Antihyperlipidemia activity of ethanol extract the stem bark of *Flacourtia rukam* on propylthiouracil-induced albino rats, *Rattus noverticus* (Wistar strain)

Muharni Muharni1*, Julinar Julinar1, Heni Yohandini1, Fitrya Fitrya2 and Rima Melati2

1Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Sriwijaya. Jl. Raya Palembang Prabumulih KM 32 Indralaya 30662 South Sumatera Indonesia
2Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Sriwijaya. Jl. Raya Palembang Prabumulih KM 32 Indralaya 30662 South Sumatera Indonesia

*E-mail: muharnikimia@unsri.ac.id

Abstract. *Flacourtia rukam* belongs to one of the traditional medicinal plants used by the people, especially in South Sumatra for the treatment of hypertension. One of the triggers for hypertension is high levels of cholesterol in the blood. The *in vivo* research of the antihyperlipidemia activity of ethanol extract the stem bark of *F. rukam* on Propylthiouracil-induced albino rats *Rattus noverticus* (Wistar Strain) has been done. The animal test was separated into 6 groups i.e. normal group, negative, positive (simvastatin), and 3 treatment groups with a variation extract dose of 100, 200, and 400 mg/kg BW. The measurement of total cholesterol, triglyceride, LDL and HDL levels using the photometric enzymatic method. The result shows that ethanol extract of *F. rukam* gives the highest anti hyperlipidemia activity at 400 mg/kg BW with decreased total cholesterol percentage of 56.28%, triglycerides 57.34%, LDL 54.29%, and increased HDL 58.77%. Statistical analysis shows that the treatment groups I, II, and III can reduce the level of total cholesterol, triglycerides, and higher-level LDL. The effective dose (ED50) is 327.83 mg/kg BW. Based on this result, the traditional plant can be used in hyperlipidemia treatment.

1. Introduction

*Flacourtia rukam* is one of the traditional medicines has been used for the treatment of various diseases. The fruit can be used to diarrhea and dysentery treatment, the juice of the leaves was used to treat swollen eyelids, the roots were used by a postpartum woman as an antiseptic [1] while the stem bark using as an antihypertensive treatment [2]. One of the triggers for hypertension is high cholesterol in the blood where causes various diseases such as coronary heart and stroke diseases [3]. Phytochemical tests the ethanol extract stem bark of *F. rukam* shown contain triterpenoid, steroid, flavonooid, and phenolic compounds [4,5]. Steroids and phenolics compounds have the effect of reducing cholesterol levels [6]. Flavonoids also can reduce hyperlipidemia levels in the blood [7]. The increased cholesterol levels in the blood are mostly occurred in people with hypertension, and increased lipid level in blood might be an important risk factor for the development of Cardiovascular disease.
Hyperlipidemia treatment can be using synthetic drugs, such as simvastatin, gemfibrozil, cholestyramine, and niacin, but the use of synthetic drugs usually caused undesirable side effects, while natural compounds usually have lower side effects than synthetic compounds. Besides that, natural compounds also can treat other diseases [8]. In our previous research, the stem bark of *F. rukam* also can reduce cholesterol levels in the blood while *in vitro* anti-cholesterol activity the ethanol extracts the stem bark of *F. rukam* obtained IC$_{50}$ values 209.092 µg/mL [9]. This research will report the potential of ethanol extract the stem bark of *F. rukam* as anti-hyperlipidemia using male albino rats Wistar strain using the enzymatic colorimetric method.

2. Methodology

2.1. Material

Sample of the stem bark of *F. rukam* used in this study was the same as we used in previous studies [4]. Thirty albino rats employed as animal model with body weight (BW) of 150-250 g were obtained from animal holdings of Bio Science Research Laboratory, Palembang city. The experiments were performed after accepting an approval letter of the protocol ethical clearance from ethics the health research review committee of Sriwijaya University (No. 074/kepkrsmhfkunsri/2020) and were carried out in accordance with current guidelines for the care of laboratory animals. The animal models were acclimatized for seven days and fed with feed and water standard [10].

2.2. Methods

2.2.1. Animal treatment. One kg of *F. rukam* was distillated in 96% ethanol using distillation apparatus distillated ethanol. The maceration for 3 days and then concentrated using rotary evaporator. Before the treatment, the body weight, total cholesterol, triglyceride, LDL, and HDL levels of each rats were measured. Animals test were divided into 6 groups, which are normal, negative control, positive control, and 3 treatment groups (Dose I, Dose II, Dose III). The normal control group were given standard foods, each given 2 mL every day with oral administration. Meanwhile, other groups were induced with solution of propylthiouracil (PTU) and high-fat foods (contained of 40% duck egg yolk, 50% cooking oil, and 10% butter). This induction was done for 14 days. On the day 15, the blood was taken to determine the total cholesterol, triglyceride, HDL, and LDL levels of the rat after induction.

The rats under hyperlipidemia were given treatment: the normal and negative control groups were given Na CMC solution 1% and positive control were given simvastatin solution at a dose of 0.193 mg/200 g BW. For the treatment group was given a solution of ethanol extract the stem bark of *F. rukam* at Dose of 100, 200, and 400 mg/kg BW. The dosage was given orally with a volume of 2 mL/200 g BW of rats for 14 days. On the day 15, blood was taken to determine the total cholesterol, triglyceride, HDL, and LDL levels of rats by using a UV-Vis spectrophotometer at the maximum wavelength respectively using a biosystem analyzer [11]. Blood of tested rats was taken from plexus retro orbitals of their eyes by microhematocrit. During the process, the bodyweight of rats was weighed before induction, after induction and after treatment.

Table 1. The treatment of group animal test.

| Groups          | Treatment                                      |
|-----------------|------------------------------------------------|
| Normal          | Na CMC 1%                                      |
| Control (-)     | Propylthiouracil 0.1% + food high-fat + 1% Na CMC |
| Control (+)     | Propylthiouracil 0.1% + food high-fat + Simvastatin 0.193 mg/200 g BW |
| Dose I          | Propylthiouracil 0.1% + food high-fat + ethanol extract 100 mg/kg BW |
| Dose II         | Propylthiouracil 0.1% + food high-fat + ethanol extract 200 mg/kg BW |
| Dose III        | Propylthiouracil 0.1% + food high-fat + ethanol extract 400 mg/kg BW |
2.2.2. Measurement of plasma lipids level. Two ml of blood was collected in EDTA tubes and centrifuged for 20 minutes at 3500 rpm for 15 min at 4°C to obtain blood serum. The serum was used to measure total cholesterol, triglyceride, LDL, and HDL concentrations by using commercial kits [12].

2.2.3. Determination of serum cholesterol. The blood serum was analyzed for total cholesterol levels using the CHOD-PAP (Cholesterol Oxidase peroxidase aminoantipyrine) method. Hundred μL of cholesterol reagent solution was added to ten μL of blood serum and mixed well. The mixture was then incubated for 20 minutes at room temperature. The absorbance was measured at λ_{max} 500 nm. Distilled water was used as a blank, while cholesterol standard was served as a standard.

2.2.4. Determination of serum triglyceride. Serum triglyceride was measured using the Glycerol Peroxidase Phosphat Acid (GPO-PAP) method. Blood serum (10 μL) was taken in a test tube, the 1000 μL solution of triglyceride reagent kit QCA (Quimica Clinica Aplicada) was added. The solution was mixed well and incubated for 20 minutes at room temperature. The absorbance was read at λ_{max} 500 nm. Distilled water was used as a blank, while cholesterol standard was served as a standard.

2.2.5 Determination of LDL and HDL. Serum (10 μL) was added to 1000 μL of precipitation reagent (polyvinyl sulphate), then the solution was mixed and incubated for 15 minutes at 37°C. Centrifugation was done for 20 min at 3500 rpm. The clear supernatant was used for the determination of LDL and measured the absorbance of the sample against reagent blank at λ max 546 nm.

Determination of serum HDL level was carried out by CHOD-PAP method. Sample serum (10 μL) was placed in a test tube and 1000 μL of cholesterol reagents solution was added. The mixture was shaken thoroughly and left to stand for 10 min at 20-25°C. The absorbance was measured at 500 nm. HDL standard was used as a standard.

2.3. Effective Dose Determination (ED_{50})
The effective dose (ED_{50}) was calculated using linear regression between decrease of total cholesterol, triglycerides, LDL levels and concentration of extract used.

2.4. Analysis Data
Analysis Statistical of data was calculated with Shapiro-Wilk normality test. If the data is normally distributed then paired simple t-test and One-Way ANOVA were tested using the SPSS 21.0 for Windows program where that p <0.05 means there is a significant difference. If the data is not normally distributed then proceed with the Kruskal-Wallis non parametric test.

3. Results and discussion
In vivo anti-hyperlipidemia activity ethanol extract of *F. rukam* using albino rats Wistar strain. The measurement of total cholesterol, triglycerides, LDL, and HDL levels were done after acclimatization process. The normal condition of total cholesterol in rats was 10 - 54 mg/dL [13]. Animal tests were fed with high-fat diets and 0.01% PTU solution to increase the total cholesterol levels. Induction of propylthiouracil will inhibit thyroid hormone production lead to hyperthyroidism where will affect lipoprotein metabolism increasing cholesterol levels, especially LDL [14]. The total level of cholesterol, triglyceride, LDL, and HDL were measured on day 15 (Table 1). The data in Table 2 shows the total cholesterol after acclimatization is in the normal range that is 35.49 ± 1.36 mg / dL – 38.93 ± 1.22 mg/dL.

3.1. Total cholesterol
The results (Figure 1) show increased total cholesterol levels for the treatment group with average cholesterol values, 80.75 ± 5.37 - 83.60 ± 0.56 mg / dL, while the normal group is 40.55 ± 1.48. This data shows that the induction process has been succeeded in increasing cholesterol levels above normal. The rats in hyperlipidemia condition were given suspension treatment of ethanol extract of *F. rukam* at...
doses of 100; 200; and 400 mg/Kg BW. On the day 15, the total level of cholesterol, triglycerides, LDL, and HDL were measured.

Figure 1 shows the decrease in total cholesterol levels for the treatment groups of 100; 200; and 400 mg/dL, and the highest decrease was given by the treatment of dose III with value of 33.74 ± 3.86. Meanwhile, simvastatin as positive control showed a decrease in total cholesterol level of 34.04 ± 4.07 mg/dL which is almost the same as the treatment dose of 400 mg/dL. The statistical analysis of the paired t-test on the total cholesterol level data, between groups before and after treatment showed a positive control, groups I, II, and III showed significant difference (p <0.05), whereas in the negative control group showed there was no significant difference (p> 0.05). Statistical analysis also shows there was no significant difference between the treatment dose of 400 mg/kg BW with positive control.

![Figure 1. Total cholesterol of animal test before and after induction and after treatment.](image)

**Table 2.** Percent decrease in total cholesterol.

| Dose   | Decrease in total cholesterol (%) | ED_{50} (mg/Kg BW) |
|--------|----------------------------------|--------------------|
| I      | 17.38                            |                    |
| II     | 34.38                            | 336.43             |
| III    | 56.28                            |                    |

Dose 1: 100 mg/kg BW; Dose II: 200 mg/kg BW; Dose III: 400 mg/kg BW

Furthermore, the determination of the effective dose (ED_{50}) value based on the linear regression the percent of decreasing the total cholesterol to the extract dose [15]. Based on the regression equation obtained ED_{50} of 336.43 mg/kg BW (Table 2).

### 3.2. Triglyceride

The triglycerides value of normal albino rats is 25-145 mg/dL. The rats induced by high fat diet and PTU showed significant (p<0.05) increment in triglyceride levels, but in the normal group show not significant (p>0.05) increases in triglyceride levels. The given ethanol extract at doses 100; 200; and 400 mg/kg BW and positive control (simvastatin 1 mg/kg BW) exhibited a significant decrease in triglyceride levels (p <0.05) compared to before treatment. Furthermore, it was calculated the value of the percent decrease in levels triglycerides (Figure 2).
Figure 2. Triglyceride of animal test before and after induction and after treatment.

Table 2. Percent decrease in triglyceride.

| Dose | %PKTG     | ED50 (mg/KgBW) |
|------|-----------|----------------|
| I    | 15.34 %   |                |
| II   | 39.58 %   | 328.09         |
| III  | 57.34 %   |                |

Dose I: 100 mg/kg BW; Dose II: 200 mg/kg BW; Dose III: 400 mg/kg BW

The statistical analysis of the paired t-test on the triglyceride level, between groups before and after treatment showed a positive control, groups I, II, and III showed significant difference (p<0.05), whereas in the negative control group possessed no significant difference (p> 0.05). The statistical analysis showed significant decrease of triglyceride between groups, and treatment groups I, II, and III could reduce triglyceride levels significantly (P<0.05). The ED50 value (Table 2) of triglyceride was determined to base on the linear regression between the dose of ethanol extract and the percent decrease in triglyceride levels. Based on linear regression ED50 the decreased of triglycerides is 328.09 mg/kg BW.

3.3. LDL Measurement
Normal LDL levels in rats is 7 - 27.2 mg/dL, high-fat diet and PTU treatment were significantly increased LDL levels compared to before induction (p<0.05). The average increase in LDL levels was (32.72 ± 2.23 - 38.82 ± 0.61 mg / dL). The decreased of LDL levels after being treated showed significant difference (p<0.05) compared before treatment. In contrast, normal and negative control groups displayed no significant difference (p>0.05) on LDL level (Figure 3). The average decrease in LDL levels in the normal group only 0.353 ± 0.249 mg / dL, whereas in the negative control group only 0.94 ± 0.66 mg /dL. The decrease in LDL levels for positive control also showed a significant decrease, where simvastatin can inhibit the biosynthesis of cholesterol in the liver, causing an increase in LDL receptor work in binding LDL particles in the liver.

The administration ethanol extract at a dose of 400 mg/kg BW also possessed an extreme difference (p<0.05) in reducing LDL levels with a decreased percentage is 54.29% (Table 3). The statistical analysis of the paired t-test on the LDL level, between groups before and after treatment showed a positive control, groups I, II, and III showed significant difference (p <0.05), whereas in the negative control group showed there was no significant difference (p> 0.05). The statistical analysis showed that were significant differences (p <0.05) in the decrease of LDL between groups, and treatment groups I,
II, and III could markedly reduce LDL levels (P <0.05). The calculation of \( ED_{50} \) LDL (Table 3) based on linear regression between concentrations ethanol extract and percent decrease in LDL levels and obtained \( ED_{50} \) LDL value 360.69 mg/kg BW.

![Figure 3. LDL of animal test before and after induction and after treatment.](image)

**Figure 3.** LDL of animal test before and after induction and after treatment.

| Dose | Decrease in LDL (%) | \( ED_{50} \) (mg/KgBW) |
|------|----------------------|-------------------------|
| I    | 13.36 %              |                         |
| II   | 31.49 %              | 360.69                  |
| III  | 54.29 %              |                         |

3.4. Determination of HDL

HDL (High Density Lipoprotein) is a protective lipoprotein that reduces the risk of coronary heart disease. Normal levels of HDL in rats is \( \geq 35 \) mg/dL [16]. The measurement of HDL levels after acclimation showed that HDL levels of rats were in the normal range (32.41 – 35.15 mg/dL). PTU and high-fat diet treatment markedly down-regulated HDL levels (10.12 - 12.78 mg/dL). Low levels of HDL can cause a risk of coronary heart disease.

![Figure 4. HDL of animal test before and after induction and after treatment.](image)

**Figure 4.** HDL of animal test before and after induction and after treatment.

The treatment with *F. rukam* ethanolic extract showed a significant increase in HDL levels (p <0.05) between before and after the treatment dose and the highest increase was indicated by the dose of 400 mg/KgBW. The positive control where the treatment with simvastatin solution did not show a significant increase in HDL levels (p> 0.05) because simvastatin does not affect HDL metabolism in the blood.
The statistical analysis of the paired t-test on the HDL level, between groups before and after treatment showed a positive control, groups positive control, I, II, and III showed significant difference (p <0.05), and more height compared positive control, whereas in the negative control group showed there was no significant difference (p > 0.05). The statistical analysis showed that were significant differences (p <0.05) in the decrease of triglyceride between groups, and treatment groups I, II, and III could reduce total cholesterol levels significantly (P <0.05). Determination of ED₅₀ HDL values (Table 4) from linear regression between doses of ethanol extract the F. rukam and percent increase in HDL levels based on linear regression equations between extract doses with percent increase HDL obtained ED₅₀ HDL 327.83 mg/kg BW.

| Dose  | Increase in HDL (%) | ED₅₀ (mg/KgBW) |
|-------|---------------------|----------------|
| I     | 12.55 %             |                |
| II    | 36.76 %             | 327.83         |
| III   | 58.77 %             |                |

Dose I: 100 mg/kg BW ; Dose II : 200 mg/ kg BW ; Dose III : 400 mg /kg BW

The given extract can reduce total cholesterol, triglyceride, LDL levels, and significantly increased HDL levels, where the effect of decreasing the level of total cholesterol, triglycerides, and LDL is greatest in the treatment group dose of 400 mg/kg. The greater the extract dose given, the greater the effect in reducing total cholesterol, triglyceride, LDL levels, and in increasing HDL levels. Base on this data ethanol extracts of F. rukam activity as anti-hyperlipidemia.

The anti-hyperlipidemia properties of the ethanol extract were related to the content of flavonoids, steroids, triterpenoids, and phenolics [4]. Flavonoids can reduce total cholesterol and LDL levels in the blood. flavonoids are also able to reduce triglyceride levels and increase HDL and another report states β-sitosterol glycoside compound work by inhibiting the absorption of cholesterol in the digestive tract [17,18].

3.5. The body weights
The weighing body weight of rats was carried out before induction, after induction, and after treatment. Statistical analysis using paired t-test showed a significant difference (p <0.05) between the bodyweight of rats before induce with after induction. This increase in body weight is due to the increase in the amount of fat and attached to adipose tissue, especially in the abdominal cavity. The Fat Deposited will cause triglyceride levels to increase in adipose tissue and the blood and cell size will increase so that there is an increase in body weight. The results measurements of body weight shown in Table 5.

| Group  | Body weight (g)         |
|--------|-------------------------|
|       | Before induction  | After induction* | After treatment |
| Normal | 155.72 ± 9.65      | 163.71 ± 7.26   | 78.25 ± 9.56    |
| Negative | 144.73 ± 0.08     | 174.85 ± 18.35  | 191.36 ± 31.06  |
| Positive | 166.94 ± 9.07     | 194.61 ± 5.02   | 183.21 ± 5.55   |
| Dose I  | 181.90 ± 4.46      | 253.79 ± 6.98   | 235.27 ± 10.92  |
| Dose II | 147.63 ± 8.67      | 173.81 ± 6.18   | 156.56 ± 5.38   |
| Dose III| 171.08 ± 9.67      | 197.83 ± 27.09  | 186.97 ± 13.41  |

* = p<0.05 a significant difference to before induction
After the treatment, the positive control group, and the treatment group doses I, II, and III demonstrated significant difference weight loss (p<0.05) compared with before treatment, whereas for the normal group and negative control showed increase body weight significant difference (p<0.05) (Table 5). This is presumably because the content of flavonoids, steroids, and phenolic in ethanol extracts stem bark of *F. rukam* causes a decrease in body fat levels so that the weight of the animal test will also go down.

4. Conclusion
Treatment groups are showing significant reduction of total cholesterol, triglyceride and LDL (P<0.05) and significant increment of average HDL (P<0.05) compared with the negative control. The ethanol extracts from the stem bark of *F. rukam* have an anti-hyperlipidemia activity that not only can reduce total cholesterol, triglyceride, and LDL levels, but also can increase HDL level in male rats with Effective dose (ED₅₀) 328.09 mg/kg BW.

Acknowledgments
The authors are thankful to the Ministry of Research and Technology of the Republic of Indonesia Basic Research Funding 2020. We also thank Department of Chemistry, University of Sriwijaya on providing facilities to carry out the research work.

References
[1] Heyne K 1987 *Tumbuhan Berguna Indonesia*. Jilid III. Jakarta: Yayasan Sarana Wana Jaya :390
[2] Yustian I, Muharni, Sukarmi S, Arbi and Zulaika 2012 Special research on the exploration of ethnomedicine and local community medicinal plants in Indonesia (ethnic Musi II). Palembang Ministry of Health of the Republic of Indonesia
[3] Adisakwattana S, Moonrat S, Srichairat M, Shim BS, Kim N, Song MC, Baek NI, Kim AH 2007 Identification of campesterol from *Calotropis gigantea* flower extract of alchohol. *Nephropharmacol J* (6) 277
[4] Muharni, Elfita, Yohandini H, Julinar, Yasrina and Miranti 2019 Chemical constituents from the stem bark of *F. rukam* Zool & Mor. And their antioxidant activities. *Sains Malays. 48*(9) 1899–906
[5] Muharni, Fitrya, Nurmaliana and Ristojo. 2016 Skrining fitokimia aktifitas antioksidan dan antibakteri dari tumbuhan obat tradisional etnis Musi, Balai Besar Obat Dan Jamu Kementrian Kesehatan Republik Indonesia, Palembang, Indonesia
[6] Choi JM, Lee EO, Lee HY, Kim KH, Ahn KS, Shim BS, Kim N, Song MC, Baek NI, Kim AH 2007 Identification of campesterol from *Chrysanthemum coronarium* L. and its antiangiogenic activities *Phytother. Res. 21* 954-9
[7] Zeka K, Ruparelia, Arroo RJJ, BB, Budriesi R, Micucci M 2017 Flavonoids and their metabolites: prevention in cardiovascular diseases and diabetes *Diseases 5*(3): 19
[8] Karimi A, Majlesi M, Rafieian-Kopaei M 2015 Herbal versus synthetic drugs; beliefs and facts *J. Nephropharmacol. 4*(1): 27–30
[9] Muharni, Heni Y, Julinar 2019 Analisa zat aktif dari tumbuhan rukam (*Flacourtia rukam*) serta prospeknya sebagai kandidat obat herbal alami baru untuk penyakit hipertensi, Laporan Penelitian Dasar Ristek Dikti 2019, Universitas Sriwijaya
[10] Kshirsagar A, Purnima, A 2008 Evaluation of Calotrops gigantea flower extract of alchohol induced hepatotoxicity *J. Cell Tissue Res. 4*(19) 1551–56
[11] Aberare OL, Okuonghae P, Mukoro N, Dirisu JO, Osazuwa F, Odigie E and Omorieg R 2011 Triglycerides, total cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol in rats exposed to premium motor spirit fumes *N. Am. J. Med. Sci. 3*(6) 277–280
[12] Sheu MJ, Hsieh YY, Lai CH, Chang CC and Wu CH 2013 Antihyperlipidemic and Antioxidant Effects of C-phycocyanin in Golden Syrian Hamsters Fed with a Hypercholesterolemic Diet *J.
Tradit. Complement. Med. 3(1) 41–7

[13] Harini MD and Astirin OP 2009 Blood cholesterol level of hypercholesterolemia rat (Rattus norvegicus) after VCO treatment Nusantara Bioscience 1(2) 53-8

[14] Perla FM, Prelati M, Lavorato M, Visicchio D and Anania C 2017 The role of lipid and lipoprotein metabolism in non-alcoholic fatty liver disease Children (Basel) 4(6) 46

[15] Adams SP, Sekhon SS and Wright JM 2014 Rosuvastatin for lowering lipids Cochrane Database Syst. Rev. 11: CD010254

[16] Nurhidajah, Astuti R and Nurrahman 2019 Black rice potential in HDL and LDL profile in Sprague Dawley rat with high cholesterol diet IOP Conf. Series: Earth and Environmental Science 292 012019

[17] Bao L, Hu L, Zhang Y and Wang Y 2016 Hypolipidemic effects of flavonoids extracted from Lomatogonium rotatum Exp Ther. Med. 11(4) 1417-24

[18] Lin X, Ma L, Racette SB, Spearie CLA and Ostlund Jr RE 2009, Phytosterol glycosides reduce cholesterol absorption in humans Am. J. Physiol. Gastrointest. Liver Physiol. 296(4) 931–35