

# PHENOLICS CONTENT AND ANTIOXIDANT ACTIVITY OF WOOD EXTRACTIVES FROM THREE CLONES OF *ACACIA* HYBRID (*Acacia mangium* × *Acacia auriculiformis*)

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## ABSTRACT

Breeding strategy of *Acacia* hybrid is being developed by Center for Forest Biotechnology and Tree Improvement Indonesia and has produced three superior clones in growth (Clone 16, 25, and 44). Understanding the extractives of the new clones might determine future development steps to improve its resistance to diseases especially heartrot. The objective of this study was to investigate the extractive content in three radial directions (SW = sapwood; OHW = outer heartwood; IHW = inner heartwood); total phenolic, flavonoid, flavanol contents (colorimetric assay); and antioxidant activity (1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay). Extractions were done with three different solvents in successive (n-hexane = H; methanol = M; hot water = W), yielded 0,69 % – 1,70 %; 1,51 % – 10,86 %; and 0,51 % – 1,16 % of extractive contents, respectively. The total phenolic content (TPC) from TPC-H, TPC-M, and TPC-W ranged between 3,68 mg of gallic acid equivalent (GAE)/g – 10,41 mg GAE/g; 76,83 mg GAE/g – 448,35 mg GAE/g; and 43,28 mg GAE/g – 198,92 mg GAE/g, respectively; the total flavonoid content (TFC) from TFC-H, TFC-M, and TFC-W between 4,23 mg of quercetin equivalent (QE)/g – 41,51 mg QE/g; 29,55 mg QE/g – 133,71 mg QE/g; and 7,70 mg QE/g – 29,37 mg QE/g, respectively; total flavanol content (TVC) from TVC-H, TVC-M, and TVC-W ranged between 28,74 mg of catechin equivalent (CE)/g – 66,90 mg CE/g; 83,39 mg CE/g – 247,18 mg CE/g; and 7,08 mg CE/g – 29,21 mg CE/g, respectively. Furthermore, the antioxidant activity was found to be significantly affected by the radial factor with the strongest activity exhibited by inner heartwood extract with an IC<sub>50</sub> value of 255,77 µg/ml (gallic acid IC<sub>50</sub> showed a value of 39,00 µg/ml). Among clones, clone 16 was determined to have the highest extractive, total flavonoid as well as flavanol contents. Thus, clone 16 was hypothesized to be more resistance against heart rot disease.

**Keywords:** *Acacia* hybrid, heartwood, phenolic contents, sapwood, wood extractives.

## INTRODUCTION

*Acacia mangium* is a multipurpose fast-growing species being used in plantations throughout some tropical countries (Sein and Mitlohner 2011). Over the last few years, its productivity has decreased due to fungal attack which causes heart and root rots (Mohammed *et al.* 2006, Coetzee *et al.* 2011). Heart rot is found in the heartwood of a tree and despite the fact it does not kill it, the merchantable wood volume is reduced by up to 24 % (Sudin *et al.* 1993). However, the less preferable species of *Acacia* genus to be planted in production forest

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is *Acacia auriculiformis* and it is known to have more resistance to heart rot (Barry *et al.* 2005).

*Acacia* hybrid is a crossbreed, either naturally or artificially, between *Acacia mangium* and *Acacia auriculiformis* which inherits several advantages from both of its parent, including faster growth, better stem form, and better resistance against pest and disease compared to its parent species (Pinso and Nasi 1991, Kha 2000, Sunarti *et al.* 2013). The Center for Forest Biotechnology and Tree Improvement Indonesia has been developing a breeding strategy for *Acacia* hybrid using the co-improvement method as one of the efforts to increase the productivity of production forest by hybridization. This was conducted using selected plus trees of *A. mangium* and *A. auriculiformis* parent trees from different family and provenance. By the co-improvement method, it produced a total of tested 44 clones, from which three clones, 44, 16, and 25, observed to have superior growth performance were selected (Sunarti *et al.* 2013).

Previous studies have identified some phenolic compounds in *A. mangium* and *A. auriculiformis* wood extractive and 3,4,7,8-tetra-hydroxy flavanone and teracacidin are the most abundant flavonoid compounds found in both species to have shown antifungal activity (Pietarinen *et al.* 2004, Barry *et al.* 2005). Furthermore, the ability of these compounds to resist heart rot was attributed to the scavenging of hydroxyl radical produced by the fungi, as correlations between antifungal activity, laccase inhibition, and antioxidant activity (Mihara *et al.* 2005).

However, the information on *Acacia* hybrid wood extractive has been found to be limited to its general extractive and lipophilic content (Yahya *et al.* 2010, Soon and Chiang 2012). Therefore, in this study, the extractive content of *Acacia* hybrid clones was measured in its radial direction for total phenolic, flavonoid, and flavanol content as well as the antioxidant activity. This information can be used to indicate each *Acacia* hybrid clone quality in many aspects regarding its extractive content, including resistance to heart rot disease and as a potential source of natural antioxidant.

## MATERIALS AND METHODS

### Wood material

Three six-year-old trees of each *Acacia* hybrid superior clones 16, 25, and 44 were harvested from Wonogiri, Central Java, Indonesia. The trees' parent family, provenance, and clone growth data are described in Table 1. Moreover, the disc was cut from a height of 15 cm above ground level and drilled in sapwood ( $\pm 1$  cm from the border of sapwood and bark), outer heartwood ( $\pm 0,5$  cm from the border of sapwood and heartwood), and inner heartwood ( $\pm 5$  cm radius of the pith) and the rough residues were collected, milled, and sieved using a 40-sized mesh.

**Table 1:** Description of each clone samples.

Clone	Average breast-height diameter (cm)	Average heartwood proportion (%)	Average total height (m)	Family and Provenance ( <i>A. mangium</i> )	Family and Provenance ( <i>A. auriculiformis</i> )
16	23,50	67,23	16,30	86 Claudi River, Iron 107 RA, Australia	107 Orchard Melville, Int, Queensland
25	19,25	63,45	19,48	114 Claudi River, Iron 112 RA, Australia	112 Orchard Melville, Int, Queensland
44	30,25	59,47	20,30	86 Claudi River, Iron 101 RA, Australia	101 Kennedy River, Queensland

### Successive extraction

Furthermore, 5 g O.D. (oven dried) wood powder samples from each clone and radial section were extracted successively through the use of n-hexane (Soxhlet, 6 h), methanol (Soxhlet, 6 h) and hot water (water

bath, 3 h) solvents. The extractive content from each of them was calculated based on the percentage of dry weight after drying with a rotary evaporator.

### Total phenolic content

The total phenolic content was calculated using Folin-Ciocalteu method according to the procedure described by Baba and Malik (2015). This was conducted by adding 0,5 ml of the extract in 1 mg/ml concentration to 2,5 ml Folin-Ciocalteu reagent (Merck, Germany) that has been diluted 10 times. After 2 min, 2 ml of 7,5 %  $\text{Na}_2\text{CO}_3$  was also added and the solution was incubated for 30 min under room temperature and analyzed using UV-VIS spectrophotometer in 765 nm wavelength. Moreover, a calibration curve was also prepared using the same procedure with gallic acid as the standard compound and total phenols were calculated as gallic acid equivalents (GAE) and expressed as mg GAE/g sample.

### Total flavonoid content

The total flavonoid content was calculated using aluminum chloride ( $\text{AlCl}_3 \cdot \text{H}_2\text{O}$ ) according to the procedure described by Diouf *et al.* (2009). This was conducted by reacting a diluted 2 ml of the extract to achieve 1 mg/ml concentration with 2 ml of 2 %  $\text{AlCl}_3 \cdot \text{H}_2\text{O}$ . The solution was shaken and incubated for 30 min under 20 °C and the absorbance was measured using UV-VIS spectrophotometer in 415 nm wavelength. Moreover, a calibration curve was also prepared using the same procedure with quercetin as the standard compound and the total flavonoids were calculated as quercetin equivalents (QAE) and expressed as mg QAE/g sample.

### Total flavanol content

The total flavanol content was calculated using vanillin -  $\text{H}_2\text{SO}_4$  according to the procedure described by Diouf *et al.* (2009). This was conducted by reacting 1 ml of the extract in 1 mg/ml concentration with 2 ml of vanillin -  $\text{H}_2\text{SO}_4$  reagent (1 g vanillin in 100 ml of 70 %  $\text{H}_2\text{SO}_4$ ). The solution was incubated for 15 min under 20 °C temperature and stopped by cooling the solution using a block of ice and the absorbance was measured using UV-VIS spectrophotometer in 500 nm wavelength. Moreover, a calibration curve was also prepared using the same procedure with catechin as the standard compound and the total flavanols were calculated as catechin equivalents (CAE) and expressed as mg CAE/g sample.

### DPPH radical scavenging assay

As much as 0,1 ml of methanol extract in varying concentrations of 100, 150, 200, and 250  $\mu\text{g}/\text{ml}$  was added to the solution of 3 ml of 0,1 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA) in methanol. The solution was shaken and kept in dark for 30 min under 22 °C temperature. Moreover, the blank solution also reacted without the addition of extract with the same procedure and the absorbance was measured at 512 nm. The calculation of inhibition was conducted using the following Equation 1:

$$\text{Inhibition}(\%) = 100 \times \frac{(A_0 - A_1)}{A_0} \quad (1)$$

Where  $A_0$  is the absorbance of the blank and  $A_1$  is the absorbance of the sample with the extract. Antioxidant activity expressed as  $\text{IC}_{50}$  (concentration inhibiting the DPPH reaction by 50 %), however, lower  $\text{IC}_{50}$  indicates higher antioxidant activity. In this experiment, gallic acid and catechin were used as a positive control and all analyses were run in triplicate and averaged.

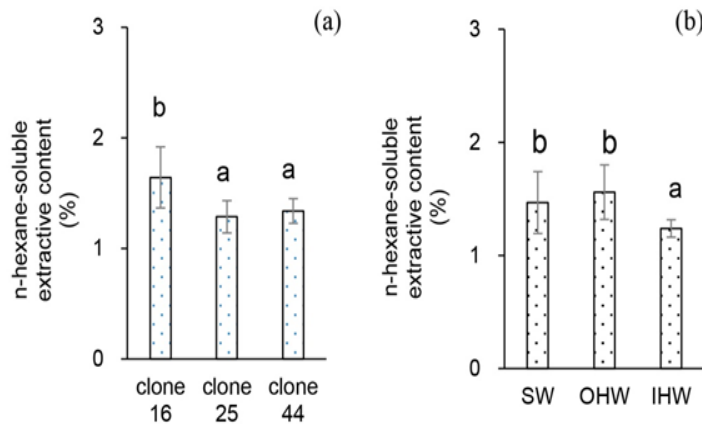
### Statistical analysis

The data were analyzed using the SPSS program (Ver. 25, IBM). A two-way analysis of variance (ANOVA) was conducted with a 95 % confidence level to determine the effect of clone (44, 25, and 16) and radial (sapwood, outer heartwood, inner heartwood) direction on the content of extractive, phenolic, flavonoid, and flavanol, as well as the antioxidant activity of the methanol-soluble fractions. All the data were assayed as a normal data distribution. Furthermore, Duncan test was performed to evaluate which factor affected significantly while Pearson's correlation was used to measure parameters correlation to each other. The coefficient of correlations was calculated in the whole wood (sapwood and heartwood together), sapwood, and heartwood parts only.

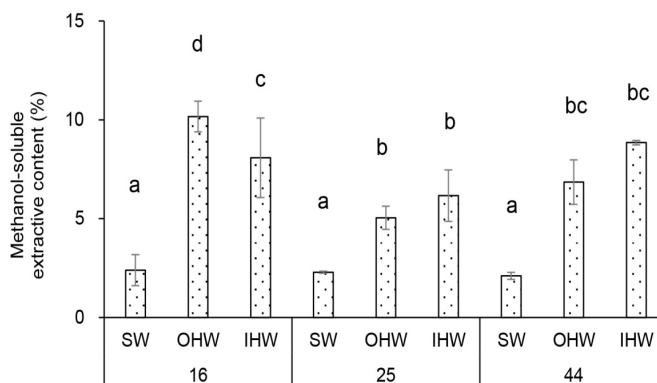
## RESULTS AND DISCUSSION

## Extractive content

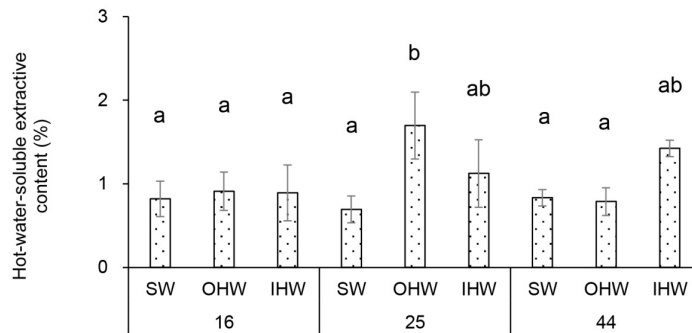
The extraction yields of each clone using solvents with increasing polarity (n-hexane, methanol, hot water) successively are shown in Figure 1, Figure 2, Figure 3. In general, clone 16 had the highest range of n-hexane-soluble extractive (HEC) and methanol-soluble extractive (MEC) but the lowest value for hot-water-soluble extractive (AEC). Meanwhile, clone 25 had the lowest value of HEC and MEC but the highest level of AEC. The ANOVA found significant differences for both clone ( $p < 0,01$ ) and radial direction ( $p = 0,02$ ) of the trees in HEC and in the interaction between clone and radial direction in MEC ( $p < 0,01$ ) and AEC ( $p < 0,01$ ) levels. Therefore, Duncan's test was conducted to evaluate the effect of these factors.



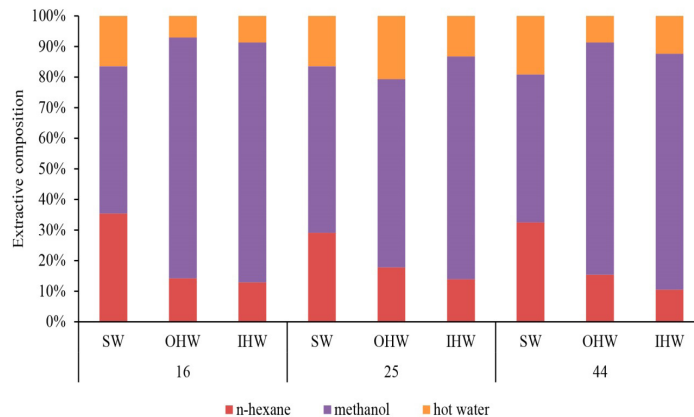
**Figure 1:** (a) Extractive content of n-hexane (% based O.D. wood) from each clone and (b) three radial directions (SW = sapwood; OHW = outer heartwood; and IHW = inner heartwood) of *Acacia* hybrid (average from three replication). The same letters on the histogram implied no significant differences ( $p < 0,05$  by Duncan's test).



**Figure 2:** Extractive content of methanol (% based on O.D. wood) from each clone (16, 25, and 44) and radial direction (SW = sapwood; OHW = outer heartwood; IHW = inner heartwood) of *Acacia* hybrid (average from three replication). The same letters on the histogram implied no significant differences ( $p < 0,05$  by Duncan's test).



**Figure 3:** Extractive content of water (% based on O.D. wood) from each clone (16, 25, and 44) and radial direction (SW = sapwood; OHW = outer heartwood; IHW = inner heartwood) of *Acacia* hybrid (average from three replication). The same letters on the histogram implied no significant differences ( $p < 0,05$  by Duncan's test).



**Figure 4:** Extractive composition from each clone (16, 25, and 44) and radial direction (SW = sapwood; OHW = outer heartwood; IHW = inner heartwood) of *Acacia* hybrid (average from three replication).

The test showed clone 16 to have the significantly highest level of HEC with  $1,64 \% \pm 0,28 \%$  while in all clones, the sapwood and outer hardwood part had the highest HEC level with  $1,47 \pm 0,27 \%$  and  $1,56 \% \pm 0,24 \%$  respectively as shown in Figure 1a and Figure 1b. Moreover, the outer heartwood of clone 16 had the highest level of MEC with  $10,17 \% \pm 0,78 \%$  as shown in Figure 2 as well as the AEC amount of the outer heartwood part of clone 25 with  $1,70 \% \pm 0,40 \%$  as shown in Figure 3. The significant difference between clones indicates the extractive contents were highly affected by the genetic factor of each clone parent's provenance and family.

In comparison with the *A. mangium* from the previous study conducted by Pinto *et al.* (2005), clone 16 had higher lipophilic extractive content, while clone 25 and 44 had a similar range. Meanwhile, the hydrophilic extractive content of all clones is generally similar to *A. auriculiformis* from the previous study (Barry *et al.* 2005). In general, wood with high extractive content, especially lipophilic extractive, is less preferable for pulp and paper material to avoid problems related to pitch deposit and yield reduction (Gutiérrez *et al.* 2004, McLean *et al.* 2014). The high lipophilic and total extractive content of clone 16 has the ability to make it less effective for pulp and paper material compared to the others. However, its high hydrophilic extractive content might indicate high bioactivity of the extract.

Furthermore, the extractive composition of each solvent showed MEC to be dominant in each clone with

a value around 48 % - 79 % based on extract weight as shown in Figure 4. In general, HEC and AEC compositions were highest in sapwood but the decreased in the heartwood. Moreover, MEC concentration was the lowest in sapwood but increased in the heartwood. Theoretically, the formation of heartwood is marked by the death of sapwood cells and the formation of secondary metabolites like phenols (Shmulsky and Jones 2011). The decreased levels of HEC and AEC also indicate the decrease of primary metabolites in the sapwood to heartwood such as starch, fats, and sugar (Taylor *et al.* 2002). A different pattern was observed in the heartwood extractive content for each clone. Clone 16 had a higher extractive content in the outer heartwood part while the others had it in the inner heartwood part. However, the higher concentration in the outer heartwood of clone 16 presumptuously indicates its maturity, as mature woods were known to have higher extractive content (Dünisch *et al.* 2010). The previous study conducted by Nugroho *et al.* (2012) also found a difference in the border of juvenile and mature wood in *A. mangium* from different provenances and there is also a possibility that each clone has a difference in the maturity of the wood.

### Total phenolic, flavonoid, and flavanol content

The total phenolic, flavonoid, and flavanol content (TPC, TFC, and TVC) of extracts from *Acacia* hybrid clones are shown in Table 2 and the highest ranges were generally exhibited by methanol-soluble extracts. Moreover, the ANOVA found significant differences in the interaction between clone and radial direction in the TPC of all solvents ( $p = 0,02$ ;  $0,03$ ; and  $<0,01$  respectively). In TFC, those for n-hexane ( $p = <0,01$ ) and methanol-soluble extracts ( $p = 0,02$ ) significantly affected by the interaction of clone and radial direction factor while those for the water-soluble extract was significantly affected by clone ( $p = <0,01$ ) and radial direction ( $p = <0,01$ ) separately. In TVC, n-hexane-soluble extract only affected by radial direction ( $p = 0,02$ ), while methanol and hot-water-soluble-extract affected by clone ( $p = 0,03$ ) and radial direction ( $p = <0,01$ ) separately.

**Table 2:** Total phenolic, flavonoid, and flavanol content of three radial parts from clone 16, 25, and 44 extracts.

Clone	Radial direction	Total phenolic content			Total flavonoid content			Total flavanol content		
		n-hexane	methanol	hot water	n-hexane	methanol	hot water	n-hexane	methanol	hot water
16	SW	3,77 ± 1,11 <sup>a</sup>	117,11 ± 46,22 <sup>a</sup>	43,92 ± 4,19 <sup>a</sup>	41,52 ± 2,87 <sup>g</sup>	29,55 ± 3,03 <sup>ab</sup>	16,18 ± 6,21 <sup>ab</sup>	36,03 ± 16,02 <sup>ab</sup>	83,39 ± 34,92 <sup>a</sup>	7,84 ± 1,92 <sup>a</sup>
	OHW	4,12 ± 0,46 <sup>a</sup>	386,56 ± 27,58 <sup>cd</sup>	138,22 ± 9,04 <sup>c</sup>	35,92 ± 2,40 <sup>fg</sup>	127,48 ± 14,19 <sup>de</sup>	21,77 ± 4,46 <sup>bc</sup>	38,66 ± 8,14 <sup>ab</sup>	200,53 ± 10,71 <sup>bc</sup>	18,71 ± 9,43 <sup>b</sup>
	IHW	3,68 ± 0,37 <sup>a</sup>	358,46 ± 86,80 <sup>cd</sup>	158,61 ± 31,26 <sup>c</sup>	15,23 ± 1,48 <sup>bc</sup>	133,71 ± 19,83 <sup>e</sup>	25,08 ± 2,61 <sup>bc</sup>	45,03 ± 12,64 <sup>b</sup>	218,21 ± 25,14 <sup>c</sup>	22,34 ± 0,78 <sup>b</sup>
25	SW	5,67 ± 1,95 <sup>ab</sup>	177,60 ± 20,95 <sup>ab</sup>	43,28 ± 14,07 <sup>a</sup>	23,49 ± 3,80 <sup>de</sup>	22,07 ± 2,84 <sup>a</sup>	7,70 ± 1,92 <sup>a</sup>	56,69 ± 13,38 <sup>bc</sup>	90,10 ± 2,84 <sup>a</sup>	7,08 ± 3,77 <sup>a</sup>
	OHW	4,24 ± 2,18 <sup>a</sup>	273,80 ± 118,29 <sup>bc</sup>	86,73 ± 41,46 <sup>b</sup>	29,34 ± 7,71 <sup>ef</sup>	110,18 ± 12,64 <sup>cd</sup>	16,35 ± 6,77 <sup>ab</sup>	28,74 ± 6,91 <sup>a</sup>	135,17 ± 21,37 <sup>ab</sup>	17,76 ± 3,93 <sup>b</sup>
	IHW	8,29 ± 1,29 <sup>bc</sup>	440,49 ± 58,87 <sup>d</sup>	154,77 ± 29,94 <sup>c</sup>	4,23 ± 0,72 <sup>a</sup>	132,66 ± 3,17 <sup>e</sup>	23,70 ± 6,39 <sup>bc</sup>	59,11 ± 14,65 <sup>c</sup>	169,97 ± 3,92 <sup>bc</sup>	25,35 ± 4,13 <sup>b</sup>
44	SW	4,18 ± 0,22 <sup>a</sup>	76,83 ± 22,06 <sup>a</sup>	58,66 ± 10,51 <sup>ab</sup>	21,73 ± 3,08 <sup>cd</sup>	41,15 ± 1,92 <sup>b</sup>	19,59 ± 6,77 <sup>bc</sup>	40,89 ± 9,38 <sup>ab</sup>	165,69 ± 49,19 <sup>bc</sup>	21,42 ± 5,30 <sup>b</sup>
	OHW	5,49 ± 1,92 <sup>ab</sup>	381,32 ± 54,78 <sup>cd</sup>	198,92 ± 20,25 <sup>d</sup>	9,78 ± 4,94 <sup>ab</sup>	101,51 ± 6,77 <sup>c</sup>	29,37 ± 9,30 <sup>c</sup>	31,19 ± 9,31 <sup>a</sup>	179,17 ± 30,63 <sup>bc</sup>	21,42 ± 4,54 <sup>b</sup>
	IHW	10,41 ± 2,90 <sup>c</sup>	448,35 ± 43,95 <sup>d</sup>	136,65 ± 7,95 <sup>c</sup>	32,19 ± 5,39 <sup>f</sup>	113,21 ± 6,39 <sup>cd</sup>	28,67 ± 1,18 <sup>c</sup>	42,58 ± 0,95 <sup>ab</sup>	188,42 ± 73,02 <sup>bc</sup>	23,99 ± 3,08 <sup>b</sup>

Values are expressed as mean ± SE of three tree each clone.

SW = sapwood; OHW = outer heartwood; IHW = inner heartwood

Equal superscripted letters indicate no significant difference between treatments ( $p < 0,05$ ) in same solvent or column.

Duncan's test was conducted to evaluate the effect of clone and radial factors on TPC, TFC, and TVC content in each solvent as shown in Table 2. The TPC value was found to be significantly highest in the inner heartwood part of clone 25 with 440,49 mg GAE/g ± 58,87 mg GAE/g and clone 44 with 448,35 mg GAE/g ± 43,95 mg GAE/g. Similarly, the highest TFC was found significantly in the inner heartwood part of clone 16 with 133,71 mg GAE/g ± 19,83 mg QE/g and 25 with 132,66 mg GAE/g ± 3,17 mg QE/g. Meanwhile, the highest value of TVC was found in the inner heartwood part of clone 16 alone with 218,21 mg GAE/g ± 25,14 mg CE/g.



The total phenolic content of *A. auriculiformis* has been reported to be five times higher than *A. mangium* (Barry *et al.* 2005) and flavonoids and proanthocyanidins (condensed tannin) are found to be the most dominant phenolic compound founds in *Acacia* heartwood extractive (Foo 1984, Barry *et al.* 2005). The higher phenolic content of *A. auriculiformis* was suspected to be contributing to its better resistance against heart rot and termites (Barry *et al.* 2005) mainly due to the monomeric flavonoids, specifically, 3,4',7,8-tetra-hydroxy flavanone and teracacidin, found in greater amount in *A. auriculiformis* (Drewes and Roux 1966, Pietarinen *et al.* 2004, Barry *et al.* 2005). In general, the TPC value of *Acacia* hybrid clones was intermediate between its two parent species and more than *A. mangium*. Moreover, the quantity of flavanol in *A. auriculiformis* was found to be threefold higher than *A. mangium* (Barry *et al.* 2005). Compared to *A. mangium* and *A. auriculiformis*, *Acacia* hybrid clones' TVC is 22 times and 6 times higher respectively. This high concentration of flavanol could be caused by the monomer of profisetinidin, a condensed tannin with flavanol monomer identified in *Acacia* species (Seigler 2003, Barry *et al.* 2005).

### Correlation between extractive and phenolic contents

Pearson's correlations between each parameter in sapwood and heartwood are shown in Table 3 and in the sapwood, the highest correlation was found between TFC and TVC levels of the methanol-soluble extracts with 0,77\*. Further, a strong correlation was found between TPC and TVC in the hot-water-soluble extract with 0,72\* and strong negative correlation was found between the TPC and TFC with -0,69\*. In the heartwood, the highest negative correlation was found between the extractive content and TPC value of the hot-water-soluble extract with -0,69\*. The other strong positive correlations were found between extractive content and TFC level of the n-hexane-soluble extracts with 0,51\*, extractive content and TVC level of the methanol-soluble extracts with 0,59\* and between TFC and TVC levels of the hot-water-soluble extracts with 0,49\*.

Several positive and negative correlations were found in this study. A positive correlation might indicate a high presence of a certain type of compound in the phenol group while a negative one might indicate a low presence. The correlations found in the methanol-soluble extractive of sapwood indicate a high presence of flavanol type compound in the flavonoid group although a low presence of flavonoid was found in the sapwood. In the heartwood part, the result suggested a tree part with higher methanol-soluble extractive content might also have a higher flavanol content. Furthermore, the presence of phenolic compounds in the n-hexane and water-soluble extract were observed not to be dominant.

**Table 3:** Pearson's correlation between extractive content and phenolics content.

Phenolic	Pearson's correlation					
	Sapwood			Heartwood		
	Extractive content	TPCH	TFCH	Extractive content	TPCH	TFCH
TPCH	-0,39			-0,56*		
TFCH	0,53	-0,45		0,51*	-0,02	
TVCH	-0,14	0,35	-0,50	-0,28	0,25	-0,35
Phenolic	Extractive content	TPCM	TFCM	Extractive content	TPCM	TFCM
TPCM	0,50			0,38		
TFCM	-0,02	-0,69*		-0,18	-0,09	
TVCM	0,17	-0,29	0,77*	0,59*	-0,31	0,33
Phenolic	Extractive content	TPCA	TFCA	Extractive content	TPCA	TFCA
TPCA	-0,12			-0,69*		
TFCA	0,47	0,60		-0,26	0,40	
TVCA	0,22	0,72*	0,47	-0,07	0,14	0,49*

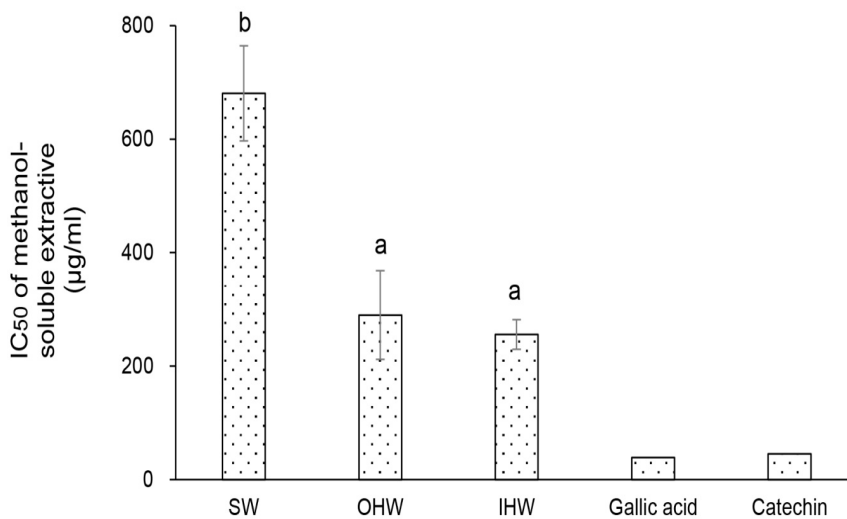
TPC = total phenolic content; TFC = total flavonoid content; TVC = total flavanol content; H = n-hexane; M = methanol; A = water;

\* marked significant correlation in 0,05 level.

The correlation between extractive and phenolic contents has also been found by Kadir and Hale (2017) in several Malaysian plant species, as total phenol increased in parallel with extractive content. Most phenolic compounds are polar due to the highly electronegative oxygen in its hydroxyl group (Rappoport 2003). Therefore, a low presence of phenolic compounds in the n-hexane-soluble extract was expected indicated by the negative correlation found in the phenol. Meanwhile, a positive correlation found between flavanol and extractive content of sapwood methanol-soluble extract is suspected to be the monomer of profisetinidin, which is a flavanol type phenol identified in previous studies on various *Acacia* species (Seigler 2003, Barry *et al.* 2005).

### Antioxidant activity

The antioxidant activities, expressed as  $IC_{50}$  (inhibitory concentration ( $\mu\text{g/ml}$ ) to scavenge the DPPH radical by 50%), are shown in Figure 5 to have only been conducted in the methanol extracts due to its high total phenolic content. The average  $IC_{50}$  values of inner heartwood, outer heartwood, and sapwood were found to be 255,77  $\mu\text{g/ml}$ ; 289,94  $\mu\text{g/ml}$ , and 680,55  $\mu\text{g/ml}$  respectively. The ANOVA only found a significant difference in the radial direction of *Acacia* hybrid clones ( $p = <0,01$ ), while Duncan's test was conducted to evaluate the significance in radial factor as shown in Figure 5. The result showed inner heartwood and outer heartwood have significantly similar strongest antioxidant activity and they are weaker than both of the positive controls of gallic acid at 39,00  $\mu\text{g/ml}$  and catechin at 45,52  $\mu\text{g/ml}$ . The  $IC_{50}$  value of outer heartwood and inner heartwood were around 7,43 and 6,56 times of gallic acid respectively, and 6,37 and 5,62 times of catechin respectively.



**Figure 5:** Inhibitory concentration to reduce DPPH radical by 50 % ( $IC_{50}$ ) in radial direction (SW = sapwood; OHW = outer heartwood; IHW = inner heartwood) of *Acacia* hybrid (average from three replication). The same letters on the histogram implied no significant differences ( $p = <0,05$  by Duncan's test).

Compared to a previous study of *A. mangium* and *A. auriculiformis* methanol extract conducted by Mihara *et al.* (2005), *Acacia* hybrid clones are in between both parent species, where *A. mangium* extract has  $IC_{50}$  value 14,53 times of the control gallic acid, and *A. auriculiformis* has 3,76 times. Mihara *et al.* (2005) also found that the antioxidant activity in both species correlated with antifungal activity and laccase enzyme inhibitory of the extract. Moreover, the phenolic compounds were suspected to have the ability to scavenge the hydroxyl radical produced by the laccase enzyme of heart rot fungi. Therefore, this finding shows *Acacia* hybrid clones has a better pest and disease resistance compared to *A. mangium*. Specifically, higher resistance to heart rot is expected from clone 16 and 25 as a great amount of flavonoid was detected in their inner heartwood. Furthermore, the polar extract might also be utilized as a natural source of antioxidants. Mihara *et al.* (2005) also found that crude extract of both species showed lower antifungal and antioxidant activity than the fractionated and isolated ones, therefore, a stronger antioxidant activity could be achieved with further fractionation and isolation of *Acacia* hybrid extract.



### Correlation between phenolics content and antioxidant activity

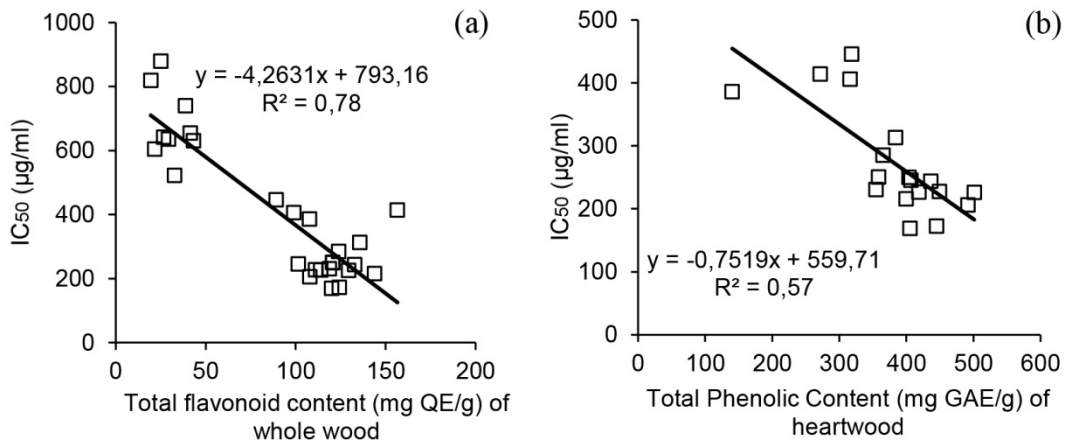
Total phenolic, flavonoid, and flavanol contents were correlated with the radical scavenging ability to estimate which compounds have more effect on the antioxidant activity. This was measured using Pearson's correlation analysis in the whole radial section, sapwood only, and heartwood only and the coefficients are as presented in Table 4. The highest correlation was found between the antioxidant activity and total flavonoid content with  $-0,89^*$  as shown in Figure 6a as well as with total phenolic and flavanol content at  $-0,87^*$  and  $-0,66^*$  respectively. In the heartwood parts only, a significant correlation was found only in the total phenolic content with  $-0,75^*$  as presented in Figure 5b and none were found between any phenolic content and the antioxidant activity of the sapwood parts.

**Table 4:** Pearson's correlation between phenolics and antioxidant activity.

Part	Whole wood			Heartwood			Sapwood		
	TPC	TFC	TVC	TPC	TFC	TVC	TPC	TFC	TVC
DPPH IC <sub>50</sub>	$-0,87^*$	$-0,89^*$	$-0,66^*$	$-0,75^*$	$-0,18$	$-0,44$	$0,23$	$-0,32$	$-0,16$

\* marked significant correlation in 0,01 level.

TPC = total phenolic content; TFC = total flavonoid content; TVC = total flavanol content.



**Figure 6:** Correlation of (a) total flavonoid content and (b) total phenolic content to IC<sub>50</sub> value of DPPH scavenging activity in whole wood and heartwood of *Acacia* hybrid clones wood extract.

Phenolic compounds are known to be able to neutralize the chain-carrying ROO\* radicals by transferring a hydrogen atom from their hydroxyl groups (Foti 2007). Previous studies have also found a correlation between phenolic and antioxidant activity levels (Franco *et al.* 2008, Wissam *et al.* 2012, Hennia *et al.* 2018). In *Acacia* species, the compounds 3,4',7,8-tetrahydroxyflavanone and teracacidin are the most abundant flavonoid compound found in *A. mangium* and *A. auriculiformis* to be showing high antioxidant and antifungal activity (Mihara *et al.* 2005). In this research, a significant correlation in heartwood was only found with total phenolic content and this shows there is a possibility some different forms of phenolic compounds other than flavonoids were responsible for the antioxidant activity of the *Acacia* hybrid.

## CONCLUSIONS

Clone 16 was determined to have the highest extractive and phenolic contents while clone 25 had the lowest. In comparison with previous studies, the extractive content, total phenolic content, total flavonoid content, and antioxidant activity of *Acacia* hybrid wood extracts intermediate between *A. mangium* and *A. auriculiformis*, while the total flavanol content of the hybrid was much higher.

Clones 25 and 44 could be effective as pulp and paper material to avoid pitch problem and maximize yield because of its lower extractive content. Furthermore, the methanol-soluble extracts of *Acacia* hybrid clones have a good radical scavenging activity and may be used as a potential source of natural antioxidant. From the results of this study, it is assumed that the *Acacia* hybrid clones, especially clone 16, may have potential against heart rot disease compared to *A. mangium*, but not as good as *A. auriculiformis*. However, it would be of interest to further investigate the antifungal activity of *Acacia* hybrid especially against heart rot as well as analyze the phenolic compounds through fractionation, isolation, and antioxidant activity assays using other radical types.

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