The Nephroprotective And Antioxidant Activity of Sterculia rubiginosa Zoll. Ex Miq. Leaves

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ABSTRACT

Background: Sterculia has an antioxidant activity. The Sterculia genus has phenols and flavonoids content, and this chemical content may be have an nephroprotective activity. Objective: The study was to investigate the in vitro study of antioxidant activity with DPPH and FRAP study and nephroprotective activity of Sterculia rubiginosa Zoll. Ex Miq. Leaves extract. Materials and Methods: The leaves was extracted using ethanol. This extract was determined for antioxidant activity by in vitro study with DPPH and FRAP methods, determined the content of total phenols, total flavonoids, and also identification of chemical content. Nephrotoxicity study done by induced gentamycin. The groups dividend 6 group, consist: negative control, positive control, normal control, and the extract with dose 50 mg/kg, 100 mg/kg, and 200 mg/kg. The parameter for nephroprotective activity was tubular necrosis, the presence of tubes casts and glomerular damage, creatinine serum, and urea. Results: The ethanol extract has IC₅₀ 162.34 µg/ml for DPPH scavenging activity and 18.65 ± 3.53 FeEAC (Mol/g) for FRAP. The secondary metabolite presence flavonoids, tannins, terpenes, alkaloids, and glycosides. The total phenols 462.36 ± 9.23 mg GAE/gr, total flavonoids content 59.44 ± 0.11 mg QE/gr extract. All the dose have an nephroprotective activity, but the best dose was 50 mg/kg. Conclusion: The ethanol extract of Sterculia rubiginosa showed antioxidant activity and nephroprotective activity.

Key words: Sterculia rubiginosa Zoll Ex. Miq., Antioxidant, Nephroprotective, Gentamicin.

INTRODUCTION

The kidneys have important functions including removing waste products from the blood and regulating water fluid levels. Nephropathy is a health problem in the world. Long-term use of drugs, such as analgesics or chemotherapy, and degenerative diseases such as diabetes mellitus and hypertension are the cause of nephropathy. Several studies have reported that some natural compound compounds such as phenol, karetenoids, polysaccharide have an effect on inhibiting reactive oxygen species (ROS), which ROS will cause pathological conditions in organs, one of which is kidney. Gentamicin is an aminoglycoside antibiotic that is widely used in negative bacterial infections. Gentamicin is excreted by the kidneys and partly reabsorbed. And accumulated in the proximal membrane, which is a major part of nephrotoxicity. So that the possibility of nephrotoxicity becomes a risk in the treatment using gentamicin.

Sterculia genus contains phenol compounds, flavonoids and their derivatives, terpenoids which are mostly as triterpenoids, coumarin, alkaloids and other compounds including phenolic acids, phenyl propanoids, fatty acids, sugars and some steroids. Based on literature studies it is known that the primary production of secondary metabolites in the genus Sterculia is phenols and flavonoids. Sterculia rubiginosa Zoll. Ex Miq. is one of the plants of the genus Sterculia. This plant has been used by people in West Java, Indonesia for the treatment of asthma. Sterculia genus plants have activities. Sterculia foetida for antibacterial and hemolytic, apoptosis,Sterculia diversifolia for immunomodulatory and anti-cancer, Sterculia villosa as fibrinolytic, sedative. Sterculia tragacantha as anti-inflammatory and analgesic. So it is interesting to study whether Sterculia rubiginosa has antioxidant and nephroprotective activity.

MATERIALS AND METHODS

Materials

Sterculia rubiginosa leaves woods collected from Botanical Garden of Bogor. This plant was determined in Botany Herbarium Research Institute, Cibinong, West Java, Indonesia. Ethanol from local supplier. Kit for urea from Sigma (Singapore). TPTZ (: 2,4,6-tripyridyl-s-triazine), Dimethyl sulfoxide (DMSO), methanol pro analysis, ethyl acetate pro-analysis, n-hexane pro analysis from Merck (Germany). Gentamycin from local supplier. Some chemical reagent for identification of the compound and determined the content of total flavonoids, total phenols and antioxidant activity by DPPH (2,2-Diphenyl-1-picrylhydrazyl) method.

Extraction

The extraction was done by maceration method using ethanol solvent. Extraction done with 200 gram of leaves powder with solvent. The extract was dried with a vacuum of rotary evaporator at temperature of 50 °C and then continued in water bath at 50 °C.
Determination of total phenols content (TPC)

TPC expressed as mg Gallic acid equivalents per gram of dried extract (mg GAE/g extract). A total of 20 µl extract added with 100 µl of Folin-C Reagent (1:10), treated for 60 seconds, and then allowed to stand for 4 min. Added with 80 µl of solution of 7.5% sodium carbonate (Na₂CO₃) in water, shake for 60 seconds. This mixture is incubated at room temperature in a dark place for 2 h. Read at 600 nm. The concentration of stock solution made was 1000 µg/ml. The concentration of stock solution made was 1000 µg/ml. The control was a sample replaced with methanol. The treatment was the same as the sample. The total phenols content using gallic acid as standards. TPC calculated as the equivalence of gallic acid (mg GAE / gram). This method according to Farasat.19

**Phytochemical screening**

The extract determined the chemical compounds and the procedure according to Indonesian Herb Pharmacopoeia and Harborne.14,15 The chemical constituent identification were alkaloids, flavonoids, tannins, saponins, and anthraquinones.

**RESULTS AND DISCUSSION**

**Antioxidant activity with DPPH method**

The antioxidant activity test was performed using DPPH and FRAP. The following results obtained. The IC₅₀ was 162.34 µg/ml. Quercetin as a positive control was 5.63 µg/ml. The result of antioxidant activity by DPPH method on the Figure 1.

**Antioxidant activity with FRAP methods**

The antioxidant activity test was performed using FRAP. The extract has antioxidant activity 18.65 ± 3.53 FeEAC(mol/g) and the positive control (quercetine) 1201.61 ± 77.89 FeEAC(mol/g). The result of antioxidant activity on the Figure 2.

**Determination of total phenols and total flavonoids**

Quercetin levels calculated as total flavonoid levels in the sample. Gallic acid levels calculated as total phenols levels in the sample. The result showed on Table 1. The total phenols in the extract was high than the flavonoids.

**Phytochemical screening**

The flavonoids, glycosides, alkaloids, tannins, terpenes, and saponins were presence in the extract and negative to anthraquinone. The test results showed in Table 2.
Figure 1: Antioxidant activities of extract *S. rubiginosa* leaves by DPPH methods.

Figure 2: Antioxidant activities of extract *S. rubiginosa* leaves by FRAP methods.

Figure 3: The creatinine serum.

Table 1: Total phenols and total flavonoids.

| Parameters       | Content            | Sd   | kV   |
|------------------|--------------------|------|------|
| Total Phenols    | 462.36 GAE/g extract | 9.23 | 1.99 |
| Total Flavonoids | 59.44 QE/g extract   | 0.11 | 0.96 |
significant differences with negative controls. Doses 200 mg/kg did not show significant differences with positive control, which means that the ability as a nephroprotective was the same as positive control. Whereas for dosages 50 and 100 mg/kg also showed no significant difference with positive control, but showed a significant difference with a dose of 200 mg/kg.

Urea serum

Urea serum showed that a dose of 50 mg/kg has the lowest creatinine level. The results of the average levels of each group showed in the figure 4.

The normality test carried out with the Kolmogorov-Smirnov test and the Sig. > 0.05 (p > 0.05). The Urea levels normally distributed. The Levene, the significant 0.124 (p > 0.05), the data homogeneously distributed. One way ANOVA statistical analysis test shows there is a significant difference between treatment groups on urea. Post hoc use Tukey analyze. The results showed that all doses have nephroprotective activity as seen from the existence of significant differences with negative controls. The dose 50 mg/kg did not show a significant difference with positive control, which means that the ability as a nephroprotective was the same as positive control whereas the highest dose of 200 mg/kg shows a decrease in activity as a nephroprotective.

Histopathology

The kidney structure observed the distance between the bowman capsule and glomerulus, tubular necrosis and the presence of casts. According to the results of research Pragati et al. Kidney damage caused by administration of gentamicin, one of which is the formation of casts, casts are a collection of proteins that result in inhibited channeling through renal tubules, also stimulates the occurrence of casts, casts are a collection of proteins that result in inhibited channeling through renal tubules, also stimulates the occurrence of tubular necrosis. 17

The accumulation of gentamicin in the kidneys, especially in proximal tubular cells, can cause oxidative stress, resulting in excessive ROS. The ROS can cause oxidative damage to mitochondria and plasma membranes, increased creatinine, urea, and uric acid may be related to loss of glomerular filtration, mesangial cell proliferation, and apoptosis induced by gentamicin. In our study, gentamicin caused kidney dysfunction, this marked by increased levels of creatinine, urea, and this similar with previous studies. 16,19,20,21 In this study, administration of Sterculia rubiginosa leaf extract significantly reduced creatinine, urea and kidney tissue damage levels. The antioxidant activity of Sterculia rubiginosa leaf extract was carried out in vitro by the FRAP and DPPH methods. The FRAP test was based on the ability of the phenol to reduce the yellow color of ferric tripyridyltriazine (Fe (III) -TPTZ) to the blue color of the ferro (Fe (II) -TPTZ complex) by antioxidant activity that contributes to electrons. The blue color produced was measured spectrophotometrically at 593. Ferric salt was used as an oxidant and its redox potential (<0.70 V), the FRAP. 22

The test required an acidic condition (non-physiological, mol of Fe (III) to Fe (II)). Previous studies conducted by previous researchers learned that treatment with medicinal plant antioxidants significantly prevented elevated creatinine levels and gentamicin-induced kidney damage. And the results obtained that phenolic compounds, flavonoids have antioxidant activity that is possibly responsible for the activity of nephroprotectors. 18,23

Kidney damage was also observed in proximal tubular necrosis. Figure 5, the casts are channeling through renal tubules, also stimulates the occurrence of necrosis in the tubules. The results showed in Figure 5, the casts are in the negative control. In treatments except normal control, founded changes in the form of proximal tubule.

Necrosis tubules

The results of the calculation of tubular necrosis showed in figure 5.

The statistical test results show that the data not homogeneously distributed The test conducted with non-parametric analysis Mann Whitney. The results of the non-parametric analysis showed on Table 3.

The distance between glomerulus and Bowman capsules

Kidney is one of the organs filled with blood vessels. If endothelial cells in blood vessels have been damaged by free radicals, then the possibility of kidney function will decrease. An imbalance in the amount of free radicals and antioxidants causes oxidative stress which causes atrophy in the glomerulus and proximal tubular necrosis. 17 In this study atrophy of the glomerulus was observed by measuring the distance between the Bowman capsule and the glomerulus. Based on observations of the distance between Bowman capsules and renal glomerulus of rats induced by gentamicin can be seen in the graph below (Figure 7):

Table 2: The chemical content of extract S. rubiginosa.

| Chemical constituents | Result |
|-----------------------|--------|
| Alkaloids             | +      |
| Flavonoids            | +      |
| Terpenes              | +      |
| Tannins               | +      |
| Glycosides            | +      |
| Anthraquinones        | -      |

Note: + = Presence, - = Absence

Table 3: The non-parametric analysis of tubulus necrosis.

| Groups    | Groups   | Sig.  |
|-----------|----------|-------|
| Normal    | Positive*| .020  |
|           | Negative*| .021  |
| 50 mg/kg  | .021     |
| 100 mg/kg | .043     |
| 200 mg/kg | .021     |
| Positif   | Negative mg/kg | .020 |
|           | 50 mg/kg  | .020  |
|           | 100 mg/kg | 1,000 |
|           | 200 mg/kg | .020  |
| Negatif   | 50 mg/kg  | .564  |
|           | 100 mg/kg | .021  |
|           | 200 mg/kg | .021  |
| 50 mg     | 100 mg/kg | .021  |
| 100 mg    | 200 mg/kg | .021  |

*= significant differences

Table 4: The urea serum.

| Group   | Urea (mg/dl) |
|---------|--------------|
| Normal  | 15.85        |
| Positive* | 23.4       |
| Negative | 25.5        |
| 50 mg/kg | 34.11       |
| 100 mg/kg| 41.78       |
| 200 mg/kg| 43.78       |

Figure 4: The urea serum.
**Figure 5:** Histopathology kidney (A) Normal control, (B) Positive control, (C) Negative control, (D) dose 50 mg/kg, (E) Dose 100 mg/kg, (F) dose 200 mg/kg, (G) Necrosis cell, (H) Narrowing / closing proximal tubules, (I) Casts.

**Figure 6:** The Tubulus necrosis.

**Figure 7:** The Distance between Glomerulus and Bowman Capsules.
CONCLUSION

The ethanol extract of *Sterculia rubiginosa* has nephroprotective and antioxidant activity. This extract potential to continue for another research to find the most active chemical constituent who is responsible to this activity.

ETHICAL ISSUES

This study was permitted by Ethic committee with number KEPK-UHAMKA 02/19.06/44.

CONFLICTS OF INTEREST

All the authors declare there is no conflicts of interest.

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REFERENCES

1. N. Tili, A. Feriani, E. Saadouri, N. Nasri, and A. Khalidi. Capparis spinosa leaves extract : Source of bioantioxidants with nephroprotective and hepatoprotective effects. Biomed Pharmacother. 2017;87:71-179.

2. M. Veljkovi, D. R. Pavlovi, N. Stojiljkovi, S. Ili, I. Jovanovi, V. Raki, and L. Veli. Bilberry : Chemical Profiling , in Vitro and in Vivo Antioxidant Activity and Nephroprotective Effect against Gentamicin Toxicity in Rats. Phytoterapi Research. 2016;1-12.

3. S. N. A. Saleh, M. el , Alia Yassin, Kaseem Mona el said, Marzouk mona mohammad, Mishara al Salwa ali. Phytochemistry, biological activities and economical uses of the genus Sterculia and the related genera: A review. Asian Pacific J Trop Dis. 2016;6:492-501.

4. A. A. Braga and R. Rodrigues, Gracy, Gregorio, Hilzet, et al. Antibacterial and Hemolytic Activity of a new Lectin Purified from the Seeds of Sterculia Foetida L. Appl Biochem Biotechnol. 2015;175:1689-99.

5. A. Jafri, S. Bano, J. Rais, F. Khan, and N. Shivnath. Phytochemical screening , safety evaluation , anti-inflammatory and analgesic studies of the leaf extracts of *Sterculia tragacantha*. J Complement Integr Med. 2016;2-7.

6. O. M. Mogbouiri, A. A. Aidedapo, and M. O. Abatan. Phytochemical screening , safety evaluation , anti-inflammatory and analgesic studies of the leaf extracts of *Sterculia tragacantha*. J Complement Integr Med. 2016;2-7.

7. A. Karadag, B. Ozcelik, and S. Saner. Review of Methods to Determine Total Flavonoid and Total Phenolic Contents of some Edible Green Seaweeds from Northern Coasts of the Persian Gulf. Iran. J Pharm Res. 2014;13(1):163-70.

8. F. Hossain, B. Talukder, M. N. Rana, R. Tasnim, T. S. Nipun, S. M. N. Uddin, and S. M. M. Hossen. In vivo sedative activity of methanolic extract of *Sterculia villosa* Roxb . leaves. BMC Complement. Altern. Med. 2016;16:10-13.

9. N. Aiswarya, R.V. Chandran, S. Teerthanath, P. Rashmi, and J. Preethi. Nephroprotective Effect of Aqueous Extract of Pimpinella anisum in gentamicin-induced acute renal toxicity in swiss albino mice. 2016;6:643-652.

10. S. Shiyan and L. R. Sari. Nephroprotective Effect of Anthocyanin Pigments Extract from Red Cabbage (Brassica oleracea L. var Capitata F. Rubra) against *Sterculia foetida* L. Appl Biochem Biotechnol. 2015;175:1689-99.

11. A. J. Figueira, J. A. M. Pereira, P. Porto-Figueira, and J. S. Câmara. Analysis Ultrasound-assisted liquid-liquid extraction followed by ultrahigh pressure liquid chromatography for the quantification of major carotenoids in tomato. J Food Compos Anal. 2017;87:87-93.

12. N. Aiswarya, R.V. Chandran, S. Teerthanath, P. Rashmi, and J. Preethi. Nephroprotective Effect of Aqueous Extract of Pimpinella anisum in gentamicin-induced acute renal toxicity in swiss albino mice. 2016;6:643-652.

13. J. Harborne, A Guide to Modern Techniques of Plant Analysis. Phytochemical Methods. Chapman and Hall London. 1998.

14. Anonymous., Farmakope Herbal Indonesia. Ed I., Departemen Kesehatan Republik Indonesia (Department of Health of RI) Jakarta, Indonesia. 2008.

15. G. Bobo-garcía, G. Davidov-pardo, C. Arroqui, and M. R. Marín-arroyo. Intra- vascular permeability and lymphatic function of the intestinal ileum in rats. Gen. J Diet Suppl. 2017;1-14.

16. M. Farasat and R. Khavari-nejad. Antioxidant Activity , Total Phenolics and Flavonoid Contents of some Edible Green Seaweeds from Northern Coasts of the Persian Gulf. Iran. J Pharm Res. 2014;13(1):163-70.

17. M. Tlili, A. Feriani, E. Saadoui, N. Nasri, and A. Khaldi. Capparis spinosa leaves extract : Source of bioantioxidants with nephroprotective and hepatoprotective effects. Biomed Pharmacother. 2017;87:71-179.

18. A. A. Braga and R. Rodrigues, Gracy, Gregorio, Hilzet, et al. Antibacterial and Hemolytic Activity of a new Lectin Purified from the Seeds of Sterculia Foetida L. Appl Biochem Biotechnol. 2015;175:1689-99.

19. O. M. Mogbouiri, A. A. Aidedapo, and M. O. Abatan. Phytochemical screening , safety evaluation , anti-inflammatory and analgesic studies of the leaf extracts of *Sterculia tragacantha*. J Complement Integr Med. 2016;2-7.

20. G. Bobo-garcía, G. Davidov-pardo, C. Arroqui, and M. R. Marín-arroyo. Intra- vascular permeability and lymphatic function of the intestinal ileum in rats. Gen. J Diet Suppl. 2017;1-14.

21. A. A. Braga and R. Rodrigues, Gracy, Gregorio, Hilzet, et al. Antibacterial and Hemolytic Activity of a new Lectin Purified from the Seeds of Sterculia Foetida L. Appl Biochem Biotechnol. 2015;175:1689-99.

22. F. Hossain, B. Talukder, M. N. Rana, R. Tasnim, T. S. Nipun, S. M. N. Uddin, and S. M. M. Hossen. In vivo sedative activity of methanolic extract of *Sterculia villosa* Roxb . leaves. BMC Complement. Altern. Med. 2016;16:10-13.

23. N. Tlili, A. Feriani, E. Saadoui, N. Nasri, and A. Khaldi. Capparis spinosa leaves extract : Source of bioantioxidants with nephroprotective and hepatoprotective effects. Biomed Pharmacother. 2017;87:71-179.

24. O. M. Mogbouiri, A. A. Aidedapo, and M. O. Abatan. Phytochemical screening , safety evaluation , anti-inflammatory and analgesic studies of the leaf extracts of *Sterculia tragacantha*. J Complement Integr Med. 2016;2-7.

25. G. Bobo-garcía, G. Davidov-pardo, C. Arroqui, and M. R. Marín-arroyo. Intra- vascular permeability and lymphatic function of the intestinal ileum in rats. Gen. J Diet Suppl. 2017;1-14.

26. M. Farasat and R. Khavari-nejad. Antioxidant Activity , Total Phenolics and Flavonoid Contents of some Edible Green Seaweeds from Northern Coasts of the Persian Gulf. Iran. J Pharm Res. 2014;13(1):163-70.

27. A. A. Braga and R. Rodrigues, Gracy, Gregorio, Hilzet, et al. Antibacterial and Hemolytic Activity of a new Lectin Purified from the Seeds of Sterculia Foetida L. Appl Biochem Biotechnol. 2015;175:1689-99.
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