

# HASIL CEK\_Mahfudz, Mantali, Sulityani\_Antihyperlipidemic, Antioxidant, Sweet Potato Leaf, Red Yeast Rice

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Antihyperlipidemic Effect Of Purple Sweet Pota

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## The Antihyperlipidemic Effect of Purple Sweet Potato Leaf Extract (*Ipomoea batatas* L.) and Red Yeast Rice Combination on Hypercholesterol Rats

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### ABSTRACT

Hyperlipidemia is a condition in which lipids in the blood, such as cholesterol and triglycerides, are present in a high level, causing a variety of other diseases. This condition also causes an increase in free radicals, resulting in oxidative stress. Purple sweet potato leaves and red yeast rice have been reported to have antihyperlipidemic and antioxidant activity in several studies. This particular study aimed to examine the effect of the combination of purple sweet potato leaf extract and red yeast rice on cholesterol and triglyceride levels as well as malondialdehyde (MDA) levels and to examine glutathione peroxidase (GPx) activity on rats induced with a high-fat diet. The experimental animals were randomly divided into 6 groups of 5 mice each: group 1 being the normal group; group 2 being the positive control; group 3 being the negative control; group 4 being treated with purple sweet potato leaf extract at 400 mg/kg BW; group 5 being treated with red yeast rice at 40 mg/kg BW; and group 6 being treated with a combination of purple sweet potato leaf extract and red yeast rice at 360 mg/kg BW and 40 mg/kg BW, respectively. The rats were given high-fat feed for 28 days, and on days 15–28 they were also given oral preparations. At the end of the study, all the rats were blood drawn to measure their cholesterol and triglyceride levels. The rats were then sacrificed, and the liver was taken to measure their MDA and glutathione peroxidase levels. The administration of purple sweet potato leaf extract (group 4), red yeast rice (group 5), and their combination (group 6) significantly ( $p < 0,05$ ) reduced cholesterol, triglyceride, and MDA levels and increased GPx activity. This finding shows that the combination of purple sweet potato leaf extract and red yeast rice had antihyperlipidemic and antioxidant activity in hypercholesterol rats.

**Keywords:** Antihyperlipidemic, Antioxidant, Sweet Potato Leaf, Red Yeast Rice..

### INTRODUCTION

Hyperlipidemia is a condition in which lipid levels in the blood are abnormally high. This condition is also known as hypercholesterolemia or hyperlipoproteinemia (Gupta *et al.*, 2011). Hypercholesterolemia is a condition in which the concentration of blood cholesterol exceeds the normal level (Durrington, 2003). Hypercholesterolemia can cause changes in the physical properties of cell membranes that trigger an increase in oxygen free radicals from mitochondria. These oxygen free radicals result in the process of lipid peroxidation in cell membranes, which produces peroxide radicals and other free

radicals (Singh *et al.*, 2017). This increase in free radicals can cause an imbalance of oxidants and antioxidants, which is better known as oxidative stress. High levels of free radicals can cause tissue damage (Murray *et al.*, 2009). Oxidative stress can be determined by looking at the increase in lipid peroxide in the body and by measuring the levels of malondialdehyde (MDA).

Purple sweet potato leaves contain carotenoids that have antioxidant abilities that reduce or inhibit mutagenesis in cells. Besides, they also contain terpenoids that can reduce low-density lipoprotein (LDL) cholesterol levels and act as anticarcinogens (Mohanraj & Sivasankar, 2014).

Purple sweet potato leaves are also known to contain anthocyanin, which also has a function as an antioxidant. Therefore, they can reduce the effects of oxidative stress, which plays a role in the process of disturbances in the cardiovascular system (Reis *et al.*, 2016). Purple sweet potato leaf extract has been found to improve lipid profile (Sumardika & Jawi, 2012). They were also reported to have antitumor activity and the ability to lower blood sugar levels (Zhao *et al.*, 2013).

Red yeast rice (*angkak*) is a food product from fermented rice produced by cultivating *Monascus purpureus* (Shi & Pan, 2011). The main content of *angkak* is monacolin K, which can inhibit cholesterol biosynthesis in humans and animals. Other contents include compounds such as flavonoids, polyphenols, carotenoids, and alkaloids, vitamins, and several secondary metabolites produced by the *Monascus* mushroom. These are components composed of polyketides, so they have antioxidant activity (Chariote *et al.*, 2009).

The combination of medicinal plants aims to increase the expected effect. The interaction between two or more medicinal ingredients can be in the form of synergism of action between the active ingredients contained in each ingredient, thereby giving a better effect. This study aimed to examine the antihyperlipidemic effect of the combination of purple sweet potato leaves and red yeast rice *in vivo* on hypercholesterol rats to produce better effects and benefits in treating hyperlipidemia and the resultant oxidative stress.

## MATERIALS AND METHODS

### Plant collection

Purple sweet potato leaves were collected from farmers in Bantul district, Yogyakarta, and red yeast rice was purchased at the Beringharjo market, Yogyakarta. They were then authenticated at the biology laboratory of the Mathematics and Natural Sciences faculty, Ahmad Dahlan University, with identification number 240/Lab.Bio/B/X/2020.

### Extraction of purple sweet potato leaves and preparation of red yeast rice

Fresh purple sweet potato leaves were sorted and washed, then dried in an oven at 40 °C. Purple sweet potato leaf extract was then subjected to extraction. Five hundred grams of purple sweet potato leaf powder was put into a container containing aquadest, then heated over a water-bath

to a temperature of 90 °C for 30 minutes. The solvent was evaporated with a vacuum rotary evaporator until it was slightly concentrated, after which it was dried using a freeze dryer. Red yeast rice was used in the powder form, mashed using a blender until it became powdered.

### Animals

The experimental animals used were 8-week old 30 male Wistar rats weighing 150–250 g. Male sex selection was intended to reduce hormonal influences. Rats were kept in a well-ventilated room with a light cycle of 12 hours light and 12 hours dark, and the humidity and temperature of the room were maintained. Before treatments were administered, all the experimental animals were allowed to adapt for 7 days. This research has been approved by the Ahmad Dahlan University Research Ethics Committee with the number 012009048.

### Hypercholesterol induction and experimental design

To create a hypercholesterol condition, the rats were fed with a high-fat diet (HFD) made with 30 g of standard feed, 20 g of chicken egg yolks, 10 g of butter, 1 g of beef fat, and 0.05 g of propylthiouracil (PTU). The feed was made into pellets by mixing all the ingredients and molding the mixture into a cylindrical form. The pellets were then oven-dried. The high-fat diet was given for 14 days before sample treatments were administered to the test and control groups. The high-fat diet feeding was continued to the 28<sup>th</sup> day for the test groups. Feed was given at 15 g/head/day, and water was given *ad libitum* during the treatments to all the test and control groups.

The rats were divided into 6 groups: a normal group given standard feed, a negative control group given HFD, a positive control group given HFD and Nutrive Benecol (Kalbe Farma, Indonesia) at 9 mL/kg BW 2 times a day, and treatment groups given HFD and purple sweet potato leaf extract at 400 mg/kg BW, red yeast rice at 40 mg/kg BW, and a combination of purple sweet potato extract and red yeast rice at 360 mg/kg BW and 40 mg/kg BW, respectively. The test preparations were first dispersed in 0.5% Na-CMC, and then they were administered orally using a probe. On the 29<sup>th</sup> day, blood was drawn for cholesterol and triglyceride testing. Then, the animals were sacrificed and dissected to take their liver.

#### Measurement of cholesterol and triglyceride levels

Cholesterol levels were tested using the CHOD-PAP (Cholesterol Oxidase-Peroxidase Aminoantipyrin) method, and triglyceride levels were tested using the GPO-PAP (Glycerol Phosphate Oxidase-Para Aminophenazone) method, with diasys reagent. Ten L of blood serum and 1 mL of reagent were blended and then incubated for 5 minutes at 37 °C and measured at 546 nm absorbance.

#### Liver homogenate preparation

Rat liver tissue was cut into small pieces, then weighed and homogenized in PBS (0.01 M, pH 7.4) on ice, with a tissue weight (g) to PBS volume (mL) ratio of 1:9. The liver tissue homogenate was then centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant was then separated and used to test the MDA levels and glutathione peroxidase activity.

#### Measurement of malondialdehyde (MDA) levels

MDA levels were determined using the elabsciens kit (E-BC-K025-S) colorimetrically according to the manufacturer's instructions. A total of 0.1 mL of liver homogenate was mixed with 0.1 mL of TBA clarivant, 3 mL of acid reagent, and 1 mL of chromogenic agent. The mixture was then incubated in a 95 water bath for 40 min, cooled with running water in a tube, and finally centrifuged at 3100 g for 10 min. The supernatant was collected and then measured by a spectrophotometer at 532 nm with a 1 cm diameter cuvette.

#### Measurement of glutathione peroxidase (GPx) activity

A measurement of glutathione peroxidase (GPx) activity was carried out using the elabsciens kit (E-BC-K096-S) according to the manufacturer's instructions. A total of 0.2 mL of liver homogenate was added with 0.2 mL of 1 mmol/L GSH, then heated in a tube together with a stock solution in a 37°C water bath for 5 min. It was added with 0.1 mL of stock solution in the tube and then reacted in a 37°C water bath for 5 min. Then, 2 mL of acid reagent was added. It was then mixed and centrifuged at 3100 g for 10 minutes. One ml of the supernatant was taken, added with 1 mL of phosphate, 0.25 mL of DTNB, and 0.05 mL of salt reagent successively. It was subsequently mixed and left for 15 min at room temperature and then measured using a spectrophotometer at 412 nm.

#### Statistical Analysis

The results of the measurement of anticholesterol and antioxidant activity were processed using a Kolmogorov-Smirnov normality test and a homogeneity of variance test, followed by parametric statistical methods using a one-way ANOVA and a post-hoc LSD test using SPSS v.22 with a 95% confidence level.

## RESULTS AND DISCUSSION

#### High-fat diet (HFD) induction

The results of the cholesterol and triglyceride level measurements for the negative control group were significantly higher than those for the normal group (Table I). They were linear with the increase in MDA levels and decrease in GPx activity (Figure 1 and Figure 2). A high-fat diet (HFD) of a mixture of standard feed, beef fat, butter, eggs, and propylthiouracil (PTU) was used. Beef fat, butter, and eggs are animal fats that contain saturated fatty acids that can be used to increase cholesterol levels. Beef fat can increase cholesterol levels to 9.5 g/10 g of ingredients, causing mice to experience dyslipidemia (Furi & Wahyuni, 2011). Propylthiouracil (PTU) is an antithyroid drug that can treat high thyroid levels in the blood, and it can cause hypothyroidism as well. Hypothyroidism conditions can affect lipoprotein metabolism by reducing the number of LDL receptors, resulting in increases in LDL and cholesterol in the blood (Fajaryanti *et al.*, 2016).

Increased cholesterol also affects the balance between oxidants and antioxidants, causing oxidative stress which is characterized by increased levels of MDA. Increased levels of MDA in animals fed with a high cholesterol diet have previously been reported: plasma total cholesterol and triglyceride concentrations were positively correlated with free radical formation (Hassan *et al.*, 2011).

#### Cholesterol and triglyceride levels

The measurements of cholesterol and triglyceride levels in the purple sweet potato leaf extract, red yeast rice, and combination treatment groups showed significant decreases compared to the negative control group ( $p < 0.05$ ), with the highest decrease resulting from the combination group. However, as compared to the negative control group, in the positive control group, a significant decrease only occurred in cholesterol levels, while the decrease in triglyceride levels was insignificant ( $p > 0.05$ ) (Table I).

Table I. The average of Cholesterol & Triglycerides levels of high-fat diet rats treated with the combination of sweet potato leaf extract and red yeast rice

Groups	Cholesterol level (mg/dL)	Triglycerides level (mg/dL)
Normal	76.64±13.17 *	154.46±44.05 *
Positive Control	82.80±13.92 *	246.15±40.58
Negative Control	105.03±12.43	265.64±38.66
Purple sweet potato leaf extract (400 mg/kg BW)	83.39±12.94 *	152.31±43.39 *
Red yeast rice (40 mg/kg BW)	83.64±18.45 *	209.03±48.31 *
Combination of sweet potato leave extract & red yeast rice (360 & 40 mg/kg BW)	75.87±12.16 *	147.95±22.02 *

Notes: \* significantly different with the negative control group (p<0,05)

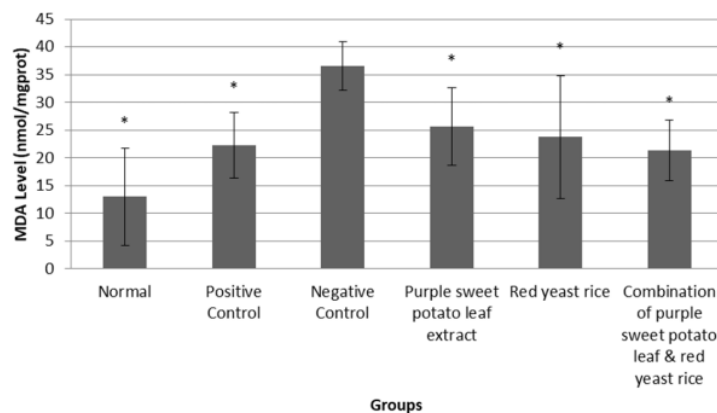


Figure 1. Malondialdehyde (MDA) Level of high-fat diet rats treated with the combination of sweet potato leaf extract and red yeast rice. \* significantly different with the negative control group (p<0.05)

This is in line with a previous clinical study finding that Nutrive Benecol, which contains plant stanol ester, could reduce total cholesterol and LDL cholesterol but could not significantly reduce triglyceride levels in people with high cholesterol levels (Lestiani *et al.*, 2018).

The finding is also consistent with another previous study, which stated that purple sweet potato leaf extract was able to significantly reduce cholesterol and triglyceride levels and that it had a protective effect on the rat liver against damages from high-fat diets induction (Mahfudh *et al.*, 2021). Purple sweet potato extract contains flavonoids that work by inhibiting cholesterol absorption, increasing bile excretion, and inhibiting HMG-CoA. The inhibited HMG-CoA will be converted to mevalonate with the help of the

HMG-CoA reductase enzyme so that flavonoids will bind to the HMG-CoA reductase enzyme. Flavonoids as inhibitors can cause mevalonic acid to decrease. Inhibition of this enzyme will inhibit the formation of cholesterol in the liver (Lairin Djala *et al.*, 2016). Flavonoids such as quercetin and anthocyanins will also inhibit the activity of the enzyme acetyl-CoA carboxylase. This enzyme functions in the synthesis of fatty acids, and consequently, the synthesis of triglycerides will be inhibited along with the enzyme diacylglycerol acyltransferase (DGAT). Inhibition of the DGAT enzyme causes the 1,2-diacylglycerol reaction not to occur into triacylglycerol or triglycerides so that triglyceride synthesis will be inhibited and result in a decrease in triglyceride levels (Elias, 2014).

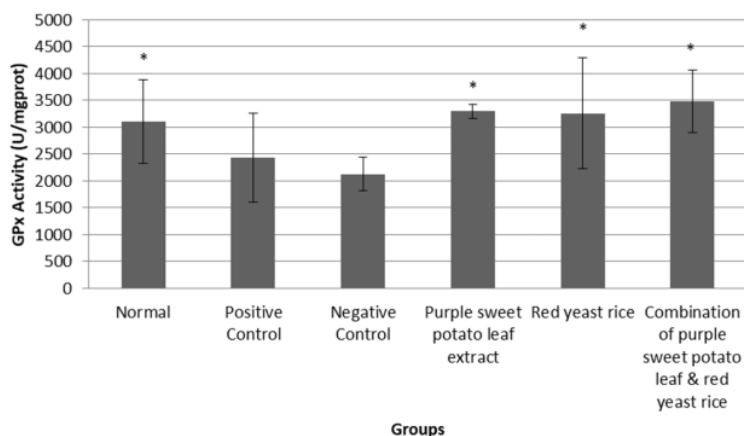


Figure 2. Glutathione Peroxidase (GPx) Activity of high-fat diet rats treated with the combination of sweet potato leaf extract and red yeast rice. \* significantly different with the negative control group ( $p < 0.05$ )

The same results were also seen in the red yeast rice group. Giving red yeast rice could reduce the total cholesterol levels in rats fed with high-fat diets (Bunnoy *et al.*, 2015; Kasim *et al.*, 2012). Several clinical trials have documented the efficacy of red yeast rice in lowering total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) (Gordon & Becker, 2011). Red yeast rice is known to be able to reduce triglyceride levels by about 13% to 44% (Shamim *et al.*, 2013). Red yeast rice contains Monacolin K, which is produced by *Monascus* sp. This compound can inhibit cholesterol biosynthesis through the inhibition of HMG-CoA reductase enzyme activity. The inhibition of this enzyme was due to the homology between the structure of monacolin K (statin), hydroxy acid, and HMG-CoA (Bunnoy *et al.*, 2015). This result also shows that combining the two ingredients could result in a better reduction in cholesterol and triglyceride levels.

#### Antioxidant activity

The purple sweet potato leaf extract, red yeast rice, and combination treatment groups showed decreases in MDA levels and increases in GPx activity significantly compared to the negative control group ( $p < 0.05$ ) (Figure 1 and 2). The group treated with the combination of purple sweet potato leaf extract and red yeast rice demonstrated the highest increases in MDA and GPx levels among the treatment groups, close to the normal group. It was discovered by a previous study that purple

sweet potato leaf extract showed a significant increase in GPx enzyme activity in rats fed with a high-fat diet (Safira, 2021). Purple sweet potato leaves contain flavonoid quercetin which has antioxidant activity. The antioxidant mechanism in flavonoids is by directly capturing free radicals, preventing the regeneration of free radicals, and indirectly increasing the antioxidant activity of cellular antioxidant enzymes (Akhlaghi & Bandy, 2009). In addition, the polyphenols contained in sweet potato leaves can increase glutathione levels by facilitating the expression of  $\gamma$ -glutamylcysteine synthetase (Moskaug *et al.*, 2005).

Red yeast rice is also able to improve this antioxidant profile. The monacholine K (lovastatin) contained in red yeast rice can activate antioxidant systems, one of which is GPx, and reduce hydrogen peroxide ( $H_2O_2$ ) (Kasim *et al.*, 2012). Glutathione peroxidase is one of the important intracellular antioxidant enzymes that can break down hydrogen peroxide in water and lipid peroxide in alcohol. Enzymes play an important role in inhibiting the process of lipid peroxidation, thereby protecting cells from oxidative stress (Francenia Santos-Sánchez *et al.*, 2019). Antioxidants play a role in neutralizing free radicals by donating their excess electrons to free radicals and reducing their destructive ability. The free radical scavenging properties of these antioxidants can delay or inhibit cell damage (Lobo *et al.*, 2010)

Oxidative stress is a phenomenon resulting from hypercholesterolemia (Lassoued *et al.*, 2014).

Following the data obtained, it was shown that hypercholesterolemia was associated with decreased antioxidant status, increased malondialdehyde (MDA) levels, and decreased glutathione peroxidase (GPx) activity. On the other hand, improvement in cholesterolemia is accompanied by improvement in this status.

#### CONCLUSION

The combination of purple sweet potato leaf extract and red yeast rice has antihyperlipidemic activity by lowering total cholesterol and triglyceride levels and has antioxidant activity by lowering malondialdehyde (MDA) levels and increasing glutathione peroxidase (GPx) antioxidant enzymes. This effect is better when compared to purple sweet potato leaf extract or red yeast rice only.

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