International Journal of Chemistry Studies
www.chemistryjournal.in
ISSN: 2581-348X
Received: 14-07-2022, Accepted: 01-08-2022, Published: 16-08-2022
Volume 6, Issue 2, 2022, Page No. 19-24

Test activity as hypolipidemia from the extract of kecombrang (*Etlingera Elatior* Jack) *flower* in obesity wistar rats

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Abstract
Kecombrang (*Etlingera elatior* Jack) is a plant with the potential for hypolipidemia. Kecombrang is reported to contain secondary metabolites in the form of tannins, saponins, alkaloids, steroids, terpenoids, phenolics, and flavonoids. This study aimed to determine the hypolipidemic activity of kecombrang flower methanol extract in obese Wistar rats fed a high-fat diet. The test animals used in this study were 18 female white rats aged two months with a weight of ± 150 grams were divided into three groups, namely normal control (only given standard feed), high-fat control (obese rats/with a high-fat diet), and flower treatment group (obese rats/with diet). high fat + flower methanol extract 100 mg/kg BW) orally for 30 days. The results were that kecombrang flowers could reduce total cholesterol levels by 55.5%; Triglycerides (TG) by 57.4%; LDL by 76.73%; and HDL by 42.96%. So we can conclude that kecombrang flowers have the potential for hypolipidemia which is characterized by decreased levels of total cholesterol, TG, and LDL and can increase cholesterol levels. HDL rat obesity (hyperlipidemia) significantly ($p<0.05$).

Keywords: hypolipidemia, kecombrang, *etlingera elatior* jack, obesity

Introduction
Riskesdas (2018) reviewed the number of obese sufferers aged > 18 years as much as 21.8%. Sufferers _ with the highest obesity were found in Riau Province 26.2%; West Papua, 26.4%; East Kalimantan, 28.7%; DKI Jakarta, 29.8%; North Sulawesi, 30.2% and other provinces. This data increased by 10.5% from 2007 to 11.5% in 2013 and grew to 21.8% in 2018. Obesity or being overweight is caused by abnormal fat accumulation that can interfere with body health (WHO, 2014). Another study explained that the term refers to excess body fat and is generally associated with weight gain that is not proportional to height (HSCl, 2014). Obesity can be one of the causes of degenerative diseases such as hypertension, coronary heart disease, diabetes mellitus, insulin resistance and arthritis. (Setiadi, 2018). Hyperlipidemia, dyslipidemia, and atherosclerosis are lipid profile disorders.

A lipid profile describes the types of lipids in the blood. The lipid profile includes total cholesterol, TG, *Low-Density Lipoprotein* (LDL), and *High-Density Lipoprotein* (HDL). In humans, normal total cholesterol values are < 200 mg/dL, triglycerides 10-190 mg/dL, LDL < 130 mg/dL and HDL > 40 mg/dL (Tortora & Bryan, 2011). LDL (bad cholesterol) is one of the main causes of atheroma formation. In contrast, HDL (good cholesterol) prevents the occurrence of atheroma or narrowing of blood vessels due to fat accumulation. In obese patients, cholesterol and triglyceride levels in the blood are high due to the accumulation of excess fat. High levels of cholesterol and triglycerides are a sign of people with hyperlipidemia.

The use of synthetic drugs can overcome the treatment of hyperlipidemia. Still, synthetic drugs can cause severe side effects such as diarrhea, fecal incontinence (the condition of the body being unable to control bowel movements or stools coming out suddenly), bloating and dyspepsia. Bogoriani *et al*. (2020) [4] reported that the leaves and rhizome of Andong (Cordyline terminalis), known as antioxidants, are effective in lowering total cholesterol, TG, LDL and can increase HDL levels. Furthermore, Bogoriani's (2022) research shows that administration of methanol extract of kecombrang flowers at a dose of 100 mg/kg BW can reduce fatty liver. Therefore, a study was conducted on the hypolipidemic activity test of 100 mg/kg BW kecombrang flowers in obese Wistar rats fed a high-fat diet.

Materials and Methods
The study was conducted for three months, where two months was the stage of high-fat feeding until the rats reached an obese condition which was indicated by the Lee index> 0.3. After one month, the obese rats were given treatment by being given kecombrang flower extract 100 mg/kg BW. Measurement of the lipid profile of rats was carried out by post-test at the Mantra Medika Laboratory.

Ingredient
Kecombrang flower, technical methanol, concentrated HCl, Mg powder, 10% NaOH reagent, Wagner reagent, Lieberman-Burchard reagent, 1% FeCl3, 10% NaCl, triglyceride reagent, cholesterol standard, cholesterol reagent, HDL precipitant kit reagent, Wistar rat female white, standard feed, high-fat feed.

Equipment
Blender, jar, laboratory equipment, serum tube, test tube (centrifugation tube), centrifuge, *rotary vacuum evaporator*, analytical balance, gauze, aluminum foil, water bath, sonde instrument, cage, rat drinking container, LCMS /MS, UV-Vis Spectrophotometer.

Procedure
Plant Setup
The collected Kecombrang flowers from the Sukawati area, Gianyar, Bali, were determined by the Head of the Plant Conservation Center for the Botanical Gardens 'Eka Karya' Bali-LIPI. The flowers obtained are then washed, cut, and mashed with a blender. The sieved Kecombrang flower powder to a fineness of 100 mesh. Furthermore, the water content of kecombrang is determined by the evaporation method. Then the powder material was extracted by maceration using methanol as a solvent.

Kecombrang Flower Extract
1 kg of kecombrang flower powder was put into a 4.5-litre beaker and then extracted with 3 litres of methanol solvent for 24 hours. They repeated the operation four times. The filtrate is collected and evaporated with an *evaporator*. This thick extract of the kecombrang flower was used for phytochemical and in *vivo* tests.

Phytochemical Test
Flavonoid Examination
1 mL of extract plus 2-3 drops of concentrated HCl and Mg powder. Positive if there is a colour change to yellow or orange.

Alkaloids Check
1 mL of extract plus 2-3 drops of Wagner's reagent. The reaction is positive if a brown precipitate is formed.

Phenolic Examination
They added 1 mL of the extract with 1% FeCl3 until the colour changed; if compared the colour to pure extract, it would look black if it were positive.

Steroid/Terpenoid Test
1 mL of extract plus Lieberman-Burchard reagent. Terpenoid positive when red or violet. And positive for steroids when a green or blue colour is formed.

Tannin Check
Boiled 1 mL of the extract with 20 mL of water, then filtered. Add 2-3 drops of 1% FeCl3. Tannin positive if the colour is greenish brown or blue-black.

Saponin check
Boiled 1 mL of the extract with 20 mL of water in a water bath. The filtrate was shaken and allowed to stand for 15 minutes. The formation of stable foam when HCl is added indicates a positive saponin.

High Fat Feed Manufacturing
Making high-fat diet feed (HFD) is done by mixing 60% standard ration/CP 550, 20% lard and 20% duck egg yolk. The composition mixture is ground and then dried in the sun. Feed is given in the form of pellets and passed for 60 days.

Experimental Animal Treatment
Eighteen female Wistar rats aged two months with a body weight of ±150 grams were divided into three groups:
- The regular control group (6 normal rats with standard feed).
- The high-fat control (6 obese rats / who had been fed a high-fat diet).
- The high-fat control group.

Treatment (6 obese rats and given kecombrang flower extract at a dose of 100 mg/kg BW/day. Obese rats were housed individually at room temperature. Rats were treated for 30 days and were free to drink (ad libitum). After 30 days of treatment, The rats fasted for 12 hours. At the end of the study, the rats were anesthetized with chloroform. In addition, blood was collected from the orbital sinus of the eye. Blood was contained in a tube and centrifuged at 3 000 rpm for 15 minutes at four °C to obtain serum. The blood serum obtained was then analyzed for levels. total cholesterol, LDL cholesterol, HDL cholesterol, and TG.

Rat Blood Serum Lipid Profile Analysis

Total Cholesterol Analysis

Prepared three test tubes for standards, blanks and samples. Put a total of 1000 µL cholesterol reagent into each test tube. Added test tube 1 (Standard) as much as 10 µL of cholesterol standard. Added test tube 2 (blank) with as much as 10 µL of distilled water. Added test tube 3 (sample) as much as 10 µL of a blood sample. Incubated the mixture at 37ºC for 10 minutes. The concentration was measured with a UV-Vis spectrophotometer at a wavelength of 546 nm.

Triglyceride Analysis

Prepared three test tubes for standards, blanks and samples. Put a total of 1000 µL of triglyceride reagent into each test tube. Added test tube 1 (average) as much as 10 µL of cholesterol standard. Added test tube 2 (blank) with as much as 10 µL *of* distilled water. Added test tube 3 (sample) as much as 10 µL of a blood sample. Incubated the mixture at 37˚C for 10 minutes. A UV-Vis spectrophotometer measured triglyceride concentration at a wavelength of 546 nm.

Analysis of HDL Cholesterol and LDL Cholesterol

Put a total of 500 µL of HDL precipitating reagent into a test tube, and 200 µL of serum was allowed to stand for 10 minutes. And centrifuged at 4000 rpm to make supernatant I. Next, put a total of 500 µL of HDL n precipitate reagent into a test tube, 200 µL of standard solution, allowed to stand for 10 minutes and centrifuged at 4000 rpm to make supernatant II. Next, prepare 3 test tubes for the standard and sample, each filled with 1000 µL of cholesterol reagent. Finally, added 100 µL of supernatant to the sample tube, 100 µL of supernatant II to the standard box and 100 µL of distilled water to the blank for the package. All three tubes were incubated for 10 minutes, and the concentration was measured by UV-Vis spectrophotometer at a wavelength of 500 nm. LDL cholesterol levels were calculated by the formula designed by Fridewald *et al*. if triglycerides were < 400 mg/dL as follows:

LDL = Total Cholesterol – (HDL Cholesterol + 1/5 Triglycerides)

Statistical Calculation

Data were analyzed by SPSS 25. First, the normality test with *Shapiro-Wilk* and then the homogeneity test. The data is usually distributed and homogeneous if the significance value is > 0.05. Finally, an analysis of differences in lipid profile data was carried out using the LSD test on One Way ANOVA. Again, the significance value > 0.05 indicates a significant difference in the data between groups.

Results And Discussion

Kecombrang Flower Methanol Extract with Mererasi Method

This maceration process was repeated four times so that it could extract the compounds contained in the kecombrang flower perfectly. The maceration results from 1 kg of kecombrang flower powder with methanol solvent obtained 116.24 grams of kecombrang flower methanol thick extract with a yield of 11.62%.

A phytochemical test of kecombrang flower extract was carried out with specific reagents. Phytochemical test results are listed in Table 1.

Table 1: Results of Phytochemical Test of Kecombrang Flower Methanol Extract.

Phytochemicals	Results	Test Reagent	Information
Polyphenol	+	$FeCl_3$	H blackish green
Flavonoids	+	Mg & HCl	Orange/red
Tannins	+	$FeCl_3$	Blue-black
Saponins	+	Foam test	Stable foam, does not disappear after drops of HCL.
Alkaloids	+	Wagner	Chocolate precipitate
Steroids	+	Liebermann-burchard	H bluish green

Information : + (detected)

- (not detected)

Kecombrang flower methanol extract showed positive reactions in all secondary metabolite tests. These metabolites have been reported to have antioxidant, anticancer, antiobesity and hypolipidemic activities.

Hypolipidemic Activity

The treatment of giving 100 mg/kg BW kecombrang flower extract to obese rats was carried out for 30 days, and the rats' body weight was observed every week. The body weight of rats for each treatment group each week is listed in Table 2 below.

Table 2: Rat Weight Every Week

Week-	Normal Control (grams)	Control Obesity /High Fat (grams)	Kcb flower (grams)
0	149.92±0.81	280.03±0.26	278.96±1.13
1	150.48±1.12	282.35±2.82	281.12±1.82
2	151.47±1.12	287.39±2.66	254.84±2.53
3	152.77±0.89	291.94±2.96	229.12±0.90
4	154.02±0.91	299.67±2.15	191.63±0.92

Remarks : Values are shown as mean weight ± Standard Deviation

Based on Table 2, the normal control group and the high-fat control group experienced weight gain. In contrast, the kecombrang flower treatment group experienced a decrease in body weight every week compared to high-fat diet control rats. ($p<0.05$). This indication shows that the administration of kecombrang flower methanol extract can suppress the increase and decrease in the body weight of mice. This weight loss of rats was due to the content of secondary metabolites contained in the kecombrang flower. The phytochemical test showed positive kecombrang flowers for secondary metabolites in the form of phenols, alkaloids, saponins, tannins, flavonoids and steroids.

Flavonoids and steroids are suspected of having the potential for anti-obesity. Flavonoids are antioxidants that can reduce appetite and pancreatic lipase inhibitors (Dzomba, 2014). Pancreatic lipase is an enzyme that catalyzes the breakdown of triglycerides into glycerol and acids fat-free absorbed by the body. The study (hydrolysis) of triglycerides is inhibited by inhibiting the lipase enzyme, which can prevent and reduce obesity. Flavonoids can also reduce glucose absorption, inhibit the breakdown of polysaccharides into simpler forms, and reduce lipid accumulation in the liver resulting in reduced fat absorption so that body weight decreases (Dheer and Bhatnagar, 2010) [6]. Steroids in the body are hypolipidemic by inhibiting cholesterol absorption in the intestine and accelerating cholesterol excretion. Decreased cholesterol levels that enter the bloodstream will minimize the accumulation of fat in the body's organs and reduce the occurrence of obesity.

Took rat blood from the orbit of the eye to measure lipid profile levels (total cholesterol, TG, LDL, and HDL) after 30 days of treatment. The results of the lipid profile levels of each group of mice are listed in Table 2.

Table 3: Results of Analysis of Total Cholesterol Levels, TG, LDL and HDL Rats.

Treatment	Total Cholesterol (mg/dL)	TG (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
K. Normal	120.83±2.32 ^{bcd}	134.17±3.31 ^{bcd}	51.85±3.99 ^{bcd}	42.15±2.53 ^{b}
K. High-fat diet	190.17±3.19 ^{acd}	209.67±2.16 ^{acd}	119.90±4.04 ^{acd}	28.33±2.16 ^{acd}
Kcb flower	84.67±3.93 ^{ab}	89.33±4.54 ^{ab}	26.30±2.65 ^{ab}	40.50±3.27 ^{b}

Remarks : Values are shown as average grade ± standard deviation.

[a] Shows a significant difference from the negative control ($p<0.05$)

[b] Shows a significant difference from the positive control ($p<0.05$)

[c] Shows a significant difference from the treatment of kecombrang flowers ($p<0.05$)

Table 2 shows that kecombrang flower extract has an effect on decreasing cholesterol levels and increasing HDL levels in obese rats with a significant difference ($p<0.05$) when compared to control rats fed a high-fat diet, there is no significant difference when compared to controls negative ($p>0.05$). The following graph of the average lipid profile level for each treatment is shown in Figure 1.

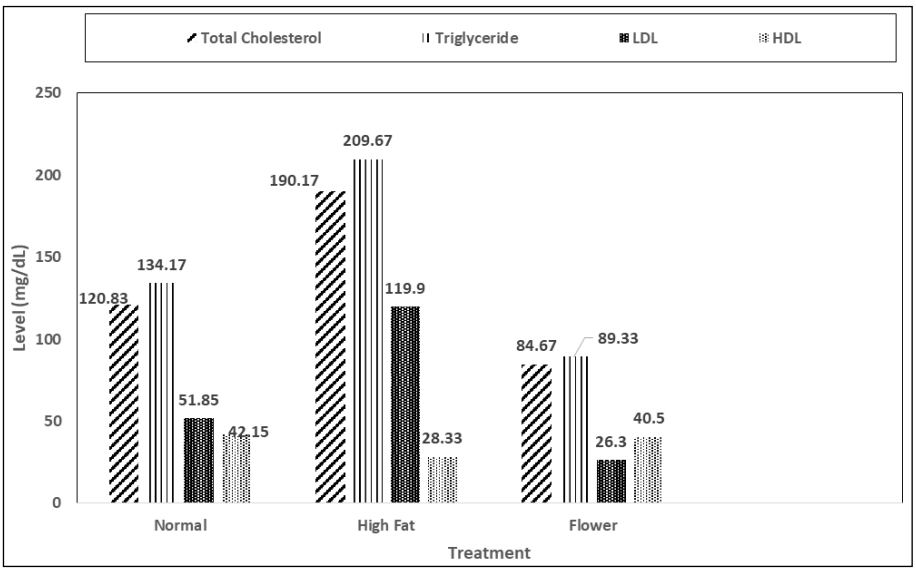

Fig 1: Graph of Average Lipid Profile Levels for Each Treatment

Kecombrang flower treatment reduced total cholesterol levels by 55.50%, TG by 57.40%, LDL by 78.06%, and HDL by 42.96%. A decrease in total cholesterol, TG, LDL and an increase in HDL is thought to be due to the presence of antioxidant compounds in kecombrang flowers such as polyphenols, flavonoids, alkaloids, tannins, saponins and steroids. Flavonoids are reported to reduce cholesterol levels because they act as a cofactor for the enzyme cholesterol esterase. In addition, flavonoids activate cytochrome P-450 enzymes so that they can increase bile secretion. In bile, cytochrome P-450 enzymes bind to several components, thereby reducing cholesterol in the body. Flavonoids can also bind to apolipoprotein B, reducing TG, increasing HDL, increasing the density of LDL receptors in the liver, and interfering with the activity of the enzyme *Acyl-Coa Cholesterol Acyl Transferase* (ACAT) in HepG2 cells (Witosari *et al*., 2014).

The antioxidant compounds found in kecombrang flowers work to inhibit HMG-CoA Reductase. Inhibition of HMG-CoA Reductase causes decreased cholesterol synthesis, and increased LDL receptors are located in extrahepatic tissues and liver cell membranes so that total cholesterol levels fall. The decrease in total cholesterol levels means that LDL levels as lipid carriers in the blood will decrease in the blood also reduces.

Polyphenols as antioxidants can neutralize free radicals by filling the electron deficiency contained in free radicals. Phenol compounds can reduce LDL levels such as preventing absorption, lowering apolipoprotein B-100, LDL biosynthesis, and oxidized LDL levels. Phenolic compounds can react with free radicals by scavenging oxygen free radicals and inhibiting the work of lipoxygenase and cyclooxygenase enzymes, which cause the formation of free radicals. Mechanism of phenol in increasing HDL. Levels, namely by the work of macrophages, to improve the process of *Reverse Cholesterol Transport* (RCT) (Heriyani, 2016). Antioxidant compounds such as phenols can encourage the increased activity of *Lecithin Cholesterol Acyl Transferase* (LCAT). LCAT is an enzyme that converts free cholesterol into cholesterol esters to form new HDL so that HDL increases.

Alkaloid compounds also play a role in reducing pancreatic lipase activity, thereby reducing the absorption of TG in the small intestine. Tannins act as an activation of antioxidant enzymes. Cholesterol and TG absorption are inhibited by tannins, where tannins can react with proteins in epithelial cells and intestinal mucosa. Tannins precipitate amino acids and protein in feed and interfere with the absorption of fat in food, resulting in the amount of cholesterol carried by chylomicrons to the liver is not the same as the amount of cholesterol taken by chylomicrons to the liver in food (Artha (2017) [8]; Nuralifah, 2020) [9].

Just like other secondary metabolites, saponins and steroids have antioxidant activity that has the potential to lower cholesterol levels. S saponins form bonds with cholesterol (from food), and bile acids form micelles or foam that the intestine cannot absorb. Saponins can also inhibit the action of the lipase enzyme (Maryani *et al*., 2016). Steroid compounds contained in plants are called phytosterols (Robinson, 1995) [10]. Phytosterol steroids inhibit cholesterol absorption through their competition in the absorption process in the intestine, thereby reducing the amount of cholesterol that enters the bloodstream and accelerating cholesterol excretion (Prahstuti, 2011).

Conclusion

The administration of methanol extract of kecombrang flower (*Etlingera elatior* Jack) at a dose of 100 mg/kg BW has the potential for hypolipidemia by reducing total cholesterol, TG, LDL and increasing HDL levels significantly ($p<0.05$). Decrease This is due to the presence of antioxidant compounds in kecombrang flowers, such as polyphenols, flavonoids, alkaloids, tannins, saponins and steroids.

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