

Comparative Phytochemical Analysis of Hydrodistilled Essential Oils of Scots Pine (*Pinus Sylvestris* L.) Needles From Central Kazakhstan

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Abstract: This study presents a detailed phytochemical investigation of hydrodistilled essential oils extracted from the needles of *Pinus sylvestris* (Scots pine), collected from fifteen natural populations located in the Karaganda and Ulytau regions of Central Kazakhstan. The extraction was performed using hydrodistillation with hexane as a co-solvent, and the chemical composition of the oils was subsequently analyzed using gas chromatography–mass spectrometry (GC-MS). The results revealed a diverse profile of volatile compounds. The major constituents consistently identified across the samples included α -pinene, β -pinene, camphene, τ -cadinol, and caryophyllene. Additionally, a range of minor compounds, such as limonene, 3-carene, and caryophyllene oxide, were also detected. Notably, many of these components are recognized in the scientific literature for their pharmacologically relevant properties, including antimicrobial, antioxidant, and anti-inflammatory effects. While this study did not conduct in vitro or in vivo bioassays, the therapeutic potential of the identified compounds is supported by prior pharmacological evidence. A significant finding of this research is the pronounced variation in the chemical composition of the essential oils between populations, which appears to correlate with environmental heterogeneity specifically, differences in soil characteristics, pollution levels, and climatic conditions across the studied regions. These results suggest that *P. sylvestris* exhibits ecological responsiveness to local stressors and may serve as a useful bioindicator species in environmental monitoring. Beyond ecological implications, the region-specific essential oil profiles of *P. sylvestris* highlight promising applications in pharmaceutical formulation, cosmetic production, and the fragrance industry. Overall, the study contributes novel data on the phytochemical diversity of Scots pine in Central Kazakhstan and establishes a valuable foundation for further investigations into its environmental adaptability and therapeutic potential.

Keywords: *Pinus Sylvestris*, Hexane Extract, Essential Oil, GC-MS, Central Kazakhstan, Phytochemical Composition

Introduction

Pinus sylvestris L. (Scots pine), a widespread coniferous species of the Pinaceae family, is native to the Northern Hemisphere and plays a vital role in maintaining ecological balance, while also holding substantial economic value (Paniagua-Zambrana *et al.*, 2024). The needles of Scots pine are a rich source of bioactive terpenoids such as α -pinene, β -pinene, limonene, and caryophyllene. These compounds have

been extensively studied for their antimicrobial, antioxidant, and anti-inflammatory activities (Delgado-Alvarado, 2022; Liang *et al.*, 2025; Li *et al.*, 2024), rendering pine needle extracts valuable in pharmaceutical, cosmetic, and aromatic applications (Lamotkin *et al.*, 2021). In particular, the essential oils derived from pine needles are known for their pronounced antioxidant (Kurti *et al.*, 2019) and antiseptic properties (Kahkonen *et al.*, 1999; Koutsaviti *et al.*, 2021).



An increasing body of research highlights that the qualitative and quantitative composition of essential oils in *P. sylvestris* is influenced by geographic origin and environmental conditions (Brichta *et al.*, 2023). Climatic factors, soil type, pollution levels, and anthropogenic stressors all affect the phytochemical profiles of plant-derived compounds. In this context, the study of biochemical indicators has become a vital tool for evaluating ecological conditions (Menshchikov *et al.*, 2020), especially in both pristine and anthropogenically modified terrestrial ecosystems (Burkhardt & Pariyar, 2014). Tree species such as *P. sylvestris* are widely used in biomonitoring studies due to their capacity to accumulate and reflect environmental pollutants (Kozlov *et al.*, 2020). Previous research has shown the potential of Scots pine as a bioindicator of aerotechnogenic pollution (Malik *et al.*, 2011). Nonetheless, there remains a need for further detailed investigations into how spatial variability and environmental stressors shape the phytochemical makeup of pine needles.

The regions of Karaganda and Ulytau in Central Kazakhstan represent an ecologically diverse territory where Scots pine is naturally distributed. These areas encompass both industrial zones and relatively protected natural landscapes, offering a unique opportunity to study the interaction between ecological factors and plant chemistry. Notably, several urban centers within these regions such as Zhezkazgan, Satpaev, Karaganda, Temirtau, and Balkhash are subject to considerable environmental stress due to intensive mining, metallurgical, and chemical industries (Askarov *et al.*,

2023; Bekseitova *et al.*, 2016; Assanov *et al.*, 2021). These activities have led to persistent air, soil, and water contamination. Even in protected areas like the Karkaraly and Ulytau mountain ranges, environmental pressures from agriculture and mineral extraction contribute to ecosystem degradation.

Given the combination of natural and anthropogenic influences in Central Kazakhstan, the Scots pine populations in these regions offer a compelling case for examining the environmental determinants of phytochemical variability. Understanding the response of *P. sylvestris* to such stressors not only contributes to environmental biomonitoring strategies but also reveals the medicinal potential of its essential oil constituents under varying ecological conditions.

The aim of this study is to conduct a comparative phytochemical analysis of Scots pine needle extracts collected from diverse locations in Central Kazakhstan, with a particular focus on evaluating how regional environmental factors may influence the composition and bioactivity of essential oil components.

Materials and Methods

The study was conducted from June 2021 to December 2024. Scots pine (*Pinus sylvestris* L.) needle samples were collected from fifteen distinct populations across two regions in Central Kazakhstan: Karaganda (latitude 49°59'N, longitude 73°08'E) and Ulytau (latitude 48°45'N, longitude 69°22'E) (Table 1). All field sampling was completed in 2024. The oil yield was calculated from 10 g of dried raw material.

Table 1: Sampling points of pine needles for research purposes

No.	Pine Sampling Location	Latitude	Longitude	Essential Oil Yield (g)	Color of Essential Oil
P1	Akimat Square, Zhezkazgan City	47.804115	67.716326	0.05	light-yellow
P2	Nauryz Park, Zhezkazgan City	47.788143	67.700632	0.033	light-yellow
P3	Akimat Square, Satpayev City	47.908344	67.527858	0.036	light-yellow
P4	Akimat Square, Ulytau Village	48.653272	67.005896	0.024	light-yellow
P5	Railwaymen's Park, Karaganda City, Sortirovka	49.967913	73.214586	0.03	light-yellow
P6	City Park, Karkaraly City	49.411991	75.467682	0.03	light-yellow
P7	Akimat Square, Karkaraly City	49.412223	75.470767	0.028	light-yellow
P8	Southeast, Ethnopark, Karaganda City	49.775016	73.126224	0.055	light-yellow
P9	Central Park, Karaganda City	49.806224	73.080321	0.034	light-yellow
P10	Victory Park, Karaganda City	49.784282	73.138977	0.032	light-yellow
P11	Vostok Park, Temirtau City	50.059233	72.997426	0.011	light-yellow
P12	Bus Station Square, Temirtau City	50.062989	72.951103	0.015	light-yellow
P13	Akimat Square, Balkhash City	46.840117	74.977181	0.020	light-yellow
P14	District Prosecutor's Office, Balkhash city	46.851308	74.975437	0.021	light-yellow
P15	Maikuduk Park, Karaganda City	49.893459	73.197725	0.05	light-yellow

Sampling was carried out during the summer months, when the concentration of chemical compounds in the needles is at its peak.

At each site, needle samples were collected from five mature, healthy trees spaced at least 10-15 meters apart to ensure genetic and environmental variability. From each tree, approximately 100 grams of current-year

needles were harvested from south-facing branches at a height of 1.3-1.5 meters. Samples from the five trees at each site were pooled to create a composite sample representative of the population.

Sampling locations were selected to encompass a broad range of ecological conditions, including industrially polluted sites (e.g., Temirtau, Zhezkazgan,

Balkhash) as well as relatively undisturbed or protected natural areas (e.g., Karkaraly Mountains, Ulytau Mountains). Selection criteria included proximity to pollution sources, vegetation cover characteristics, and site accessibility.

In addition, these fifteen sites were chosen due to the limited availability of empirical data and insufficient representation in the scientific literature. The lack of prior research in these areas highlights the need for comprehensive scientific investigations and high-quality field studies. Prioritizing these specific sites offers an opportunity to advance understanding of phytochemical diversity and contributes to addressing existing gaps in the current body of knowledge.

The diversity among populations P1-P15 in Central Kazakhstan reflect how plant secondary metabolite profiles can adapt to varying environmental conditions such as soil composition, moisture availability, and climatic factors. Figure 1 illustrates the geographic distribution of the sampling sites across the study region.

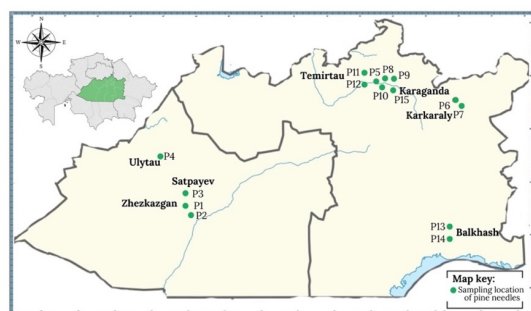


Fig. 1: Location of the study area. Scots pine populations (P1–P15)

Sample Preservation: All needle samples were placed in polyethylene bags, stored in portable coolers, and transported to the laboratory within 24 hours of collection. Samples were stored at 4°C in the dark and processed within three days to minimize the degradation of volatile compounds.

Extraction Procedure: Hydrodistillation. Essential oils were extracted from the pine needles using hydrodistillation with a Clevenger-type apparatus. Hydrodistillation was selected for its efficiency and low energy requirements in extracting volatile compounds from plant material, especially when compared to solvent-based methods such as Soxhlet extraction. To ensure reproducibility, each extraction was performed in triplicate under identical conditions. For each extraction, 10 g of dried needle material was placed into a flask with 500 mL of distilled water and heated to boiling. The hydrodistillation was carried out for 2 hours from the onset of boiling. To enhance the separation of oil from water, 10 mL of hexane was added to the receiver. The resulting hexane layer was dried using anhydrous MgSO_4 , filtered, and evaporated. The resulting essential oil was a light-yellow liquid with a characteristic pine aroma.

GC-MS Conditions: Component analysis was carried out using an Agilent 7890A gas chromatograph equipped with an Agilent 5975C mass-selective detector. This equipment undergoes annual calibration at the JSC "National Center for Expertise and Certification" of the Republic of Kazakhstan.

Column Specifications: DHA – 100; length – 30 m; inner diameter – 0.25 mm; film thickness – 0.25 μm . Injector temperature – 280°C. Column temperature program – from 60°C to 300°C at a heating rate of 8°C/min. Ion source temperature – 230°C; quadrupole temperature – 150°C. Carrier gas – helium; column pressure – 2 psi. Injection volume – 2.0 μL ; injection mode – split. Mass spectra were recorded in scan mode.

Components were identified by mass spectra and retention times using the NIST 08 library. The fraction composition calculation method was based on relative peak areas (semi-quantitative method). Analysis time – 32 minutes. The results were processed automatically using the GC-MSD Data Analysis software based on the peak areas of the total ion chromatogram. As the GC-MS was applied in a semi-quantitative mode, the reported percentages reflect relative peak areas rather than absolute concentrations, and should be interpreted as indicative rather than exact quantitative values.

Results and Discussion

One of the main methods for isolating extractive substances is solvent-based extraction. The qualitative composition and quantitative content of the identified compounds are presented in Tables 2 and 3.

As shown in Table 2 α -pinene was the dominant compound in P2 (24.04%), P4 (19.70%), P6 (23.24%), and P8 (22.33%). 1R- α -pinene was also notably present in P1 (25.66%) and P5 (9.28%). β -pinene showed higher concentrations in P5 (7.34%) and P4 (6.48%). Camphene was consistently present across all samples (2.45% to 6.67%). τ -Cadinol showed high values in P1, P2, and P7, and caryophyllene and its oxide appeared in most samples with higher levels in P5 and P6.

Other compounds such as 3-carene, limonene, and ledol were more sample-specific, with the highest 3-carene in P5 (19.51%), and limonene in P7 (11.73%) Bicyclo germacrene, caryophyllene oxide, and myrcene were also detected in smaller quantities.

The data are based on relative peak area (%) obtained via GC-MS analysis using a semi-quantitative approach.

As shown in Table 3 trend of α -pinene dominance continued in P9 (26.96%), P11 (26.89%), P13 (27.14%), and P15 (28.53%). 1R- α -pinene was high in P11 (13.79%) and P14 (16.35%). Camphene showed uniformity across all samples (3.43% to 5.00%). β -pinene reached its highest level in P9 (8.71%). τ -cadinol was again consistent, notably in P9, P11, and P13. Caryophyllene was prominent in P10 (7.47%) and P15 (6.08%). The values presented in Tables 2 and 3

correspond to the average percentages calculated from triplicate extractions.

Table 2: Compounds List of Sampling Points P1–P8

No. Compound	Area (in%)						
	P1	P2	P3	P4	P5	P6	P7
1 1S- α -Pinene	0.62	-	-	-	-	-	-
2 α -Pinene	-	24.04	21.77	19.70	-	23.24	18.35
3 1R- α -Pinene	25.66	-	-	-	9.28	-	-
4 Camphene	2.76	6.65	4.14	6.67	2.45	5.25	4.28
5 β -Pinene	3.46	-	4.48	6.48	7.34	2.52	2.14
6 β -Phellandrene	2.06	-	-	-	-	0.54	0.42
7 Bornyl acetate	2.66	-	-	-	2.51	-	1.34
8 Caryophyllene	1.93	1.92	4.46	2.64	3.64	-	1.86
9 (-)-Spathulenol	5.26	-	-	-	-	-	-
10 α -Cubebene	-	-	0.94	1.21	-	-	0.83
11 τ -Cadinol	6.55	6.50	5.55	5.82	4.09	-	6.34
12 γ -Elemene	-	-	-	5.45	-	-	2.82
13 4-Carene	-	0.85	0.96	0.49	-	-	-
14 Copaene	-	0.56	-	-	-	-	0.54
15 3-Carene	-	-	-	-	19.51	-	11.73
16 α -Cadinol	-	4.76	-	-	-	5.29	3.14
17 Limonene	-	-	2.74	-	-	1.59	11.73
18 Borneol	-	-	0.57	0.72	-	-	-
19 Bicyclo germacrene	-	-	-	-	-	4.20	-
20 α -Caryophyllene	-	-	0.77	-	-	-	0.47
21 Caryophyllene oxide	1.32	-	-	-	2.48	2.43	1.48
22 Myrcene	-	0.81	-	-	-	1.08	-
23 γ -Terpinene	-	-	-	-	-	1.38	-
24 Germacrene D	-	1.32	-	-	3.12	-	-
25 Ledol	-	-	-	4.52	5.20	-	-
26 1S- α -Pinene	-	26.93	-	-	-	-	-
27 α -Pinene	26.96	-	26.89	21.96	27.14	-	28.53
28 1R- α -Pinene	-	-	13.79	-	-	16.35	-
29 Camphene	3.49	3.43	3.96	3.80	3.47	3.52	5.00
30 β -Pinene	8.71	2.53	1.89	2.63	1.72	2.46	3.81
31 β -Phellandrene	-	-	-	-	-	-	-
32 Bornyl acetate	-	-	-	-	-	-	-
33 Caryophyllene	0.93	7.47	2.76	-	3.78	4.22	6.08
34 (-)-Spathulenol	9.07	-	-	-	-	-	-
35 α -Cubebene	-	-	0.73	1.76	0.98	0.55	-
36 τ -Cadinol	6.01	3.45	5.47	5.94	4.93	-	3.74
37 γ -Elemene	-	-	-	-	-	1.96	5.68
38 4-Carene	0.92	-	-	0.59	-	-	-
39 Copaene	-	-	-	0.80	-	-	0.92
40 3-Carene	-	15.29	-	-	-	-	0.81
41 α -Cadinol	4.45	-	4.43	-	-	5.37	2.49
42 Limonene	2.70	-	-	-	4.31	3.45	-
43 Borneol	-	-	0.63	-	-	-	-
44 Bicyclo germacrene	-	2.35	-	-	-	-	-
45 α -Caryophyllene	-	-	-	0.83	0.64	0.73	1.04
46 Caryophyllene oxide	-	-	0.79	0.57	0.80	1.52	-
47 Myrcene	-	-	-	-	0.58	-	-
48 γ -Terpinene	-	1.24	-	-	-	-	-
49 Germacrene D	-	-	-	-	-	-	-
50 Ledol	-	3.70	-	-	-	-	-

Note: ‘-’ indicates compound not detected.

Notable sample-specific compounds included γ -elemene in P15 (5.68%), (-)-spathulenol in P9 (9.07%), and ledol in P10 (3.70%). 3-carene was dominant in P10 (15.29%). The most common compounds across samples are shown in Figure 2.

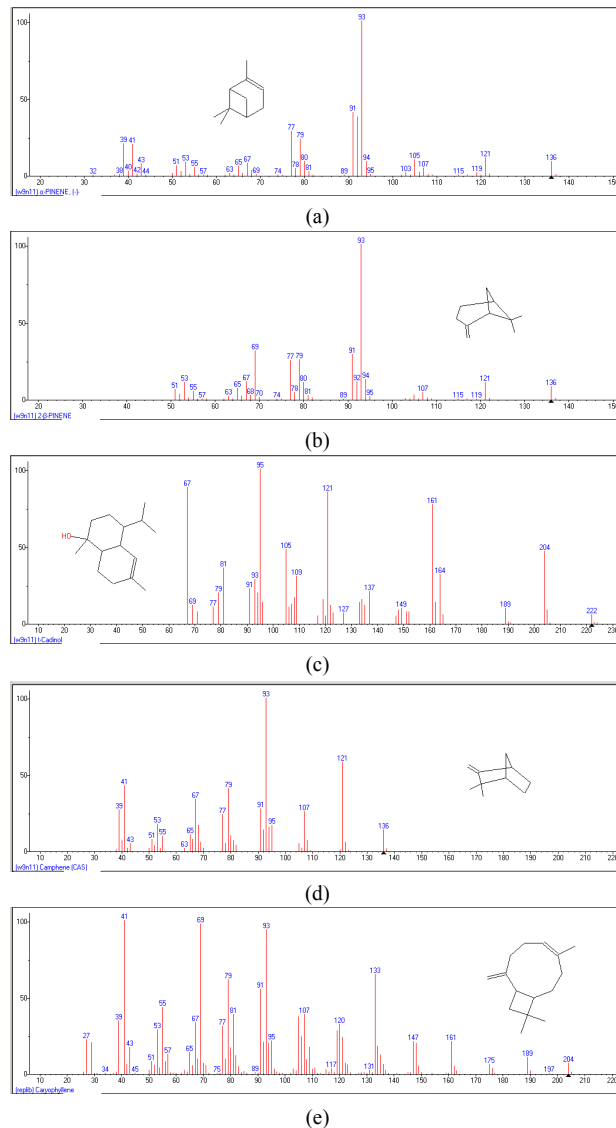


Fig. 2: Mass spectrum of compounds: (a) α -Pinene, (b) β -Pinene, (c) τ -Cadinol, (d) Camphene, (e) Caryophyllene

As shown in Figure 2, these compounds represent the most common and abundant volatiles identified across all samples. α -pinene and β -pinene displayed characteristic fragmentation patterns, while τ -cadinol and caryophyllene appeared with distinctive peaks supporting their identification.

Figure 3a illustrates the profile of P3 with moderate levels of α -pinene and camphene. Figure 3b (P15) shows strong peaks for α -pinene and caryophyllene. Figure 3c (P14) is rich in 1R- α -pinene and γ -elemene, while Figure 3d (P7) shows elevated levels of limonene and 3-carene.

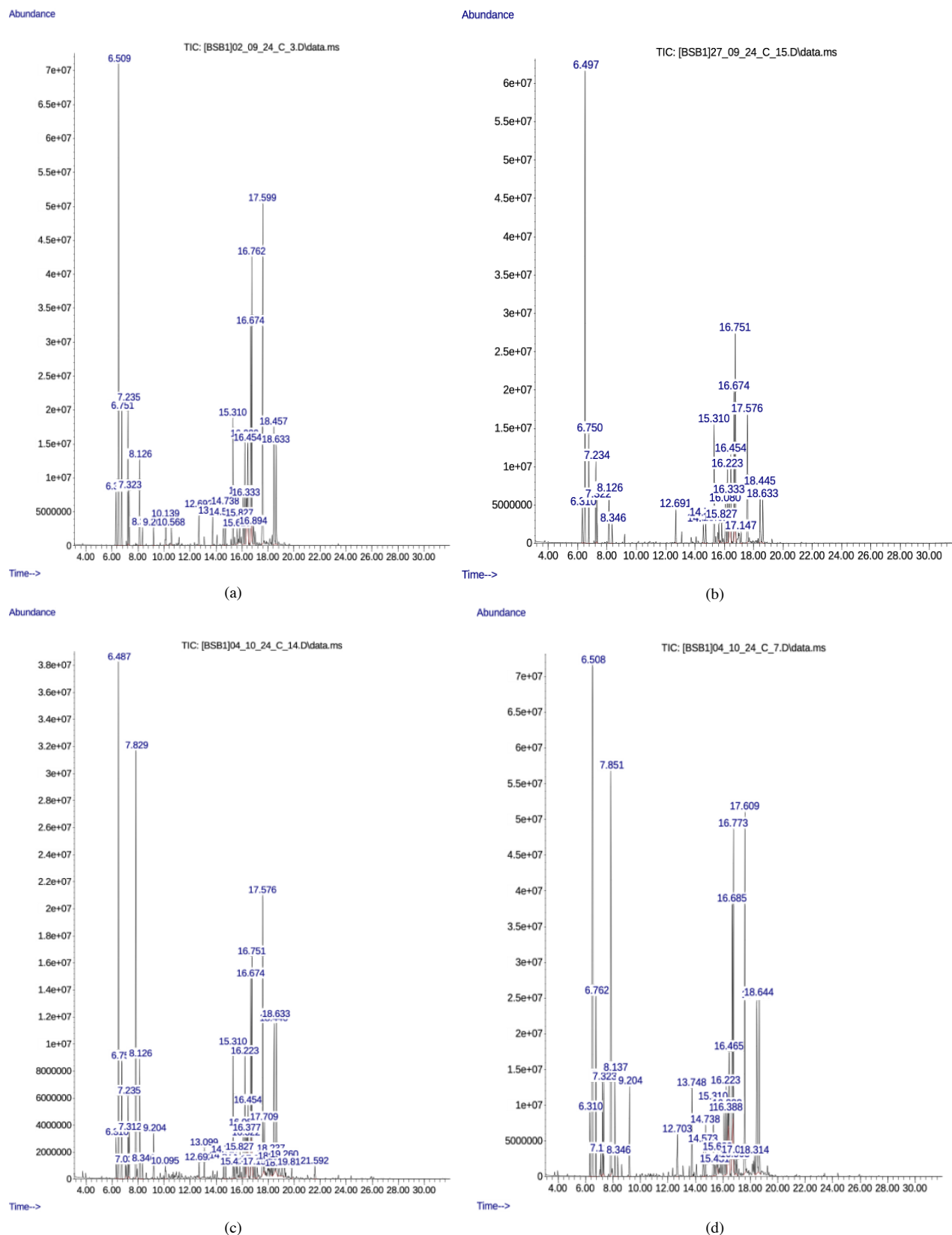


Fig. 3: The chromatographic profile of *Pinus sylvestris* L.: (a) P3 – Satpayev city, (b) P15 – Karaganda city, (c) P14 – Balkhash city, (d) P7 – Karkaraly city

Compound Distribution Analysis: Compounds such as α -pinene, 1R- α -pinene, τ -cadinol, and camphene were

consistently present, indicating their essential role in the *Pinus sylvestris* L. volatile profile. P9 and P15

(Karaganda) showed the richest compositions.

Notable Individual Compounds

4-Carene: Detected in small amounts in P2 and P9. This compound has a fresh, citrus-like odor that contributes to fragrance complexity.

3-Carene: Found at high concentrations in P5, P7, and P10. It has a sweet, pine-like aroma and is a key aromatic compound.

Bicyclo Germacrene: Present in P6 and P10. In addition to its balsamic, earthy scent, it has been investigated for potential antimicrobial and anti-inflammatory properties, though further validation is required.

Limonene: Detected abundantly in P7 and P12. It is known for its citrus scent as well as its antioxidant and gastroprotective activities, making it pharmacologically relevant.

Caryophyllene Oxide: Found in P1, P4, and P13. Apart from its woody, spicy fragrance, it has shown antifungal and cytotoxic effects in previous research, indicating medicinal potential.

Additional Findings

Bornyl Acetate: Found in P1 and P4, contributing a sweet pine aroma. (-)-Spathulenol: Detected in P1 and P9. It has a woody scent and notable antimicrobial potential. β -Phellandrene: Present in P6, adding a light, slightly citrusy fragrance.

Rare Compounds: Borneol, myrcene, and γ -terpinene appeared in limited samples but contributed to the complexity of the overall aroma profile. These compounds may also possess auxiliary bioactivities, meriting further study.

Ecological Implications

The observed variation in essential oil composition among *Pinus sylvestris* L. populations may reflect underlying environmental stressors such as air pollution, drought, or soil nutrient imbalances. Scots pine is known to respond metabolically to ecological pressures, often by altering the biosynthesis of secondary metabolites such as terpenes. For example, elevated levels of caryophyllene oxide and τ -cadinol in certain populations may be associated with oxidative stress responses or defense against microbial threats. Such biochemical profiles support the use of *Pinus Sylvestris* as a potential bioindicator species for assessing forest ecosystem health in industrial or climatically sensitive regions of Central Kazakhstan.

Comparison with Literature

Our findings are in alignment with the chemical profiles previously reported by Kupcinskiene *et al.* (2008) and Basholli-Saliho *et al.* (2017) both of which

emphasized the predominance of monoterpenes and sesquiterpenes in the essential oils of *Pinus sylvestris*. This chemical pattern has been further corroborated by more recent investigations, including those by (Erdyneeva *et al.*, 2022), (Cruz-Martins *et al.*, 2021), (Salehi *et al.*, 2019), and (Borges *et al.*, 2022), which collectively confirm the consistent occurrence and biological significance of key terpenoid constituents such as α -pinene, β -pinene, camphene, and caryophyllene across various geographic regions.

In addition to their chemical prevalence, these compounds are integral to the organoleptic characteristics of *P. sylvestris* oils, contributing to their distinctive woody, spicy, and earthy aroma profiles (Ankney *et al.*, 2022). From a pharmacological perspective, these terpenes have attracted considerable interest due to their potential therapeutic effects. For instance, caryophyllene has been identified as exhibiting notable anti-inflammatory and analgesic properties, while camphene is recognized for its antimicrobial activity (Judzentiene & Kupcinskiene, 2008; Allenspach *et al.*, 2020; Njoku *et al.*, 2022). The presence of (-)-spathulenol in samples from certain regions may suggest antimicrobial and antifungal potential, as previously reported in the literature. However, no bioactivity assays were conducted in this study, and therefore such therapeutic claims should be considered preliminary until experimentally validated.

The variations observed in the composition of essential oils from different geographical locations underscore the influence of environmental factors on the biosynthesis of these compounds and the chemotypic diversity of *Pinus sylvestris* populations in Central Kazakhstan.

Conclusion

This research provides a comprehensive analysis of the phytochemical composition of Scots pine needles collected from various locations across Central Kazakhstan. The findings confirm the ecological and pharmacological significance of this species due to its diverse profile of bioactive compounds, especially monoterpenes such as α -pinene, β -pinene, camphene, and τ -cadinol. The application of hydrodistillation followed by GC-MS analysis enabled the reliable identification of over 25 compounds, with α -pinene consistently dominating across most sampling sites.

The comparative assessment of 15 populations revealed that the chemical profiles of the essential oils are significantly influenced by geographic and environmental factors, particularly those linked to anthropogenic pressure. Sites located in heavily industrialized zones such as Zhezkazgan, Satpaev, Temirtau, and Karaganda cities exhibited variations in essential oil yield and compound composition, suggesting a clear correlation between local pollution levels and secondary metabolite biosynthesis.

Conversely, samples from relatively preserved or protected areas like Karkaraly and Ulytau Mountains demonstrated more balanced or diverse chemical profiles, although signs of ecological stress were still apparent.

These findings underscore the utility of Scots pine as a bioindicator species in monitoring air and soil pollution, as well as its potential value in the pharmaceutical and cosmetic industries. Furthermore, the study contributes to filling a significant knowledge gap concerning the chemical ecology of pine species in the Central Asian region, especially in under-researched territories such as Central Kazakhstan. Future studies should expand the range of phytochemical investigations to include other classes of compounds (e.g., phenolics and flavonoids) and explore seasonal variations, long-term ecological impacts, and the functional role of identified compounds in plant adaptation mechanisms.

Ultimately, the obtained results not only highlight the ecological plasticity of *Pinus sylvestris*, but also demonstrate the pressing need for integrating environmental monitoring with phytochemical analysis in regions undergoing intensive industrial development. The finding indicate that *Pinus sylvestris* essential oils may have promising applications in pharmaceutical, cosmetic, and fragrance industries. To further explore the ecological drivers of this chemical diversity, future research should incorporate correlation analysis with environmental parameters and apply multivariate statistical techniques such as PCA or cluster analysis.

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Author's Contributions

Kuralay Tuleshova: Concept, manuscript writing, critical revision of the manuscript, assessment, data collection.

Almagul Kali: Analysis, manuscript writing, data interpretation, identification of species, collecting of pine material, final approval.

Margarita Ishmuratova: Data interpretation, collection of pine material.

Daniyar Sadyrbekov: Extracting components from plants.

Assanali Ainabayev: Providing GC-MS analysis and identification of components.

Ethics

The authors confirm that there are no conflicts of interest related to the publication of this article. All authors have reviewed and approved the final version of the manuscript. This study did not involve human participants or animal experiments.

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