Pharmacogn J. 2018; 10(3):571-575.
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

Pharmacognosy, Phytochemical Study and Antioxidant Activity of *Sterculia rubiginosa* Zoll. Ex Miq. Leaves

Rini Prastiwi[1,2*], Berna Elya[2], Rani Sauriasari[3], Muhammad Hanafi[4], Ema Dewanti[1]

ABSTRACT

Introduction: *Sterculia rubiginosa* Zoll ex.Miq leaves have been used as traditional medicine in Indonesia. There is no report about pharmacognosy and phytochemical study with this plant.**Objective:** The main aim of this research is to establish pharmacognosy, phytochemical study and antioxidant activity of *Sterculia rubiginosa* Zoll.ex. Miq. Leaves. The plant used to cure many diseases of Indonesia. **Methods:** In the present study, pharmacognosy and phytochemical study of plant material were performed as per the Indonesian Herb Pharmacopoeia. **Results:** Microscopy powder of *Sterculia rubiginosa* Zoll.ex. Miq. Leaves shows star shape trichoma as a specific fragment. Physicochemical parameters including total ash (17.152 %), acid-insoluble ash (0.922 %), water-soluble extractive (1.610 % w/w), alcohol-soluble extractive (4.524 % w/w), hexane-soluble extractive (4.005 % w/w), and ethyl acetate-soluble extractive (3.160 % w/w) were evaluated. Phytochemical screening of ethanol extracts showed the presence of tannins, flavonoids, alkaloids, steroids-terpenoids, glycosides, and phenols. And absent of saponins and Anthraquinones. Antioxidant activity with IC_{50} 157, 4665 ppm and flavonoid total was 59.436 mg/g quercetin equivalent. **Conclusion:** The pharmacognosy, physiochemical, and phytochemical evaluation provides information for the safety, identification, and class of chemical constituent's presents in this crude extract.

Key words: *Sterculia rubiginosa* zoll. ex Miq. ,Pharmacognosy, Phytochemical, Antioxidant.

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History

- Submission Date: 02-01-2018;
- Review completed: 23-03-2018;
- Accepted Date: 27-03-2018

DOI : 10.5530/pj.2018.3.93

Article Available online
http://www.phcogj.com/v10/i3

INTRODUCTION

Indonesia Nation is rich in flora diversity. Many native plants of Indonesia that still need development for treatment, one of them from the genus *Sterculia*. The genus was present in tropical and sub-tropical regions, with a number of species 1100. The tree or shrub with simple leaves with flat or toothed edges, unisexual flowers, with 5 petals, some of which have an unpleasant odor as an example of *Sterculia foetida* in Indonesia is known by the name of *Kepuh*. Indonesia has native plants of the genus sterculia such *Sterculia hyposticta* Miq. (*Flora Indiae Batavae*, *Primum Supplementum, Prodromus Florae Sumatranae*) located in Sumatra. *Sterculia macrophylla* Vent (*Hildegardia macrophylla* (Vent.) Schott and Endl.) was present in Sumatra, Maluku, and Papua. *Sterculia parkinsonii* F.Muell was found in Papua, *Sterculia rubiginosa* Zoll. ex Miq. was in Sumatra. *Sterculia stipulate* Korth. (*Clompanus stipulate* Kuntze) was found in Borneo. *Sterculia spectabilis* (Welw.) Robert / *Sterculia spectabilis* Miq. (*Heritiera spectabilis* Baill) found in Sumatra.[1] The genus *Sterculia* contains flavonoid compounds and their derivatives, terpenoids mostly as triterpenoids, coumarins, alkaloids and other compounds such as phenolic acids, phenyl propanoids, fatty acids, sugars and some steroids.[2] From the literature study obtained data that the main product of secondary metabolites in the genus of *sterculia* was flavonoids. *Sterculia rubiginosa* Zoll. ex Miq. is one of the nutritious crops in traditional medicine that needs to be done pharmacognosy and phytochemical studies with some specific and nonspecific parameters. The pharmacognosy study can be used as the initial reference to complete the monograph data extract. In addition, it can be a description of quality standards that ensure that the simplicia as ingredients and medicinal products meet the requirements of Quality-Safety-Efficacy.[3] A variety of pharmacological activities of Sterculia such as anti- oxidant,[4] anti TB,[5] cytotoxic,[6] CNS depressant,[7] anti-inflammatory activity,[6] antibacterial and hemolitic activity,[8] antifungal, genotoxic,[9] anti-diabetic,[10] anthelmintic, gastroprotective [11] and analgesic activity.[12]

MATERIALS AND METHODS

Materials

Sterculia rubiginosa Zoll. ex Miq plant leaves. Collected in February 2017 from Botanical Garden of Bogor and determined in Botany Herbarium Research Institute, Cibinong, West Java. The solvents used in the study were n-hexane, ethyl acetate and methanol purchased from local suppliers. KLT plate (Merck, Germany), silica gel 60 (Merck, Germany), aqua bidestilata, manganese sulfate (Sigma, Singapore), methanol pro analysis (Merck, Germany), ethyl acetate pro-analysis (Merck, Germany), n-hexane pro analysis

Cite this article: Prastiwi R, Elya B, Sauriasari R, Hanafi M, Dewanti E. Pharmacognosy, Phytochemical Study and Antioxidant Activity of *Sterculia rubiginosa* Zoll. Ex miq. Leaves. Pharmacog J. 2018;10(3):571-5.

(Merck, Germany), other ingredients for identification of the compound extract and microscopic.

Collecting material

The materials used in the research are leaves of the *Sterculia rubiginosa* Zoll plant. ex Miq (SR) obtained from the Bogor Botanical Gardens, and Identified and confirmed from Bogor Botanical Garden.

Macroscopic and Microscopic

The leaves part of S. *rubiginosa* Zoll plant. ex Miq (SR) was separated from other parts, washed, cleaned and dried for further use. The macroscopic characters of the fresh leaves were noted: color, odor, taste,size and shape, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture.[13] Identification of the microscopic was done on the leaf powder to know the fragment of the identifier. Studies of morphological plants were observed based on the description given in Evans WC and Indonesian Herb Pharmacopoeia.[14,3] Organoleptic characters were observed, noted and photographs were taken in the original environment small quantity of the powdered leaves was cleared, mounted and observed for diagnostic powder characteristics. A small amount of plant material powder is placed on a glass object containing one to two drops of a chlorine hydration solution. Then closed with cover. Then observed under the microscope. Viewed fragment that observed then photographed.[13,15]

Extraction

Preparation of 70% ethanol extract by maceration of one part of *Sterculia rubiginosa* Zoll ex. Miq powder with 10 parts of ethanol 70%. This macerate was Soak for the first 6 h while stirring 3 times, then let stand for 18 h. The residue of maceration is filtered and then concentrated by using vacuum rotary evaporator until obtained by viscous extract. This extracts was used for identification of moisture content, total ash content, acid soluble ash content and identification of the compound content.

Chromatographic profile

One part of leaves powder *Sterculia rubiginosa* Zoll. ex Miq, inputted into the macerator and add 10 parts of n-heksan. Soak for the first 30 min while stirring 3 times, then let stand for 1.5 h. Maceration results filtered with filter paper. The obtained residue was macerated by using DCM for 1.5 h and filtered with filter paper. The residue was macerated using ethyl acetate for 1.5 h. The residue is macerated back using methanol for 1.5 h. The maceration results are filtered with filter paper. Each filtrat obtained by viscous extract. Then checked the chromatogram pattern. The three extracts were concentrated using a rotary evaporator and then analyzed by TLC using silica gel 60 F254 TLC plates for the chromatographic profile.[15]

Determination of Total ash value

Two (2) g of extract insert into crushed silicate crucible and tara, polish gently until the charcoal was exhausted, chill and weigh. If this way charcoal cannot be removed, add hot water, stir, and filter through filter paper free of ash. Refine the filter paper along with the rest of the filtering in the same crucible. The filtrate was insert into the crucible, steam and flush until the weight was fixed. The total ash content was calculated with the weight of the test material, presented in % w/w[3]

Determination of acid-insoluble ash value

The ash from total ash assay was boiling with 25 ml dilute hydrochloric acid for 5 min. The acid-insoluble parts were collected, strain through the ash-free filter paper, wash with hot water, grease in crucible until the weight is fixed. The acid soluble ash content was calculated on the weight of the test material, presented in% w/w[3]

Determination of water soluble content

Five (5) g of extract was put into the clogged flask, add 100 ml of chloroform saturated water, and shake it repeatedly for the first 6 h, Leave for 18 h. Strain, vaporize 20 ml of filtrate to dry in a flat with 105°C heated, heat remaining at 105°C until fixed weight. The result was calculate in % w/w water soluble[3]

Determination of Ethanol Soluble Content

Five (5) g of extract put into the clogged flask, add 100ml of 95% ethanol P, shake repeatedly for the first 6 h, leave for 18 h. Strain quickly to avoid ethanol evaporation, vaporize 20 ml of filtrate to dry in a flat with 105°C heated and tinned, heat remaining at 105°C until fixed weight. The result was calculate in % w/w ethanol soluble.[3]

Examination of Chromatographic Patterns

Prepare the TLC plate mark the lower and upper border. Each extract was dilute with each solvent. The detection by irradiated visible light and UV with wavelength 254 and 366 nm. Any spots that arise or detect are circled. The spots were observed the colors and then calculate the Rf value.

Examination of Fluorescence Characteristics

The powder of simplisia and ethanol condensed extract of 70% were dropped into drop plate and dripped by reagent solution and then seen the color change that happened using visible light and using UV light with wavelength 254 nm and 366 nm. Reagents used are aquadest, hydrochloric acid, sulfuric acid, nitric acid and in sodium hydroxide.[3]

Phytochemical screening

Phytochemical Screening test was done to investigate the presence or absence of phytochemical constituents like alkaloids, terpenoids, glycosides, steroids, tannins, flavonoids, and saponins.[3]

Determination of Total Flavonoid Content

The determination of the total flavonoid content the samples used the method of Chang (2002) and Pharmacopoeia Herbs (2012).[16] Preparation of standard parent solution of quercetin. The Quercetin weighed 10 mg, dissolve in 96% ethanol to obtain 10 ml (1000 µg / ml) solution. Created dilution 50, 40, 30, 20, and 10 µg / ml. From the concentrations taken consecutively 0.5; 0.4; 0.3; 0.2; and 0.1 ml of solution. Add 3 ml of 96% ethanol, 0.2 ml $AlCl_3$ 10%, 0.2 ml Na-acetate 1 M, and distilled up to 10 ml volume. The solution mixture was incubated for 30 min at room temperature. Measure absorbance at 437 nm wavelength using UV-Vis spectrophotometer (Chang *et al* .2002). Calculate the linear regression equation between the concentration and absorbance relationship. The standard curve equation is obtained from the linear regression between quercetin (x) and absorbance (y).

Antioxidant activity

Antioxidant activity of methanol extract was evaluated using DPPH scavenging ability assay, which was conducted in a 96-well plate according to previously used method zhang lu *et al*.,[17] with slight modification. Samples in different concentrations (100, 250, 500, 1000, 1500, 2000 ppm) and 0.114 mM DPPH solution 180 µL in methanol were added to each well. The absorbance was read at 517 nm after 30 min of reaction in dark with a micro-plate reader. The scavenging ability (%) was calculated as follows:

$$\text{Scavenging ability (\%)} = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100\%$$

AC was the absorbance of control (without sample), AS is the absorbance of sample. Ascorbic acid was used as positive standart. All tests

Figure 1: The Macroscopic leaves, fruits and seed of SR.

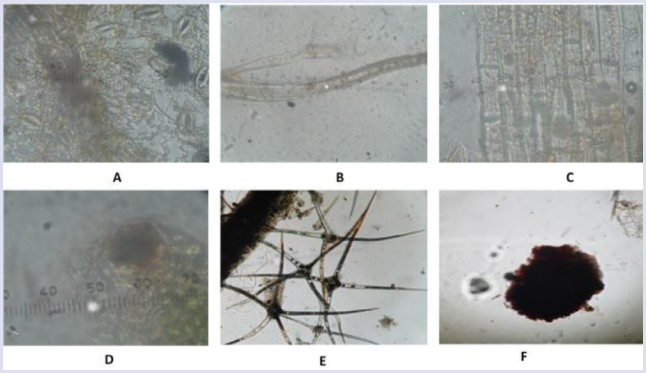

Figure 2: Diagnostic features for the powder microscopy of the leaf of SR. (A) Anomocytic stomata, (B) Sclerenchymatous fiber, (C) Parenchym, (D) Idioblast, (E) Star shape trichomes, oil cells(F).

were performed in triplicate. Concentration of samples resulting in 50% inhibition on DPPH (IC50 value) were calculated.

RESULT AND DISCUSSION

Macroscopic and microscopic organ of SR

Macroscopic testing was performed to determine the characteristic of the SR leaf. The results showed that leaves have a single leaf, uncompletus, elongated lanceolate, Apex: acuminatus, Base: acutus, nervatio: penninervis. Fruits type was schizocarpium. This macroscopic was same with the result from Kahtijah (1998).[18] The macroscopic result show in Figure 1.

Microscopic observations indicate that the powder has a star-shaped hair star recognition fragment, sclerenchyme fibers, oil cells, parenchym and stomata type anomocytic. The microscopic fragment were show in Figure 2.

Chromatogram Profile

Thin layer chromatographic profile evaluated of SR leaves extracts (hexane, ethyl acetate, methanol) constituted different colored phytochemical compounds with different Rf value. The Rf values were calculated and promising spots as shown in Table 1.

Physicochemical characteristics

The physicochemical characteristics such loss on drying, ash values, water, alcohol and ether soluble extractive, were given in the Table 2.

The organoleptic studies indicated important characteristics such as typical tongue sensitizing aromatic taste, aromatic odour, etc, which are useful diagnostic characters. The mean ash values (%) was found to be 17.152 ± 0, 0101 (total), 0.9229 ± 0, 0065 (acid insoluble ash). Total ash

Table 1: Chromatogram Profil of SR Extract.

Extract	No.	Rf	UV 366	H_2SO_4
n-Hexane	1	0.78	yellow	Brown
	2	0.85	yellow	Brown
	3	0.89	orange-red	Brown
	4	0.96	brown	Brown
	5	0.98	Red	Green
Ethyl acetate	1	0.13	yellow	Brown
	2	0.24	yellow	Brown
	3	0.40	yellow	Brown
	4	0.49	yellow	Brown
	5	0.56	yellow	Brown
	6	0.75	brown-red	Brown
	7	0.84	yellow	Brown
	8	0.85	orange-red	Brown
	9	0.89	brown-red	Brown
	10	0.98	Red	green
Methanol	1	0.11	yellow	Brown
	2	0.16	yellow	brown
	3	0.40	brown-red	Brown
	4	0.76	yellow	Brown
	5	0.84	yellow	Brown
	6	0.85	brown-red	Brown
	7	1.07	yellow	Brown

Table 2: Physicochemical Parameter of SR Powder.

No	Parameter	Average (% w/w)
1	Ash value	
	Total ash value	17.152 ± 0.01
	Acid in soluble ash	0.9229 ± 0.0065
2	Extractive value	
	Alcohol soluble	4.524 ± 0.0201
	Water soluble	1.610 ± 0.01
	Ethyl acetate soluble	3.16 ± 0.01
	n-Hexane soluble	4.005 ± 0.0150
3	Water content	8 ± 0.013

value was relatively high, which may be due to high content of carbonates, phosphates, silicates and silica. Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards.[19]

Fluorescence Characters

Fluorescence characters of powdered material were analyzed under visible and ultraviolet light (254 and 366 nm), which signifies their characteristics (Table 3).Results of fluorescene studies of leaves powder using different reagents is given in Table 3.

Phytochemical screening

The Phytochemical screening results were present for alkaloids, tannins, flavonoids, glycosides, phenols, steroid, triterpenoids and carbohydrate,

Table 3: **Fluorescence Analysis of SR Powder.**

Treatment	Visibel	UV (254 nm)	UV (366 nm)
Powder + H_2O	Dark green	Green	Greenish blue
Powder + H_2SO_4	Brown	Blueish green	Green
Powder + HCl	Yellowish brown	Greenish blue	Green
Powder + HNO_3	Yellowish green	Green	Green
Powder + NaOH	Yellowish green	Blue	Greenish blue

Table 4: **Phytochemical screening of SR extracts.**

No	Constituent	Test	Result
1	Tannin	Gelatine	+
2	Flavonoids	Shinoda	++
3	Alkaloids	Mayer	+
		Bouchardat	++
		Dragendorff	++
4	Steroids - terpenoids	Lieberman burchad	+
5	Saponins	Foam	-
6	Glycosides	Molisch	++
7	Anthraquinones	Borntrager	-
8	Fenol	Ferric chloride	+

Description: (+) = present; (-) = Absent

Table 5: **Flavonoid Total of SR Extracts.**

Extract	Flavonoid total/g	SD
Etanol	59.43625	±0.114085

and absent for saponins and anthraquinones. This result was showed in Table 4

Flavonoid Total

The result of determination of total flavonoid content was done by calculating equality with quercetin. The sample used was 200 mg. testing using UV Vis spectrophotometer. Here is the absorbance data and flavonoid levels. Total flavonoid of extract was expressed as gram quercetin equivalent/100 g extract. The amount of total flavonoid contents was from 59,43625 ±0,114085/ g. The total flavonoid content on extract was calculated by using y=a+bx. This regression from quercetin standard. The result was showed in Table 5.

Antioxidant Activity

The results of IC50 antioxidant activity of SR extract was 157, 4665 ppm. The positive control using Vitamin C obtained results 2.23 ppm. The result was show in Table 6.

Flavonoids have many biochemical properties, but the best characteristic of almost every flavonoid group is their ability to act as antioxidants. The antioxidant activity of flavonoids depends on the functional group arrangement of nuclear structures. Configurations, substitutions, and amounts of hydroxyl groups substantially affect some mechanisms of antioxidant activity such as radical scavenging and the ability of metal ion chelation.[20]

DISCUSSION

The anomocytic type of stomata observed in all the species has been reported for *S. foetida* and is common in the *Malvaceae*. Sinuous adaxial anticlinal epidermal walls are present only in *S. rubiginosa* while in other species they are either straight or straight to wavy.[7] Trichomes are epidermal

Table 6: **Antioxidant Activity of SR Extracts.**

Concentration (ppm)	Antioxidant Activity (%)	IC50 (ppm)
25	5.949337	
50	15.82276	
100	28.32276	157.4665
150	40.47466	
200	70.03164	
Vit C		2.23

out growths of considerable value for taxonomic purposes. Most of the plant species are completely devoid of trichomes while other shows in abundance. The environmental conditions influence more the length, size, than and density the types of trichomes.[2]

Total ash values of drug give an idea of earthy matter or the inorganic composition and other impurities present along with drug. The extractive values are useful for determination of crude drugs and it gives an idea about the nature of the chemical constituents present. Water-soluble indicated the presence of sugar, acids, and inorganic compounds. The alcohol soluble indicated the presence of polar constituents, and ether soluble indicated the presence of non-polar constituents. Physical constants like ash and extractive values help in establishing the pharmacopoeia standards of drug.[15]

Fluorescence is an important phenomenon indicated by various chemical elements present in plant material. Some secondary metabolites show fluorescence in the range seen in daylight. Ultra violet lamps produce fluorescence in many natural products, which do not appear to glow during the day. But if the substance itself was not fluorescent, they may often be converted into fluorescent derivatives using different reagents so some crude drugs are often qualitatively assessed in this way and these are important parameters for pharmacognostic evaluation.[19] The chronological literature survey confirmed what was originally believed, that the major production of genus *Sterculia* and related genera is indeed flavonoid metabolites.[2]

Antioxidant activity is related to the presence of a compound capable of protecting the biological system from the potentially harmful effects of the process involving reactive oxygen and nitrogen species (ROS and RNS).[21] The chemical nature of flavonoids depends on their structural class, degree of hydroxylation, other substitutions and conjugations, and degree of polymerization. Recent interest in these substances has been stimulated by the potential health benefits arising fromthe antioxidant activities of these polyphenolic compounds. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions. The chelation of metals could be crucial in the prevention of radical generation which damage target biomolecules.[22,20] *Sterculia rubiginosa* has several local languages in Indonesia such as hansep, ki hampelas. And traditionally can be used by people in Indonesia as anti-asthma.[23]

CONCLUSION

The leaves shows star shape trichoma as a specific fragment. Physicochemical parameters including total ash (17,152 %), acid-insoluble ash (0,922 %), water-soluble extractive (1,610 % w/w), alcohol-soluble extractive (4,524 % w/w), hexane-soluble extractive (4,005 % w/w), and ethyl acetate-soluble extractive (3,160 % w/w) were evaluated. Phytochemical screening of ethanol extracts showed the presence of tannins, flavonoids, alkaloids, steroids-terpenoids, glycosides, and phenols. And absent of saponins and Anthraquinones. Antioxidant activity with IC50 157, 4665 and flavonoid total was 59, 43625 mg/g quersetin equivalent.

ACKNOWLEDGEMENT

The authors are very much thank full to Botanical Garden of Bogor and determined in Botany Herbarium for the collection of *Sterculia rubiginosa* Zoll.ex Miq. Research Institute, Cibinong, West Java.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS USED

SR: *Sterculia rubiginosa* Zoll ex. Miq.

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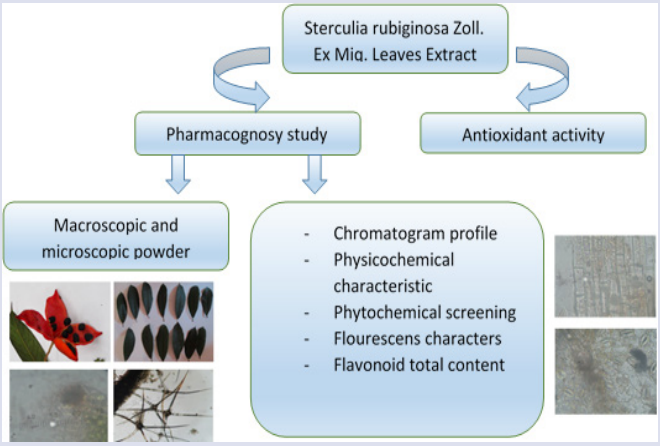
GRAPHICAL ABSTRACT



SUMMARY

- *Sterculia rubiginosa* Zoll. Ex Miq . has been called by "hansep" or "ki hampelas' in Indonesia and traditionally can be used as anti-asthma
- The pharmacognosy study and antioxidant activity of *Sterculia rubiginosa* Zoll. Ex Miq. provides useful information for quality control parameters for the crude drugs.

Cite this article: Prastiwi R, Elya B, Sauriasari R, Hanafi M, Dewanti E. Pharmacognosy, Phytochemical Study and Antioxidant Activity of *Sterculia rubiginosa* Zoll. Ex miq. Leaves. Pharmacog J. 2018;10(3):571-5.