

RESEARCH ARTICLE

Chemical composition of wild populations of *Thymus vulgaris* and *Satureja montana* in central Catalonia, Spain

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Abstract

Background: This study is devoted to determining the chemical composition of essential oils obtained from 13 wild populations of thyme (*Thymus vulgaris* L.) and 14 populations of winter savory (*Satureja montana* L.) from the Solsonès region of Catalonia, Spain. The essential oils were obtained by hydrodistillation using a Clevenger apparatus, and the volatile compounds were identified by gas chromatography coupled with mass spectrometry.

Results: Twenty-six compounds were detected in the thyme samples, including linalool, (Z)-sabinene hydrate, terpinen-4-ol, and α -terpenyl acetate. In contrast, 19 compounds were identified in the winter savory samples, with linalool, carvacrol, p-cymene, and γ -terpinene as the major components. The results facilitated comparative analyses among samples, enabling the assessment of differences related to geographical location and growing conditions. Significant variability in the chemical profile of thyme and winter savory samples was observed, underscoring the influence of several factors. Notably, the observed increase in linalool content in the northwestern areas of the region, evident in both winter savory and thyme, is attributed to a discernible position or climatic division effect. For instance, in winter savory samples, linalool content ranged from 3.9% in southern populations to as high as 75.8% in certain northern populations. Similarly, in thyme, linalool content varied significantly, reaching up to 36.7% in some northern samples compared to lower values observed in southern samples, such as 9.6%.

Conclusion: This information holds significance for the selection of populations suitable for prospective applications in the pharmaceutical, cosmetic, and gastronomic industries.

KEYWORDS

essential oil, gas chromatography, hydrodistillation, mass spectrometry, *Satureja montana*, *Thymus vulgaris*

INTRODUCTION

Medicinal and Aromatic Plants (MAPs) are characterized by containing substances or active principles with specific chemical, organoleptic, and biochemical properties.¹ Thus, MAPs such as

thyme (*Thymus vulgaris* L.) and winter savory (*Satureja montana* L.) play a significant role in various fields, with medicinal, culinary, and cosmetic applications.² *Thymus vulgaris* is an herbaceous perennial plant of the *Lamiaceae* family native to the western Mediterranean.³ It is a subshrub of up to 40 cm tall with a grayish-green

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color. Its leaves are lanceolate, and its flowers are white or pink, grouped in inflorescences.⁴ *Satureja montana* also belongs to the *Lamiaceae* family and is a perennial plant from southern Europe and northern Africa.⁵ Its flowers are white with pink spots or solid pink. The leaves are narrow, pointed, and ciliated.⁶ Both species are found in Western meso-Mediterranean calcicolous garrigues, specifically in subshrub formations formed by bushes of narrow or small leaves distributed in open, sunny, dry spaces, often flat or slightly sloping places on thin, calcareous, stony, and sedimentary soils with a high proportion of exposed soil or rock.^{7,8}

According to the Royal Spanish Pharmacopoeia,⁹ essential oils are an aromatic product, generally of complex composition, obtained from raw plant material by steam distillation, dry distillation, or an appropriate mechanical procedure. This mixture is composed of active principles synthesized by secondary metabolism¹⁰ which is tied to the plant's protective function.^{11,12} Therefore, various intrinsic or extrinsic factors of the plant provide development with differentiated chemical profiles^{5,11,13} that are responsible for their properties. For this reason, it is important to know their chemical composition to be able to characterize the plants and take advantage of their benefits.

The essential oil of *Thymus vulgaris* contains active principles with the following therapeutic properties: antifungal,¹⁴ antimicrobial,^{15,16} antioxidant,^{17,18} and as a food preservative.¹⁹ Meanwhile, the essential oil of *Satureja montana* is recognized for its antimicrobial, antioxidant, antispasmodic, antiviral, antidiarrheal,^{20–22} and antiseptic properties, which is why it is used in the treatment of respiratory and urinary infectious diseases and digestive disorders. It is also used in the treatment of wounds, nasal obstructions, and for oral hygiene.⁵ Despite the recognized importance of *Thymus vulgaris* and *Satureja montana*, there are still limited studies on the chemotypic variability of these plants in different geographical contexts. Although it is known that factors such as temperature, precipitation,^{23,24} soil composition,²⁵ and genetic factors²⁶ influence the composition of essential oils, there is a lack of studies that explore how these factors specifically affect these species.

This study involved the analysis of 13 populations of *Thymus vulgaris* and 14 populations of *Satureja montana* located in different areas of Solsonès, a region in central Catalonia. These are species widely used by the local people. The main objective of this study is to compare the chemical composition of the essential oils to evaluate the variability of populations within the same species. To achieve this, samples were taken when the plant was in the flowering stage. The plants were then dried, and their essential oils were obtained using the hydrodistillation method with a Clevenger apparatus. Finally, an analysis was performed using gas chromatography coupled with mass spectrometry to separate, identify, and quantify the different chemical compounds present in each sample. This allowed for the observation of variations in the chemical composition of different populations.

This study provides a deeper understanding of the diversity and composition of wild thyme and winter savory in the Solsonès region. It also serves as a basis for observing how climatic and edaphic factors affect the composition of different populations.

MATERIALS AND METHODS

Collection of plant material

Thirteen samples of wild *Thymus vulgaris* and 14 samples of wild *Satureja montana* were collected from various areas in the Solsonès region of Catalonia (Figure 1).

During sampling, 2–3 apical branches/inflorescences were taken from 50 different plants from each population. The thyme samples were collected in April and May of 2023 (Table 1), while the winter savory samples were collected in August and September of 2022 (Table 2). All samples were in the flowering phenological stage, as this is when plants typically have the highest concentration of essential oils^{27,28} and when they are traditionally collected.

The selection of sampling areas was based on the study of the presence of species,²⁹ geographical location, and climatic divisions defined according to precipitation and temperature (Table 3). This approach ensured that the samples would represent different conditions.

Drying plant material

After harvesting, the fresh plant samples were dried under controlled conditions with the aim of stabilizing and preserving them with the same composition of active principles.¹ Drying was carried out in an air oven at a constant temperature of 35°C for 5 days. Once dried, the leaves and flowers were removed from the stems and stored until the essential oil was obtained.

Extracting essential oils

Hydrodistillation with a Clevenger apparatus was employed to obtain the essential oil following the guidelines of the Royal Spanish Pharmacopoeia.⁹ In this case, for both thyme and winter savory, 30 g of the dry sample, a 1000-mL round-bottom flask, and 400 mL of distilled water were used as the distillation liquid. Distillation was carried out at a rate of 2–3 mL/min for 2 h.

Next, the water from the essential oil was extracted using anhydrous sodium sulfate, and a 1:50 dilution of the essential oil in dichloromethane was made. To preserve the integrity of the essential oil until chromatographic analysis, it was stored in a tightly closed vial and kept refrigerated at –20°C.

GC–MS analysis

Three µL of diluted essential oil was injected into a gas chromatograph (Fisons Instruments, model Focus GC—Thermo Scientific) operating in splitless mode, coupled to a mass spectrometer model DSQ II—Thermo Scientific (GC 8000/MD 800), operating in electron impact ionization (EI) mode (70 eV). Compounds were separated on a fused

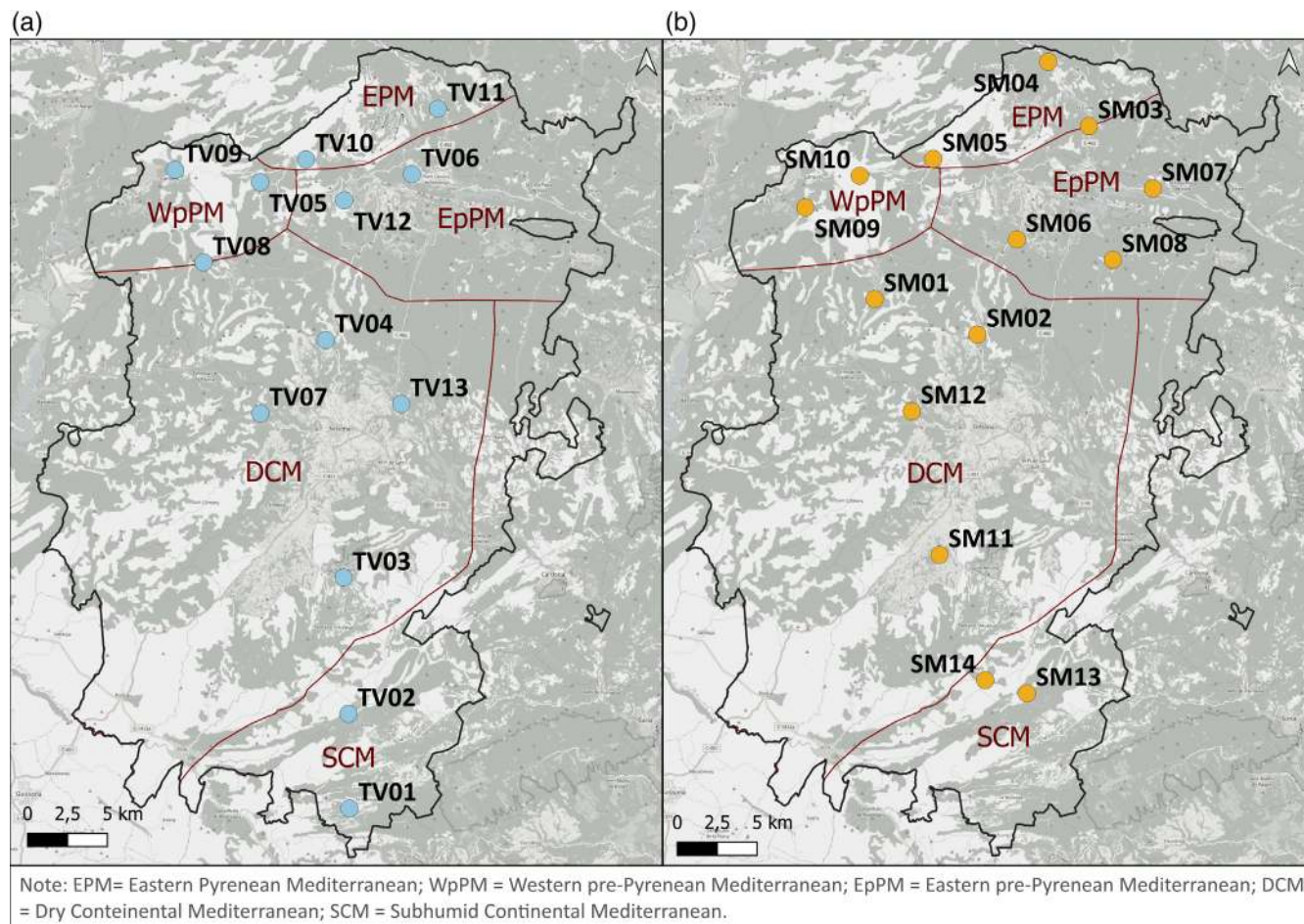


FIGURE 1 Geographic location of collection points of samples of *Thymus vulgaris* (a) and *Satureja montana* (b) in the Solsonès region.

TABLE 1 Characteristics of the samples of wild *Thymus vulgaris* used in the study.

	Location	Geographical coordinates	Climactic division	Collection date	Phenological state
TV01	La Molsosa	41.78033, 1.53054	Subhumid continental Mediterranean	11/04/2023	Flowering
TV02	Pinós	41.83366, 1.52875	Subhumid continental Mediterranean	11/04/2023	Flowering/Preflowering
TV03	Riner	41.91042, 1.52328	Dry continental Mediterranean	11/04/2023	Flowering/Preflowering
TV04	Lladurs	42.04438, 1.50668	Dry continental Mediterranean	19/04/2023	Flowering/Preflowering
TV05	Odèn	42.13277, 1.45479	Western pre-Pyrenean Mediterranean	28/04/2023	Flowering/Preflowering
TV06	Guixers	42.13881, 1.56985	Eastern pre-Pyrenean Mediterranean	28/04/2023	Flowering/Preflowering
TV07	Clarà	42.00228, 1.45812	Dry continental Mediterranean	10/05/2023	Flowering/Postflowering
TV08	Lladurs	42.08689, 1.41304	Dry continental Mediterranean	10/05/2023	Flowering
TV09	Cambrils	42.13877, 1.39019	Western pre-Pyrenean Mediterranean	10/05/2023	Flowering
TV10	Odèn	42.14604, 1.48920	Eastern pre-Pyrenean Mediterranean	10/05/2023	Flowering
TV11	La Coma i la Pedra	42.17593, 1.58869	Eastern pre-Pyrenean Mediterranean	10/05/2023	Flowering/Preflowering
TV12	Odèn	42.12340, 1.51882	Eastern pre-Pyrenean Mediterranean	10/05/2023	Flowering
TV13	Olius	42.00893, 1.56460	Dry continental Mediterranean	20/05/2023	Flowering/Postflowering

silica capillary column (DB-5 ms; length: 30 m; i.d.: 0.32 mm), coated with a low-polarity liquid-phase film with a thickness of 0.25 µm (5% methylpolysiloxane; J&W Scientific, Folsom, California). The total ion current (TIC) was acquired between 50 and 350 Da for 1 s. The oven

temperature was programmed according to the conditions described by Hanuš and Hod,³¹ as follows: start at 40°C (5 min); ramp to 150°C (5°C/min); hold the temperature at 150°C for 1 minute; then another ramp to 250°C (15°C/min); and, finally, hold at 250°C for 60 min.

TABLE 2 Characteristics of the samples of wild *Satureja montana* used in the study.

	Location	Geographical Coordinates	Climactic division	Collection date	Phenological state
SM01	Ribera Salada	42.06556, 1.43436	Dry continental Mediterranean	30/08/2022	Flowering
SM02	Lladurs	42.04657, 1.51252	Dry continental Mediterranean	01/09/2022	Preflowering/Flowering
SM03	La Coma i la Pedra	42.16525, 1.59444	Eastern Pyrenean Mediterranean	30/08/2022	Flowering
SM04	La Coma i la Pedra	42.20114, 1.56270	Eastern Pyrenean Mediterranean	30/08/2022	Flowering
SM05	Odèn	42.14525, 1.47681	Eastern Pyrenean Mediterranean	01/09/2022	Flowering
SM06	Lladurs	42.10062, 1.54125	Eastern pre-Pyrenean Mediterranean	01/09/2022	Flowering
SM07	Guixers	42.13064, 1.64372	Eastern pre-Pyrenean Mediterranean	01/09/2022	Preflowering/Flowering
SM08	Busa	42.09010, 1.61424	Eastern pre-Pyrenean Mediterranean	01/09/2022	Flowering
SM09	Cambrils	42.11649, 1.38060	Western pre-Pyrenean Mediterranean	30/08/2022	Flowering
SM10	Odèn	42.13495, 1.42130	Western pre-Pyrenean Mediterranean	30/08/2022	Preflowering/Flowering
SM11	Miracle	41.92192, 1.48683	Dry continental Mediterranean	30/08/2022	Flowering
SM12	Clarà	42.00266, 1.46394	Dry continental Mediterranean	30/08/2022	Flowering
SM13	Pinós	41.84463, 1.55469	Subhumid continental Mediterranean	30/08/2022	Flowering
SM14	Ardèvol	41.85163, 1.52302	Subhumid continental Mediterranean	30/08/2022	Preflowering

TABLE 3 Climatic classification of Solsonès based on thermometry and pluviometry.

Groups/type		Subtype	P (mm) ^a	T (°C) ^b	ΔT (°C) ^c
Mediterranean	Pyrenean	Oriental	1000–1200	3–9	13–16
		Pre-Pyrenean	850–1100	9–12	16–19
	Continental	Occidental	650–900		
		Subhumid	550–700	11–13	17–20
		Dry	350–550	12–14	17–20

Note: Adapted from the Meteorological Service of Catalonia (2023).³⁰

^aAverage annual precipitation.

^bAverage annual temperature.

^cAverage annual temperature range.

Helium was used as the carrier gas (flow rate: 1.0 mL/min). The inlet temperature was 250°C; the transfer line temperature, 250°C; the ion source temperature, 200°C; and the interface temperature, 250°C. Subsequently, compounds were identified by comparing the retention index calculated in our column with Kovats retention index published by Adams,³² as well as the analysis of mass spectra obtained in the NIST, EPS, and NIH Mass Spectral Database NBS75K using AMDIS 32 software. GC-MS was selected for its effectiveness in separating volatile compounds, while electron impact ionization provides consistent fragmentation patterns to identify compounds in complex mixtures.³³

The semiquantitative composition of the essential oils, expressed as a percentage, was determined by area normalization, enabling quantification without external standards.

Statistical analysis

Multivariate data statistical analysis was performed using the R program, version 4.3.1.³⁴ To group the samples according to their similarities, hierarchical cluster analysis was carried out. In this analysis, the values of relative abundance—expressed as a percentage of the chemical

compounds found in the analyzed samples—were used. Dissimilarity was determined using Euclidean distance, as it provides a straightforward and intuitive measure of similarity between samples. Ward's agglomeration method was chosen because it minimizes variance within clusters, ensuring that the formed groups are compact and homogeneous.

In the graphic representations, maps were used as visual backgrounds to display data points or statistical information associated with specific geographic locations. QGIS, version 3.32.1,³⁵ was used to visualize spatial data. Subsequently, R was employed to create figures displaying the distribution percentages of specific components on the maps. Specifically, the sf package version 1.0.15³⁶ was used for spatial data processing, and the ggplot2 package version 3.4.3³⁷ was used for figure representation.

RESULTS

Two characteristic chromatograms corresponding to the SM08 sample of *Satureja montana* and the TV05 sample of *Thymus vulgaris* (Figure 2) are presented. These chromatograms provide a visual

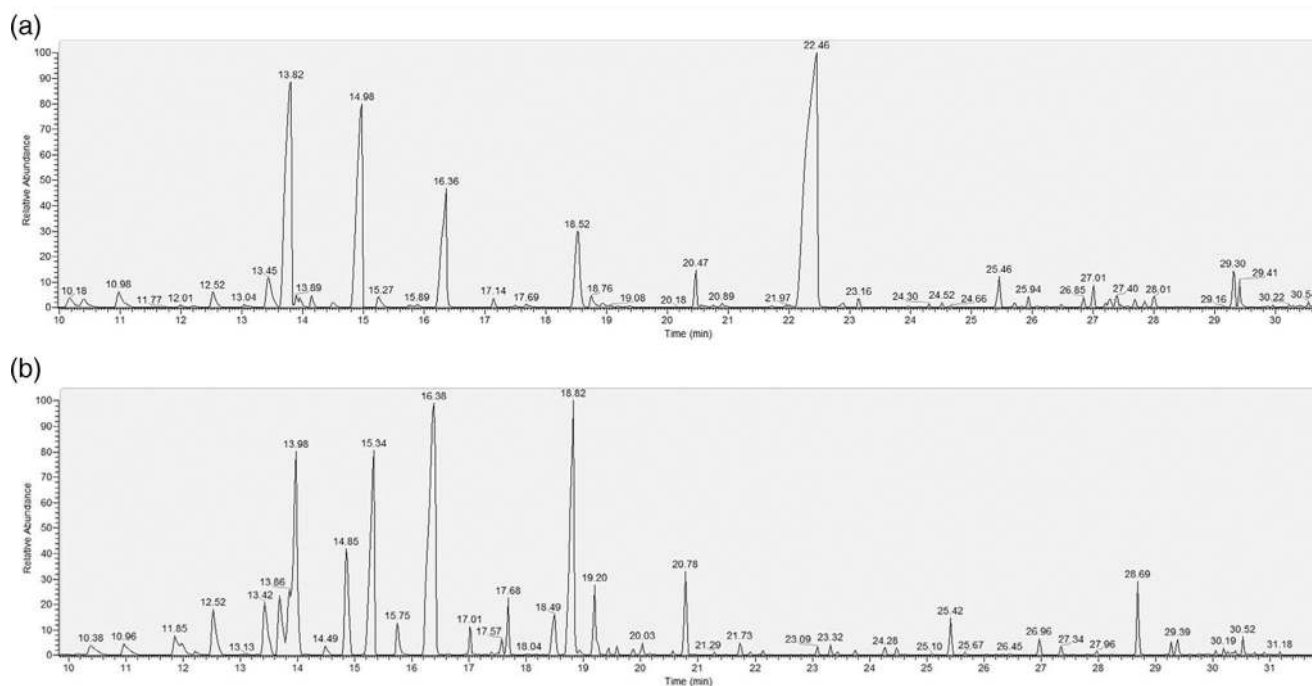


FIGURE 2 Chromatograms corresponding to the SM08 sample of *Satureja montana* (a) and the TV05 sample of *Thymus vulgaris* (b).

representation of the volatile compounds identified in the essential oils extracted from these plants.

The 26 main volatile compounds present in the essential oils of *Thymus vulgaris* samples were determined based on the analysis of the different chromatograms and mass spectra (Table 4).

The essential oil of *Thymus vulgaris* from the analyzed samples is primarily composed of monoterpenes, where oxygenated derivatives constitute the majority. Specifically, the main components found are: linalool (9.6%–36.7%), (Z)-sabinene hydrate (7.4%–21.5%), terpinen-4-ol (3.9%–18.7%), α -terpinyl acetate (0.0%–18.3%), p-mentha-1(7),8-dien-2-ol (0.3%–9.9%), α -terpineol (1.9%–12.9%), limonene (1.3%–13.2%), and 1,8-cineole (0.1%–11.3%) (Table 4). These compounds represent between 63.7% and 75.9% of the total content of the different samples.

In the samples of essential oil from *Satureja montana* analyzed, 19 major chemical compounds were identified (Table 5).

In these samples of *Satureja montana*, the most common compounds are also monoterpenes, with oxygenated monoterpenes being prominent. Additionally, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and diterpenes were identified. Among them, the main constituents are: linalool (3.9%–75.8%), carvacrol (0.2%–45.9%), p-cymene (N.D.–31.1%), γ -terpinene (1.8%–15.1%), borneol (1.3%–7.4%), and thymol (N.D.–17.5%). These compounds represent between 82.2% and 91.9% of the total content of the essential oils.

DISCUSSION

Chemical composition of *Thymus vulgaris*

Thymus vulgaris shows significant chemical polymorphism. Various studies have identified up to eight different chemotypes: thymol,

carvacrol, (Z)-sabinene hydrate/terpinen-4-ol, α -terpineol, linalool, geraniol^{38,39} borneol, and p-cymene.⁴⁰ In Spain, the 1,8-cineole chemotype has also been detected.^{41,42}

In our study, these differences in the chemical profile among the different populations of *Thymus vulgaris* in Solsonès can be observed. In the dendrogram in Figure 3, the samples are grouped into five clusters according to the cluster analysis.

Samples TV01, TV02, and TV03 (cluster IV), from the south of the region, are characterized by the presence of the following major compounds: (Z)-sabinene hydrate, α -terpinyl acetate, linalool, and terpinen-4-ol (Table 4). (Z)-Sabinene hydrate emerges as the dominant compound in all three samples, indicating a distinctive feature of this area of Solsonès. Samples TV01 and TV02 are classified within the Mediterranean continental subhumid climate division, while sample TV03 belongs to the Mediterranean continental dry division (Table 1). Despite this, the geographical proximity of sample TV03 to the climatic fringe results in similar conditions, which may contribute to this chemical similarity. Monthly maps of accumulated precipitation and average temperature from the Meteorological Service of Catalonia³⁰ show that the 5 months preceding the collection of the sample coincide in the range of accumulated precipitation and temperature in the three samples.

In the central part of the region, samples TV07 and TV13 show notable similarities, with a high percentage of terpinen-4-ol and (Z)-sabinene hydrate as the main characteristics. However, a substantial difference lies in the content of α -terpineol, which is significantly higher in sample TV13, with a relative percentage of 12.9%, compared to 3.6% in sample TV07 (Table 4). Due to their geographical proximity and shared climatic classification, the two samples present similar—though not identical—chemical profiles. This highlights the complexity of the factors influencing the chemical composition of essential oils.

TABLE 4 Relative abundance (%) of the main chemical compounds in 13 *Thymus vulgaris* populations in Solsonès.

R.T. ^a (min)	RI ^b	Constituents	Relative abundance (%) ^c												
			TV01 ^d	TV02	TV03	TV04	TV05	TV06	TV07	TV08	TV09	TV10	TV11	TV12	TV13
10.38	939	α -Pinene	1.1	1.1	1.3	0.2	1.0	1.4	0.8	1.7	1.5	1.1	0.5	1.3	0.7
10.97	954	Camphene	0.4	0.3	0.6	0.0	1.0	0.3	0.2	2.7	1.8	1.6	1.0	1.4	0.3
11.85	975	Sabinene	0.8	0.9	0.7	0.5	1.0	1.0	0.7	0.6	1.2	0.4	0.5	0.6	0.7
11.98	980	β -Pinene	0.7	0.9	0.9	0.3	0.7	1.0	0.7	0.8	1.0	0.6	0.3	0.7	0.7
12.52	990	Myrcene	3.9	4.3	4.2	1.3	3.0	6.1	2.2	3.2	5.1	2.9	1.8	3.3	2.7
13.42	1017	α -Terpinene	2.5	2.7	2.6	2.6	3.3	3.2	3.4	0.7	2.2	1.2	2.6	2.6	3.0
13.68	1024	<i>p</i> -Cymene	2.8	2.3	3.6	1.0	3.3	2.7	2.2	0.5	2.0	1.1	2.3	2.8	2.8
13.87	1029	Limonene	1.8	1.7	1.5	1.3	2.4	2.9	2.1	3.2	13.2	4.4	2.8	3.9	1.9
13.95	1031	1,8-Cineole	1.6	2.9	1.3	1.3	11.3	3.3	1.0	6.2	0.1	2.7	1.1	4.4	4.5
14.85	1059	γ -Terpinene	4.7	4.7	5.6	4.5	5.3	6.4	5.7	1.2	3.7	1.9	4.4	4.1	5.0
15.32	1070	(Z)-Sabinene hydrate	21.5	21.1	19.6	11.9	12.4	16.3	17.9	7.6	12.7	10.9	7.4	13.1	15.8
15.75	1088	Terpinolene	1.1	1.1	0.9	1.2	1.4	1.2	1.3	0.4	0.9	0.7	1.5	1.2	1.1
16.34	1096	Linalool	11.1	9.9	11.6	15.2	23.4	14.0	9.6	36.7	18.6	32.2	18.7	24.7	10.1
17.05	1133	Terpinen-1-ol	0.7	0.6	0.5	0.8	0.8	0.6	1.0	0.1	0.5	0.3	0.8	0.7	1.0
17.73	1146	Camphor	0.6	0.4	0.9	2.4	1.6	2.2	0.3	3.0	2.2	1.9	2.4	1.5	0.3
18.51	1169	Borneol	0.8	0.5	0.4	1.4	2.2	1.1	0.8	5.1	3.1	3.6	2.8	3.6	1.6
18.84	1177	Terpinen-4-ol	11.1	13.2	9.8	18.7	14.5	16.8	18.6	3.9	10.4	6.0	14.3	13.2	17.6
19.27	1188	α -Terpineol	3.0	4.2	2.4	4.4	2.7	2.7	3.6	2.4	2.7	3.2	5.1	1.9	12.9
20.14	1230	<i>p</i> -Mentha-1(7) 0,8-dien-2-ol	7.0	6.7	7.4	9.7	0.3	3.3	9.9	0.8	0.4	1.7	2.2	2.5	6.6
20.85	1257	Linalyl acetate	1.0	0.7	0.3	0.5	2.8	1.1	0.2	5.3	2.8	4.2	1.9	2.5	0.1
21.96	1289	Thymol	1.7	1.7	5.2	0.3	0.0	0.6	3.5	0.0	-	0.0	0.1	0.0	1.4
22.21	1299	Carvacrol	1.8	0.0	0.1	0.1	0.0	1.4	0.1	0.0	0.0	0.0	0.1	0.0	0.2
23.47	1349	α -Terpinyl acetate	12.4	12.6	12.1	13.6	0.0	4.5	8.7	4.6	5.8	10.0	18.3	2.2	4.2
25.43	1419	(E)-Caryophyllene	1.6	1.8	2.4	1.9	1.1	1.7	1.4	3.1	2.4	1.9	1.4	2.0	1.1
26.95	1481	Germacrene-D	0.2	0.2	0.4	0.3	0.5	0.3	0.4	1.1	0.9	0.9	0.5	0.6	-
29.39	1583	Caryophyllene oxide	0.5	0.4	0.3	0.8	0.4	0.4	0.5	0.5	0.6	0.3	0.8	1.0	0.5
		Oxygenated monoterpenes	61.9	61.8	59.4	66.5	72.3	63.2	66.5	71.2	53.4	66.8	56.8	68.1	72.1
		Monoterpene hydrocarbons	32.2	32.6	33.8	26.4	22.4	30.6	27.9	19.5	38.1	25.9	35.9	24.1	23.0
		Oxygenated sesquiterpenes	0.5	0.4	0.3	0.8	0.4	0.4	0.5	0.5	0.6	0.3	0.8	1.0	0.5
		Sesquiterpene hydrocarbons	1.8	1.9	2.8	2.2	1.5	2.1	1.8	4.2	3.3	2.8	1.8	2.5	1.1

Note: (-) Not detected (N.D.). The sum of the percentages according to the typology of the identified chemical, providing an overview of the chemical composition of the sample.

^aExperimental Retention time.

^bKovats retention index of DB-5 column³².

^cThe percentage of the composition was calculated based on GC peak areas.

^dSample code (see Table 1).

TABLE 5 Relative abundance (%) of the main chemical compounds in 14 *Satureja montana* populations in Solsonés.

R.T. ^a (min)	R.I. ^b	Constituents	Relative abundance (%) ^c													
			SM01 ^d	SM02	SM03	SM04	SM05	SM06	SM07	SM08	SM09	SM10	SM11	SM12	SM13	SM14
10.17	930	α -Thujene	0.3	0.4	0.0	0.3	0.2	0.0	0.3	0.6	0.0	0.3	0.7	0.7	0.5	0.6
10.40	939	α -Pinene	0.4	0.3	0.1	0.5	0.1	0.1	0.2	0.5	0.0	-	0.9	0.6	0.6	0.5
10.98	954	Camphene	0.7	1.0	0.2	1.2	0.6	0.3	0.6	1.1	0.2	1.9	1.0	1.1	0.7	0.7
12.52	990	Myrcene	0.5	0.4	0.5	0.5	0.4	0.6	0.5	0.8	0.5	0.8	0.9	0.7	0.9	0.6
13.45	1017	α -Terpinene	0.6	0.9	0.2	0.8	0.3	0.2	0.5	2.2	0.0	4.7	2.0	1.3	2.0	1.1
13.74	1024	<i>p</i> -Cymene	15.6	24.3	6.0	22.3	13.3	4.0	9.4	20.7	4.2	-	20.6	31.1	23.5	27.9
14.14	1037	(Z)-Beta-ocimene	0.2	0.2	0.1	1.1	0.5	1.4	0.0	0.4	1.1	0.8	0.1	0.2	0.0	0.1
14.88	1059	γ -Terpinene	5.5	6.7	2.4	5.0	4.2	2.3	4.8	15.1	1.8	2.7	12.2	10.1	12.7	7.8
15.26	1070	(Z)-Sabinene hydrate	0.6	0.6	0.3	0.6	0.5	0.6	0.4	0.6	0.9	3.3	0.5	0.5	0.6	0.8
16.39	1096	Linalool	46.8	32.3	74.5	24.0	51.4	74.8	59.4	8.1	75.8	71.6	3.9	7.5	4.1	20.1
18.49	1169	Borneol	3.00	4.0	1.3	7.4	3.0	1.5	2.1	5.0	1.4	1.6	4.3	4.1	3.7	3.0
18.75	1177	Terpinen-4-ol	0.2	0.4	0.0	0.5	0.2	0.0	0.1	0.5	0.0	-	0.6	0.5	0.8	0.5
20.75	1252	<i>p</i> -Mentha-3,6-diene-2,5-dione	0.1	0.1	-	0.8	0.0	-	0.0	-	-	0.0	0.1	0.4	0.3	0.6
22.00	1289	Thymol	-	-	-	17.5	0.8	-	-	-	0.0	7.3	-	-	0.2	0.2
22.29	1299	Carvacrol	16.3	21.9	7.2	6.0	13.4	6.1	16.2	38.1	3.6	0.2	45.9	35.9	44.9	29.3
25.45	1419	(E)-Caryophyllene	2.3	0.9	1.3	1.7	1.6	1.5	1.3	1.0	1.9	0.8	1.7	1.1	0.9	1.3
27.00	1481	Germacrene-D	1.0	0.3	1.1	1.3	1.7	1.7	1.2	0.6	2.9	0.8	0.4	0.2	0.1	0.2
29.30	1578	Spathulenol	1.8	1.7	1.1	1.4	1.4	1.7	0.6	1.2	1.5	0.7	0.9	0.8	0.6	1.2
29.40	1583	Caryophyllene oxide	1.9	1.1	0.9	2.5	1.1	0.8	0.6	0.7	0.7	0.4	1.0	1.0	0.6	1.2
		Oxygenated monoterpenes	66.9	59.2	83.3	56.0	69.3	83.0	78.2	52.2	81.7	83.9	55.2	48.5	54.3	54.0
		Monoterpene hydrocarbons	23.8	34.2	9.5	31.8	19.6	8.7	16.3	41.3	7.8	11.1	38.3	45.7	40.9	39.3
		Oxygenated sesquiterpenes	3.7	2.9	2.0	3.8	2.5	2.4	1.2	1.9	2.3	1.1	1.9	1.8	1.2	2.4
		Sesquiterpene hydrocarbons	3.3	1.2	2.3	3.0	3.3	3.1	2.5	1.6	4.8	1.6	2.1	1.3	1.0	1.6
		Diterpenes	0.1	0.1	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.4	0.3	0.6

Note: (-) Not detected (N.D.). The sum of the percentages according to the typology of the identified chemical, providing an overview of the chemical composition of the sample.

^aExperimental Retention time.^bKovats retention index of DB-5 column.³²^cThe percentage of the composition was calculated based on GC peak areas.^dSample code (see Table 2).

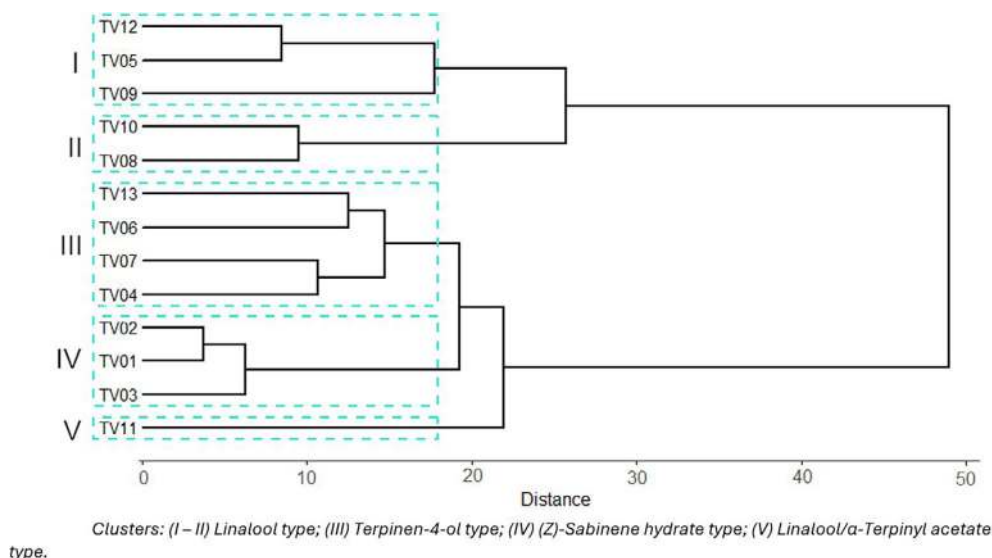


FIGURE 3 Dendrogram of the chemical compositions of *Thymus vulgaris* essential oils based on a hierarchical cluster analysis.

Although sample TV06 belongs to the eastern pre-Pyrenean region, it shows concentrations similar to those of samples from the center of the region. However, it is distinguished by an increase in linalool, a characteristic component in the rest of the samples from the pre-Pyrenean area. Similarly, sample TV04 presents a high content of terpinen-4-ol like the other samples from the center of the region, but is distinguished by an increase in linalool and a decrease in (Z)-sabinene hydrate compared to the other samples in cluster III.

The pre-Pyrenean samples (TV09, TV05, and TV12) and two samples located near the climate division boundary, TV10 from the eastern Pyrenean Mediterranean area and TV08 from the northern sector of the dry continental area (Figure 1a, Table 1), share linalool as the main component. However, they are grouped into different clusters: I and II, respectively (Figure 3). On the one hand, sample TV08 and sample TV10 stand out due to a higher percentage of linalool, with values over 30%. On the other hand, the other samples (TV05, TV09, and TV12) have a linalool percentage of around 20%. It is worth noting a significant increase in 1,8-cineole in sample TV05, with a relative percentage of 11.3%, positioning it as the fourth major component after (Z)-sabinene hydrate and terpinen-4-ol. It is also important to highlight that sample TV09 has a high limonene content (13.2%), while the other samples have percentages below 5% (Table 4). This variability in chemical components highlights the diversity of the pre-Pyrenean samples and those near the boundary, despite the evident similarities.

Finally, sample TV11 (cluster V) is characterized by an increase in α-terpinyl acetate. Its main components are linalool at 18.7%, α-terpinyl acetate at 18.3%, and terpinen-4-ol at 14.3% (Table 4).

These results provide a detailed view of the variability of chemical compounds in the Solsonès region and suggest that the specific geographical and climatic factors of each area could influence the chemical profile of the *Thymus vulgaris* essential oil (Figure 4).

Chemical composition of *Satureja montana*

Different dominant compounds were also observed in the different samples of savory. According to studies on the chemical composition of *Satureja montana* essential oil, different chemotypes exist. In Mediterranean regions, chemotypes of antioxidant compounds such as carvacrol, thymol, and their biogenetic precursors γ-terpinene and p-cymene predominate.^{22,43,44} On the other hand, linalool and β-caryophyllene are also major components in some savory plants.^{45,46}

In our case, we could define two groups in the studied samples based on the similarities in their chemical composition. The results of the cluster analysis revealed a clear distinction between carvacrol-type samples (cluster I) and linalool-type samples (cluster II) (Figure 5).

First, the samples from the southern part of the region (Figure 1b), SM11, SM12, SM13, and SM14 (cluster I), are characterized by a high carvacrol content with percentages exceeding 29.3%, along with a significant abundance of p-cymene ranging from 20% to 32%. Sample SM08 is the only one from the pre-Pyrenean climatic division that also contains a similar composition. An increase in p-cymene levels is observed in samples SM12, with 31.1%, and SM14, with 27.9%. Additionally, in sample SM14, a significant increase in linalool is detected, reaching 20.1%. This population was collected from the understory forest floor where the environmental conditions were cooler, which could influence the linalool content. The other major components remain within a similar range among these samples (Table 5).

Meanwhile, the remaining samples present linalool as the main constituent (cluster II). Samples SM02 and SM01, which are still part of the continental climatic division like the rest of the cluster I samples (Table 2), stand out for having certain similarities with the other group in major components, such as a high percentage of carvacrol and p-cymene and a lower linalool content. Finally, sample SM04, located

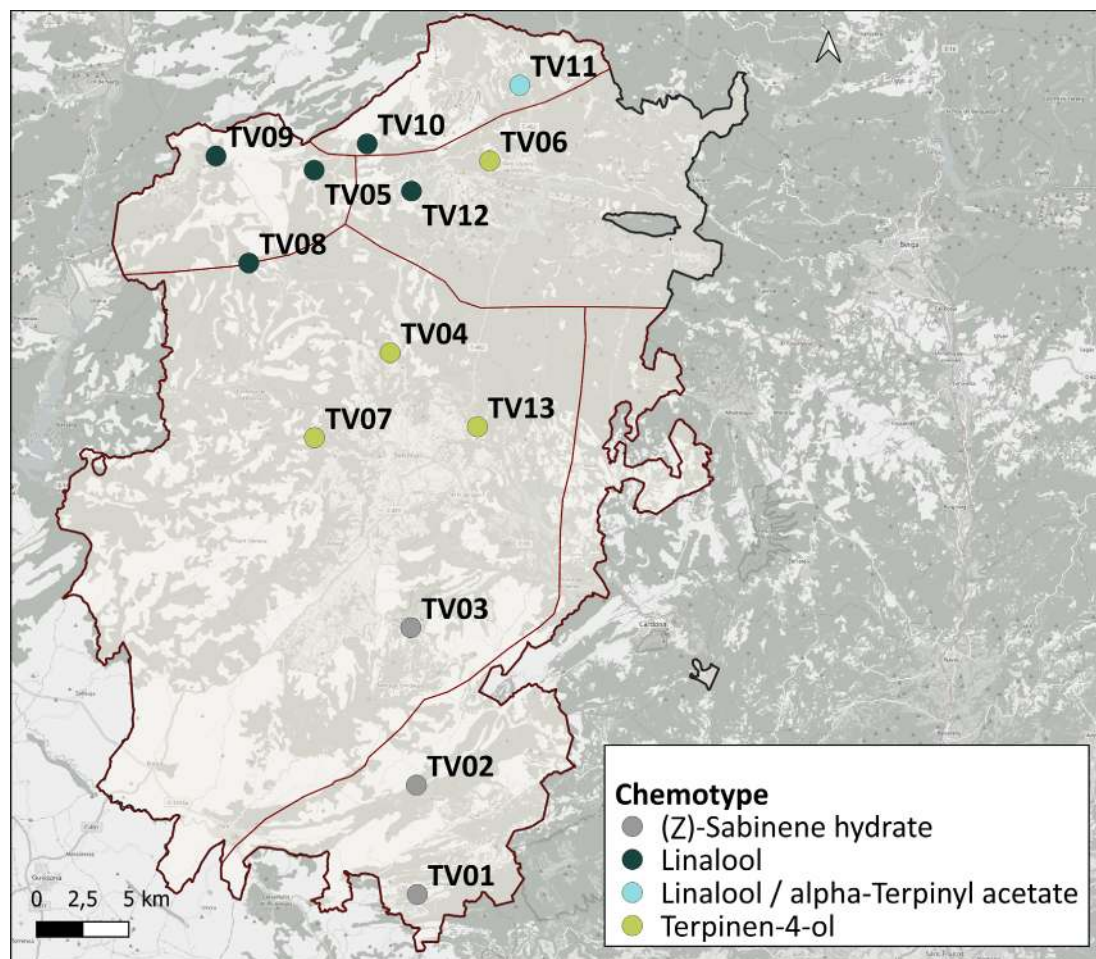


FIGURE 4 Geographical distribution of chemotypes of *Thymus vulgaris* samples in the Solsonès region.

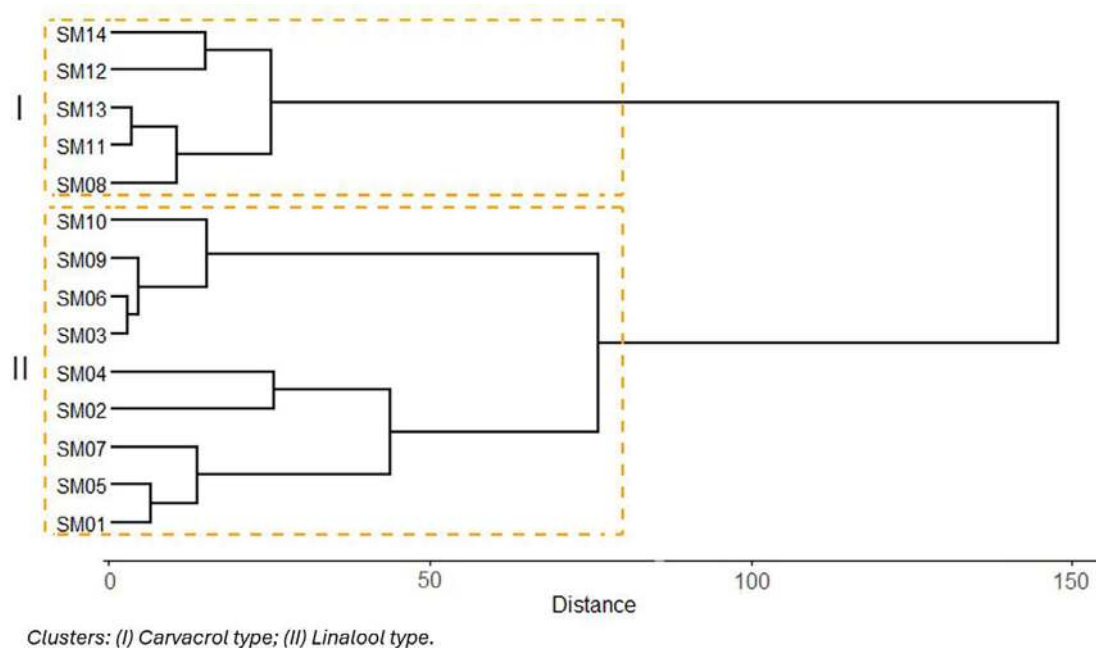


FIGURE 5 Dendrogram of the chemical compositions of *Satureja montana* essential oils based on a hierarchical cluster analysis.

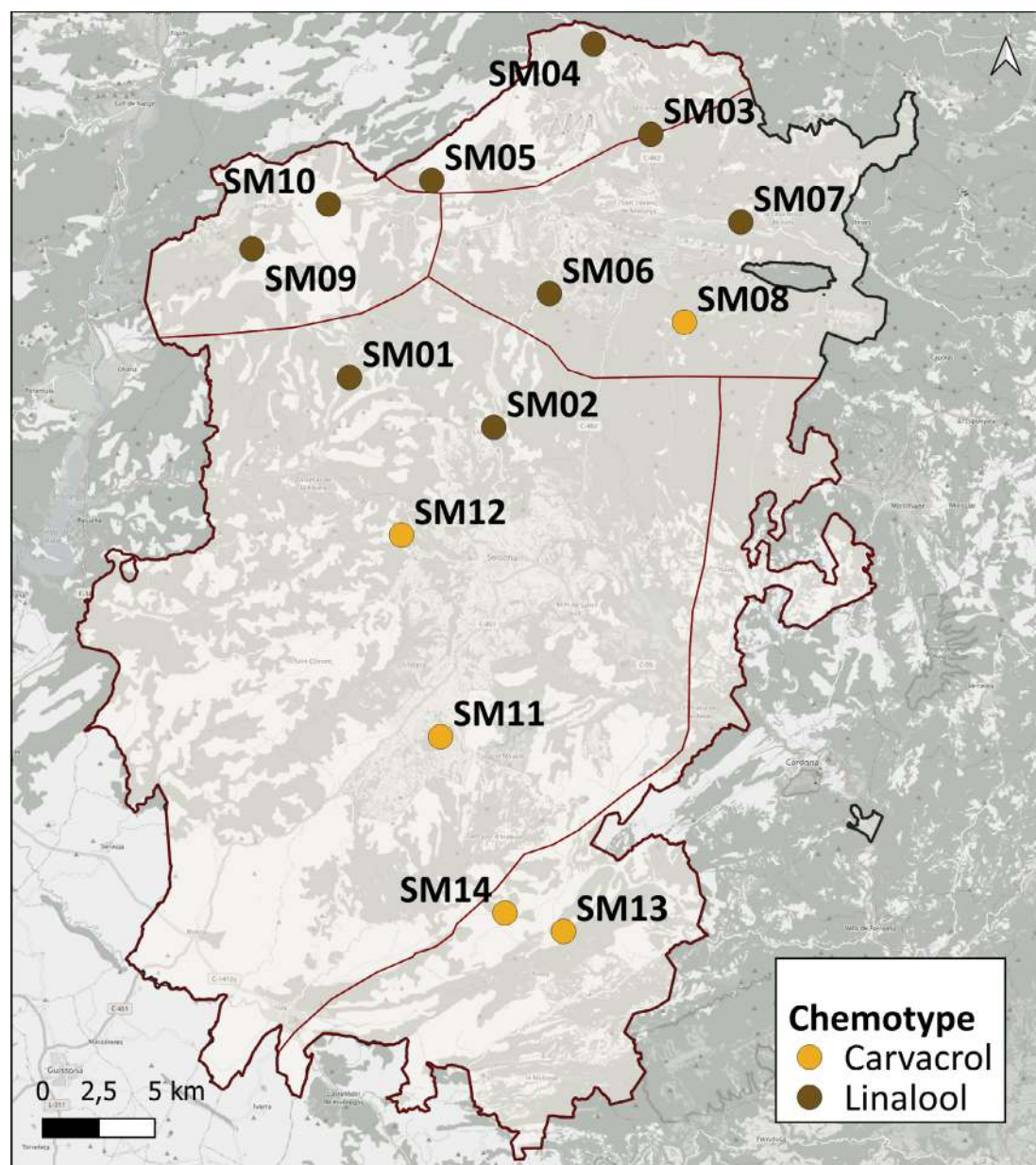


FIGURE 6 Geographical distribution of chemotypes of *Satureja montana* samples in the Solsonès region.

furthest north in the region, presents nearly the same percentage of linalool as *p*-cymene, with 24.0% and 22.3%, respectively. Furthermore, it contains a high thymol content (17.5%) as compared to the rest of the samples, where this substance was often not identified (Table 5).

These chemotypes have significant practical implications for both therapeutic and industrial applications. Carvacrol is a monoterpene phenol with biological activities such as antinociceptive, anxiolytic, antimicrobial, and anti-inflammatory effects.^{47,48} Linalool suggests therapeutic potential due to its antinociceptive effects, anxiolytic, antidepressant, and antimicrobial properties, as well as its role in behavior modulation in individuals affected by dementia.⁴⁹ It is widely used in perfumes, cosmetics, household cleaners, and food additives.⁵⁰

Overall, a relationship of similarity is observed in most of the major components depending on the proximity of these samples and, therefore, the similar environmental and climatic conditions. Figure 6 shows a map of the two clusters based on the major component of the savory samples.

Distribution according to the abundance of predominant compounds

The study by Stahl-Biskup and Sáez¹¹ demonstrates that the distribution of chemotypes in plant populations does not appear to be random, but is instead influenced by selective factors such as temperature and precipitation. In our study, the chemical similarity in

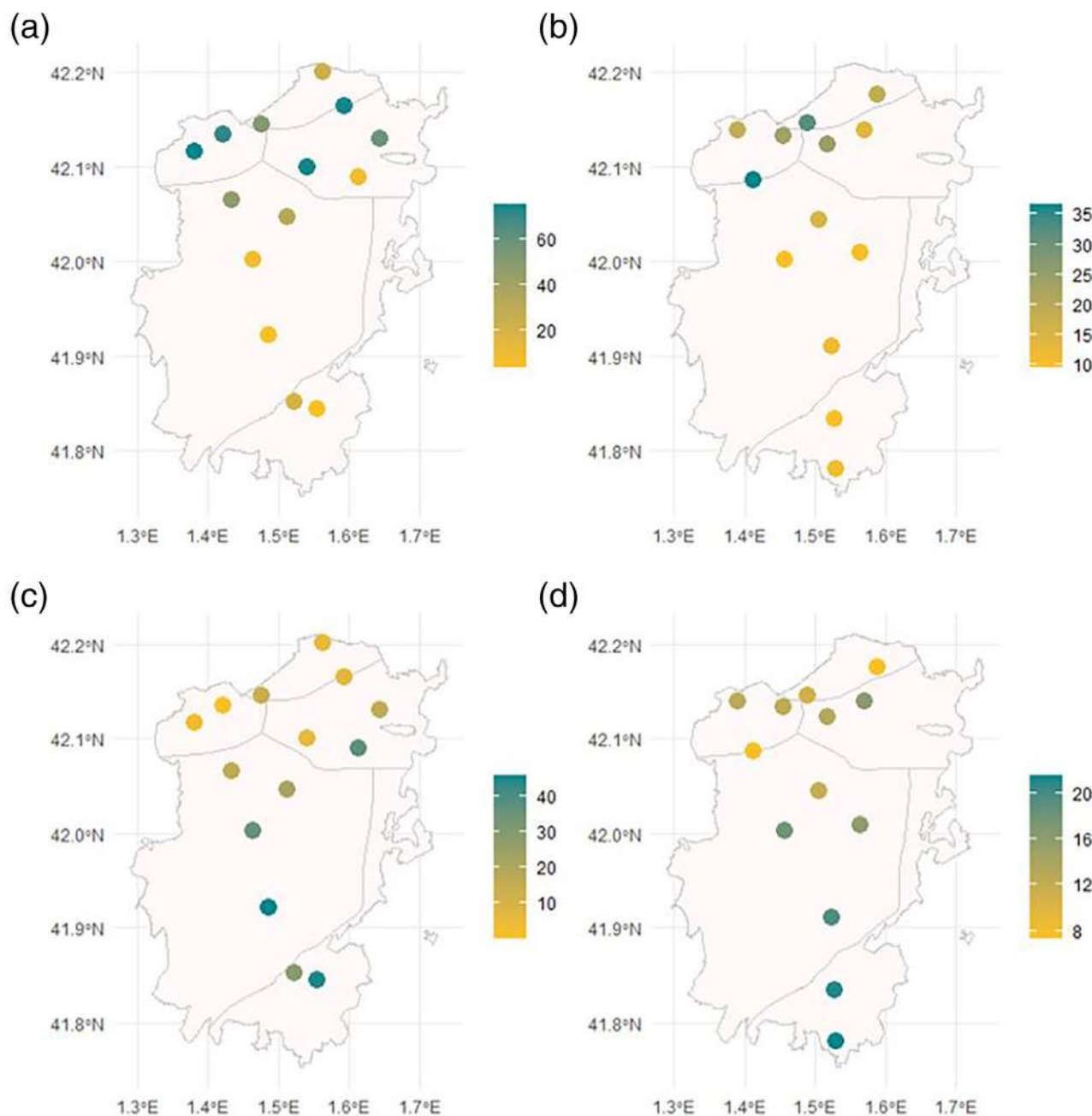


FIGURE 7 Distribution in Solsonès of the relative abundance (%) of major compounds in the analyzed essential oils: (a) linalool in samples of *Satureja montana*; (b) linalool in samples of *Thymus vulgaris*; (c) carvacrol in samples of *Satureja montana*; (d) (Z)-sabinene hydrate in samples of *Thymus vulgaris*.

populations from the same area, as observed in Figures 4 and 6, suggests an adaptation of the plants to these factors.

A variation in the content of certain predominant components in relation to geographical location has been observed. Specifically, there is an increase in the linalool content in the savory and thyme samples from the northwest area of the region (Figure 7a,b). This observation is consistent with previous results, such as those of Granger and Passet,³⁹ who established a correlation between climate and distribution, indicating that linalool chemotypes prefer humid climates. This supports the results of our investigation, as the northern part of the Solsonès region

presents higher levels of precipitation.³⁰ Furthermore, a decrease in carvacrol content has been observed in *Satureja montana* samples as we move northward, indicating a higher abundance in drier and warmer climates (Figure 7c). This trend aligns with the study by Stahl-Biskup and Sáez,¹¹ which states that phenolic compounds thrive in warm and dry regions. Nevertheless, these relationships were not detected in *Thymus vulgaris* populations. In thyme samples, the percentage of (Z)-sabinene hydrate decreased in southern areas (Figure 7d).

In contrast, no significant relationships have been found between geographical location or climatic conditions and the presence of the

major compounds terpinen-4-ol, α -terpinyl acetate, or *p*-cymene in thyme essential oil. In the case of savory samples, no relationship has been identified with (Z)-sabinene hydrate, borneol, or thymol.

Despite the scant latitudinal variations in the Solsonès region, a large number of climatic divisions converge there; they represent a range of temperatures and precipitation that varies according to the location. This may influence the diversity of chemical compositions found within a reduced geographical area.

CONCLUSIONS

This study has allowed us to better understand the chemical composition of the wild populations of *Thymus vulgaris* and *Satureja montana* in Solsonès. The results presented show compositions that vary between populations and confirm that the main components of the two species are generally classified as monoterpenes and sesquiterpenes.

The results also show a variation in the abundance of certain major components of the essential oils according to geographical location and climatic conditions. An increase in linalool content was observed in thyme and savory samples in the northwestern part of the region, which is consistent with the preference of linalool chemotypes for humid climates. Additionally, an increase in carvacrol content was observed in *Satureja montana* samples as we move southward toward warmer, drier conditions. Similarly, we observed a decrease in (Z)-sabinene hydrate in thyme samples from the south of the region. On the other hand, no significant relationships were detected in other major components. This highlights the complexity of the factors influencing the chemical composition of essential oils. In addition to the external factors affecting the plant—such as the studied climatic conditions—other intrinsic factors can also be considered and their influence analyzed. Although no significant differences in soil types between the northern and southern parts of the region were observed, further research on soil types could provide valuable insights.

This study provides a detailed view of the chemical variability of the characterized species, highlighting the influence of environmental factors on their composition and opening new avenues for future research. The knowledge generated by this study can guide the selection of *Thymus vulgaris* and *Satureja montana* populations for industrial applications in sectors such as pharmaceuticals, cosmetics, and gastronomy. By identifying populations with specific therapeutic and organoleptic properties, more efficient selections and breeding work can be made to obtain new agronomical varieties aimed at optimizing industrial products based on essential oils, allowing for a more specialized use of these natural resources.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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