



Essential oil yields and qualities of different clonal lines of *Salvia lavandulifolia* monitored in Spain over four years of cultivation



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ABSTRACT

The current knowledge about the medicinal properties and marketing possibilities of the Spanish sage (*Salvia lavandulifolia* Vahl.) requires additional studies about the management of this valuable species. To this end, essential oil yields and chemistries from eleven different mother plants collected in the wild and cultivated in a single plot were obtained and monitored during four years (2010, 2011, 2013 and 2014) at three different phenological stages ((LF) leaves formation-vegetative, (FB) full bloom and (SM) seed maturation). Significant differences have been found in the yield over the phenology being the maximum essential oil production at the stage of SM. Besides, the yield enhanced by both the shortage periods of rainfalls and the plant age with in turn a maximum average yield recorded of 4.15% v/w in SM in 2014, at the end of the experiment. However, no distinction by clonal lines could be made regarding yield. Essential oil chemistry conversely, showed deeply influenced by the genotype from which the material for hydrodistillation was taken. Thus, the main essential oil compound recorded was the oxygenated terpene 1,8-cineole (36.7% in average value for whole experiment), with other accompanying important components such as α -pinene (7.8%), β -pinene + myrcene (14.5%), limonene (6.2%) and camphor (7.1%). Besides, unlike the yield, essential oil chemistry was only partially influenced by the year of harvest with no relevant changes according to the phenological stage of the plant. Thus, phytochemical differences found in nature in the first place largely and even wider remain when plant growing under a common environment. Hence, some new and stable chemotypes were described according to their high values of α -pinene (>10%), limonene (>17%), 1,8-cineole (>50%), camphor (>20%) and β -caryophyllene (>7%). This information is highly relevant for standardizing, assuring crop quality and for the proper management of the Spanish sage cultivation.

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1. Introduction

Presently the question of searching for alternative crops which better suit the specific soil and climatic conditions is a matter of great immediate interest. The reduced soil fertility due to overexploitation and the climatic change causes that certain lands become into wastelands which resulting in a gradual productivity weaken (Lesschen et al., 2007). However, rehabilitation measures applicable to these lands which use shrubs well adapted to semiarid or other local conditions increase the chance of controlling the soil physical properties (Le Houérou, 2000) promoting their structural stability. Particularly for the Mediterranean Basin, Lamiaceae fam-

ily species that have been used in folk medicine for centuries such as genus *Lavandula*, *Rosmarinus*, *Thymus*, *Satureja* or *Salvia*, among other (Srancikova et al., 2013), are excellent candidates for the aforementioned alternative crops. Many of these species could be interesting horticultural crops for semi-arid areas and rainfed lands due to its high water-use efficiency and adaptation to drought conditions as well as its high essential oil production. In fact, these species are currently cultivated primarily by their essential oils (Lubbe and Velpoorte, 2011; Teixeira et al., 2013). Essential oils are well known natural products from the secondary metabolism of a broad range of plant species being 300 of these commonly traded on the global market (CBI, 2009). Its demand is still increasing due to novel described applications and promising pharmacological effects (Marchev et al., 2013; Radulović et al., 2013).

The genus *Salvia* comprises about 900 species, among which *Salvia lavandulifolia* (Spanish sage) has a longstanding and well

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reported reputation as a plant with remarkable pharmacological properties. By this way, the essential oils have been described as a potent cholinesterase inhibitor which improves cognitive performance and mood with additional positive pharmacological effectiveness for brain diseases such as Alzheimer or dementia (Kennedy et al., 2011; Howes and Houghton, 2012; Miroddi et al., 2014). This relevant issue brings this species as one of the most promising plants for promoting as alternative crop in the Mediterranean area. The essential oil putative active components for cytoprotection and antioxidant activities encompass a broad range of terpenoids, including α -pinene, β -pinene, myrcene, 1,8-cineole and camphor (Porres-Martínez et al., 2013, 2014, 2015) being the monoterpenes 1,8-cineole and camphor the major compounds within essential oils from Spanish endemic wild plants (Herráiz-Peñalver et al., 2010). Nonetheless, as a result of the phytochemical plant to plant variability of this species, remarkable different essential oil profiles have been found (Usano-Aleman 2014a,b). Accordingly, relying on the plant origin and the cultivation management, significant variations in the percentage content of the putative active components can be achieved. As an example of this, 1,8-cineole has been shown to be the most potent single component in terms of cholinesterase inhibition (Savelev et al., 2003) although its relative percentage in the essential oil may vary from 8.2% up to 75% mainly by genotype differences (Usano-Aleman et al., 2014b). Thus the genotype of the plant seems able to play a huge role in how well it responds to the environment or growing conditions although there is an obvious lack of long-term monitoring works. Furthermore, due to this phytochemical heterogeneity provided for most of the aromatic plants (Franz and Novak, 2010) the reported Latin binomial names of the species in manuscripts describing biological properties of diverse plant extracts or phytochemical profiles from a single or few samples might not be enough to link the putative pharmacological effectiveness with a well-defined herbal product. The systematic review of the available literature shows that *S. lavandulifolia* essential oils exert beneficial effects against oxidative stress in neurons enhancing cognitive performance (Kennedy and Wightman, 2011). However, most of these promising beneficial effects are debased by spot samples or not well identified raw-materials with lack of details of cultivation managements and conditions. Reliable and predictable quality is required for any practical use of plant secondary metabolites which must be developed with the appropriate crop genotypes that can be cultivated, harvested and treated accordingly. The cultivation of these species currently faces important challenges: first of all, the cultivation of many species for commercial purposes is widely underexplored since the market is largely satisfied by either collection of natural populations or from very small cultivated areas. The variation among and within crops cultivars of aromatic and medicinal plants is a limitation if studies about potential applications of diverse plant extracts do not consider this genetic variation but it is also an opportunity to develop superior genotypes that meet with a higher product quality (Bruce, 2014). By this way, cultivated species with no previous work of selection and breeding of adapted cultivars are subjected to the same uncertainty regarding quality which may be defined as the sum of all factors which contribute directly or indirectly to the safety, effectiveness and acceptability of the product.

The Spanish sage has been so far scarcely cultivated due to the lack of appropriate plant material, so breeding and selection programs as well as life cycle follow-up works are needed. *S. lavandulifolia* wild populations show high morphological and chemical variability to the extent that only in the Iberian Peninsula five different subspecies have been described on the basis of certain morphological characters (Saez, 2010). Monitoring crops with different phytochemical profiles is a key tool for providing information about a proper management that eventually entails

a better quality product. The present work is the most comprehensive follow-up study of the essential oil production in *S. lavandulifolia*. We monitored during four years the essential oil yield and chemical composition of eleven clonal lines caught in previously characterized wild populations (Herráiz-Peñalver et al., 2010; Usano-Aleman et al., 2014a) and transferred to a single plot under cultivation conditions. Despite of growing interest and the commercial importance of essential oils, little is still known about the interaction between different genotypes and the environment as well as the evolution of main essential oil components through the life cycle of the plants. Therefore, the aims of this study were the following: (1) to corroborate if Spanish sage wild phytochemical differences of individual plants are kept when growing in a single and common environment, (2) to evaluate essential oil yields from all clonal lines and their interactions with climatic conditions, (3) to identify the main factors affecting essential oil yield and chemical compositions for the Spanish sage as well as the stability over time of the essential oil quality in different clonal lines established as a single crop.

2. Material and methods

2.1. Origin of the cultivated *Salvia lavandulifolia* eleven clonal lines and their cultivation conditions

A total of four populations of *S. lavandulifolia* located in the Central Spain were previously prospected for characterization and subsequent plant material collection in 2008 (further details in Herráiz-Peñalver et al., 2010; Usano-Aleman et al., 2014a,b). From each of these populations (named A, B, C and D), cuttings from three individual plants were transferred to a greenhouse for a vegetative propagation. After propagation, eleven different clonal lines were developed (one mother plant from location A had no surviving cuttings) for cultivation in the experimental field plot of the Centro de Investigación Agroforestal de Albaladejito (ES-Cuenca). Each clonal line was named according to the population which they came from followed by one number between 1 and 3. The experimental design of the culture consisted of the installation of clonal lines in rows in a single plot. A period of one year (2009) was left after the beginning of the cultivation to allow clones adapting to the new environmental conditions. Inputs as watering and fertilizers were applied only during this adaptation year leaving the crop in rainfed conditions in the years thereafter. Weather conditions of rainfall and temperature in the experimental plot were recorded by a weather station Davis Vantage Pro2™ (Hayward, Ca, U.S.A.).

2.2. Harvest times of the cultivated clonal lines

Aerial parts from the cultivated clones were harvested manually three times per year during 2010, 2011, 2013 and 2014. The year 2012 could not be considered for reasons beyond our control. The three harvest events per year were conducted when plants grew new leaves in vegetative stage in April (LF), when full bloom was achieved (FB) at the end of June and when seeds were mature at the end of September (SM).

2.3. Isolation and chemical analysis of the essential oils

The aerial parts (leaves, stems, and flowers, if any) from each clonal line were dried at room temperature to a constant weight and split in two subsamples for the isolation of the essential oils by hydrodistillation, yield quantification and lastly for the chemical determination. The hydrodistillation was carried out in a Clevenger-type apparatus for 3 h, as described in the European Pharmacopoeia. Each value of an individual sample was considered as the main value for the mentioned two sub-samples. After

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