

# The effect of mundu fruit *Garcinia dulcis* Roxb Kurz methanol extract on lyphoprotein profile and trygliseride white rat *Rattus norvegicus* L

*By* Arum Setiawan

## THE EFFECT OF MUNDU FRUIT (*Garcinia dulcis* (ROXB.) KURZ) METHANOL EXTRACT ON LYPHOPROTEIN PROFILE AND TRYGLISERIDE WHITE RAT (*Rattus norvegicus* L)

Arum Setiawan\*, Laila Hanum\* and Elvi Rusmiyanto Pancaning Wardoyo\*\*

\*Departement of Biology, Faculty of Mathematic and Natural Science, Sriwijaya University, Jl. Palembang-Prabumulih KM 32, Indralaya (Ol) South Sumatera, Indonesia, 30662

\*\*Departement of Biology, Faculty of Mathematic and Natural Science, Tanjungpura University, Jl. Prof. Hadari Nawawi, Pontianak, West Kalimantan, Indonesia 78124

Corresponding Author email: [lailahanum@gmail.com](mailto:lailahanum@gmail.com)

### ABSTRACT

*Garcinia dulcis* (Roxb.) Kurz (Guttiferae) is a tropical fruit tree native to Indonesia and its known as an Asian medicinal plant used in folk medicines. In ethno pharmacology, *Garcinia dulcis* has been used in traditional medicine, such as anti-inflammatory, anti-tumor, anti-malaria and anti-bacterial. The scientific studies of biological activity of *Garcinia dulcis* on lipide profile and triglyceride are relatively measily. Therefore, the objectives of this research were to know the effect of methanol extract of *Garcinia dulcis* fruit on total cholesterol, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol and triglyceride rat hyperlipidemic. These studies using *Garcinia dulcis* fruit from Banyuasin, South Sumatra. In this observation, 30 male rat from Laboratory and Research Institute for Integrated Testing, Gadjah Mada University, 2 months old were used and divided into five groups randomly. The first group was given high cholesterol and lipid diet, as a normal group. The second group was given high cholesterol, lipid diet and simvastatin 3.6 mg/kg bow/day dosage, as a simvastatin group. The third, fourth and fifth groups was given high cholesterol, lipid diet and methanol extract 1.8; 2.7 and 3.6 mg/kg bow/day dosage. Total cholesterol, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol and triglyceride contains using spectrophotometry method, were observed 1 and 30 days. The differences among groups were analyzed using Anova followed by LSD to locate the differences. LSD analysis showed that *Garcinia dulcis* methanol extract treatment doses of 3.6 mg/kg bw and simvastatin lowers total cholesterol levels highest in the amount of 32.17 mg/dL and 24.1 mg/dL, significantly different compared with the treatment dose of 1.8 mg/kg bw. *Garcinia dulcis* fruit methanol extract treatment doses of 3.6 mg/kg bw and simvastatin lowers triglyceride levels by 7.22 mg/dL and 24.58 mg/dL, significantly different compared with the treatment dose of 1.8 mg/kg bw and controls. *Garcinia dulcis* fruit extract dose of 3.6 mg/dL lower low density lipoprotein-cholesterol highest of 16.45 mg/dL. *Garcinia dulcis* fruit methanol extract dose of 3.6 mg/kg bw was also able to increase the levels of high density lipoprotein-cholesterol by 5.62 mg/dL. The results showed that the fruit of *Garcinia dulcis* contain compounds that have potential as agents antihyperlipidemia and presumably through inhibition of the enzyme HMG-CoA reductase.

**Key words:** cholesterol, *Garcinia dulcis*, triglyceride

### INTRODUCTION

Mangosteen is a plant that grow and spread in the lowland tropical forests of Southeast Asia, starting from the region of Thailand, Peninsular Malaysia and Indonesia and is known as the queen of fruits. In Indonesia, genus *Garcinia* are common in Sumatra, Java, Kalimantan and Maluku. The genus *Garcinia* (Familia: Guttiferae) is a tropical plant and has approximately 350 species, in-cluding *Garcinia dulcis*, *Garcinia bancana*, *Garcinia bor-neensis*, *Garcinia parvifolia*, *Garcinia rostrata*, and *Garcinia* spp (Swecency, 2008). Besides eaten, this plant has been widely used by people for a variety of traditional treatments, such as limphatis, parotid, anti-inflammatory, anti-tumor and anti-bacterial activity on *Staphylococcus aureus*, *Shigella dysenteriae*, *Shigella flexneri*, *Escherichia coli*, *Vibrio cholerae* (Deachathai *et al.*, 2005).

The content of chemical compounds that have been found to vary widely in the genus *Garcinia* are flavonoids, xanton, triterpenoids, and quinone (Deachathai *et al.*, 2005). Flavonoid compounds that have been found in the genus *Garcinia* mostly biflavonoid types. *Garcinia scorte-chinii* fruit contains compounds biflavonoid (Sukpondma *et al.*, 2005) and kolafllavanon (Adaramoye & Muritala, 2013).

Research has been done on the genus *Garcinia* shows there biflavonoid other compounds, such as amentoflavon, agathisflavon, robustaflavon, hinokiflavon, volkensiflavon, rhusflavanon, succedaneiflavon of *Garcinia multiflora* which shows antidiabetic and antiviral activity and hepatoprotective (Lin *et al.*, 1997; Iwu *et al.*, 1990). Five compounds xanton i.e dihidroksixanton, dihidroxydes-D-garcigerrin, metilsimpoxanton, simpoxanton and

garciniaxanton apparently showed antimalarial activity (Likhwiwiyawuid *et al.*, 1998). Benzophenone derivative compounds, namely garcinol, isogarcinol, xantosimol, isoxantosimol and sikloxantosimol showed antibacterial activity against *Staphylococcus aureus* (Inuma *et al.*, 1996).

Several compounds of *Garcinia xanthon* showed antioxidant activity, namely dihydroxy furan and 1,4,5-trihidroksixanton (Minami *et al.*, 1995). The compound 3-hydroxy- $\beta$ -20,29,30-trinorlupan-9-on garciniellipton and Garsubellin A isolated from *Garcinia subelliptica* showed cytotoxic activity, antibiotics, and increase the activity of choline asetiltransferase (Lin *et al.*, 2012; Zhang *et al.* 2010; Asano *et al.*, 1996; Fukuyama *et al.*, 1997; Kosela *et al.*, 2000).

Until now, research on the activity of the anti-hyperlipidemia methanol extract of the fruit *Garcinia dulcis* from South Sumatra on lipoprotein profile and triglycerides are still rarely found. Antihyperlipidemia activity can be known through the inhibition of HMG-CoA reductase compounds. HMG-CoA reductase is an enzyme that catalyzes the conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonic acid, one of the important stages in the pathway of cholesterol synthesis. Inhibition of the enzyme leads to increased density of LDL receptors in the liver cells resulting in decreased levels of cholesterol, LDL-cholesterol and triglycerides (Delgado & Remers, 1991).

Based on research conducted by Qudsiyah & Ward (2007), the methanol extract of *Garcinia dulcis* stem can reduce total cholesterol by 65% rats at levels of 3.6 mg/kg/ day. According Olaiya & Omolekan (2013), ethanol ex-tract contained in *Buchholzia coriacea* at dose

of 250 mg/ kg bw has anti-hyperlipidemia activity via decreased levels of total cholesterol, LDL-cholesterol and triglycerides hyperlipidemic mice at 45.9%, 73.5%, and 48.4 %. *Vernonia amygdalina* methanol extract dose of 200 mg/kg bw able to reduce levels of cholesterol, LDL-cholesterol and triglycerides hyperlipidemic mice by 29%, 49% and 28% (Ada-ramoye *et al.*, 2008). *Brucea javanica* seed methanol extract dose of 2.7 mg/kg bw could reduce levels of LDL-cholesterol and triglycerides were hyperlipidemic white mice by 43.8% and 32.2% (Wulandari *et al.*, 2011).

Therefore, this study aims to determine the activity of fruit extracts Mundu (*G. dulcis*) at different doses on levels of total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol white rat (*Rattus norvegicus* L.).

## MATERIAL AND METHOD

### Extraction of Mundu (*Garcinia dulcis*)

Mundu (*G. dulcis*) fruit derived from Banyuasin Regency, South Sumatera Province, and has been determined by Herbarium Bogoriense LIPI, Bogor. Extraction is done by maceration using methanol pa. 250 gram samples that have been dried fruits Mundu, incorporated into maserator then add 500 mL methanol pa. Maceration is done for 3 x 24 hours. The extract obtained was concentrated by using a rotary evaporator for 3 hours.

### Test Anti-hyperlipidemia

Test animals used were male Wistar rats (*Rattus norvegicus*), 2 months old with body weight of 150-250 g were obtained from preclinical Laboratory and Research Institute for Integrated Testing (LPPT) UGM. Thirty male white rats of Wistar male (*R. norvegicus*) are healthy and have normal activities were randomly divided into 5 groups with 6 replicates. The control group is the group fed a standard diet and feed high cholesterol and fat for 4 weeks; Statin group is a group fed a standard diet and feed high in fat and cholesterol and simvastatin at a dose of 3.6 mg/kg bw; Group M1, M2 and M3 are the group fed a standard diet and feed high in fat and cholesterol and methanol extract at a dose of 1.8, respectively; 2.7 and 3.6 mg/kg bw/day.

Before treatment, white male rats adapted for 1 week in the animal cages at the temperature and humidity of 50-60% 26-27°C. During the adaptation period, male rats were fed a standard 15 gr/150 gr BB mature rats and drinking water ad libitum (Gad and Chengelis, 1992). According Anonim (1993), the composition of foods to increase levels of lipid and cholesterol of the rats per day are: egg yolks 5%, 10% beef tallow, palm oil and food standard 1% to 100%.

Determination of the dose refers to Dubey *et al.* (2005), the standard drug dose conversion for human consumption simvastatin dose to rats, at 0.018. Methanol extract dose used was 1.8; 2.7; 3.6 mg/kg rat. On days 1 and 30 rat blood drawn through orbital sinus. Before blood sampling rats were fasted for 16 hours with the aim of completing the process of lipid metabolism that comes from food (Felig & Frohman, 2001). Blood is drawn and then centrifuged at 3000 rpm for 15 minutes until the serum and blood cells separate. Blood serum obtained was used to determine total cholesterol, triglycerides, HDL-

cholesterol and LDL-cholesterol using analysis kit (Azima *et al.*, 2004).

### Research Parameter

Analysis of cholesterol, HDL and triglycerides performed enzymatically using the cholesterol kit, kit triglycerides, HDL precipitant kit. Absorbance measurements performed using a spectrophotometer at a 500 nm wavelength.

Total cholesterol levels were measured enzymatically using CHOD-PAP method (cholesterol oxidase-p-amino-phenozone). A total of 5 mL and 5 mL of blood serum standard, each 500 mL mixed reagent. Then, serum, standards and blanks were incubated for 20 minutes at a 25-27°C temperature and the absorbance read at a 500 nm wavelength by using a spectrophotometer. Total cholesterol level was calculated using the formula:

$$\text{Total Cholesterol} \left( \frac{\text{mg}}{\text{dL}} \right) = \frac{\Delta A_{\text{sampel}}}{\Delta A_{\text{Standar}}} \times \text{Standard concentration} \left( \frac{\text{mg}}{\text{dL}} \right)$$

Triglyceride levels were measured by the GPO-PAP method (glycerol phosphate oxidase-p-aminophenozone). A total of 5 mL of blood serum and 5 mL of each standard 500mL mixed reagent. Then, serum, standards and blanks were incubated for 20 minutes at a temperature of 25-27°C and the absorbance read at a wavelength of 500 nm. Triglyceride levels calculated by the formula:

$$\text{Triglycerida} \left( \frac{\text{mg}}{\text{dL}} \right) = \frac{\Delta A_{\text{sampel}}}{\Delta A_{\text{Standar}}} \times \text{Standard concentration} \left( \frac{\text{mg}}{\text{dL}} \right)$$

HDL-cholesterol was measured by precipitation chylomicrons, VLDL and LDL plasma by adding a precipitant reagent composed by fosfotungstat acid and magnesium chloride. A total of 100 mL of blood serum plus 250 mL precipitant and incubated for 10 min at room temperature. After that, the mixture was centrifuged at a speed of 4000 rpm for 10 minutes so that chylomicrons, VLDL and LDL forms a precipitate. Clear supernatant was separated and tested levels of HDL-cholesterol CHOD-PAP method as in the measurement of total cholesterol. LDL-cholesterol levels obtained from the Friedewald equation as follows:

$$\text{LDL-cholesterol} \left( \frac{\text{mg}}{\text{dL}} \right) = (\text{Total Cholesterol} - \text{HDL cholesterol}) - \frac{1}{5} \text{triglycerides}$$

Data measurements of total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were analyzed by ANOVA, treatment showed no significant difference followed by LSD test at 5% confidence level.

## RESULT

Total cholesterol and triglyceride white rats at the beginning of treatment and end of treatment is shown in Table 1. Decrease in total cholesterol levels at most Mundu fruit methanol extract treatment doses of 3.6 mg/kg bw of (32.17 mg/dL). This decrease was greater than the addition of simvastatin (24.1 mg/dL). Decreased levels of tri-glycerides treatment doses of 2.7 and 3.6 mg / kg bw same relatively large (6.36 and 7.22 mg/dL), and is lower when compared with addition of simvastatin is able to reduce levels of triglycerides by 24.58 mg/dL.

HDL-cholesterol and LDL-cholesterol mice at the beginning of treatment and end of treatment can be seen in Table 2 HDL-cholesterol is known as good cholesterol. Increased levels of HDL-cholesterol was greatest in the methanol extract of the fruit Mundu treatment dose of 3.6 mg/kg bw, which amounted to 5.62 mg/dL, compared to doses of 1.8 and 2.7 mg/kg bw which only amounted to 1.61 and 1.07 mg/dL. Increased levels of HDL-cholesterol in control is relatively small in the amount of 0.75 mg/dL. Increased levels of HDL-cholesterol following addition of

simvastatin (0.9 mg/dL) lower than the third methanol extract treatment. Neal (2005) suggest that simvastatin is a hypolipidemic drug that can increase levels of HDL-cholesterol by about 5%.

LDL-cholesterol is known as bad cholesterol. Addition of the methanol extract of the fruit Mundu treatment dose of 3.6 mg/kg bw able to reduce levels of LDL-cholesterol by 14.65 mg/dL, greater than the dose of 2.8 mg/kg bw (10.58 mg/dL) and simvastatin (8.33 mg/dL). Increased levels of LDL-cholesterol occurs in the control.

Table 1: Levels of total cholesterol and triglycerides (mg / dL) rats at the beginning of treatment and end of treatment

Treatment (mg / kg bw)	Total cholesterol (mg / dL)		Triglycerides (mg / dL)	
	Beginning treatment	End of treatment	Beginning treatment	End of treatment
Control	91.34±4.46 <sup>a</sup>	93.97±5.21 <sup>a</sup>	65.05±4.22 <sup>a</sup>	73.24±4.27 <sup>b</sup>
3,6 Statin	110.25±4.31 <sup>b</sup>	86.15±11.07 <sup>a</sup>	56.32±5.18 <sup>a</sup>	31.74±1.59 <sup>a</sup>
1,8 extract	99.22±4.67 <sup>a</sup>	112.38±8.99 <sup>b</sup>	58.36±4.28 <sup>a</sup>	68.71±4.76 <sup>b</sup>
2,7 extract	105.77±6.22 <sup>a</sup>	99.76±38.82 <sup>a</sup>	43.19±4.19 <sup>a</sup>	36.83±15.88 <sup>a</sup>
3,6 extract	112.54±5.45 <sup>b</sup>	80.37±29.69 <sup>a</sup>	39.28±4.28 <sup>a</sup>	32.06±16.79 <sup>a</sup>

Note: numbers followed by the same letter within a column indicate no significant difference ( $\alpha = 5\%$ )

Table 2. Levels of HDL-cholesterol and LDL-cholesterol (mg / dL) white rats at the beginning of treatment and end of treatment

Treatment (mg / kg bw)	HDL (mg/dL)		LDL (mg/dL)	
	Beginning treatment	End of treatment	Beginning treatment	End of treatment
Control	25.46±7.35 <sup>a</sup>	26.21±7.21 <sup>a</sup>	87.38±7.29 <sup>a</sup>	89.58±8.66 <sup>a</sup>
3,6 Statin	37.44±4.67 <sup>a</sup>	38.34±6.28 <sup>a</sup>	81.75±6.47 <sup>a</sup>	73.42±6.39 <sup>a</sup>
1,8 extract	38.21±6.53 <sup>a</sup>	39.82±6.24 <sup>a</sup>	78.56±8.28 <sup>a</sup>	81.24±4.67 <sup>a</sup>
2,7 extract	32.86±7.88 <sup>a</sup>	33.93±7.35 <sup>a</sup>	98.21±7.46 <sup>a</sup>	87.63±7.37 <sup>a</sup>
3,6 extract	36.29±5.78 <sup>a</sup>	41.91±7.43 <sup>a</sup>	82.47±6.43 <sup>a</sup>	67.82±6.43 <sup>a</sup>

Note: numbers followed by the same letter within a column indicate no significant difference ( $\alpha = 5\%$ )

## DISCUSSION

Mundu fruit extraction is done by maceration using methanol solvent. The resulting methanol extract of purple yellow and is estimated to contain compounds that are polar. Based on the phytochemical analysis, the methanol extract containing flavonoids, polyphenols, quinones and alkaloids. Deachathai *et al.* (2005), the content of chemical compounds in the genus *Garcinia* i.e flavonoids, xanths, triterpenoids, and quinones.

The composition of chemical compounds contained in extracts of methanol is still very much. The methanol extract of the Mundu fruit contains many bioactive compounds, each compound has a different biological activity. Under these conditions, the bioactive compounds contained in the methanol extract of the fruit Mundu can interact hypolipidemic produce a complex mechanism.

According Deachathai *et al.*, 2005, the Mundu fruit contains flavonoids, xanths and quinones. This compound is expected to have an activity that can lower total cholesterol and triglycerides (Qudsiyah & Ward, 2007). Total cholesterol and triglycerides in the control treatment tended to be increased. This condition may occur due to the intake of carbohydrates that will induce the synthesis of fatty acids by fatty acid synthase enzyme into triglycerides.

Simvastatin (statin derivative) is hypolipidemic drug that inhibits cholesterol synthesis in the liver. Statins have active functional groups in the form of the lactone ring which is a dihydroxy acid as its active form. In the body of the lactone ring functional groups will be hydrolyzed to produce dihydroxy acid form of active compounds that

can inhibit the enzyme competitively with HMG-CoA reductase. According to Kawano *et al.* (2007), in addition to works by blocking the synthesis of endogenous cholesterol, statins can also increase the activity of the enzyme lipoprotein lipase (LPL). LPL enzyme is able to hydrolyze triglycerides into free fatty acids, and causes a decrease in blood triglycerides. The mechanism of reduction in triglyceride levels may be related to the inhibition of lipolysis in the liver and inhibition of endogenous cholesterol biosynthesis. Total cholesterol cannot give an exact picture of the effect of the methanol extract of the fruit Mundu.

Total cholesterol is cholesterol from food (chylomicrons), VLDL, LDL and HDL. Under these conditions, the mechanism of reduction in total cholesterol levels correlated with more specific lipoproteins, such as HDL-cholesterol and LDL-cholesterol.

Conditions are expected because of high cholesterol levels in the liver resulting in liver reduces the synthesis of LDL receptors remove excess cholesterol hepatocytes and hepatocytes in the form of VLDL into the blood stream. Catabolism of VLDL in the blood lead levels into LDL. This process causes the levels of LDL-cholesterol to rise (Felig and Frohman, 2001).

Simvastatin is a hypolipidemic drug that works by blocking an enzyme standard of HMG-CoA reductase. Inhibition of this enzyme causes a decrease in VLDL synthesis and increasing LDL receptors in the liver. Decrease in VLDL synthesis which brings triglycerides indirectly cause a decrease in blood triglyceride levels, whereas the increase in LDL receptors in the liver causes a decrease in blood levels of LDL-cholesterol (Neal, 2005). Another

consequence of the addition of simvastatin is synthesized VLDL cholesterol ester poor heart that this VLDL cholesterol esters from HDL to get. This process can lead to dec-reased levels of HDL-cholesterol following addition of simvastatin.

Methanol extract containing flavonoids, xanton, tri-terpenoids, and quinone groups are estimated to contain lactone ring. In the body, the active lactone groups will be hydrolyzed to form dihydroxy acid, the active compounds that can inhibit the enzyme competitively with HMG-CoA reductase. Barriers occur because dihydroxy acid form similar to the form of HMG-CoA reductase.

The mechanism of the biological activity of compounds of the research work by inhibiting the enzyme HMG-compete with CoA reductase. HMG-CoA reductase is an enzyme that catalyzes the conversion of 3-hydroxy-3-methyl-glutaril CoenzimA to mevalonic acid, one of the important stages in the pathway of cholesterol synthesis. Barriers of this enzyme causes an increase in the density of LDL receptors in the liver cells resulting in dec-reased levels of cholesterol, LDL cholesterol and increase HDL number.

From this study it can be concluded that the methanol extract of Mundu fruit (*Garcinia dulcis*) can reduce total cholesterol, triglycerides and LDL-cholesterol and the methanol extract of Mundu fruit (*Garcinia dulcis*) can increase levels of HDL-cholesterol.

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