

# 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  $\beta$ 2-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<sub>26</sub>H<sub>39</sub>O<sub>4</sub>) and  $\beta$ 2-solanine (C<sub>39</sub>H<sub>63</sub>NO<sub>11</sub>) 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 (*Annona muricata* L) (Uneputty et al. 2013), temulawak rhizomes (*Curcuma xanthorrhiza*) (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  $\beta$ 2-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  $\beta$ 2-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 novogicus*) 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  $\beta$ 2-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 novogicus*) 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-FMI-PA/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 36<sup>th</sup> 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  $\beta$ 2-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 ( $M \pm SD$ ) 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  $\pm$  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  $77.33 \pm 3.21$  mg/dL and LDL levels of  $0.46 \pm 0.30$  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 $\pm$ 3.5	86.66 $\pm$ 1.52	72.00 $\pm$ 2.00	17.00 $\pm$ 1.50
	High Fat Diet Induction (2)	116.66 $\pm$ 1.52	87.33 $\pm$ 1.22	78.66 $\pm$ 3.21	18.53 $\pm$ 1.50
	Extract Administration (3)	114.00 $\pm$ 1.00 <sup>a</sup>	85.66 $\pm$ 2.07 <sup>a</sup>	79.66 $\pm$ 1.52 <sup>a</sup>	17.20 $\pm$ 1.48 <sup>a</sup>
	Percentage Change (%) (Period 2-3)	2.27 $\pm$ 1.20	1.90 $\pm$ 2.11	1.34 $\pm$ 1.88	12.84 $\pm$ 7.9
K1 (Positive control, Simvastatin 0.25 mg/200 g BW)	Initial (1)	96.00 $\pm$ 4.00	82.33 $\pm$ 1.52	64.66 $\pm$ 2.51	14.86 $\pm$ 1.21
	High Fat Diet Induction (2)	156.00 $\pm$ 4.58	56.00 $\pm$ 2.00	43.00 $\pm$ 2.00	101.46 $\pm$ 2.11
	Extract Administration (3)	92.33 $\pm$ 3.05 <sup>b</sup>	82.00 $\pm$ 2.64 <sup>a</sup>	74.66 $\pm$ 1.52 <sup>b</sup>	0.26 $\pm$ 0.11 <sup>b</sup>
	Percentage Change (%) (Period 2-3)	40.80 $\pm$ 1.20	46.44 $\pm$ 2.88	73.77 $\pm$ 4.61	99.73 $\pm$ 0.10
K2 (Ethanol extract of <i>S. blumei</i> 100 mg/Kg BW)	Initial (1)	97.00 $\pm$ 7.00	81.00 $\pm$ 4.00	66.66 $\pm$ 5.50	14.13 $\pm$ 0.74
	High Fat Diet Induction (2)	150.66 $\pm$ 4.50	69.00 $\pm$ 3.00	50.33 $\pm$ 4.50	85.13 $\pm$ 1.65
	Extract Administration (3)	97.66 $\pm$ 3.21 <sup>c</sup>	84.66 $\pm$ 3.50 <sup>a</sup>	77.00 $\pm$ 1.00 <sup>a</sup>	2.73 $\pm$ 1.44 <sup>c</sup>
	Percentage Change (%) (Period 2-3)	35.16 $\pm$ 1.23	22.70 $\pm$ 0.47	53.66 $\pm$ 12.66	96.75 $\pm$ 1.81
K3 (Ethanol extract of <i>S. blumei</i> 200 mg/Kg BW)	Initial (1)	89.66 $\pm$ 5.5	75.00 $\pm$ 2.00	58.66 $\pm$ 2.51	15.66 $\pm$ 2.66
	High Fat Diet Induction (2)	155.00 $\pm$ 4.00	59.00 $\pm$ 2.00	46.33 $\pm$ 2.45	98.20 $\pm$ 1.24
	Extract Administration (3)	77.33 $\pm$ 3.21 <sup>d</sup>	74.33 $\pm$ 1.77 <sup>b</sup>	62.33 $\pm$ 2.50 <sup>c</sup>	0.46 $\pm$ 0.30 <sup>b</sup>
	Percentage Change (%) (Period 2-3)	50.09 $\pm$ 1.35	26.02 $\pm$ 1.81	34.59 $\pm$ 1.86	99.52 $\pm$ 0.27
K4 (Ethanol extract of <i>S. blumei</i> 300 mg/Kg BW)	Initial (1)	88.33 $\pm$ 4.72	80.33 $\pm$ 2.51	61.00 $\pm$ 2.00	9.93 $\pm$ 1.36
	High Fat Diet Induction (2)	155.00 $\pm$ 3.00	58.66 $\pm$ 2.51	50.33 $\pm$ 2.07	88.93 $\pm$ 2.00
	Extract Administration (3)	88.66 $\pm$ 3.05 <sup>b</sup>	78.66 $\pm$ 1.52 <sup>a</sup>	70.33 $\pm$ 2.51 <sup>b</sup>	3.26 $\pm$ 1.21 <sup>c</sup>
	Percentage Change (%) (Period 2-3)	41.66 $\pm$ 1.52	34.17 $\pm$ 3.18	39.76 $\pm$ 2.26	96.30 $\pm$ 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  $\pm$  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 ( $50.09 \pm 1.35\%$ ), followed by *S. blumei* ethanol extract 300mg/Kg BW ( $41.66 \pm 1.52\%$ ) and ethanol extract of *S. blumei* 100mg/Kg BW ( $35.16 \pm 1.23\%$ ) ( $P < 0.01$ ). The administration of simvastatin caused a decrease in cholesterol levels ( $40.80 \pm 1.20\%$ ) which was not significantly different from the administration of ethanol extract of *S. blumei* 300mg/KgBW ( $40.80 \pm 1.20\%$ ) ( $P > 0.01$ ), furthermore 200mg/KgBW (K3) resulted in a decrease in serum LDL levels by  $99.52 \pm 0.27\%$ , an increase in HDL levels by  $34.59 \pm 1.86\%$  and an increase in serum triglyceride levels by  $26.02 \pm 1.81\%$  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 Extract Administration			
	Decreasing percentage of Cholesterol (%)	Decreasing percentage of LDL (%)	Increasing percentage of HDL (%)	Increasing percentage of Triglycerida
K0	$2.27 \pm 1.20^a$	$12.84 \pm 7.90^a$	$1.34 \pm 1.88^a$	$1.90 \pm 2.11^a$
K1	$40.80 \pm 1.20^b$	$99.73 \pm 0.10^b$	$73.77 \pm 4.61^b$	$46.44 \pm 2.88^b$
K2	$35.16 \pm 1.23^c$	$96.75 \pm 1.81^b$	$53.66 \pm 12.66^c$	$22.7 \pm 0.47^c$
K3	$50.09 \pm 1.35^d$	$99.52 \pm 0.27^b$	$34.59 \pm 1.86^d$	$26.02 \pm 1.81^c$
K4	$41.66 \pm 1.52^b$	$96.3 \pm 1.51^b$	$39.76 \pm 2.26^d$	$34.17 \pm 3.18^d$

## 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%

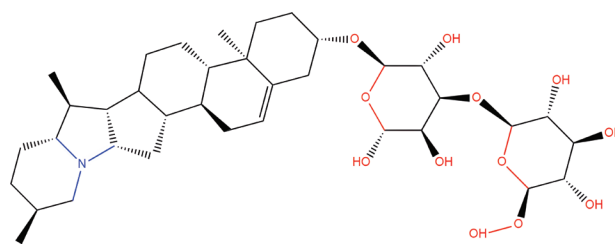
The anticholesterol activity of the ethanol extract of *S. blumei* may be due to several mechanisms including inhibition of the cholesterol biosynthetic enzyme HMG-CoA reductase, stimulating the enzyme cholesterol-7- $\alpha$ -hydroxylase to convert cholesterol into bile acids and inhibition of cholesterol 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.

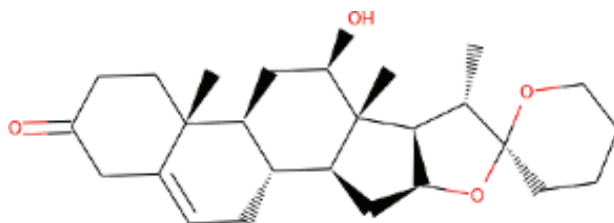
Some of the chemicals identified as having hypocholesterolemic potential include phytosterols, saponins, flavonoids, tannins and water-soluble dietary fiber. The ethanol extract of the leaves of *S. blumei* contains a steroid saponenin compound derived from diosgenin ( $C_{26}H_{39}O_4$ ) which has natural immunostimulant activity (Patent, IDP0000448080, 2017; Simorangkir et al. 2013b) and steroidal alkaloid glycoside  $\beta$ 2-solanine or [Solanid-5-ene-(1 $\rightarrow$ 3)- $\beta$ -D-galactopyranoside-(1 $\rightarrow$ 3')- $\beta$ -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  $\beta$ 2-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  $\beta$ 2-solanine ( $C_{39}H_{63}NO_{11}$ ) and saponenin ( $C_{26}H_{39}O_4$ ) 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  $\beta$ 2-solanine and saponenin 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  $\beta$ 2-solanine (Simorangkir et al. 2016) and a steroidal saponenin derivative of diosgenin ( $C_{26}H_{39}O_4$ ) which has natural immunostimulant activity (Patent, IDP0000448080, 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.



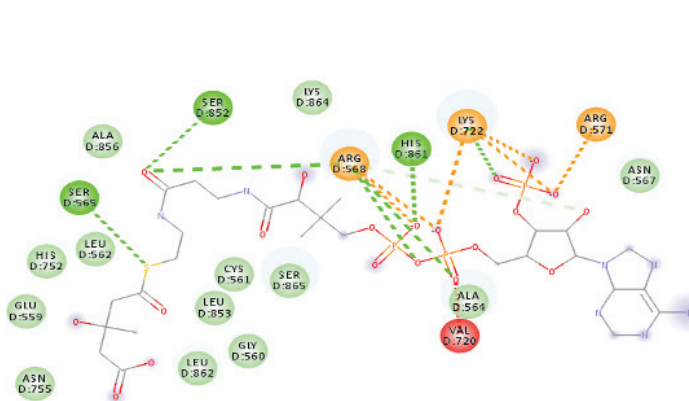
**Figure 1.** Molecule of steroid glycoside alkaloids  $\beta$ 2-solanin, [Solanid-5-ene-(1 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 3')- $\beta$ -D-glucopyranoside] ( $C_{39}H_{63}NO_{11}$ ) (Simorangkir et al. 2016) (Compound 1)



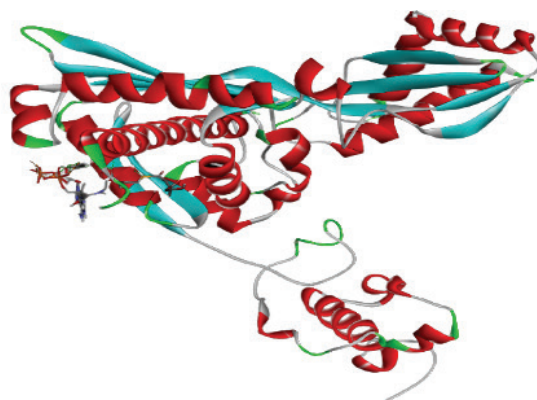
**Figure 2.** Diosgenin-derived steroid saponenin molecule (2'R,6aR,8aS, 8bR, 9S,11aS)-8-hydroxy-6a,8a,9-trimethyl-1,3'4',5,5'6,6,6a,6b,6',7,8,8a,8b,9,11a,12a,12b-Octadecahydro-spiro[naptho 2;1':4,5] indeno [2,1-b]furan-10,2'-pyran]-4(3H)-one. (Patent, IDP0000448080, Simorangkir 2017) (Compound 2)

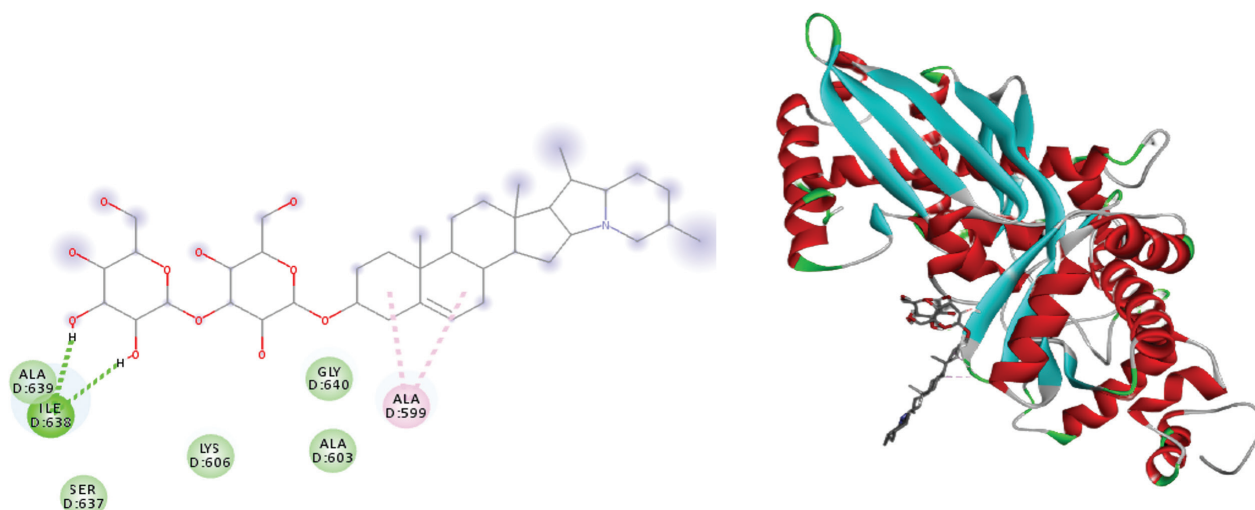
The results of in silico test in inhibition of HMG-CoA reductase enzyme by natural ligand compounds, steroidal glycoside alkaloids  $\beta$ 2-solanine ( $C_{39}H_{63}NO_{11}$ ) (compound 1) and steroidal saponenin compounds derived from diosgenin ( $C_{26}H_{39}O_4$ ) (compound 2) from *S. blumei* are 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

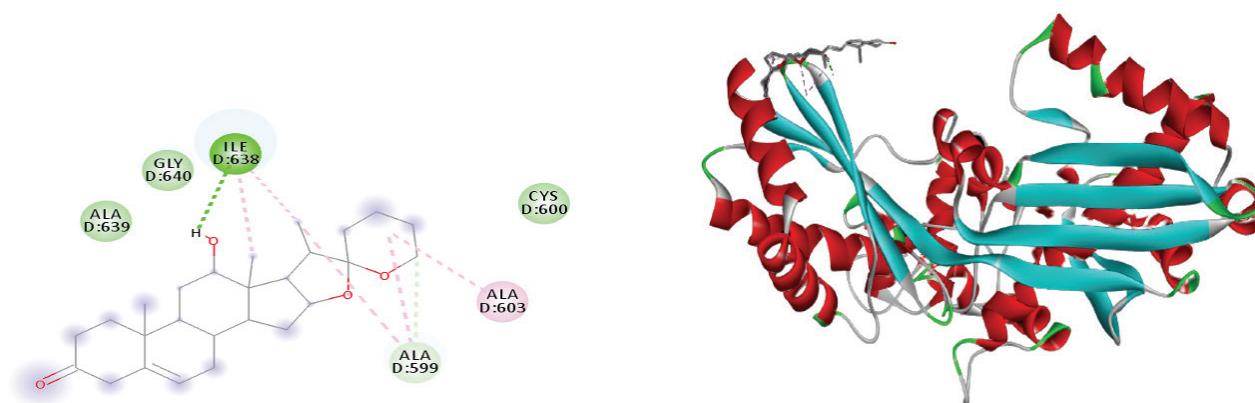


**Figure 3.** Natural Ligand Interaction with HMG-CoA Reductase





**Figure 4.** Interaction of Compound 1 ( $\beta$ 2-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 Å	-5.23	147.6 $\mu$ M
Compound 1 ( $\beta$ 2-Solanin)	2.0 Å	-0.21	702.47mM
Compound 2 (Disogenin)	2.0 Å	-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) (Siswando 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  $\beta$ 2-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 diosgenin and steroidal glycoside  $\beta$ 2-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 diosgenin contained in the ethanol extract of *S. blumei* leaves has more negative free energy (-3.78 kcal/mol) than compound (1) steroid alkaloid glycoside  $\beta$ 2-solanine (-0.21 kcal/mol). It shows that the steroid sapogenin compound derived from diosgenin has the ability to inhibit the HMG Co-A reductase enzyme which is greater than the steroid alkaloid glycoside  $\beta$ 2-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  $\beta$ -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  $\beta$ -solanine ( $C_{39}H_{63}NO_{11}$ ) showed inhibition activity to HMG-CoA reductase enzyme by silico method.

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## References

- Aljenedil S, Ruel I, Watters K, Genest J (2018) Severe xanthomatosis in heterozygous familial hypercholesterolemia. *Journal of Clinical Lipidology* 12(4): 872–877. <https://doi.org/10.1016/j.jacl.2018.03.087>
- Alloubani A, Nimer R, Samara R (2021) Relationship between hyperlipidemia, cardiovascular disease and stroke: a systematic review. *Current Cardiology Reviews* 17(6): 52–66. <https://doi.org/10.2174/1573403x16999201210200342>
- Aznam N, Atun S (2016) Pharmacological test of herbal products from temulawak (*Curcuma xanthorrhiza*) as antihypercholesterol by in vivo. *International Journal of Pharmacognosy and Phytochemical Research* 8(5): 807–811.
- Bissantz C, Folkers G, Rognan D (2000) Protein-Based Virtual Screening of Chemical Databases. 1. Evaluation of Different Docking/Scoring Combinations. *Journal of Medicinal Chemistry* 43(25): 4759–4767. <https://doi.org/10.1021/jm001044l>
- Bo MS, Cheah WL, Lwin S, Moe Nwe T, Win TT, Aung M (2018) Understanding the Relationship between Atherogenic Index of Plasma and Cardiovascular Disease Risk Factors among Staff of an University in Malaysia. *Journal of Nutrition and Metabolism* 2018: 1–6. <https://doi.org/10.1155/2018/7027624>
- Cameron TMD (2021) Atherogenic index: defenition, atherogenic diet and measurement of the atherogenic index. *Hematological Diseases, Scope Heal*, 67 pp.
- Ezekwesili CN, Obidoo O, Nwodo OFC (2008) Effects of ethanol extract of *Acalypha torta* leaves on the lipid profile and serum electrolytes of rabbits. *Nigerian Journal of Biochemistry and Molecular Biology* 23(1): 15–19.
- Gaamoussi F, Israili ZH, Lyoussi B (2010) Hypoglycemic and hypolipidemic effects of an aqueous extract of *Chamaerops humilis* leaves in obese, hyperglycemic and hyperlipidemic Meriones shawi rats. *Pakistan journal of pharmaceutical sciences* 23(2): 212–219.
- Ghasi S, Nwobodo E, Ofili J (2000) Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed wistar rats. *Journal of Ethnopharmacology* 69(1): 21–25. [https://doi.org/10.1016/s0378-8741\(99\)00106-3](https://doi.org/10.1016/s0378-8741(99)00106-3)
- Guyton AC, Hall JE (1997) *Textbook of Medical Physiology*, 9<sup>th</sup> edn., EGC, Jakarta.
- Hartanti L, Yonas SMK, Mustamu JJ, Wijaya S, Setiawan HK, Soegiarto L (2019) Influence of extraction methods of bay leaves (*Syzygium polyanthum*) on antioxidant and HMG-CoA Reductase inhibitory activity. *Heliyon* 5(4): e01485. <https://doi.org/10.1016/j.heliyon.2019.e01485>
- Iyamu OA, Ugheoke JE, Ozor MO, Airhomwanbor KO, Eidangbe AP, Idehen IC, Okhiai O, Akpede N (2014) The effect of nutritional lipid supplementation on serum lipid levels and effectiveness of antitubercular chemotherapy. *American Journal of Biomedical Research* 2(2): 36–41. <https://doi.org/10.12691/ajbr-2-2-4>
- Jain AN, Nicholls A (2008) Recommendations for evaluation of computational methods. *Journal of Computer-Aided Molecular Design* 22(3–4): 133–139. <https://doi.org/10.1007/s10822-008-9196-5>
- Karamustafalioglu N, Kalelioglu T, Unalan P, Kok B, Sozen S, Yuksel O, Cihnioglu R (2017) Atherogenic index of plasma in manic, depressive and euthymic stages of bipolar disorder. *European Neuropsychopharmacology* 27: S818–S819. [https://doi.org/10.1016/s0924-977x\(17\)31480-3](https://doi.org/10.1016/s0924-977x(17)31480-3)
- Li F, Xiao J, Yun Z, Cai G (2021) Atherogenic index of plasma is related to coronary atherosclerotic disease in elderly individuals: a cross-sectional study. *Lipids in Health and Disease* 20(1): 68. <https://doi.org/10.1186/s12944-021-01496-8>
- Miras MB, Sabater JAB, Pedreno MA, Almagro L (2016) Bioactivity of phytoosterols and their production in plant in vitro cultures. *Journal of Agricultural and Food Chemistry* 64(38): 7049–7058. <https://doi.org/10.1021/acs.jafc.6b02345>
- Mongi R, Simbala HEI, De Queljoe E (2019) Activity test of reducing blood sugar levels ethanol extract of Yaki (*Areca vestiaria*) leaves against male white rats wistar (*Rattus norvegicus*) induced by alloxan. *Pharmacon* 8(2): 449. <https://doi.org/10.35799/pha.8.2019.29312>
- Olantunji LA, Adebayo JO, Oguntoye OB, Olatunde NO, Olantunji VA, Soladoye AO (2005) Effects of aqueous extracts of petals of red and greenhibiscus sabdariffa on plasma lipid and hematological variables in rats. *Pharmaceutical Biology* 43(5): 471–474. <https://doi.org/10.1080/13880200590963934>
- Purukan JA, Kusmardi, Laksmiawati DR, Syamsudin (2019) Comparison of Lipid profile and determination of atherogenic index in white rats werw given crude fucoidan from brown seaweed (*Sargassum polycystum*) that induced high-fat diet. *Journal Ilmu Kefarmasian Indonesia* 17(1): 46–55. <https://doi.org/10.35814/jifi.v17i1.648>
- Roza D, Selly R, Munzirwan R, Fadhilah G (2021) Molecular docking of quinine derivative as inhibitor in Sars-Cov-2. *Journal of Physics:*

- Conference Series 1819 (2021) 012053, AISTSSE 2020: 1–8. <https://doi.org/10.1088/1742-6596/1819/1/012053>
- Sekhon S (2012) Antioxidant, antiinflammatory and hypolipidemic properties of apple flavonols, undergraduate thesis, Nova Scotia Agricultural College Truro, Nova Scotia, 144 pp.
- Simorangkir M (2017) The isolation process of black ranti leaf extract (*Solanum blumei* Nees ex Blume) and its composition containing immunostimulant active substances. 2017; Paten No. IDP000048080.
- Simorangkir M, Barus T, Surbakti R, Simanjuntak P (2016) Isolation and toxicity of steroidal alkaloid glycoside from fruits of ranti hitam (*Solanum blumei* Nees ex Blume). Asian Journal of Chemistry 28(1): 203–206. <https://doi.org/10.14233/ajchem2016.19345>
- Simorangkir M, Surbakti R, Barus T, Simanjuntak P (2013a) Aktivitas antioksidan ekstrak n-heksana, etil asetat dan etanol ranti hitam dengan metode peredaman DPPH. Prosiding Seminar Nasional Kimia, Universitas Mulawarman, Samarinda, 9 November 2013a, 182–185.
- Simorangkir M, Sinaga E, dan Riwayati (2013b) Ekstraksi dan Aktivitas Imunostimulan Metabolit Sekunder Daun Ranti Hitam (*Solanum blumei* Nees ex Blume), Prosiding Seminar Hasil Penelitian, Lembaga Penelitian UNIMED, Cetakan 1, 1 Desember 2013, 137–141.
- Simorangkir M, Surbakti R, Barus T, Simanjuntak P (2017) Secondary metabolites phytochemical analysis of leaves and fruit extract *Solanum blumei* Nees ex Blume local. Jurnal Pendidikan Kimia 9(1): 244–248. <https://doi.org/10.24114/jpkim.v9i1.6186>
- Siswandono S (2016) Kimia Medisinal 1<sup>st</sup> and 2<sup>nd</sup> edn., Surabaya, Airlangga University Press, 35 pp.
- Sun F, Yang X, Ma C, Zhang S, Yu L, Lu H, Zhang F (2021) The effects of diosgenin on hypolipidemia and its underlying mechanism: a review. Diabetes, metabolic syndrome and obesity: targets and therapy 14: 4015. <https://doi.org/10.2147/dmso.s326054>
- Trinder P (1969) Enzymatic colorimetric determination of triglycerides by GOP-PAP method. Annals of Clinical Biochemistry 6: 24–27. <https://doi.org/10.1177/000456326900600108>
- Ugwu Okechukwu PC, Nwodo Okwesili FC, Joshua Parker E, Odo Christian E, Ossai Emmanuel C (2013) Effect of ethanol leaf extract of *Moringa oleifera* on lipid profile of mice. Research Journal of Pharmaceutical, Biological and Chemical Sciences 4(1): 1324–1332.
- Unepetty JP, Yamlean PV, Kojong N (2013) Potency of soursop leaf infusion (*Annona muricata* L.) on blood cholesterol levels of male white rats (*Rattus norvegicus*). Pharmacon 2(2): 1–6.
- Zhao D-K, Zhao Y, Chen S-Y, Kennelly EJ (2021) Solanum steroidal glycoalkaloids: structural diversity, biological activities, and biosynthesis. Natural Product Reports 38(8): 1423–1444. <https://doi.org/10.1039/d1np00001b>