



Research Article

## Chemical composition of essential oils obtained from *Picea glehnii* (F. Schmidt) Mast. grown in Hokkaido, Japan

Mitsuki Takeyama<sup>1</sup>, Yoshihito Kohari<sup>2\*</sup>  and Miki Murata<sup>1,2</sup> 

1. Graduate School of Manufacturing Engineering, Kitami Institute of Technology, 165 Koen-Cho, Kitami, Hokkaido 090-8507, Japan.
2. School of Earth, Energy and Environmental Engineering, Faculty of Engineering, Kitami Institute of Technology, 165 Koen-Cho, Kitami, Hokkaido 090-8507, Japan.

### Abstract

The chemical composition of essential oils obtained using hydrodistillation of shoots, leaves, and branches of Sakhalin spruce (*Picea glehnii*), which exclusively grows in northeastern Hokkaido, Japan, and southern Sakhalin Oblast, Russia, was quantitatively and qualitatively determined. Yields of the essential oils were  $0.15 \pm 0.01\%$  in the shoots,  $0.08 \pm 0.03\%$  in leaves,  $0.05 \pm 0.02\%$  in branches, and  $0.03 \pm 0.01\%$  in dried leaves obtained via vaporization of essential oil components during the drying process. Bornyl acetate, which is unique to the pine family and is the main contributor to the fragrance of pine leaves, was found to be present at  $42.95 \pm 3.35\%$  in the shoots and  $41.20 \pm 8.27\%$  in the leaves, while the branches contained a lesser amount ( $4.79 \pm 1.32\%$ ). By contrast, other aerobic components such as  $\alpha$ -pinene, 3-carene,  $\beta$ -phellandren and longifolene were found to be more abundant in the branches.

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### Corresponding Author

Dr. Kohari Yoshihito  
E-mail: [kohari@mail.kitami-it.ac.jp](mailto:kohari@mail.kitami-it.ac.jp)  
Tel.: +00966114677262

### Keywords

Essential oils, Sakhalin spruce, *Picea glehnii*, Hydrodistillation, Bornyl acetate, quantitative analysis.

## 1. Introduction

Pines, which belong to the gymnosperm and cormophyte families, are widely distributed throughout the Northern Hemisphere. They represent the conifers and are important in cultural and religious practices in many of these regions. Pines are one of the most abundant conifers and are divided into many species, which include subfamilies such as Pinus, Larch, Fir, and Spruce [1, 2]. Plants classified in the spruce subfamily have brown, scaly bark and branches, with "leaf sinks" from which pointed leaves grow. Spruce trees are commonly used as lumber for building materials and manufacturing musical instruments. Essential oils obtained by hydrodistillation of non-woody parts of spruce are widely used in perfumes, cosmetics, and food

additives. The essential oils of spruce plants are useful resources because of their excellent antimicrobial activity [3-5]. The components of spruce essential oil are mostly composed of monoterpenes. The main component is borneyl acetate, which is a characteristic aroma of pine plants, and pinenes, ocimene, camphors, calenes, *p*-menthane, and thujanes are also known. Unlike *Picea mariana* and *Picea abies*, which are widely distributed members of the spruce subfamily, *Picea glehnii*, also known as sakhalin spruce, first introduced in 1880 [6], is concentrated in southern Sakhalin, Russia, and eastern Hokkaido, Japan [7]. In Hokkaido, Japan, it is favored for artificial plantation because it is easy to grow from seedlings, is resistant to disease, and sprouts late in spring, making it

suitable for afforestation in high-latitude, extremely cold, and heavily snow-covered areas. The objective of this study is to determine the chemical composition of essential oils obtained from the rare spruce plant *Picea glehnii* using GC/MS and GC/FID to determine the usefulness of these essential oils and to promote their widespread use.

## 2. Materials and methods

### 2.1 Plant materials

*Picea glehnii* used in this study was collected in September 2022 from three sites planted in residential and mountainous areas of the Okhotsk area of eastern Hokkaido, Japan, to exclude the effects of differences in growth environments of these plants on essential oil components. The collected *Picea glehnii* samples were cut into different parts such as shoots (leaves and branches), leaves, and branches, and some leaves were dried in a cool, dark place until they weighed less than 1% of the initial weight to determine the effect of drying on the essential oils. The moisture content of the leaves was  $36.8 \pm 1.5\%$  ( $n = 4$ ). In this study, as described for other pine families, 200 g of *Picea glehnii* shoots (without separating the leaves and branches) were hydrodistilled. After approximately 40 min of distillation, the essential oils were obtained at  $0.15 \pm 0.01\%$  ( $n = 3$ ). To further clarify the essential oils contained in each part, leaves and branches were separated and 200 g of each was distilled.

### 2.2 Hydrodistillation (HD) [8]

A total of 200 g each of *Picea glehnii* shoots, leaves, branches, and dried leaves ( $n = 3$ ) were steam-distilled using a Clevenger apparatus (Tokyo Seisakushiyo, Japan) until 200 mL of the aqueous part was removed. The extracted essential oils were then separated from the distilled water and stored in a refrigerator at 6°C for further analysis.

### 2.3 Gas chromatography-mass spectrometry and gas chromatography-flame ionization detector analysis

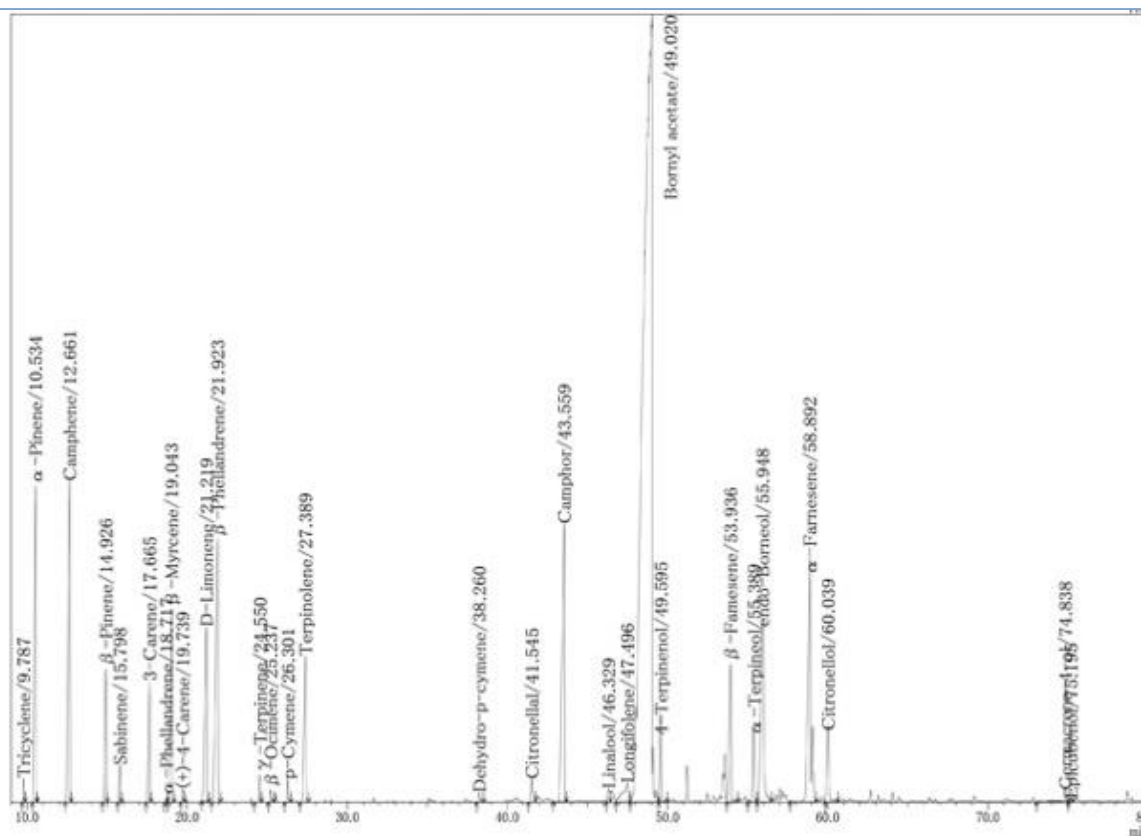
Qualitative analyses of the chemical composition of essential oils were carried out using ultra-gas chromatography-mass spectrometry (GC/MS, QP-2010, Shimadzu, Japan) equipped with an InertCap Pure-WAX (polyethylene glycol)-fused silica capillary column (60 m  $\times$  0.25 mm; 0.25  $\mu$ m film thickness). The injector and interface temperatures were maintained at 250°C, and helium was used as the carrier gas at a flow rate of 1 mL/min. The essential oils were diluted

with n-hexane and injected using 1.0  $\mu$ L aliquots to perform GC. The column temperature was programmed for heating from 50 to 180°C at 2°C/min, with initial and final hold times of 10 min with a split ratio of 100. Mass spectra were scanned from  $m/z$  30 to 1000 amu. Identification of peaks was carried out using NIST14 library data supplied with the GC/MS system (NIST 14: Mass Spectral Library & Search Software, 2014) and via co-injection of commercially available  $\alpha$ -pinene,  $\beta$ -pinene, sabinene, *d*-limonene, bornyl acetate, and caryophyllene.

Quantitative analyses of the chemical composition of the essential oils were performed using GC equipped with a flame ionization detector (GC-FID, GC-2014, Shimadzu, Japan). The capillary column, flow of helium gas, temperature conditions, and split ratio for GC-FID analysis were the same as those described for GC/MS. The injector and detector temperature were maintained at 250°C. The essential oils were analyzed using tridecane as an internal standard, diluted with n-hexane, and injected in 1.0  $\mu$ L aliquots. The retention index (RI) of the essential oil components was calculated using standard alkane solutions (C8-C20 and C20-C40). The percentage and concentration of bornyl acetate were calculated using an internal standard based on the GC-FID peak areas, and the contents of other chemical components were calculated based on the GC-FID peak areas with FID response factors [9].

## 3. Results and discussion

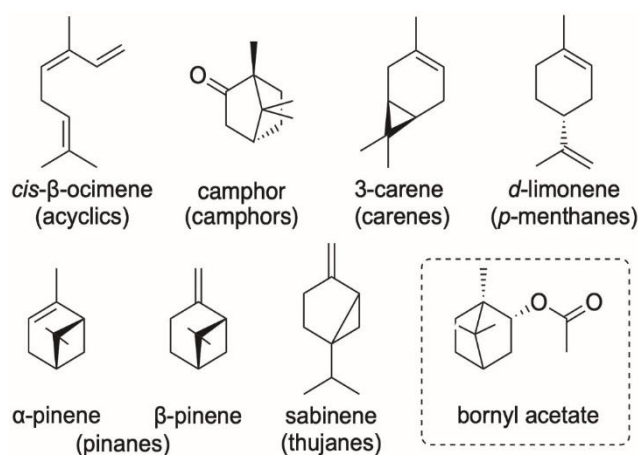
Essential oils from pine plants are usually produced by hydrodistillation of shoots. Essential oils were obtained from leaves and branches at yields of  $0.08 \pm 0.03\%$  ( $n = 3$ ) and  $0.05 \pm 0.02\%$  ( $n = 3$ ), respectively. Although the season of collection has been reported to have a significant effect on yield of oils [10], it is clear that the yield of essential oils obtained from *Picea glehnii* was lower than that obtained from other *Picea* subfamilies (1.01% [11] in *Picea abies* L, 0.96% [12] in *Picea pungens* and 0.91% [13] in *Picea engelmannii*). The combined amounts of essential oils obtained from leaves and branches were almost identical to those obtained from shoots, indicating that, unexpectedly, more essential oils were extracted from the branches. The dried leaves were then hydrodistilled to determine the effect of the drying process, which was performed to efficiently obtain essential oils from the



**Figure 1.** MS chromatogram of essential oils obtained from *Picea glehnii* shoots

plant. The percentage of essential oils obtained from the dried leaves was  $0.03 \pm 0.01\%$ , indicating that, in *Picea glehnii*, a large portion of the essential oil is lost through the drying process. Such results were obtained probably because essential oils in other essential oil-producing plants such as *Mentha* accumulate in oil cells present on the leaf surface, whereas *Pinus* plants secrete essential oils to the outside through secretory ducts [14]. Next, to determine the chemical composition of the essential oils obtained from each part of *Picea glehnii*, GC-MS was used for qualitative analysis. (Fig. 1).

The composition of the obtained essential oils was found to be dominated by monoterpenes derived from geranyl diphosphate (GPP), which is produced by condensation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) derived from the mevalonic acid pathway, which is universally found in common plants [15]. The following compounds were identified: *cis*- $\beta$ -ocimene (acyclic), camphor (camphors), 3-carene (carenes), *d*-limonene (*p*-menthanes),  $\alpha$  and  $\beta$ -pinene (pinenes), sabinene (thujanes); bornyl acetate, a characteristic aromatic component of pine plants, was also found in the essential oils of *Picea glehnii* shoots. (Fig. 2) The results



**Figure 2.** Structure of compounds in *Picea glehnii* shoots essential oil

are similar to those reported for other essential oils of the spruce family.

Next, to determine the chemical composition of the essential oils obtained from each site, each component was qualitatively determined using GC-MS and quantified using an internal standard employing GC-FID. (Table 1). The results revealed 31 components and their contents. Interestingly, the content of borneol acetate, a component of the aroma of plants belonging to the spruce subfamily that is used as a

**Table 1.** Chemical composition of essential oils obtained from *Picea glehnii*

Compounds	RI	Oil content (%)									
		Shoots		Leaves		Branches		Dried leaves			
Tricyclene	1003	0.11	± 0.03	0.01	± 0.01	0.09	± 0.07	0.02	± 0.01		
α-pinene	1017	2.43	± 0.72	0.25	± 0.18	15.87	± 8.72	0.34	± 0.25		
Camphene	1057	3.00	± 0.62	0.46	± 0.36	0.57	± 0.28	0.57	± 0.39		
β-Pinene	1100	0.87	± 0.11	0.16	± 0.10	2.48	± 0.84	0.21	± 0.11		
Sabinene	1113	0.00	± 0.00	0.00	± 0.00	0.80	± 0.38	0.00	± 0.00		
3-Carene	1139	0.12	± 0.04	0.02	± 0.00	7.70	± 3.76	0.01	± 0.01		
β-Myrcene	1159	0.99	± 0.08	0.27	± 0.16	2.18	± 0.78	0.36	± 0.26		
D-Limonene	1191	6.42	± 0.29	1.28	± 1.31	1.87	± 0.86	0.56	± 0.30		
β-Phellandren	1201	3.12	± 0.29	0.84	± 0.53	9.62	± 2.69	1.11	± 0.69		
γ-Terpinene	1238	0.00	± 0.00	0.01	± 0.00	0.00	± 0.00	0.00	± 0.00		
β-Ocimene	1247	0.06	± 0.01	0.03	± 0.01	0.41	± 0.31	0.04	± 0.02		
p-Cymene	1262	0.05	± 0.00	0.03	± 0.01	0.00	± 0.00	0.02	± 0.02		
Terpinolene	1278	0.77	± 0.00	0.41	± 0.10	2.25	± 1.17	0.49	± 0.24		
dehydro-p-cymene	1425	0.09	± 0.00	0.08	± 0.01	0.17	± 0.03	0.08	± 0.01		
Citronellal	1472	0.05	± 0.01	0.12	± 0.07	0.38	± 0.15	0.07	± 0.03		
Camphor	1502	0.20	± 0.12	1.06	± 0.56	0.14	± 0.11	1.34	± 0.52		
Linalool	1545	0.11	± 0.06	0.14	± 0.03	0.92	± 0.38	0.13	± 0.03		
Longifolene	1562	0.34	± 0.01	0.46	± 0.18	12.89	± 4.78	0.60	± 0.03		
Bornyl acetate	1585	44.07	± 3.14	44.00	± 7.74	5.34	± 1.04	46.94	± 0.85		
4-Terpineol	1594	0.04	± 0.02	0.21	± 0.16	1.95	± 0.35	0.33	± 0.07		
β-Famesene	1664	0.03	± 0.03	0.50	± 0.35	5.32	± 1.44	1.55	± 0.28		
α-Terpineol	1688	0.03	± 0.03	0.11	± 0.13	0.65	± 0.49	0.52	± 0.67		
endo-Borneol	1697	0.04	± 0.06	0.99	± 0.49	0.03	± 0.04	0.77	± 0.55		
α-Farnesene	1737	0.24	± 0.08	0.91	± 0.58	0.31	± 0.23	2.37	± 0.31		
δ-Cadinene	1740	0.83	± 0.06	1.27	± 0.50	1.84	± 1.31	2.85	± 0.35		
Citronellol	1759	0.06	± 0.01	0.27	± 0.18	0.28	± 0.40	0.17	± 0.13		
Germacrene-4-ol	2009	0.05	± 0.01	0.17	± 0.02	0.45	± 0.63	0.24	± 0.04		
τ-Cadinol	2041	0.25	± 0.06	0.24	± 0.06	0.00	± 0.00	0.44	± 0.03		
δ-Cadinol	2050	0.07	± 0.03	0.06	± 0.02	0.02	± 0.04	0.10	± 0.01		
α-Cadinol	2060	0.59	± 0.16	0.53	± 0.13	0.12	± 0.10	0.61	± 0.04		
Citronellic acid	2066	0.03	± 0.01	0.04	± 0.01	0.00	± 0.00	0.06	± 0.01		
Total		65.07	± 1.14	54.92	± 8.06	74.67	± 13.97	62.92	± 2.59		

food additive and flavoring agent, was 44.07% in the shoots (44.00% in leaves, 5.34% in branches, and 46.94% in dried leaves). Moreover, most of this compound was present in the needle-like leaf parts but was present in lower amounts in the branches. Notably, this value is very high compared to that of other members of the spruce subfamily such as *Picea pungens* (2.4%) [13], *Picea pungens* (29.40%), *Picea mariana* (21.64%), and *Picea glauca* (31.25%) [16]. When the chemical composition of the leaves and branches were compared, it was observed that a

higher content of the precursor of borneol acetate, borneol, was present in the leaves (0.99% in leaves compared to 0.03% in branches). These results suggest that borneol acetyltransferase, which synthesizes borneol and acetyl-CoA to bornyl acetate, may be abundant in the leaves. In addition, the leaves had a high content of camphor (1.06% in leaves compared to 0.14% in branches). Nevertheless, higher contents of many other components are thought to be produced in branches. For example, the bicyclic monoterpene, sabinene, which is found in a variety of plants, has been suggested to be derived from branches and not



from leaves (1.06% in leaves compared to 0.14% in branches). Furthermore, a comparison of dried and non-dried leaves confirmed that drying decreased the yield of essential oils, but increased the content of most other quantifiable components. However, the content of d-limonene decreased after the drying process (1.28% in fresh leaves compared to 0.56% in dried leaves). The reason for this is presumably due to its transformation into other compounds, i.e., carvone and carbonyl, through oxidation during the drying process.

#### 4. Conclusions

In summary, essential oils obtained from *Picea glehnii* contained higher concentrations of borneyl acetate (44.07% in the shoots, 44.00% in leaves and 5.34% in branches), a main component that contributes to fragrance, than those obtained from other pine species, suggesting its use as a food additive or flavoring agent. It was also found that the drying process is not suitable as a method to produce essential oils because it significantly reduces the yield. As a future development of this study, the biological activity of the essential oils obtained from other *Picea* and *Picea glehnii* will be compared. In addition, seasonal variations in the composition of *Picea glehnii* essential oil will be studied to ensure its stable use.

#### Authors' contributions

Investigation, T.M.; Methodology and writing—original draft, K.Y.; Project administration, M.M.

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#### Availability of data and materials

All data will be made available on request according to the journal policy.

#### Conflicts of interest

The authors declare no conflict of interest.

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