Hidden bioactive of caryophyllene inside Keruing wood

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Abstract. In the utilization of natural resources, there is often something problematic on one side, but there are benefits on the other side. Utilization of natural resources in the form of wood should be directed at a variety of efforts that are more diverse, not focused on wood only. Bearing in mind that trees contain not only lignocellulosic components, but other high-value materials. Research was conducted on one species of wood that is considered problematic in wood processing, namely Keruing (Dipterocaropus hastelii). The results showed that the Keruing wood contained extractive substances with the main compound of bioactive caryophyllene which is widely used by the pharmaceutical, food and cosmetics industries. The total caryophyllene content in extractive wood reached 47.68%.

Keywords: problematic wood, keruing wood, solubility, extractives

1. Introduction
Indonesia's abundant natural resources can be optimally utilized with the known ingredients contained therein. In terms of biodiversity, Indonesia also has a large number of tree species. Forestry Research and Development Agency [1] listed 4000 species of trees growing in Indonesia. The utilization of the forest resources still focuses on the wood only until now because it is the tangible one of wood as a biomaterial with the main component of lignocellulose.

In addition to lignocellulose, in wood, there is another material called as extractive because it can be extracted using solvents both polar and non-polar, without damaging the structure of cellulose or lignin of the wood. In the narrow sense, extractive substances are compounds that can dissolve in organic solvents. In this meaning extractives are used in wood analysis [2]. Several types of extractives material in wood are tannins and other polyphenols, dyes, essential oils, fats, resins, wax, sap and starch. The literature on extractive substances in wood mentioned that the amount is ranging from less than 1% to more than 30%, depends on various factors in relation to tree growth and season when the tree is logged [3]. Extractive substances affect some wood properties such as colour, odour, taste, resistance to microorganism attack, density, hygroscopicity, and combustibility.

The presence of extractive substances in several types of wood is often considered problematic matter because it sometimes interferes in the processing and is considered to reduce product quality. Among the types of wood that are considered problematic is Keruing wood (Dipterocarpus
This paper presents the results of research on Wood Keruing which contained high extractive substances. It is expected that by knowing the material content in the extractive, the use of this wood species is not limited to the wood only, but also on the material contained in it which probably to be of high value.

2. Methods and materials

2.1. Materials and equipment
The material used was Keruing wood (*Dipterocarpus hasseltii* Bl.) which is considered problematic wood due to the discharge of the sap, either from the logs or from the processed wood. The equipment used are woodworking machinery for preparing test samples, water baths, soxlet distillation equipment for solubility, Py-GCMS Shimadzu QP 2010 to determine the composition of types of extractive substances, ovens, and digital scales. To observe the extractive position on wood vessels the equipment including sliding microtome, object glass, cover glass and a microscope were used.

2.2. Samples preparation
Wood samples were cut from the logs into square form measuring 30 x 6 x 3 cm. The wood samples were then dried in an oven at 60 °C until the sap came out (Figure 1). After the sap came out in sufficient quantities, wood is conditioned in the open air with room temperature to facilitate the collection of the sap / oil. The sap was scraped off and weighed as much as 5 g to be analyzed in py-GCMS.

![Figure 1. The sap comes out from the logs and sawn timber (see the arrow) of Keruing wood (*Dipterocarpus hasseltii* Bl.)](image)

2.3. Observation on extractives position
The position of oily extractive substances in Keruing wood was observed through the anatomical structure of the wood which includes macroscopic and microscopic structures. Macroscopic structure of wood in general characteristics was done on wood samples that have been smoothed surface, which are relevant to the purpose of the study include: fiber direction, gloss, touch impression, and odour [4]. Microscopic characteristics of wood observed include characteristics recommended by the International Committee of the Association of Wood Anatomists [5].

2.4. Wood extractives analysis
In general, to find out the presence of extractive substances in wood, wood solubility in cold and hot water, as well as in alcohol-benzene were undertaken [6,7]. Meanwhile, to determine the composition of the dominant substances of extractives from Keruing wood, an analysis was carried out on the sap by identifying the chemical components of extractive substances using the pyrolysis-GCMS method.
3. Result and discussion

3.1. Extractives location in wood

Extractive substances occupy certain morphological places in wood structures. For example, resin acids are present in resin canals, while fats and wax are in the parenchymal cells of the radius. Extraction of phenolic groups is found mainly in sapwood and bark [8]. Based on its position in wood, extractives are divided into two main groups namely: 1) extractives stored in capillary structures and 2) extractives stored in cell wall structures [9].

Based on field observations, the veneer sheets and sawn timber produced from Keruing wood (D. hasseltii) under study have many stains, but the percentage of extractive content is unknown. The initial prediction of the high extractives content is the arrangement and frequency of axial intercellular or scattered vessels. The collection of extractives in vessels in the form of white granules (Figure 2) can be understood because according to Achmadi [10], in broad leaf wood, extractive substances are in the parenchyma cells of the ray cells associated with vessels. The high extractive content in this Keruing wood can be identified from the macroscopic analysis of the wood with a slippery and sticky touch and a pungent or extractive odour. Visually, the extractives can be seen as granular located in the vessels (Figure 2).

![Figure 2. Granular ekstractives appear (g) on the cross section of the Keruing wood (Dipterocarpus hasseltii Bl.)](image)

There are many species of oily Keruing. Soerianegara and Lemmens [11] mention several species, such as D. borneensis, D. caudatus, D. chartacus, D. conferts, D. cornutus, D. costatus, D. crinitus, D. dyeri, D. gracilis, D. grandiflorus, D. hasseltii, D. kerrii, D. kunstleri, D. lowii, D. palembanicus, D. retusus, D. submellatus, D. validus, and D. verrucosus contain very high oils. Due to the high content of the extractives, the wood species above are limited in use. Anatomically, up to now there has never been any research to differentiate on the members of the Dipterocarpus genus, both Keruing groups which are oily or those which are not or less oily. Key identification based on anatomical structure is very necessary to get effective results considering each species has unique characteristics.

3.2. Solubility

The solubility values of the Keruing wood studied were presented in Table 1. Based on the solubility value, the keruing wood studied was classified as wood that had a high extractive content because the solubility value was more than 4% [12]. However, the high level of dissolved material - as indicated by the high solubility rate - has not been indicate the specific substance therein.
According to Mantanis et al [9] in general the content of extractive substances in wood (non-tropical) varies between 2 - 10% while in some types of tropical wood can reach 20-25%. Pettersen [13] states that the levels of extractive substances in wood range from 1 - 20%. Extractives are usually divided into three sub groups, namely: (i) aliphatic groups, (ii) terpenes and terpenoids, and (iii) phenolics.

### Table 1. Solubility of Keruing wood (D. Hasseltii)

| Solvent                      | Solubility (%) |
|------------------------------|----------------|
| Cold water                   | 4.6            |
| Hot water                    | 6.9            |
| Alcohol : benzene (1:2)      | 4.4            |

3.3 Substance composition in Keruing wood extractives

Analysis with Py-GCMS succeeded in identifying 63 names from 70 materials that were hydrolyzed (Figure 3). Some of them, such as caryophyllene, appear with different names even though they are actually the same substance. Figure 3 shows that at the retention time of 24.679 minutes the peak of the graph occurred for trans-caryophyllene. Table 2 shows extractive ingredients containing more than 1%.

![Figure 3. Chromatograph of extractives of the Keruing wood (Dipterocarpus hasseltii Bl.)](image)

Table 2 shows that the dominant substances contained in the extractive oily Keruing wood that have been studied successively from the highest are trans-Caryophyllene, Dinorergost acetate, alpha-humulene, Caryophyllene oxide, Androstan ethyl hydroxy, Cedranone, Tetracyclo-tridecan dimethyl and duvatrieniol. The total of eight major substances reached 70.82%.

Trans-caryophyllene is a synonym of β-caryophyllene, Caryophyllene oxide or Cedranone, while α-caryophyllene is the old name of α-humulene [14]. Table 3 also shows that the caryophyllene groups appeared at minutes 24.413, 24.679, 25.164, 27.057, and 50.178 of retention times, respectively with the total content of 47.68%. The two substances in nature are often found together in a mixture. They are sesquiterpenes which have three isoprene units.
5.247  Acetic acid (CAS) Ethylic acid  1.36  
24.413  CIS-Caryophyllene  1.29  
24.679  trans-Caryophyllene  27.19  
25.164  alpha.-Humulene  7.52  
26.877  Unidentified  1.13  
27.057  (-)-Caryophyllene oxide  6.39  
27.385  1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl- 2.21  
27.689  Tetracyclo[6.3.2.0E2,5.0E1,8]tridecan-9-OL, 4,4-dimethyl- 4.82  
27.896  Duvatriendiol  4.77  
28.043  Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.).-(CAS) 3-ethyl-3-hydro  5.89  
41.614  1,4-Methano-1H-indene, octahydro-1,7A-dimethyl-4-(1-methylene)  2.82  
43.249  Illurinic acid  1.14  
50.178  Cedranone (CAS) 9-cedranon  5.29  
51.147  2(1H)-Naphthalenone, 4a,5,6,7,8a-hexahydro-7.alpha.-isopropyl-4a.beta.,8  1.00  
58.295  26,27-Dinorergost-23-en-3-ol, acetate, (3.beta.,5.alpha.).-(CAS)  8.95  

| Retention time (minutes) | Substances name | Total (%) |
|--------------------------|-----------------|-----------|
| 5.247                    | Acetic acid (CAS) Ethylic acid | 1.36 |
| 24.413                   | CIS-Caryophyllene | 1.29 |
| 24.679                   | trans-Caryophyllene | 27.19 |
| 25.164                   | alpha.-Humulene | 7.52 |
| 26.877                   | Unidentified | 1.13 |
| 27.057                   | (-)-Caryophyllene oxide | 6.39 |
| 27.385                   | 1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl- | 2.21 |
| 27.689                   | Tetracyclo[6.3.2.0E2,5.0E1,8]tridecan-9-OL, 4,4-dimethyl- | 4.82 |
| 27.896                   | Duvatriendiol | 4.77 |
| 28.043                   | Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.).-(CAS) 3-ethyl-3-hydro | 5.89 |
| 41.614                   | 1,4-Methano-1H-indene, octahydro-1,7A-dimethyl-4-(1-methylene) | 2.82 |
| 43.249                   | Illurinic acid | 1.14 |
| 50.178                   | Cedranone (CAS) 9-cedranon | 5.29 |
| 51.147                   | 2(1H)-Naphthalenone, 4a,5,6,7,8a-hexahydro-7.alpha.-isopropyl-4a.beta.,8 | 1.00 |
| 58.295                   | 26,27-Dinorergost-23-en-3-ol, acetate, (3.beta.,5.alpha.).-(CAS) | 8.95 |

**Figure 4.** The structure formula of α- and β-caryophyllene

3.4. The use of caryophyllene

Sjostrom [8] explains that different extractive types required to maintain diverse biological functions of trees. For example, fat is an energy source for wood cells, whereas low terpenoids, resinic acids and phenol compounds protect wood against damage caused by microbiology or insect attack. Specifically, Robinson [15] mentions that terpenoid compounds act as protectors against insects.

As a member of sesquiterpenoids [15], caryophyllene is the basic compound of many essential oils, especially clove oil (Syzygium aromaticum) [16]. This material is also found in cinnamon and black pepper. Caryophyllene is widely researched and developed in the pharmaceutical, food and
cosmetics worlds because it provides benefits in all three sectors [17, 18]. Furthermore [16] explained that β-caryophyllene can be used for local anesthesia. While α-caryophyllene because it provides aroma and flavor, is widely used by the food and perfume industry [17, 18]. Beta-caryophyllene has been tested as an anti-inflammatory [19]. Caryophyllene is also the source of antioxidant which is very beneficial in the world of health, food and cosmetics [17, 18]. Recently, antioxidant is also used in fuel research and development biodiesel to give stability effect [20].

4. Conclusion
The problematic Keruing wood (Dipterocarous hasseltii) contains extractive substances with the main compound of bioactive caryophyllene which is widely used by the pharmaceutical, food and cosmetics industries. The total caryophyllene content in the extractive wood reached 47.68%. By knowing the contents of these substances, the wood species should have more added value.

This research needs to be continued on the extraction and isolation or purification aspects to obtain pure caryophyllene. Research also needs to be undertaken on other wood species that are considered problematic because it can be used as an initial indication of the presence of certain materials inside the wood.

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