Investigation of Flavonoid Extractives and their Contribution to Color of *Dalbergia latifolia* Roxb Wood

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Abstract

Dalbergia latifolia wood is a beautiful wood with color of dark purple to dark brown and rich in flavonoid compounds. This study aimed to investigate the presence of flavonoid compounds from *D. latifolia* wood and their contribution to its color. The *D. latifolia* wood sample was powdered and extracted with ethanol-toluene (1/2, v/v). The ethanol- toluene extract then was column chromatographed to obtain fraction 1 to fraction 12. Methylation was conducted to each fraction to detect flavonoid compounds by GC-MS. The results of GC-MS analysis showed that ethanol- toluene extract gave Fr- 1- Fr. 3 as soluble flavonoids (34.2%) and Fr. 4- Fr. 12 as insoluble polymers (62.6%). Furthermore, 12 flavonoids i.e latifolin, dalbergiphenol, chalcone, dalbergin, and their derivatives were characterised and these compounds might be significance in contributing to color of *D. latifolia* wood.

Keywords: *D. latifolia*, color, extractives, flavonoids, latifolin, dalbergiphenol, chalcone, dalbergin

Introduction

Dalbergia latifolia Roxb, known as *sonokeling* in Indonesia is a member of Fabaceae family. In Indonesia*, D. latifolia* is found growing naturally in the forest of East and Central Java, favoring moist and well drained soils but also known to be drought resistant (Krishnamurthy *et al.* 2010; Praciak 2013). *D. latifolia* is known for its high-quality wood (Dwianto *et al*. 2019). Further, *D. latifolia* wood is highly valued for its beautiful dark purplish pattern with reddish brown color and also rich in fragrant. By those qualities, it made *D. latifolia* wood become popular for furniture, musical instruments, and various high-class home furnishing material. (Khalid *et al.* 2015; UNEP-WCMC 2017).

Color is one of an important aspect of wood quality. The color of dark purple to dark brown in the wood of *D. latifolia* was strong suggested by the presence of flavonoids and quinones. The earlier reports, the heartwood of *D. latifolia* have shown the presence of katinone, neoflavonoid dalcriodon, latinone, latifolin, 4-methoxydalbergione, and obtusaquinol (Rastogi and Mehrotra 1993a; and 1993b; Thurlough *et al.* 1981; Sekine *et al.* 2009a; Khalid *et al.* 2015). Latifolin from *D. latifolia* has been assayed for its antitermite and antifungal activity (Sekine *et al.* 2009b). Recent study has also identified a new diaryl 1, 2-diketone from the heartwood of *D. latifolia* and assessed its antibacterial activity (Liu *et al.* 2018).

Wood color variation is associated with the composition of its extractive components. Flavonoid type compounds have been mentioned for its contribution to coloration of plant parts (Giusti and Wrolstad 2003; Iwashina 2013; Panche *et al.* 2016). Previous researchers (Imamura *et al.* 1980; Yazaki *et al.* 1985; Kai and Swan 1990) showed some flavonoids to be responsible for coloration of wood. Several compounds with color have been isolated from *D. latifolia* wood such as dalbergiphenol, 5-O-methyllatifolin, and 3'-hydroxy-2 4 5-trimethoxydalbergiquinol which are yellow color (Meng *et al.* 2019). However, study of the relationship of flavonoids on their contribution to the color of *D. latifolia* has not been reported. In this study, the fractionation of ethanol- toluene extract of *D. latifolia* wood through column chromatography was conducted and each fraction were further examined for their contributions to color of *D. latifolia* wood.

Materials and Methods

Sample Collection and Extraction

The sample of *D. latifolia* wood (15-year-old) was collected and purchased from a woodworking industry in Bantul, while the tree origin is from Community Forest in Bantul, Yogyakarta, Indonesia. The heartwood and sapwood (2/1, w/w) were mixed and milled to powder then 10 g of sample was extracted by ethanol/ toluene (2/1, v/v) for 6 h in Soxhlet apparatus.

Column Chromatography and GC-MS Analysis

The ethanol- toluene extract (3.4 g) was loaded into Si-gel (60 N, spherical 63-210 µm, neutral Kanto Chemical Co., Inc., Japan) column chromatography. For the fractionation, the solvent of *n*-hexane, ethyl acetate (EtOAc), acetone, methanol (MeOH), and water were used as eluent. Each fraction then was evaporated and weighed as results (percentage of dry extract).

The GC-mass spectrometry (GC-MS) data were collected with a GCMS-QP 2010 (Shimadzu, Japan). The 1 µl of sample (1 mg/ml) was prepared for direct injection sample and methylation sample. The methylation protocol: to 2 mg of sample was dissolved into 1 ml of MeOH. Further, 100 µl of TMAH (tetramethylamoniumhydroxide, Sigma Aldrich, Germany) was added. The GC condition: Rtx-5MS

capillary column (30 m x 0.25 mm I.D. and 0.25 μm; GL Sciences, Tokyo, Japan); detection temperature of 285 °C; column temperature from 70 °C (1 min) to 290 °C at 5 °C/min; injection temperature of 250 °C; acquisition mass range from of 50-500 amu using helium as the carriers gas. The sample was compared to NIST library and literatures (Balakhrisna *et al.* 1962; Khan *et al.* 2006; An *et al.* 2008; Sekine *et al.* 2009a, and 2009b; Meng *et al.* 2019; Cuong *et al.* 2019).

Color Properties Determination (λ= 300-700 nm)

The sample (1 mg/ml) from 12 fractions was read at wave length of 300-700 nm for color measurement by an Ultraviolet (UV)/ Visible spectrophotometer (model SP-3000 Nano, Optima, Tokyo, Japan).

Results and Discussion

Extraction, Fractionation, and GC-MS Analysis

The woodmeal of *D. latifolia* was extracted by ethanol-toluene (2:1, v/v) and then was fractionated (Masendra *et al*. 2020). The fractionation was conducted by column chromatography with solvent *n*-hexane and increasing the polarity with EtOAc, acetone, MeOH, and water. Figure 1 showed the result of fractionation of ethanol-toluene extract (in total 3.28 g or 96.8% of initial sample). The results showed Fr. 1, Fr 2, Fr. 11, and Fr. 12 had great amount of extractive contents (≥10%). The Fr. 1 was eluted with *n*-hexane, Fr. 2 with *n*-hexane- EtOAc (8/2), Fr. 11 and Fr. 12 were obtained with polar solvent such as MeOH and water.

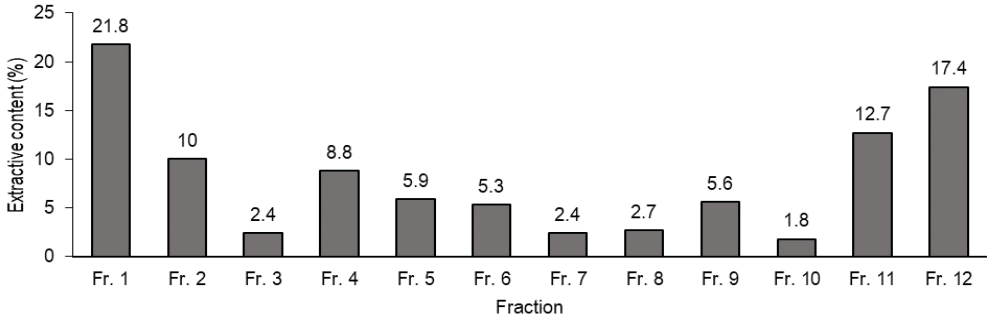

Figure 1. Fractionation results of ethanol- toluene extract of *D. latifolia* wood; Fraction (eluent): Fr. 1 (n-hexane), Fr. 2 (n-hexane- EtOAc; 8/2), Fr. 3 (n-hexane- EtOAc; 7/3), Fr. 4 (n-hexane- EtOAc; 5/5), Fr. 5 (n-hexane- EtOAc; 3/7), Fr. 6 (n-hexane- EtOAc; 2/8), Fr. 7 (n-hexane- EtOAc; 1/9), Fr. 8 (EtOAc), Fr. 9 (EtOAc- acetone; 5/5), Fr. 10 (acetone), Fr. 11 (MeOH), Fr. 12 (MeOH- water; 5/5).

The analysis of GC-MS by direct injection showed the Fr. 1- Fr. 3 have one peak and it was identified as latifolin (Masendra *et al*. 2020). Therefore, the methylation was conducted in all fractions. By methylation, many other flavonoids were observed (Figure 2 and Table 1) and were appeared at retention of 30- 40 minutes. That result was similar with that of direct injection of Fr. 1- Fr. 3. However, there were no peaks were detected in the Fr. 4- Fr. 12. The percentage of latifolin was dominant in Fr. 2 and Fr. 3, while 3-hydroxy-2,4,5-trimethoxydalbergiquinol was in a high concentration in Fr. 1.

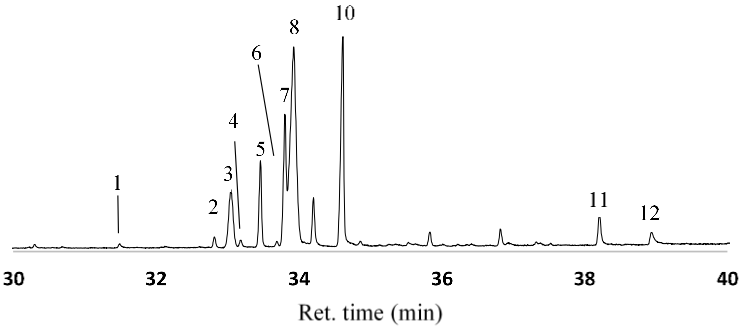

Figure 2. Chromatogram of GC-MS from Fr. 1; **1**. dalbergiphenol (retention time/ RT (min): 31.5), **2**. latifolin dimethyl ether (32.8), **3**. 2,3-dimethoxy-2,4-dihydroxychalcone (33.1), **4**. latifolin dimethyl ether isomer (33.2), **5**. 5-O- methyl latifolin (33.5), **6**. 2-O- methyl latifolin isomer (33.7), **7**. 2-O- methyl latifolin (33.8), **8**. 3-hydroxy-2,4,5-trimethoxydalbergiquinol (33.9), **9**. 2,4,5-trimethoxydalbergiquinol (34.2), **10**. latifolin (34.6), **11**. O-methyl dalbergin (38.2), **12**. dalbergin (38.9).

Table 1. Flavonoids from *D. latifolia* wood detected by GC-MS

Compound	Rt (min)	Constituents	Relative abundance (%)			Color
			Fr. 1	Fr. 2	Fr. 3	
1	31.5	dalbergiphenol	0.29	0.86	-	brown
2	32.8	latifolin dimethyl ether isomer	0.91	2.56	14.32	colorless
3	33.1	2,3-dimethoxy-2,4-dihydroxychalcone	8.78	1.29	-	yellow
4	33.2	latifolin dimethyl ether	0.64	-	21.61	colorless
5	33.5	5-O- methyl latifolin	7.35	3.47	-	yellow
6	33.7	2-O- methyl latifolin isomer	0.36	-	-	yellow
7	33.8	2-O- methyl latifolin	13.23	4.43	-	yellow
8	33.9	3-hydroxy-2,4,5-trimethoxydalbergiquinol	38.83	14.52	-	yellow
9	34.2	2,4,5-trimethoxydalbergiquinol	4.00	0.59	8.17	yellow
10	34.6	latifolin	21.33	58.30	25.48	colorless
11	38.2	O-methyl dalbergin	2.98	2.53	30.41	yellow
12	38.9	dalbergin	1.32	11.46	-	yellow

Remarks : the identification and color of constituents were assigned based on previous studies

Characterization of Fr.1- F. 3

In this study, flavonoids of *D. latifolia* wood were detected by GC-MS with direct injection and methylation method and the mass spectra of flavonoids detected in the sample were compared with previous works (Balakhrisna *et al.* 1962; Khan *et al.* 2006; An *et al.* 2008; Sekine *et al.* 2009a, and 2009b; Meng *et al.* 2019; Cuong *et al.* 2019). The chemical structure of compound 1- 12 was displayed in Figure 3.

Dalbergiphenol (**1**); MS, m/z (relative abundance): 270 [M^+] (100), 253 (15.4), 237 (14.6), 223 (21.2), 209 (15.8), 195 (6.9), 177 (12.7), 165 (23.3), 153 (14.6), 139 (123.3), 115 (52.9), 103 (14), 91 (47.3), 77 (28.8), 69 (25.7), 51 (27.2), 39 (5). The MS compound **1** was compared to previous works (Khan *et al.* 2006; Sekine *et al.* 2009b). Dalbergiphenol has a light brown oil color and it was isolated from *D. odorifera*, *D. latifolia* and *Ranunculus repens* (Khan *et al.* 2006; An *et al.* 2008; Sekine *et al.* 2009b). *Latifolin dimethyl ether* (**2**); MS, m/z (relative abundance): 314 [M^+] (100), 299 (13.6), 285 (31.5), 268 (10.2), 253 (7.3), 239 (9.3), 225 (6), 209 (6), 193 (4), 181 (11.2), 165 (9), 151 (21), 128 (11), 115 (16.8), 105 (9.7), 91 (10.4), 77 (14.7), 69 (10.5), 43 (7). The MS compound **2** was compared with a previous literature and this compound was isolated from *D. latifolia* heartwood (Sekine *et al.* 2009a). *2,3-Dimethoxy-2,4-dihydroxychalcone* (**3**); MS, m/z (relative abundance): 300 [M^+] (48.2), 285 (2.2), 269 (100), 253 (6.4), 239 (4.1), 225 (8.5), 210 (2.6), 181 (6.3), 168(6.6), 153 (9.6), 131 (7.4), 115 (8), 103 (4.4), 91 (6.6), 77 (7.2), 69 (10.1), 39 (3.9). The compound **3** was predicted from a chalcone group which have yellow color. *Latifolin dimethyl ether isomer* (**4**); MS, m/z (relative abundance): 314 [M^+] (100), 299 (19.8), 283(20.8), 269 (5.2), 257 (6.7), 241(4.6), 221 (4.7), 208 (6.9), 191 (7.5), 181 (15), 165 (11.6), 145 (16.1), 131 (14.4), 121 (21.6), 105 (8.3), 91 (21), 77 (11), 69 (12), 51 (8.3), 41 (5.5). Compound **4** was suggested to close with compound **2** with m/z 314 as molecule intake. *5-O- methyl latifolin* (**5**); MS, m/z (relative abundance): 300 [M^+] (53.6), 285 (3.3), 269 (27.5), 253 (5.5), 241 (4.1), 225 (6.6), 210 (4.4), 194 (5.9), 168 (100), 167 (9.1), 153 (39.5), 131 (16.1), 107 (11), 91 (8), 77 (16.6), 69 (12.1), 51 (6.6), 39 (7.8). The compound **5** was similar to 5-O- methyl latifolin that isolated from *D. latifolia* heartwood and *D. odorifera* heartwood with yellow color (Sekine *et al.* 2009a; An *et al.* 2008; Meng *et al.* 2019). *2-O- methyl latifolin isomer* (**6**); MS, m/z (relative abundance): 300 [M^+] (100), 285 (13.7), 271 (30.8), 253 (10.4), 239 (10.2), 225 (10), 211 (5.2), 194 (12.2), 181 (8), 153 (12.2), 137(25.4), 126 (7.6), 105 (18), 91 (25), 79 (10.5), 69 (15.4), 55 (12.5), 39 (8). The MS of compound **6** was compared to a previous work (Sekine *et al.* 2009a), it was isolated from *D. latifolia* heartwood. *2-O- Methyl latifolin* (**7**); MS, m/z (relative abundance): 300 [M^+] (100), 285 (9.3), 283 (9.8), 271 (20.3), 253 (7.6), 237 (8.1), 209 (5.6), 192 (10.3), 177 (7.6), 167 912.7), 137 (19.3), 115 (24.3), 105 (8.6), 91 (29.6), 77 (15.6), 65 (8.8), 53 (6.2), 41 (2.9). Same as compound **6**, compound **7** was similar to 2-O- methyl latifolin that was isolated from *D. latifolia* heartwood (Sekine *et al.* 2009a). *3-hydroxy-2,4,5-trimethoxydalbergiquinol* (**8**); MS, m/z (relative abundance): 300 [M^+] (19.4), 286 (35.2), 271 (5.4), 255 (100), 240 (8.5), 225 (4.7), 211 (6.2), 197 (3.7), 181 (6.1), 165 (10), 152 (7.2), 128 (11.2), 115 (14.2), 105 (6.2), 91 (11.7), 77 (7.7), 69 (13.5), 43 (6), 35 (5). The MS of compound **8** was similar with previous studies, and it also was isolated from the heartwood of *D. tonkinensis* and *D. latifolia* in yellow color (Cuong *et al.* 2019; Meng *et al.* 2019). *2,4,5-trimethoxydalbergiquinol* (**9**); MS, m/z (relative abundance): 284 [M^+] (100), 269 (7), 253 (3.5), 239 (8.3), 237 (35.2), 223 (3), 209 (22), 183 (4.3), 181 (16.4), 152 (10.9), 128 (13), 115 (9.1), 102 (4.7), 91 (3.3), 77 (6.2), 69 (14.9), 43 (11), 35 (5). This compound was isolated from heartwood of *D. odorifera* (Yun *et al.* 2015). *Latifolin* (**10**); MS, m/z (relative abundance): 286 [M^+] (59.2), 269 (6.2), 255 (37.5), 239 (5), 227 (5), 211 (6.4), 197 (4), 180 (9), 154 (100), 153 (15.5), 139 (23), 107 (12), 91 (10.3), 77 (20), 69 (17.2), 53 (9), 39 (11). The compound **10** was compared and reported from previous works as a colorless compound (Balakhrisna *et al.* 1962; Sekine *et al.* 2009a). *O-Methyl dalbergin* (**11**); MS, m/z (relative abundance): 282 [M^+] (100), 267 (11), 254 (44.4), 239 (25.7), 235 (32.4), 207

(7.6), 183 (13.1), 181 (3.3), 155(17), 152 (18.5), 139 (30.2), 115 (4.8), 102 (6.4), 89 (3.7), 77 (19.5), 69 (21.1), 51 (8), 39 (3.9). The compound **11** was suggested as O-methyl dalbergin that was isolated from the heartwood of *D. sissoo* (Ahluwalia *et al.* 1957; Ahluwalia and Seshadri 1956). *Dalbergin* (**12**); MS, m/z (relative abundance): 268 [M^+] (100), 253 (3.2), 225 (93.3), 197 (9), 183 (3), 169 (3), 155 (7), 141 (12), 127 (18), 115 (8), 101 (7), 89 (5), 77 (12), 69 (32), 53 (9), 39 (6). The compound **12** was suggested to similar with dalbergin, a yellow color compound that isolated from heartwood of *D. latifolia*, *D. odorifera*, *D. melanoxylon*, and *D. sissoo* (Ahluwalia and Seshadri 1956; Chan *et al.* 1997; Mutai *et al.* 2013; Meng *et al.* 2019).

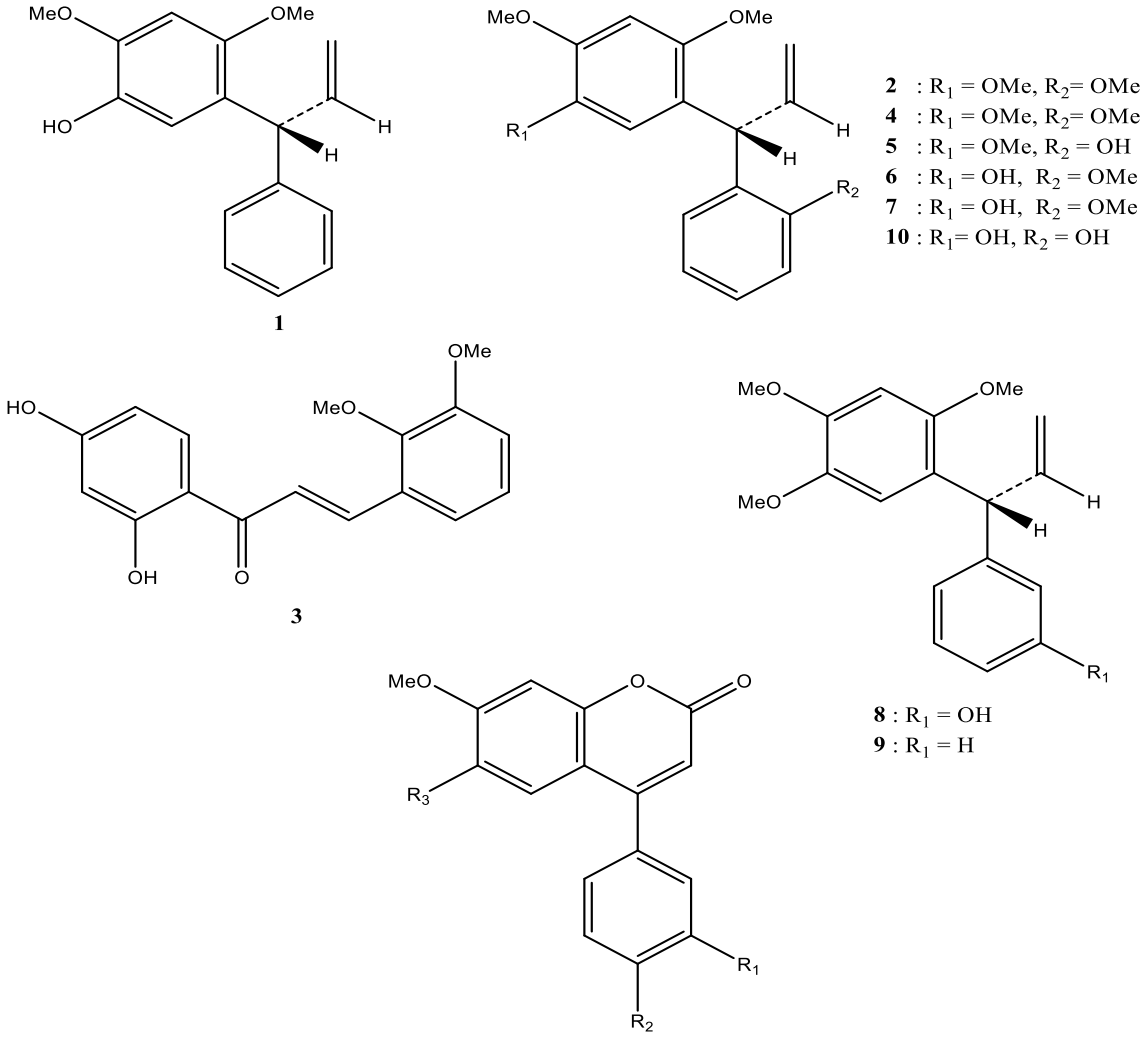

Figure 3. Chemical structure of flavonoids detected in *D. latifolia* wood; **1**. dalbergiphenol, **2**. latifolin dimethyl ether isomer, **3**. 2,3-dimethoxy-2,4-dihydroxychalcone, **4**. latifolin dimethyl ether, **5**. 5-O- methyl latifolin, **6**. 2-O- methyl latifolin isomer, **7**. 2-O- methyl latifolin, **8**. 3-hydroxy-2,4,5-trimethoxydalbergiquinol, **9**. 2,4,5-trimethoxydalbergiquinol, **10**. Latifolin,**11**. O- methyl dalbergin, **12**. Dalbergin.

Color Properties and Extractives Contributing to Color

In some wood species, flavonoids significantly involved in coloration and discoloration of wood (Yazaki *et al.* 1985; Kai and Swan 1990) and might decrease the quality of wood. Thus, the study of correlation between flavonoids and their contribution to the wood color is to support and develop the prevention of wood from damage when it is utilized. The fractionation of ethanol-toluene extracts in Figure 1 obtained 12 fractions which three fractions (Fr. 1- Fr.3) as soluble flavonoids and nine fractions (Fr.4- Fr. 12) as insoluble polymers. The yield of soluble flavonoids was 34.2% of ethanol- toluene extract dried sample. The solubility of flavonoids from Fr.1- Fr.3 generally obtained from solvent with a low polarity. By GC-MS analysis, flavonoids were almost from neoflavonoid group (Table 1). In comparison, neoflavanoids also were able to dissolve in solvent of *n*-hexane and dichloromethane (Sekine *et al.* 2009a, and 2009b; Wu *et al.* 2011).

The GC-MS analysis showed latifolin, dalbergin, dalbergiphenol, chalcone, and their derivatives were in Fr. 1- Fr. 3. The absorbance measurement of Fr. 1 and Fr. 2 resulted the high absorption in the beginning of UV-Vis spectra (Figure 4). This finding in Fr. 1 and Fr. 2 might be correlated with the presence of soluble flavonoids. On the contrary, the Fr. 11 and Fr. 12, as insoluble polymers, showed the absorption in a higher wavelength. With regard to a previous study, Santos-Buelga *et al*. (2003) reported that flavonoids with maximum absorption at 310- 370 nm is might from quercetin and maximum absorption at around 450-550 nm is from anthocyanins.

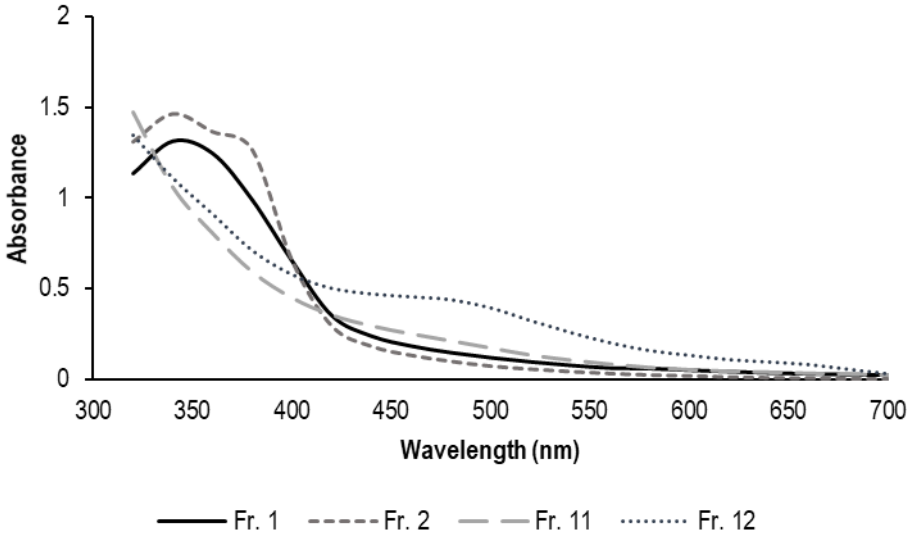

Figure 4. Absorbance of Fr. 1, Fr. 2, Fr. 11, and Fr. 12 from *D. latifolia* wood extract.

Based on yield extraction, the color properties of *D. latifolia* wood was dominated by Fr. 1 (21.8 %) and Fr. 12 (17.4%). Further, after GC-MS analysis of direct injection and methylation in the 12 fractions, only 3 fractions (Fr.1-Fr.3) showed the information of flavonoids. In the case of other fractions (Fr. 4- Fr. 12), the absent of flavonoids in GC-MS detection indicates that insoluble polymers were exist. The percentage of flavonoids in Fr. 1- Fr. 3 and insoluble polymers in Fr. 4- Fr. 12 were 34.2% and 62.6% (Figure 1), and thus the contribution of insoluble polymers was higher than soluble polymer to color of *D. latifolia* wood.

In this study, the absorbance measurement was conducted to each fraction using UV-Vis spectrophotometry. Due to Fr. 1, Fr. 2, Fr. 11 and Fr. 12 that represent soluble flavonoids and insoluble polymers had a high extractive content, the absorption of these fractions were further discussed. The appearance of color and absorbance for some fractions have been captured in Figure 4 and 5. The Fr. 1 and Fr. 2 had the high absorbance at 300- 400 nm, while Fr. 11 and Fr. 12 had a high absorbance at 400-550 nm.

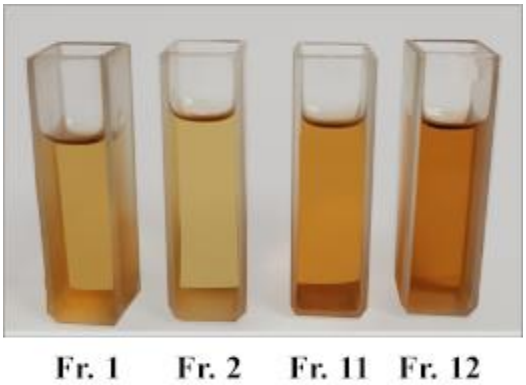

Figure 5. Color appearance of fractions from *D. latifolia* wood extract.

In the appearance (Figure 5), the color of Fr. 12 has the darkest color (brown) following by Fr. 11, Fr. 1, and Fr. 2. With regard to flavonoid presences, the Fr. 1 contained 3-hydroxy-2,4,5-trimethoxydalbergiquinol or compound **8** as the main compound (38.83%). In Fr. 2, compound **8** was 14.52% and it was a predominant compound. This compound (**8**) has a yellow color and it was derivative from dalbergiphenol, a compound with light brown oil color (Khan *et al.* 2006). In the case of Fr. 2, latifolin or compound 10 (58.3%) was the major compound (Table 1). However, due to the compound **10** is colorless, a single compound **10** may not contribute to the color of *D. latifolia* wood. This colorless compound (**10**) also may contribute the color of Fr. 2 that was not too dark as other fractions (Figure 5). Further, a comparison between color and colorless compounds was made in Figure 6. The Fr. 1 was dominated by color compounds, while the colorless compounds were in considerable proportion in Fr. 2. Thus, the presence of compound **8** and other colored compounds (**1, 3, 5, 6, 7, 9, 11,12**) in Fr. 1 and Fr. 2 might contribute to the color of *D. latifolia* wood.

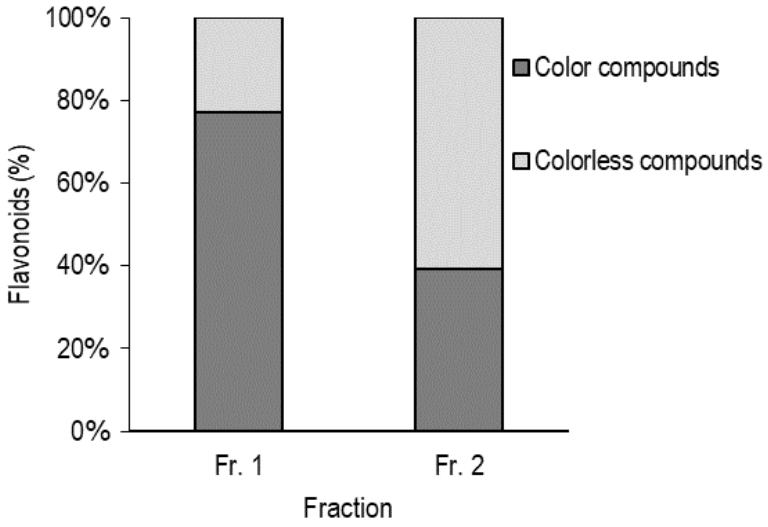

Figure 6. Comparison of extracts with color and colorless flavonoids detected in *D. latifolia* wood.

However, it should be considered that latifolin was detected in a high concentration with direct method injection of GC-MS, especially found in Fr. 1- Fr. 3. This suggests latifolin is a precursor in insoluble polymers (Fr. 4- Fr.12), which subsequently affected considerably to the color of *D. latifolia* wood. This phenomenon similar to previous works on catechin, a colorless compound and a precursor of condensed tannin in some wood species (Roux 1972; Yazaki *et al.* 1985). To the best of our knowledge, this is the first information of the correlation between flavonoids and color of *D. latifolia* wood. Thus, the identification of insoluble polymers from *D. latifolia* wood is necessary to be studied in the future work in order to find out the compounds that represent 62.6% of color contribution such as by acidolysis methods (Kai and Swan 1990).

Conclusions

The investigation of flavonoids from the ethanol-toluene extract of the *D. latifolia* wood and their contribution to the color has been conducted. The correlation showed that soluble flavonoids from Fr.1- Fr. 3 gave attribution of 34.2% to color of *D. latifolia* wood, while insoluble polymer was 62.6% in Fr. 4- Fr. 12. By methylation, 12 flavonoids that were grouped into latifolin, dalbergiphenol, chalcone, dalbergin were identified and their presence may contribute to the color of *D. latifolia* wood.

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