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Comparative Analysis of Bioactive Compounds in Pine Resin: Headspace/GC-MS and Direct Injection/GC-MS

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Abstract: This study, the chemical composition of pine resin collected from three different locations (Kaburgediği, Karabucak and Mavisilifke) of Mersin province in the Mediterranean Region of Türkiye was investigated using headspace/GC-MS and direct injection/GC-MS techniques. A commercial essential oil was used as a control sample in the study. The analysis focused on key volatile compounds, including α-pinene, delta-3-carene, camphene, DL-limonene (mixture of D- and L-form), and caryophyllene. The headspace/GC-MS method was more effective for detecting compounds with higher volatility, such as α-pinene, which was more abundant in Karabucak (66.12% in headspace vs. 53.13% in direct injection). On the other hand, direct injection/GC-MS provided higher sensitivity for less volatile compounds, such as caryophyllene (4.82% in direct injection vs. 0.67% in headspace in Kaburgediği). The most significant difference between the methods was observed in the detection of DL-limonene, which showed higher concentrations in direct injection/GC-MS (3.76% in Mavisilifke) compared to headspace/GC-MS (1.51%). This suggests that direct injection/GC-MS is better for quantifying compounds with lower volatility, while headspace/GC-MS is more effective in capturing the overall aroma profile, especially for volatile compounds. This highlights the difference in efficiency and sensitivity based on the compound's volatility. Therefore, choosing the right method depends on the specific compounds of interest and their volatility characteristics. These findings highlight the unique volatile profiles of each resin, shaped by both the species and the analytical method used, with implications for their aromatic and potential pharmacological properties.

Keywords: Pine resin, headspace/gas chromatography-mass spectrometry, bioactive compounds, aroma profile, volatile oil

1. Introduction

Pine resin is a natural substance with a complex content that plays an important role in the defense mechanisms of coniferous trees, acting as a protective barrier against microbial infections, insect infestations and environmental stress factors (Trapp and Croteau, 2001). Its chemical composition consists of monoterpenes, sesquiterpenes, diterpenes and phenolic compounds that contribute to its biological activities, including antimicrobial, antioxidant, anti-inflammatory and insect repellent properties (Alonso-Esteban et al., 2022; Ancuceanu et al., 2024). Among the most important bioactive compounds identified in pine resin are α-pinene, camphene, δ-3-carene, limonene and caryophyllene, which have important pharmacological and industrial applications (Ioannou et al., 2014). One of the most abundant monoterpenes in pine resin, α-pinene, is known for its antimicrobial, anti-inflammatory, bronchodilator and neuroprotective effects, and has pharmaceutical value especially in the treatment of respiratory disorders and cognitive disorders (Antonelli et al., 2020). On the other hand, camphene has been identified to have antioxidant and cardioprotective properties, and studies have emphasized that it has the potential to lower blood cholesterol levels and reduce damage associated with oxidative stress (Alves-Silva et al., 2021). D-3-carene, a naturally occurring bicyclic monoterpene, has antifungal and anti-inflammatory effects and widely used in

essential oils and aromatherapy due to its therapeutic benefits (Siddiqui et al., 2024). Another important terpene, limonene, is widely used for its anticancer, anxiolytic and anti-inflammatory activities, as well as its applications in the food, pharmaceutical and cleaning industries due to its pleasant citrus-like odor and broad-spectrum antimicrobial effects (Francezon and Stevanovic, 2018). Unlike monoterpenes, caryophyllene is a sesquiterpene with effective anti-inflammatory and analgesic properties, as it is the only known terpene that can interact with the CB2 cannabinoid receptor, making it promising for potential therapeutic applications in pain management, neuroprotective therapies, and inflammatory diseases (Ricardi et al., 2024).

The composition of pine resin is highly dependent on environmental conditions such as altitude, temperature, soil composition, and climatic factors, and there is significant variability in the concentration and diversity of its bioactive compounds in different geographical locations. This variability requires detailed chemical analysis to accurately characterize the composition of pine resin and to assess its potential biological activities (Velasco-García and Hernández-Hernández, 2024). One of the most effective techniques to identify and quantify volatile and semi-volatile organic compounds in pine resin is gas chromatography-mass spectrometry (direct injection/GC-MS), which provides high sensitivity and selectivity in detecting monoterpenes and sesquiterpenes. In addition, headspace gas chromatography-mass spectrometry (headspace/GC-MS) is widely used for the analysis of volatile compounds without direct sample extraction, minimizing the risk of compound degradation and providing a more accurate representation of the natural volatile profile (Bleton and Tchapla, 2009; Kim et al., 2014; Nakas et al., 2024). Considering the economic and medical importance of pine resin, comparative studies on its bioactive composition from different locations are essential to optimize its industrial applications and increase its use in pharmaceutical and therapeutic formulations (Clark et al., 2014; Swamy et al., 2023). However, despite the well-documented pharmacological potential of pine resin, there are limited comparative studies on the locational diversity of its bioactive compounds in Türkiye, especially in Mersin, a Mediterranean coastal location known for various pine species such as *Pinus brutia*, *Pinus nigra* and *Pinus pinea*.

This study aims to investigate the bioactive composition of pine resins collected from three different locations of Mersin, Türkiye, and compare them using two analytical methods were headspace/GC-MS and direct injection/GC-MS.

2. Materials and Methods

2.1. Tapping pine resins and clevenger-based extraction

The pine resin samples collected from three different location Kaburgediği, Karabucak, and Mavisilifke districts of Mersin province. Kaburgediği location was 37° 09′ 00″ N 34° 47′ 49″ E, Karabucak location was 36° 52' 22" N 34° 52' 51" E and Silifke location was latitude 36° 37' 64" N and longitude 33° 92' 57" E (Figure 1). Resin extraction from pine trees in the Mersin location is known as "tapping" where resin is collected by making incisions in the bark (Figure 2).

Figure 1. Geographic location of samples taken for resin extraction (Anonymous, 2024)

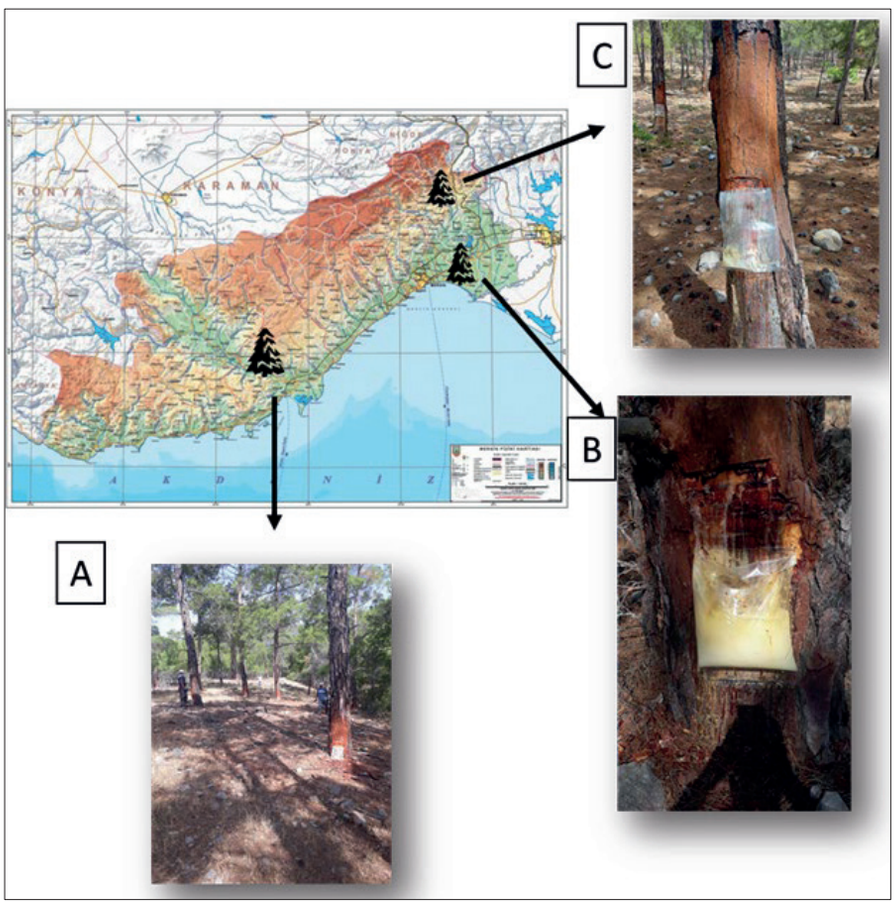

Figure 2. Resin extraction from pine trees in the Mersin location (A: Mavisilifke, B: Kaburgediği and C: Karabucak) is known as "tapping" where resin is collected by making incisions in the bark

The conventional tapping method widely used for resin extraction was used to obtain pine resin samples. A section of the bark was carefully removed to create a rectangular cut (20-30 cm in length) on the trunk of the tree. Within this window, controlled linear cuts were made to stimulate resin exudation. A collection bag was securely attached to the tree with a metal clip. This allowed the resin to accumulate over a period of 2-3 months. Harvested resin was then collected, transported under controlled conditions to the laboratory and stored.

Clevenger-based extraction, 61.5 grams for each sample were taken and placed in a 1liter volumetric flask. To this, 600 mL of distilled water was added, and the mixture was subjected to steam distillation in a Clevenger apparatus for 3 hours to extract the essential oils. The resulting essential oils were then filtered using a syringe through calcium chloride ($CaCl_2$) desiccant and a 0.22 µm polyvinylidene difluoride (PVDF) filter. Following filtration, 200 µL of the essential oil was transferred into a vial, and 1.4 mL of dichloromethane (CH_2Cl_2) was added for direct injection/GC-MS analyses.

2.2. Direct injection/GC-MS analyses

Direct injection/GC-MS is widely recognized as the standard method for analyzing semivolatile organic compounds. For analyzing by direct injection/GC-MS, the sample vial was subjected to heating at 130 °C for 30 minutes in a Triplus rotating slotted heater (RSH) oven. Afterward, 2.5 mL of the heated sample was injected into the GC-MS system. The analysis was performed using a single quadrupole (ISQ) mass spectrometer (Thermo Fisher Scientific, Austin, TX) coupled with a Trace 1310 gas chromatograph. The chromatographic conditions were set as follows: the initial oven temperature was held at 80 °C for 2 minutes, then ramped up to 240 °C at a rate of 4 °C per minute, followed by a 25-minute hold at 240 °C. The ion source and detector were both maintained at 250 °C, and the sample injection volume was adjusted to 1.5 mL. Helium (1.2 mL min^{-1}) served as the carrier gas. A Thermo TG-WAXMS GC column (60 m × 0.25 mm ID × 0.25 µm) was utilized for high-sensitivity separation. The mass spectral scan range was set to 55-300 (m z^{-1}), mass-to-charge ratio (m z^{-1}). Component identification was achieved by comparing the spectral data against

the National Institute of Standards and Technology (NIST) demo, Wiley7, Wiley9, redlip, mainlip, and WinRI libraries (Gök and Başar, 2023).

2.3. Headspace/GC-MS analyses

Headspace/GC-MS is widely regarded as the primary technique for analyzing volatile organic compounds. The analysis was performed using a Thermo Trace 1310 GC ISQ single quadrupole mass spectrometer (Thermo Fisher Scientific, Austin, TX). Dried resin samples were ground using a blender (IKA brand), and 0.5 grams of the ground sample were placed into a 25 mL Chromacol 20-HSV vial. The vial was then loaded onto the GC-MS device (THERMO, Triplus RSH Trace1310-ISQ LT).

Headspace/GC-MS conditions involved heating the vial at 130 °C for 90 minutes in the Triplus RSH headspace oven, followed by injecting a 2.5 mL volume from the heated vial into the GC-MS. The GC oven temperature program began at 60 °C for 6 minutes, followed by an increase to 230 °C at a rate of 2 °C per minute. The analysis was concluded with 30 minutes wait at 230 °C. Helium was used as the carrier gas at a flow rate of 1.2 mL min^{-1}. The analysis utilized a Thermo TG-WAXMS GC column (60 m x 0.25 mm ID x 0.25 µm). The MS conditions were set with the ion source and detector at 250 °C, and the mass scanning range was from 55 to 550 amu. Identifications of the obtained peaks were made using the NIST, Wiley 9, Redlip, Mainlip, and WinRI libraries (Gök and Başar, 2023).

3. Results and Discussion

Headspace/GC-MS technique improves the separation, identification and sensitivity of volatile compounds, making it a reliable tool in plant analysis. For example, headspace/GC-MS offers 35-55 times higher extraction efficiency than static methods (Ross, 2012). Monoterpenes were identified in the pine resin samples from Kaburgediği and other locations such as Karabucak and Mavisilifke (Figure 3).

3.1. Kaburgediği location analysis

In headspace analysis, α-pinene appeared with 66.12% at a retention time of 7.88 min, while in direct injection/GC-MS, it was found to be the most dominant component with 53.13% at 8.03 min (Table 1). This indicates that α-pinene is an important component contributing to the characteristic pine aroma in the Kaburgediği sample. Compared to the control as commercial sample, α-pinene was detected at a lower percentage of 48.55% at 7.82 min (Table 2). The higher α-pinene concentration in the Kaburgediği was observed to make this sample have a more dominant pine odor compared to the control, which had a slightly less pronounced pine aroma.

For camphene concentration in Kaburgediği, both methods showed at 2.14% in headspace/GC-MS at 9.13 min and 1.37% in direct injection/GC-MS at 9.20 min (Table 1). On the other hand, higher amount of camphene was detected in the control sample at 3.32% at 9.18 min (Table 2). This

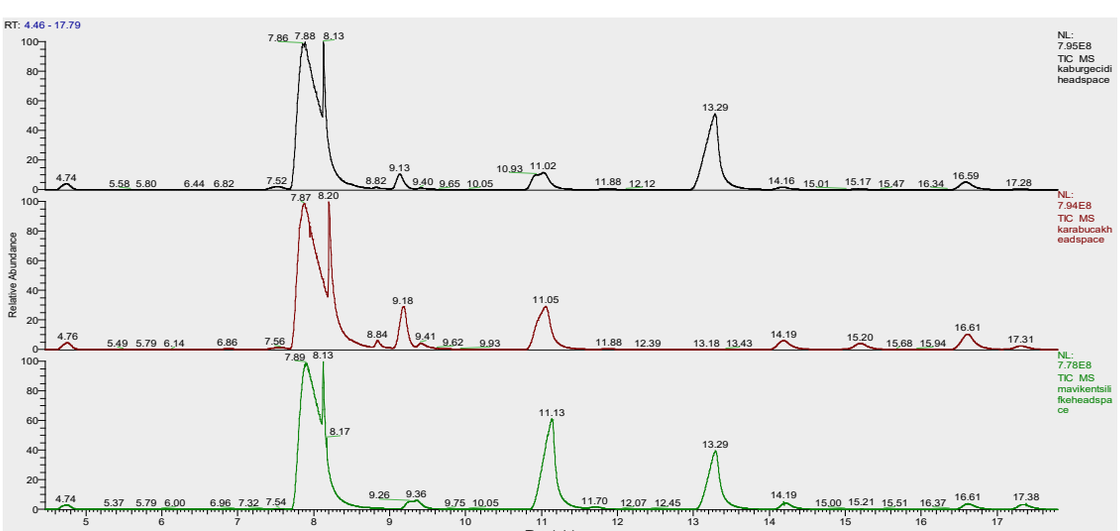

Figure 3. Comparative headspace/GC-MS analysis of pine resin samples from three Mersin location

suggested that the control sample was a stronger herbal or camphor note, while the Kaburgediği resin exhibits a more subtle camphene aroma.

Delta-3-carene, a sesquiterpene with a characteristic citrus odor, appeared significantly in the Kaburgediği sample at 13.29 min at 20.97% in

Table 1. Compounds identified through headspace/GC-MS and direct injection/GC-MS analyses-Kaburgediği location

Compound name (Chemical name)	Headspace/GC-MS		Direct injection/GC-MS		Molecular formula
	RT (min)	Area (%)	RT (min)	Area (%)	
2-propanamine, 2-methyl- (CAS)	4.74	1.08	-	-	$C_4H_{11}N$
α-pinene, (-)-	7.88	66.12	8.03	53.13	$C_{10}H_{16}$
Camphene	9.13	2.14	9.20	1.37	$C_{10}H_{16}$
2-α-pinene	11.02	5.67	10.94	5.32	$C_{10}H_{16}$
Delta-3-carene	13.29	20.97	13.33	27.13	$C_{10}H_{16}$
DL-limonene	16.59	2.21	16.56	3.59	$C_{10}H_{16}$
α-terpinolene	24.55	1.15	24.57	2.98	$C_{10}H_{16}$
Caryophyllene	67.89	0.67	-	-	$C_{15}H_{24}$
Tricyclene	-	-	7.37	0.31	$C_{10}H_{16}$
Ocimene	-	-	6.64	0	$C_{10}H_{16}$
α-humulene	-	-	15.10	0.24	$C_{15}H_{24}$
4,7-methano-1H-indene, 2,4,5,6,7,7a-hexahydro	-	-	17.94	0.01	$C_{10}H_{14}$
1,3,6-octatriene	-	-	19.83	0.02	$C_{10}H_{16}$
γ-terpinene	-	-	20.63	0.47	$C_{10}H_{16}$
Ylangene	-	-	31.61	0.01	$C_{15}H_{24}$
Longipinene	-	-	28.98	0.13	$C_{15}H_{24}$
10,13-octadecadiynoic acid, methyl ester	-	-	40.13	0.01	$C_{19}H_{30}O_2$
Perilla alcohol	-	-	46.18	0.01	$C_{10}H_{16}O$
Benzene, (2-methyl-1-propenyl)	-	-	48.24	0.03	$C_{10}H_{12}$

RT: Retention time

headspace/GC-MS and 27.13% at 13.33 min in direct injection/GC-MS (Table 1). This compound was significantly higher in the Kaburgediği sample compared to the control, where it was only present at 13.09 min at 6.46% (Table 2). The higher delta-3-carene concentration in the Kaburgediği sample suggests a more citrus-heavy profile, contrasting with the slightly more balanced aroma of the control. Headspace/GC-MS has the potential to identify a large number of volatile compounds in plant materials.

Table 2. Compounds identified through direct injection/GC-MS analyses-commercial sample*

Compound name (Chemical name)	Direct injection/GC-MS		Molecular formula
	RT (min)	Area (%)	
α-pinene	7.82	48.55	$C_{10}H_{16}$
Camphene	9.18	3.32	$C_{10}H_{16}$
2-α-pinene	10.9	5.2	$C_{10}H_{16}$
Delta-3-carene	13.09	6.46	$C_{10}H_{16}$
2-α-pinene	14.08	1.44	$C_{10}H_{16}$
α-terpinene	15.08	2.79	$C_{15}H_{24}$
Humulene	15.09	2.79	$C_{15}H_{24}$
DL-limonene	16.58	10.51	$C_{10}H_{16}$
γ-terpinene	20.61	1.56	$C_{10}H_{16}$
α-terpinolene	24.67	12.48	$C_{10}H_{16}$
Junipene	64.53	1.3	$C_{15}H_{24}$
Caryophyllene	67.91	1.77	$C_{15}H_{24}$
α-terpineol	77.48	4.41	$C_{10}H_{18}O$

*: Commercial sample table was used as control data, RT: Retention time

The other significant compound, DL-limonene, was detected at 2.21% in headspace/GC-MS and 3.59% in direct injection/GC-MS at 16.59 and 16.56 min respectively. The DL-limonene was found at 10.51% in control at 16.58 min, showing similar concentrations to the samples (Table 2).

The presence of caryophyllene, a sesquiterpene contributing to woody and spicy notes, was detected in Kaburgediği at 67.89 min with 0.67% in headspace/GC-MS and was not detected in GC-MS (Table 1). In contrast, caryophyllene was observed at 67.91 min with 1.77% in the control (Tablo 2).

The results confirm that α-pinene is the dominant volatile compound in Kaburgediği, contributing to its characteristic pine aroma. The lower camphene concentration in Kaburgediği compared to the control suggests a less intense herbal profile. Differences in monoterpene and sesquiterpene concentrations between the two methods likely arise from the distinct extraction mechanisms, as headspace/GC-MS relies on gas-phase partitioning, whereas direct injection/GC-MS enables direct solvent extraction (Wang and Guo, 2004).

The significantly higher delta-3-carene concentration in Kaburgediği highlights a strong citrus note, in contrast to the control sample's more balanced composition. Previous studies on Pinus species have similarly identified α-pinene, camphene, and limonene as key volatile constituents shaping the aromatic profile (Kim et al., 2014). The lower detection of caryophyllene in headspace/GC-MS is consistent with its lower volatility, making direct injection/GC-MS a more suitable method for sesquiterpene quantification (Górecki and Pawliszyn, 1995).

Overall, headspace/GC-MS effectively captured the primary volatile compounds in Kaburgediği resin, emphasizing its potential for aroma profiling. However, direct injection/GC-MS provided a more comprehensive quantification, particularly for less volatile compounds, demonstrating the complementary nature of both analytical techniques.

3.2. Karabucak location analysis

In this part (Table 3), α-pinene was detected with 67.47% in headspace/GC-MS at 7.87 min and 47.13% in direct injection/GC-MS at 7.82 min; α-pinene was found to be similar to the control sample content with 48.55% at 7.82 min (Table 2). Significant levels of α-pinene were present in both samples, indicating a dominant pine aroma. A stronger pine odor was present in the control sample, while a similar but less intense pine aroma was present in the sample taken from Karabucak.

The highest DL-limonene concentration in Karabucak (Table 3) was 4.11% at 16.61 min in headspace/GC-MS and 2.28% at 16.53 min in direct injection/GC-MS whereas 10.51% at 16.58 min and in the control (Table 2). The volatile composition of essential oils and plant extracts is very important in defining their aromatic and functional properties. Another important monoterpene, DL-limonene, is known for its characteristic citrus aroma and has been investigated for its antimicrobial and antioxidant properties. The almost identical concentration of DL-limonene in both Karabucak and the control suggests a common metabolic pathway potentially affected by similar growing conditions or genetic factors.

Caryophyllene in Karabucak was detected only in direct injection GC-MS at 67.94 min, with a relative abundance of 1.11% (Table 3). In the control, caryophyllene was present at 1.77% at 67.91 min (Table 2). This suggests that Karabucak has a lower caryophyllene presence compared to the control where it was found at 1.5 fold, potentially resulting in a less pronounced spicy, woody note.

Headspace/GC-MS proved to be an effective technique for extracting and analyzing volatile compounds in *Pinus* species, providing insights into their chemical diversity and potential contributions to plant quality and pharmacological properties. These findings are consistent with previous studies validating headspace/GC-MS as a reliable method for plant volatile analysis (Wang and Guo, 2004; Yu et al., 2017).

A similar approach was employed by Raber et al. (2021) to investigate interspecific variations in terpene profiles of *Picea pungens* and *Picea abies* using headspace/GC-MS, demonstrating distinct chemical compositions with ecological and physiological implications.

The volatile composition of essential oils plays a crucial role in defining their aromatic and functional properties. The comparable DL-limonene concentrations in Karabucak and the control suggest the influence of a common metabolic pathway, potentially shaped by genetic or environmental factors. Caryophyllene, a bioactive sesquiterpene with anti-inflammatory properties, was found at higher levels in Karabucak, indicating an enhanced aromatic profile and a possible adaptive response to environmental stress conditions.

Table 3. Compounds identified through headspace/GC-MS and direct injection/GC-MS analyses-Karabucak location

Compound name (Chemical name)	Headspace/GC-MS		Direct injection/GC-MS		Molecular formula
	RT (min)	Area (%)	RT (min)	Area (%)	
Butane (CAS)	4.75	1.11	-	-	C_4H_{10}
α-pinene	7.87	67.47	7.82	47.13	$C_{10}H_{16}$
Camphene	9.18	6.88	9.21	2	$C_{10}H_{16}$
2-α-pinene	11.06	11.65	11.07	19.67	$C_{10}H_{16}$
α-myrcene	14.19	2.28	14.12	2.09	$C_{10}H_{16}$
α-humulene	15.20	1.78	74.20	0.72	$C_{15}H_{24}$
DL-limonene	16.61	4.11	16.53	2.28	$C_{10}H_{16}$
1,4-cyclohexadiene	20.69	0.56	-	-	$C_{10}H_{16}$
γ-terpinene	24.56	2.06	24.55	2	$C_{10}H_{16}$
1,4-methanoazulene, decahydro-4,8,8-trimethyl-9-methylene, [1S(1α,3aα,4α,8aα)]	64.52	1.21	64.56	1.42	$C_{15}H_{24}$
Caryophyllene			67.94	1.11	C_{15} H_{24}
3-cyclohexene-1-methanol, α,α,4-trimethyl-(S)	77.43	0.49	77.48	0.48	$C_{10}H_{18}O$
Benzene, 1,2-dimethoxy-4-(2-propenyl)	96.50	0.4	-	-	$C_{11}H_{14}O_2$
Junipene	-	-	64.56	4.46	$C_{15}H_{24}$

RT: Retention time

3.3. Mavisilifke location analysis

In the comparative analysis of Mavisilifke samples (Table 4), α-pinene, a monoterpene associated with a characteristic pine-like aroma, was detected in 7.91 min (60.03%) in the headspace/GC-MS analysis of Mavisilifke and 8.10 min (68.25%) in the direct injection/GC-MS analysis. Mavisilifke (Table 4) α-pinene concentration was found the highest value in control (Table 2) and other samples (Table 1 and Table 3), indicating that it has a more pronounced pine aroma compared to Mavisilifke. On the other hand, this discrepancy in detection levels may be due to differences in sample preparation and volatilization efficiency in headspace/GC-MS and direct injection/GC-MS.

In Table 4, the camphene, which contributes to the herbal and camphor notes, exhibited similar concentrations in Mavisilifke (3.31% at 9.23 min via direct injection/GC-MS) compared to the control (3.32% at 9.18 min via direct injection/GC-MS) (Table 2), indicating that Mavisilifke has an intense herbal profile.

Delta-3-carene (Table 4), a bicyclic monoterpene associated with citrus-like notes, showed significant differences between the two methods and samples, being detected at 13.29 min 13.56% concentration in headspace/GC-MS at 14.10 min and 2.15% concentration in direct injection/GC-MS in Mavisilifke, whereas it was only present at 6.46% at 13.09 min in the control (Table 2). This suggests that Mavisilifke exhibits a more pronounced citrus aroma, while the control has relatively less presence of this compound. In headspace analysis, the amount of delta-3-carene was higher, while it was detected at lower levels in direct injection/GC-MS.

The DL-limonene, another important citrus-related terpene, was present in Mavisilifke 1.51% in headspace/GC-MS at 16.61 min and 3.76% in direct injection/GC-MS at 16.54 min (Table 4), while it appeared in the control at 10.51% at 16.58 min (Table 2).

Finally, caryophyllene, a sesquiterpene providing woody and spicy notes, was detected at 0.53% in the headspace/GC-MS at 67.89 min and 4.82% at 64.65 min in direct injection/GC-MS in Mavisilifke (Table 4), while in the control, caryophyllene was found at a lower concentration of 1.77% at 67.94 min (Table 2).

The differences in retention times and compound concentrations highlight the influence of the analytical method used. Matrix effects in headspace/GC-MS can affect the partitioning of compounds, thereby affecting detection levels (Górecki and Pawliszyn, 1995). Differences between headspace/GC-MS and direct injection/GC-MS are due to the volatility of the compounds and their interaction with the sample matrix. Headspace/GC-MS is best for the analysis of volatile compounds because it works with vapour phase equilibrium (Zhang et al., 2023), but it is less effective for compounds with low vapour pressure, such as sesquiterpenes (Górecki and Pawliszyn, 1995).

The similar DL-limonene concentrations in Mavisilifke and the direct injection/GC-MS control suggest that they follow a similar metabolic pathway, influenced by genetic or environmental factors. The lower detection of DL-limonene in headspace/GC-MS for Mavisilifke is likely due to limited volatilisation efficiency due to matrix interactions (Wardencki et al., 2013). Direct injection/GC-MS provides more accurate quantification through solvent extraction, but may alter compound concentrations due to differences in solubility (Kristenson et al., 2005).

However, headspace/GC-MS is a non-destructive method that better reflects the aroma profile by analysing the equilibrium between the

Table 4. Compounds identified through headspace/GC-MS and direct injection/GC-MS analyses-Mavisilifke location

Compound name (Chemical name)	Headspace/GC-MS		Direct injection/GC-MS		Molecular formula
	RT (min)	Area (%)	RT (min)	Area (%)	
Butane (CAS)	4.74	0.77	-	-	C_3H_6O
α-pinene	7.91	60.03	8.1	68.25	$C_{10}H_{16}$
Camphene	-	-	9.23	3.31	$C_{10}H_{16}$
2-α-pinene	11.03	21.11	11.02	13.71	$C_{10}H_{16}$
Delta-3-carene	13.29	13.56	14.10	2.15	$C_{10}H_{16}$
DL-limonene	16.61	1.51	16.54	3.76	$C_{10}H_{16}$
1,4-cyclohexadiene	17.38	1.37	-	-	$C_{10}H_{16}$
α-phellandrene (Sabinene)	17.38-	1.37-		-	$C_{10}H_{16}$
γ-terpinolene	24.58	1.12	24.54	1.72	$C_{10}H_{16}$
Caryophyllene	67.89	0.53	64.65	4.82	$C_{15}H_{24}$

RT: Retention time

sample and the gas phase, making it valuable for fragrance and flavour analysis (Jin et al., 2019). Matrix composition can affect the equilibrium distribution, resulting in differences in detection and retention times. Therefore, while GC-MS is better for accurate volatile quantification, headspace/GC-MS (Figure 3) is a useful tool for studying flavour profiles that match sensory perception.

4. Conclusions

This study provides valuable insights into the volatile composition of *Pinus* species from Kaburgediği, Karabucak, and Mavisilifke, highlighting significant differences in key compounds like α-pinene, delta-3-carene, camphene, DL-limonene, and caryophyllene. These findings highlight the distinct aroma profiles of the samples and the value of combining different analytical techniques to fully characterize the chemical composition of plant-based essential oils. The study also emphasizes the effectiveness of headspace/GC-MS in capturing volatile compounds, while recognizing its limitations with sesquiterpenes. For future research, it is recommended to explore the broader ecological and physiological implications of these compounds. Particularly their potential applications in fragrance, flavor, and medicinal fields. Further refinement of analytical methods could enhance the detection of less volatile compounds, offering a more comprehensive understanding of the aromatic profiles.

Ethical Statement

The authors declare that ethical approval is not required for this research.

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Declaration of Author Contributions

Conceptualization, Material, Methodology, Investigation, Data Curation, Visualization, Writing- Original Draft Preparation, Writing-Review & Editing, *N. YILMAZ*; Data Curation, Formal Analysis, Writing-Review & Editing, *M.O. KAYA*; Data Curation, Formal Analysis, Writing-Review & Editing, *E. COŞKUN DAĞGEÇEN*. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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