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Terpene Compound Composition and Antioxidant Activity of Essential Oils from Needles of *Pinus densiflora*, *Pinus koraiensis*, *Abies holophylla*, and *Juniperus chinensis* by Harvest Period

Eunjin Kim [1], Suyeon Yang [2], Byeong Bae Jeon [3], Eugene Song [4] and Hwayong Lee [5,*]

1 Department of Forest Therapy, Chungbuk National University, Cheongju 28644, Republic of Korea; kimej@chungbuk.ac.kr
2 Department of Food Science and Biotechnology, Kyungpook National University, Daegu 41566, Republic of Korea; didtndus1128@knu.ac.kr
3 Suwon R & D Center, Hanbit Flavor and Fragrance, Suwon 16681, Republic of Korea; bbjeon@hffaroma.com
4 Department of Consumer Science, Chungbuk National University, Cheongju 28644, Republic of Korea; eugenesong@chungbuk.ac.kr
5 Department of Forest Science, Chungbuk National University, Cheongju 28644, Republic of Korea
* Correspondence: leehy@chungbuk.ac.kr

Abstract: Plant resources are used as raw materials in various industries related to humans, such as edible, medicinal, taste, and flavor industries, depending on processes such as drying, processing, and collection period. In this study, we investigated the terpene compound composition and antioxidant activity of essential oils extracted from the needles of *Pinus densiflora*, *Pinus koraiensis*, *Abies holophylla*, and *Juniperus chinensis* collected in the harvest period (February, April, July, and October) planted on the campus of Chungbuk National University. The essential oil was separated by hydrodistillation. According to the analysis results of gas chromatography–mass spectrometry, the terpene compounds changed depending on the season and tree species. The proportions of monoterpene and sesquiterpene classes in the needles of *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* changed depending on the harvest period. The antioxidant activity by DPPH and ABTS assays varied depending on the species and seasons, and needles harvested from *P. koraiensis* showed the highest activity in all harvest periods. High antioxidant activity has been confirmed even at low concentrations in pine trees, so it is expected to play a role as a natural antioxidant. Additionally, since the composition of terpene compounds varies depending on the harvesting time and species, it is expected to have various uses in the pharmaceutical, cosmetics, and food industries.

Keywords: essential oils; monoterpene; sesquiterpene; antioxidant

Citation: Kim, E.; Yang, S.; Jeon, B.B.; Song, E.; Lee, H. Terpene Compound Composition and Antioxidant Activity of Essential Oils from Needles of *Pinus densiflora*, *Pinus koraiensis*, *Abies holophylla*, and *Juniperus chinensis* by Harvest Period. *Forests* **2024**, *15*, 566. https://doi.org/10.3390/f15030566

Academic Editors: Biljana M. Nikolić, Zorica S. Mitić and Dalibor Ballian

Received: 26 February 2024
Revised: 14 March 2024
Accepted: 19 March 2024
Published: 20 March 2024

1. Introduction

Essential oils are a mixture of plant secondary metabolites [1] and are volatile aromatic substances composed of bioactive compounds synthesized for self-defense against external environmental threats [2]. Unlike humans and animals, plants have a unique defense mechanism that protects themselves from harmful microorganisms, including herbivores, by secreting low-molecular-weight compounds called secondary metabolites. This compound is concentrated in the essential oil of the plant, and its medicinal properties also treat human diseases [3]. Essential oils are obtained from plant organs such as leaves, bark, roots, flowers, fruit, and seeds [4], and the yield of essential oils is generally known to be less than 1% of the plant [2,5]. Essential oils are mostly composed of terpenes and can be divided into alcohols, esters, aldehydes, ketones, phenols, and ethers, depending on the functional group [6].

Terpenes, the largest group of essential oils, are used for various medicinal purposes, and their uses are not limited to scent, taste, or color [7]. In modern medicine, terpene compounds are used to achieve antiplasmodial [8], antioxidant [9], hypoglycemic and

hypolipemic [10], antiviral [11], anticancer [12], and antidepressant [13] activities. Because of these effects, terpenes derived from medicinal plants have been widely used in natural folk remedies as antipyretics, cough suppressants, analgesics, antiemetics, and antidiarrheals [14]. In particular, there are commonly used plants, such as tea, thyme, Spanish sage, citrus fruit, etc., which offer a variety of medicinal values [15]. In addition, tea tree's volatile essential oil is known for its antibacterial properties and acts as an active ingredient in the treatment of skin infections [16], and terpenes synthesized from cannabis have also been used medicinally for a long time [15]. In addition to their medicinal effects, essential oils are used in the cosmetics industry. Fragrances play a particularly important role in increasing the attractiveness of cosmetics, and a pleasant smell affects the comfort and effectiveness of the product and has a significant impact on the overall evaluation of the cosmetic product [17]. Because the use of aromatic and chemical compounds in natural vegetable oils requires high costs, most industries use synthetic fragrances that imitate them, and these synthetic fragrances may not be safe for application to humans [18]. Essential oils have an attractive scent and no side effects; therefore, they are used to treat skin diseases, and their demand is increasing in the cosmetics industry because they are suitable as skin care products [19]. Consumers are expecting minimal side effects from skin care products and effective prevention and treatment effects [20]. More recently, consumers have demanded a switch to natural cosmetics with natural ingredients compared to conventional cosmetics derived from chemical products [21]. In addition, essential oils have antibacterial [22] and anti-fungal [23] properties against pathogenic fungi and bacteria; therefore, they have the potential to replace synthetic additives [24]. Essential oil is a masking agent that prevents unpleasant odors and is used in folk medicine and aromatherapy as a natural treatment for depression, anxiety, insomnia, and stress caused by changes in the living environment [19]. Famous cosmetic brands use a variety of essential oils in their cosmetics, and the most expensive perfumes contain pure essential oils, giving them a special and unique scent, making them unique as exclusive perfumes [25]. The main markets for essential oils are pharmaceuticals (15%), cosmetics and aromatherapy (29%), and beverages and foods (35%) [17]. In the pharmaceutical industry, medicines in the form of capsules, syrups, ointments, creams, and sprays contain some essential oil ingredients, and their production is continuously increasing [26]. Essential oils are used in the cosmetics industry as antioxidants [27,28] and for care [29], cleansing [30], and protection of skin [31]. In addition, we are attempting to apply it to the food industry as a natural preservative using nanoencapsulation, active packaging, and polymer-based coating [32].

One of the important sources of essential oils is coniferous trees, which have significant economic value in terms of wood production and are important for construction and other uses, such as pulp and paper [33]. Coniferous species such as *Pinus densiflora*, *P. koraiensis*, *Abies holophylla*, and *Juniperus chinensis* are distributed throughout Northeast Asia, including Korea [34]. *P. densiflora* is used in medicine [2], food [35], and cosmetics industries [36]. Essential oil extracted from *P. densiflora* wood has been reported to exhibit anti-inflammatory activity [2]. In *P. koraiensis*, essential oil extracted from the needles has been reported to have anti-fatigue activity [37], essential oil extracted from wood has anti-inflammatory properties [38], and essential oil extracted from cones has been reported to have anti-tumor activity [39]. Essential oil extracted from *A. holophylla* needles has been reported to have antibacterial activity against respiratory bacteria [40], while essential oil extracted from *J. chinensis* needles is known to induce apoptosis [41] and has also been reported to exhibit antibacterial activity [42]. Therefore, essential oils derived from *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* have antibacterial properties and excellent industrial value.

Antioxidation is the action of preventing or removing excessive production of free radicals. Active oxygen is oxygen that is created when oxygen enters the body through breathing and undergoes metabolic processes within the body. When it increases rapidly, it promotes cellular oxidation and attacks human tissues, causing damage to cell structure, loss of cell function, DNA damage, aging, inflammation, and various diseases [43,44]. In a previous study, essential oils were evaluated for their natural antioxidant effects

in neutralizing unstable substances called free radicals [45], and Ruberto & Baratta [46] compared the antioxidant activities of 98 essential oil components and reported differences in the activity of each component.

The composition of essential oil compounds varies depending on the growth stage of the plant [47,48]. Additionally, climate changes such as rapid temperature rise and drought also affect the production of secondary metabolites [49]. Therefore, for the use of essential oils, research on the seasonal changes in their compounds is necessary.

In this study, to explore the applicability of essential oils, essential oils were extracted seasonally from the needles of evergreen coniferous species *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis*, and changes in seasonal compounds and antioxidant activity of each extracted essential oil were investigated.

2. Materials and Methods

2.1. Sampling and Essential Oil Extraction

The sample needles of *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* used in this experiment were collected from the major branches of trees about 12 m tall that were previously planted and managed at the College of Agriculture and Life Sciences of Chungbuk National University (N 36°37′42.42″, E 127°27′13.08″) during 8–11 February (February), 19–22 April (April), 4–7 July (July), 24–27 October (October), and in 2022. Since the production of secondary metabolites in plants is the result of interactions between various factors, in this study, essential oils from needles of *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* planted in the same location were collected at different times. The sample was finely crushed immediately after collection, then 500 g of the crushed sample was mixed with 1.5 L of distilled water, distilled at 100 °C for 2 h using hydrodistillation, and the essential oil was separated through a cooling process.

2.2. Compounds Analysis of Essential Oils

Identification and quantification of essential oil compounds extracted from the needles of *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* were used by modifying the method of Guleria et al. [50], and Mothana et al. [51]. The temperature conditions of the GC (Agilent 8890 GC, Agilent Technologies, Inc., Santa Clara, CA, USA) were from an initial temperature of 50 °C, raised to 230 °C at a rate of 3 °C/min, and maintained at 230 °C for 5 min. The column used was DB-5MS UI (30 m length, 0.25 mm dia., 0.25 μm Film). A GC-MSD (Agilent 5977 B GC/MSD, Agilent Technologies, Inc., Santa Clara, CA, USA) was used to identify the components. The operating conditions of the MSD were ion source temperature of 350 °C, ionization voltage of 70 eV, and mass scan range (mass/charge) of 40~500 m/z. Each component was identified through comparative analysis with the NIST17 library and compared with information reported in the literature [52] (Table 1).

Table 1. The analysis conditions of essential oil using GC/MSD.

Column	DB-5MS UI, 30 m length, 0.25 mm dia., 0.25 μm Film
Mass range	40~500 m/z
Inlet Temp.	250 °C
Ion source Temp.	350 °C
Carrier gas	He gas, 1.0 mL/min
Injection volume	0.5 mL
Spilt ratio	20:1
Ionization	EI(electron impact) 70 eV
Temp. program	50 °C (4 min) → 3 °C/min → 230 °C (5 min)

Essential oils were identified by the relative amount (%) of each compound and expressed as a percentage of the peak area relative to the total peak area. The relative peak area (%) of all compounds was greater than 0.1%, and the quality was greater than 90%.

2.3. HaCaT Cell Viability

HaCaT cells (HaCaT-immortalized human keratinocytes) were purchased from AddexBio Technologies (San Diego, CA, USA) and subcultured at 37 °C and 5% CO_2 in DMEM medium containing 10% FBS and 1% P/S. Cell viability was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay. HaCaT cells were dispensed into a 96-well plate at 1×10^5 cells/mL, and samples of different concentrations were mixed and cultured at 37° C and 5% CO_2 incubator for 24 h. When the cells were 80%–90% confluent, the essential oil extracted from *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* needles were diluted and treated at concentrations of 12.5, 25, 50, and 100 µL/mL. After 24 h of incubation, the culture medium was removed, washed twice with PBS, and the washed cells were treated with 100 µL of MTT solution (0.5 mg/mL) and further cultured in a 37 °C and 5% CO_2 incubator for 60 min. The MTT solution of cells after additional incubation was removed, treated with 100 µL of DMSO, and incubated for 30 min. Afterward, the absorbance was measured at 595 nm using a microplate reader (Mobi, Microdigital, Seongnam, Republic of Korea).

2.4. Antioxidant Activity

For antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) free radical scavenging assay were used. The DPPHand ABTS assay was a modification of the method used by Torres-Martínez et al. [53]. Considering industrial use through HaCaT cell viability, each sample was prepared by diluting it in ethanol at concentrations of 12.5, 25, and 50 µL/mL [54]. For the DPPH assay, 0.2 mM DPPH solution and sample were mixed in the same ratio (1:1), cultured in the dark for 30 min, and absorbance was measured at 517 nm using a microplate reader (Mobi, Microdigital, Seongnam, Republic of Korea). ABTS assay was performed by mixing 7 mM ABTS solution and 2.45 mM Potassium persulfate solution in the same ratio (1:1) and reacting for 16 h at room temperature in dark conditions, and 20 µL of the sample of each concentration and 180 µL of ABTS solution were mixed, cultured in the dark for 30 min, and measured at 734 nm. Each measurement was repeated three times, and each method was calculated using the formulas shown below. The control group was treated with ascorbic acid at the same concentration as the sample.

$$\% \text{ of antioxidant activity} = [(Ac - As)/Ac] \times 100 \quad (1)$$

where Ac: Control reaction absorbance; As: Testing specimen absorbance.

2.5. Statistical Analysis

The collected data were analyzed using the SPSS Statistics ver.18 program. ANOVA analysis was performed to compare the antioxidant activity of each coniferous species by concentration, and Tukey HSD was used as a post hoc test to compare the antioxidant activity of each coniferous species.

3. Results

3.1. Research Site and Essential Oil Extraction

The location where the trees used in this experiment were planted (N 36°37′42.42″, E 127°27′13.08″) is a temperate continental climate area with four distinct seasons, with a hot and humid summer and a cold and dry climate in the winter, with an annual average of The temperature is 13.1 °C and the average annual relative humidity is 64%. In 2022, the average temperature was −0.9 °C, and the average relative humidity was 52% in February. The average temperature was 15.2 °C, and the average relative humidity was 48% in April. The average temperature was 27.6 °C, and the average relative humidity was

72% in July, and the average temperature was 15.1 °C, and the average relative humidity was 64% in October [55]. The soil in this area was sandy loam (60.3%), silt (24%), clay (15.4%), pH 6.0, organic matter 17.9 g/kg, and available phosphorus 355.6 mg/kg [56]. The average recovery rate of each essential oil was ca. 0.35%, and the recovery rate of essential oil extracted in October was the highest at ca. 0.48%. The essential oil recovery rate by species was highest in *A. holophylla* species, with an average essential oil recovery rate of 0.49 ± 0.25. The weather conditions and essential oil recovery rates for each harvest day are as follows (Table 2).

Table 2. Yields of essential oils from needles of *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* and weather conditions on each harvest period.

Species	Harvest Day	Oil Yield (%)	Temperature (°C)	Relative Humidity (%)
Pinus densiflora	8 February 2022	0.14	0.2	45.8
	19 April 2022	0.2	16.2	35.3
	4 July 2022	0.3	28.8	71
	24 October 2022	0.28	10.9	46.9
Pinus koraiensis	9 February 2022	0.3	1.4	43
	20 April 2022	0.4	16.3	33.4
	5 July 2022	0.5	28.5	74.5
	25 October 2022	0.7	11.3	53.8
Abies holophylla	10 February 2022	0.24	3.3	44.9
	21 April 2022	0.3	15.8	41
	6 July 2022	0.7	30.3	68.5
	26 October 2022	0.7	12.7	53.9
Juniperus chinensis	11 February 2022	0.14	3	50.5
	22 April 2022	0.14	17.5	59.1
	7 July 2022	0.26	30.5	67.4
	27 October 2022	0.24	13.8	63.5

Oil yield: mL/1000 g wet weight (%).

3.2. Terpene Compound by Each Harvest Period of Essential Oils Extracted from Needles of *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis*

The chemical compounds that accounted for more than 0.1% of the essential oils extracted from the needles of *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* by harvest period are shown in Table S1 and Figure 1. From essential oil compounds extracted from *P. densiflora* needles, the proportion of monoterpenes was 71.41% in February, 49.79% in April, 56.48% in July, and 66.86% in October, and the proportion of sesquiterpenes was 28.59% in February, 47.21% in April, 42.06% in July, and 33.14% in October. Among the essential oil compounds extracted from *P. koraiensis* needles, the proportions of monoterpenes were 56.86% in February, 55.49% in April, 49.13% in July, and 41.81% in October, and the proportions of sesquiterpenes were 38.82% in February, 44.06% in April, 50.18% in July, and 56.77% in October. Among the essential oil compounds extracted from *A. holophylla* needles, the proportions of monoterpene and sesquiterpene were 45.49% and 54.51 in February, 63.05% and 36.95% in April, 61.17% and 38.83% in July, and 72.15% and 27.85 in October, respectively. Among the essential oil compounds extracted from juniper needles, the proportions of monoterpenes were 56.29% in February, 73.66% in April, 77.29% in July, and 73.33% in October, and the proportions of sesquiterpenes were 43.71% in February, 25.96% in April, 22.71% in July, and 26.67% in October (Figure 1).

Among the essential oils extracted from the needles of *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis*, the top 5 compounds were considered to be the main compounds and were compared based on the most abundant amounts for each harvest period. The main terpene compounds in essential oil extracted from *P. densiflora* needles were 3-Carene, β-Phellanderene, β-Pinene, D-Limonene, and Caryophyllene, and the main terpene compounds in essential oil extracted from *P. koraiensis* needles were γ-Terpinene, α-Pinene,

Germacrene D, γ-Muurolene, and D-Limonene. The main terpene compounds in essential oil extracted from *A. holophylla* needles were 3-Carene, β-Bisabolene, D-Limonene, α-Pinene, and γ-Muurolene, and the main terpene compounds in essential oil extracted from the needles of the *J. chinensis* were 3-Carene, Terpineol, γ-Muurolene, α-Pinene, and Caryophyllene (Table 3).

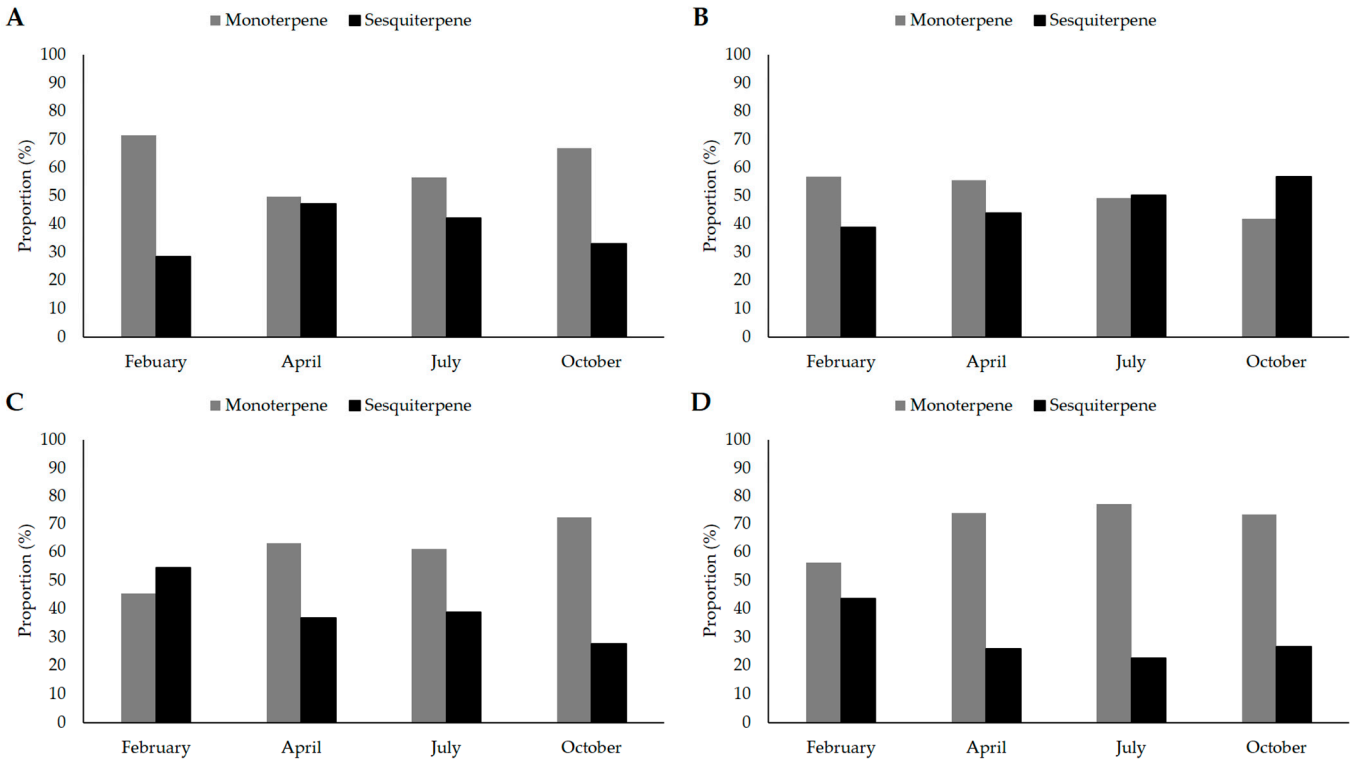

Figure 1. Proportion of monoterpenes and sesquiterpenes by harvest period of essential oils extracted from needles of *P. densiflora* (**A**), *P. koraiensis* (**B**), *A. holophylla* (**C**), and *J. chinensis* (**D**) (Relative rate (%)).

Table 3. Major compounds in essential oils extracted from needles of *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* (Relative rate (%)).

Tree Species	No.	Compounds	February	April	July	October
Pinus densiflora	1	3-Carene	28.55	1.94	2.10	1.16
	2	β-Phellanderene	0.51	10.61	10.43	26.57
	3	β-Pinene	0	6.56	10.69	25.82
	4	D-Limonene	15.23	0	0	0
	5	Germacrene D	0	10.50	11.36	11.87
Pinus koraiensis	1	γ-Terpinene	16.92	0.93	0	0.70
	2	α-Pinene	0	12.73	9.16	8.29
	3	Germacrene D	5.50	9.33	12.33	13.30
	4	γ-Muurolene	3.47	2.09	7.45	11.96
	5	3-Carene	4.22	6.42	12.63	4.50
Abies holophylla	1	3-Carene	6.02	12.24	8.44	14.28
	2	β-Bisabolene	0	12.78	4.54	0
	3	D-Limonene	12.70	11.46	11.95	10.52
	4	α-Pinene	0	11.37	10.19	12.23
	5	γ-Muurolene	11.52	5.60	5.90	5.99

Table 3. *Cont.*

Tree Species	No.	Compounds	February	April	July	October
Juniperus chinensis	1	3-Carene	20.43	2.72	8.91	1.02
	2	Terpineol	2.46	16.61	16.16	19.61
	3	γ-Muurolene	4.17	15.15	10.89	11.53
	4	α-Pinene	13.94	10.91	0	11.82
	5	Caryophyllene	13.94	0	1.76	2.07

Among the compounds in essential oils extracted from *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis*, β-Myrcene, 3-Carene, Terpineol, α-Humulene, and γ-Muurolene were commonly identified in all tree species (Figure 2).

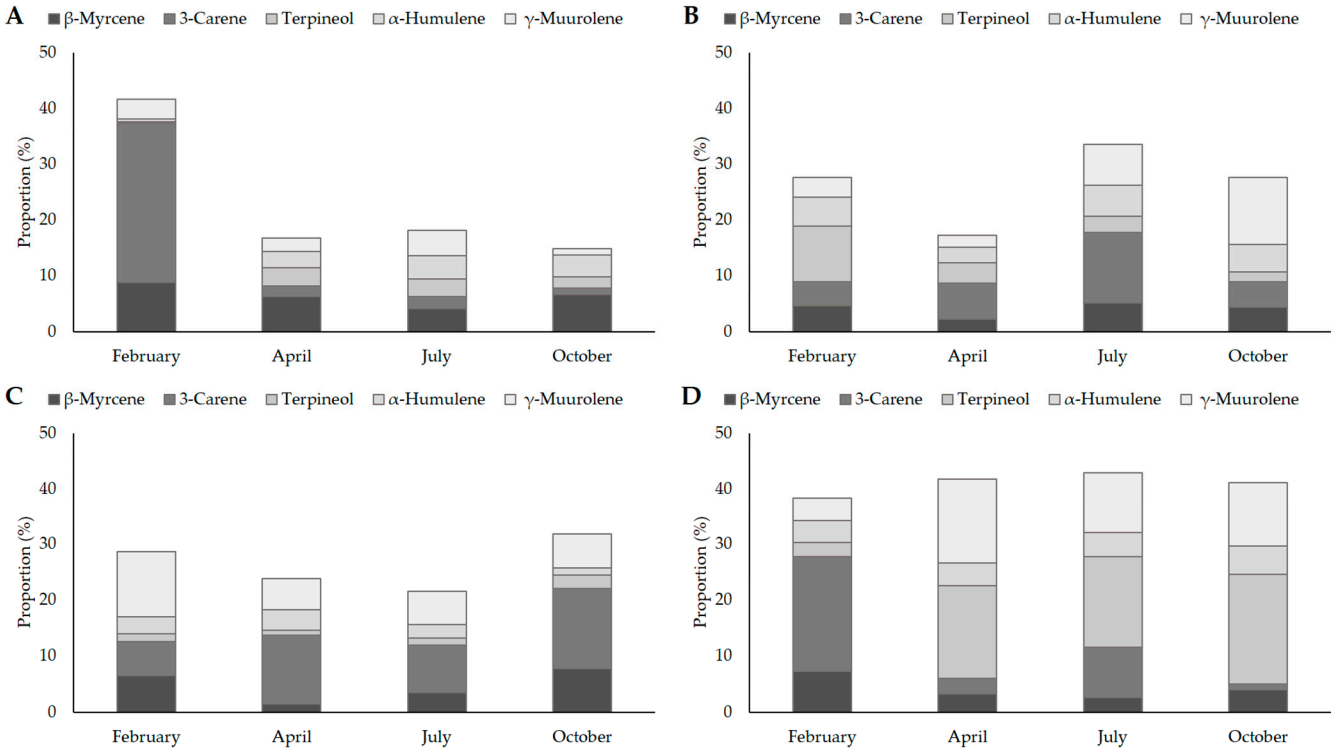

Figure 2. Overlapping compounds in essential oils depending on harvest period (Relative rate (%)). (**A**) *P. densiflora*, (**B**) *P. koraiensis*, (**C**) *A. holophylla*, (**D**) *J. chinensis*.

3.3. HaCaT Cell Viability

To confirm the concentration range of the antioxidant activity analysis of essential oil extracted from *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* needles, cell viability was measured by MTT assay using HaCaT cells. The cell viability was high at essential oil concentrations of 12.5 to 50 μL/mL, but when the concentration was higher than 100 μL/mL, essential oil extracted from *P. koraiensis*, *A. holophylla*, and *J. chinensis* needles showed a cell viability of 49 to 74%. According to ISO 10993-5, if the cell viability exceeds 80%, it is considered non-cytotoxic, 60%~50% is classified as weak cytotoxicity, 40%~60% as moderate cytotoxicity, and under 40% as strong cytotoxicity [57]. Therefore, toxicity was confirmed at a concentration of 100 μL/mL. As a result, an antioxidant activity test was conducted at the non-toxic range of 12.5, 25, and 50 μL/mL concentrations of essential oil extracted from *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* needles (Figure 3).

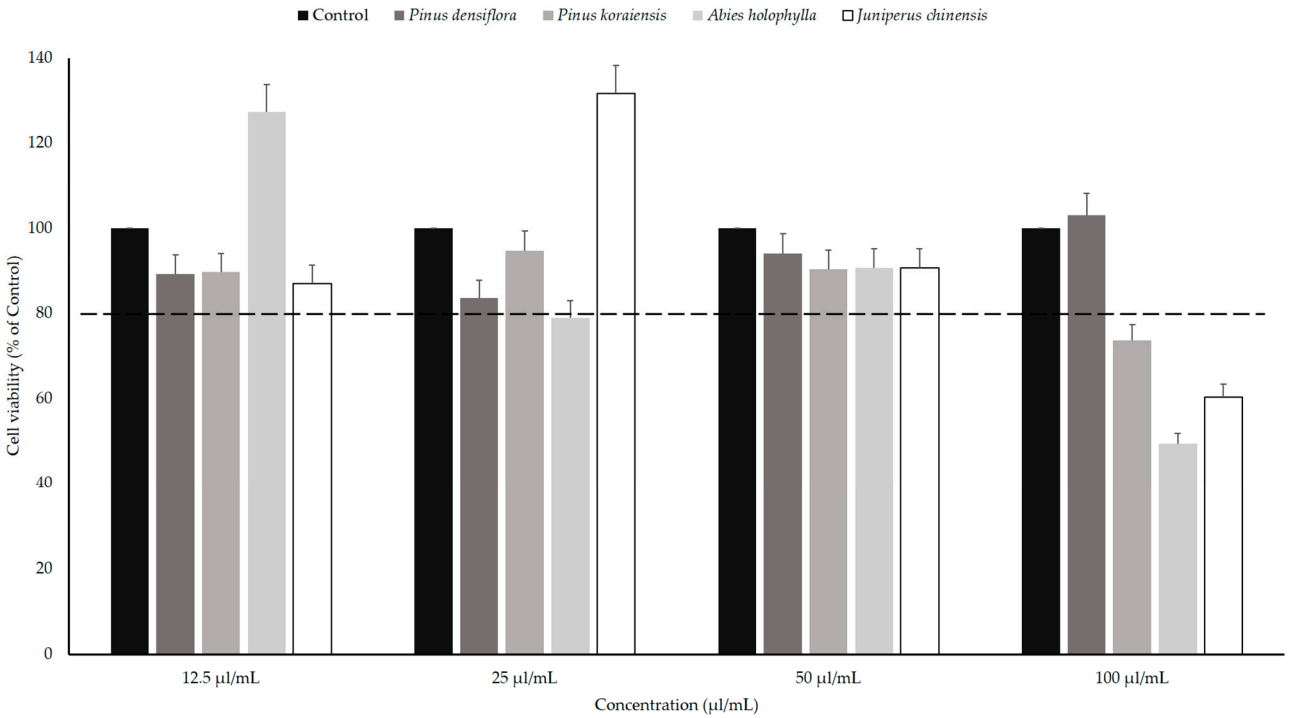

Figure 3. HaCaT cell viability treated with essential oils extracted from needles harvested over time from *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis*. Statistical analysis was performed using ANOVA with the Tukey HSD test. Values are presented as mean ± S.D. The dotted line represents 80% cell viability.

3.4. Antioxidant Activity of Essential Oils Extracted from Needles of P. densiflora, P. koraiensis, A. holophylla, and J. chinensis by Each Harvest Period

The antioxidant activity of essential oils extracted seasonally from needles of *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* was analyzed using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) and 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay and compared with ascorbic acid.

In the DPPH assay, the essential oil from *P. densiflora* needles harvested in July showed antioxidant activity similar to that of ascorbic acid at a concentration of 25 and 50 μL/mL, and July had the highest concentration at 12.5 μL/mL. In essential oil *P. koraiensis* needles, antioxidant activity was similar to that of ascorbic acid in July at a concentration of 12.5 μL/mL and in April, July, and October at a concentration of 25 μL/mL. Also, at concentrations of 25 and 50 μL/mL, the antioxidant activity was very high at over 80%, except for February at the 25 μL/mL concentration. Essential oils extracted from *A. holophylla* and *J. chinensis* needles had antioxidant activity similar to that of ascorbic acid in oils extracted from October and July at concentrations of 50 μL/mL, respectively (Figure 4).

Antioxidant activity using ABTS, *P. koraiensis* needles had similar activity to ascorbic acid at concentrations of 25 and 50 μL/mL at all harvest times. Essential oil extracted from *P. densiflora* needles had similar activity to ascorbic acid at concentrations of 25 and 50 μL/mL only in July. Essential oil harvested from needles of *A. holophylla* had lower activity than ascorbic acid at all harvest times and concentrations, and essential oil extracted from *J. chinensis* needles harvested and extracted in July and October had similar activity to ascorbic acid (Figure 5).

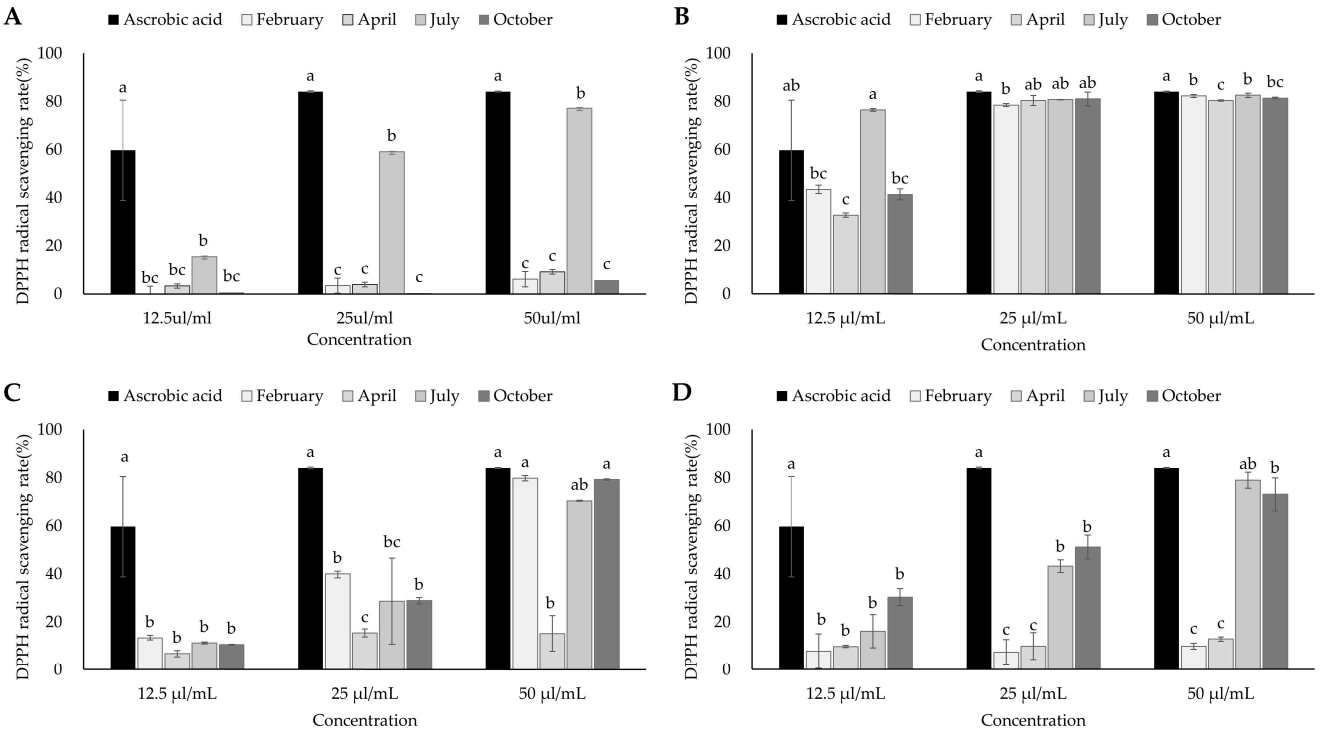

Figure 4. Antioxidant activity of essential oils extracted from leaves harvested over time from *P. densiflora* (**A**), *P. koraiensis* (**B**), *A. holophylla* (**C**), and *J. chinensis* (**D**) by DPPH assay. Statistical analysis was performed by ANOVA with Tukey HSD test. Values are presented as mean ± S.D. Different superscripts are significantly different ($p < 0.05$).

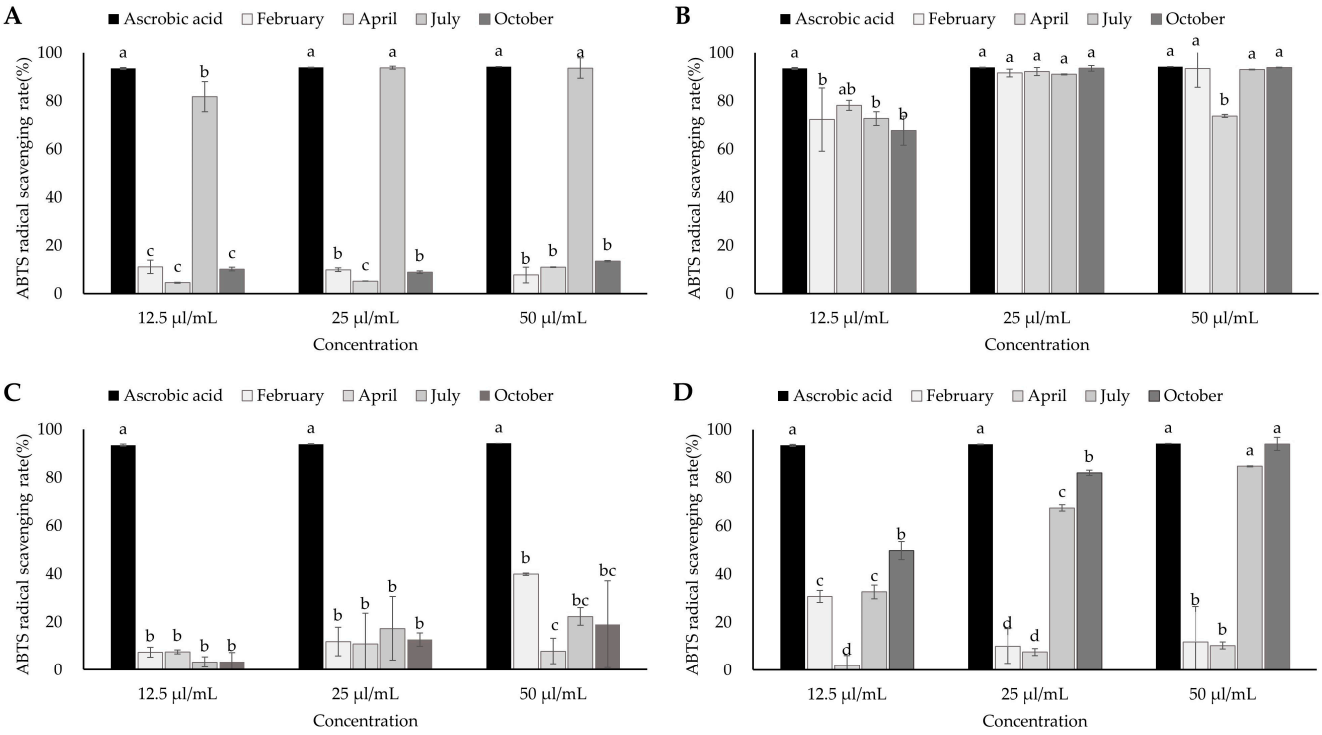

Figure 5. Antioxidant activity of essential oils extracted from leaves harvested over time from *P. densiflora* (**A**), *P. koraiensis* (**B**), *A. holophylla* (**C**), and *J. chinensis* (**D**) by ABTS assay. Statistical analysis was performed by ANOVA with Tukey HSD test. Values are presented as mean ± S.D. Different superscripts are significantly different ($p < 0.05$).

4. Discussion

In this study, essential oils were extracted from evergreen coniferous trees such as *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis*, and the objective was to investigate changes in the composition and antioxidant activity of the essential oils depending on the collection period.

Essential oil extraction methods include the Soxhlet method, hot-air drying process (HD), and supercritical fluid extraction (SFE) [58]. In this study, samples were collected and extracted using hydrodistillation for two hours to extract essential oils. The harvest rate of essential oil from the seeds of *Tamarindus indica* was higher with Soxhlet extraction than with hydrodistillation, but high-quality essential oil was obtained [59]. In the leaves of *Eucalyptus camaldulensis*, the yield was high using hydrodistillation, but the proportion of eucalyptol, a major compound, and antioxidant activity was high using supercritical fluid extraction (SFE) [60]. Depending on the condition of the leaves, 40 and 54 compounds were identified in fresh and dried leaves of *Citrus sinensis*, accounting for 91.5% and 99.6%, respectively, and the main compounds in fresh leaf essential oil were Sabinene (20.4%) and terpinene-4-ol (13.2%), and β-element (16.3%) and Sabinene (10.7%) were confirmed in the dried leaf essential oil [61]. Essential oils are considered to have different extraction cycles and compositions depending on the extraction method and sample conditions. In this study, fresh needles were extracted using hydrodistillation to confirm seasonal changes in essential oil composition and yield. In this study, the essential oil yield rate was highest when *P. densiflora* and *J. chinensis* were collected in the summer, *P. koraiensis* in fall, and *A. holophylla* in summer and fall. The composition of essential oils varies depending on the plant development stage [47,48], and climate changes such as rapid temperature rise and drought, also affect the production of secondary metabolites [49]. This study also shows changes in the production of essential oil compounds depending on the collection time and weather factors, so continuous monitoring is required.

Terpenes constitute the largest group of essential oils [7]. Terpenes are transparent in color, highly volatile, and have antibacterial, analgesic, and sterilizing effects [62]. Monoterpenes have several properties, including antiplasmodial activity, anti-inflammatory, antioxidant, anticancer, preservative, astringent, digestive, and diuretic properties [7]. Sesquiterpenes are used in the perfume and cosmetics industry and have antioxidant [63,64], anticancer, and anti-inflammatory properties [65]. In this study, monoterpene and sesquiterpene components were separated from the essential oils of four coniferous tree species, and monoterpenes were found to be more abundant than sesquiterpenes [2]. Differences in essential oil compounds were confirmed to depend on tree species and season [42,66–69].

When comparing the main compounds of essential oils by tree species and harvest period in this study with other studies, while there were different trends, similar results were also found. Kim et al. [70] and Park & Lee [67], reported that the main compounds of *P. densiflora* essential oil in summer are α-Humulene and Champhene, respectively, but in this study, Germacrene D was highest in July, the summer season. In this study, the main component of *J. chinensis* in April, July, and October, corresponding to spring, summer, and fall, was Terpineol, and the main component in winter was 3-Carene. However, in the study by Raina et al. [71], Sabinene was analyzed as the main compound in summer. The above research results showed a different trend from that of this study. In this study, in *P. Koraiensis* needles collected in spring, the main compounds in October (fall) and February (winter) were confirmed to be Germacrene D and r-Terpinene, respectively. However, in other studies, the main compounds in fall and winter were α-Pinene [72–74] and Limonene [75]. In *A. holophylla*, in this study, D-Limonene was identified as the main substance in needles in April and February, which corresponds to spring and winter, and 3-Carene in fall. In other studies, Limonene was identified as the main compound in summer and winter [40], and 3-Carene was identified as the main compound in fall [76], showing a similar trend to this study.

The major terpene compounds were examined by harvest period in the needles of *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* essential oils analyzed in this study.

3-Carene was also commonly identified in the needles of four coniferous tree species and was identified during all harvest periods. It was identified at a high rate in *P. densiflora* and *J. chinensis* needles extracted in February, *P. koraiensis* needles extracted in July, and *A. holophylla* needles extracted in October. Pinene is the main compound of coniferous essential oil and is composed of two isoprenes with fresh pine and woody scents [77]. In this study, α-Pinene was analyzed as a major compound in needles of *P. koraiensis* and *A. holophylla* except in February and needles of *J. chinensis* except for July, and β-Pinene was analyzed as a major compound in *P. densiflora* except in February. In this study, the highest amount of γ-Muurolene was analyzed in *P. koraiensis* needles in October and *A. holophylla* needles in February, and the lowest amount was analyzed in *J. chinensis* needles in February. D-Limonene was very high, accounting for 15.23% of the essential oil in *P. densiflora* needles harvested in February, and was the main compound in *A. holophylla* needles, accounting for more than 10% in all four harvest periods. Previous studies have also confirmed the high content of D-Limonene in fir essential oil compounds, so it is believed to be a characteristic of these trees in producing secondary metabolites [69]. Germacrene D was the main compound in the essential oil harvested from needles of *P. densiflora* in April, July, and October, and in the essential oil of *P. koraiensis* needles, it was identified as the main compound in all four harvest periods and was highest in October. The main compound identified only in the essential oil of *P. densiflora* needles is β-Phellanderene. γ-Terpinene was identified as a major compound only in *P. koraiensis* and was analyzed in the highest amount in February. β-Bisabolene was identified at a high rate in essential oil *A. holophylla* needles collected in April. Terpineol and Caryophyllene were analyzed as major compounds only in *J. chinensis*.

In this study, when comparing essential oils extracted from *P. densiflora* needles by harvest period, it was found that essential oils extracted from needles harvested in July had higher antioxidant activity compared to other periods. The essential oil extracted from *P. koraiensis* needles showed high antioxidant activity at concentrations above 25 μL/mL, regardless of harvest period. It is considered that the high antioxidant activity, regardless of the season, is a characteristic of the essential oil extracted from *P. koraiensis* needles [78–80]. The antioxidant activity of essential oil extracted from *A. holophylla* needles did show significant antioxidant activity at 50 μL/mL concentrations essential oil extracted from needles of harvested in October; however, other studies have also shown that the essential oil of *A. holophylla* has antioxidant activity [81]. Essential oils extracted from the needles of *J. chinensis* had high antioxidant activity when harvested in summer and fall, and previous studies have shown similar results [82,83].

In this study, changes in the composition of essential oil compounds and differences in antioxidant activity were found in each tree species depending on the harvesting time. There was a significant result in antioxidant activity when the essential oil of *P. densiflora* needles was harvested in July, and the essential oil of *J. chinensis* needles was harvested in July and October. The essential oil of *P. koraiensis* needles showed changes in the composition of compounds depending on the harvest time, and there was a difference in antioxidant activity depending on the harvest time, but high antioxidant activity was confirmed at all harvest times. In a previous study, the antioxidant activity of 423 essential oils was measured, and the results showed that phenolic terpenes (carvacrol, thymol, eugenol, linalool, Pinene) were the main compounds with the best antioxidant activity, followed by 1,8-cineol, borneol, and terpinen-4-ol, several sesquiterpene, D-Limonene [84], and Germacrene D compounds also removed free radicals [45,85]. Since natural essential oils are a mixture of several compounds, they are the result of various types of complex interactions, so it is difficult to regard them as simply the antioxidant properties of a single compound [86]. Therefore, the antioxidant activity of oxygenated monoterpenes and sesquiterpenes has been reported [87]. In other studies on antioxidant activity, high synergy effects are also reported in complex mixtures rather than single compounds and by mixing with non-phenolic oils [88]. The antioxidant activity of pine tree essential oil shown in this study includes the high content of the single compounds Pinene, D-Limonene, and Germacrene confirmed

in previous studies, the composition of various and complex essential oil compounds, monoterpenes, monoterpenoids, and sesquiterpenes. It is believed that a synergistic effect occurred through a harmonious mixture of sesquiterpenoids and diterpenes.

5. Conclusions

In this study, essential oil was extracted from evergreen coniferous trees such as *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis*, and the extraction yield, terpene content, major compounds, common compounds, and antioxidant activity of the essential oil by harvest period were investigated. The yield of essential oil extraction by collection period of *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* needles was confirmed to be high when harvested in July and October. The high content of monoterpene was confirmed in most essential oil compounds, and the high content of sesquiterpene was confirmed in the essential oil of *P. koraiensis* needles harvested in October and the essential oil of *A. holophylla* needles harvested in February. Among essential oil compounds, 3-Carene (*P. densiflora*: 28.55%, *J. chinensis*: 20.43%) was found to have a high content in pine and juniper essential oils collected in February, and D-Limonene (10.52%–12.7%) was found in all fir trees. It was confirmed to be a major compound at the time of harvest. The main compounds of *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* essential oils were identified differently depending on the harvesting period. Therefore, additional research is needed to clearly reveal that the production of terpene compounds is related to the development stage of the plant and weather conditions. When examining the antioxidant activity of essential oils from *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* needles by harvesting time, there were differences in antioxidant activity by harvesting time and concentration for all tree species used in the study. In particular, *P. koraiensis* needle essential oil has been confirmed to have high antioxidant activity regardless of the collection time, and antioxidant activity has been confirmed even at low concentrations, so it is expected to play a role as a natural antioxidant. In addition, changes in the essential oil compound composition of pine, pine, fir, and juniper needles are observed depending on the harvesting time, suggesting the possibility of being utilized in various industries.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/f15030566/s1, Table S1: Chemical composition of the *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* essential oil (Relative rate (%)); Figure S1: Chromatograms of essential oils extracted from needles of *P. densiflora*, *P. koraiensis*, *A. holophylla*, and *J. chinensis* by seasons (spring, summer, and autumn, and winter).

Author Contributions: Conceptualization, H.L., B.B.J., E.S. and E.K.; methodology, H.L., B.B.J., S.Y. and E.K.; investigation, H.L., B.B.J., S.Y. and E.K.; writing—original draft preparation, H.L. and E.K.; writing—review and editing, H.L., B.B.J., E.S. and E.K.; visualization, E.K.; supervision, H.L.; project administration, E.S.; funding acquisition, E.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Korea Environment Industry & Technology Institute (KEITI) through the Technology Development Project for Safety Management of Household Chemical Products, funded by the Korea Ministry of Environment (MOE) (No. 2021002970004 and 1485019515).

Data Availability Statement: All data are available within the paper.

Conflicts of Interest: The authors declare no conflicts of interest.

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