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

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Abstract

The dark red color of *Swietenia machrophylla* 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. machrophylla* 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. machrophylla*, 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 (Bourdy *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*, *Syzgium 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 dying 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 swietemacrophyllanin 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_2CO_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% $AlCl_3.6H_2O$ 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 (λ= 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).

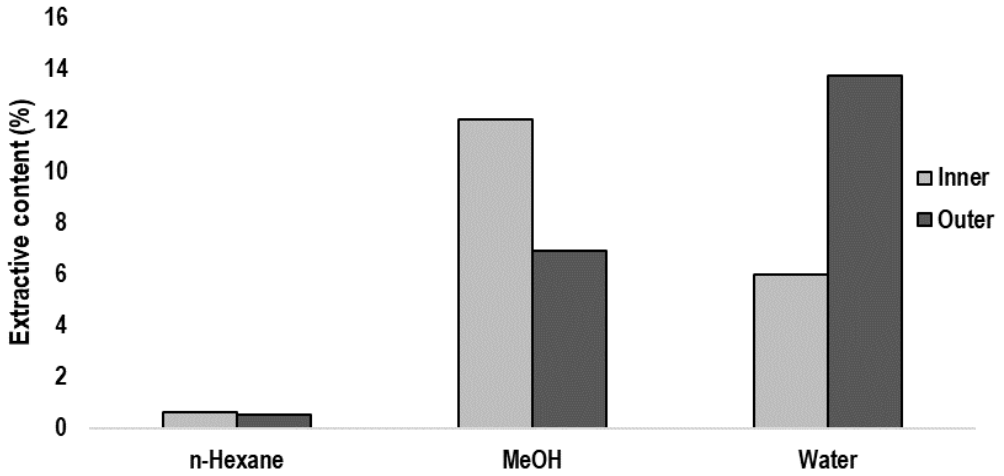

Figure 1. *n*- Hexane, methanol (MeOH), and hot water extractive content of the inner and outer bark of *Shorea macrophylla*

Polyphenols, Sugar, Saponin, and Alkaloid

In the first screening using the qualitative method, the bark of *S. 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	IB	OB	IB	OB
Hexane	+	+	-	-	-	-	-	-	-	-
Methanol	-	-	+++	+++	++	+	+++	+++	-	+++
Water	-	-	++	+++	+	+	+++	+++	+	++

(-): 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 Mollisch 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).

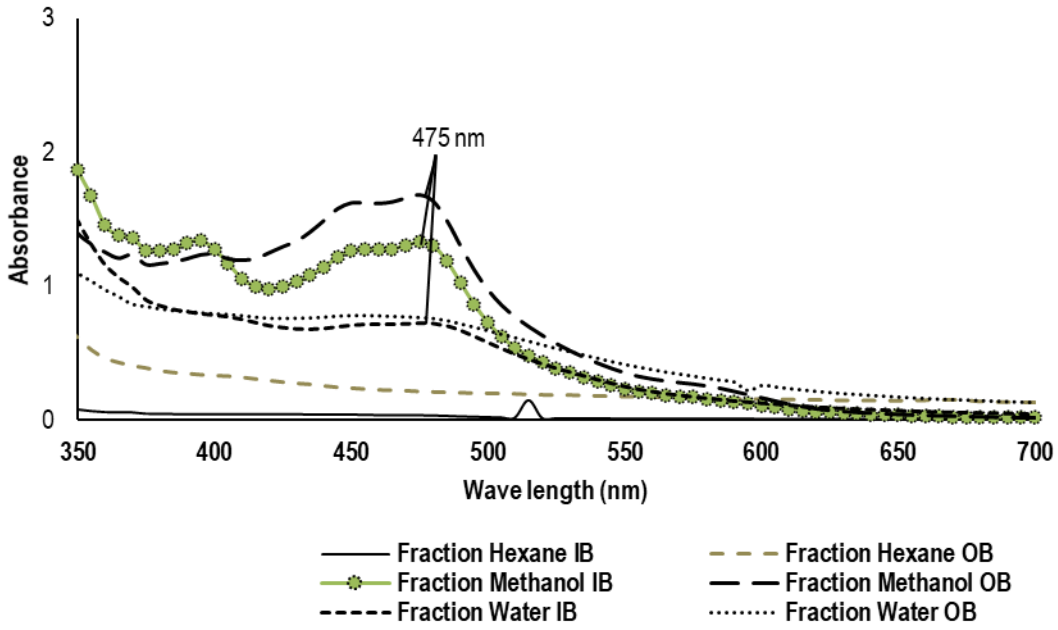

Figure 2. Absorbance of the *n*-hexane, methanol, and hot water extractives from inner bark (IB) and outer bark (OB) of *Swietenia macrophylla*

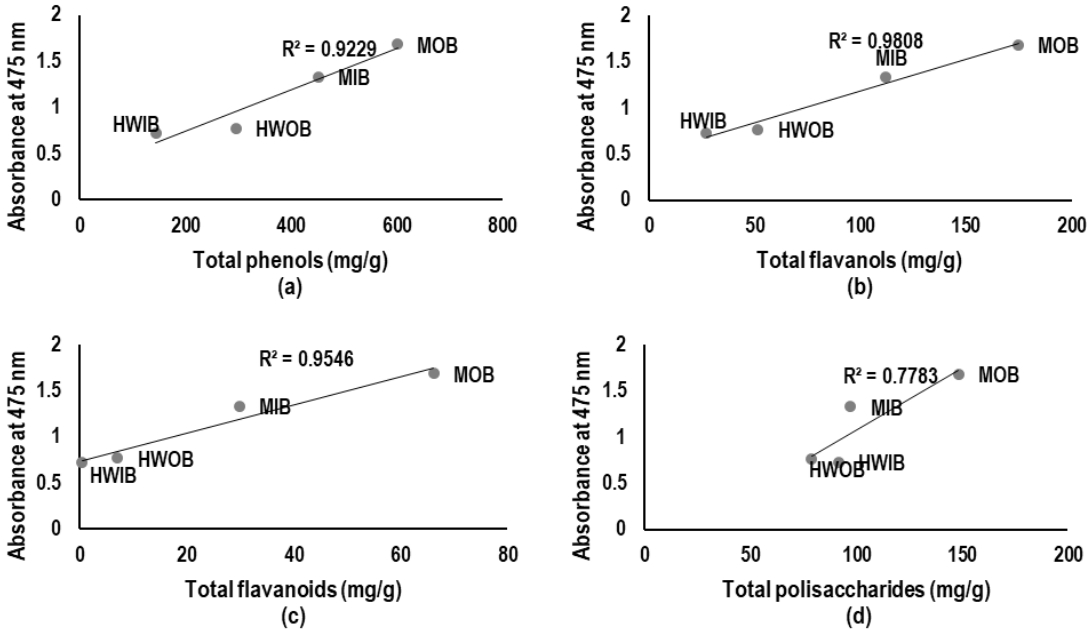

Figure 3. Correlation between absorbance at 475 nm and total phenols (a), total flavanols (b), total flavonoids (c), and total sugars (d). MIB (methanol extract inner bark), MOB (methanol extract outer bark), HWOB (hot water extract outer bark), HWIB (hot water extract inner bark).

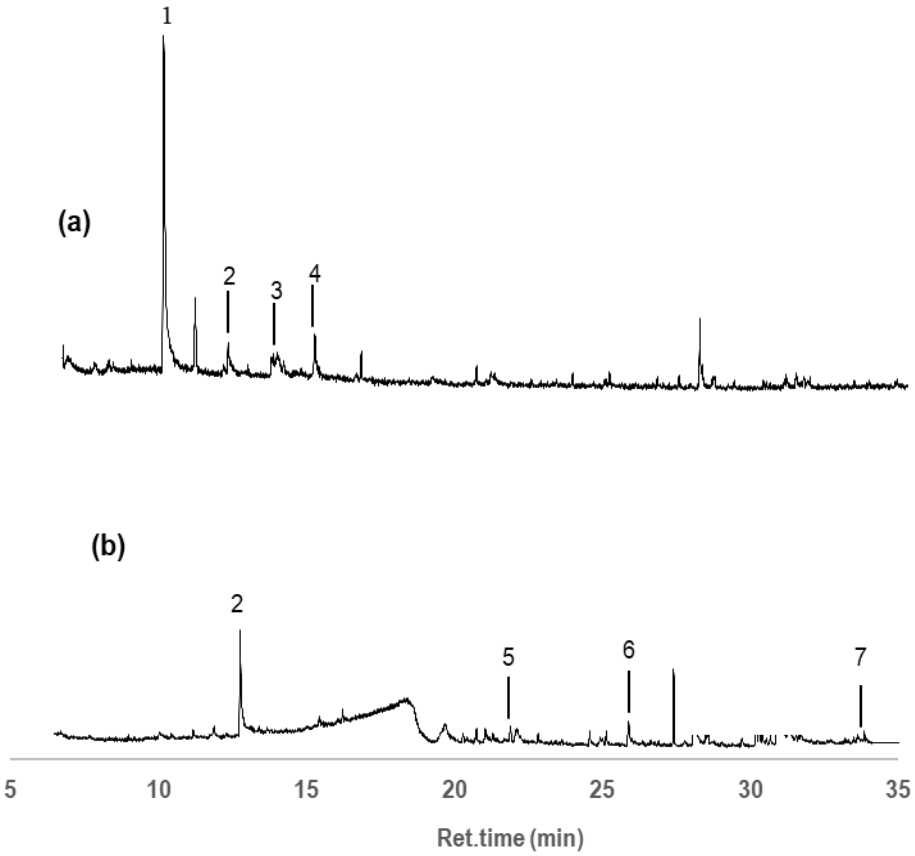

Figure 4. GC-MS chromatogram of outer bark (a) and inner bark (b) methanol extract of *Swietenia macrophylla*; 1. Catechol, 2. Resorcinol, 3. 4-Methylcatechol, 4. Syringol, 5. Antiarol, 6. Syringic acid, 7. Trimethoxycinnamic acid

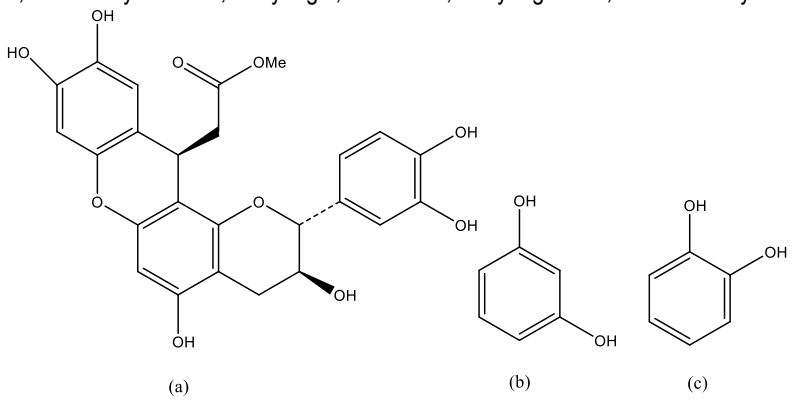

Figure 5. Chemical structure of swieteniemacrophyllanin (a), resorcinol (b), and catechol (c).

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 swieteniemacrophyllanin (Figure 5a) and two other flavonoids from soluble extracts. The presence of swieteniemacrophyllanin, 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 extractives of *S. machrophylla*. 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. Machrophylla*. 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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References

Adeel, S.; S. Ali; I.A. Bhatti; F. Zsila. 2009. Dyeing of Cotton Fabric using Pomegranate (*Punica granatum*) Aqueous Extract. Asian Journal of Chemistry. 21(5): 3493-3499.

Al-Huqail, A.A.; S.I. Behiry; M.Z.M Salem; H.M. Ali; M.H. Siddiqui; A.Z.M. Salem. 2019. Antifungal, Antibacterial, and Antioxidant Activities of *Acacia saligna* (Labill.) H.L. Wendl. Flower Extract: HPLC Analysis of Phenolic and Flavonoid Compounds. Molecules 24(4): 700-714.

Arisandi, R.; Masendra; B.A.V. Purba; F. Z. Wati; F. V. Ihda; F. Sumantri. 2019. Lipophilic Extractives of Mahogany (*Swietenia macrophylla* King) Barks. Conference: Proceeding of 9th International Symposium of Indonesian Wood Research Society At: Denpasar, 26-27 September 2017. Pp 192-201.

Brighente, I.M.C.; M. Dias; L.G. Verdi; M.G. Pizzolatti. 2007. Antioxidant Activity and Total Phenolic Content of Some Brazilian Species. Pharmaceutical Biology 45(2): 156-161

Bourdy, G.; S.J. De Walt; L.R. Chavez De Muchel; A. Roca; E. Deharo. 2000. Medicinal Plants Uses of the Tacana, an Amazonian Bolivian Ethnic Group. Journal of Etnopharmacology 70(2): 87-109.

Brown, N.; S. Jennings; T. Clements. 2003. The Ecology, Silviculture and Biogeography of Mahogany (*Swietenia macrophylla*): A Critical Review of Evidence. Plant Ecology and Evolution System 6(1): 37-49.

Browning, B. L. 1967. Methods of Wood Chemistry. Volume 1. Interscience Publisher. New York.

Bukhari, M.D.; Shahid-ul-islam; M. Shabir; L.J. Rather; M. Shahid; U. Singh; M. A. Khan; F. Mohammad. 2017. Dyeing Studies and Fastness Properties of Brown Naphtoquinone Colorant Extracted from *Juglans regia* L on Natural Protein Fiber Using Different Metal Salt Mordants. Textiles and Clothing and Sustainability 3 (3): 1-9.

Burtin, P.; C. Jay-Allemand; J.P. Charpentier; G. Janin. 1998. Natural Wood Colouring Process in *Juglans* sp. (*J. nigra, J. regia* and Hybrid *J. nigra 23 ´ J. regia)* Depends on Native Phenolic Compounds Accumulated in the Transition Zone Between Sapwood and Heartwood. Trees 12(5): 258-264.

Diouf, P.N.; T. Stevanovic; A. Cloutier. 2009. Antioxidant Properties and Polyphenol Contents of Trembling Aspen Bark Extracts. Wood Science and Technology 43(5): 457-470

DuBois, M.; K.A. Gilles; J.K. Hamilton; P.A. Rebers; F. Smith. 1956. Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chemistry 28(3): 350-356.

Emiliana, K.; Widhiati. 2002. The Effect of Using Natural Dyes from Woods Extract to the Physical Properties of Red Snapper Fish Skins (in Indonesia). Majalah Barang Kulit Karet dan Plastik 18(1): 3-9.

Falah, S.; T. Suzuki; T. Katayama. 2008. Chemical Constituents from *Swietenia macrophylla* Bark and Their Antioxidant Activity. Pakistan Journal of Biological Sciences 11(16): 2007-2012.

Haque, M.A.; G.M.A. Khan; S.M.A. Razzaque; K. Khatun; A.K. Chakraborty; M.S. Alam. 2013. Extraction of Rubiadin Dye from *Swietenia mahagoni* and Its Dyeing Characteristics onto Silk Fabric Using Metallic Mordants. Indian Journal of Fibre & Textile Research 38(3): 280-284.

Kadir, R. 2017. Toxic Effects of Three Selected Malaysian Timbers Plant Extracts Against subterranean Termites. Maderas Ciencia y Tecnologia 19(4): 417-432.

Kadota, S.; L. Marpaung; T. Kikuchi; H. Ekimoto. 1990. Constituents of the Seed of *Swietenia mahagoni* Jacq. III. Structure of Mahonin and Secomahoganin. Chemical and Pharmaceutical Bulletin 38(6): 1495-1500.

Kelebek, H.; A. Canbas; M. Jourdes; P.L. Teissedre. 2010. Characterization of Colored and Colorless Phenolic Compounds in Öküzgözüwines from Denizli and Elazig Regions Using HPLC-DAD–MS. Industrial Crops and Products 31(3): 499-508

Kokate, C.K. 1999. Phytochemical methods. Phytotherapy 78: 126-129.

Mir, M.A.; K. Parihar; U. Tabasum; E. Kumari. 2016. Estimation of Alkaloid, Saponin and Flavonoid Content in Various Extracts of *Crocus sativa*. Journal of Medicinal Plants Studies 4(5): 171-174.

Masendra; T. Ashitani; K.Takahashi; G. Lukmandaru. 2018. Lipophilic Extractives of Inner and Outer Barks from Six Different *Pinus* species Grown in Indonesia. Journal of Forestry Research 29(5): 1329-1336.

Masendra; T. Ashitani; K. Takahashi; M. Susanto; G. Lukmandaru. 2019. Hydrophilic Extracts of the Bark from Six *Pinus* species. Journal of The Korean Wood Science and Technology 47(1): 80-89.

Popa, V. I. 2015. Wood Bark as Valuable Raw Material for Compounds with Biological Activity. Celuloză şi Hârtie 64(4): 1-14.

Seki, K.; K. Orihashi; M. Sato. 2012. Accumulation of Constitutive Diterpenoids in the Rhytidomeand Secondary Phloem of the Branch Bark of *Larix gmelinii* var. Japonica. Journal of Wood Science 58(5): 437-445.

Sjöstrom, E. 1993. Wood Chemistry: Fundamentals and Applications. Second Edition. Academic Press. New York.

Trease, G.E; W.C. Evans. 2002. Pharmacognosy. 15th Ed. Saunders Publishers. London.

Valette, N.; T. Perrot; R. Sormani; E. Gelhaye; M. Morel-Rouhier. 2017. Antifungal Activities of Wood Extractives. Fungal Biology Reviews 31(3): 113-123.

Wanyama, P.A.G.; B.T. Kiremire; J.E.S. Murumu. 2014. Extraction, Characterization and Application of Natural Dyes from Selected Plants in Uganda for Dyeing of Cotton Fabrics. African Journal of Plant Science 8(4): 185-195.

Wijayanto, A.; S. Dumacay; C. Gerardin-Charbonnier; R.K. Sari; W. Syafii; P. Gerardin. 2015. Phenolic and Lipophilic Extractives in *Pinus merkusii* Jungh. Et de Vries Knots and Stemwood. Industrial Crops and Products 69: 466-471.

Yazaki, Y. 2014. Wood Colors and Their Coloring Matters: Review. Natural Product Communications 10(3): 505-512.

Yusuf, M.; M. Shahid; M.I. Khan; S.A. Khan; M.A. Khan; F. Mohammad. 2015. Dyeing Studies with Henna and Madder: A Research on Effect of Tin (II) Chloride Mordant. Journal of Saudi Chemical Society 19(1): 64-72.

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