Anti-hypercholesterolemic effect of Ethyl acetate extract from stem bark of Artocarpus dasyphylla toward Rattus norvegicus Wistar strain

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Anti-hypercholesterolemic effect of Ethyl acetate extract from stem bark of *Artocarpus dasyphylla* toward *Rattus norvegicus Wistar* strain

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Abstract

Artocarpus dasyphylla with the local name "cempedak utan" is a member of Artocarpus Genus from Moraceae family. It is a rare and endemic plant from east region of Indonesia. The purpose of this study was to determine the antihypercholesterolemic effect of ethyl acetate extract from *A. dasyphylla* toward the level of total cholesterol, LDL, and HDL of hypercholesterolemic Rattus norvegicus as the prevention effort of atheroschlerosis and CVD. It was due to antioxidant activities of phenolic compounds. The powder of stem bark of *Artocarpus dasyphylla* was extracted by maceration and partition method. Phenolic total of ethyl acetate extract from stem bark of *A. dasyphylla* was determined with Folin Ciocalteu reagent, it was 9,86 mg GAE/g of extract. In vivo experiment toward *Rattus norvegicus* with hypercholesterol diet used randomized post test only control group design. Ethyl acetate extract with the treatment doses 75, 150, 225 mg/kg body weight showed antioxidant activity by decreasing total cholesterol and LDL level to normal level. Paradox result occured to HDL level, the level of HDL decreasing as the increase of dose of sample, but still above the threshold. The best anti-hypercholesterolemic activity was shown by treatment with the dose of ethyl acetate extract from stem bark of *A. dasyphylla* 150 mg/kg body weight of *Rattus norvegicus*.

Keyword: *Artocarpus dasyphylla*, antihypercholesterolemic effect, total cholesterol, LDL, HDL, *Rattus norvegicus*.

1. INTRODUCTION

Artocarpus is one of genus belong to Moraceae family beside *Ficus* and *Morus*. *Artocarpus* has at least 50 species and some are endemic of Indonesia. Several studies reported the biological activity of *Artocarpus*, such as antioxidant, antibacterial, antimalaria, antitubercular, antivirus, cytotoxic, antiplatelet, and antiinflamasi [1].

Artocarpus dasyphylla or cempedak utan is a member of genus *Artocarpus* that is a rare and endemic plant from east region of Indonesia [2]. Phenolic compound had been isolated from dichloromethane extract of stem bark of *A. dasyphylla* were norartocarpetin, oxyresveratrol, catechin, and afzelechin-3-O-rhamnosida [3]. Based on the toxicity test toward *Arthemia salina* Leach, the phenolic compounds from ethyl acetate and chloroform extract of *A. dasyphylla*'s stem bark were non-toxic[2].

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Cardiovascular Disease (CVD) is the blood vessels illness that directly related to heart activity. According to World Health Organization (WHO), on 2005, approximated about 17,5 million people died caused by CVD or it is about 30% from the whole death causal factor in the world. On 2015, approximated about 20 million people will die caused by CVD [4]. Hypercholesterolemia is one of causal factor of CVD. The level of LDL in the hypercholesterolemic blood over to 200 mg/dL. LDL in the blood should be broken down in the macrofag in peripheral tissues to be transported to the liver. But, by the presence of free radical (ROS and RNS) so LDL will be oxydized to Ox-LDL that unrecognized by LDL receptor. Ox-LDL will accumulate in blood vessel walls and cause atherosclerosis. The atherosclerosis cause cardiovascular disease [5].

Phenolic compounds is well known of its antioxidant activity [6]. Flavonoids from a variety of sources have been reported to prevent LDL oxidation *in vitro* and show markedly hypolipidemic activity *in vivo*. Those suggesting the effectiveness of flavonoids for the prevention and treatment of hypercholesterolemia and artherosclerosis. Epidemiological studies have exposed an association between increased consumption of antioxidant-rich vegetables and fruits and the decreased risk of coronary heart disease [7].

The purpose of this study was to determine the antihypercholesterolemic effect of ethyl acetate extract from *A. dasyphylla* toward the level of total cholesterol, LDL, and HDL of hypercholesterolemic *Rattus norvegicus* as the prevention effort of atheroschlerosis and CVD.

2. MATERIAL AND METHOD

2.1 Plant material

The stem bark of *A. dasyphylla* were collected from Purwodadi Botanic Garden, East Java, Indonesia. Plants materials were dried at room temperature and ground in a mortar.

2.2 Chemicals and reagents

Methanol, n-hexane, ethyl acetate, Follin-Ciocalteau reagent, Gallic acid, Na_2CO_3, $AlCl_3$, Na-CMC, and Ketamine HCl.

2.3 Extraction

Stem bark powder were extracted in methanol by maceration for 3 x 24 hours in a room temperature. The methanol extracts were concentrated using vacuum rotary evaporator and then partitioned with hexane-water (1:1). Water extracts were concentrated using rotary vacuum evaporator and then partitioned with ethyl acetate (1:1). Then ethyl acetate extracts were used for next step.

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2.4 Determination of total phenolic

Total phenolic contents of each crude extracts from stem bark of *A. dasyphylla* were determined by spectrophotometry using Follin-Ciocalteu reagents. The ethyl acetate extract of *A. dasyphylla*'s stem bark was dilluted in 95% ethanol to make 100 ppm of 25 ml solution. Then the solution was added 7.5 mL of Folin-Ciocalteu reagents (previously diluted with water 1:10 v/v). The mixture allowed to stand for 5 min then added 7.5 mL of Na_2CO_3 (60 mg/ml). The mixture was homogenized and incubated in the dark room for a hour. The standard solution used gallic acid. 100 ppm of gallic acid solution were divided into variety concentrations, 0, 2, 4, 6, 8, and 10 ppm. The resulting absorbance was measured by a spectrophotometer (UV-VIS Spectrophotometer, Shimadzu) at a wavelength of 725 nm. Phenolic content was expressed in milligram per gram of dry weight samples based on a standard curve of gallic acid (GA), which was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract.

2.5 Experimental animal

This experiment used 35 male white rats *Rattus norvegicus*, *Wistar* strain, at age 2-3 months, with body weight 180-200 grams. This study used *Randomized Post Test Only Control Group Design*. Before being used in the experiment, white rats were being adapted for 7 days in order to get habitual to the food and environment.

Hypercholesterol dietary was diet with high fat and cholesterol, consist of yolk and lard, given with standard diet. The provision of food and water was in *ad libitum* way.

The rats were divided into 5 treatment groups; **K0**: control groups (non-hypercholeserolemic rats), treated with standard diet; **K1**: control groups (hypercholesterolemic rats), treated with hypercholesterol diet; **K2**: treatment groups, treated with hypercholesterol diet beside standard diet and ethyl acetate extract from stem bark of *A. dasyphylla* with dose 75 mg/kg body weight (BW); **K3**: treatment groups, treated with hypercholesterol diet and ethyl acetate extract from stem bark of *A.dasyphylla* with dose 150 mg/kg BW; **K4**: treatment groups, treated with hypercholesterol diet and ethyl acetate extract from stem bark of *A. dasyphylla* with dose 225 mg/kg BW.

The experiment carried out for 4 weeks. At the end of treatment (at the beginning of fifth week), the rats from K0, K1, K2, K3, and K4 stunned by using ketamine HCl, their blood was taken intracardial to be examinated the level of its total cholesterol, LDL, and HDL.

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2.6 Data analysis

The normally distributed data, total cholesterol and LDL, performed using Analysis of Variance (ANOVA) test, followed by Post Hoc test using LSD (Least Significant Difference) test.the abnormally distributed data, HDL, used Kruskal-Wallis, followed by Mann-Whitney U with a significance level $p<0.05$. Data analysis was performed by using a computerized method of SPSS version 17.

3. RESULT

Total phenolic correlated to the antioxidant activity and their effect to reduce superoxide radical and lipid peroxidation [8]. Based to the measurement of total phenolic used Follin-Ciocalteau reagent, yielded regression equation of gallic acid $y = 0.683x + 0.0779$. The absorbance of ethyl acetate extract was 0,752. The calculation was done by subtitute y of gallic acid equation with the absorbance of ethy acetate extract, it was x= 9.86. This result shown that in dried weight of ethyl acetate from stem bark of *A. dasyphylla* there is 9.86 mg GAE / g of extract. This data was used to estimate the bioactivity potential of this extract, although there are some study showed that there is no corelation between phenolic total and biological activity of the sample. The influence of hypercholesterol diet induction was determined by comparing K0 (non-hypercholesterol diet treatment) and K1 (hypercholesterol diet treatment). The analysis results shown in **Table 1** show the significant enhancement of the total cholesterol level (p=0.001) and LDL level (p=0.042) of K1 which means that the rats on K1 undergone hypercholesterolemia.

The treatment of ethyl acetate extract from stem bark of *A. dasyphylla* with variety doses to K2 (75 mg/kg BW), K3 (150 mg/kg BW), and K4 (225 mg/kg BW) significantly can decrease the level of total cholesterol of K2 (p=0.001), K3 (p=0.001), and K4 (p=0.001), and also significantly decrease the level of LDL to K3 (p=0.0016), and not significantly of LDL to K2 (p=0.096) and K4 (p=0.136). For HDL, occured paradox phenomenon, there was the decreasing of HDL level from K1 (p=0.001) not significantly to K2 (p=0.165), significantly to K3 (p=0.001) and K4 (p=0.001). The analysis result of HDL level shown that the treatment of ethyl acetate from stem bark of *A. dasyphylla* was decreased but still above the normal level (>20 mg/dL) on the blood of rats.

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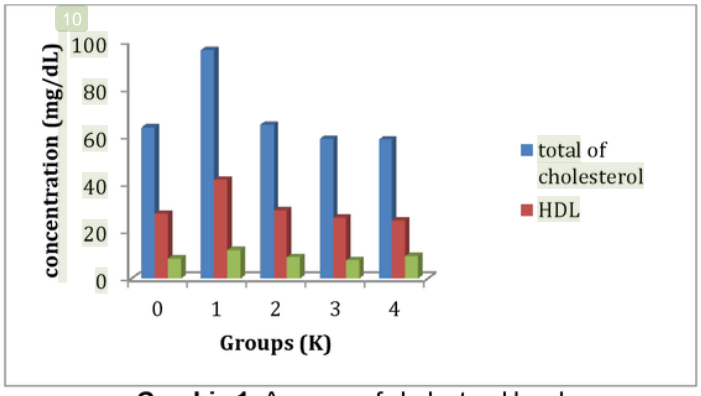
Table 1. Average and standard deviation of cholesterol level (mg/dL)

Variable	Groups				
	K0	K1	K2	K3	K4
	average ± SD	average ± SD	average ± SD	average ± SD	average ± SD
total of cholesterol	63.86 ± 6.3	96 ± 13.6	65 ± 10.9	59.0 ± 8.9	58.71 ± 24.43
LDL level	8.43 ± 2.4	12.0 ± 3.4	9.0 ± 4.3	7.71 ± 1.9	9.43 ± 3.3
HDL level	27.43 ± 1.7	41.71 ± 4.2	28.8 ± 2.6	25.71 ± 3.6	24.4 ± 4.6



Graphic 1. Average of cholesterol level

4. DISCUSSION

Artocarpus dasyphylla contains phenolic compounds in the ethyl acetate extract about 9.86 mg GAE/g of extract. By this result, it being estimated that the extract has the antioxidant activity. Phenolic compounds acting as antioxidant may function as terminator of chain reaction of free radicals and as metal ions chelator agent that catalyze lipid peroxidation. Phenolic oxidant (PhOH) interfere with the lipid oxidation by hydrogen atom donation to free radicals ($ROO^{\cdot}$) by the reaction $ROO^{\cdot} + PhOH \rightarrow ROOH + PhO^{\cdot}$ [6]. The reaction produces the phenoxy radical intermediates that are relatively stable because of its structure resonance so they do not initiate further chain reaction of free radicals. In a CVD prevention, phenolic compounds capable to protect LDL from oxidation of radical oxygens in the blood vessels, so it can prevent atherosclerosis [9]. Besides, phenolic compounds also capable to inhibite lipid biosynthesis [10].

In vitro, there is no report about antioxidant activity of the extract or chemical compounds of *A. dasyphylla*. In vivo experiment toward *Rattus norvegicus* showed that the treatment of ethyl acetate extract from stem bark of *A. dasyphylla* with dose 75, 150, and 225 mg/kg BW can decrease the level of total cholesterol and LDL, also maintain the

HDL level over the normal level. The comparation of three different dose variations of treatment showed that the highest anti-hypercholesterolemic effect was given by 150 mg/kg BW of ethyl acetate extract from stem bark of *A. dasyphylla*. Paradox phenomenon of HDL level, it because another mechanism was probably working. It appeared that there was no need for an increased production of HDL because there was no excess of cholesterol that must be returned to the liver [11]. This phenomenon of HDL to total cholesterol level also occured to the previous research [12].

5. CONCLUSION

Based to the result of this study, it was concluded that the treatment of ethyl acetate extract from stem bark of *A. dasyphylla* with dose variations 75, 150, and 225 mg/kg BW showed the improvement of serum lipid profile by the decrease of total cholesterol and LDL level, and maintained the HDL over the normal level of hypercholesterolemia *rattus norvegicus* as prevention effort toward atherosclerosis and CVD. The comparation between three treatment dose variations concluded that the highest antihypercholesterolemic effect shown in treatment dose of 150 mg of extract/kg BW of rats.

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