

Quality of Sage (*Salvia officinalis* L.) Essential Oil Grown in Egypt

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Abstract

Salvia officinalis (Lamiaceae) is one of the most important medicinal plants has powerful biological activity and pharmacological properties. This work reports the essential oil content and its composition of *Salvia officinalis* grown in Egypt, and determines their active constituents. The volatile oil content has been analyzed by GC/MS. The GC/MS revealed the presence of camphore (23.38%), α -thujone (22.82%), sclareol (10.46%), β -thujone (9.96%), 1,8-cineole (7.83%), γ -selinene (7.73%), α -humulene (5.59%), caryophyllene, (3.16%), borneol (3.06%), limonene (1.74%) and humulene epoxide (1.02%) which represents the main compounds of volatile oil extracted from *Salvia officinalis* fresh herb.

Keywords

Salvia Officinalis, Essential Oil, Camphor, α -Thujone, β -Thujone, Sclareol, 1,8-Cineole

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1. Introduction

Medical and aromatic herbs have been essential components of healthcare throughout human history (Schippmann et al. 2002). Recently, there has been a general opinion that synthetic materials that are commonly used in food and drug industry cause many diseases, such as cancer. This has led to increased global demand for natural and organic forms of medication. As a result of this demand, healing herbs and medicines of herbal origins are commonly used worldwide as a part of traditional culture worldwide, and prefer alternative medicine methods at least once a year, and consume herbal drugs and healing herbs. Previously, local usage of these herbs was common as part of the traditional culture, but today, these herbs have become an important source of national and international trade. It is known that 50,000–75,000 plant species are used in traditional and modern medicine worldwide (Schippmann et al. 2006). Medical and

aromatic herbs are used in such domains as cosmetics, medicine, dye, herbal tea, nutritional supplements, liquor, pesticide and fungicide, essential oil products, perfumes, flavoring liquids and cleaning products.

The Lamiaceae (Labiatae) is one of the most diverse and widespread plant families in terms of ethnomedicine and its medicinal value is based on the volatile oils concentration (Sarac and Ugur, 2007). The Lamiaceae plant family is one of the largest families among the dicotyledons, many species belonging to the family being highly aromatic, due to the presence of external glandular structures that produce volatile oil (Giuliani and Maleci Bini, 2008). This oil is important in pesticide, pharmaceutical, flavouring, perfumery, fragrance and cosmetic industries (Ozkan, 2008). Medicinal plants have an important value in the Socio-cultural, spiritual and medicinal use in rural and tribal lives of the developing countries (Hendawy et al., 2010). People around the world use between 50,000 to 80,000 flowering plants for medicinal

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purposes (Naguib, 2001). Medicinal and aromatic plants are known to be used by 70% to 80% of global population for their medicinal-therapeutic effects as estimated by WHO (2008). Many members of the family are widely cultivated, owing not only to their aromatic qualities but also their ease of cultivation: these plants are among the easiest plants to propagate by stem cuttings. Besides those grown for their edible leaves, some are grown for decorative foliage, such as Coleus. The enlarged Lamiaceae contains about 236 genera and 6,900 to 7,200 species. The largest genera are *Salvia* (900). The most common purpose of medical and aromatic herbs is *Salvia* spp.

Sage, garden sage, or common sage (*Salvia officinalis*, Lamiaceae) is a perennial, evergreen subshrub, with woody stems, grayish leaves, and blue to purplish flowers. It is native to the Mediterranean region, being currently cultivated in various countries (Mirjalili *et al.*, 2006; Raal *et al.*, 2007). It is one of the oldest medicinal plants and also has been used for a long time in folk medicine as medication against fever, rheumatism, perspiration, sexual debility, and in the treatment of chronic bronchitis, as well as mental and nervous diseases (Kamatou *et al.*, 2005). Sage is largely used as a savory food flavouring either as dried leaves or essential oil (Perry *et al.*, 1999). Sage leaves and its essential oil possess carminative, antispasmodic, antiseptic, astringent, and antihidrotic properties (Raal *et al.*, 2007). The predominant medicinally valuable metabolites are monoterpenes (e.g., α - and β -thujone, 1,8-cineole, camphor), diterpenes (e.g., carnosic acid) triterpenes (oleanic and ursolic acids), and phenolic compounds like rosmarinic acid (Lawrence, 1983; Lamaison *et al.*, 1991; Cuvelier *et al.*, 1994). Essential oil of sage and their preparations are externally used for inflammations and infections of the mucous membranes of throat and mouth (stomatitis, gingivitis, and pharyngitis). Internally, the essential oil is used for dyspeptic symptoms and excessive perspiration (Raal *et al.*, 2007). *Salvia officinalis* produces monoterpenes with a broad range of carbon skeletons, including acyclic, monocyclic, and bicyclic compounds. Three distinct monoterpene synthases are responsible for the first steps in the formation of the most characteristic monoterpenes of *S. officinalis* essential oil. The (+)-sabinene synthase catalyzes the production of sabinene, which undergoes further rearrangements leading to the two major monoterpenes, α - and β -thujone. The 1,8-cineole synthase produces in one-step 1,8-cineole. Finally, (+)-bornyldiphosphate synthase produces bornyl diphosphate, which is subsequently hydrolyzed to borneol and then oxidized to camphor (Croteau and Karp, 1977; Croteau *et al.*, 1978; Croteau and Karp, 1979 a,b; Croteau *et al.*, 1994; Wise *et al.*, 1998).

Many studies have focused on the chemical composition of *S.*

officinalis and large variations have been reported from different countries (Boelens and Boelens, 1997; Chalchat *et al.*, 1998; Lawrence, 2001). The essential oil composition differs significantly, depending on the individual genetic variability, different plant parts and development stages (Perry *et al.*, 1999).

The oil is obtained by steam distillation from fresh or dried leaves and flowering tops of the plant. The composition of oil of *S. officinalis* from different countries has been studied by several authors (Fellah *et al.*, 2006; Kanas *et al.*, 1998; Pino *et al.*, 1997; Radulescu *et al.*, 2004; Tsankova *et al.*, 1994; Velickovic *et al.*, 2002). It is composed mainly of monoterpene and sesquiterpene hydrocarbons and their oxygen derivatives. Seasonal variation of oil of *S. officinalis* has also been reported (Asllani, 2000; Mirjalili *et al.*, 2006; Perry *et al.*, 1999; Pitarevic *et al.*, 1984; Santos-Gomez and Fernandez-Ferreira, 2001).

The aim of this study was to establish the chemical composition of the essential oil of *Salvia officinalis* growing in Egypt.

2. Materials and Method

2.1. Plant Material

Seeds of *Salvia officinalis* were obtained from the Komarov Botanical Institute, Saint Petersburg, Russia. Seeds were sown in the nursery on 25th November, 2013. On February 10, 2014, uniform seedlings were transplanted into the experimental farm of the Faculty of Pharmacy, Cairo University, Giza, Egypt, which represents clay-loamy soil. The fresh herb was collected at the end of July.

2.2. Gas Chromatography/Mass Spectrometry (GC/MS)

Extraction of essential oil: Fresh herb (100 g) was extracted by water distillation using Clevenger apparatus for 2 h according to Guenther (1961). GC/MS Analytical Condition: The volatile oil analysis was carried out using gas chromatography-mass spectrometry instrument stands at the Central Laboratories, National Research Center with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC/MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 μ m film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 40 C for 1 min; rising at 4.0 $^{\circ}$ C/min to 160 $^{\circ}$ C and held for 6 min; rising at 6 C/min to 210 C and held for 1min. The injector

and detector were held at 210 °C. Diluted samples (1:10 hexane, v/v) of 0.2 μ L of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using two different analytical methods: relative retention time and mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

3. Results and Discussion

The essential oil of *Salvia officinalis* growing in Egypt was subjected to detailed GC/MS analysis. Exactly 31 compounds, mostly aromatic, were identified, representing 99.59 % of the total oil. The major compounds were camphor 26.38%, 1,8-cineole 17.83 % and α -thujone 13.82%. Other important compounds were β -thujone (5.96%), manoyl oxide (5.46%), humulene (4.59%), caryophyllene (3.86%), γ -selinene (3.73%), limonene (3.54%), borneol (3.06%), α -terpinyl acetate (2.02%), α -terpineol (1.50%), caryophyllene (I3) (1.10%), caryophyllene oxide (1.06%) and humulene epoxide (1.02%), most findings were in agreement with the results presented in this study but with variable concentrations, as indicated that the essential oils obtained from *Salvia officinalis* showed significant variability in their chemical composition depending on location and stages of development (Lawrence, 1981; Tucker and Maciarello, 1990; Mockute et al., 2003). The percentage of oxygenated monoterpenes such as camphor, 1,8-cineole, α -thujone and β -thujone proved that sage essential oil quality (Table 1). Good quality sage oils should contain α -thujone + β -thujone > 50% and camphor < 20% (Guenther, 1949; Putievsky, 1992). According to the ISO specification (ISO, 1997) 18-43% α -thujone and 3-8.5% β -thujone are allowed. For assessing the safety of botanicals and botanical preparations used in food and drug, leaves of common sage containing essential oil is α -thujone 12-65%, β -thujone 1.2-35.6% (total thujone content 30-60%), eucalyptol (1,8-cineole) 8-22.5%, camphor 4.4-30% (CoE, 2008; Raal et al., 2007).

Qualitative composition is similar to that described in literature (Perry et al., 1999; Pino et al., 1997; Santos-Gomes and Fernandes-Ferreira, 2001), not so the quantitative content. The constituents α -pinene, β -pinene, 1,8 cineole, α -thujone, β -thujone, camphor, borneol, α -humulene, viridiflorol, and manool account at least 81% of weight in all samples. This quantitative composition of the samples is quite related to samples reported in Arouca, Portugal (Santos-Gomes and Fernandes-Ferreira, 2001) and New Zealand (Santos-Gomes and Fernandes-Ferreira, 2001).

The oil composition of *S. officinalis* was found to be rich in oxygenated monoterpenes, with its range varying from 59.43

to 70.68%. Main constituents identified were α -thujone, β -thujone, 1,8-cineole, camphor and borneol. The oil composition was found to meet the standards given by ISO 9909 for use of *S. officinalis* for medicinal purposes (Berotiene et al., 2007). Good quality sage oils contain a high percentage (> 50%) of epimeric α - and β - thujones and a low proportion (< 20%) of camphor (Raal et al., 2007). Raina et al. (2013) showed that sage had oil rich in thujone content which ranged from 41.31 to 47.51%. The α -thujone constituted the major constituent, varying from 36.06 to 40.10% in it. α -Thujone is known to be more toxic than β -thujone, and is reported to attribute biological property to the sage oil. The maximum thujone as well as essential oil content in aerial plant parts. In another study, sage was found to be camphor-rich (29.45% to 37.67), with thujone as the second major compound (> 10%). Sage was found to contain comparatively higher sesquiterpenes.

A perusal of earlier results on oil composition of *S. officinalis* from various countries suggested that Italian, French, Romanian, Czech, Portuguese and Turkish sage oils are characterized by camphor (22.0 to 31.79%) as the most important component (Dob et al., 2007). In contrast, samples from many countries are represented by α -thujone (21.5 to 31.5%) as the major compound in their oils. The chemical composition of sage oil from Uttarakhand, India was found to be closely similar to that reported from Italy, Yugoslavia, Bulgaria and Iran. It was characterized by its large amount of oxygenated monoterpenes, with α -thujone, 1,8-cineole and camphor as the principal compounds which are known to exhibit antimicrobial, anti-inflammatory and antioxidant features (Radulescu et al., 2004). Sage leaves and essential oils are known to possess carminative, antispasmodic, antiseptic, and astringent properties. Hence, the essential oils from these sage collections can be used for these applications by the medical industry.

Table 1. Principal constituents of *Salvia officinalis* essential oil.

Compound	%	Compound	%
limonene	3.54	myrtenol	0.26
1,8-cineole	17.83	carveol	0.10
linalool	0.20	ledol	0.95
α -thujone	13.82	γ -selinene	3.73
β - thujone	5.96	caryophyllene oxide	1.06
β -pinene	0.27	santolina triene	0.19
caryophyllene	3.86	spathulenol	0.47
γ -elemene	0.20	humulene epoxide	1.02
(Z)-pinocamphone	0.31	myristicin	0.13
camphor	26.38	isoaromadendrene epoxide	0.31
humulene	4.59	α -santalol	0.11
camphene	0.25	trans-Z- α - bisabolene epoxide	0.25
α -terpineol	1.50	Caryophyllene (I3)	1.10
borneol	3.06	manoyl oxide	5.46
α -terpinyl acetate	2.02	β -selinene	0.45
geranyl acetate	0.21	Total identified compounds	92.45

4. Conclusion

It can be concluded that the major constituents of *Salvia officinalis* L. essential oil grown in Egypt from the aerial parts were camphore (23.38%), α -thujone (22.82%), sclareol (10.46%), β -thujone (9.96%), 1,8-cineole (7.83%), γ -selinene (7.73%), α -humulene (5.59%), caryophyllene, (3.16%), borneol (3.06%), limonene (1.74%) and humulene epoxide (1.02%).

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