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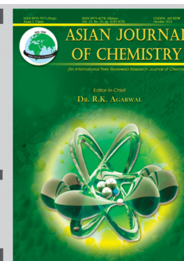

Sage (*Salvia aucheri* Bentham var. *canescens* Boiss. and Heldr.): Essential Oil Composition, Phenolics and Antioxidant Activity

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The oil yield of dried plants (v/dw) (0.9 %) was determined by hydrodistillation. The essential oil composition of *S. aucheri* was analyzed by GC-MS. Thirteen compounds were identified, representing 93.95 % of the oil. The main constituents of the oils were: 1,8-cineole (44.43 %), camphor (19.04 %), α-pinene (6.38 %), borneol (5.64 %), (5.82), camphene (5.82 %) and β-pinene (5.12 %), respectively. 1,8-Cineole and camphor were the major components of *S. aucheri*. var. *canescens*. Antiradical activity (IC_{50}) of the essential oil was established as 41.31 = μg/mL. In addition, phenolic components and antioxidant effect of extracts from *S. aucheri* were determined. The extract yield (%) of *S. aucheri* was 13.58. Main phenolic components (mg/100 g dried herb) were found as rosmarinic acid and carvacrol. Total phenolic content and antiradical activity (IC_{50}) of the extract were also determined as 112.14 mg GAE/g extract and IC_{50} = 75.69 μg/mL.

Key Words: *Salvia aucheri* var. *canescens*, Essential oil, Phenolics, Antioxidant.

INTRODUCTION

The sage plant, *Salvia*, belongs to the family Lamiaceae, which also includes the mints, many species and varieties of which grow wild ora recultivated in many parts of the world[1,2]. Many species of the genus *Salvia* are to be found in Turkey. Due to the various usage of this genus, we intend to determine the oil and extracts of *S. aucheri* which grows in Turkey. *Salvia aucheri* Bentham var. *canescens* Boiss. and Heldr., a member of the family Labiatae, is a perennial plant endemic to Turkey[3]. In recent years, several studies have been performed on the chemical composition of *Salvia* spp. oils of different origins[4-8]. An infusion of aerial parts of *Salvia* spp. is used as a tonic, carminative, antiseptic, spasmolytic, astringent, haemostatic and diuretic[9]. The antioxidant activity of herb and spices, caused mainly by phenolic compounds, has been demonstrated in many studies during recent years[10-14]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides[15].

The purpose of this study was to determine total extract and oil yield, total phenolics, antiradical activity, phenolic compounds and essential oil composition of aerial parts of *Salvia aucheri* growing wild in Kavakolugu location (Gülnar-Mersin) in Turkey.

EXPERIMENTAL

Aerial parts of *Salvia aucheri* Bentham var. *canescens* Boiss. and Heldr. was collected during their flowering periods (June-August 2009) near Mersin (Kavakolugu-Gülnar) and South area of Turkey. Voucher specimens (Ozcan-102) were deposited at the Herbarium of the Department of Food Engineering, Selçuk Universty, Konya, Turkey.

Isolation of essential oil: Dried and powdered herb material (200 g, each) was distilled for 3 h using Clevenger-type apparatus. The essential oils dried over anhydrous sodium sulphate and, after filtration, stored at -20 ºC until use.

Preparation of the extract: Dried and powdered herb material (15 g) was extracted with 150 mL methanol for 4 h at 60 ºC by using an Soxhlet. The extracts were filtered and the solvent mixtures were concentrated by using rotary evaporator (Rotavator, T < 40 ºC) under vacuum to get crude extracts. The residue was stored at -20 ºC until use.

Analysis of essential oil components: Analyses of the essential oil components were performed on GC-MS/ Quadropole detector analyses, using a Shimadzu QP 5050 system, fitted with an FFAP (50 m × 0.32 mm (i.d.), film thickness: 0.25 μm) capillary column. Detector and injector temperatures were set at 240 ºC. The temperature program for FFAP column was from 60 ºC (1 min) to 220 ºC at a rate of 5 ºC min^{-1} and than held at 220 ºC for 35 min. Helium was used as a

carrier gas at a flow 14 psi. (Split 1:20) and injection volume of each sample was 5 µL. The identification of the components was based on comparison of their mass spectra with those of Wiley and Nist, Tutore Libraries. The ionization energy was set at 70 eV.

Analysis of phenolic constituents: The procedure for quantitaion of the phenolic compounds has previously been described by Capanio *et al.*[16]. The reversed phase-high performance liquid chromatography (RP-HPLC) was used. Detection and quantification was carried out with a SCL-10 Avp System controller, a SIL-10AD vp Autosampler, a LC-10AD vp pump, a DGU-14a degasser, a CTO-10 A vp column heater and a diode array detector set at 278 nm. Agilent Eclipse XDB C_{18} column (250 mm × 4.6 mm), 5 µ was used. The flow rate was 0.8 mL/min, injection volume was 10 µL and the column temperature was set at 30 ºC. Gradient elution of two solvents was used: Solvent A consisted of acetic acid-water (2:98, v/v), solvent B: methanol and the gradient programme used is given in Table-1. The data were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software System (Chiyoda-ku, Tokyo, Japan). The extract samples, standard solutions and mobile phases were filtered by a 0.45 µm pore size membrane filter (Vivascience AG, Hannover, Germany). The amount of phenolic compounds in the extract was calculated as mg/100 g herb using external calibration curves, constructed for each pure phenolic standard. All determinations were carried out in triplicate and the results were averaged.

TABLE-1
SOLVENT GRADIENT CONDUTIONS
WITH LINEAR GRADIENT

Final time	A (%)	B (%)
(Initial)	95	5
3	84	16
18	84	16
20	84	16
30	84	16
40	84	16
50	60	40
55	55	45
65	50	50
70	45	55
75	0	100
80	0	100

A (solvent): Acetic–water (2:98 v/v), B (solvent): Methanol.

Determination of total phenolics and free-radical scavenging activity: Total phenolics were determined according to the method adapted by the Folin-Ciocalteu colorimetric method[17]. Estimations were carried out in triplicate and calculated from a calibration curve obtained with gallic acid and total phenolics were expressed as gallic acid equivalent (mg GAE g^{-1} extract).

Free-radical scavenging activity was measured by DPPH method of and calculated according to the following formula: free-radical scavenging activity = 100 × (absorbance of control sample - absorbance of sample/absorbance of control sample). Extract concentration providing 50 % inhibition (IC_{50}) was calculated from the plot of inhibition percentage against extract concentration.

Statistical analyses: Results of the study were evaluated by variance analysis and the differences between the groups were established by Duncan multiple comparison test[18].

RESULTS AND DISCUSSION

The total yield, total phenolics and antioxidant activity of *S. aucheri* were established as 13.58 %, 112.14 mgGAE/g extract and 75.69 IC_{50} = g/µmL, respectively. In additon, the essential oil yield and antiradical activity values of *S. aucheri* were determined as 0.90 % and 41.33 IC_{50} = g/µmL, respectively. However, total phenolic content of essential oil could not be determined (Table-2). Gallic acid, (+)-catechin, caffeic acid, vitexin, hesperidin, rosmarinic acid, quercetin, naringenin, luteolin, carvacrol were established in *S. aucheri* extract by HPLC. Main phenolic components (mg/100 g dried herb) were established as rosmarinic acid (860.33) and carvacrol (1523.73) (Table-3). The main antioxidative effect of sage was reported to relate to the presence of carnosic acid, carnosol and rosmarinic acid[19]. Lu and Foo[20] studied the antioxidant activity of flavonoids and phenolic acids isolated from sage, using three different test methods, such as DPPH free radical, superoxide anion radical. The antioxidant activity of the flavonoids was variable and that those with a catechol B-ring (luteolin glycosides) were more active than those without (apigenin glycosides). The activity of the extracts is attributed to their hydrogen donating ability[21]. It is well known that free radicals cause autoxidation of unsaturated lipids in feed. Antioxidant molecules present or inhibit these harmful reactions[22,23]. Özcan *et al.*[14], established that the total phenolic contents of several herb extracts were found between 1.2848 mgGAE/g extract (basil) to 10.5832 mg GAE/g extract (oregano). The total phenol content of extract was found to be higher when compared with literature values[14]. The percentage composition of the oils is given in Table-4. Thirteen compounds were identified, representing 93.95 % of the oil. The main constituents of the oils were: 1,8-cineole (44.43 %), camphor (19.04 %), α-pinene (6.38 %), borneol (5.64 %), (5.82), camphene (5.82 %) and β-pinene (5.12 %), respectively. 1,8-Cineole and camphor were the major components of *S. aucheri*. var. *canescens*. The oil contained mainly oxygenated monoterpenes.

Özcan *et al.*[23], reported that fourty-four were identified in the oil constituting 96.3 % of the total components detected. 1,8-cineole (38.9 %), camphor (18.3 %), camphene (7.9 %) and β-pinene (4.3 %) were found to be major components in the oil. In other study, Özcan *et al.*[24], established 1.4 and 1.7 % oil yield at the *S. aucheri* collected two different locations (Gülnar and Silifke). The main constituents of the oils were 1,8-cineole (32.3 %, 28.6 %), camphor (18.9 %, 22.8 %), borneol (8.2 %, 8.9 %), α-pinene (6.3 %, 9.0 %) and β-pinene (5.3 %, 6.2 %), respectively. In a previous investigation on *S. aucheri*, Holemen *et al.*[25], mentioned for a Moroccon sample in postflowering stage that the oil contained camphor (44.1 %), α-pinene (12.3 %) and 1,8-cineole (8.8 %) as the major components. The essential oils of *Salvia* spp. have been the subject of various analyses. Tha main component of *S. fruticosa* oil was reported to be 1,8-cineole (55.5 %)[1]. From *S. cryptantha* oil borneol (24.8), camphor (17.5 %) and 1,8-cineole (10.4 %) have been the major constituents[1], whereas from the same

TABLE-2
TOTAL YIELD, TOTAL PHENOLICS AND ANTIRADICAL ACTIVITY OF S.AUCHERI

	Total yield (%)	Total phenolics (mg GAE/g extract)	Antiradical activity (IC_{50} = μg/mL)
Extract	13.58 ± 0.08*	112.14 ± 3.88	75.69 ± 0.59
Essential oil	0.90 ± 0.01	–	41.31 ± 0.01
BHT	–	–	96.13 ± 0.01

*The values are the average of three determinations (± SD).

TABLE-3
PHENOLIC CONTENTS S.AUCHERI EXTRACT BY HPLC

Phenolic compounds	Retention time (min)	Amount (mg/100 g)
Gallic acid	6.776	2.07 ± 0.12
(+)-Catechin	17.59	361.00 ± 9.54
Caffeic acid	22.508	2.50 ± 0.17
(–)-Epicatecin	25.22	Not detected
Vitexin	41.671	Not detected
Rutin	49.261	44.63 ± 6.40
Naringin	49.847	Not detected
Hesperidin	52.968	21.13 ± 2.11
Apigenin 7-glucoside	55.557	Not detected
Rosmarinic acid	56.656	860.33 ± 11.50
Eriodictiol	60.239	Not detected
Quercetin	71.017	79.43 ± 1.61
Naringenin	71.707	7.60 ± 0.30
Luteolin	74.37	7.47 ± 0.21
Genistin	74.849	Not detected
Apigenin	77.872	Not detected
Acecetin	80.761	Not detected

TABLE-4
ESSENTIAL OIL COMPOSITION OF *S. aucheri*

Compounds	RT	Compositions
α-Pinene	8.82	6.38 ± 0.21*
Camphene	10.59	5.82 ± 1.00
β-Pinene	13.60	5.12 ± 0.99
β-Myrcene	14.81	1.13 ± 0.07
Limonene	17.01	1.55 ± 0.05
Eucalyptol (1.8-cineole)	17.66	44.43 ± 3.20
γ-Terpinene	18.30	0.21 ± 0.00
Camphor	38.60	19.04 ± 0.08
Bornyl Acetate	41.43	0.34 ± 0.08
Caryopyllene	40.60	3.91 ± 0.05
α-Humulene	45.20	0.38 ± 0.07
Borneol	46.90	5.64 ± 1.06

*The values are the average of three determinations (± SD).

species from different locations in Turkey the dominating component was 1,8-cineole (15.7-37.1 %)[26]. The oil of *S. caespitosa* was characterized by the presence of camphor (20.7 %), 1,8-cineole (16.1 %) and α-pinene (13.5 %)[27]. β-pinene (34.4 %) and α-pinene (22.6 %) were the main components of the oil of *S. candissima*[1]. *S. pomifera* essential oil was found to contain β-thujene (50-67 %) as the dominating component[28].

Conclusion

As a result, it can be said that plant extract are good phenol resources. Also, several derivatives such as essential oil and extracts of medicinal and aromatic plants can be used as preservatives for food. Further studies should be carried out on biofunctional properties of these plant derivatives *in vitro*.The analyzed *S. aucheri* var. *canescens* oil has the typical high content in oxygenated compounds of *Salvia* oil. When the results were compared with the literature, the oil showed significant differences or climatological factors or development stages or plant parts analyzed.

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REFERENCES

1. A. Bayrak and A. Akgül, *Phytochemistry*, **26**, 846 (1987).
2. F. Sefidkon and M.S. Khajavi, *Flav. Fragr. J.*, **14**, 77 (1999).
3. P.H. Davis, Flora of Turkey and the Aegean Islands, Edinburgh University Press: Edinburgh, Vol. 7, p. 947 (1982).
4. M. Couladis, O. Tzakou, D. Stojanovic, N. Mimica-Dukic and R. Rancic, *Flav. Fragr. J.*, **16**, 227 (2001).
5. P. Salehi, F. Sefidkon, L.B. Tolami and A. Sonboli, *Flav. Fragr. J.*, **20**, 525 (2005).
6. D. Pitarokili, O. Tzakou and A. Loukis, *Flav. Fragr. J.*, **21**, 670 (2006).
7. R. Kowalski and J. Wawrzykowski, *Flav. Fragr. J.*, **24**, 31 (2009).
8. M.B. Taarit, K. Msaada, K. Hosni, T. Chahed and B. Marzouk, *J. Food Biochem.*, **34**, 142 (2010).
9. C.A. Newall, L.A. Anderson and J.D. Phillipson, Herbal Medicines: A Guide for Healthcare Professionals, Pharmaceutical Press: London, p. 231 (1996).
10. M. Özcan and A. Akgül, *Acta Aliment.*, **24**, 81 (1995).
11. C.A. Rice-Evans, N.J. Miller, P.G. Bolwell, P.M. Bramley and J.B. Pridham, *Free Radic. Res.*, **22**, 375 (1995).
12. V. Exarchou, N. Nenadis, M. Tsimidou, I.P. Gerothanasis, A. Troganis and B. Boskou, *J. Agric. Food Chem.*, **50**, 5294 (2002).
13. M.P. Skerget, M. Kotnik, A.R. Hadolin, M. Hras Simonic and Z. Knez, *Food Chem.*, **89**, 191 (2005).
14. M.M. Özcan, Ö. Erel and E.E. Herken, *J. Med. Food*, **12**, 198 (2009).
15. T. Osawa, In eds.: I. Uritani, V.V. Garcia and E.M. Mendoza, Novel Natural Antioxidants for Utilization in Food and Biological Systems, In: Postharvest Biochemistry of Plant Food Materials in the Tropics Leds, Japan Scientific Societies Press, Tokyo, pp. 241-251 (1994).
16. F. Capanio, V. Alloggio and T. Gomes, *Food Chem.*, **64**, 203 (1999).
17. V.L. Singleton and J.R. Rossi, *Am. J. Enol. Vitis*, **16**, 144 (1965).
18. O. Düzgünes, T. Kesici, O. Kavuncu and F. Gürbüz,, Research and Experiment Methods, Arastirma ve Deneme Metotlari, Ankara Üniv. Zir. Fak. Yay. No: 295, Ankara (1987).
19. M.E. Cuvelier, H. Richard and C. Berset, *J. Am. Oil Chem. Soc.*, **73**, 645 (1996).
20. Y. Lu and L.Y. Foo, *Food Chem.*, **75**, 197 (1996).
21. K.K. Shimada, K.Y. Fujikawa and T. Nakamura, *J. Agric. Food Chem.*, **40**, 945 (1992).
22. H. Kaur and J. Perkins, In eds.: O.I. Aruoma and B. Halliwell, The Free Radical Chemistry of Food Additives, In: Free Radicals and Food Additives, Taylor & Francis Ltd., London, pp. 17-35 (1991).
23. M. Özcan, A. Akgül and J.C. Chalchat, *J. Essent. Oil Res.*, **14**, 339 (2002).
24. M. Özcan, O. Tzakou and M. Couladis, *Flav. Fragr. J.*, **18**, 325 (2003).
25. M. Holemen, M. Berrada, J. Bellakhdar, A. Ilidrissi and R. Pinel, *Fitoterapia*, **55**, 143 (1984).
26. K.H.C. Baser, S.H. Beis and T. Özek, *J. Essent. Oil Res.*, **7**, 113 (1995).
27. K.H.C. Baser, M. Kürkçüoglu, T. Özek and S. Sarikardasoglu, *J. Essent. Oil Res.*, **7**, 229 (1995).
28. K.H.C. Baser, T. Özek, N. Kirimer and G. Tümen, *J. Essent. Oil Res.*, **5**, 347 (1993).