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# Oleoresin Production and Turpentine Component of *Pinus oocarpa* and *Pinus merkusii*

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# Oleoresin Production and Turpentine Component of *Pinus oocarpa* and *Pinus merkusii*

**Agus Sukarno[1], Sutarman[2*], Yani Quarta Mondiana[1], Dedy Wahyu Irawan[1], Yudo Aria Wiranegara[1], and Muhammad Abror[2]**

[1]Malang Agriculture University, Malang, Indonesia
[2]Universitas Muhammadyah Sidoarjo, Sidoarjo, Indonesia

*sutarman@umsida.ac.id

**Abstract.** This study aims to determine differences in oleoresin production and the type and content of turpentine *P. oocarpa* and *P. merkusii*. The first stage of the research activity was to collect oleoresin obtained from 15 plants of each type of pine aged 14 years which were determined randomly. Determination of the type and content of phytochemicals, especially turpentine using Gas Chromatography-Mass Spectrometry by injecting gas-phase chemical isolates. The oleoresin tapping data were analyzed by paired t-test with a test level of 5% to determine the difference in the amount of oleoresin. The results showed that there was no difference in production capacity ($p<0.05$) between the two stands with an average oleoresin production per tree of 0.0127±0.002 $g.d^{-1}$ (*P. oocarpa*) and 0.0183±0.003 $g.d^{-1}$ (*P. merkusii*). The most important species in *P. oocarpa* consisted of: α-pinene (5.2%), ß-pinene (5.8%), and delta 3 carena (13.8%); while in *P. merkusii* are α-pinene (8.2%), ß-pinene (11.5%), limonene (5.2%), α-terpinolene (32.7%), benzenemethanol (4.3%), and trans-pinocarveol (3.5%). All turpentine compounds produced by the two types of pine can be used for various pharmaceutical, cosmetic, and pesticide industries.

## 1. Introduction

*Pinus oocarpa* is one of the pine species in the Perum Perhutani forest area, its presence is in minor numbers because most of the pine plantations in Perum Perhutani are *P. merkusii*. *P. oocarpa* is native to North America, growing from Mexico to Southern Nicaragua. In the last two to three decades, this type of pine has begun to be cultivated to meet the needs of pine resin in Indonesia. In almost all over the world, pine stands are highly oleoresin for various industrial needs [1-2]. Some of the forest areas used for the development of *P. oocarpa* pine forests include the Gunung Walat Education Forest, Sukabumi (West Java Province), Kintamani Bali (Bali Province), and Perum Perhutani KPH Jember in Garahan and Perum Perhutani KPH Probolinggo in Pronojiwo-Lumajang (East Java Province).

The role of non-timber forest products derived from pine resin is now becoming increasingly important, both in the form of resin, turpentine, and even more so its derivatives. Indonesia is the world's largest producer of pine resin after China and Brazil [3]. In the past, the use of resin was still very limited, for example only for batik, as well as turpentine only for paint thinner. However, currently, the use of resin is much more, for example as a sizing agent in the paper industry, viscosity enhancer, adhesive enhancer, and a mixture of eye shadow ingredients. Turpentine contains several components, including α-pinene, ß-pinene, and delta 3 carene. These components are much needed by the industry and have a higher selling value than resin and turpentine. The two types of pine developed in Indonesia are part of about 250 species that are widely distributed throughout the world [4] whose essential oil products are widely used as an anti-inflammatory, anti-microbial, analgesic, and antistress

[5]. Product derivatives produced by pine are now widely used for various purposes in the cosmetic and pharmaceutical industries [6-7].

Until now, research and utilization regarding the turpentine content of *P. merkusii* have often been carried out, but the same thing for the turpentine content of *P. oocarpa* has not been widely carried out. Information related to latex productivity and turpentine content in *P. Oocarpa* is still very scarce. On the other hand, research comparing the possibility of differences in the quantity aspect of oleoresin production and the diversity of turpentine compounds between *P. oocarpa* as an exotic plant and *P. merkusii* as an Indonesian indigenous pine has not been widely carried out. Although genetically *P. oocarpa* there are differences in the two species, the possibility of similarities and differences in the production of sap and turpentine types produced by the two types of pine grown in the same habitat cannot be predicted [8]. The purpose of the study was to determine the differences in sap production and turpentine content of *P. oocarpa* and *P. merkusii* through oleoresin tapping.

## 2. Methodology

### 2.1. Rubber Tapping

Oleoresin tapping using a drill with a drill bit size of 16 mm [9] was carried out on 14 years old *P. oocarpa* and *P. merkusii* trees which were selected as a random sample of 15 trees. Pine oleoresin collection point is in plot 2k, RPH Sumberowo, BKPH Pronojiwo, KPH Probolinggo, with an altitude of 742.8 m above sea level, geographical location 8° 12'LS, 112° 55'E. The oleoresin that comes out is directly channeled through the gum from a 20 mm diameter PVC pipe into a plastic bag. The oleoresin collection was carried out two days after the tapping. The oleoresin obtained is then distilled in the laboratory to obtain turpentine. The amount of sap needed to meet the capacity of the tool is 1,400 g. Cooking oleoresin using a distillation apparatus with direct heating, a temperature of 160-180°C. The turpentine that comes out is at the top, while the water is at the bottom because the density of water is greater than turpentine. Furthermore, turpentine is accommodated in a 250 mm measuring cup which is placed on a Vibra brand electric scale with a capacity of 6 kg, so that the volume and weight can be immediately known. Analysis of the difference in the production of oleoresin data using the t-test at a real rate of 5%. The turpentine content analysis used Gas Chromatography Mas-spectrometry.

### 2.2. GCMS Analysis

The turpentine fraction was analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS) Shimadzu QP 5000. A sample of 1 L was injected into the GC-MS which was operated using a glass column 25 m long, 0.25 mm in diameter, and 0.25 m thick with a stationary phase with programmed oven temperature between 70-270 °C [10]. The number of chemical isolates contained in turpentine was recorded through a chromatogram which showed the percentage.

## 3. Results and Discussion

### 3.1. Oleoresin Production

The test results showed that there was no difference in oleoresin production between *P. merkusii* and *P. ocarpa* ($p>0.05$); each tree produces 0.0127±0.0014 and 0.0183±0.0029 $g.d^{-1}$.

**Table 1.** Results of t-test on the volume of oleoresin production between *P. merkusii* and *P. oocarpa*

| | *Pinus merkusii* | *Pinus oocarpa* |
|---|---|---|
| Mean | 0.0126667 | 0.0183333 |
| Variance | 3.167E-05 | 0.0001345 |
| Observations | 15 | 15 |
| Pooled Variance | 8.31E-05 | |
| Hypothesized Mean Difference | 0 | |
| Df | 28 | |
| t Stat | -1.702434 | |
| P(T<=t) two-tail | 0.0997527 | |
| t Critical two-tail | 2.0484071 | |

The genetic differences between the two pine plants did not show the different performance of oleoresin production, including chitinase activity in the production of important latex. Chitinase plays a key role in modifying cell wall structure. Chitinase can affect the rate of oleoresin transport from living cells to resin channels where cytochrome P450s can catalyze oxidation in the biosynthetic pathway of various resin acid diterpenes as the main component of oleoresin [11-12].

### 3.2. GCMS Analysis

The results of the analysis on the turpentine of *P.oocarpa* and *P. merkusii* sap of each pine resin are presented on the chromatogram as shown in Figures 1 and 2.

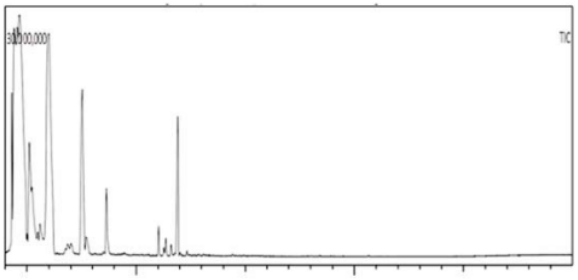

**Figure 1.** Chromatogram of turpentine content of *P. oocarpa*

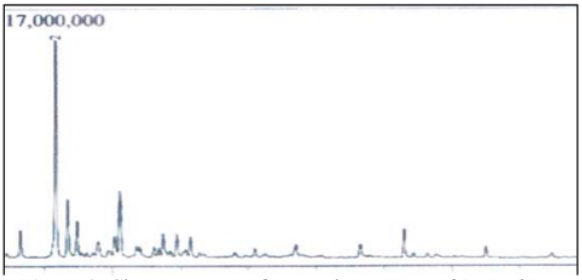


**Figure 2.** Chromatogram of turpentine content of *P. merkusii*

Based on the data presented in Figures 1 and 2, the similarities and differences in types and their respective concentrations and gas retention times are shown in Table 2 as well as information on the molecular formula, molecular weight, and structural formula of each compound detected through GCMS analysis (Table 3).

**Table 2.** Turpentine components of *P. oocarpa* and *P. merkusii*

| No. | Components | *P. oocarpa* | | *P. merkusii* | |
|---|---|---|---|---|---|
| | | % | R.time | % | R.time |
| 1. | α-pinene | 5.2 | 4.38 | 8.2 | 9.30 |
| 2. | β-pinene | 5.8 | 5.07 | 11.5 | 10.10 |
| 3. | Limonene | | | 5.2 | 9.50 |
| 4. | delta 3 carene | 13.8 | 4.60 | - | - |
| 5. | α-terpinolene | | | 32.7 | 9.20 |
| 6. | Benzenemethanol | | | 4.3 | 10.70 |
| 7. | Trans-Pinocarveol | | | 3.5 | 10.03 |

**Table 3.** Molecular formulas, molecular weights, and formulas of compounds contained in pine turpentine as a result of GCMS analysis

| No. | Compound Name | Molecular Formula and Molecular Weight (g.mol$^{-1}$ ) | Chemical Structure |
|---|---|---|---|
| 1 | α-pinane [13] | $C_{10}H_{16}$; 138.25 |  |
| 2 | ß-pinane [14] | $C_{10}H_{16}$; 136.23 |  |
| 3 | limonene [15] | $C_{10}H_{16}$ ; 136.23 |  |
| 4 | delta 3 carene [16] | $C_{10}H_{16}$ ; 136.24 |  |
| 5 | α-terpinolene [17] | $C_{10}H_{16}$; 136.23 |  |
| 6 | Benzene methanol [18] | $C_7H_8O$; 108.14 |  |
| 7 | trans-pinocarveol [19] | $C_{10}H_{16}O$; 152.23 |  |

Various compounds produced by pine have important biological activities, including monoterpene derivatives used for analgesia, sterilization, antiviral, and sedation [20-21], and anti-cancer [22]. Compound α-Pinene is the main monoterpene from pine tree essential oil whose vapor can induce non-rapid eye movement sleep (NREMS) without side effects [23] and significantly increase hypnotic sleep duration of NREMS [24]. This compound also has a clear bactericidal and bacteriostatic effect on *Candida albicans* and prevents atherosclerosis [25]. In addition, it was also reported that one of the compounds from the monoterpene group is a plant essential oil that has pharmacological activity modulating antibiotic resistance, anticoagulant, antitumor, antimicrobial, antimalarial, antioxidant, anti-inflammatory, and analgesic effects [26], as well as antifungal and antibacterial even at low concentrations of 625 g.mL$^{-1}$ [27]. The results also showed that the monoterpene ß-pinene provided protection against Cr(VI) toxicity in maize (*Zea mays*) where treatment with ß-pinene (10 M) significantly reduced the accumulation of Cr(VI) causing decreased root and coleoptile growth in maize [28].

Limonene and its derivatives are mostly used in various applications in the food, pharmaceutical, and cosmetic industries; even because of the increased need for this compound to be produced through microbial engineering as well as through the biosynthetic process, limonene derivation is produced [29]. Limonene has the functions of sterilization, insect repellent [30] central nervous system sedative, expectorant, cough, antiasthmatic, and dissolution of gallstones [31]. Terpinolene and camphene have anthelmintic and antibacterial effects [32]. The compound delta 3 carene is a perfume ingredient; widely found in various plants such as banana (*Musa sapientum* L.), chestnut (*Castanea* species), *Cinnamomum* species, *Curcuma* species, Ginger (*Zingiber* species), grape (*Vitis* species), Kiwifruit (*Actinidia chinensis*, syn. *A. deliciosa*), Pepper (*Piper nigrum* L.), potato (*Solanum tuberosum* L.), marmelo (*Cydonia oblonga* Mill.), Rice (*Oryza sativa* L.), soybean (*Glycine max*. L. Merr.), and strawberry (*Fragaria* species) [ 33]. Meanwhile, the results of other studies showed that terpinolene's anti-cancer effect promotes higher rates of apoptotic and necrotic cell death and induces cell death and intracellular oxidative stress in breast cancer cells [34]. The compound benzene methanol is an essential oil resin that has the bioactive ability as a strong antibacterial against *Escherichia coli* and *Staphylococcus aureus* [35]. This trans-pinocarveol compound is similar to the compound isolated from *Cyperus rotundus* L and exhibits anti-inflammatory activity [36] so it has prospects for development in the pharmaceutical industry.

## 4. Conclusion

The results of the oleoresin production test showed that there was no difference in production capacity ($p$<0.05) between *Pinus oocarpa* and *P. merkusii* stands with an average oleoresin production per tree of 0.0127±0.002 g.d$^{-1}$ and 0.0183±0.003 g.d$^{-1}$. Turpentine type *P. oocarpa*

consisted of: α-pinene (5.2%), ß-pinene (5.8%), and delta 3-carena (13.8%); while in *P. merkusii* are α-pinene (8.2%), ß-pinene (11.5%), limonene (5.2%), α-terpinolene (32.7%), benzenemethanol (4.3%), and trans-pinocarveol (3.5%). All of the turpentine compounds produced by the two types of pine can be used for various pharmaceutical, cosmetic, and pesticide industries.

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