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Age-related variation in the volatile compounds and phenolic profile of *Pinus sylvestris* L. needles

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ABSTRACT **Research Article**

Background and aims Scots pine (*Pinus sylvestris* L.) is an ecologically and economically important species due to its wide distribution and rich content of volatile compounds. The aim of this study was to identify the volatile compounds present in the needles of Scots pine individuals of different age groups and to determine the effect of age on these compounds.
Methods The study material consisted of needle samples collected from 5-, 25-, and 70-year-old Scots pine trees growing in Gölcük Nature Park. The GC-MS method was used to identify volatile compounds, and the RP-HPLC method was used to identify phenolic compounds; differences among age groups were evaluated using statistical analyses and Principal Component Analysis (PCA).
Results The analyses identified a total of 81 volatile compounds. α-pinene (18.79%), caryophyllene (11.10%), germacrene D (10.94%), and β-pinene (7.47%) were identified as the major compounds. Monoterpene hydrocarbons constituted the major compound class across all age groups. Additionally, it was determined that the levels of some sesquiterpene compounds tended to increase with age. While catechin was the phenolic compound present in the highest amount, epicatechin and p-coumaric acid were detected only in the 25-year-old age group. PCA results revealed that the age groups were distinguishable from one another in terms of their volatile compound profiles and that age is a significant factor influencing chemical composition.
Conclusions The findings indicate that the volatile compound profile of Scots pine needles changes with age. These results contribute to a better understanding of the species' chemical properties. Furthermore, they provide a basis for studies aimed at determining the appropriate age group for the extraction of volatile compounds for pharmacological, aromatic, and industrial applications.

Key Words: Age-related variation, essential compounds, GC-MS, RP-HPLC, Scots pine

Pinus sylvestris L. ibrelerinin uçucu bileşik ve fenolik profillerinde yaşa bağlı değişimler

ÖZ

Giriş ve Hedefler Sarıçam (*Pinus sylvestris* L.), geniş yayılış alanı ve zengin uçucu bileşik içeriği nedeniyle ekolojik ve ekonomik açıdan önemli bir türdür. Bu çalışmada, farklı yaş gruplarındaki sarıçam bireylerinin iğne yapraklarında bulunan uçucu bileşiklerin belirlenmesi ve yaşın bu bileşikler üzerindeki etkisinin ortaya konulması amaçlanmıştır.
Bulgular Analizler sonucunda toplam 81 uçucu bileşik tespit edilmiştir. α-pinene (%18,79), caryophyllene (%11,10), germacrene D (%10,94) ve β-pinene (%7,47) başlıca bileşenler olarak belirlenmiştir. Tüm yaş gruplarında monoterpen hidrokarbonlar baskın bileşik sınıfını oluşturmuştur. Ayrıca, bazı seskiterpen bileşiklerin yaşla birlikte artış eğilimi gösterdiği belirlenmiştir. Kateşin en yüksek miktarda bulunan fenolik bileşen olurken, epikateşin ve *p*-kumarik asit yalnızca 25 yaş grubunda belirlenmiştir. PCA sonuçları, yaş gruplarının uçucu bileşik profilleri bakımından birbirinden ayrıldığını ve yaşın kimyasal kompozisyon üzerinde etkili bir faktör olduğunu ortaya koymuştur.
Sonuçlar Elde edilen bulgular, sarıçam iğnelerinin uçucu bileşik profilinin yaşa bağlı olarak değiştiğini göstermektedir. Sonuçlar, türün kimyasal özelliklerinin daha iyi anlaşılmasına katkı sağlamaktadır. Ayrıca farmakolojik, aromatik ve endüstriyel amaçlarla değerlendirilecek uçucu bileşiklerin elde edilmesinde uygun yaş grubunun belirlenmesine yönelik çalışmalara temel oluşturabilecek niteliktedir.

Anahtar Kelimeler: Yaşa bağlı değişim, uçucu bileşen, GC-MS, RP-HPLC, Sarıçam

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1. Introduction

Scots pine (*Pinus sylvestris* L.) is the coniferous tree species with the widest distribution in the Northern Hemisphere (Matías and Jump, 2012). This species ranges from northern Spain and Scotland to Russia and Mongolia, and southward to Turkey and the Caucasus (Boratynski, 1991). It can grow in various ecological conditions from sea level up to an elevation of approximately 2,700 m and extends as far as the northern forest boundary (Yaltırık, 1988; Eckenwalder, 2009; Farjon, 2010). It is also one of the primary species for wood production in Northern European countries and is of great economic importance (Matías and Jump, 2012).

Due to the Scots pine's wide distribution, ecological tolerance, and economic importance, several studies have been conducted on the species' growth characteristics, genetic diversity, adaptation mechanisms, and wood quality (Brichta et al., 2023; Lebedev, 2023; Okoń et al., 2024; Nacakci and Gülcü, 2025). Furthermore, studies on the species' chemical composition and bioactive compounds have also increased in recent years (Njoku et al., 2022; Mirković et al., 2024; Pachura-Hanusek et al., 2026). In particular, the essential oils and phenolic compounds found in the needle-like leaves have attracted the attention of researchers due to their biological activities.

Essential oil derived from pine species are obtained through the hydrodistillation of various plant parts primarily needles but also branches and cones and consist of a complex mixture of more than 50 compounds (Lawlees, 1992; Góra J. and Lis, A., 2000; Dziedziński et al., 2021). The composition of these oils may vary depending on the species, plant part, harvesting conditions, and extraction method. The major compounds commonly found in pine oils include α-pinene, β-pinene, β-fellandrene, β-caryophyllene, camphene, α-terpineol, germacrene D, bornyl acetate, citronellol, and tricyclene (Koukos et al., 2000; Ustun et al., 2006; Hajdari et al., 2021). Among these compounds, α-pinene and β-pinene are bicyclic monoterpenes found among the characteristic constituents of the genus *Pinus* (Borges et al., 2019). In particular, the essential oil obtained from the needles of *P. sylvestris* is characterized by balsamic and turpentine-like aroma and ranges in color from colorless to light yellow. It has been reported that Scots pine essential oil exhibits antibacterial, antifungal, antiviral, and antiseptic properties and is traditionally used for its expectorant and diuretic effects (Lawless, 1992; Góra and Lis, 2000). Although there are numerous studies on the chemical profiles of essential oils compounds in Scots pine, research aimed at demonstrating age-related changes in these compounds is limited (Ustun et al., 2006; Tümen and Reunanen, 2010; Karan and Usta, 2024; Kännaste et al., 2026). Determining age-related changes in the chemical composition of Scots pine needles may contributes to a better understanding of the species' physiological characteristics and facilitates the evaluation of its potential applications. The chemical composition of plants is not limited to volatile compounds, phenolic compounds also constitute a significant portion of this chemical profile (Luna-Guevara et al., 2018). Polyphenols constitute a broad group of compounds that includes flavonoids, tannins, phenolic acids, and their polymeric derivatives; to date, more than 10,000 phenolic compounds have been identified (Khalil and Tazeddinova, 2020). It has been reported that they play a role in plant growth, development processes, and defence against insects and protection against various biotic and abiotic stress factors (Aggarwal et al., 2010; Ren et al., 2021; Ghahremani et al., 2020).

The aim of this study was to identify the volatile and phenolic compounds in needle samples collected from 5-, 25-, and 70-year-old Scots pine (*P. sylvestris* L.) trees growing in Gölcük Nature Park and to determine the effect of age on the profile of these compounds. Within this framework, volatile compounds were analysed using GC–MS, while phenolic compounds were analysed using RP-HPLC.

2. Materials and Methods

2.1 Study area

The study area, Gölcük Lake Nature Park, is in Türkiye's Lake Region, between 37°43′50″ north latitude and 30°29′38″ east longitude. The park is bordered by Gelincik Village to the east, Darıderesi Village to the west, Yakaören Village to the north, and Ağlasun District of Burdur Province to the south (Figure 1).

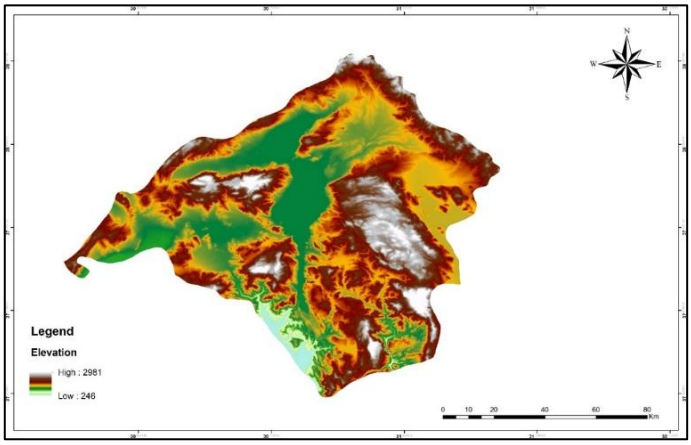


Figure 1. Map of study area

Located 11.5 km southwest of Isparta Province, Lake Gölcük is a crater lake situated at an elevation of 1,380 m above sea level. The lake's surroundings are characterized by hills rising 150–300 m above the lake's surface and covered with volcanic ash deposits. The lake is fed by springs supplied by precipitation and groundwater (Yavuz, 2010). The soil around the lake are mostly sandy loam and soils are characterized by low concentrations of. organic matter, total nitrogen, phosphorus, calcium, and salt (Gül et al., 2002; Yavuz, 2010). According to data from the Isparta Meteorological Station for the year 2026, the average annual temperature in the study area is 12.5 °C, and the average annual precipitation is 526.3 mm (TSMS, 2026). The vegetation surrounding the lake consists mainly of planted stands of Calabrian pine (*P. brutia*), Scots pine (*P. sylvestris*), Black locust (*Robinia pseudoacacia*), and Taurus cedar (*Cedrus libani*) trees, which were planted as part of afforestation programs (Osmanoğlu and Özdemir, 2014).

2.2 Methods

Needle samples were collected from Scots pine (*P. sylvestris* L.) trees aged 5, 25, and 70 years. For each age group, two healthy trees were randomly selected, and tree ages were determined based on the records of the field management. A total of 100 g of healthy needles were collected from each tree. Sampling was conducted in February, at midday, at the beginning of the vegetation period. To minimize the effect of environmental variability, samples were collected from the north side of the trees. Immediately after collection, the samples were placed in insulated containers containing ice packs and transported to the laboratory.

2.3 Volatile compound analysis

Volatile compounds were isolated by hydrodistillation according to the method described by Yılmazer et al., 2016. 5 g of needle material was mixed with distilled water at a 1:5 (weight) ratio in a 1-L round-bottom flask and subjected to hydrodistillation for 1 hour using a Clevenger-type apparatus (Yılmazer et al., 2016; Tekeş et al., 2024). The collected volatile compounds were gathered via a cooling system, dried over anhydrous sodium sulfate, filtered, and then stored in test tubes at -20 °C until analysis. GC–MS analysis was performed using a Shimadzu GC-2010 coupled with an MS-QP2010 Plus mass spectrometer and an Rxi-5Sil MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a linear velocity of 44.2 cm/s, and 1 µL samples were injected in split mode (10:1). The oven temperature was programmed from 40 °C (2 min) to 250 °C at 4 °C min^{-1} and held for 5 min. Electron ionization (EI) was used, and mass spectra were recorded over an m/z range of 35–450. The identification of the volatile compounds was performed using the Flavor 2, NIST05, and Wiley7n mass spectrum libraries. The relative abundance (%) of each volatile compound was calculated from the corresponding peak area and expressed as a percentage of the total chromatographic peak area.

2.4 Phenolic compound analysis

Phenolic compounds were determined from dried needle samples extracted with an acetonitrile:methanol acid (50:49.5:0.5, v/v/v) solvent mixture. Following ultrasonic extraction, filtration, and solvent evaporation under reduced pressure, the extracts were re-dissolved in methanol and analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC). The chromatographic system consisted of an SCL-10Avp system controller, SIL-10ADvp autosampler, LC-10ADvp pump, DGU-14A degasser, CTO-10Avp column oven, and a diode-array detector (DAD; Shimadzu Corporation, Kyoto, Japan), with detection performed at 278 nm. (El-Sherbiny et al., 2024; Nacakcı, 2026).

2.5 Statistics

All data are presented as mean ± standard deviation (SD) based on biological replicates. Before conducting statistical analyses, the assumptions of normality and homogeneity of variance were tested. Differences among age groups were assessed using one-way analysis of variance (ANOVA). When significant differences were detected, Tukey's Honestly Significant Difference (HSD) test was applied for pairwise comparisons among groups. Statistical significance was determined at the 5% probability level ($p < 0.05$). Means sharing different letters were considered significantly different. Principal Component Analysis (PCA) was performed to determine the relationships between tree age and volatile compounds. All analyses were conducted in RStudio using the FactoMineR and ggplot2 packages (Lee et al., 2008; Wilkinson, 2011; Tekeş et al., 2025; R Core Team, 2026).

3. Results and Discussion

Within the framework of this study, the volatile compound profiles of needle samples collected from Scots pine trees of different age groups were determined, and letter groups were formed based on the Tukey HSD test; the results are presented in Table 1 as mean ± standard deviation. Additionally, Principal Component Analysis (PCA) was performed to evaluate the variability in volatile compounds among age groups. The PCA results showed that the first two principal components explained 99.55% of the total variance in the dataset. Figure 2, it was observed that samples from different age groups were distributed at different positions along the principal components. This distribution indicates that there are changes in the composition of volatile compounds among the age groups. When the positions of the age groups on the principal components were examined, it was observed that nearly all groups were highly correlated with the PC1 axis (Table 2).

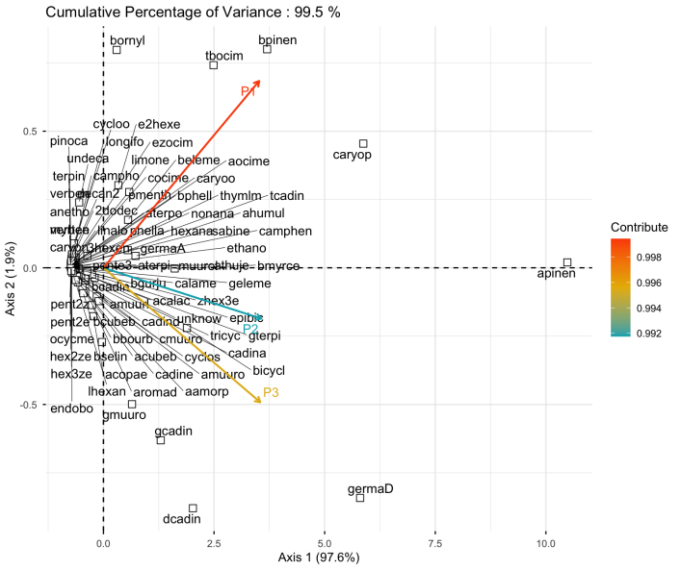


Figure 2. Multidimensional view of needle volatile compounds, and age groups relationships using PCA biplot

Table 2. Age groups and principal component axes of needle volatile compounds and pearson correlation axes

Variables	PC1	PC2	PC3
P1	0.981	0.191	0.024
P2	0.995	-0.052	-0.091
P3	0.988	-0.137	0.068

Table 1. Volatile compounds and relative contents of compounds in *P. sylvestris* needles

No	Compounds	Code	RI	Relative Content (%)		
				P1	P2	P3
1	Pent-2(E)-enal	pent2e	748	0.01±0.01a	0.04±0.00a	0.00±0.00a
2	Pent-2(Z)-enol	pent2z	765	0.01±0.00a	0.04±0.00a	0.00±0.00a
3	3-hexenal	3hexen	797	0.11±0.04a	0.16±0.15a	0.04±0.02a
4	Hexanal	hexana	800	0.16±0.06a	0.09±0.07a	0.15±0.06a
5	(E)-2-hexenal	e2hexe	850	2.49±0.71a	1.78±1.45a	1.17±0.48a
6	(Z)-Hex-3-en-1-ol	zhex3e	853	0.61±0.37a	0.58±0.27a	0.69±0.28a
7	Hex-2(Z)-enol	hex2ze	864	0.00±0.00a	0.04±0.00a	0.00±0.00a
8	1-hexanol	lhexan	868	0.00±0.00a	0.18±0.00a	0.04±0.02a
9	Tricyclene	tricyc	919	0.52±0.08a	0.37±0.10a	0.76±0.08a
10	α- thujene	athuje	923	0.04±0.00ab	0.03±0.00b	0.06±0.01a
11	α-pinene	apinen	935	18.95±2.77a	19.23±2.06a	18.18±1.89a
12	Camphene	camphen	945	2.81±0.36a	1.60±0.68a	2.99±0.58a
13	Verbenene	verbee	947	0.03±0.00a	0.00±0.00a	0.00±0.00a
14	Sabinene	sabine	970	0.17±0.02a	0.08±0.04a	0.17±0.08a
15	β- pinene	bpinen	976	9.59±2.64a	6.28±4.66a	6.53±0.16a
16	β- myrcene	bmyrce	990	4.12±0.41a	3.41±1.04a	4.30±0.59a
17	l-phellandrene	phella	1004	0.09±0.01a	0.06±0.02a	0.06±0.01a
18	Hex-3(Z)-enyl acetate	hex3ze	1005	0.00±0.00b	0.00±0.00b	0.08±0.01a
19	α- terpinene	aterpi	1015	0.04±0.00a	0.03±0.00b	0.05±0.00a
20	o-cymene	ocycme	1023	0.02±0.01a	0.02±0.00a	0.02±0.00a
21	Limonene	limone	1028	2.80±1.01a	2.37±0.45a	1.49±0.23a
22	β- phellandrene	bphell	1029	0.74±0.24a	0.59±0.14a	0.57±0.02a
23	cis-ocimene	cocime	1035	0.29±0.13a	0.13±0.01a	0.17±0.01a
24	trans-β-ocimene	tbocim	1048	7.02±0.97a	5.66±1.38a	3.58±1.73a
25	γ- terpinene	gterpi	1057	0.05±0.00b	0.04±0.01b	0.11±0.00a
26	α- terpinolene	aterpo	1084	0.22±0.04a	0.16±0.06a	0.16±0.01a
27	Linalool	linalo	1100	0.06±0.04a	0.01±0.01a	0.01±0.00a
28	Nonanal	nonana	1103	0.04±0.01a	0.03±0.01a	0.02±0.00a
29	Campholenal	campho	1124	0.11±0.00a	0.00±0.00a	0.00±0.00a
30	Allo-ocimene	aocime	1127	0.05±0.01a	0.03±0.01a	0.02±0.00a
31	p-Mentha-1,5,8-triene	pmenth	1129	0.13±0.03a	0.08±0.03a	0.05±0.02a
32	(4E,6Z)-allo-ocimene	ezocim	1139	0.22±0.10a	0.14±0.03a	0.07±0.02a
33	Pinocarvone	pinoca	1159	0.06±0.00a	0.00±0.00a	0.00±0.00a
34	Decan-2-one	decan2	1160	0.17±0.00a	0.00±0.00a	0.00±0.00a
35	endo-borneol	endobo	1170	0.02±0.01a	0.06±0.00a	0.02±0.00a
36	Myrtenal	myrten	1192	0.05±0.00a	0.00±0.00a	0.00±0.00a
37	Terpineol <α->	terpin	1193	0.06±0.00a	0.00±0.00a	0.01±0.00a
38	Verbenone	verben	1205	0.05±0.00a	0.00±0.00a	0.00±0.00a
39	Thymyl methyl ether	thymlm	1228	0.06±0.01a	0.03±0.02a	0.04±0.00a
40	Carvone	carvon	1242	0.01±0.00a	0.00±0.00a	0.00±0.00a
41	Cyclooctyl acetate	cycloo	1276	0.35±0.00a	0.00±0.00a	0.04±0.00a
42	Bornyl acetate	bornyl	1282	3.50±3.47a	1.74±1.67a	0.04±0.00a
43	Anethole	anetho	1286	0.05±0.00a	0.00±0.00a	0.00±0.00a
44	Undecan-2-one	undeca	1291	0.93±0.00a	0.01±0.00a	0.06±0.00a
45	γ-elemene	geleme	1331	0.28±0.05a	0.36±0.07a	0.27±0.00a
46	α- cubebene	acubeb	1346	0.25±0.03a	0.58±0.28a	0.50±0.10a
47	2-dodecanone	2dodec	1363	0.08±0.00a	0.00±0.00a	0.00±0.00a

RI: Retention index, P1: 5-year-old, P2: 25-year-old, P3: 70-year-old

Table 1. Continued

No	Compounds	Code	RI	Relative Content (%)		
				P1	P2	P3
48	Cyclosativene	cyclos	1369	0.07±0.02a	0.26±0.15a	0.24±0.06a
49	α- copaene	acopae	1375	0.39±0.05a	1.16±0.66a	1.00±0.17a
50	β- bourbonene	bbourb	1383	0.35±0.03a	0.57±0.22a	0.71±0.26a
51	β- elemene	beleme	1389	2.61±0.91a	2.06±0.34a	1.87±0.02a
52	Longifolene	longifo	1407	0.15±0.00a	0.00±0.00a	0.00±0.00a
53	Caryophyllene	caryop	1423	12.56±1.01a	9.78±0.77a	10.96±0.61a
54	β- cubebene	bcubeb	1430	0.25±0.01a	0.57±0.22a	0.61±0.13a
55	β-gurjunene	bgurju	1434	0.15±0.01a	0.17±0.02a	0.17±0.02a
56	Aromadendrene	aromad	1439	0.42±0.06a	1.14±0.43a	0.84±0.04a
57	β- cadinene	bcadin	1445	0.33±0.04a	0.41±0.08a	0.45±0.06a
58	cis-muurola-3,5-diene	cmuuro	1449	0.16±0.03a	0.25±0.10a	0.31±0.04a
59	α-humulene	ahumul	1459	2.38±0.28a	1.96±0.18a	2.16±0.09a
60	(+)-Epi-bicyclosesquiphellandrene	epibic	1461	0.75±0.03a	0.83±0.12a	0.84±0.09a
61	Cadina-1(6),4-diene <10βH->	cadina	1472	0.20±0.03a	0.37±0.13a	0.31±0.02a
62	γ- muurolene	gmuuro	1478	1.16±0.04a	2.66±1.24a	3.10±0.86a
63	Germacrene-D	germaD	1485	9.40±1.03a	10.39±0.47a	13.03±2.01a
64	β-selinene	bselin	1490	0.52±0.03a	1.46±0.65a	1.54±0.27a
65	α-amorphene	aamorp	1493	0.77±0.04a	1.17±0.38a	1.23±0.22a
66	Bicyclogermacrene	bicycl	1498	3.78±0.78a	5.08±0.30a	4.30±0.21a
67	α-muurolene	amuuro	1499	0.72±0.09a	1.16±0.61a	1.08±0.20a
68	Cadinene	cadine	1504	0.15±0.03a	0.29±0.07a	0.38±0.08a
69	Germacrene A	germaA	1508	0.08±0.03a	0.08±0.00a	0.06±0.00a
70	γ- cadinene	gcadin	1515	1.85±0.58a	4.16±1.96a	4.18±0.63a
71	δ- cadinene	dcadin	1519	2.49±0.81a	5.58±2.72a	5.71±0.90a
72	Calamenene	calame	1521	0.18±0.02a	0.20±0.05a	0.20±0.05a
73	Cadina-1,4-diene	cadind	1533	0.16±0.02a	0.26±0.08a	0.30±0.07a
74	α-muurolene-(-)	amuurl	1537	0.45±0.08a	0.61±0.14a	0.63±0.09a
75	α-calacorene	acalac	1541	0.08±0.00a	0.13±0.05a	0.12±0.02a
76	Unknown	unknow	1576	0.82±0.57a	0.95±0.16a	0.95±0.04a
77	Caryophyllene oxide	caryoo	1578	0.04±0.00a	0.04±0.00a	0.00±0.00a
78	Muurolol <α-,epi->	muurol	1643	0.18±0.00a	0.20±0.08a	0.18±0.02a
79	tau- cadinol	tcadin	1656	0.11±0.00a	0.10±0.03a	0.08±0.02a
80	Penten-3-one	pente3	<700	0.06±0.02a	0.13±0.10a	0.03±0.00a
81	Ethanol	ethano	<700	0.04±0.00a	0.03±0.00a	0.04±0.01a

RI: Retention index, P1: 5-year-old, P2: 25-year-old, P3: 70-year-old

To examine differences among age groups, a PCA was performed using the major compounds that accounted for more than 5% of the total compound. The Pearson correlation coefficients are presented in Table 3, while the positions of all these variables across the two principal components are illustrated in Fig 3.

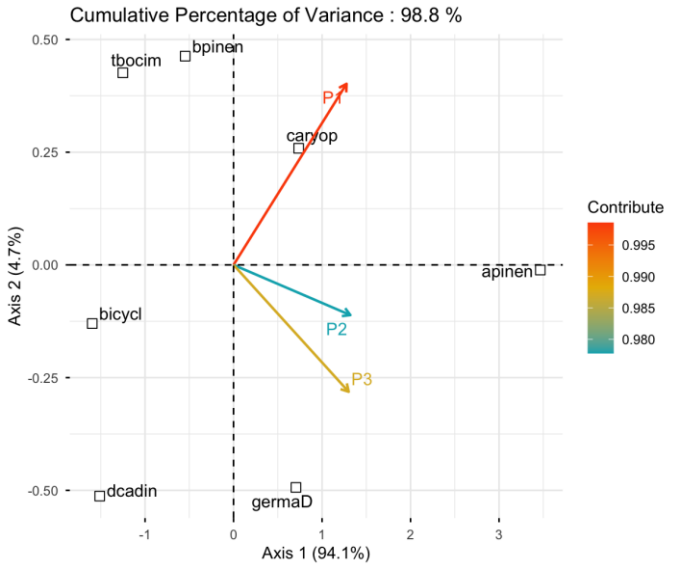


Figure 3. Multidimensional view of needle major (5%) volatile compounds, and age groups relationships using PCA biplot

When the PCA biplot was examined, it was observed that the samples were distributed at different positions along the principal components. While sample P1 was in the positive direction of the PC2 axis, samples P2 and P3 were positioned in the negative direction. The age variable examined in Table 3 showed a high positive value (0.904) on the PC1 axis. It was determined that the compounds δ-cadinene (0.965), germacrene D (0.887), and bicyclogermacrene (0.590) which were aligned in the same direction as age tended to increase with increasing age. In contrast, β-pinene (-0.933), trans-β-ocimene (-0.942), caryophyllene oxide (-0.740), and α-pinene (-0.535) showed a negative correlation with age.

Table 3. Pearson correlation coefficients between major (5%) volatile compounds, and the principal component axes

Variables	PC1	PC2
apinen	-0.535	0.845
germaD	0.887	-0.461
caryop	-0.74	-0.673
bpinen	-0.933	-0.361
dcadin	0.965	0.262
bicycl	0.59	0.807
tbocim	-0.942	0.336
age	0.904	-0.427

The phenolic composition of *P. sylvestris* needles showed differences among the three age groups (Table 4). Among the investigated phenolic compounds, catechin was the predominant compound and was detected in all age groups. Its mean concentration was 478.1 ± 49.8 µg/g in P1, 658.05 ± 72.75 µg/g in P2, and 748.8 ± 369 µg/g in P3. Although catechin showed a numerical increase with tree age, the differences among age groups were not statistically significant ($p > 0.05$).

Among the flavonols, kaempferol was detected in P1 and P3 at concentrations of 24.6 ± 1.1 and 33.1 ± 3.9 µg/g, respectively, whereas it was not detected in P2. There wasn't significant difference was observed between P1 and P3 for kaempferol. Quercetin was not detected in any of the age groups. Among the flavan-3-ols, epicatechin was detected only in P2 (30.75 ± 6.15 µg/g), while catechin occurred in all three groups. Regarding phenolic acids, p-coumaric acid was detected exclusively in P2 (6.9 ± 0.1 µg/g), with a significant difference between P2 and the other age groups. Overall, catechin was consistently present across all ages, whereas epicatechin, p-coumaric acid, and kaempferol showed age-group-specific occurrence patterns.

Table 4. Phenolic compounds of needles from *P. sylvestris*

Flavonoid compounds (µg/g)	P1	P2	P3
Flavonols			
Quercetin	0.0±0.0	0.0±0.0	0.0±0.0
Kaempferol	24.6±1.1a	0.0±0.0b	33.1±3.9a
Flavan-3-ols			
Epicatechin	0.0±0.0a	30.75±6.15a	0.0±0.0a
Catechin	478.1±49.8a	658.05±72.75a	748.8±369a
Phenolic acids			
p-coumaric acid	0.0±0.0b	6.9±0.1a	0.0±0.0b

Plant secondary metabolite composition is influenced by a combination of genetic, environmental, and developmental factors (Charrier et al., 2015; Gülsoy et al., 2013, 2014, 2017; Viehweger, 2014; Öğüt et al., 2026). In the present study, α-pinene was the predominant volatile compound in *P. sylvestris* needles across all age groups, while monoterpene hydrocarbons constituted the major chemical class. The predominance of monoterpenes and α-pinene is in agreement with previous reports on pine species and *P. sylvestris* (Kubeczka and Schultze, 1987; Leung and Foster, 1996; Lau, 1998; Venskutonis et al., 2000; Lawrence, 2003; Naydenov et al., 2005; Rinne et al., 2000; Judzentiene and Kupcinskiene, 2008). Nevertheless, variation in the relative abundance of individual volatile compounds has been reported among studies. Such differences may be associated with genetic background, geographical origin, environmental conditions, developmental stage, sampling conditions, and analytical procedures, all of which can influence the volatile profile of conifer needles.

In the present study, the volatile compound profile of Scots pine needles varied among age groups. A total of 81 volatile compounds were identified, with α-pinene, caryophyllene, germacrene D, and β-pinene as the major constituents. Monoterpene hydrocarbons constituted the predominant chemical class across all age groups, consistent with previous reports on the volatile composition of pine species (Kubeczka and Schultze, 1987; Leung and Foster, 1996; Lau, 1998; Venskutonis et al., 2000; Lawrence, 2003; Naydenov et al., 2005). Among the individual compounds, α-pinene was predominant in all age groups, although the relative abundance of the other major compounds varied with age. The predominance of α-pinene has also been reported in previous studies on *P. sylvestris* (Rinne et al., 2000; Judzentiene and Kupcinskiene, 2008). Nevertheless, differences in the relative

abundance of α-pinene among studies may be related to geographical and climatic conditions, genetic variation, developmental stage, sampling conditions, and differences in extraction and analytical methods. Therefore, variation among previous reports and the present study should not be attributed to tree age alone.

Age-related variation was observed in the relative abundance of volatile compounds, with some compounds increasing and others decreasing with tree age. In particular, several sesquiterpenes, including germacrene D, γ-muurolene, and cadinene derivatives, tended to increase with age. Previous studies on *P. sylvestris* have also reported germacrene D, γ-cadinene, and δ-cadinene among the major sesquiterpene components (Venskutonis et al., 2000; Judzentiene and Kupcinskiene, 2008). These findings suggest that the relative contribution of sesquiterpenes to the volatile profile of Scots pine needles may change with tree age. The relatively high abundance of germacrene D observed in the present study may reflect the combined effects of tree age, site conditions, and genetic variation. Environmental factors such as temperature, water availability, altitude, and soil properties may influence carbon allocation and secondary metabolite production, potentially altering the balance between monoterpenes and sesquiterpenes (El-Sawi et al., 2007; Hojjati et al., 2009). In addition, differences in sampling and analytical procedures among studies should be considered when comparing terpene profiles.

PCA further supported the age-related variation in the volatile compound profiles of Scots pine needles. The contrasting associations of germacrene D and δ-cadinene with β-pinene and trans-β-ocimene suggest that different volatile compounds may exhibit distinct patterns during tree development. Overall, the multivariate analysis supports the contribution of tree age to the variation in volatile composition. The chemical composition of volatile compounds is influenced by genetic factors, environmental conditions, plant organ, developmental stage, and analytical methods (El-Sawi et al., 2007; Hojjati et al., 2009; Nacakcı, 2025; Öğüt et al., 2026). Since the samples in this study were collected from the same location under similar environmental conditions, environmental variability was partially controlled, suggesting that tree age may have contributed to the observed chemical differences. The age-related increase in certain sesquiterpenes and decrease in some monoterpenes further suggest that the volatile composition of Scots pine needles may vary during tree development.

Phenolic compounds, including flavanols and flavonoids, have been widely investigated in coniferous species (Saleem et al., 2003; Slimestad, 2003; Naeem et al., 2010; Karapandzova et al., 2015; Kim et al., 2018; Nacakcı, 2026). In pine species, catechin, epicatechin, quercetin, and taxifolin have been reported as common phenolic metabolites, and phenolic profiles have been characterized in different plant parts, including bark, needles, and cones (Saleem et al., 2003; Slimestad, 2003; Yesil-Celiktas et al., 2009; Naeem et al., 2010; Karapandzova et al., 2015; Kim et al., 2018; Ramos et al., 2022; Szmechtyk and Małecka, 2024).

Previous studies have demonstrated considerable variation in the phenolic composition of *Pinus* species. Karapandzova et al. (2015) identified kaempferol glycosides and quercetin rutinoside (rutin) in several *Pinus* species, including *P. sylvestris*, and reported p-coumaric acid in *P. nigra*. Similarly, Naeem et al. (2010) reported several flavonols, including quercetin and kaempferol, in *P. wallichiana* and *P. roxburghii*, while Slimestad (2003) identified kaempferol- and isorhamnetin-derived flavonols in *P. cembra*. These findings indicate that phenolic profiles may differ among *Pinus* species and provide a useful basis for comparison with the age-related patterns observed in the present study.

In the present study, quercetin was not detected in any age group, whereas previous studies have reported quercetin mainly in glycosylated forms, such as quercetin rutinoside (rutin) (Slimestad, 2003; Karapandzova et al., 2015). This difference may be related to the chemical form of quercetin detected, suggesting that quercetin in Scots pine needles may occur predominantly as glycoside derivatives rather than in its free form.

Epicatechin and p-coumaric acid were detected only in 25-year-old individuals, while they could not be detected in 5- and 70-year-old individuals. Although the literature reports the presence of these compounds in various pine species, no detailed information is provided regarding their age-dependent distribution. Therefore, the results suggest that the accumulation of epicatechin and p-coumaric acid may vary with age; however, they also indicate that more comprehensive studies encompassing different age groups are needed to establish this relationship.

Catechin, on the other hand, was detected in all age groups and was found particularly at high concentrations. This result is consistent with previous studies reporting that catechin is one of the predominant phenolic compounds in pine species (Saleem et al., 2003; Kim et al., 2018).

From an applied perspective, the age-related differences observed in the chemical profiles of *P. sylvestris* needles may be relevant to the selection of plant material for different industrial purposes. Younger trees showed relatively higher proportions of some monoterpenes, whereas older trees were characterized by higher levels of several sesquiterpenes. The 25-year-old group was particularly notable for the occurrence of epicatechin and p-coumaric acid, while the 70-year-old group showed the highest numerical catechin content. These differences suggest that the most suitable age class may depend on the target compound and intended application rather than on a single optimal age. Therefore, younger or intermediate-aged trees may be of interest when monoterpene-rich aromatic profiles or specific phenolic compounds are targeted, whereas older trees may be considered when sesquiterpene-rich profiles are desired. However, since essential oil yield and biological activities were not directly evaluated, the suitability of particular age groups for pharmaceutical, cosmetic, or aromatic applications should be confirmed by further bioactivity and yield studies.

A limitation of the present study is the small number of biological replicates (n = 2) within each age group. Although the observed patterns provide preliminary evidence of age-related variation in the volatile and phenolic profiles of *P. sylvestris* needles, the limited sample size reduces the statistical power and may restrict the generalization of the ANOVA and Tukey HSD results. Therefore, the statistical differences and age-related trends observed in this study should be interpreted with caution. Future studies with larger sample sizes and a broader

representation of individuals within each age group are needed to confirm these patterns.

3. Conclusions

The results demonstrated age-related variation in the volatile and phenolic profiles of *P. sylvestris* needles. Among the major volatile compounds, germacrene D and δ-cadinene showed an increasing tendency with age, whereas α-pinene, β-pinene, and trans-β-ocimene were negatively associated with age. PCA further supported the differentiation of volatile profiles among the age groups, indicating that tree age contributes to chemical variation in Scots pine needles. The phenolic profile also varied among age groups, with epicatechin and p-coumaric acid detected only in 25-year-old trees and catechin present in all age groups.

From an applied perspective, the most suitable age class may depend on the target compounds and intended use. Younger or intermediate-aged trees may be more relevant when monoterpene-rich profiles or specific phenolic compounds are targeted, whereas older trees may be of interest for sesquiterpene-rich profiles. However, further studies on essential oil yield and biological activity are required before specific age groups can be recommended for industrial, pharmaceutical, cosmetic, or aromatic applications.

This study provides evidence that tree age is an important source of chemical variation in *P. sylvestris* needles and demonstrates age-related differentiation of volatile profiles using both compound-level analyses and PCA. Future studies should include a wider range of age classes, larger sample sizes, different seasons, and populations from different ecological regions to better distinguish age-related effects from environmental and individual variation.

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