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Chemical Composition of the Essential Oils of Five *Salvia* Species Growing Wild or Cultivated From Lebanon

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ABSTRACT

Salvia (sage) species have been used for centuries worldwide used as culinary herbs as well as traditional remedies. In Lebanon, Salvia species: S. fruticosa Mill.; S. judaica Boiss.; S. officinalis L.; S. palaestina Benth.; and S. sclarea L. stand among the most important medicinal and aromatic plants that have high potential to contribute to healthcare and livelihoods. The aim of this study was to assess the chemical composition of the essential oils of these 5 Salvia species by GS-MS analysis. The results displayed significant qualitative and quantitative chemical profiles characterized mainly by 1,8-Cineol, Germacrene-D, α-Thujone, Caryophyllene, Sclareol. The main compounds of tested oils were 1,8-Cineole (21.55%) and β-Pinene (10.1%) in S. fruticosa; Germacrene-D (16.1%) and α-Cadinene (8.3%) in S. judaica; α-Thujone (21.04%) and Caryophyllene (12.48%) in S. officinalis; Sclareol (20.2%) and Caryophyllene (15.6%) in S. palaestina and Terpineol (8.9%) and Germacrene-D (8.81%) in S. sclarea. In spite of the dominance of α-Thujone, the profile of the cultivated S. officinalis was found not to be in accordance with the profile defined by standard ISO 9909 official which necessitates the need for more comprehensive investigation through the various developmental stages of the plant and influence of growing and climatic conditions. Nevertheless, the high contents of the 1,8-Cineol, Germacrene-D, α-Thujone, Caryophyllene and Sclareol in the essential oils confirm their potential as natural remedies, cosmetic and food industries. However, with the growing emphasis on quality control, it is necessary to examine the safety of these oils being a key factor in their suitability for consumption and use.

Keywords
Salvia, Lamiaceae, Essential oils, 1.8-Cineol, Sclareol, Terpineol, Germacrene D.

Introduction

Salvia genus, commonly called Sage, is the largest genus of Lamiaceae (formerly Labiatae) that includes more than 900 shrub and tree species. Members of this genus are spread throughout Eurasia and the Americas and is especially diverse in Central America and in the Mediterranean region (https://www.britannica.com/plant/Salvia). Some members of Salvia are well recognized for their culinary use, herbal medicine, aromatherapy, perfumery and cosmetic applications. In Lebanon, Salvia is represented in the new Flora of Lebanon and Syria [1] by 34 species, two of which are recognized as endemic species. While the essential oils of a few representative of the genus, e.g. *S. officinalis L., S. sclarea L.* and *S. fruticosa* Mill. are recognized as commercially important sources for different purposes [2] the potential of other less known species such as *S. palaestina* Benth. and *S. judaica Boiss*. as good sources for novel compounds and products remain a promising area for exploration.

S. officinalis, known as Common Sage and as Kass'iine in Arabic, is one of the most important medicinal plant worldwide known for its use as diuretic, tonic, antiseptic and antispasmodic as well as to treat large number of diseases [3]. In Lebanon and the Middle East, the plant is used for fever and digestive disorders [4]. In the case of prolonged use, however, or following large doses of extracts and volatile oil of *S. officinalis*, some undesirable effects such as vomiting, tachycardia, allergic reactions, convulsions and other effects may occur [3]. The essential oils of *S. officinalis*, both wild and cultivated, have been extensively investigated in many countries including Europe, Mediterranean region and Middle East. α-Thujone, β-Thujone, 1,8-Cineole, Camphor, α-Humulene, Linalool, Germacrene D, Viridiflorol, α-Pinene, Limonene, and Borneol have been reported as the major constituents of oils of three cultivated varieties in Italy [5] 1,8-Cineole, Camphor, Borneol, α-Humulene and α-Thujone were found to be the major oil components in the plant from Germany [2]. Six chemotypes have been described for European *S. officinalis* displaying different profiles and a subsequent variability in their quality [6,7]. In Jordan, the main compounds were 1,8-Cineole and Camphor [4]. In spite of the considerable traditional importance and economic potential of the plant in Lebanon, the profile of the essential oil of the cultivated *S. officinalis* has to date not been examined.

S. fruticosa (*syn. S. libanotica*) is an endemic species of the Eastern Mediterranean basin. It also stands as a commercial herb for its popularity in culinary use and herbal tea and in traditional medicine in the Middle East [4,8,,9]. In Lebanon, locally known as Kass'iine, Quwayseh, 'Ayzaqan or Kayzahan, Maryamiya, hydrodistillate of the flowering aerial parts of *S. fruticosa* is prepared throughout most of Lebanese villages and used for the treatment of ailments of the digestive system and diabetes. Nevertheless, the reproductive toxicity potentials of *S. fruticosa* was reported [10]. The chemical composition of the essential oils in *S. fruticosa* from different countries including Lebanon was reported to have 1,8-Cineole, α-Thujone, β-Thujone, Camphor, and (E)-Caryophyllene as main volatile components [11-14]. However, different populations displayed high variations in the oil profiles. Skoula et al. reported that certain populations growing wild in Crete exhibited significant differences in oil yield and composition and this variability was mainly attributed to genetic diversity. In addition, in a study from Jordan attempting to develop cultivation protocols of the plant and to compare chemical profiles of propagated individuals, the essential oil was found to contain α-Pinene, 1,8-Cineole, Camphor, and Borneol. No α-Thujone or β-Thujone was detected [8]. In Lebanon, in spite of the widespread popularity of the plant and its economic potential, limited research has been conducted to characterize the chemical profile oil of the plant and there is still a need for more investigations [15].

S. sclarea locally known as Kaff al-dubb and commonly as Clary Sage is also one of the most popular Sage species for its medicinal and aromatic properties as well as its economic importance in liquor, cosmetic and pharmaceutical industries. The plant occurs in the Mediterranean basin mainly and is one of the most important aromatic plants cultivated worldwide as a source of essential oils. The essential oils or extracts of the aerial parts of the *S. sclarea* plant have a broad spectrum of effects: analgesic, anti-inflammatory, antioxidant, antifungal, and antibacterial properties. Essential oil of *S. sclarea* is also used as antiseptic and to clean wounds and as an eyewash. A cluster analysis of the compositions of 39 essential oils of *S. sclarea* from the published literature has shown that essential oils are rich in Linalyl acetate, Linalool and Geraniol/Geranyl acetate, Methyl Chavicol, Germacrene-D, α-Thujone, Thujene, and Manool oxide/Phytol [2]. Due to its scarcity in Lebanon, the plant is less popular in comparison to *S. officinalis* and *S. fruticosa* and the composition of its essential oil has, to date, not been investigated.

S. palaestina, locally known as Kharnah, is also one of the Sage species in the East Mediterranean countries known mainly for culinary use for its distinct pleasant flavor and aroma [9]. The crude methanol extract of the aerial parts of *S. palaestina* has been reported to exhibit antimicrobial, antioxidant and antiproliferative effects [9,16]. The chemical profile of the oil from Turkey was reported to contain Caryophyllene oxide, (E)-Caryophyllene, Pulegone, Terpineol-4, Farnesyl acetone, Carvacrol and α-Copaene as the major constituents. Whereas Eucalyptol (1,8-Cineol) and Camphor were reported as dominant in the oil from Palestine [9]. In Iran, β-Caryophyllene was reported as the only predominant compounds in one study [17] while Germacrene D, β-Bisabolene, 1-Epi-Cubenol, Decanal, β-Caryophyllene and Isobornyl butanoate were the main compounds in another report [18]. In Lebanon, a single previous study from the North of the country reported Sclareol, β-Caryophyllene and Linalool as the main compounds [19]. To date, there has been no further studies on the oil composition from other regions.

S. judaica (Judean Sage), locally known as Quwayseh Yahoudieh, is also another Sage species that is envisaged to potentially possess important therapeutic properties being a member of the Salvia genus. Very little work has been done on the plant [20]. A single study only has to date reported β-Cubebene and Ledol as the main constituents of the oil [21]. Despite the progress in modern approaches in drug discovery, plant derived compounds continue to be valuable sources for therapeutic agents. Further research to examine the chemical profiles of *S. judaica* from other regions presents a very promising endeavor.

Material and Methods

Plant material

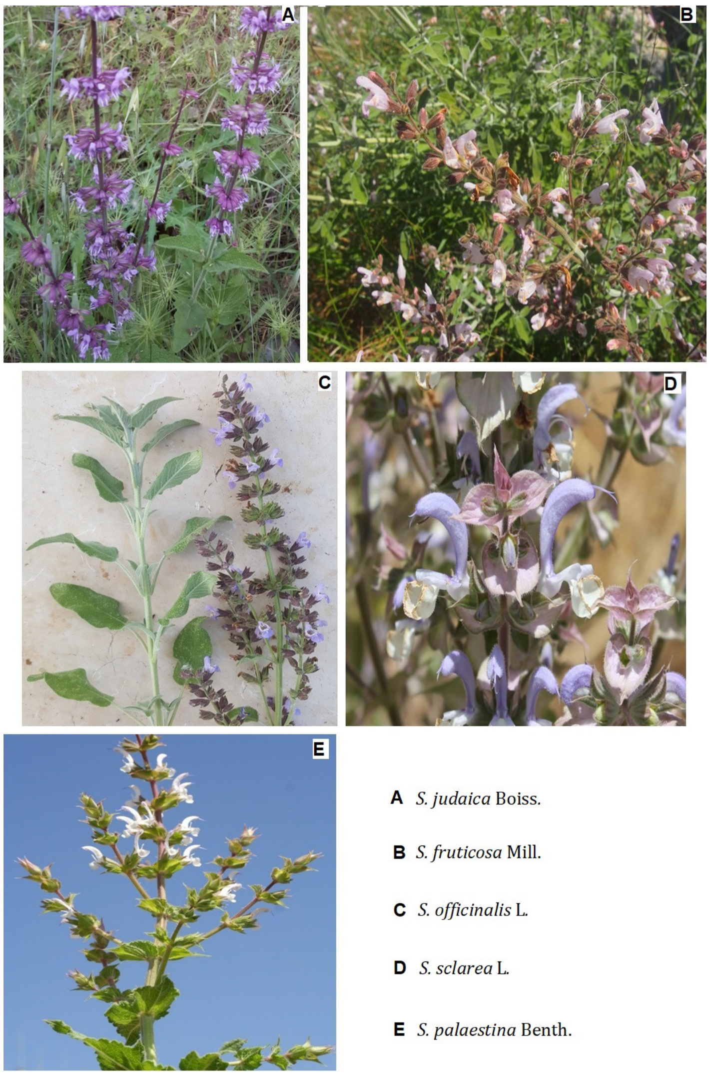
Aerial flowering parts of *S. sclarea*, *S. fruticosa*, *S. palaestina* and *S. judaica* under study were collected from various regions and altitudes where they grow wild in Lebanon. Whereas, *S. officinalis* was collected from the Herbal Garden of Beirut Arab University, Ta'anayel, Bekaa where it is cultivated for experimental purposes. Species identification was performed by Professor Arnold, N. using the determination keys of the New Flora of Lebanon and Syria [1]. Voucher specimens were deposited at the Faculty of Agriculture and Food Sciences, USEK, Kaslik.

Essential oil isolation

The essential oils from the five fresh aerial parts materials were hydrodistilled separately by Clevenger type apparatus for 4 hours [22]. The volatile oils were then dried over anhydrous magnesium sulfate and stored in dark vials at 4°C until analysis. The yield percentages were calculated based on fresh weight of aerial plants parts.

GC and GC/MS analysis

Agilent Technologies 7890 gas chromatography equipped with a Flame Ionization Detector (FID) and a HP-5MS 5% capillary column was used to assess chemical composition of obtained oils. Mass spectra were recorded at 70 e-V of electron energy and a mass range of 50-550 m/Z. The carrier gas was Helium at a flow of 0.8 mL/min. The initial column temperature was 60ºC programmed to increase to 280ºC at a rate of 4oC/min. The split ratio was 1:40. The injection temperature was set at 300oC.The purity of Helium gas was 99.99 %. A sample of 1 µL was injected manually in the split mode. Components identification was based on retention indices and comparison with mass spectral data of authentic standards and computer matching with Wiley 229, Nist 107, Nist 21 libraries as well as by comparing the fragmentation patterns of the mass spectra with those reported in the literature.



Results and Discussion

The yield percentages and main compound groups of each essential oil obtained from the five Salvia sp. under study are presented in Table 1. As indicated, high variations in the content of the different chemical groups are found with monoterpenes recording highest amounts in *S. fruticosa* (68.07%) followed by *S. officinalis* (55.57%), and *S. sclarea* (42.93%), *S. palaestina* (19.00%) and *S. judaica* (11.96%). On the contrary, the content of sesquiterpenes was strikingly high in *S. judaica* (83.72%) followed by *S. palaestina* (77. 4%), *S. sclarea* (52 .77%), *S. officinalis* (38.15%) and *S. fruticosa* (25.67%).

Chemical profile	*S. fruticosa*	*S. judaica*	*S. officinalis*	*S. palaestina*	*S. sclarea*
% yield	0.026	0.055	0.405	0.027	0.233
Number of components	52.00	69.00	52.00	29	75.00
Hydrogenated Monoterpenes (%)	30.76	3.49	11.57	0.3	24.65
Oxygenated Monoterpenes (%)	37.31	8.47	44.00	18.7	18.28
Hydrogenated Sesquiterpenes (%)	24.12	62.12	29.04	41.8	42.37
Oxygenated Sesquiterpenes (%)	1.55	21.6	9.11	35.6	10.40
Others (%)	3.38	4.08	4.43	3.5	3.56
Total (%)	98.12	99.76	98.15	99.9	99.26

Table 1: Main compound groups in the essential oils of Salvia species under study.

The main compounds of the GC and GC/MS analysis of the Salvia species under study are presented in Table 2. As indicated, *S. fruticosa* oil revealed the identification of 52 compounds representing 98.12% of total oil. The major components are 1,8-Cineol (21.55%), β-Pinene (10.1%), Terpineol (9.21%) and Caryophyllene (7.3%). These results are in the line with several studies reporting 1,8-Cineole as the component of highest amount [12,14]. However, considerable variations are noted with the remain profile in terms of quality and quantity. For example, Camphor reported in the cited above studies as a main compound second to 1,8-Cineole was found in much lesser amounts. Neither, α-Thujone being a major compound in many populations of different countries was not detected among the major compound in the herein study [13]. Taking into consideration the high 1,8-Cineole and Camphor contents that are well known for their antibacterial properties, the potential use of antimicrobial activity of the essential oil of *S. fruticosa* from Lebanon supports its high potential of the plant for pharmaceutical, food and cosmetic industries.

In *S. judaica* oil, 69 compounds representing 99.76 % of total volatile oil were identified. The major components were Germacrene-D (16.1%), α-Cadinene (8.3%), Caryophyllene (6.96%). The essential oil from the aerial parts of *S. judaica* showed major differences of that reported from Hungary which was characterized by Ledol (12.00%), β-Cubebene (9.45%), α-Humulene (8.52%), Sclareol (7.01%) [20].

In *S. officinalis* oil, 52 compounds representing 98.15% were identified. The major components were α-Thujone (21.04%), Caryophyllene (12.48%), γ-Gurjunene (9.85%), 1,8-Cineole (9.83%), Epimanoyl oxide (8.2%), Borneol (6.32%), β-Pinene (4.98%) and Camphor (4.3%). In spite of the high content α-Thujone and 1,8-Cineole, the profile doesn't match with the profile defined by the standard ISO 9909 (ANSI, 1997) described as follows: α-Thujone (18–43%), β-Thujone (3–8.5%), Camphor (4.5–24.5%), 1,8-Cineole (5.5–13%), Humulene (0–12%), α-Pinene (1–6.5%), Camphene (1.5–7%), Limonene (0.5–3%), Linalool [free and esterified (1% maximum)], and Bornyl acetate

(2.5% maximum). The presence of Caryophyllene (12.48%) and γ-Gurjunene (9.85%) among the main compounds of the oil stands as a unique feature to be further investigated. A comparison with oil from Jordan having 1,8-Cineole and Camphor as the main compounds and Thujones in very low amounts indicates also high variations between oils [4].

Salvia sp.	Oil Yield (%)	Main components
S. fruticosa	0.026	1,8-Cineol (21.55%), β-Pinene (10.1%), Terpineol (9.21%), Caryophyllene (7.3%), Camphor (6.3%), Camphene (5%) and γ-Gurjunene (4.4%)
S. Judaica	0.055	Germacrene-D (16.1%), α-Cadinene (8.3%), Caryophyllene (6.96%), Epimanoyl oxide (5.06%), δ-Cadinene (4.04), γ-Eudesmol (2.97%) and Rimuene (2.27%)
S. officinalis	0.405	α-Thujone (21.04%), Caryophyllene (12.48%), γ-Gurjunene (9.85%), 1,8-Cineole (9.83%), Epimanoyl oxide (8.2%), Borneol (6.32%), β-Pinene (4.98%) and Camphor (4.3%)
S. palaestina	0.027	Sclareol (20.2%), Linalyl acetate (5.2%), Sclareol oxide (4.5%), Copaene (4.2%), Caryophyllene oxide (3.8%), Isoamyl isovalerate (3.5%), Spathulenol (3.4%) and Bicyclogermacrene (3%)
S. sclarea	0.233	Terpineol (8.9%), Germacrene-D (8.81%), Copaene (3.6%), Sclareol (3.2%), Cis-Abienol (3.2%), Caryophyllene (3.05%), Ocimene (2.6%) and δ-Cadinene (2.5%)

Table 2: The main compounds of the essential oils of Salvia species under study.

The result of *S. Palaestina* analysis showed 29 identified components representing 99.9 % of the total oil. Sclareol (20.2%) represented the major compounds confirming our previous results of the same plant collected from another region in Lebanon. However, the profile was different to that reported from Iran with Germacrene D (14.0%), β-Bisabolene (11.9%), 1-Epi-Cubenol (9.8%) being the main compounds [17] and that of Turkey with Caryophyllene oxide (16.1%) as the major components. With its anti-inflammatory and immunomodulatory properties, Sclareol is suggested as a therapeutic treatment for inflammation associated with rheumatoid arthritis [23].

In *S. sclarea* oil, 75 compounds were identified representing 99.26% of the total oil. The main oil components were Terpineol (8.9%), Germacrene-D (8.81%). The essential oil of *S. sclarea* reported from Spain [13] indicated Linalool (29.36%), Linalyl acetate (18.35%) and α-Terpineol (11.18%) as major compounds, while small amounts of Thujones, Camphor and 1,8-Cineole were found. Higher content of α-Terpineol (47.4%) and lower quantity of ester α-Terpinyl acetate (22.1%) and Linalyl acetate (12.7%) characterized the oil from Sardinia [24].

As can be seen from the results given above, all compositions of our various Salvia species from Lebanon showed great diversity and results are not often agreed with the literature data. As well evident with other medicinal and aromatic plants, the chemical profile of Salvia species essential oils may be influenced by genetic, environmental and ecological factors, growing conditions if cultivated and the plant development stages at collection as well as methods of drying and extraction of the essential oil of the plant [9,25].

Conclusion

This study represents the characterization of chemical profiles of essential oils of five Salvia species from Lebanon. The results displayed significant qualitative and quantitative variations characterized mainly by 1,8-Cineol, Germacrene-D, α-Thujone, Caryophyllene and Sclareol. The presence of these constituents justifies the use of the essential oils under study in a wide range of applications; namely pharmaceutical, food and cosmetic industries. Scientific evidence is still needed to further identify the chemotypes of the wild species growing in different Lebanese regions and to examine the influence of environmental conditions on the chemical profiles of oils. Further, assessing the physical characteristics of the oils as useful references, points of comparison to ensure quality standards in the commercial transactions as well as their efficacy and safety are foreseen as important areas for future research. In addition, Salvia species in this study may serve as good sources for important bioactive compounds and stand the potential to be integrated into development programs to enhance resilience rural communities.

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