BIODIVERSITAS
Volume 26, Number 7, July 2025
Pages: 3343-3355

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260726

Diversity and ecological determinants of essential oil composition of *Achillea millefolium* from various geobotanical areas of Samarkand, Uzbekistan

MOHIGUL ISTAMKULOVA[1,♥], KHUDOYOR KELDIYAROV[1], ASADULLO DAMINOV[2], NURALI MUKHAMADIEV[1], GAVHAR DUSHANOVA[1], DAVRON KHAYITOV[1], YUNUS RUZIEV[1], ISKANDAR RAYIMQULOV[2], ROKHILA JURAEVA[3], UMIDA NORBOYEVA[4]

[1]Samarkand State University named after Sharof Rashidov. University Boulevard 15, 140104 Samarkand, Uzbekistan. Tel.: +998-66-2391305, [♥]email: moxistamkulova@gmail.com

[2]Samarkand State University of Veterinary Medicine, Livestock and Biotechnologies. Mirzo Ulugbek St. 77, 140103 Samarkand, Uzbekistan

[3]Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan. Tashkent, Shaykhantakhur District, 7 B100128, Uzbekistan

[4]Department of Ecology and Geography, Bukhara State University. Mukhammad Iqbol St. 11, 705018 Bukhara, Uzbekistan

Manuscript received: 21 April 2025. Revision accepted: 10 July 2025.

Abstract. *Istamkulova M, Keldiyarov K, Daminov A, Mukhamadiev N, Dushanova G, Khayitov D, Ruziev Y, Rayimqulov I, Jurayeva R, Norboyeva U. 2025. Diversity and ecological determinants of essential oil composition of* Achillea millefolium *from various geobotanical areas of Samarkand, Uzbekistan. Biodiversitas 26: 3343-3355. Achillea millefolium* is a well-known medicinal plant, widely distributed in Asia, Europe and North America. It is extensively used in traditional medicine and is valued for its broad spectrum of therapeutic properties. One of the key elements of its pharmacological value is its essential oil, which exhibits antimicrobial, antiparasitic, antioxidant, and other properties. This study explores the variation in the chemical composition of essential oils extracted from *A. millefolium* flowers collected in four distinct geobotanical areas of the Samarkand region, Uzbekistan: Amonkutan, Korabulok, Ilonsoy and Sazagan. Essential oils were obtained using the hydrodistillation method and their compositions were analyzed using gas chromatography-mass spectrometry. The results revealed significant qualitative and quantitative differences in essential oil compositions, primarily influenced by environmental factors. Essential oil variation reflects ecotypic local adaptation, highlighting its relevance for biodiversity and conservation efforts. These findings emphasize the influence of environmental factors such as altitude, precipitation, and soil pH on the chemotypic variability of *A. millefolium*. A total of 48 compounds were identified, with region-specific dominance: Ascaridole (20.43%) in Sazagan, Terpinolene (20.06%) in Amonkutan, m-Cymene (21.05%) in Korabulok, Camphor (2.86%) and 2-Carene (26.33%) in Ilonsoy. These constituents appear to play key roles in plant adaptation to local stress conditions. The identified relationships between environmental factors and the composition of plant essential oils enable the differentiation of chemotypes based on geographic characteristics and provide a basis for developing recommendations on optimal cultivation conditions, selective cultivation programs and standardization of raw materials for pharmacological applications.

Keywords: *Achillea millefolium*, biological activity, chemical components, environmental variability, hydrodistillation

INTRODUCTION

Medicinal plants refer to species used in traditional or modern medicine for preventing and treating diseases in humans and animals (Khojimatov et al. 2023; Shukla et al. 2025). Various plant parts—leaves, flowers, buds, fruits, seeds, bark, roots, rhizomes, tubers, and bulbs—serve as medicinal raw materials (Salehi et al. 2020; Khojakulov et al. 2024; Bussmann 2025). For preparing tinctures, extracts, and phytopreparations, plant parts with the highest concentrations of bioactive compounds are typically selected (Khojimatov et al. 2023; Ranasinghe et al. 2023).

Environmental conditions strongly influence the biosynthesis of secondary metabolites by regulating biochemical pathways at the cellular level (Ilieva 2020; Ruziev et al. 2024). In light of climate change and environmental degradation, understanding how external factors affect essential oil composition is critical, given their wide applications in pharmaceuticals, cosmetology, agriculture, and veterinary medicine (Štrbac et al. 2020; Guzmán et al. 2021; Angane et al. 2022; Dalili et al. 2022). Studying the impacts of soil, climate, and topography on bioactive compounds such as terpenes helps identify plants with specific chemical profiles and reveals adaptation strategies (Kurchenko et al. 2019; Istamkulova et al. 2024b; Losapio et al. 2024).

Soil composition plays a crucial role. Acidic, neutral, or alkaline soils vary in mineral availability, influencing metabolic activity. For instance, nitrogen deficiency reduces amino acid synthesis, whereas excess nitrogen may promote the accumulation of phenolics or alkaloids with antiparasitic or antioxidant properties (Angelova et al. 2023; Vakhidova et al. 2024). Soil moisture also affects metabolism; drought induces lipids and phytohormones regulating water balance. Pollution from heavy metals and pesticides triggers antioxidant production and other defense-related secondary metabolites (Istamkulova et al. 2024b).

Stress adaptation enhances survival by triggering the production of protective compounds like essential oils. The essential oils key products of secondary metabolism, mediate responses to environmental factors (El-Kalamouni et al.

2017; Howyzeh et al. 2019) and contribute to chemotypic variation, driving ecological and metabolic diversity in plant populations (Istamkulova et al. 2024a; Farhadi et al. 2020).

Essential oils from aromatic plants have garnered increasing interest due to their pharmacological properties—antibacterial, antiparasitic, antiviral, antioxidant, and more (Nazir et al. 2022). Within this context, the genus *Achillea* has drawn significant scientific attention. This genus in the Asteraceae family includes numerous perennial species distributed across Europe, Asia, and North America (Kaffash et al. 2020; Sharova et al. 2021; Toplan et al. 2022; Güçlü et al. 2023).

Achillea millefolium L., the best-known species in the genus, is widely used for its therapeutic potential. Its essential oil demonstrates antimicrobial, anti-inflammatory, antioxidant, and wound-healing activities (Mohammadhosseini et al. 2017; Farhadi et al. 2020; Yener et al. 2020; Stojanović et al. 2022; Noedoost et al. 2024). The species shows broad ecological tolerance, growing in habitats from lowland meadows to mountain slopes. This adaptability leads to significant ecotypic variation in essential oil composition.

Environmental factors—altitude, soil pH, and precipitation—affect the biosynthesis of terpenoids and oxygenated compounds in Asteraceae species (Güçlü et al. 2023; Noedoost et al. 2024). Higher altitudes often correlate with increased monoterpene production, while lowland habitats favor complex sesquiterpenes (Nazir et al. 2022; Das et al. 2023; Losapio et al. 2024). Stressful conditions such as drought, high temperature, solar radiation, pollution, and nutrient-poor soils activate biosynthetic pathways related to essential oil production and defense (Farhadi et al. 2020).

Understanding this chemical diversity is essential for biodiversity conservation, resource management, and identifying potential phytochemical applications. In this context, analyzing essential oils of *A. millefolium* across ecological gradients in the Samarkand region provides scientific and applied insights (Istamkulova et al. 2021, 2023). Observed variations may reflect local adaptation to diverse environmental conditions including altitude, drought severity, soil chemistry, and mineral availability (Farhadi et al. 2020; Istamkulova et al. 2024b).

This study aims to investigate the diversity of essential oil composition in *A. millefolium* flowers collected from multiple geobotanical zones in Samarkand and to assess the environmental factors influencing the biosynthesis of bioactive compounds. The objective is to clarify adaptation mechanisms and evaluate the species' potential applications in medicine, phytotherapy, cosmetology, and veterinary practices. The novelty lies in this being the first comprehensive comparative study of *A. millefolium* essential oils from distinct geobotanical areas of Samarkand, thus addressing a significant regional phytochemical knowledge gap.

MATERIALS AND METHODS

Study area

Samarkand region is an important economic and cultural area of the Republic of Uzbekistan, located in the central part of the country. According to its natural and climatic conditions, the territory belongs to a zone of continental arid climate. Winters are relatively mild, with an average January temperature of -2°C in the northern part of the region, 1.9°C in Samarkand city and -4.8°C in the mountainous areas. The average temperature in July is +26.7°C in Samarkand and may reach up to +32+38°C in the southwestern part of the region (Tojibaev et al. 2018). The research is conducted in different geobotanical areas of the Samarkand region, Uzbekistan (Figure 1).

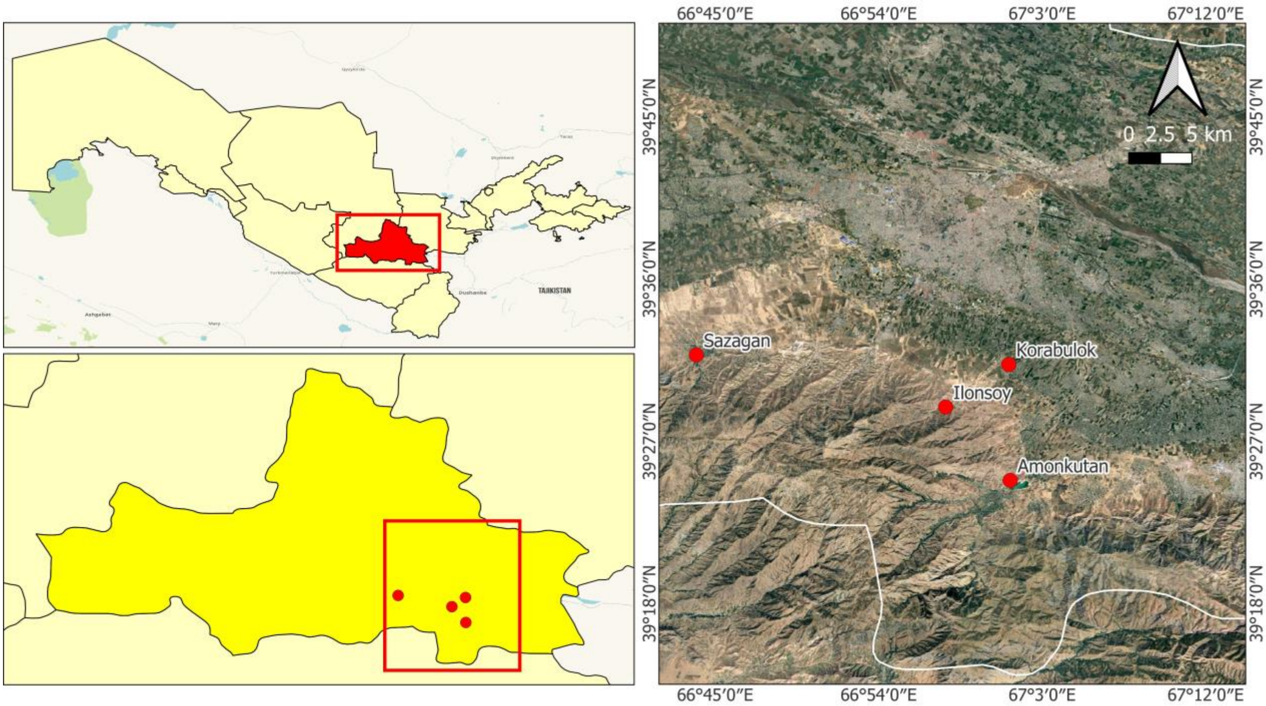

Figure 1. Map of the study area in the Samarkand Region, Uzbekistan

The average annual precipitation in the study areas up to 355 mm (Tojibaev et al. 2018) and the soil types vary from gravelly rocky in mountainous zones to moderately fertile gray soils in the lowlands. Soil pH values range from 7.2 to 8.2 (Khojimatov et al. 2023) with elevation spanning from 833 m to 950 m above sea level (Table 1). These conditions influence water availability, mineral content and microclimatic stability, potentially affecting the biosynthesis of essential oils.

This study was conducted using *A. millefolium*, a widely distributed species of the genus *Achillea*. Variation in the composition and properties of the plant in different geographical areas of the Samarkand region was analyzed by collecting plant material from sites with distinct climatic and soil conditions (Table 1).

The ecological description and soil characteristics of the sampling sites were determined based on field observations and supplemented with data from topographic maps. The sampling sites are located in arid regions, where the average annual precipitation up to 355 mm and the soil pH ranges from neutral to alkaline (7.2-8.2) (Tojibaev et al. 2018; Khojimatov et al. 2023).

Plant material

To investigate the diversity of essential oil composition in *A. millefolium* flowers, fresh plant samples were collected during May-June, from different geobotanical areas of the Samarkand region, where the species grows under varying environmental conditions (Figure 2).

At each collection site, three biological samples were gathered and two technical measurements were conducted per sample to guarantee the reliability and consistency of data. The analysis revealed significant variations in quality as well as quantity of essential oil components.

Essential oil isolation

Essential oils were isolated from fresh *A. millefolium* flowers collected from different areas of the Samarkand region by hydrodistillation using a Clevenger-type apparatus (Karami et al. 2022). Hydrodistillation is one of the most traditional and widely used methods for obtaining essential oils. This method is particularly suitable for extracting high-boiling compounds that are insoluble in water. Due to its simplicity and efficiency, hydrodistillation is especially appropriate for heat-stable essential oils.

Table 1. Biogeographical characteristics of the collection sites

Collection site	Climate	Soil type	Altitude (m asl.)	Geographical coordinates	
				Latitude	Longitude
Amonkutan	Arid, mountainous, with sharp temperature changes	Rocky, gravelly	945	39°24′50.62″	67°01′14.93″
Korabulok	Sharply continental, with hot summers	Gray soil, moderately fertile	833	39°31′16.12″	67°01′10.24″
Ilonsoy	Semi-arid	Light gray soil (Bozsor), moderately fertile	950	39°28′54.51″	66°57′41.74″
Sazagan	Arid	Rocky, gravelly	945	39°31′48.01″	66°43′58.13″

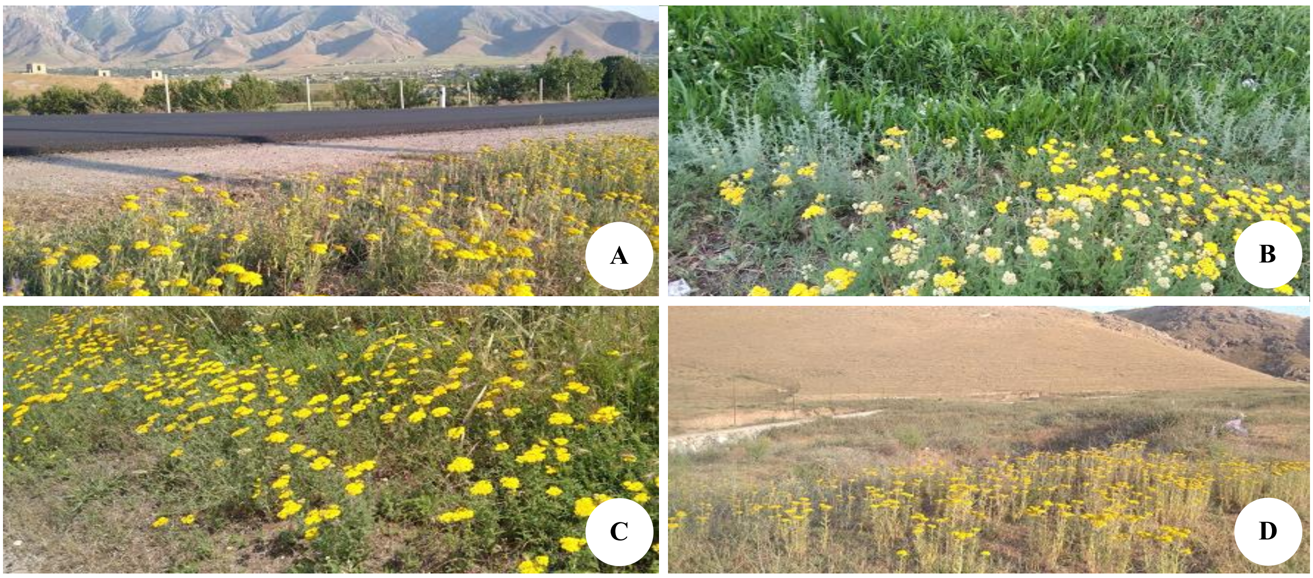

Figure 2. *Achillea millefolium* L. growing under different conditions. A. Amonkutan, B. Korabulok, C. Ilonsoy, D. Sazagan

In this hydrodistillation process, 500 g of fresh flower material were immersed in 1000 mL of water and additionally exposed to externally supplied steam. The water at the bottom of the flask was brought to a boil, producing steam that passed through the plant material and carried the volatile components upwards. The steam enables vaporization of essential oil components at temperatures below their normal boiling points, thereby preventing thermal degradation of the oil. The steam mixture containing the essential oil components was directed to a condenser, where it was cooled and condensed into two separate layers: the essential oil and the aqueous phase, known as hydrosol. The essential oil was carefully separated from the hydrosol, transferred to dark glass vials and stored at +4°C until further analysis. The hydrodistillation process lasted from 4 to 8 hours (Efremov 2013; Istamkulova et al. 2024a).

GC-MS analysis

Gas chromatography with mass spectrometry is chosen to investigate the composition of the essential oil (Saleh 2023; Istamkulova et al. 2024b). The study was conducted using an Agilent 8890 GC gas chromatograph equipped with both split and splitless injection modes, operated in conjunction with an Agilent 5977B Series GC-MS system in SCAN mode with electron impact ionization. In order to prepare a sample for analysis, dilution of the essential oil with hexane was used.

GC-MS analytical process

The sample is introduced into the chromatograph's injector, where it is instantly vaporized, mixed with an inert carrier gas and delivered under pressure into the column. As the sample passes through the chromatographic column, it is separated into individual components, which further transferred to the mass spectrometer and analyzed by its spectrometric unit. To obtain the spectrum, the molecules of the sample components are ionized and a specialized detector records changes in the ion current, based on data a chromatogram is generated. Chromatogram processing software allows the resulting peaks to be compared with previously registered spectra, enabling precise qualitative and quantitative identification of the components.

Chromatography conditions

Column thermostat temperature - initial - 60°C 3 min (isothermal mode); heating at a rate of 15°C/min (temperature programming mode) up to 250°C and at 250°C (isothermal mode) 3 min. Injector temperature - 250°C, helium gas flow -1 mL/min, SplitRatio -1/100. Detector mass parameters - solvent delay-3 min, emission-50mA, scanning range -30-350 a.u.m., scanning speed-1600 a.u.m./sec, ion source temperature - 230°C, transfer temperature - 280°C. Time of analysis - 21min. The components were identified by comparing the mass spectra obtained with the NIST mass spectra library and by retention time and quantified by internal normalization method (Novak 2021).

Data analysis

Data analysis was performed using standard botanical protocols, including the assessment of external morphological traits and comparative evaluation of ecological site characteristics. Statistical processing was conducted using Excel 2019 and STATISTICA v.10 software. Parametric differences among sampling sites were evaluated using Student's t-test, with statistical significance considered at $p<0.05$.

RESULTS AND DISCUSSION

Chemical diversity of essential oils from *Achillea millefolium*

The essential oils extracted from the flowers of *Achillea millefolium* collected in four areas of the Samarkand region - Amonkutan, Korabulok, Ilonsoy, Sazagan were analyzed. Chromatograms of the oils (Figures 3-6) and the identified components (Tables 2-5) demonstrate notable variation in chemical composition depending on the collection site. Key constituents such as Terpinolene, m-Cymene and Ascaridole were found in all areas, albeit in differing concentrations.

Influence of ecological factors on essential oil composition

Environmental conditions—such as altitude, humidity, and soil pH have a significant impact on the qualitative and quantitative profile of *A. millefolium* essential oils (Table 6). Several key correlations were identified. A strong positive correlation was observed between altitude and γ-Terpinene content ($r \approx 0.85$), indicating that higher elevations are associated with increased levels of γ-Terpinene. Conversely, a moderate negative correlation between soil pH and Ascaridole content ($r \approx -0.70$) suggests that more alkaline soils lead to a reduction in Ascaridole concentration. Additionally, a positive correlation was found between precipitation and m-Cymene content ($r \approx 0.65$), meaning that wetter conditions favor higher levels of m-Cymene. In contrast, altitude showed no significant correlation with β-Thujone levels ($r \approx 0.10$), implying a negligible relationship. Finally, a negative correlation between precipitation and Sabinene content ($r \approx -0.68$) was established, suggesting that drier environments are associated with higher Sabinene concentrations (Figure 7). These findings underscore the critical role of ecological parameters in shaping the essential oil composition of the studied plant populations.

Considering the identified ecological correlations is essential for selecting optimal conditions for harvesting plant material intended for pharmacognostic studies and phytochemical analysis.

Comparative analysis of common and unique components

Based on the data obtained, a comparative analysis was carried out to evaluate the chemical composition of essential oils extracted from *A. millefolium* collected in the four studied areas-Amonkutan, Korabulok, Ilonsoy and Sazagan. Table 7 presents the common and unique components, along with their percentage content, in relation to the habitat-specific environmental conditions. According to the data presented in Table 7, several chemical components were identified across all collection sites: m-Cymene, Ascaridole, Terpinolene, Camphor, Terpinen-4-ol and Linalool. The concentrations of these components vary depending on the region. For instance, Terpinolene was most abundant in Amonkutan, Korabulok and Ilonsoy. Ascaridole had highest value in Sazagan 20.43%. m-Cymene was a dominant constituent in all areas, ranging from 12.62% in Ilonsoy to 21.05% in Korabulok. Camphor was also detected in all regions, with concentrations ranging from 1.06% in Sazagan to 2.86% in Ilonsoy. Terpinen-4-ol was present in all samples, with concentrations ranging from 2.02% in Ilonsoy to 2.96% in Sazagan. The presence of Linalool was consistent in all studied samples. Moreover, analysis of the essential oil samples revealed the presence of Tetrachloroethylene and Bis(2-ethylhexyl) terephthalate. These organic compounds are not typically found in plants under natural conditions and their detection may indicate anthropogenic contamination. This necessitates further investigation to identify the sources and mechanisms of accumulation.

The essential oil composition of *A. millefolium* includes both common and unique components depending on the habitat. Common compounds such as m-Cymene, Ascaridole, Terpinolene, Camphor, Terpinen-4-ol and Linalool were detected in all studied regions. Unique compounds such as 2-Carene, Germacrene D, Nerolidol and Eugenol were present only in certain samples (Figure 7).

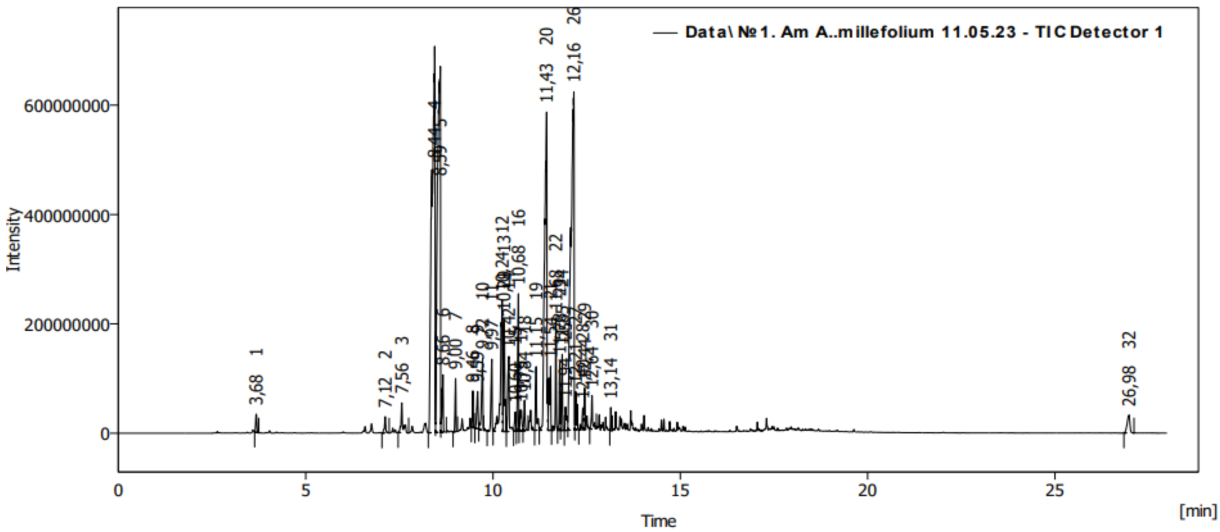

Figure 3. Chromatogram of the essential oil of *Achillea millefolium* collected from Amonkutan

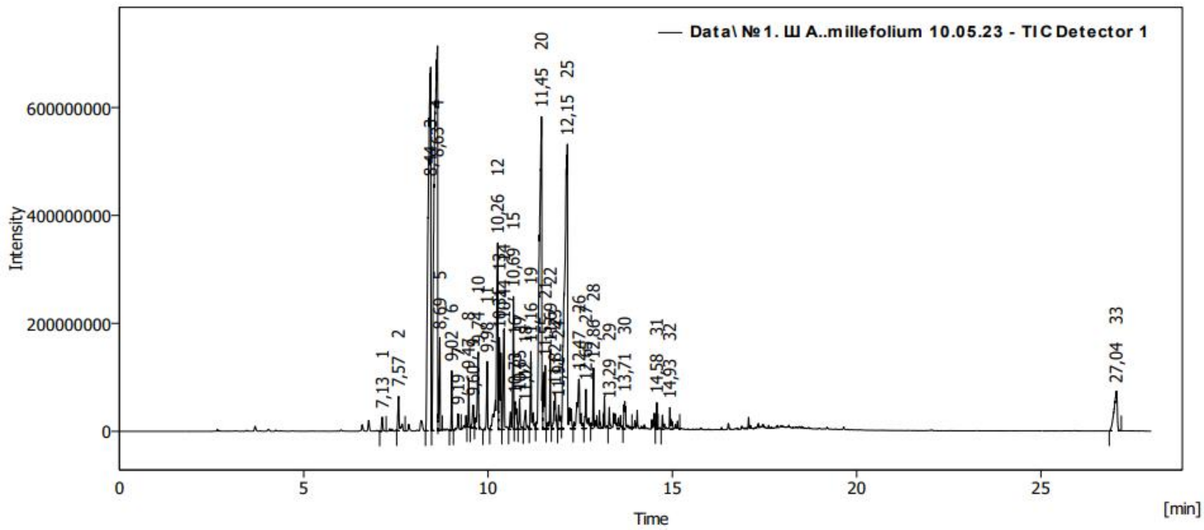

Figure 4. Chromatogram of the essential oil of *Achillea millefolium* collected from Korabulok

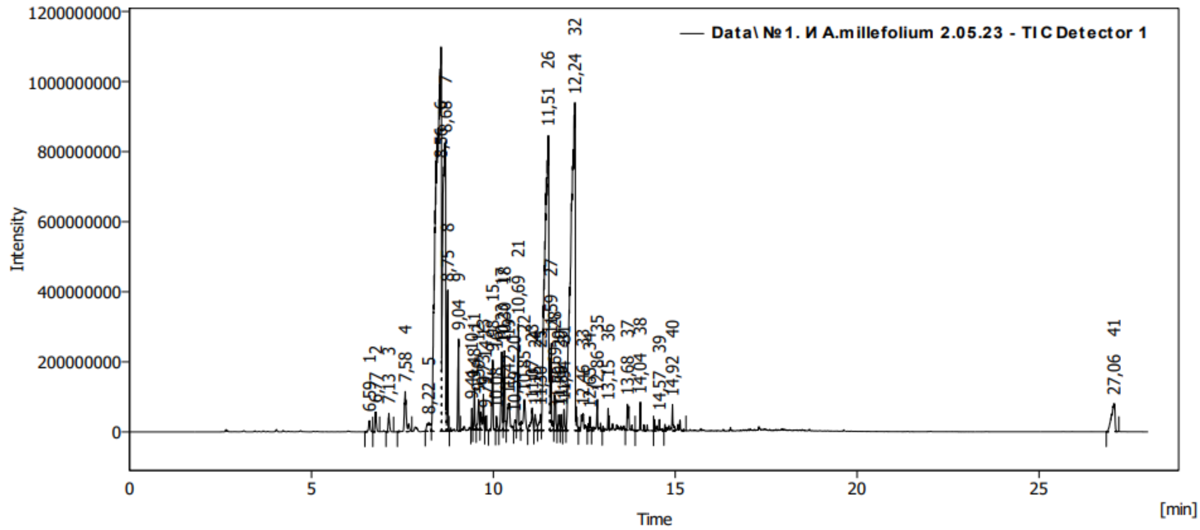

Figure 5. Chromatogram of the essential oil of *Achillea millefolium* collected from Ilonsoy

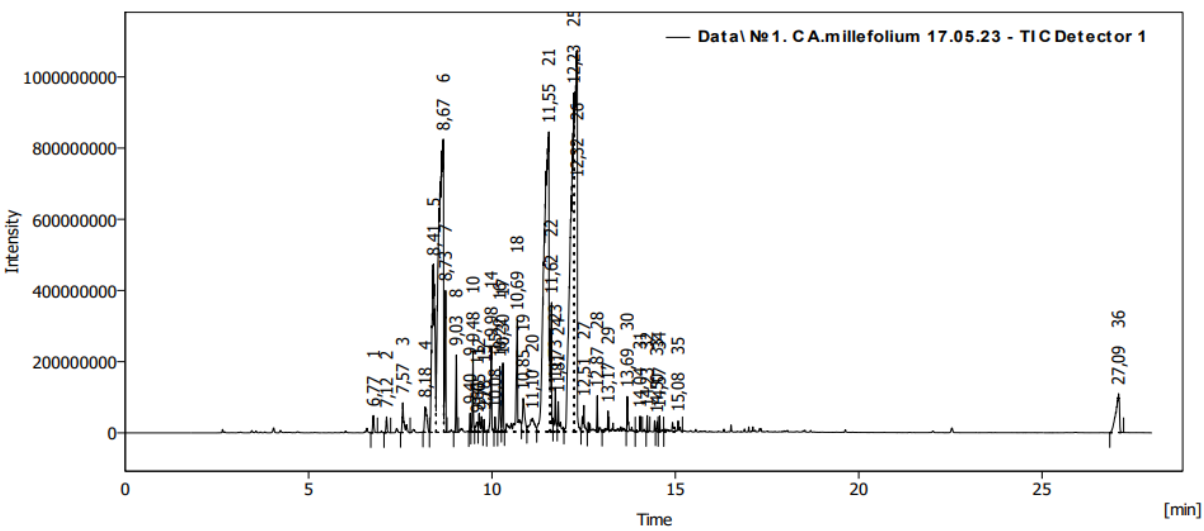

Figure 6. Chromatogram of the essential oil of *Achillea millefolium* collected from Sazagan

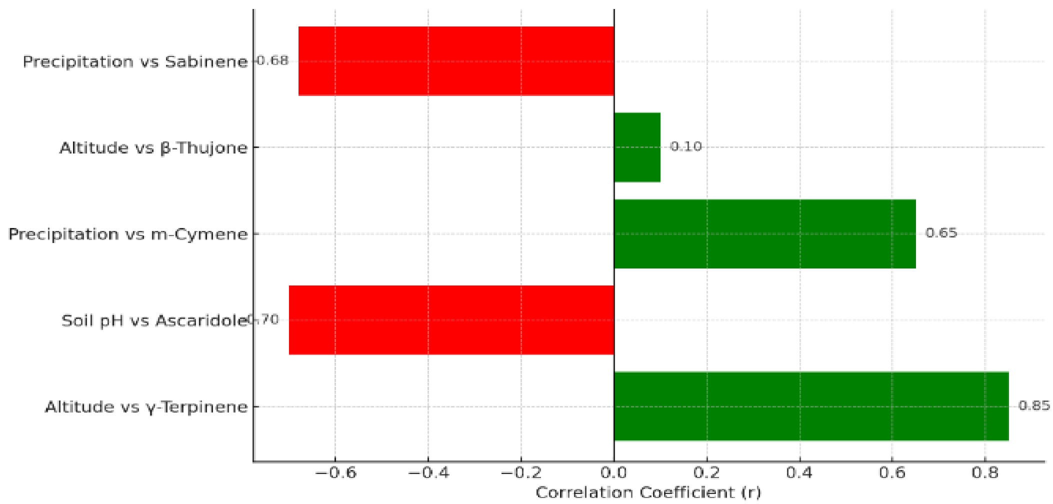

Figure 7. Correlation between environmental factors and essential oil components

Table 2. Component content of *Achillea millefolium* essential oil collected from Amonkutan

Component name	Retention time (min)	Content (%)
Tetrachloroethylene	3.680	0.35
Camphene	7.124	0.34
Sabinen	7.564	0.88
Terpinolene	8.442	20.06
m-Cymene	8.593	18.41
Eucalyptol	8.665	1.16
γ-Terpinene	9.001	0.76
p-Cymenene	9.464	0.49
Linalool	9.589	0.82
m-Cymene	9.722	1.55
2-p-Menthen-1-ol	9.969	1.34
cis-p-Mentha-2,8-dien-1-ol	10.245	4.24
Camphor	10.295	2.52
Lavandulol	10.424	1.85
(-)-cis-Carveol	10.596	0.36
Terpinen-4-ol	10.678	2.33
Benzenemethanol, α, α,4-trimethyl-	10.754	0.69
trans-Piperitol	10.843	0.59
Ascaridole	11.151	1.25
Ascaridole	11.431	12.46
Ascaridole	11.538	1.69
Lavandulyl acetate	11.682	1.53
Bornyl acetate	11.782	0.85
3-Hexyne-2,5-diol, 2,5-dimethyl-	11.847	1.16
Carvacrol	11.936	0.57
Ascaridole	12.158	17.87
2(1H)-Naphthalenone, octahydro-8a-hydroxy-4a-methyl-, cis-	12.208	0.73
Ascaridole	12.402	0.45
Jasmone	12.445	0.99
cis-p-Mentha-2,8-dien-1-ol	12.642	0.66
Caryophyllene	13.144	0.26
Bis(2-ethylhexyl) terephthalate	26.983	0.80
	Total	100.00

Table 3. Component content of the essential oil of *Achillea millefolium* collected from Korabulok

Component name	Retention time (min)	Content (%)
Camphene	7.131	0.29
Sabinen	7.572	0.80
Terpinolene	8.442	15.81
m-Cymene	8.629	21.05
Eucalyptol	8.693	1.46
γ-Terpinene	9.016	0.70
Hexanoic anhydride	9.188	0.28
p-Cymenene	9.474	0.61
Linalool	9.600	0.55
m-Cymene	9.736	1.63
2-p-Menthen-1-ol	9.976	1.14
cis-p-Mentha-2,8-dien-1-ol	10.263	4.89
Camphor	10.309	2.51
Lavandulol	10.438	2.11
Terpinen-4-ol	10.689	2.22
cis-p-Mentha-2,8-dien-1-ol	10.732	0.75
trans-Piperitol	10.854	0.56
trans-Piperitol	11.019	0.56
Ascaridole	11.162	1.55
Ascaridole	11.449	12.31
Ascaridole	11.553	1.66
Lavandulyl acetate	11.689	1.04
Dimethylhexynediol	11.821	1.05
Carvacrol	11.915	0.58
Ascaridole	12.151	14.09
Limonene dioxide	12.470	2.12
cis-p-Mentha-2,8-dien-1-ol	12.653	0.96
Jasmone	12.861	1.34
Globulol	13.291	1.19
Linalyl isovalerate	13.710	0.88
Caryophyllene oxide	14.581	0.29
Rosifoliol, (+)-	14.932	0.94
Bis(2-ethylhexyl) terephthalate	27.043	2.08
	Total	100.00

Geographical and ecological characteristics of the sampling sites

Below is a description of the ecological and chemical characteristics of the sample collection sites: In Amonkutan, Urgut district, Samarkand region, the climate is arid and mountainous with sharp temperature fluctuations. The slopes are steep (35-40°) and the mid-mountain soil is stony and rubbly. High levels of Camphene, γ-Terpinene and Terpinolene were recorded. Tetrachloroethylene 0.35% was also detected, which may be linked to the local soil characteristics.

In Korabulok, Samarkand district, Samarkand region, the climate is sharply continental with hot summers. The soil is gray soil and moderately fertile. Terpenes were moderately abundant, while Camphor, Eucalyptol, γ-Terpinene, m-Cymene 21.05% and Caryophyllene oxide 0.29% were prominent. Unique compounds such as Limonene dioxide, Hexanoic anhydride and Dimethylhexynediol were also identified and may be related to soil composition. In Ilonsoy, Samarkand district, the climate is semi-arid and less extreme than in Sazagan. The area has a moderate slope (~10°) and light gray soil that is moderately fertile. Linalool and Eucalyptol were detected in higher concentrations.

In Sazagan, Eyrugul village, Nurobod district, Samarkand region, the climate is arid with sharp daily temperature fluctuations. The site is located on medium-high mountain slopes (around 40°) with stony, rubbly soil. The highest concentration of Ascaridole 20.43% was recorded in this region. The observed variations may reflect local adaptation, indicating ecotypic responses to diverse environmental factors (Figure 8).

Table 4. Component content of the essential oil of *Achillea millefolium* collected from Ilonsoy

Component name	Retention time (min)	Content (%)
α-Thujene	6.593	0.21
(+)-α-Pinene	6.765	0.36
Camphene	7.127	0.31
β-Phellandrene	7.575	1.05
α-Thujene	8.220	0.40
2-Carene	8.561	26.33
M-Cymene	8.682	12.62
Eucalyptol	8.747	1.51
γ-Terpinene	9.044	1.12
Terpinolene	9.406	0.19
p-Cymenene	9.478	0.67
Linalool	9.593	0.55
M-Cymene	9.725	0.70
1,3,8-p-Menthatriene	9.786	0.13
2-p-Menthen-1-ol	9.983	1.13
1,3,8-p-Menthatriene	10.084	0.19
cis-Sabinene hydrate	10.230	1.40
Camphor	10.302	2.86
Lavandulol	10.424	0.95
(+)-Sabinol	10.589	0.23
Terpinen-4-ol	10.689	2.02
cis-Piperitol	10.854	0.95
trans-Piperitol	11.065	0.57
Ascaridole	11.151	0.35
Ascaridole	11.298	0.39
Ascaridole	11.510	15.39
Ascaridole	11.592	2.27
Lavandulyl acetate	11.685	0.61
Bornyl acetate	11.793	0.25
Safranal	11.861	0.18
cis-p-Mentha-2,8-dien-1-ol	11.943	0.31
Ascaridole	12.241	18.93
Jasmone	12.463	0.61
cis-p-Mentha-2,8-dien-1-ol	12.653	0.23
Jasmone	12.861	0.57
Caryophyllene	13.154	0.27
β-Cubebene	13.685	0.58
Terpenyl propionate	14.040	0.37
Caryophyllene oxide	14.566	0.14
β-Eudesmole	14.925	0.75
Bis (2-ethylhexyl) terephthalate	27.061	1.35
	Total	100.00

Table 5. Component content of the essential oil of *Achillea millefolium* collected from Sazagan

Component name	Retention time (min)	Content (%)
(+)-α-Pinene	6.765	0.28
Camphene	7.124	0.25
β-Phellandrene	7.568	0.69
α-Thujene	8.177	0.80
Terpinolene	8.406	6.78
M-Cymene	8.672	18.82
Eucalyptol	8.733	1.94
γ-Terpinene	9.026	0.84
Terpinolene	9.403	0.19
1-Methyl-4-(prop-1-en-2-yl) benzene	9.482	0.89
Linalool	9.600	0.28
Nonanal	9.646	0.47
1,3,8-p-Menthatriene	9.783	0.15
2-p-Menthen-1-ol	9.983	1.33
1,3,8-p-Menthatriene	10.084	0.19
2-p-Menthen-1-ol	10.216	0.98
Camphor	10.299	1.06
(-)-Terpinen-4-ol	10.689	2.96
cis-Piperitol	10.850	1.02
trans-Piperitol	11.101	0.92
Ascaridole	11.549	20.43
Ascaridole	11.624	1.94
Ascaridole	11.728	0.71
Bornyl acetate	11.807	0.62
Ascaridole	12.233	16.78
Ascaridole	12.323	13.65
Eugenol	12.506	0.54
Jasmone	12.871	0.71
Caryophyllene	13.165	0.28
Germacrene D	13.688	0.51
Terpinyl propionate	14.040	0.17
Nerolidol	14.233	0.14
(+)-Spathulenol	14.502	0.11
Caryophyllene oxide	14.570	0.17
Oxacyclopentadecan-2-one	15.075	0.49
Bis(2-ethylhexyl) terephthalate	27.086	1.93
	Total	100.00

Table 6. The influence of environmental factors on the quantitative composition of essential oil components

Collection site	Alt. (m asl.)	Soil type	Precipitation	Soil pH	Essential oil components (%)				
					γ-Terpinene	m-Cymene	Ascaridole	β-Thujone	Sabinene
Amonkutan	945	Rocky, gravelly	280	7.5	0.76	18.41	17.87	0.58	0.82
Korabulok	833	Gray soil, moderately fertile	300	7.8	0.70	21.05	14.09	0.39	0.49
Ilonsoy	950	Light gray soil moderately fertile	320	8.2	1.12	12.62	18.93	0.25	0.34
Sazagan	945	Rocky, gravelly	340	8.0	0.84	18.82	20.43	0.19	0.22

Table 7. Common and unique components and the highest percentage content in the essential oil of *Achillea millefolium* depending on the habitat conditions

Chemical class	Components	Amonkutan (%)	Korabulok (%)	Ilonsoy (%)	Sazagan (%)	Component status
Terpenes	Camphene	0.34	0.29	0.31	0.25	Common
	Sabinen	0.88	0.80	1.05	0.69	Common
	Terpinolene	20.06	15.81	0.19	6.78	Common
	γ-Terpinene	0.76	0.70	1.12	0.84	Common
	Caryophyllene	0.26	-	0.27	0.28	Unique
	Germacrene D	-	-	-	0.51	Unique
	α-Thujene	-	-	0.21	0.80	Unique
	2-Carene	-	-	26.33	-	Unique
Oxygenated terpenes	Eucalyptol	1.16	1.46	1.51	1.94	Common
	Linalool	0.82	0.55	0.55	0.28	Common
	Terpinen-4-ol	2.33	2.22	2.02	2.96	Common
	Camphor	2.52	2.51	2.86	1.06	Common
	Nerolidol	-	-	-	0.14	Unique
Aromatic compounds	m-Cymene	18.41	21.05	12.62	18.82	Common
	p-Cymenene	0.49	0.61	0.61	-	Unique
	Eugenol	-	-	-	0.54	Unique
Lactones and ketones	Jasmone	0.99	1.34	0.61	0.71	Common
Oxygenated sesquiterpenes	Caryophyllene oxide	-	0.29	0.14	0.17	Unique
Peroxides	Ascaridole	17.87	14.09	18.93	20.43	Common
Other connections	Limonene dioxide	-	2.12	-	-	Unique
	Tetrachloro ethylene	0.35	-	-	-	Unique
	Bis(2-ethylhexyl) terephthalate	0.80	2.08	1.35	1.93	Common

Discussion

The results of this study show (Figure 9) that the chemical composition of *A. millefolium* essential oil varies significantly depending on the environmental conditions of the collection sites. The consistent presences of major compounds such as m-Cymene, Ascaridole, Terpinolene, Camphor, Terpinen-4-ol and Linalool across all samples suggest their stable biosynthesis in the species, regardless of location. These compounds are widely reported in the literature as key contributors to the biological activity of essential oils (Nazir et al. 2022).

The high level of Ascaridole 20.43% in Sazagan may be associated with the plant's adaptation to arid conditions and nutrient-poor, rocky soils. According to published studies, Ascaridole exhibits antiparasitic, antimicrobial and anti-inflammatory properties (Mohammadhosseini et al. 2017; Stojanović et al. 2022), which may suggest its ecological function in stress tolerance.

The accumulation of terpenoids and peroxide compounds, may be stimulated by environmental stressors, including drought, high temperatures and nutrient-poor rocky soils. These conditions are likely to induce oxidative stress responses, thereby promoting the biosynthesis of secondary metabolites that enhance plant survival.

The detection of Tetrachloroethylene at a concentration of 0.35% and the presence of Bis (2-ethylhexyl) terephthalate were also recorded in the essential oil samples. These compounds are not typically found among naturally occurring plant metabolites and have been reported in previous studies as environmental contaminants resulting from industrial activity and plastic degradation (Angelova et al. 2023; Istamkulova et al. 2024a). Their presence in the samples may therefore suggest anthropogenic influence or contamination from surrounding soil, air or water sources. Further investigation is required to confirm the origin of these compounds.

Unique components, including 2-Carene, Germacrene D, Nerolidol and Eugenol, were found only in specific samples, suggesting that localized environmental factors—such as microclimate, altitude and soil type-may influence the biosynthesis of these compounds. For example, Linalool and Eucalyptol were more abundant in Ilonsoy, where the climate is milder and the soil more fertile, possibly favoring the production of more aromatic compounds suitable for medicinal and aromatherapeutic uses. The observed chemical diversity in the essential oil composition of *A. millefolium* reflects the ecological variability of the Samarkand region and underscores the importance of environmental factors in shaping the plant's secondary metabolism.

In contrast to samples from Turkey (Yener et al. 2020), where α-Thujone was dominant (up to 32.6%), our samples were characterized by significantly lower α-Thujone levels (ranging from 0.19% to 0.58%) and higher concentrations of m-Cymene (12.62-21.05%) and Ascaridole (14.09-20.43%).

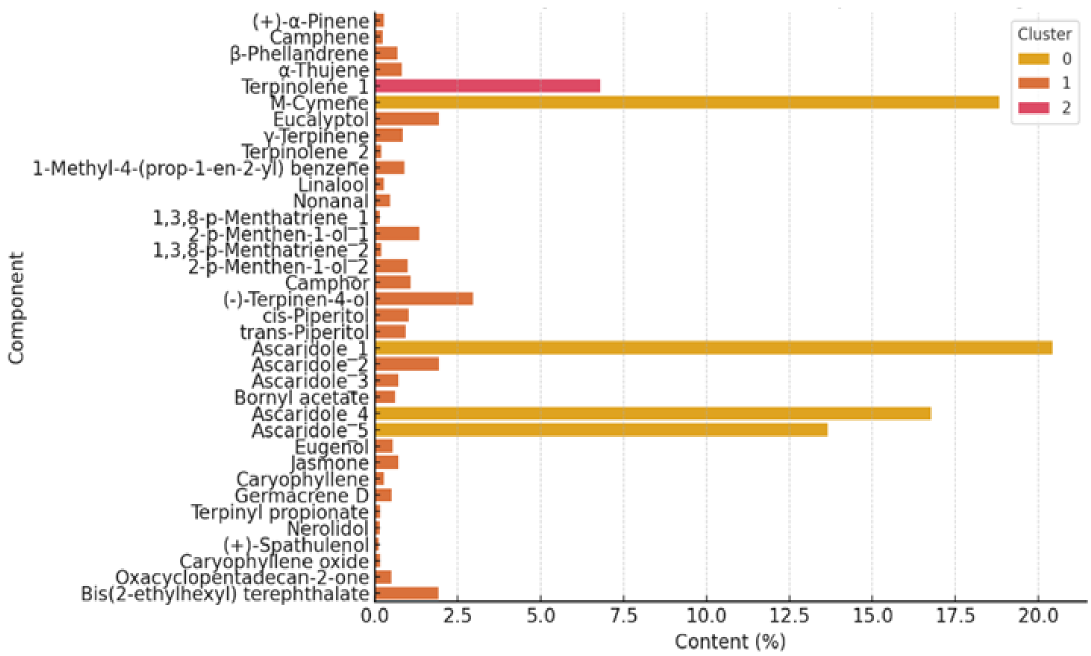

Figure 8. Cluster analysis of essential oil components (Sazagan)

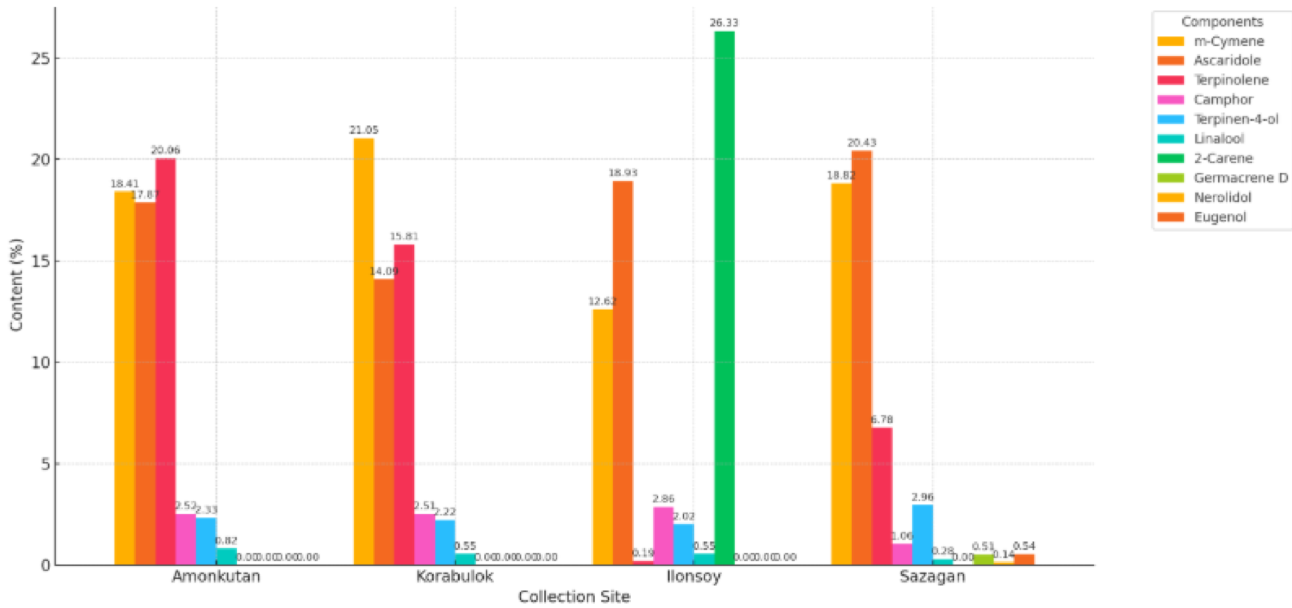

Figure 9. Content of common and unique chemical components of essential oil of *Achillea millefolium* depending on habitat conditions

As a result of comparing the obtained data on the diversity of the essential oil composition of *A. millefolium* collected from four areas of the Samarkand region with the essential oil composition of *A. millefolium* growing in Eastern Turkey, as reported by Toplan et al. (2022), both similarities and significant differences were identified. For example, the content of Eucalyptol in our samples ranged from 0.28% to 1.94%, whereas Toplan et al. (2022) reported 12.3%. This difference may be attributed to variations in geographical origin or differences in extraction methods (Toplan et al. 2022). Camphor and Terpinen-4-ol showed similar concentrations in both studies: 1.06-2.86% and 2.02-2.96%, respectively, in the Samarkand samples, compared to 1.3% and 2.2% reported by Toplan et al. (2022). Linalool content was also comparable: 0.28-0.82% in our samples versus 0.5% in their study. The most notable discrepancy concerned the content of p-Cymene and m-Cymene. In our study, m-Cymene reached up to 21.05% (especially in the Korabulok sample), while Toplan et al. (2022) reported only 2.4% p-Cymene. Sabinene was detected at up to 1.05% in our samples, slightly lower than the 1.8% reported by Toplan et al. (2022). Additionally, they recorded high concentrations of β-Eudesmol 8.9% and α-Pinene 4.3%, while we detected β-Eudesmol only in Ilonsoy 0.75% and α-Pinene at 0.36% in Ilonsoy, 0.28% in Sazagan. These findings indicate a clear variation in terpene profiles between populations. Terpinolene was one of the dominant component in our samples, reaching up to 20.06% in Amonkutan, compared to only 0.3% in Toplan et al. (2022). Ascaridole, absent from their study, was detected in significant amount in our samples (up to 20.43%), suggesting region-specific phytochemical traits possibly driven by environmental or genetic factors (Toplan et al. 2022). Caryophyllene oxide also showed divergence: our samples contained up to 0.29%, while Toplan et al. (2022) reported 4.2%.

Further comparison with the study by Nazir et al. (2022), which analyzed essential oils of *A. millefolium* from Kashmir, India revealed differences at the chemical class level. While both studies agree that terpenes dominate the oil, their distribution varied: our samples had higher level of monoterpene hydrocarbons (e.g., Terpinolene 20.06%, m-Cymene 21.05%), especially in Amonkutan and Korabulok, while Nazir et al. (2022) found high level of oxygenated monoterpenes—Camphor 35.15% and Eucalyptol 30.80%-with monoterpene hydrocarbons constituting only 14.91%.

In our samples, oxygenated monoterpenes such as Terpinen-4-ol and Lavandulol ranged from 2.0% to 2.96% and Camphor ranged from 1.06% to 2.86%. According to Nazir et al. (2022), oxygenated monoterpenes comprised over 76% of the essential oil composition, with notable constituents including α-Terpineol, Linalool and Bornanone. In contrast, our analysis revealed only trace amounts (<1%) of aromatic compounds such as Carvacrol and Eugenol, which were not mentioned among the identified components in Nazir et al. (2022). Camphor ketone was a major marker in Nazir et al. (2022), while in our data, Camphor appeared in lower amounts. Lactones were not prominent in either study, suggesting a minor role in *A. millefolium* oil chemistry under different environmental conditions. In terms of oxygenated sesquiterpenes, Nazir et al. (2022) identified Copaen-4-α-ol 3.43%, Elemol and Eudesmol (total 5-6%). In our samples, these were either absent or present in trace amounts Eudesmol 0.75%, Spathulenol 0.11%, suggesting lower expression of these pathways in a continental climate. The most striking difference was the high concentration of Ascaridole (up to 20.43%) in the Samarkand samples, especially in Sazagan, while this compound was completely absent in Nazir et al. (2022). Since Ascaridole is a peroxide, its accumulation may be associated with oxidative stress typical of hot, arid climates (Mohammadhosseini et al. 2017; Stojanović et al. 2022; Istamkulova MM et al. 2024a).

Angelova et al. (2023) investigated the impact of heavy metal contamination on the essential oil composition of *A. millefolium* and reported an increased presence of oxygenated sesquiterpenes, particularly α-Bisabolol, Borneol and Camphor. The experiment was conducted on plots located at different distances (0.5 km and 15 km, respectively) from the source of pollution - the Non-Ferrous-Metal Works (MFMW) near Plovdiv, Bulgaria.

In contrast to the findings of Angelova et al. (2023) from Bulgaria, where oxygenated sesquiterpenes predominated under heavy metal stress, our samples were dominated by monoterpene hydrocarbons such as Terpinolene, m-Cymene, 2-Carene, and γ-Terpinene, comprising up to 45% of the total composition, particularly in Amonkutan and Korabulok areas. These differences may reflect contrasting environmental stressors, which regulate biosynthetic pathways differently. Oxygenated monoterpenes such as Eucalyptol, Camphor and Terpinen-4-ol were present in our samples up to 2.96%, while Angelova et al. (2023) reported much higher level under metal stress. Oxygenated sesquiterpenes like Caryophyllene oxide were present in trace amount in our data (<1%) but were more abundant in Angelova et al. (2023). A unique feature of our samples was the presence of Ascaridole up to 20.43% in Sazagan, absent in Angelova et al. (2023). Ascaridole is known for its antiparasitic properties and is considered a marker of oxidative stress (Stojanović et al. 2022).

A comparison with the essential oils profile of *Achillea millefolium* reported by Howyzeh et al. 2019 from Iran revealed notable differences in both the qualitative and quantitative compositions. While the Iranian samples were dominated by sesquiterpenes such as Caryophyllene oxide, Camphor, Borneol and γ-Eudesmol, our samples from the Samarkand region exhibited a higher proportion of monoterpene hydrocarbons. The major constituents identified in our samples included m-Cymene up to 21.05%, Ascaridole up to 20.43%, Terpinolene up to 20.06% and 2-Carene up to 26.33%. Camphor was also present but in significantly lower concentrations maximum 2.86% compared to the Iranian samples. These differences likely reflect ecological influences, such as variations in altitude, precipitation, and soil pH, which are known to modulate terpenoid biosynthesis. This chemotypic divergence supports the hypothesis that geographically distinct populations of *A. millefolium* develop unique adaptive metabolic profiles in response to environmental pressures.

Achillea millefolium has a long history of medicinal use worldwide. Reports from India, Pakistan, Iran describe its applications against microbial infections, digestive and respiratory conditions and as a diuretic and antihelminthic agent (Mohammadhosseini et al. 2017; Khojimatov et al. 2023). In Turkey and Italy, it is traditionally used to alleviate abdominal pain and flatulence (Khojimatov et al. 2023). In Brazil and Greece, it is used to manage menopausal symptoms, support lactation and improve sleep (Mohammadhosseini et al. 2017). In Uzbekistan, *A. millefolium* is employed in the treatment of asthma, dysentery, tuberculosis and various infections, often in combination with honey (Khojimatov 2021; Khojimatov et al. 2023).

The results of this study, supported by comparative analysis with prior research (Nazir et al. 2022; Toplan et al. 2022; Angelova and Ihtyarova 2023), demonstrate that the chemical profile of *A. millefolium* essential oil varies significantly depending on geographical origin, climate, soil conditions, extraction method and possible environmental contamination. These findings offer insights into optimizing the production of oils with specific biological properties for applications in medicine, cosmetics, agriculture and veterinary practice.

In conclusion, the chemical composition of *Achillea millefolium* essential oils is significantly shaped by geographic, climatic, and environmental factors, as well as collection time and method. These conditions influence plant metabolic pathways, leading to variability in bioactive compounds. Unexpected detection of substances like Tetrachloroethylene and Bis(2-ethylhexyl) terephthalate underscores the need for stricter environmental monitoring and quality control. Moreover, extraction methods affect both the yield and pharmacological value of essential oils, which possess antiparasitic, anti-inflammatory, and antiseptic properties. These findings highlight the importance of integrating chemical diversity into biodiversity assessments and genetic resource conservation. Identifying region-specific chemotypes supports the targeted cultivation of *A. millefolium* to enhance its medicinal, therapeutic, and industrial potential, offering a basis for sustainable and site-specific applications in pharmaceutical and veterinary contexts.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Vivary Laboratory of Biophysiological and Biochemical Research at the Biochemistry Institute of Samarkand State University named after Sharof Rashidov for providing the necessary equipment and reagents for conducting scientific experiments. Special thanks are also extended to the Institute of Bioorganic Chemistry in Tashkent named after O.S. Sodiqov and to Candidate of Chemical Sciences Mamadrahimov Azimjon Akparaliyevich from the Center for Collective Use of Scientific Equipment at the same institute for his direct involvement in performing the chemical analyses.

REFERENCES

Angane M, Swift S, Huang K, Butts CA, Quek SY. 2022. Essential oils and their major components: An updated review on antimicrobial activities, mechanism of action and their potential application in the food industry. Foods 11 (3): 464. DOI: 10.3390/foods11030464.

Angelova V, Ihtyarova M. 2023. Heavy metal accumulation and chemical composition of the essential oils of yarrow (*Achillea millefolium* L.) cultivated on heavy metal-contaminated soils. Agric Sci 15 (36): 67-76. DOI: 10.22620/agrisci.2023.36.006.

Bussmann RW. 2025. *Achillea millefolium* L. Asteraceae. Ethnobotany of the Mountain Regions of Eastern Europe. Springer, Cham. DOI: 10.1007/978-3-031-87802-2_4

Dalili A, Milani SE, Kamali N, Mohammadi S, Pakbaz M, Jamalnia S, Sadeghi M. 2022. Beneficial effects of *Achillea millefolium* on skin injuries: A literature review. J Essent Oil Res 34 (6): 479-489. DOI: 10.1080/10412905.2022.2104392.

Das S, Prakash B. 2023. Effect of environmental factors on essential oil biosynthesis, chemical stability and yields. In: Plant essential oils: From traditional to modern-day application. Springer Nature, Singapore. DOI: 10.1007/978-981-99-4370-8_10.

Efremov AA. 2013. Method of exhaustive hydroparodistillation in obtaining essential oils of wild plants. Uspekhi Sovremennoi Naukh 7: 88-94. [Russian]

El-Kalamouni C, Venskutonis PR, Zebib B, Merah O, Reynaud C, Talou T. 2017. Antioxidant and antimicrobial activities of the essential oil of *Achillea millefolium* L. grown in France. Medicines 4 (2): 30. DOI: 10.3390/medicines4020030.

Far BF, Behzad G, Khalil H. 2023. *Achillea millefolium*: Mechanism of action, pharmacokinetic, clinical drug-drug interactions and tolerability. Heliyon 9 (12): e22841. DOI: 10.1016/j.heliyon.2023.e22841.

Farhadi N, Babaei K, Farsaraei S, Moghaddam M, Pirbalouti AG. 2020. Changes in essential oil compositions, total phenol, flavonoids and antioxidant capacity of *Achillea millefolium* at different growth stages. Ind Crops Prod 152: 112570. DOI: 10.1016/j.indcrop.2020.112570.

Güçlü G, İnanır M, Uçar E, Eruygur N, Ataş M, Uskutoğlu T, Şenkal BC. 2023. Biological activities of different plant species belonging to the *Asteraceae* family. Intl J Second Metab 10 (1): 11-22. DOI: 10.21448/ijsm.1107819.

Guzmán E, Lucia A. 2021. Essential oils and their individual components in cosmetic products. Cosmetics 8: 114. DOI: 10.3390/cosmetics8040114.

Howyzeh MS, Aslani S, Pooraskari O. 2019. Essential oil profile of an Iranian yarrow (*Achillea millefolium*). J Essent Oil Bear Plants 22 (1): 295-300. DOI: 10.1080/0972060x.2019.1586589.

Ilieva I. 2020. Specific epithet-A denomination of geographical region, particular place of growth, spread or origin of the plants. Biomed J Sci Tech Res 24: 18685-18713. DOI: 10.26717/bjstr.2020.24.004126.

Istamkulova MM, Mukumov IU, Keldiyarov HO. 2021. The genus *Achillea* in the flora of Samarkand region. Vestnik Nauki 10 (43): 114-120. [Russian]

Istamkulova MM, Mukhamadiev NK, Keldiyarov OX, Makhmudova GN, Mukhamadiev AN. 2023. GC-MS analysis of the chemical composition of essential oil isolated from *Achillea millefolium* L. Cent Asian J Med Nat Sci 4 (4): 46-54.

Istamkulova MM, Mukhamadiev AN, Mukhamadiev NK, Keldiyarov HO, Istamkulov MS. 2024a. GC-MS analysis of essential oils of species of species of the genus *Achillea* L. Am J Biomed Sci Res 24 (1): 50-61. DOI: 10.34297/ajbsr.2024.24.003160.

Istamkulova MM, Mukhammadiyev NK, Keldiyarov KHO, Dushanova GA. 2024b. Comparative analysis of the content of the essential oils of *Achillea millefolium* L. and *Achillea arabica* Kotschy. Chin J Evid Based Med 24 (3): 821-831.

Kaffash S, Sefidkon F, Mafakheri S. 2020. Comparing essential oil composition of cultivated and wild samples of *Achillea biebersteinii* Afan. in Kurdistan Province. J Med Plants Prod 9 (2): 149-158. DOI: 10.22092/jmpb.2020.123875.1073.

Karami P, Zandi M, Ganjloo A. 2022. Evaluation of key parameters during ohmic-assisted hydro-distillation of essential oil from aerial part of yarrow (*Achillea millefolium* L.). J Appl Res Med Aromat Plants 31: 100425. DOI: 10.1016/j.jarmap.2022.100425.

Khojakulov D, Khaydarov K, Rabbimov A, Mukimov T, Matkarimova G, Davronqulova F, Ochilov U, Alikulov B. 2024. Biological, physiological and economic characteristics of *Onobrychis chorasanica* Bunge ex Bois. (Sainfoin) under sowing conditions. Plant Sci Today 11 (3): 14-21. DOI: 10.14719/pst.3180.

Khojimatov OK, Gafforov Y, Bussmann RW. 2023. Ethnobiology of Uzbekistan: Ethnomedicinal Knowledge of Mountain Communities. Springer, Cham. DOI: 10.1007/978-3-031-23031-8.

Khojimatov OK. 2021. Lekarstvennie Rasteniya Uzbekistana. Ma'naviyat, Tashkent. [Russian]

Kurchenko VP, Sushinskaya NV, Chubarova AS, Tarun EI, Kupriyanov AN, Khrustaleva IA, Bondaruk AM, Tsygankov VG, Zhurihina LN, Filonyuk VA, Shabunya PG. 2019. The composition of biologically active substances of extracts of yarrow flowers of the native flora of Belarus, Kazakhstan and Russia, their antioxidant properties and toxicity. Èkobioteh 2 (3): 286-292. DOI: 10.31163/2618-964X-2019-2-3-286-292.

Losapio G, Doussot B, Aranit F, Bruno L, Guevara R, Dirzo R. 2024. Population persistence, phenotypic divergence and metabolic adaptation in yarrow (*Achillea millefolium* L.). Ecosphere 15 (12): e70146. DOI: 10.1002/ecs2.70146.

Mohammadhosseini M, Sarker SD, Akbarzadeh A. 2017. Chemical composition of the essential oils and extracts of *Achillea* species and their biological activities: A review. J Ethnopharmacol 199: 257-315. DOI: 10.1016/j.jep.2017.02.010.

Nazir I, Gangoo SA, Sofi PA, Singh A. 2022. GC-MS analysis and antioxidant activity of essential oil from *Achillea millefolium* L. flowers in temperate climatic conditions of Kashmir. J Essent Oil Bear Plants 25 (2): 290-296. DOI: 10.1080/0972060X.2022.2083456.

Noedoost F, Behroozian M, Karami S, Joharchi MR. 2024. Potential impacts of climate change on the geographic distribution of *Achillea* *eriophora* DC., a medicinal species endemic to Iran in southwestern Asia. Ecol Evol 14 (4): e11241. DOI: 10.1002/ece3.11241.

Novak J. 2021. Quantitative analysis by gas chromatography. In: Giddings JC (eds). Advances in Chromatography. CRC Press, Boca Raton.

Ranasinghe S, Armson A, Lymbery AJ, Zahedi A, Ash A. 2023. Medicinal plants as a source of antiparasitics: An overview of experimental studies. Pathog Glob Health 117 (6): 535-553. DOI: 10.1080/20477724.2023.2179454.

Ruziev Y, Kushiev K, Ismayilova M, Khayitov D, Ruziyev F. 2024. Sweet potato (*Ipomoea batatas* (L.) Lam.): A study on physiological and biochemical properties. Bio Web Conf 130: 01026. DOI: 10.1051/bioconf/202413001026.

Saleh B. 2023. Phytochemical analysis of two *Achillea* (Asteraceae) species using GC/MS technique. J Stress Physiol Biochem 19 (1): 97-104.

Salehi B, Selamoglu Z, Sevindik M, Fahmy NM, Al-Sayed E, El-Shazly M, Csupor-Löffler B, Csupor D, Yazdi SE, Sharifi-Rad J, Arserim-Uçar DK, Arserim EH, Karazhan N, Jahani A, Dey A, Azadi H, Vakili SA, Sharopov F, Martins N, Büsselberg D. 2020. *Achillea* spp.: A comprehensive review on its ethnobotany, phytochemistry, phytopharmacology and industrial applications. Cell Mol Biol 66 (4): 78-103. DOI: 10.14715/cmb/2020.66.4.13.

Sharova EA, Sharova AD. 2021. Ecological and biological features of *Achillea millefolium*, which grows in the Baikalov district. AIP Conf Proc 2388: 030041. DOI: 10.1063/5.0069974.

Shukla S, Patra D, Sinha A, Saha R, Tripathi Y, Borah N, Shukla SK. 2025. Medicinal and aromatic plant cultivation, improvement and conservation for sustainable development. In: Industrial Crops Improvement: Biotechnological Approaches for Sustainable Agricultural Development. Cham: Springer Nature Switzerland. DOI: 10.1007/978-3-031-75937-6_11.

Stojanović JP, Stojanović GS, Stojanović-Radić ZZ, Zlatković BK, Ickovski JD, Zlatanović IG, Jovanović SČ, Mitić ZS. 2022. Essential oils of six *Achillea* species: Chemical profiles, antimicrobial potential and toxicity toward crustaceans. Chem Biodivers 19 (3): e202100905. DOI: 10.1002/cbdv.202100905.

Štrbac F, Bosco A, Amadesi A, Rinaldi L, Stojanović D, Simin N, Orčić D, Pušić I, Krnjajić S, Ratajac R. 2020. In vitro ovicidal activity of two chemotypes of the Yarrow (*Achillea millefolium* L.) essential oil against ovine gastrointestinal nematode eggs. Arch Vet Med 13 (2): 59-76. DOI: 10.46784/e-avm.v13i2.246.

Tojibaev KS, Beshko NY, Kodirov UH, Batoshov AR, Mirzalieva DU. 2018. Cadastre of the Flora of Uzbekistan: Samarkand Region. Fan, Tashkent. [Russian]

Toplan GG, Taşkın T, İşcan G, Göger F, Kürkçüoğlu M, Civaş A, Ecevit-Genç G, Mat A, Başer KHC. 2022. Comparative studies on essential oil and phenolic content with in vitro antioxidant, anticholinesterase, antimicrobial activities of *Achillea biebersteinii* Afan. and *A. millefolium* subsp. *millefolium* Afan. L. growing in Eastern Turkey. Molecules 27 (6): 1956. DOI: 10.3390/molecules27061956.

Vakhidova A, Daminov A, Muradova E, Nurullayev A. 2024. Changes in the content of certain macro-and microelements in the blood of karakul sheep exposed to helminth infestation. Bio Web Conf 95: 01008. DOI: 10.1051/bioconf/20249501008.

Yener I, Yilmaz MA, Olmez OT, Akdeniz M, Tekin F, Hasimi N, Alkan MH, Ozturk M, Ertas A. 2020. A detailed biological and chemical investigation of sixteen *Achillea* species' essential oils via chemometric approach. Chem Biodivers 17: e1900484. DOI: 10.1002/cbdv.201900484.