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Radial Distribution of Lipophilic Extractives in Walikukun (*Schoutenia ovata* Korth.) Wood

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ABSTRACT

Walikukun (*Schoutenia ovata* Korth.) is a commercially significant wood species in Indonesia. Heartwood, in particular, is important due to the natural durability and aesthetics, while lipophilic extractives play a crucial role in the formation. Therefore, this study aimed to determine the levels of lipophilic compounds in different radial positions associated with the formation of Walikukun heartwood. The results showed that the mean of *n*-hexane, methanol, and hot water extractive content ranged from 0.97% to 1.35%, 7.18% to 14.4%, and 1.73% to 2.23% in oven-dried wood, respectively. In general, the total extractive content increased from sapwood to heartwood. This pattern was similar to the methanol extractive content. The highest extractive contents of *n*-hexane level were in transition zone, followed by heartwood and sapwood. Furthermore, the highest lipophilic compounds were found in alkenes, followed by fatty alcohols, fatty acids, and sterols. Fatty acids and sterols decreased radially from heartwood to sapwood, while fatty alcohols increased from heartwood to transition zone, then decreased to sapwood. The decrease in the proportion of fatty acids, sterols and fatty alcohols from heartwood to sapwood is probably due to the heartwood formation process where lipid compounds are metabolised in the sapwood or transition zone.

Keywords: sapwood; heartwood; lipophilic; extractive; Walikukun

INTRODUCTION

Walikukun (*Schoutenia ovata* Korth.) is one of the quite popular commercial wood species in Indonesia. This species with strength class I and durability class II is widely found in Java as well as to a lesser extent in the Sunda Islands (Kartasujana and Martawijaya 1973). Although the primary habitat of Walikukun is usually hot lowlands, it also grows on less fertile soils and is tolerant of shade. A previous study reported that the tree is used for wood and has religious value (Sujarwo and Keim 2017), with the specific gravity ranging from 0.90 to 1.08. Despite being hard, the wood is easy to work with and can be used for construction, flooring, panelling, building musical instruments as well as shipbuilding (Kartasujana and Martawijaya 1973).

Heartwood of Walikukun has a reddish-brown color and a specific gravity of up to 0.98 (Kartasujana and Martawijaya 1973). Specific gravity is a highly important parameter in the selection of wood for numerous applications such as furniture manufacture, cabinet making, construction of frames, bridges, building structures, etc. (Meghwal et al. 2020). Wood density or specific gravity is strongly correlated with physical and mechanical properties. It has been widely used as a determinant of wood quality for its utilization (Zobel and van Buijtenen 1989). The heartwood of Walikukun also has a high specific gravity, which is an interesting area of research. According to the International Association of Wood Anatomists (IAWA 1964), heartwood is the inner woody layer of a standing tree that, as the tree grows, no longer contains living cells (parenchyma). The reserve materials such as starch are also either removed or converted into heartwood-specific materials. Meanwhile, sapwood is located in the outer wood zone of the secondary xylem. It is a physiologically active area containing living cells that transport xylem sap through tracheary elements (Słupianek et al. 2021). Parenchyma cells in transition zone

between sapwood and heartwood experience metabolic changes before cell death (Piqueras et al. 2020). This leads to increased synthesis of secondary metabolites such as extractives. As stated by Darmawan et al. (2018), extractive is a naturally occurring minor component of wood, essential in protection against decay by harmful organisms.

Heartwood formation is a specific process that transforms sapwood into heartwood. This process is often accompanied by physiological progressions such as depletion of storage compounds (Magel et al. 1994), xylem dehydration (Kuroda et al. 2009), programmed cell death (PCD) (Nakaba et al. 2012), and accumulation of heartwood extractives (Magel et al. 1991). The accumulation of heartwood extractives is the most important investment in heartwood formation due to the influence on natural durability. Previous reports have shown that heartwood is free of storage material. This is because during the formation, starch, and lipids are degraded, which then play an important role as a carbon skeleton for the extractive phenolic biosynthesis and the senescence of the ray parenchyma cells (Hillis 1987, Magel 2000, Song et al. 2011). Heartwood substances are the products of the secondary metabolism of trees. The substrates metabolized are mainly non-structural carbohydrates (NSC) (Cui et al. 2020). The NSCs in trees are the major storage compounds of photosynthesis and are transported inward by the cells of the ray parenchyma in the formation of secondary compounds in the heartwood (Hillis 1987). The main components of NSCs are starch and soluble sugars (e.g., sucrose, glucose, arabinose, fructose, galactose, stachyose). However, it is not only the NSCs that are important in the process of heartwood formation, but also the lipid groups. The lipid content of the xylem has also been shown to be involved in the formation of heartwood (Bergström 2003).

According to previous reports, heartwood of Walikukun has a good quality. These properties underscore the need to examine the mechanism of heartwood formation, specifically from the perspective of lipophilic compounds. Lipids such as fatty acids, resin acids, and sterols are often analyzed to determine the role in heartwood formation process. Some species such as *Pinus radiata* D. Don., *Pinus sylvestris* L., *Picea abies* (L.) H. Karst., and *Sweitenia mahagoni* (L.) Jacq. reportedly increased the amounts of fatty acids and sterols from sapwood to heartwood (Hemingway and Hillis 1971, Höll and Goller 1982, Saranpää and Nyberg 1987, Bergstrom 2003, Arisandi et al. 2024b). However, there is no study of the extractive lipophilic compounds in Walikukun wood associated with heartwood formation. Therefore, this study aimed to analyse lipophilic compounds in relation to heartwood formation in Walikukun wood.

MATERIALS AND METHODS

Sample Collection and Preparation

Three Walikukun trees were felled from the Special Purpose Forest Area (KHDTK) Wanagama 1 (07°90'S, 110°05'E) in Bunder Village, Patuk District, Gunungkidul. Disk with 4 cm thickness was taken from the 1.3 m diameter at breast height (DBH) and then transported to Universitas Gadjah Mada for laboratory testing. The disc samples were stored at room temperature and air-dried. The diameter width ranged from 25.6 cm to 29.4 cm and the disk was radially divided into three parts, namely heartwood (H, 2 cm from the pith), transition zone (T, between the outer heartwood and sapwood), and sapwood (S, 1.5 cm from the bark) (Figure 1). The range of heartwood proportion was from 15.7% to 22.7%. Each wood section was drilled in four directions, and then mixed for each heartwood section (H1–H4), transition zone (T1–T4) and sapwood (S1–S4) to avoid radial variations. The powder was ground and sieved to 40–60 mesh for analysis. After collecting the powder sample, the sample was then put into zip lock bag and stored at room temperature.

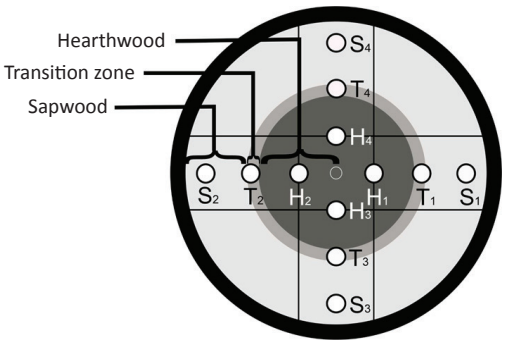

Figure 1. Radial section of Walikukun wood sample in the heartwood (H), transition zone (T), and sapwood (S).

Chemicals

Standard compounds, including, palmitic (≥99), oleic (≥99), linoleic (≥99), stearic (≥99), and behenic acid (≥99) were obtained from Shanghai Chemical Industry (Shanghai, China). Meanwhile, veratric acid (≥99) and β-sitosterol (≥99) were purchased from Kanto Chemical (Tokyo, Japan).

Moisture Content Determination

The powder of 1 g (the initial weight, W_0) was put into the oven at a temperature of 103°C ± 2°C. Every two hours, the powder was weighed after being put into a desiccator for ±15 minutes until the constant oven dried weight (W_1). Moisture content (MC, in %) was calculated using the following equation:

$$MC = \frac{W_0 - W_1}{W_1} \cdot 100\% \quad (1)$$

where W_0 is the initial weight (g), and W_1 is oven dried weight (g).

Extractive Content Determination

A 5 g powdered sample (40–60 mesh size) was successively extracted using *n*-hexane (150 mL) and methanol (150 mL) for 6 hours in a Soxhlet apparatus (250 mL), followed by hot water (100 mL) extraction through refluxing in water bath for 3 hours. Furthermore, the solvent was evaporated by a rotary evaporator at a temperature of 40°C for *n*-hexane and 60°C for

methanol. Meanwhile, the hot water solution was evaporated on the hot plate until all the solvent had evaporated. The extract was dried in an oven (103°C ± 2°C) and the extractive content was quantified (Lukmandaru 2012).

Lipophilic Compounds Determination

Gas chromatography-mass spectrometry (GC-MS)-QP 2010 (Shimadzu, Japan) was used to analyze dry *n*-hexane extracts of the samples. The analysis was performed under the conditions of RTX-5MS capillary column (30 m · 0.25 mm I.D. and 0.25 µm, GL Sciences, Tokyo, Japan), column temperature from 70°C (2 min) to 290°C at 5°C·min^{-1}, as well as injection and detection temperature of 200°C, and 285°C, respectively. The collection mass ranged from 50 to 666 amu using helium as the carrier gas. By using derivatization with trimethylchlorosilane (TMCS) method, samples were derivatized before being injected according to a modification of the method described by Moldeveanu and David (2018). The main purpose of silylation in chromatography is to reduce the polarity of the analyte, to increase the stability of the analyte and to improve the behavior of the GC. A common silylating mixture is BSTFA with 1% TMCS. The silylation reaction is usually carried out in a solvent containing no active hydrogen. Dimethylformamide (DMF), pyridine and acetonitrile are the most commonly used solvents for silylation (Moldeveanu and David 2018). The main role of the solvent is to dissolve the analyte and the reagents in the sample. In this study, about 100 µL in a mixture of N, O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA), and 1% trimethylchlorosilane (TMCS) was added to 3 mg of each dry *n*-hexane extract diluted using 100 µL pyridine. The mixtures obtained were ultrasonicated at room temperature and heated for 30 minutes at 103°C. Subsequently, 1 mL of veratric acid (1 mg·mL^{-1}, dissolved in *n*-hexane) was added to the mixtures as an internal standard followed by ultra-sonication at an ambient temperature. Components detected in GC-MS were compared with standard compounds, NIST MS Library (NIST 2011), and literature. Compounds that have the same similarity were assumed to be compounds contained in the sample. For quantification, each detected component was compared with an internal standard (veratric acid). Veratric acid is relatively stable under various analytical conditions, including derivatization processes and high temperatures in GC-MS. In previous studies, it was chosen as an internal standard for identifying lipophilic compounds in the derivatization method (Masendra et al. 2018, Arisandi et al. 2020, Arisandi et al. 2024b).

Statistical Analysis

Data were statistically processed using SPSS (version 25 IBM, New York, USA). Analysis of variance (ANOVA) was performed, and statistically significant differences were determined at the 95% confidence level. One-way ANOVA was applied to determine the effect of radial position (heartwood, transition zone, and sapwood) on extractive content that had been assayed as a normal data distribution (Shapiro-Wilks test). Furthermore, Duncan's test was performed to determine specific differences between pairs of means. Lipophilic compounds were analyzed descriptively and the analysis was performed as the mean ± standard deviation of three replicate measurements of trees.

RESULTS AND DISCUSSION

Composition of Extractive and Lipophilic Compounds

Fats, waxes, resins, and sterols are theoretically removed using a nonpolar solution (*n*-hexane) (Lukmandaru 2011). Meanwhile, polar solvents such as methanol, cold and hot water dissolve mineral salts, sugars, tannins, phenolic compounds, dyes, pectins, free acids, and others (Fengel and Wegener 1989, Sjöström 1993, Han and Rowell 1997). Based on the results, the mean of *n*-hexane, methanol, and hot water extractive content from sapwood to heartwood ranged from 0.97% to 1.35%, 7.18% to 14.4%, and 1.73% to 2.23% in the oven-dried wood, respectively (Table 1). The extractive content was higher compared to the results of previous studies in dichloromethane, ethanol, and hot water extracts for 40- and 36-year-old *Eucalyptus globulus* Labill. wood (Gominho et al. 2012, Gominho et al. 2014, Gominho et al. 2015). In addition, the results were also higher compared to 42-year-old teak (*Tectona grandis* L. f.) wood extracted using *n*-hexane, ethyl acetate, methanol, and hot water solutions (Lukmandaru 2009). The differences are presumably due to differences in tree age, species, solvents, and extraction methods used (Pereira 1988, Freire et al. 2005, Meszaros et al. 2007). Methanol dissolved 70.1–80.5% of the total material extracted, while the extractive solubilized in *n*-hexane contributed to a low percentage of the total amount. Polar solvents such as methanol and hot water represented approximately 90% to 93% of the total extractive (Table 1, Figure 2). This study was in line with previous studies on *E. globulus* wood (Miranda and Pereira 2002, Morais and Pereira 2012).

Table 1. The extractive content in heartwood, transition zone, and sapwood Walikukun wood (based on % oven dried wood).

Solvents	Radial position		
	Heartwood (%)	**Transition zone** (%)	**Sapwood** (%)
n-hexane	1.26 ± 0.10^{a}	1.35 ± 0.07^{a}	0.97 ± 0.10^{b}
Methanol	14.40 ± 3.17^{a}	11.90 ± 2.40^{ab}	7.18 ± 0.14^{b}
Hot water	2.23 ± 0.09^{a}	1.73 ± 0.24^{a}	2.09 ± 0.57^{a}
Total	**17.9**	**15.0**	**10.2**

Note: Average of three trees ± standard deviation, the same letters in the same row are not significantly different at p<5% by Duncan's test.

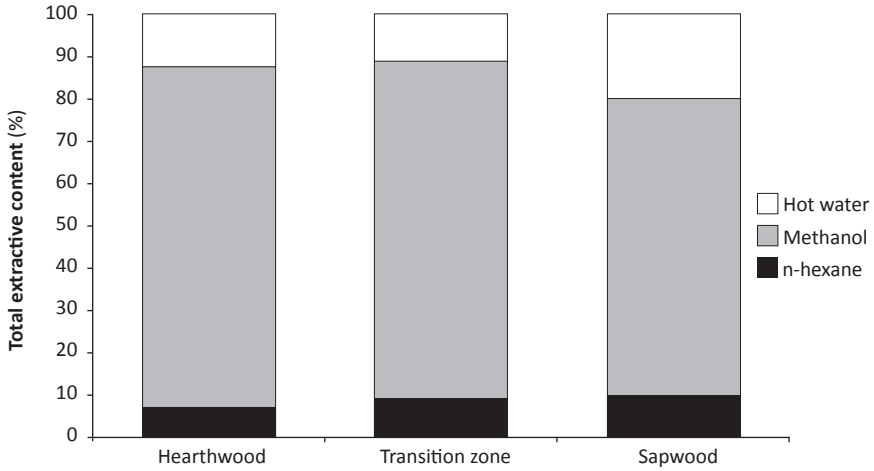

Figure 2. Total extractive content of Walikukun wood based on oven-dried extractive weight.

Extractive content comprises compounds extracted using solvents in wood tissues, including phenols, sterols, fatty acids, and others (Rowell et al. 2005). These compounds play a very important role in wood decay, resistance to fungi and termites, as well as environmental stress. In this study, *n*-hexane extract was analysed by GC-MS to detect lipophilic compounds in heartwood, transition zone, and sapwood. Four lipophilic groups were found in the examined Walikukun samples, namely acyclic alkenes, fatty acids, alcohols, and sterols (Table 2, Figure 3). Acyclic alkenes were found as the largest fraction, while the smallest were sterol groups (Figure 4). The abundant acyclic alkenes include 1-pentadecene, while 1-dodecene compound was the smallest content. The 1-pentadecene compound has been reported in other species such as *Swietenia mahagoni* (L.), *Anthocephalus cadamba* Roxb. Miq., and *Anthocephalus macrophyllus* (Roxb.) Havil. (Arisandi et al. 2024a, Arisandi et al. 2024b). The acyclic alkenes in this study represented the largest fraction. This fraction belongs to lipophilic group with a large amount in the wood, as reported by some studies in other species such as *E. globulus* and *S. mahagoni, A. cadamba,* and *A. macrophyllus* (Gutiérrez et al. 1999, Arisandi et al. 2024a, Arisandi et al. 2024b).

Fatty alcohols represented the second largest fraction after acyclic alkenes. Compounds of *n*-nonadecanol-1, *n*-eicosanol, *n*-tetracosanol-1 were dominant, while hexacosanol and 1-heptacosanol were found in small amounts. These compounds have also been detected in several other species such as *A. cadamba, A. macrophyllus, E. globulus,* and *E. urograndis* W. Hill clone (Gutierrez et al. 1999, Silvério et al. 2007, Arisandi et al. 2024a).

Regarding fatty acids groups, palmitic, oleic, linoleic, and stearic acids were the main compounds, while behenic acid was found in small amounts. These results were in line with those reported in previous studies where compounds such as palmitic, linoleic, oleic, and stearic acids were dominant in other species including *S. mahogani, E. pellita* F.Muell*, E. globulus, A. cadamba,* and *A. macrophyllus* wood (Arisandi et al. 2020, Arisandi et al. 2024a, Arisandi et al. 2024b). Small amount of behenic acid was detected in *E. pellita* and *S. mahagoni* (Arisandi et al. 2020, Arisandi et al. 2024b). Although the smallest lipophilic fraction were sterols, several studies have found that these compounds play a crucial role in the process of heartwood formation. β-sitosterol was the main compound, while stigmasterol was found in small amounts. Similar results were also reported in previous studies on *E. pellita, S. mahagoni, A. cadamba*, and *A. macrophyllus* (Arisandi et al. 2020, Arisandi et al. 2024a, Arisandi et al. 2024b).

Radial Distribution

Extractive Content

In radial position, the highest total extractive content was found in heartwood (17.9%) followed by transition zone (15.0%) and sapwood (10.2%) part (Table 1). This level was due to the accumulation of methanol and hot water-soluble extractives in heartwood, which accounts for 90% of the total extractive (Figure 1). The trend was in accordance with previous studies in some species such as teak wood, *E. globulus,* and larch wood (Gierlinger and Wimmer 2004, Morais and Pereira 2012, Lukmandaru et al. 2021). The higher extractive content in heartwood was due to accumulation during heartwood formation (Hillis 1971). The extractive content of *n*-hexane and methanol was also significantly larger in heartwood compared to sapwood (Table 1). Meanwhile, no significant difference was found in the amount of hot water extractive between heartwood and sapwood, although the value was slightly higher in heartwood.

Lipophilic Compounds

Fatty Acids

Lipophilic content increased from sapwood to heartwood except for alkene fraction. The highest number of fatty acids was found in heartwood (1.80%) followed by transition zone (1.59%) and sapwood (1.20%) (Table 2, Figure 4). The content of the main compounds such as oleic and stearic acids decreased from heartwood to sapwood.

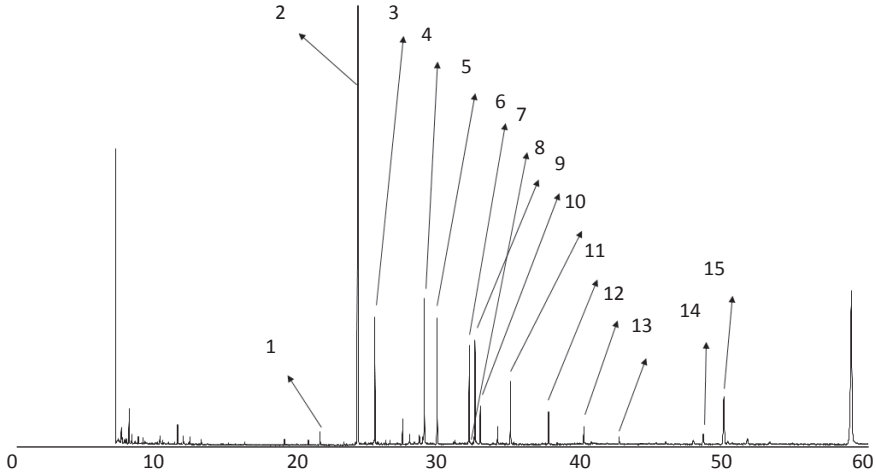

Figure 3. GC-MS chromatogram of heartwood *n*-hexane extract (tree no. 3) from Walikukun (TMCS derivatization method). Peak 1. 1-dodecene (21.3 min), 2. veratric acid (internal standard, 24.0 min), 3. 1-tertadecene (25.2 min), 4. 1-pentadecene (28.7 min), 5. palmitic acid (29.6 min), 6. *n*-nonadecanol (31.8 min), 7. linoleic acid (32.2 min), 8. oleic acid (32.3 min), 9. stearic acid (32.6 min), 10. 1-eicosanol (34.8 min), 11. *n*-tetracosanol-1, 12. 1-hexacosanol (39.9 min), 13. 1-heptacosanol (42.4 min), 14. stigmasterol (48.3 min), 15. β-sitosterol (49.8 min).

Table 2. Lipophilic extractive in heartwood, transition zone, and sapwood of Walikukun wood (% based on dried *n*-hexane extract).

Compounds	Formula	Radial Position		
		Heartwood (%)	**Transition Zone** (%)	**Sapwood** (%)
Acyclic Alkenes		**1.64**	**2.07**	**1.92**
1-Dodecene**	$C_{12}H_{24}$	0.09 ± 0.01	0.12 ± 0.02	0.08 ± 0.03
1-Tetradecene**	$C_{14}H_{28}$	0.67 ± 0.11	0.95 ± 0.11	1.05 ± 0.42
1-Pentadecene**	$C_{15}H_{30}$	0.88 ± 0.10	1.00 ± 0.09	0.79 ± 0.20
Fatty acids		**1.80**	**1.59**	**1.20**
Palmitic acid*	$C_{16}H_{32}0_2$	0.96 ± 0.30	0.89 ± 0.05	1.01 ± 0.65
Linoleic acid*	$C_{18}H_{32}O_2$	0.04 ± 0.00	0.12 ± 0.10	n.d.
Oleic acid*	$C_{18}H_{34}O_2$	0.42 ± 0.32	0.16 ± 0.05	n.d.
Stearic acid*	$C_{18}H_{36}O_2$	0.24 ± 0.01	0.29 ± 0.07	0.19 ± 0.05
Behenic acid*	$C_{22}H_{44}O_2$	0.05 ± 0.01	n.d.	n.d.
Fatty alcohols		**1.49**	**1.88**	**1.34**
n-Nonadecanol-1**	$C_{19}H_{40}O$	0.67 ± 0.06	0.80 ± 0.10	0.61 ± 0.10
n-Eicosanol**	$C_{20}H_{42}O$	0.41 ± 0.03	0.52 ± 0.10	0.38 ± 0.05
n-Tetracosanol-1**	$C_{24}H_{50}O$	0.23 ± 0.02	0.30 ± 0.07	0.20 ± 0.03
1-Hexacosanol**	$C_{26}H_{54}O$	0.13 ± 0.01	0.17 ± 0.04	0.10 ± 0.01
1-Heptacosanol**	$C_{27}H_{56}O$	0.05 ± 0.00	0.09 ± 0.02	0.05 ± 0.00
Sterols		**0.60**	**0.49**	**0.40**
Stigmasterol**	$C_{29}H_{48}O$	0.11 ± 0.06	0.11 ± 0.05	n.d.
β-Sitosterol*	$C_{29}H_{50}O$	0.49 ± 0.25	0.38 ± 0.05	0.40 ± 0.07

Note: n.d. not detected, means of three trees, * compared with standard compound, ** compared with similarity index > 80% (NIST Library 2011) and/or with data from the literature.

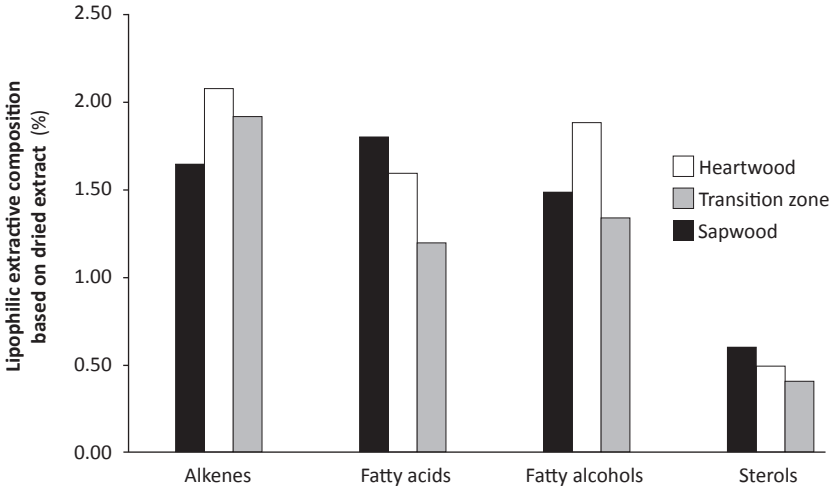

Figure 4. Lipophilic extractive composition (%) based on dried *n*-hexane extract from Walikukun wood.

Similar trends have been reported by some previous studies in several other species such as *Pinus sylvestris* (Saranpää and Nyberg 1987, Bergstrom 2003), *Picea abies* (Bertaud and Holmbom 2000), *E. camaldulensis* (Benouadah et al. 2017), and *E. globulus* (Gominho et al. 2020). The majority of lipids in heartwood are converted into extractive compounds. As stated in a previous study, plastids are the sole source of fatty acids in the plant (Stumpf 1980). Therefore, fatty acids and lipids found in wood are partly synthesized in living wood cells and not transported from the photosynthetic cells of the tree, since ray parenchyma cells have starch-containing plastids (Parameswaran and Bauch 1975).

Linoleic was only found in heartwood and transition zone, while behenic acid was only detected in heartwood. In a previous study, long-chain fatty acids such as behenic and lignoceric acids were more abundant in heartwood than in sapwood of *E. pellita* and *E. camaldulensis* Dehnh. (Benouadah et al. 2017, Arisandi et al. 2020). According to Metsä-Kortelainen and Viitanen (2009), the high concentration of fatty acids in the heartwood samples, especially the long-chain fatty acids, suggests that these compounds are produced at high levels during the formation of heartwood, where the heartwood is actually more resistant to wood-destroying organisms such as microorganisms, insects, or decay fungi. Karimi et al. (2015) also reported that several compounds such as palmitic, stearic, oleic, linoleic, and linolenic acids have antioxidant and antibacterial activities. Fatty acids appear to have antibacterial activity (Desbois and Smith 2010). Fatty acids can act as ionic surfactants. They have antibacterial and antifungal properties at low pH (Hayes and Berkovitz 1979). Sermakkani and Thangapandian (2012) stated that palmitic acid had been used as an antioxidant, hypocholesterolemic agent, antimicrobial agent, and anti-inflammatory agent. Carrillo et al. (2012) also mentioned that oleic acid is an antibacterial and anti-tumor agent.

Sterols

The highest amount of sterols was found in heartwood (0.60%), followed by transition zone (0.49%) and sapwood (0.40%) (Table 2, Figure 4). The main compound in sterol fraction was β-sitosterol (0.38% to 0.49%), while stigmasterol (0% to 0.11%) was the minor component. A similar trend was also found in several previous studies such as *Acacia* hybrid, *E. globulus, E. camaldulensis, E. pellita,* and *S. mahagoni* (Soon and Chiang 2012, Benouadah et al. 2017, Gominho et al. 2020, Arisandi et al. 2020, Arisandi et al. 2024b). Hillinger et al. (1996) stated that the high amount of sterols in heartwood may be due to the crucial role in self-defense mechanisms. In addition, β-sitosterol has been reported to inhibit the growth rate of the fungi *P. chrysosporium* (Masendra et al. 2020) and *Fusarium spp.* (Kiprono et al. 2000). The development of parenchymal cells has certain characteristics in lipid metabolism. Kampe and Magel (2013) explained that the number of phospholipids and parenchymal cell integrity parameters in *Robinia pseudoacacia* L. wood decreased towards transition zone with only a small amount found in heartwood. The amount of free sterols decreased from sapwood to transition zone but reached a maximum in heartwood. Interestingly, the highest concentrations of sterols (Figure 4) were found in heartwood, in which mostly the cells are dead.

Sterols are most commonly found in cell membranes, and presumably in all organelles (Grunwald 1980). Thus, their presence in heartwood, where the membranes are mostly degraded, is not easily explained. The senescence processes are generally accompanied by changes in cell membranes in all plant tissues studied to date (Hillinger et al. 1996). Catabolism of membrane phospholipids by deacylation and peroxidation is considered to be a major contributor to senescence. It does not only affect the physical properties of the plasma membrane, such as fluidity and microviscosity, but it also creates an altered lipid environment for enzyme

activities and translocation processes (Beja-Tal and Borochov 1994, Borochov et al. 1994). In addition, a variety of changes in the metabolic state of the cell coincide with the increasing disintegration of the cellular organization.

During the transition from sapwood to heartwood, there are metabolic shifts toward the synthesis of secondary products, including the consumption of storage carbohydrates and their conversion into heartwood substances, i.e., phenolic compounds (Hillis 1987, Magel et al. 1991, Magel et al. 1994). The degradation of reserve lipids and the accumulation of hydrolysis products and intermediates also appear to be closely correlated with the formation of heartwood (Saranpää and Nyberg 1987, Fischer and Höll 1992, Hillinger et al. 1996). In addition, Höll and Pieczonka (1978) stated that heartwood sterols in spruce heartwood may be part of a non-metabolic pool that may play a role in disease resistance in young trees, since spruce heartwood does not contain phospholipids and thus membranes.

Alkenes and Fatty Alcohols

The number of alkenes in heartwood (1.64%) increased to transition zone (2.07%) and then decreased to sapwood parts (1.92%). Hydrocarbons have been commonly reported in wood such as eucalyptus, *S. mahagoni, A. cadamba,* and *A. macrophyllus*. Del Río et al. (1998) reported that *E. globulus* wood extractive consisted mainly of hydrocarbons, fatty acids, waxes, steroids, and triglycerides. Hydrocarbons were also found to be the largest fraction in wood of *S. mahagoni, A. cadamba*, and *A. macrophyllus* (Arisandi et al. 2024a, Arisandi et al. 2024b). Furthermore, the level of fatty alcohols in transition zone (1.88%) was greater than in heartwood (1.49%) and sapwood (1.34%). This trend was supported by the dominant compounds of fatty alcohols such as *n*-nonadecanol-1, *n*-eicosanol, and *n*-tetracosanol-1. The compounds have also been detected in *E. globulus, A. cadamba*, and *A. macrophyllus*, where *n*-eicosanol and *n*-tetracosanol-1 were the most abundant in the two species of red and white jabon, as well as *E. globulus* wood (Gutierrez et al. 1999, Arisandi et al. 2024a).

Alkenes and fatty alcohols in sapwood and heartwood have been reported by Gominho et al. (2020) and Arisandi et al. (2024b). Considering that both belong to the same lipid class, the function of alkenes and fatty alcohols in relation to heartwood formation process may be similar to fatty acids and sterols. However, further investigation is required to determine the more specific role of alkenes and fatty alcohols in heartwood formation. Furthermore, this study is limited to the identification of lipophilic compounds in *n*-hexane extract. Therefore, the identification of other groups of compounds (e.g., phenolics and sugars) in methanol and hot water extracts is also necessary in further studies to obtain more detailed data in heartwood formation.

CONCLUSIONS

In conclusion, this study was the first to report on the extractive and lipophilic components found in sapwood, transition zone, and heartwood of Walikukun wood in relation to heartwood formation. This trend was consistent with the patterns of *n*-hexane, methanol, and hot water extractive, with methanol contributing most to the differences between the three parts. Based on the GC-MS analysis, the *n*-hexane extract of the Walikukun wood had the potential to contain four lipophilic fractions, namely alkenes, fatty acids, fatty alcohols, and sterols. The major groups consisted of alkenes (1-petradecene and 1-pentadecene), fatty acids (palmitic, oleic, linoleic, and stearic acids), fatty alcohols (*n*-nonadecanol-1, *n*-eicosanol, and *n*-tetracosanol-1), and sterols (β-sitosterol). Meanwhile, 1-dodecene, behenic acid, 1-hexacosanol, 1-heptacosanol, and stigmasterol were minor constituents. Acyclic alkenes were also the largest fractions followed by fatty alcohols, fatty acids, and sterols. The distribution of lipophilic extractives, specifically fatty acids and sterols increased from sapwood to heartwood. Furthermore, the role of acyclic alkenes and fatty alcohols in relation to heartwood formation needs to be further investigated.

Author Contributions

RA conceptualized, designed the study and wrote the original draft of the manuscript, RA, BPH, FYP and VAKP collected and processed the data, RA, AF, GL secured the research funding, supervised the research and helped to draft the manuscript, GL performed methodological discussions and gave final approval of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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