Extractives Contributing to the Color of *Swietenia macrophylla*’s Bark

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Abstract

The dark red color of *Swietenia macrophylla* King bark is correlated with the extractive constituents such as phenolic compounds. This study, therefore, aimed to investigate extractives from the inner and outer bark of *S. macrophylla* and their effects to color properties. The results showed that the extractive content in the inner bark was higher than the outer except for hot water soluble. In addition, the polyphenols and sugar levels from inner to the outer bark were increased, except in the soluble-sugar of hot water extractive. The highest correlation between the absorbance of methanol, hot water-soluble extracts, and total polyphenols were observed using the visual spectrophotometer. The extractives that contributed to the bark’s color were indicated from flavonoids with a precursor such as monophenol of catechol and resorcinol.

Keywords: polyphenols, *S. macrophylla*, polysaccharides, coloration.

Introduction

*Swietenia macrophylla* King, also known as mahogany, is a species of wood that commonly found in Indonesia, which originated from Central and South America (Brown *et al.* 2003). In Jepara of Central Java, home furnitures such as chairs, bed frames, and other products are manufactured from this wood. The utilization of *S. macrophylla* discards the bark as residues. The bark contains more polyphenol and lignin, which produces important medicinal extractives. Previous studies reported that *S. macrophylla* bark has been investigated as an astringent for the wound (Falah *et al.* 2008). The leaf was used for leishmaniasis, abortion medicine (Bourdly *et al.* 2000), cancer, amoebiasis, diabetic, and malaria as a folk medicine in Indonesia (Kadota *et al.* 1990).

Beside its potency for medicinal, it is also used for dye materials. According to Adeel *et al.* (2009), the increasing utilization of plant materials for dyeing is due to the growing awareness of the environment, healthcare and natural colorants that consists of properties such as antimicrobial activity (Yusuf *et al.* 2015). Furthermore, its use has been reported in the species of *Albizia coriaria*, *Morinda lucida*, *Syzygium cordatum*, *Vitellaria paradoxa*, and *Juglans regia* (Wanyama *et al.* 2014; Bukhari *et al.* 2017). Haque *et al.* (2013) reported that the potential use of an anthraquinone compound, known as rubiadin, isolated from *S. mahagoni* bark was for silk fabric dyeing. Emiliana and Widhiati (2002) also stated that a satisfactory result of using *S. macrophylla* bark extract is dyeing the red snapper fish skin.

The presence of color in the wood of plants is usually related to the extractives content such as flavonoids (Yazaki 2014). Three flavonoid type compounds, namely catechin, epicatechin, and a pale reddish swietenmacrophyllin were isolated from *S. macrophylla* bark and its antioxidant activity was assessed (Falah *et al.* 2008). The chemical composition of its inner and the outer bark lipophilic extractive was also investigated (Arisandi *et al.* 2019). However, there are no present study on the coloring compounds of *S. macrophylla*, especially in its separated inner and outer bark, which are known to be differed in their chemical composition (Masendra *et al.* 2018; Masendra *et al.* 2019; Seki *et al.* 2012). This study aimed to observe the total amount of phenols, flavanols, flavonoids, and polysaccharides contained in the inner and outer bark of *S. macrophylla* and their correlation with the color.

Materials and Methods

Bark Collection and Extraction

The bark was collected from Srikandiratu, furniture industry in Jepara, Central Java, Indonesia, and the leaves was identified in Faculty of Forestry Universitas Gadjah Mada as *S. macrophylla*. The characteristic of inner bark with light red color with thickness of 0.5-1.0 mm was easily peeled and separated from outer bark with dark red color and 1.5-3.0 mm thickness. It was grounded to powder with the inner and outer barks (500 g) successively refluxed for 6 h using n-hexane, methanol, and water. The solution was evaporated and the resulting crude extract was weighed.

Phytochemical Tests of the Plants

The bark extracts were subjected to phytochemical screening to identify the main classes of secondary metabolites. The tests were Mollisch for carbohydrates (Browning 1967), frothing for saponins (Kokate 1999), Mayer for alkaloids (Mir *et al.* 2016), ferric chloride from tannins (Trease and Evans 2002), and sodium hydroxide for flavonoids (Browning 1967).

Total Phenols

Total phenols were investigated by the Folin-Ciocalteu method with modification (Diouf *et al.* 2009). Approximately 0.5 ml of an ethanol solution of the sample (0.25 mg/ml) was mixed and incubated for 2 minutes with 2.5 ml of the Folin-
Ciocalteu reagent (10 times dilution). Furthermore, 2 ml of 7.5% aqueous sodium carbonate (Na$_2$CO$_3$) was added to the solution, and the mixture was allowed to stand for 30 min at room temperature. The absorbance of the sample was read at 765 nm and the results were expressed as gallic acid equivalents (mg GAE/g based on dry extract weight).

**Total Flavanols**

Total flavanols were observed by vanillin-HCl assay as described by Diouf et al. (2009). In addition, 0.5 ml (0.25mg/ml) of ethanol solution was mixed with 3 ml of vanillin reagent (4% vanillin in methanol) and 1.5 ml of HCl. After 15 minutes incubation of a sample, the absorbance was read at 500 nm, with the results expressed in (+)-catechin equivalents (mg CE/g based on dry weight).

**Total Flavonoids**

The AlCl$_3$ method is used to determine the total flavonoids (Brighente et al. 2007). First, 2 ml of the sample at 1 mg/ml concentration was added to 2% AlCl$_3$.6H$_2$O solution and stood after 1 h incubation at 20°C. After that, the absorbance was read at 415 nm, and the results expressed in quercetin equivalents (m QE/extract).

**Total Soluble Polysaccharides**

The polysaccharides contents were determined by using the DuBois method (DuBois et al. 1956), with 1 ml of hot-water extract mixed with 1 ml of phenol (5%) and 5 ml of concentrated sulfuric acid (98%). The mixture was maintained for 20 min at 25°C, with the absorbance of the sample was read at 490 nm and calculated in glucose equivalents (mg GE/g sample).

**Color Measurements ($\lambda$ = 300-700 nm)**

The absorbance of n-hexane, methanol, and hot water extracts (each 1 mg/ml) was read at a wavelength of 300-700 nm for color measurement.

**Gas Chromatography-mass Spectrometry (GC-MS)**

The GC-MS data were collected using a GCMS-QP 2010 (Shimadzu, Japan), with 1 µl of silylated sample injected to the GC-MS machine. The GC condition are as follows: Rtx- 5MS capillary column (30 m x 0.25 mm I.D. and 0.25 μm), column temperature from 70°C (2 min) to 290°C at 5°C/min, injection temperature of 200°C, detection temperature of 285°C, and acquisition mass ranging from 50-800 amu using helium as the carrier gas. The mass spectra of samples were compared to the NIST11 library.

**Results and Discussion**

**Extractive Content**

The extractive content of outer and inner bark using three different solvents is shown in Figure 1. Previous research by Arisandi et al. (2019) found that the n-hexane soluble extracts of the inner bark was higher than the outer part. In addition, the methanol extractive content in the inner bark also had a higher value, while the hot water extractive content showed the opposite result. The higher extractive content in the inner bark indicated that this part contains more constituents such as lipophilic or phenolic. Previous studies also reported that the inner bark of six Pinus species contained more lipophilics, phenolics, and sugar compounds than the outer bark (Masendra et al. 2018; Masendra et al. 2019).
Polyphenols, Sugar, Saponin, and Alkaloid

In the first screening using the qualitative method, the bark of *Swietenia macrophylla* was found to contain alkaloid, saponin, tannin, flavonoid and carbohydrate. However, the alkaloids were only detected in the inner and outer bark of *n*-hexane extracts (Table 1). The presence of saponin was easily detected in the methanol extract of the inner bark than other fractions, while tannin detected on inner bark hot water extracts led to a higher concentration compared to outer bark. Furthermore, the carbohydrate test showed that methanol and hot water extract of inner bark were in lower concentration and undetected, compared to the outer bark. Due to this result, the total polyphenol and soluble polysaccharide content were quantitatively analysed.

**Table 1. Qualitative measurement of alkaloid, flavonoid, saponin, and carbohydrate test of *Swietenia macrophylla***

| Fraction    | Alkaloid | Flavonoid | Saponin | Tannin | Carbohydrate |
|-------------|----------|-----------|---------|--------|--------------|
|             | IB       | OB        | IB      | OB     | IB           | OB |
| Hexane      | +        | +         | -       | -      | -            | -  |
| Methanol    | -        | -         | +++     | +++    | +            | +++ |
| Water       | -        | -         | ++      | +++    | +            | +++ |

(\(\cdot\): not detected, (+): low detected, (++): moderately detected, (+++): highly detected.

**Table 2. Total phenols, total flavanols, total flavonoids, and total polysaccharides from the bark of *Swietenia macrophylla***

| Fraction | Total phenols (mg GAE/g extract) | Total flavanols (mg CE/g extract) | Total flavonoid (mg QE/g extract) | Total polysaccharides (mg GE/g extract) |
|----------|----------------------------------|----------------------------------|----------------------------------|----------------------------------------|
|          | IB     | OB     | IB     | OB     | IB     | OB     | IB     | OB     |
| Methanol | 451.6 ± 15.5 | 601.3 ± 10.5 | 112.3 ± 11.8 | 174.9 ± 6.8 | 29.8 ± 5.4 | 66.2 ± 3.3 | 97.3 ± 38.3 | 148.2 ± 29.2 |
| Water    | 143.8 ± 5.4  | 295.8 ± 2.4   | 27.3 ± 2.9 | 51.6 ± 0.5 | 0.5 ± 0.5 | 7.1 ± 2.6 | 91.5 ± 5.8 | 78.7 ± 3.4 |

IB: inner bark, OB: outer bark.

The polyphenol measurement showed that the total phenols dominated the composition of extractive in the bark samples compared to total flavanols, flavonoids, and polysaccharides (Table 2). In addition, their concentrations in methanol and hot water content were lower in the inner bark. However, the levels of total polysaccharides in hot water extract from inner bark were higher than the outer.

Phenolic is a main class of secondary metabolites found in plants with broad compounds and known for its bioactivity (Valette et al. 2017; Kadir 2017; Al-Huqail et al. 2019) with the ability to remove the color of a material (Burtin et al. 1998; Kelebek et al. 2010). Therefore, the outer bark is often associated with a high amount of phenolic compounds, including flavonoid and flavanol with protective function against pathogens (Popa 2015). Similar patterns between phenolics were found in a study conducted by Masendra et al. (2019) in six species of *Pinus*, with a high concentration of polysaccharides measured in hot water-soluble fraction of the inner bark. This result was, however, inconsistent with the phytochemical screening carried out by the Molisch test as the carbohydrate was detected in low concentration in the inner bark. Theoretically, the presence of carbohydrate can be found in both inner and outer bark. However, the present study showed carbohydrate reaction in the inner bark was lower than outer bark. Further, the presence of polysaccharide in hot water-soluble extract of inner bark was expected due to its function in the distribution and storage of nutrients from the root to other parts of the tree (Sjöstrom 1993).

**Extractives for Color**

The outer and inner colors of the bark extractive were dark brown. The wavelength measurement from 300-700 nm by spectrophotometer showed that a higher solubility was presented by methanol and hot water extract of outer bark followed by the inner bark, respectively (Figure 2). The highest shoulder was observed at 475 nm where the absorbance of methanol and hot water extracts in both barks were 1.684, 1.33, 0.77, and 0.73, respectively.

The presence of more intense shoulder at 475 nm matched with the color properties in the methanol and hot water extracts. The correlations between absorbance at 475 nm, polyphenols measurements (total phenols, flavanols, and flavonoids), and sugar content are linear as shown in Figure 3. However, the correlation between absorbance at 475 nm and polyphenols content was stronger than total polysaccharides. Therefore, methanol and hot water extract color are affected by polyphenols (\(R^2 > 0.9\)) (Figure 3b).
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Figure 2. Absorbance of the n-hexane, methanol, and hot water extractives from inner bark (IB) and outer bark (OB) of *Swietenia macrophylla*

Figure 3. Correlation between absorbance at 475 nm and total phenols (a), total flavanols (b), total flavonoids (c), and total sugars (d). MIB (methanol extract inner bark), MOB (methanol extract outer bark), HWOB (hot water extract outer bark), HWIB (hot water extract inner bark).
The methanol extract absorbance was analysed by GC-MS for phenolic compounds detection. To determine the GC-MS analyses, the methanol was extracted with ethyl acetate, which was in-turn trimethylsilylated (Wijayanto et al. 2015). In Figure 4, the chromatogram of methanol extract, along with the detected monophenols in the inner and outer barks of S. macrophylla is shown. Catechol and resorcinol were dominant monophenols detected in the outer and inner bark methanol extract, respectively, and responsible for their coloration.

Research carried out by Fallah et al. (2008) successfully isolated a reddish pale compound known as swieteniemacrophylalanin (Figure 5a) and two other flavonoids from soluble extracts. The presence of swieteniemacrophylalanin, isolated from the bark of S. macrophylla by Falah et al. (2008), has the ability to affect the coloration. Therefore, the extractive responsible for coloration in the inner and outer barks are flavonoid with resorcinol structure (Figure 5b), and flavonoids that contain catechol (Figure 5c). Further studies are needed to identify...
the polyphenol compounds or flavonoids that contain catechol and resorcinol by HPLC or NMR analyses.

Conclusions

In conclusion, preliminary phytochemical screening was used to detect alkaloids, saponins, tannins, flavonoids, and carbohydrates in the inner and outer bark extracts of *Swietenia macrophylla*. The total values of phenols, flavonoids, and flavanols were higher in the outer bark. In addition, the absorbance of methanol and hot water-soluble extract was highest at 475 nm and linearly correlated by polyphenols. The stronger absorbance and the detection of phenolic compounds by GC-MS was in the methanol extract for inner and outer bark of *S. macrophylla*. Therefore, we suggested that extractives contributing to color in the bark were flavonoids with monophenols structure such as catechol and resorcinol.

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