SHORT REPORT

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Differential Essential Oil Composition and Morphology between Perennial *Satureja* species Growing in Spain

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Abstract: Chemical composition of the essential oils obtained by hydrodistillation from the aerial parts of thirty six samples of perennial Spanish savouries (*Satureja montana* L., *Satureja innota* (Pau) G. López, *Satureja cuneifolia* Ten. and *Satureja intricata* Lange), was investigated by GC and GC-MS. A total of 72 compounds accounting between 98.25-99.55% of the total oil were identified. High content of carvacrol (59.72±1.50%) followed by γ-terpinene (17.40±1.11%) were found in *S. montana* essential oils. *S. cuneifolia* yielded an oil rich in camphor (45.04±1.67%) and camphene (12.42±1.71%) whereas *S. innota* produces an essential oil with linalool (23.94±7.58%) or geraniol (8.62±3.45%) according to the locality of collection and *S. intricata* showed chemical polymorphism with camphor (16.02±1.75%), as the main compound followed with populations with myrcene (8.46±1.46%) and populations with γ-terpinene (8.22±1.33%). Although the morphological affinity between *S. innota*, *S. cuneifolia* and *S. intricata* could lead to consider the subspecies level, the phytochemical discriminant analysis support the taxonomic classification of Flora Iberica which ranks these taxa into species.

Keywords: *Satureja* L.; morphometry; essential oils; GC; GC/MS; chemotaxonomy.

1. Plant Source

The genus *Satureja* (Lamiaceae family) with 38 species, is widely distributed throughout the Mediterranean Area, Caucasus and West Asia. Includes semi-bushy or herbaceous annual and perennial herbs that grow in arid, sunny, stony and rocky habitats. As aromatic plants produce essential oils that are secreted in glandular hairs [1] on the leaf surface and flowers being a specific anatomical characteristic of this family. The essential oils of some Lamiaceae spices (*Origanum*, *Satureja* or *Thymus*), play an important role in the Mediterranean diet, being widely used in food-flavoring, cosmetic and pharmaceutical industries.

2. Previous Studies

The anatomy of the glandular apparatus (glandular cells and adjoined epidermal cells) as well as the essential oil composition are proposed to be elements for the recognition of *Satureja* species [2,3]. In Flora Iberica [4], an annual species (*Satureja hortensis* L.) and four perennial species (*S. montana* L., *S. innota* (Pau) G. López, *S. cuneifolia* Ten. and *S. intricata* Lange) have been reported.

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The classification is based on morphologic characteristics particularly in the form, size and pubescence of the leaves. The Flora Europaea [5] confirms this classification whereas other authors consider these taxa as subspecies [6].

The essential oil composition of *S. montana* (winter savory) has been reported by many authors. Thymol, carvacrol or their biogenetic precursors *p*-cymene and γ-terpinene were found the main compounds from many Mediterranean countries. The essential oil composition of *S. cuneifolia* (wild savory) has been studied in Turkey, Italy and Spain, being the qualitative and quantitative composition very different according to the country of origin. There are few studies about the essential oil composition of *S. intricata* and *S. innota*. The major components in the essential oil of *S. intricata* collected at the flowering stage from different localities in the south-east of the Iberian Peninsula [7] were thymol and its biogenetic precursors *p*-cymene and γ-terpinene, along with borneol, whereas the main compounds of this species (previously known as *S. cuneifolia* Ten. subsp. *gracilis* (Willk.) G. López) in Valencia (Spain) were *p*-cymene, α-terpinene, linalool, γ-terpinene, borneol, camphor and myrcene [8]. Up to now, the only study about the essential oil composition of *S. innota*, in a population located in Castellón (Spain), obtained camphor, α-pinene and camphene as the main constituents [8]. As the yield and chemical composition of the essential oils are affected by exogenous (geographical origin, climate, altitude or soil composition) and endogenous (ontogenetic development stage) factors as well as by the drying and extraction methods, in this study to minimize variability, all the essential oil from the thirty six samples analysed were obtained from fresh plant material in a Clevenger-type apparatus and in the same vegetative stage (when blooming stage begins). The aim of this work was to determine the essential oil composition of four perennial Spanish savouries: *S. montana, S. cuneifolia, S. innota,* and *S. intricata,* with morphological similarities, in order to corroborate their taxonomical classification in Flora Iberica as well as provide information about its essential oil composition in an area not previously studied, contributing to their protection and exploitation of genetic resources used in food or pharmaceutical industries.

3. Present Study

Thirty-six *Satureja* samples, growing between 10 and 1282 m.a.s.l. were collected (Supplementary Table 1, S1) in representative localities of this genus in the Comunidad Valenciana. The essential oils obtained by hydrodistillation (Supplementary S2) showed variability in both yield and chemical composition. The high yield (v/w) was found for *S. cuneifolia* (1.05±0.13) followed by *S. montana* (0.85±0.05 and 0.79±0.01), *S. intricata* (0.60±0.07; 0.60±0.08; 0.50±0.04 and 0.40±0.09) and *S. innota* 0.30±0.05 and 0.39±0.09). Morphological comparison (Supplementary S2) between *S. montana* at different altitude and the three other species shows that leaf length, length-width ratio and hairs length (Supplementary Table 2, S3) are morphological characters that can separate this species from closer species. *S. cuneifolia* had the shortest leaves, significantly lower than the species *S. innota* and *S. intricata*, which showed intermediate values. The ANOVA analysis shows similarities in length-width ratio with *S. innota.* All *S. intricata* populations are separated from the other species by the lower values of hair density but also have similarities with *S. innota* in calyx length.

Discriminant analysis with morphometric characters (Supplementary Figure 1, S4) showed that *S. montana* is separated from other species, whereas there was a morphological affinity between *S. cuneifolia* and *S. intricata*. The leaf morphology (both length and width), in agree with Flora Iberica, has proved to be the most important factors.

The results of the qualitative and quantitative analysis (Supplementary S2) of the perennial *Satureja* essential oils showed a high content in the monoterpene fraction with different oxygenated monoterpenes as the main compounds which have been particularly helpful in taxonomic relationships. The 72 identified compounds [9] are listed (Table 3) in order of their elution on a methylsilicone HP-1 column.

To evaluate whether the identified compounds may be useful in taxonomic relationships a discriminant analysis (Supplementary Figure 2, S5) from the matrix of the main identified compounds was performed. The first group corresponding with *S. montana* samples, is characterized by a high content of carvacrol (59.72±1.50%), followed by its biogenetic precursor γ-terpinene (17.40±1.11%) (Table 3), in the analysed populations. In this species carvacrol have obvious taxonomic significance because this oxygenated monoterpene are not found in large amount in the other analysed species. These results are consistent with several previous studies that also found carvacrol as the main compound [2,10-12]. Other authors obtained their isomer thymol as the principal component [13] whereas the biogenetic precursor *p*-cymene was the dominant constituent in other works [14].

Table 3. Essential oil composition of perennial *Satureja* species.

RI[a]	Compound	Peak area (%)			
		S. montana	***S. cuneifolia***	***S. innota***	***S. intricata***
929	tricyclene	-	0.35 ± 0.08	0.01 ± 0.01	0.13 ± 0.02
933	α-thujene	0.68 ± 0.07	0.42 ± 0.08	0.06 ± 0.03	0.38 ± 0.05
941	α-pinene	0.37 ± 0.04	6.40 ± 1.24	0.88 ± 0.42	2.95 ± 0.41
958	camphene	0.20 ± 0.03	12.42 ± 1.71	1.12 ± 0.52	5.04 ± 0.62
979	sabinene	0.11 ± 0.01	0.60 ± 0.04	0.29 ± 0.09	0.45 ± 0.05
980	β-pinene	0.11 ± 0.01	1.49 ± 0.20	0.21 ± 0.07	0.75 ± 0.09
989	octen-3-ol	0.28 ± 0.02	0.18 ± 0.02	0.11 ± 0.04	0.21 ± 0.02
995	myrcene	1.44 ± 0.08	1.78 ± 0.19	6.37 ± 1.62	8.46 ± 1.46
1010	α-phellandrene	0.18 ± 0.01	0.02 ± 0.02	-	0.04 ± 0.01
1013	δ-3-carene	0.05 ± 0.00	0.03 ± 0.01	-	-
1024	α-terpinene	1.84 ± 0.11	1.02 ± 0.11	0.14 ± 0.05	0.63 ± 0.10
1035	*p*-cymene	3.02 ± 0.16	1.24 ± 0.07	0.27 ± 0.06	3.82 ± 0.47
1039	limonene	0.33 ± 0.03	3.08 ± 0.45	2.49 ± 1.06	4.27 ± 0.55
1041	1,8-cineole	0.19 ± 0.01	0.08 ± 0.04	0.25 ± 0.06	0.05 ± 0.05
1044	*cis*-ocimene	0.41 ± 0.13	0.58 ± 0.07	1.08 ± 0.37	1.54 ± 0.30
1054	*trans*-ocimene	0.19 ± 0.04	0.37 ± 0.03	0.78 ± 0.27	1.14 ± 0.22
1068	γ-terpinene	17.40 ± 1.11	2.16 ± 0.17	0.51 ± 0.13	8.22 ± 1.33
1091	terpinolene	0.07 ± 0.00	0.56 ± 0.05	0.04 ± 0.02	0.26 ± 0.02
1080	*cis*-sabinene hydrate	0.46 ± 0.02	1.07 ± 0.32	0.76 ± 0.13	0.83 ± 0.09
1090	*trans*-linalool oxide	-	-	0.08 ± 0.03	-
1109	*trans*-sabinene hydrate	-	2.95 ± 0.45	-	0.01 ± 0.00
1114	linalool	0.90 ± 0.17	-	23.94 ± 7.58	6.66 ± 0.86
1133	*cis*-*p*-menth-2-en-1-ol	-	-	0.02 ± 0.01	0.05 ± 0.02
1161	camphor	-	45.04 ± 1.67	6.95 ±1.30	16.02 ± 1.75
1185	borneol	0.91 ± 0.11	1.25 ± 0.19	0.46 ± 0.15	5.50 ± 0.89
1190	terpinen-4-ol	0.40 ± 0.01	5.91 ± 0.49	1.41 ± 0.23	2.29 ± 0.17
1200	*p*-cymen-8-ol	-	0.12 ± 0.02	-	-
1200	*p*-methyl-acetophenone	-	-	-	0.01± 0.01
1204	α-terpineol	-	1.26 ± 0.32	1.76 ± 0.28	3.67 ± 1.00
1212	*cis*-piperitol	-	0.25 ± 0.00	-	-
1223	*trans*-dihydro-carvone	-	-	-	0.04 ± 0.01
1232	*trans*-piperitol	-	0.08 ± 0.00	-	-
1235	nerol	-	0.02 ± 0.02	0.88 ± 0.38	-
1246	isobornyl formate	-	-	-	0.01 ± 0.01
1249	neral	-	-	0.34 ± 0.15	0.01 ± 0.01
1261	geraniol	-	0.12 ± 0.12	8.62 ± 3.45	-
1266	carvacrol methyl eter	0.39 ± 0.18	0.02 ± 0.02	-	0.05 ± 0.04
1278	geranial	-	-	0.38 ± 0.16	-
1291	bornyl acetate	0.08 ± 0.07	0.15 ± 0.03	0.04 ± 0.03	0.12 ± 0.04
1291	thymol	1.44 ± 0.65	-	0.01 ± 0.01	0.39 ± 0.26
1304	carvacrol	59.72 ± 1.50	0.01 ± 0.01	0.72 ± 0.42	3.53 ± 1.05
1356	thymol acetate	0.03 ± 0.02	-	-	-
1366	neryl acetate	-	-	0.12 ± 0.06	-
1375	carvacrol acetate	1.24 ± 0.26	-	-	0.07 ± 0.01
1379	α-copaene	-	0.03 ± 0.02	0.17 ± 0.02	-
1386	geranyl acetate	-	-	2.08 ± 1.40	-
1387	β-bourbonene	0.01 ± 0.01	0.27 ± 0.05	0.43 ± 0.19	0.12 ± 0.03
1393	β-elemene	-	-	0.13 ± 0.06	0.08 ± 0.01
1411	α-gurjunene	-	-	0.18 ± 0.06	0.25 ± 0.04
1427	β-caryophyllene	3.97 ± 0.46	1.89 ± 0.04	8.57 ± 1.16	6.47 ± 0.67
1433	β-copaene	-	-	0.05 ± 0.02	-
1442	aromandrene	0.06 ± 0.02	-	0.05 ± 0.02	-
1460	α-humulene	0.14 ± 0.02	-	0.47 ± 0.09	0.27 ± 0.04
1465	*allo*-aromadendrene	0.03 ± 0.01	0.07 ± 0.00	0.24 ± 0.04	0.18 ± 0.04
1486	germacrene D	0.62 ± 0.06	0.95 ± 0.16	5.55 ± 0.98	1.83 ± 0.41
1492	β-selinene	-	-	0.07 ± 0.03	0.04 ± 0.01
1502	bicyclogermacrene	1.27 ± 0.15	2.86 ± 0.48	7.92 ± 1.31	6.56 ± 1.79
1511	germacrene A	-	-	0.26 ± 0.08	0.04 ± 0.01
1511	β-bisabolene	0.60 ± 0.07	-	-	0.07 ± 0.02
1512	γ-cadinene	-	0.03 ± 0.02	0.23 ± 0.03	0.05 ± 0.02
1524	δ-cadinene	0.02 ± 0.01	0.25 ± 0.06	1.00 ± 0.17	0.36 ± 0.09
1576	palustrol	-	-	0.19 ± 0.10	0.08 ± 0.02
1577	ledol	-	-	-	0.08 ± 0.04
1590	spathulenol	0.27 ± 0.04	0.72 ± 0.15	3.41 ± 0.44	1.30 ± 0.24
1592	caryophyllene oxide	0.16 ± 0.01	0.32 ± 0.05	1.35 ± 0.22	0.96 ± 0.28
1595	viridiflorol	0.02 ± 0.01	0.16 ± 0.05	0.52 ± 0.12	0.34 ± 0.09
1643	caryophylla-4(14),	-	0.10 ± 0.01	0.21 ± 0.04	0.18 ± 0.04

	8(15)-dien-5-ol				
1654	*epi*-α-muurolol	0.01 ± 0.01	0.05 ± 0.03	0.38 ± 0.06	0.16 ± 0.04
1657	α-muurolol	-	0.07 ± 0.03	0.47 ± 0.09	0.10 ± 0.04
1666	α-cadinol	-	0.31 ± 0.10	1.47 ± 0.21	0.52 ± 0.11
1692	germacra-4(15)-5, 10(14)-trien-1-α-ol	-	0.04 ± 0.02	0.15 ± 0.04	0.06 ± 0.02
1701	shyobunol	0.04 ± 0.01	0.01 ± 0.01	1.45 ± 0.67	1.03 ± 0.21
Total identified %		**99.55 ± 0.11**	**99.16 ± 0.14**	**98.25 ± 0.47**	**98.70 ± 0.29**
Monoterpene hydrocarbons %		**26.38 ± 1.53**	**32.52 ± 3.78**	**14.22 ± 4.43**	**38.08 ± 3.33**
Oxygenated monoterpenes %		**65.67 ± 1.76**	**58.33 ± 2.90**	**48.80 ± 4.72**	**39.28 ± 2.87**
Sesquiterpenes hydrocarbons %		**6.72 ± 0.57**	**6.35 ± 0.68**	**25.32 ± 3.49**	**16.32 ± 2.82**
Oxygenated sesquiterpenes %		**0.51 ± 0.05**	**1.78 ± 0.34**	**9.59 ± 1.23**	**4.80 ± 0.88**
Others %		**0.28 ± 0.02**	**0.18 ± 0.02**	**0.11 ± 0.04**	**0.21 ± 0.02**

Compounds listed in order of elution in the HP-1 column; [a] RI: retention index relative to C8-C32 n-alkanes on the HP-1 column.

Since the next group (Supplementary Figure 2, S5) was formed by the eight samples of *S. innota* collected in Culla and Sueras. This taxon could be separated into two subcluster, *S. innota* elaborate an essential oil rich in linalool (up to 42.60±5.82 in Culla populations) and geraniol (up to 16.06±4.26 in Suera populations). Up to now several monoterpenes and sesquiterpenes [8] have been described in the essential oil of *S. innota* but not essential oils of this species have been reported with large amounts of linalool or geraniol as the populations here analyzed. In the only previous study about this species, camphor (11.14%), α-pinene (8.26%) and camphene (7.94%) were identified as the main compounds. The compound linalool represented only 1.68% while geraniol was not detected. The main compounds reported by Velasco-Negueruela and Pérez-Alonso in 1983 [8] are more similar to those obtained in this study for *S. cuneifolia* (Table 3) and could explain their consideration as subspecies by other authors, but the chemical discriminant analysis clearly shows two separate groups (Supplementary Figure 2, S5) and support the classification in Flora Europaea in two different species. The differences in the chemical composition could provide evidences for the existence of different chemotypes in this species. Ecological factors of *Satureja* species studied have been carried out (Supplementary Table 4, S6), showing differences between populations of the same species, however further studies are necessary in order to establish the relationships between chemical variability and ecological factors.

The essential oils with high content in camphor (45.04±1.67) and camphene (12.42±1.71) (Table 3), of *S. cuneifolia* samples constituted the third group (Supplementary Figure 2, S5). Discriminant morphological analysis positioned *S. cuneifolia* between *S. innota* and *S. intricata* but discriminant chemical analysis shows a greater separation between these species. Previous studies in Spain confirm that the essential oil of *S. cuneifolia* showed camphor as the main compounds [7-8]. In Italy [15], were tested 36 samples of *S. cuneifolia* being most of them borneol chemotype (camphor´s precursor) and minor linalool and α-pinene chemotypes. However, carvacrol was the major component in samples tested in Turkey [16] whereas in Croatia there was a great variability with β-cubebene [10], α-pinene [3] or carvacrol [2] as the main constituents.

Finally the morphological analysis about *S. intricata* shows this taxon closely related with *S. cuneifolia* but again the results based on the chemical composition of the sixteen essential oils analysed difference this species (Supplementary Figure 2, S5). *S. intricata* showed chemical polymorphism, probably due to the different ecological conditions where each population inhabits, with camphor (16.02±1.75%) as the main compound, followed by myrcene (8.46±1.46%) or γ-terpinene (8.22±1.33%) populations.

In conclusion morphometric analysis revealed that the species *S. montana* is clearly separated from the other species, whereas there was a morphological affinity between the species *S. cuneifolia* and *S. intricata*. Chemical composition of perennial *Satureja* essential oils showed greatest differences between the analysed species, supporting the taxonomic classification of the Flora Iberica [4], which ranks these taxa into species.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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