Antihypercholesterolemic Activity of Water Fraction, Ethyl Acetate Fraction and *n*-Hexane Fraction of Jawer Kotok Leaves (*Plectranthus scutellarioides* L.) towards Hypercholesterolemic Rats

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Abstract
Hypercholesterolemic is a risk factor for atherosclerosis leading to heart disease and stroke. Hypercholesterolemic is the most common cause of death in Indonesia causing the death of 328,500 people.[13] Ethanolic extract from jawer kotok leaves (Plectranthus scutellarioides L.) of 500 mg/kg BW was reported to have significantly antihyperlipidemic activity on normal and propylthiouracil induced hyperlipidemic rats.[4] The present study investigated antihypercholesterolemic activity of waterfraction, ethyl acetate fraction and n-hexane fraction of jawer kotok leaves (Plectranthus scutellarioides L.) on wistar male white rats (Rattus novergicus).

Plectranthus scutellarioides leaves were extracted with ethanol 70% by macerator apparatus. The concentrated extract is then carried out as liquid liquid extraction with solvents n-hexane, ethyl acetate and water. Propylthiouracil and a high-fat diet were used to induced hypercholesterol rats followed by oral administration of simvastatin 1 mg/kg BW, n-hexan fraction 300 mg/kg BW, ethyl acetate fraction 300 mg/kg BW and water fraction 300 mg/kg BW. The result showed that n-hexan fraction 300 mg/kg had better antihypercholesterolemic activity. n-hexane fraction from jawer kotok leaves contains alkaloid, monoterpenoid and sesquiterpenoid.

Keywords: *Plectranthus scutellarioides* L., Antihypercholesterolemic, Propylthiouracil, Cholesterol, Fraction.

Introduction

Cholesterol is a steroid that biochemically plays an important role as the basic material of steroid hormone synthesis. Normal total cholesterol level in the blood is below 200 mg/dL. When it exceeds the normal limit, it is referred to as hypercholesterolemia.[1] Hypercholesterolemic is a risk factor for atherosclerosis leading to heart disease and stroke. Hypercholesterolemic is the most common cause of death in Indonesia causing the death of 328,500 people.[13]

Simvastatin as a synthetis drug can lower cholesterol levels in the blood. However, simvastatin has serious side effects if used in the long term. Common side effects of simvastatin are gastrointestinal disorders, blurred vision, insomnia and myopathy. The use of simvastatin in patients with liver and kidney disorders should be closely monitored.[1,5]

The use of herbal medicine can be an alternative in patients who have tolerance to synthesis drugs. In addition, herbal medicine is relatively safer than synthetis drugs. One of herbal medicine can lower cholesterol levels in the blood by jawer kotok leaves (*Plectranthus scutellarioides* L.). Ethanolic extract from jawer kotok leaves (*Plectranthus scutellarioides* L.) of 500 mg/kg BW was reported to have significant antihyperlipidemic activity on normal and propylthiouracil induced hyperlipidemic rats.[4]

The present study investigated antihypercholesterolemic activity of *n*-hexane fraction, ethyl acetate fraction and water fraction of jawer kotok leaves (*Plectranthus scutellarioides* L.) on Wistar male white rats (*Rattus novergicus*).

Material and Methods

Plant Material: Three kg of dry leaves of jawer kotok (*Plectranthus scutellarioides* L.) were purchased at Indonesian Spices and Medicinal Crops Research Institute, Bogor, West Java in October 2016. Confirmatory identification of the plant was done at *Herbarium Bandungense*, School of Life Science and Technology, Bandung Institute of Technology, West Java, Indonesia. *Plectranthus scutellarioides* leaves were extracted with ethanol 70% by macerator apparatus, then kept 3x24 hours by changing ethanol every 24 hours. Liquid extract was then separated by using flannel cloth and the filtrate was concentrated by evaporator.

The concentrated extract is then carried out by liquid liquid extraction with solvents *n*-hexane, ethyl acetate and water. The concentrated extract used was weighed in a ratio 1:1 with the solvent. The first solvent used was water, then *n*-hexane was added. Shake and replace *n*-hexane solvent until *n*-hexane fraction was cleared. Furthermore, the water fraction is mixed with ethyl acetate solvent. Shake and replace ethyl acetate solvent until ethyl acetate fraction was cleared. Then from each fraction remove the solvent using

rotary evaporator and water bath until the fraction was concentrated. The concentrated *n*-hexane fraction from jawer kotok leaves will be referred as JKH (Jawer Kotok *n*-hexane). The concentrated ethyl acetate fraction from jawer kotok leaves will be referred as JKE (Jawer Kotok Ethyl Acetat). The concentrated water fraction from jawer kotok leaves will be referred as JKW (Jawer Kotok Water).

Other materials: Other materials used are 70% ethanol, aquadest, ethil acetate, n-hexane, Dragendorff reagent, Mayer reagent, Liebermann-Burchard reagent, propylthiouracil, simvastatin, pulvis gummi arabicum (PGA), Total Cholesterol Kit, Triglycerides Kit, HDL-Cholesterol Kit from Biolabo.

Apparatus: Macerators, Evaporators, Waterbath, Blood Collection Tube No Additive, Centrifuge (Hettich) and Visible Spectrophotometer (Thermo Genesys).

Experimental Design: Thirty wistar male white rats with 2-3 months and weighing between 170-200 gram were used for this investigation. They were obtained from D' Wistar, Bandung, West Java, Indonesia. They were acclimated for 10 days before the experiment. They were divided into six groups, each consisting of five Wistar male white rats.

Group I: Normal control group in which the rats were daily administered vehicle PGA 2% for 14 days.

Group II: Negative control group in which the rats were daily administered propylthiouracil 2 mg/kg BW and high-fat diet for 14 days.

Group III: Positive control in which the rats were daily administered propylthiouracil 2 mg/kg BW, high-fat diet and simvastatin 1 mg/kg BW for 14 days.

Group IV: Test group in which the rats were daily administered propylthiouracil 2 mg/kg BW, high-fat diet and JKW 300 mg/kg BW for 14 days.

Group V: Test group in which the rats were daily administered propylthiouracil 2 mg/kg BW, high-fat diet and JKE 300 mg/kg BW for 14 days.

Group VI: Test group in which the rats were daily administered propylthiouracil 2 mg/kg BW, high-fat diet and JKH 300 mg/kg BW for 14 days.

High-fat diet was made in the form of emulsions, all the ingredients are mixed and stirred until homogeneous and made fresh every day. High-fat diet was given 2 mL/200gram by oral to induce hypercholesterolemic rats. High-fat diet is made with a total volume of 100 mL; egg yolk 80 gram, glucose 65% 15 gram, cow fat 5 gram. Propylthiouracil 2 mg/kg BW was suspended in a vehicle PGA 2% orally to induce hypercholesterolemic rats. Simvastatin, JKW, JKE and JKH were suspended in a vehicle PGA 2% by oral. Propylthiouracil and high-fat diet were administered daily at relatively the same time. Simvastatin, JKW, JKE and JKH were given to test group one hour after induced hypercholesterolemic.[4,7,10,11]

At the end of experimental study, rats were fasted for 12-14 hours and then ether anesthesia. The blood was collected by intracardial with syringe 3 mL and put into vacutainer. Vacutainer contains blood centrifuged for 10 minutes with 3.000 rpm. The blood was separated into serum and plasma. Serum was transferred into Eppendorf tube and then stored in the refrigerator 20^0C before analysis.[4,7,10,11]

Estimation of Serum Lipid Profile: Serum of total cholesterol, triglycerides and HDL cholesterol was estimated using kit Biolabo. VLDL cholesterol and LDL cholesterol were calculated.[4]

$$\text{VLDL cholesterol} = \frac{\text{Triglycerides}}{5}$$

$$\text{LDL cholesterol} = \text{Total cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL cholesterol}$$

$$\text{Atherogenid Index (AI)} = \frac{\text{LDL cholesterol}}{\text{HDL cholesterol}}$$

$$\text{Coronary Risk Index (CRI)} = \frac{\text{Total cholesterol}}{\text{HDL cholesterol}}$$

Statistical Analysis: The result was analyzed using Statistical Package for the Social Sciences 21 by one way ANOVA followed by Tukey's multiple comparion test. The P value <0.05 was set for statistical significant.

Thin Layer Chormatography for the Most Actively Fraction: Thin layer chromatography is applied to the most actively fraction for the presence of secondary metabolite by spraying the specifics of the spot.

Results and Discussion

The fractionation results showed that the compounds in jawer kotok leaves (*Plectranthus scutellarioides* L.) are dissolved in the polar solvent (table 1).

Antihypercholesterolemic Activity of Fractions: In testing antihyperolesterolemic activity of fractions, use only one dose in each fraction. The selected dose is 300 mg/kg BW. All the test groups had significant differences with negative control group. All the test groups did not have significant differences with control group. This proves that water fraction, ethyl acetate fraction and *n*-hexane fraction of jawer kotok leaves can decrease total cholesterol level in hypercholesterolemic rats and can give effect of decreasing total cholesterol almost equal to normal control group. The most actively fraction is *n*-hexane fraction (figure 1).

All the test groups had significant differences with negative control group. All the test groups did not have significant differences with control groups. This proves that water fraction, ethyl acetate fraction and *n*-hexane fraction of

jawer kotok leaves can decrease triglycerides level in hypercholesterolemic rats and can give effect of decreasing triglycerides almost equal to normal control group. The most actively fraction is ethyl acetat fraction (figure 2).

All the test groups did not have significant differences with negative control group. All the test groups did not have significant differences with control groups. This proves that water fraction, ethyl acetate fraction and n-hexane fraction of jawer kotok leaves cannot increase HDL cholesterol level in hypercholesterolemic rats (figure 3).

All the test groups had significant differences with negative control group. All the test groups did not have significant differences with control groups. This proves that water fraction, ethyl acetate fraction and *n*-hexane fraction of jawer kotok leaves can decrease LDL cholesterol level in hypercholesterolemic rats and can give effect of decreasing LDL cholesterol almost equal to normal control group. The most actively fraction is *n*-hexane fraction (figure 4).

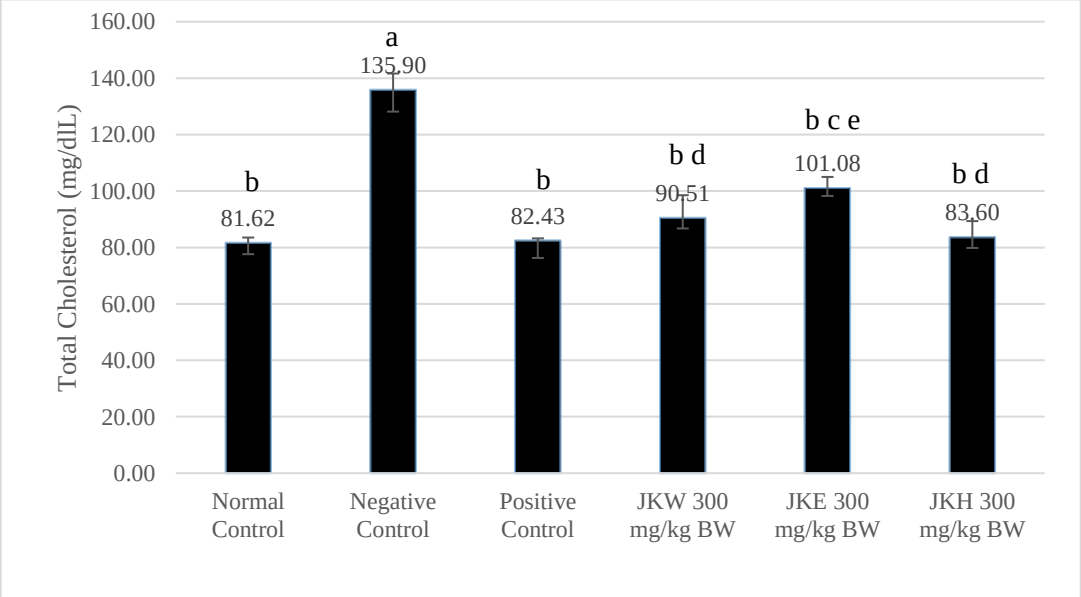
All the test groups had significant differences with negative control group. All the test groups did not have significant differences with control groups. This proves that water fraction, ethyl acetate fraction and *n*-hexane fraction of jawer kotok leaves can decrease VLDL cholesterol level in hypercholesterolemic rats and can give effect of decreasing VLDL cholesterol almost equal to normal control group. The most actively fraction is ethyl acetat fraction (figure 5).

Atherogenic Index and Coronary Risk Index: All the test groups had significant differences with negative control group. All the test groups did not have significant differences with control groups. This proves that water fraction, ethyl acetate fraction and *n*-hexane fraction of jawer kotok leaves can decrease atherogenic index and coronary risk index in hypercholesterolemic rats (figure 6).

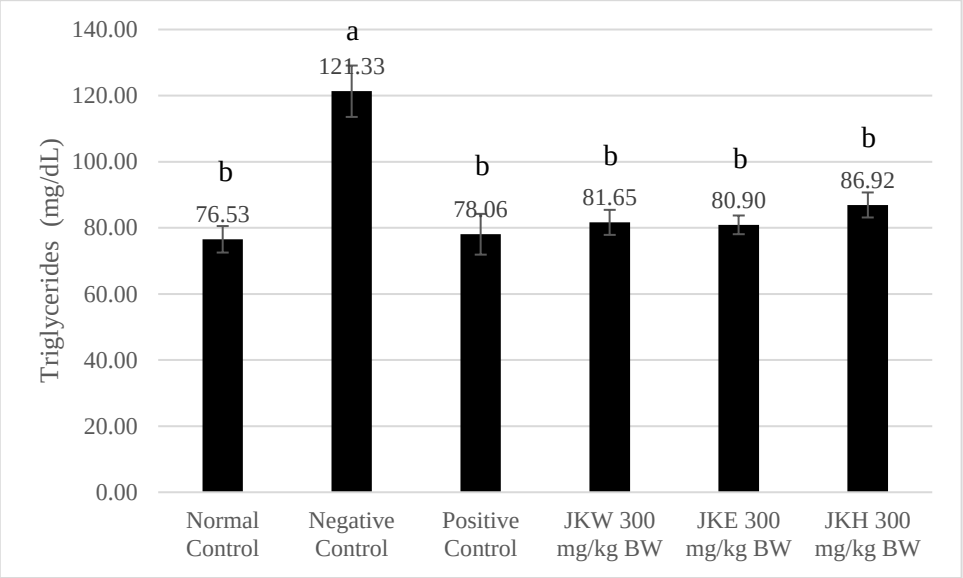
Thin Layer Chormatography For the Most Actively Fraction: Thin Layer Chormatography for the most actively fraction was applied to *n*-hexane fraction. The mobile phase is *n*-hexane:ethyl acetate in ratio 8:2. Room temperature at the time of bottling and development is 25^0C with humidity of 50%. The elution system used is isocratic. Type of plate used uses Silica Gel F254 with plate size 10x2 cm. The spotting viewer is Dragendorff for alkaloid, vaniline sulphate for monoterpenoid and sesquiterpenoid. $FeCl_3$ for phenol and Liebermann-Burchard for steroid.

Table 1
Rendemen Fraction of Jawer Kotok Leaves (*Plectranthus scutellarioides* L.)

Fraction	Concentrated Fraction (g)	Rendemen Fraction (%)
Water	495,54	15,49
Ethyl Acetat	68,97	2,15
n-Hexane	39,75	1,24



a: Significantly different compared with normal rats (p<0,05)
b: Significantly different compared with hyperlipidemic rats (p<0,05)
c: Significantly different compared with water fraction dose 300 mg/kg BW (p<0,05)
d: Significantly different compared with ethyl acetat fraction dose 300 mg/kg BW (p<0,05)
e: Significantly different compared with *n*-hexane fraction dose 300 mg/kg BW (p<0,05)
Figure 1: The graph of measurement results of total cholesterol levels of jawer kotok leaves fractions



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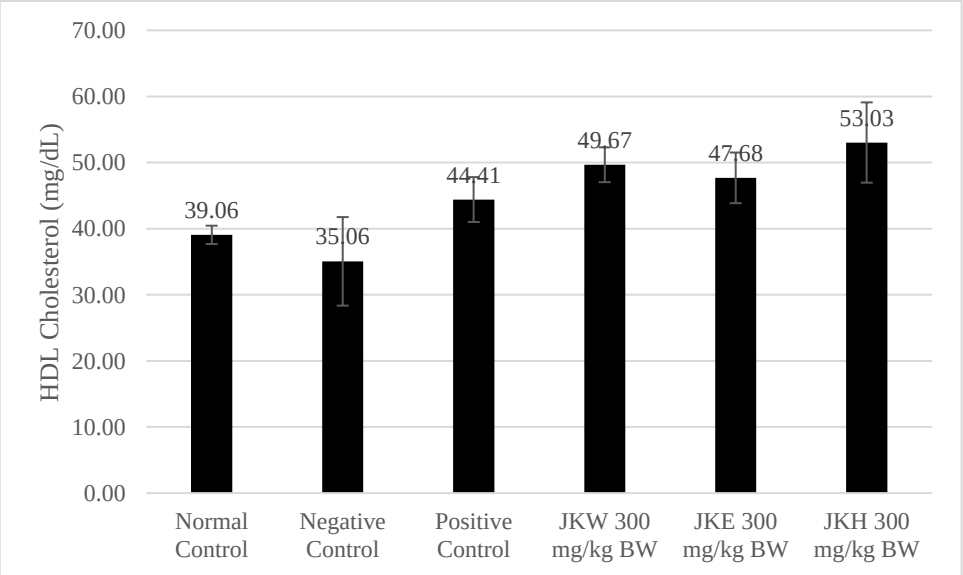
e: Significantly different compared with *n*-hexane fraction dose 300 mg/kg BW (p<0,05)
Figure 2: The graph of measurement results of triglycerides levels of jawer kotok leaves fractions



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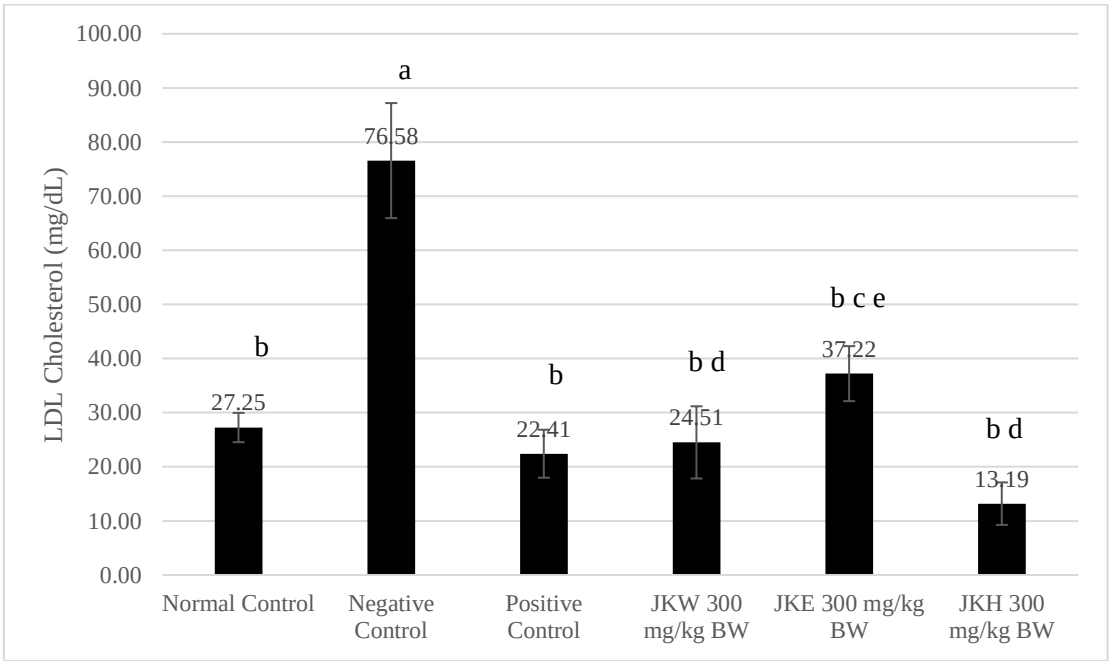
e: Significantly different compared with *n*-hexane fraction dose 300 mg/kg BW (p<0,05)
Figure 3: The graph of measurement results of HDL cholesterol levels of jawer kotok leaves fractions

Dragendorff can detect the presence of nitrogen base in general and alkaloid compounds in the presence of a change in orange-brown.[8] In the spot number 1-4, the color of spots was changed to brown after sprayed with Dragendorff. This shows that the *n*-hexane fraction contains alkaloid compounds. Vaniline sulphate can detect the presence of monoterpenoid and sesquiterpenoid compounds by change to blue.[12] In the spot number 7, the color of spot was changed to blue after sprayed with vaniline sulphate. This shows that the *n*-hexane fraction contains monoterpenoid and sesquiterpenoid compounds (figure 7).

$FeCl_3$ can detect the presence of phenol compounds by change in color to blue.[12] TLC results did not change color to blue after sprayed with $FeCl_3$. This shows that the *n*-

hexane fraction does not contain phenol compounds. Liebermann-Burchard can detect the presence of steroid compounds by change in color to yellow.[12] In the TLC results did not change color to yellow after sprayed with Lieberman-Burchard. This shows that the *n*-hexane fraction does not contain steroid compounds (figure 7).



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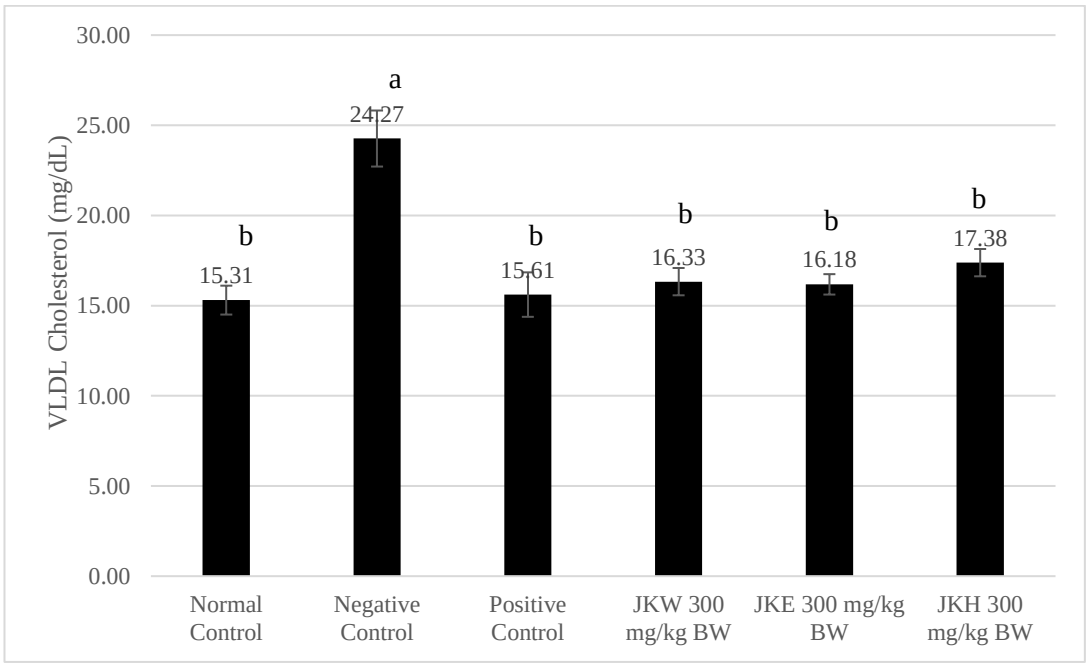
d: Significantly different compared with ethyl acetat fraction dose 300 mg/kg BW (p<0,05)
e: Significantly different compared with *n*-hexane fraction dose 300 mg/kg BW (p<0,05)
Figure 4: The graph of measurement results of LDL cholesterol levels of jawer kotok leaves fractions



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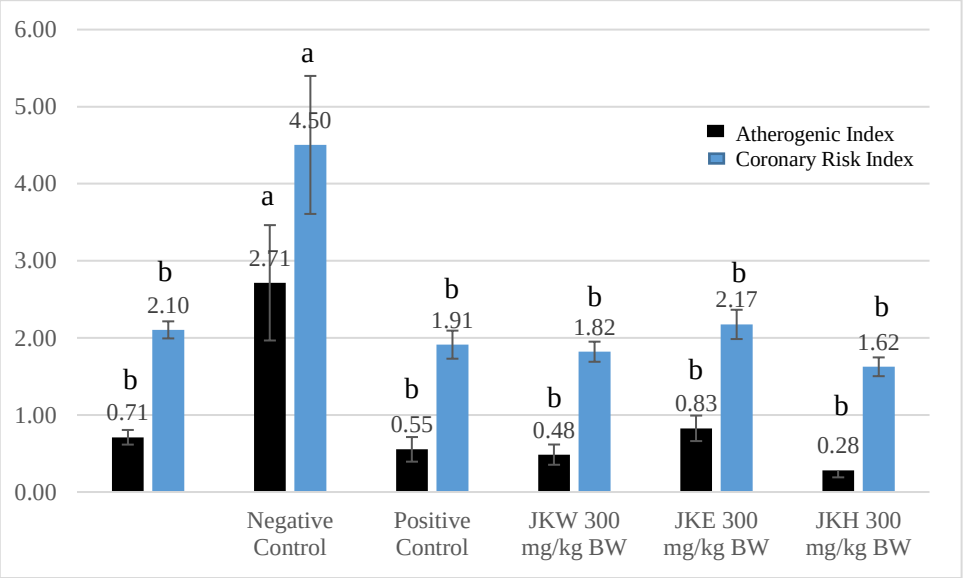
c: Significantly different compared with water fraction dose 300 mg/kg BW (p<0,05)
d: Significantly different compared with ethyl acetat fraction dose 300 mg/kg BW (p<0,05)
e: Significantly different compared with *n*-hexane fraction dose 300 mg/kg BW (p<0,05)
Figure 5: The graph of measurement results of VLDL cholesterol levels of jawer kotok leaves fractions



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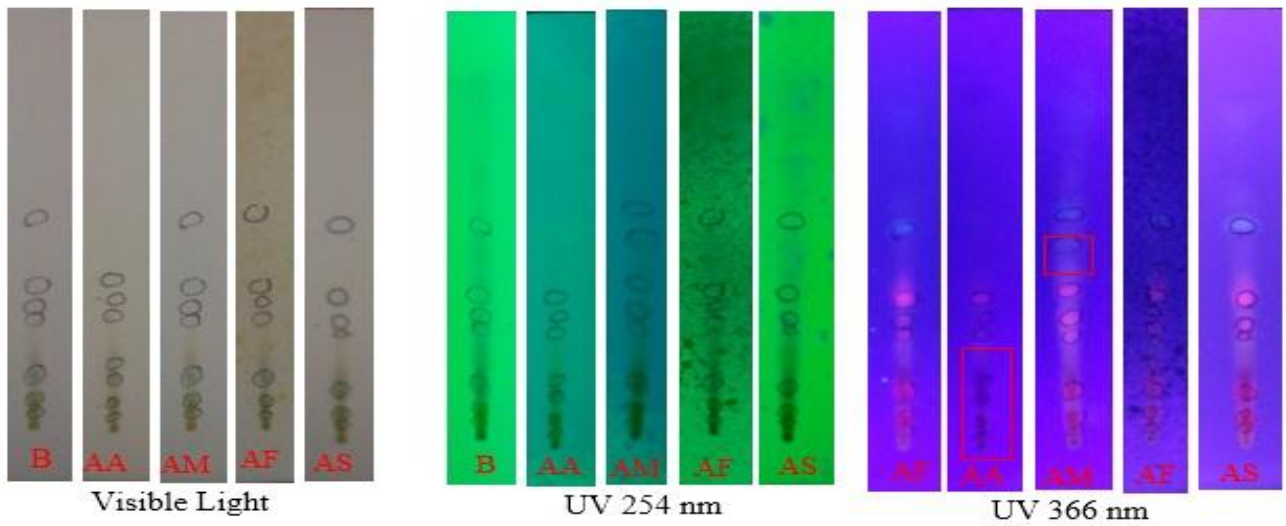
b: Significantly different compared with hyperlipidemic rats (p<0,05)
c: Significantly different compared with water fraction dose 300 mg/kg BW (p<0,05)
d: Significantly different compared with ethyl acetat fraction dose 300 mg/kg BW (p<0,05)
e: Significantly different compared with *n*-hexane fraction dose 300 mg/kg BW (p<0,05)
Figure 6: The graph of measurement results of atherogenic index and coronry risk index of jawer kotok leaves fractions



B: before sprayed with the spotting viewer
AA: after sprayed with Dragendorff (for Alkaloid)
AM: after sprayed with Vaniline Sulphate (for Monoterpenoid and Sesquiterpenoid)
AF: after sprayed with $FeCl_3$ (for Phenol)
AS: after sprayed with Liebermann-Burchard (for Steroid)
Figure 7: Thin Layer Chormatography For the Most Actively Fraction

Conclusion

n-hexane fraction dose 300 mg/kg BW has better antihypercholesterolemic activity. *n*-hexane fraction of jawer kotok leaves (*Plectranthus scutellarioides* L.) contains alkaloid, monoterpenoid and sesquiterpenoid.

Acknowledgement

This present study was supported by Padjadjaran University on program Academic Leadership Grant (ALG).

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