

Comparison of Aroma Compounds in Distilled and Extracted Products of Sage (*salvia officinalis* L.)

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Abstract: *Salvia* (*Salvia officinalis*), a member of Lamiaceae family, is valuable medicinal and aromatic plant. Although extraction products like concrete, absolute, and hydrosol from the oil of sage have an economical interest and there are very limited studies on these products. Sage oil and sage water (hydrosol) were released from the fresh herb of sage plants by using steam distillation. Sage concrete was extracted from the fresh herb by using *n*-hexane as a solvent. Also sage absolute was produced from the concrete with ethyl alcohol extraction. Essential oils from these products were analysed by a gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Fresh herb essential oil yield was 0.55 % while hydrosol (aromatic water) oil yield was 0.17 %. The yield of sage concrete was 1.05 % and the concrete had 68.77 % sage absolute. Camphor, *cis*-thujone, 1,8-cineole, and *trans*-thujone were the major components of sage oil, hydrosol oil, sage concrete and sage absolute. Higher amount of viridiflorol was found in the concrete and absolute oils (8.72 % and 10.95 %, respectively) compared with steam distillation oil (2.21 %).

Key words: Sage, *Salvia officinalis* L., Essential oil, Hydrosol, Concrete, Absolute.

Introduction

The Genus *Salvia* L. (sage) of the family Lamiaceae comprises nearly 900 species spread widely throughout the world. *Salvia* is represented in Turkey by 94 taxa belonging to 89 species with 50 % endemism. In Turkey, commercial *Salvia* species belong to the following groups: camphor/1,8-cineole group: *S. fruticosa*, pinene group: *S. tomentosa*, and thujone group: *S. officinalis* ¹.

Sage is well known as a common medicinal and aromatic plant widely used in food, perfumery, herbal medicine and products ²⁻³. Some scientific studies on *Salvia* species showed that the presence of many compounds belonging mainly to the groups of phenolic acids, phenolic glycosides,

flavonoids, anthocyanins, coumarins, polysaccharides, sterols, terpenoids and essential oils ⁴. These compounds in sage extracts and essential oils have strong antiradical activities and antioxidant capacities ⁵, and also antibacterial properties ⁶. Since the polar extracts of *Salvia* species were exhibited excellent antioxidant activities when compared to BHT, it seems possible to keep perishable fat-containing food longer by direct addition of an extract of sage ⁷.

Sage hydrosol is released from a by-product during the distillation in Turkey and is used for herbal drink as natural antibiotic against various bacterial diseases and digestion disorders. Hydrosol contains a part of the essential oil

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compounds dissolved in and remain in the distillation water. It has a very pleasant aroma and is recognized as having commercial applications. A concrete and an absolute can be obtained through extraction with various solvents. Concretes are extracted from aromatic plant materials using volatile solvents such as *n*-hexane, benzene and petroleum ether. After the solvents are evaporated the residues called concretes which is a waxy mass used in cosmetics, but primary usage is to obtain absolutes⁸.

Concrete contains fragrance related compounds like terpenes and sequi-terpenes but also fatty acids and their methyl esters, paraffin and other high molecular weight compounds⁹. Absolutes (wax-free residues which are more widely used in perfumery) are produced with ethanol extraction from the concretes. The ethanol solution is then cooled to about -20°C, filtered to eliminate waxes and then concentrated by vacuum distillation⁸. The advantage of the absolute which is more concentrated with aromatic compounds than the concrete.

Clary sage (*Salvia sclarea* L.) concrete is a valuable product which is used for obtaining absolute. Also isolation of sclareol from absolute is used to make a raw material at the origin of ambrox production, for perfume compositions and cosmetics items. Although concrete and absolute are commonly produced from clary sage¹⁰, there are very limited studies on these products with Dalmatian sage (*Salvia officinalis* L.). This study was aimed to investigate the comparison of aroma compounds of distillation and extraction products from fresh herb of sage.

Materials and methods

Plant material

Sage (*Salvia officinalis* L.) plants were grown under cultivation and harvested above 10 cm soil level on June 21st in 2007 from the research field of Süleyman Demirel University, Faculty of Agriculture, Department of Field Crops in Isparta, Turkey (latitude 37°45' N, longitude 30°33' E, elevation 950 m). The samples were transported in polypropylene bags to the laboratory and held at room temperature to extract. Voucher specimens were kept at the herbarium of the Department of Field Crops in Suleyman Demirel University,

Isparta, Turkey.

Production of the essential oil extraction and hydrosol

The essential oils in the fresh herb were extracted by steam distillation in a pilot plant designed by Rose and Rose Products Research and Implementation Center of Süleyman Demirel University. For each experiment, 25 kg of fresh sage herb was loaded into the distillation vessel and steam was produced from the boiling water (75 L) in the bottom of the vessel. The water steam with essential oil was carried to a water-cooled, parallel-piped multibular steel condenser, where the steam is condensed. The essential oil and hydrosol (the distillate water) were then separated in a 10 L Florentine flask^{8,11}.

Production of concrete and absolute

50 g of fresh herb of sage was extracted with 500 ml of *n*-hexane in a Soxhlet apparatus. Then *n*-hexane was evaporated from the extract by a rotary evaporator. A waxy, greenish semi-solid material (Sage concrete) was obtained. The concrete was dissolved in analytical grade ethyl alcohol (Merck, 96 %) and alcoholic solution was cooled at -20°C in a deep freezer; the crystallized stearoptens were filtered through black band filter paper under vacuum. The separated stearoptens that remained on the filter paper was re-dissolved in 50 mL ethyl alcohol to obtain the liquid filtrates. The filtrate left to stand overnight in a deep freezer and evaporated in vacuum using a rotary evaporator to yield the absolute. During the process of absolute, the concretes were treated with ethyl alcohol for approximately 10 times their own respective weights. Then sage products were stored at 4°C for GC and GC-MS analyses^{8, 12-14}.

GC and GC-MS analysis

The hydrodistilled volatiles, concrete and absolute of sage were analyzed by GC-FID and GC-MS. The GC analysis was carried out on Varian Gas Chromatograph equipped with an FID and 25 m × 0.25 mm × 0.25 µm WCOT column coated with 5 % diphenyl dimethyl siloxane (HP-5). Helium was used as carrier gas at a flow rate of 1.2 mL/min at a column pressure of 22 kPa.

Samples (0.2 µL) were injected into the column with a split ratio of 50:1. Component separation was achieved following a linear temperature program of 60-200°C at 2°C/min and then held at 200°C for 50 min, with a total run time of 120 min. The percentage composition was calculated using peak normalization method assuming equal detector response. The samples were then analyzed on a Shimadzu QP5050 GC-MS system equipped with a Quadrupole detector. GC-MS system operating in the EI mode at 70eV, equipped with a capillary column, CP-Wax 52 CB (50 m x 0.32 mm; film thickness = 0.25 µm). The initial oven temperature of the column was 60°C and was raised to 220°C with a rate of 10°C/min and then kept at 220°C for 10 min. The total run time 60 min, injector temperature 240°C, detector temperature 250°C, carrier gas He, flow rate 20 ml/min. 1 µL of essential oil diluted with *n*-hexane was injected into the GC-MS system. As a reference compounds, pure commercial oil components were purchased from the Sigma Chemical Co., St. Lois, MO, USA.

Peak identification of the constituents was carried out by with the help of retention times of standard substances by composition of mass spectra with the data given in the NIST/TUDOR and Wiley libraries¹⁵⁻¹⁶ (Stein, 1990, Adams 2001). The compound identification was finally confirmed by comparison of their relative retention indices with literature values.

Results and discussion

Table 1 presents the compounds of the distillation and extraction products of sage. The sage oil, hydrosol, concrete and absolute were analysed by GC and GC-MS, and total 26 compounds were detected above the trace level (Table 1). Sage oil yield of the fresh herb was 0.55 % and the oil yield of hydrosol (aromatic water) was 0.17 %. 1,8-cineole (17.93 %), *cis*-thujone (20.06 %), *trans*-thujone (9.11 %) and camphor (19.93 %) in sage oil, and 1,8-cineole (23.99 %), *cis*-thujone (15.46 %), *trans*-thujone (4.41 %) and camphor (43.38 %) in sage hydrosol oil were the main components (Table 1). Problems of the crystallization of camphor, 1,8-cineole and borneol in the condenser¹⁷ were not observed

under the conditions used in this study and therefore can be neglected as the same reported by Langer *et al.*¹⁸.

Variations in the essential oil yield and compositions in sage may be due to the quality of the plant material (influence of harvest time and plant parts, different genetic and chemical types, employment of cultural practices, affects of climate and soil characteristics etc.) as well as to the methods used for their production and analyses^{5, 18, 19-24}. For example, the dominant constituents of the essential oils hydrodistilled from the aerial parts of *Salvia officinalis* collected in 8 gardens in Eastern Lithuania by Bernotiene *et al.*²³ were manool (14.4-20.9 %, 4 oils), 1,8-cineole (12.4-17.6 %, 2 oils), *cis*-thujone (12.6 %, 2 oils).

Steam distillation may be recommended in extraction of essential oils from sage. The steam distillation time is shorter than that of the hydro distillation in the same conditions. Note in this research that over 90 % of the oil was extracted at 90 min, and that no further accumulation of oil was observed after 120 min. A similar result on steam distillation time for lavender (*Lavandula x intermedia* Emeric ex Loisel.) was reported by Tannous *et al.*¹¹ and Baydar and Kineci⁸. Essential oil yield of steam distillation is also much more than that of hydro distillation in the aromatic plants²⁵. For example, while the yield of essential oil obtained by the hydro distillation is much lower at only 0.44 %, the yield of essential oil obtained by the steam distillation is 1.2 % in rosemary (*Rosmarinus officinalis* L.)²⁶.

ISO 9909 for medicinal uses regulates the amounts of the following constituents in the sage essential oils: *cis*-thujone (8.0-43.0 %), camphor (4.5-24.5 %), 1,8-cineole (5.5-13.0 %), *trans*-thujone (3.0-8.5 %), α -humulene (<12.0 %), α -pinene (1.0-6.5 %), camphene (1.5-7.0 %), limonene (0.5-3.0 %), bornyl acetate (<2.5 %) and linalool+linalyl acetate (<1.0 %)²³. The chemical composition of the *S. officinalis* essential oils under study nearly matched the requirements of ISO 9909. The sage oils from the plants cultured in Isparta region of Turkey contained a bit higher amounts of 1,8-cineole and *trans*-thujone than required by the mentioned acts (Table 1).

The hydrosol, which included 0.17 % essential

Table 1. Composition of the distilled and extracted products of sage

RI ^a	Components	Distillation and extraction products (%)			
		Essential oil	Hydrosol	Concrete	Absolute
1059	α -Pinene	2.80	tr	tr	0.20
1135	β -Pinene	2.53	tr	tr	tr
1221	Camphene	3.52	tr	0.98	0.27
1259	1,8-Cineole	17.93	23.99	18.31	6.51
1298	Sabinene	0.11	tr	tr	0.56
1324	γ -Terpinene	0.34	tr	tr	tr
1379	Myrcene	1.18	tr	tr	tr
1429	α -Terpinene	0.11	tr	tr	tr
1471	Limonene	1.83	tr	tr	tr
1691	p-Cymene	0.48	tr	tr	tr
1988	Camphor	19.93	43.38	27.00	19.92
2009	Linalool	1.10	2.70	6.84	11.44
2100	Linalyl acetate	0.73	tr	7.59	11.49
2163	<i>cis</i> -Thujone	20.06	15.46	13.97	11.16
2235	<i>trans</i> -Thujone	9.11	4.41	1.68	3.03
2275	Caryophyllene	4.41	tr	1.77	3.30
2498	α -Humulene	1.78	tr	tr	tr
2541	Borneole	4.56	7.66	7.93	8.12
2593	Terpineol	0.18	tr	tr	tr
2624	Bornyl acetate	1.28	tr	tr	0.90
2854	Myrtenol	0.51	0.55	tr	tr
2920	Verbenone	0.11	tr	tr	0.71
2938	Caryophyllene oxide	0.88	tr	3.55	4.55
2956	Humulene oxide	0.43	1.12	tr	1.94
2978	Viridiflorol	2.21	tr	8.72	10.95
	Total detected (%)	98.11	99.27	98.34	95.05
	Yield (%)	0.55	0.17	1.05	68.77

^a The retention index (RI) relative to C9-C30 *n*-alkanes from electronic integration measurements using mass quadrupole detector (GC/MS) on CP-Wax 52 CB column.

tr trace, (<0.01%)

%, relative percentage

oil that remained the water fraction, was highly aromatic and its composition was rather different than the prime essential oil. While sage essential oil from steam distillation contained 28 oil compounds, hydrosol from a by-product during the steam distillation contained only 8 compounds which were *cis*-thujone, *trans*-thujone, 1,8-cineole, camphor, linalool, borneole, myrtenol and viridiflorol (Table 1). Camphor was the dominant compound found in the sage hydrosol (43.38 %).

This can be explained that camphor is highly soluble in distillation water.

The yield of sage concrete was 1.05 % and 68.77 % of the concrete was the sage absolute. Twelve compounds were detected in concrete essential oil, and 16 components were in absolute essential oil. The detected components in sage concrete were 1,8-cineole (18.31 %), α -thujone (13.97 %), β -thujone (1.68 %) and camphor (27.00 %). 1,8-cineole (6.51 %), α -thujone (11.16 %), β -thujone

(3.03 %) and camphor (19.92 %) were the major components in sage absolute (Table 1). The differences between concrete and absolute compositions were mainly due to the polarity of the organic solvents used in the extraction (*n*-hexane is non-polar, and ethyl alcohol is polar solvent).

A remarkable characteristic difference of the extraction products from the steam distillation products were linalool, linalyl acetate and viridiflorol. These compounds were found in higher levels than that of other constituents in the concrete and absolute essential oils (Table 1). No fatty acids or fatty acid methyl esters were detected in sage concrete. Essential oil composition of sage absolute is suited to the ISO 9909 standards for *cis*-thujone, *trans*-thujone, 1,8-cineole, camphor, bornyl acetate and α -humulene, but contained much more values especially for linalool+linalyl acetate. The volatile solvents used in the extraction can influence the yield and composition of concrete and absolute. The present study showed that sage could be an alternative product for oil-bearing rose. Since G ller region of Turkey, especially Isparta province, is the center of oil-bearing rose (*Rosa damascena* Mill.) products such as rose oil, rose water, rose concrete and rose absolute in the world ²⁷. The factories that processing oil-bearing rose need alternative

aromatic plants to keep on working out off oil-bearing rose season.

Conclusion

In conclusion, sage could be a valuable aromatic material for not only essential oil production, but also for hydrosol, concrete and absolute production with higher yield and quality. The oil yield was 0.55 % in the fresh herb (1.92 % in the dried herb). 95.2 kg of fresh herb was needed to produce 1 kg of concrete, and 1 kg concrete had 68.8 g absolute. The essential oil was dominated by *cis*-thujone (20.06 %), hydrosol oil was dominated by camphor (43.38 %). Sage concrete and absolute were characterized by the higher contents of camphor (27.0 % and 19.92 %, respectively) and lower contents of *cis*-thujone (13.97 % and 11.16 %, respectively) and *trans*-thujone (1.68 % and 3.03 %, respectively) in comparison with essential oil components. Although thujones is a toxic compound found in sage essential oils ²⁸, sage concrete and absolute have an advantage from this point of view. The results could potentially be beneficial, since essential oil producers need alternative essential oil crops producing marketable aromatic products, and this study might open new opportunities to the exploitation of sage distillation and extraction products with high quality in the world.

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