

# Influence of extraction methods of bay leaves (*Syzygium polyanthum*) on antioxidant and HMG-CoA Reductase inhibitory activity

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# Influence of extraction methods of bay leaves (*Syzygium polyanthum*) on antioxidant and HMG-CoA Reductase inhibitory activity

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## Abstract

**Objective:** Bay leaf, one of the plants in Indonesia that has been shown to have activities to reduce cholesterol in the blood. HMG-CoA Reductase inhibition is one of many mechanisms in lowering the level of cholesterol in the blood. Here, we reported the inhibitory activity of HMG-CoA Reductase of bay leaves ethanol extracts that we suspected to be the mechanism of action of bay leaves in reducing cholesterol in the blood. In this research we also investigated the correlation between the inhibitory activities, the total phenol content and antioxidant activities of bay leaves (*Syzygium polianthum*) ethanol extracts.

**Methods:** The inhibitory activity of HMG-CoA Reductase was determined kinetically at 340 nm using simvastatin as positive control. *In vitro* scavenging assays of 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric ion reducing antioxidant power (FRAP), and beta-carotene method were used to determine the antioxidant activities. The total phenolic content was determined by Folin-Ciocalteu's method.

**Results:** The IC<sub>50</sub> of bay leaves ethanolic extract obtained by percolation and soxhlet extraction method towards HMG-CoA Reductase enzyme activity were

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49.50  $\pm$  0.700  $\mu$ g/mL and 15.50  $\pm$  0.707  $\mu$ g/mL, respectively, while the  $IC_{50}$  of simvastatin was 0.00238  $\pm$  0.00004  $\mu$ g/mL. The antioxidant activity and total phenolic content of bay leaves ethanolic extract obtained by Soxhlet extraction method was higher compared to the percolation method (DPPH and beta-carotene assay results). The 3D linear analysis showed that there was a high correlation between the inhibition activities of HMG-CoA Reductase pattern of both extract types and the total phenol pattern and also the antioxidant pattern of these extracts.

**Conclusion:** The result showed that the bay leaves ethanolic extract have a potent activity to reduce the cholesterol serum level by inhibition of HMG-CoA Reductase activity. The activity was due to the phenolic compounds in the extracts as well as the antioxidant activity of the extracts.

**Keywords:** Biochemistry, Molecular biology, Natural product chemistry

## 1. Introduction

Cardiovascular disease contributed largely to the high mortality rate worldwide year by year. Based on the research in epidemiology, the risk factor of cardiovascular disease is a combination of two or more risk factors. The risk factors of cardiovascular disease are classified into two groups, which are the modifiable risk factors (dyslipidemia, hypertension, smoking, diabetes mellitus, stress, obesity) and the non-modifiable risk factors (heredity, age, gender). A common risk factor of cardiovascular disease is high serum cholesterol level [1, 2, 3].

Cholesterol is a lipid produced in the liver with a number of important roles, such as a membrane constituent and the parent molecule for steroid hormones [4]. Cholesterol can be synthesized by the body and also can be derived from daily food. The increase of cholesterol level in the bloodstream can cause hypercholesterolemia [1]. Hypercholesterolemia is one of the risk factors for the emergence of atherosclerosis, which is inflammatory disorders in artery walls characterized by the formation of atheroma [5]. Atherosclerosis plaque could clog the heart's blood vessel area. This blockage then leads to cardiovascular disease [6]. The increase in cholesterol level can be caused by excessive cholesterol synthesis, the excess of cholesterol absorption, and high cholesterol intake from daily food. Decreasing the cholesterol level can be done by inhibiting cholesterol synthesis through inhibiting the activity of HMG-CoA Reductase which converts Acetyl-CoA into mevalonate [1, 7]. This enzyme is a pharmacological treatment target for group of drugs called HMG-CoA Reductase inhibitor (statins) [8]. However, anti-cholesterol drugs usually are used in combination, and this may increase the chance of unexpected side effects in long-term use.

Bay leaves (*Syzygium polyanthum*) is one of the plants that can be used to decrease the cholesterol level [9]. Bay leaves contain secondary metabolites, such as saponin, terpenoid, flavonoid, polyphenol, alkaloid, and essential oil. Some previous *in vivo* studies showed that the extract of bay leaves could lower cholesterol levels in the animal blood [10, 11]. It is believed that flavonoid (phenolic compound) as one of the chemical content of the bay leaves plays a role in the decrease of cholesterol levels in the blood. In addition, the research conducted by Lee et al. [12] proved that flavonoids can lower cholesterol levels by inhibiting the action of HMG-CoA Reductase. Several experiment showed that flavonoids and phenolic acids, which are classes of polyphenolic compounds have antioxidant properties, including induction of anti-inflammatory actions, inhibition of oxidative enzymes, and scavenging of free radicals [13].

Based on the researches that have been done to the animals treated with bay leaves, further research about the potency of bay leaves as the anti-hypercholesterolemia *in vitro* is needed with the enzymatic measurement. The extract of bay leaves used was obtained by Soxhlet extraction and percolation method. The measurement of antioxidant activities in each extract was also done to seek the correlation of antioxidant activities and HMG-CoA Reductase inhibition activities. This research covers the taxonomy of Biochemistry and Molecular Biology.

## 2. Materials and methods

### 2.1. Equipment and materials

The equipment used during the study were analytical scales (Sartorius, Germany); oven (Binder); infrared moisture balance (Kett, China); 5  $\mu$ L capillary tubes; microtubes (Mini spin, USA); vortex; micropipettes; blue tips; white tips; membrane filters; glasswares; chamber; soxhlet; water bath; spectrophotometer (Multiscan Go, Thermo Scientific, USA); cuvettes (Bio-Rads Lab, 2000 Alfred Nobel Drive Hercules, Catalog number 9109250).

Dried bay leaves (*Syzygium polyanthum*) obtained from PT. HRL International Indonesia, Pasuruan, East Java, the enzyme used was the HMG-CoA Reductase Assay Kit (Catalog number CS 1090, Sigma, Germany), 96% ethanol, phytochemical screening reagents, water for injection, sodium hydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ) (Merck, Indonesia), sodium dihydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) (Merck, Indonesia), simvastatin tablet, antioxidant assay reagents.

### 2.2. Preparation of extract

Standardization was done to the dried bay leaves prior to the extraction. The extraction was done with percolation and Soxhlet extraction method using ethanol 96% as

the solvent. The mass of the dried bay leaves used for percolation method was 1 kg in total 3.6 liter of solvent, while the mass used for Soxhlet extraction was 0.5 kg in total 3.03 liter of solvent divided in several steps, which was 20 gram of dried bay leaves in 120 ml solvent for each process. The rendement of extract obtained from percolation method was 25.05%, while from soxhlet extraction method was 23.62%.

The extract was then evaporated on a water bath then was stored in a sterile bottle. The dried extract was further standardized to determine the organoleptic characteristics, total ash content, water content, and the solubility in ethanol to ensure the quality. Phytochemical screening was also done to the dried extract prior to antioxidant and enzymatic assay.

### 2.3. HMG-CoA Reductase activity assay

366 µl 1x assay buffer was mixed with 24 µl HMG-CoA substrate, 8 µl NADPH, and 2 µl enzyme. The mixture was then measured at 37 °C with a spectrophotometer UV (Multiscan Go, Thermo Scientific, USA) at 340 nm wavelength and the absorbance was read every 15 seconds for 5 minutes [14].

125 mg of bay leaves ethanol extract was dissolved in 25 ml of sterile water to make the standard solution 5000 ppm. The solution was further made into different concentration: 0 ppm, 10 ppm, 25 ppm, 50 ppm, 150 ppm, 300 ppm, 600 ppm, and 1200 ppm. The solution was centrifuged and filtered using a 0.45 µm filter membrane to remove the residual sediment from the extract. 364 µl 1x assay buffer was mixed with 24 µl HMG-CoA substrate, 8 µl NADPH, 2 µl extract from each concentration and 2 µl enzyme. The mixture was then measured at 37 °C with a spectrophotometer UV (Multiscan Go, Thermo Scientific, USA) at 340 nm wavelength and the absorbance was read every 15 seconds for 5 minutes.

Standard solution of simvastatin was taken 2 µl from each concentration 0 ppm, 0.0010 ppm, 0.0014 ppm, 0.0018 ppm, 0.0022 and 0.0026 ppm. Each 2 µl solution was mixed with 364 µl 1x assay buffer, 24 µl HMG-CoA substrate, 8 µl NADPH and 2 µl enzyme. The mixture was then measured at 37 °C with a spectrophotometer UV (Multiscan Go, Thermo Scientific, USA) at 340 nm wavelength and the absorbance was read every 15 seconds for 5 minutes.

The data from spectrophotometric was analyzed to determine the enzyme activity (Sigma-Aldrich, 2013), using this equation:

$$\text{Specific activity} = \frac{(\Delta A(\text{sample}/\text{min}) \times \text{TV})}{12.44 \times V \text{ enzyme} \times 0.6 \times \text{LP}}$$



where  $\Delta A$ : Change of absorbance,  $T_V$ : Total volume of the reaction in ml, 12.44: coefficient of NADPH,  $V_{\text{enzyme}}$ : volume of enzyme used in the assay, 0.6: Enzyme concentration in mg-protein, LP: Lightpath in cm.

## 2.4. Statistical analysis

All test scores were presented as mean values of inhibition  $\pm$  standard deviation from two replications. The percent of inhibition was obtained from the activity without inhibitor minus activity with inhibitor divided by activity without inhibitor. For statistical data analysis, each group was compared using independent sample T-test with 95% level of confidence.

## 2.5. Antioxidant assays

Antioxidant activities of the extracts were assayed by three different methods, which were the DPPH method, the FRAP method, and beta-carotene method. The DPPH method states the antioxidant activity as the oxidation inhibition by referring to Chandra and Dave [15] and Shafazila et al. [16]. The antioxidant potency was measured using % Scavenging effect. The antioxidant assay using FRAP reagent refers to Benzie and Strain [17] where the antioxidant capacity stated as  $\mu$ moles Trolox/g dry powder. The beta-carotene assay was done according to Utami et al. [18]. The antioxidant potency of the sample was expressed as the concentration with exhibit 50% of the antioxidant activity ( $EC_{50}$ ).

## 2.6. Total phenolic content

Extract solution of bay leaves was prepared in different concentration: 0 ppm, 10 ppm, 25 ppm, 50 ppm, 150 ppm, 300 ppm, 600 ppm, and 1200 ppm. Each solution of bay leaves extract was pipetted 100  $\mu$ l and was mixed with 300  $\mu$ l of 2% sodium carbonate, 1.58 ml of deionized water, and 100  $\mu$ l of 10% Folin-Ciocalteu reagent. The absorbance of the reaction mixture was observed at 750 nm (Multiscan Go, Thermo Scientific, USA) after 30 min incubation at room temperature. Gallic acid was used as a standard [19]. The data were expressed as ppm gallic acid equivalents.

## 3. Results & discussion

Choosing the right extraction method is one of the supporting factors in the success of a therapy, including lowering cholesterol level in the blood. This can be caused by the solubility of secondary metabolites in plants depending on the type of solvent and temperature used during extraction. From the phytochemical screening results, both bay leaves ethanol extract (percolation method and soxhletation method) contain alkaloid, flavonoid, saponin, tannin, steroid.

The results of inhibition potency and  $IC_{50}$  of bay leaves ethanolic extract obtained by percolation and soxhlet extraction method towards HMG-CoA Reductase enzyme activity can be seen in Tables 1 and 2. Simvastatin, the first generation of statins, was used as a reference compound in this research. The inhibition potency of simvastatin toward HMG-CoA Reductase enzyme is shown in Fig. 1. The  $IC_{50}$  value of simvastatin measured in this study was  $0.00238 \pm 0.00004$   $\mu\text{g/mL}$ , which is smaller than the values found in the former researches which were about  $0.00376$ – $0.00778$   $\mu\text{g/mL}$  [7, 20, 21]. These values ( $49.50 \pm 0.700$   $\mu\text{g/mL}$  for extract obtained by percolation, and  $15.50 \pm 0.707$   $\mu\text{g/mL}$  for extract obtained by Soxhlet extraction) were significantly different ( $p > 0.05$ ) if compared to the  $IC_{50}$  of simvastatin. The potency of ethanolic extract of bay leaves in inhibiting HMG-CoA Reductase is smaller when compared with simvastatin, where the ability of simvastatin in inhibiting HMG-CoA Reductase about six thousand to twenty thousand times greater than the ethanolic extract of bay leaves.

Several other reports have also reported the potency of plant extracts in HMG-CoA Reductase inhibition. *Opuntia ficus-indica* (L) Miller extract was reported by Re-saissi et al. [22] to have  $IC_{50}$  20.3  $\mu\text{g/ml}$  and said as to have moderate potency. Ademosun et al. [23] reported that grapefruit peels had an  $IC_{50}$  on HMG-CoA Reductase activity 0.11  $\mu\text{g/ml}$ . *Vernonia condensata* extract showed the  $IC_{50}$  value of 271.7  $\mu\text{g/ml}$  [24] and *Gnetum gnemon* extract had an  $IC_{50}$  value on HMG-CoA Reductase of 400  $\mu\text{g/ml}$  [25]. There are also studies that have assayed the potency of several isolated chemical contents of the plants in HMG-CoA Reductase inhibition, and it was reported that the compounds inhibit the enzyme activity with the  $IC_{50}$  value 8.34–149.6  $\mu\text{g/ml}$  [22, 26]. Based on these several studies it can be stated that certain plant extract is said to have HMG-CoA Reductase inhibition potency in the range value of  $IC_{50}$  between 0.1 to 400  $\mu\text{g/ml}$  [22, 23, 25, 27]. Thus, the ethanol extracts of bay leaves are also a potent HMG-CoA Reductase inhibitor.

**Table 1.** The inhibition of HMG-CoA Reductase of ethanol extract of bay leaves obtained by percolation method.

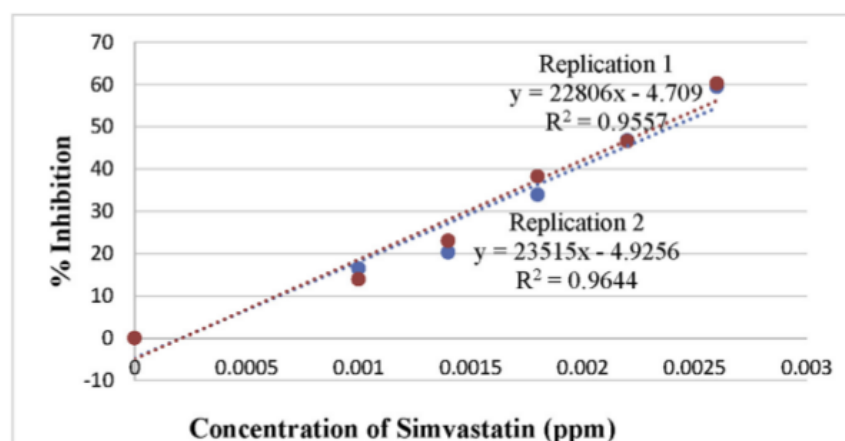
Concentration ( $\mu\text{g/ml}$ )	% of Inhibition		Mean	SD	$IC_{50}$ ( $\mu\text{g/ml}$ )
	n1	n2			
0	0	0	0	0	n1 = 50.00
10	28.49	21.03	24.760	5.275	n2 = 49.00
25	47.10	42.59	44.845	3.189	
50	57.10	57.03	57.065	0.049	
150	64.40	67.02	65.710	1.853	
300	66.24	74.66	70.450	5.954	
600	82.24	83.28	82.760	0.735	
Mean $\pm$ SD = $49.50 \pm 0.700$					

**Table 2.** The inhibition of HMG-CoA Reductase of ethanol extract of bay leaves obtained by Soxhlet method.

Concentration (μg/ml)	% of Inhibition		Mean	SD	IC50 (μg/ml)
	n1	n2			
0	0	0	0	0.000	n1 = 15.00
10	47.17	48.55	47.860	0.976	n2 = 16.00
25	54.72	56.16	55.440	1.018	
50	69.81	66.67	68.240	2.220	
150	79.25	76.09	77.670	2.234	
300	88.68	84.42	86.550	3.012	
600	101.9	97.10	99.500	3.394	

Mean ± SD = 15.50 ± 0.707

The potency of ethanol extract of bay leaves obtained by Soxhlet extraction is three times higher than the potency of ethanol extract of bay leaves obtained by percolation. This showed that the Soxhlet process was able to extract more active constituent that responsible for the inhibition of HMG-CoA Reductase and that the active constituents are stable under heating. It is suspected that these active constituents are polyphenolic compounds such as gallic acid, eugenol, kaempferol and quercetin [28]. Some studies have shown that polyphenolic compounds (luteolin, quercetin, and isorhamnetin) contained in many plant extracts play a role in inhibiting HMG-CoA Reductase activity [22, 27]. The phenolic compound of grapefruit peels (genistein and daidzein) showed inhibition of HMG-CoA Reductase activity competitively against HMG-CoA as substrate [23]. Flavonoids, in specific, are stated by Lee et al. [12] to have the ability to inhibit the activity of the HMG-CoA Reductase. The research conducted by Anggraeni [29] which states that at the same concentration

**Fig. 1.** Graphic of HMG-CoA Reductase inhibition by simvastatin.



(10 µg/ml) quercetin and rutin are able to inhibit the activity of HMG-CoA Reductase respectively 41.10% and 60.17 % also support this hypothesis. However, other studies have not mentioned the inhibition kinetics of other flavonoid groups.

The hypothesis that the inhibition of HMG-CoA Reductase in ethanol extract of bay leaves was due to the polyphenolic content was proved by searching the correlation between the inhibition activity and the total phenolic content in the extract. Besides that, we also measured the antioxidant activity of each extract to study the correlation of it to inhibition activity and types of extract. The total phenolic content and antioxidant activity of each concentration involved in the measurement of HMG-CoA Reductase inhibition activity were reported in Tables 3 and 4. The total phenol in the soxhlet extract is greater than the total phenol in the percolation extract, which in accordance with the inhibition of HMG-CoA Reductase activity pattern. The antioxidant activity of each extract, measured by DPPH, FRAP and beta-carotene method, was compared to gallic acid and quercetin (Table 5). The DPPH and beta-carotene method gave the same pattern results, which showed that the antioxidant activity of Soxhlet extract was higher when compared to the percolation extract. These results also in line with the inhibition of HMG-CoA Reductase activity pattern. The FRAP method in the other way gave a different result, which showed that the antioxidant activity of the percolation method is higher than that of the Soxhlet method. This could be caused by the difference in the mechanism of the assay. FRAP method was based on the reduction of ferric ion to ferrous ion. Not all of the  $Fe^{3+}$  reductants are antioxidant, and some antioxidants are not able to reduce  $Fe^{3+}$  [30].

**Table 3.** Total phenolic content and antioxidant activity of ethanol extract of bay leaves obtained by percolation method.

Concentration (µg/ml)	Total phenol content (ppm)	Antioxidant activity		
		DPPH method <sup>a</sup>	FRAP method <sup>b</sup>	Beta-Carotene method <sup>c</sup>
0	0.0A	1.9960A	0A	0.0000A
10	53.6B	3.5532B	0A	10.2513B
25	56.0B	3.8627B	0A	8.0186C
50	61.4C	5.1647C	0.9625A	13.1217D
150	99.0D	8.5790D	8.3633B	7.9707E
300	150.4E	24.9729E	21.3933C	12.5075F
600	193.7F	43.3887F	22.2472C	21.2928G

Data were obtained from three independent experiments, each performed in triplicates (n = 9) and represented as mean ± SD.

Values with the same letter are not significantly different (P < 0.05).

<sup>a</sup> EC<sub>50</sub> was the concentration of substance that provides 50% inhibition.

<sup>b</sup> FRAP value was calculated as Ferrous Equivalents, the concentration of trolox/quercetin or extracts which induced an absorbance value equal to that of 1 mM  $Fe_2SO_4$ .

<sup>c</sup> EC<sub>50</sub> represents the effective concentration at 50% of total antioxidant activity.

**Table 4.** Total phenolic content and antioxidant activity of ethanol extract of bay leaves obtained by Soxhlet method.

Concentration (µg/ml)	Total phenol content (ppm)	Antioxidant activity		
		DPPH method <sup>a</sup>	FRAP method <sup>b</sup>	Beta-Carotene method <sup>c</sup>
0	0.0A	2.2224A	0A	0A
10	35.4B	4.1808B	0A	14.9736B
25	90.8C	5.1574C	0A	15.2237C
50	92.4C	10.0685D	0A	18.4625D
150	139.0D	20.1246E	0A	20.6429E
300	187.9E	46.5714F	4.2877B	27.6990F
600	201.8F	66.9863G	19.1348C	29.0379G

Data were obtained from three independent experiments, each performed in triplicates (n = 9) and represented as mean ± SD.

Values with the same letter are not significantly different (P < 0.05).

<sup>a</sup>IC<sub>50</sub> was the concentration of substance that provides 50% inhibition.

<sup>b</sup>FRAP value was calculated as Ferrous Equivalents, the concentration of trolox/quercetin or extracts which produced an absorbance value equal to that of 1 mM Fe<sub>2</sub>SO<sub>4</sub>.

<sup>c</sup>EC<sub>50</sub> represents the effective concentration at 50% of total antioxidant activity.

The correlation analysis between each factor in this research was done by 3D linear analysis using SigmaPlot 12.5. The results of the analysis were shown in Tables 6, 7, and 8. Table 6 showed the correlation between extraction method (expressed in concentration, x-axis) and total phenolic content (y-axis) towards antioxidant activity. The level of correlation was shown by the R<sup>2</sup> value. The results showed that there is a high correlation between the extraction method and total phenolic content towards antioxidant activity. The higher to total phenolic content in both extracts will cause the increase in the antioxidant activity.

**Table 5.** Antioxidant activity value of ethanol extract of bay leaves obtained by Soxhlet Method.

Samples	Antioxidant activity		
	DPPH method (IC <sub>50</sub> – ppm) <sup>a</sup>	FRAP method (FRAP value – ppm) <sup>b</sup>	Beta-Carotene method (EC <sub>50</sub> – ppm) <sup>c</sup>
Gallic Acid	23.87 ± 0.00A	10.60 ± 0.01A	24.87 ± 0.24A
Quercetin	48.87 ± 0.00B	21.94 ± 0.00B	98.44 ± 0.39B
Bay leaves ethanolic extract - percolation	888.08 ± 0.05C	295.00 ± 0.02C	2965.62 ± 0.65C
Bay leaves ethanolic extract - soxhlet	437.89 ± 0.03D	684.00 ± 0.03D	2230.35 ± 1.20D

Data were obtained from three independent experiments, each performed in triplicates (n = 9) and represented as mean ± SD.

Values with the same letter are not significantly different (P < 0.05).

<sup>a</sup>IC<sub>50</sub> was the concentration of substance that provides 50% inhibition.

<sup>b</sup>FRAP value was calculated as Ferrous Equivalents, the concentration of trolox/quercetin or extracts which produced an absorbance value equal to that of 1 mM Fe<sub>2</sub>SO<sub>4</sub>.

<sup>c</sup>EC<sub>50</sub> represents the effective concentration at 50% of total antioxidant activity.

**Table 6.** Correlation between extraction method and total phenolic content towards antioxidant activity.

Extraction method	Antioxidant method	Function	R <sup>2</sup>
Percolation	DPPH	$f = 0.8310 + 0.445x + 0.0742y$	0.9890
	FRAP	$f = 0.7649 + 0.0419x + 0.0196y$	0.9663
	Beta – Carotene Bleaching	$f = 3.7012 + 0.0068x + 0.0652y$	0.8511
Soxhlet	DPPH	$f = 5.2176 + 0.0288x + 0.20083y$	0.9137
	FRAP	$f = 1.2690 + 0.0465x - 0.0501y$	0.9949
	Beta – Carotene Bleaching	$f = 5.1409 + 0.0032x + 0.1196y$	0.9156

**Table 7.** Correlation between extraction method and total phenolic content towards percent of HMG-CoA Reductase inhibition.

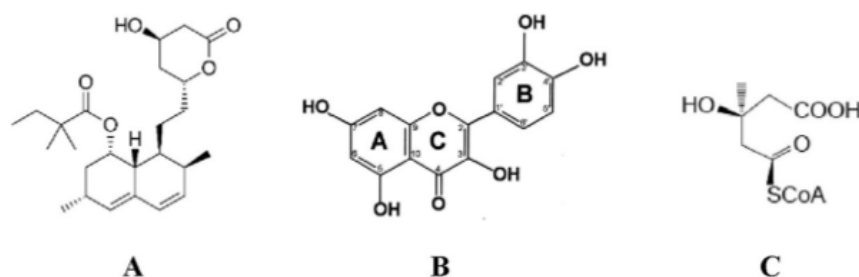
Extraction method	Function	R <sup>2</sup>
Percolation	$f = 3.9241 - 0.0955x + 0.6945y$	0.8688
Soxhlet	$f = 15.4733 - 0.0299x + 0.4829y$	0.8871

**Table 8.** Correlation between extraction method and antioxidant activity towards percent of HMG-CoA Reductase inhibition.

Extraction method	Antioxidant method	Function	R <sup>2</sup>
Percolation	DPPH	$f = 38.8052 - 0.3180x - 3.1319y$	0.6154
	FRAP	$f = 32.6035 + 0.0486x + 1.1740y$	0.6006
	Beta – Carotene Bleaching	$f = 15.5054 + 0.0362x + 2.6778y$	0.7075
Soxhlet	DPPH	$f = 43.3496 + 0.2689x - 2.2742y$	0.5670
	FRAP	$f = 43.5523 + 0.0533x + 1.3197y$	0.5750
	Beta – Carotene Bleaching	$f = 1.4981 - 0.0057x + 3.4218y$	0.9759

Table 7 showed the correlation between extraction method (concentration, x-axis) and total phenolic content (y-axis) towards percent of HMG-CoA Reductase inhibition. There was also a strong correlation between each factor towards the inhibition of HMG-CoA Reductase activity, but the concentration of extract gave a different effect against the inhibition of HMG-CoA Reductase activity when compared to the total phenolic content. It can be explained that the increase of the concentration of extract will cause the increase also in the total phenolic content, but not all of the phenolic compounds in the extract act as an inhibitor of HMG-CoA Reductase. Thus, some of the phenolic compounds in the extract may act as an activator of the HMG-CoA Reductase.

Correlation between extraction method (concentration, x-axis) and antioxidant activity (y-axis) towards percent of HMG-CoA Reductase inhibition was shown in Table 8. The results of the 3D linear analysis showed a poor correlation between the concentration of extract and antioxidant activity towards the inhibition of HMG-CoA



**Fig. 2.** Structure of simvastatin (A), flavonoid (B), and HMG-CoA (C) [31, 32, 33].

Reductase activity. Thus, though the HMG-CoA Reductase catalyze the reduction-oxidation activity, its inhibition mechanism was not related to the antioxidant mechanism. We conclude that antioxidant compounds might be contributes to inhibit HMG-CoA Reductase but does not go through in the reduction-oxidation mechanisms.

<sup>19</sup>Based on these results, it can be concluded that the inhibition of HMG-CoA Reductase activity by the percolation and soxhlet extracts are caused by the phenolic compounds in the extracts, and it was suspected due to the flavonoids compounds. Further research needs to be done to confirm this report. The relationship between the flavonoid structure (Fig. 2B) with its activity as an enzyme inhibitor of HMG-CoA Reductase is due to the presence of -OH groups in C3', C4', and C5. It is also caused by the C=O group at C4. These groups play a role in forming hydrogen bonds with amino acids from HMG-CoA Reductase through hydrophobic interaction [26]. It is suspected that these groups play a role in their activity inhibiting the HMG-CoA Reductase enzyme because they have similarities in the pharmacophores group of the simvastatin. In the simvastatin structure (Fig. 2A) there is an -OH group and a C=O group (a pharmacophore group) that will form a bond with the enzyme, so that the enzyme work becomes inhibited. The C=O group in lactone ring of simvastatin will be hydrolyzed to become an active form (acid). The hydrolyzed simvastatin will then bind <sup>12</sup>the HMG-CoA Reductase by hydrogen bonding with the amino acids located on the active site of the enzyme. The structure of the hydrolyzed simvastatin in the lactone ring corresponds to the structure of the HMG-CoA substrate (Fig. 2C) so that the enzyme is able to bind with simvastatin and form the complex of enzymes.

## Declarations

## Author contribution statement

<sup>10</sup>Lanny Hartanti, Sumi Wijaya: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Stefania Maureen Kasih Yonas, Josianne Jacqlyn Mustamu: Performed the experiments; Wrote the paper.

Henry Kumia Setiawan: <sup>24</sup>Conceived and designed the experiments; Analyzed and interpreted the data; <sup>11</sup>Contributed reagents, materials, analysis tools or data.

Lisa Soegianto: Contributed reagents, materials, analysis tools or data.

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### <sup>6</sup>Competing interest statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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