

SUPERCritical CO₂ EXTRACTION AND GC-MS PROFILING OF GERANIUM MACRORRHIZUM AND PELARGONIUM RADULA CULTIVATED IN BULGARIA

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ABSTRACT

Supercritical carbon dioxide extraction (SC-CO₂) is a promising modern extraction method increasingly used to extract lipophilic bioactive compounds from aromatic and medicinal plants. This study aimed to compare the extraction yields and chemical compositions of supercritical CO₂ extracts from *Geranium macrorrhizum* L. and *Pelargonium radula* (Cav.) L'Hér., cultivated in Bulgaria. The dried aerial parts of both species were subjected to SC-CO₂ extraction at 200 bar and 40 °C using a laboratory extraction system. The extraction yield was determined gravimetrically, and the chemical composition of the extracts was characterized by gas chromatography-mass spectrometry (GC-MS). It was found that the extraction yield of *Geranium macrorrhizum* (1.80%) was significantly higher than that of *Pelargonium radula* (0.25%) under identical operating conditions. GC-MS analysis

identified 25 constituents, mainly long-chain hydrocarbons, oxygenated sesquiterpenoids, and other lipophilic compounds. The main constituents in both extracts were n-nonacosane and n-tetratriacontane. However, significant species-related differences in composition were also observed. *Geranium macrorrhizum* contained higher levels of germacrone, curcuminone, and bisabolol derivatives, while *Pelargonium radula* was characterized by the presence of eugenol and a higher relative content of vitamin E. Also, phytol was found exclusively in the extract from *Geranium macrorrhizum*. The results indicate that botanical origin significantly

influences both the extraction efficiency and the phytochemical composition under identical supercritical extraction conditions. This study provides new data on the chemical profiles of SC-CO₂ extracts of *Geranium macrorrhizum* and *Pelargonium radula* grown in Bulgaria and contributes to further evaluation of their potential biological and industrial applications.

KEYWORDS: *Geranium macrorrhizum*; *Pelargonium radula*; supercritical carbon dioxide extraction; GC–MS; phytochemical profiling; lipophilic compounds.

1. INTRODUCTION

The Geraniaceae family includes numerous aromatic plant species known for their volatile, lipophilic secondary metabolites, which have potential applications in the pharmaceutical, cosmetic, and food industries. Among them, *Geranium macrorrhizum* L. and *Pelargonium radula* (Cav.) L'Hér. are recognized for their distinctive aroma and rich phytochemical profile. Essential oils and plant extracts from these species have attracted scientific interest due to the presence of both terpenoids and many other bioactive constituents that support traditional and industrial applications.^[1-3] The qualitative and quantitative composition of plant extracts is strongly influenced by the extraction technique used. Conventional methods such as hydrodistillation remain widely used to isolate volatile constituents; however, prolonged exposure to elevated temperatures can degrade, transform, or cause the loss of thermolabile compounds. Therefore, increasing attention is being paid to alternative extraction technologies that preserve the natural chemical profile of aromatic plants.^[4-5]

Supercritical carbon dioxide (SC-CO₂) extraction has emerged as a leading green extraction technique and a method for extracting lipophilic and volatile compounds from plant materials. In this process, carbon dioxide is converted into a supercritical fluid above its critical temperature and pressure, combining gas-like diffusivity with liquid-like solvation ability. This allows for efficient penetration of plant matrices and selective extraction of target compounds under relatively mild operating conditions. Furthermore, SC-CO₂ extraction eliminates the need for organic solvents and yields high-purity extracts suitable for pharmaceutical and cosmetic applications.^[6-8] Numerous studies have shown that extraction parameters, such as pressure, temperature, and carbon dioxide flow rate, strongly influence the extraction yield and chemical composition. Depending on the chosen conditions, supercritical extraction can preferentially recover terpenes, oxygen derivatives, long-chain hydrocarbons, and other lipophilic constituents, resulting in chemical profiles that differ

significantly from those obtained by conventional extraction techniques.^[9-11] In addition to the extraction yield, supercritical carbon dioxide extraction can significantly affect the relative abundance of individual phytoconstituents, including terpenoids, phenolic compounds, and other lipophilic metabolites. This further correlates with one of the most widely used analytical techniques for the characterization of supercritical plant extracts, namely Gas Chromatography with Mass Detection.^[12-14] The composition of the essential oil of *Geranium macrorrhizum* has been extensively studied, revealing significant variability related to geographical origin, environmental conditions, and plant genotype.^[1,3] Studies on Bulgarian populations further confirmed the presence of characteristic volatile constituents and demonstrated the species' phytochemical potential.^[15] Similarly, *Pelargonium radula* has attracted attention as an aromatic species rich in volatile compounds^[16], again with significant variability in chemical composition depending on species identity, environmental conditions, and extraction methods, highlighting the importance of detailed phytochemical characterization of individual species and of extracts obtained under specific processing conditions.^[17]

Despite the growing interest in supercritical extraction of aromatic plants, comparative information on SC-CO₂ extracts of *Geranium macrorrhizum* and *Pelargonium radula* obtained under identical extraction conditions remains limited. Furthermore, data on the chemical composition of supercritical extracts from plants cultivated in Bulgaria are scarce.

Therefore, the aim of the present study was to obtain supercritical CO₂ extracts of *Geranium macrorrhizum* and *Pelargonium radula* cultivated in Bulgaria and to characterize their chemical composition using gas chromatography-mass spectrometry (GC-MS). Particular attention was paid to the extraction yield, chromatographic profiles, and distribution of major constituents in order to assess species-related differences in the composition of the recovered lipophilic fractions.

2. MATERIALS AND METHODS

2.1. Plant Material

Aerial parts of *Geranium macrorrhizum* L. and *Pelargonium radula* (Cav.) L'Hér. were harvested during the flowering period from cultivated populations in the Gabrovo region of Central Bulgaria. Botanical identification of both species was conducted by Assoc. Prof. Iliya Zhelev (Department of Biology, Faculty of Pharmacy, Medical University of Varna, Bulgaria).

After collection, the plant material was dried under ambient conditions in a shaded, well-ventilated area until a constant weight was achieved. The dried material was then ground in a laboratory mill to obtain a homogeneous powder with an approximate particle size of 0.5 mm. The resulting herbal substances were used for supercritical carbon dioxide extraction and subsequent GC–MS analysis.

2.2. Supercritical CO₂ Extraction

Supercritical carbon dioxide extraction was performed using a laboratory-scale supercritical extraction system (SUPEREX F-500, Superex, Turkey). Thirty grams of dried and pulverized plant material from each species were placed in a fabric extraction basket and loaded into the extraction vessel.

The extraction process was conducted at a pressure of 200 bar and a temperature of 40 °C. Carbon dioxide was supplied at a constant flow rate of 5 L/min. Extraction continued for approximately 2–4 h until no additional extract was visually observed in the collection vessel.

The extracts were collected in pre-weighed glass tubes, and their masses were determined using an analytical balance. Extraction yield was calculated as a percentage of the initial dry plant material using the following equation:

$$\text{Yield (\%)} = (\text{mass of extract} / \text{mass of dry plant material}) \times 100$$

The extracts were stored at –20 °C until further analysis.

2.3. GC–MS Analysis

The chemical composition of the supercritical CO₂ extracts was determined using an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 5975C mass selective detector. GC–MS conditions were applied as previously described, with slight modifications.^[18] Chromatographic separation was achieved on an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness).

The oven temperature was initially held at 35 °C for 3 min, then increased at 5 °C/min to 250 °C, followed by a final 3-min hold. The total run time was 49 min. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. Sample injection was performed with a split ratio of 30:1. Identification of individual constituents was accomplished by comparing retention times and mass spectral data with those available in commercial mass spectral libraries and in the published literature. The identified compounds were arranged by retention

time, and their relative abundances were expressed as percentages of the total ion chromatogram.

3. RESULTS AND DISCUSSION

3.1. Extraction Yield

The extraction conditions and extraction yields of the investigated species are presented in Table 1.

Table 1: Supercritical CO₂ extraction conditions and extraction yield of *Geranium macrorrhizum* and *Pelargonium radula*.

Plant species	Plant material (g)	Extract mass (g)	Yield (%)
<i>Geranium macrorrhizum</i>	30.0	0.5385	1.80
<i>Pelargonium radula</i>	30.0	0.0756	0.25

Under identical supercritical CO₂ extraction conditions (200 bar, 40 °C, and a CO₂ flow rate of 5 L/min), the two investigated species exhibited markedly different extraction yields. *Geranium macrorrhizum* yielded 1.80%, whereas the yield obtained from *Pelargonium radula* was only 0.25%, indicating substantially greater recovery of CO₂-soluble constituents from *G. macrorrhizum*. Since all extraction parameters were kept constant, the observed difference is most likely associated with intrinsic species-specific characteristics rather than methodological variability.

Extraction efficiency in supercritical CO₂ processes depends not only on the applied pressure and temperature but also on the plant matrix's chemical composition, the localization of extractable metabolites within plant tissues, and the affinity of individual compounds for the supercritical solvent.^[4–6,9] Previous studies have shown that modifying extraction parameters can substantially affect both extraction yield and chemical composition by altering the density and solvent power of supercritical carbon dioxide.^[5,8–11] In the present study, however, these variables were standardized for both species, suggesting that the considerably higher extraction yield of *G. macrorrhizum* primarily reflects differences in its phytochemical composition and the abundance of lipophilic constituents.

The observed differences are consistent with previous studies highlighting considerable phytochemical variability within the Geraniaceae family. The chemical composition of *Geranium macrorrhizum* has been reported to vary by genotype, geographical origin, and environmental conditions^[1,3,15], whereas *Pelargonium* species likewise show pronounced

interspecific differences in their phytochemical profiles, with extraction technique representing an additional source of compositional variation.^[16,17] Such species-dependent variability may influence both the abundance and distribution of CO₂-soluble lipophilic constituents within plant tissues and, consequently, their recovery under supercritical extraction conditions. Therefore, the substantially higher extraction yield from *G. macrorrhizum* is likely to reflect intrinsic phytochemical differences between the two investigated species rather than differences in the extraction process itself.

Although extraction yield is an important technological parameter, it should not be the sole indicator of extract quality. Supercritical CO₂ selectively recovers lipophilic constituents, and differences in yield do not necessarily reflect greater phytochemical diversity but may instead reflect differences in the abundance of specific metabolite classes.^[6,8] Therefore, comprehensive chromatographic characterization is essential to determine whether the higher extraction yield of *G. macrorrhizum* is accompanied by qualitative differences in phytochemical composition. Accordingly, both extracts were subsequently characterized by GC–MS analysis.

3.2. GC–MS Composition of the Supercritical CO₂ Extracts

The chemical composition of the supercritical CO₂ extracts obtained from *Geranium macrorrhizum* and *Pelargonium radula* is presented in Table 2.

Table 2: Chemical composition of the supercritical CO₂ extracts of *Geranium macrorrhizum* and *Pelargonium radula* determined by GC–MS analysis.

Peak	RT	RI	Name	% of TIC	
				s1	s2
1	21,81	1333	Eugenol	nd	1,06
2	25,91	1525	5-Allyl-2-methoxyphenyl acetate	nd	0,28
3	27,81	1607	β -Elemenone	nd	0,34
4	28,88	1662	<i>epi</i> - β --Bisabolol	1,39	nd
5	29,17	1671	β -Bisabolol	1,02	0,50
6	29,37	1685	<i>epi</i> - α --Bisabolol	0,57	nd
7	29,85	1689	α -Bisabolol	0,89	0,38
8	30,03	1701	Germacrone	3,26	1,19
9	33,03	1844	Curcumenone	1,80	0,40
10	33,12	1850	Phenyl ethyl octanoate	2,08	0,75
11	33,45	1866	(<i>E</i>)- β -Santalol acetate	1,04	0,40
12	34,51	1913	Farnesyl acetone	0,68	0,20
13	34,98	1945	Phytol	0,83	nd
14	42,37	2360	5-Methyl-5-(4,8,12-trimethyltridecyl)dihydro-2(3H)-furanone	0,66	0,28

15	43,82	2498	<i>n</i> -Pentacosane	0,99	0,60
16	45,28	2600	<i>n</i> -Hexacosane	0,73	0,44
17	47,84	2701	<i>n</i> -Heptacosane	2,63	2,41
18	49,76	2800	<i>n</i> -Octacosane	1,73	1,10
19	50,04	2875	α -Tocospiro B	1,89	1,14
20	50,71	2902	<i>n</i> -Nonacosane	35,08	37,10
21	51,95	3140	Vitamin E	1,07	1,81
22	52,97	3201	<i>n</i> -Dotriacontane	2,21	1,33
23	53,31	3400	<i>n</i> -Tetratriacontane	26,61	35,92
24	54,54	3486	3-Methyltetratriacontane	6,19	1,64
25	55,75	3603	<i>n</i> -Hexatriacontane	6,54	10,58

A total of 25 constituents were identified in the extracts, including oxygenated sesquiterpenoids, phenylpropanoids, diterpenoids, long-chain hydrocarbons, and other lipophilic compounds. The chromatographic profiles of both species were dominated by long-chain hydrocarbons, indicating that the selected extraction conditions favored the recovery of nonpolar constituents. This observation aligns with previous studies showing that supercritical carbon dioxide preferentially extracts hydrophobic metabolites because of its low polarity and tunable solvent properties, yielding extracts enriched with lipophilic compounds.^[4–8]

Among the identified constituents, *n*-nonacosane and *n*-tetratriacontane were the predominant compounds in both extracts. In *G. macrorrhizum*, *n*-nonacosane and *n*-tetratriacontane accounted for 35.08% and 26.61% of the total ion current, respectively, whereas in *P. radula* their relative abundances were 37.10% and 35.92%. In addition, *n*-hexatriacontane was detected in both species, representing 6.54% of the extract from *G. macrorrhizum* and 10.58% of the extract from *P. radula*. The predominance of these long-chain hydrocarbons aligns with previous reports describing hydrocarbon-rich supercritical extracts from aromatic plants, reflecting the selective solubilization of nonpolar constituents by supercritical CO₂.^[5,6,8]

Although the overall chromatographic profiles of the two species were broadly similar, marked differences were observed in the distribution of oxygenated constituents. *Geranium macrorrhizum* contained substantially higher levels of germacrone (3.26%) and curcumenone (1.80%) than *P. radula* (1.19% and 0.40%, respectively). Likewise, α -bisabolol, β -bisabolol, and their epimeric derivatives were considerably more abundant in *G. macrorrhizum*, with a cumulative content of 3.87%, compared with only 0.88% in *P. radula*. Previous investigations have demonstrated considerable phytochemical variability in *G. macrorrhizum*

associated with genotype, geographical origin, and environmental conditions.^[1,3,15] Although those studies mainly focused on essential oils, the present results indicate that species-specific differences are also reflected in supercritical CO₂ extracts.

Distinct qualitative differences were also evident among the species examined. Eugenol, 5-allyl-2-methoxyphenyl acetate, and β -elemenone were detected exclusively in *P. radula*, whereas phytol, together with epi- β -bisabolol and epi- α -bisabolol, was identified only in *G. macrorrhizum*. These qualitative differences are consistent with previous reports that document pronounced interspecific variation among *Pelargonium* species and emphasize the influence of both botanical origin and extraction technique on phytochemical composition.^[16,17] The exclusive occurrence of these constituents further supports the species-specific character of the recovered lipophilic fractions.

Vitamin E was detected in both extracts, though its relative abundance was higher in *P. radula* (1.81%) than in *G. macrorrhizum* (1.07%). In contrast, phytol was identified exclusively in *G. macrorrhizum*. This diterpene alcohol is widely recognized as a biologically relevant lipophilic constituent of higher plants and has been reported in numerous plant-derived extracts obtained using nonpolar extraction techniques.^[19] Its selective occurrence in the present study further differentiates the phytochemical profiles of the two investigated species and highlights the ability of supercritical CO₂ extraction to preserve species-specific metabolites.

The distribution of the major compound groups identified in the analyzed extracts is summarized in Table 3.

Table 3: Distribution of major constituent groups in the supercritical CO₂ extracts of *Geranium macrorrhizum* and *Pelargonium radula*.

Compound group	<i>Geranium macrorrhizum</i> (%)	<i>Pelargonium radula</i> (%)
Bisabolol derivatives*	3.87	0.88
Oxygenated sesquiterpenoids**	5.06	1.59
Long-chain hydrocarbons	82.71	91.12
Other identified compounds	8.25	6.26

*Bisabolol derivatives = α -bisabolol, β -bisabolol, epi- α -bisabolol and epi- β -bisabolol.

**Oxygenated sesquiterpenoids = germacrone and curcumenone.

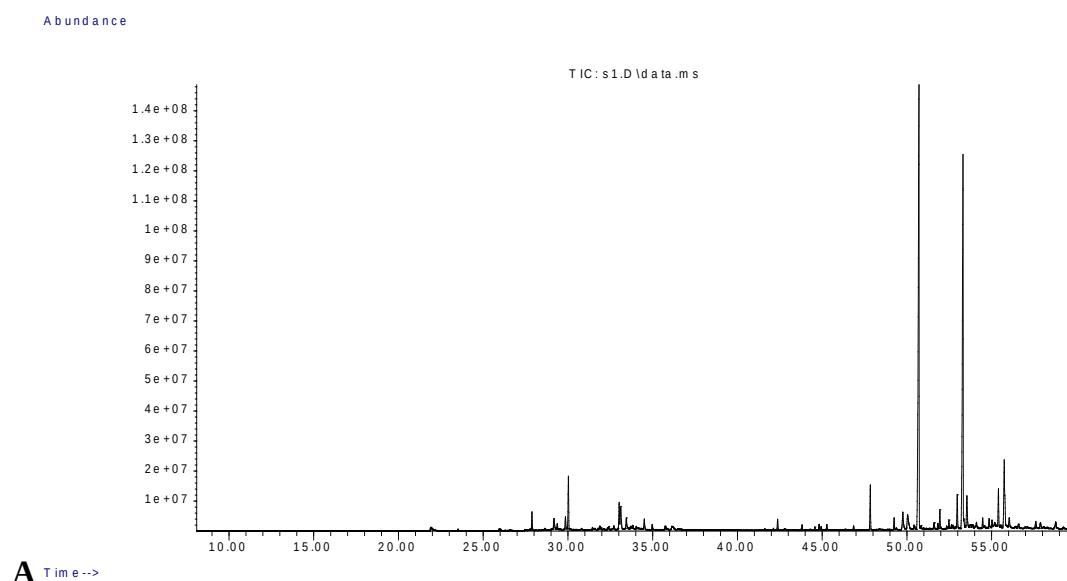
As shown in Table 3, long-chain hydrocarbons were the predominant chemical class in both extracts, accounting for 82.71% and 91.12% of the identified constituents in *G.*

macrorrhizum and *P. radula*, respectively. In contrast, *G. macrorrhizum* contained considerably higher proportions of oxygenated sesquiterpenoids (5.06%) and bisabolol derivatives (3.87%) than *P. radula*. These differences demonstrate that, although identical extraction conditions produced hydrocarbon-rich extracts in both species, botanical origin substantially influenced the relative abundance of oxygenated metabolites. These observations align with previous studies indicating that species identity and extraction methodology are among the principal factors determining the phytochemical composition of aromatic plant extracts.^[5,8,16,17]

Overall, the GC–MS analysis demonstrated that supercritical CO₂ extraction produced chemically distinct lipophilic extracts from the two Geraniaceae species investigated. While both extracts were dominated by long-chain hydrocarbons, *G. macrorrhizum* showed higher abundance of oxygenated sesquiterpenoids and bisabolol derivatives, whereas *P. radula* had higher relative levels of hydrocarbons and species-specific constituents such as eugenol. These findings confirm that botanical origin plays a decisive role in determining the phytochemical composition of supercritical CO₂ extracts, even when extraction parameters are identical.

3.3. Comparative Chromatographic Analysis

Representative GC–MS chromatograms of supercritical CO₂ extracts from *Geranium macrorrhizum* and *Pelargonium radula* are shown in Figure 1.



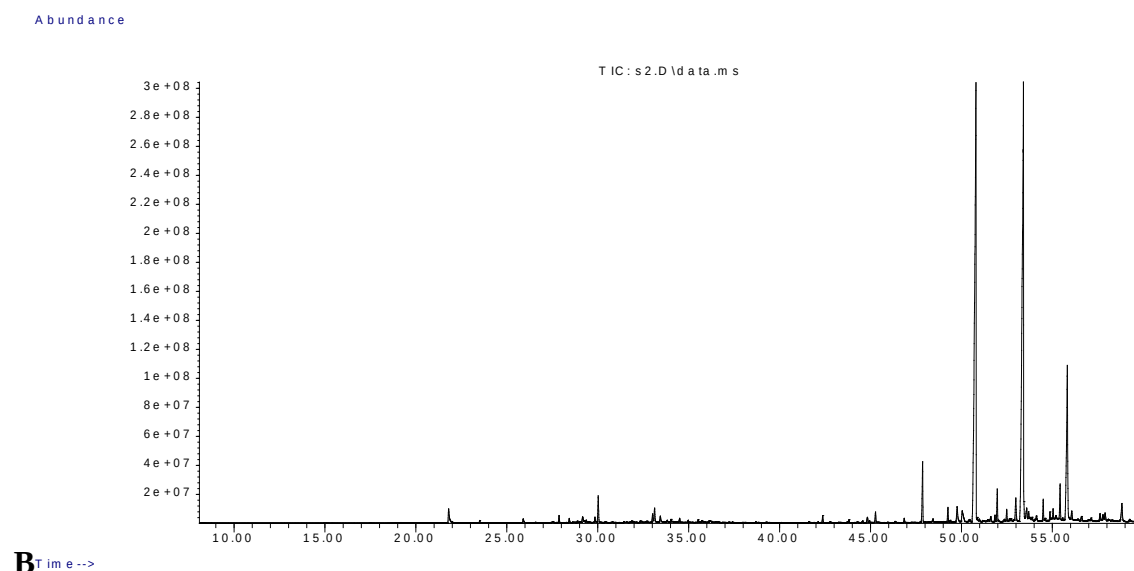


Figure 1: GC–MS chromatograms of the supercritical CO₂ extracts obtained from (A) *Geranium macrorrhizum* and (B) *Pelargonium radula*.

Visual comparison of the chromatograms showed similar overall chromatographic profiles for both species, with several dominant peaks eluting at longer retention times. These peaks corresponded predominantly to *n*-nonacosane, *n*-tetratriacontane, and *n*-hexatriacontane, confirming that long-chain hydrocarbons were the major constituents of both supercritical extracts. The predominance of these nonpolar compounds is consistent with the well-established selectivity of supercritical CO₂ for lipophilic metabolites under the applied extraction conditions.^[5,6,8]

Despite the overall similarity of the chromatographic patterns, noticeable differences were observed in the relative intensities of several peaks corresponding to oxygenated constituents. In particular, the chromatogram of *G. macrorrhizum* exhibited more intense signals for germacrone, curcumenone, and bisabolol derivatives, whereas *P. radula* was distinguished by the presence of eugenol and relatively higher abundances of several hydrocarbon constituents. These observations closely align with the quantitative GC–MS data presented in Table 2 and further confirm that the principal differences between the investigated species are associated with minor oxygenated metabolites rather than with the dominant hydrocarbon fraction.

The comparative chromatographic analysis demonstrates that, although identical supercritical extraction conditions produced hydrocarbon-rich extracts with broadly similar chemical profiles, botanical origin remained the primary factor governing the qualitative and

quantitative distribution of individual constituents. This finding aligns with previous studies reporting that species identity is a major determinant of phytochemical composition in aromatic plants, irrespective of the extraction technique employed.^[1,3,15–17] Collectively, the chromatographic and GC–MS results confirm that supercritical CO₂ extraction effectively preserves species-specific phytochemical characteristics while selectively recovering lipophilic constituents.

4. CONCLUSION

The present study demonstrated the applicability of supercritical carbon dioxide extraction for recovering lipophilic constituents from *Geranium macrorrhizum* and *Pelargonium radula* cultivated in Bulgaria. Under identical extraction conditions, *G. macrorrhizum* yielded substantially more extract than *P. radula*, indicating pronounced species-dependent differences in the recovery of CO₂-soluble compounds.

GC–MS analysis identified 25 constituents, predominantly long-chain hydrocarbons, oxygenated sesquiterpenoids, phenylpropanoids, diterpenoids, and other lipophilic metabolites. Although both extracts were dominated by *n*-nonacosane and *n*-tetratriacontane, distinct qualitative and quantitative differences were observed. *Geranium macrorrhizum* was characterized by higher levels of germacrone, curcumenone, and bisabolol derivatives, along with the exclusive occurrence of phytol, whereas *Pelargonium radula* contained eugenol and exhibited higher relative abundances of vitamin E and several long-chain hydrocarbons.

The results demonstrate that, even under identical supercritical extraction conditions, botanical origin remains the primary factor shaping both extraction efficiency and the phytochemical composition of the recovered lipophilic fractions. By providing a comparative GC–MS characterization of supercritical CO₂ extracts from *G. macrorrhizum* and *P. radula* cultivated in Bulgaria, this study expands the available phytochemical knowledge of these species and provides a scientific basis for future investigations into their biological properties and for optimizing supercritical CO₂ extraction for pharmaceutical, cosmetic, and other industrial applications. These findings provide a basis for future optimization of supercritical extraction parameters and targeted recovery of biologically active lipophilic compounds from Geraniaceae species.

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