

Chemical profile of *Ramonda serbica* populations from Albania

Aurel Nuro^{1*}, Bledar Myrtaj¹, Efigjeni Kongjika², Valbona Sota^{2,3}, Bekim Gashi⁴, Ariola Mesiti⁵, Kleva Shpati⁶, Aida Dama⁶, Aurora Napuce⁶

¹Department of Chemistry, Faculty of Natural Sciences, University of Tirana, Albania

²Research center of Biotechnology and Genetics, Albanian Academy of Sciences, Albania

³Department of Biotechnology, Faculty of Natural Sciences, Tirana University, Albania

⁴Department of Biology, Faculty of Natural Sciences and Mathematics, Pristina University, Kosovo

⁵Research Center of Flora and Fauna, Faculty of Natural Sciences, Tirana University, Albania

⁶Department of Pharmacy, Faculty of Medicinal Sciences, Albanian University, Albania

*Corresponding author: aurel.nuro@fshn.edu.al

(Received: 23 December 2025 / Accepted: 7 October 2026)

Abstract

Ramonda serbica (Gesneriaceae) is a rare and relict resurrection plant endemic to the Balkan Peninsula and represents one of the few European members of the Gesneriaceae family. This species is widely recognized for its exceptional desiccation tolerance, being able to survive prolonged periods of complete dehydration and to fully recover upon rehydration. Owing to these unique physiological traits, species of the genus *Ramonda* have been extensively investigated from biological, genetic, and biochemical perspectives, particularly with regard to stress tolerance mechanisms, dehydration–rehydration dynamics, genome size, ploidy-level variation, and genetic polymorphism. In contrast, information concerning the chemical composition of *R. serbica*, especially its essential-oil profile, remains scarce. The aim of the present study was to characterize, for the first time, the chemical profile of the essential oil obtained from leaves of *R. serbica* populations growing in Central Albania. Leaf samples were collected in July 2025 from two representative sites, Petrelë and Krujë. After air-drying, the plant material was subjected to hydro-distillation using a Clevenger-type apparatus. The resulting essential oils were analyzed by gas chromatography–mass spectrometry (GC–MS) for qualitative identification and by gas chromatography with flame ionization detection (GC–FID) for quantitative evaluation. Chromatographic analysis revealed a complex chemical profile dominated by oxygenated compounds, particularly oxygenated sesquiterpenes and alcohols, while non-oxygenated hydrocarbons were present only in minor amounts. The prevalence of high-molecular-weight, oxygen-containing metabolites clearly differentiates the chemical signature of *R. serbica* from essential-oil profiles typically dominated by hydrocarbon terpenes. Moreover, the overall similarity in the main constituents between samples from different locations indicates a relatively stable chemical pattern within Central Albania, despite local environmental differences. These findings provide the first reference data on the essential-oil composition of *R. serbica* in Albania and establish a valuable baseline for future phytochemical, ecological, and bioactivity-oriented studies on this unique Balkan endemic species.

Keywords: ramonda serbica, essential oil, hydro-distillation, GC/FID, GC/MS

Introduction

Ramonda serbica Panč., is a rare perennial species of the family Gesneriaceae and one of the few representatives of the genus *Ramonda*, a relict taxon of significant biogeographical value in Europe. While Gesneriaceae is primarily tropical, *R. serbica* has persisted in south-eastern Europe, reflecting long-term survival in climatically stable of the Balkan Peninsula [1-5]. First described in the nineteenth century near Niš, Serbia, the species has attracted attention due to its limited distribution and remarkable physiological adaptations. A key feature of *R. serbica* is its desiccation tolerance, which allows the plant to survive near-complete loss of water and subsequently recover full metabolic activity upon rehydration [2, 5-8]. This places it among a small group of vascular “resurrection plants.” Unlike most flowering plants, which are damaged irreversibly under severe water deficit, *R. serbica* can endure prolonged drought periods while maintaining cellular integrity [4, 6]. The species’ distribution is confined to the Balkan Peninsula, including Serbia, Montenegro, North Macedonia, Albania, Bulgaria, and northern Greece [5, 7, 9-12]. Populations are fragmented and restricted to shaded limestone cliffs, deep gorges, and ravines that provide reduced solar

radiation, moderated temperatures, and relatively high humidity [3]. Typically, the species grows on north- or northwest-facing rock surfaces at elevations of 400–1,500 m.

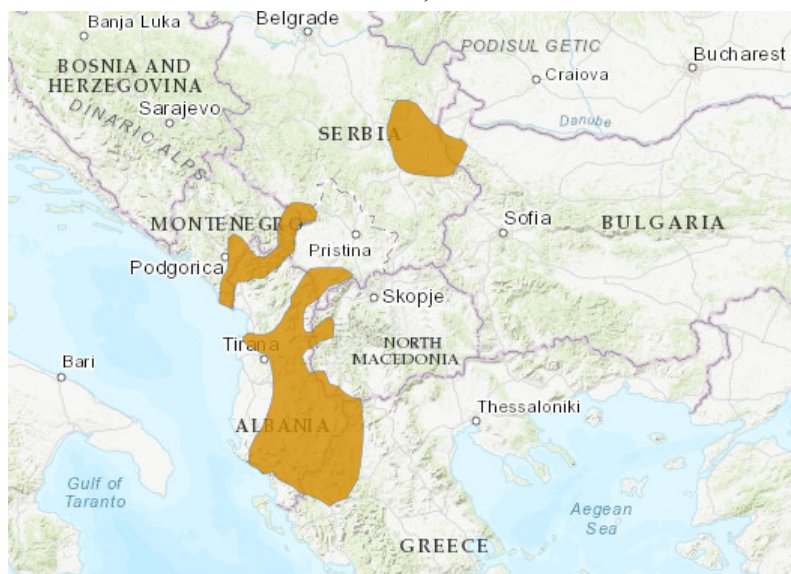


Figure 1. The habitat of *Ramonda serbica* in the Balkan area [5].

Morphologically, *R. serbica* is a low-growing perennial herb forming a compact basal rosette. The leaves are thick, coriaceous, and crenate, allowing flexibility during dehydration and rehydration cycles. Compared with other *Ramonda* species, *R. serbica* has a flatter rosette, which aids its persistence on vertical limestone surfaces [1, 4-9]. Flowering occurs from mid-April to late May, with violet to bluish flowers usually consisting of five or six lobes, and is influenced by local microclimatic conditions. During dry periods, particularly in summer, *R. serbica* enters a reversible state of metabolic dormancy known as anabiosis, characterized by reduced physiological activity and complete desiccation [8-14]. Even during flowering, severe spring drought can trigger this state, highlighting the species' survival-first strategy under extreme stress [3]. Physiological studies show that the plant responds to dehydration by enhancing antioxidative defence systems, including enzymes that scavenge reactive oxygen species [11-17]. Changes in cellular redox balance act as signals coordinating protective responses, ensuring membrane and protein stability during desiccation and rehydration.

Phytochemical analyses indicate that *R. serbica* contains a variety of bioactive compounds contributing to its stress resilience. High levels of phenolic compounds, such as flavonoids and phenolic acids, act as antioxidants neutralizing reactive oxygen species generated during dehydration cycles [8]. Soluble sugars, including sucrose and raffinose-family oligosaccharides, stabilize membranes and proteins, while accumulation of specific amino acids and stress-related proteins supports osmotic balance and protects cellular structures under water deficit. Rehydration triggers a rapid but controlled recovery. Oxidative stress markers typically decline within 24 hours, and relative water content is restored to near-normal levels within 48 hours. Complete recovery, including repair of membranes and fine-tuning of metabolism, requires several additional days [8-15]. In summary, *Ramonda serbica* is a Balkan relict species with narrow ecological requirements, fragmented populations, and remarkable desiccation tolerance. Its combination of physiological adaptations and protective chemical compounds makes it a valuable model for studying plant survival under extreme environmental stress, while its restricted habitat underscores its conservation importance in the Balkan Peninsula [4, 7, 9-16].

Materials and Methods

Sampling of Ramonda serbica plants

The samples of *Ramonda serbica* plants (aerial parts) was taken from the wild populations of the Central Albania: 4 stations near the Petrela (6 km in the South of Tirana) and 5 stations to the Kruja region (17 km in the East-North of Tirana). Leaves of *R. serbica* plants were sampled in July 2025. The plant material of each station were air dried in the shadow for conserving their morphological characteristics. The plant material

after drying was grinded into small pieces < 0.5 cm for further analysis. Plant of each station were analyzed together as a representative sample (9 samples in total) by using HD followed by GC/FID technique.

Isolation of essential oils by using HD

The plant material of *Ramonda serbica* (50 g of dry leaves) was subjected to hydro-distillation (HD) for 4 hours by using a Clevenger type apparatus (recommended by European Pharmacopeia) for the isolation of the essential oil. The essential oil was collected in 2 ml toluene as extraction solvent. The extract was dehydrated by adding 1 g anhydrous sodium sulphate. It was stored in dark vials at +4°C. The essential oil of *R. serbica* was subjected to qualitative analyse by using GC/MS technique and quantitative analysis using the GC/FID technique [15, 17-19].

The qualitative analyses of R. serbica on GC/MS spectra's

The *Ramonda serbica* samples were analysed qualitatively by using a Shimadzu 2030 Nexis gas chromatograph equipped with a fused silica capillary column BP-5MS (5% phenyl methyl siloxane, 30 m x 0.25 mm x 0.25 µm), and coupled with a GCMS-QP 2020NX mass spectrometer detector from the same company. The injector and the interface temperature were set at 280°C and 300°C, respectively. 1 µL of the essential oil (dissolved in toluene), was injected in a split ratio 50:1). The oven temperature firstly was held for 1 minute at 70°C, after that with 5°C/min was increased to 150°C. In the next step temperature was increased at 280°C with 10°C/min, held for 4 minutes. The MS conditions were: ionization voltage 70 eV, acquisition mass range 50–500, scan time 0.32 s. The chromatograms were obtained from the minute 2.5 (excluding toluene peak) to the 32 minute. The extracts constituents of *R. serbica* were identified by comparing their linear retention indices (relative to C6–C32 alkanes on the BP-5MS column) with literature values and their mass spectra with those of authentic standards, as well as those from NIST02, Mass Finder 2.3, and electronic library. Some pure substances (Limonene, Geraniol, and Camphene) were used for the confirmation of detected compounds [17-19].

The quantification analyse of R. serbica on GC/FID spectra

Gas chromatographic analysis of *Ramonda serbica* samples was performed on a Varian 450 GC apparatus, equipped with a PTV injector and a flame ionization detector (FID). Injector and detector temperatures were set at 280°C and 300°C, respectively. 1 µL of essential oil of *R. serbica* extract dissolved in Toluene was injected in split mode (1:50). The same capillary column BP-5MS (30 m x 0.25 mm x 0.25 µm), injector, oven temperatures and the gas flow were selected the same as GC/MS apparatus. Helium was used as carrier gas (1 ml/min) and nitrogen as make-up gas (25 ml/min). Hydrogen and air were the flame gases in the detector at 30 ml/min and 300 ml/min, respectively. VF-1ms capillary column (30 m x 0.33 mm x 0.25 µm) was used to isolate the essential oil compounds. Each compound was reported in percentage based on peak area [17-19].

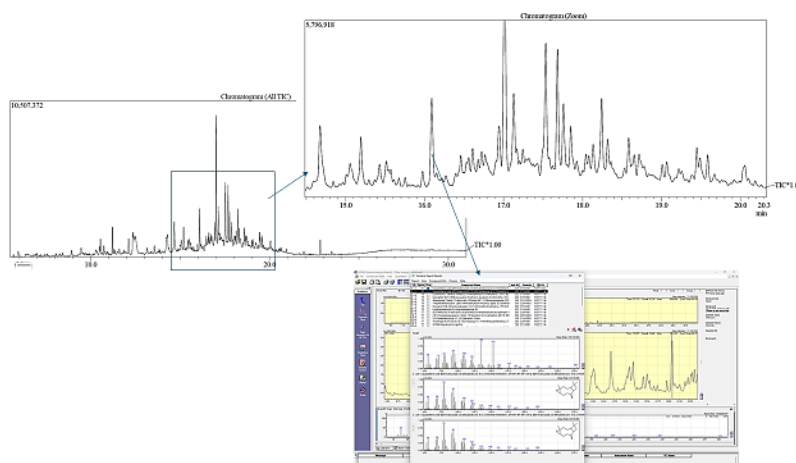


Figure 2. Scan mode GC/MS spectra and peak identification by using NIST library spectral library

Results and Discussion

In the present study, the chemical profile of *Ramonda serbica* was elucidated for the first time in Albania. This species represents a rare and relict plant not only within the national flora but also at the European level, with a restricted geographical distribution primarily confined to the Balkan Peninsula. Although *R. serbica* is sporadically distributed across several regions of Albania, the plant material investigated in this work was collected from two representative locations in Central Albania, namely Petrela and Kruja, which provide distinct ecological and microclimatic conditions. The leaves of *R. serbica* were air-dried under controlled conditions and subsequently subjected to hydro-distillation (HD) in order to obtain the volatile fraction. Qualitative analysis of the extracts was performed using gas chromatography coupled with mass spectrometry (GC–MS), while quantitative determination of the individual components was carried out by gas chromatography with flame ionization detection (GC–FID). This combined analytical approach allowed a comprehensive characterization of both the qualitative composition and the relative abundance of the detected constituents.

The chromatographic analysis revealed a high chemical complexity of the volatile profile. Depending on the analyzed sample, between 65 and 92 individual compounds were detected in the chromatograms. In the present work, particular attention was devoted to the identification and discussion of compounds occurring at relative concentrations higher than 0.1%. A total of 35 compounds fulfilled this criterion and were consistently identified across all analyzed samples, accounting on average for 97.7% of the total detected composition. For each of these compounds, the minimum, maximum, mean values, and standard deviations were calculated based on samples collected from Petrelë and Krujë, thus allowing an assessment of both intra- and inter-site variability within Central Albania. From a chromatographic perspective, the distribution of compounds along the retention time axis revealed distinct chemical patterns. In the early portion of the chromatograms (up to approximately 10 minutes), monoterpenes, low-molecular-weight alkanes, aldehydes, alcohols, and esters containing up to ten carbon atoms were predominantly detected. In contrast, the later part of the chromatograms was dominated by higher-molecular-weight compounds, including alcohols, sesquiterpenes, their oxygenated derivatives, and other compounds containing more than ten carbon atoms. Notably, compounds bearing hydroxyl functional groups—namely alcohols and phenols—represented the most abundant chemical category, accounting for more than 75% of the identified constituents. Moreover, more than 85% of all detected compounds were oxygenated molecules, highlighting the strong predominance of oxygen-containing constituents in the chemical profile of *R. serbica* (Figure 3). This compositional feature suggests a biosynthetic preference toward oxygenated secondary metabolites, which may be related to the plant's ecological adaptations and stress tolerance mechanisms. Among the identified compounds, globulol was found to be the most abundant constituent, with an average relative content of 15.1%. Globulol is a bicyclic sesquiterpene alcohol characterized by the presence of a hydroxyl group, which is consistent with the overall dominance of oxygenated sesquiterpenes in the profile. The second most abundant compound was furfural (8.2%), a heterocyclic aldehyde, followed by oxygenated derivatives of caryophyllene and aromadendrene, which were detected at average relative concentrations of 5.7% and 5.6%, respectively. Additional major constituents included 1,4-dihydroxy-2-methyl-5-(hydroxy-1)-isopropyl-3-cyclohexene

(4.4%), 8-methylenecyclooctene-3,4-diol (4.3%), isoaromandendrene epoxide (3.2%), 5-carneol (3.1%), and trimethyl-3,4,4-octahydroazulene (3.0%). Importantly, all these major constituents belong to the class of oxygenated compounds, predominantly alcohols and epoxides, further emphasizing the oxygen-rich nature of the chemical profile.

Figure 4 summarizes the distribution of the main chemical classes identified in the analyzed *R. serbica* samples. Sesquiterpenes and their oxygenated derivatives constituted the dominant class, accounting for approximately 41.6% of the total composition. These compounds were mainly eluted after 15 minutes, reflecting their higher molecular weights and structural complexity, and corresponded to the most intense and densely populated regions of the chromatograms. Alcohols and phenols represented the second most abundant class (approximately 25%), although it should be noted that several compounds classified under other categories also contained hydroxyl functional groups. Aldehydes and ketones constituted the third major group, with a relative contribution of approximately 18.4%. Minor classes included heterocyclic compounds (2.1%), diterpenes, and other high-molecular-weight constituents (5.5%), which were also predominantly oxygenated. In contrast, only a limited fraction of the identified compounds consisted of non-oxygenated hydrocarbons, such as alkanes, alkenes, and non-oxygenated terpenes and sesquiterpenes (6.1%). These hydrocarbon compounds were mainly detected between 2.8 and 12 minutes of retention time. Overall, purely hydrocarbon constituents represented only a small portion of the chemical profile of *R. serbica*.

The marked predominance of oxygenated compounds, particularly those containing hydroxyl functional groups, constitutes the defining characteristic of the chemical profile of *Ramonda serbica* as revealed in this study. To the best of our knowledge, this is the first comprehensive investigation of the chemical profile of *R. serbica* conducted in Albania. Available data from other regions are scarce and are generally limited either to partial volatile profiles or to datasets that do not allow direct comparison with the present results. Consequently, meaningful inter-regional comparisons remain challenging at this stage. Nevertheless, the findings presented herein provide a solid reference framework and a valuable baseline for future phytochemical, ecological, and bioactivity-oriented studies on this highly distinctive and biologically intriguing species of the Balkan Peninsula.

Table 1. Data on percentages of compounds found in *Ramonda serbica* from Central Albania, June 2025

	RT	Min	Max	Mean	STDEV
Tricyclene	2.89	0.85	1.02	0.94	0.12
3-Methyl-2-buten-1-ol	4.05	0.38	0.62	0.50	0.17
Furfural	4.81	7.14	9.24	8.19	1.48
(E)-3-Hexenol	4.83	0.36	1.21	0.79	0.60
3-(Methylthio)propanal	5.16	1.25	2.45	1.85	0.85
Benzaldehyde	5.36	1.63	3.04	2.34	1.00
1-Octen-3-ol	5.45	0.52	1.22	0.87	0.49
2-Acetylthiazole	5.84	0.72	1.74	1.23	0.72
Phenylacetaldehyde	5.99	1.25	3.24	2.25	1.41
5-Methyldecane	7.02	0.15	0.85	0.50	0.49
4-Methyldecane	7.56	0.62	0.87	0.75	0.18
Acetophenone	8.02	0.13	0.25	0.19	0.08
Undecane	8.54	0.57	0.69	0.63	0.08
Linalool	8.95	0.61	0.81	0.71	0.14
o-Hydroxyacetophenone	9.52	0.53	0.88	0.71	0.25
5-Caranol	10.51	2.13	4.05	3.09	1.36
5,9-Dimethyl Decanol	10.75	1.12	2.26	1.69	0.81
2,6-Dimethoxy Phenol	11.19	1.54	2.54	2.04	0.71
Phytol (E-2-Hexadecen-1-ol)	11.25	0.54	0.69	0.62	0.11

	RT	Min	Max	Mean	STDEV
Cyclotridecanone	11.31	0.48	0.67	0.58	0.13
alfa-Bisabolene epoxide	11.81	0.44	0.58	0.51	0.10
5-Isopropenyl-2-methyl-7-oxabicyclo[4,1,0]heptan-2-ol	12.11	2.24	3.21	2.73	0.69
8-Methylenecyclooctene-3,4-diol	12.35	3.02	5.52	4.27	1.77
3,6-Dimethyl-2-octanone	12.42	0.85	2.49	1.67	1.16
[(2-Ethyl, hexyl) methyl]oxirane	12.47	0.64	1.05	0.85	0.29
Z-7-Hexadecenal	13.18	0.41	0.95	0.68	0.38
trans-Verbenol	13.52	0.24	0.52	0.38	0.20
alfa-Limonene diepoxide	14.29	0.68	1.88	1.28	0.85
1-Heptatriacontanol	14.33	0.71	1.93	1.32	0.86
1,4-Dihydroxy-2-methyl-5-(hidroxy-1)-isopropil-3-cyclohexene	14.65	2.59	6.24	4.42	2.58
[2,2,3,3-Tetramethyl-1-(3-methyl-1-pentyl) cyclopropil]-1-butanol	15.08	1.62	3.24	2.43	1.15
alfa-Cadinol	15.15	1.77	2.65	2.21	0.62
1-[1-Propenyl]-3-propyladamantene	15.42	0.85	2.47	1.66	1.15
alfa-Bisabolene epoxide	15.51	0.56	1.06	0.81	0.35
Retinal	15.55	0.34	0.74	0.54	0.28
Aromadendrane-4,10-diol	15.94	0.42	0.77	0.60	0.25
Caryophyllene oxide	16.09	4.12	7.21	5.67	2.18
Thurbergol	16.91	0.82	1.55	1.19	0.52
Globulol	17.01	11.16	19.25	15.21	5.72
Trimethyl-3,4,4-octahydroazulene	17.12	1.66	4.24	2.95	1.82
Aromadendrene oxid	17.51	3.85	7.27	5.56	2.42
Isoaromandendrene epoxide	17.66	2.18	4.15	3.17	1.39
Calarene epoxide	17.75	1.73	3.24	2.49	1.07
Cycloanostan-3-ol acetate	17.84	0.72	2.29	1.51	1.11
Longfolenaldehyde	18.25	0.63	1.05	0.84	0.30
Kauran-18-al	20.02	0.51	1.87	1.19	0.96
1-Heptatriacotanol	21.82	0.32	0.63	0.48	0.22
alfa-Bisabolen oxide	22.26	0.33	0.55	0.44	0.16
7-Methanoazulene-6-ol	22.75	0.61	1.73	1.17	0.79
Alkanes/ Alkenes /Terpene's	-	3.96	8.30	6.13	3.07
Alcohols/ Phenols	-	16.84	32.85	24.85	11.32
Aldehydes and Ketones	-	13.67	23.21	18.44	6.75
Heterocycles	-	1.36	2.79	2.08	1.01
Sesquiterpenes and their derivate	-	29.46	53.75	41.61	17.18
Diterpenes/ High molecular mass compounds	-	3.25	7.77	5.51	3.20

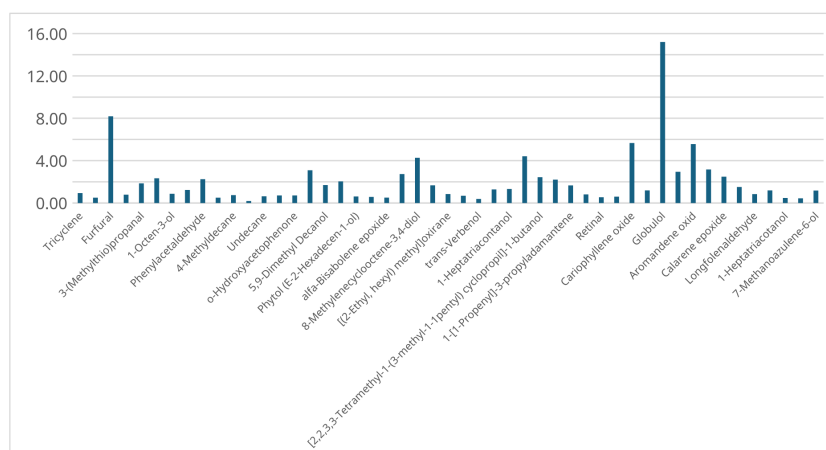


Figure 3. Profile of organic compounds found at *Ramonda serbica* plants from Central Albania

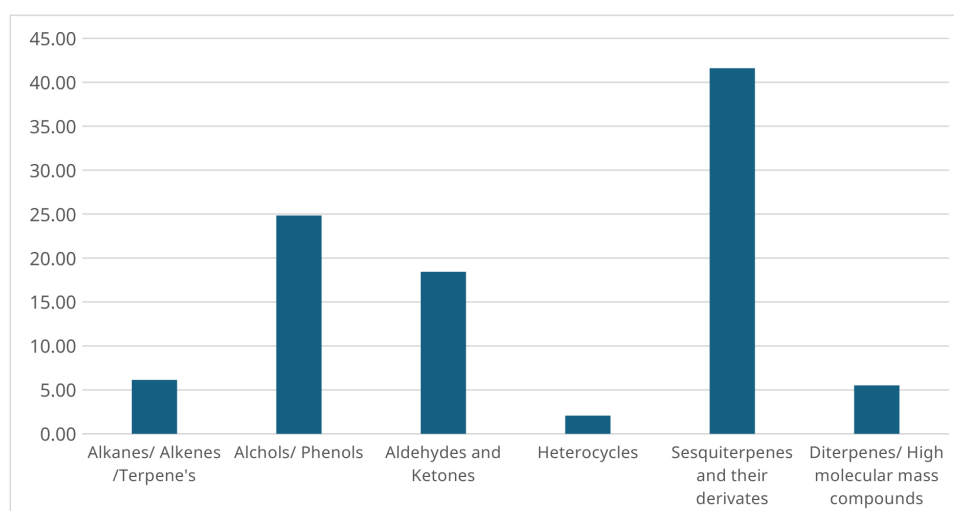


Figure 4. Classes of organic compounds found at *Ramonda serbica* plants from Central Albania

Conclusion

This study presents the first comprehensive characterization of the chemical profile of *Ramonda serbica* collected from Albania, providing novel and original data for a rare and relict species of the Balkan Peninsula. The combined application of GC–MS and GC–FID techniques enabled both qualitative and quantitative assessment of the volatile fraction obtained from leaf samples, revealing a chemically rich and highly structured profile. A total of 65 to 92 individual compounds were detected depending on the sampling site, while 35 compounds occurring at relative concentrations above 0.1% were consistently identified across all samples, accounting for an average of 97.7% of the total composition. The chemical profile of *R. serbica* was dominated by oxygenated constituents, with more than 85% of the identified compounds containing oxygen in their molecular structure. In particular, compounds bearing hydroxyl functional groups—such as alcohols and phenols—represented the most abundant class, exceeding 75% of the total identified composition. Oxygenated sesquiterpenes constituted the predominant chemical class, with globulol emerging as the major constituent, followed by furfural and several oxygenated derivatives of caryophyllene and aromadendrene. This marked prevalence of oxygenated metabolites clearly distinguishes the chemical signature of *R. serbica* from profiles dominated by hydrocarbon terpenes. The distribution of compounds along the chromatographic retention time further highlighted the predominance of high-molecular-weight constituents, while non-oxygenated hydrocarbons represented only a minor fraction of the total profile. The consistency of the main components across samples from different locations suggests a relatively stable chemical pattern within Central Albania, despite local environmental variability. Overall, these findings provide a robust baseline for future phytochemical investigations and contribute valuable reference data for comparative studies, ecological interpretation, and the exploration of potential biological activities of *Ramonda serbica*. This work lays the groundwork for further multidisciplinary research on this unique and biologically significant Balkan endemic species.

Acknowledgement

The authors thank the Albanian Academy of Sciences for the financial support of this study within the framework of the project entitled: "Innovative in vitro strategies in bioreactors for the sustainable utilization of aromatic medicinal plants (AMPs) for the production of secondary metabolites (MDs) used in pharmacology, as food additives and cosmetics" (VITROMBA)".

Conflict of Interest

The authors declare that they do not have any conflict of interest.

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