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Original Article

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

Rini Prastiwi[1,2], Berna Elya[1,*], Muhammad Hanafi[3,4], Yesi Desmiaty[2], Rani Sauriasari[5]

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 of the activity antioxidants 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

Rini Prastiwi[1,2], Berna Elya[1,*], Muhammad Hanafi[3,4], Yesi Desmiaty[2], Rani Sauriasari[5]

[1]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy Universitas Indonesia, Depok 16424, West Java, INDONESIA.
[2]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy and Science Muhammadiyah Prof. Dr. Hamka University, 1340 Jakarta, INDONESIA.
[3]Research Center for Chemistry, Indonesian Institute of Science, Serpong (LIPI Indonesia), INDONESIA.
[4]Department of Pharmacognosy Phytochemistry, Faculty of Pharmacy Universitas Pancasila, Jakarta, West Java, INDONESIA.
[5]Department of Pharmacology, Faculty of Pharmacy Indonesia University, Depok 16424, West Java, INDONESIA.

Correspondence

Berna Elya

Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy Universitas Indonesia, Depok 16424, West Java, INDONESIA.

E-mail: Berna.elya@gmail.com

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INTRODUCTION

The genus *Sterculia* rich of metabolite secondary flavonoids and phenolic compounds. The phenolic compounds such as phenolic acid, propanoic phenyl, coumarins, lignans, and lignin with much smaller amounts 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 Ferro ions whose concentration is known.[3-5] 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.[6] Some *sterculia* genus plants are known to have antioxidant activity, including methanol extract *Sterculia foetida* stems with IC_{50} values of 20 µg/ml.[7] *Sterculia villosa* bark ethyl acetate fraction with IC_{50} 23.99 µg/ml.[8]

MATERIALS AND METHODS

Material

TPTZ (2,4,6-tripyridyl-s-triazine) from Sigma (Singapore), Hexane pa, ethyl acetate pa, and methanol pa 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 fot *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). From The stock made different concentration for determined FeEAC. Piping 30 µl samples into the well. The sample dissolved in methanol. Then added 270 µL FRAP (reagents Buffer: 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: 1 reagent (buffer acetate: TPTZ: FeCl3.6H2O). The standard curve uses AFS. This method refers to the research of Pereira *et al.* and Wong *et al.*[9,10]

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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$$FeEAC = \frac{\Delta A}{GRAD} \times \frac{Av}{Spv} \times D \times \frac{1}{Cext} \times 10^5$$

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), C_{ext} = 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.

Determination of total flavonoids content

The total flavonoids content determined by the method described by Farasat *et al.*[11] 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, $AlCl_3$ replaced with aquadest. The calibration curve used was 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).

Determination of total phenols content

The total phenol content in the extract determined using the method from Farasat *et al.*, with slight modifications. Twenty (20) µl of extract was added with 100 µL of Folin-C Reagent (1:10), shaking for 60 seconds and allowed to stand for 4 minutes. Added with 80 µl solution of Sodium carbonate (Na_2CO_3) 7.5% in water, shaking for 60 s. This mixture was incubated at room temperature in a dark place for 2 hours. Furthermore, it read at 600 nm. The concentration of extract in the sample made at 100 µg/ml. The concentration of stock solutions was 1000 µg/ml. Blank was samples replaced with methanol. The treatment was the same as sample. In total phenols determination using gallic acid as the standard, total phenols calculated as equality of gallic acid (mg GAE/gram).

RESULTS AND DISCUSSION

Total phenols compound

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

Flavonoids compound

Determination of total flavonoids levels in *Sterculia stipulata* results in greater flavonoids content in leaves compared to wood. The results showed in Table 2.

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.

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

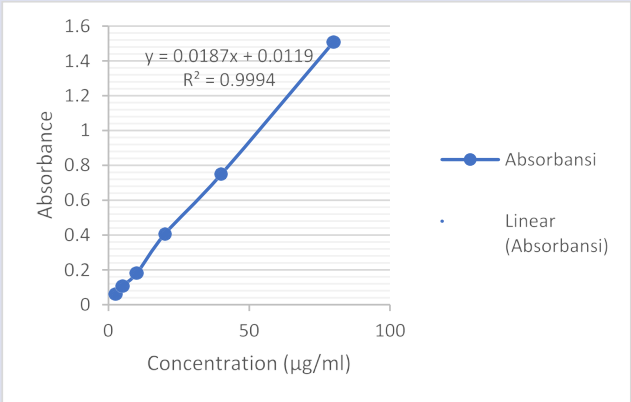

Figure 1: AFS standart.

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)).[10,12] 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.[2] Fe (II) was a prooxidant that can react with H_2O_2 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.[13-15] The total phenols, total flavonoids and antioxidant activity in leaves better than woods. Phenols and flavonoids have an antioxidant activity.[11]

Table 4: Identification chemical compound of *Sterculia stipulata* extract.

Chemical compound	leaves	Woods
Terpenoids/steroids	Terpenoid +	Steroid +
Alkaloids		
Dragendorff	+	+
Mayer	+	+
Tanins		
FeCl3	+	+
Folin	+	+
Gelatine	+	+
Flavonoids	+	+
Antraquinons	-	-
Saponins	+	+

Note : + = presence, - = absence

CONCLUSION

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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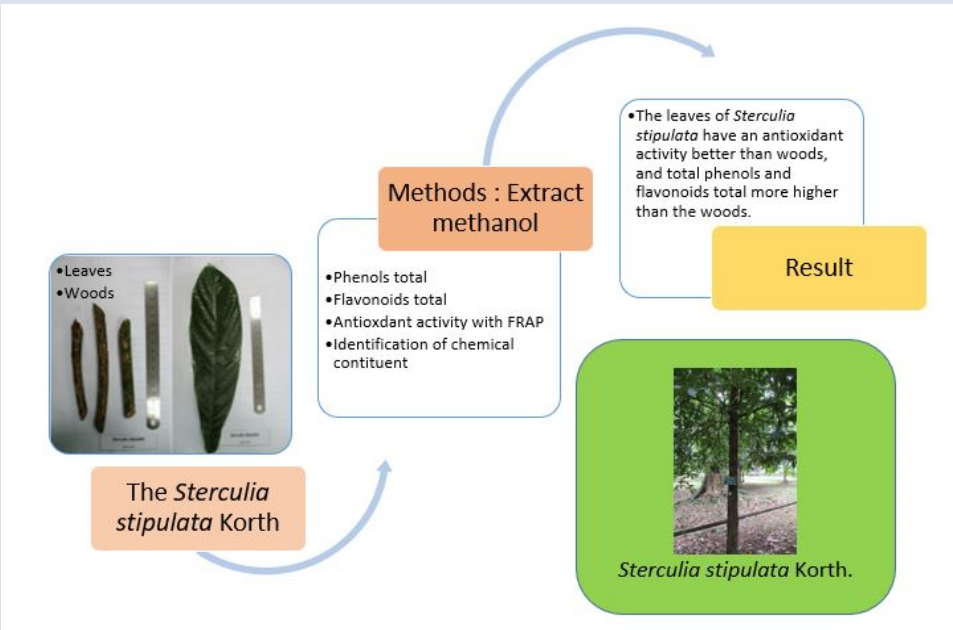
GRAPHICAL ABSTRACT



ABOUT AUTHORS

Rini Prastiwi: Lecturer and researcher at Faculty of Pharmacy and Sciences, Universitas Muhammadiyah Prof. Dr. Hamka, Klender, Jakarta 13460, Indonesia, and student at Faculty of Pharmacy, Universitas Indonesia.

Berna Elya: Lecturer and researcher at Faculty of Pharmacy Universitas Indonesia, Depok 16424, West Java, Indonesia.

Rani Sauriasari: Lecturer and researcher at Faculty of Pharmacy Universitas Indonesia, Depok 16424, West Java, Indonesia.

Muhammad Hanafi: Researcher at Research Center for Chemistry, Indonesian Institute of Science, Serpong, (LIPI) Indonseia and lecturer at Faculty of Pharmacy Universitas Pancasila, Depok, West Java, Indonesia.

Yesi Desmiaty: Lecturer and researcher at Faculty of Pharmacy Universitas Pancasila, Depok, West Java, Indonesia and student at Faculty of Pharmacy, Universitas Indonesia.

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