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Total Phenolic Content, Radical Scavenging Properties, and Essential Oil Composition of *Origanum* Species from Different Populations

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The aim of this work was to compare the antiradical activity, total phenol content (TPC), and essential oil composition of *Origanum vulgare* spp. *virens*, *Origanum* × *applii*, *Origanum* × *majoricum*, and *O. vulgare* spp. *vulgare* cultivated in Argentina in different localities. The experiment was conducted in the research station of La Consulta (INTA-Mendoza), the research station of Santa Lucia (INTA-San Juan), and Agronomy Faculty of National University of La Pampa, from 2007 to 2008. The composition of the essential oils of oregano populations was independent of cultivation conditions. In total, 39 compounds were identified in essential oils of oregano from Argentina by means of GC−MS. Thymol and *trans*-sabinene hydrate were the most prominent compounds, followed by γ-terpinene, terpinen-4-ol, and α-terpinene. *O. vulgare vulgare* is the only *Origanum* studied which is rich in γ-terpinene. Among tested oregano, *O.* × *majoricum* showed the highest essential oil content, 3.9 mg g^{-1} dry matter. The plant extract of *O.* × *majoricum* had greater total phenol content values, 19.36 mg/g dry weight, than the rest of oregano studied. To find relationships among TPC, free radical scavenging activity (FRSA), and climate variables, canonical correlations were calculated. The results obtained allow us to conclude that 70% of the TPC and FRSA variability can be explained by the climate variables (R^2 = 0.70; $p = 8.3 \times 10^{-6}$), the temperature being the most important climatic variable.

KEYWORDS: *Origanum*; essential oils; phenol content; antiradical activity

INTRODUCTION

Oregano plays a primary role among temperate culinary herbs in world trade (*1*). Of the species commercially known as oregano, most of the production comes from species of the genus *Origanum*. *Origanum vulgare* L. is the most variable species of the genus and the only one commonly known as oregano in most countries. Six subspecies have been recognized within *O. vulgare* on the basis of morphological and chemical characters (*2*−*4*).

The areas under cultivation of *O. vulgare*, in Argentina, are the central and southwest regions (*5*, *6*). The commercial oregano of Argentina comes from *O. vulgare* ssp. *vulgare*, *O. vulgare* ssp. *virens*, *O.* × *applii*, and *O.* × *majoricum* (*5*, *7*). However, these subspecies are poor in essential oil (*8*, *9*). Thus, none of the oil rich subspecies appears to be present in the cultivated land of Argentina. However, the exportation of vegetable oregano from Argentina is expanding (*10*). So far, there is no report on the yield and composition of the essential oil of these cultivated populations in the literature.

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The composition of essential oil of *O. vulgare* ssp. *vulgare* was analyzed. The principal constituents of this essential oil were sesquiterpenes, such as β-bisabolene, germacrene D, spathulenol, and β-caryophyllene, while the monoterpenes were represented by terpinen-4-ol, α-terpineol, 1,8-cineole, sabinene, and thymol (*11*, *12*). *O. vulgare* subsp. *virens* plants produced essential oils with linalool, β-caryophyllene, α-terpineol, terpinen-4-ol, carvacrol, sabinene, and germacrene D as the main components (*3*, *9*, *11*, *13*). *Origanum* × *majoricum* Cambess is a known hybrid of *O. majorana* L. × *O. vulgare* L. ssp. *virens* (Hoffm. et Link) Ietswaart and is also cultivated in Argentina. *Origanum* × *majoricum* has a high essential oil yield whose principal components are the *trans*- and *cis*-sabinene hydrate and terpinen-4-ol, followed by sabinene, γ-terpinene, and carvacrol (*14*, *15*). The other hybrid growing in Argentina is *O.* × *applii* (*O. vulgare* ssp. *vulgare* × *O. majorana*) (*5*). This hybrid, however, is not homogeneous in its essential oil composition, as it includes two main chemotypes: thymol and *p*-cymene (*16*, *17*). As previously demonstrated, the content of essential oil and antioxidant properties may change depending on the differences in cultivation, origin, vegetative stage, and growing season of the plants (*12*, *15*).

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For these reason, in this work we present an analysis of the essential oil compounds, total phenolic content, and radical-scavenging properties from three populations of *Origanum vulgare* spp. *virens, Origanum* × *applii, Origanum* × *majoricum*, and *O. vulgare* spp. *vulgare* that grow in Argentina.

MATERIALS AND METHODS

Plant Material. The studied species were *Origanum vulgare* spp. *virens* (Hoffm. et Link) letswaart; *Origanum* × *applii* (Domin) Boros; *Origanum* × *majoricum* Cambess, and *O. vulgare* L. spp. *vulgare*. The experiment was conducted in the research station of INTA La Consulta, Argentina from 2007 to 2008. However, the plants were collected from various sites: research station of Santa Lucia (INTA-San Juan), La Consulta (INTA−Mendoza), and Agronomy Faculty of National University of La Pampa. Two harvests were carried out at the full flowering stage in November 2007 and 2008. For harvesting, the plant material was cut 5 cm above the soil surface. After determination of fresh matter, plant samples were air-dried at room temperature, and then, the dry matter was determined. Air drying at ambient temperature seems to be an appropriate method in preserving most of the phenolic compounds present in plant materials (*18*). To warrant a good comparison, plants were uniformly cut shortly before the beginning of the second growing season.

Essential Oil Extraction. Samples of at least 200 g of dried leaves were hydrodistilled in triplicate for 1 h using a Clevenger-type apparatus (*19*). The EO content was gravimetrically quantified. Each sample was analyzed three times, and the average content of EO was used for further statistic evaluation. The EOs were dried over anhydrous sodium sulfate and stored at 4−6 °C until further analysis.

GC and GC-MS Analyses. *GC-FID*. For the quantification of individual components, the EO was analyzed using a Perkin-Elmer Clarus 500 gas chromatograph equipped with a flame ionization detector (GC-FID). A capillary column DB-5 (30 m × 0.25 mm i.d. and 0.25 m coating thickness) was used for the separation of individual components of the EO. Helium was employed as the carrier gas with a flow rate of 0.9 mL/min. The temperature program was 60 °C for 5 min, from 60 to 250 at 5 °C/min, with a final hold time of 10 min. The injector and detector were maintained at 260 and 280 °C, respectively. The sample, 0.2 μL, was injected with a 1:100 split ratio.

GC/MS. For the determination of the composition, EO samples were diluted with hexane. The injection volume was 1 μL. The identification of the components of the EO was realized by GC−MS. A Perkin-Elmer Q 700 GC-MS coupled with an ion trap mass detector was employed for the identification. A capillary column DB-5 (30 m × 0.25 mm i.d. and 0.25 m coating thickness) was used for the separation of the components. Helium was used as carrier gas with a flow rate of 0.9 mL/min. The temperature program for the oven and injector was the same as that for the GC-FID. Ionization was realized by electron impact at 70 eV. Mass spectral data were acquired in the scan mode in the m/z range 35−450.

Retention indices (RI) of the sample components were determined on the basis of homologous *n*-alkane hydrocarbons under the same conditions. The compounds were identified by comparing their retention indices and mass spectra with published data (*20*) and libraries NIST and Adams. The main components were further identified by coinjection of authentic standards (SIGMA, USA). Fenchone was used as internal standard at a concentration of 0.1 mg/mL dichloromethane. The quantitative composition was obtained by peak area normalization, and the response factor for each component was considered to equal 1.

Plant Material Extract. Dry oregano plant material (200 mg) was extracted with 5 mL of deionized water (24 °C) (concentration: 40 mg of dry weight/mL). The extracts were left to stay at room temperature for 24 h and then were filtered, and a liquid portion was analyzed for its total phenol content and its antioxidant capacity. Each sample was tested in triplicate.

Free Radical Scavenging Activity Determination. Antioxidant activity was measured on the basis of the scavenging of the stable free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), using a modified version of a previously described method (*21*). Each sample (10 μL of plant material extract) was mixed with 900 μL of 100 mM Tris-HCl buffer (pH 7.4), 40 μL of ethanol, and 50 μL of 0.5% (w/w) Tween 20 solution and then added to 1 mL of 0.5 mM DPPH in ethanol (250 μM in the reaction mixture). The control sample was prepared using water instead of plant extract. The mixture was shaken with a mechanical shaker and left to stand for 30 min at room temperature in a dark room. After 30 min, the absorbance was measured at a wavelength of 517 nm. The free radical scavenging activity was expressed as follows: DPPH scavenging activity (%) $= [(Ac - As/Ac)] \times 100$where Ac is the absorbance of the control sample, and As is the absorbance of the test sample (*22*).

Total Phenols Determination. Total phenols were determined by Folin−Ciocalteu reagent (*23*). Each plant extract (0.5 mL) or gallic acid (standard phenolic compound) was mixed with Folin−Ciocalteu reagent (0.5 mL, diluted with 8 mL of distilled water) and aqueous Na_2CO_3 (1 mL, 1 M). The mixtures were allowed to stand for 1 h, and the total phenols were determined by colorimetry at 760 nm. Total phenol values are expressed in terms of miligram of gallic acid equivalent per gram of dry weight of plant, which is a common reference compound (*24*).

Statistical Analyses. Experimental values are the means ± SD or SE of the number of experiments indicated in the legends. A one-way analysis of variance was used. Significance (at $P \leq 0.05$) was assessed using Duncan's multiple range test. Principal component analysis (PCA) was performed to determine the influence of the genotype and the climate characteristics on the total phenol content (TPC) and free radical scavenging activity (FRSA) in the oregano species studied. Canonical correlations were calculated to find relationships between the linear combinations of primary (TPC and FRSA) and secondary (temperature and rainfall) variables, and were evaluated using chi-square test. All statistical analyses were calculated by using InfoStat software (*25*).

Table 1. Essential Oil Content of the *Origanum* Species Examined[a]

samples	Essential oil content (mg g^{-1} dry wt.)
O. × *applii*	1.83 ± 0.27 a
O. vulgare subsp. *vulgare*	1.97 ± 0.22 a
O. vulgare subsp. *virens*	2.17 ± 0.32 a
O. × *majoricum*	3.90 ± 0.25 b

[a] Values (means ± SE) with different letters are significantly different from each other according to Duncan's multiple range test at $P \leq 0.05$ ($n = 3$).

RESULTS AND DISCUSSION

Origanum × *majoricum* is known in Argentina as oregano type Mendocino. It is generally accepted that it has the best quality (*5*−*7*). During our studies on the EO content of oregano plants from Argentina, we have found that *O.* × *majoricum* is rich in EO (3.90 mg g^{-1} dry matter). However, plants belonging to the other two subspecies and *O.* × *applii* contain a much lower amount of EOs (1.83 to 2.17 mg g^{-1} dry matter) (**Table 1**).

The major components in oregano oil from Argentina are listed in **Table 2**, and only those components with concentrations greater than 0.05% are reported. The chemical profile of the different subspecies of *Origanum vulgare* from Argentina is quite similar. The principal components in the oils were the monoterpenes *trans*-sabinene hydrate (27.77−36.77%) and thymol (17.77−30.77%). There were smaller amounts of α-terpinene (3.13−4.63%), limonene (2.1−3.6%), *cis*-hydrate sabinene (1.43−3.37%), terpinen-4-ol (3.23−5.03%), and carvacrol (trace 3.57%). The principal sesquiterpenes encountered were the hydrocarbons β-caryophyllene (1.07−2.9%) and germacrene D (1.30−1.50%).

In a quantitative aspect, some noticeable differences can be observed in the EO from oregano of Argentina, such as sabinene hydrate and thymol relative percentages. The major component in EO of *O. vulgare* ssp. *vulgare*, in addition to *trans*-sabinene hydrate and thymol, was γ-terpinene (15.47%). The composition of the volatile fraction of *O.* × *applii* is intermediate to its parental species, *O. vulgare* ssp. *vulgare* and *O. majorana*. The major compounds were thymol (30.7%) and *trans*-sabinene hydrate (29.53%), the thymol being characteristic of *O. vulgare* ssp.

Table 2. Relative Percentage Concentrations of the Terpenoid Constituent of the Four *Origanum* Species, According to Their Elution Order in the GC Analysis[a]

retention index	compounds[b]	*O.* × *appli* "criollo"	*O. vulgare vulgare* "compacto"	*O. vulgare vulgare virens* "cordobés"	*O.* × *majoricum* "mendocino"	methods of identification
930	α-thujene	0.5 (0.06) a	0.5 (0.06) a	0.7 (0.06) a	0.6 (0.06) a	GCMS
939	α-pinene	0.43 (0.18) a	0.6 (0.06) a	0.5 (0) a	0.53 (0.13) a	GCMS−Co
954	camphene	0.13 (0.04) a	tr	0.23 (0.03) b	tr	GCMS
975	sabinene	0.33 (0.03) a	0.2 (0) a	1.2 (0.9) a	0.17 (0.07) a	GCMS−Co
979	β-pinene	0.3 (0) b	0.27 (0.03) b	0.33 (0.03) b	0.13 (0.07) a	GCMS−Co
991	β-myrcene	1.3 (0.47) a	1.37 (0.09) a	1.5 (0.1) a	1.73 (0.09) a	GCMS
1003	α-phellandrene	0.23 (0.09) a	0.2 (0) a	0.17 (0.03) a	0.2 (0) a	GCMS
1017	α-terpinene	3.13 (0.35) a	4.63 (0.23) b	4.17 (0.29) ab	3.9 (0.61) ab	GCMS
1025	*p*-cymene	1.66 (0.79) a	2.73 (0.18) a	2.5 (0.3) a	1.4 (0.06) a	GCMS−Co
1029	limonene	2.1 (0.56) a	2.73 (0.03) a	2.73 (0.2) a	3.6 (0.84) a	GCMS−Co
1031	1,8-cineole	tr		tr		GCMS
1037	β-*cis*-ocimene	1.3 (0.12) bc	0.23 (0.09) a	1.63 (0.34) c	0.7 (0.06) ab	GCMS
1050	β-*trans*-ocimene	0.13 (0.03) a	tr	0.23 (0.03) b	0.1 (0) a	GCMS
1060	γ-terpinene	4.4 (0.46) a	15.47 (0.49) c	5.4 (0.55) ab	5.9 (0.06) b	GCMS−Co
1070	*cis*-sabinene hydrate	2.03 (0.34) a	1.43 (0.18) a	2.73 (0.03) b	3.37 (0.03) c	GCMS−Co
1089	terpinolene	0.6 (0.12) ab	0.47 (0.07) a	0.57 (0.07) ab	0.73 (0.03) b	GCMS
1098	*trans*-sabinene hydrate	29.63 (0.69) a	32.47 (0.7) b	27.77 (0.52) a	36.77 (0.62) c	GCMS−Co
1113	octen-3-yl acetate			0.73 (0.09) a	0.7 (0.06) a	GCMS
1144	*cis*-β-terpineol	0.5 (0.12) a	0.47 (0.09) a	0.3 (0.1) a	0.33 (0.03) a	GCMS
1163	*trans*-β-terpineol	0.2 (0)				GCMS
1169	borneol	1.17 (0.15) b	tr	1.23 (0.18) b	0.2 (0) a	GCMS
1177	terpinen-4-ol	3.23 (0.3) a	5.03 (0.23) b	3.5 (0.21) a	4.63 (0.09) b	GCMS−Co
1189	α-terpineol	1.07 (0.12) b	0.17 (0.03) a	1.5 (0.21) bc	1.77 (0.2) c	GCMS
1196	*cis*-piperitol	0.3 (0) a	tr	0.4 (0.1) a	0.3 (0.06) a	GCMS
1235	thymol methyl ether	0.27 (0.07) a	tr	0.13 (0.03) a	0.53 (0.38) a	GCMS
1245	carvacrol methyl ether	1 (0.06) a	1.77 (0.2) b	1.07 (0.03) a	1.4 (0.25) ab	GCMS
1290	thymol	30.77 (1.04) d	20.5 (0.64) b	26.1 (0.23) c	17.77 (0.48) a	GCMS−Co
1299	carvacrol	0.53 (0.03) a	tr	0.47 (0.09) a	3.57 (0.09) b	GCMS−Co
1362	neryl acetate	0.7 (0.3) a		0.6 (0.1) a		GCMS
1381	geranyl acetate	0.77 (0.17) a	0.83 (0.03) a	0.8 (0.06) a		GCMS
1388	β-bourbonene	tr	tr	tr		GCMS
1419	β-caryophyllene	2.9 (0.36) b	1.07 (0.09) a	2.63 (0.17) b	2.5 (0.62) b	GCMS
1437	γ-elemene	1.9 (0.51) c	0.47 (0.12) ab	1.37 (0.18) bc	0.06 (0.03) a	GCMS
1455	α-humulene	0.13 (0.03) a	tr	0.1 (0) a	0.17 (0.03) a	GCMS
1480	γ-muurolene	0.13 (0.04) a	tr	tr	0.06 (0.03) a	GCMS
1485	germacrene D	1.50 (0.21) a	1.30 (0.12) a	1.33 (0.09) a	1.37 (0.35) a	GCMS
1506	β-bisabolene	0.83 (0.24) b	0.53 (0.07) b	0.73 (0.03) b	0.06 (0.03) a	GCMS
1578	spathulenol	0.53 (0.07) c	0.2 (0) a	0.37 (0.07) b	0.13 (0.03) a	GCMS
1583	caryophyllene oxide	0.2 (0) b	0.1 (0) a	0.13 (0.03) a	0.1 (0) a	GCMS
	total	96.77 (0.58)	96.63 (1.49)	96.17 (1.79)	96.63 (0.24)	

[a] Values with different letters are significantly different from each other according to Duncan's multiple range test at $P \leq 0.05$ ($n = 3$). GCMS: peak identifications are based on MS comparison with file spectra. Co: peak identification are based on standard comparison with relative retention time. [b] Each cell in the table is formatted according to the scheme mean (standard error of the mean). The abbreviation tr stands for trace amounts of the compound and is used when the average amount of a particular compound is 0.05% or less while the standard error of the mean is very close to zero.

vulgare and sabinene hydrate being typical for *O. majorana* (*3*, *14*). Although EOs compositions of *Origanum vulgare* ssp. *vulgare* and ssp. *virens* have been found rich in acyclic compounds and sesquiterpenoids (*3*, *9*, *11*−*13*) the samples from Argentina were found to be rich in sabinyl compounds (**Table 2**). The dominant components in the essential oil of *O.* × *majoricum* were also *trans*-sabinene hydrate and thymol but in different ratios (**Table 2**), together with γ-terpinene and 4-terpineol. These results are in accordance with the previously published data on *O.* × *majoricum* (*14*,*15*). Relatively high sabinene hydrate content in the oils of the hybrids appears to have been inherited from the European type of *O. majorana* since carvacrol has been reported as the main constituent of the Turkey type of this parent (*3*,*14*,*15*).

In order to find out if any ordination of the studied oils exists, a PCA was applied using as variables the percentage of α-terpinene, *p*-cymene, limonene, γ-terpinene, *trans*-sabinene hydrate, terpinen-4-ol, thymol, and β-caryophyllene. In **Figure 1**, the first two components, accounted for 76.8% of the total variance. As can be seen, in our samples no geographical pattern could be observed. This means that all *Origanum* species are grouped by individual species. The plot of PCA showed that *O.* × *majoricum* and *O. vulgare* spp. *vulgare* are separated from the rest.

Phenolic compounds are a class of antioxidant agents which act as free radical terminators. **Table 3** shows also the contents of TPC that were measured by Folin−Ciocalteu reagent in terms of the gallic acid equivalent. There are no data regarding the environmental effect on the total phenolic contents of the oregano from Argentina. We have found differences in the TPC of the samples ranging from 8.84 ± 0.46 to 19.36 ± 0.10 mg/g of dry weight. The greatest DPPH scavenging capacity of the tested oregano samples was to quench 75.3% DPPH, which was observed in *O. vulgare* ssp. *vulgare* (**Table 3**). This significant differentiation may be attributed not only to the genotype of each subspecies tested but also to environmental factors. As can be seen from **Table 4**, each population is characterized by different climate conditions, temperature, humidity, and rainfall. This difference might have played a vital role in the accumulation of phenolic compounds and, subsequently, in the TPC in the

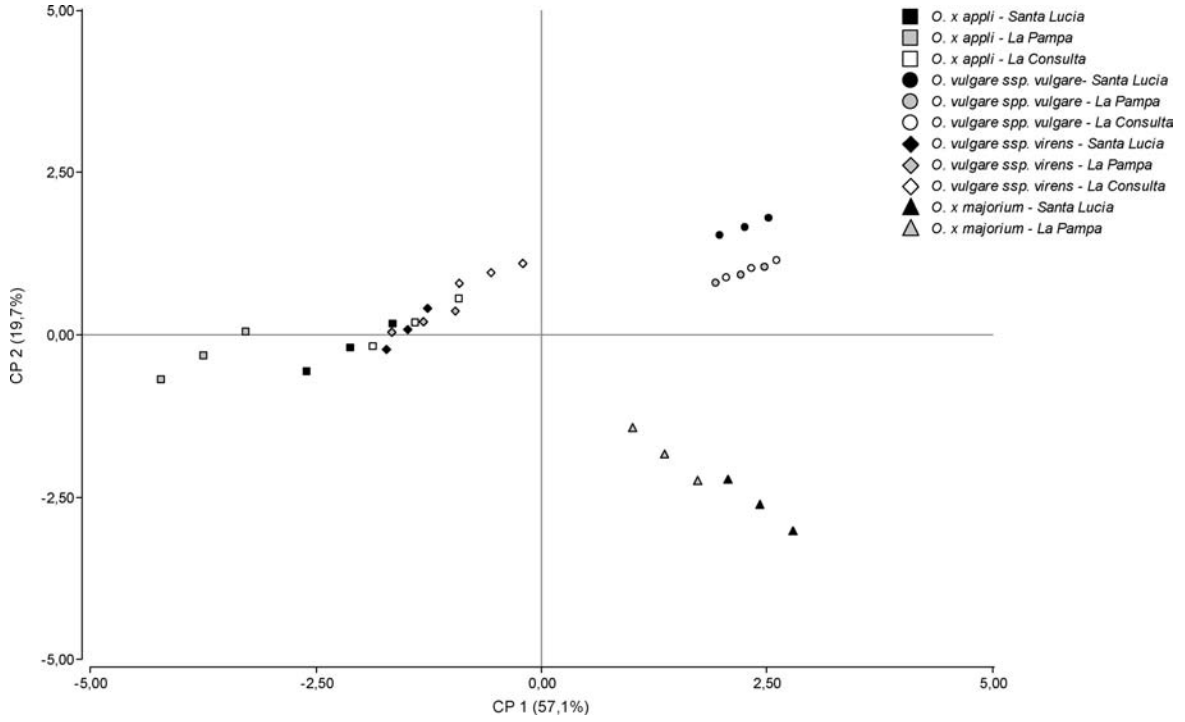

Figure 1. Principal component analysis of the main essential oil compounds of *Origanum* species studied.

Table 3. Total Phenolic Content (Expressed as mg Gallic acid/g of Dry Weight) and Free-Radical (1,1-Diphenyl-2-picrylhydrazyl)-Scavenging Activity (%) (FRSA) of the *Origanum* Extracts at 40 mg of Plant/mL

Origanum species	locality	phenol content (mg/ g of dry weight)[a]	FRSA (%)[a]
O. × *appli* "criollo"	Santa Lucia	18.09 ± 0.20 de	67.3 ± 0.6 e
	La Pampa	18.19 ± 0.14 de	34.9 ± 1.7 c
	La Consulta	17.46 ± 0.31 d	74.5 ± 0.4 f
O. × *majoricum* "mendocino"	Santa Lucia	19.36 ± 0.10 f	54.6 ± 1.7 d
	La Pampa	19.23 ± 0.13 f	73.6 ± 1.0 f
O. vulgare ssp. *virens* "cordobés"	Santa Lucia	18.21 ± 0.43 de	17.5 ± 1.1 a
	La Pampa	8.84 ± 0.46 a	25.2 ± 0.1 b
	La Consulta	13.94 ± 0.37 b	74.7 ± 0.1 f
O. vulgare ssp. *vulgare* "compacto"	Santa Lucia	18.88 ± 0.40 ef	75.3 ± 0.1 f
	La Pampa	18.86 ± 0.38 ef	74.8 ± 0.1 f
	La Consulta	16.04 ± 0.33 c	74.6 ± 0.1 f

[a] Values with different letters are significantly different from each other according to Duncan's multiple range test at $P \leq 0.05$ ($n = 3$).

Table 4. Collection Sites of *Origanum* Species and Their Climate Characteristics

locality	elevation (m)	GPS S	GPS W	mean annual temperature (°C)	relative humidity (%)	annual rainfall (mm)
Santa Lucia	1000	68°5′	31°5′	17.3	41−68	17.8
La Pampa	200	64°3′	36°5′	16	59−70	600
La Consulta	1117	69°1′	33°5′	7.6	39−76	267

plant extracts. Climatic data relative to localities of interest demonstrate that the lowest TPC yield is from the oregano collected in La Consulta, where mean annual temperature is the lowest all the year, while the lowest free radical scavenging activity is from the samples of oregano collected in Santa Lucia, where precipitation is the lowest. There are two exceptions, *O. vulgare* ssp. *vulgare* from Santa Lucia and *O.* × *appli* from the La Pampa population. The order of antioxidant activity for the oregano extracts did not seem to depend on TPC. It is worth noting that the relationship between the content of particular antioxidants and antioxidant activity is difficult to explain on the basis of only a quantitative analysis, as synergistic action taking place among the phenolic constituents present in natural extracts may contribute to differences in the antioxidant ability of plant extracts. Rice-Evans et al. (*26*) suggest that characteristics of the phenolic compounds may affect the antioxidant activity. Thus, the ortho-substitution with electron donating alkyl or methoxy groups of phenols increases the stability of the free radical and hence its antioxidant potential. The position and degree of hydroxylation of phenolic compounds is of primary importance in determining the antioxidant activity of phenolic compounds. The ortho and para positions of hydroxyl groups contribute markedly to the antioxidant activity, while the meta position has little or no effect on the antioxidant (*27*). This is a point that needs further investigation (*18*, *28*−*30*).

To determine the influence of the genotype and the climate characteristics on the total phenol content (TPC) and free radical scavenging activity (FRSA), a PCA analysis was made.

The PCA, in which the first component explains the 66.4% of total variance, showed that *O. vulgare* ssp. *virens* from the La

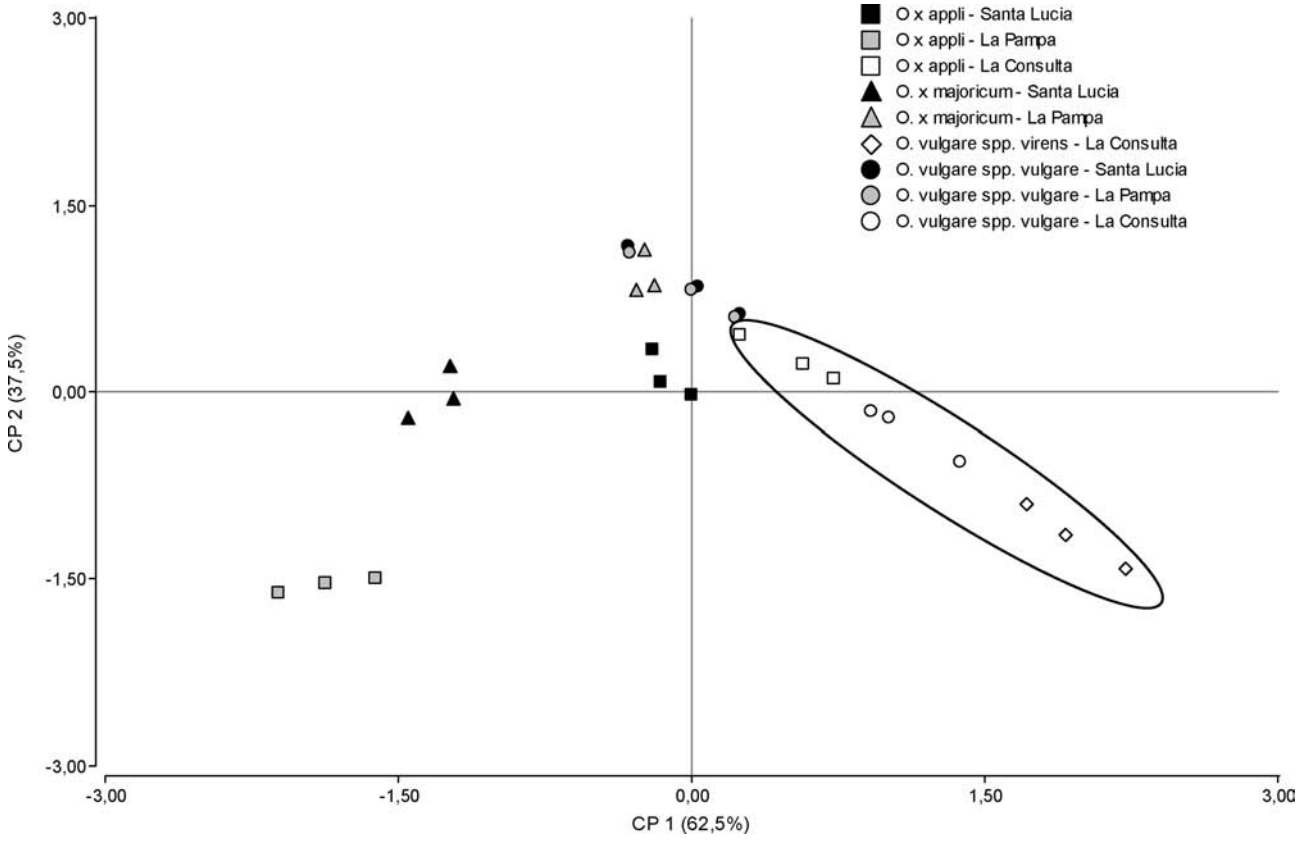

Figure 2. Principal component analysis of TPC (total phenol content) and FRSA (free radical scavenging activity) variables of *Origanum* species studied (with the exception of *O. vulgare* ssp. *virens* from the La Pampa and Santa Lucia localities; see text). The ellipse represents all of the species from the La Consulta locality.

Pampa and Santa Lucia localities are clearly separated from the rest of the species studied (data not shown). The separation is due mainly to PC1. Investigation of the loading plot of PC1 indicated that the first component explained the variance in FRSA components. *O. vulgare* ssp. *virens* from those localities showed the lowest activity in this variable (**Table 3**) and suggest the importance of the genotypical characteristics on the TPC and FRSA. However, this tendency was not shown in *O. vulgare* ssp. *virens* from La Consulta. The high pattern of separation of this species from these two localities does not enable us to differentiate among the other species studied. Thus, we removed those populations to observe any groupings in the rest of the species that could be masked in the first PCA, and therefore, we carried out another PCA (**Figure 2**). The first component in the second PCA analysis explains the 62.5% of total variance. Examination of the plots for PC1 versus PC2 showed that all oregano species cultivated in the La Cosulta locality are clearly separated from the rest (**Figure 2**). The separation is due mainly to PC1. These populations showed the highest values of FRSA and the lowest in TPC. It means that climate characteristics of the La Consulta locality are important in the expresion of those variables. It is important to emphasize that within this group of La Consulta, *O. vulgare* ssp. *virens* is separated from the other oregano species cultivated in this region to have lower TPCs, indicating again the genotypical importance of this species.

To find relationships between TPC and FRSA and climate variables, canonical correlations were calculated. The results obtained allow us to conclude that 70% of the TPC and FRSA variability can be explained by the climate variables ($R^2 = 0.70$; $p = 8.3 \times 10^{-6}$), the temperature being the most important climatic variable.

In conclusion, the results of this study demonstrated that the different *Origanum* species cultived in Argentina contained different levels of TPC and antioxidant properties by environmental factors. The EOs of oregano species studied in this work showed variability in the relative percentage of the two main components, while no geographical pattern could be observed.

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