Chemical Composition of the Stemwood from *Eucalyptus pellita*

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ABSTRACT

The objectives of this research were to investigate the chemical composition of *Eucalyptus pellita* F. Muell stemwood. Wood powder from the middle part of two trees was successively extracted by dichloromethane, ethanol, and hot water. The content of dichloromethane, ethanol, and hot water extracts were 0.17 to 0.47 %, 2.56 to 3.16 %, and 0.68 to 1.20 %, respectively. Total phenolics content (TPC) were from 549.05±25.75 to 570.35±137.05 mg GAE/ g dried extract (ethanol extract) and 175.9±50.4 to 465.2±16.0 mg GAE/ g dried extract (hot water extract). Further, total flavanols content (TFC) from ethanol and hot water solution varied from 257.45±24.45 to 301.25±73.25 mg CE/ g dried extract and 52.5±2.75 to 113±32.9 mg CE/ g dried extract, respectively. It was observed that, TFC of ethanol extract increased from bark to heartwood. By GC-MS, the lipophilic constituents composed of fatty acids, sterols, steroids, and other components. Short-chain fatty acids and sterols were the most abundant of the lipid compositions. With the regard to cell wall components, the content of holocellulose, alpha-cellulose, and lignin were from 68.33 to 69.47 %, 45.63-47.27 %, and 32.31-32.80 %, respectively.

Keywords : extractives, flavanols, lipophilics, lignin, phenolics

Introduction

One of the most popular tropical tree plantation species is eucalyptus. There are more than 700 varieties of eucalyptus trees, the vast majority of which come from Australia. A commercially successful plantation tree should include rapid growth under plantation conditions, straight stems with limited branching, and decent wood quality for particular uses and products (Dombro 2010). In Indonesia, *Eucalyptus pellita* F. Muell *(E. pellita)* is one of the fast-growing species that has a great potential for industrial tree plantation (HTI) development. *E. pellita* is the native from New South Walles, Queensland (Australia), Bupul-Muting, Papua (Indonesia) and also are found in Morehead District (Papua New Guinea).

Chemical composition is linked to wood quality parameters and determines the suitability of the pulp (Pereira et al. 2003). It is known that cellulose, lignin, and extractives affect pulping behavior and determine the quality of pulp products (Valente et al. 1992). As a raw material for pulp and paper manufacturing, it is not only the

content of the cell wall components which affected the pulp quality but also the extractives composition. In the earlier studies, the chemical composition of *E. pellita* wood from progeny trials in Indonesia have been published (Fatimah et al. 2013; Lukmandaru et al. 2016). Additionally, the chemical composition of *E. pellita wood* from other countries also have been reported (Igarza et al. 2006; Oliveira et al. 2010). Hence, the objective of this work is to explore the chemical composition of *E. pellita* trees from natural forest.

Materials and Methods

Sample collection and extraction

The samples from 2 individuals of *E. pellita* trees were obtained from natural forest in Malind district (8.13^0'S, 140.05^0'E), Merauke, Papua. Bark, sapwood (± 0.5 cm from bark), and heartwood (± 1.0 cm from sapwood) parts were cut from the cross-section at the middle part of the trees. The tree diameters the middle part were 15 cm and 26 cm with the heartwood proportion of 53.78 % and 59.17 %. The bark and wood were separately milled and sieve screened to pass a 1 mm sieve. The 5 g powder in 40–60 mesh size fractions were successively extracted by dichloromethane, ethanol, and hot water solvent for 6 h in Soxhlet apparatus (Freire et al. 2002; Morais & Pereira 2012). Further, the solvent was evaporated by rotary evaporator. Then, the extract was dried in the oven and the extractive content was calculated.

Chemical analysis

Total phenolics and flavanols content

Total phenolics content (TPC) was measured according to Folin-Ciocalteu method (Nunes et al. 1999; Diouf et al. 2009) with modification. Further, total flavanols content (TFC) was done by vanillin-sulfuric acid method (Miranda et al. 2016). Visible spectrophotometer (model UV-1800, Shimadzu, Tokyo, Japan) was used. TPC and TFC were performed as the mean ± standard deviation of the duplicate measurements and expressed in gallic acid for TPC (mg GAE/g dried extract) and catechin equivalents for TFC (mg CE/g dry extract).

Lipophilic constituents

GC-FID: DB-5 capillary column (30 m x 0.25 mm I.D. and 0.25 μm; GL Sciences, Tokyo, Japan). Column temperature: 100 ^{0} C (1 min) to 320 ^{0} C at 5 ^{0} C/min. Injection: temperature at 250 ^{0} C. Carrier gas: He. GC-MS: DB-1 capillary column (30 m x 0.25 mm I.D. and 0.25 μm; GL Sciences, Tokyo, Japan). Column temperature: 50 ^{0} C (1 min) to 320 ^{0} C at 5 ^{0} C/min. Injection: temperature at 250 ^{0} C. Acquisition mass: range 50-800 amu. Carriers gas: He.

Identification of components: comparison with standard compounds and the NIST MS library. Quantification of components were conducted by calculating the relative peak area.

Cell wall components

The determination of wood of holocellulose and alpha-cellulose content was done with chlorite acid modification of Wise method (Browning 1967). Further, Klason lignin content was measured according to TAPPI T 222 os 1978 (1992).

Results and Discussion

Extractive content

The average values of dichloromethane extract (DEE), ethanol extract (ETA), and hot water extract (HTW) were 0.17 to 0.47 %, 2.56 to 3.16 %, and 0.68 to 1.20 %, respectively (Table 1). From the physical observation of the colour extract, the DEE was yellow-brown and oily-like appearance as apolar solvents will dissolve the oil compounds, waxes, fats, and terpenes. ETA and HTW extracts were dark and reddish in colour. Polar solvents, theoretically, will dissolve the phenolic compounds (Fengel & Wegener 1989; Sjostrom 1995). The levels of DEE and HTW showed that extractive content in the bark part was higher than in the stemwood. On the other hand, the content of ETA in the heartwood part was highest. In this study, it was found that extractive content in the heartwood was higher than sapwood part (3.15 % and 2.55 %, respectively).

Table 1. Extractive content of *E. pellita* stemwood (n=2, mean ± standar deviation)

Extractive content (%)	Radial		
	Bark	Sapwood	Heartwood
Dichloromethane	0.47±0.20	0.17±0.00	0.22±0.03
Ethanol	2.68±0.75	2.55±1.76	3.15±1.24
Hot water	1.20±0.10	0.69±0.34	0.67±0.11
Total	4.35±0.65	3.41±1.58	4.04±1.90

The amounts of ETA and HTW were slighty lower compared to ethanol-toluene (ETO) extract of *E. pellita* wood from progeny trials i.e. from Wonogiri (Fatimah et al. 2013) or from South Borneo (Lukmandaru et al. 2016) which their values ranged from 3.0 – 6.4 % (ETO) and 0.8 – 3.5 % (HTW). Further, the levels of the ETA and HTW in this study were also smaller than the levels of ETO (6.19-13.22 %) and HTW (13.96%) of *E. pellita* woods from Brazil (Igarza et al. 2006; Oliveira et al. 2010; Andrade et al. 2010). Thus, the comparatively low amounts of extractives in this study is an advantage for pulping process. On the other hand, the content in this present study were larger than that of *E. globulus* from Pepper Hill, Tasmania (Miranda & Pereira 2002) as the amounts

of DEE, ETA, and HTW of their woods were 0.2-0.3 %, 1.1-1.6 %, and 1.2-1.4 %, respectively.

Total phenolics and flavanols content

Total phenolic and flavanol contents were measured in ethanol and hot water soluble extracts. It was found that the composition of the extracts was differed among the various solvents. Content of total phenolics varied from 549.05±25.75 to 570.35±137.05 mg GAE/g dried extract and 175.9±50.4 to 465.2±16.0 mg GAE/ g dried extract (respectively for ethanol and hot water extract). Flavanols contents from the ethanol and hot water extracts were 257.45±24.45 to 301.25±73.25 mg CE/ g dried extract and 52.5±2.75 to 113±32.9 mg CE/ g dried extract, respectively (Table 2). The phenolic content from hot water solvent increased from the bark to the heartwood part. Further, stem wood extract presented the highest concentration of phenolic contents in the heartwood part. These indicate that particularly the heartwood part is potential sources of phenolic molecules for antioxidant activity. Luis et al. (2014) have been reported that stem wood has a great potential source of phenolic molecules for medicinal purposes.

Table 2. Total phenolics and flavanols content from *E. pellita* stemwood

Extracts	Parts	Phenol (mg GAE/ g dried extract)	Flavanol (mg CE/ g dried extract)
Ethanol	Bark	549.05±25.75	257.45±24.45
	Sapwood	570.35±137.05	260.65±94.45
	Heartwood	565.5±37.3	301.25±73.25
Hot water	Bark	175.9±50.4	52.5±2.75
	Sapwood	298.6±4.5	113±32.9
	Heartwood	465.2±16.0	65.85±2.55

GAE : Galic acid equivalents, CE : Catechin equivalents

The similar results have been reported that the levels of the total phenolics content from was extracts in the stemwood part (460 ± 5.61 mg GAE/g) were higher than in stem bark part (253.07 ± 4.94 mg GAE/g) of *E. globulus* (Luis et al. 2014). Further, other works mentioned that the phenolics content of the bark samples of 11 different eucalypts ranged from 282.5±0.8 to 916.7±50 mg GAE/g dried extract (Lima et al. 2017). It was reported that the content of flavanols in *E. urophylla* hybrid bark samples was ranged from 77 to 184 CE/g in ethanolic extracts of (Satori et al. 2016) and in *E. globulus* stump was 29 mg GAE/g in acetone-water extracts (Luis et al. 2014).

Lipophilic constituents

The lipid compositions of *E. pellita* stemwood i.e short-chain fatty acids(C<16), long-chain fatty acids(C>19), sterols, steroids, and other compounds were obtained (Table 3). Short-chain fatty acids can be detected (ret. time 6-24 min), along with long-

chain fatty acids (ret. time 27-37 min), steroids and sterols (ret. time 40-52 min). The long-chain fatty acids such as arachidic acid (28.0 min), docosanoic acid (30.9 min), lignoceric acid (33.9 min), and hexacosanoic acid (36.5 min) have been investigated in previous studies of wood and bark of *E. globulus, E.urograndis, E. grandis* and *E. maidenii* (Guttierez et al. 1999; Freire et al. 2006; Domingues et al. 2011).

Short-chain fatty acids such as myristic acid (17.0 min), palmitic acid (21.0 min), heptadecanoic acid (21.7 min), linoleic acid (23.9 min), oleic acid (24.1 min), and stearic acid (24.6 min), were mentioned also in wood, bark and pulp mill of *E. globulus* (Guttierez et al. 1999, 2006; Freire et al. 2006), *E. grandis* and *E. urograndis* clones (Silverio et al. 2007; Domingues et al. 2011). Further, palmitic acid, oleic acid, and linoleic acid were the most abundant lipophilic fraction of fatty acids (Guttierez et al. 1999; Freire et al. 2002, 2006; Silverio et al. 2007; Domingues et al. 2011). Additionally, sterols were the second most abundant of the lipid compositions, particularly for β-sitosterol. The similar pattern was reported in the outer bark from *E.globulus* and 3 other eucalypts (*E. grandis*, *E. urograndis, and E. maidenii*) (Freire et al. 2002; Dominguez et al. 2011). It should be noticed that the high amounts of long-chain fatty acids (29%) tend to cause a pitch formation (Silvestre et al. 1999). Furthermore, the considerable levels of sterols (21%) would cause a higher viscosity in the liquor than those of fatty acids and would induce a formation of pitch problem in a kraft process (Qin et al. 2003).

Table 3. Composition of lipids from *E. pellita* stemwood (*n* = 2%, based on dried extract)

Compounds	Formula	Maximum	Minimum	Average
Short Chain Fatty Acids				
Butanoic acid (**)	$C_4H_8O_2$	0.24	Tr	0.10 (0.11)
Nonanoic acid (**)	$C_9H_{18}O_2$	0.4	Tr	0.09 (0.16)
Dodecanoic acid (**)	$C_{12}H_{24}O_2$	0.63	Tr	0.15 (0.26)
Myristic acid (**)	$C_{14}H_{28}O_2$	0.44	0.25	0.44 (0.18)
Palmitic acid (*)	$C_{16}H_{32}O_2$	13	2.84	6.92 (3.57)
9-hexadecanoic acid (**)	$C_{16}H_{30}O_2$	0.16	Tr	0.03 (0.07)
Heptadecanoic acid (**)	$C_{17}H_{34}O_2$	0.49	Tr	0.11 (0.20)
Stearic acid (*)	$C_{18}H_{36}O_2$	3.41	0.46	1.19 (1.11)
Oleic acid (*)	$C_{18}H_{34}O_2$	12.1	1.02	3.79 (4.17)
Linoleic acid (*)	$C_{18}H_{36}O_2$	2.76	0.25	1.64 (0.98)
Long-Chain Fatty Acids				
Arachidic acid (*)	$C_{20}H_{40}O_2$	0.56	0.11	0.35 (0.17)
Docosanoic acid (**)	$C_{22}H_{44}O_2$	0.48	Tr	0.34 (0.18)
Lignoceric acid (*)	$C_{24}H_{48}O_2$	1.05	0	0.70 (0.40)
Hexacosanoic acid (**)	$C_{26}H_{52}O_2$	6.12	0.35	3.05 (2.43)
Sterols				
β-sitosterol (*)	$C_{29}H_{50}O$	7.77	2.55	3.94 (1.93)
Stigmastanol (**)	$C_{29}H_{52}O$	1.57	0	1.09 (0.55)
Olean-12-ene-3,28-diol(**)	$C_{30}H_{50}O_2$	6.11	0	1.02 (2.49)
Steroids				
Stigmast-4-en-3-one (**)	$C_{29}H_{48}O$	1.49	1.1	1.26 (0.14)
Urs-12-en-28-al (**)	$C_{30}H_{48}O$	3.93	0	0.93 (1.67)

Other compounds				
Squalene (*)	$C_{30}H_{50}$	0.28	0	0.13 (0.14)
Glycerol (*)	$C_3H_8O_3$	1.64	0.16	0.57 (0.55)
2,5-furandione (**)	$C_4H_2O_3$	0.50	0	0.26 (0.20)

(*) compared with standard compound, (**) compared with similarity index ≥ 80 % (NIST MS Library), tr: trace, the standard deviations are given in parentheses.

Carbohydrates and Klason lignin

The carbohydrate portion of the cell wall is composed of holocellulose and minor amounts of other sugar polymers such as pectin and starch. The carbohydrate fraction constituted 70-75 % of the wood cell wall. Further, lignin is the most abundant natural non-carbohydrate organic compound in fibrous materials. The content of holocellulose, alpha cellulose, Klason lignin ranged from 68.33 to 69.47 %, 45.63 to 47.27 %, and 32.31 to 32.80 %, respectively (Table 4).

This study showed slightly lower values when compared to the values of holocellulose (72.89 - 79.91%), alpha cellulose (41.84 - 54.85%), and Klason lignin (32.12 - 36.61%) that have been reported in the previous studies of *E. pellita* (Fatimah et al. 2013; Lukmandaru et al. 2016). On the other hand, the content of lignin was slightly larger when compared to that of *E. globulus* (Feria et al. 2012) and other eucalypts such as *E. botroyides*, *E. sideroxylon, E. rudis, E. maculata, E. resinifera, E. grandis, E. camaldunensis, E. saligna,* and *E. propinqua* (Neiva et al. 2014). Thus, compared to other eucalypts, the high lignin content of *E. pellita* is not expected as for its negative effects toward pulp properties. No considerable differences was observed in the cell wall content of the sapwood and heartwood.

Table 4. Chemical properties cell wall components of *E. pellita*

Chemical Component (%)	Sapwood	Heartwood
Holocellulose	69.47±2.65	68.33±2.57
Apha-cellulose	47.27±0.57	45.63±5.05
Klason lignin	32.8±0.65	32.31±2.17

Conclusions

Ethanol solution removed the highest portion of extractives (2.56 to 3.16 %), followed by hot water (0.68 to 1.20 %,), and dichloromethane (0.17 to 0.47 %). Thus, it showed that the polar fraction was largest in the stemwood. The extractive content in the heartwood part was higher than sapwood part. Further, *E. pellita* had a great potential source of antioxidant activity due to high levels the total phenolics and flavanols content, especially in the heartwood part. With regard to the composition of the lipids, the major compound such as long-chain fatty acids (29 %) and sterols (22 %) based of the weight extract were observed. It should be noticed that sterols was second

most abundant that may cause in pitch deposits on pulp and paper production. Furthermore, with regard to the cell wall component, both in heartwood and sapwood were an approximately similar percentage. However, the high content of Klason lignin was unfavorable for pulp and paper productions.

Acknowledgements

This work was supported by JASSO (Japan Student Services Organization) and DPP Grant 2017 (Faculty of Forestry, UGM).

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