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Comparative Analysis of Volatile Components in Commercial Japanese Hinoki Cypress (*Chamaecyparis obtusa*) Essential Oils

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Hinoki cypress (*Chamaecyparis obtusa*) is an evergreen conifer endemic to Japan. The benefits of hinoki fragrance for the central nervous system have garnered increasing attention, and its inhalation is gaining popularity. While hinoki essential oils are available from various manufacturers, the growing regions of the trees and plant parts used for extraction differ among products, and the characteristic benefits of each product are not clearly defined. In this study, we performed gas chromatography with flame ionization detection (GC-FID) and GC-MS analysis on ten commercial hinoki essential oils, one from leaf and nine derived from wood, to examine differences in volatile components between plant parts and among wood-derived samples. The results showed that sabinene, bornyl acetate and α-terpinyl acetate were the major components in leaf-derived oils, while α-pinene and δ-cadinene predominated in wood-derived oils. Among the wood-derived samples, the ratio of α-pinene to δ-cadinene was categorized into three distinct patterns: samples with higher α-pinene content, samples with higher δ-cadinene content, and samples in which both components were present in nearly equal amounts. Differences in chemical composition and the relative proportion of components may influence the physiological effects of hinoki essential oil. Evaluating how these variations affect health benefits could contribute to product quality assessment and help establish tailored applications for each specific chemical profile.

Key words *chamaecyparis obtusa*, essential oil, gas chromatography, terpenoids

INTRODUCTION

Odorous substances, especially plant-derived fragrances, are known to induce various physiological, emotional, and behavioral responses in humans and other mammals.[1] Studies have found that inhaling plant essential oil vapors can influence psychological states.[2,3]

Hinoki (*Chamaecyparis obtusa* [Siebold et Zucc.] Endl., Cupressaceae), is an evergreen conifer endemic to Japan, and its fragrance, like that of many plant-derived essential oils, is believed to provide benefits for psychological functions. The distinctive woody aroma of hinoki has long been appreciated in Japanese culture, with hinoki wood used in Japanese architecture since the Nara period (710 AD)[4] and in traditional hinoki baths. Additionally, hinoki forests are integral to Shinrin-yoku (forest bathing), a practice that promotes relaxation and emotional well-being. Recent studies have shown that spending time in hinoki forest environments can significantly improve mood, reduce depressive symptoms, and increase levels of beneficial biological markers like serotonin and oxytocin.[5] Inhaling hinoki's natural aroma in daily settings, such as homes, baths, or nature walks, may also contribute to comfort and psychological ease. However, the scientific evidence supporting the beneficial health effects of hinoki fragrance is insufficient. A wide variety of hinoki essential oils is commercially available from different manufacturers, with differences among products potentially arising from variations in growing regions, plant parts used for extraction, and manufacturing processes such as extraction methods, all of which can affect the chemical composition. Despite this variability, it is consistently claimed that inhaling hinoki fragrance provides relaxation benefits. This raises the possibility that differences in chemical composition may affect both the intensity and nature of these benefits. In this study, we primarily focused on analyzing wood-derived hinoki essential oils, emphasizing the characteristic fragrance of hinoki wood used in various

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applications. Because leaf-derived oils are also commercially available, we additionally aimed to clarify the differences in constituent composition between plant parts under identical analytical conditions from a quality control perspective. Therefore, one leaf-derived and nine wood-derived commercial hinoki essential oils were included in the analysis. These samples were examined using gas chromatography with flame ionization detection (GC-FID) and GC-MS to assess both qualitative and quantitative differences in their volatile components from a quality perspective. We also examined variation in the chemical profiles of the wood-derived oils, which are widely distributed in Japan.

By evaluating the activity of individual components, this study aims to strengthen the scientific evidence for the health benefits of hinoki fragrance. Additionally, we seek to determine how variations in component composition influence these benefits, thereby contributing to product quality assessment and proposing appropriate applications for each product.

MATERIALS AND METHODS

Hinoki Essential Oil In this study, ten commercially available hinoki essential oils were used. Sample 1 was obtained from Frontier Japan Co., Ltd. (wood part, cultivated in the Shimanto region, Lot No. 230401), sample 2 from Tree of Life Co., Ltd. (wood part, Japan (cultivation region not specified), Lot No. 92), sample 3 from Tree of Life Co., Ltd. (wood part, cultivated in the Yoshino region, Lot No. 27K), sample 4 from Inscent Co. (wood part, cultivated in the Yoshino region, Lot No. 138), sample 5 from Inscent Co. (wood part, cultivated in the Kiso region, Lot No. 138), sample 6 from Inscent Co. (wood part, cultivated in the Shimanto region, Lot No. 138), sample 7 from Ash Inc. (wood part, cultivated in the Yoshino region, Lot No. 230731), sample 8 from Green Flask Co., Ltd. (wood part, cultivated in the Kiso region, Lot No. GF120), sample 9 from hinoki LAB Co., Ltd. (wood part, cultivated in the Okayama prefecture, Lot No. 7154) and sample 10 from hinoki LAB Co., Ltd. (leaf part, cultivated in the Okayama prefecture, Lot No. 7152).

GC-FID and GC-MS Analysis Quantitative analysis of the volatile components: GC-FID analyses were performed on a GC-2014 (Shimadzu Corporation) with a flame ionization detector. The operating conditions were as follows: column: Stabilwax (Restek Corporation), 30 m × 0.25 mm, 0.25 μm film thickness; column temperature: 50 °C to 250 °C (ramped at Δ5 °C/min); carrier gas: helium; injector temperature: 250 °C; detector temperature: 250 °C.

Qualitative analysis of the volatile components: GC-MS analyses were performed on a GCMS-QP2010 (Shimadzu Corporation). The operating conditions were as follows: column and column temperature as above; carrier gas: helium; injector temperature: 250 °C; ion source temperature: 250 °C. MS was operated in the electron ionization (EI) mode at an ionization voltage of 70 eV over an *m/z* range from 35 to 400 atomic mass units (amu). The mass spectrum was compared with the Wiley Registry 12th edition/NIST 2020 mass spectral library.

RESULTS AND DISCUSSION

GC Analysis of Hinoki Leaf Essential Oil Figure 1 shows the GC-MS chromatogram of sample 10 (leaf-derived oil). Based on similarity search results, a total of 19 peaks were identified. The chemical structures of these compounds are also presented in Fig. 1. The major constituents identified were sabinene (21.2%), bornyl acetate (11.7%), and α-terpinyl acetate (18.1%), all of which are monoterpenes. These main components were consistent with previous reports,[6] where sabinene, bornyl acetate, and α-terpinyl acetate were identified at approximately 12.34%, 10.78%, and 18.78%, respectively.

GC Analysis of Hinoki Wood Essential Oil Figure 2 shows the GC-MS chromatogram of sample 9 (wood-derived oil). Based on similarity search results, a total of 16 peaks were identified. The chemical structures of these compounds are also presented in Fig. 2. The major constituents were α-pinene, a monoterpene, and δ-cadinene, a sesquiterpene. In sample 9, α-pinene accounted for 37.4% and δ-cadinene for 14.5% of the total peak area. These components are consistent with previous findings,[7] although their proportions differed, with α-pinene and δ-cadinene present at approximately 15.3% and 17.5%, respectively.

The GC analysis results for the ten samples are presented in Table 1. In hinoki leaves, the characteristic components were identified as the monoterpenes sabinene, bornyl acetate, and α-terpinyl acetate. In contrast, hinoki wood primary contains α-pinene, a monoterpene, along with sesquiterpenes such as δ-cadinene and α-muurolene. As reported by Yatagai, while trees such as hinoki and ceder mainly contain terpenes, leaf oils are richer in low-boiling, highly volatile monoterpene hydrocarbons, whereas wood oils contain a higher proportion of high-boiling, less volatile sesquiterpenes. Consequently, leaf oils produce a light, refreshing scent, while wood oils provide a heavier, calming aroma.[8] The present findings suggest that the identified compounds serve as distinctive constituents defining the fragrance of each plant part.

Comparison of Chemical Composition Ratios in Hinoki Wood Essential Oils In Japan, wood-derived hinoki essential oil is generally considered to be more widely produced and distributed than leaf-derived oil. GC analysis was performed on nine commercially available hinoki wood essential oils, and the qualitative composition of compounds was consistent across all samples (Table 1). Figure 3 illustrates the compositional ratios of α-pinene and δ-cadinene, which are representative monoterpene and sesquiterpene compounds characteristic of hinoki wood and exhibited high peak intensities. Comparison of their relative contents among the samples revealed three distinct categories based on the ratio of these compounds.

(1) α-pinene exceeded δ-cadinene (Sample 1, 3, 6, 9)
(2) both compounds were present at approximately equal levels (Sample 2, 4)
(3) δ-cadinene exceeded α-pinene (Sample 5, 7, 8)

These differences may be due to variations in the growing conditions of the trees and differences in the essential oil production processes. Regarding the influence of growing region, Kang *et al*. reported that hinoki trees cultivated in warm, humid climates with nutrient-rich soils produced essential oils with higher yields and significantly greater contents of α-terpinene and cedar acetate. Their findings suggested the importance of environmental management and site selection for optimizing essential oil quality.[9] Additionally, differences in steam distillation processes, including distillation time, can significantly affect essential oil composition. Since α-pinene is a monoterpene and δ-cadinene is a sesquiterpene,

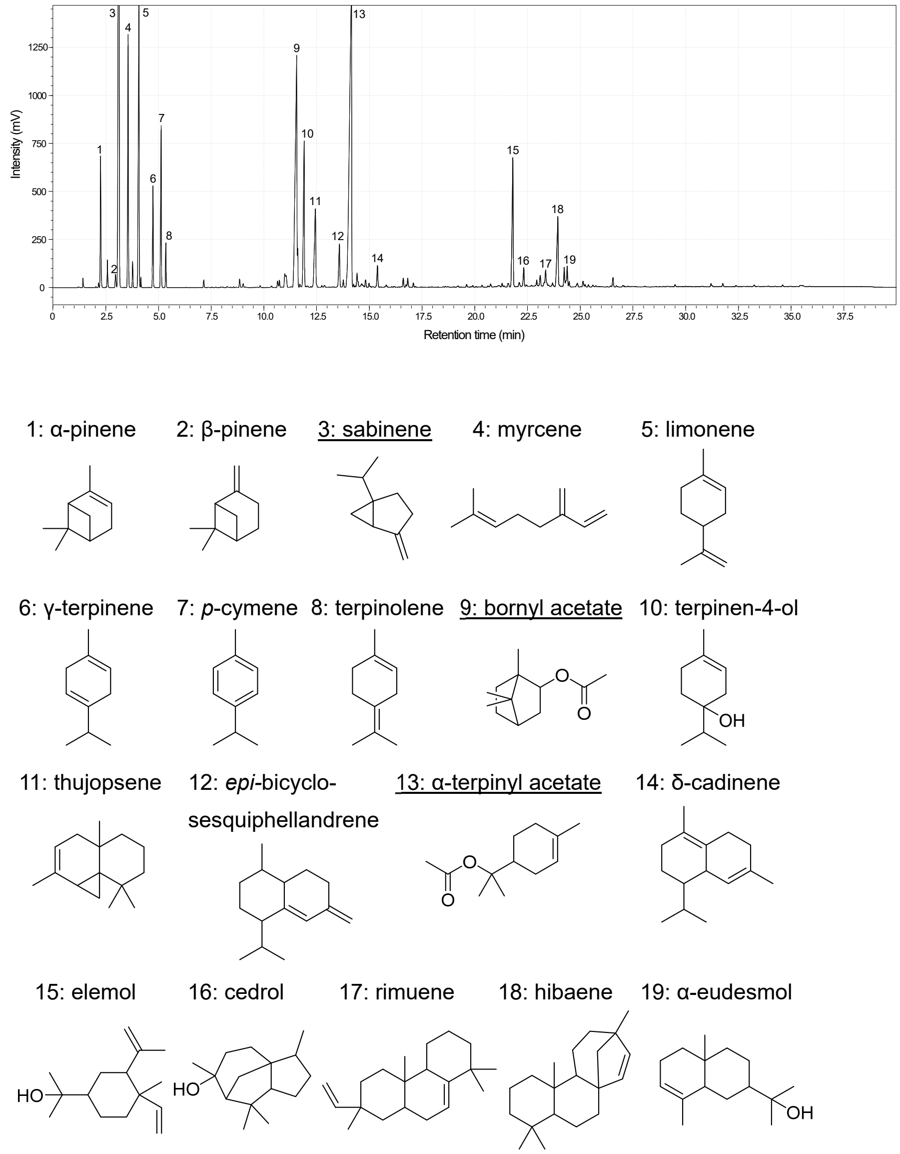

Fig. 1. GC Chromatogram of Hinoki Leaf Essential Oil (Sample 10) and Chemical Structures of the Compounds as Numbered in the Chromatogram

The composition of the 19 identified peaks is shown below:

1: α-pinene, 2.7%; 2: β-pinene, 0.3%; 3: sabinene, 21.2%; 4: myrcene, 4.9%; 5: limonene, 7.5%; 6: γ-terpinene, 2.0%; 7: *p*-cymene, 3.3%; 8: terpinolene, 0.8%: 9: bornyl acetate, 11.7%; 10: terpinen-4-ol, 4.3%; 11: tsujobsen, 3.1%; 12: *epi*-bicyclo-sesquiphellandrene, 1.3%; 13: α-terpinyl acetate, 18.1%; 14: δ-cadinene, 0.6%; 15: elemol, 4.0%; 16: cedrol, 0.6%; 17: rimuene, 0.8%; 18: hibaene, 2.7%; 19: α-eudesmol, 0.6%.

the latter's higher boiling point means it is generally extracted later in the distillation process. Roger *et al*. demonstrated using *Picea mariana* (Pinaceae) as a model that shorter distillation time favor the extraction of monoterpenes like α-pinene, whereas longer distillation results in increased sesquiterpene content, such as δ-cadinene.[10] Thus, the compositional differences observed in this study may reflect not only biological or ecological variability in the plant material but also differences in distillation parameters.

On the other hand, although the behavioral effects of inhaled compounds such as α-pinene [11] and sabinene [12] have been reported in animals, the effects of δ-cadinene on the central nervous system via inhalation have not yet been investigated, and the molecular mechanisms underlying the psychological effects of individual constituents in hinoki essential oil remain unclear. Understanding the action mechanisms of these constituent compounds will not only contribute to building scientific evidence for the health benefits of hinoki fragrance, but also help link the relative proportions of these compounds to the intensity and nature of their effects. Future research evaluating how variations in the composition ratios affect health benefits could contribute to product quality assessment and help establish optimal applications tailored to specific chemical profiles.

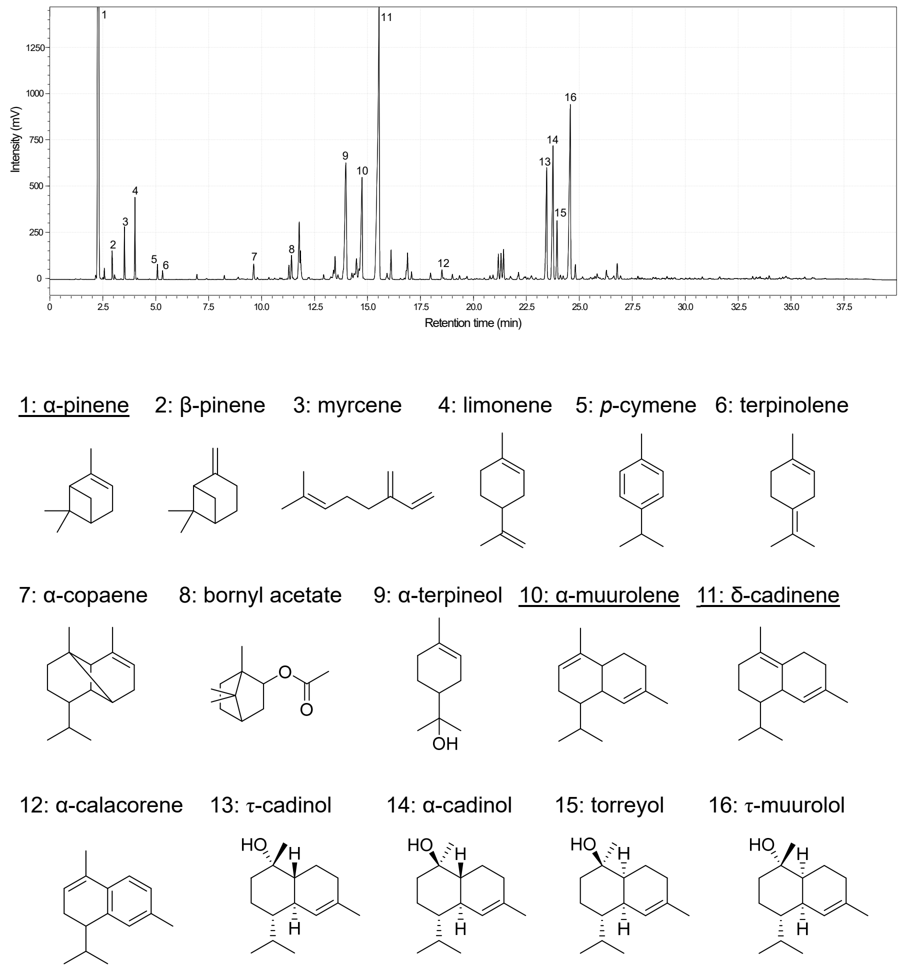

Fig. 2. GC Chromatogram of the Hinoki Wood Essential Oil (Sample 9) and Chemical Structures of the Compounds as Numbered in the Chromatogram

The composition of the 16 identified peaks is shown below:

1: α-pinene, 37.4%; 2: β-pinene, 0.5%; 3: myrcene, 0.9%; 4: limonene, 1.4%; 5: *p*-cymene, 0.3%; 6: terpinolene, 0.2%; 7: α-copaene, 0.4%: 8: bornyl acetate, 0.7%; 9: α-terpineol, 5.7%; 10: α-muurolene, 3.9%; 11: δ-cadinene, 14.5%; 12: α-calacorene, 0.3%; 13: τ-cadinol, 3.6%; 14: α-cadinol, 4.6%; 15: torreyol, 1.5%; 16: τ-muurolol, 7.0%.

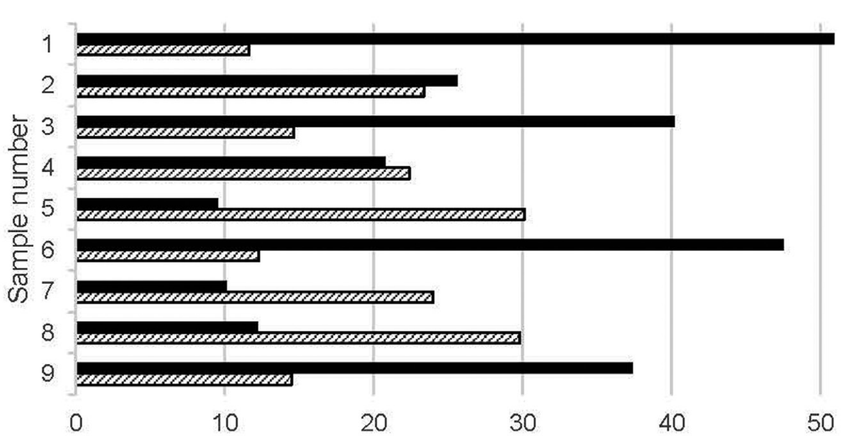

Fig. 3. Relative Contents of α-Pinene and δ-Cadinene in Hinoki Wood Essential Oil Samples 1–9

Table 1. Chemical Composition of Ten Commercially Available Hinoki Essential Oils

Compound	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
α-pinene	50.9	25.6	40.2	20.8	9.5	47.5	10.1	12.2	37.4	2.7
β-pinene	0.4	0.3	0.4	0.2	0.1	0.5	0.1	0.2	0.5	0.3
sabinene	ni	ni	ni	ni	ni	ni	ni	ni	ni	21.2
myrcene	0.6	0.3	0.8	0.3	0.1	0.7	0.2	0.4	0.9	4.9
limonene	1.2	0.7	1.1	0.8	0.2	1.0	0.4	0.7	1.4	7.5
γ-terpinene	ni	ni	ni	ni	ni	ni	ni	ni	ni	2.0
p-cymene	0.2	0.1	0.2	0.2	0	0.2	0.1	0.4	0.3	3.3
terpinolene	0.1	0.2	0.2	0.8	0.1	0.1	0.1	0.2	0.2	0.8
α-copaene	0.1	0.5	0.4	0.5	0.9	0.3	0.5	0.9	0.4	ni
bornyl acetate	0.5	0.5	0.8	0.6	0	0.5	0.4	0.1	0.7	11.7
terpinene-4-ol	ni	ni	ni	ni	ni	ni	ni	ni	ni	4.3
tsujobsen	ni	ni	ni	ni	ni	ni	ni	ni	ni	3.1
epi-bicyclo-sesquiphellandrene	ni	ni	ni	ni	ni	ni	ni	ni	ni	1.3
α-terpineol	5.2	6	5.4	7.5	4.9	4.0	5.8	6.3	5.7	ni
α-terpinyl acetate	ni	ni	ni	ni	ni	ni	ni	ni	ni	18.1
α-muurolene	3.4	5.6	3.9	6.5	7.1	3.3	5.6	7.0	3.9	ni
δ-cadinene	11.6	23.4	14.6	22.4	30.1	12.3	24	29.8	14.5	0.6
α-calacorene	0.3	0.4	0.3	0.6	0.4	0.2	0.4	0.4	0.3	ni
elemol	ni	ni	ni	ni	ni	ni	ni	ni	ni	4.0
cedrol	ni	ni	ni	ni	ni	ni	ni	ni	ni	0.6
τ-cadinol	3.1	4.9	4.2	4.8	6.2	3.5	7.4	4.6	3.6	ni
rimuene	ni	ni	ni	ni	ni	ni	ni	ni	ni	0.8
α-cadinol	3.9	6.1	5.3	6.1	6.8	4.5	9.3	4.8	4.6	ni
torreyol	1.3	2.1	1.9	2	2.4	1.5	3.3	1.9	1.5	ni
hibaene	ni	ni	ni	ni	ni	ni	ni	ni	ni	2.7
α-eudesmol	ni	ni	ni	ni	ni	ni	ni	ni	ni	0.6
τ-muurolol	5.6	9.9	8.8	9.1	9.3	7.3	16.3	6.7	7.0	ni

ni, not identified

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Conflict of interest The authors declare no conflict of interest.

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