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Research Article

Phytochemical Profile of *Achillea Millefolium* Extracted by Supercritical CO_2

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Abstract

Achillea millefolium is a medicinal plant widely used in traditional and modern medicine. This study characterised the chemical composition of essential oils obtained from *A. millefolium* collected in four regions of Albania (Tirana, Pogradeci, Kukësi and Kruja) using supercritical CO_2 extraction. Volatile compounds were analysed using gas chromatography with flame ionization detection (GC-FID). A total of 23 volatile compounds were identified, representing 88–93% of the total chromatographic area. The major constituent was β-caryophyllene, ranging from 18.02 ± 0.03% in Tirana to 24.40 ± 0.53% in Kukësi. Germacrene D ranged from 9.23 ± 0.05% in Kruja to 13.00 ± 0.01% in Tirana. The monoterpene 1,8-cineole showed regional variation, with the highest level in Tirana (9.39 ± 0.01%) and lower levels in Kukësi (6.45 ± 0.13%). Sabinene showed the highest values in Kukësi (7.65 ± 0.14%) and Kruja (6.28 ± 0.07%), while linalool was most abundant in Kruja (3.62 ± 0.07%). In addition, α-thujone showed the highest value in Kukësi (3.95 ± 0.23%) compared to the lowest in Tirana (0.90 ± 0.01%). Statistical analysis (one-way ANOVA and Tukey's HSD test) revealed significant differences in compound composition among regions. Principal component analysis (PCA) showed clear clustering of replicate samples within each region, with Tirana associated with relatively higher monoterpene content, Kukësi and Kruja associated with higher sesquiterpene abundance, and Pogradeci showing an intermediate profile. The observed variation in essential oil composition may be influenced by regional environmental conditions. The major compounds identified have been reported in the literature to exhibit anti-inflammatory, neuroprotective, antioxidant, and antimicrobial properties. These findings provide the first region-specific phytochemical dataset of *A. millefolium* essential oils in Albania obtained by supercritical CO_2 extraction and contribute to the chemotypic characterisation of the species.

Keywords: Achillea Millefolium, Essential Oil, Medicinal Plant, Supercritical CO_2 Extraction.

INTRODUCTION

Medicinal plants have an important role in traditional and modern medicine and phytopharmaceutical research [1-5]. They contain a wide range of chemical constituents and a broad spectrum of biological activities [6-13]. Among these medicinal plants, *Achillea millefolium* L., a member of the Asteraceae family, is commonly known as yarrow in

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European, Asian, and North American populations. This herbaceous perennial plant is native to the Northern Hemisphere and has been used in traditional medicine for thousands of years [3, 14-18]. The genus name "*Achillea*" is derived from the legendary Greek warrior Achilles [19-21]. Ethnobotanical studies indicate that this plant is used worldwide for wound healing, gastrointestinal disorders, inflammatory conditions, and respiratory problems, and for its sedative effects [3, 21]. The broad therapeutic effects of *A. millefolium* are largely attributed to its secondary metabolites. Chemical analyses have identified several classes of pharmacologically active compounds, including volatile terpenes mainly monoterpenes and sesquiterpenes [22-27]. Other compounds are glycosylated flavonoids such as apigenin, luteolin and quercetin, phenolic carboxylic acids such as caffeoylquinic acid and proazulene which are converted into the blue-pigmented anti-inflammatory compound chamazulene during thermal processes [1]. These phytochemicals are responsible for the antimicrobial, anti-inflammatory, antioxidant [28-33] and cytoprotective properties [14]. Previous studies have demonstrated that the chemical composition of *A. millefolium* essential oil varies considerably and is influenced by factors including geographical origin [13]. These factors also include soil and climatic conditions [2, 10]. To isolate and identify the phytochemical constituents of *Achillea millefolium*, several conventional extraction methods, such as maceration, Soxhlet extraction, and hydrodistillation, have been widely employed.

However, traditional extraction methods have disadvantages such as heat degradation of thermosensitive constituents, low selectivity, and use of environmentally harmful organic solvents [29]. In recent years, supercritical carbon dioxide extraction (SFE-CO_2) has become a more environmentally friendly, sustainable, selective method of extracting bioactive constituents. In supercritical extraction, carbon dioxide reaches a supercritical state, thus it exhibits properties of both a gas and a liquid, i.e. there is a high diffusion rate, gas-like properties and high solvation power, liquid-like behaviour and the ability to extract thermosensitive and lipophilic compounds with minimal degradation [34]. When *Achillea millefolium* is extracted by this method under moderate operating conditions (90 bar and 40 °C), thermolabile compounds such as chamazulene are preserved. Results are pale yellow oils with high content of sesquiterpene hydrocarbons (73.63–94.3%), such as (E)-caryophyllene, γ-murolene and caryophyllene oxide and of mono- and sesquiterpenes and oxygenated derivatives, such as bisabolol, α-bisabolol oxide A and B, and α-cadinol [20, 32]. These compounds show extended stability and enhanced activity [20, 32]. The extraction process can be optimized to achieve target specific outcomes such as maximum yield, by manipulation of extraction variables such as pressure, temperature and flow rate [31], for pharmaceutical, cosmeceutical and nutraceutical applications. While *Achillea millefolium* oils have been studied extensively throughout the world, there is a paucity of data on the specific phytoconstituents in Albania. Moreover, studies on the phytochemicals of plants in Albania utilizing advanced extraction technologies are limited. To meet the requirements of European regulations for herbal medicinal products, there is a need for defined phytochemical data specific to the region, to support sustainable harvesting, and access to standardisation of herbal raw materials for industry.

In this context, the present study aims to evaluate and compare the phytochemical composition of A. millefolium essential oils from four ecologically distinct regions of Albania (Tirana, Pogradeci, Kukësi, and Kruja) using supercritical CO_2 extraction and GC-FID analysis, and to assess whether regional environmental conditions are associated with distinct terpene profiles. Volatile compounds were identified by comparison of experimentally determined retention indices (RI), calculated using the Van Den Dool and Kratz equation from a C8–C18 n-alkane standard series, with published literature values from Adams (2007) on a non-polar column, GC-MS confirmation was not performed. From a biochemical perspective, ecological gradients may influence terpene biosynthesis through differential regulation of the methylerythritol phosphate (MEP) pathway, primarily associated with monoterpene formation, and the mevalonate (MVA) pathway, associated with sesquiterpene biosynthesis [10, 24]. Environmental factors such as altitude, temperature fluctuations, solar radiation, and water availability may modulate metabolic flux toward protective volatile compounds. Therefore, regional variability in A. millefolium essential oil composition may reflect adaptive biochemical responses rather than simple compositional differences.

Research Hypothesis

The chemical composition of *Achillea millefolium* essential oils is known to be strongly influenced by location and environmental factors [13, 24, 25]. Environmental effects such as altitude, temperature, sun radiation, and soil quality have been found to influence, through varied regulation of the methylerythritol phosphate (MEP) and mevalonate (MVA) terpene biosynthetic pathways, volatile secondary metabolite pathways [10, 23, 24]. Ecological factors such as those described may also induce formation of different phytochemical profiles in geographically dispersed plant populations. With this in mind, the study described here was designed to test the following hypotheses:

- Main hypothesis**:** Regional ecological conditions influence the terpene composition of *Achillea millefolium* essential oils collected from four regions of Albania differing in altitude, climate, and local environmental conditions.
- H1: The ratio of monoterpene to sesquiterpene varies strongly along the 4 Albanian regions, indicating different ways of terpene biosynthesis in different ecological environments.
- H2: Principal component analysis (PCA) reveals distinct clustering of essential oil samples by region, indicating compositional variation among populations that can be distinguished through multivariate analysis.

H1 was evaluated using one-way analysis of variance (ANOVA, $\alpha = 0.05$) followed by Tukey's HSD post-hoc test for pairwise comparisons. H2 was evaluated using PCA on autoscaled data ($n = 12$, $p = 23$), with Ward's hierarchical clustering method used to validate the compositional groupings observed in the score plot.

he four sampling locations encompass a diversity of habitats. Pogradeci (Valamara Mt 2370 m) and Tirana (Dajti Mt 1613 m) are higher altitude mountain sites subject to increased UV exposure, low temperatures, high diurnal variability; Kruja (Qafe Shtama 1240 m) is a mid-altitude forested mountain pass; Kukësi (Bicaj 460 m) was ground level elevation sampled, though it sits at the foot of the (Gjallica Mt 2,489 m) and experiences a continental mountain climate with cold winters, high seasonal temperature variation and relatively high surrounding relief. The high sesquiterpene concentrations in the Kukësi population relative to the other sampling locations suggests that local microclimate, in addition to collection altitude, may be an important factor driving chemotypic divergence.

Advantages and Limitations of Supercritical CO_2 Extraction in Achillea Millefolium

Supercritical CO_2 extraction has been used in the phytochemical analysis of *Achillea millefolium* as an environmentally friendly alternative to traditional methods of botanical extraction. Supercritical CO_2 extraction involves the application of carbon dioxide at pressures and temperatures above its critical temperature and pressure, creating a solvent with liquid-like solvating power and gas-like diffusivity, which aids penetration into plant matrices and facilitates the recovery of bioactive constituents [6, 8]. A key benefit of this procedure is the enhanced recovery of thermolabile and volatile essential oil components, due to the mild thermal conditions of supercritical extraction compared to hydrodistillation, which can cause thermal degradation of sensitive constituents [30].

Retention of chemical integrity in the extract - including tocopherols, antioxidants, essential oils, and isoprene-derived compounds - is essential for complete phytochemical profiling of *Achillea millefolium* [30]. Supercritical CO_2 extraction maximises recovery of thermolabile compounds such as camphor and 1,8-cineole by avoiding the hydrolytic and thermal degradation associated with hydrodistillation [6, 34]. The method also excels in the selectivity and purity achievable through manipulation of pressure and temperature, offering successful differential extraction of sesquiterpene components and yielding organic solvent-free extracts suitable for pharmaceutical, cosmeceutical, and nutraceutical applications, while reducing the time and effort required for subsequent isolation procedures [4, 8, 15]. Despite these advantages, supercritical CO_2 extraction has limitations for extracting polar phenolic compounds and flavonoids abundant in *A. millefolium*. Addition of co-solvents such as ethanol can improve solubility of polar constituents but introduces additional process complexity [4]. Comparative studies have shown that the chemical profile of *A. millefolium* extracts obtained by supercritical CO_2 extraction differs from those obtained by hydrodistillation, with lower proportions of chamazulene and higher sesquiterpene content [11,20], indicating method-dependent selectivity. Another limitation is the dependence on precise control of extraction parameters. Studies on Bulgarian *A. millefolium* have shown that variables such as pressure, temperature, and CO_2 flow influence overall yield but have a limited effect on volatile profile [34]. Furthermore, the equipment required represents a significant capital investment and greater operational complexity than conventional methods.

MATERIALS AND METHODS

Plant Collection and Preparation

Achillea millefolium (whole aerial parts) was collected from May to July 2023 in four regions of Albania. Figure 1 depict the location of sampling sites of *Achillea millefolium* in Albania

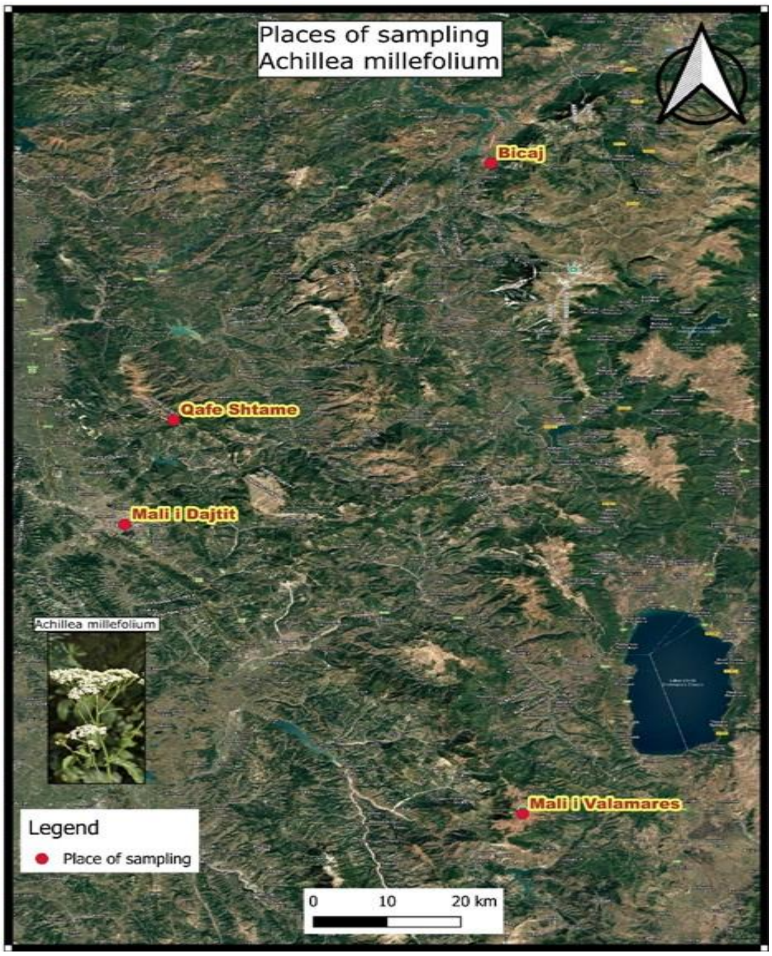

Table 1. location of sampling sites of *Achillea millefolium* in Albania

The first region was near Kukësi, in the village of Bicaj (41.9943°N, 20.4128°E), at an elevation of approximately 460 m above sea level.

The second sample was collected in the Kruja region at Qafë Shtama (41.5207°N, 19.8922°E), 1,240 m above sea level; samples from this site were collected in June. The third samples were taken in June from Tirana, specifically at Dajti Mountain (41.3674°N, 19.9226°E), 1,613 m above sea level. The fourth region was in the Pogradeci region in Valamara mountain (40.7947°N, 20.4647°E), at an elevation of approximately 2,370 m, where sampling was carried out in July. After collection, the plant material was dried at 22-25°C for 10 days. Moisture content was measured using a gravimetric oven-drying method (105°C until constant weight) and maintained below 6%. The dried material was milled using a laboratory grinder and sieved to obtain a uniform particle size of 0.3 mm.

Extraction

The volatile constituents of *Achillea millefolium* were extracted using supercritical CO_2. Extraction was performed at 40°C and 150 bars. The flow rate was maintained at 20 g/min, and the total extraction time was 2 hours. The extraction yield, determined gravimetrically as the mass of extract recovered per 100 g of dry plant material, was 4.11 g/100 g. Gas chromatographic analysis of the essential oil was performed on a Varian 450 GC equipped with a PTV injector and a flame ionization detector (FID). The injector and detector temperatures were set at 280°C and 300°C, respectively. A volume of 2 µl of essential oil diluted in toluene was injected in split mode (1:50). Nitrogen was used as carrier gas (1 ml/min) and as make-up gas (25 ml/min). Hydrogen and air were used as detector flame gases at 30 ml/min and 300 ml/min, respectively. A VF-1ms capillary column (30 m × 0.33 mm × 0.25 µm) was used to separate the essential oil compounds. The oven temperature programme was as follows: from 40°C (held for 2 minutes) to 150°C at 4°C/min, then to 280°C at 10°C/min, held at 280°C for 2 minutes. Compound identities were assigned based on comparison of experimentally determined retention indices (RI), calculated using the Van Den Dool and Kratz equation from a C8–C18 n-alkane standard series co-injected under the same chromatographic conditions, with published literature values from Adams (2007) on a non-polar column (VF-1ms), GC-MS was not performed in the present study and will be included in future work. Quantitative data are expressed as a percentage of the total chromatographic peak area.

Statistical Analysis

Quantitative differences in essential oil composition among the four regions were assessed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc test (α = 0.05). Results are expressed as mean ± standard deviation (SD; n = 3). Significant differences among regions are indicated by different superscript letters in Table 1. Chemometric analysis was performed using PAST v4.0 (Hammer et al., 2001). Principal component analysis (PCA) was conducted on auto scaled data (mean-centered, unit-variance scaled) using a data matrix of 12 observations (3 replicates × 4 regions) and 23 variables (volatile compounds). Ward's method (hierarchical clustering) with Euclidean distance was applied to validate the compositional groupings observed in the PCA score plot.

RESULTS

Table 1 depict the chemical composition of A. *millefolium* essential oils from Albanian regions.

Table 1. Chemical composition of *A. millefolium* essential oils from four Albanian regions. RT: retention time (min); RI (exp.): experimental retention index; RI (lit.): literature RI from Adams (2007). Values are mean ± SD (n = 3). Different superscript letters indicate significant differences

Compound	RT (min)	RI (exp.)	RI (lit.)	Tirana (%)	Pogradec (%)	Kukësi (%)	Kruja (%)	F (3,8)
α-Thujone	7.25	1101	930	$0.90{\pm}0.01^d$	$2.80{\pm}0.02^b$	$3.95{\pm}0.23^a$	$1.50{\pm}0.05^c$	403.03
α-Pinene	3.69	982	932	$4.96{\pm}0.06^a$	$2.98{\pm}0.02^b$	$0.93{\pm}0.38^c$	$2.50{\pm}0.13^b$	200.67
Sabinene	4.52	974	975	$4.19{\pm}0.01^d$	$4.90{\pm}0.01^c$	$7.65{\pm}0.14^a$	$6.28{\pm}0.07^b$	1175.64
1,8-Cineole	8.63	1030	1026	$9.39{\pm}0.01^a$	$6.80{\pm}0.02^c$	$6.45{\pm}0.13^d$	$7.66{\pm}0.05^b$	1011.60
α-Phellandrene	9.33	1005	1002	$2.79{\pm}0.01^b$	$2.90{\pm}0.01^a$	$2.03{\pm}0.15^c$	$1.70{\pm}0.10^d$	121.52
p-Cymene	9.61	1020	1020	$4.37{\pm}0.03^b$	$4.11{\pm}0.07^c$	$4.76{\pm}0.08^a$	$3.85{\pm}0.05^d$	132.96
α-Terpinene	10.05	1015	1014	$2.90{\pm}0.01^a$	$2.80{\pm}0.01^b$	$2.00{\pm}0.11^d$	$2.24{\pm}0.03^c$	186.49
Terpinolene	10.91	1085	1086	$2.08{\pm}0.08^b$	$2.19{\pm}0.01^b$	$2.39{\pm}0.01^a$	$1.60{\pm}0.03^c$	204.83
Pinocarvone	11.25	1200	1161	$2.05{\pm}0.05^c$	$2.37{\pm}0.03^a$	$2.21{\pm}0.04^b$	$1.29{\pm}0.03^d$	507.24
Camphor	11.92	1125	1141	$2.90{\pm}0.01^a$	$2.79{\pm}0.01^b$	$1.56{\pm}0.04^d$	$2.14{\pm}0.04^c$	1705.85
Linalool	12.36	1108	1095	$1.21{\pm}0.01^d$	$2.79{\pm}0.01^b$	$1.80{\pm}0.05^c$	$3.62{\pm}0.07^a$	2012.29
Borneol	12.95	1165	1165	$4.02{\pm}0.08^a$	$1.89{\pm}0.02^d$	$3.53{\pm}0.08^b$	$2.85{\pm}0.05^c$	716.76
Geraniol	14.86	1255	1249	$2.08{\pm}0.03^d$	$3.14{\pm}0.04^a$	$2.55{\pm}0.05^c$	$2.81{\pm}0.14^b$	101.52
Dodecanol	16.35	1550	1272	$2.10{\pm}0.01^c$	$2.80{\pm}0.02^b$	$1.77{\pm}0.15^d$	$4.05{\pm}0.14^a$	292.00
Borneyl acetate	17.25	1280	1284	$4.07{\pm}0.06^d$	$4.80{\pm}0.02^b$	$6.20{\pm}0.10^a$	$4.25{\pm}0.05^c$	696.37
β-Caryophyllene	24.25	1420	1417	$18.02{\pm}0.03^d$	$21.17{\pm}0.29^c$	$24.40{\pm}0.53^a$	$23.73{\pm}0.38^b$	198.69
β-Pinene	4.21	974	979	$5.07{\pm}0.06^b$	$6.41{\pm}0.07^a$	$6.35{\pm}0.05^a$	$6.39{\pm}0.04^a$	428.86
Longipinene	25.36	1475	1352	$4.09{\pm}0.01^a$	$2.09{\pm}0.01^b$	$2.16{\pm}0.06^b$	$2.15{\pm}0.05^b$	2033.64
Germacrene D	26.33	1490	1480	$13.00{\pm}0.01^a$	$10.44{\pm}0.06^b$	$10.43{\pm}0.15^b$	$9.23{\pm}0.06^c$	1028.00
Cedrene	26.95	1425	1409	$1.97{\pm}0.06^d$	$2.58{\pm}0.03^c$	$2.74{\pm}0.07^b$	$3.82{\pm}0.08^a$	490.77
Humulene	27.45	1460	1452	$2.93{\pm}0.06^b$	$4.03{\pm}0.06^a$	$2.25{\pm}0.13^c$	$2.16{\pm}0.05^c$	327.69
Cadinene	28.65	1510	1513	$2.90{\pm}0.01^a$	$1.07{\pm}0.03^c$	$1.65{\pm}0.06^b$	$2.95{\pm}0.05^a$	1465.59
Caryophyllene oxide	30.15	1585	1582	$2.03{\pm}0.05^b$	$2.15{\pm}0.05^a$	$0.23{\pm}0.03^d$	$1.25{\pm}0.05^c$	1119.00

among regions (Tukey's HSD, p < 0.0001). All F-values significant at p < 0.0001.

A total of 23 volatile compounds were identified in the essential oils of *Achillea millefolium* collected from four regions of Albania (Tirana, Pogradeci, Kukësi and Kruja), representing approximately 88–93% of the total chromatographic area. The chromatograms contained 40–50 peaks in total; compounds present at less than 0.1% are not included in the above table. The standard deviation was generally below 0.1%, indicating high analytical reproducibility. Despite this stability, regional differences were observed. The findings are shown in Table 1. The most abundant compound was the sesquiterpene β-caryophyllene, which showed statistically significant regional differences ($F(3,8) = 198.69$, $p < 0.0001$). The highest mean value of β-caryophyllene was in Kukësi (24.40 ± 0.53%) followed by Kruja (23.73 ± 0.38%), Pogradeci (21.17 ± 0.29%) and Tirana

(18.02 ± 0.03%). 1,8-Cineole, a monoterpene, showed regional variation with the highest level in Tirana (9.39 ± 0.01%) and lower values in Kukësi (6.45 ± 0.13%; $F(3,8) = 1011.60$, $p < 0.0001$). Sabinene showed the highest mean value in Kukësi (7.65 ± 0.14%) and Kruja (6.28 ± 0.07%), confirming significant regional differentiation in monoterpene distribution ($F(3,8) = 1175.64$, $p < 0.0001$). α-Thujone showed the highest value in Kukësi (3.95 ± 0.23%) and the lowest in Tirana (0.90 ± 0.01%; $F(3,8) = 403.03$, $p < 0.0001$). The oxygenated monoterpene linalool had the highest value in Kruja (3.62 ± 0.07%) and the lowest in Tirana (1.21 ± 0.01%; $F(3,8) = 2012.29$, $p < 0.0001$). Bornyl acetate reached its maximum in Kukësi (6.20 ± 0.10%; $F(3,8) = 696.37$, $p < 0.0001$). Germacrene D showed moderate variability, with the highest content in Tirana (13.00 ± 0.01%) and the lowest in Kruja (9.23 ± 0.06%; $F(3,8) = 1028.00$, $p < 0.0001$). All pairwise comparisons were significant at $p < 0.0001$ (Tukey's HSD) unless otherwise indicated by shared superscript letters in the above table.

A comparative visualization of the regional distribution of volatile compounds is presented as a heatmap in Figure 2.

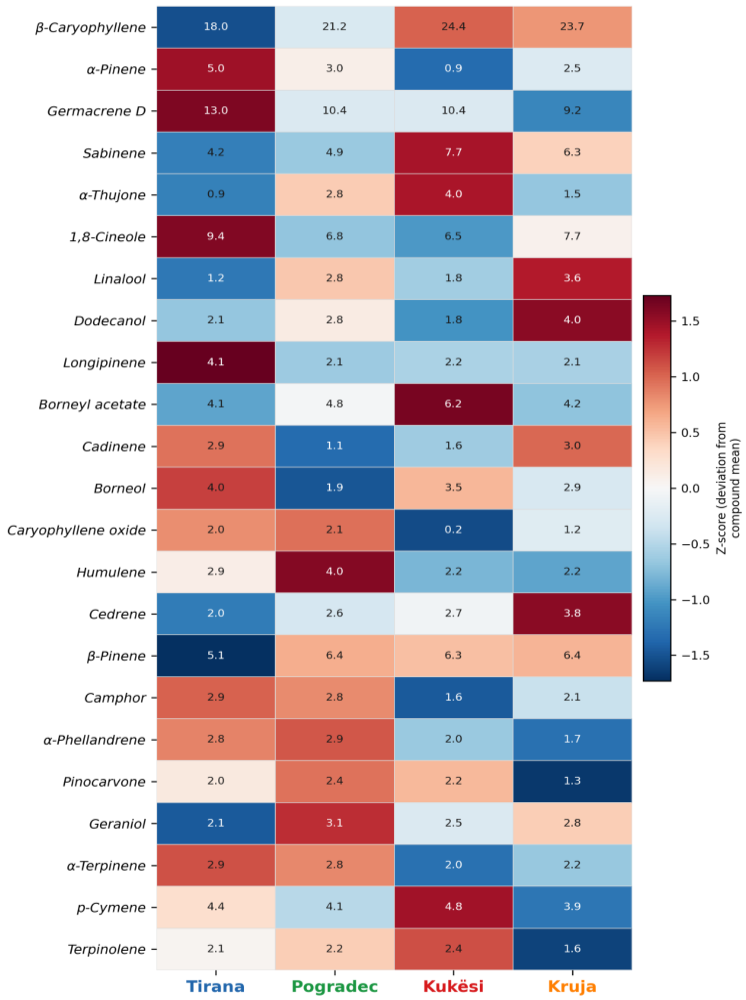

Figure 2. Heatmap of volatile compound content in *A. millefolium* from four Albanian regions. Values represent mean percentages (n = 3). Colour scale = z-score deviation from compound mean (red = above average; blue = below average).

Chemometric Analysis of Regional Variability

To further explore similarities and differences among the analyzed samples, principal component analysis (PCA) was performed based on the relative abundances of the 23 identified volatile compounds. The first principal component (PC1) explained 48.4% of the total variance and the second principal component (PC2) accounted for 29.1%, together representing 77.5% of the total variability in the dataset. The scree plot (Figure 4) shows a pronounced drop in variance after PC2, confirming that a two-dimensional PCA model adequately describes the major patterns of phytochemical variation among the analyzed *Achillea millefolium* samples. The PCA biplot (Figure 3) reveals that PC1 separates populations characterized by higher abundances of β-caryophyllene together with monoterpene hydrocarbons such as β-pinene and sabinene (positive loadings) from populations characterized by higher proportions of oxygenated monoterpenes and other monoterpene hydrocarbons such as α-pinene, 1,8-cineole, longipinene and camphor (negative loadings). Samples from Tirana are positioned on the negative side of PC1, reflecting relatively higher proportions of oxygenated monoterpenes.

Samples collected from Kukësi and Kruja are positioned toward the positive side of PC1, characterized by higher relative abundances of β-caryophyllene and monoterpene hydrocarbons such as β-pinene and sabinene. The Pogradeci population occupies an intermediate position between these two groups, indicating a mixed terpene profile. These results highlight clear regional differentiation in essential oil composition, suggesting that ecological conditions may influence phytochemical variability among *Achillea millefolium* populations in Albania.

The PC1 loadings (Table 2) confirm this pattern quantitatively. Compounds with the strongest positive loadings on PC1 include β-caryophyllene (+0.2967), sabinene (+0.2712), β-pinene (+0.2707) and α-thujone (+0.2092), indicating that these compounds are the primary drivers of separation toward the positive PC1 axis associated with Kukësi and Kruja populations. In contrast, compounds with the strongest negative loadings on PC1 include α-pinene (−0.2867), longipinene (−0.2720), α-terpinene (−0.2641), 1,8-cineole (−0.2568) and camphor (−0.2554), which are characteristic of the Tirana population. This loading pattern confirms that PC1 represents a compositional gradient between hydrocarbon-rich terpene profiles and oxygenated monoterpene-rich profiles across the four populations.

PC2 loadings are dominated by terpinolene (+0.3728), p-cymene (+0.3679) and pinocarvone (+0.3133) on the positive side, and dodecanol (−0.3700) and linalool (−0.2969) on the negative side. This axis primarily separates Kruja characterized by elevated linalool and dodecanol from Kukësi, which shows higher terpinolene and p-cymene content. Pogradeci occupies the central position on both axes, confirming its intermediate chemical profile. The eigenvalues and variance explained by each principal component are presented in Table 3.

The compositional groupings observed in the PCA were validated by Ward hierarchical clustering with Euclidean distance on the same autoscaled dataset (Figure 5). All three replicates of each region consistently clustered together, confirming high within-region analytical reproducibility and clear between-region differentiation in essential oil composition.

Table 2. PC1 and PC2 loadings and percentage contributions for the 23 volatile compounds identified in *Achillea millefolium* essential oils from four Albanian regions.

Compounds	PC1 (48.4%)		PC2 (29.1%)	
	Loading	Contrib. (%)	Loading	Contrib. (%)
α-Thujone	+0.2092	4.38	+0.2516	6.33
α-Pinene	−0.2867	8.22	−0.1069	1.14
Sabinene	+0.2712	7.36	+0.1020	1.04
1,8-Cineole	−0.2568	6.60	−0.1058	1.12
α-Phellandrene	−0.2215	4.91	+0.1212	1.47
p-Cymene	+0.0136	0.02	+0.3679	13.54
α-Terpinene	−0.2641	6.97	−0.0479	0.23
Terpinolene	−0.0062	0.00	+0.3728	13.90
Pinocarvone	−0.0678	0.46	+0.3133	9.82
Camphor	−0.2554	6.52	−0.1096	1.20
Linalool	+0.1699	2.89	−0.2969	8.82
Borneol	−0.1035	1.07	+0.1356	1.84
Geraniol	+0.1670	2.79	−0.1184	1.40
Dodecanol	+0.0786	0.62	−0.3700	13.69
Borneyl acetate	+0.1962	3.85	+0.2901	8.42
β-Caryophyllene	+0.2967	8.80	−0.0073	0.01
β-Pinene	+0.2707	7.33	−0.0509	0.26
Longipinene	−0.2720	7.40	+0.0442	0.20
Germacrene D	−0.2661	7.08	+0.1614	2.61
Cedrene	+0.2212	4.89	−0.2542	6.46
Humulene	−0.1289	1.66	+0.0331	0.11
Cadinene	−0.0993	0.99	−0.2037	4.15
Caryophyllene oxide	−0.2280	5.20	−0.1503	2.26

Table 3. Eigenvalues and variance explained by each principal component.

PC	Eigenvalue	Variance (%)	Cumulative (%)
PC1	11.1303	48.39%	48.39%
PC2	6.6918	29.10%	77.49%
PC3	5.0287	21.86%	99.35%
PC4	0.0510	0.22%	99.57%
PC5	0.0390	0.17%	99.74%
PC6	0.0233	0.10%	99.84%

The scree plot (Figure 4) shows a pronounced drop in variance after PC2, confirming that a two-dimensional PCA model adequately describes the major patterns of compositional variation. PC1 and PC2 together account for 77.5% of the total variance, indicating that the two-component model captures the dominant sources of phytochemical differentiation among the four populations

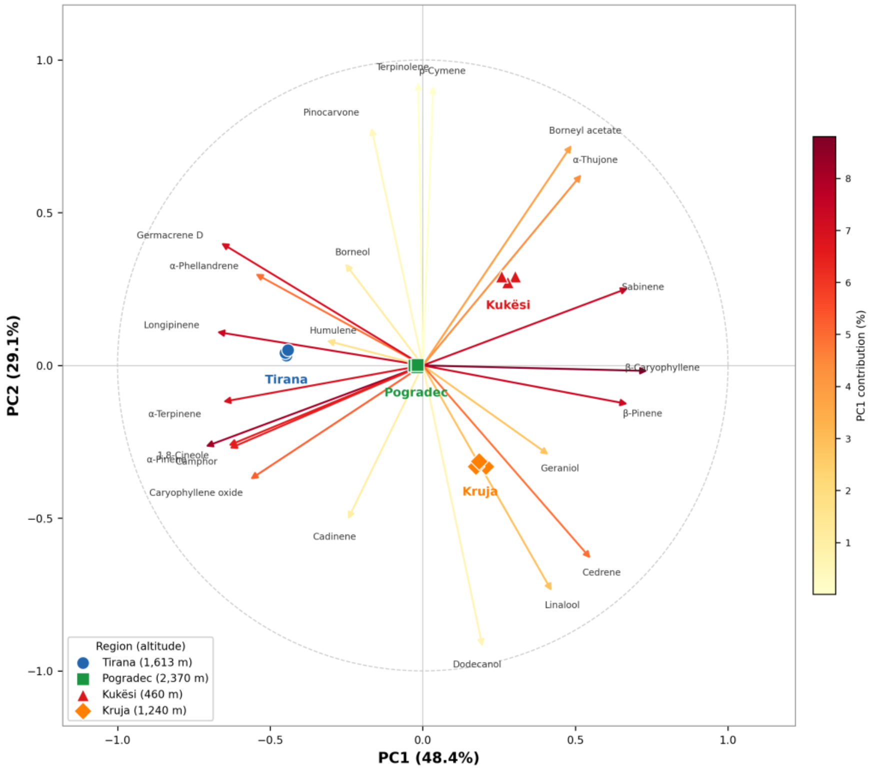

Figure 3. PCA biplot of essential oil composition of *Achillea millefolium* populations from four regions of Albania.

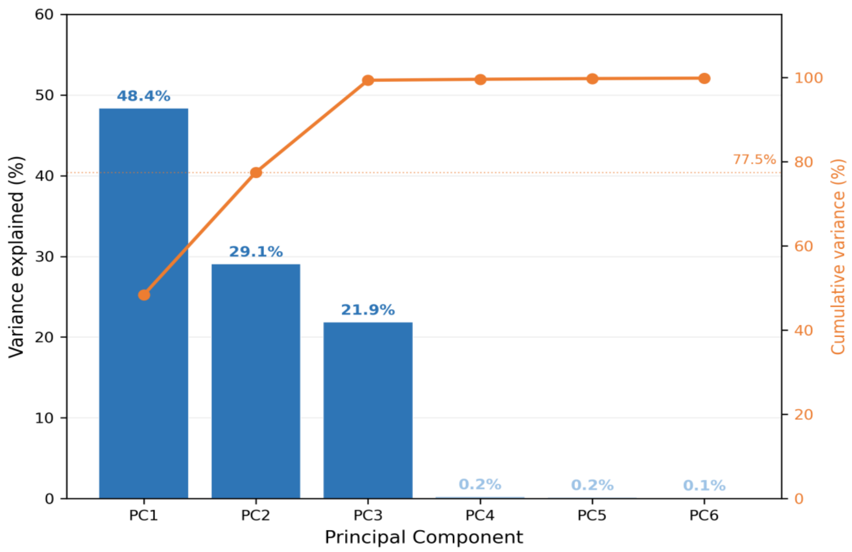

Figure 4. Scree plot showing variance explained by each principal component.

The compositional groupings observed in the PCA were validated by Ward hierarchical clustering with Euclidean distance on the same auto scaled dataset (Figure 5). All three replicates of each region consistently clustered together, confirming high within-region analytical reproducibility. The clear separation between regional clusters further supports the conclusion that essential oil composition differs significantly among Albanian populations of *Achillea millefolium*.

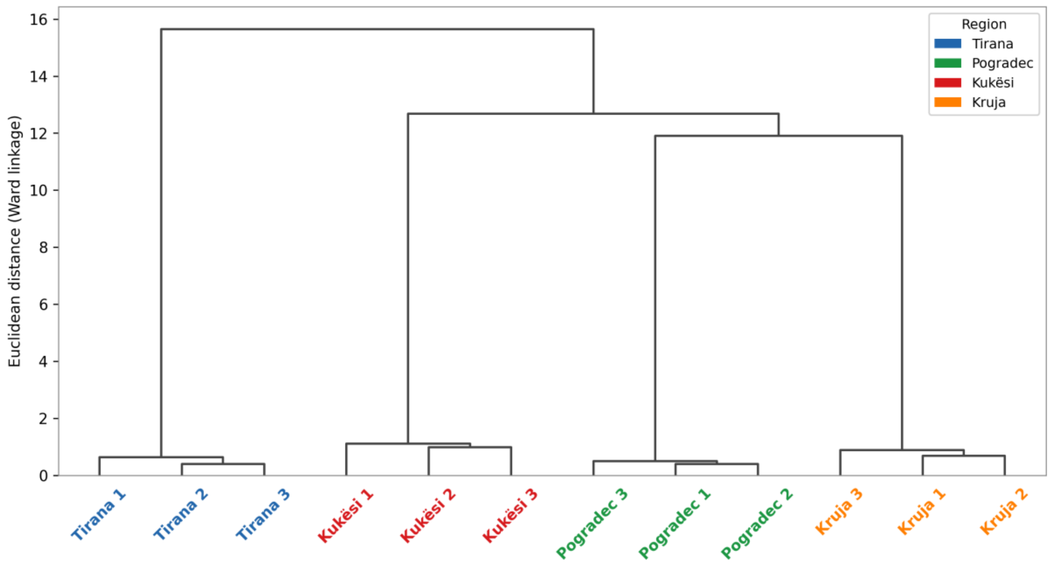

Figure 5. Ward hierarchical clustering dendrogram of *Achillea millefolium* essential oil composition from four Albanian regions. Auto-scaled data; Euclidean distance; Ward linkage.

DISCUSSIONS

Chemotype Classification

Based on the most abundant compound in the four regions the investigated populations of *Achillea millefolium* can be classified within a β-caryophyllene - germacrene chemotype. β-caryophyllene represented the principal compound (>18%), followed by germacrene (9-13%), indicating the dominance of sesquiterpene. Kukesi and Kruja populations may be described as β-caryophyllene-rich variants (> 23%). Tirana shows a higher value of oxygenated monoterpenes particularly 1,8 cineole suggesting monoterpene enrichment. Pogradeci population shows sesquiterpene dominance and moderate monoterpene presenting an intermediate profile. The other compounds a thujone and 1.8- cineole showed less difference in their values. Therefore, all four Albanian populations belong to a common sesquiterpene-dominant chemotype characterized by β-caryophyllene as the primary compound.

Ecological Interpretation

The highest β-caryophyllene values were observed in Kukësi and Kruja. Although the Kukësi population was collected at valley floor elevation (460 m), the site is situated at the foot of Gjallica Mountain (2,489 m) and is characterised by a continental mountain climate with cold winters and pronounced seasonal temperature fluctuations. Kruja (1,240 m) represents a mid-altitude forested environment. Both sites may therefore experience ecological conditions associated with increased sesquiterpene abundance. Elevated altitudes and continental climates are often characterised by lower mean temperatures, higher UV radiation, and greater temperature variation - factors reported to influence sesquiterpene biosynthesis as part of plant protection mechanisms [25].

The observed variability in terpene composition may also be related to environmental regulation of terpene biosynthesis. Terpenes in *Achillea millefolium* are synthesised through two primary pathways: the methylerythritol phosphate (MEP) pathway, responsible for monoterpenes, and the mevalonate (MVA) pathway, responsible for sesquiterpenes. Environmental factors such as temperature, radiation, and ecological stress may influence these biosynthetic pathways, leading to shifts in the relative production of monoterpenes and sesquiterpenes [10, 24]. Sesquiterpenes like *β*-caryophyllene are frequently associated with plant responses to environmental stress, including temperature fluctuations and increased ultraviolet radiation [10, 23]. Thus, the continental mountain climate of the Kukësi region and mid altitude climate of Kruja might be linked with higher amounts of these compounds.

Conversely, other oxygenated monoterpenes e.g. 1.8-cineole, showed higher values in plants collected from the vicinity of Tirana (616 m Dajti Mountain). These latter results may be indicative of environmental factors that are associated with increased monoterpene production, even though the relationship between elevation and composition is not linear. Plants originating from the Pogradeci population exhibited a balanced monoterpene–sesquiterpene composition analogous to an intermediate phytochemical profile, which might be more representative of mixed environmental factors. The results tend to support the argument that environmental factors may be responsible for the observed variation in Achillea millefolium population phytochemistry, but that microclimatic conditions may be more significant than elevation itself.

Pharmacological Relevance

β-Caryophyllene (24.40 ± 0.53%), the most abundant compound in the Kukësi essential oil, has been reported in the literature to exhibit anti-inflammatory, analgesic, neuroprotective, and anticancer properties. Recent reviews confirm its therapeutic potential in liver, neurological, and cancer applications [7, 17]. The essential oil from the Kruja region was enriched in linalool (3.62 ± 0.07%), which has reported anxiolytic and sedative properties [19]. Recent studies confirm that linalool has a broad spectrum of antimicrobial activity against resistant pathogens and biofilms, supports wound healing, and is used in dermatology [26]. Toxicological reviews reveal its safety and therapeutic potential [28]. *α*-Pinene, most abundant in the Tirana region (4.96 ± 0.06%), has reported anti-inflammatory, bronchodilatory, and neuroprotective properties [27]. 1,8-Cineole (eucalyptol), also most abundant in Tirana (9.39 ± 0.01%), is used in respiratory therapy; recent reviews reveal its antioxidant, antimicrobial, and neuroprotective effects and its use in asthma, sinusitis and cancer therapy [16, 18, 35]. Geraniol (3.14 ± 0.04%), with the highest percentage in the Pogradeci essential oil, is a free radical scavenger with antioxidant and cytoprotective potential [9]. Recent studies confirm its modulation of oxidative stress, anti-inflammatory activity, and Nrf2/HO-1 pathway activation, supporting anticancer and neuroprotective applications [5, 36].

Positioning Within State-of-the-Art

The variability in Achillea millefolium essential oil composition has been recorded along the rest of Europe and Asia, where different ecotypes have been shown to contain contrasting chemotypes [4, 11]. Its chemotypic profiles recorded in Albania are absent when using supercritical CO_2 extraction, which is a deficiency in the literature. Hydrodistillation is one of the most common extraction methods, as seen for the highland region of Dagestan in [25] and the Italian Alps [14]. Both reveal that highland populations are rich in sesquiterpenes with lowland populations containing a higher efficacy of oxygenated monoterpenes, similar to the population in Kukësi and Tirana from this study. Contents of *β*-caryophyllene in Dagestan ranged from 0.45% to 5.42% [25], while [14] recorded Italian Alpine values of 3.77% to 7.73%. Authors in [24] recorded the same variation seen here, between *Achillea millefolium* populations distributed down over different geographical gradients, for readers to recognize abiotic influences on phytochemical composition. Microwave assisted hydrodistillation [22] is used to increase efficiency in oil extraction with no noticeable alteration of chemical composition, with supercritical CO_2 helping to increase isolation of sesquiterpenes and protection of thermolabile elements as discussed in this study. Authors in [11] contrasts supercritical CO_2 extraction with hydrodistillation, noting compositional differences and stating benefits of supercritical CO_2 extraction.

This research work contributes region-specific data to the European chemotype landscape, documenting ecologically driven variation in Albanian populations. The phytochemical composition observed in Albanian populations shows similarities with previously reported European populations of *A. millefolium*. Studies conducted in mountainous areas of Dagestan reported higher concentrations of sesquiterpenes in high-altitude populations [25], which is consistent with the elevated β-caryophyllene levels observed in the Kukësi and Kruja populations in the present study (18.02–24.40%). Similarly, investigations carried out in the Italian Alps demonstrated altitude-dependent variation in essential oil composition, with higher monoterpene concentrations observed in lower-altitude environments [14]. Compared with Mediterranean populations reported in the literature, the Albanian populations display comparable terpene profiles but a markedly stronger sesquiterpene dominance in certain regions. In particular, the β-caryophyllene content observed in this study (18.02–24.40%) is substantially higher than that reported in most European populations and approaches the value reported by (26.0%) for supercritical CO_2 extracts [20]. In contrast, supercritical extracts of Bulgarian *Achillea millefolium* reported by [34] were dominated by oxygenated monoterpenes such as camphor (8.2–12.6%) and borneol (9.3–12.5%), with very low β-caryophyllene content

(~0.84–1.58%), highlighting substantial chemotypic variation even under similar extraction conditions. Table 4 depict the comparative overview of the chemotypic variability of *Achillea millefolium* from several world regions and its potential implications for Albanian populations.

This trend may also be explained in terms of a plant adaptative response to environmental conditions, where environmental variables such as increased solar radiation and lower temperatures and increased diurnal variability could impact the secondary metabolism, inducing the synthesis of sesquiterpenes which afford protective and adaptive advantages during environmentally stressful conditions [10, 23, 24]. Whether these similarities and differences can be attributed to differences in environmental conditions and their subsequent influence on chemotaxonomic variation across European A. millefolium populations remains to be seen. PCA results indicated population clustering into region-specific groups, and further reinforces the contention that environmental factors may have an impact on the chemotypic variation observed.

Table 4. Comparative overview of the chemotypic variability of *Achillea millefolium* from several world regions and its potential implications for Albanian populations.

Study	**Region / Method**	**Key Findings**	**β-caryophyllene (%)**	**Relevance to Albanian Populations**
[25]	Dagestan, Russia / Hydrodistillation	Essential oil composition varies with altitude, increasing sesquiterpene proportion at higher elevation	0.45–5.42%	Comparable ecological trend but lower β-caryophyllene than Albania
[14]	Italian Alps / Hydrodistillation	Altitude-dependent variation in essential oil yield and composition	3.77–7.73%	Lower values confirm stronger sesquiterpene dominance in Albanian populations
[22]	Iran / Microwave-assisted hydrodistillation vs. HD	Extraction method affects yield and relative composition of essential oil components	Not specified	Supports methodological baseline for conventional HD comparisons

[34]	Bulgarian / Supercritical CO_2 extraction	Essential oil dominated by oxygenated monoterpenes (camphor, borneol, artemisia ketone); low sesquiterpene content	0.84–1.58%	Contrast with Albanian samples, indicating different chemotype despite similar extraction method
[24]	Europe / Phytochemical profiling across geographical gradients	Significant variation in phenolic profiles of *Achillea millefolium* populations was observed across different geographical regions.	Not specified	Confirms that geographical gradients influence phytochemical composition, supporting regional variability observed in Albanian populations.
[11]	Sardinia, Italy / HD vs. Supercritical CO_2	Differences in chemical composition; antimicrobial activity	Not specified	Underscores pharmacologic al relevance of chemotypes
[20]	Lithuania / Supercritical CO_2	Essential oil dominated by sesquiterpenes, particularly β-caryophyllene	26.0%	Comparable upper range to Albanian samples
Present Study		Kukesi: β-caryophyllene–germacrene dominance; Tirana: relatively higher monoterpenes	18.02–24.40%	Indicates sesquiterpene-enriched Albanian chemotype

SUMMARY AND CONCLUSION

This study evaluated the phytochemical profile of *Achillea millefolium* essential oils from four Albanian regions (Tirana, Pogradeci, Kukësi, and Kruja) using supercritical CO_2 extraction coupled with GC-FID analysis. All three populations showed clear geographical effects on essential oil composition. It has been shown the dominance of a *β*-caryophyllene/germacrene *D* chemotype but concentrations of sesquiterpenes tended to be higher at Kukësi and Kruja which are located at sites with continental mountain climate and at sites with mid altitude ecological conditions, respectively. Conversely, the populations from Tirana showed higher levels of oxygenated monoterpenes, especially 1,8-cineole and α-pinene. Such adaptations are compatible with previous observations in which environmental effects such as altitude and climate have shown to affect the chemical composition of A. millefolium essential oils [14, 25]. Intermediate compositions have been observed in the Pogradeci population which occupies a transitional region between the continental mountain climate and the mediterranean climate. However, from a methodological point of view, supercritical extraction achieves selectively the recovery of specific components such as sesquiterpenes, while avoiding degradation of thermally sensitive and volatile ones. The presence of major bioactive compounds such as β-caryophyllene, 1,8-cineole, linalool, and α-pinene supports the pharmacological relevance of the investigated populations, in agreement with previously reported biological activities including anti-inflammatory, antioxidant, and antimicrobial effects [7, 16, 19, 27].

Additionally, this study was undertaken with a very limited number of sampling sites and a single sampling season where seasonal or interannual variability may have been exaggerated. Also, abiotic data (i.e. soil characteristics and microclimate variables) were not quantitatively recorded and therefore causal relationships were difficult to determine.

It should be noted, however, that this work offers the very first comprehensive profile of *A.millefolium* essential oils as collected from Albania, utilizing supercritical CO_2 extraction, as well as affirms the position of the four studied populations within the overall European chemotype model. The delineation of a sesquiterpene enriched chemotype derived from mid altitude ecological variables and a continental mountain climate further implies the significance of local ecological factors in determining terpene profiles and supports the promise of Albanian populations as a viable resource of bioactive composites for pharmaceutical and industrial purposes. The PCA clustering in this work mirrors qualitative differences in terpene composition as indicated by comparative contribution of sesquiterpenes versus monoterpenes, which is in agreement with the regional difference seen across the four populations.

The findings of this study have an important implication for herbal raw material standardization and European regulatory requirements. Demonstrating chemotype variations of *Achillea millefolium* through supercritical CO_2 extraction this study underscores the need for national and regional policies to promote sustainable harvesting of medicinal plants. Documenting chemotype variability also provides a scientific basis for regulatory authorities to establish more precise quality standards for herbal medicines and essential oils, ensuring consistency in therapeutic efficacy. On the industrial side, the use of supercritical CO_2 extraction offers a clean, solvent free, and selective method that preserves thermolabile compounds, thereby producing higher quality oils for pharmaceutical, cosmetic, and nutraceutical applications. The rich profile of the sesquiterpene of Kukësi and the oxygenated monoterpene profile of Tirana can create differentiated products. Integrating phytochemical data into national bioeconomy strategies would promote value-added processing within Albania and strengthen the long-term sustainability and industrial relevance of *A. millefolium* resources.

In the next phase, the doctoral research will expand the dataset by incorporating larger number for multivariate chemometric analyses for PCA and hierarchical clustering, will integrate more detailed climatic data, and will compare different extraction methods, including SFE-CO_2 and hydrodistillation. GC-MS confirmation of compound identities should be prioritised in future work Linking chemical profiles with bioactivity assays will further strengthen the evaluation of the applicative potential of Albanian *Achillea millefolium* chemotypes.

AUTHOR CONTRIBUTION

Conceptualization, E.K., and L.K.; methodology, E.K., and K.S.; software, E.K., and A.D.; validation, M.A., and A.B.; formal analysis, E.K.; investigation, E.K.; resources, L.K.; data curation, E.K., and L.K.; writing—original draft preparation, E.K.; writing—review and editing, E.K., and L.K.; visualization, A.B., and K.S.; supervision, L.K.; project administration, L.K.

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CONFLICT OF INTERESTS

The authors should confirm that there is no conflict of interest associated with this publication.

REFERENCES

1. Ali, S.I., Gopalakrishnan, B., & Venkatesalu, V. Pharmacognosy, phytochemistry and pharmacological properties of Achillea millefolium L.: A review. *Phytotherapy Research*, **2017**, *31*(8), 1140–1161.
2. Alvarenga, I.C.A., Boldrin, P.F., Pacheco, F.V., Silva, S.T., Bertolucci, S.K.V., & Pinto, J.E.B.P. Effects on growth, essential oil content and composition of the volatile fraction of Achillea millefolium L. cultivated in hydroponic systems deficient in macro- and microelements. *Scientia Horticulturae*, **2015**, *197*, 329–338.
3. Applequist, W.L., & Moerman, D. E. Yarrow (Achillea millefolium L.): A neglected panacea? A review of ethnobotany, bioactivity, and biomedical research. *Economic Botany*, **2011**, *65*(2), 209–225.
4. Arumugham, T., Rambabu, K., Hasan, S.W., Show, P.L., Rinklebe, J., & Banat, F. Supercritical carbon dioxide extraction of plant phytochemicals for biological and environmental applications. *A review. Chemosphere*, **2021**, 271, 129525.

5. Ben Ammar, R. Potential effects of geraniol on cancer and inflammation-related diseases: *A review of the recent research findings, Molecules,* **2023**, *28*(9), *3669.*
6. Bocevska, M., & Sovová, H. Supercritical CO_2 extraction of essential oil from yarrow. *The Journal of Supercritical Fluids*, **2007**, *40*(3), 360–367.
7. Cardeal dos Santos, A.N., da Cruz Freire, J.E., & Coelho-de-Souza, A.N. Pharmacokinetic and toxicological profile in pharmaceutical bioprospecting of caryophyllene molecules using computational biology for therapeutic purposes. *In Silico Pharmacology*, **2025**, *13*(2), 105.
8. Čechovičienė, I., Kazancev, K., Hallmann, E., Sendžikienė, E., Kruk, M., Viškelis, J., Tarasevičienė, Ž. Supercritical CO_2 and Conventional Extraction of Bioactive Compounds from Different Cultivars of Blackberry (*Rubus fruticosus* L.) Pomace. *Plants* **2024**, *13*, 2931.
9. Chen, W., & Viljoen, A. M. Geraniol-A review of a commercially important fragrance material. *South African Journal of Botany*, **2010;** *76*(4), 643–651.
10. Das, S., & Prakash, B. Effect of environmental factors on essential oil biosynthesis, chemical stability, and yields. *In B. Prakash, N. K. Dubey, & J. Freitas Brilhante de São José (Eds.), Plant essential oils. Springer.* **2024**, pp. 225-247.
11. Falconieri, D., Piras, A., Porcedda, S., Marongiu, B., Gonçalves, M.J., Cavaleiro, C., & Salgueiro, L. Chemical composition and biological activity of the volatile extracts of Achillea millefolium. *Natural Product Communications* **2011**, *6*(10), 1527–1530.
12. Garzoli, S.; Cicaloni, V.; Salvini, L.; Trespidi, G.; Iriti, M.; Vitalini, S. SPME-GC-MS Analysis of the Volatile Profile of Three Fresh Yarrow (*Achillea millefolium* L.) Morphotypes from Different Regions of Northern Italy. *Separations* **2023**, *10*, 51.
13. Farhadi, N., Babaei, K., Farsaraei, S., Moghaddam, M., & Ghasemi Pirbalouti, A. Changes in essential oil compositions, total phenol, flavonoids and antioxidant capacity of Achillea millefolium at different growth stages. *Industrial Crops and Products*, **2020;** *152*, 112570.
14. Giorgi, A., Bononi, M., Tateo, F., Cocucci, M. Yarrow (*Achillea millefolium* L.) Growth at Different Altitudes in Central Italian Alps: Biomass Yield, Oil Content, and Quality. *J. Herbs Spices Med. Plants* **2005**, *11*(3), 47–58.
15. Gómez de Cedrón, M., Siles-Sanchez, M. D. N., Martín-Hernandez, D., Jaime, L., Santoyo, S., & Ramírez de Molina, A. Novel bioactive extract from yarrow obtained by the supercritical antisolvent-assisted technique inhibits lipid metabolism in colorectal cancer. *Frontiers in Bioengineering and Biotechnology*, **2024**, 12, 1256190.
16. Hermanowicz, K., Oleksy, D., Doman, K., Nowak, J., Cioch, M. J., Najdek, A., &Mycyk, M. Therapeutic potential of 1,8-cineole in respiratory diseases with a focus on asthma, sinusitis, and upper respiratory tract infections: *A comprehensive review. Journal of Education, Health and Sport* **2025**, *78*, 57692.
17. Ivanova, S., Dzhakova, Z., Todorova, V., Boyuklieva, R., Simeonov, P., & Katsarov, P. Advancing brain health naturally: β-caryophyllene and xanthohumol as neuroprotective agents. *Molecules*, **2025;** *30*(18), 3702.
18. Juergens, L.J., Worth, H., & Juergens, U.R. New perspectives for mucolytic, anti-inflammatory and adjunctive therapy with 1,8-cineole in COPD and asthma: *Review on the new therapeutic approach. Advances in Therapy*, **2020**; *37*, 1737–1753.
19. Linck, V.M., da Silva, A.L., Figueiró, M., Piato, A.L., Herrmann, A.P., Dupont Birck, F., et al. Inhaled linalool-induced sedation in mice. *Phytomedicine* **2009**, *16*(4),303–307.

20. Marzouki, H., Piras, A., Porcedda, S., Falconieri, D., & Bagdonaite, E. Influence of extraction methods on the composition of essential oils of Achillea millefolium L. from Lithuania. *Journal of Biodiversity Management & Forestry* **2014**, *3*(4), 1000133.
21. Mohammadhosseini, M., Sarker, S. D., & Akbarzadeh, A. Chemical composition of the essential oils and extracts of Achillea species and their biological activities: A review. *Journal of ethnopharmacology* **2017**, *199*, 257–315.
22. Mollasalehi, S., & Kashefi, B., & Hashemi-Moghaddam, Hamid. Comparison of Microwave-Assisted and Hydrodistillation Methods for Extraction of Essential Oil from Achillea millefolium. *Journal of Chemical Health Risks*. **2013**, *3*. 39-46.
23. Öner, E. K., & Yeşil, M. Effects of altitude on secondary metabolite contents of Origanum majorana L. *Scientific Reports* **2023**, *13*, 10765.
24. Radušienė, J., Karpavičienė, B., Raudone, L., Vilkickyte, G., Çırak, C., Seyis, F., Yayla, F., Marksa, M., Rimkienė, L., &Ivanauskas, L. Trends in Phenolic Profiles of Achillea millefolium from Different Geographical Gradients. *Plants* **2023**, *12*(4), 746.
25. Radzhabov, G., Aliev, A., Musaev, A.M., & Islamova, F. Variability of the constituent composition of Achillea millefolium essential oils in the wild flora of Dagestan. *Pharmaceutical Chemistry Journal* **2022**, *56*, 1–6.
26. Rai, A., Subramaniyan, Y., Fathima, F., et al. Broad-spectrum antimicrobial properties of linalool: Supporting its pharmacological use in chronic wound infections by pathogens within the ESKAPE group and polymicrobial biofilms. *World Journal of Microbiology and Biotechnology*, **2025**, *41*, 99.
27. Salehi, B., Upadhyay, S., Erdogan Orhan, I., et al. Therapeutic Potential of α- and β-Pinene: A Miracle Gift of Nature. *Biomolecules* **2019**, *9*(11), 738.
28. Soulimani, R., & Joshi, R.K. Toxicological aspects and pharmaco-therapeutic properties of linalool, a natural terpene derivative of essential oils: Literature studies. *American Journal of Essential Oils and Natural Products*, **2020;** *8*(4), 24–34.
29. Usman, I., Hussain, M., Imran, A., Afzaal, M., Saeed, F., Javed, M., Saewan, S. Traditional and innovative approaches for the extraction of bioactive compounds. *International Journal of Food Properties*, **2022;** *25*(1), 1215–1233.
30. Uwineza, P. A., &Waśkiewicz, A. Recent advances in supercritical fluid extraction of natural bioactive compounds from plant materials. *Molecules* **2020,** *25(17), 3847. https://doi.org/10.3390/molecules25173847*
31. Vidović, S., Vasić, A., Vladić, J., Jokić, S., Aladić, K., Gavarić, A., &Nastić, N. Carbon dioxide supercritical fluid extracts from yarrow and rose hip herbal dust as valuable source of aromatic and lipophilic compounds. *Sustainable Chemistry and Pharmacy* **2021**, *22*, 100494.
32. Villalva, M., Jaime, L., Villanueva-Bermejo, D., Lara, B., Fornari, T., & Reglero, G. Supercritical anti-solvent fractionation for improving antioxidant and anti-inflammatory activities of an Achillea millefolium L. extract. *Food Research International*, **2019**, *115*, 128–134.
33. Villalva, M., Silván, J. M., Alarcón-Cavero, T., Villanueva-Bermejo, D., Jaime, L., Santoyo, S., & Martínez-Rodríguez, A. J. Antioxidant, anti-inflammatory, and antibacterial properties of an Achillea millefolium L. extract and its fractions obtained by supercritical anti-solvent fractionation against Helicobacter pylori. *Antioxidants* **2022**, *11*(10), 1849.

34. Villanueva-Bermejo, D., Rodríguez-García Risco, M., Reglero, G., & Fornari, T. Supercritical CO_2 extraction of Achillea millefolium: Optimization and characterization. *The Journal of Supercritical Fluids*, **2017**, *128*, 20–28.

35. Yao, B., He, B., Peng, J., et al. The comprehensive review of eucalyptol: Synthesis, metabolism, and therapeutic applications in disease treatment. *Molecular Biology Reports* **2025**, *52*, 346.

36. Younis, N.S., Abduldaium, M.S., & Mohamed, M.E. Protective effect of geraniol on oxidative, inflammatory and apoptotic alterations in isoproterenol-induced cardiotoxicity: Role of the Keap1/Nrf2/HO-1 and PI3K/Akt/mTOR pathways. *Antioxidants (Basel)*, **2020,** *9*(10), 977.