

# Properties of Included Phloem in Teakwood

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## Abstract

In some areas of Indonesia, the heartwood of teak tree (*Tectona grandis* L.f.) contains included phloem, which is categorized as defects. This paper characterized the colour and chemical properties of such abnormal wood. Three selected trees from Perhutani plantation, Randublatung region, were assessed. The heartwood colour properties were measured by CIELAB system. Result showed that the included phloem-containing heartwood (IPHW) was darker ( $L^*$ ), but less red ( $a^*$ ) and yellow ( $b^*$ ) compared to the adjacent normal heartwood (NHW). The lignin and ash contents were not significantly different in the wood radial direction. In contrast, the level of extractive contents were significantly different between sapwood and heartwood. The amount of ethanol-benzene extractive and solubility in 1% NaOH in the IPHW region were significantly higher than that in normal tissues. The analysis of extractive components using gas chromatography-mass spectrometry showed that the deoxylapachol and lapachol was highly marked in the IPWH region. The obtained results suggest that naphthaquinone compounds were related to the protection against wood-destroying organisme attack.

**Keywords:** *Tectona grandis* L.f, included phloem, chemical properties, colour properties, extractives.

## Introduction

Teak is undoubtedly the main commodities of hardwood in the world for its advantageous properties and its silvicultural aspect. In Indonesia, teak is mainly planted in the Java Island and become naturalized along the time. Supply of teak timber for industries in Indonesia has been mainly provided by the state company, i.e. Perhutani Enterprise. However, cultivation of teak has been intensified by community forests to meet the high demand in the last decades.

Superiority of teak wood is due to its natural durability, strength, weather resistance as well as its beautiful grain and colour. However, in the field, such as in Perhutani forest, there are some abnormalities or defects caused by nature on the teak timber. One of them is included phloem or phloem tissue lying within the secondary xylem. The included phloem is categorized as defects based on the Indonesian National Standard of log or sawn-wood (BSN 2010a; 2010b). The frequent appearances of included phloem in the transverse surface of the wood could indicate the strength properties of the wood. The more frequent it occurs, the less strength wood does (Lee and Shum 1962; Chow *et al.* 1990). Also, it causes the inhomogenous appearances in the wood surface, such as the appearance of a darker heartwood colour compared to the normal ones.

The included phloem phenomenon appears also in several woody species, such as *Calycopteris floribunda* (Rajput *et al.* 2009), *Combretum erythrophyllum* and *Strychnos madagascariensis* (Carlquist 2013). However, there is not much information available in describing the existence of included phloem in teak and its frequency in the teak plantation. Based on our limited observation in the

fields, the included phloem in the teak stem wood occurs along with the attacks of *Neotermes tectonae*, a dry-wood termite. The attack of *Neotermes tectonae* itself, which is called 'inger-inger' or 'gembol' in Indonesian, is marked by the tumorous-shape apperances in the stem. Thus, the appearance of included phloem formation in teak has indicated a plant defense mechanism against termites.

Previous studies about the included phloem in several species were more focused to the wood formation and its anatomical properties (Outer and Veenendaal 1995; Rajput *et al.* 2008; Rajput *et al.* 2009; Veenendaal and Outer 1993). One of the studies has adressed the decay resistance of the included phloem in *Koompassia excelsa* wood and has compared it to the normal tissues (Wong 1988). Our previous works have explored the chemical characterization of abnormal wood in teak (Lukmandaru *et al.* 2009; Lukmandaru 2011; Lukmandaru 2015). Therefore, this study investigated the colour and chemical properties of the included phloem stem wood in radial direction.

## Materials and Methods

### Sample Collection and Preparation

Samples of teak wood were harvested from three trees (class age of 41 to 50 years) grown in Perhutani plantation, Randublatung Region, Central Java Province. Those trees were marked by the attacked of *Neotermes tectonae* termites and the appearance of thin black streaks around the annual ring. The included phloem-contained samples were selected. The wood blocks were sawn from trees in 5 cm thicknesses. Those blocks were selected from the middle part (4 to 7 m) of the trees. They were carefully

divided into three different zones from outside to inside (toward the pith): sapwood (SW), included phloem containing heartwood (IPHW) and the adjacent normal heartwood (NHW) as shown in Fig. 1. The wood samples of each region were ground to size 40-60 mesh (0.4 mm) sieve in order to obtain homogeneous particle sizes and to remove fine particles. This wood meal was used for analysis of colour and chemical properties.

### Colour Measurement

The colour of the specimens was determined from the scanned specimens and expressed in CIE  $L^* a^* b^*$  system by NF777 spectrophotometer (Nippon Denshoku). The -absorbance read at the wavelength interval of 400-700 nm. The illuminant A type (tungsten halogen light) was used and a  $10^\circ$  standard observer was employed. The aperture of the sensor head was 6 mm. The brightness of the colour was represented by  $L^*$ -axis, while the chromaticity coordinates were represented by  $a^*$ -axis and  $b^*$ -axis. In the CIE  $L^* a^* b^*$  coordinates,  $+a^*$  stands for red,  $-a^*$  for green,  $+b^*$  for yellow,  $-b^*$  for blue, and  $L^*$  varies from 100 (white) to zero (black). All measurements were carried out in triplicate.

### Chemical Analyses

Chemical properties, such as ash content, 1% NaOH, hot-water, cold-water and ethanol-benzene solubility were determined according to ASTM (1984a; 1984b; 1984c). Klason lignin values (TAPPI 1992) were also determined. All assays were carried out in duplicate.

### Gas Chromatography-Mass Spectrometry Analyses

The extracts of ethanol-benzene were dissolved in concentration of 25 mg/mL. The GC-MS analyses were carried out using an Shimadzu QP500 fitted with a NB-1 capillary column (30 m, 0.25 mm i.d.; 0.25  $\mu$ m film thickness). GC: oven temperature was programmed from 120 to 300°C rising with a 4°C/min rate and held isothermally at 300°C for 5 min. Injector temperature was 250°C. The carrier gas was helium at a 1 mL/min flow. The injection volume was 1  $\mu$ L with a split ratio 1:80. EI-MS: the electron energy was 70 eV. Ion source and the connection parts temperature were 250°C. Constituents were identified by comparing experimental retention indices with those of reference compounds (2-methyl anthraquinone, lapachol, 2-hydroxymethyl anthraquinone, squalene, palmitic acid) run under identical conditions and literature data (Lemos *et al.* 1999; Perry *et al.* 1991; Windeisen *et al.* 2003). For the quantification, 2-methyl anthraquinone (tectoquinone) was used as internal standard. The concentration of each

component is expressed as mg/g of the total amount of oven dry wood powder.



Figure 1. Sampling position on a cross section of heartwood containing included phloem in teak from three trees. Remarks: SW = sapwood, IPHW = included phloem-containing heartwood, NHW = normal heartwood.

### Statistical Analyses

The data were statistically calculated by one-way analysis of variance (ANOVA) and Duncan's post hoc test with  $p < 0.05$  was considered to be statistically significant. Statistical analyses were done by means of SPSS 16 under Windows.

## Results and Discussion

### Colour Measurement

The colour of wood powder samples in radial direction was measured and the results are described in Fig. 2. The ranges for brightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) in the sapwood region were 51-61, 9-11 and 21-24, whereas the ranges in the heartwood were 32-45, 8-15, and 15-24, respectively. All colour measurements were significantly affected ( $p > 0.01$ ) by radial direction. The IPHW region was significantly darker than the NHW region ( $L^*$  less than 3-7 units), although the  $a^*$  and  $b^*$  levels in IPHW were about 3-4 units lower than the NHW. The  $a^*$  levels between the sapwood and heartwood regions were not significantly different. On the other hand, the  $L^*$  value of heartwood was lower to 9-10 units than the value in sapwood.

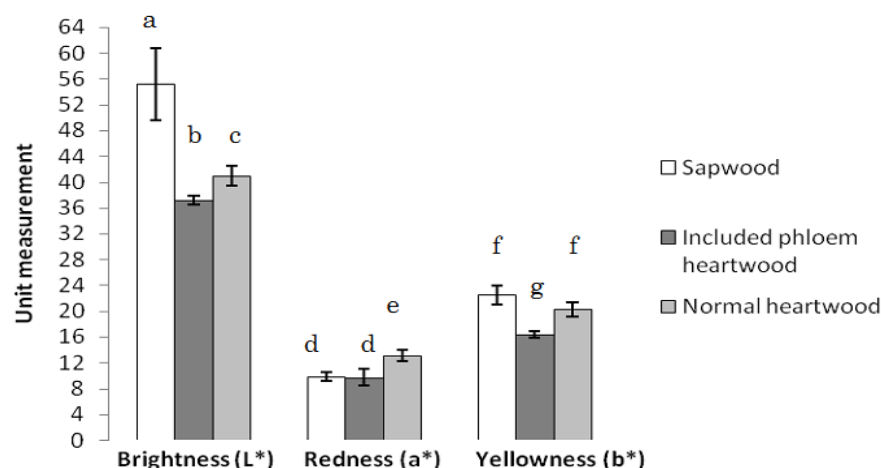


Figure 2. Colour properties in L\* (brightness), a\* (redness) and b\* (yellowness) of teakwood containing included phloem by radial position. Mean of three trees with error bar as standard deviations. The same letters on the same graphic are not statistically different at  $p < 0.05$  by Duncan's test.

Based on the field observation at the same location, the stem with black-streak frequently occurs without the included phloem. Previous findings showed a lesser value of brightness (L\*) to 12-15 units and yellowness (b\*) to 3-4 units in the black streak part (Lukmandaru *et al.* 2009). This raised question whether the occurrence of included phloem is related to the black-streak phenomenon in teak. Measurement of the L\* values in three replicates of trees could not firmly conclude the effect of blackening as shown by the difference of merely 3 units value. To assess the effect of blackening, the heartwood samples with included phloem but without the black-streaked are necessary to study at the same site.

### Chemical Analyses

Measurements of extractive and ash contents were displayed in Fig. 3. The range of ethanol-benzene soluble extractive, hot-water solubility and cold-water solubility contents in the sapwood were 4.73-6.72%, 2.86-3.92%, and 5.78-7.98%, whereas the ranges in the heartwood were 5.78-20.16%, 0.85-2.16% and 0.98-2.43%, respectively. The ANOVA revealed that the effect of radial direction was highly significant to ethanol-benzene soluble extractive and cold-water solubility contents ( $p > 0.01$ ) and was significant to hot-water solubility contents ( $p = 0.01$ ).

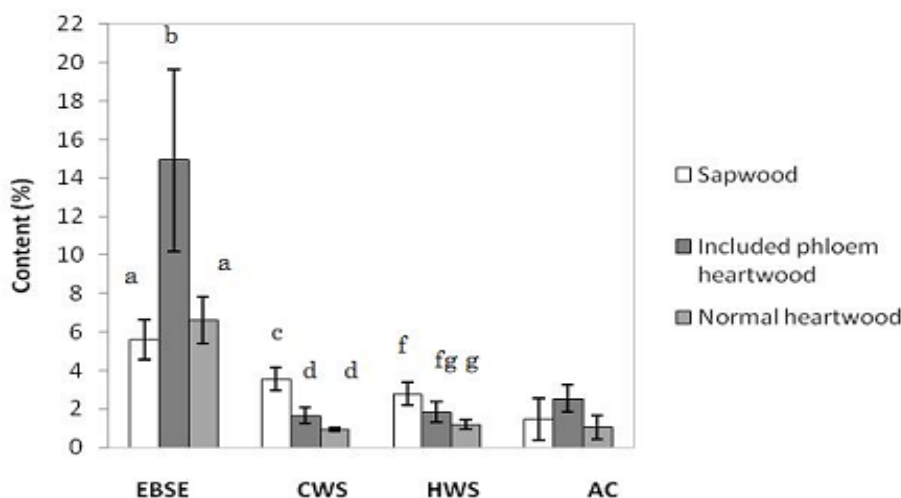


Figure 3. The extractive and ash contents of teakwood containing included phloem by radial position. Mean of three trees with error bar as standard deviation. The same letters on the same graphic are not statistically different at  $p < 0.05$  by Duncan's test. EBSE : ethanol-benzene soluble extractive content, CWS = cold-water solubility content, HWS = hot-water solubility content, AC = ash content.

The ethanol-benzene soluble extractive amounts were significantly different ( $p < 0.05$ ) between the IPHW and NHW. The mean of ethanol-benzene soluble extractive content in the IPHW was 14.90% compared to 6.69% in the NHW. This is possibly due to the higher extractive contents of the phloem (bark) mixed in the wood. The comparatively high content of ethanol-benzene soluble extractive in the IPHW was not observed in the amounts of cold-water and hot-water solubility. It is an unexpected finding as barks normally has a higher water solubility content than the woods (Fengel and Wegener 1989). The extractive contents by successive extraction were similarly found in the black-streaked heartwood of teak (Lukmandaru *et al.* 2009). Thus, contribution of blackening phenomenon to the colour properties of the black-streak heartwood is remain unknown. The high levels of cold-water and hot-water solubility in sapwood regions indicate the high content of primary extractives such as sugars.

The range of ash content in the sapwood and heartwood was 0.54-2.68% and 0.48-3.33%, respectively. Radial direction has no significant effect ( $p = 0.15$ ) in this matter. Similar to the cold-water and hot-water solubility, the comparatively high ash content levels in the sapwood is due to its physiological function, i.e. absorbing the nutrients from the soil. There is no significant difference in ash contents between sapwood and heartwood, which may probably due to the presence of phloem in the heartwood. In general, barks posses a higher ash content compared to woods (Sjostrom 1993). However, it could not adequately explain the insignificancy between the IPHW and NHW values.

The lignin content and 1% NaOH solubility are presented in Fig. 4. The lignin content of the sapwood and

heartwood regions ranged from 35.1-37.0% and 34.8-37.6%, whereas, the 1% NaOH solubility contents ranged from 19.43-24.12% and 12.90-28.24%, respectively. Radial direction was not significantly affect lignin content ( $p = 0.57$ ). However, it was significantly affect 1% NaOH solubility content ( $p = 0.04$ ). Lignin is known as a protection component against microbial wood degradation (Henriksson 2009). The insignificant of lignin content indicates the presence of included phloems which was not affected the composition of cell wall component. The level of NaOH solubility in the IPHW (24.62%) was significantly higher than the level in the NHW (16.77%). Previous studies have reported the high content of 1% NaOH solubility in the barks (Kofujita *et al.* 1999; Usta and Kara 1997; Voulgaridis *et al.* 1985). This result indicates the presence of higher amount of low-molecular weight sugar in the IPHW region, as the short-chain sugars could be derived from its phloem (Hillis 1987).

#### Gas Chromatography-Mass Spectrometry Analysis

GC-MS analysis of ethanol-benzene extracts are reported in Fig. 5. The chromatograms presented about 10 main peaks. Identification of the products using standard components and literatures indicated the presence of quinones (lapachol, deoxylapachol and its isomer, tectoquinone and tectol), palmitic acid and the dominants triterpene, squalene, along with two unidentified compounds (Un1 and Un2). Those identified compounds were also detected in previous reports (Lukmandaru 2012; Lukmandaru and Takahashi 2009; Niamké *et al.* 2011; Windeisen *et al.* 2003).

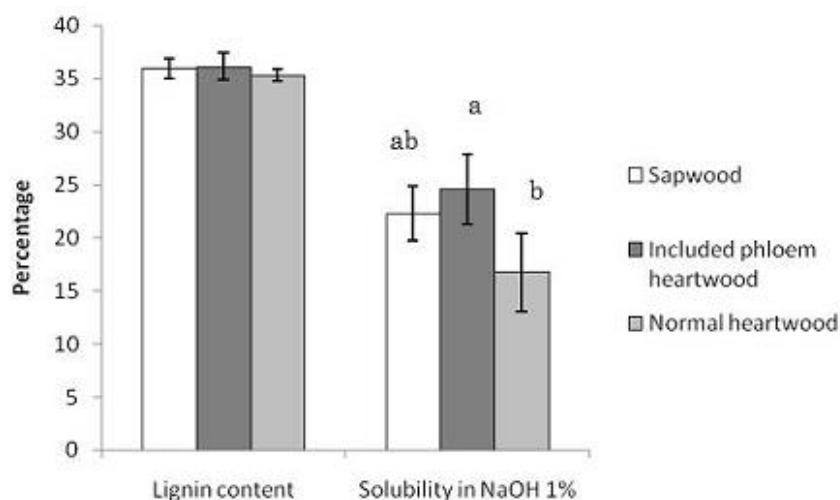


Figure 4. The lignin content and solubility in 1 % NaOH of teakwood containing included phloem by radial position. Mean of three trees with error bar as standard deviation. The same letters on the same graphic are not statistically different at  $p < 0.05$  by Duncan's test.

The quantification of major compounds is summarized in Table 1. There was a statistically significant difference in deoxylapachol and its isomer, lapachol, Un1 and squalene. The amounts of deoxylapachol and its isomer, lapachol and Un1 were significantly higher in the IPHW region than those in the NH. While, the squalene level in the sapwood was significantly different to the level in heartwood. Chemical structures analysis has characterised lapachol, deoxylapachol and its isomer as napthaquinone, while tectoquinone as an anthraquinone and tectol as a dimeric napthaquinone. The molecular masses of unidentified compound (Un1) was found to be m/e (base peak) for 244.

The formation of included phloem could be a kind of protection of phloem against insects and other pests attacks in one to several layers of wood (Mauseth 2014). The natural decay resistance of included phloem was higher than that of normal tissues in *Koompassia excelsa* wood as reported in Wong (1988). Previous reports demonstrated that deoxylapachol effectively inhibited the growth of some fungi (Sumthong *et al.* 2006, 2008; Lukmandaru 2013). Thus, the formation of included phloem might be related to the *Neotermes tectonae* termite attacks (Lukmandaru 2015).

However, one study of termiticidal activities showed that both lapachol and deoxylapachol were not as effective as tectoquinone (Sandermann and Simatupang 1966). In this present experiment, the amount of tectoquinone in the IPHW and NHW was not significantly different. This result is contrast to the previous report of black-streak parts of teak heartwood which contains high tectoquinone amounts (Lukmandaru *et al.* 2009). It is due to the wide variations in the amount of tectoquinone and other major constituents found in different trees by examining standard deviations (Table 1).

Deoxylapachol is hypothesized as the precursor of both tectoquinone and tectol (Sandermann and Simatupang 1966). Comparatively high level of the napthaquinone derivatives in the IPHW has been interpreted as a defense mechanism by slowing the conversion of napthaquinone into more complex components. Future studies with larger number of tree samples and different locations, as well as further bioassay testing and characterization of unknown compound (Un1) would be beneficial to clarify the assumptions above.

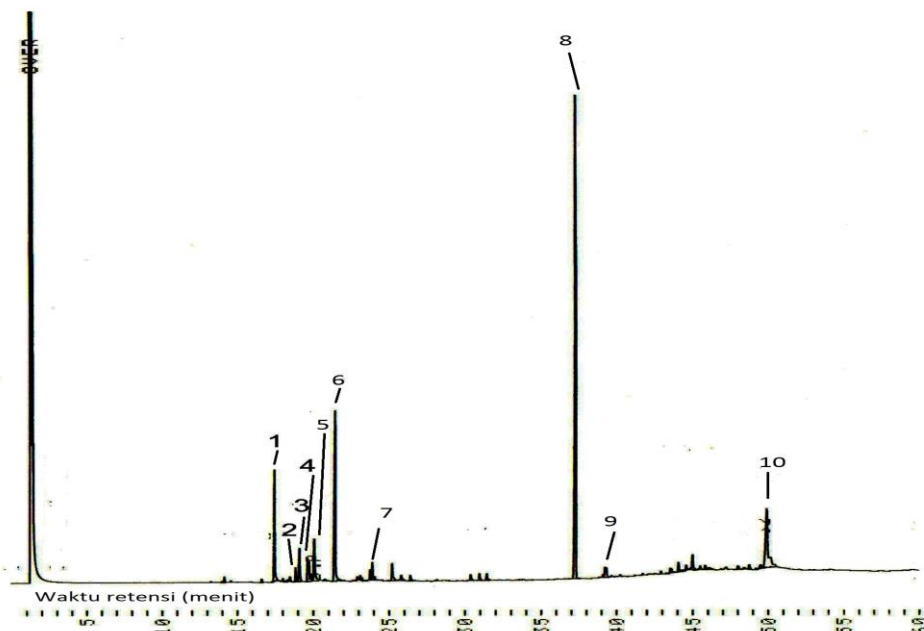


Figure 5. Gas chromatogram of the ethanol-benzene extracts from heartwood containing included phloem in teak (1 & 5. Deoxylapachol or its isomer; 2. Palmitic acid; 3. Unknown; 4. Lapachol; 6. Tectoquinone; 7. Unknown; 8. Squalene; 9. Unknown; 10. Tectol).

Table 1. The content of extractive components (mg/g oven dry wood) in teakwood containing included phloem by radial position.

GC-MS analysis			Radial position			
No.	Component	Ret. time (minutes)	Sapwood	Included phloem - heartwood	Normal heartwood	Sig.
1.	Deoxylapachol	17.41	5.46 (4.90)a	23.2 (7.92)b	0.15 (0.08)a	0.01>**
2.	Palmitic acid	18.62	0.03 (0.05)	0.33 (0.30)	0.23 (0.15)	0.25
3.	Un1	18.93	0.36 (0.46)a	2.03 (0.92)b	0.23 (0.15)a	0.01*
4.	Lapachol	19.48	0.73 (0.25)a	7.73 (5.68)b	1.30 (0.36)a	0.04*
5.	Isodeoxylapachol	19.84	3.73 (4.02)a	11.20 (4.61)b	1.76 (0.40)a	0.03*
6.	Tectoquinone	21.17	1.83 (1.10)	25.56 (23.18)	7.10 (0.95)	0.15
7.	Un2	23.65	2.60 (3.89)	2.20 (1.70)	0.66 (0.25)	0.62
8.	Squalene	37.27	6.60 (3.37)a	16.40 (9.60)b	26.53 (11.70)b	0.04*
9.	Un3	39.35	0.80 (0.80)	1.10 (1.15)	2.66 (4.35)	0.66
10.	Tectol	50.06	3.36 (3.25)	3.46 (2.97)	4.90 (1.56)	0.74

Remarks: Mean of three trees with standard deviation in parentheses. The same letters on the same row are not statistically different at  $p < 0.05$  by Duncan's test.

### Conclusions

The characteristics of the included phloem-containing heartwood (IPHW) parts of teak were examined for colour and chemical properties. IPHW showed a darker but less red and yellow colour than the adjacent normal heartwood. The IPHW portion also contained ethanol-benzene extractives. Its 1% NaOH solubility contents was significantly higher than the content of normal heartwood parts. Between the sapwood and the IPWH, significant differences were observed in the ethanol-benzene and cold-water soluble extractives. Lignin contents in radial direction did not vary significantly. Among the extractive components, the lapachol, deoxylapachol and its isomer, as well as the content of unknown compound (Un1) were particularly high in the IPWH portion, which suggests some relation of napthaquinones with the defense mechanism of teak.

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