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Short Communication

ANTI DYSLIPIDEMIC ACTIVITY OF KATUK LEAVES SAPONINS FRACTION (*SAUROPUS ANDROGYNUS* (L) MERR) IN RATS INDUCED WITH FAT-RICH DIET

NI KADEK WARDITIANI[1], MILAWATI[1], NI MADE PITRI SUSANTI[1]

[1]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Udayana University, Bali, Indonesia 80361
Email: kadek.warditiani@gmail.com

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ABSTRACT

Objective: The present study was to evaluate the anti dyslipidemic activity and the atherogenic indicator of saponin compound from katuk leaves (*Sauropus androgynus* (L).

Methods: This compound separated from Katuk leaves crude extract by column chromatography (a mobile phase: silica GF 254; mobile phase = gradient polarity of solvent (chloroform: methanol = 1:9-9:1). Male Wistar rats were fed with fat-rich diet (15% pig fats, 5% duck egg yolk) for 30 d to increase its total cholesterol level, triglyceride, LDL and to decrease blood HDL level compared to normal lipid level. Saponins fraction of Katuk leaves dose 10 and 30 mg/kg BW was given for 2 w and then its lipid level in blood was measured. Lipid level difference from each group was analyzed statistically.

Results: Total cholesterol, triglyceride, LDL and HDL level from a group of rats that were given saponins fraction of Katuk leaves was significantly different with negative control rats group and was not significantly different compared to normal control rats. Saponins fraction was able to reduce CRR, AR, and IAP value in treated rats.

Conclusion: Katuk leaves saponins fraction can become an anti dyslipidemic and also prevent the cardiovascular disorder in Wistar male rats.

Keywords: Dyslipidemia, Katuk leaves saponins fraction, Lipid profile

Increased low-density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C) are risk factors for cardiovascular diseases (CVD) [1]. Smaller LDL-C more easily oxidized compared LDL-C particles so small LDL-C are much more atherogenic [2]. HDL-C particles play a protective role against the development atherosclerosis [3]. The AIP (atherogenic index value accurately reflects the presence of atherogenic small LDL-C particles and HDL-C particles and it also a sensitive predictor of coronary atherosclerosis and cardiovascular risk [4].

In Indonesia, Katuk leaves (*S. androgynus* (L) Merr) were easily found. Based on the previous research, ethanol extract of Katuk leaves could have activity as antihyperlipidemic that decreasing total cholesterol, triglyceride, LDL-C and increasing HDL-C in the blood [5]. Katuk leaves contained secondary metabolites such as saponins, triterpene, polyphenol, flavonoid, and isoflavone. Yamamoto *et al.*, stated that saponins, triterpene, and polyphenol had an activity of anti-obesity because it could block both pancreatic lipase enzyme in small intestine and absorption of triglyceride in the body [6]. Han *et al.*, also stated that the saponins contained in tea leaves could block the pancreatic lipase enzyme so it could decrease body weight and adipose tissue in rabbits fed with fat-rich diet [7]. Saponins fraction of *Ilex paraguariensis* at dose 1.75±0.2 mg/day/animal could induced lipogenesis in muscle and adipose tissue [8]. The aim of this work is to assess the effects of saponin fraction of Katuk leaves on decreasing total cholesterol, triglyceride, LDL-C, and increasing HDL-C and calculate atherosclerosis index value in rats induced with fat-rich diet.

Katuk leaves (*S. androgynus* (L) Merr.) obtained from the local area of Girimulyo village, Nanggulan area, Kulonprogo regency, Yogyakarta, Indonesia. This plant was identified and authenticated by a botanist at the Pharmaceutical Biology Laboratory, Faculty of Pharmacy, Gadjah Mada University, Indonesia.

Katuk leaves powder was defatted with *n*-hexane and the *n*-hexane insoluble part was macerated. The ethanol soluble part was separated, and its solvent was evaporated. Fractionation was conducted with column chromatography, the solvent ratio of chloroform: methanol 9:1 until 1:9 for 20 ml. Each 5 ml of the fraction was stored.

Saponins identification was conducted with foam height test; some fraction was mixed with water and shaken vigorously. It would form the persistent appearance of foam with 1-10 cm height and when added with HCl 2N, the foam would still present [9].

Wistar male albino rats, aged 4 w, 150-200 g of weight were used for the antidyslipimic study. The rats were maintained under controlled condition, were used in the experiment. The rats were fed with standard chow diet and given drink ad libitum. Ethical Clearance of the animal's handling was provided by the Faculty of Veterinary, Udayana University no: 189/KE-PH-Lit-1/V/2015. Environment condition adaptation for animals was conducted for 1 w. Animals were then grouped as following:

- Normal group: rats were fed with normal diet.
- Negative control group: rats were fed with fat-rich diet.
- Positive control group: rats were fed with fat-rich diet and treated with 1.8 mg/kg BW simvastatin [10].
- Saponins fraction group 1: rats were fed with fat-rich diet and treated with Katuk leaves saponins fraction with 10 mg/kg BW.
- Saponins fraction group 2: rats were fed with fat-rich diet and treated with Katuk leaves saponins fraction with 30 mg/kg BW.

After environment adaptation, dyslipidemia induction was done by giving fat-rich diet (15% lard, 10% yolk) for 30 d and blood lipid level was then measured [11]. After blood lipid level measurement, treated rats were given Katuk leaves saponins fraction and simvastatin in treatment groups for 2 w. Katuk leaves saponins fraction was given at doses 10 and 30 mg/kg BW, its higher than saponins fraction of *Ilex paraguariensis* doses 1.75±0.2 mg/day/animal [8].

Total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) measured with standard reagent (DiaSys, Holzeim, Germany).

The atherogenic indices were calculated as follows: [12]

Cardiac Risk Ratio (CRR) = TG/HDL

Atherogenic Coefficient (AC) = (TG-HDL)/HDL

Atherogenic Index of Plasma (AIP) = log (TG/HDL)

The obtained data was absorbance data. The obtained absorbance value was changed into blood lipid level (mg/dL). Blood lipid level data homogeneity and normality from all animal groups were analyzed and then Mann-Whitney test was used.

Column chromatography was used to saponin separation compound from a crude extract of Katuk leaves. We use combination solvent chloroform and methanol (9:1 until 1:9 each 10 ml). The fraction was collect each 5 ml. The fraction obtained until 20 fractions. TLC staning with H_2SO_4 spray and H_2SO_4. vanilline used to detect the content of terpenoid compound in each fraction. The result shown that fraction number 11 until 20 contained terpenoid compound. Foaming test of the fraction was used to detect the content of saponin compound. fraction no 15-17 was positive formed foam.

Based on rat's blood lipid measurement, it was known that the treated animals fed with fat-rich diet for 6 w had an elevation of total cholesterol level, LDL and triglyceride and reduction of HDL compared to normal control group (fig. 1). There was a significant lipid profile difference between groups fed with fat-rich diet (negative control, positive control, 10 and 30 mg/kg BW saponins fraction group) towards the normal control group ($p<0.05$). Dyslipidemia condition characterized by hypercholesterolemia and hypertriglyceridemia is a most prevalent indicator of cardiovascular diseases [13].

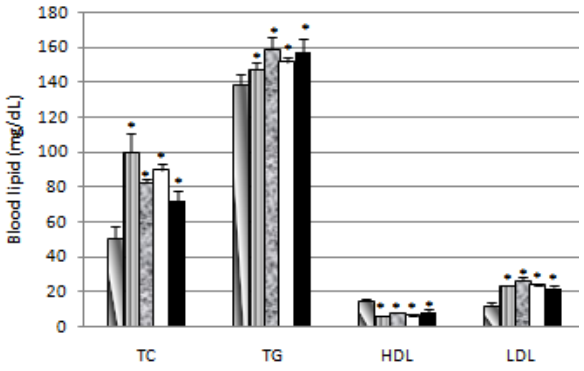

Fig. 1: Alteration graph of blood TC (total cholesterol), TG (triglyceride), LDL and HDL after fat-rich diet (n=5; mean±SD). * signs show that there are significant difference with normal control group ($p<0.05$)
TC = blood total cholesterol level; TG= blood triglyceride level; LDL= blood LDL level; HDL= blood HDL level; = normal control; = negative control; = 1.8 mg/kg BW simvastatin group; = 10 mg/kg BW saponins fraction group; = 30 mg/kg BW saponins fraction group

Treatments (simvastatin, 10 and 30 mg/kg BW saponins fraction) were conducted for 3 w. Blood measurement of rats show that saponins fraction and simvastatin could decrease TC, TG, and LDL and increase blood HDL level (fig. 2). There was a significant lipid profile difference between treatment groups (simvastatin, 10 and 30 mg/kg BW saponins fraction) towards negative control group. Increased level of LDL-C and triglyceride in serum is a risk factor for cardiovascular disease [14]. The antidyslipidemic activity of saponins fraction is not significantly different with positive control group ability (simvastatin). Saponins have the ability in block pancreatic lipase enzyme so that cholesterol absorption in the intestine will decrease [15, 16]. Saponins of sea cucumber can decreased lipids by enhancing β-oxidation via activating PPARα [8]. Saponins fraction of Ilex paraguariensis at dose 1.75±0.2 mg/day/animal were able to influence the lipid metabolism of male Wistar rats fed with a high-fat diet by induce lipogenesis in muscle and adipose tissue. It's also could induced a 40% increase fecal fat excretion. These results may be correlated with saponins ability to inhibited pancreatic lipase enzyme [8].

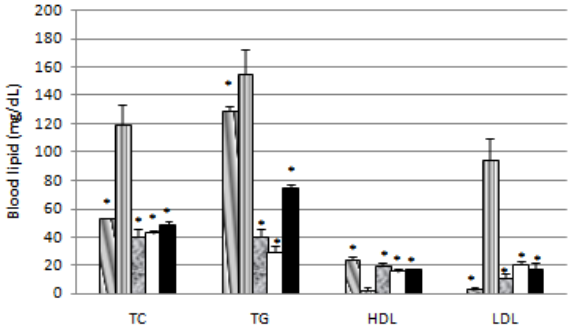

Fig. 2: Alteration graph of blood TC (total cholesterol), TG (triglyceride), LDL and HDL treatment (n=5; mean±SD). * signs show that there are significant difference with negative control group ($p<0.05$)
TC = blood total cholesterol level; TG= blood triglyceride level; LDL= blood LDL level; HDL= blood HDL level; = normal control; = negative control; = 1.8 mg/kg BW simvastatin group; = 10 mg/kg BW saponins fraction group; = 30 mg/kg BW saponins fraction group

Atherogenic is an indicator that shows cardiovascular disorder. The higher the value, the risk of cardiovascular disorder will increase [17-19]. In this research, saponin fraction could decrease atherogenic indicators such as CRR, AC and AIP (table 1) according to Ikewuchi and Ikewuchi [11]. There was an elevated ratio of TG: HDL (CRR) in treated rats fed with fat-rich diet for 6 w and this indicates risk of cardiovascular disorder. There were significant difference of CRR, AC and AIP value between normal control group and other groups ($p<0,05$). Saponin fraction could reduce TG level and increase HDL level in blood and it made the CRR value decrease. Atherogenic index is a powerful indicator of heart disease risk. The ability of saponin to decrease atherogenic index can be used as one way to prevent cardiovascular disorder due to its ability as anti dyslipidemic agent. All of results show the indication of protective mechanism of saponin fraction of *Sauropus androgynus* (L) merr. against the development of atherosclerosis as well as the dyslipidemia condition.

Table 1: Atherogenic index alteration before and after treatment in rats

Groups	CRR		AC		AIP	
	Before	After	Before	After	Before	After
Normal control	9.1602	5.4049	8.1602	4.4049	0.9604	0.7318
Negative control	22.8104*	59.6515	21.8104*	58.6515	1.3577*	1.7401
Positive control	19.8102*	2.0327+	18.8102*	1.0327+	1.2949*	0.2989+
Saponins 10 mg/kg BW	22.8528*	1.9322+	21.8528*	0.9322+	1.3582*	0.2729+
Saponins 30 mg/kg BW	19.3786*	4.4372+	18.3786*	3.4372+	1.2790*	0.6365+

CRR = Cardiac Risk Ratio; AC = Atherogenic Coefficient; AIP = Atherogenic Index of Plasma; * significantly different with normal control group ($p<0.05$);+significantly different with before treatment ($p<0.05$), Katuk leaves saponins fraction (*Sauropus androgynus* (L) merr.) can reduce TC, TG and LDL level and elevate HDL level in blood. Saponins fraction also able to decrease atherogenic indicators (CRR, AC and AIP) so that it can prevent cardiovascular disorder.

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CONFLICTS OF INTERESTS

All authors have none to declare

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