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THE CONTENTS OF PHENOLICS AND CELL WALL COMPONENT OF *EUCALYPTUS PELLITA* F. MUELL STEMWOOD AND BARK

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ABSTRACT

Eucalyptus pellita is the fast-growing species which is being developed for a raw material of pulp and paper in Indonesia. The aim of this research was to evaluate the total phenolics (TPC) and flavanols contents (TFC) in the stemwood and bark parts from four individual trees. Another purpose was to determine its cell wall contents. Wood and bark materials in two vertical positions (bottom and top) were successively extracted using dichloromethane, ethanol and hot water as the solvents. Axial factor affected significantly in the values of hot water extract, TPC, and TFC but no significantly affected the cell wall component contents. The ethanol extract levels in the heartwood part was the significantly highest. It is noticed that the heartwood part had high levels of the TPC and TFC and low level in lignin content. From this experiment, the comparatively high levels of TPC and TFC in the heartwood indicate the potential antioxidative properties that should be explored in the future. Further, the low content of Klason lignin in the heartwood part would be an advantage for pulp production.

KEYWORDS: Extractive content, phenolics, cell wall components, pulp, *E. pellita*.

INTRODUCTION

Eucalyptus is a native genus from Australia and belongs to a family of Myrtaceae and consists of 900 species (Brooker and Keing 2004). *Eucalyptus* species are important raw materials for pulp, timber and charcoal industries, e.g. *E. globulus, E. urophylla, E. grandis* and various

hybrids (Neiva et al. 2014, Sartori et al. 2016). One of the selected species for plantation forest of *Eucalyptus* species is *E. pellita*. It has a natural distribution in Papua New Guinea (PNG), Irian Jaya (Indonesia), and north Queensland (Australia) (Harwood et al. 1997).

E. pellita has shown great potential for plantation forest, where 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 (Pinyopusarerk et al. 1992, Dombro 2010). Nowadays, in Indonesia, *E. pellita* substituted *Acacia mangium* as an industrial forest plantation (Hutan Tanaman Industri or HTI) as this species would be more tolerant with a variety of soils and location conditions; distinct to be resistant to common pest and diseases such as insect and fungi attacks (Harwood 1998, Dombro 2010).

Parts of the eucalyptus trees are known as a potent antioxidant activity such as terpenoids, tannins, flavonoids, and phloroglucinol derivative (Boulekbache-Makhlouf et al. 2013). Many of these compounds have therapeutic properties and are known for their anticarcinogenic, antimutagenic, cardioprotective, anti-neurodegenerative, and antimicrobial activities (Babich and Visioli 2003, Gursoy et al. 2009, Rababah et al. 2011). Some studies of *Eucalyptus* species were reported that the woods contain biologically activity such as stump wood and bark parts of *E. globulus* (Luís et al. 2014), 11 *Eucalyptus* species bark (*E. botryoides, E. camaldulensis, E. globulus, E. grandis, E. maculata, E. ovata, E. propinqua, E. resinifera, E. rudis, E. saligna, E. viminalis*) (Lima et al. 2017).

However, to our knowledge, investigations related to *E. pellita* are still limited, particularly in phenolic contents. Only a few studies were conducted with regard to the chemical composition of *E. pellita* wood which was from progeny trials in Indonesia (Fatimah et al. 2013, Lukmandaru et al. 2016) and heart-rotted mature wood (Lukmandaru 2018). Hence, this study is to explore the extractives, phenolic contents, and cell wall components on the axial and radial direction of *E. pellita* stem wood and bark from natural forest.

MATERIALS AND METHODS

Wood specimen

E. pellita trees (4 individuals) were collected in May 2012 at the natural forest (tree age was unknown) in Malind district (8.13o'S, 140.05°'E), Merauke, Papua. The amounts of total diameter, heartwood proportion and total merchantable height ranged from 15.2 to 27 cm, 58.28 to 72.75%, 19 to 27 m, respectively. Each of *E. pellita* tree was cut at the bottom part (10% of total height) and the top part (80% of total height) in disc form (± 5 cm in thickness) to obtain 8 discs form. Each disc was divided into a bark, sapwood (± 0.5 cm from bark), and heartwood (± 1.0 cm from the sapwood-heartwood border) to obtain 32 samples, which were taken from the cardinal direction on the trunk to avoid radial variation, if any.

By visual inspection, the heartwood has a distinctive brown colour compared with lighter sapwood colour. The bark and wood were separately milled to powder meal and were sieve-screened (to pass a 1 mm sieve).

Extraction

The 5 g powder in 40-60 mesh size fractions was successively extraction by dichloromethane and ethanol for 6 hours in a Soxhlet apparatus as well as hot water (refluxing in separated extraction for 3 hours) (Morais and Pereira 2012). The temperature was set at 60°C for dichloromethane, 90° for ethanol, and 110°C for hot water. Further, the solvent was evaporated

by a rotary evaporator. Then, the extract was dried in an oven (103±2°C) and the extractive content was quantified.

Chemicals

Aluminium chloride, sodium carbonate, sodium hydroxide, Folin-Ciocalteu's phenol reagent were purchased from Merck (Darmstadt, Germany) and standard components of gallic acid, and (+)-catechin were purchased from Sigma-Aldrich (Chemie GmbH, USA) to analytical grade.

Determination of total phenolic content

Total phenolic content (TPC) was measured by Folin-Ciocalteu method (Singleton et al. 1999). Briefly, 2.5 ml of diluted Folin-Ciocalteu phenol reagent and an aqueous solution (1:9, v/v) was mixed with 0.5 ml of the sample solution (0.25 $mg{\cdot}ml^{-1}$) in a 9 ml glass. After an interval of 2 min, 2 ml of 7.5% aqueous sodium carbonate (Na_2CO_3) was added to the glass, and the mixture was allowed to stand for 30 min at ambient temperature. The absorbance for testing of standard solutions was performed against the blank at 765 nm with an Ultraviolet (UV) / Visible spectrophotometer (model UV-1800, Shimadzu, Tokyo, Japan). The standard curve was prepared using 0.03125, 0.0625, 0.125, 0.25 $mg{\cdot}ml^{-1}$ solutions of gallic acid in methanolic ($y = 0.0947 x - 0.0225$; $R^2 = 0.9991$) and hot water ($y = 0.1927 x - 0.0085$; $R^2 = 0.9998$). TPC was performed as the mean ± standard deviation of four trees replication measurements and an expressed as milligrams gallic acid equivalents (mg GAE/g dried extract).

Determination of total flavanol content

Total flavanol content (TFC) was quantified by vanillin HCl assay as described by Miranda et al. (2016) with modification. To 0.5 ml (0.25 $mg{\cdot}ml^{-1}$) of the sample solution, 3 ml vanillin reagent (4% vanillin methanol) and 1.5 ml HCl were added and the reaction is performed for 15 min at ambient temperature. The blank solution was prepared with the same procedure without vanillin. The absorbance for testing of standard solutions was performed against the blank at 500 nm with an Ultraviolet (UV) / Visible spectrophotometer (model UV-1800, Shimadzu, Tokyo, Japan). A set of reference standard solutions of (+)-catechin (0.03125, 0.0625, 0.125, 0.25 $mg{\cdot}ml^{-1}$) with (+)-catechin in methanol ($y = 0.3591x - 0.0141$; $R^2 = 0.9995$) and hot water ($y = 0.3426 x - 0.0079$; $R^2 = 0.9998$). TFC were performed as the mean ± standard deviation of the four trees replication measurements and an expressed in (+)-catechin equivalents (CE) (mg CE/g dried extract).

Cell wall component in the heartwood and sapwood

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 by standards of T 222 os - 1978 (TAPPI Standard 1992).

Statistical analysis

The data were statistically handled using the SPSS program (version 16 IBM, New York, USA). Analysis of variance (ANOVA) was carried out and statistically significant differences were set at a 95% confidence level. Two-way ANOVA was applied to determine the effect of axial (bottom and top) and radial (bark, sapwood, and heartwood) direction on extractive content, TPC, and TFC, and cell wall components. Further, Duncan test was performed to determine specific differences between pairs of means. The analysis performed as the mean ± standard deviation of the four tree replications measurements.

RESULT AND DISCUSSION

Extractive content

By observing the colours, ethanol and hot water extracts showed dark and reddish colours. Theoretically, those polar solvents will dissolve the phenolic compounds (Fengel and Wegener 1989, Sjöström 1993). Average of dichloromethane, ethanol, and hot water extractives content were ranged from 0.19 to 0.38%, 1.58 to 4.95%, and 0.64 to 1.89% based on oven-dried wood, respectively. The amounts of ethanol and hot water extract were slightly lower compared to the values of ethanol-toluene extract of young *E. pellita* wood from progeny trials, Wonogiri (Fatimah et al. 2013) and from South Borneo (Lukmandaru et al. 2016) with the values from 3 to 6.4% (ethanol-toluene extract) and from 0.8 to 3.5% (hot water extract). However, it showed lower amounts compared to ethanol extract (3.98 to 16.86%) and hot water extract (1.92 to 3.40%) in a mature tree (Lukmandaru 2018). Further, the levels of the ethanol and hot water extracts were also smaller than of ethanol-toluene (6.19 - 13.22%) and hot water extract (13.96%) values of *E. pellita* from Brazil (Igarza et al. 2006, Oliveira et al. 2010, Andrade et al. 2010).

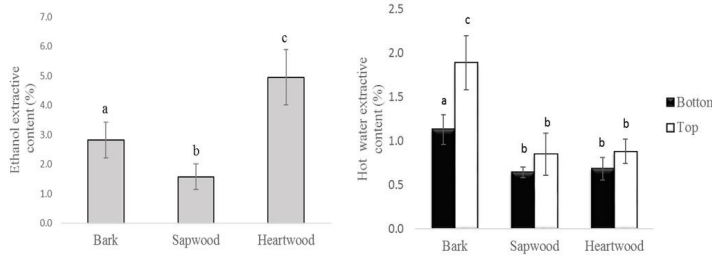
The variation of the dichloromethane extract was reported in the parallel work (Arisandi et al. 2019), which the bark part had the highest levels (0.42 ± 0.18%) for radial direction, and in the top parts (0.33 ± 0.14%) for longitudinal direction. Further, the two-way ANOVA analysis was performed to obtain the effects of axial and radial direction (Tab. 1). Two-way ANOVA showed that the radial factor affected ($p < 0.01$) the ethanol extract amount, as well as interaction on axial and radial direction affected hot water extract amount ($p < 0.05$).

Tab. 1: Results from two- way ANOVA for ethanol and hot water extract of E. pellita.

Source of variation	df	Solvent	
		Ethanol	Hot water
Axial (A)	1	n.s.	***
Radial (R)	2	**	***
A x R	2	n.s.	*
Error	18		
Total	23		

df degrees of freedom;

n.s. not significant at 5% level, * P<0.05, **P<0.01, ***P<0.001.



(a) (b)

Fig. 1: (a), (b): The extractive content of ethanol and hot water (% based on oven-dried wood) from E. pellita (means of four trees) on a radial direction for ethanol and interaction on an axial and radial direction for hot water with error bar as standard deviation. The same letters on the histograms implied no significant differences (p <0.05 by Duncan's test).

The levels of ethanol extract in the heartwood part were significantly higher than those of bark and sapwood part (Fig. 1 a-b). Further, the amount of hot water extract in bark at the top part was significantly found to be the highest. No systematic difference was measured in the heartwood and sapwood parts for axial direction, except in the bark part (Fig. 1b). In other eucalypt species, these trends are similar to those described in the previous works with regard to the ethanol extract in *E. globulus* (Miranda et al. 2007, Lourenco et al. 2010, Morais and Pereira 2012), *E. urograndis* hybrid (Gominho et al. 2001), and *E. grandis* and *E. pilularis* (Higgins 1984, Mariana et al. 2005). Furthermore, this present finding showed that the ethanol extract of the bark part was lower compared to heartwood part. It was a similar trend with ethanol extract of *Juglans regia* L. (Hosseini Hashemi 2012). Previously, Kai (1991) reported that heartwood part is richer in polyphenols and resin acids (diterpenes) than bark part. In the heartwood part (with lower pH condition), the most of the soluble sugars (xylose, mannose, and arabinose) were derived from the hydrolyses.

Further, the portion of hot water extract in this experiment was similar to previous works where the extractive content in the bark was considerably higher compared to other wood parts (Fengel and Wegener 1989, Sjöström1993). In other eucalypts, the total amount of extractives content had only a few percents in bole but the amount can be much larger in certain parts of the tree, e.g., bark, branches, and topwood part (Pereira 1988, Domingues et al. 2010). The content and composition of extractives can vary in response to age (Pereira 1988), species (Wilkes 1984), growth rate (Hillis 1971), site (Miranda and Pereira 2002) and wood storage (Gutiérrez et al. 1998). The highest levels of hot water content in the top bark indicated more amounts of sugars, such as starches (Gominho et al. 2015).

Total phenolic and flavanol contents

The total phenolic contents in the ethanol and hot water extracts ranged from 368.4 to 632.5 and from 199.9 to 429.6 mg GAE/g dried extract, respectively. On the other hand, the values of flavanols content from the ethanol and hot water extracts were 165.6 to 373.8 and 47.5 to 132.5 mg CE/mg dried extract, respectively. Two-way ANOVA analysis was carried out to observe the effects of axial and radial direction (Tab. 2).

Tab. 2: Results from two-way ANOVA for ethanol and aqueous extracts for total phenolic and flavanol contents of E. pellita.

Source of variation	df	Total phenolics content		Total flavanols content	
		Ethanol	Hot water	Ethanol	Hot water
Axial (A)	1	n.s.	**	n.s.	n.s.
Radial (R)	2	**	**	***	***
A x R	2	n.s.	n.s.	*	**
Error	18				
Total	23				

df degrees of freedom;

n.s. not significant at 5% level, * P<0.05, **P<0.01, ***P<0.001.

Two way ANOVA showed that the TPC values of ethanol extract were significantly affected by radial direction, whereas hot water extract was influenced by both axial and radial directions. On the other hand, the interaction between axial and radial direction significantly affected the amount of TFC in both ethanol and hot water extracts (Tab. 2).

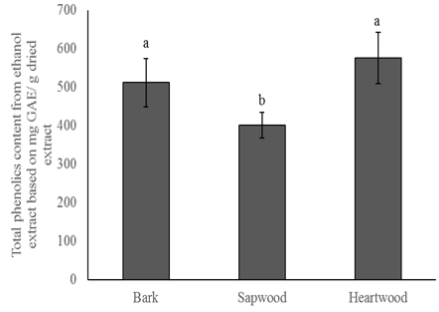


Fig. 2: Total phenolic content of ethanol extract based on mg GAE/g dried extract from E. pellita (means of four trees) on radial direction with error bar as standard deviation. The same letters on the histograms implied no significant differences (p <0.05 by Duncan's test).

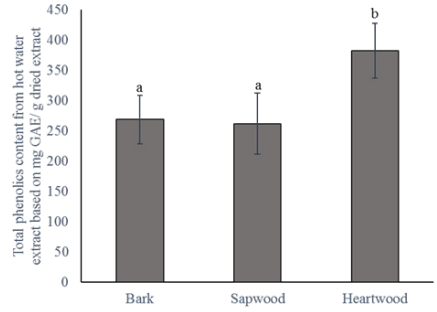


Fig. 3: Total phenolic content of hot water extract based on mg GAE/g dried extract from E. pellita (means of four trees) on radial direction with error bar as standard deviation. The same letters on the histograms implied no significant differences (p <0.05 by Duncan's test)

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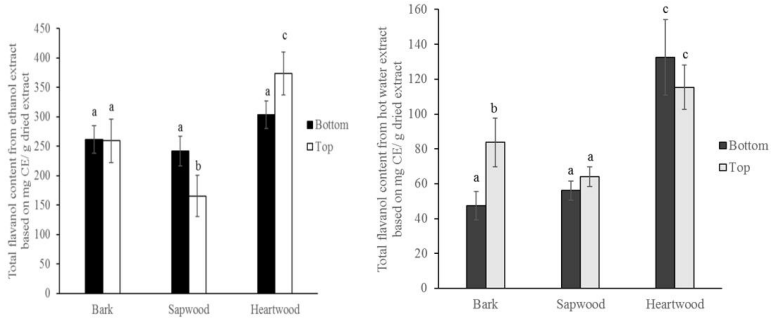

(a) (b)

Fig. 4: (a), (b): Total flavanols content of ethanol and hot water extract based on mg CE/g dried extract from E. pellita (means of four trees) interaction on axial and radial direction with error bar as standard deviation. The same letters on the histograms implied no significant differences (p <0.05 by Duncan's test).

By Duncan test, TPC of ethanol extract in the sapwood part was significantly lower than in the bark and heartwood parts (Fig. 2). Additionally, TPC amounts of hot water extract in the heartwood part was significantly larger compared to other parts in the radial direction (Fig. 3). Further, TPC in the top part was significantly higher (347.2 ± 32.4 mg GAE/g dried extract) than in the bottom part (261.6 ± 12.6 mg GAE/g dried extract) on axial direction (p<0.05). These findings implied that in the heartwood part are potential sources of phenolic molecules for antioxidant activities as well as antimicrobial and antifungal activities. Luís et al. (2014) reported that stump wood has a great potential of phenolic molecules for medicinal purposes. Gao et al. (2007) reported that sapwood is rich in nutrients such as sucrose and glycosides, unlike heartwood and outer bark which are typically deficient in these nutrients but are rich in secondary metabolites such as the flavanols and the other phenolic contents to protect the living tissues against biological attacks.

A comparison of the TPC of *E. pellita* in stem wood and bark with previously published works in other eucalypt species is interesting. In most cases, TPC in the stem wood and bark observed here were either similar or below the reported values: in the bark of some *Eucalyptus,* such as *E. sideroxylon, E. grandis, E. urograndis*, and *E. maidenii* barks, the values were 441, 386, 347, and 204 mg GAE/g ethanol and ethanol–hot water extract, respectively (Santos et al. 2012, Miranda et al. 2016). Further, Vázquez et al. (2008) and Santos et al. (2011) also mentioned that TPC values of *E. globulus* bark of the ethanolic, methanolic, and hot water extracts were 223, 410 and 155 to 201 mg GAE/g dried extract, respectively. Additionally, these results are also in agreement with those obtained for *E. tereticornis* (198 mg GAE/g methanolic extract) (Puttaswamy et al. 2014), 11 eucalyptus species ranged from 283 to 917 mg GAE/g of extract (Lima et al. 2017) and in *E. camaldulensis*, *E. globulus*, and *E. rudis* (93, 23, and 3 mg GAE/g of bark), respectively (Conde et al. 1995, Cadahía et al. 1997). Furthermore, the values of phenolic contents of some *Eucalyptus* wood, such as *E. globules* was 262.67 ± 3.06 mg GAE/g dried ethanolic extract (Luís et al. 2014), *E. grandis, E. urograndis*, and *E. maidenii* were 825.47 ± 26.75, 775.59 ± 34.51, 687.89 ± 30.58 mg GAE/g dried extract methanolic / hot water extract (Santos et al. 2013) and *E. camaldulensis, E. globulus*, and *E. rudis* were in the range from 5.22 to 25.63 $mg \cdot g^{-1}$ (Conde et al. 1995). Compared to other species, TPC of the heartwood, sapwood, inner bark and outer bark extract of Port Orford Cedar were 136.9, 257.7, 537.5, 489.1 mg GAE/g dried extract (Gao et al. 2007).

With regard to flavanol contents, some studies were published for other eucalyptus species. Luís et al. (2014) reported the values of flavanols content in the wood and stump bark varied from 13.93 to 17.00 mg GAE/g dried extract. The content of flavanols in the different eucalyptus barks were also observed, i.e. *E. sideroxylon* (395 mg CE/g ethanolic extract) (Miranda et al. 2016), *E. urophylla* hybrids (77 to 184 mg CE/g in ethanol extract) (Sartori et al. 2016), *E. tereticornis* bark (103 mg tannic acid equivalents / g in hot water extract) (Puttaswamy et al. 2014), and *E. camaldulensis, E. globulus*, and *E. rudis* barks (0.13 to 39.21, 3.28 to 7.43, 0.16 to 2.09 mg GAE/dried of bark, respectively) (Cadahía et al. 1997), as well as for 11 other eucalyptus species in bark (94 mg to 545 mg CE/g dried extract) (Lima et al. 2017). Further, other species showed that the values TFC trembling aspen bark was 5.2 to 18.3 mg CE/g dried extract (Diouf et al. 2009) whereas the values in methanol / hot water extract of *Maclura tinctoria* wood and bark were 5.1 ± 0.6 and 3.9 ± 0.1 mg CE/dried wood / bark (Lamounier et al. 2012). This study finding demonstrated the differences between the levels of TPC and TFC on the axial and radial direction. It should be noted that this study observed the high levels of TPC and TFC, particularly in the heartwood and bark parts (e.g. Fig. 2 and Fig. 4a-b). Phenolic compounds have received much attention for their effective antioxidant properties (Lima et al. 2017). Therefore, identification of those compounds will be necessary in the future work.

Cell wall components

The carbohydrate portion of a cell wall is composed of holocellulose and minor amounts of other sugar polymers such as pectins and starches. The carbohydrate fraction constitutes 70-75% of wood cell wall. Further, lignin is the most abundant natural non-carbohydrate organic compound in fibrous materials. The content of holocellulose, α-cellulose, and Klason lignin ranged from 67.37 to 70.57%, 42.55 to 50.97%, and 28.8 to 32.94%, respectively. Two-way ANOVA analysis was performed to observe the effects of axial and radial direction (Tab. 3).

Tab. 3: Results from two-way ANOVA for cell wall components of E. pellita.

Source of variation	df	Cell wall		
		Holocellulose	α-cellulose	Klason lignin
Axial (A)	1	n.s.	n.s.	n.s.
Radial (R)	1	n.s.	n.s.	**
A x R	1	n.s.	n.s.	n.s.
Error	12			
Total	15			

df degrees of freedom;

n.s. not significant at 5% level, *P<0.05, **P<0.01.

Tab. 4: Cell wall content on the axial and radial direction of E. pellita.

Cell wall	Radial	
	Sapwood (%)	Heartwood (%)
Holocellulose (b)	70.31 ± 5.44	70.57 ± 3.57
Holocellulose (t)	67.49 ± 3.87	67.37 ± 1.23
α-cellulose (b)	50.97 ± 4.68	48.34 ± 7.09
α-cellulose (t)	42.55 ± 4.52	44.77 ± 5.30
Klason lignin	32.54 ± 1.82 (a)	29.42 ± 1.79 (b)

Note: Average of four trees ± the standard deviation; b = bottom, t = top.

Values followed by the same letter within a column are not significantly different (*P<0.05) as determined by two-way ANOVA. In the radial position, there was no significant difference in holocellulose and α-cellulose contents. Difference results were reported by Mariana et al. (2005) in *E. nitens* that the heartwood part had lower holocellulose and α-cellulose contents than in the sapwood part. On the other hand, the amount of Klason lignin content in sapwood part was significantly larger compared to the heartwood part (Tab. 4). With regard to lignin content, the value was different compared to a previous work in a mature wood of *E. pellita* (Lukmandaru 2018). It was observed that the content of Klason lignin in the heartwood part was higher than that of in the sapwood part. However, similar trends were found in *E. nitens, Acacia melanoxylon*, and poplar I-69 (Mariana et al. 2005, Lourenco et al. 2010, Gao et al. 2011).

Compared to other studies of *E. pellita* wood, the range levels of the holocellulose in this study was similar to previous studies (Andrade et al. 2010, Oliveria et al. 2010, Fatimah et al. 2013, Lukmandaru et al. 2016, Lukmandaru 2018). For comparison, in other species, holocellulose and Klason lignin of 12 *eucalyptus* species varied from 55.4 to 70.1% and 21.6 to 30.8% (Neiva et al. 2014), as well as α-cellulose, and Klason lignin content in 6 other *eucalyptus* species ranged from 46.1 to 48.8% and 28.8 to 31.4%, respectively (Pereira et al. 2013). Thus, from the point of view of pulp and paper production, the comparatively low lignin content in the heartwood part would be beneficial regardless of its extractive content.

CONCLUSIONS

The yield of ethanol extract of *E. pellita* wood was significantly affected by radial direction. It was found that the levels of ethanol extracts in the heartwood were significantly higher than in the bark and sapwood. Further, hot water extract was significantly influenced by interaction on axial

and radial direction. The levels of hot water extract in the bark at the top part were the highest whereas no significant difference was observed for axial and radial directions with the exception for the bark part. Further, either in ethanol or hot water extracts, i.e. content the heartwood part showed a high TPC and TFC levels which indicate a great potential of antioxidative activities. It was observed that the heartwood part contained the highest levels of both extractive and phenolic contents. With regard to cell wall components, Klason lignin content of the heartwood part was significantly lower compared to sapwood part. As the heartwood generally has great proportion, this condition is an advantage as a raw material for pulp and paper manufacturing.

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