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Original Article

IN SILICO, *IN VITRO* AND *IN VIVO* STUDY OF BAWANG DAYAK (ELEUTHERINE BULBOSA (L) MERR.) POTENTIAL AS ANTI-CHOLESTEROL

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ABSTRACT

Objective: This study aimed to determine the potential of Bawang Dayak extract as an anti-cholesterol.

Methods: *In silico* study was carried out using Gas Chromatography-Mass Spectroscopy (GC-MS) analysis and continued with screening using Lipinski's Rule of Five. *In vitro* study was carried out using a cholesterol reagent that read in Multimode Reader. *In vivo* study was carried out by cholesterol level measurement against egg yolk-induced rats. The doses chosen by initial dose orientation and prior efficacy study of Bawang Dayak extract anti-cholesterol effect.

Results: The results of *in silico* study using the Lipinski's Rule of Five was active compound in Bawang Dayak was Undecanoic Acid. Undecanoic acid is ethyl ester compound with Log P<5. Molecular validation produced reference ligand compounds on the IHW9 receptor. Based on previously oriented concentrations and doses, the highest percentage of cholesterol decrease was at 50 ppm (40.04 %) *in vitro*, and 250 mg/70 kgBW (24.70 %) *in vivo*.

Conclusion: Bawang Dayak has an activity as anti-cholesterol that proven by *in silico*, *in vitro* and *in vivo* study. The absence of evaluation focused on mechanistic studies and anti-cholesterol pathways becomes limitation of this study.

Keywords: Anti-cholesterol, Bawang dayak extract, *In silico*, *In vitro*, *In vivo*

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INTRODUCTION

Cardiovascular disease has increased every year in Indonesia. It comes from the unhealthy lifestyle of the people, such as consuming fatty and fast food. The longer they consume unhealthy food, the more dangerous it will be for their health. One of the diseases that can occur in this condition is coronary heart disease, which is correlated with atherosclerosis caused by high cholesterol levels in the blood [1]. Cholesterol is a fatty substance that is an important part of the outer lining of cells in the body of the animals. Cholesterol is also found in the blood circulation of humans. It is also a precursor for the synthesis of steroid hormones [2]. Cholesterol is a waxy lipid, which 80% is produced in the liver and the rest is obtained from foods rich in cholesterol, such as meat, eggs and dairy products. Total cholesterol is the amount of cholesterol that is carried in cholesterol-carrying particles in the blood, including High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), and Very Low-Density Lipoprotein (VLDL) [1].

Cholesterol biosynthesis occurs in the endoplasmic reticulum, starting with the condensation of two acetyl-Coenzyme A (CoA) molecules into acetoacetyl-CoA catalyzed by the enzyme thiolase. This acetoacetyl CoA then combines with acetyl CoA to form 3-Hydroxy-3-Methylglutaryl (HMG) CoA catalyzed by HMG CoA reductase. The HMG CoA formed then converted into mevalonate, which goes through several processes to form squalene. Enzyme converts squalene into squalene epoxide, which is then converted into lanosterol by the oxidosqualene cyclase enzyme. The lanosterol that formed then goes through further stages to produce cholesterol. The cholesterol that is produced then transported to the plasma as LDL. LDL transports cholesterol from the liver to tissues or cells, then if LDL levels are excessive, it will be secreted to the liver through HDL [3].

One of the plants that can be utilized as source of medicine is Bawang Dayak *(Eleutherine bulbosa merr.).* Comprehensive study of anti-cholesterol activity and related pharmacology activities of Bawang Dayak have been carried out. Bawang Dayak is traditionally used to treat sexual disorders, stroke, hypertension, breast cancer, diabetes, menstrual pain, coronary disorder, wound healing, and to enhance breast milk production [4]. Previous study of Bawang Dayak impact to high cholesterol levels and arterial stiffness in an animal model stated that the ethanol extract of Bawang Dayak at a dose of 100 mg/kgBW, successfully prevented the rise in lipid profiles and the progression of atherosclerosis [5]. Nevertheless, there is no study about the anti-cholesterol effect of Bawang Dayak that carried out *in vitro.*

Bawang Dayak contains several active compounds that potentially effective to treat high cholesterol, including eleutherin, gallic acid, chlorogenic acid, quercetin, kaempferol, rutin, epicatechin gallate, and myricetin. The most notable compound groups isolated from Bawang Dayak are Naphthalene, anthraquinone, and naphthoquinone. The compounds are hongconin, eleutherin, eleutherol, isoeleutherol, isoeleutherine, elecanacin, eleutherinoside A, eleuthoside B, and four polyketides including (R)-4-hydroxyeleutherin, eleuthone, eleutherinol-8-O-β-D-glucoside and isoeleuthoside C (dihydroisoeleutherin-5-O-β-D-gentiobioside), 9,9′-dihydroxy8,8′-dimethoxy-1,1′-dimethyl-1H, 1H′-[4,4′]bis[naphtha[2, 3-c]funanyl]-3,3′-dione, 6,8-dihydroxy-3,4-dimethoxy1-methyl-anthraquinone-2-carboxylic acid methyl ester and 2-acetyl-3,6,8-trihydroxy-1-methyl anthrax-quinone [4].

Because of there is a gaps in prior study related to the anti-cholesterol effect of Bawang Dayak, we represent the first multi-method validation of Bawang Dayak's anti-cholesterol effects combining GC-MS profiling, *in silico* docking, *in vitro* enzyme inhibition, and *in vivo* animal models.

MATERIALS AND METHODS

Equipment and equipment

The equipment used were a set of glassware (Pyrex), stirring rods (IWAKI), watch glass (Normax), beakers (Pyrex), scissors (Joyko), human analyzer Microlab 300 and 300 lX (MRK Diagnostic), Multimode reader (Synergy HTX S/N22050919), centrifuge

(Biocen), analytical scales (Fujitsu FS-AR), animal scales, rotary evaporator (IKA RV10), vortex (Gemmy VM300), Lipinski's Rule of Five software. The materials used were distilled water, 96% ethanol (Merck), Bawang Dayak, filter paper (Whatman), 0.5 % Na CMC (Merck), quail egg yolk, cholesterol reagent kit (Sigma-Aldrich).

Methods hardware and software preparation

Moleculardocking is a computational method that can be used as the basis for drug discovery [6]. Hardware used in this research was a laptop with Windows 10 Intel(R) Core (TM) i5-8250U CPU @ 1.60GHz 1.80 GHz, 8 GB RAM, 64-bit operating system and x64 based processor. Software used in this research were AutoDockTools-1.5.7 that used for docking simulation and receptor binding visualization; Discovery Studio 2021 that used for receptor preparation, ligand preparation, binding analysis and 2D interaction interpretation.

2D structure of ligand from GC-MS search for 3D structure on PubChem website

The structure of the compounds found in PubChem website saved in 3D sdf format. Then the format was converted to. pdb with the help of Discovery Studio 2021 by saving as then selecting the Protein Data Bank format [7].

Protein preparation

The 3D structure of the anti-cholesterol protein (simvastatin) has Protein Data Bank (PDB) code of 1HW9. Validation Root mean Square Deviation (RMSD) Reference<2A, which was 1.7058 Å was obtained from the Protein Data Bank site: https://www.rcsb.org/.

Ethical clearance

This process aimed to measure the ethical feasibility of the research and to ensure that the research met applicable ethical standards and did not harm the research subjects. The ethics examination is carried out by the Health Research Ethics Committee of STIFAR Yayasan Pharmasi Semarang. The ethics approval number was 706/EVM-NA/KEPK/STIFAR/EC/X/2024.

***In vitro* cholesterol test with multimode reader**

Samples were made with concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm using assay buffer. To make a reaction mix, put 48.5 µl** assay buffer, 0.5 µl** Probe, 0.5 µl** enzyme mix and 0.5 µl** cholesterol esterase per well and mix properly. 50 µl** of each sample, cholesterol standar and blank was put into each well. The plate was incubated for 30 min at temperature of 37 °C then the absorbance readings was carried out at wavelength of 535 nm [8].

***In vivo* cholesterol test with human analyzer microlab 300 simvastatin suspension preparation**

Simvaststin tablets @weighed as many as 10 tablets and calculated the average weight of the tablets. Then the tablets were ground in a mortar until they became powder. Simvaststin powder was weighed and then suspended with 10 ml of Na CMC 0.5 % w/v.

High fat diet (HFD) preparation

Induction of increased cholesterol was carried out using raw quail egg yolk which was then given to test animals using a 5 ml syringe for each mouse [9]. Quail eggs fall into the category of very high cholesterol levels, with the fat content of 11.1% and the cholesterol level of 844 mg/dl [10]. Choosing quail egg yolks also has several advantages, such as being easy to obtain and cost-effective.

Bawang dayak ethanol extract suspension preparation

Ethanol extract suspension of Bawang Dayak at doses of 75 mg/70 kgBW, 150 mg/70 kgBW and 250 mg/70 kgBW was made by weighing each extract as much as 75 mg, 150 mg, and 250 mg, then suspended with 10 ml of Na-CMC 0.5% b/v [11]. Dose selection was based on initial dose orientation and prior efficacy study of Bawang Dayak extract anti-cholesterol effect.

Preparation and treatment of test animals

This research selected male Wistar rats as test animals. If female rats were used, there would be errors in measuring cholesterol levels because the hormonal system of the test animals influenced them. In addition, female rats have an increase in estrogen hormone [12]. Cholesterol levels were measured before induction on day 0. Then, the mice were divided into 5 groups. Mice were induced by quail egg yolk for 21 d. On the 22nd d, total cholesterol levels were measured after the induction. Then the mice were given suspension tests orally and quail egg yolk once a day until 35th d. The animals test was given:

- Egg Yolk+Na-CMC 0.5% for group 1 (Negative control)
- Egg Yolk+Simvastatin suspension for group II (positive control)
- Egg yolk+Bawang Dayak ethanol extract with a dose of 75 mg/70 kgBW for group III
- Egg yolk+Bawang Dayak ethanol extract with a dose of 150 mg/70 kgBW for group IV
- Egg yolk+Bawang Dayak ethanol extract with a dose of 250 mg/70 kgBW for group V

On the 36th day, total cholesterol and triglyceride levels were measured after being given the suspension test. Before blood sampling was conducted, the mice were fasted for 14-18 h.

Blood sampling process

Blood was taken through the lateral vein in the tail by cutting the tip of the tail 0.2 cm. The blood was collected in an Eppendrof tube as much as 0.5 ml, then centrifuged for 10 min at a speed of 3000 rpm and then the blood serum (clear layer) was collected [11].

Cholesterol levels measurement

Blood serum that collected was taken as much as 3 µl then added 300 µl of cholesterol and triglyceride reagents in each. The solution was homogenized using vortex and was incubated for 25 seconds. Measurement of total cholesterol and triglyceride levels was carried out using *the Human Analyzer* (Microlab 300) with wavelength of 300 nm [13]. This study was conducted at the Pharmacology Laboratory in STIFAR Yayasan Pharmasi Semarang.

RESULTS

***In silico* identification results of bawang dayak extract**

Result of Bawang Dayak extract chromatogram can be seen in fig. 1 and table 1.

Table 2: Results of active compounds and the formula in bawang dayak using GC-MS

S. No.	Compounds name	Formula	AH (Min)	RT (Min)
1	Undecanoic acid, ethyl ester	$C_{13}H_{26}O_2$	9.12	10.189
2	Hexadecanoic acid	$C_{16}H_{32}O_2$	2.60	10.795
3	7-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	5.76	10.975
4	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	4.51	11.105
5	9-Octadecenoic acid	$C_{18}H_{34}O_2$	5.87	11.355
6	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	7.63	11.483
7	(Z,Z)-6,9-cis-3,4-epoxy-nonadecadiene	$C_{19}H_{34}O$	9.06	11.995
8	9-Octadecenal, (Z)	$C_{18}H_{34}O$	5.67	29.576
9	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy	$C_{30}H_{52}O_2$	8.33	32.794

Description: AH: Adjusted retention time, RT: Retention time, Min: Minute

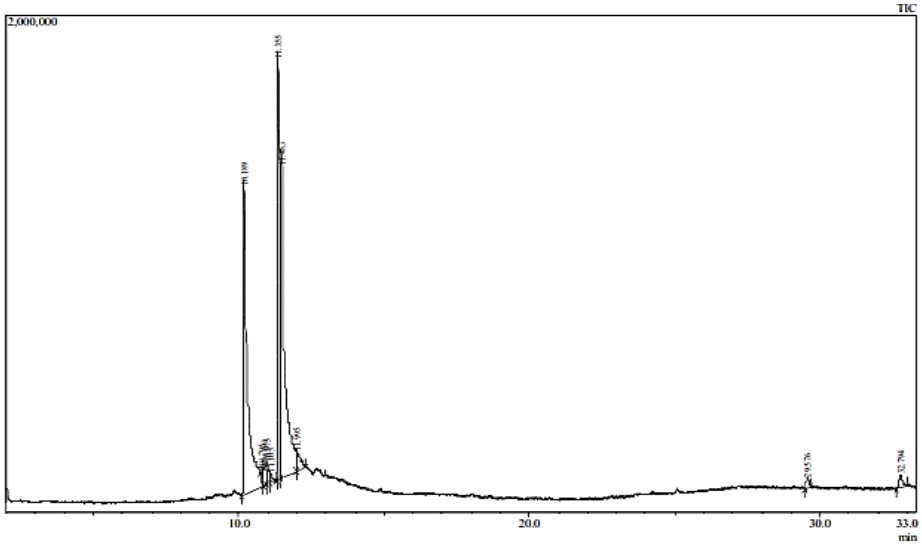


Fig. 1: Chromatogram of bawang dayak extract, active compounds found in bawang dayak extract through GC-MS analysis based on reference are shown in table 1

Table 3: Lipinski's rule of five screening results of active compounds in bawang dayak

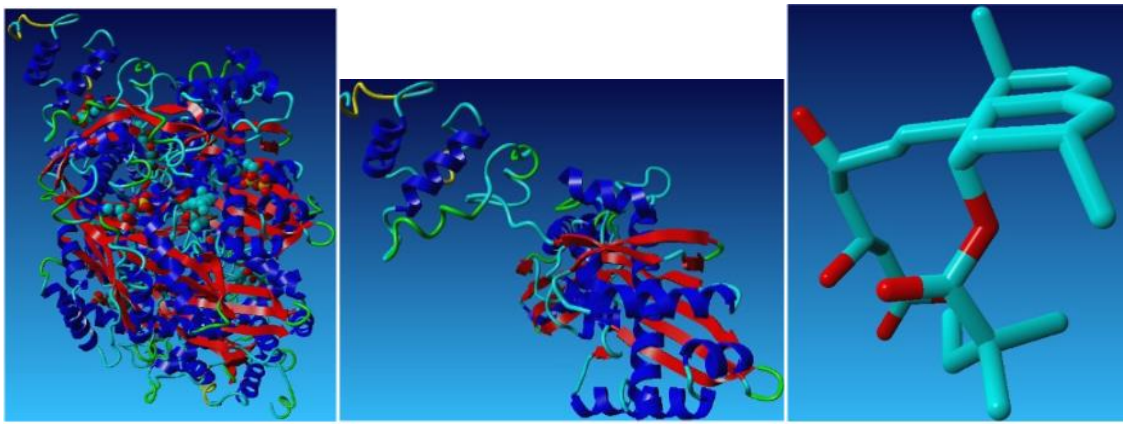
S. No.	Compounds name	Molecular weight	Hydrogen bonds		Log P	Molar refractive	Description
			Donor	Acceptor			
1	Undecanoic acid, Ethyl ester	214	0	2	4.08	63.85	√
2	Hexadecanoic acid	256	1	2	5.55	77.94	-
3	7-Hexadecenoic acid, Methyl ester	268	0	2	5.41	82.23	-
4	Octadecanoic acid, Methyl ester	298	0	2	5.64	104.47	-
5	9-Octadecenoic acid	282	1	2	6.10	87.08	-
6	Octadecanoic acid, Ethyl ester	312	0	2	5.90	110.60	-
7	(Z,Z)-6,9-Cis-3,4-Epoxy-nonadecadiene	278	0	1	6.19	89.07	-
8	9-Octadecenal, (Z)	266	0	1	6.22	85.51	-
9	Tricyclo[20.8.0.0(7,16)]Triacontane, 1(22),7(16)-Diepoxy	444	0	2	9.18	133.13	-

Description: (√) = Qualified, (-) = Not Qualified

Table 4: Results of pharmacokinetic screening of bawang dayak active compounds

Compound name	Parameter			Description
	Caco-2 (nm/sec)	HIA (%)	PPB (%)	
Undecanoic Acid, Ethyl Ester	54.98	100	100	√

Description: Caco-2:<4 = low permeability; 4-70 = moderate permeability;>70 = high permeability, Human Intestinal Absorption (HIA): 0-20 = not well absorbed; 20-70 = moderately absorbed; 70-100 = well absorbed, Plasma Protein Binding (PPB):>90 = strongly bound;<90 = weakly bound (√) = Qualified; (-) = Not Qualified.



(a) **(b)** **(c)**

Fig. 2: Structure of the anti-cholesterol: receptor complex (1HW9) (a); Protein (1HW9) (b); Simvastatin (c) (Source: Personal document)

Table 5: Results of toxicity screening of bawang dayak active compounds

Compound name	Toxicity prediction		
	Mutagens	Carcinogen	
	Ames test	Carcino mouse	Carcino rat
Undecanoic Acid, Ethyl Ester	-	+	-

Description: (+) = predicted to be mutagenic/carcinogenic, (-) = predicted to be non-mutagenic/non-carcinogenic

Table 6: Protein optimization

S. No.	Validation	Anti-cholesterol
1	Compound	Simvastatin
2	PDB Code	1HW9
3	*Grid Box*	X = 4.03081
		Y =-9.43187
		Z =-11.5016

Table 7: Validation of molecular anti-cholesterol methods

S. No.	Validation	Anti-cholesterol
1	Compound	Simvastatin
2	PDB Code	1HW9
3	RMSD Reference	1.7058 Å

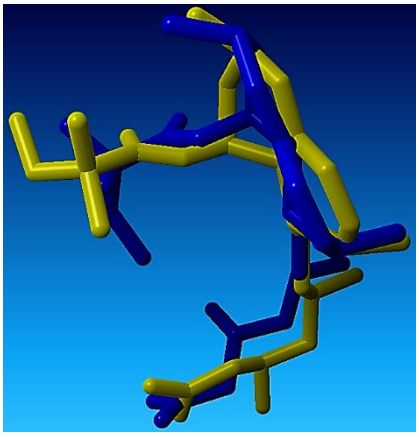


Fig. 3: Molecular validation of initial conformational *overlay and conformational* form after molecular docking simulation. Blue color of *reference ligand compound* on 1HW9 receptor and yellow color of *reference ligand compound* on 1HW9 receptor that has been *relocked*

Table 8: Results of *comparative* activity of undecanoic acid ethyl ester site with 1HW9

No	Conformation	1HW9	
		Simvastatin (Reference ligand)	Undecanoic acid ethyl ester
1	HMG-COA Reductase Conformation 1	-75.1301	-59.6474
2	HMG-COA Reductase Conformation 2	-78.1763	-63.3378
3	HMG-COA Reductase Conformation 3	-72.9728	-60.2474
4	HMG-COA Reductase Conformation 4	-71.5213	-62.3162
5	HMG-COA Reductase Conformation 5	-71.8975	-61.6943
6	HMG-COA Reductase Conformation 6	-72.1852	-64.5758
7	HMG-COA Reductase Conformation 7	-72.6779	**-64.8993**
8	HMG-COA Reductase Conformation 8	-73.9162	-63.0358
9	HMG-COA Reductase Conformation 9	-76.3301	-57.1942
10	HMG-COA Reductase Conformation 10	-78.3429	-63.3875
Average		-74.31503	-62.03357

Table 9: Results of energy binding locations in anticholesterol activity

S. No.	Compound name	Hydrogen bonds	Hydrophobic bonds
1	Undecanoic Acid Ethyl Ester	Series: 852	Ala: 564, Ala: 856, Arg: 568, Asn: 755, Cys: 561, Glu: 559, Gly: 560, His: 752, Leu: 562, Leu: 853, Ser: 565
2	Simvastatin (Comparator)	Ala: 751, Asn: 755, Lys: 735, Glu: 559	Ala: 756, Cys: 561, His: 752, Leu: 562, Leu: 853

Description: Ala: AlanineGlu: Glutamic Acid, Arg: ArginineGly: Glycine, Asn: AsparagineHis: Histidine, Cys: CysteineLeu: Leucine, Ser: SerineLys: Lysine

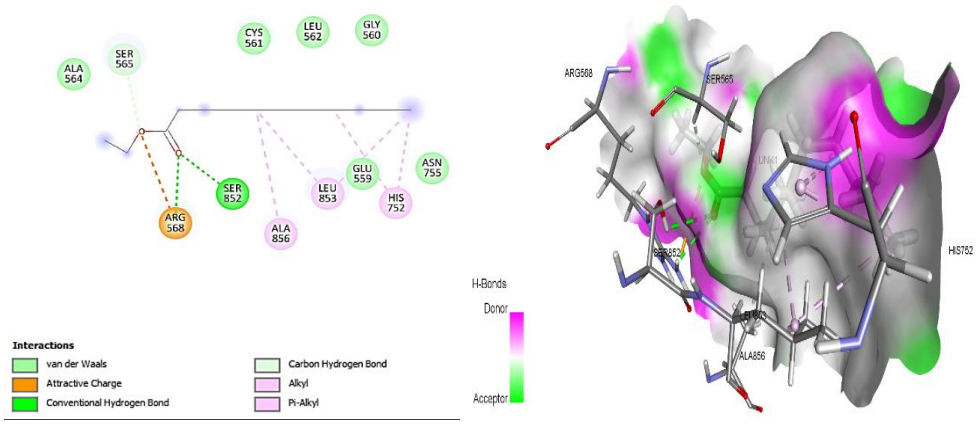


Fig. 4: Two-dimensional and three-dimensional visualization between ligands and receptors (Undecanoic acid, Ethyl ester)

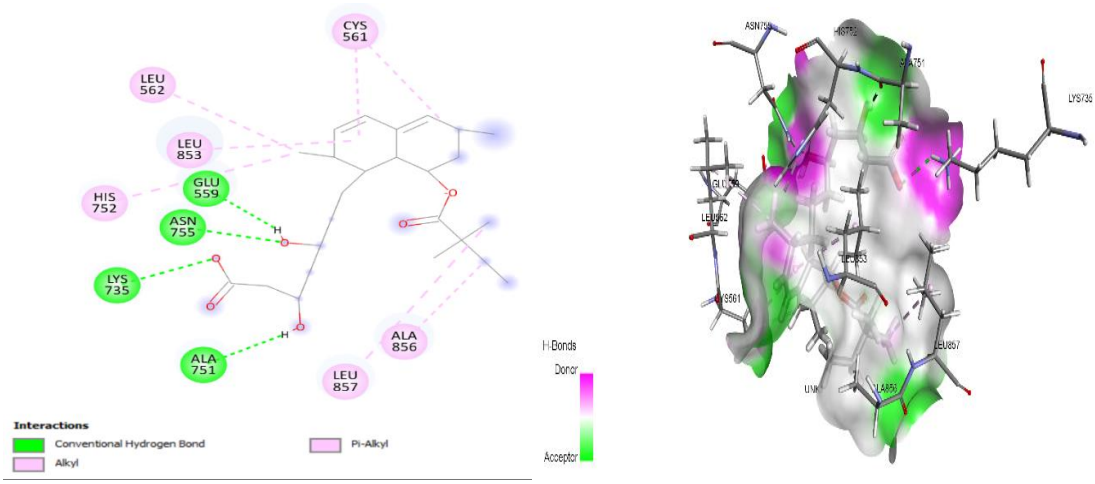


Fig. 5: Two-dimensional and three-dimensional visualization between ligands with (Simvastatin)

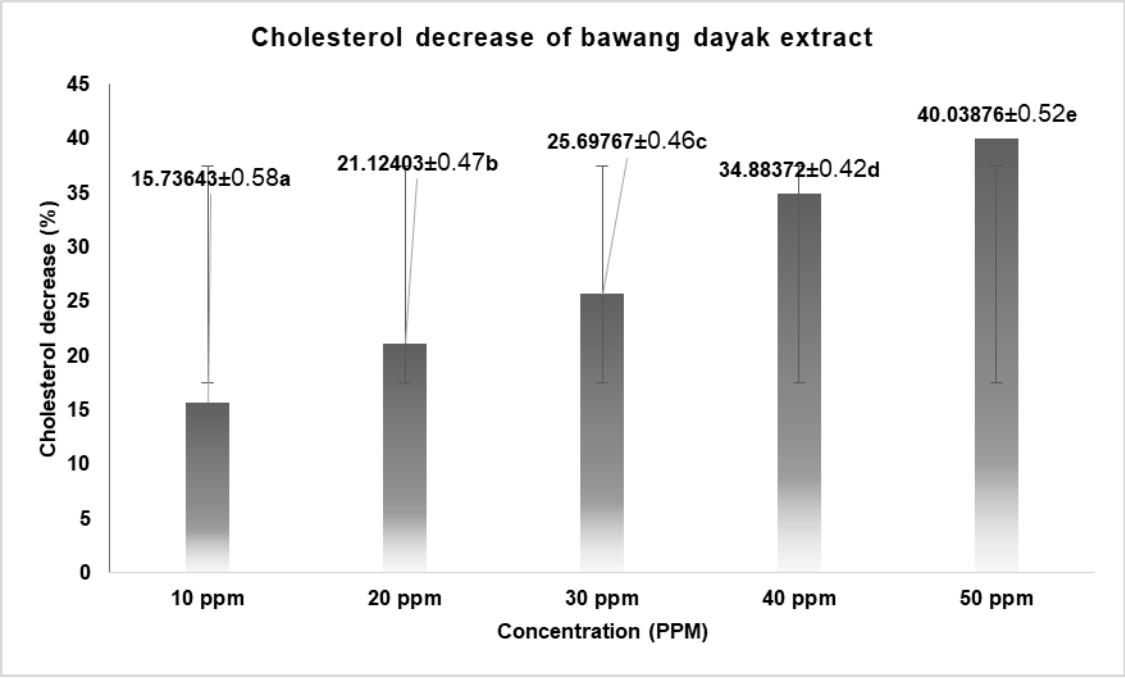


Fig. 6: *In vitro* results of anti-cholesterol activity of bawang dayak extract, Description: Numbers followed by the same letter are not significantly different in the tukey HSD test (0.05)

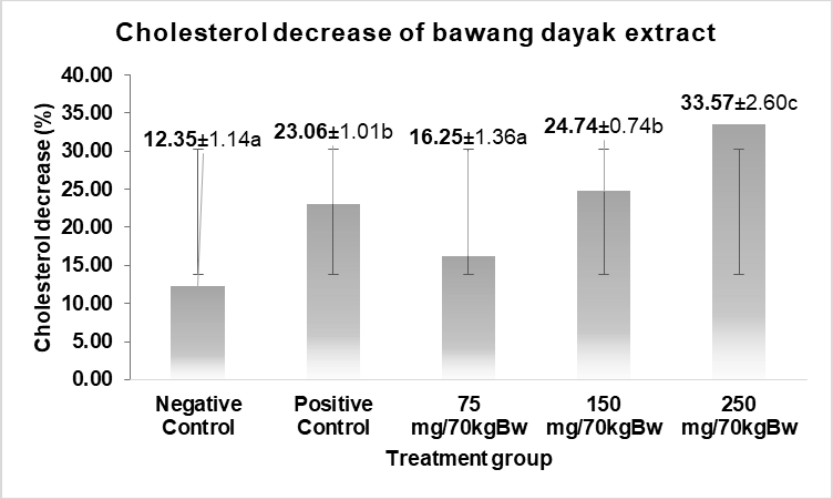


Fig. 7: *In vivo* result of anti-cholesterol activity of bawang dayak extract, description: Numbers followed by the same letter are not significantly different in the tukey HSD test (0.05)

DISCUSSION

Active compounds found in Bawang Dayak extract through GC-MS analysis based on reference was shown in table 1**.** The compounds are mostly found in the terpenes and the fatty acid esters group. Terpenes and fatty acid esters can be extracted using a wide range of solvents. Ethanol used as a solvent in this study can effectively extract terpene compounds and fatty acid esters so that they can be analyzed using GC-MS. The selection of this solvent is in line with research from [14, 15]. In drug discovery, a good drug solubility and permeability profile is certainly needed, the *Lipinski's Rule of Five method* has the function of accelerating the drug discovery process based on the permeability of drug candidates. Compounds identification that was carried out using the GC-MS method obtained 9 active compounds formulas. Determination of compounds can be determined as proof of the formula obtained in several active compounds by obtaining AH values and RT values [16]. The compounds screening in Bawang Dayak extract based on Lipinski's Rule of Five to determine which compounds that have potential to be a drug candidate was shown in table 2**.**

Based on Lipinski's rule, compounds that can be used as drug candidates are those that meet the criteria for hydrogen donor parameters value<5, hydrogen acceptor value<10, molecular weight<500, and logP value<5 [17]. This information explains that compounds with a molecular weight>500 can reduce permeability of the compound in the intestines, blood circulation, and the central nervous system. Log P or can be called the solubility value of a compound<5. If there are many hydrogen donors (the value>5 and hydrogen acceptors (the value>10) in a compound, it will make the compound difficult to penetrate the lipid bilayer membrane because it tends to be partitioned in a strong hydrogen bond solvent such as water. Table 2 showed the results of compound identification using the *Lipinski's Rule of Five method* of Bawang Dayak, that had 9 active compounds from the GC-MS method with an AUC value of<1%(18). Among the 9 compounds, 1 compound qualified the requirements was Undecanoic Acid, Ethyl Ester compound. The absorption, distribution and toxicity levels of the selected drug compounds were observed so that provisions that the compound had good bioavailability, thus avoiding failure in the next step [19].

The software used in this study was *Lipinski's Rule of Five* method. This method was used for structure-based drug design modeling where the biological target of the disease can be known. Drug compounds that meet Lipinski's criteria are reviewed using the Pre-Absorption, Distribution, Metabolism, Excretion and Toxicity (Pre-ADMET) tool for ADME characteristics and toxicity [20]. The pharmacokinetic screening of undecanoic acid, ethyl ester as an active compound in Bawang Dayak that can be seen in table 3 by observing the parameters of Caco-2 absorption, HIA and PPB had results that the compound qualified the requirements as a good drug candidate [21]. Besides, toxicity aspects of undecanoic acid, ethyl ester with the parameter of carcinogenic and mutagenic that can be seen in table 4 showed that the undecanoic acid compound, ethyl ester can be predicted in carcino mice was carcinogenic. This identification aimed to reduce the possibility of drug development failure so that the compound considered as selected drug candidates are based on their pharmacokinetics and toxicity profile of the compound [19]. The safety of Bawang Dayak extract in acute and chronic conditions in mice model has not been studied. It is thus required to assess the safety of Bawang Dayak extract in mice model to ensure the result of in vivo study related to Bawang Dayak extract using mice model are not disturbed or biased. The results in table 5**.** protein optimization from anticholesterol validation data showed the compound simvastatin, PDB code Protein 1HW9 with a grid box (X=4.03081, Y=-9.43187, Z=-11.5016). The results in table 6**.** Regarding the validation of the molecular anti-cholesterol method show the compound simvastatin with PDB code protein 1HW9, RMSD Reference 1.7058A.

The active site of Undecanoic Acid Ethyl Ester with 1HW9 binding energy showed the stability of a ligand in binding to its receptor (table 7). The higher the negative value, the higher the binding energy, so the more stable the conformation between the ligand and the target protein. The best ligand produced by virtual screening is the ligand with the highest binding energy that approaches the actual ligand binding energy value. HMG-COA Reductase Conformation 7 in the undecanoic acid, ethyl ester compound had a value of-64.8993. This value was the largest value approaching the reference value of the simvastatin ligand. A large value indicates strong and stable binding [22].

The relationship between ligands and active sites of certain residue micro molecules (amino acids) and macro-molecules could be seen in table 8. The presence of interactions that follow the formation of bonds has the potential for internal reactions. To facilitate the handling of certain ligands and encourage ligand-receptors binding. Hydrogen bonds is very important in determining how the ligand aligns with the receptor [19].

Hydrogen bonds can be predicted by measuring the separation between the contacts. Hydrophilic interactions can significantly affect the binding energy results [23]. Complex molecules with molecular interactions in the form of chemical bonds are created when ligands attach to proteins. The presence of chemical bonds that occur between ligand atoms and amino acid residues in the target protein, including interactions such as hydrogen bonds and hydrophobic bonds [24].

Simulation of the interaction between the tested chemical and the active site of the receptor can be seen in fig. 1 and fig. 2. This

simulation is an anticipation of the candidate drug that is being tested with an optimal interaction configuration. Table 9 showed the findings of the simulation of the test drug molecule which produces many characteristics such as free energy, inhibition constant bonds, and the nature of the interaction between the ligand and the receptor [25]. Van der Waals bonds, hydrogen bonds, and other bonds are used to predict the presence of interactions (fig. 3). The comparator is then used to compare the parameters generated by the molecular docking simulation of the test compound. The simvastatin compound or the original ligand is the comparison used (fig. 4) [26].

The results showed the smallest free energy value of the interaction of test compounds derived from Undecanoic Acid, Ethyl Ester has a hydrogen bond (Ser: 852), gyrophobic bond (Ala: 564, Ala: 856, Arg: 568, Asn: 755, Cys: 561, Glu: 559, Gly: 560, His: 752, Leu: 562, Leu: 853, Ser: 565). Simvastatin as comparator has a hydrogen bond (Ala: 751, Asn: 755, Lys: 735, Glu: 559), gyrophobic bond Ala: 756, Cys: 561, His: 752, Leu: 562, Leu: 853. The strength of the ligand bond with the 1HW9 receptor is indicated by its binding energy. Greater stability is indicated by the lack of its binding energy value. This value is influenced by various interactions between the ligand and the receptor [27].

The inhibition constant is another parameter that can be detected in the results of molecular simulations. One parameter that shows the interaction between a ligand and its receptor is the inhibition constant. Binding energy and inhibition constant values have a close correlation. The lower the inhibition constant value, the more stable the binding. Hydrogen bonds of Undecanoic Acid, Ethyl Ester compound was found in amino acids SER 852 and ARG 568. Simvastatin comparison test on amino acids obtained GLU 559, ASN755, LYS735, ALA751. The results could be seen in fig. 5 and 6 [28].

The results of the *in vitro* anti-cholesterol activity study can be seen in fig. 5. The result showed the largest percentage decrease at a concentration of 50 ppm with a result of 40.04 % [29]. Based on statistical analysis, every concentration gives a significant anti-cholesterol effect. The results of the *in vivo* study of anti-cholesterol activity can be seen in fig. 6. The result showed the largest percentage decrease in the extract group with a dose of 250 mg/70 kgBw with a value of 33.57%. The percentage of cholesterol decrease in the positive control group was 23.06 %. This result is not significantly different from Bawang Dayak extract in a dose of 150 mg/70 kgBw. The *in vitro* and *in vivo* results prove that Bawang Dayak can lower the total cholesterol level. Based on the prior research of another natural anti-cholesterol agent like garlic (*Allium sativum*), Bawang Dayak extract is more effective in cholesterol reduction. The study from [30] briefly stated that garlic extract with a dose of 500 mg/70 kgBw can give 16% cholesterol reduction in rats. Another study from [31] showed that 100 ppm of Bawang Dayak extract only decrease the cholesterol less than 20%.

Bawang Dayak extract has secondary metabolite compounds of flavonoids and active compounds of Undecanoic Acid, Ethyl Ester that potentially play a role in reducing cholesterol levels *in vivo.* The mechanism of action of undecanoic acid ethyl ester in lowering total cholesterol is not been fully discovered. The potential mechanism of undecanoic acid ethyl ester, like other fatty acid ethyl esters, such as increased plasma lipoprotein lipase activity, decreased lipogenesis in the liver, increased mitochondrial and peroxisomal β-oxidation in the liver and inhibition of acyl-CoA: 1,2-diacylglycerol acyltransferase [32]. Further research is thus required to fully understand the enzyme inhibition mechanism specific to HMG-CoA reductase to validate docking predictions, and molecular dynamics simulations to assess ligand-receptor interaction stability.

CONCLUSION

However, this research had some limitations as follows: the founding compounds have not been validated with a proper method such as similarity retention index, calibration curves, or LOD/lOQ. The absence of direct mechanistic studies, such as Western blotting, to assess HMG-CoA reductase expression. The presence of approximately 20% unidentified compounds, which could influence biological activity.

In conclusion, the active site of Undecanoic Acid Ethyl Ester with 1HW9 binding energy showed the stability of a ligand in binding to its receptor. The best ligand produced by virtual screening is the ligand with the highest binding energy that approaches the actual ligand binding energy value. HMG-COA Reductase in the undecanoic acid, ethyl ester compound had a value of-64.8993. This value was the largest value approaching the reference value of the simvastatin ligand the *in vitro* study showed the largest percentage of cholesterol decrease was at a concentration of 50 ppm with a value of 40.04 %. The *in vivo* study showed the largest percentage of cholesterol decreasing were in the positive control group (simvastatin) with a value of 23.06% and in Bawang Dayak extract group with a dose of 250 mg/70 kgBW with a value of 33.57 %. Bawang dayak has an activity as anti-cholesterol that proven by *in silico*, *in vitro* and *in vivo* study.

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AUTHORS CONTRIBUTIONS

First and second authors contribute to drafting, supervision, investigation, review, resource, and conceptualization. Third, fourth, and fifth authors contribute to drafting and reviewing.

CONFLICT OF INTERESTS

The authors have no conflicts of interest regarding this investigation.

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