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Characterization of *Origanum* species grown in Quebrada de Humahuaca, Jujuy, Argentina, through the study of the essential oils

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ABSTRACT

In order to contribute to the characterization of oregano implanted in Quebrada de Humahuaca (Jujuy, Argentina) physical properties and chemical composition of essential oils (EO) were determined. Pilot and laboratory scale EO were obtained by steam distillation and hydrodistillation (using a Clevenger type trap) respectively from samples of oregano collected from a small production: Oregano negro(On), Oregano compacto(Oc), Oregano mendocino(Oxm) y Oregano criollo(Oxa). Influence of storing period and extraction techniques were also evaluated. All EO were dextrorotatory (+5 to +15) with 0.92 mean specific gravity and 1.483 mean refractive index. Both hybrids (Oxm and Oxa) and Oc belong to A group characterized by Skoula *et al.* (1999) because the major compounds are aromatic monoterpenes and/or their precursors. O. Negro is closely related to less common sesquiterpene type.

It is necessary a complete work on botanical identity correlated with the physical and chemical characterization of essential oils from different *Origanum* species grown in our country to have a contrasting quality tool.

Keywords: Essential oils, *Origanum*

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Introduction:

The genus *Origanum* (family Lamiaceae) is an annual, perennial and shrubby herb (Sahin *et al.*, 2004). It comprises about 38 species widespread in the Mediterranean, Euro-Siberian and Irano-Siberain regions (Kokkini, 1997).

Until 1980 there was no satisfactory classification of the genus, but since then the Ietswaart system has become widely accepted (Kokkini, 1997). This postulates that *Origanum vulgare* L. has six subspecies with differences in the indumentums, the number of sessile glands on the leaves, bracts and calyces, and the size and colour of the bracts and flowers. The most widely used and consumed subspecies *Origanum vulgare* ssp. *vulgare* L., is native throughout Europe, Iran, India, and China and *Origanum vulgare* ssp. *hirtum* (Link) Ietswaart is indigenous to Albania, Greece, and Turkey (Kokkini, 1997), but alien in Central European Hungary. Ecologically these species prefer warm, sunny habitats and loose, often rocky, calcareous soils, low in moisture content.

Origanum vulgare L. is the most variable species of the genus and the only one commonly known as 'oregano' in most European countries (D'Antuono *et al.*, 2000). Bertelli *et al.* (2003) stated that it is the most important species in Europe and is widely distributed in Europe and Asia up to a height of 2000 m. The subspecies most widely used and consumed is *Origanum vulgare* ssp *vulgare* L.

Origanum plants are widely used in agriculture and the pharmaceutical and cosmetic industries as a culinary herb, flavouring substances of food products, alcoholic beverages and perfumery for their spicy fragrance (Sahin *et al.*, 2004). The essential oils obtained from *Origanum vulgare* L. have been shown to possess antimicrobial and antioxidant activities (Burt and Reinders, 2003; Sartoratto *et al.,* 2004; Capecka *et al*., 2005)

The *Origanum* composition depends on the climate, altitude, time of recollection and the stage of growth (Arcila-Lozano *et al*., 2004). *O. vulgare* growing in a Mediterranean climate or a continental one contains a higher amount of phenols (Hristova *et al*., 1999; Montes *et al*., 1998) or terpenic alcohols, respectively (Tkachenko *et al.,* 2002). However, the variability between commercial and wild plants inside the same climate remains high, as shown by Hristova *et al.* (1999) in terms of yield and phenolic contain. In general, essential oils (EO) yields peak under hot summer conditions species producing oil containing 60–75% phenols, mostly carvacrol (McGimpsey, 1993). This compound together with thymol, p-cymene and γ-terpinene are commonly reported as the main components of origanum EO (Arnold *et al.*, 2000; Daferera, *et al.*, 2003; Hristova *et al.*, 1999; Kulisica *et al.*, 2004; Marino *et al.*, 2001; Montes *et al.,* 1998; Nostro *et al.*, 2004).

The following genetic material (named by the common names) is widespread in Argentina: Mejorana (*Origanum majorana*), Negro o vulgare (*Origanum vulgare* ssp *vulgare*) Compacto (*Origanun vulgare* ssp *virens*), Híbrido Criollo (*Origanum* x *applii*), Híbrido Cordobés (*Origanum* x *applii*), Híbrido Mendocino (*Origanum* x *majoricum*) (Di Fabio, 2005).

In order to contribute to the characterization of oregano implanted in Quebrada de Humahuaca (Jujuy, Argentina) physical properties and chemical composition of essential oils (EO) were determined. Pilot and laboratory scale EO were obtained by steam distillation and hydrodistillation (using a Clevenger type trap) respectively from samples of oregano collected from a small production: Oregano negro (On), Oregano compacto(Oc), Oregano mendocino(Oxm) y Oregano criollo(Oxa). Influence of storing period and extraction techniques were also evaluated.

Experimental

Plant material:

Aerial parts were collected in Tilcara (2460 m), Jujuy, Argentina. Voucher specimens were deposited in the PRONOA-UNJu Herbarium, Jujuy, Argentina (numbers: 06-20, 06-21, 06-22, 06-24, 06-26, 06-27, 06-28, 06-29).

All samples were collected at the beginning of the flowering stage except 06-24 which was collected at full flowering.

The aerial parts were dried and stored during different time (from 10 months to 2 years) before analysis.

Essential oils

Laboratory scale EOs were obtained by hydrodistillation (using a Clevenger type trap) and steam distillation was used to obtain EOs at pilot scale.

Analysis of the essential oils

The analysis of the essential oils was carried out by GC/FID and GC/MS analysis. The analyses were carried out on Hewlett Packard GC 6890 / MSD 5972 using a HP-5 MS capillary column (30 m x 0.25 mm; film thickness 0.25 μm) and a hydrogen carrier gas flow of 0.7 mL/min.

The oven temperature program was: 60 ºC (5 min), 60º-230ºC (6 ºC/min); the temperature of

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the detector (300 ºC) and the injector (250 ºC) were held constant during analyses. The quantitative results were obtained by electronic integration of the FID responses.

Component identification

The Retention Indices (RI) of compounds were determined relative to the retention times of C_7-C_{24} *n*-alkanes.

Compound identification

The constituents were identified using literature data (Adams, 2007; Jennings and Shibamoto, 1980; Davies, 1990; Formácek and Kubeczka, 1982) and spectra recorded in the MS library (NBS 75K) and a library spectra built up from pure substances and components of known oils. In some cases co-chromatography with authentic standards was used.

Physical properties

Specific gravities (20ºC/20ºC), refractive indices (20ºC) and polarimetric deviation (20ºC) of the essential oils were measured according to IRAM (Instituto Argentino de Normalización y Certificación) standards (16504, 18505 and 18507 respectively). Weights were measured using a sensitive balance with an accuracy of ±0.0001 g (Sartorius AG, Gottingen, Germany). Refractive index was measured using the Karl Zeiss 8311 refractometer (Jena, Germany). Polarimetric deviation was measured using the Karl Zeiss polarimeter (Jena, Germany). All measurements were undertaken in triplicate at 20ºC.

Results and Discussion:

Twenty seven compounds have been identified in the volatile fraction of the four *Origanum* species and hybrids (Table 1).

Physical properties of EO were determined when enough volume was available (Table 2).

Oregano Negro or vulgare: *Origanum vulgare* ssp. *vulgare* (Di Fabio, 2005) EO yield was low (≤0.034 mL 100 g^{-1} dry wt). For this reason it was not possible to measure the physical properties of EO.

Two samples collected a year apart at the beginning of the flowering stage and distilled at laboratory scale with different drying times were analyzed. The sample distilled 22 months after harvest (OnE) contains 14.2% *p*-cymene, 5.7% *Z*-caryophyllene, 17.8% caryophyllene oxide, 25.7% β-bourbonene and only 1.5% germacrene D, while the sample distilled 10 months after harvest (OnD) contains 2.8%, 22.9,1%, 5.8%. 16.7% and 16.1% respectively. The increase in *p*-cymene and caryophyllene oxide indicates oxidative damage. The concerted retrobicyclation that transforms tricyclesesquiterpene β-bourbonene in monocyclic germacrene-D may have been produced.

The EO is rich in sesquiterpenes (60-70%) with only traces of thymol (0-0.3%). Main components are *Z*-caryophyllene and β-bourbonene, with percentages related to the time of airing of harvested material.

Sesquiterpenes such as *E*-caryophyllene and γ-muurolene are also informed in literature as main components of *Origanum vulgare* ssp. *vulgare* (Skoula *et al.*, 1999), (Veres *et al.*, 2003). This EO should be type caryophyllene – germacrene-D (Chalchat y Pasquier, 1998)

O. compacto is *O. vulgare* ssp. *virens*. EO yield dry wt is 1.10 %. The EO is dextrorotatory (Table 2). The percentages of principal components of the analyzed EO (terpinen-4-ol (30.2%) and γ-terpinene (14.5%)) are within the intervals determined by Chalchat and Pasquier (1998) for E type but *p*-cymene (14.4%) and thymol (6.2%) exceed the ranges informed by these authors for E type probably because of the aging of the material distilled two years after harvest. During storage the aromatization of *p*-menthanic terpenic structures might have occurred.

The composition here determined differs from that reported by Alves-Pereira, Fernandez-Ferreira (1998) on specimens from northern Portugal with linalool (16.44%), δ-elemene (12.85%), β-caryophyllene (11.07%) and *E-β*-ocimene as main compounds.

EO from two hybrids: *Origanum* x *majoricum* (hybrid from *Origanum majorana* x *Origanum vulgare* ssp. *virens*) and *Origanum* x *applii* (hybrid from *Origanum majorana* x *Origanum vulgare* ssp. *vulgare*) were also analyzed.

In previus analysis (Gonzalez *et al.*, 2007) of samples of EO from *Origanum* x *majoricum* from Mendoza, *trans*-sabinene hydrate was the principal component (36.8%) followed by thymol (14.6%).

In the EO from the hybrids grown in Quebrada de Humahuaca collected two years apart the principal components are different: terpinen-4-ol (19.2 – 20.3%), γ-terpinene (13.4 – 16.9%), while thymol and *trans*-sabinene hydrate have percentages lower than 14 and 10% respectively.

High *p*-cymene content (9 – 20%) was observed in all analyzed samples of *Origanum* x *applii* while the sum of γ-terpinene and *p*-cymene, related compounds (the last one can be generated through aromatization of the first), ranges between 23.3 and 34.0%. Thymol content varies from 9.5 to 26.8%. EO obtained by steam distillation pilot scale (T1) is richer in volatile

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aliphatic hydrocabon monoterpenes and has less *p*-cymene percentage than EO obtained by hydrodistillation using a Clevenger type trap (T2) from the same sample. Therefore T1 technique seems to produce EO of better quality. EO composition data of this hybrid were not found in literature.

The highest EO yield (1.29-2.12%) was that of Oxm and the composition is similar to marjoram, with lower phenol content and higher terpinene and terpinene-4-ol percentages. In Oxa EO thymol is the principal component (mean content 20%) and similar to oregano EO. Oxa presented lower EO yields (0.5 – 1.4%)

All EO were dextrorotatory (+5 to +15) with 0.92 mean specific gravity and 1.483 mean refractive index.

According to Skoula *et al.* (1999) there are two major biochemically related compound families present in *Origanum* species: aromatic monoterpenes and sabinyl compounds. Three other biochemical groups of less importance are present in the genus: acyclic monoterpenoids, bornane type compounds and sequiterpenes such as β-caryophyllene, β-bisabolene, germacrene-D. Hydroxyl monocyclic monoterpenoid terpinene-4-ol should also be added to the above as biochemically related to the first type because it was found in *Origanum* EO analyzed previously in Jujuy as main component (Gonzalez *et al*., 2007)

Conclusions:

Both hybrids (Oxm and Oxa) and Oc belong to A group characterized by Skoula *et al.* (1999) because the major compounds are aromatic monoterpenes and/or their precursors. O. Negro is closely related to less common sesquiterpene type. It is necessary a complete work on botanical identity correlated with the physical and chemical characterization of essential oils from different *Origanum* species grown in our country to have a contrasting quality tool

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Table 1: Composition of the essential oil of *Origanum* species growing in Quebrada de Humahuaca, Jujuy (Argentina)[a]

Compound	**KI**	**Oxm**		**Oc**	**On**		**Oxa**					
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**
α-Thujene	931	0.6	0.4	0.3			0.6	1.2	0.5	0.5	0.8	1.1
α-Pinene	948	1.5	1.6	1.3			0.6	2.7	1.4	1.1	1.8	2.2
Sabinene	977	3.8	4.1	2.3	1.0	7.3	0.7	2.8	2.4	2.0	0.9	2.6
Myrcene	992	2.2	2.0	0.9	0.5	1.4	2.2	2.1	2.3	2.1	2.0	2.3
α-Terpinene	1020	8.0	6.2	6.8	0.3		1.6	3.1	4.8	2.8	6.6	4.6
p-Cymene	1028	2.5	2.3	14.4	1.3	14.2	19.8	9.4	4.6	16.6	14.5	8.9
β-Phellandrene	1034	6.2	4.5	4.9	1.4		4.4	5.9	8.1	2.9	5.2	6.0
(*Z*)-β-Ocimene	1040	1.4	2.1		3.0			3.5	3.6	3.8	3.9	4.0
(*E*)-β-Ocimene	1051				1.5			0.6			0.7	0.8
γ -Terpinene	1061	16.9	13.4	14.5		0.5	7.6	13.9	16.8	8.0	19.5	16.1
Terpinolene	1088	2.9	2.3	2.7	0.3		1.1	1.4	1.8	1.5	2.4	1.5
trans-Sabynene hydrate	1098	4.9	9.3	2.5	0.5		4.8	6.6	8.5	4.4	2.8	4.2
Terpinen-4-ol	1175	20.3	19.2	30.2	2.0	0.4	13.9	3.6	13.5	15.6	12.3	2.7
α-Terpineol	1187	2.8	2.8	3.2			2.6	1.1	2.5	3.0	1.9	0.8
Thymol methyl ether	1230	0.5	0.5	2.0	0.5		0.4	0.4			0.3	0.4
Carvacrol methyl ether	1239	2.1	2.0	0.9			1.7	2.0	1.1	1.2	1.4	1.9
Linalyl acetate	1251	2.6	2.3				1.7	2.0	2.6	2.6	1.2	2.0
Thymol	1288	10.5	13.1	6.2	0.4		26.8	21.6	11.6	17.8	9.5	20.4
Carvacrol	1295	1.1	1.2				0.8	0.4		0.5	0.2	0.5
β-Bourbonene	1376	0.2			16.7	25.7		0.1			0.2	0.3
(*Z*)-Caryophyllene	1406	2.5	3.2	0.8	22.9	5.7	1.1	3.5	4.2	2.8	2.6	3.8
(*E*)-Caryophyllene	1417				2.2	2.0		0.2			0.2	0.3
Germacrene-D	1470				16.1	1.5	0.3	1.4			0.5	1.3
Bicyclogermacrene	1484	0.7	1.4		0.8			1.5	0.2		0.3	1.4
β-Bisabolene	1496			0.4	3.4	0.4	0.9	0.9	0.7	0.8	0.4	0.7
Caryophyllene oxide	1569				5.8	17.8	2.0	0.1	0.4	1.6	0.1	0.2
	Sum	94.4	93.9	94.2	80.6	75.4	95.6	92.0	91.7	91.2	92.0	90.8
Hydrocarbons C10 + C15		49.5	43.6	49.2	71.4	57.2	40.8	54.2	51.5	44.8	62.4	57.8

[a]Plants collected at the beginning of flowering except sample **C** (full flowering)
Oxm: *Origanum* x *majoricum*
Ov: *Origanum vulgare* ssp. *virens*
On: *Origanum vulgare* ssp. *vulgare*
Oxa: *Origanum* x *applii*
A collected in 2004 – **E. F** and **G** collected in 2005 – **B. C. D. H** and **I** collected in 2006.
All the essential oil were obtained by hydrodistillation in the Clevenger type apparatus except **G** and **K** (steam distillation pilot scale)

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Table 2: Physic properties of the essential oil of *Origanum* species growing in Quebrada de Humahuaca. Jujuy (Argentina)

	Oxm		Ov	On		Oxa					
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**
Relativy density at 20ºC	0.902	0.904	0.908	vol ~ 0	vol ~ 0	0.971	0.912	0.915	0.927	0.905	0.928
Optical rotation at 20ºC	+ 11.5º	+ 15.0º	+ 9,5º	vol ~ 0	vol ~ 0	+ 9.0º	+ 5.0º	+ 11.0º	+ 6.0º	+ 6.5º	+ 5.5º
Refractive Index al 20ºC	1.4809	1.4841	1.4829	vol ~ 0	vol ~ 0	1.4888	1.4843	1.4791	1.483	1.4798	1.4841