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Lipid Profile Improving Effect of Tenggulun Leaf (*Protium javanicum*) Tea Powder in Rats Fed with High-Fat Diet

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ABSTRACT

The objective of this research was to determine the effect of tenggulun leaf tea powder in improving lipid profile in rats fed with high fat diet. We developed a tenggulun leaf tea powder and did a biochemical analysis. The in vivo study was conducted using 18 male Wistar rats grouped into three different diets, Control-Standard Feed (CSF) group, Hypercholesterol-Standard Feed (HSF) group and Hypercholesterolemia-Tenggulun Feed (HTF) group. The treatment was given for 30 days in all groups. Biochemical analysis showed that tenggulun leaf tea powder contains various cholesterol lowering substance such as dietary fibre (42.73%), phenolic content (9.42 mg GAE/g), tannins (10.80 mg TAE/g), flavonoids content (1.81 mg QE/g), and it also showed antioxidant activity (IC_{50} value of 67.20 ppm). In vivo analysis after treatment showed that there was no significant difference in total cholesterol levels between the HSF and HTF groups. However, in the HTF group there was a decrease in total cholesterol levels from the initial level by 16.48%. In addition, the HTF group had significantly higher HDL and lower LDL level compared to the HSF. The administration of tenggulun leaf tea powder for 30 days showed a significant effect on Low-Density Lipoprotein (LDL) and High-Density Lipoprotein (HDL) cholesterol levels, but not on serum Triglyceride (TG) levels. Therefore, the tenggulun leaf tea powder showed a significant effect in improving lipid profile in rats fed with high fat diet.

Keywords: cholesterol, *protium javanicum,* tea, tenggulun

INTRODUCTION

Herbal tea is products made from the leaves, stems, flowers, or seeds of one or a mixture of several herbal plants (Ravikumar 2014). Recently, herbal teas have received a lot of attention because of their beneficial physiological effects on health. One of the plants that have the potential to be developed into herbal teas is tenggulun (*Protium javanicum*). Tenggulun is a tropical plant with leaf morphology that is not too wide, tapered, and pink on very young leaves and dark green on old leaves. In Bali, young tenggulun leaves are often consumed as vegetables and used for traditional medicine.

Tenggulun leaves contain various bioactive compounds such as phenolics, flavonoids, and tannins (Simamora *et al.* 2021), α and β amyrin, and β sitosterol (Puspawati *et al.* 2019). Santos *et al.* (2012) reported that protium genera such as *Protium heptaphyllum* has antihyperglycemic and hypolipidemic effects. The content of triterpene compounds (α and β amyrin mixture) in these genera could be a leading compound for drug development effective in diabetes and atherosclerosis. The content of other bioactive compounds such as dietary fibre, flavonoids and tannins can prevent and treat degenerative diseases. Maheshwari (2020) also reported that various mixtures of phytochemical (plant sterols, flavonoid, lignan) extracts that can help in reducing blood cholesterol levels.

Tenggulun leaf tea powder is an herbal product made from young tenggulun leaves. The leaves are steamed, dried, grinded, and sifted to obtain a fine powder. It can be consumed by dissolving it in water without being filtered. Rohiqi *et al.* (2021) reported that the best tenggulun leaf tea powder comes from young leaves. Other researchers reported that steaming method was the best processing for producing tenggulun leaf tea powder (Yusasrini & Permana 2021).

Various studies have been carried out to explore the potential of herbal tea products for the treatment or prevention of hypercholesterolemia

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such as *Curcuma zedoaria* Roscoe tea (Tariq *et al.* 2016) and green grass jelly leaf tea (Rizki *et al.* 2015). Kuchta *et al.* (2021) reported that administration of *Cistus incanus* tea containing phenols and flavonoids could improve lipid profiles in healthy volunteers. Giving a combination of white tea and Moringa leaves has also been reported to reduce blood triglycerides in vivo (Martini *et al.* 2019). *Cang salak* tea (Karta *et al.* 2021) and chamomile tea (Kaseb *et al.* 2018) have also been reported to improve lipid profiles in hypercholesterolemic conditon.

Despite the vast body of evidence for other herbal tea products for improving cholesterol profile, studies on the physiological effects of tenggulun leaf tea powder in vivo have been absent. It is important to explore more on the potential of this local plants for the prevention or treatment of hypercholesterolemia. Therefore, this research aimed to determine the effect of tenggulun leaf tea powder in improving lipid profile in rats fed with a high-fat diet.

METHODS

Design, location, and time

This research was carried out at the Food Processing Laboratory, and at the Biochemistry and Nutrition Laboratory, Faculty of Agricultural Technology, Udayana University from March to September 2021. This is an experimental study with a post-test control group design. The completely randomized design used 3 treatment groups and 6 replications so that 18 experimental units were obtained. This research has been approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University with a Certificate of Approval of Animal Ethics Number: B/146 /UN14.2.9/ PT.01.04/2021.

Material and tools

Young tenggulun leaves with characteristic light green color, limp and spotless, that grows wild in the Bukit Jimbaran area were collected. The standard feed ingredients according to AIN 93 consists of 62.069% corn starch, 5% Carboxymethyl Cellulose (CMC), 4% soybean oil, 10% sucrose, 14% casein, 1% vitamin mix, 3.5% mineral mix, 0.18% L-cystine, and 0.25% choline bitartrate. The reagents for analysis were cholesterol FS (Diasys), triglycerides FS (Diasys), HDL Precipitant (Diasys), 70% ethanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), $NaNO_2$, $AlCl_3$, NaOH, H_2SO_4, boric acid, HgO, Na_2SO_4, HCl, and hexane.

The equipment used for the research included a vortex, oven, small centrifuge (Hettich EBA III), Eppendorf tubes, blender, a set of individual mouse cages, muffle furnace (Heraeus Instrument), oven, analytical balance (Sartorius), injection syringe, micro hematocrite tube (Becton Dickinson & Company), micro-pipette and glassware.

Procedure

Material preparation. Tenggulun leaf tea powder was obtained using the modified steaming method according to Topuz *et al.* (2014). A batch of 100 g of young tenggulun leaves were collected and then steamed at 100°C for 90 seconds. After the tenggulun leaves had been cooled for 5 min, it was dried in an oven at 50°C for 4 h. The dried tenggulun leaves was then ground and sieved through a 100-mesh sieve.

Feed formulation. Standard feed was made by mixing ingredients according to the standard AIN 1993. The standard feed composition per kg consisted of 620.69 g corn starch, 140 g casein, 100 g sucrose, 40 g soybean oil, 50 g Carboxymethyl Cellulose (CMC), 35 g mineral mixture, 10 g vitamin mix, 1.8 g L-cystine, and 2.5 g of choline bitartrate. High-fat feed was prepared by adding 2% cholesterol and 10% beef fat per kg of standard feed. Tenggulun leaf tea powder feed was made by substituting 10% tenggulun leaf tea powder into a standard feed mixture with isocaloric considerations. To obtain the isocaloric value, the amount of corn starch, casein, soybean oil, CMC, and mineral mix in the tenggulun leaf tea powder feed was determined based on the results of the proximate analysis of the tenggulun leaf tea powder. Each feed ingredient was mixed homogeneously, put into a printing machine, and dried in an oven at a temperature of 50^0C for 4 h.

Preparation of experimental animals. Eighteen (18) male Wistar rats with initial weight of 100±5 g were placed in individual cages and acclimatized for one week with standard feed and drinking water ad libitum. Furthermore, the rats were divided into three groups (each group consisted of 6 rats) namely: 1). Control-Standard Feed (CSF), 2). Hypercholesterol-Standard Feed

(HSF), and 3). Hypercholesterol-Tenggulun Feed (HTF). The CSF group was given standard feed from the beginning to the end of the bioassay. The HSF group was fed a high-fat diet for two weeks, followed by a standard diet for 30 days. The HTF group was fed a high-fat diet for two weeks, then fed tenggulun leaf tea powder feed for 30 days. Bioassays were carried out for 30 days. At the end of the bioassay, blood was collected for analysis.

Blood collection. Blood was collected after two weeks of high-fat feeding for initial cholesterol level determination, and at the end of the bioassay for lipid profile analysis. Before blood collection, rats were fasted overnight. Blood was taken from the orbital sinus of the eye, collected in Eppendorf tubes, and centrifuged at 4,000 rpm for 10 min. Blood serum was accommodated in microtubes and analysed for lipid profiles.

Proximate analysis. Proximate analysis was carried out according to AOAC (2005). The moisture and ash content analysis were carried out according to the gravimetric method, the protein content analysis was carried out using the semi-micro Kjeldahl method and the fat analysis was carried out according to the Soxhlet method. Carbohydrate content was calculated by difference. Analysis of dietary fibre was carried out using the multienzyme method according to AOAC (2005).

Total phenolic content analysis. Analysis of total phenolic content was carried out using the spectrophotometric method according to Sakanaka *et al.* (2005) with slight modifications. A total of 0.1 g of the sample was put into a tube and diluted with 85% ethanol to a volume of 5 ml. The solution was vortexed until homogeneous and centrifuged at 2,500 rpm for 15 min. A total of 50 µl of filtrate was transferred into a test tube and added with 350 µL of distilled water. The mixture was added with 400 ml of folin ciocalteu reagent. Incubated for 6 min, and added with 4.2 ml of 5% Na_2CO_3. The solution was vortexed and incubated again for 90 min. The absorbance was read at a wavelength of 760 nm.

Total flavonoid analysis. Total flavonoid analysis was carried out using the spectrophotometric method according to da Silva *et al.* (2015) with slight modifications. A total of 5 g sample was dissolved in 100 ml of ethanol. A total of 0.1 g of dissolved sample was weighed and added with 50% ethanol until the volume was 5 ml. The solution was vortexed until homogeneous and then centrifuged at 2,500 rpm for 15 min. A total of 50 µl of filtrate was transferred into a test tube and added with 450 µl of ethanol and 1 ml of $AlCl_3$. The solution was incubated for 30 min. The absorbance was read at a wavelength of 415 nm.

Tannin analysis. Tannin analysis was carried out using the spectrophotometric method according to Nair *et al.* (2015) with slight modified. A total of 5 g of the sample that has been mashed was added with distilled water to a volume of 100 ml. The solution was shaken until homogeneous and filtered. A total of 1 ml of clear solution was added with 0.5 ml of follin denis solution, 1 ml of saturated $NaCO_3$ solution, and distilled water until it reached 10 ml in volume. The solution was vortexed until homogeneous and absorbance was read at a wavelength of 730 nm.

Antioxidant activity. Determination of antioxidant activity was carried out by the DPPH (1,1,2,2-Diphenyl Picryl Hydrazyl) method according to Molyneux (2004). Samples were made with certain concentrations. A total of 1 ml of each solution was put in a test tube and added with 1 ml of the 200 mm DPPH. The solution was incubated in the dark for 30 min and diluted with methanol to a volume of 5 ml. The absorbance of the solution was measured at a wavelength of 517 nm.

Total cholesterol analysis. Total cholesterol was measured according to the CHOD-PAP enzymatic photometric method following the procedure from Kit DiaSys®, Germany (Diagnostic System International). A total of 10 µl of serum was added to 1,000 µl of reagent, then incubated for 20 min at 25°C. The absorbance was read at a wavelength of 500 nm. Total cholesterol was calculated based on the formula:

$$Total\ cholesterol\left(mg/dl\right) = \frac{Sample\ absorbance}{Standard\ absorbance}\ X\ Standard\ concentration\left(\frac{mg}{dl}\right)$$

HDL cholesterol analysis. Analysis of HDL cholesterol using the CHOD-PAP enzymatic photometric method based on the procedure from Kit DiaSys®, Germany (Diagnostic System International). A total of 200 µl of serum was added with precipitate reagent, then incubated for 15 min at room temperature, centrifuged for 20 min at a speed of 2,500 rpm. A total of 100 µl of

supernatant was added to 1,000 µl of cholesterol reagent, then vortexed and incubated for 10 min at ambient temperature. The absorbance was read at a wavelength of 500 nm. HDL cholesterol was calculated based on the formula:

$$HDL-cholesterol\,(mg/dl) = \frac{Sample\ absorbance}{Standard\ absorbance}\ X\ Standard\ concentration\left(\frac{mg}{dl}\right)$$

LDL cholesterol analysis. LDL cholesterol was calculated using the formula compiled by Fridewald.

$$LDL-cholesterol = Total\ cholesterol - \left(HDL + \frac{1}{5}\ Triglycerides\right)$$

Total triglyceride analysis. Total triglyceride analysis was determined by the GPO-PAP method using the ReiGed Diagnostic Kit. A total of 10 µl of serum was added to 1,000 µl of reagent, then vortexed and incubated for 10 min at 25^{O}C. The absorbance was read at a wavelength of 505 nm. Total triglycerides were calculated based on the formula:

$$Total\ triglyceride\,(mg/dl) = \frac{Sample\ absorbance}{Standard\ absorbance}\ X\ Standard\ concentration\left(\frac{mg}{dl}\right)$$

Data analysis

The data on proximate analysis, dietary fiber, total phenol, total flavonoid, total tannin and antioxidant activity of tenggulun leaf tea powder was presented in descriptive analysis. Data on total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides between groups were analysed using ANOVA followed by Duncan post-hoc test by using the IBM SPSS 23.

RESULTS AND DISCUSSION

The chemical composition of tenggulun leaf tea powder

Tenggulun leaf tea powder has physical characteristics of yellowish green color, uniform particle size, and distinctive aroma of tenggulun leaves. The results of the proximate analysis of tenggulun leaf tea powder are presented in Table 1.

Tenggulun leaf tea powder has a low moisture content of 7.66% and complying to the national standard SNI 3836:2013 regarding packaged dry tea which requires a maximum moisture content of 8%. Moisture content is closely related to product quality. High water content affects the shelf life and, sensory properties such as the taste and aroma of steeping tea and causes microbiological contamination. The ash content of tenggulun leaf tea powder is 7.07%, which met the SNI requirements of maximum 8%.

Table 1. Chemical composition of tenggulun leaf powder

Chemical composition	Content (%)
Moisture content	7.66±0.07
Ash content	7.07±0.10
Protein content	12.2±0.04
Lipid content	0.53±0.01
Carbohydrate (by difference)	72.53±0.02

Tenggulun leaf tea powder contains 42.73% dietary fiber with the main components being insoluble dietary fiber 40.19% and soluble dietary fiber 2.53% (Table 2). The content of other bioactive compounds in tenggulun leaf tea powder is listed in Table 2.

Tenggulun leaf tea powder contains phenols, tannins, and flavonoids of 9.42, 10.8, and 1.815%, respectively. The level of phenol in this herbal tea product is lower than the polyphenol compound required by SNI 3945:2016, which is at least 15%. Bioactive components such as phenols, tannins, and flavonoids are related to the antioxidant activity of tenggulun leaf tea powder. The IC_{50} results of tenggulun leaf tea powder showed a value of 67.205 ppm, which indicates strong antioxidant activity. Antioxidant activity is classified as "strong" if the IC_{50} value is between 50–100 ppm (Molyneux 2004).

Phenolic compounds are the biggest group of phytochemicals. Their bioavailability is

Table 2. Dietary fiber, total phenol, total flavonoid, total tannin and antioxidant activity of tenggulun leaf powder

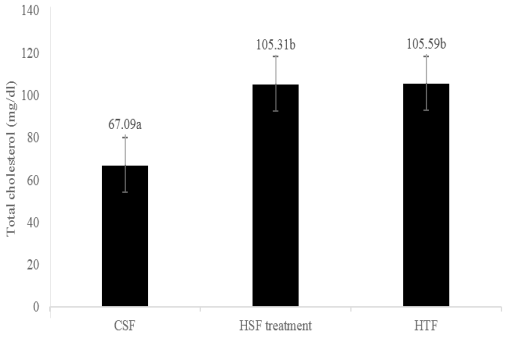
Bioactive compounds	Content
Total dietary fiber content (%)	42.73 ± 0.16
Soluble dietary fiber	2.53 ± 0.07
Insoluble dietary fiber	40.19 ± 0.09
Total phenolic content (mg GAE/g)	9.42 ± 0.08
Tannin content (mg TAE/g)	10.8 ± 0.08
Total flavonoids (mg QE/g)	1.815 ± 0.13
Antioxidant activity (IC_{50}) (ppm)	67.205 ± 1.71

decisive in exerting useful consequences in vivo and is encouraged through the molecular length and complexity of their chemical structure, consisting of conjugation with different phenols, polymerization, glycosylation, acylation, or hydroxylation (Sobhani *et al.* 2021). Flavonoids (consisting of flavonols, flavones, flavanones, flavanols, isoflavones, and anthocyanidins) are a collection of polyphenols that exert a couple of useful consequences. A current examination indicates that flavonols, particularly quercetin , alters the intestine microbiota and decreases the atherogenic lipids, including cholesterol and lysophosphatidic acids, with these types of consequences being related to the diminution of atherosclerotic lesions area (Nie *et al.* 2019). When compared with similar products, the total phenol content of tenggulun leaf tea powder is higher than African leaf tea powder (Putri *et al.* 2021) but lower than the total phenolic content of matoa leaf tea (Dewi *et al.* 2021).

Total cholesterol

Analysis of variance showed that rats fed with a high-fat diet had a significantly higher total cholesterol level compared to the control. The data is illustrated in Figure 1.

The HSF and HTF groups had higher baseline cholesterol levels (56.96–57.37%) than CSF group. The addition of 2% cholesterol and 10% beef fat into the standard feed was effective in increasing the cholesterol levels of rats. In mice *R. norvegicus* Wistar strain, normal blood cholesterol levels is 10–54 mg/dl. Beef fat contains higher saturated fatty acids than pork (Prabawati & Fajriati 2019). The dominant effect of these saturated fatty acids is an increase in total cholesterol level. The outcomes of this examination are in line with the ones reported by Dahlia *et al.* (2017) and Harsa (2014) that the administration of a high-fat diet was able to increase the total cholesterol levels of rats.



Bars with different letters show a significant difference at $p<0.05$ (n=6)

CSF: Control-Standard Feed; HSF: Hypercholesterolemia-Standard Feed; HTF: Hypercholesterolemia-Tenggulun Feed

Figure 1. Effect of high-fat diet on initial cholesterol levels

Analysis of variance showed that feeding of tenggulun leaf tea powder for 30 days could improve lipid profile in rats (Table 3). CSF group had the lowest total cholesterol level, while the HSF group had the highest, but there was no significant difference between the HSF and HTF groups. However, HTF group showed a decrease of 16.48% in total cholesterol compared to the initial cholesterol level.

Analysis of variance also showed that despite consuming the same high fat diet, the HTF group had significantly lower LDL level compared to the HSF group and it did not differ significantly with the CSF group that received normal diet. Therefore, it indicates that the feeding of tenggulun leaf tea powder had a significant effect on lowering LDL cholesterol levels in rats with high fat diet. In addition, the HTF group also had significantly higher HDL level compared to both the HSF and CSF groups. On the other hand, tenggulun leaf tea powder showed no significant effect on blood triglyceride levels in rats. Although statistically there has been no significant difference in serum triglyceride

Tabel 3. Effect of high-fat diet on the lipid profile of rats after 30 days of intervention

Treatments	Total cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Triglyceride (mg/dl)
CSF	66.67±16.73[a]	6.46±5.26[a]	51.65±16.95[a]	43.26±10.35[a]
HSF	94.87±5.79[b]	28.76±16.54[b]	57.05±13.28[a]	45.26±24.73[a]
HTF	88.18±11.78[b]	3.45±3.14[a]	77.75±12.76[b]	34.86±7.80[a]

[a,b] in the same column show a significant difference at $p<0.05$ (n=6)

LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; CSF: Control-Standard Feed; HSF: Hypercholesterolemia-Standard Feed; HTF: Hypercholesterolemia-Tenggulun Feed

between the three groups, the administration of tenggulun leaf tea powder tended to lower serum triglyceride level. The HTF group had 22.97% lower triglyceride levels than the HSF group and 19.41% lower than the CSF group.

The high content of dietary fibre and the presence of bioactive compounds such as phenols, tannins, and flavonoids in food can help improve blood lipid profile. Tenggulun leaf tea powder contains a high dietary fibre of 42.73% and is dominated by Insoluble Dietary Fibre (IDF). Dietary fibre has antihyperlipidemic properties through bulking effect, low ranges of energy, viscosity, fermentation, and binding capacity. Insoluble dietary fibre presents bulking effect, consequently growing stool mass, assuaging constipation, and enhancing regularity. Due to the extended bulk and water content, the nutrients including sugar and lipids, are diluted and their migration to the intestinal partitions slows down. The IDF also relates to a slower intestinal transit time that enables a lower absorption time of those sugars and lipids. Hence, it lowers the absorption of macronutrients, particularly carbohydrates and cholesterol, both through delaying gastric emptying or through shortening small intestinal transit time, similarly to a discounted glycaemic response, which can help the discount in insulin stimulation of hepatic cholesterol synthesis (Dai & Chau 2017; Nie *et al.* 2021). Dietary fiber also inhibits the absorption of cholesterol in the intestine, which causes increased excretion of bile acids through faeces so that more cholesterol is converted into bile acids to emulsify fats. These reasons explain lower overall LDL levels of cholesterol in the blood (Soliman 2019).

Tenggulun leaf tea powder also contains phenols, tannins, and flavonoids that function as antioxidants and are hypocholesterolaemic. Flavonoids have been reported to reduce oxidative stress and regulate blood lipid levels, exhibit anti-inflammatory and activities activity, and improve endothelial function (Siasos *et al.* 2013). As mentioned earlier, phenolic compounds in foods, especially in herbal teas are also reported to have hypocholesterolemic effect by inhibiting LDL oxidation, thereby minimizing the possibility of blood vessel damage caused by LDL oxidation (Amarowicz 2016). Dahlia *et al.* (2017) found that the administration of white tea extract can reduce LDL cholesterol in the blood. Likewise, as reported by Karta *et al.* (2021), *Cang salak* tea was able to normalize LDL cholesterol levels in hyperlipidemic rats. In addition, tannins, such as contained in the tenggulun tea also increases lipoprotein lipase activity resulting in decreasing total serum cholesterol (Kothari *et al.* 2011).

On the other hand, HDL has an antagonistic function to LDL. In the metabolic process, LDL primarily transports lipids from the intestine and liver to the peripheral tissues, while the HDL is primarily involved in reverse cholesterol transport (Bali & Utaal 2019). LDL is atherogenic because it causes calcification of the coronary vessels, in contrast to HDL that prevents the occurrence of calcification. HDL contains less than 25% cholesterol so it minimizes the danger of atherosclerosis. Our study showed that rats treated with high fat diet and tenggulun leaf tea powder had significantly higher HDL compared to rats receiving high fact diet without tenggulun tea. It is proposed that dietary fibre might indirectly able to increase blood HDL levels by lowering cholesterol absorption. In addition, protective function of flavonoid intake is related to the bioactivity of flavonoids as antioxidants and anti-inflammatory compounds. These characteristics can increase HDL or reverse cholesterol transport and provide protection against HDL dysfunction in the context of inflammatory disease states such as atherosclerosis or obesity (Millar *et al.* 2017).

CONCLUSION

The administration of tenggulun leaf tea powder at 10% of standard feed for 30 days to a group of rats fed with a high-fat diet improves the overall blood lipid profile. Rats fed with high fat diet containing tenggulun leaf tea powder had significantly lower LDL and higher HDL levels compared to groups that did not receive the herbal tea. However, it did not show significant difference in total cholesterol level and triglycerides. Hence, further research is needed to determine the appropriate dose of tenggulun leaf tea powder that are able to significantly reduce total cholesterol and triglycerides levels.

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DECLARATION OF INTERESTS

The authors declare that there is no conflict of interest with other person or institution.

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