

Anredera Cordifolia Leaves Sub-fraction as Anti Hyperlipidemia

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Abstract

This study aimed to evaluate the anti-hyperlipidemic properties of the sub-fraction of the Binahong (Madeira vine, *Anredera cordifolia*) extract in male Wistar rats. The rats were given high-fat food every day for 3 months and pure cholesterol on weeks 0, 3, 7, and 11. Furthermore, Ethyl acetate sub-fraction of binahong leaves with 0, 12 mg/kg BW, and 0, 24 mg/kg BW doses were given orally for 3 weeks and observed for lipid profile on day 21. The result showed that sub-fractions of Madeira vine leaves decreased in triglyceride, cholesterol, and low-density lipoprotein (LDL) levels and did not influence the high-density lipoprotein level (HDL). Meanwhile, statin decreased total triglyceride, cholesterol, and the LDL level, and increased the HDL level. It concluded that Madeira vine sub-fraction with 0, 24 mg/kg BW dose presented improved results in the lipoprotein profile.

Keywords: Binahong, Madeira vine, *Anredera cordifolia*, hyperlipidemia, ethyl acetate fraction

Introduction

Cardiovascular diseases (CVDs) are the first death cause in the world, estimated at 17.9 million lives each year. CVDs mean a group of heart and blood vessel disorders and include cerebrovascular disease, rheumatic heart disease, coronary heart disease, and other conditions. CVD which causes death is caused by heart attacks and strokes; one-third of them occur prematurely in people under 70 years old [1].

The main adjustable risk factor in CVD development is low-density lipoprotein cholesterol (LDL-C). Statin as the first-line pharmacotherapy to reduce LDL-C in patients with a great risk of cardiovascular risk was suggested by the Japan Atherosclerosis Society (JAS) in 2012 [2]. A circumstance included in the Terplay of several factors like lipoproteins oxidation and the formation of atherosclerotic plaque known as atherosclerosis [3].

Binahong (*Anredera cordifolia* (Tenore) Steen) is perennial (old age) and also a creeper. It reaches 5 m long which was first discovered in China under the name of Dheng san chi. The plant has cylindrical, soft, and long stems with single leaf heart shape; 5-10 cm long, 3-7 cm wide with flower-shaped compound bunches which can be eaten [4]. Atherosclerosis is identified as a disease characterized by the thickening of arteries, which is starts in endothelial cells.

The complication related to atherosclerosis that influences blood supply may result in stroke [5] and then *A.cordifolia* influenced blood cholesterol level of fraction extracts of *A.cordifolia*. This study aimed to evaluate the reduction of blood cholesterol levels as an anti-hyperlipidemic expression of the binahong (*A. cordifolia*) sub-fraction extract in male Wistar rats.

Methods

Materials

Binahong (*A. cordifolia*) leaves, sulfuric acid, aquadest, hydrochloric acid, amyl alcohol, sodium carboxymethyl cellulose, ethyl acetate, ethanol, water, mercuric chloride, potassium iodide, bismuth nitrate, statin, propylthiouracil, sodium oxide, vanillin liquid in sulfuric acid, pure cholesterol, chloroform, gelatin, acetic anhydride, triglyceride, reagent kit for LDL (low-density lipoprotein), HDL (high-density lipoprotein), and cholesterol. If not stated otherwise, all ingredients were analytical grade

Preparing Plant

A. cordifolia leaves were obtained from Lembang, Bandung, Indonesia. It was perfectly cleaned using water from the tap and sorted while it was wet, then cut, dried, and ground into powder. Binahong was identified at Herbarium Bandungense, School of Life Science and Technology, Bandung Institute of Technology. The extraction of a crude drug was obtained by the reflux method using ethanol 96%, the next fraction was carried by Liquid-liquid extraction using ethyl acetate and n-hexane. Thus three fractions were identified: water fractions, ethyl acetate, and n-hexane. The ethyl acetate fraction was liquid sub-fractionated using a combination of n-hexane, ethyl acetate, and methanol eluents, to obtain ethyl acetate subfraction [6].

Experimental Animals

Male Wistar rats weighed 200-250 g with an

approximate age of 3 months. This research was based on code by ITB Animal Research Ethics Committee (No. 02/KEPHP-ITB/11-2014).

Phytochemical Screening

Phytochemical screening of n-hexane, ethanolic extract, ethyl acetate, crude drug, and water fraction of binahong leaves (*A.cordifolia*) was conducted to identify the existence of tannin, saponins, quinone, alkaloid, flavonoid, and steroid/triterpenoid group [7, 8].

Animal Models for Endothelial Fat Content

Animals were stimulated using high cholesterol food with 1% of cholesterol, fat goat 10%, duck egg yolk 5%, coconut oil 1%, and 83% of regular food for 12 weeks. At the 0, 3rd, 7th, and 11th week, pure cholesterol was given to the rat orally. Next, they were taken for 21 days of test extract administration. Moreover, the rats were classified into four groups; a group of statin (simvastatin 3.6 mg/kg BW), binahong leaves subfractions with 0,12 mg/kg BW (as the first dose), and 0,24 mg/kg BW (as the second dose), and negative control group (matrix) [5].

Results and Discussion

The study was initiated with a phytochemical screening of binahong leaves fractions, crude drug, and extract. The phytochemical screening result is displayed in Table 1. The reduction percentage of total cholesterol is shown below.

Table 1: Phytochemical screening of crude drug, extract and ethyl acetate fraction

Group	Crude Drug	Ethanol Extract	Ethyl Acetate Fraction
Saponin	+	+	+
Quinone	-	-	-
Flavonoid	+	+	+
Tannin	+	+	+
Alkaloid	+	+	+
Steroid /Triterpenoid	+	+	+

Prior studies expressed that binahong (*A. cordifolia*) leaves extract and fraction of binahong have an effect on antihyperlipidemic [5, 10]. The acute and subchronic toxicity study of ethanol Binahong extract by Salasanti *et.al* [11] showed that the extract was safe. Past studies explained that binahong leaves have multiple biological effects for instance anti-inflammatory [12], antihyperuricemic [13], antibacterial [14], antifungal [15], hematoma [16], and

improving kidney failure in rats [17]. Demonstrated that leaves of binahong could be one of the options to administer hypercholesterolemia and hypertension risk that lead to heart diseases which is coronary [18]. Based on the results of the anti hypercholesterolemic test after administration of binahong leaf fraction (water, ethyl acetate, and n-hexane).In conclusion, the ethyl acetate fraction gave the best effect in improving blood lipoprotein

levels. Thus, the fraction was selected to proceed to the subfractionation stage. The subfraction process was carried out by vacuum liquid chromatography with gradient elution using a mix of n-hexane-ethyl acetate-methanol eluent, sub fractions obtained were then checked by TLC, and apigenin and apigenitrin were used as a comparison. Based on the results of previous studies, apigenin affected reducing blood cholesterol [19]. Furthermore, using in silico methods, Apigenin could be developed as anticholesterol [20]. The goal of this study

was to find the effect of binahong leaves extract in the reduction of fat accumulation and hypercholesterolemia on endothelial cells of male Wistar rats 200-250 g. On 0, 3rd, 7th, 11th week, the rats were stimulated orally using pure cholesterol throughout 7 days. This cholesterol induction was carried out to stimulate the increased level of cholesterol and optimize the level of cholesterol. The optimal level of cholesterol caused impairment and triggered fat accumulation on endothelial cells. Next, the sub-fraction sample was distributed for 3 weeks

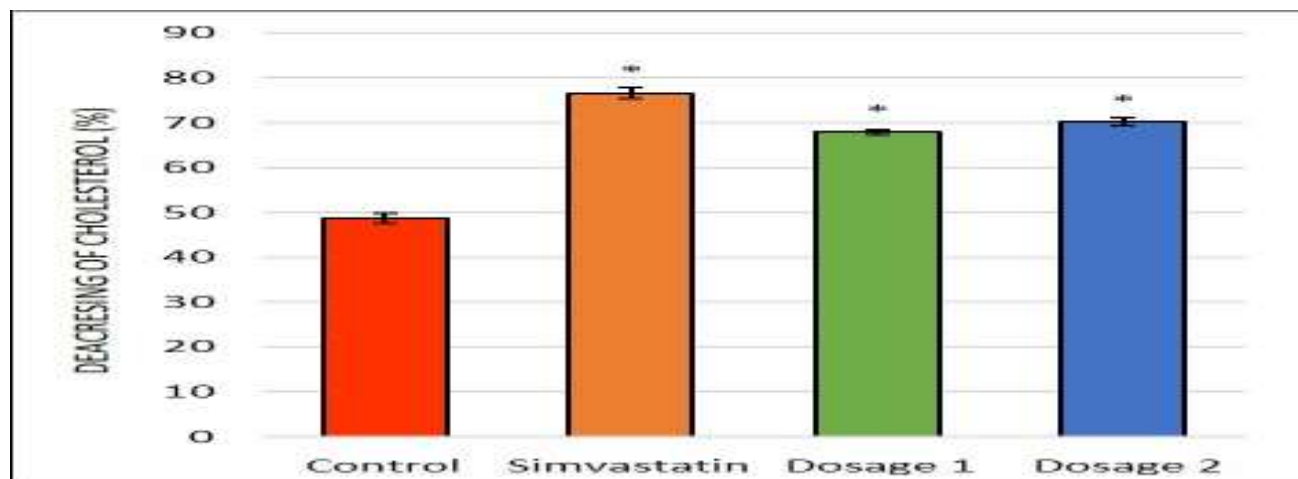


Fig. 1: Percentage reduction in total cholesterol after administration of binahong leaf subfraction, * = significantly different from the control group ($p < 0.05$), control = Na-CMC 1%; simvastatin = 3.6 mg/kg bw, dose 1 = 0.12 mg/kg bw, dose 2 = 0.24 mg/kg bw

From Fig. 1 it could be seen that dose 2 (0.24 mg/kg BW) gave a greater percentage of cholesterol reduction than dose 1 (0.12 mg/kg BW). The dose group 2 was no doubt distinct compared to the control group ($p < 0.05$). In the control group (CMC) also decreased cholesterol levels due to changes in food,

which was initially given three months of high cholesterol food, but during therapy (the next 3 weeks) was given normal food. This could be applied in everyday life, by regulating diet, then cholesterol levels in the blood could be reduced.

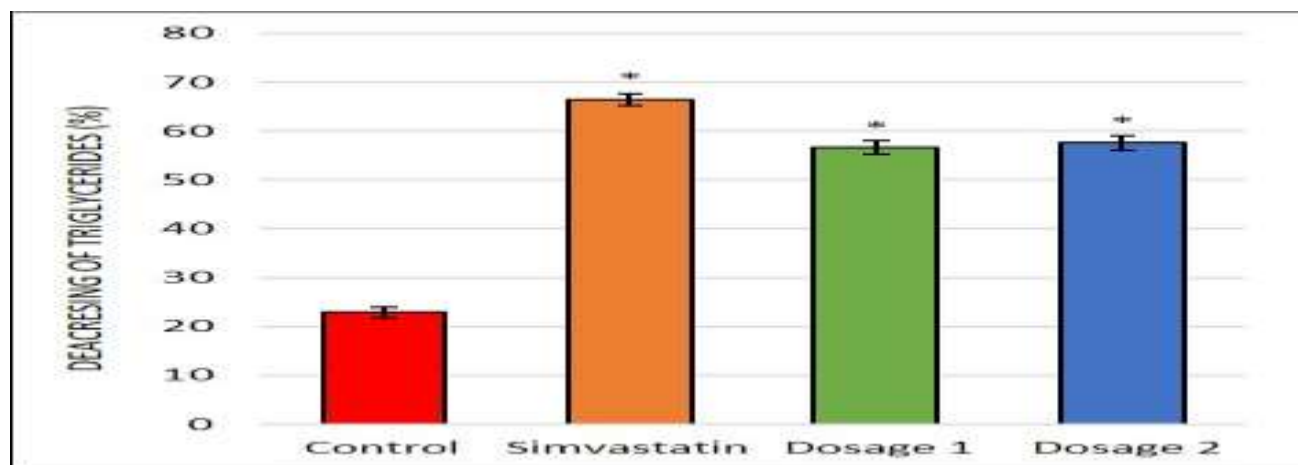


Fig. 2: Percent decrease in triglycerides after administration of binahong leaf subfraction, * = significantly different from the control group ($p < 0.05$), # = not significantly different from simvastatin group, control = Na-CMC 1%, simvastatin = 3.6 mg/kg BW, dose 1 = 0.12 mg/kg bw, dose 2 = 0.24 mg/kg bw

From fig. 2, for triglycerides, sub-fraction dose 2 showed the ability to approach the simvastatin group in reducing triglycerides,

and not significantly different from the simvastatin group. This implied the ability of subfraction dose 2 (0.24 mg/kg BW) was

almost equivalent to simvastatin in reducing triglycerides. The control group gave a

reduction in triglyceride levels because of the high cholesterol food substitution with regular food.

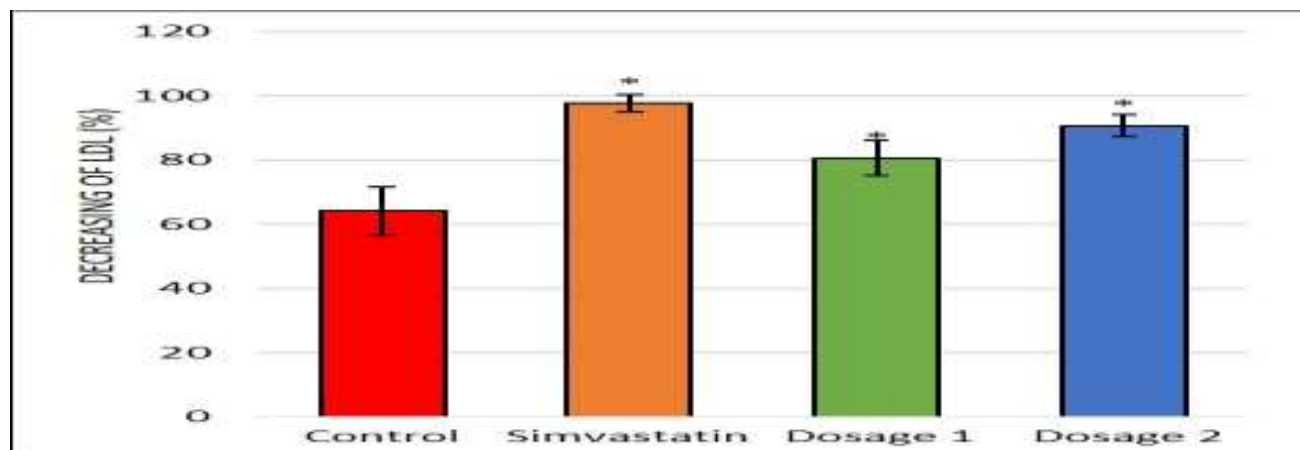


Fig. 3: Percentage reduction in LDL after administration of binahong leaf subfraction * = significantly different in the control group ($p < 0.05$), # = not significantly different in the statin group, control = Na-CMC 1%, simvastatin = 3.6 mg/kg body weight, dose 1 = 0.12 mg/kg body weight, dose 2 = 0.24 mg/kg body weight

Sub fraction dose 1 (0.12 mg/kg BW) and dose 2 (0.24 mg/kg BW) gave a drastic decrease in LDL to the control group ($p < 0.05$). Sub fraction dose 2 has the ability that is almost equivalent to simvastatin in reducing LDL.

The control group also showed a decrease in the LDL level of 64.17% which might be caused by the same factors as before (see Fig. 3).

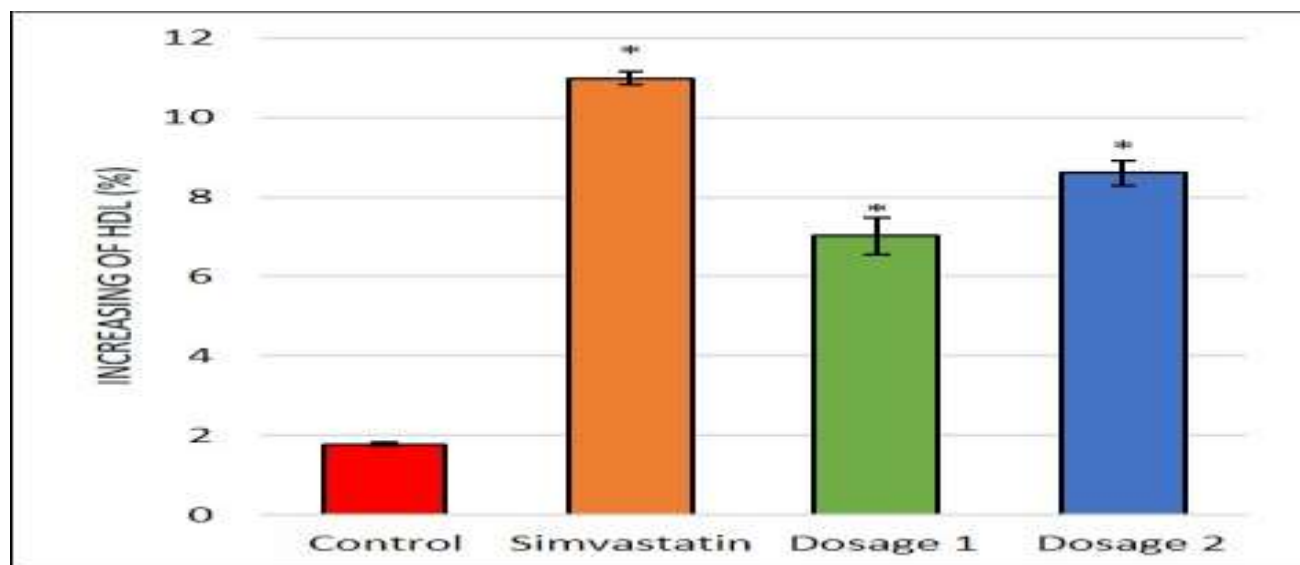


Fig. 4: Percentage increase in HDL after administration of binahong leaf subfraction * = significantly different in the control group ($p < 0.05$), control = Na-CMC 1%, simvastatin = 3.6 mg/kg bw, dose 1 = 0, 12 mg/kg bw, dose 2 = 0.24 mg/kg bw

Statin could increase HDL level sub-fraction showed only slightly increased in the HDL level. This result supported the previous study that *A. cordifolia* leaves ethanol extract could act as anti-hyperlipidemic [5] and that *A. cordifolia* leaves fraction could act as anti-hyperlipidemic as shown in Fig. 4.

Conclusion

From this study, it concluded that Madeira

vine ethyl acetate subfraction with a dose of 0, 24 mg/kg BW indicated improved results in the lipoprotein profile.

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