

## Effect of Methanol Extract of *Arcangelisia flava*(L.) in High-fat diet-induced Hyperlipidemic Mice

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**Abstract:** *Arcangelisia flava* is one of the plants used as traditional medicine to treat hypercholesterolemia. The aim of this study. To determine the effect of anti-hypercholesterolemia extract of *Arcangelisia flava* In male mice induced by fat diet. This study used plant of akar kayu kuning extracted macerated with methanol solvent and tested on 24 male mice divided into 6 groups, ie treatment control dose 9,5 mg / 20gBB, 19 mg / 20gBB, 38 mg / 20gBB positive control, Negative control, and normal control with each group consisting of 5 mice. Induction of fatty feed is administered orally with quail egg yolks. Measurement of total cholesterol level was done 3 times, the measurement of early cholesterol (H0), cholesterol after induced fat feed (H7) and cholesterol after treatment (H15). Total cholesterol levels were measured by GCU test. The results of statistical analysis of the study showed that *arcangelisia flava* extract at 9.5 mg / 20gBB, 19 mg / 20gBB, 38 mg / 20gBB decreased total cholesterol by 10%, 13%, 17%, respectively That extract dose of 38 mg / 20gBB gives the effect of decreasing total cholesterol total thebiggest, but still different from simvastatin.

**Keywords:** *Arcangelisia flava*, Cholesterol, Mice

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### I. Introduction

Hyperlipidaemia is a heterogeneous disorder with total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL), triglycerides, and decreased levels of high density lipoprotein (HDL). In addition, hyperlipidemia is also defined as serum cholesterol at least 200 mg / dl or serum triglyceride of at least 150 mg / dl (Maryani et al., 2016). LDL levels <160 mg / dl and HDL in men > 35 mg / dl, in women > 45 mg / dl (Fathila et al., 2015).

According to the World Health Organization (WHO) in 2008 the prevalence of hyperlipidemia increased in adults, ie 37% for men, and 40% for women. According to the World Health Organization, the highest prevalence of hyperlipidemia in Western Europe is around 54% for both sexes, followed by 48% for both sexes. The regions of Africa and Southeast Asia show the lowest percentage of 23% and 30% (Wijaya, 2015). Prevalence of hyperlipidemia in Indonesia from year to year, in 2008 increased by 35.1% Then in the year 2013 increased to 35.9% (Oktomalioputri et al., 2016).

Cholesterol is basically a very important fat for life because cholesterol is a cell membrane-forming agent and a number of hormones (Subinarto, 2004). The main function of cholesterol is to provide effective membrane components, which help you to work, create a major inhibitor of hormone production in life, is one of the ingredients needed by the body to make vitamin D, and help coat Nerves and anti airborne engineering at arterial surface (Povey, 2002).

One type of plant that concerns current research is yellow wood (*Arcangelisia flava* (L)). Plants belonging to the Menispermaceae family are found by saponins, terpenoids, flavonoids, and berberine alkaloids found in roots, stems, leaves, and plant stalks. Berberine is an alkaloid that has a wide pharmacological effect and is very different from hyperlipidemic (decreased lipid levels). Berberine as a hypolipidemic substance can significantly lower blood cholesterol, triglyceride, LDL, and atherogenic lipoproteins (Maryani et al., 2016).

Berberine that is able to alter lipid concentrations (total cholesterol, triglycerides, and LDL-C) by increasing transcriptional activity of LDL receptor promoters via JNK (c-Jun N-terminal kinase) pathway and stabilizing LDL-C receptor via pathways regulated by extracellular kinase (ERK) -depends. Moreover, affecting 5' AMPK and MAPK / ERK pathway blockage leads to inhibition of lipid synthesis in hepatocyte cells (Pang et al., 2015). Antioxidant activity *Arcangelisia flava* In addition there are flavonoid compounds that can inhibit LDL oxidation. This leads to the endothelial integrity of the yoga vessels and the dancing of the rishi

## II. Method

This research is a laboratory experimental study that describes the activity of yellow wood roots (*Arcangelisia flava* (L.)) in lowering total blood cholesterol levels in mice. Tools and materials used are Geranda cut, blender, beaker glass, measuring glass, glass funnel, brown bottle, white cloth, al.foil, rotary evaporator, analytical scales, reaction tube, pumpkin, chemical glass, stirrer, , dropper dropper, measuring pipette, erlenmeyer flask, bunsen, tricycle, filter paper, tube clamp, Mice scales, oral sonde, total cholesterol test stick, markers, paper plaster. The roots of yellow wood, N-Hexan, methanol, H<sub>2</sub>SO<sub>4</sub>, MnO<sub>4</sub>, Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>, H<sub>3</sub>PO<sub>4</sub>, chromatropic acid, toluene, ethanol, NH<sub>4</sub>OH, dragendorf, methanol, borate citrate, aquadest, mecit, standard feed, quail egg yolk, CMC-Na, aquadest , tablet simvastatin 10 mg. The experimental animals used were male mice weighing 20-30 grams, with the age of 2-3 months.

### **Preparation Extract of Arcangelisia flava (L.)**

The root of yellow wood (*Arcangelisia flava* (L.)) Was washed with running water to clean the roots of foreign objects such as soil, gravel and damaged parts of the plant, after the roots were dried and then chopped using gerenda, this was done to simplify the process maceration. The simplisia powder is macerated using methanol (1: 7.5) for 48 hours. The maseration results are filtered to separate between the filtrate and the dregs. Then do the remaseration twice using the same method and comparison with maceration. The filtrate of the maceration and remaseration was concentrated using a rotary evaporator with a temperature of 500 C, until all methanol evaporated, the yolk root extract of the yellow root was concentrated using a horner stove on a fume holder with a temperature of 500 C until thickened.

### **Screening Phytochemistry**

#### **Alkaloid Assay**

Extracts of ± 1 mL were mixed with 1 mL of chloroform and 1 mL of ammonia were fed into the test tube, then heated on top of a water bath, shaken and filtered. The obtained filtrate is divided into three equal parts, then insert it into the test tube, and add each 3 drops of 2N sulfuric acid, shake and let stand a few minutes apart. The top of each filtrate is taken and tested by Wagner and Dragendorf reagents. The formation of orange and cokeat deposits in each test result indicated the presence of alkaloids (Harborne, 1987).

#### **Flavonoid Assay**

Extracts of ± 1 mL mixed with 3 mL of ethanol 70%, then shaken, heated, and shaken again and then filtered. The obtained filtrate, then added Mg 0.1 g and 2 drops of concentrated HCl. The formation of red color in the ethanol layer indicates the presence of flavonoids (Harborne, 1987)

#### **Triterpenoid Assay**

Extracts of ± 1 mL were mixed with 3 ml of chloroform or 3 ml of 70% ethanol and added 2 mL of concentrated sulfuric acid and 2 mL acetic acid anhydrous (Liebermann-Burchard reagent). The formation of brownish red color on the intercrops shows the presence of triterpenoids (Harborne, 1987).

#### **Saponins Assay**

Extracts of ± 1 mL boiled with 10 mL of water in a water bath. The filtrate is shaken and allowed to stand for 15 minutes. The formation of a stable foam (lasting longer) means positive there is saponin (Harborne, 1987).

### **Free Test of Methanol**

How to check the presence of methanol is by the way, yellow wood root extract plus 1 drop of concentrated sulfuric acid and 1 drop of concentrated permanganate solution, let stand for 10 minutes. Add dropwise the concentrated sodium bisulfite solution into the mixture until the permanganate color (brown) is lost. If there is still a Chocolate Color add 1 drop of phosphoric acid solution. In a non-abrasive solution add 5 ml of fresh chromatropic acid and then heat over the water bath at 500C for 10 minutes (M.Noor et al., 2006).

### **Preparation of Suspension of Methanol Arcangelisia flava (L.) Merr Extract**

The thick yellow root root extract (*Arcangelisia flava* (L.) Merr.) Was each weighed as much as 200 mg; 400 mg, 800 mg were inserted into the mortar then crushed and added 1% w / v CMO-Na colloidal solution, 10 mL bit by bit to homogeneous.

### **Preparation of Simvastatin Suspension**

Simvastatin of 20 tablets weighed and calculated the average weight. After that, all simvastatin tablets are inserted into the mortar and crushed to a powder. Weighed 36.764 mg of simvastatin powder was then suspended in 1% CMC-Na piecemeal while stirring, volume up to 10 mL (Atvinda et al.,).

### **Preparation of CMC-Na Suspension 1%**

To prepare a 1% CMC-Na solution, weighed CMC-Na of 1g which is then subjected to a 50-mL hot aquades (70°C) and stirred with a stirrer until a colloidal solution is formed and sufficiently volume up to 100 mL with inner aquades 100 mL beaker.

### **The Treatment of Animal Trials**

Total of 24 male mice were placed in cages with standard feeding and drinking. After adaptation for 1 week, all mice weighed, given the identification on the tail. Mice test then divided into 6 groups, each group consists of four mice. Five groups of test mice were induced to feed the quail egg whilst, while one other group was used as normal controls with feed and drinking drinking standard for seven days. After the total cholesterol level increased, checked the total cholesterol level on the eighth day. Furthermore, three groups of test rats were each administered a suspension of methanol extract of yellow wood root (*Arcangelisia flava* (L)) dose of 9.5 mg / 20g BB; 18 mg / 20g BB; 38 mg / 20g BB. One group induced standard simvastatin (control (+)), and CMC-Na 1% (control (-)) for 7 days, and re-examined total cholesterol levels.

### **Total Cholesterol Testing with Strip Test**

Measurements are made using the GCU Easy Touch tool tool calibrated first with a code number adapted to the test strip used. Test strips inserted in a special place on the tool, then on the screen will appear a picture of "drops of blood" that indicates ready for ready to use. After the tail of the mouse was disinfected with 70% ethanol, the tail tip was cut, the first drop of blood was discarded, the next drop was dripped onto the test strip tucked into the tool. A certain amount of blood will be absorbed according to the absorbent capacity of the test strip until it can be heard on the screen, after which the mice bleeding is stopped. Results will be visible on the screen after 150 seconds (Umami et al., 2016).

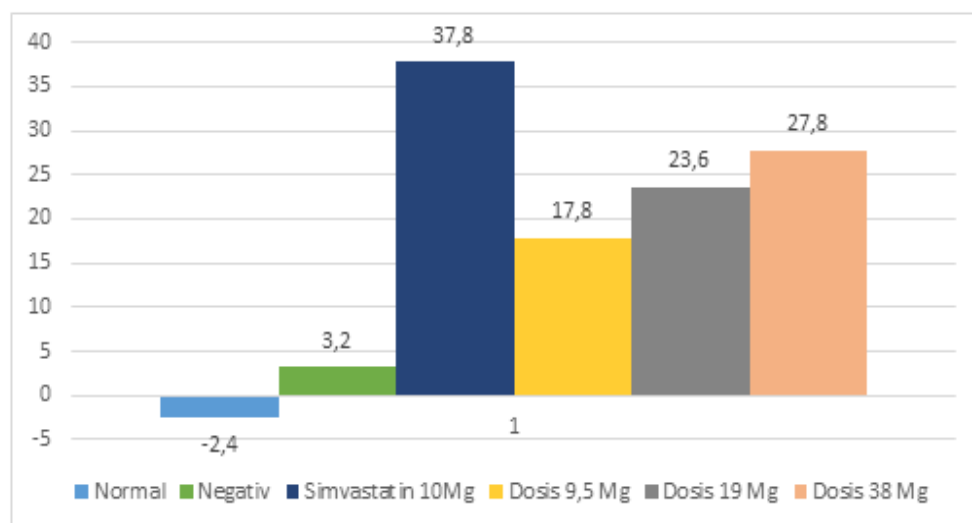
### **Data analysis**

Data analysis of research result using descriptive analysis and inferential analysis with SPSS program. Descriptive statistical analysis is done by means of Total Cholesterol data, measured from each group is described in tabular form. To test the difference of total cholesterol, one-way ANOVA test was used, price  $p < 0,05$  with 95% confidence level ( $\alpha = 0,05$ ).

## **III. Result and Discuss**

The root sample of *Arcangelisia flava* (L.) Merr wood plants was weighed, obtained by weight of 1868 grams undergoing washing, drying, and chopping process, to obtain 1459 gram of simplicia powder. The powder of simplicia weighed 1345 gram was extracted with methanol solvent 10,0875 L with maseration and remasari method, then evaporated in temperature range 50°C until methanol evaporated, then concentrated using acid cabinet in temperature range, so obtained 186 gram extract, yield of 13,82%.

Methanol *Arcangelisia flava* (L.) Merr Extract contains flavonoids, alkaloids, triterpenoids and saponins. This study was conducted for 21 days by first acclimatization of animals try for 7 days then measured cholesterol levels consisting of the measurement of initial cholesterol levels, cholesterol levels after induced fat diet for 7 days and cholesterol levels after treatment of yellow root root extract dose 9,5 mg / 20gBB, 19 mg / 20gBB and 38 mg / 20gBB positive control (Simvastatin), negative control (Na-CMC) and not treated, performed for 7 days. In the group fed high fat diet increased by 62.75 %, when compared with the normal control group. After a high-fat diet was given, all experimental animals were given standard oral medications and extracts at several concentrations for 7 days and examined total cholesterol levels. Based on the results of the examination, it can be seen that there is a decrease in total cholesterol levels in the group of experimental animals given oral sonde of yellow wood roots methanol extract and also standard drug simvastatin (control (+)).



**Figure 2.** Effect of Methanol *Arcangelisia flava* (L.) Merr Extract against Total Cholesterol Levels

Methanol *Arcangelisia flava* (L.) Merr Extract is also known to have sufficiently effective capability in lowering total cholesterol levels which can be seen from the large percentage reduction in total cholesterol levels produced. In each increasing dose of the extract, the percentage decrease in total cholesterol level was also increased, where the dose of 38 mg / g BW showed the best decrease of 17%, but when compared with the control (+) by 21% the decrease was still less effective. Decrease in total cholesterol levels is due to the content of alkaloids, saponins and flavonoids contained in the Methanol *Arcangelisia flava* (L.) Merr Extract. Giving simvastatin for 7 days in this study has not been able to reduce total cholesterol levels of animal try to reach normal levels. This may be due to the inappropriateness of drug administration, in which simvastatin is administered during the day. Simvastatin is better consumed at night when the synthesis of endogenous cholesterol in a higher number when compared to during the day. In addition, simvastatin is also known to lower total cholesterol levels better if consumed at night.

#### IV. Conclusion

Based on the results of research that has been done, it can be concluded that yellow wood root extract can lower blood cholesterol levels in male mice. And there was a significant difference in total cholesterol level of mice blood after administration of yellow wood root extract when compared with simvastatin 10 mg.

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