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ANTIHYPERLIPIDEMIC ACTIVITY OF PLECTRANTHUS SCUTELLARIOIDES (L.) R.Br. n-HEXANESUBFRACTION ON NORMAL AND PROPYLTHIOURACIL INDUCED HYPERLIPIDEMIC RATS

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A B S T R A C T

This study examined the activity of n-hexane subfraction Plectranthus scutellarioides on normal and propylthiourasil induced hyperlipidemic rats. Propylthiourasil and high fat diet were used to induced hyperlipidemic, and also given Simvastatin 1 mg/kg body weight of rats. Fraction n-hexane of Plectranthus scutellarioides was separated by using Vacum Liquid Chromatography method (VLC) using percentage gradient eluent of n-hexane : ethyl acetat (100:0 ; 90:10 ; 80:20 ; 70:30 ; 60:40 ; 50:50 ; 40:60 ; 30:70 ; 20:80 ; 10:90 ; 0:100) and 100% ethanol. The result of separation is obtained by five subfraction (SF a, SF b, SF d, SF e, and SF f) and 3 (SF b, SF c, and SF d) of 5 subtractions were done testing with oral administration for 2 weeks. The result show that SF b provides the best results in lowering cholesterol total levels, trygliserid, LDL-C, and VLDL-C. SF c provide the best result to incrase HDL-C level.

INTRODUCTION

Hypercholesterolemia is a condition in hich total cholesterol in the blood increases. It is a form of "hyperlipidemia" (elevated lipid levels in the blood) and "hyperlipoproteinemia"(elevatedlipoproteinlevelsintheblood). Hypercholesterolaemia results in accumulation, oxidation and lipid modification in the endothelium of blood vessels leading to endothelial dysfunction, chronic inflammation and cardiovascular disease (CVD). Epidemiological studies have shown that high LDL-c concentrations are associated with increased CVD risk and progression of atherosclerosis (Catapano, 2009). Atherosclerosis is a major cause of morbidity and mortality in developing countries. Atherosclerosis is caused by dyslipidemia. Dyslipidemia is a fat metabolism disorder characterized by increased or decreased levels of fat in blood. Increased levels of fat in the blood are causes occurrence of atherosclerosis. Hyperlipidemia is classified into primary hyperlipidaemia and secondary hyperlipidaemia. Primary hyperlipidemia is caused by a disorder of fat metabolism, whereas secondary hyperlipidemia is caused due to complications from other diseases (Anjani *et al*, 2015). Statins are the most widely prescribed drug for all age groups. Hypercholesterolemia is a condition that requires patients to take drugs in the long term. Use of statins in the long term is known to cause adverse effects. The most common adverse effects found are muscle pain, fatigue, weakness as well as rhabdomyolysis (Beatrice and Macella, 2008). Based on adverse effect of long term use statin. Need alternative therapies that have minimal side effects with optimal therapeutic effect. The use of herbal medicine has grown rapidly because it can be an alternative in patients who have tolerance to synthesis drugs and also its use in the long term is relatively safer than synthesis drugs (Kaliora, *et al*., 2006). Research conducted by Faizah (2017) states that ethanol extract from iler leaf with a dose of 400 mg / kgBB is known to have antihyperipidemia activity on normal and propylthiouracil induced hyperlipidemic rats (Faizah, 2017). *Plectranthus scutellarioides* belongs to an annual plant, has a herbaceous stem, erect or lying on its

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base and creeps in height ranging from 30-150 cm, height 0.5-1.5 meters and belongs to the category of wet plants whose stems are easily broken. Single-colored purple brown to blackish purple, and leaf blobs oval (Depkes RI, 2008). Based on the above exposure, it is suspected that iler leaf subfraction has activity as antihyperlipidemic.

MATERIALS AND METHODS

Material

Leaves of *Plectranthus scutellarioides* which has been dried and pureed. Obtained from Balai Penelitian Tanaman Rempah dan Obat (Balittro) Bogor, West Java in October 2016 (*Plectranthus scutellarioides leaves* were determined in Herbarium Bandungese, School of Life Science and Technology, Bandung Institut of Technology withthedocument number 3802/I1.CO2.2/PL/2017). Wistar male white rats with the age of about 2-3 months, with weight 170-200 gram, were taken from D' Wistar, Bandung, West Java (The research was agreed by the Health research Ethics Committee of Faculty of Medicine of Padjadjaran University, with the document number 900/UN6.C10/PN/2017). Other materials used are 70% ethanol, aquadest, ethil acetate, n-hexane, propylthiouracil, simvastatin, pulvis gummi arabicum (PGA), Total Cholesterol Kit, Triglycerides Kit, HDLCholesterol Kit from Biolabo.

Apparatus

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

Preparation of Extract

Plectranthus scutellarioides leaves were extracted with ethanol 70% by macerator apparatus. Kept 9x24 hours then replace ethanol every 72 hours. Liquid extract was then separated by using flannel cloth and the filtrate was concentrated by evaporator. Then viscous extract over the water bath until get constant weigh.

Fractination by Liquid-Liquid Extraction

Ethanol extract (30 mg) reviled in destilled water (300 ml) and then placed in separatory funnel. First, solution was extraction with n-hexane 300 ml. Separated non organic layer (top) and organic layer (bottom), catch extract non organic and add n-hexane to extract organic. Repeated until extract non organic changes the colour. Next, the extract organic was extraction with ethyl acetate. All fraction obtained were concentrated using the rotary evaporator.

Subfraction of the n-hexane fraction

The n-hexane fraction was separated by using Vacum Liquid Chromatography method (VLC) using percentage gradient eluent of n-hexane : ethyl acetat (100:0 ; 90:10 ; 80:20 ; 70:30 ; 60:40 ; 50:50 ; 40:60 ; 30:70 ; 20:80 ; 10:90 ; 0:100) and 100% ethanol. The result can be in TLC with eluen n-hexane: ethyl acetat (5:5). The subfractions further will be referred as SF.

Protocols

Before the treatment wistar male white rats were acclimated for 14 days. Rats divided into six groups with each group containing five rats.

Group I Normal group in which the rats were daily administered vehicle PGA2% for 14 days.
Group II Hyperlipidemic group in which the rats were daily administered propylthiouracil 2 mg/kg and high-fat diet for 14 days.
Group III Positive group in which the rats were daily administered propylthiouracil 2 mg/kg, high-fat diet and simvastatin 1mg/kg for 14 days.
Group IV Test group in which the rats were daily administered propylthiouracil 2 mg/kg, high-fat diet and mg/kg for 14 days.
Group V Test group in which the rats were daily administered propylthiouracil 2 mg/kg, high-fat diet and PSE 500 mg/kg for 14 days.
Group VI Test group in which the rats were daily administered propylthiouracil 2 mg/kg, high-fat diet and PSE 600 mg/kg for 14 days.

High-fat diet is made by egg yolk 80 gram, glucose 65% 15 gram, cow fat 5 gram with total volume is 100 ml. All ingredient is mixed and stirred until homogeneous and made freshly every day. It's was given in 2mL/200 gram by oral. Propylthiouracil 2 mg/kg bw were suspended in a vehicle PGA 2% orally. Simvastatin and PES were suspended in a vehicle PGA 2% by oral. Propylthiouracil and high-fat diet were administered dailyat relatively the same time to induce hyperlipidemia. Simvastatin and PSE were given to test group one hour after induced hyperlipidemic. On the 14th day, rats were fasted for 12-14 hours. Before take the blood, rats were given the ether anesthesia. The blood was collected by intracardial with syringe 3 mL and put intovacutainer. Vacutainer were contains blood centrifuged for 10 minutes with 6.000 rpm. The blood would separated into serum and plasma. Serum was transferred into Eppendorf tube and then stored in the refrigerator 20^0C before analyzed (Faizah, 2017).

Estimation of Serum Lipid Profile

Serum of total cholesterol, triglycerides and HDLcholesterol were estimated using kit form Biolabo. VLDLcholesterol and LDL cholesterol was

$$\text{calculated.} VLDL\ \text{kolesterol} = \frac{\text{Trigliserida}}{5}$$

$$\text{LDL kolestero} = \text{Kolesterol tota} - \frac{\text{Trigliserida}}{5} - \text{HDL kolesterol}$$

Statistical Analysis

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

RESULT

Table 2 Rendemen Extract of Plectranthus scutellarioides

Dry leaves	Concentrated Extract	Rendemen Extract
2900 gram	517,5 gram	17, 83 %

The results of the 517,5 g ethanol extract separation were obtained 7,5 g (0,25 %) *n*-hexane fraction, 240 g (8,27 %) ethyl acetate fraction, and 270 g (9,31%) water fraction. Subtraction of the n-hexane fraction yields 12 subfractions.

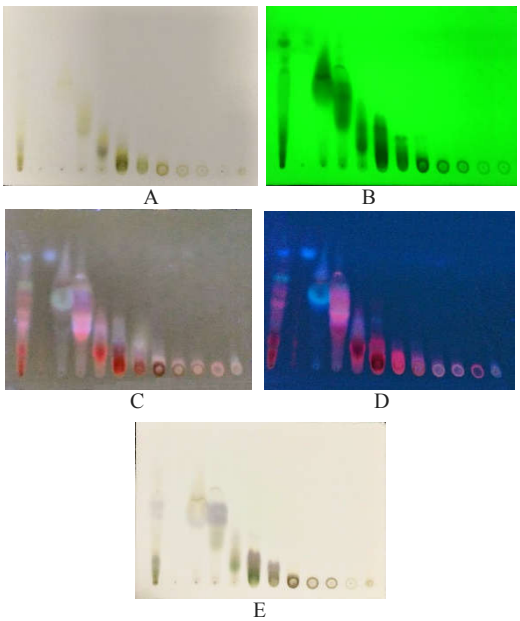

Figure 1 TLC of *n*-hexane subfraction (SF 12).

Stationary phase : Silica gel 60 F_{254}
Mobile phase : *n*-hexane : ethyl acetate (8:2)
Detection : A = Visible,
B = UV 254
C = UV 366
D = Visible + H_2SO_4
E = UV 366 +H_2SO_4

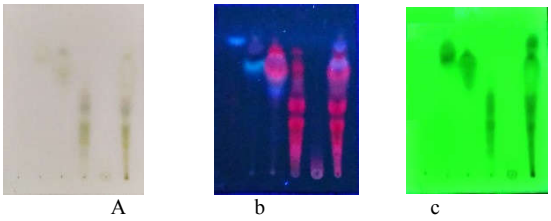

Figure 2 TLC of *n*-hexane subfraction combined (SF a-e).

Stationary phase : Silica gel 60 F_{254}
Mobile phase : *n*-hexane : ethyl acetate (8:2)
Detection : A = Visible,
B = UV 254
C = UV 366
Note : a = SF a ; b = SF b ;
c = SF c ; d = SF d ;
e = SF e ; f = SF

DISCUSSION

Phytochemical screening of *Plectrathus scutellarioides* leaf extract showed this extract contained secondary metabolite compounds that is alkaloids, polyphenol, tanin, flavonoid and triterpenoid (Moektiwardoyo, 2011). Flavonoid, polyphenols, steroid and triterpenoid known has activity to decrease cholesterol level (Rajasekaran *et al*, 2013). The results of the F-I fraction in KCV to separate the compounds contained in F-I. This separation is based on the difference of the polarity level of the compound, so that the fraction obtained will have a simpler component. Eluen used with a gradient system, ie by the ratio of n-hexane: ethyl acetate. KCV results were seen in the profile of the compound using TLC with eluen n-hexane: toluene (6: 4). Based on TLC results of compounds having the same profiles combined and obtained 5 combined subfractions (SF a, SF b, SF c, SF d, SF e) from the initial 12 subfractions.

The results of testing antihypercholesterol activity against SF b, SF c, and SF d. SF a and SF e were not tested for antihypercolesterol because the weight gain was not sufficient to be tested. Determination of dosage used based on dose optimization. The dosage optimization using SF b was chosen based on the highest number of weights. The dosage used in the optimization is dose 25 mg / kg BW, 50 mg / kg BW, and 100 mg / kg BW. The results of the optimization showed that dosing 25 mg / kg BW had decreased cholesterol level from 317 to 219, based on the optimum dose of antihypercolesterol test using 25 mg / kg BW.

Antihypercholesterol testing using high-fat feed and PTU as inducer of hypercholesterolemia. Administration of PTU can increase cholesterol levels by decreasing thyroid hormone synthesis. Thyroid hormones in the blood can lower cholesterol, phospholipid, and triglyceride levels by increasing the speed of cholesterol secretion, so the amount of cholesterol released through the feces increases. Thyroid hormones induce LDL receptors in hepatic cells thus causing accelerate the removal of LDL from

Simvastatin given on the positive control group is a drug that has activity to lower cholesterol. Simvastatin belongs to statins that play a role in synthesizing cholesterol. The mechanism of simvastatin is by inhibiting HMG-CoA reductase and also increasing LDL receptors in blood. Inhibition of HMG-CoA reductase convert acetyl-CoA to mevalonic acid which acts as a cholesterol precursor (Cesare RS, 2014).

Identification of independent risk factors for cardiovascular disease clinically measured from LDL-C, total cholesterol, trigliseride, and VLDL (Razvan TD and Christie MB *et al*. 2014). HDL is a heterogeneous subpopulation of discrete different particles quantitatively and qualitatively with other lipid compositions. Although HDL conventionally characterized by including cholesterol(HDL-C), but HDL cholesterol particles do not participate directly in a variety of atheroprotective functions Epidemiology, plasma HDL-C is a marker or predictor of plasma by the liver. (Sitti R *et al*, 2014).The use of high-fat diet and PTU will have a synergistic effect in rapidly increasing levels of cholesterol.

Cardiovascular disease risk, but the role of HDL as the underlying cause of atherosclerotic is not yet confirmed (Robert *et al*, 2017). Test of activity as antihypercholesterolemia show that SF b provides the best results in lowering cholesterol total levels, trygliserid,

Table 3 Effect of Plectranthus scutellarioides on Serum Lipid Profile in Normal and Hyperlipidemic Rats (Mean±SEM)

Group	Total Cholesterol (mg/dl)	Triglyserides (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	VLDL Cholesterol (mg/dl)
Normal	83,07±1,63[b]	76,53±0,80[b]	39,06±1,39	28,70±2,34[b]	15,31±0,80[b]
Hyperlipidemic	135,90±5,73[a]	121,33±7,77[a]	35,06±6,70	76,58±10,63[a]	24,27±1,55[a]
Positive	82,43±0,78[b]	78,06±6,16[b]	44,41±3,39	22,41±4,43[b]	15,61±1,23[b]
SF b	59,22±2,58[a]	49,32±3,18[b]	43,29±2,62	6,07±0,85[b]	9,86±0,64[b]
SF c	98,14±3,07[b]	121,95±2,48[a]	66,05±2,04[b]	7,70±2,52[b]	24,39±0,50[a]
SF d	81,56±2,74[b]	200,75±0,74[b]	29,06±2,34	12,35±1,47[b]	40,15±0,74[b]

LDL-C, and VLDL-C. SF c provide the best result to incrase HDL-C level. Statistically showed a significant difference between the SF b group and the hyperlipidemic control group on the reduction of LDL-C, triglycerides, and VLDL. Whereas, SF c gives significant differences with hyperlipidemic control groups in elevated HDL-C levels.

CONCLUSION

The results of this study concluded that the subfractions from *n*-hexane fraction *Plectranthus scutellarioides* (L.) R.Br. leaves has activity as antihypercholesterolemia with dose 25 mg/kg bw of SF b on normal and propylthiourasil induced hyperlipidemic rats.

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