

Supporting Information

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

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S1: Number of samples studied with coordinates, altitude, locality, species and voucher number

Samples	Coordinates	Altitude	Locality	Specie	Voucher
1-4	40° 19.457' N 0° 8.166' W	801 m	Barranco Culla	<i>S. montana</i> 1	VALA 9543
5-8	40° 15.043' N 0° 21.339' W	1282 m	San Juan de Penagolosa	<i>S. montana</i> 2	VALA 9544
9-12	40° 19.826' N 0° 6.671' W	812 m	Culla	<i>S. innota</i> 1	VALA 9545
13-16	39° 56.167' N 0° 22.645' W	312 m	Sueras	<i>S. innota</i> 2	VALA 9546
17-20	39° 11.835' N 0° 14.498' W	10 m	Cullera	<i>S. cuneifolia</i>	VALA 9547
21-24	39° 27.733' N 0° 43.983' W	327 m	Chiva	<i>S. intricata</i> 1	VALA 9548
25-28	39° 28.383' N 0° 46.800' W	616 m	Chiva	<i>S. intricata</i> 2	VALA 9548
29-32	38° 53.783' N 0° 46.966' W	641 m	Navalón	<i>S. intricata</i> 3	VALA 9550
33-36	38° 54.783' N 0° 50.583' W	811 m	Navalón	<i>S. intricata</i> 4	VALA 9551

S2: Analysis methods

Plant Materials

Aerial parts of *Satureja* samples were collected in June 2009, when the savory blooming stage begins. All samples were collected from their natural habitat (Table S1). From each locality four samples were collected. Voucher specimens (VALA9543-VALA9551) was authenticated by the Botany Unit of the Universidad Politécnica de Valencia (UPV) and deposited in the Herbarium of Agronomy belonging to the Universidad Politécnica de Valencia (VALA).

Morphometric analyses:

Plants of the nine *Satureja* L. localities (270 samples), representatives of the studied taxa were used for the morphometric analysis. Based on the descriptions of each species in the literature and in their own observation, we established the following characters: length and width of leaves, length-width ratio, length and density leaf hairs, calyx length and length of the calyx teeth. With the data obtained a matrix was developed in which the samples for each taxon were arranged in rows and the handled characters in columns as the basis for the subsequent discriminant analysis. Data processing was performed using the statistical program Statgraphics Centurion XVI (2011).

Extraction of the essential oils:

Fresh plant material of each sample was submitted to hydrodistillation for three hours using a Clevenger-type apparatus to obtain the essential oils, that were dried over anhydrous sodium sulphate and stored in vials at 4°C until chromatographic analysis.

GC/FID and GC-MS analyses:

Gas chromatography was performed using a Perkin-Elmer Clarus 500GC apparatus equipped with a flame ionization detector (FID) and a Hewlett-Packard HP-1 (cross-linked methyl silicone) capillary column (30 m long and 0.2 mm i.d., with 0.33 µm film thickness). The column temperature program was 60 °C during 5 min, with 3 °C/min increases to 180 °C, then 20 °C/min increases to 280 °C, which was maintained for 10 min. The carrier gas was helium at a flow-rate of 1 mL/min. Both the FID detector and injector port temperature were maintained at 250 and 220 °C, respectively. Gas chromatography-mass spectrometry analysis were carried out with a Varian Saturn 2000 equipped with a Varian C.S VA-5MS capillary column (30 m long and 0.25 mm i.d. with 0.25 µm film thickness). The same working conditions used for GC and split mode injection (ratio 1:25) were employed. Mass spectra were taken over the m/z 28–400 range with an ionizing voltage of 70 eV. Kovat's retention index was calculated using co-chromatographed standard hydrocarbons. The individual compounds were identified by MS and their identity was confirmed by comparison of their RIs, relative to C_8 - C_{32} *n*-alkanes, and by comparing their mass spectra and retention times with those of authentic samples or with data already available in the NIST 2005 Mass Spectra Library.

Statistical analysis:

In data processing, a matrix was developed with the major components identified in columns and the different samples analyzed in rows. Subsequently, the related variables were eliminated (correlation coefficient ≥ 0.9) and the resulting matrix was subjected to a discriminant analysis. The development and data processing were performed using Statgraphics Centurion XVI (2011).

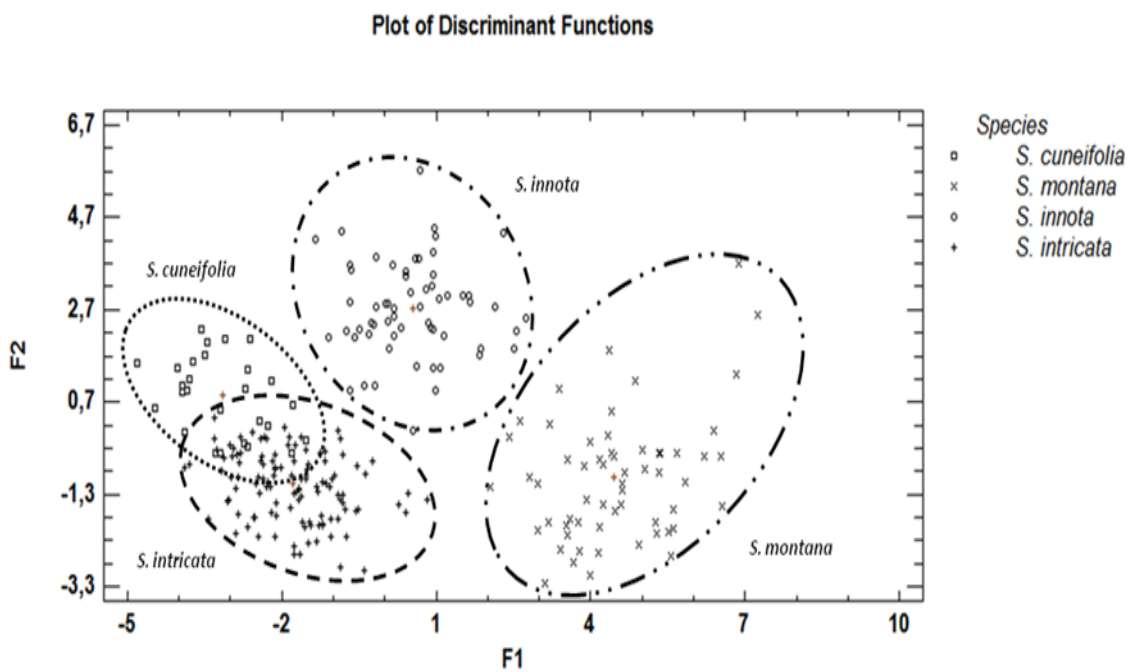
Results were submitted to analysis of variance. Percentage values were previously arcsin transformed. The means were compared by Fisher's least significant difference (LSD) test ($P < 0.05$).

S3: Average values of the morphometric data of perennial *Satureja* species

	LL	LW	LWR	CL	LCT	HD	HL
<i>S. montana</i>	18.48 ± 0.24a	3.45 ± 0.08b	5.49 ± 0.13a	5.89 ± 0.06a	1.77 ± 0.05c	23.85 ± 0.54a	0.49 ± 0.02b
<i>S. cuneifolia</i>	9.34 ± 0.36d	3.37 ± 0.10b	2.80 ± 0.11c	3.80 ± 0.08b	1.41 ± 0.05d	15.90 ± 1.28c	0.11 ± 0.01d
<i>S. innota</i>	12.66 ± 0.24c	4.70 ± 0.11a	2.76 ± 0.05c	5.72 ± 0.08a	2.12 ± 0.06b	18.47 ± 0.60b	0.53 ± 0.02a
<i>S. intricata</i>	13.33 ± 0.17b	3.91 ± 0.09b	3.54 ± 0.07b	5.71 ± 0.07a	2.26 ± 0.04a	8.22 ± 0.40d	0.16 ± 0.01c

LL: leaf length, LW: leaf width, LWR: length-width ratio of the leaf, CL: calyx length ; LCT: length of calyx teeth, HD: hair density; HL: hairs length. Measures in mm±standard error. *S. montana* (n=60 samples); *S. cuneifolia* (n=30 samples); *S. innota* (n=60samples); *S. intricata* (n=120 samples). Within each row, different letters in the same line indicates that the mean values are different at the 95% level of probability ($P \leq 0.05$) using Fisher's least significant difference test (LSD).

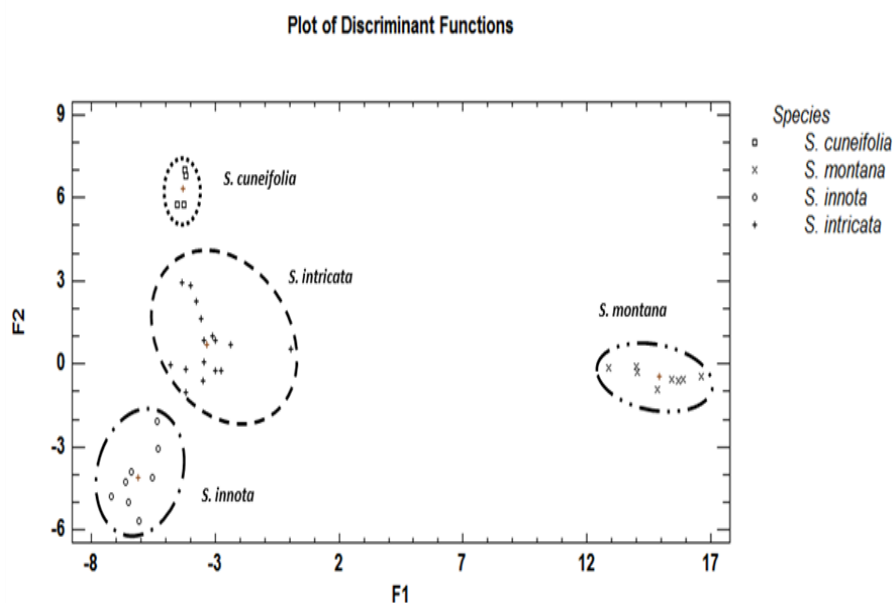
S4: Distribution of the samples in the plane of the first two discriminant functions. The principal functions (F1 and F2) contained 85% of morphological information



$$F1 (x) = 1.01*LL - 0.81*WL - 0.32*LWR + 0.33*CL - 0.20*LCT + 0.54*HD + 0.51*HL$$

$$F2 (y) = -0.89 *LL + 1.03*WL + 0.16*LWR - 0.23*CL - 0.04*LCT + 0.30*HD + 0.59*HL$$

S5: Distribution of the samples in the plane of the first two discriminant functions. The principal functions (F1 and F2) contained 96.64% of the phytochemical information.



$$\mathbf{F1} (x) = 0.08*\text{camphene} + 0.25*\gamma\text{-terpinene} - 0.48*\text{linalool} - 0.09*\text{camphor} - 0.20*\text{geraniol} + 1.10*\text{carvacrol} - 0.48*\beta\text{-caryophyllene} + 0.64*\text{bicyclogermacrene}$$

$$\mathbf{F2} (y) = 0.77*\text{camphene} + 0.39*\gamma\text{-terpinene} - 0.16*\text{linalool} + 0.85*\text{camphor} - 0.61*\text{geraniol} + 0.20*\text{carvacrol} + 0.35*\beta\text{-caryophyllene} + 0.64*\text{bicyclogermacrene}$$

S6: Ecological factors of the perennial *Satureja* species studied.

	Soil	pH	OM %	Ca CO ₃ %	AL %	EC (dS/m)
<i>S. montana</i> 1	Sandy clay loam	8.37 ± 0.07	6.26 ± 0.95	17.47 ± 3.64	4.40 ± 0.31	2.36 ± 0.09
<i>S. montana</i> 1	Sandy clay loam	8.09 ± 0.11	5.85 ± 3.17	23.98 ± 1.72	3.24 ± 0.30	2.84 ± 0.51
<i>S. cuneifolia</i>	Sandy clay loam	8.24 ± 0.06	3.73 ± 0.53	18.95 ± 2.46	6.13 ± 0.12	2.15 ± 0.04
<i>S. innota</i> 1	Sandy clay loam	8.12 ± 0.03	3.15 ± 0.41	12.79 ± 4.72	3.25 ± 0.51	2.43 ± 0.17
<i>S. innota</i> 2	Clay loam	8.58 ± 0.06	2.37 ± 0.94	22.45 ± 1.46	3.70 ± 0.31	2.30 ± 0.19
<i>S. intricata</i> 1	Sandy clay loam	8.26 ± 0.10	0.57 ± 0.32	69.82 ± 3.11	8.68 ± 0.87	6.24 ± 2.99
<i>S. intricata</i> 2	Clay loam	8.31 ± 0.11	2.67 ± 0.38	20.31 ± 1.86	4.40 ± 0.31	4.46 ± 1.12
<i>S. intricata</i> 3	Loam	8.39 ± 0.04	5.11 ± 0.52	73.21 ± 3.39	2.21 ± 0.24	2.93 ± 0.23
<i>S. intricata</i> 4	Sandy loam	8.23 ± 0.06	3.39 ± 0.62	66.65 ± 1.16	0.23 ± 0.02	3.60 ± 0.50

K ⁺ (meq/L)	Ca ²⁺ (meq/L)	Na ⁺ (meq/L)	Ic	It	Itc	Tp	Pp
4.62 ± 0.35	51.97 ± 6.69	23.01 ± 0.23	17.58	200.2	200.2	1479.3	748
8.56 ± 3.24	73.02 ± 17.52	26.32 ± 3.24	19.49	119.5	126.95	1175.4	748
3.24 ± 0.60	42.20 ± 8.18	26.22 ± 1.40	14.4	389	389	2062	576
5.33 ± 1.61	52.70 ± 1.13	26.12 ± 1.42	17.91	233.2	233.2	1607.1	748
7.10 ± 1.78	35.52 ± 12.57	32.24 ± 0.20	16.2	319	319	1878	506
7.08 ± 0.93	127.06 ± 32.74	34.92 ± 3.45	18.55	280.7	283.5	1796	498
11.78 ± 2.52	39.26 ± 11.96	32.83 ± 1.07	18.55	280.7	283.5	1796	498
14.70 ± 1.97	73.62 ± 5.39	28.44 ± 1.20	15.8	299	299	1752	516
7.80 ± 1.77	91.25 ± 24.34	25.09 ± 1.00	15.8	299	299	1752	516

OM: organic material; CaCO₃: calcium carbonate equivalent; AL: active limestone; EC: electrical conductivity; Ic: continentality index; It: thermicity index; Itc: compensated thermicity index; Tp: yearly positive temperature; Pp: yearly positive precipitation; Io: ombrothermic index