

Phenol Contents and Antioxidant Activity of Sonokeling (*Dalbergia latifolia* Roxb) Wood

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Abstract

Dalbergia latifolia or sonokeling is a native species of Java, Indonesia, used as an important wood for furniture and building materials, due to the high of durability and beautiful color. This study, therefore, was aimed to investigate the phenol composition, represented by total phenolic, flavonoid, and flavanol content, as well as antioxidant activity, conducted by DPPH (1, 1-diphenyl-2-picrylhydrazyl) method on *D. latifolia* wood. The sample was extracted using ethanol-toluene solvent in a Soxhlet apparatus, and subsequently subjected to column chromatography. This treatment yielded 12 fractions, which were then evaluated for phenol contents and antioxidant activity. The results showed a high antioxidant activity and total phenolic content in Fr.1- Fr.3, while latifolin was detected and characterized by GC-MS and a literature comparison. Therefore, it was established that the antioxidant activity of *D. latifolia* wood extractives properly correlated with the total phenolic, but not with the total flavonoid and flavanol contents.

Keywords: Sonokeling, DPPH activity, phytomedicine, neoflavonoid, extractives

Introduction

Dalbergia latifolia called sonokeling (Javanese) or Java palisander (English) is a native species from Indonesia, known to possess beautiful wood, with a brown to dark brown color (Orwa *et al.* 2009). In addition, they are classified as highly resistant, naturally durable (Kalynasundaran and Ganti 1975), placed in strength class II (Dwianto *et al.* 2019), and also deliver good acoustical properties (Karlinasari *et al.* 2012). Hence, the wood is commonly used in the manufacture of furniture and building materials.

Based on the chemical properties, Sekine *et al.* (2009) isolated some neoflavonoids compounds from the heartwood, *D. latifolia*, characterized as latifolin and its derivatives, which were then tested for antitermite and antifungi activities (Sekine *et al.* 2009). The wood, bark, and leaves extracts were also reported to confer anticancer and antioxidant effect (Khalid *et al.* 2011; Niraimathi and Sundaraganapathy 2014; Liu *et al.* 2018; Tripathi 2018). Other investigations performed on the genus *Dalbergia* demonstrated the propensity for the leaf extract of *D. saxatilis* to increase kidney toxicity (Ismail *et al.* 2015), *D. sisoo* to function as a photoprotective and DNA protective agents (Yasmeen and Gupta 2016), while *D. parviflora* contained antioxidant isoflavonoids (Castellano and Torrens 2015).

This antioxidant activity is affiliated with the protection of cell body from free radicals continuously which is produced internally, where the excess quantities are responsible for various disease manifestations (Young and Woodside 2001). Numerous radicals are known to be highly reactive with other molecules, e.g., DPPH (1, 1-diphenyl-2-picrylhydrazyl), which is unstable in dark purple color. Meanwhile, phenolic compounds as antioxidants play the

role of donating proton to reduce DPPH-H to the nonradical form of DPPH, which is an activity of polyphenols from plants (Ku *et al.* 2007; Gan *et al.* 2010). This study, therefore, investigated the wood extractives obtained from *D. latifolia* wood, in order to determine the phenol contents and antioxidant activity.

Materials and Methods

Sample Collection and Extraction

The sample of *D. latifolia* wood was purchased and collected from a wooden industry in Bantul, Yogyakarta, Indonesia. The 10 g of the heartwood and sapwood were mixed and milled to powder, followed by drying at oven temperature 40°C for a week, and then extraction was conducted using ethanol-toluene (2/1, v/v) in soxhlet apparatus for 6 h.

Column Chromatography and Gas Chromatography Mass Spectrometry (GC-MS) Analysis

Si-gel 60 with size of 63-210 µm (Kanto Chemical Co., Inc., Japan) was used for column chromatography, where *n*-hexane, ethyl acetate (EtOAc), acetone, and methanol (MeOH) were loaded as eluent. Conversely, a GC-MS-QP 2010 (Shimadzu, Japan) machine was implemented to detect the compound, as 1 µl of the sample (1 mg/ml) was directly injected with column temperature from 100° C (1 min) to 320°C at 5°C/min; while that for injection and detection were 250°C and 320°C, respectively. In addition, DB-1 capillary column (30 m x 0.25 mm I.D. and 0.25 µm; GL Sciences, Tokyo, Japan) was used in the machine, using helium as the carrier gas, and the acquisition mass were set from 50-800 amu. Subsequently, the mass

spectrum obtained for each sample was compared with data from the NIST library and the literature (Sekine *et al.* 2009).

Total Phenolic Content (TPC)

The Folin-Ciocalteu method by Diouf *et al.* (2009) was used as a reference during the investigation of TPC. Approximately 2.5 ml of Folin-Ciocalteu phenol reagent (10 times dilution) was mixed with 0.5 ml of the sample (0.25 mg/ml) and incubated for 2 min, then 2 ml of 7.5% aqueous sodium carbonate was added and incubated again for 30 min. Finally, the mixture was placed in the equipment, followed by the sample absorbance reading at 765 nm, and the results of TPC were expressed as (+)- gallic acid equivalents (mg GAE/g extract).

Total Flavonoid Content (TFC)

TFC evaluation involved the AlCl_3 method (Brighente *et al.* 2007), where 2 ml of the sample prepared at 1 mg/ml concentration was reacted with 2% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution (2 ml). This mixture was then incubated for 1 h at 20°C, followed by the absorbance reading at 415 nm, and the results expressed in quercetin equivalents (mg QE/g extract).

Total Flavanol Content (TVC)

The TVC was determined using the vanillin-HCl method (Richard *et al.* 1978), where 0.5 ml of the sample (1 mg/ml concentration) was mixed with 3 ml of 4% vanillin reagent and 1.5 ml of HCl. This reaction was performed for 15 min at ambient temperature, followed by the absorbance reading at 500 nm, and standard calibration used was of (+)-catechin (mg CE/g extract).

Determination of DPPH Radicals Scavenging Activity

The determination of DPPH radicals scavenging or antioxidant activity was conducted according to Gao *et al.* (2006), where each 0.1 ml methanolic extract at different concentrations were mixed with 5 ml of 0.004% DPPH in methanol and incubated for 30 minutes. Therefore, the sample absorbance was read at 517 nm, using UV-Vis spectrophotometer, and the antioxidant activity was calculated using the following equation:

$$\text{DPPH scavenged (\%)} = 100 \times (\text{Ao} - \text{A1}) / \text{Ao}$$

Where Ao is the absorbance of blank and A1 is absorbance of sample. The antioxidant activity also was represented as IC_{50} , which is an expression for the concentration responsible for inhibiting 50% activity.

Chemicals

(+)- Gallic acid (97.5%), (+)- catechin ($\geq 95\%$), quercetin ($\geq 95\%$), and 1,1-diphenyl-2-picrylhydrazyl were purchased from Sigma Aldrich (Germany), while heneicosane ($\geq 95\%$) was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan).

Results and Discussion

Extraction and Isolation

The yield of *D. latifolia* ethanol-toluene extract was not mentioned in this report, although Table 1 shows the result of sample fractionation. Conversely, the isolation process involved the use of column chromatography with *n*-hexane as solvent, whose polarity was increased with EtOAc, acetone, MeOH, and water. At the inception, Fr. 1 and Fr. 2 had the highest yield of 0.74 g and 0.34 g (Table 1), which were collected in the eluent of *n*-hexane 100% and *n*-hexane/EtOAc 80%, respectively. Therefore, it is established that *D. latifolia* wood extractives is dominated by apolar compounds, although Fr. 12 yielded 0.59 g in the MeOH-water soluble fraction (polar compounds). This fraction is observed to possess a comparably higher content, and predicted to comprise of more polar components, including tannins.

Characterization of Fr.1- Fr.12

The 12 fractions were analyzed using GC-MS by direct injection, where only Fr. 1, Fr. 2, and Fr. 3 demonstrated a compound with a higher peak at a similar retention time of 41.5 mins (Figure 1), suggesting the tendency of similar components. Meanwhile, none was detected from Fr. 4- Fr. 12, as the presence of polar compounds possibly requires further processing by silylation or methylation.

Further discussions were performed to characterize Fr. 1- Fr. 3, through a comparison with the mass spectra of latifolin, as demonstrated in Table 2. Furthermore, the molecular weight obtained for Fr. 1- Fr. 3 was at m/z 286, alongside a base relative intensity at m/z 154. Therefore, a similarity was established between the fragmentations and latifolin, as reported by Sekine *et al.* (2009), making it the main compound, based on literature comparisons. This was also demonstrated in previous investigations performed on the genus *Dalbergia*, as an isolate from *D. parviflora* (Muangnoicharoen and Frahm 1982), with the molecular structure displayed in Figure 2.

Table 1. Yield of fractionation of *D. latifolia* wood

Column chromatography of ethanol toluene fraction (3.39 g)		
Eluting solvents	Fraction number	Weight (g)
<i>n</i> -Hexane	1	0.74
<i>n</i> -Hexane-EtOAc (8/2)	2	0.34
<i>n</i> -Hexane- EtOAc (7/3)	3	0.08
<i>n</i> -Hexane- EtOAc (5/5)	4	0.30
<i>n</i> -Hexane- EtOAc (3/7)	5	0.20
<i>n</i> -Hexane- EtOAc (2/8)	6	0.18
<i>n</i> -Hexane- EtOAc (1/9)	7	0.08
EtOAc	8	0.09
EtOAc -acetone (5/5)	9	0.19
Acetone	10	0.06
MeOH	11	0.43
MeOH- water (1/1)	12	0.59
Total		3.28

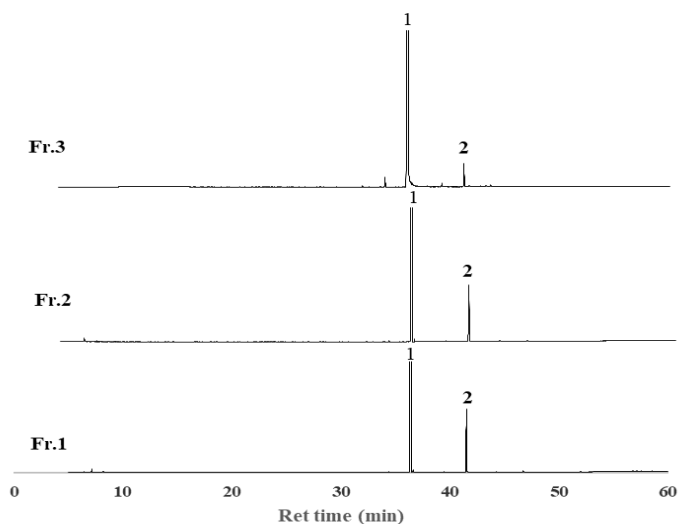


Figure 1. Chromatograms of GC- MS from Fr. 1- Fr. 3; 1. Heneicosane (Internal Standard (retention time: 36.4 min)), 2. Targeted compound (ret. time: 41.5 min) of *Dalbergia latifolia* wood

Table. 2. Comparison mass spectra of latifolin with Fr.1- Fr.3 of *Dalbergia latifolia* wood

Samples	Mass spectra fragmentations
<i>R</i> - (-) - <i>latifolin</i> ^a	286 [M] ⁺ (47), 269 (4), 255 (25), 240 (3), 227 (3), 211 (4), 193 (2), 180 (9), 167 (12), 154 (100), 139 (16), 133 (13), 131 (13), 115 (10), 107 (12), 105 (6), 91 (7), 77 (13), 69 (15), 65 (7), 51 (8)
Fr. 1	286 [M] ⁺ (83), 269 (7), 255 (42), 240 (5), 227 (4), 211 (6), 193 (3), 180 (9), 167 (11), 154 (100), 139 (19), 133 (18), 131 (18), 115 (14), 107 (19), 105 (10), 91 (14), 77 (22), 69 (21), 65 (10), 51 (10)
Fr. 2	286 [M] ⁺ (87), 269 (8), 255 (42), 240 (5), 227 (4), 211 (6), 193 (3), 180 (10), 167 (12), 154 (100), 139 (19), 133 (18), 131 (18), 115 (14), 107 (20), 105 (10), 91 (13), 77 (22), 69 (19), 65 (10), 51 (9)
Fr. 3	286 [M] ⁺ (86), 269 (7), 255 (40), 240 (7), 227 (7), 211 (7), 193 (3), 180 (9), 167 (10), 154 (100), 139 (17), 133 (15), 131 (19), 115 (20), 107 (23), 105 (12), 91 (15), 77 (22), 69 (19), 65 (10), 51 (10)

(a): Sekine *et al.* 2009

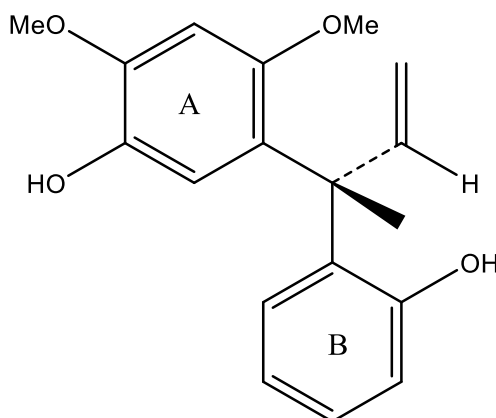


Figure 2. Chemical structure of *R*- (-)-*latifolin* from *D. latifolia*

Phenol Contents and Antioxidant Activity

The phenol content and antioxidant activity of Fr. 1- Fr. 12 were displayed in Table 3, as the highest TPC concentration was observed in Fr. 1 and Fr. 2, while Fr. 1 and Fr. 4 demonstrated the most significant level of TFC, and high TVC values were identified in Fr. 7 and Fr. 9. A comparison with previous works showed a markedly lower amount of TPC, especially in the Fr. 1 (469.8 mg/g) and Fr. 2 (415.5 mg/g), than in the bark of *D. latifolia* at 641.8 mg/g (Khalid *et al.* 2011), although higher than reported by Tripathi (2018) in the leaves (29.1 mg/g). Conversely, the TFC value for Fr. 1 (171.6 mg/g), Fr. 4 (173.2 mg/g), and Fr.

9 (170.5 mg/g) were higher than previous reports on the bark of *D. latifolia*, at 46 µg/ml (Khalid *et al.* 2011).

The test conducted with DPPH demonstrated a higher level of antioxidant activity in Fr. 1- Fr. 3 (Table 3), which was affiliated with the presence of latifolin as the main compound. Therefore, the high values in these fractions were assumed to have been affected by the neoflavonoids, despite the comparably higher level of the positive control, encompassing catechin, quercetin, and gallic acid. This study also demonstrated lower antioxidant activity in Fr. 1- Fr. 3 when compared to the bark of *D. latifolia* (Khalid *et al.* 2011). However, the values recorded were higher than *D. saxatilis* woody roots (Isyaka *et al.* 2015).

Table 3. Total phenolic content, total flavanoid, and antioxidant activity of fractions of *D. latifolia* wood

Fraction	TPC ^a	TFC ^b	TVC ^c	DPPH scavenging activity (%)		IC50 (µg/ml)
				500 µg/ml	250 µg/ml	
Fr. 1	469.8	171.6	28.3	72.2	37.6	340.4
Fr. 2	415.5	128.4	28.8	82.0	41.7	303.3
Fr. 3	191.3	144.4	52.8	59.6	26.8	393.1
Fr. 4	187.4	173.2	48.4	40.8	23.2	663.8
Fr. 5	82.2	101.1	31.8	24.5	13.8	1179.2
Fr. 6	109.0	166.4	54.0	31.0	15.8	7848.0
Fr. 7	113.3	162.4	61.6	28.0	15.6	1041.4
Fr. 8	128.8	158.8	52.1	28.1	12.1	944.9
Fr.9	142.8	170.5	56.8	37.3	21.9	592.0
Fr. 10	152.1	97.1	44.9	41.9	26.4	604.6
Fr. 11	69.0	96.6	8.3	26.0	6.7	1110.5
Fr. 12	51.6	104.7	31.4	20.4	11.3	1328.4
Catechin	-	-	-	93.8	92.0	83.3
Quercetin	-	-	-	96.1	93.0	28.7
Gallic acid	-	-	-	94.6	94.5	88.5

(-): not determined, DPPH: 1,1-diphenyl-2-picrylhydrazyl

^aDetermined by Folin-Ciocalteu assay, in units of milligrams (+)-gallic acid equivalent per gram sample^bDetermined by AlCl₃ assay, in units of milligrams quercetin equivalent per gram sample^cDetermined by vanillin-HCl assay, in units of milligrams (+)-catechin equivalent per gram sample

Correlation between Phenol Contents and Antioxidant Activity

Figure 3 shows the plots between antioxidant activity and phenol contents, which displayed a good pattern against TPC, suggesting the dependence of *D. latifolia* on TPC for effectiveness (3a), while Figure 3b and 3c were resulted in random plots. This relationship is in agreement with several prior studies (Eddebbagh *et al.* 2016; Guedes *et al.* 2017; Amamra *et al.* 2018; Hossain *et al.* 2019).

The measurement of TPC indicates the presence of phenols, hence a better understanding of the particular compound responsible in the antioxidant activity requires the conduction of specific phenol evaluation, through TFC and TVC. Figure 3b showed a low correlation with TFC, although fractions demonstrating high effectivity were generally observed to possess high concentrations. Furthermore, GC-MS data in the current study and a previous work (Sekine *et al.* 2009) reported on the presence of neoflavonoids on the extracts of *D. latifolia*, as the common fractions with more significant activity also possessed higher total flavonoids. The neoflavonoids identified in this research, including latifolin probably does not refer to the total flavonoids measured by the unit of standard, quercetin (Figure 4a). Based on the analysis of regression, the direct correlation against TFC was also weak, which is inconsistent with the previous reports by Eddebbagh *et al.* (2016) and Amamra *et al.* (2018), although

in agreement with the study by Ghasemi *et al.* (2009) on peels and tissues of 13 citrus species.

Further determination on more specific phenols was also conducted for TVCs, and the values obtained correlated properly with antioxidant activities (Figure 3c). Similar with TFC, it was impossible to establish a good correlation against TVC, due on the generally opposing values recorded, as shown in Table 3. This outcome suggests the weak dependence of antioxidant activity on TVC, which was expressed in catechin unit, possessing latifolin as the main compound and a different type flavonoid. Conversely, flavanols or catechins are flavanones 3-hydroxy derivatives, also referred to as flavan-3-ols, due to the bound of the hydroxyl group with the C ring at position 3 (Figure 4b). This compound is classified as a neoflavonoid, possessing the 4-phenylchromone skeleton, which is different from the 2-phenylchromen-4-one backbone (Phance *et al.* 2016). The varying concentration of catechins and latifolin as the main compounds in *D. latifolia* wood possibly lead to the reduced value of TVC in Fr. 1- Fr. 12, and is also associated with the absence of a good correlation against antioxidant activity. Meanwhile, the flavanols responsible for the donation of proton in the sample were not detected, which is different from the study of Henning *et al.* (2003) conducted on green tea extract, but in agreement with the research on tea extracts by Gao *et al.* (2013).

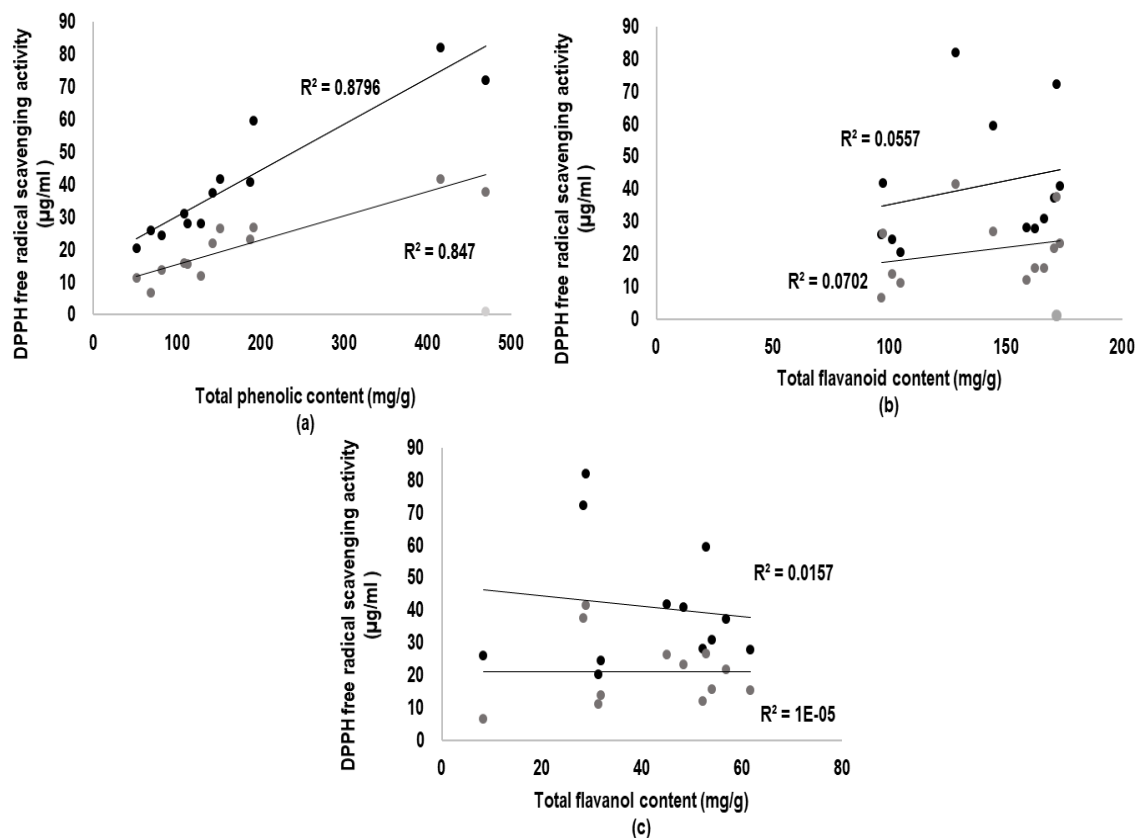


Figure 3. Correlation between antioxidant activity and total phenolic content (a), total flavonoid content (b), and total flavanol content (c). Black circles, 500 µg/ml; gray circles, 250 µg/ml of *Dalbergia latifolia*.

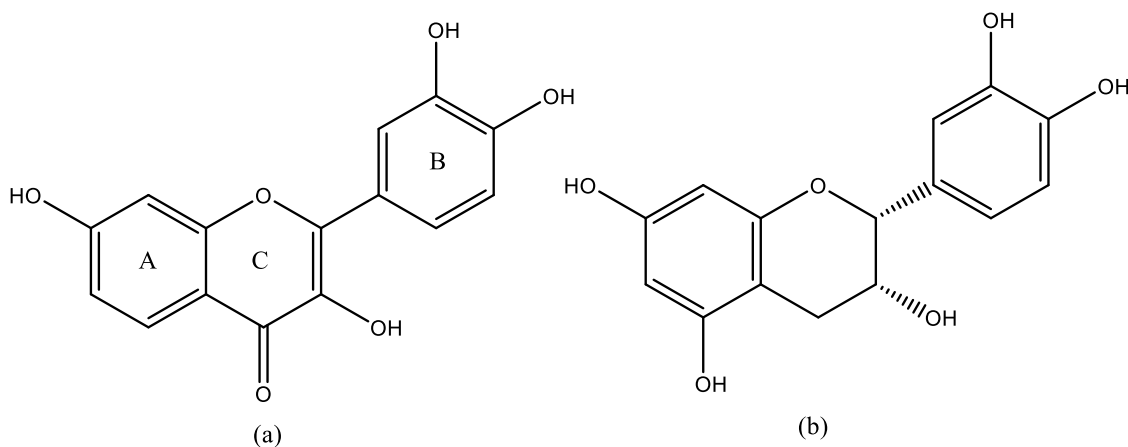


Figure 4. Common chemical structure of quercetin (a) and catechin (b).

Conclusions

Based on the results and discussion, it was established that Fr. 1- Fr. 3 showed comparably higher TPC and antioxidant activity than other fractions. Conversely, more significant levels of TFC were observed in Fr. 1 and Fr. 4, while Fr. 7 and Fr. 9 demonstrated relatively better TVC

concentrations. The GC-MS analysis detected latifolin in Fr. 1- Fr. 3, which was assumed to be responsible for antioxidant activity. Furthermore, the differences observed in the results of correlation against TPC, TFC, and TVC suggests the dependence of *D. latifolia* wood on TPC for effectiveness.

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