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Proof of geographical origin of Albanian sage by essential oil analysis

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ABSTRACT

Salvia officinalis L. and the closely related *Salvia fruticosa* Mill. (Lamiaceae) are amongst the economically most important medicinal and aromatic plants. Both species are rich in essential oil, which can vary significantly and is partly responsible for their bioactivity and sensorial properties. Therefore we studied its variability within Albania, one of the main exporters of both species. In *S. officinalis* a clear geographical gradient could be observed from North to South, based mainly on a trade-off between the thujones (α- and β-thujone) and camphor. The differences between the provinces were so pronounced that the essential oil profile could be used to identify the origin of unknown samples from within Albania to a high degree of certainty. The variability within *S. fruticosa* was not as pronounced due to its restricted distribution to coastal regions in the South–West only. No hybrids between the two species could be found in the overlapping distribution areas.

1. Introduction

Salvia officinalis L. (Dalmatian, common or garden sage) and *Salvia fruticosa* Mill. (syn. *Salvia triloba* L.; *Salvia libanotica* Boiss & Gaill.; *Salvia cypria* Kotschy; *Salvia lobryana* Aznav., English: Greek, Turkish or three-lobed sage), both members of the Lamiaceae family, are amongst the most important aromatic and medicinal herbs (Kintzios, 2008). The US imported in 2009, e.g., 2295 metric tons of *S. officinalis*, over 55% of which was exported by Albania (United States Department of Commerce, 2009). The real percentage is in fact much higher (up to 85%) by re-export of Albanian sage by other countries to the US (Herbert Bohrer, personal communication). In trade, *S. fruticosa* is better known as *S. triloba* and in the European Pharmacopoeia the drug is still denominated 'Salviae trilobae folium' (Salviae trilobae folium, 2012).

The two species are rich in essential oil, which accounts for part of their bioactive and aromatic qualities. The essential oil of *S. officinalis* is known for its remarkable variability in the main monoterpene constituents β-pinene, 1,8-cineole, α-thujone, β-thujone and camphor (Boelens and Boelens, 1997; Lawrence, 1998). Compared to *S. officinalis*, *S. fruticosa* is characterized by a higher content of 1,8-cineole and lower contents of α- and β-thujone (Karousou et al., 1998a, b; Länger et al., 1996; Skoula et al., 1999).

Although the thujones are of sensorial importance, their toxic potential was always a matter of debate (Tucker and Maciarello, 1990). While the European Council deregulates the use of thujones in food (Demyttenaere, 2012), EMA decreases the respective limits in medicine (EMA, 2011a, b) which leads in the moment to a contradictory situation in the assessment by different European regulatory bodies. Thujones are GABA antagonists and can produce muscle spasms when

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taken in large doses and they were also suspected to cause absinthism. However, there is not sufficient evidence supporting this hypothesis (Padosch et al., 2006).

Sometimes the differences of a distinct compound in the essential oil between individuals are so big, that they form distinct chemical entities (chemotypes). In the essential oil of *S. officinalis* chemotypes are rather frequent, about 18 essential oil chemotypes have been described so far (Jug-Dujaković et al., 2012; Lamien-Meda et al., 2010; Novak et al., 2006; Perry et al., 1999; Tucker and Maciarello, 1990). Besides the genetic background (Jug-Dujaković et al., 2012) – phenotypically expressed as 'chemotypes' – the essential oil composition is influenced by other factors like ontogenesis (Grausgruber-Gröger et al., 2012; Mirjalili et al., 2006; Putievsky et al., 1986; Santos-Gomes and Fernandes-Ferreira, 2001), plant hormones (Schmiderer et al., 2010) and the environment (Li et al., 1996; Piccaglia and Marotti, 1989; Santos-Gomes and Fernandes-Ferreira, 2001).

S. officinalis and *S. fruticosa* are both occurring in Albania, where *S. fruticosa* has a rather small distribution area in the South–West of the country, while *S. officinalis* is found in the coastal mountain ranges from North to South. The aim of this work was to study the natural variability of both *Salvia* species in Albania. The study also addresses possible hybridizations between the two species in overlapping distribution areas leading to deviating essential oil compositions. Furthermore, discriminant analysis was used to check the possibility to identify the origin of the plant material within Albania by the complex essential oil profile.

2. Material and methods

2.1. Sample collection

Four hundred seventy three samples of *S. officinalis* L. and 32 samples of *S. fruticosa* Mill. (Lamiaceae) were collected at the beginning of flowering from 26 Albanian populations in June 2011 (Table 1). Populations 1 to 10 were originating from the North of Albania (province Shkodra). Populations 11 and 12 were coming from an isolated mountain stock between the center and the South (Shpiraq Mountain, province Berat). All other populations (no. 13–26) including the three *S. fruticosa* populations were sampled in the South of Albania (provinces Vlora and Gjirokastra).

The plant material was dried at room temperature. Voucher specimens are stored at the herbarium of the Institute for Animal Nutrition and Functional Plant Compounds, University of Veterinary Medicine, Vienna, Austria (registration numbers Al_001–Al_626). The specimens were identified by J. Novak according to Tutin et al. (1972).

2.2. Extraction and gas-chromatography

Approximately 200 mg dried leaves were extracted in 2 ml dichloromethane for 30 min in an ultrasonic water bath. The extracts were filtered with cellulose wadding on a Pasteur pipette and transferred to GC-vials.

Table 1
Location of sampled Albanian populations of *Salvia officinalis* and *S. fruticosa*.

Pop. No.	Location	Province	GPS N	GPS E	msm	Sample No.	Species
1	Zagorë	Shkodra	42°18.079′N	19°29.614′E	939	18	*S. officinalis*
2	Drogovoja	Shkodra	–	–		18	*S. officinalis*
3	Koplik, cultivation	Shkodra	42°13.575′N	19°27.416′E	154	23	*S. officinalis*
4	Goraj Budishtë	Shkodra	42°19.640′N	19°28.949′E	536	15	*S. officinalis*
5	Reç	Shkodra	42°14.771′N	19°30.794′E	310	21	*S. officinalis*
6	Reç	Shkodra	42°14.403′N	19°32.056′E	377	24	*S. officinalis*
7	Hot	Shkodra	42°22.061′N	19°26.900′E	206	18	*S. officinalis*
8	Rapsh – Stare	Shkodra	42°22.623′N	19°28.371′E	507	19	*S. officinalis*
9	Llesha	Shkodra	–	–		21	*S. officinalis*
10	Tarabosh Mountain	Shkodra	42°04.048′N	19°25.574′E	40	20	*S. officinalis*
11	Shpiraq Mountain, Sinja	Berat	40°38.667′N	19°52.398′E	731	24	*S. officinalis*
12	Shpiraq Mountain, Paftal	Berat	40°40.584′N	19°52.674′E	581	19	*S. officinalis*
13	Llogara Pass, Dukat	Vlora	40°13.226′N	19°34.736′E	733	23	*S. officinalis*
14	Llogara Pass, Llogara	Vlora	40°11.081′N	19°35.604′E	650	19	*S. officinalis*
15	Llogara Pass	Vlora	40°10.783′N	19°36.802′E	420	15	*S. officinalis*
16	Kopaçez	Vlora	39°59.414′N	20°02.544′E	318	21	*S. officinalis*
17	Muzinë Pass, Krongji	Vlora	39°54.844′N	20°10.503′E	250	31	*S. officinalis*
18	Muzinë Pass, Muzinë – Grapsh	Gjirokastra	39°56.641′N	20°13.900′E	550	23	*S. officinalis*
19	Muzinë Pass, Muzinë – Grapsh	Gjirokastra	39°56.697′N	20°14.098′E	492	11	*S. officinalis*
20	Mali i Gjerë, Libohova	Gjirokastra	40°02.304′N	20°16.567′E	697	17	*S. officinalis*
21	Hormovë	Gjirokastra	40°14.461′N	20°05.487′E	370	26	*S. officinalis*
22	Picar	Gjirokastra	40°10.177′N	20°02.675′E	350	32	*S. officinalis*
23	Bënçë	Gjirokastra	40°16.900′N	20°00.896′E	230	15	*S. officinalis*
24	Llogara Pass, Palasë	Vlora	40°10.304′N	19°36.866′E	313	8	*S. fruticosa*
25	Ilias	Vlora	40°08.511′N	19°40.208′E	310	11	*S. fruticosa*
26	Gjashta Pass	Vlora	39°54.665′N	19°59.509′E	250	13	*S. fruticosa*