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HYPOLIPIDEMIC ACTIVITY OF MUNDU FRUIT (*Garcinia dulcis* (Roxb.) Kurz) IN WHITE RAT (*Rattus norvegicus* L.)

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ABSTRACT - The objectives of this research were to identify compound and hypolipidemic activity of *Garcinia dulcis* fruit on lyphoprotein profile and triglyseride in white rat hyperlipidemic. These studies using *Garcinia dulcis* fruit from Banyuasin, South Sumatra. In this observation, 30 male white 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 upper isolate 1.8; 2.7 and 3.6 mg/kg bow/day dosage. Total cholestrol, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol and triglyseride contains using spectrophotometry method, were observed 1 and 30 days. The result of the hypolipidemic activity showed that phenolic compound treatment doses of 3.6 mg/kg bw and simvastatin lowers lyphoprotein and triglyceride levels, significantly different compared with the treatment dose of 2.7 and 1.8 mg/kg bw. The purification of compound was done by recrystalitation with CH_2Cl_2. The structure was elucidated by spectroscopic methods with UV, IR, 1H-NMR and 13C-NMR. Based on spectral data of LC-ESI-ToF-MS showed that the compound was dulcisxanthone.

Keywords **:** dulcisxanthone, *Garcinia dulcis,* hypolipidemic, lyphoprotein, triglyceride

INTRODUCTION

The Mangosteen has been known to the public since a long ago as a fruit that has a sweet taste and beautiful shape. Besides being able to be consumed as a sweet fruit, rural communities also take advantage of the mangosteen to treat the obesity, hyperlipidemia (Koriem , 2014; Adiputro et al., 2013), lymphatic, parotic and inflammation (Deachathai et al., 2005). Until now, the mangosteen has been known as one type of forest plants that have efficacy as a medicinal plant. The Mangosteen plant can grow and spread in the tropical forests, such as the Philippines, Thailand, Malaysia and Indonesia. The diversity of types of mangosteen (Garcinia Genus, Family Guttiferae) comprises more than 350 species, among others *Garcinia dulcis, Garcinia bancana, Garcinia borneensis, Garcinia parvifolia,* and *Garcinia rostrata* (Sweeney, 2008).

The content of chemical compounds that have been found in the genus of Garcinia among other flavonoid, xanton, triterpenoids, and quinones. The Flavonoid compounds that have been found in the genus Garcinia mostly kind biflavonoid. The *Garcinia scortechinii* fruit contains of biflavonoid and kolaflavanon (Adaramoye & Muritala, 2013; Deachathai et al., 2005), amentoflavon, agathisflavon, robustaflavon, hinokiflavon, volkensiflavon, rhusflavanon, succedaneflavon from *Garcinia multiflora* (Lin et al., 1997; Iwu et al., 1990), dihidroxyxanton, dihidroxy-des-D-garcigerrin, metil simpoxanton, simpoxanton dan garciniaxanton (Likhiwitayawuid *et al*., 1998), garcinol, isogarcinol, xantosimol, isoxantosimol and sikloxantosimol (Linuma *et al*., 1996).

Some of the garciniaxanton compounds showed antioxidant activity that are dihydroxyfuran and 1,4,5-trihidroksixanton of *Garcinia subelliptica* (Minami et al., 1995). The Compounds of 3-β-hydroxy-20,29,30-trinorlupan-9-in garciniellipton and Garsubellin A isolated from *Garcinia subelliptica* showed cytotoxic activity, antibiotics and increasing the activity of choline asetiltranferase (Lin et al., 2012; Zhang et al., 2010; Asano et al., 1996; Fukuyama et al., 1997; Kosela et al., 2000). According to Koriem (2014) the mangosteen fruit has hypolipidemic activity by decreasing levels of total cholesterol and LDL cholesterol. Methanol extract of mangosteen capable of lowering cholesterol levels from hiperlipidemik white rats (Setiawan et al., 2014).

Until now, the mangosteen fruit has been widely used in traditional medicine, including utilized to reduce obesity and hyperlipid (Koriem , 2014; Adiputro *et al*., 2013). Hyperlipidemia is a condition caused by elevated levels of cholesterol and triglycerides in the blood serum. One effort that is often done by people to reduce hyperlipid condition is through a low-fat diet and consumption of drug antihyperlipidemic (Setiawan et al., 2014). Anti-hyperlipidemic drug is a synthetic compound that is used to lower plasma lipid levels, but these drugs typically used for patients who have acute hyperlipidemic.

The Mangosteen fruit is consumed as an estimation of *Garcinia dulcis* hypolipidemic activity shown by the flavonoid and steroid compounds and quinone. These compounds can inhibit the action of the HMG-CoA reductase enzyme because it has an active group of lactone that is analogous with statins in antihyperlipid drugs (Guo et al, 2005). Antihyperlipidemia activity can be known through the inhibition of HMG-CoA reductase compound. HMG-CoA reductase is an enzyme that catalyzes the conversion of 3-hydroxy-3-methyl-glutaril coenzyme A (HMG-CoA) to mevalonic acid, one important step in the cholesterol synthesis pathway.

The previous research showed that the methanol extract of the *Garcinia dulcis* fruit showed antihyperlidemic activity. Therefore, further research used fractionation methanol extract by vaccum liquid chromatography method (VLC) using several solvents. Until now, the research on the effect of the fractions obtained from the fractionation of the methanol extract of the *Garcinia dulcis* fruits to the lipoprotein and triglyceride profiles has never been found. Atherogenic index analysis also determined to assess the risk of atherosclerosis. Atherogenic index can be determined based on the levels of cholesterol. The lower the atherogenic index value, the greater the risk of atherosclerosis down. This study aims to determine hypolipidemic activity of methanol extract fractions of mundu fruit (*Garcinia dulcis* (Roxb.) Kurz) in white rat (*Rattus norvegicus* L.) after oral administration.

MATERIALS AND METHODS

Study area

The tools used are vessel macerator, blender (Philips), *rotary evaporator* (Eyela), sintered glass (Iwaki), vacuum pump, mikropipet 5-20 µL (eppendorf) , animal cage test, gastric sonde, scales (Metler), glass tools and spectrophotometer (UV-vis Genesys 6). UV-Vis (Perkin Elmer), FT-IR (*Fourier Trnasform-Infrared*) (Perkin Elmer), NMR (*Nuclear Magnetic Resonance*) 500 MHz (JEOL), HPLC (*High Performance Liquid Chrmatography*) Alliance 2695 (Waters) with PAD (*Photodiode Array Detector)* 2996 (Waters), UPLC-ToF-MS (*Ultra Performance Liquid Chromatography Time of Flight Mass Spectrometry*) Acquity SDS (Waters), Acquity column UPLC BEH C18 1,7µm, 2,1x50mm, *Mass Spectrometry* (MS) (Xevo-G2QTOF (Waters).

The materials used are the Mundu fruit (*G. Dulcis*). Species accuraty of Mundu (*G. Dulcis*) identified by *Herbarium Bogoriense* LIPI, Bogor. Test animal used were male Wistar rats (*R. norvegicus*), 2 months old and weighing 150-250g were obtained from preclinical Laboratory of Integrated Research and Testing Institute (LPPT) UGM. Methanol pa (Merck), reagent kits cholesterol (Rajawali Nusindo), reagent kits triglycerides (DiaSys), HDL precipitant (Randox), foods high in cholesterol and fat diet, a standard diet, distilled water and medicine simvastatin.

Procedures

Sub-procedures-1

Based on the research that has been done by Setiawan et al. (2014) showed that the methanol fraction of mundu fruit is an active fraction which has the most excellent hypolipidemic activity. The next research step is Peparatif Thin Layer Chromatography (KLTP). Orientation eluent made to obtain a suitable eluent for KLTP. KLTP goal is to isolate the bioactive compounds obtaining purer. For this KLTP, KLTP plate prepared by using the tools. The principle of this tool is applying absorbent layer (stationary phase) on a glass plate with a layer thickness that can be set. In the layers of glass measuring 20 cm x 20 cm, added to a solution of silica gel thickness of 0.5 mm. Silica gel solution made by mixing 40 grams of powdered silica gel 60 PF254 in 85 ml of distilled water for manufacturing 6 TLC plates. After the solution was added to the silica gel on the glass plate, the plate allowed to stand for at least overnight. Before use, the plates are first activated by heating in an oven at a temperature of 100 ° C for 1 hour.

Methanol fraction spotted on TLC plates lengthwise (spotted 2 cm distance from the lower limit and 1 cm from the right and left of the plate glass). KLTP mobile phase used comes from the orientation and monitoring performed on the combined fractions. Once the mobile phase reaches the upper limit, the plates are removed from the developer vessel and put in a fan oven at 40 ° C for 15 minutes to dry.

TLC chromatogram profile visualized by using visible light, UV light with λ = 254 nm and 366 nm. Profile that shows the same chromatogram, then grouped into one. Based on the classification obtained three zones (top, middle and bottom) and marked the boundary with a pencil. Tape on the plates that have been marked scraped off with a spatula and placed in erlenmeyer. Each powder scrapings results extracted by using the same solvent with KLTP, then homogenized with a magnetic stirrer for 15 minutes, then filtered with sintered glass. Each will filter out the new solution, the silica gel residue discarded and sintered glass is washed with the same solution. The filtrate is collected in a porcelain dish and dried.

Bioactive compounds contained in isolates results monitoring by TLC. The chromatogram Profile was visualized using visible light, UV light λ = 254 nm and UV λ = 366 nm, sprayed with the cerium (IV) sulfate, is heated in an oven of 100 ° C for 5-10 minutes.

Sub-procedures-2

Thirty male white rats Wistar (*R. norvegicus*) were healthy and had normal activity were randomly divided into 8 treatment groups with 8 replications.

1. The control group is a group fed a standard diet and food high in cholesterol and fat for 4 weeks;
2. The Statin group is a group fed a standard diet and food high in cholesterol and fat and simvastatin at a dose of 3.6 mg/kg bw;
3. The group F2.1 is a group fed a standard diet and food high in cholesterol and fat and fractions F2 with each dose of 1.8 mg/kg bw/day.
4. The group F2.1 is a group fed a standard diet and food high in cholesterol and fat and fractions F1 with each dose of 2.7 mg/kg bw/day.
5. The group F2.1 is a group fed a standard diet and food high in cholesterol and fat and fractions F1 with each dose of 3.6 mg/kg bw/day.

Before the treatment, male rats adapted for 1 week in an animal enclosure at a temperature of 26-27^{o}C and humidity of 50-60%. During the period of adaptation, male rats were fed a standard 15 gr / 150 gr bw mature rats and drinking water ad libitum (Gad and Chengelis, 1992). According Phytomedica (1993), the composition of the diet to improve cholesterol and lipid levels of the white rats per day are: chicken egg yolk 5%, 10% beef tallow, palm oil 1% and the standard of food to 100%.

Determination of the dose refers to Dubey et al. (2005), which is the conversion of a standard dose of the drug for human consumption simvastatin dose to rats, at 0.018 mg/kgbw. Doses of methanol extract used was 1.8; 2.7; 3.6 mg/kg bw in rats. On days 1 and 30 mouse blood taken through orbital sinus. Before blood sampling the white rats were fasted for 16 hours with the aim of completing the process of lipid metabolism are derived from food (Felig and Frohman, 2001). Blood is taken and then centrifuged at 3000 rpm for 15 minutes until the serum and blood cells separate. Blood serum obtained is used to determine total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol analysis using a kit (Azima et al., 2004).

Research parameter

Cholesterol, HDL and triglycerides were analyzed enzymatically using cholesterol kit, triglycerides kit, HDL precipitant kit. Absorbance measurement is done by using a spectrophotometer at a wavelength of 500 nm.

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

$$\text{Total Cholesterol(mg/dL)} = \frac{\Delta A \text{ sampel}}{\Delta A \text{ standard}} \times \text{standard C. (mg/dL)}$$

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

$$\text{Triglyceride (mg/dL)} = \frac{\Delta A \text{ sampel}}{\Delta A \text{ standar}} \times \text{standard C. (mg/dL)}$$

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

LDL-cholesterol (mg/dL) = (Total cholesterol-HDL-cholesterol) -1/5 Triglycerides

Data analysis

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

RESULTS AND DISCUSSIONS

Preparative Thin Layer Chromatography (PTLC) active fraction

Table 1. Total cholesterol levels (mg/dL) dan trygliceride (mg/dL) white rats at the beginning of treatment and end of the treatment isolates F2.1

Treatment (mg/kg bw)	Total cholesterol (mg/dL)		Trygliceride (mg/dL)	
	Early treatment	End treatment	Early treatment	End treatment
Control	89,25±6,11[a]	94,13±4,06[a]	73,12±3,57[a]	75,13±6,19[b]
Simvastatin	109,62±5,24[b]	79,87±6,47[a]	62,34±6,35[a]	42,62±3,21[a]
1,8 isolat	106,15±5,11[a]	110,18±6,25[b]	61,73±5,22[a]	72,12±5,29[b]
2,7 isolat	112,51±6,09[a]	89,34±7,21[a]	51,32±6,28[a]	43,21±8,11[a]
3,6 isolat	113,98±6,12[b]	74,23±8,88[a]	42,26±7,18[a]	38,21±9,21[a]

Description: The numbers followed by the same letter in the column showed no significant difference (α=5%)

Table 2. Levels of HDL-cholesterol and LDL-cholesterol (mg/dL) white rat at the beginning and end of the treatment isolates treatment F2.1

Treatment (mg/kg bb)	HDL (mg/dL)		LDL (mg/dL)	
	Early treatment	End treatment	Early treatment	End treatment
Kontrol	42,14±5,63[a]	34,62±6,42[a]	98,32±5,47[a]	99,25±6,07[a]
Simvastatin	56,12±3,78[a]	59,13±8,46[a]	92,71±8,89[a]	87,05±7,82[a]
1,8 isolat	54,11±8,63[a]	57,67±8,38[a]	94,55±6,78[a]	98,45±6,88[a]
2,7 isolat	63,25±9,65[a]	43,17±6,34[a]	99,86±9,02[a]	88,14±6,47[a]
3,6 isolat	64,11±6,45[a]	58,54±9,12[a]	93,73±7,34[a]	63,02±7,83[a]

Description: The numbers followed by the same letter in the column showed no significant difference (α=5%)

Preparative Thin Layer Chromatography (PTLC) active fraction (F2) Mundu fruit is done by using the eluent chloroform: methanol (10: 1). Based on phytochemical analysis, active fractions containing the class of flavonoids, polyphenols, quinones and alkaloids. This is consistent with research Deachathai et al. (2005), the content of chemical compounds in the genus Garcinia that the flavonoid, xanton, triterpenoids, and quinones.

Based on the results PTLC can be predicted that the composition of chemical compounds contained in the active fraction is still a lot. The active fraction Mundu fruit contains many bioactive compounds, each compound has a different biological activity. Under these conditions, the bioactive compounds contained in the active fraction Mundu fruit can interact to produce a hypolipidemic complex mechanism.

Total cholesterol and triglycerides white rats at the beginning of treatment and end of the treatment can be seen in Table 1. The decrease in total cholesterol levels at most isolates F2.1 fruit Mundu treatment dose of 3.6 mg / kg bw. This decrease is greater than that of simvastatin. Decreased levels of triglycerides dosage of 2.7 and 3.6 mg / kg bw relatively equal, and lower when compared with simvastatin administration that can lower triglyceride levels. Total cholesterol and triglycerides in the control treatment tends to be increased. This condition may occur due to the intake of carbohydrates that will induce the synthesis of fatty acids by the fatty acid synthase enzyme into triglycerides.

Simvastatin is a hypolipidemic drug that inhibits cholesterol synthesis in the liver. Statins have active functional groups in the form of the lactone ring which is dihydroxy acid as its active form. In the body of the lactone ring functional groups will be hydrolyzed to produce the dihydroxy acid form of the active compounds can competitively inhibit the enzyme HMG-CoA reductase.

Levels of HDL-cholesterol and LDL-cholesterol white rats at the beginning of treatment and end of the treatment can be seen in Table 2. HDL-cholesterol is known as good cholesterol. Increased levels of HDL-cholesterol treatment is greatest in methanol extracts of fruit Mundu dosage of 3.6 mg / kg bw, ie, compared doses of 1.8 and 2.7 mg / kg bw. Increased levels of HDL-cholesterol in the control is relatively small (Table 2). 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. F1 isolates giving treatment dose of 3.6 mg / kg bw able to reduce levels of LDL-cholesterol, greater than the dose of 2.8 mg / kg bw and simvastatin. Increased levels of LDL-cholesterol occurs in control. This condition is expected because of high levels of cholesterol in the liver resulting in liver hepatocytes reduce the synthesis of LDL receptors and remove excess cholesterol 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.

Simvastatin is a standard hypolipidemic drugs that inhibit the enzyme HMG-CoA reductase. Inhibition of this enzyme causes a decrease in VLDL synthesis and increase LDL receptors in the liver. A decrease in VLDL synthesis which carry triglycerides indirectly cause a decrease in blood triglyceride levels, while the increase in LDL receptors in the liver lead to decreased blood levels of LDL-cholesterol. Another result of the administration of simvastatin is synthesized poor liver VLDL cholesterol ester thus gaining VLDL cholesterol esters from HDL. This process can lead to decreased levels of HDL-cholesterol after administration of simvastatin.

Isolates purification carried through recrystallization method using a solvent CH_2Cl_2, then put in the freezer. Recrystallization results obtained yellow isolates. Determination and identification of bioactive compounds which is done by using spectroscopic methods. The instrument used is UV spectroscopy, FT-IR, NMR^{13}C and 1H and LC-MS.

UV spectrum (CH_3OH) indicated that λmax (nm) at 370, 315, 263, and 207. Analysis of the IR spectrum (KBr) showed a strong absorption at the wave numbers 3406 CM^{1} and 1642 CM^{1}. ^{1}H-NMR spectra indicate that the hydrogen resonance singlet 1-OH (d 13.45); H-4 (d 6.29); H-5 (d 6.69) and 3-OCH3 (d 3.81). Methoxy group contained in the C-3 (3-OMe). Two groups prenyl d 5.16 (H-20) and 5.24 (H-200), two doublet at d 3.28 (H - 10) and 4.27 (H - 100) and four singlet at d 1.72 (H-50 and H-400), 1.60 (H-40) and 1.82 (H-500). This is consistent with research Deachathai *et al.* (2005) which states that the two prenyl groups attached to C-2 and C-8 of the xanthone ring.

Based on the information spectrum of UV-vis, FT-IR, NMR ^{13}C and ^{1}H, and LC-ESI-ToF-MS, the active compounds including flavonoid which has a ring group xanthones. According to the IUPAC the compound has the name 1, 6, 7-trihydroxy-3-methoxy-2,8-bis (3-methyl-2-butenyl) xanthone (dulcis xanthones) with the structural formula $C_{24}H_{26}O_6$ (Anonymous, 2014). The compounds coined the m/z of 411,70 + (M + H $^{+}$) with a compound structure can be seen in Figure 1.

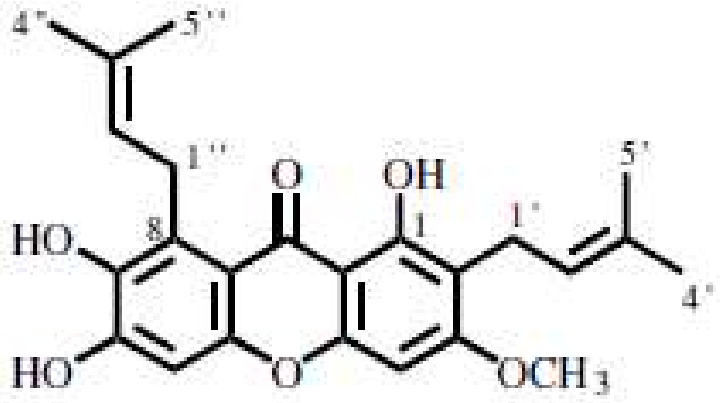

Figure 1. The structure of bioactive compounds 1,6,7- trihydroxy-3-methoxy-2,8-bis (3-methyl-2-butenyl) xanthone (dulcisxanthone)

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ABSTRACT - The objectives of this research were to identify compound and hypolipidemic activity of *Garcinia dulcis* fruit on lyphoprotein profile and triglyseride in white rat hyperlipidemic. These studies using *Garcinia dulcis* fruit from Banyuasin, South Sumatra. In this observation, 30 male white 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 upper isolate 1.8; 2.7 and 3.6 mg/kg bow/day dosage. Total cholestrol, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol and triglyseride contains using spectrophotometry method, were observed 1 and 30 days. The result of the hypolipidemic activity showed that phenolic compound treatment doses of 3.6 mg/kg bw and simvastatin lowers lyphoprotein and triglyceride levels, significantly different compared with the treatment dose of 2.7 and 1.8 mg/kg bw. The purification of compound was done by recrystalitation with CH_2Cl_2. The structure was elucidated by spectroscopic methods with UV, IR, 1H-NMR and 13C-NMR. Based on spectral data of LC-ESI-ToF-MS showed that the compound was dulcisxanthone.

Keywords : dulcisxanthone, *Garcinia dulcis,* hypolipidemic, lyphoprotein, triglyceride

INTRODUCTION

The Mangosteen has been known to the public since a long ago as a fruit that has a sweet taste and beautiful shape. Besides being able to be consumed as a sweet fruit, rural communities also take advantage of the mangosteen to treat the obesity, hyperlipidemia (Koriem , 2014; Adiputro et al., 2013), lymphatic, parotic and inflammation (Deachathai et al., 2005). Until now, the mangosteen has been known as one type of forest plants that have efficacy as a medicinal plant. The Mangosteen plant can grow and spread in the tropical forests, such as the Philippines, Thailand, Malaysia and Indonesia. The diversity of types of mangosteen (Garcinia Genus, Family Guttiferae) comprises more than 350 species, among others *Garcinia dulcis, Garcinia bancana, Garcinia borneensis, Garcinia parvifolia,* and *Garcinia rostrata* (Sweeney, 2008).

The content of chemical compounds that have been found in the genus of Garcinia among other flavonoid, xanton, triterpenoids, and quinones. The Flavonoid compounds that have been found in the genus Garcinia mostly kind biflavonoid. The *Garcinia scortechinii* fruit contains of biflavonoid and kolaflavanon (Adaramoye & Muritala, 2013; Deachathai et al., 2005), amentoflavon, agathisflavon, robustaflavon, hinokiflavon, volkensiflavon, rhusflavanon, succedaneflavon from *Garcinia multiflora* (Lin et al., 1997; Iwu et al., 1990), dihidroxyxanton, dihidroxy-des-D-garcigerrin, metil simpoxanton, simpoxanton dan garciniaxanton (Likhiwitayawuid *et al*., 1998), garcinol, isogarcinol, xantosimol, isoxantosimol and sikloxantosimol (Linuma *et al*., 1996).

Some of the garciniaxanton compounds showed antioxidant activity that are dihydroxyfuran and 1,4,5-trihidroksixanton of *Garcinia subelliptica* (Minami et al., 1995). The Compounds of 3-β-hydroxy-20,29,30-trinorlupan-9-in garciniellipton and Garsubellin A isolated from *Garcinia subelliptica* showed cytotoxic activity, antibiotics and increasing the activity of choline asetiltranferase (Lin et al., 2012; Zhang et al., 2010; Asano et al., 1996; Fukuyama et al., 1997; Kosela et al., 2000). According to Koriem (2014) the mangosteen fruit has hypolipidemic activity by decreasing levels of total cholesterol and LDL cholesterol. Methanol extract of mangosteen capable of lowering cholesterol levels from hiperlipidemik white rats (Setiawan et al., 2014).

Until now, the mangosteen fruit has been widely used in traditional medicine, including utilized to reduce obesity and hyperlipid (Koriem , 2014; Adiputro *et al.*, 2013). Hyperlipidemia is a condition caused by elevated levels of cholesterol and triglycerides in the blood serum. One effort that is often done by people to reduce hyperlipid condition is through a low-fat diet and consumption of drug antihyperlipidemic (Setiawan et al., 2014). Anti-hyperlipidemic drug is a synthetic compound that is used to lower plasma lipid levels, but these drugs typically used for patients who have acute hyperlipidemic.

The Mangosteen fruit is consumed as an estimation of *Garcinia dulcis* hypolipidemic activity shown by the flavonoid and steroid compounds and quinone. These compounds can inhibit the action of the HMG-CoA reductase enzyme because it has an active group of lactone that is analogous with statins in antihyperlipid drugs (Guo et al, 2005). Antihyperlipidemia activity can be known through the inhibition of HMG-CoA reductase compound. HMG-CoA reductase is an enzyme that catalyzes the conversion of 3-hydroxy-3-methyl-glutaril coenzyme A (HMG-CoA) to mevalonic acid, one important step in the cholesterol synthesis pathway.

The previous research showed that the methanol extract of the *Garcinia dulcis* fruit showed antihyperlidemic activity. Therefore, further research used fractionation methanol extract by vaccum liquid chromatography method (VLC) using several solvents. Until now, the research on the effect of the fractions obtained from the fractionation of the methanol extract of the *Garcinia dulcis* fruits to the lipoprotein and triglyceride profiles has never been found. Atherogenic index analysis also determined to assess the risk of atherosclerosis. Atherogenic index can be determined based on the levels of cholesterol. The lower the atherogenic index value, the greater the risk of atherosclerosis down. This study aims to determine hypolipidemic activity of methanol extract fractions of mundu fruit (*Garcinia dulcis* (Roxb.) Kurz) in white rat (*Rattus norvegicus* L.) after oral administration.

MATERIALS AND METHODS

Study area

The tools used are vessel macerator, blender (Philips), *rotary evaporator* (Eyela), sintered glass (Iwaki), vacuum pump, mikropipet 5-20 µL (eppendorf) , animal cage test, gastric sonde, scales (Metler), glass tools and spectrophotometer (UV-vis Genesys 6). UV-Vis (Perkin Elmer), FT-IR (*Fourier Trnasform-Infrared*) (Perkin Elmer), NMR (*Nuclear Magnetic Resonance*) 500 MHz (JEOL), HPLC (*High Performance Liquid Chrmatography*) Alliance 2695 (Waters) with PAD (*Photodiode Array Detector)* 2996 (Waters), UPLC-ToF-MS (*Ultra Performance Liquid Chromatography Time of Flight Mass Spectrometry*) Acquity SDS (Waters), Acquity column UPLC BEH C18 1,7µm, 2,1x50mm, *Mass Spectrometry* (MS) (Xevo-G2QTOF (Waters).

The materials used are the Mundu fruit (*G. Dulcis*). Species accuraty of Mundu (*G. Dulcis*) identified by *Herbarium Bogoriense* LIPI, Bogor. Test animal used were male Wistar rats (*R. norvegicus*), 2 months old and weighing 150-250g were obtained from preclinical Laboratory of Integrated Research and Testing Institute (LPPT) UGM. Methanol pa (Merck), reagent kits cholesterol (Rajawali Nusindo), reagent kits triglycerides (DiaSys), HDL precipitant (Randox), foods high in cholesterol and fat diet, a standard diet, distilled water and medicine simvastatin.

Procedures

Sub-procedures-1

Based on the research that has been done by Setiawan et al. (2014) showed that the methanol fraction of mundu fruit is an active fraction which has the most excellent hypolipidemic activity. The next research step is Peparatif Thin Layer Chromatography (KLTP). Orientation eluent made to obtain a suitable eluent for KLTP. KLTP goal is to isolate the bioactive compounds obtaining purer. For this KLTP, KLTP plate prepared by using the tools. The principle of this tool is applying absorbent layer (stationary phase) on a glass plate with a layer thickness that can be set. In the layers of glass measuring 20 cm x 20 cm, added to a solution of silica gel thickness of 0.5 mm. Silica gel solution made by mixing 40 grams of powdered silica gel 60 PF254 in 85 ml of distilled water for manufacturing 6 TLC plates. After the solution was added to the silica gel on the glass plate, the plate allowed to stand for at least overnight. Before use, the plates are first activated by heating in an oven at a temperature of 100 ° C for 1 hour.

Methanol fraction spotted on TLC plates lengthwise (spotted 2 cm distance from the lower limit and 1 cm from the right and left of the plate glass). KLTP mobile phase used comes from the orientation and monitoring performed on the combined fractions. Once the mobile phase reaches the upper limit, the plates are removed from the developer vessel and put in a fan oven at 40 ° C for 15 minutes to dry.

TLC chromatogram profile visualized by using visible light, UV light with λ = 254 nm and 366 nm. Profile that shows the same chromatogram, then grouped into one. Based on the classification obtained three zones (top, middle and bottom) and marked the boundary with a pencil. Tape on the plates that have been marked scraped off with a spatula and placed in erlenmeyer. Each powder scrapings results extracted by using the same solvent with KLTP, then homogenized with a magnetic stirrer for 15 minutes, then filtered with sintered glass. Each will filter out the new solution, the silica gel residue discarded and sintered glass is washed with the same solution. The filtrate is collected in a porcelain dish and dried.

Bioactive compounds contained in isolates results monitoring by TLC. The chromatogram Profile was visualized using visible light, UV light λ = 254 nm and UV λ = 366 nm, sprayed with the cerium (IV) sulfate, is heated in an oven of 100 ° C for 5-10 minutes.

Sub-procedures-2

Thirty male white rats Wistar (*R. norvegicus*) were healthy and had normal activity were randomly divided into 8 treatment groups with 8 replications.

1. The control group is a group fed a standard diet and food high in cholesterol and fat for 4 weeks;
2. The Statin group is a group fed a standard diet and food high in cholesterol and fat and simvastatin at a dose of 3.6 mg/kg bw;
3. The group F2.1 is a group fed a standard diet and food high in cholesterol and fat and fractions F2 with each dose of 1.8 mg/kg bw/day.
4. The group F2.1 is a group fed a standard diet and food high in cholesterol and fat and fractions F1 with each dose of 2.7 mg/kg bw/day.
5. The group F2.1 is a group fed a standard diet and food high in cholesterol and fat and fractions F1 with each dose of 3.6 mg/kg bw/day.

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

Determination of the dose refers to Dubey et al. (2005), which is the conversion of a standard dose of the drug for human consumption simvastatin dose to rats, at 0.018 mg/kgbw. Doses of methanol extract used was 1.8; 2.7; 3.6 mg/kg bw in rats. On days 1 and 30 mouse blood taken through orbital sinus. Before blood sampling the white rats were fasted for 16 hours with the aim of completing the process of lipid metabolism are derived from food (Felig and Frohman, 2001). Blood is taken and then centrifuged at 3000 rpm for 15 minutes until the serum and blood cells separate. Blood serum obtained is used to determine total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol analysis using a kit (Azima et al., 2004).

Research parameter

Cholesterol, HDL and triglycerides were analyzed enzymatically using cholesterol kit, triglycerides kit, HDL precipitant kit. Absorbance measurement is done by using a spectrophotometer at a wavelength of 500 nm.

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

$$\text{Total Cholesterol(mg/dL)} = \frac{\Delta A \text{ sampel}}{\Delta A \text{ standard}} \times \text{standard C. (mg/dL)}$$

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

$$\text{Triglyceride (mg/dL)} = \frac{\Delta A \text{ sampel}}{\Delta A \text{ standar}} \times \text{standard C. (mg/dL)}$$

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

LDL-cholesterol (mg/dL) = (Total cholesterol-HDL-cholesterol) -1/5 Triglycerides

Data analysis

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

RESULTS AND DISCUSSIONS

Preparative Thin Layer Chromatography (PTLC) active fraction

Table 1. Total cholesterol levels (mg/dL) dan trygliceride (mg/dL) white rats at the beginning of treatment and end of the treatment isolates F2.1

Treatment (mg/kg bw)	Total cholesterol (mg/dL)		Trygliceride (mg/dL)	
	Early treatment	End treatment	Early treatment	End treatment
Control	89,25±6,11[a]	94,13±4,06[a]	73,12±3,57[a]	75,13±6,19[b]
Simvastatin	109,62±5,24[b]	79,87±6,47[a]	62,34±6,35[a]	42,62±3,21[a]
1,8 isolat	106,15±5,11[a]	110,18±6,25[b]	61,73±5,22[a]	72,12±5,29[b]
2,7 isolat	112,51±6,09[a]	89,34±7,21[a]	51,32±6,28[a]	43,21±8,11[a]
3,6 isolat	113,98±6,12[b]	74,23±8,88[a]	42,26±7,18[a]	38,21±9,21[a]

Description: The numbers followed by the same letter in the column showed no significant difference (α=5%)

Table 2. Levels of HDL-cholesterol and LDL-cholesterol (mg/dL) white rat at the beginning and end of the treatment isolates treatment F2.1

Treatment (mg/kg bb)	HDL (mg/dL)		LDL (mg/dL)	
	Early treatment	End treatment	Early treatment	End treatment
Kontrol	42,14±5,63[a]	34,52±6,42[a]	98,32±5,47[a]	99,25±6,07[a]
Simvastatin	56,12±3,78[a]	59,13±8,46[a]	92,71±8,89[a]	87,05±7,82[a]
1,8 isolat	54,11±8,63[a]	57,67±8,38[a]	94,55±6,78[a]	98,45±6,88[a]
2,7 isolat	63,25±9,65[a]	43,17±6,34[a]	99,86±9,02[a]	88,14±6,47[a]
3,6 isolat	64,11±6,45[a]	58,54±9,12[a]	93,73±7,34[a]	63,02±7,83[a]

Description: The numbers followed by the same letter in the column showed no significant difference (α=5%)

Preparative Thin Layer Chromatography (PTLC) active fraction (F2) Mundu fruit is done by using the eluent chloroform: methanol (10: 1). Based on phytochemical analysis, active fractions containing the class of flavonoids, polyphenols, quinones and alkaloids. This is consistent with research Deachathai et al. (2005), the content of chemical compounds in the genus Garcinia that the flavonoid, xanton, triterpenoids, and quinones.

Based on the results PTLC can be predicted that the composition of chemical compounds contained in the active fraction is still a lot. The active fraction Mundu fruit contains many bioactive compounds, each compound has a different biological activity. Under these conditions, the bioactive compounds contained in the active fraction Mundu fruit can interact to produce a hypolipidemic complex mechanism.

Total cholesterol and triglycerides white rats at the beginning of treatment and end of the treatment can be seen in Table 1. The decrease in total cholesterol levels at most isolates F2.1 fruit Mundu treatment dose of 3.6 mg / kg bw. This decrease is greater than that of simvastatin. Decreased levels of triglycerides dosage of 2.7 and 3.6 mg / kg bw relatively equal, and lower when compared with simvastatin administration that can lower triglyceride levels. Total cholesterol and triglycerides in the control treatment tends to be increased. This condition may occur due to the intake of carbohydrates that will induce the synthesis of fatty acids by the fatty acid synthase enzyme into triglycerides.

Simvastatin is a hypolipidemic drug that inhibits cholesterol synthesis in the liver. Statins have active functional groups in the form of the lactone ring which is dihydroxy acid as its active form. In the body of the lactone ring functional groups will be hydrolyzed to produce the dihydroxy acid form of the active compounds can competitively inhibit the enzyme HMG-CoA reductase.

Levels of HDL-cholesterol and LDL-cholesterol white rats at the beginning of treatment and end of the treatment can be seen in Table 2. HDL-cholesterol is known as good cholesterol. Increased levels of HDL-cholesterol treatment is greatest in methanol extracts of fruit Mundu dosage of 3.6 mg / kg bw, ie, compared doses of 1.8 and 2.7 mg / kg bw. Increased levels of HDL-cholesterol in the control is relatively small (Table 2). 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. F1 isolates giving treatment dose of 3.6 mg / kg bw able to reduce levels of LDL-cholesterol, greater than the dose of 2.8 mg / kg bw and simvastatin. Increased levels of LDL-cholesterol occurs in control. This condition is expected because of high levels of cholesterol in the liver resulting in liver hepatocytes reduce the synthesis of LDL receptors and remove excess cholesterol 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.

Simvastatin is a standard hypolipidemic drugs that inhibit the enzyme HMG-CoA reductase. Inhibition of this enzyme causes a decrease in VLDL synthesis and increase LDL receptors in the liver. A decrease in VLDL synthesis which carry triglycerides indirectly cause a decrease in blood triglyceride levels, while the increase in LDL receptors in the liver lead to decreased blood levels of LDL-cholesterol. Another result of the administration of simvastatin is synthesized poor liver VLDL cholesterol ester thus gaining VLDL cholesterol esters from HDL. This process can lead to decreased levels of HDL-cholesterol after administration of simvastatin.

Isolates purification carried through recrystallization method using a solvent CH_2Cl_2, then put in the freezer. Recrystallization results obtained yellow isolates. Determination and identification of bioactive compounds which is done by using spectroscopic methods. The instrument used is UV spectroscopy, FT-IR, NMR^{13}C and 1H and LC-MS.

UV spectrum (CH_3OH) indicated that λmax (nm) at 370, 315, 263, and 207. Analysis of the IR spectrum (KBr) showed a strong absorption at the wave numbers 3406 CM^1 and 1642 CM^1. ^{1}H-NMR spectra indicate that the hydrogen resonance singlet 1-OH (d 13.45); H-4 (d 6.29); H-5 (d 6.69) and 3-OCH3 (d 3.81). Methoxy group contained in the C-3 (3-OMe). Two groups prenyl d 5.16 (H-20) and 5.24 (H-200), two doublet at d 3.28 (H - 10) and 4.27 (H - 100) and four singlet at d 1.72 (H-50 and H-400), 1.60 (H-40) and 1.82 (H-500). This is consistent with research Deachathai *et al.* (2005) which states that the two prenyl groups attached to C-2 and C-8 of the xanthone ring.

Based on the information spectrum of UV-vis, FT-IR, NMR ^{13}C and ^{1}H, and LC-ESI-ToF-MS, the active compounds including flavonoid which has a ring group xanthones. According to the IUPAC the compound has the name 1, 6, 7-trihydroxy-3-methoxy-2,8-bis (3-methyl-2-butenyl) xanthone (dulcis xanthones) with the structural formula $C_{24}H_{26}O_6$ (Anonymous, 2014). The compounds coined the m/z of 411,70 + (M + H $^+$) with a compound structure can be seen in Figure 1.

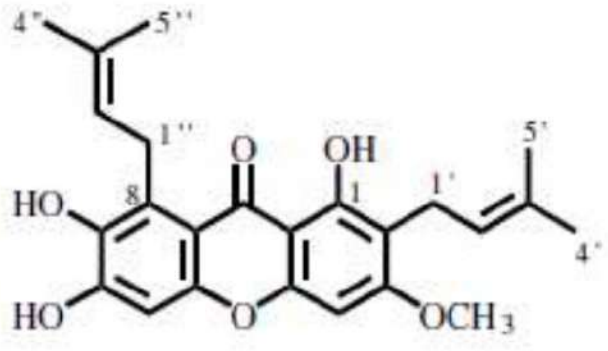

Figure 1. The structure of bioactive compounds 1,6,7- trihydroxy-3-methoxy-2,8-bis (3-methyl-2-butenyl) xanthone (dulcisxanthone)

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HYPOLIPIDEMIC ACTIVITY OF MUNDU FRUIT (Garcinia dulcis (Roxb.) Kurz) IN WHITE RAT (Rattus norvegicus L.)

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Kecukupan dan Kemutahiran data/Informasi dan metodologi (30 %)	6						5
Kelengkapan unsur dan Kualitas penerbit / prosiding (30 %)	6						5
Total = (100 %)	20						15
Kontribusi Pengusul (Penulis Pertama/Anggota Utama)	Penulis Utama=(0,6x15)= **9**						

KOMENTAR/ULASAN PEER REVIEW	
Kelengkapan dan Kesesuaian Unsur	Paper terkait aktivitas hipolidemik buah mundu pada tikus putih. Isi paper sudah memenuhi kaidah-kaidah karya ilmiah namun kurang sesuai dengan bidang biologi konservasi
Ruang Lingkup dan Kedalaman Pembahasan	Hasil penelitian dibahas cukup komprehensif dengan penyampaian pembanding dari temuan-temuan penelitian lainnya dan teori terkait. Referensi yang diacu dalam pembahasan sudah cukup update untuk bidang kajian ini.
Kecukupan & Kemutakhiran Data & Metodologi	Data-data hasil penelitian sudah baik dan didukung peta lokasi sampling, tabel dan gambar yang ditampilkan menarik. Data didapatkan dengan menggunakan metode yang sudah standard.
Kelengkapan Unsur & Kualitas Penerbit	Penerbit Jurusan Biologi FMIPA UNS berkualitas baik, tidak termasuk predatory publisher, dan prosiding tidak terindeks di scopus

Surabaya, 15 Mei 2020
Penilai 1

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2.8.

LEMBAR
HASIL PENILAIAN SEJAWAT SEBIDANG (*PEER REVIEW*)
KARYA ILMIAH: PROSIDING

Judul Karya Ilmiah : HYPOLIPIDEMIC ACTIVITY OF MUNDU FRUIT (Garcinia dulcis (Roxb.) Kurz) IN WHITE RAT (*Rattus norvegicus* L.)

Jumlah Penulis :, **Arum Setiawan**, Laila Hanum, Elvi Rusmiyanto PW

Identitas Prosiding :
a. Nama Prosiding : International Conference on Biodiversity for Sustainable Industries
b. ISBN/ISSN : 978-602-61830-0-2
c. Nomor/Volume/Hal : 1/1/88-93
d. Penerbit : Jurusan Biologi FMIPA UNS
e. Jumlah Halaman : 6

Kategori Publikasi Jurnal Ilmiah (Beri √ pada kategori yang tepat) :
☑ Prosiding Forum Ilmiah Internasional
☐ Prosiding Forum Ilmiah Nasional
☐ Makalah tidak disajikan dalam seminar/symposium.lokakarya, tetapi dimuat dalam prosiding internasional
☐ Makalah tidak disajikan dalam seminar/symposium.lokakarya, tetapi dimuat dalam prosiding nasional
☐ Makalah disajikan dalam seminar internasional (Tetapi tidak dimuat dalam prosiding)
☐ Makalah disajikan dalam seminar nasional (Tetapi tidak dimuat dalam prosiding)

I. Hasil Penilaian Validasi:

No.	ASPEK	URAIAN/KOMENTAR PENILAIAN
1	Indikasi Plagiasi	8 %
2	Linieritas	

II. Hasil Penilaian *Peer Review*:

Komponen Yang Dinilai	Nilai Maksimal Jurnal Ilmiah (Isikan di kolom yang sesuai)						Nilai Akhir Yang DIperoleh
	Prosiding Forum Ilmiah Internasional (Maks. 30)	Prosiding Forum Ilmiah Nasional (Maks. 10)	Makalah TIdak diseminarkan tetapi dimuat dalam prosiding internasional (Maks. 10)	Makalah TIdak diseminarkan tetapi dimuat dalam prosiding nasional (Maks. 5)	Makalah disajikan dalam seminar internasional (Tetapi tidak dimuat dalam prosiding) (Maks. 5)	Makalah disajikan dalam seminar nasional (Tetapi tidak dimuat dalam prosiding) (Maks. 3)	
Kelengkapan dan Kesesuaian unsur isi paper (10 %)	2						2
Ruang Lingkup dan kedalaman pembahasan (30 %)	6						6
Kecukupan dan Kemutahiran data/Informasi dan metodologi (30 %)	6						6
Kelengkapan unsur dan Kualitas penerbit / prosiding (30 %)	6						6
Total = (100 %)	20						20
Kontribusi Pengusul (Penulis Pertama/Anggota Utama)	International Conference on Biodiversity for Sustainable Industries ISBN: 978-602-61830-0-2 Vol. 1(1):88-93 Penulis pertama dari tiga penulia. Nilai 100%. Nilai Pengusul: 0,6 x 20 : 12						

KOMENTAR/ULASAN PEER REVIEW	
- Kelengkapan dan Kesesuaian Unsur	Format lengkap dari Abstrak hingga referensi.
- Ruang Lingkup dan Kedalaman Pembahasan	Ruang lingkup masih didalam bidang ilmu terkait.
- Kecukupan & Kemutakhiran Data & Metodologi	Data sudah cukup banyak dan metode tidak ada yang baru.
- Kelengkapan Unsur & Kualitas Penerbit	Penerbit Jurusan Biologi FMIPA UNS berkualitas dan lengkap.

Yogyakarta, 6 Juli 2020
Penilai 2

tanda tangan .....
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