



Acute Toxicity Study of Standardized Faloak Bark (*Sterculia quadrifida* R. Br.) Extract on Wistar Rats

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ABSTRACT

The bark of Faloak (*Sterculia quadrifida* R. Br., *Malvaceae*) is used for traditional medicine in Indonesia. This study aims to examine the level of toxicity of the Faloak bark standardized ethanolic extract. The barks were collected from East Nusa Tenggara, Indonesia. TLC profile was determined by TLC-densitometry, total phenolic and flavonoid content were determined in ethanolic extract from bark of faloak (*S. quadrifida*) by UV-V is method. The acute toxicity was performed according to the TD₄₂₀ method by OECD (2001) on Wistar rats. The animals were grouped into control and treatment groups (2000 mg/kg body weight). All animals were evaluated for possible toxicity signs by measuring body weight, food and water consumption, and histopathological observation. The result of mean concentration of total phenolic content was found to be 17,69±2,01 (%GAE) and concentration of total flavonoid content was found to be 8,56 ± 0,02 (%NE). The results showed that no mortality occurs after 14 days of treatment. In general, no significant changes in animal behavior, body weight, food and water consumption compared to the control group. Histological observation found no sign of toxicity on vital organs. LD₅₀ values obtained from the acute toxicity test results for Faloak ethanol extract (*Sterculia quadrifida* R. Bark) are higher than 2,000 mg/kg BW and categorized as having a low level of toxicity.



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INTRODUCTION

Faloak (*Sterculia quadrifida* R. Br., *Malvaceae*) is a medicinal plant used by local people of Timor, East Nusa Tenggara, Indonesia, for generations. Faloak bark is used for the treatment of hepatitis, kidney, rheumatism, anemia, and restoring stamina, but the primary use is for the treatment of hepatitis (Siswadi *et al.*, 2014). A preliminary study on the bark indicates the presence of alkaloids, triterpenoids, phenolics, and saponins. Those groups of compounds may be responsible for the pharmacological activity (Rollando and Siswadi, 2016). Reported that the phenolics and flavonoids corre-

late to the macrophage stimulation activity, further confirmed by (Munawaroh *et al.*, 2020). Ethanol extract of faloak bark was also reported as a potent DPPH radical scavenger, having an IC₅₀ of 4.8101 ppm (IC₅₀ vitamin C, 3.4873 ppm) (Amin *et al.*, 2016). (Dash *et al.*, 2007) states that there is a correlation between hepatoprotective and antioxidant activity. The activity of flavonoid compounds found in the bark of the Faloak can be potential Faloak for activity hepatoprotective. (Sulistiyani and Hasim, 2004) reported that secondary metabolite compounds as flavonoids and phenolic could report function-impaired rat blood lipid peroxide when using paracetamol.

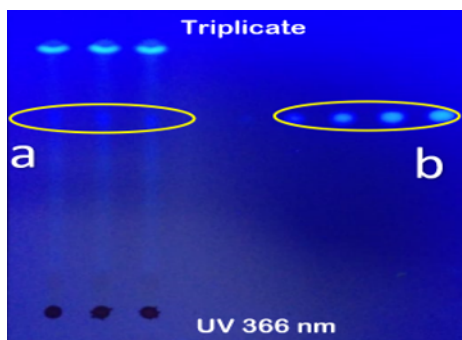


Figure 1: Chromatogram profile (a) Ethanolic extract of Faloak bark (b) Scopoletin standard. Stationary phase silica gel F₂₅₀ and mobile phase chloroform: ethyl acetate (8:2 v/v).

The decocted faloak bark powder given to male mice orally once a day for six days has reported inhibiting CCl₄ hepatotoxicity with the ED₅₀ value of 5.24 g/kg BW. (Winanta *et al.*, 2019) have reported the decocted faloak bark's immunomodulatory potential in Balb/c mice.

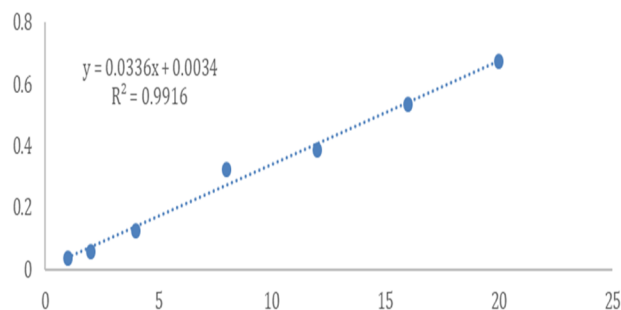


Figure 2: Calibration curve of Gallic acid for total phenol calculation.

(Siswadi and Saragih, 2018) has informed the LD₅₀ of faloak ethanolic extract was > 5,000 mg/kg BW on Sprague Dawley rats but liver necrosis was observed on the treatment groups. This toxicity study was performed on Wistar rats, according to OECD guidelines (OECD, 2001) guidelines to complement the report, as mentioned above.

MATERIALS AND METHODS

Plant material preparation

The faloak barks were obtained from Kupang, East Nusa Tenggara, Indonesia, and shipped to Yogyakarta. Dr. Joko Santosa (Dept of Pharmaceutical Biology, Faculty of Pharmacy, UGM) has identified the plant taxonomy under Reg No. UGM/FA/1917/M/03/ 02. Barks were thoroughly washed, weighed, and dried in a drying oven at 50°C to dryness. The dried barks of Faloak were ground to a fine powder by a mill machine.

Ethanolic extract preparation

A total of 600 grams of faloak powder were extracted using the maceration method with 6 liters of 96% ethanol solvent, carried out three times. The filtrates were concentrated by rotary evaporator. The % yield in the final product was 20.68 grams of red-brown solid ethanol extract.

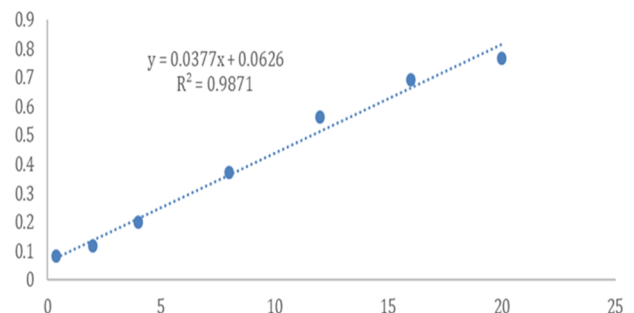


Figure 3: Calibration curve of naringin for total flavonoid content.

TLC profile

A stock solution of *S. quadricida* extract (10 mg/mL) was prepared by dissolving 1 mg extract in methanol to reach 10 mL. The sample was applied to the stationary phase of silica gel F₂₅₀ plate using densitometry and the mobile phase using chloroform: ethyl acetate (8:2 v/v). Spots were detected under UV light UV 366 nm.

Determination of total phenolic contents

The total phenolic content was determined using the Folin-Ciocalteu reagent solution (Singleton *et al.*, 1999). Gallic acid (Sigma-Aldrich, Germany) was employed as the reference standard, and the result was reported as g of gallic acid per 100 ml of extract (% GAE).

Determination of total flavonoid contents

The total flavonoid content was determined by the method described by (Chang *et al.*, 2002). Measurement of total flavonoid content was carried out using the 2,4-dinitrophenylhydrazine colorimetric method and used naringin as a standard compound.

Table 1: Observation of Toxicity Signs.

Observation	30 min	4 hour	24 hour	48 hour	7 day	14 day
Skin and Fur	N	N	N	N	N	N
Eyes	N	N	N	N	N	N
Lethargy	-	-	-	-	-	-
Convulsion	-	-	-	-	-	-
Tremor	Y	-	-	-	-	-
Diarrhea	-	-	-	-	-	-
Dead	-	-	-	-	-	-

*Exp: (N) = Normal; (-) = Not Occur; (Y) = Yes.

Naringin as much as 20 mg was dissolved in methanol and diluted to 500, 1000, and 2000 $\mu\text{g/mL}$. One milliliter of each diluted standard solution was reacted with 2mL of 1% 2,4-dinitrophenylhydrazine reagent and 2mL of methanol at 50°C for 5 minutes. After cooling at room temperature, then reacted with 5 mL of 1% potassium hydroxide in 70% methanol and incubated at room temperature for 2 minutes. Then 1 mL is taken from the solution and mixed with 5 mL methanol and centrifuged 1000x for 10 minutes to remove the sediment. The supernatant was measure at 495 nm absorbance (%NE).

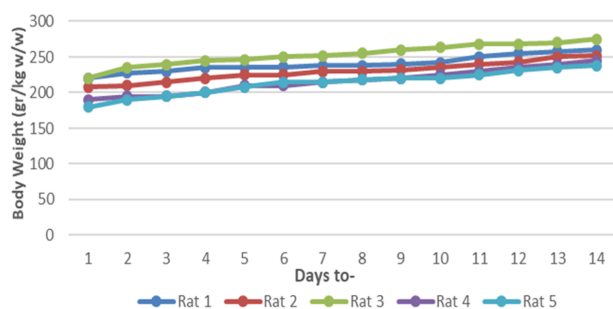


Figure 4: Bodyweight measurement following CMC 0.5%.

Experimental animal

Ten male rats (Wistar) obtained from the Pharmacology and Toxicology Laboratory, Faculty of Pharmacy, Universitas Gadjah Mada. The animals were kept in a temperature-controlled environment (22-28°C), humidity 70-80%, and with a regular 12 h light/12 h dark cycle. The adjustment was made for five days. Before being treated, the rats were fasted for 14-18 hours to avoid a reaction between the test animal feed and the test preparation. During fasting, the mice were only given water and not given food. The principal of animal handlings was followed, whereas the study design was approved by the Institutional of Animal Ethics Committee approved the use of animals and the study design (Approval No. KE/FK0363/EC/2019).

Dosage determination for toxicity study

Acute oral toxicity was carried out according to the Organization for Economic Cooperation Development (OECD) guidelines for testing chemical number 420 (OECD, 2001). The study was initiated with a preliminary study aimed to determine the dose for the acute toxicity study. Ten Wistar rats (*Rattus norvegicus*) were divided into two groups, where group 1 was the control group, and group 2 was given the graded doses of 500, 1000, 2000 mg/kg body weight. All the rats were monitored and observed once daily within 14 days. This study revealed no mortality found on rats orally administered with a 500, 1000, 2000 mg/kg BW dose. Based on this observation, the highest dose of 2000 mg/kg BW was selected for the acute toxicity study.

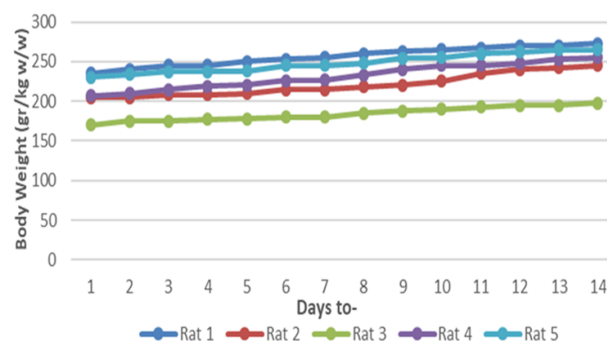


Figure 5: Bodyweight measurement following S. quadrifida extract 2000 g/kg BW.

Acute toxicity study

For the main study, Wistar rats (*Rattus norvegicus*) were randomly divided into two groups, consisting of five rats. Group 1 served as normal control and was orally given pure water and CMC 0,5%. Group 2 served as treatment and was orally given 2000 mg/kg extract. For the sighting study taken by one rat from each group and observed symptoms of toxicity within 24 hours. The rest of the rats from each group were kept for four hours after treatment and continued until 14 days. After 14 days, the rats will

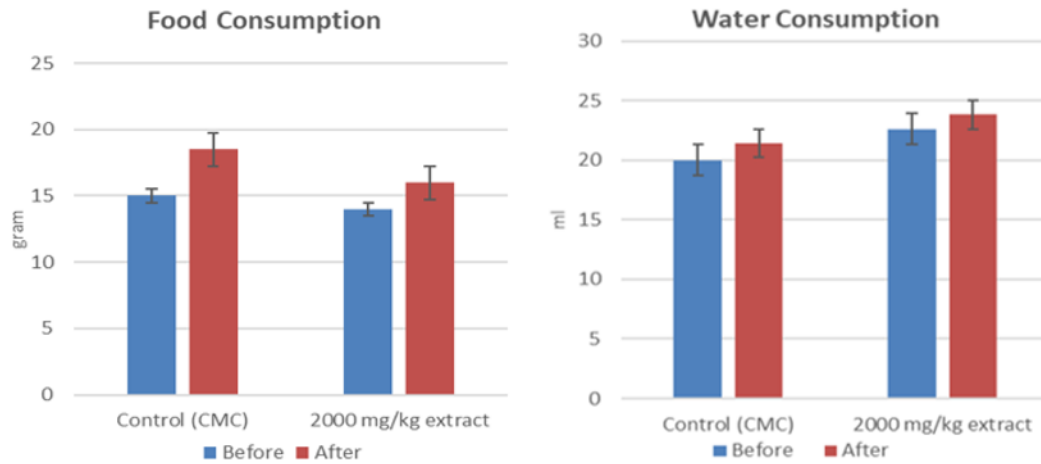


Figure 6: Observation of food and water consumption following *S.quadrifida* extract application.

be sacrificed, and the vital organ was immediately taken out and washed with NaCl, then weighed and stored in formalin 10% for histopathological observation.

Statistical Analyses

The results are expressed as a mean \pm standard of the mean. To determine whether the data were normally distributed or not, the normality test was carried out using *Shapiro Wilk* with a significance of P^3 0.05. To determine whether there is a significant difference between the control group and the 2000 mg/kg body weight dose group, if the data is normally distributed and homogeneous, the statistical test used is the Independent Sample T-test with a significance level of 0.05. If the data is not normally distributed and heterogeneous, then it is continued with non-parametric statistical tests. The statistical test used is the *Mann-Whitney* test with a significance level of 0.05.

Histopathology Observation

There are seven vital organs (heart, liver, lungs, kidneys, spleen, stomach, gut) were fixed with 10% formalin buffer solution (pH 7.4) for 24 h and dehydrated with a sequence of ethanol solution and embedded in paraffin. The serial sections were cut into 5 mm thick and stained with hematoxylin-eosin (HE), and then observed the organ change by photo microscope (*Olympus BX53* at 100 magnification).

RESULTS AND DISCUSSION

Phytochemical analyses of extract

A total of 600 g of *S.quadrifida* bark powder was macerated using 96% ethanol, and a thick extract of 20.68 g (3.46 % yield) was obtained. The TLC profiles using the mobile phase chloroform: ethyl acetate (8:2 v/v) as described in Figure 1.

Total Phenolic Determination

The quantitative determination of total phenol was carried out using Folin Ciocalteu reagent in terms of gallic acid equivalent. The Folin-Ciocalteu reagent will react with a hydroxy group on a phenolic compound to form a purple complex that can be detected with a UV-vis spectrophotometer (*Singleton et al., 1999*). In Figure 2 correlation between gallic acid concentration and its absorbance is expressed in the equation $y = 0.0336x + 0.0034$ with a correlation coefficient ($R = 0.9916$). The total phenolic was determined as 17.69 ± 2.01 %GAE.

Total Flavonoid Determination

The total flavonoid content was calculated by the naringin equivalent mg/100 mL sample (% b/b equivalent of the naringin). In this study, a standard used was naringin (a flavanone). The relationship between naringin concentration and its absorbance is expressed in the equation $y = 0.0377x + 0.0626$ with a correlation coefficient ($R^2 = 0.9871$) Figure 3. (*Winanta et al., 2019*) reported that the total flavonoid content of 96% ethanol extract from Faloak bark with height <300 meters above sea level and a diameter of 15-30 cm was 0.374% w/w QE (quercetin equivalent). These results are slightly different because the standard used is different, and also the total flavonoid measurement methods used are different. The total flavonoid content was determined as 8.56 ± 0.02 %NE.

Observation of Toxicity following sample application

Observation of signs of toxicity includes the condition of the skin and hair, eyes, lethargy (lethargy), convulsions (convulsions), tremors (trembling), diarrhea and mortality. No sign of toxicity was observed following the application of 2000 mg/kg BW of faloak ethanolic extract in Wistar rats Table 1.

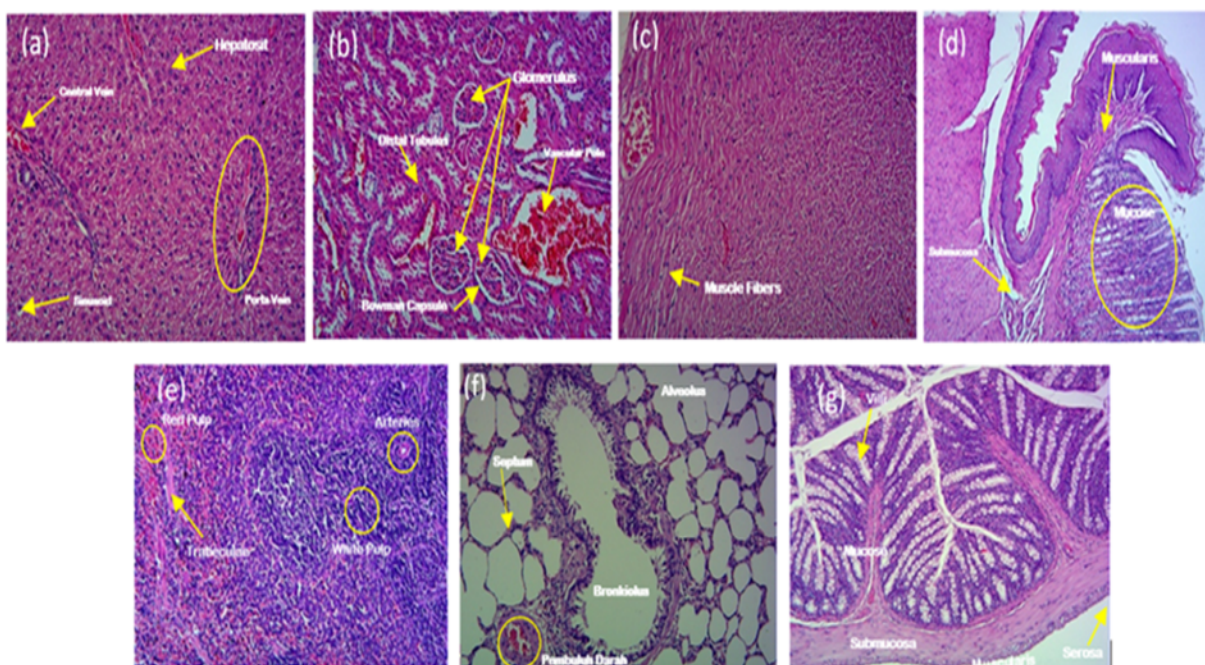


Figure 7: Histopathological observation of vital organs of experimental animals following *S. quadrifida* application, (a) Liver (x200); (b) Kidney (x200); (c) Heart (x200); (d) Stomach(x100); (e) Spleen (x200); (f) Lung (x200); (g) Gut (x100).

Measurement of Bodyweight

The mean of rat's body weight was measured daily for 14 consecutive days. The weight changes of the Wistar strain rats were analyzed using a one-way analysis of variance and showed that the weight of the Wistar rats in the control group (CMC) Figure 4 and the treatment group (administered of ethanol extract of faloak) was 2,000 mg/kg body weight Figure 5, not significantly different. All Wistar strain rats used in the toxicity test at a dose of 2,000 mg/kg body weight did not experience death so that the LD₅₀ value of the ethanol extract of faloak was more than 2,000 mg/kg body weight.

Consumption of Food and Water

The amount of food and water consumed was measured daily from the quantity of food and water supplied, and the amount remaining after 24h. It is estimated that the amount of food and water in a day the group can consume 100 grams of food and water as much as 90 ml. A profile graph of the amount of food and water averages for each group can be made to illustrate the changes over 14 days from the weighing results. All data represent the value of n=5 for each group Figure 6. These data showed that the ethanol extract of Faloak does not affect food and water consumption in experimental animals.

Histopathology

Vital organs of experimental animals (liver, heart, kidney, spleen, lung, stomach and gut) were examin-

ed macroscopically. The morphology of the internal organs was visually observed for signs of toxicity Figure 7. Hematoxylin and eosin (H&E) were used as staining. Histological examination of liver, heart, kidney, spleen, lung, stomach, and gut revealed normal morphology characteristics in all extract groups, and following 14 days of observation. In the present study, the results of histopathological observation of vital organs show no sign of toxicities. A single dose of faloak (*S. quadrifida*) ethanol extract 2000 mg/kg body weight given orally did not cause significant changes in the lungs, heart, kidney, liver, spleen, stomach and gut of the Wistar rat.

It is interesting to find out that no sign of toxicity following the sample application was observed. At the same time, (Siswadi and Saragih, 2018) reported liver necrosis on Sprague-Dawley (SD) rats following faloak ethanolic extract (200 – 5,000 mg/kg BW). The result of the toxicity study on Wistar rats (*Rattus norvegicus*) will add preclinical scientific data. The difference in results can be caused by various factors, one of which is the compound contained in the extract. Although the plant species are the same, climate change, soil, and water factors can modify the chemical composition of plants.

CONCLUSIONS

The standardized ethanol extract of faloak bark (*S. quadrifida*) has a total phenolic content 17.69 ± 2.01 %GAE and total flavonoid content 8.56 ±

0.02 %NE. The extract administrated orally 2,000 mg/kg BW caused no mortality on Wistar rats (*Rattus norvegicus*). No sign of toxicity was observed. The extract is categorized as having a low level of toxicity. Further study on the sub acute and chronic toxicity of *S.quadrifida* ethanolic extract in order to evaluate its long term effect as herbal medicine should be conducted.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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