMSD value indicates a more stable structure (Maruyama et al., 2023). The RMSD All of the values, was estimated to be below 3Å (Figure 4a), indicating that the protein conformational changes induced by CAF are relatively small, suggesting the stability of the interaction (Widyananda et al., 2023c). RMSD value of ligand movement was similar to simvastatin, suggesting that CAF may have good interaction stability with HMG-CoA reductase (Figure 4b). The RMSF value showed that CAF tended to remain fixed at most of the key residues of the HMG-CoA reductase binding domain (≤3 Å). However, at residue His866 and Ser865, CAF experiences more significant fluctuations (6.66 Å) than statin (4.08 Å) (Figure 4c).

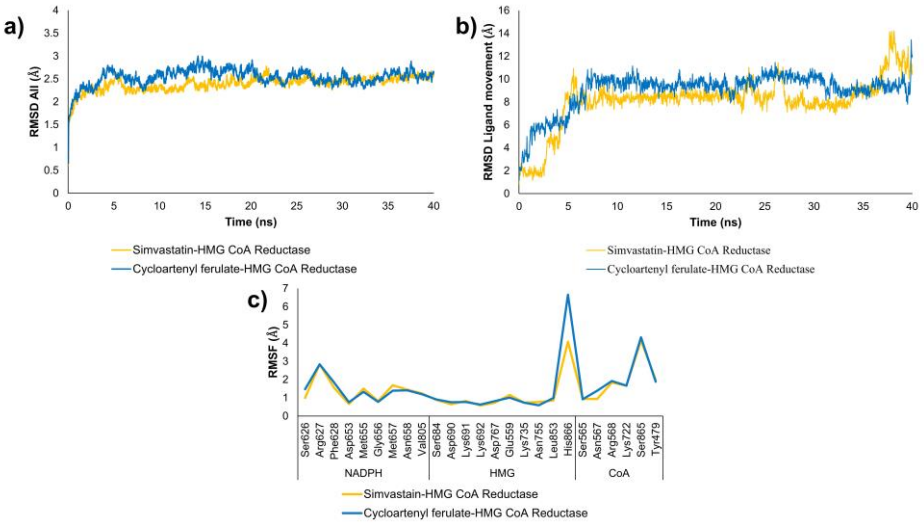

Figure 4. Molecular dynamics simulation result for cycloartenyl ferulate and simvastatin on HMG-CoA reductase. a) RMSD All, b) RMSD Ligand movement, c) RMSF.

Discussion

Cholesterol biosynthesis is an important pathway that controls intracellular cholesterol synthesis and extracellular cholesterol transport in the body (Litvinov et al., 2018). Dysregulation of cholesterol biosynthesis can lead to several pathological disorders, especially hypercholesterolemia (Marcuzzi et al., 2015). Recent studies have highlighted the significant potential of HMG-CoA reductase as one of the most reported mechanisms of bioactive compound from nutrition food to decrease cholesterol levels close to normal conditions (Su et al., 2024). By inhibiting HMG-CoA reductase through inhibition of HMG-CoA reductase, the production of mevalonate as a cholesterol precursor can be suppressed This, in turn, triggers downregulated downstream enzyme cholesterol biosynthesis (Zhang et al., 2021).

γ-Oryzanol is an active compound that is considered one of the most effective antioxidants. Derivatives of CAF, 24-MCF, β-SF, and ferulic acid (metabolic products of γ-oryzanol) show strong free radical scavenging from lipid peroxidation comparable to tocopherol (Zduńska et al., 2018). Under its strong antioxidant properties, γ-oryzanol may exert its cholesterol-lowering effects by targeting reduction-oxidation enzymes, such as HMG-CoA reductase. This enzyme is pivotal in the cholesterol biosynthesis pathway and contributes to increased plasma LDL-cholesterol levels. Based on the docking result, the ferulic acid component of γ-oryzanol on the hydroxyl group (-OH) hydrogen interacted with the binding pocket residue of HMG-CoA reductase. Ferulic acid, a component of γ-oryzanol, possesses a CH=CH-COOH group that enhances its antioxidant activity. The hydroxyl group (-OH) on the phenolic ring, along with the electron delocalization, plays a crucial role in the antioxidant activity of γ-oryzanol (Minatel et al., 2016). γ-Oryzanol is predicted to interact directly and inhibit by blocking the active site of HMG-CoA reductase. This *in silico* study showed that γ-oryzanol derivatives interacted at key residues Val805, Met655, Phe628, and Asn658 in the S-domain, which is the binding pocket of NADPH in Figure 3 and Table 2. The compound cyanidin-3-O-glucoside was found to interact with three crucial amino acid residues of HMG-CoA reductase: Asn658, Lys691, and Asp767 (Fatchiyah et al., 2020). The interaction triggers a change in enzyme conformation that closes the binding pocket of other active sites (Gesto et al., 2020).

CAF is the most abundant derivative in brown rice compared to other γ-oryzanol derivatives (Wu et al., 2023). CAF has antioxidant abilities that can protect neuronal cells, inhibit alpha-glucosidase and amylase, and induce apoptosis (Sari et al., 2021; Liu et al., 2021 ; Yasuda et al., 2019). Analysis of the RMSD values demonstrated that the inhibition of HMG-CoA reductase was stable (Figure 4). This stable inhibition by CAF might decrease intracellular cholesterol levels. Low cholesterol will activate the transcription factor SREBP-2, increasing LDL receptor (LDLR) expression. This indirectly takes up and reduces LDL cholesterol from blood plasma (Hwang et al., 2017). Enhancing LDLR expression can lead to lowering LDL-cholesterol levels in blood plasma. However, the bioavailability value of γ-oryzanol is relatively low (Malik et al., 2018). This decreases the permeability of γ-oryzanol across the target cell membrane (Khan & Singh, 2016). Previous study found that low bioavailability improved by formulating chitosan-mediated γ-oryzanol nanoparticles in novel drug delivery systems (Rawal et al., 2018). Chitosan nanoparticles allow them to interact with cell membranes more efficiently, which increases the membrane permeability (Aibani et al., 2021). Despite being evaluated based on their pharmacokinetics profiles, the side effects of γ-oryzanol and its derivatives are still unknown. However, a prior preliminary research suggests that γ-oryzanol may have fewer side effects than simvastatin (Ali & Devarajan, 2017). There were no observed side effects with applying γ-oryzanol at 1000 and 2000 mg/kgBW per day (Sulaiman et al., 2021). With this limitations, future research is needed to evaluated the optimum dosage and bioavailability in treating hypercholesterolemia.

Conclusion

This in-silico study concluded that γ-oryzanol derivatives from brown rice, specifically cycloartenyl ferulate, were identified as lowering cholesterol inhibitor agents from brown rice which blocked stable interaction of NADH binding domain of HMG-CoA reductase. Cyloartenyl ferulate was found to stably block the NADPH binding site of HMG-CoA reductase, thus potentially preventing the enzyme from catalyzing further cholesterol biosynthesis.

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References

Abbasi, S., Khan, A., & Choudhry, M. W. (2024). New Insights Into the Treatment of Hyperlipidemia: Pharmacological Updates and Emerging Treatments. *Cureus*, *16*(6). https://doi.org/10.7759/cureus.63078

Aibani, N., Rai, R., Patel, P., Cuddihy, G., & Wasan, E. K. (2021). Chitosan nanoparticles at the biological interface: implications for drug delivery. *Pharmaceutics*, *13*(10), 1686. https://doi.org/10.3390/pharmaceutics13101686

Ali, A., & Devarajan, S. (2017). Nutritional and health benefits of rice bran oil. *Brown Rice*, 135–158. https://doi.org/10.1007/978-3-319-59011-0_9

Amirus, K., Muhani, N., Sari, F. E., Saputri, F. A., & Terta, R. L. (2024). Counseling And Screening for Risk Factors for Hypercholesterolemia in Class II A Women's Correctional Institutions Bandar Lampung City in 2024. *Abdi Dosen: Jurnal Pengabdian Pada Masyarakat*, *8*(1), 311–318. https://doi.org/10.32832/abdidos.v8i1.2255

Benito-Vicente, A., Uribe, K. B., Jebari, S., Galicia-Garcia, U., Ostolaza, H., & Martin, C. (2018). Familial hypercholesterolemia:

the most frequent cholesterol metabolism disorder caused disease. *International Journal of Molecular Sciences*, *19*(11), 3426. https://doi.org/10.3390/ijms19113426

Chen, Y., Ku, H., Zhao, L., Wheeler, D. C., Li, L.-C., Li, Q., Varghese, Z., Moorhead, J. F., Powis, S. H., & Huang, A. (2014). Inflammatory stress induces statin resistance by disrupting 3-hydroxy-3-methylglutaryl-CoA reductase feedback regulation. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *34*(2), 365–376. https://doi.org/10.1161/ATVBAHA.113.301301

Crismaru, I., Pantea Stoian, A., Bratu, O. G., Gaman, M.-A., Stanescu, A. M. A., Bacalbasa, N., & Diaconu, C. C. (2020). Low-density lipoprotein cholesterol lowering treatment: the current approach. *Lipids in Health and Disease*, *19*, 1–10. https://doi.org/10.1186/s12944-020-01275-x

Fatchiyah, F., Meidinna, H. N., & Suyanto, E. (2020). The cyanidin-3-O-glucoside of Black Rice inhibits the interaction of HMG-CoA and HMG-CoA Reductase: three-and two-dimension structure. *Journal of Physics: Conference Series*, *1665*(1), 12005. https://doi.org/10.1088/1742-6596/1665/1/012005

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. https://doi.org/10.3390/molecules25173891

Hwang, K.-A., Hwang, Y.-J., & Song, J. (2017). Cholesterol-lowering effect ofAralia elata (Miq.) Seemvia the activation of SREBP-2 and the LDL receptor. *Journal of the Chinese Medical Association*, *80*(10), 630–635. https://doi.org/10.1016/j.jcma.2017.06.007

Ivanović, V., Rančić, M., Arsić, B., & Pavlović, A. (2020). Lipinski's rule of five, famous extensions and famous exceptions. *Popular Scientific Article*, *3*(1), 171–177. https://doi.org/10.46793/ChemN3.1.171I

Jiang, S.-Y., Li, H., Tang, J.-J., Wang, J., Luo, J., Liu, B., Wang, J.-K., Shi, X.-J., Cui, H.-W., & Tang, J. (2018). Discovery of a potent HMG-CoA reductase degrader that eliminates statin-induced reductase accumulation and lowers cholesterol. *Nature Communications*, *9*(1), 5138. https://doi.org/10.1038/s41467-018-07590-3

Jukema, J. W., Cannon, C. P., de Craen, A. J. M., Westendorp, R. G. J., & Trompet, S. (2012). The controversies of statin therapy: weighing the evidence. *Journal of the American College of Cardiology*, *60*(10), 875–881. https://doi.org/10.1016/j.jacc.2012.07.007

Khan, A. D., & Singh, L. (2016). Various techniques of bioavailability enhancement: a review. *Journal of Drug Delivery and Therapeutics*, *6*(3), 34–41. https://doi.org/10.22270/jddt.v6i3.1228

Kong, L., Lin, Y., Liang, J., Hu, X., Ashraf, U., Guo, X., & Bai, S. (2022). Dynamic Changes in Vitamin E Biosynthesis during Germination in Brown Rice (*Oryza sativa* L.). *Foods*, *11*(20), 3200. https://doi.org/10.3390/foods11203200

Lemus, C., Angelis, A., Halabalaki, M., & Skaltsounis, A. L. (2014). γ-Oryzanol: An attractive bioactive component from rice bran. In *Wheat and rice in disease prevention and health*: Elsevier, 409-430. https://doi.org/10.1016/B978-0-12-401716-0.00032-5

Lesma, G., Luraghi, A., Bavaro, T., Bortolozzi, R., Rainoldi, G., Roda, G., Viola, G., Ubiali, D., & Silvani, A. (2018). Phytosterol and γ-oryzanol conjugates: synthesis and evaluation of their antioxidant, antiproliferative, and anticholesterol activities. *Journal of Natural Products*, *81*(10), 2212–2221. https://doi.org/10.1021/acs.jnatprod.8b00465

Litvinov, D. Y., Savushkin, E. V, & Dergunov, A. D. (2018). Intracellular and plasma membrane events in cholesterol transport and homeostasis. *Journal of Lipids*, *2018*(1), 3965054. https://doi.org/10.1155/2018/3965054

Liu, C., Xi, X., Liu, Y., Lu, Y., Che, F., Gu, Y., Yu, Y., Li, H., Liu, J., & Wei, Y. (2021). Isolation of four major compounds of γ-oryzanol from rice bran oil by ionic liquids modified high-speed countercurrent chromatography and antimicrobial activity and neuroprotective effect of cycloartenyl ferulate in vitro. *Chromatographia*, *84*(7), 635–644. https://doi.org/10.1007/s10337-021-04044-9

Luo, H., Mattes, W., Mendrick, D. L., & Hong, H. (2016). Molecular docking for identification of potential targets for drug repurposing. *Current Topics in Medicinal Chemistry*, *16*(30), 3636–3645. https://doi.org/10.2174/1568026616666160530181149

Malik, A.-Q., Hailat, W., Bouchekara, H. R. E. H., & Javaid, M. S. (2018). Gamma oryzanol loaded microspheres with improved bioavailability. *African Journal of Pharmacy and Pharmacology*, *12*(17), 202–207. https://doi.org/10.5897/AJPP2018.4914

Marcuzzi, A., Piscianz, E., Loganes, C., Vecchi Brumatti, L., Knowles, A., Bilel, S., Tommasini, A., Bortul, R., & Zweyer, M. (2015). Innovative target therapies are able to block the inflammation associated with dysfunction of the cholesterol biosynthesis pathway. *International Journal of Molecular Sciences*, *17*(1), 47. https://doi.org/10.3390/ijms17010047

Maruyama, Y., Igarashi, R., Ushiku, Y., & Mitsutake, A. (2023). Analysis of protein folding simulation with moving root mean square deviation. *Journal of Chemical Information and Modeling*, *63*(5), 1529–1541. https://doi.org/10.1021/acs.jcim.2c01444

Moore II, B. M., & Cook, G. A. (2022). Medicinal chemistry and pharmacology of statins. In *Cholesterol*: Academic Press, 903-926.. https://doi.org/10.1016/B978-0-323-85857-1.00012-2

Palis, C. N., Safitri, A., Wijayanti, E. D., & Fatchiyah, F. (2023). Virtual prediction of Brown Rice Gamma Oryzanol as anti-inflammation via COX-1 and COX-2 inhibitions. *Berkala Penelitian Hayati*, *29*(2), 57–66. https://doi.org/10.23869/bphjbr.29.2.20233

Phunikhom, K., Sattayasai, J., Tiamkao, S., & Gaysonsiri, D. (2021). A Randomized, Double Blind Clinical Study to Assess the Effects of a Gamma-oryzanol-enriched Rice Bran Oil on Lipid Profile in the Hypercholesterolemic Patients. *Journal of the Medical Association of Thailand*, *104*. https://doi.org/10.35755/jmedassocthai.2021.S01.12239

Ramazani, E., Akaberi, M., Emami, S. A., & Tayarani-Najaran, Z. (2021). Biological and pharmacological effects of gamma-oryzanol: An updated review of the molecular mechanisms. *Current Pharmaceutical Design*, *27*(19), 2299–2316. https://doi.org/10.2174/1381612826666201102101428

Ravichanthiran, K., Ma, Z. F., Zhang, H., Cao, Y., Wang, C. W., Muhammad, S., Aglago, E. K., Zhang, Y., Jin, Y., & Pan, B. (2018). Phytochemical profile of brown rice and its nutrigenomic implications. *Antioxidants*, *7*(6), 71. https://doi.org/10.3390/antiox7060071

Rawal, T., Mishra, N., Jha, A., Bhatt, A., Tyagi, R. K., Panchal, S., & Butani, S. (2018). Chitosan nanoparticles of gamma-oryzanol: Formulation, optimization, and in vivo evaluation of anti-hyperlipidemic activity. *Aaps Pharmscitech*, *19*, 1894–1907. https://doi.org/10.1208/s12249-018-1001-8

Reiner, Ž. (2014). Resistance and intolerance to statins. *Nutrition, Metabolism and Cardiovascular Diseases*, *24*(10), 1057–1066. https://doi.org/10.1016/j.numecd.2014.05.009

Sari, D. R. T., Paemanee, A., Roytrakul, S., Cairns, J. R. K., Safitri, A., & Fatchiyah, F. (2021). Black rice cultivar from Java Island of Indonesia revealed genomic, proteomic, and anthocyanin nutritional value. *Acta Biochimica Polonica*, *68*(1), 55–63. https://doi.org/10.18388/abp.2020_5386

Schumacher, M. M., & DeBose-Boyd, R. A. (2021). Posttranslational regulation of HMG CoA reductase, the rate-limiting enzyme in synthesis of cholesterol. *Annual Review of Biochemistry*, *90*(1), 659–679. https://doi.org/10.1146/annurev-biochem-081820-101010

Su, X., Zhang, L., Hu, K., An, Y., Zhang, Q., Tang, J., Yan, B., Li, X., Cai, J., & Li, X. (2024). Discovery of Natural Potent HMG-CoA Reductase Degraders for Lowering Cholesterol. *Angewandte Chemie International Edition*, *63*(6), e202313859. https://doi.org/10.1002/anie.202313859

Sulaiman, A., Sulaiman, A., Sert, M., Khan, M. S. A., & Khan, M. A. (2021). Functional and Therapeutic Potential of γ-Oryzanol. *Functional Foods: Phytochemicals and Health Promoting Potential*, *259*. https://doi.org/10.1002/anie.202313859

Szcześniak, K. A., Ostaszewski, P., Ciecierska, A., & Sadkowski, T. (2016). Investigation of nutriactive phytochemical–gamma-oryzanol in experimental animal models. *Journal of Animal Physiology and Animal Nutrition*, *100*(4), 601–617. https://doi.org/10.1111/jpn.12428

Szklarczyk, D., Gable, A. L., Nastou, K. C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N. T., Legeay, M., Fang, T., & Bork, P. (2021). The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Research*, *49*(D1), D605–D612. https://doi.org/10.1093/nar/gkab835

Valko, K., Butler, J., & Eddershaw, P. (2013). Predictive approaches to increase absorption of compounds during lead optimisation. *Expert Opinion on Drug Discovery*, *8*(10), 1225–1238. https://doi.org/10.1517/17460441.2013.815613

Widyananda, M. H., Wicaksono, S. T., Rahmawati, K., Puspitarini, S., Ulfa, S. M., Jatmiko, Y. D., Masruri, M., & Widodo, N. (2022). A potential anticancer mechanism of finger root (Boesenbergia rotunda) extracts against a breast cancer cell line. *Scientifica*, *2022*. https://doi.org/10.1155/2022/9130252

Widyananda, M. H., Pratama, S. K., Ansori, A. N. M., Antonius, Y., Kharisma, V. D., Murtadlo, A. A. A., Jakhmola, V., Rebezov, M., Khayrullin, M., Derkho, M., Ullah, E., Susilo, R. J. K., Hayaza, S., Nugraha, A. P., Proboningrat, A., Fadholly, A., Sibero, M. T., & Zainul, R. (2023a). Quercetin as an anticancer candidate for glioblastoma multiforme by targeting AKT1, MMP9, ABCB1, and VEGFA: an *in silico* study. *Karbala International Journal of Modern Science*, *9*(3), 10. https://doi.org/10.33640/2405-609X.3312

Widyananda, M. H., Kurniasari, C. A., Alam, F. M., Rizky, W. C., Dings, T. G. A., Ansori, A. N. M., & Antonius, Y. (2023b). Exploration of potentially bioactive compounds from Fingerroot (*Boesenbergia rotunda* L.) as inhibitor of atherosclerosis-related proteins (CETP, ACAT1, OSC, sPLA2): an *in silico* study. *Jordan Journal of Pharmaceutical Sciences*, *16*(3), 550-564. https://doi.org/10.35516/jjps.v16i3.1609

Widyananda, M. H., Kurniasari, C. A., Alam, F. M., Rizky, W. C., Dings, T. G. A., Ansori, A. N. M., & Antonius, Y. (2023c). Exploration of potentially bioactive compounds from Fingerroot (*Boesenbergia rotunda* L.) as inhibitor of atherosclerosis-related proteins (CETP, ACAT1, OSC, sPLA2): an *in silico* study. *Jordan Journal of Pharmaceutical Sciences*, *16*(3), 550-564. https://doi.org/10.35516/jjps.v16i3.1609Wu, H., Nakamura, T., Guo, Y., Matsumoto, R., Munemasa, S., Murata, Y., & Nakamura, Y. (2023). Cycloartenyl ferulate is the predominant compound in brown rice conferring cytoprotective potential against oxidative stress-induced cytotoxicity. *International Journal of Molecular Sciences*, *24*(1), 822. https://doi.org/10.3390/ijms24010822

Yasuda, S., Sowa, Y., Hashimoto, H., Nakagami, T., Tsuno, T., & Sakai, T. (2019). Cycloartenyl ferulate and β-sitosteryl ferulate-steryl ferulates of γ-oryzanol-suppress intracellular reactive oxygen species in cell-based system. Journal of Oleo Science, 68(8), 765-768. https://doi.org/10.5650/jos.ess19054

Zduńska, K., Dana, A., Kolodziejczak, A., & Rotsztejn, H. (2018). Antioxidant properties of ferulic acid and its possible application. *Skin Pharmacology and Physiology*, *31*(6), 332–336. https://doi.org/10.1159/000491755

Zhang, C., Jin, D.-D., Wang, X.-Y., Lou, L., & Yang, J. (2021). Key enzymes for the mevalonate pathway in the cardiovascular system. *Journal of Cardiovascular Pharmacology*, *77*(2), 142–152. https://doi.org/10.1097/FJC.0000000000000952

Zhao, Z., Zhong, L., He, K., Qiu, C., Li, Z., Zhao, L., & Gong, J. (2019). Cholesterol attenuated the progression of DEN-induced hepatocellular carcinoma via inhibiting SCAP mediated fatty acid de novo synthesis. *Biochemical and Biophysical Research Communications*, *509*(4), 855–861. https://doi.org/10.1016/j.bbrc.2018.12.181