

Yellow Pumpkin (*Cucurbita maxima D.*) Extract As Anti-Hypercholesterolemia

Erna Kustiyaningsih, Istianatus Sunnah, Dian Oktianti*

Pharmacy Study Program, Faculty of Health Sciences, Universitas Ngudi Waluyo, Ungaran, Jl. Diponegoro No.186, Ngablak, Gedanganak, Ungaran Timur, Semarang, Jawa Tengah 50512.

Abstract

Hyperlipidemia is a risk factor for various diseases, which is still a big problem in Indonesia. Yellow pumpkin (*Cucurbita maxima D.*) is a plant containing flavonoids and terpenoids, which can be used as an anti-hypercholesterolemic agent. This study aims to analyze the activity and dosage of yellow pumpkin extract, which can be used as an anti-hypercholesterolemic agent to reduce total cholesterol levels comparable to simvastatin by 1.8mg/kg BW/day. This research is pure experimental research with a pre and post-test group design approach. The number of samples was 25 male Wistar rats induced by high-fat feed, quail egg yolk: pork oil (2:1) by 5 ml/200 gramBW/day for 7 days. The extract dosage range was 200, 400, 600 mg/kg BW/ day for 7 days. Data were analyzed using One Way ANOVA. The results showed that the administration of yellow pumpkin extract could reduce rats' total blood cholesterol levels with a dose of 600 mg/kg BW/day, comparable to simvastatin 1.8 mg/kg BW/day. The secondary metabolites of the extract were flavonoids and terpenoids. Extract of yellow pumpkin (*Cucurbita maxima D.*) could reduce total blood cholesterol levels of rats. The dose of 600 mg/kg BW/day could reduce blood cholesterol levels in rats comparable to simvastatin 1.8 mg/kg BW / day.

Keywords: Antihypercholesterolemia; *Cucurbita maxima D*; Flavonoid; Terpenoid

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INTRODUCTION

Hyperlipidemia is a condition of excess lipids or cholesterol metabolism disorders consisting of total cholesterol, triglycerides, HDL, and LDL caused by cholesterol levels in the blood exceeding normal limits¹. One of the plants that can be used as medicine is yellow pumpkin (*Cucurbita maxima D.*). Yellow pumpkin flesh contains carbohydrates, protein, fiber, amino acids, tocopherols, and carotenoids². In addition, yellow pumpkin

flesh also contains flavonoids³. Isoflavone, flavones, and flavanones are a group of flavonoids compounds that can reduce total cholesterol levels⁴. Apart from flavonoids, terpenoids also have an activity to lower total serum cholesterol levels.

METHOD

This research is purely a pre and post-test group design using the test animals model by taking blood samples before and after

* Corresponding author, e-mail: istianahizna29@gmail.com

treatment. The pre-test data were taken from the blood sample after induced with high-fat feed, while the post-test data were taken from the blood sample after induced with the extract. The animals were divided into 5 groups, showed in Table (1).

Table 1. Grouping of test animals model

Groups	Information
Normal Group (KN)	Animals with standard feeding + CMC Na
Positive Control (K +)	Animals with standard feeding + simvastatin suspension 1.8mg/KgBW/day
P 1	Animals with Standard feeding + extract suspension 200 mg / KgBW /day
P 2	Animals with Standard feeding + extract suspension 400 mg / KgBW /day
P3	Animals with Standard feeding +extract suspension 600 mg / KgBW /day

Making of Yellow Pumpkin Extract

Yellow pumpkin (*Cucurbita maxima* D.) was obtained from the Kopeng area of Semarang Regency, made in simplicia and powdered. The extract was made from 500 grams of the pumpkin flesh, 500 grams of maceration with 96% ethanol solvent in a 1:10 ratio. Maceration was carried out for 3 days, and re-maceration was done for 2 days. It was then evaporated using a rotary evaporator at a temperature of 60 °C. Phytochemical screening of the extract was carried out. The extract of pumpkin flesh detected flavonoids on TLC with silica GF254 stationary phase, which was then eluted using the mobile phase of n-butanol: acetic acid: water (3: 1: 1). As for terpenoids, the mobile phase of chloroform: methanol (9: 1) was selected. The selection of stationary and mobile phases was based on the polarity and properties of the flavonoids and terpenoids.

Induction of high cholesterol feed

Hypercholesterolemic feed was made from quail egg yolks and pork oil. The ratio of quail egg yolk: lard was 2:1. High cholesterol feed induction was given to rats for 7 days, and their cholesterol levels were monitored.

Pumpkin extract induction

The experiment animals in this research were white male rats (*Rattus novergicus*) aged 2-3 months, with an average weight of 200 grams. The number of samples was 25 male Wistar rats induced with high-fat feed, quail egg yolk: pork oil (2: 1) 5 ml /200 gramBW/day, for 7 days. The extract dosage ranges used were 200 mg/kg BW/day, 400 mg/kg BW/day, 600 mg/kg BW/day for 7 days.

Rat weighing

Weighing the rats was carried out to determine the effect of high-cholesterol feeding on their body weight.

Data analysis

The data was in the form of the difference in total blood cholesterol levels in rats from pre and post-test. Data were analyzed using One Way ANOVA.

RESULTS AND DISCUSSION

Table 1. Identification ethanol-free test

Samples	Reagents	Result
Yellow pumpkin extract	H ₂ SO ₄ pekat + CH ₃ COOH was heated	Not smell ester
Yellow pumpkin extract	K ₂ CR ₂ O ₇ +H ₂ SO ₄ pekat	No color change

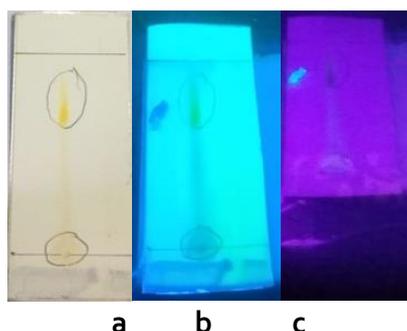


Figure 1. The results of TLC flavonoids (a) observations on visible light, (b) observations at 254 nm UV after spraying ammonia vapor, (c) observations on UV light 366 nm after being sprayed with ammonia reagent

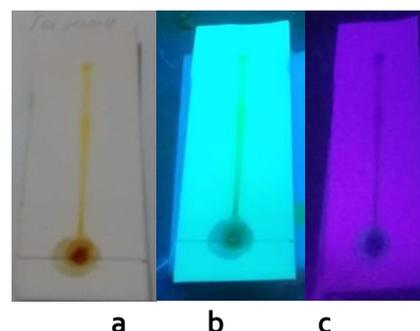


Figure 2. TLC results for the terpenoid class compounds. (a) observing visible light, (b) observing UV light at 254 nm after being sprayed with Liberman-Buchard, (c) observing UV light at 366 nm after being sprayed with Liberman-Buchard

Table 2. Identification of secondary metabolites

Secuder metabolities	Reagents	Color spot	UV 254 nm	UV 366 nm	Rf Standart	Value Rf	Informati on
Flavonoid	Ammonia	Yellow (254nm) Blue (366nm)	Yellow	Blue	0.54-0.92	0.78	(+)
Terpenoid	Liberman-Buchard	Yellow (254nm) Green - blue (366)	Yellow	Green-blue	0.08-0.96	0.95	(+)

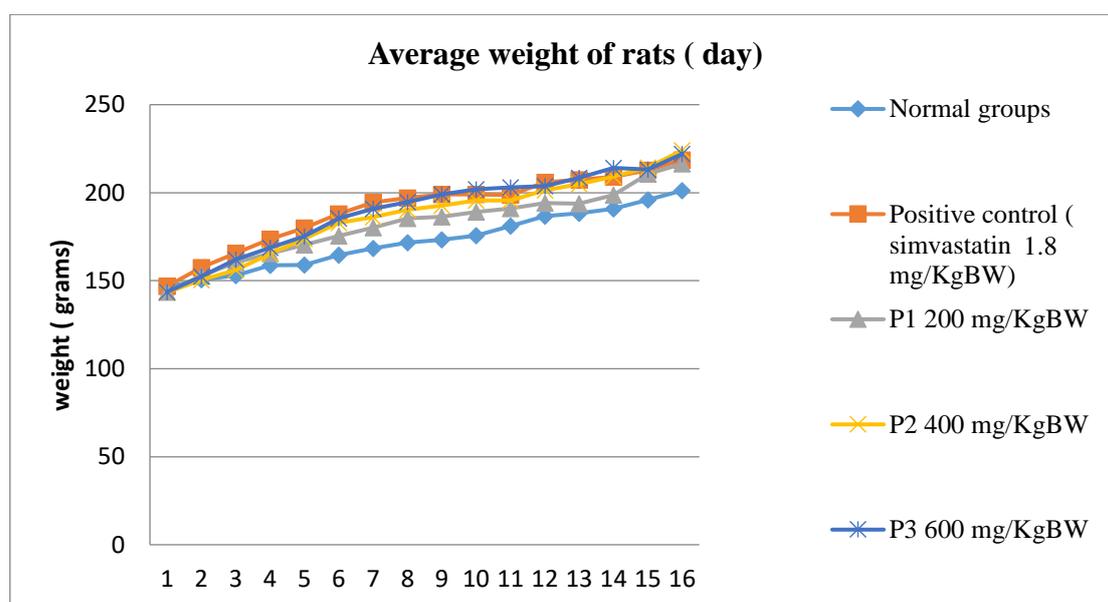


Figure 3. Graph of average weight gain of rats

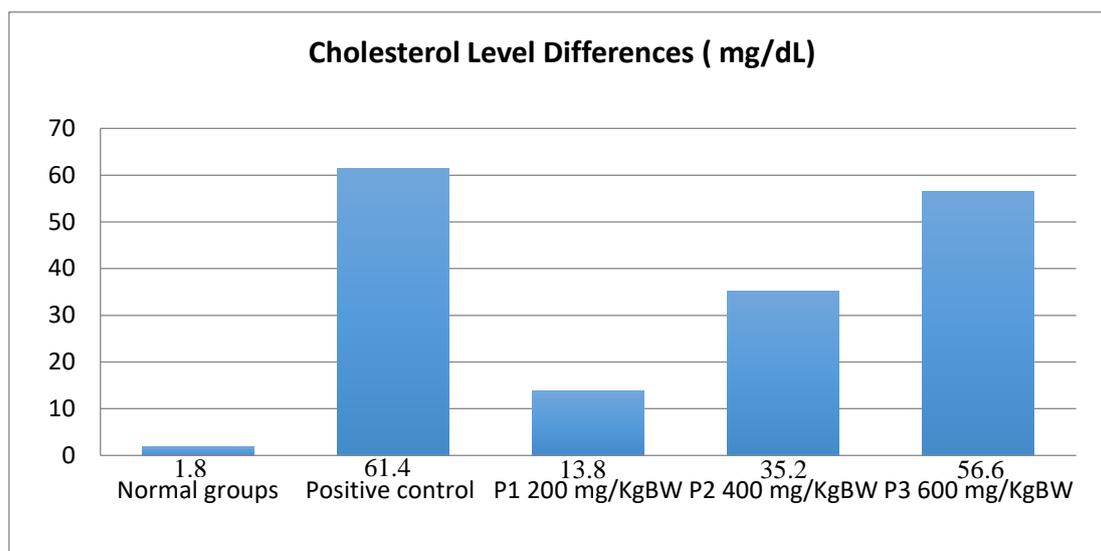


Figure 4. Diagram of Difference in Total Cholesterol Levels Each Group

Table 3. Cholesterol level percentage

Groups	Cholesterol Level at Pretest	Cholesterol level at Post-test	Cholesterol level Differences	% decrease level
Normal	113.800±3.493	112.000±2.345	1.800±1.924	1.58
Positive	186.400±15.437	125.000±17.479	61.400±4.827	32.94
P1	190.000±15.572	176.200±18.606	13.800±3.421	7.26
P2	180.800±22.466	145.600±23.394	35.200±4.970	19.47
P3	182.000±22.249	125.400±23.734	56.600±3.286	31.90

Yellow pumpkin has been widely known for its pharmacological benefits due to its metabolite content in the form of flavonoids and terpenoids. In this study, the pumpkin extraction was carried out to analyze the presence of anti-hypercholesterolemic activity. The extract was prepared by maceration of *Simplicia* using ethanol 96% (1:10). Maceration results obtained a thick reddish-brown extract of 90.149 grams, and the yield was 18.029%. This number has met the requirements of the Indonesian Herbal Pharmacopeia (FHI), which is not less than 10.0%, as the higher the yield value, the greater the extract produced.

Phytochemicals screening

The thick extract of pumpkin flesh was tested with the ethanol-free test to determine whether there was ethanol content in the extract not to affect the test. The test results showed that the thick extract of pumpkin flesh was free from ethanol as it did not smell ester, and there was no color change⁵ (Table 1).

The result of phytochemical screening showed the presence of secondary metabolite compounds in the form of flavonoids and terpenoids in pumpkin extract (Figure 1 and 2). In Table (2), the result showed a presence of flavonoids with an Rf value of 0.78, seen after sprayed

with ammonia vapor reagent, and an Rf value of 0.95 for terpenoids after sprayed with Liberman Buchard reagent. The mobile phase used to identify flavonoids was n-butanol: acetic acid: water (3: 1: 1), and the mobile phase for terpenoids used chloroform: methanol (9: 1). The solvent influence determined the metabolite compound extraction due to 'like dissolve like'.

Rat Weighing

The rats fed high cholesterol in figure (3) showed a higher body weight gain compared to the normal control group. The increase in body weight in the normal group (KN) was 37.43%, the positive group (K+) was 48.91%, groups with 200 mg/kg BW/ day dose (P1) was 50.69%, groups with 400 mg/kg BW/ day dose (P2) was 56.20%, and groups with 600 mg/kg BW/ day dose (P3) was 54.59%. This result showed that high-fat feeding increased the weight of the test rats higher and faster. During the study, there were differences in the weight gain of the rats. The difference in weight gain occurred as these white rats had genetic differences, causing different treatment responses. Increasing body weight was influenced by diet and lots of fat⁶. Weight gain and increased cholesterol levels in rats were seen during fed fatty foods⁷. In the first stage of the study, the animal models, except the normal control group, were induced with a high cholesterol feed, raw quail egg yolk, and pork oil (2: 1) 5 ml/200 gram BW/day for 7 days to be hypercholesterolemic. Cholesterol levels could increase as quail egg yolks contained very high cholesterol compared to other eggs, which had 3,640 mg/100 grams of eggs, while pork oil contained 200 mg/100 grams of cholesterol. Apart from containing very high cholesterol, quail eggs also had a composition of 31.85%

saturated fatty acids⁸. In addition, pork oil also contained 21 % saturated fatty acids⁶.

Cholesterol Level

Blood sampling for measuring total cholesterol levels was carried out through the tail, measured using the easy touch device. The advantage of using this tool is that it only requires a small amount of blood sample, is easy to use, and the results are obtained quickly (150 seconds). The results of measuring blood cholesterol levels after feeding high cholesterol were defined as pre-test total cholesterol levels, as showed in figure (4). The results showed a significant increase in the total blood cholesterol levels of rats. It means that the induction of high cholesterol feed using quail egg yolk and lard (2: 1) could increase the total cholesterol level of rats, indicated by total cholesterol level 98-148 mg/dL⁹. Quail has 30.63% lipid content⁸. Cholesterol levels of the tested animals showed a decrease after treatment with pumpkin extract and simvastatin. The difference between the highest and lowest decreases in a row was: the positive group was 61.40 mg/dL, the P3 group was 56.60 mg/dL, the P2 group was 35.20 mg/dL, the P1 group was 13.80 mg/dL, and the normal group was 1.80 mg/dL. The research data obtained in the pre and post test had a fairly large standard deviation, due to the limitations of researchers in controlling food intake in each tested animal.

The results of LSD in the positive control group and the P3 group showed no significant difference ($p = 0.062$). In the positive group, the decrease in total blood cholesterol levels was 32.94%, which was the highest compared to other groups. Whereas, in the P3 group, the decrease in total blood cholesterol levels was 31.09%, as showed in table (3).

Based on the result, the ability of yellow pumpkin extract in reducing total blood cholesterol levels of rats was due to secondary metabolites, such as flavonoids and terpenoids. Flavonoids had a mechanism of action to inhibit the enzyme HMG Co-A reductase⁴.

The mechanism of flavonoids has similarities with the mechanism of action of simvastatin in reducing cholesterol levels. Flavonoids also have a role as antioxidants that act to reduce LDL in the body. Yellow pumpkin contains a flavonoid of 4.433 mg/mL quercetin. It also contains flavon and flavonols as an antioxidant¹⁰ and antihyperlipidemic. Flavonoid as antihyperlipidemic includes isoflavones, flavones and flavonols⁴. Flavones groups in yellow pumpkin are vitexin, isovetexin, krisoeriol and apigenin¹⁰ as antihyperlipidemia⁴. Quercetin and azaleatin are included in flavonols groups in yellow pumpkin extract¹⁰, having a role as antihyperlipidemic. Apart from flavonoids, terpenoids also have activities to lower total blood cholesterol levels. It has antihypercholesterolemic activity by acting as ligands for PPAR (Peroxisome Proliferator-Activated Receptor)⁷.

CONCLUSION

Extract of yellow pumpkin (*Cucurbita maxima* D.) flesh affected the reduction of total cholesterol levels in hyperlipidemic male rats. A 600 mg/kg BW/day dose could reduce total cholesterol levels compared to a dose of simvastatin 1.8 mg/kg BW/day. This anti-hypercholesterolemic pharmacological activity was influenced by the content of secondary metabolite compounds in the form of flavonoids and terpenoids in yellow pumpkins.

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