The Antioxidant Activity of Sterculia stipulata Korth Woods and Leaves by FRAP Method

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ABSTRACT
Background: Phenol compounds and flavonoids are known have antioxidant activity. Sterculia genus has secondary metabolite rich of phenols and flavonoids. Objective: The aim of this study was to assess the antioxidant activity of Sterculia stipulata Korth. Woods and leaves by FRAP method. Materials and methods: Extraction done using n-hexane, ethyl acetate, and methanol. The methanol extract was determined antioxidant activity using the FRAP method and also determined the total phenols content, total flavonoids, and phytochemical screening. Results: The antioxidant activity of wood extract was 4.74 ± 1.03 FeEAC (mol/g) while leaves extract 41.17 ± 1.99 FeEAC (mol/g). Total phenols content for wood extract 16.46 ± 3.51 mg GAE/g, for leaves extract 141.62 ± 10.54 mg GAE/g. The total flavonoids content for woods extract was 27.99 ± 0.62 mg QE/g for leaf extract 41.45 ± 5.83 mg QE/g. The compounds of woods and leaves are the same; it is consist of terpenoids, alkaloids, phenols, flavonoids, saponins, terpenoids, and negatives for anthraquinone. Conclusion: The antioxidant activity of the leaves of Sterculia stipulata Korth. is greater than its wood activities. Keywords: Antioxidant; Sterculia stipulata Korth.; FRAP; Phenols; Flavonoids

INTRODUCTION
The genus Sterculia rich of metabolite secondary flavonoids and phenolic compounds. The phenolic compounds such as phenolic acid, propanoic acid, flavonoids, lignans, and lignin with much smaller amount than flavonoids.1 The iron-reducing antioxidant power (FRAP) test was a method used to determine antioxidant activity by reducing Fe (III) to Fe (II) by complexation with 2,4,6-Tripyridyl-s-Triazine (TPTZ). The test is carried out at acidic pH 3.6 to maintain iron solubility.2 FRAP value obtained with comparing changes in the absorption of the test mixture at 593 nm with the mixture, which contains Ferrozine whose concentration is known.2-3,6 The FRAP test based on electron transfer. FRAP cannot detect compounds that work by radical reduction (hydrogen transfer). Method FRAP testing is simple, fast, inexpensive, and requires no special equipment.2 In this study, the antioxidant activity of S. Stipulata determined. Antioxidants play an important role in the prevention and treatment of metabolic disorders caused by oxidative stress.8 Some sterculia genus plants are known to have antioxidant activity, including methanol extract Sterculia foetida stems with IC50 values of 20 μg/ml.1 Sterculia villosa bark ethyl acetate fraction with IC50 23.99 μg/ml.8

MATERIALS AND METHODS
Material
TPTZ, (2,4,6-tripyridyl-s-triazine) from Sigma (Singapore), Hexane p.a, ethyl acetate p.a, and methanol p.a from Sigma (Singapore). Solvent from local supplier. Quercetine from Sigma (Singapore). Sterculia stipulata leaves and woods from Botanical Garden of Bogor and determined in Botany Herbarium Research Institute, Cibinong, West Java. The voucher specimen for Sterculia stipulata VIII.G.199.

Extraction
Woods and leaves of Sterculia stipulata extracted by n-hexane, ethyl acetate, and the final solvent was methanol. Extraction by maceration method. The methanol extract identified the antioxidant activity and also the phenols total and flavonoids total.

Antioxidant activity with FRAP
The antioxidant activity with the FRAP method for sample preparation was carried out by five (5) mg sample (extract/quercetine) of 5 mg dissolve in 2 ml methanol p.a, (Concentration to 2500 μg/ml). The AFS solution was diluted and stocked with various concentration (1200; 600; 300; 150; 75; and 37.5 μM). The absorbance measured at a wavelength of 593 nm. As a blank methanol used to replace the sample. Solvent for TPTZ was methanol 300 μl. The absorbance measured at a wavelength of 593 nm. As a blank methanol used to replace the sample, which contains of 30 μl methanol and 270 μl FRAP reagent. The plate blank contains methanol 300 μl. FRAP reagent = 10: 1 reagent (buffer acetate: TPTZ: FeCl3.6H2O = 10:1:1) shaken and incubated at 37 °C for 30 min. The absorbance measured at a wavelength of 593 nm. As a blank methanol used to replace the sample, which contains of 30 μl methanol and 270 μl FRAP reagent. The plate blank contains methanol 300 μl. FRAP reagent = 10: 1 reagent (buffer acetate: TPTZ: FeCl3.6H2O). The standard curve used AFS. This method refers to the research of Pereira et al. and Wong et al.10 AFS solution as a calibration curve. AFS standard solution used 1.200 μM. The AFS solution was diluted and stocked with various concentration (1200; 600; 300; 150; 75; and 37.5 μM). The formula used to calculate antioxidant activity is according to Wong et al.

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Based on the formula, FeEAC was the equality of ferric ions with antioxidant activity (µmol/g). ΔA = absorbance of samples that have been reduced by blank. GRAD (M⁻¹) was the gradient of the AFS calibration graph. Av = total volume for the test (300 µl), Spv = sample volume (30 µl), Cext = concentration of sample stock, weight (gram) in volume (g/l), D = dilution factor for sample before analysis (D = 1 if sample not diluted). GRAD (gradient) determined from the calibration curve on AFS.

**RESULTS AND DISCUSSION**

### Total Phenols Content

Determination of total phenols content in the extract determined using the method described by Farasat et al.²² Twenty (20) µl of methanol extract added with 20 µl of added AlCl₃.6H₂O 10%, then 20 µl sodium acetate 1 M and 180 µl aquadest. The solution was shaken 60 seconds and incubated at room temperature for 30 min. The color intensity read at 415 nm. The concentration of the sample was 100 µg/ml. As a blank, AlCl₃ was added with aquadest. The calibration curve was used quercetine. The total flavonoids determined as equality with quercetine (mg QE/gram). The calibration curve used quercetine, and treatment was the same as the sample, only replaced with quercetine in various variations (100; 50; 25; 12.5; 6.25; 3.75 µg/ml).

### Flavonoids Content

Determination of total flavonoids content in leaves of *Sterculia stipulata* showed that the total flavonoids content in wood was greater than that found in the leaves. The results showed in Table 1.

### Antioxidant activity with FRAP (Ferric reducing antioxidant power)

In testing antioxidant activity using the FRAP method using the AFS comparison. The results of the regression curve for AFS standard chart results shown in the Figure 1.

The antioxidant activity with the FRAP method gives the results that wood extract has a greater activity compared leaves extract 41.17 ± 1.99 FeEAC (mol/g), while for woods it was 4.74 ± 1.03 FeEAC (mol/g). This value is still far compared to the positive control quercetine of 1201.61 ± 77.89 FeEAC (mol/g). The antioxidant activity showed on Table 3.

### Phytochemical screening

Identification of the content of chemical compounds is carried out on terpenoids/steroids, alkaloids, tannins, flavonoids, saponins and anthraquinones. The results of their identification showed on Table 4.

For the result of chemical content showed that there is no differences between woods and leaves.

The FRAP test 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 used as an oxidant and its redox potential (<0.70 V). The FRAP test required an acidic condition (non-physiological, low pH value = 3.6) to maintain iron solubility. One FRAP unit defined as a reduction of 1 mol of Fe (III) to Fe (II) \( \Delta A \). The advantages of FRAP Test are simple, fast, cheap, and strong and do not require special equipment. FRAP tests can done using automatic, semi-automatic, or manual methods.²³ Fe (II) was a prooxidant that can react with H₂O₂ to produce hydroxyl radicals (•OH), dangerous free radicals found in vivo. The ability of compounds to produce Fe (II) from Fe (III) is defined as "antioxidant strength" in the FRAP test. The ability to reduce Fe (III) is possible reflecting the ability to reduce reactive species. However, not all reductants capable of reducing Fe (III) are antioxidant.²⁴ The total phenols, total flavonoids and antioxidant activity in leaves better than woods. Phenols and flavonoids have an antioxidant activity.²⁵

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**Table 1: Phenols total of *Sterculia stipulata* woods and leaves.**

| No | Extract | Phenols total (mg GAE/g) extract | kv |
|----|---------|---------------------------------|----|
| 1  | Leaves  | 141.62 ± 10.54                  | 16.47 |
| 2  | Woods   | 16.46 ± 3.51                    | 10.70 |

**Table 2: Flavonoids total of *Sterculia stipulata* woods and leaves.**

| No | Extract | Flavonoids Total (mg QE/g) Extract | kv |
|----|---------|----------------------------------|----|
| 1  | Leaves  | 41.45 ± 5.83                     | 9.60 |
| 2  | Woods   | 27.99 ± 0.62                     | 14.69 |

**Table 3: Antioxidant activity with FRAP.**

| Extracts | Antioxidant Activity FeEAC (mol/g) | kv |
|----------|-----------------------------------|----|
| Leaves   | 41.17 ± 1.99                      | 5.42 |
| Woods    | 4.74 ± 1.03                       | 3.67 |
| Quercetine | 1201.61 ± 77.89                 | 6.48 |

**Figure 1: AFS standart.**
The leaves of *Sterculia stipulata* have an antioxidant activity better than woods, and total phenols and flavonoids total more higher than the woods.

**CONFLICT OF INTEREST**

All the authors declare there is no conflict of interest.

**ABBREVIATIONS**

S: *Sterculia*; TPTZ: 2,4,6-tripyridyl-s-triazine; FRAP: Ferric Reducing Antioxidant Power; AFS: Ammonium ferrous sulfate

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**CONCLUSION**

The leaves of *Sterculia stipulata* have an antioxidant activity better than woods, and total phenols and flavonoids total more higher than the woods.
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