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In-Depth Investigation of the Chemical Profile of *Pelargonium odoratissimum* (L.) L'Hér. Hydrolate by SPME-GC/MS, GC/MS, LVI-GC/MS and PTR-Tof-MS Techniques

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Abstract

Hydrolates are aromatic aqueous solutions saturated with volatile water-soluble compounds of essential oil. Despite their potential, hydrolates remain less explored than essential oils. In this work, the hydrolate of *Pelargonium odoratissimum* (L.) L'Hér. has been analyzed by multiple analytical techniques in order to describe its chemical composition. Headspace (HS-) and Direct Immersion-Solid Phase Microextraction-Gas Chromatography/Mass spectrometry (DI-SPME-GC/MS) and Proton Transfer Reaction Time-of-Flight Mass Spectrometry (PTR-ToF-MS) were employed to reveal the VOC emission from the hydrolate. Further, a direct injection of the pure hydrolate and of the hydrolate after extraction with hexane was performed by Large-Volume Injection Gas Chromatography/Mass Spectrometry (LVI-GC/MS) and GC/MS. The results obtained by HS- and DI-SPME-GC/MS highlighted a nearly overlapping chemical profile with linalool, isomenthone, and α-terpineol as the main volatiles. On the other hand, analysis of the hydrolate by GC/MS after solvent extraction revealed a lower overall number of compounds but allowed the detection of thujone and cis-linalool oxide. In comparison, LVI-GC/MS was the technique that allowed the identification of a higher number of volatiles with citronellol, linalool, and α-terpineol as the principal compounds. Finally, PTR-ToF-MS was a fundamental approach to quantify and evaluate total terpene emissions from this complex matrix starting from low-molecular-weight compounds such as acetylene, methanol, acetaldehyde, acetone, and ethanol, which were the most abundant. Among the detected compounds, dimethyl sulfide and small amounts of dimethyl-furan and 2-butylfuran were also identified. Overall, the findings showed that the hydrolate was rich in monoterpene compounds while sesquiterpene compounds were missing. A very low intensity relating to sesquiterpenes was recorded only by PTR-ToF-MS technique.

Keywords: chemical analysis; mass spectrometry; gas chromatography; secondary metabolites; volatiles

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1. Introduction

Hydrolate, also known as hydrosol, is a distilled aromatic water that remains after the process of hydrodistillation or steam distillation of plant material to obtain essential oil (EO) (ISO 9235:2013) [1]. In detail, hydrolates are heterogeneous mixtures consisting

of essential oil droplets and water-soluble components. Hydrolates are easy and cheap to obtain, but since the main distillation product is the EO, they are often discarded because they are considered a by-product [2,3]. Moreover, being more delicate in their properties, because they are more diluted than the corresponding essential oils, they can be widely used [4,5]. For example, due to their antioxidant properties, hydrolates are widely applied in cosmetology as well as in the agri-food and pharmaceutical sectors [6,7]. Hydrolates, thanks to the fact that they contain a small percentage of essential oil, are safer to use, so much so that they can find application in the dermatological field or used in the food sector as they have a pleasant and not too aggressive odor [4]. Over the years, with a view to sustainability and adding value to this essential oil byproduct, interest in hydrolates has increased significantly, thanks also to scientific studies conducted to highlight their bioactive compound content and verify their biological properties [4,8,9].

Hydrolates are suspensions of essential oil droplets and other water-soluble substances, so oxygen-containing compounds are usually dominant among the volatiles detected. Most studies on the composition of hydrolates have focused on their volatile constituents. Frequently, hydrolates have been extracted with solvents before chemical analysis [10–12]. Headspace solid-phase microextraction (HS-SPME) is a solvent-free method used to describe the volatile chemical profile, which allows the extraction step to be avoided [13].

Despite the efforts made so far, information on how to maximize the potential of hydrolates, which are long-undervalued natural products, is still limited, especially regarding knowledge of their phytochemical profile.

Pelargonium odoratissimum (L.) L'Hér., originally from South Africa and Eswatini and belonging to the Geraniaceae family, is a particularly aromatic plant with very scented leaves. It produces small thin flowers of a pale pink, almost white, and grouped in small umbels. It is a plant particularly rich in bioactive metabolites, widely used in traditional medicine for the treatment of many diseases (to relieve the pain of hemorrhoids, dysentery and inflammation), and is less studied than other species of *Pelargonium* [14]. The chemical composition of *P. odoratissimum* essential oil has been extensively investigated, demonstrating how the prevalence of some compounds over others can vary especially depending on the growth region and the distillation process [15–18]. On the other hand, to the best of our knowledge, the hydrolate has never been chemically investigated until now. In our previous work, *P. odoratissimum* essential oil was investigated in terms of both its chemical composition and biological activity [18]. Chemical analysis revealed a volatile profile with significant qualitative and quantitative differences compared to the hydrolate.

The aim of this work was to describe a detailed characterization of the chemical composition of *Pelargonium odoratissimum* hydrolate through a metabolomic approach based on different analytical investigation techniques. In detail, the analysis performed by Headspace- and Direct Immersion Solid-Phase Microextraction-Gas Chromatography/Mass Spectrometry ((HS- and DI-) SPME-GC/MS), GC/MS, Large Volume Injection- Gas Chromatography/Mass Spectrometry (LVI-GC/MS) and Proton Transfer Reaction-Time of Flight-Mass Spectrometry (PTR-ToF-MS) techniques provided relevant information on the volatile and semi-volatile compositional profile of this unexplored matrix.

2. Materials and Methods

2.1. Plant Material

The hydrolate of *P. odoratissimum* obtained by steam distillation of the aerial parts (flowers and leaves) for 2 h at a pressure of 0.25 Atm, growing in Tuscany, Italy, was provided directly by "èssenziale" Azienda Agricola, San Donato in Poggio (FI), Italy.

2.2. Headspace Solid-Phase Microextraction (HS-SPME) Sampling

To characterize the chemical volatile profile of *P. odoratissimum* hydrolate, a SPME fiber was used for the extraction of the analytes. Approximately, 2.0 mL of the sample was placed into a 15 mL glass vial with PTFE-coated silicone septum. A thermostatic bath with constant magnetic stirring was utilized to reach thermal equilibrium. For the adsorbtion of the components, a SPME device from Supelco (Bellefonte, PA, USA) with 1 cm fiber coated with 50/30 µm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) was used. Before use, the fiber was conditioned at 270 °C for 30 min. Subsequently, the fiber was inserted into the vial and exposed to the headspace for 10 min at 60 °C to collect the volatiles. Finally, the compounds were desorbed from the fiber by insertion into the GC injector held at 250 °C (splitless mode).

2.3. Direct Immersion Solid-Phase Microextraction (DI-SPME) Sampling

For DI-SPME, the fiber was immersed directly in the aqueous solution of *P. odoratissimum* for 10 min, thermally conditioned at 60 °C to ensure the extraction of both volatile and semi-volatile compounds. The analyte extraction process was performed as described in the previous section (Section 2.2).

2.4. Extraction Procedure of the P. odoratissimum Hydrolate

P. odoratissimum hydrolate (15 mL) was extracted using the method described by Zoccolillo et al. (1996). Briefly, 0.5 mL of hexane was added to the sample in a round-bottom flask. The resulting solution was mixed using a magnetic stirrer for 15 min (Figure 1a). After another 15 min of rest, the upper phase was recovered using a microextractor device (Figure 1b) by introducing a sufficient amount of water in tube B to allow the hexane layer to rise to the top of tube A (Figure 1c). Without reconcentrating the hexane phase, it is possible to take the sample and inject it into the GC-MS system.

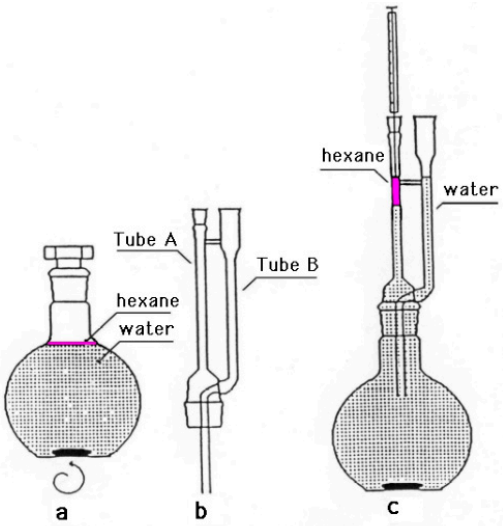


Figure 1. Microextractor used for organic component of *P. odoratissimum* hydrolate.

2.5. Gas Chromatography/Mass Spectrometry (GC-MS) Analysis of P. odoratissimum Hydrolate

To perform GC/MS analysis, a Clarus 500 model Perkin Elmer (Waltham, MA, USA) gas chromatograph equipped with a FID (flame ionization detector) and coupled with a mass spectrometer was used. The chromatographic capillary column was a Varian Factor Four VF-5 (60 m, 0.25 mm, 1.00 µm, 7-inch cage) and helium was used as gas carrier at a flow rate of 1.0 mL min^{-1}. The programmed temperature applied started at 40 °C then increased to 220 °C at 6 °C/min, and held for 10 min. The injector was set at 250 °C for SPME-GC/MS analysis and at 270 °C for injection of the hexane extract. The

mass spectra were obtained in the electron ionization (EI) at 70 eV in scan mode in the range 35–400 m/z. The identification of the detected compounds was performed by the matching their mass spectra with those reported in the Wiley 2.2 and Nist 11 mass spectra libraries. In addition, linear retention indices (LRIs) were calculated using a homologous series of *n*-alkanes (C_8–C_{30}), injected in the column at the same operating conditions, and compared with the available literature values. Relative amounts of each component were expressed as percentage of its peak area in the total ion chromatogram without the use of an internal standard and any factor correction. The analyses were carried out in triplicate to ensure reproducibility.

2.6. LVI-GC-MS Analysis of P. odoratissimum Hydrolate

P. odoratissimum hydrolate was injected into the GC/MS (Konik, Sant Cugat del Vallés, Barcelona, Spain) using a TOTAD (Through Oven Transfer Adsorption Desorption) interface following Aragón et al., [19] with slight modifications. The adsorption temperature was set at 60 °C for 7.67 min under a nitrogen gas flow of 150 mL/min to eliminate the solvent (water) present in the hydrosol sample (20 µL), which was transferred to the interface with a methanol flow of 0.1 mL/min for 1 min. Adsorption of the compounds occurred at 260 °C for 3 min.

The GC/MS apparatus (Konik 5000C Sant Cugat del Vallés, Barcelona, Spain) consisted of a gas chromatograph at 5000 °C coupled to a Q12 MS mass spectrometer. The capillary column was a Merck SLB-5ms fused silica column (30 m × 0.25 mm, 1.0 µm) operating with helium carrier gas at 2.0 mL/min. During the adsorption phase (7.67 min), the oven was held at 38 °C, then raised to 280 °C at a rate of 5 °C and held for 5 min. The mass spectrometer interface was maintained at 260 °C, while the ion source (electron ionization mode at 70 eV) was set to 180 °C. The quadrupole operated in full scan mode from 41 to 450 m/z.

2.7. PTR -ToF- MS Analysis of P. odoratissimum Hydrolate

VOC acquisition was performed using a PTR-ToF 8000 from IONICON Analytik GmbH (Innsbruck, Austria). PTR-ToF-MS is widely used in many fields thanks to many advantages such as short response time, low limit of detection (LOD), direct sampling, and speed of analysis [20]. The advantages, limitations, and specific operational details of this tool are extensively discussed in [21,22]. In this study, the tool was used to investigate the chemical composition of *P. odoratissimum* hydrolate which was analyzed in triplicate. Raw data were recorded using TofDaq software (Tofwerk AG, Switzerland). To calibrate the instrument, we used a commercial gas mixture containing known compounds, such as acetone (m/z 59), limonene (m/z 137), and trichlorobenzene (m/z 181), at known concentrations, with the aim of generating a transmission curve (calibration curve) that allows for the quantification of each measured compound [23]. VOCs spontaneously emitted by hydrolate were acquired and measured using the same protocol reported by Caparrotta et al., 2019 [24]. In detail, 0.1 mL of hydrolate was put in a sealed glass vessel (2/3 L glass jar) and incubated for 100 sec at room temperature (22 °C). The drift tube operating parameters were set as follows: FC inlet 50 sccm, pressure 2.3 mbar, inlet and drift temperature 60 °C, voltage 600 V, extraction voltage (Udx) 35V which corresponded to an E/N value of 140 Td, optimizing the balance between water cluster formation and product ion fragmentation [25]. Each run involved a 180-s measurement to allow the acquisition of 180 spectra (acquisition of 1 spectra per sec) and was conducted in a conditioned room. To ensure data accuracy and prevent sample cross-contamination, the tool apparatus was cleaned using a zero-air generator, and a new, clean glass vessel was used for each replicate. Once each replicate was measured, the instrument was calibrated off-line using reference

peaks detected at m/z 21.022 ($H_3{}^{18}O^+$), 59.049 ($C_3H_7O^+$), and 137.132 ($C_{10}H_{17}{}^+$). At the end of the analysis, we converted the signal intensities of each VOC from counts per second (cps) to parts per billion by volume (ppbv) using the primary ion signal ($H_3{}^{18}O^+$). Subsequently, the dataset was reduced to remove peaks associated with water chemistry and filtered out signals with average concentrations below 1 ppbv. So, the resulting cleaned dataset was used for statistical analysis. Finally, by comparison with the GC-MS literature for hydrolate and PTR-MS studies of fragmentation patterns, we tentatively identified each VOC detected.

3. Results and Discussion

3.1. P. odoratissimum Hydrolate Chemical Composition

A total of 16 compounds were detected and identified by HS-SPME/GC-MS and by DI-SPME/GC-MS analysis (Table 1). The results show that *P. odoratissimum* hydrolate (Hy) is characterized by the presence of monoterpene compounds. No sesquiterpenes were found. Linalool (29.6%; 30.7%), isomenthone (21.1%; 19.9%), α-terpineol (18.9%; 18.7%), and p-menthan-3-one (6.6%; 6.9%) were the principal components detected by HS-SPME and DI-SPME, respectively. The volatile chemical profile obtained by applying the two extraction techniques was almost superimposable as there were no significant qualitative–quantitative differences. The DI- and HS-SPME-GC-MS chromatograms are reported in Figures 2 and 3.

Table 1. Chemical composition (percentage mean value ± standard deviation) of *P. odoratissimum* Hy as determined by HS-SPME-GC/MS and DI-SPME-GC/MS.

N°	Component [1]	LRI [2]	LRI [3]	Hy [4] (%)	Hy [5] (%)
1	2H-piran, 2-ethenyl tetrahydro-2,6,6-trimethyl-	958	960	0.29 ± 0.02	0.41 ± 0.02
2	*β*-myrcene	990	987	2.2 ± 0.06	3.0 ± 0.06
3	limonene	1025	1022	1.2 ± 0.04	0.69 ± 0.02
4	1,8-cineole	1029	1027	1.4 ± 0.05	1.6 ± 0.05
5	*cis*-*β*-ocimene	1040	1037	0.81 ± 0.04	0.91 ± 0.03
6	*trans*-linaool oxide	1085	1082	1.0 ± 0.05	1.1 ± 0.04
7	linalool	1090	1089	29.6 ± 2.10	30.7 ± 2.64
8	rose-oxide	1110	1112	2.0 ± 0.06	2.1 ± 0.04
9	camphor	1132	1139	2.3 ± 0.05	2.2 ± 0.05
10	*p*-menthan-3-one	1139	1142	6.6 ± 0.09	6.9 ± 0.11
11	*trans*-pinocamphone	1144	1148	0.59 ± 0.02	0.59 ± 0.03
12	isomenthone	1155	1159	21.1 ± 1.52	19.9 ± 0.15
13	isocamphopinone	1164	1162	2.8 ± 0.04	2.7 ± 0.06
14	*α*-terpineol	1195	1198	18.9 ± 0.15	18.7 ± 0.11
15	citronellol	1209	1212	8.8 ± 0.10	8.3 ± 0.09
16	methyleugenol	1355	1360	0.41 ± 0.02	0.30 ± 0.02
	SUM			100.0	100.0
	Monoterpenes			95.0	95.0
	Sesquiterpenes			-	-
	Others			5.0	5.0

[1] The components are reported according to their elution order on non-polar column; [2] Linear Retention Indices measured on non-polar column; [3] Linear Retention Indices from the literature (NIST); Hy [4]: percentage mean values of "*Pelargonium*" hydrolate as determined by HS-SPME/GC–MS; Hy [5]: percentage mean values of "*Pelargonium*" hydrolate as determined by DI-SPME/GC–MS.; –not detected.

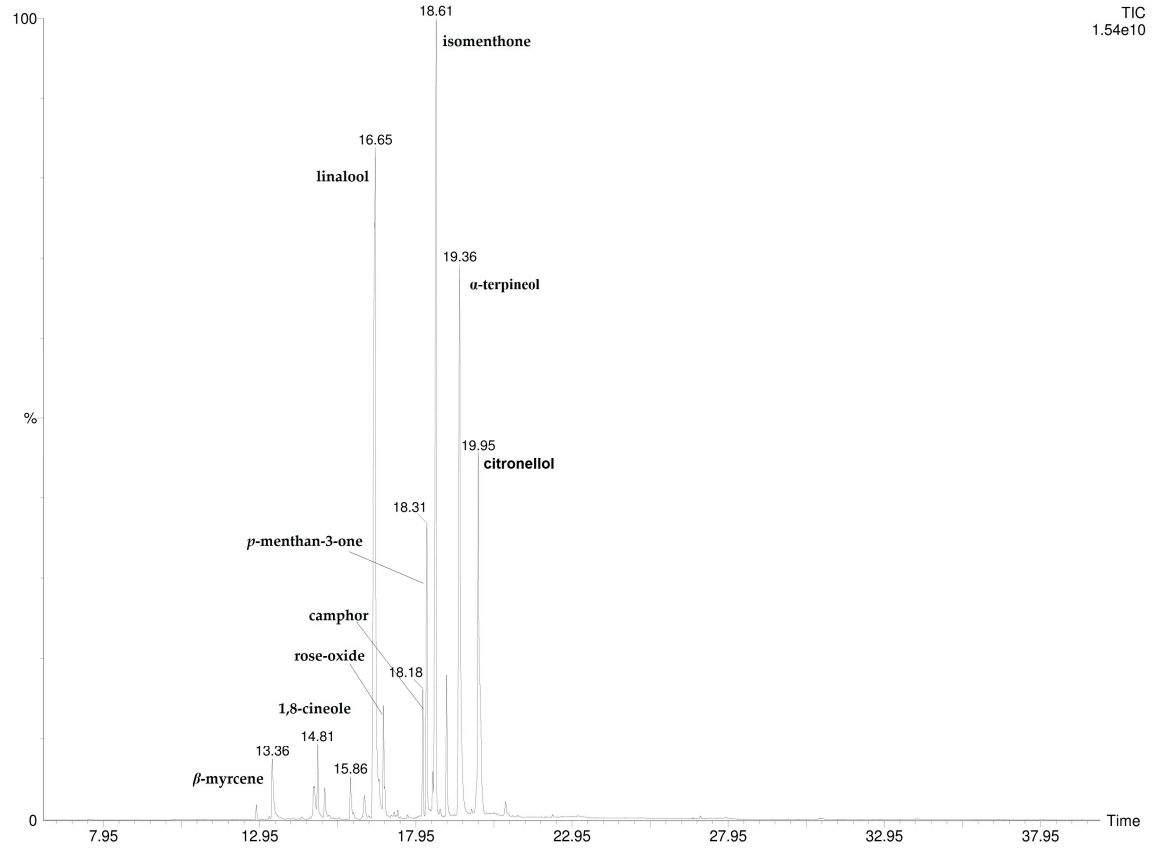


Figure 2. HS-SPME-GC-MS chromatogram of *P. odoratissimum* hydrolate.

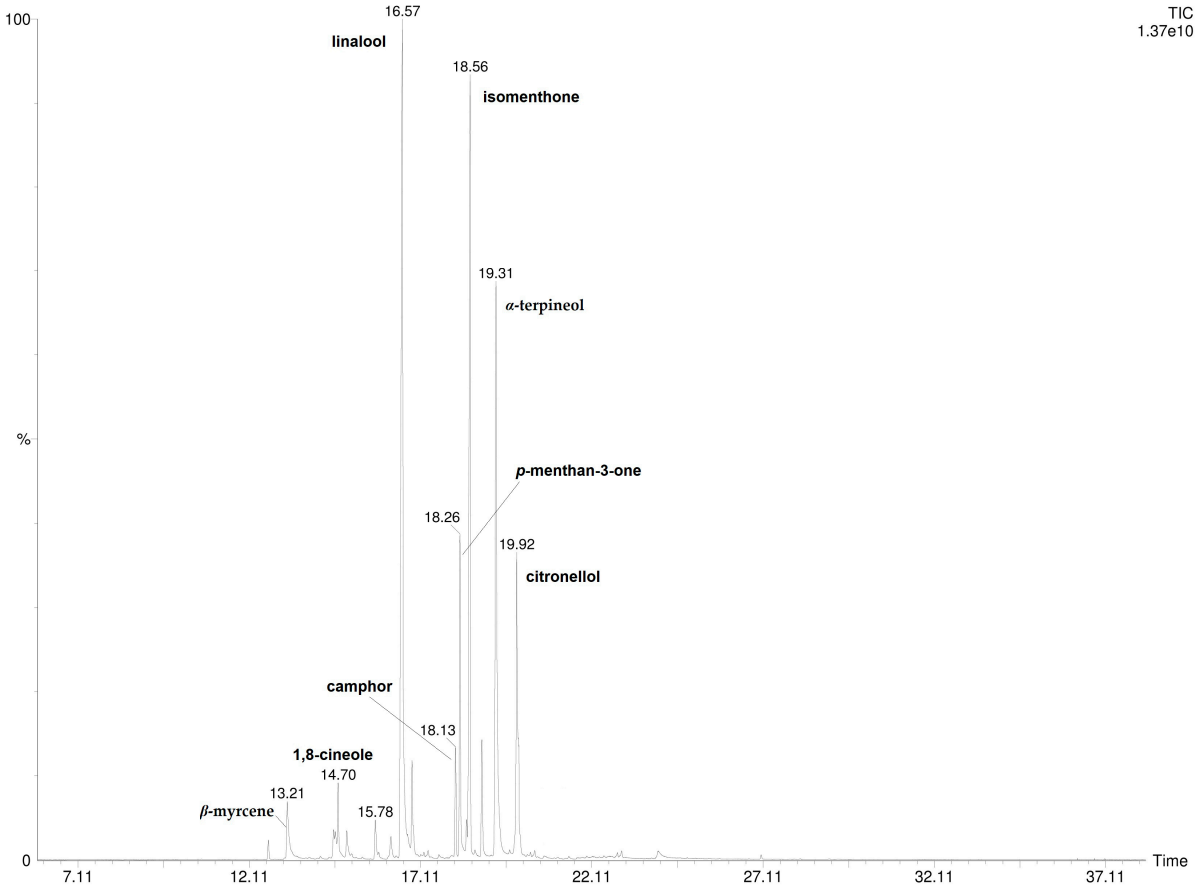


Figure 3. DI-SPME-GC-MS chromatogram of *P. odoratissimum* hydrolate.

Generally, the volatile content of hydrolates is lower, both qualitatively and quantitatively, than the corresponding essential oils. In fact, monoterpene and sesquiterpene hydrocarbons, more hydrophobic, are present in reduced percentages in the hydrolate. On the other hand, the content of some oxygenated monoterpenes is higher in the hydrolate [26–28]. Our previous study conducted on *P. odoratissimum* essential oil showed that it was rich in oxygenated monoterpenes (81.2%), while sesquiterpenes accounted for only 11% [14]. The main compounds were citronellol (40.0%), nerol (15.3%), and citronellyl formate (12.6%) followed by (-)-aristolene (9.8%), isomenthone (7.4%), linalool (4.8%), and geranyl isobutyrate (2.6%). Among these major components, citronellol, isomenthone, and linalool were also detected in the hydrolate. On the contrary, α-terpineol, the third more abundant volatile in the hydrolate, was absent in the essential oil [18].

There are no previous works in the literature on the chemical composition of *P. odoratissimum* hydrolate or different Pelargonium species; therefore, comparative evaluations are not feasible. Only Rao et al. [2] discuss the chemical composition of *P. graveolens* hydrolate but only after extraction with hexane and subsequent distillation to produce a secondary essential oil in order to recover the oil dispersed in the hydrolate.

3.2. *P. odoratissimum* Hydrolate Hexane Extract Chemical Composition

The injection into GC/MS of the hexane extract of *P. odoratissimum* hydrolate revealed the presence of 13 compounds, listed in Table 2. Among them, linalool was the most abundant (33.6%) followed by α-terpineol (20.9%), *trans*-linalool oxide (15.8%), and isomenthone (15.1%). Two compounds, thujone (0.9%) and *cis*-linalool oxide (3.5%), were not detected by SPME-GC/MS (both HS- and DI-) analysis. These components were also missing in the correspondent essential oil [18]. The GC-MS chromatogram is reported in Figure 4.

Table 2. Chemical composition (percentage mean value ± standard deviation) of hexane extract of *P. odoratissimum* Hy as determined by GC/MS.

N°	Component [1]	LRI [2]	LRI [3]	Hy (%)
1	β-myrcene	985	987	1.1 ± 0.04
3	1,8-cineole	1031	1027	2.0 ± 0.06
4	*cis*-β-ocimene	1042	1037	0.70 ± 0.03
5	*cis*-linalool oxide	1058	1062	3.5 ± 0.07
6	*trans*-linaool oxide	1085	1082	15.8 ± 1.03
7	linalool	1091	1089	33.6 ± 4.05
8	rose oxide	1115	1112	1.3 ± 0.07
9	thujone	1092	1099	0.90 ± 0.04
10	camphor	1142	1139	2.7 ± 0.05
11	isomenthone	1155	1159	15.1 ± 1.06
12	isocamphopinome	1160	1162	2.3 ± 0.06
13	α-terpineol	1189	1198	20.9 ± 3.22
	SUM			99.9
	Monoterpenes			98.6
	Sesquiterpenes			-
	Others			1.3

[1] The components are reported according to their elution order on non-polar column; [2] Linear Retention Indices measured on non-polar column; [3] Linear Retention Indices from the literature (NIST).

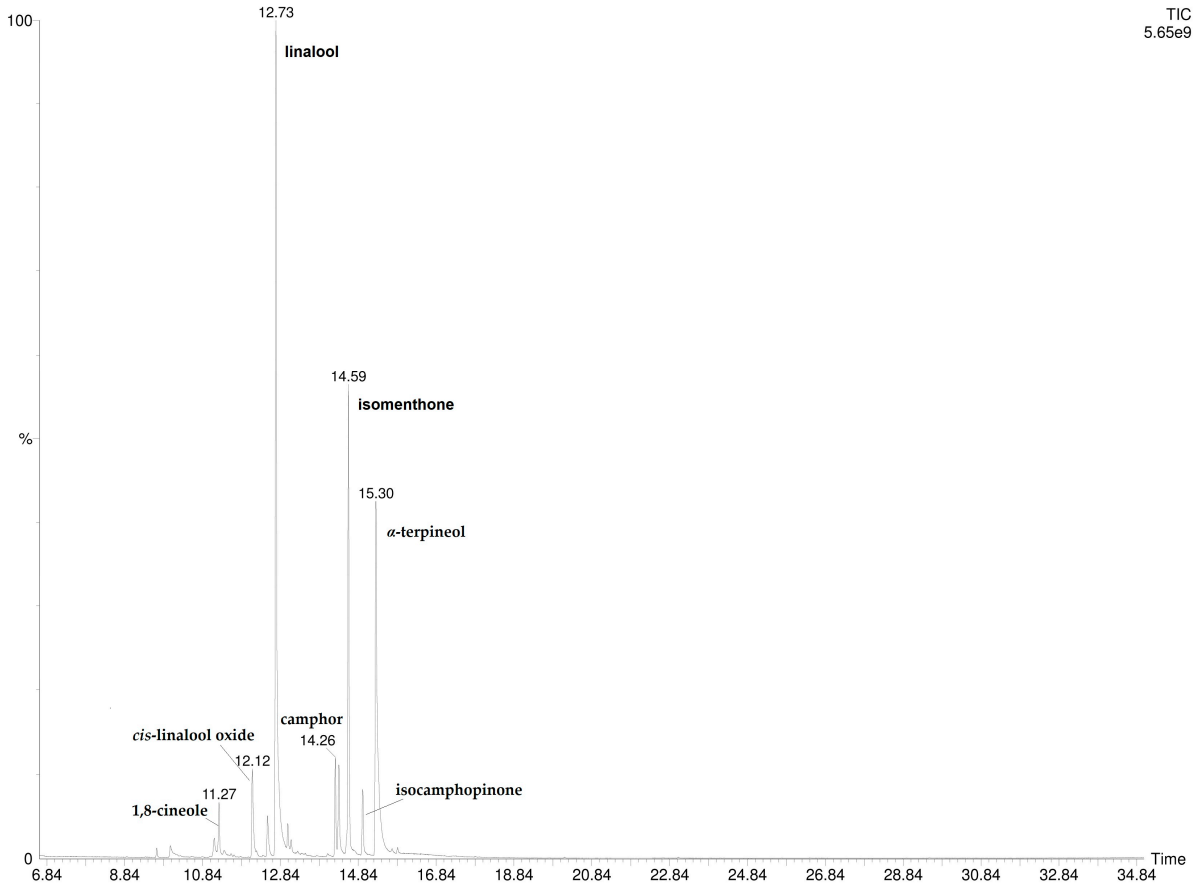


Figure 4. GC-MS chromatogram of *P. odoratissimum* hydrolate hexane extract.

3.3. LVI-GC/MS Chemical Composition

P. odoratissimum hydrolate chemical composition obtained applying the LVI-GC-MS method is reported in Table 3. Monoterpene compounds covered 79% of the total composition and no sesquiterpenes were detected.

Table 3. Chemical composition (percentage mean value ± standard deviation) of *P. odoratissimum* Hy as determined by LVI-GC/MS.

N°	Component [1]	LRI [2]	LRI [3]	Hy (%)
1	hexenol (3z)	857	857	1.1 ± 0.05
2	2h-pyran, 2-ethenyltetrahydro-2,6,6-trimethyl-	978	971	2.1 ± 0.07
3	sulcatone	986	986	0.40 ± 0.03
4	β-myrcene [4]	992	989	4.3 ± 0.09
5	1-hexanol, 2-ethyl	1030	1031	0.80 ± 0.03
6	limonene[4]	1040	1039	0.69 ± 0.04
7	β-phellandrene	1044	1041	0.11 ± 0.01
8	β-ocimene [4]	1050	1044	1.3 ± 0.06
9	seudenone	1068	1046	0.20 ± 0.01
10	acetophenone	1078	1073	1.2 ± 0.05
11	*trans*-linalool oxide [4]	1081	1082	1.4 ± 0.06
12	benzenemethanol, α,α-dimethyl-	1095	1090	9.5 ± 0.12
13	linalool [4]	1102	1099	12.8 ± 0.13
14	6-methyl-3,5-heptadiene-2-one	1109	1107	2.3 ± 0.06
15	rose-oxide [4]	1118	1113	0.20 ± 0.01
16	myrcenol	1122	1118	2.8 ± 0.06
17	cis-ocimenol	1156	1157	1.2 ± 0.04

Table 3. *Cont.*

N°	Component [1]	LRI [2]	LRI [3]	Hy (%)
18	unknown	1169	/	4.9 ± 0.07
19	menthone	1172	1163	1.1 ± 0.05
20	Isomenthone [4]	1182	1182	2.5 ± 0.06
21	*p*-mentha-1,5-dien-8-ol	1185	1185	1.0 ± 0.04
22	α-terpineol [4]	1211	1210	11.1 ± 0.10
23	citronellol [4]	1230	1228	25.8 ± 1.19
24	geraniol	1253	1255	6.6 ± 0.12
25	hydroxy citronellol	1368	1362	4.0 ± 0.11
26	methyl eugenol [4]	1406	1401,8	0.60 ± 0.05
	SUM			100.0
	Monoterpenes			79.0
	Sesquiterpenes			-
	Others			21.0

[1] The components are reported according to their elution order on non-polar column; [2] Linear Retention Indices measured on non-polar column; [3] Linear Retention Indices from the literature (NIST); [4] components also detected by SPME analysis (Table 1).

The results show important qualitative and quantitative differences with respect to SPME-GC/MS analysis.

In total, 26 components were detected and identified, 10 more than those identified by HS-SPME/GC–MS and by DI-SPME/GC-MS analysis (Table 1), and 13 more than those identified in hexane extract (Table 2). Among these, the monoterpenes linalool (12.8%), α-terpineol (11.1%), and citronellol (25.8%) were the main detected compounds also relieved in both SPME analyses (HS- and DI-) but with different relative amounts. Particularly significant were the differences for linalool (29.6% in HS-, 30.7% in DI-) and for citronellol (8.8% in HS-, 8.3% in DI-). The relative amount of isomenthone (2.5%) also showed a remarkable difference compared to the SPME analysis, where rather higher quantities (21.1% in HS-, 19.9% in DI-) were found. Among compounds present in LVI analysis and absent in SPME analysis, the most abundant were benzenemethanol, α,α-dimethyl- (9.5%), geraniol (6.6%), myrcenol 2.8%), and hydroxy citronellol (4.0%). All other compounds (note 4) detected by SPME and LVI analysis, did not show significant variation. The LVI-GC-MS chromatogram was reported in Figure 5.

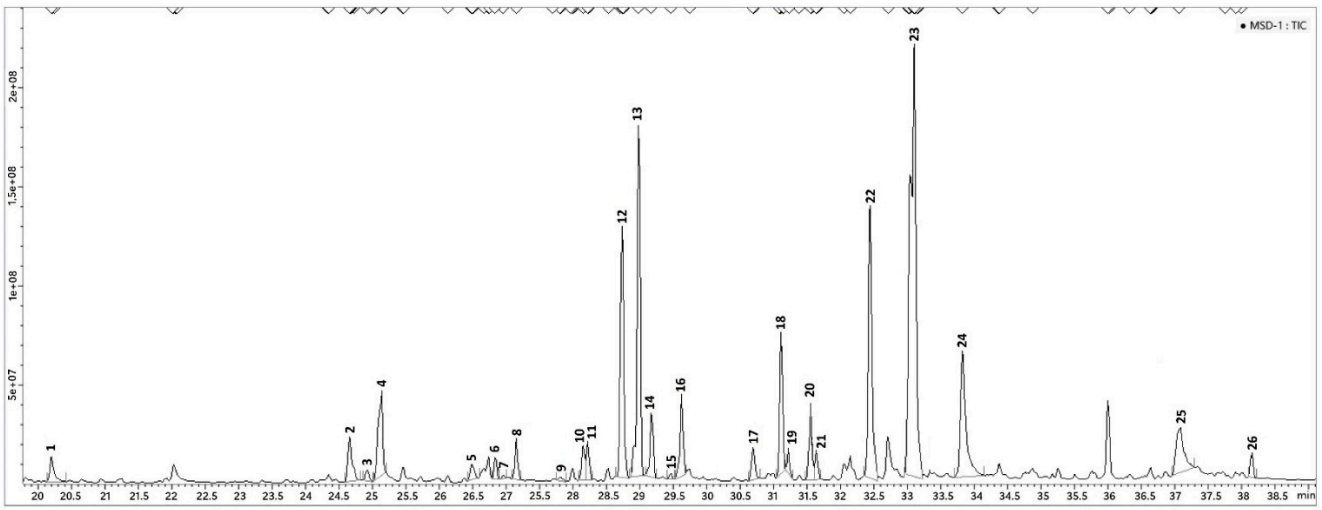


Figure 5. LVI-GC-MS chromatogram of *P. odoratissimum* hydrolate. For peaks assignation, see Table 2.

3.4. PTR-ToF-MS

PTR-ToF-MS analysis of *P. odoratissimum* hydrolate identified 46 distinct peak signals with emission intensities exceeding 1 ppbv. Table 4 provides a comprehensive list of these compounds, including aldehydes, ketones, esters, sulfur-containing compounds, terpenes, and other unidentified compounds, detected within the mass range of m/z 20 to 220. PTR-ToF-MS is a powerful tool for high-frequency detection and quantification of gas-phase volatile organic compounds (VOCs). However, its soft ionization technique makes it difficult to distinguish structural isomers, limiting the ability to specifically identify individual terpene species [29]. Despite this limitation, total terpene concentrations expressed as the sum of isomers can still be estimated using characteristic terpene fragmentation patterns described in previous studies [29–32]. These studies highlight that sesquiterpenes, monoterpenes, and oxygenated terpenes can be assessed by targeting key m/z channels corresponding to their known fragmentation pathways. This approach provides viable means to quantify and evaluate total terpene emissions from complex natural matrices such as hydrolates.

Table 4. VOC profile of *P. odoratissimum* hydrolate as determined by PTR-ToF-MS.

Number of Detected Signals	m/z	Protonated Chemical Formula	Tentative Identifications	Hy	SD	% on the Total
1	27.022	$C_2H_3^+$	Acetylene	401	119	10.2
2	30.046	$C_2H_6^+$	Ethylene (isotope)	42.8	6.06	1.09
3	31.018	CH_3O^+	Formaldehyde	116	22.4	2.96
4	33.033	CH_5O^+	Methanol	605	217	15.4
5	41.038	$C_3H_5^+$	Alkyl fragments	87.9	35.1	2.23
6	43.018	$C_2H_3O^+$	Alkyl fragments (ethenone/incensole acetate)	229	88.1	5.84
7	43.050	$C_3H_7^+$	Alkyl fragments (propene)	59.7	29.6	1.52
8	45.033	$C_2H_5O^+$	Acetaldehyde	684	415	17.4
9	47.049	$C_2H_7O^+$	Ethanol	409	123	10.4
10	55.055	$C_4H_7^+$	C4 aldehydes fragment (Butanal)	18.6	10.0	0.47
11	57.033	$C_3H_5O^+$	Alkyl fragments (hexanal/1-butanol/1-octanol)	5.63	0.67	0.14
12	57.069	$C_4H_9^+$	Alkyl fragments (hexanol/valeric acid)	12.8	5.84	0.33
13	59.049	$C_3H_7O^+$	Propanal, acetone	701	131	17.8
14	61.027	$C_2H_5O_2^+$	Acetates	130	66.1	3.31
15	63.027	$C_2H_7S^+$	Dimethyl sulfide	78.5	15.8	2.00
16	65.038	$C_5H_5^+$	Alkyl fragments	4.73	2.07	0.12
17	67.054	$C_5H_7^+$	Terpene fragments (e.g., myrcene)	3.89	1.54	0.10
18	69.069	$C_5H_9^+$	Isoprene/terpene fragments	8.92	4.64	0.23
19	71.049	$C_4H_7O^+$	Methyl vinyl ketone	10.5	7.05	0.27
20	73.065	$C_4H_9O^+$	Methyl ethyl ketone	26.3	6.12	0.67
21	75.044	$C_3H_7O_2^+$	Methyl acetate	3.57	2.32	0.09
22	79.054	$C_6H_7^+$	Terpene fragments	11.9	1.65	0.30
23	81.069	$C_6H_9^+$	Terpene fragments/C6 fragments	116	21.2	2.96

Table 4. *Cont.*

Number of Detected Signals	m/z	Protonated Chemical Formula	Tentative Identifications	Hy	SD	% on the Total
24	83.049	$C_5H_7O^+$	3-methyl furan	3.43	1.90	0.09
25	83.086	$C_6H_{11}^+$	C6 compounds/hexenol fragment	6.18	3.29	0.16
26	85.065	$C_5H_9O^+$	Methyl-butenal/ pentenone	2.98	1.87	0.08
27	87.044	$C_4H_7O_2^+$	2,3-Butandione/diacetyl	13.7	3.91	0.35
28	87.080	$C_5H_{11}O^+$	Pentanal/methylbutanal	11.5	5.05	0.29
29	89.059	$C_4H_9O_2^+$	Ethyl acetate/methyl-propanoate	2.32	1.48	0.06
30	93.069	$C_7H_9^+$	Terpenes fragments	25.6	3.54	0.65
31	95.086	$C_7H_{11}^+$	Terpene fragments	19.9	4.01	0.51
32	97.064	$C_6H_9O^+$	Dimethyl-furan	2.43	0.90	0.06
33	99.080	$C_6H_{11}O^+$	Hexenals /methyl-pentenone	8.75	1.64	0.22
34	101.059	$C_6H_{13}O^+$	Hexenol	3.25	1.39	0.08
35	107.086	$C_8H_{11}^+$	Terpenes fragment	1.51	0.85	0.04
36	109.085	$C_8H_{11}^+$	Terpenes fragments	3.63	2.54	0.09
37	111.080	$C_7H_{11}O^+$	Dimethyl-2-cyclopenten-1-one	1.98	0.64	0.05
38	113.096	$C_7H_{13}O^+$	Methylcyclohexanone	2.51	0.61	0.06
39	115.054	$C_9H_7^+$	C9- aromatics	2.28	0.67	0.06
40	123.101	$C_9H_{15}^+$	Terpenes fragments	2.66	0.39	0.07
41	125.096	$C_8H_{13}O^+$	2-Butylfuran	1.94	0.59	0.05
42	135.117	$C_{10}H_{15}^+$	p-cymene	4.91	0.51	0.12
43	137.132	$C_{10}H_{17}^+$	Monoterpenes (e.g., limonene)	19.6	2.41	0.50
44	143.145	$C_8H_{15}O_2^+$	Nonanal	2.94	0.51	0.07
45	153.127	$C_{10}H_{17}O^+$	Terpenoid-like compound (e.g., camphor)	14.6	5.10	0.37
46	155.143	$C_{10}H_{19}O^+$	Terpenoid-like compound (e.g., 1,8-cineole)	6.67	3.47	0.17
		Total Terpene Emission		326.6	86.1	8.30
		Total VOC Emission		3933.7	1379	100

Number of compounds detected, along with their corresponding m/z values, chemical formulas, tentative identifications (TI), and average emission intensities (ppbv) with standard deviations (n = 3). Tentative identification was based on exact mass values provided by the instrument, comparison fragmentation patterns (when available), and reference to the existing PTR–MS literature. Only signals with emission intensities greater than 1 ppbv were included. Total terpene emissions were calculated as the sum of signal intensities for m/z 67, 69, 79, 81, 93, 95, 109, 123, 135, 137, 153, and 155. Hy: *P. odoratissimum* hydrolate; SD: standard deviation.

Analysis of the *P. odoratissimum* hydrolate revealed that the most abundant volatile compounds were detected at m/z 27 (fragments), 33 (methanol), 45 (acetaldehyde), and 59 (acetone), collectively accounting for over 60% of the total emissions (Table 4). These results suggest that water-soluble alcohols, aldehydes, and other polar compounds are effectively transferred into the aqueous phase during steam distillation. Moreover, a sulfur-containing compound, although present at a low concentration, was in line with the findings of Amel et al. [33]. This compound detected at m/z 63 was identified as dimethyl sulfide, known for its distinctive odor and potential contribution to the hydrolate's sensory profile. Despite its low-level presence (approximately 2% of the total emissions), dimethyl sulfide may play an important role in defining the characteristic scent and potential bioactivity of the hydrolate.

In contrast, nitrogen-containing compounds, often considered important contributors to the aromatic profile of geranium essential oils, were not detected in this study. In addition to the presence of dimethyl sulfide, which may contribute unique sensory nuances, another key class of volatiles identified in the *P. odoratissimum* hydrolate was terpenes.

Despite exhibiting low emission intensities, terpenes, known for their low perception thresholds, are characteristic of many plant hydrolates [34]. Indeed, the aroma profile of the *P. odoratissimum* hydrolate appeared to be strongly influenced by the presence of terpene compounds, as evidenced by the detection of characteristic mass-to-charge ratios (m/z) such as 135, 137, 153, and 155, along with their corresponding fragment ions (m/z 67, 69, 79, 81, 93, 95, 109). The detection of signals at m/z 135 and 137 is indicative of the presence of monoterpenes, while signals at m/z 153 and 155 are characteristic of oxygenated monoterpenes. However, no peaks corresponding to sesquiterpene compounds were detected with an intensity exceeding 1 ppbv. Consistent with the findings of Tavares et al. (2022) [34], hydrolates obtained from various plant species exhibit a diverse array of primary and secondary metabolites, making them valuable sources of biologically active compounds. Despite typically possessing a strong aroma, hydrolates usually contain low concentrations of essential oil and often exhibit a chemical resemblance to the corresponding essential oil composition, albeit with quantitative differences [34].

4. Conclusions

In this study, the chemical composition of *P. odoratissimum* hydrolate was explored by different techniques to describe an in-depth compositional framework. The findings highlighted the presence of a high number of monoterpenes. The comparison of the data obtained from the analyses demonstrates how the approach based on the different applied analytical techniques was useful for the detection of the individual secondary metabolites including volatiles and semi-volatiles.

SPME, a solvent-free technique, has proven to be a highly sensitive method for collecting analytes spontaneously emitted from the hydrolate. The monoterpene fraction dominated the volatile profile of *P. odoratissimum* hydrolate, with linalool, isomenthone, and α-terpineol being the most abundant. However, no significant differences were observed between the HS- and DI-SPME techniques. In contrast, the analysis of the hydrolate hexane extract by GC/MS showed a lower number of compounds but allowed the detection of thujone and cis-linalool oxide.

PTR-ToF-MS, with its exceptional sensitivity and selectivity, ensured comprehensive chemical characterization across a wide range of molecular weights, including compounds belonging to different chemical classes such as aldehydes, alcohols, terpenes, a sulfur containing compound, and traces of furan derivatives. On the other side, TOTAD interface allowed for the injection of large quantities (up to a few hundred microliters) of the hydrolate (predominantly aqueous sample) directly into the GC capillary column, thus ensuring a significant increase in sensitivity. Indeed, it was possible to detect other compounds such as benzenemethanol, α,α-dimethyl-, geraniol, myrcenol, and hydroxy-citronellol.

Overall, the applied analytical techniques proved invaluable for in-depth study of the chemical profile of the hydrolate, allowing for the precise identification and accurate quantification of VOCs.

In conclusion, the findings of this research provide important compositional information for potential use of *P. odoratissimum* hydrolate as a natural source of bioactives.

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