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Research Article

Anticholesterol activity of ethanol extract of Ranti Hitam (*Solanum blumei* Nees ex Blume) Leaves: In vivo and In silico study

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Abstract

Ranti Hitam known as local name of (*Solanum blumei* Nees ex Blume) found in the Dairi, Sumatera Utara, Indonesia. It is used by the community as traditional medicine which contains of various phytochemical constituent of steroidal alkaloids of β2-solanin, diosgenin, flavonoids, saponins and tannins. The purpose of the study was to investigate the anticholesterol activity of the ethanol extract of (*Solanum blumei* Nees ex Blume) by in vivo and insilico methods. A number of 15 rats were divided into 5 treatment groups as in vivo high fat diet model, otherwise insilico study was carried out to determine the activity of main compound of *S. blumei* in inhibiting HMG Co-A reductase. The bioactive compounds of *S. blumei*, diosgenin ($C_{26}H_{39}O_4$) and β2-solanine ($C_{39}H_{63}NO_{11}$) showed inhibition activity to HMG-CoA reductase by in silico and invivo test and it was indicated that 2 bioactive compounds of *S. blumei* had anticholesterol activity.

Keywords

Anticholesterol, *Solanum blumei* Nees ex Blume, In vivo, In silico

Introduction

Hyperlipidemia can lead to a variety of health problems, including atherosclerosis, coronary artery disease, and high blood pressure (Alloubani et al. 2021). Hypercholesterolemia is a condition in which blood cholesterol levels surpass the usual range (Aljhenedil et al. 2018). Hypercholesterolemia is caused by an increase in LDL cholesterol and a reduction in HDL cholesterol (Ghasi et al. 2002; Ugwu et al. 2013). One example is consuming high fat diet such as quail egg yolk and lard at the same time promotes hypercholesterolemia , because it is a source of exogenous cholesterol and triglycerides. Reducing blood LDL concentrations and increasing HDL concentrations is one technique for preventing or delaying the onset of chronic hypercholesterolemia-related diseases in humans (Olantunji et al. 2005; Ezekwesili et al. 2008). The atherogenic index is a risk indicator for atherosclerosis, which is the leading cause of coronary heart disease. The plasma atherogenic index is a sensitive criterion for assessing cardiovascular high-risk individuals (Su Bo et al. 2018; Yuksel et al. 2018; Li et al. 2021). Some of Indonesia's abundant natural plant resources, such as tamarind leaves (*Tamarindus indica* Linn) (Assagaf et al. 2015), sirsak leaves (*Annova muricata* L) (Uneputty et al. 2013), temulawak rhizomes (*Curcuma xanthorhiza*) (Aznam and Atun 2016), have been used as an alternative medication to treat hyperlipidemia.

According Simorangkir et al. (2016), they detected a steroidal glycoside alkaloid molecule β2-solanine ($C_{39}H_{63}NO_{11}$) in the ethanol extract of *S.blumei* fruit, as well as a steroidal sapogenin compound generated from diosgenin $C_{26}H_{39}O_4$ in the leaf extract of *S. blumei* (Simorangkir et al. 2016). The phytosterol molecule β2-solanine is a member of this category. Phytosterol compounds can reduce blood cholesterol by lowering cholesterol solubility in the oil phase and blocking bile acid reabsorption. Flavonoids could lower blood cholesterol by blocking the HMG Co-A reductase enzyme (Sekhon 2012). This enzyme increases cholesterol-7-hydroxylase, which converts cholesterol to bile acids, and prevents cholesterol absorption from the digestive tract (Gaamoussi et al. 2010). *S. blumei* leaves ethanol extract demonstrated stronger antioxidant activity than ethyl acetate and n-hexane extract, as well as immunostimulant activity (Simorangkir et al. 2013a, b). In addition, antioxidants play a function in preventing the growth of cholesterol in the body, hence *S.blumei* leaves have the potential to act as anti-cholesterol agents.

In this study the anticholesterol activity of ethanol extract of *S.blumei* leaves were evaluated by determine the atherogenic index of hypercholesterolemic male white rats (*Rattus novegicus*) that induced by a high-fat diet, and also to determine the potential inhibition activity to HMG-CoA reductase enzyme by in silico analysis using 2 compound of *S.blumei*, that are β2-solanine and steroidal sapogenin derivatives from the diosgenin $C_{26}H_{39}O_4$ plant *S. blumei*. The inhibition test of the HMG-CoA reductase enzyme by the plant bioactive compound *S.blumei* was carried out by molecular docking procedure.

Materials and methods

Materials

The tools used are rotary evaporator (Heidolph), blender, analytical balance, vacuum pump, refrigerator, centrifuge, oral sonde, NGT (Naso Gastric Tube) size 5 mL (Terumo), syringe size 5 mL, 3 mL (Terumo), micropipette, microtube, hematocrit microcapillary, eppendorf tube, vortex (SBS), 3 mL vaculab, beaker (Pyrex) and spectrophotometer microlab 300 (Elitech). The chemicals used were n-hexane, ethyl acetate, ethanol (Merck) solvent and cholesterol, HDL, triglyceride test kit reagents (Dialab) and Na-CMC.

Plant collection and Identification

The plant samples were fresh leaves of *S. blumei* obtained from the village of Kuta Nangka, Dairi, North Sumatera Regency, Indonesia and has been determined by Herbarium Bogoriense, Botany Division of Biology Research Center, Bogor (Identification No: 546/IPH.1.02 /1f.8/III/2013).

Animal model

The animal model used were male white rats (*Rattus novergicus*) Wistar strain, aged about 2–3 months, weighing about 180–200 g, which were obtained from the USU Biology Laboratory. Rats were kept in a typical room temperature and relative humidity setting with 12-hour light/dark cycles. The animals were fed a regular laboratory pellet diet (PC202) while drinking tap water. Prior to the trial, the tested animals were acclimatized for one week. This study was approved by the Animal Research Ethics Committees (AREC), FMIPA, University of North Sumatra, No. 0350/KEPH-FMIPA/2016.

Extraction procedure

Fresh leaves (5.01 kg) were dried at room temperature and ground into a 60 mesh powder (434.23 g). Leaf powder (430.00 g) were macerated for 48 hours with three replications in a solvent having polarity, specifically n-hexane, followed by ethylacetate, and finally ethanol (Simorangkir et al. 2017). At a temperature of 50 °C, the maceration results were concentrated using a vacuum rotary evaporator.

High fat diet preparation

The high-fat diet feed given was a mixture of lard and quail egg yolk (1: 5) and 0.1% propylthiouracil (PTU) (Assagaf 2015).

Treatment regime

At the first day, rats were divided into 5 groups, each group consisted of 3 rats. On day 8, blood was taken and total cholesterol, HDL and serum triglyceride levels for initial data were measured. Furthermore, rats were induced hypercholesterolemic with a high-fat diet and PTU as much as 2 mL/day by sonde for 14 days, except for the normal control group (K0). On day 22, The blood were collected, and the levels of cholesterol, HDL and serum triglycerides were measured. Furthermore, the rats were given simvastatin 1.25 mg/kg body weight as much as 1 mL/day as positive control (group K1), ethanol extract of *S. blumei* leaves 100 mg/kg BW (group K2), extract 200 mg/kg BW (group K3) and extract 300 mg/kg BW (K4 group) and 0.1% Na-CMC (K0), by orally as much as 1 mL/day, for 14 days. On the 36th day, the rat's blood were collected and the serum cholesterol, HDL and triglyceride levels were measured. Determination of total serum cholesterol levels, HDL-cholesterol and triglycerides were carried out using the colorimetric enzymatic method (Trinder 1969; Iyamu et al. 2014; Dialab 2016) The high-fat diet feed given was a mixture of lard and quail egg yolk (1: 5) and 0.1% propylthiouracil (PTU) (Assagaf 2015).

Atherogenic index

The atherogenic index is an indicator to determine the risk of atherosclerosis, the main cause of coronary heart disease. The atherogenic index is calculated using the formula (Purukan et al. 2019):

$$\text{Atherogenic Index} = \frac{\text{Total Cholesterol} - \text{HDL}}{\text{HDL}}$$

HMG Co-A reductase inhibition test In silico by *Solanum blumei* bioactive compound

Previous research has demonstrated that the ethanol extract of *S. blumei* fruit (*Solanum blumei* Nees ex Blume) contains the steroidal glycoside alkaloid compound β2-solanine ($C_{39}H_{63}NO_{11}$) (Simorangkir et al. 2016) and a steroidal sapogenin compound derived from diosgenin $C_{26}H_{39}O_4$ (PATENT) IDP0000448080, 2017). As a result, an insilico study was conducted to ascertain the mechanism by which the active compound in *S. blumei* (*Solanum blumei* Nees ex Blume) inhibits the HMG-CoA reductase enzyme. The molecular docking process begins with the preparation of the HMG-CoA reductase enzyme's three-dimensional structure, optimization of the test compound's three-dimensional structure, validation of the molecular docking method, and docking of the test compound to the HMG-CoA reductase enzyme using the AutoDockTools-1.5.6 and AutoDock vina 1 1 2 software. The data were analyzed using bond energy parameters. The lower the bond energy between the test compound and the enzyme, the stronger and more stable the bond is.

Data analysis

The data obtained were in the form of mean and standard deviation (M ± SD) and were analyzed by one-level Analysis of Variance (ANOVA), followed by LSD test to see if there was a significant effect between groups with a significance of 0.05.

Results and discussion

Lipid profile assessment

The average lipid profile was shown three times, namely the initial period before a high-fat diet, after the induction of a high-fat diet and after administration of the ethanol extract of *S. blumei* (Table 1).

Data expressed as Mean ± SD, n=3. Different superscripts in the same vertical row showed significant differences ($P < 0.05$). The coefficient of diversity (CD) cholesterol 3.02%; CD LDL 25.30%; CD HDL 2.63%; CD triglycerides 2.93%.

The results of this study showed that the administration of ethanol extract of *S. blumei* leaves to hypercholesterolemic rats for 14 days of treatment, caused changes in the lipid profile of rat serum (Table 1). the administration of ethanol extract of *S. blumei* leaves had a very significant effect on rat serum cholesterol levels ($p<0.01$), HDL levels ($p<0.01$), LDL levels ($p<0.01$) and triglyceride levels ($p<0.01$) compared to with the control class (Table 1). Administration of ethanol extract of *S. blumei* leaves at dose of 200 mg/kg BW (K3) caused rat serum cholesterol at level of 77.33±3.21 mg/dL and LDL levels of 0.46±0.30 mg/dL which were the lowest

Table 1. Result of lipid profile assessment.

Groups	Period	Serum Lipid Profile (mg/dL)			
		Cholesterol	Triglycerides	HDL	LDL
K0 (Normal control, without high-fat diet and extract)	Initial (1)	105.33±3.5	86.66±1.52	72.00±2.00	17.00±1.50
	High Fat Diet Induction (2)	116.66±1.52	87.33±1.22	78.66±3.21	18.53±1.50
	Extract Administration (3)	114.00±1.00[a]	85.66±2.07[a]	79.66±1.52[a]	17.20±1.48[a]
	Percentage Change (%) (Period 2-3)	2.27±1.20	1.90±2.11	1.34±1.88	12.84 ± 7.9
K1 (Positive control, Simvastatin 0.25 mg/200 g BW)	Initial (1)	96.00±4.00	82.33±1.52	64.66±2.51	14.86±1.21
	High Fat Diet Induction (2)	156.00±4.58	56.00±2.00	43.00±2.00	101.46±2.11
	Extract Administration (3)	92.33±3.05[b]	82.00±2.64[a]	74.66±1.52[b]	0.26±0.11[b]
	Percentage Change (%) (Period 2-3)	40.80±1.20	46.44±2.88	73.77±4.61	99.73±0.10
K2 (Ethanol extract of *S. blumei* 100 mg/Kg BW)	Initial (1)	97.00±7.00	81.00±4.00	66.66±5.50	14.13±0.74
	High Fat Diet Induction (2)	150.66±4.50	69.00±3.00	50.33±4.50	85.13±1.65
	Extract Administration (3)	97.66±3.21[c]	84.66±3.50[a]	77.00±1.00[a]	2.73±1.44[c]
	Percentage Change (%) (Period 2-3)	35.16±1.23	22.70±0.47	53.66±12.66	96.75±1.81
K3 (Ethanol extract of *S. blumei* 200 mg/Kg BW)	Initial (1)	89.66±5.5	75.00±2.00	58.66±2.51	15.66±2.66
	High Fat Diet Induction (2)	155.00±4.00	59.00±2.00	46.33±2.45	98.20±1.24
	Extract Administration (3)	77.33±3.21[d]	74.33±1.77[b]	62.33±2.50[c]	0.46±0.30[b]
	Percentage Change (%) (Period 2-3)	50.09±1.35	26.02±1.81	34.59±1.86	99.52±0.27
K4 (Ethanol extract of *S. blumei* 300 mg/Kg BW)	Initial (1)	88.33±4.72	80.33±2.51	61.00±2.00	9.93±1.36
	High Fat Diet Induction (2)	155.00±3.00	58.66±2.51	50.33±2.07	88.93±2.00
	Extract Administration (3)	88.66±3.05[b]	78.66±1.52[a]	70.33±2.51[b]	3.26±1.21[c]
	Percentage Change (%) (Period 2-3)	41.66±1.52	34.17±3.18	39.76±2.26	96.30±1.51

compared to the extract at dose of 100mg/kg BW and 300 mg/kg BW ($p < 0.05$).

The percentage of changes in serum lipid levels after administration of *S. blumei* leaf ethanol extract in hypercholesterolemic rats can be seen in Table 2.

Data expressed as mean ± SD, n=3. The coefficient of diversity (CD) of cholesterol reduction was 19.99%. CD LDL reduction 8.90%; CD HDL increase 15.45%; CD Triglyceride increase 9.29%. Different superscripts in the same vertical row, showed significant differences ($P<0.05$)

The administration of ethanol extract of *S. blumei* leaves for 14 days of treatment in hypercholesterolemic rats had a significant effect on reducing cholesterol, LDL levels and increasing HDL levels and serum triglyceride levels in rats ($p < 0.01$). The administration of ethanol extract of *S. blumei* leaves 200mg/Kg BW (K3) resulted in the highest decrease in serum cholesterol levels (50.09±1.35%), followed by *S. blumei* ethanol extract 300mg/Kg BW (41.66±1.52%) and ethanol extract of *S. blumei*.100mg/Kg BW (35.16 ±1.23%) ($P<0.01$). The administration of simvastatin caused a decrease in cholesterol levels (40.80±1.20%) which was not significantly different from the administration of ethanol extract of *S. blumei* 300mg/KgBW (40.80±1.20%) ($P> 0.01$), furthermore200mg/KgBW (K3) resulted in a decrease in serum LDL levels by 99.52±0.27%, an increase in HDL levels by 34.59±1.86% and an increase in serum triglyceride levels by 26.02±1.81% towards normal levels.

Table 2. Percentage of changes in serum lipids after administration of *S. blumei* extract.

Groups	Mean Value Percentage Change in Serum Lipid After Extarct Administration			
	Decreasing percentage of Cholesterol (%)	Decreasing percentage of LDL (%)	Increasing percentage of HDL (%)	Increasing percentage of Triglyserida
K0	2.27±1.20[a]	12.84±7.90[a]	1.34±1.88[a]	1.90±2.11[a]
K1	40.80±1.20[b]	99.73±0.10[b]	73.77±4.61[b]	46.44±2.88[b]
K2	35.16 ±1.23[c]	96.75±1.81[b]	53.66±12.66[c]	22.7±0.47[c]
K3	50.09±1.35[d]	99.52±0.27[b]	34.59±1.86[d]	26.02±1.81[c]
K4	41.66±1.52[b]	96.3±1.51[b]	39.76±2.26[d]	34.17±3.18[d]

Atherogenic index

The atherogenic index of rats in the early stages, after being given a high-fat diet and after giving the extract is presented in Table 3. The atherogenic index is an indicator to determine the risk of atherosclerosis, the main cause of coronary heart disease.

After rats were induced by high-fat diet, there was an increase in the atherogenic index of rats (Table 3). However, after being given *S. blumei* extract, the atherogenic index of rats decreased. The lowest atherogenic index of rats was at a dose of K3, 200 mg/kg (0.24), followed by K2, 100 mg/kg (0.26) and K4, 300 mg/kg (0.26) extracts. The atherogenic index of rats at a dose of 200 mg/kg was the same as the administration of simvastatin. The highest decrease in the atherogenic index of rats were in simvastatin administration, K1(90.87%), followed by 200 mg/kgBW extract, K3 (89.74%), 300 mg/kgBW extract, K4 (87.43%), 100 mg/kgBW extract, K2 (86.93%) and normal control, K0 (10%). Plasma atherogenic index is a sensitive parameter that can be used to assess cardiovascular high-risk groups (Su Bo et al. 2018; Yuksel et al. 2018; Li et al. 2021). Individuals who have an atherogenic index value of less than 0.11 are classified as low risk of cardiovascular disease, when it between 0.11 and 0.24 are classified as moderate risk of heart disease and those more than 0.24 are classified as high risk of cardiovascular disease (Cameron 2021).

Table 3. Rat atherogenic index in early stage, induction of high fat diet and administration of *S. blumei* leaf extract.

Groups	Atherogenic Index			Reduction percentage in Atherogenic index (2–3)	
	Initial (1)	High fat diet induction (2)	Extract administration (3)		
K0	0.46	0.48	0.43	0.05	10%
K1	0.48	2.63	0.24	2.39	90.87%
K2	0.45	1.99	0.26	1.73	86.93%
K3	0.52	2.34	0.24	2.10	89.74%
K4	0.44	2.07	0.26	1.81	87.43%

The anticholesterol activity of the ethanol extract of *S. blumei* may be due to several mechanisms including inhibition of the cholesterol biosynthetic enzyme HMG-CoA reductase, stimulating the enzyme cholesterol-7-α-hydroxylase to convert cholesterol into bile acids and inhibition of cholesterol absorption from the intestine due to the formation of complexes with compounds such as glycosides and saponins (Gaamoussi et al. 2010).

The results from relevant research showed that the ethanol extract of bay leaf has potent activity to reduce serum cholesterol levels through inhibition of HMG-CoA reductase activity due to the presence of phenolic compounds in the extract as well as the antioxidant activity of the extract. The presence of -OH groups on the C3', C4', C5' and C=O groups on the C4 flavonoid structure will form hydrogen bonds with amino acids on the active site of the HMG-CoA reductase enzyme, which results in inhibited enzyme activity. This atomic group is thought to play a role in inhibiting the activity of the HMG-CoA reductase enzyme because it has similarities to the pharmacophore group of simvastatin. The -OH and C=O pharmacophore groups in simvastatin can form bonds with the HMG-CoA reductase enzyme so that the enzyme work is inhibited (Hartanti et al. 2019). Flavonoids can also reduce cholesterol synthesis by inhibiting the activity of the enzyme acyl-CoA cholesterol acyl transferase (ACAT) in HepG2 cells which play a role in reducing cholesterol esterification in the intestine and liver. Secondary metabolites of alkaloids, flavonoids, tannins and saponins contained in the ethanol extract of the leaves of *S. blumei* Nees ex Blume (Simorangkir et al. 2017) may cause a decrease in serum cholesterol in hypercholesterolemic rats after being given the ethanol extract of *S. blumei* leaves 200 mg/kg BW/day for 14 days.

Some of the chemicals identified as having hypocholesterolemic potential include phytosterols, saponins, flavonoids, tannins and water-soluble dietary fiber. The ethanol extract of the leaves of *S. blumei* contains a steroid sapogenin compound derived from disogenin ($C_{26}H_{39}O_4$) which has natural immunostimulant activity (Patent, IDP0000448080, 2017; Simorangkir et al. 2013b) and steroidal alkaloid glycoside β2-solanine or [Solanid-5-ene-(1'→3)-β-D-galactopyranoside-(1"→3')-β-D-glucopyranoside],($C_{39}H_{63}NO_{11}$) (Simorangkir et al. 2016). Solanum steroidal glycoalkaloid compounds have pharmacological activities such as anticholesterol. The carbohydrate side chains of solanum steroidal glycoalkaloids act as cholesterol binders. About 107 steroidal glycoalkaloid solanum have been identified from the Solanum plant (Zhao et al. 2021). In this study, the steroidal glycoside β2-solanine ($C_{39}H_{63}NO_{11}$) contained in *S. blumei* (Simorangkir et al. 2016) may bind cholesterol and cause a decrease in blood cholesterol. The two compounds β2-solanine ($C_{39}H_{63}NO_{11}$) and sapogenin ($C_{26}H_{39}O_4$) contained in this *S. blumei* plant (Simorangkir et al. 2016) belong to the group of phytosterol compounds. Phytosterols are sterols found in plants that can reduce plasma cholesterol by reducing the solubility of cholesterol in the oil phase and inhibiting the reabsorption of bile acids (Miras et al. 2016). Tannins are polyphenolic compounds that can form complexes with insoluble proteins. The two secondary metabolites β2-solanine and sapogenin tannins and saponins found in *S. blumei* have the activity of lowering blood cholesterol in rats induced by a high-fat diet.

HMG Co-A reductase inhibition test In silico by *Solanum blumei* bioactive compound

The ethanol extract of *S. blumei* fruit contains a steroidal glycoside alkaloid compound β2-solanine (Simorangkir et al. 2016) and a steroidal sapogenin derivative of disogenin ($C_{26}H_{39}O_4$) which has natural immunostimulant activity (Patent, IDP000048080, Simorangkir et al. 2013b; Simorangkir 2017). The depiction of the structure of the two molecules using the Discovery Studio application is shown in Figs 1, 2.

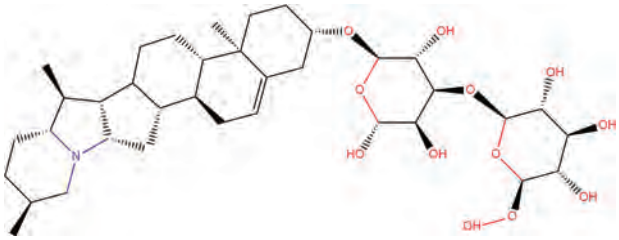

Figure 1. Molecule of steroid glycoside alkaloids β2-solanin, [Solanid-5-ene-(1'→3)-β-D-galactopyranosyl-(1" →3')-β-D-glucopyranoside] ($C_{39}H_{63}NO_{11}$) (Simorangkir et al. 2016) (Compound 1)

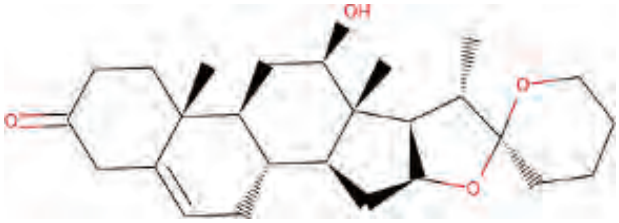

Figure 2. Diosgenin-derived steroid sapogenin molecule (2'R,6aR,8aS, 8bR, 9S,11aS)-8-hydroxy-6a,8a,9-trimethyl-1 ,3'4',5,5'6,6,6a,6b,6',7,8,8a,8b,9,11a,12a,12b-0ctadecahydro-spiro[naptho 2',1':4,5] indeno [2,1-b]furan-10,2'-pyran]-4(3H)-one. (Paten, IDP000048080, Simorangkir 2017) (Compound 2)

The results of in silico test in inhibition of HMG-CoA reductase enzyme by natural ligand compounds, steroidal glycoside alkaloids β2-solanine ($C_{39}H_{63}NO_{11}$) (compound 1) and steroidal sapogenin compounds derived from disogenin ($C_{26}H_{39}O_4$) (compound 2) from *S. blumei* are presented in Figs 3–5, Table 4.

Based on the results of molecular docking of compounds 1 and 2 to the HMG-CoA reductase receptor, it was found that the interaction between HMG-CoA reductase protein receptor and compound 1 &2 had a higher binding energy by -0.21 kcal/mol and -3.78 kcal /mol respectively compared to binding energy with natural ligands -5.23 kcal/mol (Table 4). As shown in Fig. 3, natural ligands had more bonds than compound 1 (Fig. 4) and compound 2 (Fig. 5). Natural ligands have 7 hydrogen bonds, whereas compounds 1 and 2 only have 2 hydrogen bonds each. Besides that, natural ligands also have other

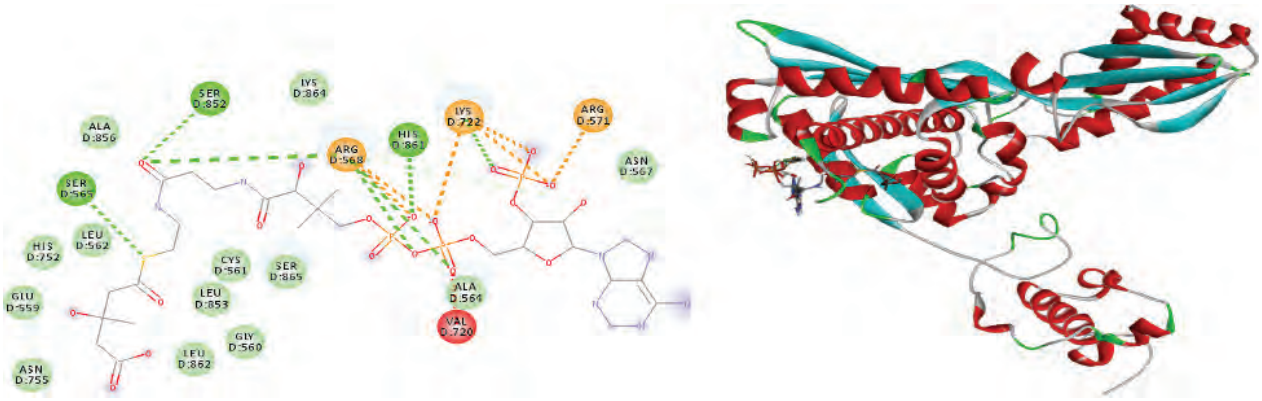

Figure 3. Natural Ligand Interaction with HMG-CoA Reductase

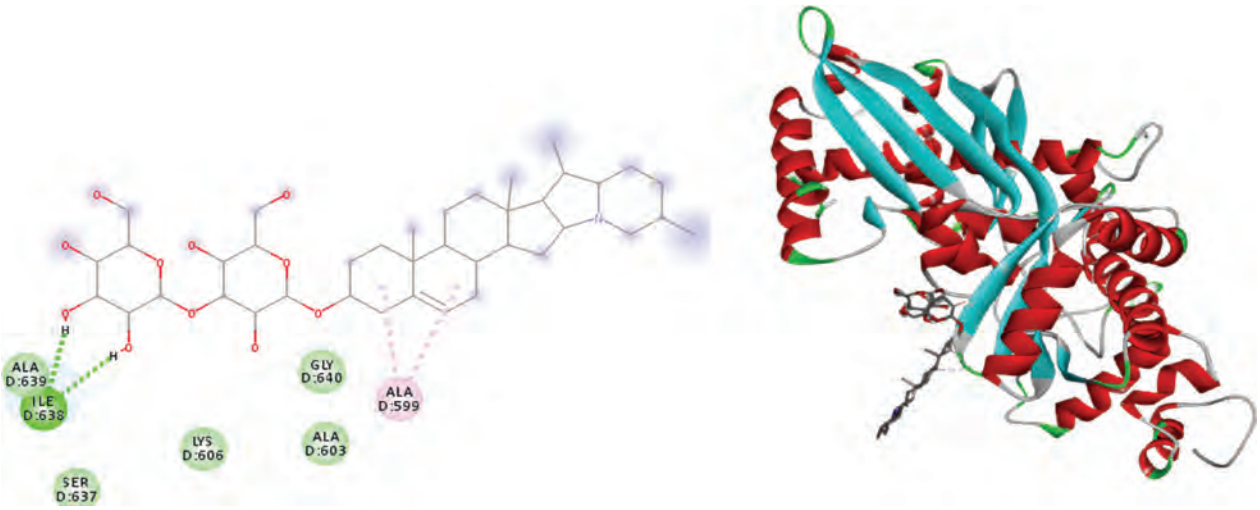

Figure 4. Interaction of Compound 1 (β2-Solanine) with HMG-CoA Reductase (Docking conformation of Compound 1 with HMG-CoA reductase protein)

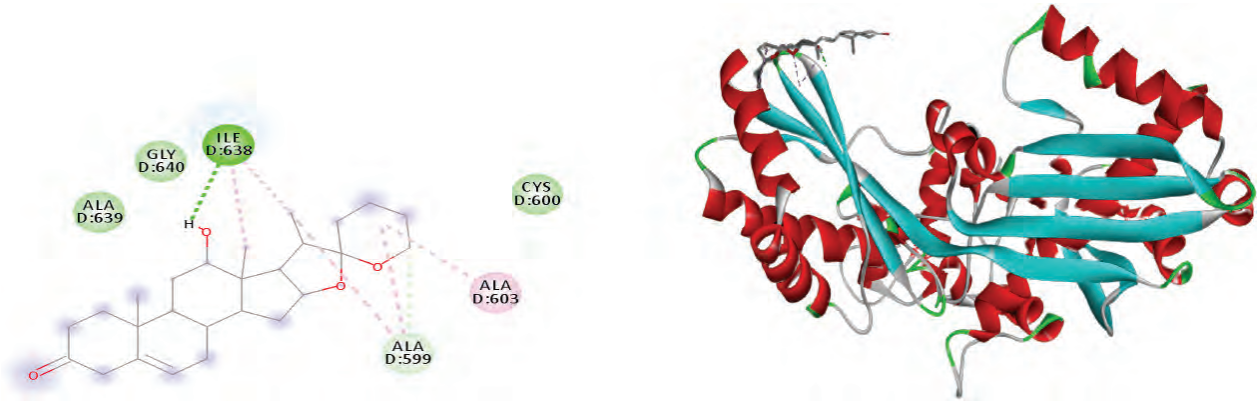

Figure 5. Interaction of Compound 2 (Diosgenin) with HMG-CoA Reductase

Table 4. Molecular docking results of natural ligands, compound 1 and compound 2 against HMG-CoA Reductase Protein.

Ligand	Value of RMSD	Lowest binding energy (kcal/mol)	Inhibition score
Natural Ligand	2.0 A	-5.23	147.6uM
Compoun 1 (β2-Solanin)	2.0 A	-0.21	702.47mM
Compound 2 (Disogenin)	2.0 A	-3.78	1.68mM

hydrogen bonds (salt bridge) which compounds 1 and 2 do not have. However, compounds 1 and 2 have hydrophobic bonds. Compound 2 has more hydrophobic bonds (4 bonds) than compound 1 (2 bonds) so that the binding energy of compound 2 is lower than compound 1.

Hydrophobic bonding is one of the important forces in the process of merging the non-polar region of the drug and the non-polar receptor. Non-polar regions of drug molecules that are insoluble in water will combine through hydrogen bonds to form a quasi-crystalline structure (icebergs) (Siswandono 2016). The lower the bond energy between the test compound and the enzyme, the stronger and more stable the bond. Thus, although not as strong as natural ligands, compounds 1 and 2 have potential as inhibitors of the HMG-CoA Reductase enzyme. Compound 2 (a steroid sapogenin) is a compound derived from diosgenin can inhibit the HMG-CoA Reductase enzyme which is greater than compound 1 as a steroidal glycoside alkaloid compound β2-solanine with a binding energy of -3.78 kcal/mol less than -0.21 kcal/mol. This finding was relevant to other study that reported several pharmacological activity of diosgenin including anti-cholesterol activity (Sun et al. 2021)

The in silico test using the molecular docking method showed that the steroidal sapogenin compounds derived from disogenin and steroidal glycoside β2-solanine compounds found in *S. blumei* leaves have affinity to bind to the active site of the HMG Co-A reductase enzyme. Compound (2) steroidal sapogenin derived from disogenin contained in the ethanol extract of *S. blumei* leaves has more negative free energy (-3.78 kcal/mol) than compound (1) steroid alkaloid glycoside β2-solanine (- 0 ,21 kcal/mol). It shows that the steroid sapogenin compound derived from disogenin has the ability to inhibit the HMG Co-A reductase enzyme which is greater than the steroid alkaloid glycoside β2-solanine. However, the two compounds found in *S. blumei* leaves have the potential as inhibitors of the HMG Co-A reductase enzyme that functions in endogenous

cholesterol biosynthesis. The inhibition of the HMG Co-A reductase enzyme will influence lipid levels in the blood. The ability of the ethanol extract of *S. blumei leaves* to reduce blood cholesterol levels in male white rats (*Rattus norvegicus*) (Tables 2, 3) induced by high fat may be due to the activity of the bioactive compounds disogenin and β2- solanine found in *S. blumei* inhibits the HMG CoA reductase enzyme.

Conclusion

Solanum blumei ethanol extract showed anticholesterol activity and significantly reduced the atherogenic index of hypercholesterolemic rats. The bioactive compounds of *Solanum blumei* that is sapogenin derived from disogenin ($C_{26}H_{39}O_4$) and β2-solanine ($C_{39}H_{63}NO_{11}$) showed inhibition activity to HMG-CoA reductase enzyme by silico method.

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