A Comparison of Brutian Pine (*Pinus brutia* Ten.) Root Volatile Compounds *vs.* the Stem Wood

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Recently, tree stumps have attracted attention as a source of woody biomass. This study investigated the practicability of brutian pine (*Pinus brutia* Ten.) root as an alternative raw material in the essential oil industry. The composition of volatile compounds of brutian pine root was investigated and compared with that of brutian pine stem wood. In the wood sample, the major volatile compound was α-pinene (32.69%) and in the root sample it was β-caryophyllene (25.41%). Terpenes (α-pinene, β-pinene, Δ^3-carene, β-caryophyllene, and α-humulene) constituted the major compounds in the wood sample, whereas oxygen-containing components (linalool and (E)-anethole) were the main compounds in the root sample, in addition to terpenes (limonene, β-caryophyllene, and α-humulene). Monoterpenes, sesquiterpenes, terpene oxides, alcohols, aldehydes, ethers, esters, and ketones were identified as the main groups of volatile compounds in the wood and root samples of brutian pine. Except for monoterpenes, the amounts of the other main groups of volatile compounds were higher in the root sample than in the wood sample. The variation and number of detected volatile compounds indicate that the root of brutian pine might be a useful raw material for some industrial processes that use these volatile compounds in their production.

Keywords: Wood root; Volatile compounds; α-Pinene; β-Caryophyllene

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INTRODUCTION

Human social, technological, and economic development depend primarily on the efficient use of existing resources. Human beings have used wood-based products as important resources since ancient times. Due to the rapid growth in both the world population and the process of industrialization, the demand for wood products has increased in recent decades. However, a growing environmental consciousness, an unprecedented consumption of forest resources, and global warming have pushed mankind to adopt alternative wood resources (Yasar *et al*. 2010).

Many countries in Europe have plans to increase the use of sustainable energy sources to meet their energy demands. For countries with forests, woody products can provide significant amounts of bioenergy by the harvest of logging residues after logging operations have ceased. Most tree parts excluding stumps are widely used for bioenergy. The tree stumps left behind after harvesting have generally been ignored as a source of woody biomass until the last decade, but the recent need for more renewable energy has increased interest in the exploitation of this fairly large source of energy. Removing stumps can be a problem for the soil, and it can cause erosion if it is done in the wrong way. The slope should be less than 40%. Also, industrial plantation plans can minimize negative aspects of removal of roots. Therefore, countries have adopted new management plans to harvest tree stumps for bioenergy purposes (Walmsley and Godbold 2009; Persson 2012; Uri *et al*. 2015).

In particular, Brutian pine (*Pinus brutia* Ten.) is a species used to obtain raw material for wood and non-wood industries in Turkey (Deniz 2013; Oz *et al*. 2015; Ozdemir and Ucar 2016). It is distributed widely around the Mediterranean, Aegean, and Marmara regions, and in some localities of the western Black Sea region, for a total area of approximately 5.8 million ha in Turkey (General Directorate of Forestry in Turkey 2016). Therefore, besides wood, stumps of brutian pine may attract attention as an alternative source for raw materials due to their high numbers in some industrial areas. In many European countries, wood stumps and roots are uprooted for use in the production of bioenergy (Walmsley and Godbold 2009), but they may also hold potential for utilization as raw material for valuable industrial products.

α-Pinene and β-pinene, which are the main volatile compounds in pine woods, are commonly used as starting materials in the preparation of more valuable components. In addition to pinenes, there are other terpenes such as camphene, Δ^3-carene, tricyclene, myrcene, pcymene, limonene, β-caryophyllene, and aromadendrene in the pine volatiles. (McMorn *et al*. 2000; Rezzi *et al*. 2005; Wang *et al*. 2006; Limberger *et al*. 2007; Mercier *et al*. 2009). Such compounds are used as raw materials for the manufacture of pharmaceuticals, repellents, solvents, plasticizers, insecticides, food additives, and antiviral, antimicrobial, and antioxidant constituents (Sun 2007; Burdock and Carabin 2008; Mayekiso *et al*. 2008; Behr and Johnen 2009; Zulak and Bohlmann 2010; Adams *et al*. 2011; Teshome 2011; Back *et al*. 2012; Rudback *et al*. 2012; Gillette *et al*. 2012; Limberger *et al*. 2012). Previous works have shown that conifer species can be significant sources of volatile compounds (Ludley *et al.* 2009; Kivimäenpää *et al.* 2012; Semiz *et al.* 2017).

In this study, the volatile compounds of brutian pine roots were investigated. To differentiate between these compounds, the composition of roots and those of wood from the stems were compared, and the main groups of volatile compounds were identified.

EXPERIMENTAL

Materials

Stem wood (here called "wood") and root samples were taken from Aglasun-Burdur in Turkey (37°38'24.6" N, 30°31'30.5" E). The three felled trees were aged 40 years, 45 years, and 48 years. The heights and diameters of the trees were 22 m and 45 cm, 25 m and 47 cm, and 23 m and 51 cm, respectively. Discs were taken from breast-high felled trees with the thickness of 15 cm. After felling the wood, the tree stumps were uprooted, and the fresh disc and root materials were immediately transported in frozen bags to the laboratory. The diameter of root samples were 5 cm and more. After debarking and chipping, the wood discs and roots were separately milled to pass through 40- to 100-mesh in a SK 1 (Retsch, Haan, Germany) mill for solid-phase microextraction (SPME) and gas chromatography-mass spectroscopy (GC/MS) analysis.

Methods

SPME Adsorption

For SPME, the author used an apparatus with a fiber coated with a 75-μm-thick layer of carboxen/polydimethylsiloxane (CAR/PDMS). Then, the equivalent of 2.5 g each of wood and root sample were added to a 10-mL vial. The vial was then sealed using a silicone septum and crimp cap, and the sealed vial was conditioned for 30 min at 60 °C. The SPME fiber was then pushed through the headspace of the conditioned vial to adsorb the volatile compounds.

GC/MS analysis

The SPME fiber with the adsorbed volatile compounds was introduced directly into the injection port of a 2010 Plus GC-MS (Shimadzu, Kyoto, Japan). Then, chromatographic separation was performed using a Rx-5Sil MS (Restek Corporation, Bellefonte, PA, USA) capillary column (30 m × 0.25 mm i.d., 0.25-µm film thickness) coupled to a mass selective detector produced by the same company and operated in electron impact ionization mode (70 eV) using helium as the carrier gas at a flow rate at 1.61 mL/min. For injection and detection, a temperature of 250 °C was applied. The mass of the samples analyzed ranged between 35 m/z and 450 m/z. The temperature of the column was programmed to 40 °C for 2 min and then increased to 250 °C at a rate of 4 °C/min and maintained at 250 °C for 5 min.

Identification of compounds

The author calculated the retention indices of the determined volatile compounds by injecting a series of saturated n-alkanes using the same gas chromatographic conditions described above. The volatile compounds were identified by comparing their mass spectra to those of compounds listed in the Wiley, NIST, Tutor, and FFNSC libraries.

Statistical analysis

Statistical software (Minitab Inc., Version 16, State College, PA, USA) was used to perform an independent-sample t-test analysis on each variable to determine the statistical significance of the results.

RESULTS AND DISCUSSION

Table 1 lists the volatile compounds in the SPME extracts of the root and wood of the brutian pine, as detected using GC/MS. The root sample contained 46 volatile compounds, and the wood sample contained 41. Twenty-six of the volatile compounds were determined to be the same in both samples of brutian pine.

According to the results of the independent-samples t-test, there was a significant difference between the root and wood samples for 22 of the same volatile compounds ($p < 0.05$), but not α-terpinene, hexyl butanoate, α-copaene, or α-muurolene.

High-content volatile compounds in the wood sample included α-pinene, β-pinene, β-caryophyllene, Δ^3-carene, and α-humulene in the respective amounts of 32.69%, 16.91%, 8.72%, 7.79%, and 7.23% (Fig. 1). In the root sample, the distinctive volatile compounds were β-caryophyllene, limonene, linalool, (E)-anethole, and α-humulene in the respective amounts of 25.41%, 15.99%, 10.17%, 9%, and 5.82% (Fig. 2).

In the brutian pine wood sample, α-pinene, β-pinene, β-caryophyllene, Δ^3-carene, and α-humulene represented 73.34% of the total quantity of volatile compounds. These are known to be the main components of the turpentine oil of oleoresin obtained from brutian pine (Oz *et al*. 2015; Yasar *et al*. 2018).

Table 1. Volatile Compounds of Root and Wood Samples from Brutian Pine

No	RI^1	Compound	Main Group	Wood (%)^2	Root (%)	*t*-test Results		
						F	*t*	*p*
1	869	*n*-Hexanol	Alcohol	-	0.30 (0.03)	-	-	-
2	902	*n*-Heptanal	Adehyde	0.50 (0.01)	-	-	-	-
3	946	*α*-Pinene	Monotertepene	32.69 (0.48)	2.19 (0.17)	1.482	103.743	<0.0001
4	949	Camphene	Monotertepene	0.63 (0.02)	-	-	-	-
5	956	Benzaldehyde	Adehyde	-	0.75 (0.04)	-	-	-
6	968	1-Heptanol	Alcohol	-	0.39 (0.07)	-	-	-
7	973	Sabinene	Monotertepene	0.18 (0.03)	-	-	-	-
8	980	*β*-Pinene	Monotertepene	16.91 (0.37)	-	-	-	-
9	989	6-Methyl-5-hepten-2-one	Ketone	-	0.27 (0.03)	-	-	-
10	992	*β*-Myrcene	Monotertepene	1.20 (0.09)	0.97 (0.06)	0.307	3.682	0.0211
11	1010	Δ^3-Carene	Monotertepene	7.79 (0.28)	0.25 (0.05)	2.615	45.915	<0.0001
12	1013	*α*-Phellandrene	Monotertepene	0.07 (0.02)	0.24 (0.04)	0.800	-6.584	0.0027
13	1020	*α*-Terpinene	Monotertepene	0.27 (0.04)	0.27 (0.02)	0.800	0.000	1.0000
14	1025	*p*-Cymene	Monotertepene	0.74 (0.05)	2.00 (0.11)	0.986	-18.061	<0.0001
15	1027	Limonene	Monotertepene	6.39 (0.41)	15.99 (0.23)	0.586	-35.370	<0.0001
16	1031	1,8-Cineole	Terpene oxide	0.36 (0.12)	1.82 (0.08)	0.307	-17.534	<0.0001
17	1060	*γ*-Terpinene	Monotertepene	0.26 (0.04)	0.48 (0.05)	0.976	-5.951	0.0040
18	1074	1-Octanol	Alcohol	-	0.16 (0.03)	-	-	-
19	1088	*α*-Terpinolene	Monotertepene	1.07 (0.04)	0.15 (0.02)	0.800	35.631	<0.0001
20	109	trans-Sabinene	Monotertepene	0.15 (0.04)	-	-	-	-
21	1101	Linalool	Alcohol	0.87 (0.06)	10.17 (0.35)	2.667	-45.631	<0.0001
22	1106	Nonanal	Adehyde	0.12 (0.01)	-	-	-	-
23	1109	Nonanol	Alcohol	0.16 (0.06)	0.31 (0.05)	0.065	-3.326	0.0292
24	1111	*α*-Thujone	Ketone	-	0.37 (0.02)	-	-	-
25	1139	trans-Limonene oxide	Terpene oxide	-	0.16 (0.03)	-	-	-
26	1150	Camphor	Ketone	-	0.20 (0.02)	-	-	-
27	1159	Isomenthone	Ketone	-	0.37 (0.05)	-	-	-
28	1172	Neomenthol acetate	Ester	-	0.48 (0.03)	-	-	-
29	1182	4-Terpineol	Alcohol	-	0.39 (0.03)	-	-	-
30	1189	*α*-Terpineol	Alcohol	0.13 (0.04)	0.56 (0.09)	1.030	-7.562	0.0016
31	1191	Hexyl butanoate	Ester	0.20 (0.05)	0.60 (0.30)	2.702	-2.277	0.0850
32	1199	*p*-Allylanisole	Ether	1.17 (0.08)	5.58 (0.36)	2.305	-20.712	<0.0001
33	1236	Cuminic aldehyde	Adehyde	0.12 (0.02)	-	-	-	-
34	1207	(Z)-carvone	Ketone	-	0.87 (0.06)	-	-	-
35	1231	2-Methylbutanoate	Ester	-	0.33 (0.04)	-	-	-
36	1242	D-carvone	Ketone	0.28 (0.05)	2.81 (0.27)	2.568	-15.959	<0.0001
37	1252	Linalyl acetate	Ester	0.13 (0.03)	1.20 (0.04)	0.160	-37.066	<0.0001

38	1289	(E)-anethole	Ether	1.15 (0.1)	9.00 (0.23)	1.074	-54.213	<0.0001
39	1291	Menthyl acetate	Ester	-	0.22 (0.02)	-	-	-
40	1293	Pentyl hydroxy(phenyl) acetate	Ester	0.17 (0.06)	-	-	-	-
42	1329	*α*-Longipinene	Sesquiterpene	1.32 (0.07)	0.89 (0.22)	1.688	3.226	0.0321
43	1345	Ylangene	Sesquiterpene	0.19 (0.04)	-	-	-	-
44	1351	*α*-Terpinenyl acetate	Ester	-	0.57 (0.06)	-	-	-
45	1355	(+)-Longicyclene	Sesquiterpene	0.30 (0.07)	0.15 (0.04)	0.553	3.222	0.0322
46	1380	*α*-Copaene	Sesquiterpene	0.38 (0.08)	0.34 (0.04)	0.800	0.775	0.4818
47	1382	*β*-Bourbonene	Sesquiterpene	-	0.19 (0.05)	-	-	-
48	1387	Hexyl hexanoate	Ester	-	0.30 (0.03)	-	-	-
49	1396	(+)-Sativen	Sesquiterpene	0.20 (0.02)	-	-	-	-
50	1415	Junipene	Sesquiterpene	5.78 (0.31)	4.56 (0.23)	0.172	5.474	0.0054
51	1418	*β*-Caryophyllene	Sesquiterpene	8.72 (0.41)	25.41 (0.27)	0.325	-58.885	<0.0001
52	1435	*α*-Bergamotene	Sesquiterpene	-	0.16 (0.01)	-	-	-
53	1439	Aromadendrene	Sesquiterpene	0.55 (0.08)	-	-	-	-
54	1452	*α*-Humulene	Sesquiterpene	7.23 (0.30)	5.82 (0.49)	0.437	4.251	0.0131
55	1456	(E)-*β*-farnesene	Sesquiterpene	-	0.40 (0.03)	-	-	-
56	1480	Germacrene D	Sesquiterpene	0.28 (0.04)	-	-	-	-
57	1499	*α*-Muurolene	Sesquiterpene	0.33 (0.04)	0.27(0.04)	0.000	1.837	0.1401
58	1506	(E,E)-*α*-farnesene	Sesquiterpene	-	0.88 (0.14)	-	-	-
59	1508	*β*-Bisabolene	Sesquiterpene	0.10 (0.02)	-	-	-	-
60	1518	*δ*-Cadinene	Sesquiterpene	0.14 (0.03)	-	-	-	-
61	1520	*γ*-Cadinene	Sesquiterpene	0.10 (0.01)	-	-	-	-
62	1581	Caryophyllene oxide	Terpene oxide	0.67 (0.10)	0.21 (0.08)	0.976	6.221	0.0034

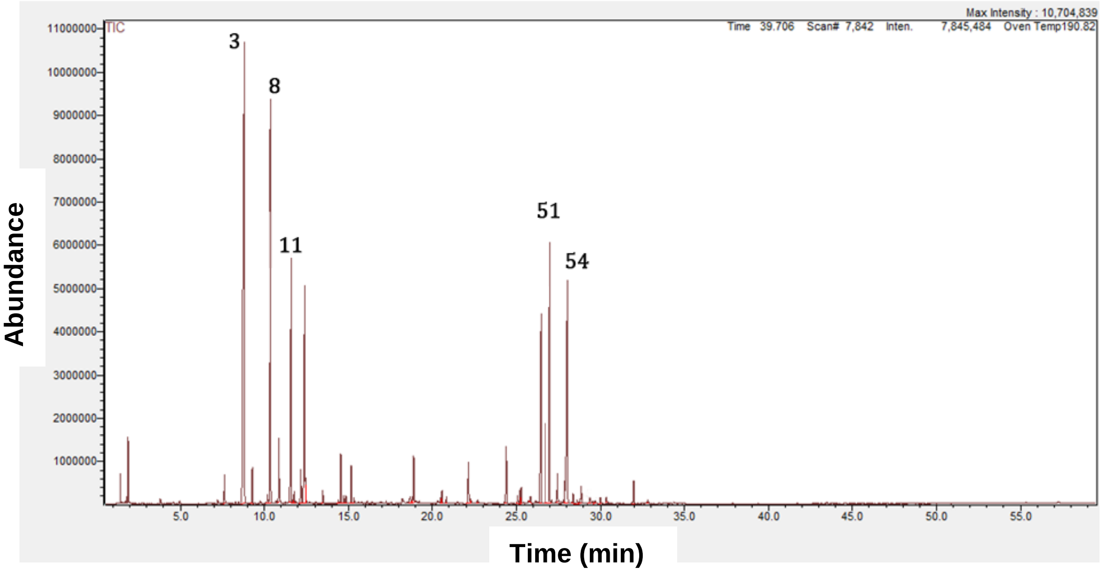

Fig. 1. GC/MS chromatogram of wood from *Pinus brutia* Ten. (For the name of compounds, see the corresponding numbers in Table 1)

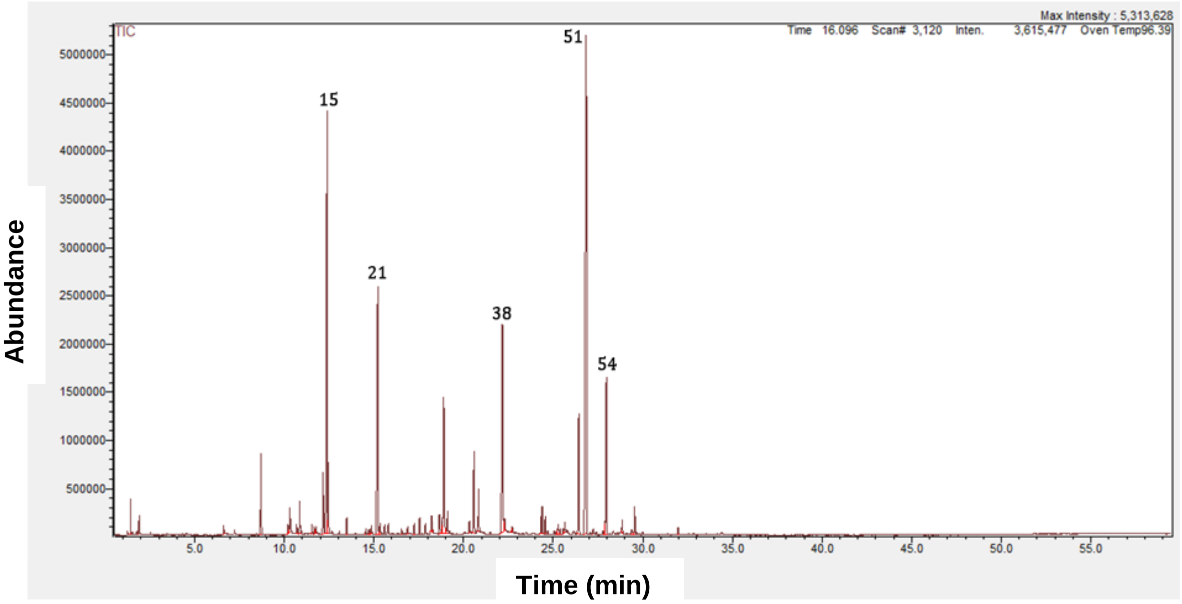

Fig. 2. GC/MS chromatogram of root from *Pinus brutia* Ten. (For the name of compounds, see the corresponding numbers in Table 1.)

Oz *et al*. (2015) reported the quantities of α-pinene, β-pinene, β-caryophyllene, Δ^3-carene, and α-humulene to respectively represent 19.7%, 13.3%, 7.8%, 6.9%, and 3.3% (representing 51% of the turpentine oil of oleoresin from brutian pine).

Yasar *et al*. (2018) determined these quantities to be α-pinene (49.12%), β-pinene (19.12%), β-caryophyllene (2.8%), Δ^3-carene (15.33%), and α-humulene (1.71%), representing 88.08 % of the turpentine oil of oleoresin from brutian pine. Of the volatile compounds of oleoresin from brutian pine, Yasar *et al*. (2018) detected α-pinene, β-pinene, β-caryophyllene, Δ^3-carene, and α-humulene at respective amounts of 44.35%, 18.59%, 1.73%, 14.58%, and 1.67%, representing 80.92% of the total volatile compounds.

The findings regarding the main volatile compounds of brutian pine wood in this study are supported by Oz *et al*. (2015) and Yasar *et al*. (2018), who reported α-pinene, β-pinene, β-caryophyllene, and Δ^3-carene as the notable main components in the volatile compounds obtained from brutian pine.

Although α- and β-pinenes were the main volatile compounds obtained from brutian pine wood, α-pinene was only a minor compound with a quantity of 2.19%, whereas β-pinene was not even detected in the root sample of brutian pine. Limonene, linalool, and (E)-anethole were significant volatile compounds in the root sample of the brutian pine, but were found in lower amounts of 6.39%, 0.87%, and 1.15%, respectively, in the wood sample of brutian pine. β-caryophyllene and α-humulene were major compounds in the volatiles of both samples of brutian pine.

In the wood sample of brutian pine, the major volatile compounds were monoterpenes (α-pinene, β-pinene, and Δ^3-carene) and sesquiterpenes (β-caryophyllene and α-humulene), whereas in the root sample the main volatile compounds were from oxygen-containing components (linalool and (E)-anethole) as well as a monoterpene (limonene) and sesquiterpenes (β-caryophyllene and α-humulene).

Some of the major components' usage areas are explained below:

α-Pinene has anti-inflammatory and antimicrobial properties and acts as an antibiotic of broad spectrum. It also works as an inhibitor of acetyl cholinesterase as well as a bronchodilator (Mercier *et al.* 2009; Hunter 2009; Rodrigues-Corrêa *et al.* 2012).

β-pinene has been used as a flavoring and perfume agent due to its woody-green pine-like odor. It is very widespread in essential oils and is also used as a preservative in cooking to provide the food with flavoring impacts. β-pinene also has antibacterial, cytotoxic, and antimicrobial effects (Hunter 2009; Mercier *et al.* 2009; Rodrigues-Corrêa *et al.* 2012).

Δ^3-carene is used as a raw material in perfumes, cosmetics, flavors, and terpene resins. Δ^3-carene is used to distinguish and stimulate calcium productivity in bone cells, thereby contributing to bone growth (Rodrigues-Corrêa *et al.* 2012).

D-Limonene is widely used as a basis for the manufacture of cleaning product as a solvent; it is also used as a precursor to carvone in chemical synthesis (Hunter 2009).

β-caryophyllene is an FDA approved food additive, and is the first dietary cannabinoid. β-caryophyllene contributes to the unique fragrance linked with plant oils. It also has anti-inflammatory, antibacterial, antidepressant, and antioxidant effects (Hunter 2009; Rodrigues-Corrêa *et al.* 2012).

Table 2. Main Groups of Volatile Compounds Identified in Wood and Root Samples from Brutian Pine

No	Main Group	Wood (%)	Root (%)	*t*-test results		
				F	*t*	*p*
1	Monotertepene	68.35 (1.83)	22.54 (0.01)	3.956	43.357	<0.0001
2	Sesquiterpene	25.62 (0.86)	39.07 (1.56)	0.617	-13.077	0.0002
3	Terpene oxide	1.03 (0.22)	2.19 (0.19)	0.426	-6.912	0.0023
4	Alcohol	1.16 (0.04)	12.28 (0.55)	3.421	-34.927	<0.0001
5	Adehyde	0.74 (0.02)	0.75 (0.04)	0.800	-0.387	0.7183
6	Ether	2.32 (0.02)	14.58 (0.59)	3.729	-35.971	<0.0001
7	Ester	0.50 (0.14)	3.70 (0.44)	1.689	-12.004	0.0003
8	Ketone	0.28 (0.05)	4.89 (0.45)	3.122	-17.635	<0.0001
( ): Standard deviations						

Table 2 shows the main groups of volatile compounds identified in the wood and root samples of brutian pine. Monoterpenes, sesquiterpenes, terpene oxides, alcohols, aldehydes, ethers, esters, and ketones comprised the main groups of volatile compounds in both samples of brutian pine.

According to the results of an independent-samples t-test, there was a significant difference between the wood and root samples of brutian pine with respect to monoterpenes, sesquiterpenes, terpene oxides, alcohols, ethers, esters, and ketones ($p < 0.05$). However, there was no significant difference between the wood and root samples of brutian pine with respect to aldehydes ($p > 0.05$). The root sample contained 34.43%, 52.97%, 90.55%, 84.09%, 88.38%, and 94.27% more sesquiterpenes, terpene oxides, alcohols, ethers, esters, and ketones, respectively, than the wood sample, whereas the wood sample had 67.02% more monoterpenes than the root sample.

CONCLUSIONS

1. In the wood sample, α-pinene (32.69%) was determined to be the dominant volatile compound, whereas β-caryophyllene (25.41%) was the distinctive volatile compound in the root sample.
2. Although terpenes were identified as the major compound in the wood sample, in the root sample, oxygen-containing components were the main compounds, in addition to terpenes.
3. It was determined that monoterpenes, sesquiterpenes, terpene oxides, alcohols, aldehydes, ethers, esters, and ketones were the main groups of volatile compounds in both samples of brutian pine. The wood sample was rich in monoterpenes, whereas the root sample contained a considerable amount of the other main groups of volatile compounds.
4. These study results indicate that the root of brutian pine offers a variety of valuable volatile compounds and might be more effectively used as an industrial raw material than a bioenergy source.

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