CONTRIBUTIONS to SCIENCE, 8 (2): 77–84 (2012)
Institut d'Estudis Catalans, Barcelona
DOI: 10.2436/20.7010.01.137 ISSN: 1575-6343
www.cat-science.cat

Ethnobotanical study of the sages used in traditional Valencian medicine and as essential oil: Characterization of an endemic *Salvia* and its contribution to local development*

Vanessa Martínez-Francés,[1,2*] Emeline Hahn,[2] Jorge Juan-Vicedo,[1] Roser Vila,[2] Segundo Ríos,[1] Salvador Cañigueral[2]

1. Torretes Field Station, Research University Institute CIBIO, University of Alacant, Alacant

2. Unity of Pharmacology and Pharmacognosy, Faculty of Pharmacy, University of Barcelona, Barcelona

Resum. Set espècies de *Salvia* i dues de *Phlomis*, emprades en la medicina tradicional valenciana en preparats d'ús intern i extern per a tractar diferents malalties, han sigut estudiades. S'aporten noves dades etnobotàniques obtingudes mitjançant la realització d'entrevistes semiestructurades a trenta-quatre informants. Es presenta la caracterització estacional de l'oli essencial d'una sàlvia silvestre *Salvia blancoana* Webb & Heldr. subsp. *mariolensis* Figuerola, per GC-FID i GC-MS, com una eina per a assegurar un control de qualitat a les espècies endèmiques d'ús tradicional com aquesta, que eventualment són comercialitzades per indústries locals. La comparació del seu oli essencial amb el de la *Salvia lavandulifolia* Vahl subsp. *lavandulifolia* permet la seua comercialització sota el nom de *sàlvia espanyola*.

Paraules clau: *Salvia* · etnobotànica · identificació cromatogràfica · medicinal · País Valencià

Abstract. Seven wild and cultivated *Salvia* species and two *Phlomis* species, used traditionally in Valencian medicine to treat a variety of external and internal ailments, were studied. New ethnobotanical data are provided, obtained from semi-structured interviews with 34 people in the Valencian area. A seasonal characterization of the essential oil of a wild sage, *Salvia blancoana* Webb & Heldr. subsp. *mariolensis* Figuerola, by GC-FID and GC-MS was carried out as a means to ensure quality control of endemic traditional species such as this one, which has been commercialized by local industries. A comparison with the essential oil of *Salvia lavandulifolia* Vahl subsp. *lavandulifolia* allowed inclusion of the wild sage within the commercial 'Spanish sage' oil.

Keywords: *Salvia* · ethnobotany · chromatographic identification · medicinal · Valencia region

Abbreviations

EMA: European Medicines Agency
FDA: Food and Drug Administration
FFT: Full flowering time
GC-FID: Gas chromatography-flame ionization detector
GC-MS: Gas chromatography-mass spectrometry
HS-GC-MS: Headspace gas chromatography-mass spectrometry
HPLC: High performance liquid chromatography
HPLC/MS/MS: High performance liquid chromatography-tandem mass spectrometry
HPTLC: High performance thin layer chromatography
MS: Mass spectrometry
NBS: National Bureau of Standards library
NMR: Nuclear magnetic resonance
WHO: World Health Organization

Introduction

Sage has been considered an important medicinal plant since earliest times. Its name comes from the Latin *salvere*, which reflects the relevance of sage to human health. A proverb in the Tabula Salerni, also included in other medicinal treaties, evidences the significance of sage in ancient medicine, which persists even today: *Cur moriatur cui salvia crescit in horto?* (Why should someone die whilst sage grows in his garden?). *Salvia officinalis* L. and *S. fruticosa* Mill. were the reference species in those medicinal treaties [11,22], accounting for the reputation of officinal sages as a panacea, with a wide range of medicinal effects [4,6].

In the Mediterranean and Irano-turanic regions, ~40 of the approximately 900 of known *Salvia* species are found. Some of

* This contribution was funded by a grant obtained in 2010 from the Institute for Catalan Studies for the study 'Chemical characterization of the main sages used as a spice or for medicinal purposes in the Valencia region.'

Correspondence: V. Martínez-Francés, Estació Biològica Torretes, I.U. de la Biodiversiat CIBIO, Universitat d'Alacant, Crtra. Sant Vicent del Raspeig, s/n Sant Vicent del Raspeig E-03690, Alacant, EU. Tel. +34-965903400 (ext. 3343). Fax +34-965903815. E-mail: vanessa.martinez @ua.es

them are included in two important world-wide commercial groups [21]. One, comprising *S. officinalis* and similar species, usually cultivated, covers the oriental distribution of this genus in the Mediterranean region. The other is made up of the occidental sage group *S. lavandulifolia* and other closely related wild species (sometimes cultivated). The lack of consensus among taxonomists regarding the correct traits and their value in accurate species identification has resulted in a misidentification of the raw materials used in the marketing of sage-based products [21].

With the aim of understanding the importance of sages in Valencian folk medicine, an ethnobotanical study was performed. As part of that study, data from seven wild and cultivated sages of the Valencia region, often referred to as coarse sages, were compiled: *S. blancoana* Webb & Heldr. subsp. *mariolensis* Figuerola, *S. lavandulifolia* Vahl subsp. *lavandulifolia*, *S. microphylla* Kuntz, *S. officinalis* L., *S. sclarea* L., *S. verbenaca* subsp. *controversa* (Ten.) Arcang, *S.* x *auriculata* Mill., and two species of *Phlomis: P. crinita* Cav.and *P. purpurea* L. In Valencian folk medicine, several wild-cultivated sages included in the occidental group are used with the same popular purposes and with greater importance than *S. officinalis* or *S. fruticosa*. One of them, *S. blancoana* Webb. & Heldr. subsp. *mariolensis* Figuerola, is endemic in Valencia and Alicante provinces. This plant is commonly collected in the wild and sold by local companies together with a more widely distributed sage, *S. lavandulifolia* subsp. *lavandulifolia*. The reduced distribution area of the former and the continuous taxonomic discussions regarding *S. lavandulifolia* and *S. blancoana* taxa have resulted in the two species being either grouped together or separated depending on the criteria of the botanist. In the present study, the knowledge of the local human population was used to compare agronomic information, such as the best places and season to collect these sages or the permissible storage time, with the data obtained in the laboratory for two of these species. *S. lavandulifolia* subsp. *lavandulifolia* and *S. blancoana* subsp. *mariolensis*, which are marketed as 'Spanish sage.'

Many of the plants used in traditional medicine over the centuries have not been officially recognized in most countries due to a shortage in the amount and the quality of the relevant data [6]. The aim of this study was to supplement current knowledge as well as to offer an approach to resolving adulteration problems in sage preparations. As noted above, these problems arise due to the difficulty of botanical identification during commercialization. For *S. blancoana* subsp. *mariolensis*, we propose the phytochemical characterization of this endemic medicinal plant in order to contribute to establishing quality-control parameters for the raw material and its essential oils. This and similar characterizations would clearly define which plants can be referred to as 'Spanish sage.'

To characterize the Valencian sages included in the 'Spanish sage' group, quantitative and qualitative investigations of their essential oil in different phenological phases were carried out using gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) analyses (Fig. 1).

Fig. 1. Study area located in the Valencia region in Spain.

Thus, our study sought to demonstrate the importance of characterizing locally distributed or exploited plant resources in order to make available information essential to quality control for local industries. In addition, data supporting the culture of these species, as a complement to wild collections and to ensure good management practices for natural resources, are provided herein.

Materials and methods

Ethnobotanical study. Semi-structured interviews among older Valencian, randomly selected, men and women, were carried out. A few younger people were also interviewed in order to determine whether herbal-medicine knowledge has been transmitted. Some of these interviewees joined us in collecting the sample material from the field.

Phytochemical study

Plant material. Aerial parts of *Salvia blancoana* subsp. *mariolensis* were collected during the full blooming period from a home garden in Banyeres de Mariola, in Alacant (*Sp*. Alicante) Province, in June 2009 and 2010. In addition, a sample was collected before the flowering stage, also in June 2010. Flowering aerial parts of *S. lavandulifolia* subsp. *lavandulifolia* were collected in Cinctorres, in Castelló Province, in June 2010. Both provinces are in the Valencia region, Spain. Plant material was identified and a sample of each one was deposited in the Herbarium BCF of the University of Barcelona. The plant material was dried according to traditional usage, i.e., at room temperature in a dark, dry place, in the Biological Station of Torretes (Ibi, Alacant).

Essential oil preparation. Air-dried plant material was submitted to hydrodistillation with a Clavenger-type apparatus, following the method described in the European Pharmacopeia [8]. The yield was calculated as the average of three replicates and the essential oil obtained was stored at 4°C until analysis.

Analysis of the essential oils. The essential oils were analyzed by GC-FID and GC-MS using two fused silica capillary columns (60 m × 0.25 mm i.d.; 0.25-μm film thickness) of different stationary phases: Supelcowax^{TM} 10 and methylsilicone SE-30. GC-FID analyses were performed on a Hewlett-Packard 6890 instrument, equipped with HP ChemStation data processor software, under the following analytical conditions: carrier gas, helium; flow rate, 1 ml/min; oven temperature programmed, 60°C (2 min); 60–180°C at 2°C/min, 180°C (7 min), 180–230°C at 4°C/min, 230°C (15 min), 230–260°C at 10°C/min, 260°C (8 min); injector temperature, 250°C; detector temperature, 270°C; split ratio 1:100. The amount of undiluted essential oil injected was 0.1 μl. Mass spectra were obtained with a computerized system consisting of a GC Hewlett-Packard 6890 coupled to a mass selective detector Hewlett-Packard 5973N, using the same analytical conditions as above. Mass spectra were taken over *m/z* 35–400 at an ionizing voltage of 70 eV.

The plant components were identified by means of: (a) comparison of their GC linear retention indices in two stationary phases, determined in relation to a homologous series of *n*-alkanes (8–23 carbons) and a homologous series of fatty acid methyl esters (FAME indices), with those of authentic compounds or literature data; (b) comparison of the fragmentation patterns in the mass spectra with those stored in our own library or in the GC-MS mass spectral library. Each compound was quantified on the basis of its GC peak areas on the two columns, using the normalization procedure without corrections for a response factor.

Results

Ethnobotanical study. Prior to the collection of plant material for chemical characterization, local people were asked about the following:

1. If they recognized the different sages of the area
2. The popular names of these plants
3. The best place and time to collect them
4. Who in the family goes to collect the wild sage
5. Their main use (medicinal or as an herb)
6. Mode of preparation and posology
7. Whether the type of use was traditional or recent

Semi-structured interviews were held with 34 individuals (19 women and 15 men) between 24 and 83 years of age and from different localities of the Valencia region: Herbers and Llucena del Cid, in Castelló Province; Bocairent, Corbera, and València in València Province; and Alcoi, Alfafara, Banyeres de Mariola, Benifato, Cocentaina, Ibi, Onil and Xixona in Alacant Province. The different sages cited by the informants and the main uses reported are shown in Table 1.

Two sages and its hybrids, *S. officinalis*, *S. fruticosa* and *S.* x *auriculata*, are widely known among Eastern Mediterranean cultures [21,22]. In Valencian home gardens they are mostly cultivated for ornamental purposes. Closer examination of the reported uses revealed that wild (sometimes cultivated) sages appear to be more popular in folk-medicine than cultivated sages (Fig. 2).

S. officinalis, *S. lavandulifolia*, *S. verbenaca* were determined to be the most commonly used species throughout the Valencia region and surrounding areas whereas use of *S. microphylla* and *S. blancoana* subsp. *mariolensis* is more local. This was especially the case in the mountainous regions between northern Alacant Province and southern Valencia Province. We determined a loss of folk knowledge, as exemplified by *S. sclarea*. Some of the interviewees grew it in their gardens but did not recognize the plant as a sage and therefore, did not use it either as an herb or medicinally. The two *Phlomis* species are considered as coarse sages, which is reflected in their popular name 'Salvió' (Table 1). Although people still remembered the medicinal properties of both species, they were no longer in use. Two morphologically very similar sages, *S. blancoana* subsp. *mariolenis* and *S. lavandulifolia* subsp. *lavandulifolia*, had common uses in all the studied areas. Their distribution in the Valencian region is complementary but not overlapping (except at their respective boundaries) and they are apparently used for the same purposes in folk medicine. Nonetheless, people who used one were able to distinguish it from the other. Most of the medicinal uses reported involved a single plant type. When the plants were prepared in mixtures, an examination of which was beyond the scope of this study, the uses and the amount of the preparations significantly increased.

Except for *S. verbenaca* and the two *Phlomis* species, most of the formulations made with sages in the Valencian area are for oral administration, to treat ailments such as indigestion, colds, hypertension, insomnia, and anxiety. As external preparations, such as plasters, ointments, alcohols and washes, they are used as vulneraries and for their anti-inflammatory properties, or to treat hyperhydrosis (Table 1, Fig. 2).

During the ethnobotanical interviews some of the questions were aimed at determining the best places to collect wild sages as well as the optimal time period. These discussions sometimes revealed toponymic names (e.g., 'El Tossal de la Sàlvia' and 'El Salviar') that identified the places where these plants can be found in abundance, although there are other sites, without toponymic identification, where people go every year to collect wild sages. These collection areas are selected considering two important factors: (i) the place where the best plant material can be obtained and (ii) accessibility. Some of the collectors were elderly and unable to travel long distances from their homes. To circumvent this problem, some of them grew these plants, routinely used for their medicinal properties, in small home gardens, after having selected the best plants from the wild and adapting them to culture. In fact, nowadays such cultures provide an important agronomic resource and merit further investigation.

Essential oil content and constituents. The study of volatile oils and other secondary metabolites found in nature, with the aim of identifying novel natural products for different therapeutic and industrial purposes, is rapidly increasing. Variations in the chemical composition and dynamics of accumulation of

Table 1. Ethnobotanical results. Prov: province, A: Alacant, Ab: Albacete, Cs: Castelló, V: Valencia, L: leaves, I: Inflorescence, S: seeds, E: essential oil

Species	Prov	Popular names	Popular uses (*)
Salvia blancoana Webb & Heldr subsp *mariolensis* Figuerola	A	Sàlvia[1], sàlvia de la Mariola[1], sàlvia de serra[1]	Spice (L)[1], digestive tea (I)[1,11], emmenagogue tea (I)[1,11], antitussive and anti-cold tea (I)[1,11], antipyretic tea (I)[1], nervous sedative inhalation and tea (I)[1], tonic-digestive liquour (I)[1], detoxifying tea (L)[1], hypotensive tea (L)[1,11], dermal anti-hyperhidrosis washes (L)[1], perfumes (E)[1], flavoring snuff (L)[1], floral carpets and bouquets in religious rituals (I)[1,11], ornamental plant[1]
Salvia lavandulifolia Vahl subsp. *lavandulifolia*	Ab, V, Cs	Sàlvia[1], sàlvia de serra[1], sàvia[12], sava[12], sèrvia[12], sèlvia[12], sàlvia d'Aragó[12], sàlvia de Sant Joan de Penyagolosa[12], estepera[12]	Spice (L)[1], digestive tea (I)[1], antitussive tea (I)[1,12], anti-cold tea (I)[1,10,12], emmenagogue tea (I)[1,12], hypotensive tea (I)[1,12], antipyretic tea (I)[1,12], tonic-digestive liquour (I)[1], nervous sedative tea (I)[1,12], oftalmic antiinflammatory, antirheumatic and antimigraine fumes (I)[12], dermal antiinflammatory washes (I)[12], dermal antiinflamatory alcohol (I)[12], antidote plaster to scorpion bites (I)[12], ornamental plant[1]
Salvia microphylla Kuntz.	A, V	Sàlvia roja[1], sàlvia vera[1]	Nervous sedative tea (I)[1], digestive tea (I)[1], emmenagogue tea (I)[1], tonic-digestive liquour (I)[1], ornamental plant[1]
Salvia officinalis L.	A	Sàlvia[1], sàlvia de jardí[1]	Spice (L)[1] , digestive tea (I)[1], emmenagogue tea (I)[1], detoxifying tea (L)[12], hypotensive tea (L)[12], anti-cold tea (L)[10], dermal anti-hyperhidrosis washes (L)[1]
Salvia sclarea L.	A	Unknown	Ornamental plant[1]
Salvia verbenaca subsp. *controversa* (Ten.) Arcang	A, Cs	Tàrrec[1,10,11,12], tèrrec[11], tèrric[11], terri[11], tàrrega[12], tàrrego[12], tàrrago[12], herba de Santa Llúcia[12]	Oftalmic antiseptic and hypertensive (S)[1,10,11,12], dermal antiinflammatory washes (I)[12], wax or oil antiinflammatory ointment (I)[12] , vulnerary and antiinflammatory plaster (L)[11], snuff substitute (L)[1,11,12], forage for rabbits (L)[11]
Salvia x *auriculata* Mill.	A	Sàlvia[1], sàlvia de jardí[1]	Spice[1], digestive tea (I)[1,2], emmenagogue tea (I)[1,2], ornamental plant[1]
Phlomis crinita Cav.	V	Salvió[1]	Detoxifying tea (I)[1], snuff substitute (L)[1]
Phlomis purpurea L.	V	Salvió[1]	Detoxifying tea (I)[1], snuff substitute (L)[1], wicks and scourers (L)[1]

[1] Own data; [2] Rivera et al. (1994); [10] Fresquet et al. (1994); [11] Pellicer (2001); [12] Mulet (1991). (*) Popular uses as determined from our interviews as well as from the literature are reported.

essential oils during plant ontogenesis are characteristic of each taxon [19]. These changes determine the harvest time of each species or even of the many cultivars [10,19,24]. For example, in some species of the Lamiaceae family, essential oil accumulation reaches a maximum at the flowering stage. However, this maximum is influenced by ecological, climatological, and agrotechnological factors [19].

In order to investigate the seasonal variability of the essential oil composition of *S. blancoana* subsp. *mariolensis*, plants grown in a home garden were analyzed at different time periods. Two samples were collected at the optimal time (according to the person who grew them) in 2009 and 2010. Additionally, in 2010, one sample of the same sage was gathered a few weeks before it had fully bloomed. Precipitation and temperature data were obtained from a climatological station located in the same village, less than 1 km from where the sampled plants were grown [17]. This Ibero-levantine sage has a very small distribution area, located between three important mountains of northern Alacant Province and southern Valencia Province. This species was selected because the plant, as a dry bulk tea, and its essential oils are locally sold, in both forms often as a mixture with *S. lavandulifolia* subsp. *lavandulifolia*, depending on the collection area. It was therefore deemed important to characterize the raw material and the essential oils, especially if they are to be used by the food and cosmetic industries or for medicinal purposes.

The main components identified in the essential oils analyzed from *S. blancoana* subsp. *mariolensis* are shown in Table 2. Samples collected in 2009 and 2010 at full flowering time (FFT) were quite similar, except in the significantly higher

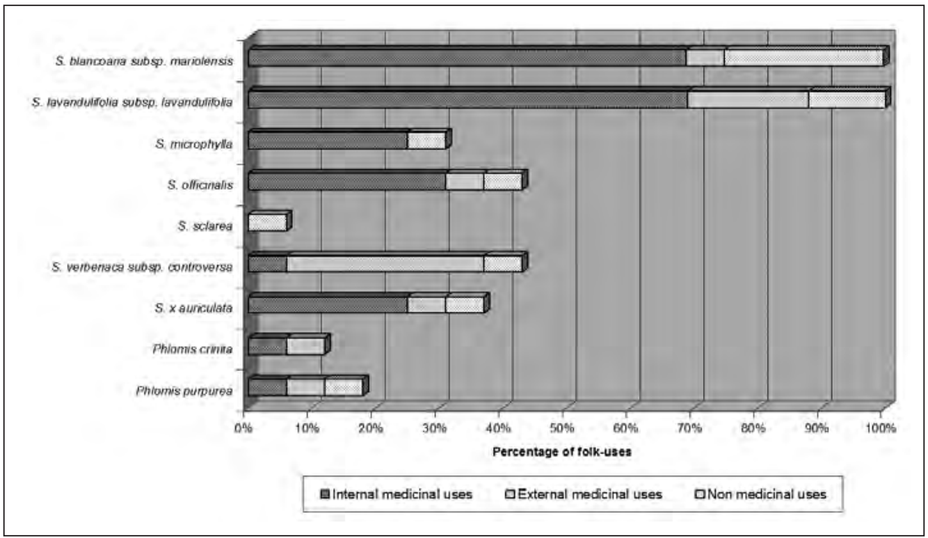

Fig. 2. Valencian folk uses compiled for the different sages studied.

amount of 1,8-cineole and the significantly lower amount of borneol in 2009 than in 2010. Only small differences were measured in the amounts of the other compounds in 2009 vs. 2010, e.g., the concentrations of camphor were slightly higher whereas those of camphene, β-pinene, bornyl acetate and β-caryophyllene were slightly lower.

Additionally, the variations between samples in 2010 with respect to the pre-flowering time (PFT) and FFT included increases in 1,8-cineole, β-pinene, and bornyl acetate and a decrease in camphor during the optimum FFT. Interestingly, however, in terms of their biosynthesis, camphor and 1,8-cineole are nearly identical, as both involve the actions of cyclase enzymes and a menthone skeleton [9]. The observed differences in their amounts may be related to plant defense systems. A number of studies have focused on the activity of 1,8-cineole, confirming its potent antibacterial [1,13,24,26], antifungal [1,13,24,26,27], biocidal [16], and herbicidal [5] activities. In addition, this compound is allelopathic [7], which improves the competitiveness of the plant in its ecosystem. Camphor is less active than 1,8-cineole but nonetheless has important antimicrobial [1,2,24] and biocidal [16] properties.

It was suggested that alterations in the essential oil composition reflect structural changes in the accumulation of organelles or modifications in the biosynthesis and metabolism of synthesized products [19]. According to the informants, sun-grown plants differ in their yields of essential oils from those that are shade-grown, with the highest yields in the former. They also reported that, during years with large amounts of rainfall, the yield of essential oils decreases.

During the 2-year study period (2009–2010), the plants used for sampling were grown in the sun. The climatological data are shown in Table 4. During the first hydrological year (2008–2009), rainfall was about 170 mm less than in the second hydrological year (2009–2010). The average essential oil yield at FFT in 2009 was 6.2 % (Table 5) vs. 3.2 % in FFT 2010. These data support the assertion that increases in annual precipitation decrease the essential oil yield.

Temperatures were slightly higher in the second hydrological year, but with a progression very similar to the previous year (Figs. 3 and 4, Table 3). Although extreme temperatures (cold and hot) occurred more often in the second year (Table 4), the data are insufficient to determine whether these fluctuations affected the yield. However, empirically, field data from different studies and traditional knowledge support the conclusion that high temperatures a few weeks before FFT accelerates optimal blooming and therefore the collection date as well.

The yield of essential oil from *S. blancoana* subsp. *mariolensis* is typically very high (Table 5), albeit with large variations from one year to another. If the plant is collected 2 weeks before the recommended date, the oil yield is 40 % lower.

Table 2. Percentage variation of the main components (>1 % minimum in one studied sample) of *S. blancoana* subsp. *mariolensis* during different times and years. Abbr.: FFT: full flowering time (25.06.2009, 20.06.2010), PFT: pre-flowering time (06.06.2010), t (trace) < 0.05 %

Component	FFT 2009	FFT 2010	PFT 2010
	%	%	%
α-Pinene	5.0	4.2	4.8
Camphene	4.3	7.3	8.3
β-Pinene	5.0	9.6	6.4
β-Myrcene	5.8	5.0	3.7
Limonene	3.5	3.4	3.3
1,8-Cineole	39.0	24.2	18.2
cis-Ocimene	1.2	0.6	1.2
Camphor	21.0	18.0	23.7
Bornyl acetate	1.5	4.3	2.3
β-Caryophyllene	t	3.3	4.0
α-Terpenyl acetate	1.0	t	t
Borneol	3.3	10.2	11.2

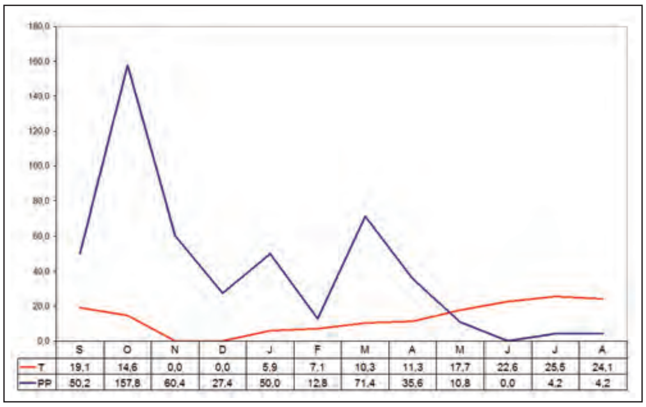

Fig. 3. Data for the hydrological year 2008–2009. PP: total monthly precipitation; T: medium monthly temperature. Total annual precipitation: 485 mm.

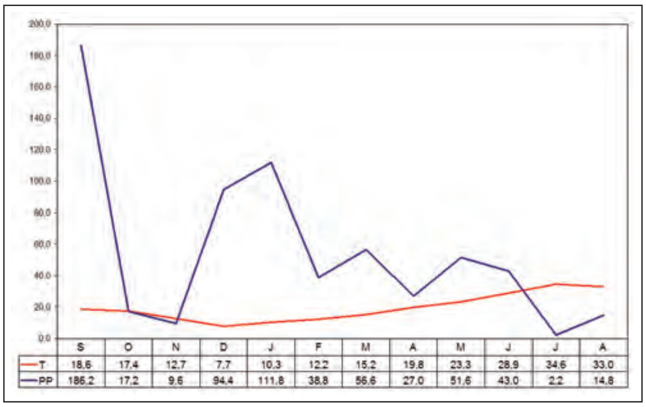

Fig. 4. Data for the hydrological year 2009–2010. PP: total monthly precipitation; T: medium monthly temperature. Total annual precipitation: 653 mm.

Table 3. Results of annual precipitation and temperature during 2008–2010 years. Abbr.: Total PP: Total precipitation; PP-S-D: Precipitation between September to December, PP-J-A: Precipitation between January to August

Precipitation				Temperature			
				Months: S-D		Months: J-A	
Year	Total PP	PP S-D	PP G-A	Day < 0°C	Day > 32°C	Day < 0°C	Day > 32°C
2008	540	296		13	2		
2009	496	307	189	7	6	8	52
2010	495		346	9	3	20	55

Table 4. Comparison of precipitation and temperature between the two hydrological years studied with agronomic purposes

Hydrological year	Hydrological year PP	Day < 0°C	Day > 32°C
2008–2009	485	21	54
2009–2010	653	27	61

Furthermore, the informants related the importance of collecting the plant every year. They also stated that to maintain the quality of the plant material the storage time should not exceed 2 years. The sage collected in FFT 2009 was hydrodistilled 18 months later, resulting in an essential oil yield of 4.8 % v/w. This is > 20 % lower than the yield obtained by hydrodistilling the plant material within 12 months of its collection.

There are currently three standards to assess the quality of the essential oil of 'Spanish sage' (*Salvia lavandulifolia*): standard ISO 3526 [14], from the International Organization for Standardization; standard UNE84310 [3], from the Spanish Association for Standardization (AENOR); and monograph 1849 of the European Pharmacopeia [8]. Table 6 compares the experimental values obtained for the samples analyzed and the above-mentioned standards. In the two sages studied (*S. blancoana* subsp. *mariolensis* and *S. lavandulifolia* subsp. *lavandulifolia*), the percentages of most of the analyzed compounds were within the ranges accepted by national and international standards. Slightly higher or lower percentages for certain compounds, such as borneol, sabinyl acetate, and thujone in *S. blancoana* subsp. *mariolensis*, and linalool, linalyl acetate, α-terpenyl acetate, sabinyl acetate, and thujone in *S. lavandulifolia* subsp. *lavandulifolia*, were negligible. The ranges included in the standards are established for industrial products whereas the laboratory samples were obtained by the hydrodistillation from a small volume of plant material. Although in Lamiaceae, infra-specific variability has been reported [19], Table 6 shows the high degree of agreement between the oils of the two sages studied and the standards.

Consequently, both *S. blancoana* subsp. *mariolensis* and *S. lavandulifolia* subsp. *lavandulifolia* could be used for the production of 'Spanish sage' oil. However, additional studies fingerprinting the non-volatile constituents are necessary before these two species can interchangeably be used as a source of herbal dry material marketed for teas and other purposes.

Table 5. Changes in essential oil yield of *Salvia blancoana* subsp. *mariolensis*

Salvia blancoana Webb & Heldr. subsp. *mariolensis* Figuerola	
Collection date	Essential oil yield (%) v/w
25.06.2009	6.2
06.06.2010	1.9
20.06.2010	3.2

Table 6. Comparison between percentages obtained in our samples of *S. blancoana* subsp. *mariolensis* and *S. lavandulifolia* subsp. *lavandulifolia* and % range of each compound included in ISO/DIS 3526 (2005) and UNE84310 (2001) for 'Spanish sage' group [3]. Abbr.: nd: non detected

Component	*S. blancoana* subsp. *mariolensis*	*S. lavandulifolia* subsp. *lavandulifolia*	UNE-84310 ISO 3526	Ph.Eur
	% (20.06.10)	%	% range	% range
α-Pinene	4.2	9.4	4.0–11.0	4.0–11.0
Sabinene	0.8	0.5	0.1–3.0	0.1–3.5
Limonene	3.4	6.3	2.0–5.0	2.0–6.5
1,8-Cineole	24.2	20.0	11.0–30.0	10.0–30.5
linalool	0.3	0.2	0.3–4.0	0.3–4.0
camphor	18.0	16.2	15.0–36.0	11.0–36.0
Borneol	10.2	5.0	1.0–5.0	1.0–7.0
Terpinen-4-ol	0.2	0.5	<2.0	<2.0
Linalyl acetate	nd	0.04	0.1–5.0	<5.0
α-Terpenyl acetate	0.7	0.1	0.5–9.0	0.5–9.0
Sabinyl acetate	nd	nd	0.5–9.0	0.5–9.0
Thujone	nd	nd	-	<0.5

Conclusions

Ethnobotanical data are a good starting point for further research studies of wild and cultivated material with medicinal, food, or cosmetic interest. In the area of Valencia, sages are still used, either alone or as complex herbal formulae, for daily health care. The current use keeps alive traditional knowledge. Yield data from sage grown in a home garden but originally selected from wild material showed that local people were aware of the value of their resources and of means to maximize their harvest. Plants collected from the wild may vary in their volatile oil composition due to changes in climate or soil conditions, hybridizations, or introgressions, among other aspects, but the essential oil pattern is for the most part maintained. There is no consensus among Spanish botanists regarding the taxonomic status of *S. lavandulifolia* and *S. blancoana* sages. Both are collected from the wild or cultured and are sold mixed as dry bulk teas, while their essential oils are used, under the name 'Spanish sage,' in the perfume and cosmetic industries.

This study highlights the similarity between the essential oil composition pattern of the endemic Levantine sage *Salvia blancoana* subsp. *mariolensis* and the Iberian sage *S. lavandulifolia* subsp. *lavandulifolia*. Our results make it clear that the two sages can indeed be used interchangeably for the production of essential oils while complying with the current standards for 'Spanish sage oil.' Furthermore, they attest to the importance of studying our traditions, which enable us to select the best species for characterization, which allows them to be sustainably exploited for local, national, and international industries, thereby boosting the economy of the producing regions. To prevent the overexploitation of these species, it is necessary to promote their cultivation based on agricultural studies of the cultivars of interest.

Acknowledgements. The authors are grateful to the Institute for Catalan Studies for financial support through the *Borsa d'estudi Països Catalans*. Emeline Hahn acknowledges financial support from an ERASMUS Internship (FsTRASBO48).

References

1. Akin M, Demirci B, Bagci Y, Baser KHC (2010) Antibacterial activity and composition of the essential oils of two endemic *Salvia* sp. from Turkey. African Journal of Biotechnology 9:2322-2327
2. Anand AK, Mohan M, Haider Z, Sharma A (2011) Essential oil composition and antimicrobial activity of three *Ocimum* species from Uttarakhand (India). International Journal of Pharmacy and Pharmaceutical Sciences 3:223-225
3. Asociación Española de Normalización y Certificación (2001) Aceites esenciales. Aceite esencial de salvia de España (*Salvia lavandulifolia* Vahl). UNE84310
4. Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils – A review. Food and Chemical Toxicology 46:446-475
5. Barton AFM, Dell B, Knight AR (2010) Herbicidal activity of cineole derivatives. J Agric Food Chem 58:10147-10155
6. Bouaziz M, Yangui T, Sayadi S, Dhouib A (2009) Desinfectant properties of essential oils from *Salvia officinalis* L. cultivated in Tunisia. Food and Chemical Toxicology 47:2755-2760

7. Chain F, Loandos MH, Fortuna AM, Villecco MB (2007) Síntesis y actividad alelopática de derivados del 1,8-cineol sobre semillas de mono y dicotiledóneas. Bol Latinoam Caribe Plant Med Aromaticas 6:335-336
8. Council of Europe (2010) European Pharmacopeia, vol 1, 7th ed. Directorate for the Quality of Medicines and Health Care of the Council of Europe, Strasbourg
9. Dewick PM (2002) The mevalonate and deoxyxylulose phosphate pathways: terpenoids and steroids in medicinal natural products. John Wiley & Sons, Ltd., pp. 167-289
10. Dudai N, Lewinsohn E, Larkov O, Katzir I, Ravid U, Chaimovitsh D, Sa'adi D, Putievsky E (1999) Dynamics of yield components and essential oil production in a commercial hybrid sage (*Salvia officinalis* x *Salvia fruticosa* cv. *Newe Ya'ar No.4*). Journal of Agricultural Food Chemistry 47:4341-4345
11. Dweck AC (2000) The folklore and cosmetic use of various *Salvia* species. In: Kintzios SE (ed) Sage. The genus *Salvia*. Haword Academic Publishers, pp. 1-27
12. Fresquet JL, Tronchoni JA, Ferrer F, Bordallo A (1994) Salut, malaltia i terapèutica popular. Els municipis riberencs de l'Albufera. Col·lecció Josep Servès, de documentació i recerca. Ajuntament de Catarroja, 223 pp.
13. Hendry ER, Worthington T, Conway BR, Lambert PA (2009) Antimicrobial efficacy of eucalyptus oil and 1,8-cineole alone and in combination with chlorhexidine digluconate against microorganisms grown in planktonic and biofilm cultures. Journal of Antimicrobial Chemotherapy 64:1219-1225
14. International Standards Organization (2005) Oil of sage, Spanish (*Salvia lavandulifolia* Vahl). ISO 3526
15. Liang Y-Z, Xie P, Chan K (2004) Quality control of herbal medicines. Journal of Chromatography B 812:53-70
16. Liska A, Rozman V, Kalinovic I, Ivezic M, Balicevic R (2010) Contact and fumigant activity of 1,8-cineole, eugenol and camphor against *Tribolium castaneum* (Herbst). 10th International Working Conference on Stored Product Protection, Julius-Kühn-Archiv, 425 pp.
17. MeteoData (http://www.meteobanyeres.com/drupal_meteobanyeres/node/10)
18. Mulet L (1991) Estudio etnobotánico de la provincia de Castellón. Diputación de Castellón, 596 pp.
19. Németh É (2005) Changes in essential oil quantity and quality influenced by ontogenic factors. In: Bernáth J, Németh É, Craker LE, Gardner ZE, (eds) Proc WOCMAP III, vol I: Bioprospecting & Ethnopharmacology, Acta Hort 675:159-165
20. Pellicer J (2001) Costumari botànic. Recerques etnobtàniques a les comarques centrals valencianes, 2a ed. Edicions Bullent, 253 pp.
21. Reales A, Rivera D, Palazón JA, Obón C (2004) Numerical taxonomy study of *Salvia* sect. *Salvia* (Labiatae). Botanical Journal of the Linnean Society 145:353-371
22. Rivera D, Obón C, Cano F (1994) The botany, history and traditional uses of three-lobed sage (*Salvia fruticosa* Miller) (Labiatae). Economic Botany 48:190-195
23. Rzepa J, Wojtal L, Staszek D, Grygierczyk G, Labe K, Hajnos M, Kowalska T, Waksmundzka-Hajnos M (2009) Fingerprint of selected *Salvia* species by HS-GC-MS analysis of their volatile fraction. Journal of Chromatographic Science 47:575-580
24. Safaei-Ghomi J, Batooli H (2010) Determination of bioactive molecules from flowers, leaves, stems and roots of *Perovskia abrotanoides* Karel growing in central Iran by nano scale injection. Digest Journal of Nanomaterials and Biostructures 5:551-556
25. Sajewicz M, Rzepa J, Hajnos M, Wojtal Ł, Staszek D, Kowalska T, Waksmundzka-Hajnos M (2009) GC-MS study of the performance of different techniques for isolating the volatile fraction from sage (*Salvia* L.) species, and comparison of seasonal differences in the composition of its fraction. Acta Chromatographica 21:453-471
26. van Vuuren SF, Viljoen AM (2007) Antimicrobial activity of limonene enantiomers and 1,8-cineole alone and in combination. Flavour and Fragance Journal 22:540-544
27. Vilela GR, de Almeida GS, Reginato D'Arce MAB, et al. (2009) Activity of essential oil and its major compound, 1,8-cineole, from *Eucalyptus globulus* Labill., against the storage fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. Journal of Stored Products Research 45:108-111