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# Variations in Essential Oil, Phenolic Compounds, and Antioxidant Activity of Tunisian Cultivated *Salvia officinalis* L.

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The variation in the chemical composition of the essential oil of *Salvia officinalis*, growing in different habitats, was studied. GC-MS analysis revealed 57 compounds representing 94.68−96.80% of total oils. The major components were α-thujone (11.55−19.23%), viridiflorol (9.94−19.46%), 1,8-cineole (8.85−15.60%), camphor (5.08−15.06%), manool (5.52−13.06%), β-caryophyllene (2.63−9.24%), α-humulene (1.93−8.94%), and β-thujone (5.45−6.17%), showing significant differences between different collection sites. Analysis of some representative polyphenolic compounds and antioxidant activity was performed using postdistilled dry samples. Rosmarinic acid, carnosol, and carnosic acid were the prevalent compounds of *S. officinalis* methanolic extracts. The results revealed differences in the polyphenolic composition and also exhibited antioxidant and radical-scavenging activities at different magnitudes of potency. However, within the used methods, only the DPPH• assay showed significant differences ($p < 0.05$) in free radical scavenging activity among samples collected in different regions. Plants collected in the coastal regions Soliman and Kelibia accumulate more polyphenolic compounds, known to be responsible for the main antioxidant activity of sage (rosmarinic acid, carnosol, and carnosic acid), than those growing inland at Bou Arada and Sers. Moreover, the former presented a higher radical-scavenging activity. The methanolic extracts of postdistilled *S. officinalis* might be valuable antioxidant natural sources and seemed to be applicable in both the health medicine and food industries.

**KEYWORDS: *Salvia officinalis* L.; essential oil; polyphenolic compounds; antioxidant activity; DPPH; ABTS; FRAP**

## INTRODUCTION

*Salvia officinalis* is a perennial woody subshrub native to the Mediterranean region. The popular species is largely cultivated for culinary and medicinal purposes. The curative properties of sage have long been known; it is used as an antihydrotic, spasmolytic, antiseptic, and anti-inflammatory and in the treatment of mental and nervous conditions (*1*). Recently, it has been demonstrated that sage essential oil can improve the memory, showing promise in the treatment of Alzheimer's disease (*2*), and it has also a potential in treating cancer as it shows strong antitumorigenic activities (*3*).

Terpenoids and phenolics have been identified as the two major typical products of *S. officinalis* secondary metabolites (*4*). Among the terpenoids, volatile oils have been largely investigated (*4*−*6*) because of their broad range of applications in culinary, cosmetic, pharmaceutical, and industrial fields. The essential oil composition of *Salvia* species is highly influenced by genetic and environmental factors (*7*), organ age (*8*), climate conditions (*9*), and organ, season, and culture site (*10*, *5*). Because of such variation, the sage essential oil composition sometimes does not match the profile defined by standard ISO 9909 (*11*).

On the other hand, sage polyphenolic compounds showed multiple biological effects including antioxidant, antiplatelet, antitumor, and antiviral activities (*12*). The search for natural antioxidants in aromatic plant byproducts has become an alternative to synthetic antioxidants in the food and pharmaceutical industries (*13*−*16*). These authors considered the study of the remaining distilled material potentially interesting as a result of the water-soluble properties of phenolic compounds that rarely form part of the essential oils. Phenolic compounds tend to be water-soluble, because they frequently occur combined as glycosides, and they are usually located in the cell vacuole (*17*).

Consequently, these compounds constitute an interesting target in the search for health-beneficial phytochemicals and also offer a possibility to use phenolic compounds or phenolic extracts to stabilize fat and fat-containing foods (*18*). The beneficial effects of those molecules are related to their antioxidant activity, particularly their ability to scavenge free radicals, to donate hydrogen atoms or electrons, or to chelate metal cations (*19*).

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**Table 1.** Collection Sites of Cultivated *Salvia officinalis* and Their Eco-geographical Characteristics

| no. | collection site | code | bioclimatic stage | soil pH | rainfall (mm/year) | temperature (°C/year) | geographical location | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | longitude (N) | latitude (E) | altitude (m) |
| 1 | Kelibia | OK | subhumid | 7.66 | 450 | 17.3 | 36° 51′ 00″ | 11° 05′ 12″ | 17 |
| 2 | Soliman | OS | semiarid superior | 8.02 | 500 | 19.2 | 36° 41′ 48″ | 10° 29′ 32″ | 16 |
| 3 | Bou Arada | OB | semiarid moderate | 8.15 | 450 | 17.8 | 36° 21′ 02″ | 9° 37′ 48″ | 252 |
| 4 | Sers | OR | semiarid moderate | 7.15 | 700 | 16.8 | 36° 04′ 38″ | 9° 01′ 16″ | 487 |

The main antioxidant activity of *S. officinalis* was attributed to rosmarinic acid and the diterpene phenolics carnosol and carnosic acid (*20*). However, sage extracts also contain flavonoids and other phenolics that may contribute to the total antioxidant activity (*21*). Variation in environmental factors affected the composition of sage phenolic extracts and consequently their antioxidative power (*20*, *22*).

To the best of our knowledge, variations in essential oils, postdistilled aerial parts extracts, and antioxidant activities of methanolic extracts of Tunisian *S. officinalis* growing in different habitats have not yet been reported. This study was undertaken with the aim to identify the composition of essential oils and some representative polyphenolic compounds of the methanolic extracts from postdistilled sage and to test extracts for antioxidant capacity to valorize *S. officinalis* as a source of bioactive molecules, according to its collection site.

## MATERIALS AND METHODS

**Plant Material.** *S. officinalis* L., which does not occur wild in Tunisia, has been cultivated in different regions of the country for a long time. Aerial parts from 10 cultivated plants of *S. officinalis* were randomly collected from different regions in northern Tunisia. Plant material from different genotypes was harvested at the flowering period in March and April 2008. Details of collection sites are provided in **Table 1**. A voucher specimen was deposited at the Herbarium of the Laboratory of Biochemistry and Molecular Biology at the Faculty of Sciences of Bizerte under the numbers SO 2008-121, SO 2008-122, SO 2008-123, and SO 2008-124), respectively, for the Kelibia, Soliman, Bou Arada, and Sers sites.

**Chemicals.** 2,2-Diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt [ABTS$(NH_4)_2$], 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium persulfate, homologous series of $C_6-C_{17}$ *n*-alkanes, and high-purity standards were purchased from Sigma-Aldrich (Madrid, Spain). Methanol, acetonitrile, petroleum ether, formic acid, ethanol, glacial acetic acid, hydrochloric acid, anhydrous sodium carbonate, $FeCl_3 \cdot 6H_2O$, sodium acetate, anhydrous sodium sulfate were supplied from Scharlau Chemie S. A. (Sentmenat, Spain). 2,4,6-Tripyridyl-*s*-triazine (TPTZ) was obtained from Fluka (Madrid, Spain). Methanol was of HPLC grade, and other reagents were of analytical grade.

**Essential Oil Extraction.** Plant material was dried at room temperature (20−25 °C) until it reached a constant weight. Aerial parts of each sample were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The oil obtained was separated from water and dried over anhydrous sodium sulfate and kept in amber vials at 4 °C until chromatographic analysis (*23*). Essential oil extractions were done in triplicate for each *S. officinalis* collection site. Yield percentage was calculated as volume (mL) of essential oil per 100 g of plant dry matter.

**Gas Chromatography−Mass Spectrometry (GC-MS) Analysis.** Samples of 0.1 μL were subjected to analysis by GC-MS. A Hewlett-Packard 5890 series II Plus gas chromatograph (GC), equipped with a 30 m × 0.25 mm HP-5 column with 0.25 μm film thickness, and a DB-Wax (30 m × 0.32 mm i.d.) and 1.0 μm film thickness was used. Both stationary phases were supplied by Agilent Technologies (Palo Alto, CA). Helium was used as the carrier gas (constant pressure, β-ionone eluting at 27.6 min for HP-5MS column and 41.38 min for DB-Wax column), and the split ratio was set to 100:1. The GC was linked to an Agilent model 5972 inert mass spectrometry detector. For both stationary phases, the initial oven temperature was set at 60 °C, then increased at 2.5 °C/min to 155 °C, and finally raised to 250 °C at a rate of 10 °C/min; the injection port and the transfer line to the mass selective detector were kept at 250 and 280 °C, respectively. The mass spectrometer was operated in electron impact ionization mode with an ionizing energy of 70 eV, scanning from *m*/*z* 50 to 500 at 3.21 scan/s. The quadrupole temperature was 150 °C, and the electron multiplier voltage was maintained at 1300 V (*23*).

The individual peaks were identified by retention times and retention indices (relative to $C_6-C_{17}$ *n*-alkanes), compared with those of known compounds, and by comparison of mass spectra using the NBS75K library (U.S. National Bureau of Standards, 2002) and spectra obtained from the standard. Percentage compositions of samples were calculated according to the area of the chromatographic peaks using the total ion current.

**Extraction of Polyphenolic Compounds.** Distilled plant material was dried in an oven at 35 °C until it reached a constant weight and then finely ground to pass a 2 mm sieve. For the extraction, dried samples of 0.5 g were first homogenized with 30 mL of petroleum ether under magnetic stirring for 5 min and taken to dryness at room temperature. Second, they were extracted using 150 mL of methanol in a Soxhlet extractor (B-811) (Büchi, Flawil, Switzerland) for 2 h under a nitrogen atmosphere. Methanolic extracts were taken to dryness at 40 °C under vacuum conditions in an evaporator system (Syncore Polyvap R-96) (Büchi). The residue was redissolved in methanol and made up to 5 mL (*23*). The concentration of the extracts was expressed in terms of milligrams of dry methanolic extract weight per gram of dry plant weight. The extracts were kept in vials at −80 °C until their corresponding analysis. Two extracts were prepared for each sample.

**HPLC Analysis.** For the HPLC analysis, a method adapted from Zheng and Wang (*24*) was performed on a reverse phase Zorbax SB-C18 column (4.6 mm × 250 mm, 5 μm pore size, Hewlett-Packard) using a guard column (Zorbax SB-C18 4.6 mm × 125 mm, 5 μm pore size, Hewlett-Packard) at ambient temperature. Extracts were passed through a 0.45 μm filter (Millipore SAS, Molsheim, France), and 20 μL was injected in a Hewlett-Packard system equipped with a G1311A quaternary pump and G1315A photodiode array UV−vis detector. The mobile phase was acetonitrile (A) and acidified water containing 5% formic acid (B). The gradient was as follows: 0 min, 5% A; 10 min, 15% A; 30 min, 25% A; 35 min, 30% A; 50 min, 55% A; 55 min, 90% A; 57 min, 100% A, which was held for 10 min before returning to the initial conditions. The flow rate was 1.0 mL/min, and the wavelengths of detection were set at 280 and 330 nm. The identification of the phenolic components was made by comparison of retention times and spectra with those of commercially available standard compounds. For the purpose of quantifying, linear regression models were determined using standard dilution techniques. Linear regression equations calculated for the standards were as follows: caffeic acid, $y = 60.4716x + 18.2482$, $r^2 = 0.9996$; ferulic acid, $y = 64.6192x - 172.1063$, $r^2 = 0.9987$; rosmarinic acid, $y = 31.9033x + 73.4244$, $r^2 = 0.9996$; gallic acid, $y = 32.2926x - 1.8639$, $r^2 = 0.9998$; carnosic acid, $y = 2.2477x + 0.6104$, $r^2 = 0.9938$; carnosol, $y = 2.7335x - 0.3012$, $r^2 = 0.9993$; luteolin, $y = 36.9107x + 14.4913$, $r^2 = 0.9997$; apigenin, $y = 42.4503x + 1.6876$, $r^2 = 0.9997$; genkwanin, $y = 39.4661x - 1.7896$, $r^2 = 0.9998$; and naringin, $y = 14.9731x - 1.6478$, $r^2 = 0.9999$. Phenolic compound contents were expressed in micrograms per gram of dry plant material weight.

**DPPH$^{\bullet}$ Radical-Scavenging Activity.** The ability of the methanolic extracts to scavenge DPPH$^{\bullet}$ free radicals was determined according to the method described by Brand-Williams and co-workers (*25*). Briefly, 500 μL of methanolic extracts at different concentrations (from 2 to 15 μL/mL) were added to 1 mL of DPPH$^{\bullet}$ methanolic solution (0.1 mM). Decolorations were measured using a Shimadzu (UV-2401PC) spectrophotometer at 517 nm after incubation for 20 min at room temperature in the dark. Absorbance was measured against a blank of 500 μL of sample plus 1 mL of methanol. The absorbance of the control consisting of 500 μL of

methanol and 1 mL of DPPH$^{\bullet}$ solution was measured daily against a blank of 1.5 mL of methanol. Measurements were performed in triplicate.

The percentage activity for the DPPH$^{\bullet}$ was calculated according to

$$\% \text{ decoloration} = [1-(\text{absorbancesample}/\text{absorbancecontrol})]\times 100$$

The results were expressed as the inhibitory concentration of the extract necessary to decrease 50% ($IC_{50}$) of the DPPH$^{\bullet}$ absorbance. Concentrations are expressed in micrograms of dry plant methanolic extract per milliliter of methanol.

**ABTS$^{\bullet+}$ Radical Cation Decoloration Assay.** The ABTS free radical-scavenging activity of each sample was determined according to the method described by Re and co-workers (*26*). ABTS$^{\bullet+}$ radical cation was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 16 h before use. A working solution was diluted with ethanol to an absorbance of 0.70 (±0.02) nm (constant initial absorbance value used for standard and samples) at 734 nm and 30 °C. An aliquot (15 $\mu$L) of each sample (with appropriate dilution) or Trolox standard was mixed with the working solution (1.5 mL) of ABTS$^{\bullet+}$, and the decrease of absorbance was measured after 6 min at 734 nm using a Shimadzu (UV-2401PC) spectrophotometer. Measurements were performed in triplicate. The ABTS$^{\bullet+}$ scavenging rate was calculated to express the antioxidant ability of the sample, and results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC, $\mu$M Trolox equivalents per mg of dry plant methanolic extract).

**Ferric Reducing Antioxidant Power (FRAP).** The ability to reduce ferric ions was measured using the method described by Benzie and Strain (*27*). The FRAP reagent was freshly prepared from 300 mM acetate buffer, pH 3.6, 10 mM TPTZ made up in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. All three solutions were mixed together in the ratio of 10:1:1 (v/v/v). An aliquot of 40 $\mu$L of each sample (with appropriate dilution) was added to 1.2 mL of FRAP reagent. The absorption of the reaction mixture was measured at 593 nm after 2 min of incubation at 37 °C. Measurements were performed in triplicate. Fresh working solutions of known Fe(II) concentrations ($FeSO_4 \cdot 7H_2O$ of 0−2 mM) were used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of samples was calculated from the linear calibration curve and expressed as millimolar $FeSO_4$ equivalents per milligram of dry plant methanolic extract.

**Statistical Analysis.** All data were reported as mean ± standard deviation of three experiments. Data were analyzed by an analysis of variance ($p < 0.05$), and the means were separated by Duncan's multiple-range test (ANOVA procedure). Results were processed by computer programs Excel and STATISTICA software (*28*).

## RESULTS AND DISCUSSION

**Essential Oil Composition.** Fifty-seven volatile components were identified on the basis of their mass spectra characteristics, retention indices, and cochromatography with available standards using two columns of different polarities. **Table 2** shows the composition of sage essential oils from four different localities, amounting to a total percentage of 94.68−96.80%.

The oil yields of aerial parts range from 1.1 to 1.2% based on dry weight. In this case, obtained yields are higher than previously investigated samples cultivated in Tunisia (*6*). However, our values are included in the range (0.4−2.2%) of sage essential oil yields from a variety of European sources (*10*, *29*).

The essential oils of analyzed populations contained large proportions of oxygenated components (67.25−77.24%), represented by oxygenated monoterpenes, oxygenated sesquiterpenes, and oxygenated diterpenes. Oxygenated monoterpenes were shown to be the most abundant chemical class for all studied samples (33.89−60.45%). Our results are in agreement to those of Santos-Gomes and Fernandes-Ferreira (*5*), Avato and co-workers (*4*), and Pinto and co-workers (*29*), who reported the oxygenated monoterpenes as the major compounds in *S. officinalis* essential oils.

The monoterpene fraction presented the lowest level for the collection site Kelibia (41.18%), which is located in the subhumid bioclimate at low altitude, increased for the samples collected in the semiarid superior locality Soliman (57.14%), and reached the highest percentages in the semiarid moderate sites, located in high altitudes, Bou Arada (69.82%) and Sers (72.18%). The opposite was observed for the sesquiterpene fraction; in fact, samples of Bou Arada and Sers showed the lowest proportions (19.92%) and (16.48%), respectively. The percentage of sesquiterpenes increased for Soliman's samples (27.28%) and showed the highest levels for the Kelibia collection site located at low altitude. Similar findings have been reported by Oliveira and co-workers (*30*), who found that sesquiterpenes were mainly produced at lower altitudes, whereas monoterpenes were produced at higher ones.

Viridiflorol (9.94−19.46%), α-thujone (11.55−19.23%), 1,8-cineole (8.85−15.60%), camphor (5.08−15.06%), manool (5.52−13.06%), β-caryophyllene (2.63−9.24%), α-humulene (1.93−8.94%), and β-thujone (5.45−6.17%) were the major compounds of sage essential oil, whereas β-pinene (1.81−3.80%), borneol (1.35−2.87%), camphene (0.43−2.22%), and α-pinene (1.05−1.63%) were found in reasonable amounts. The analyzed data illustrated significant differences in the amount of some compounds in sage cultivated in different regions. In agreement with our findings, Lima and co-workers (*31*) reported the same prevalent constituents in *S. officinalis* essential oil cultivated in Portugal, whereas Marino and co-workers (*32*) obtained a similar result except for viridiflorol and manool, which were present in high amounts in our study and not detected by the latter authors. In earlier studies, viridiflorol was found in high amounts in sage cultivated in Tunisia (19%) and Croatia (14.2%) (*9*, *33*). Also, manool was obtained in relatively large proportions in *S. officinalis* originating from Cuba (14.7%) (*34*).

On the other hand, Hayouni and co-workers (*6*) revealed qualitative and quantitative differences, compared with our findings. The major constituents reported for *S. officinalis* cultivated in another Tunisian locality were 1,8-cineole (33.27%), β-thujone (18.40%), α-thujone (13.45%), borneol (7.39%), β-elemene (4.82%), camphor (3.31%), α-pinene (2.74%), fenchyl acetate (1.6%), and α-muurolol (1.41%).

Compared with the *S. officinalis* essential oil profile defined by the standard ISO 9909 (*11*), the levels of α-thujone and camphene obtained for samples collected in Kelibia and Soliman were lower than the corresponding minimum values (18−43%) and (1.5−7%), respectively. However, the essential oils of Bou Arada and Sers collection sites afforded higher amounts of 1,8-cineole than the level (5.5−13%) mentioned in standard ISO 9909 (*11*).

Samples of Bou Arada and Sers, cultivated in high altitudes, in the same bioclimatic conditions, showed large similarities in their essential oil quantitative compositions; they afforded the highest amounts of α- and β-thujones, 1,8-cineole, and camphor. According to Sur and co-workers (*35*), high concentrations of these compounds were responsible of the antimicrobial activity of sage.

Sage samples collected in Kelibia showed significant differences in the quantitative composition of its essential oil compared to the remaining collection sites. It was characterized by the largest proportions of viridiflorol, manool, β-caryophyllene, and α-humulene. It has been reported that β-caryophyllene has antitumor, anti-inflammatory, anesthetic, and immunomodulatory activities (*36*) and that α-humulene is cytotoxic against several solid tumor cell lines (*37*).

As reported in the literature, many factors such as geographical origin, ecological conditions (*5*), and genetic factors (*7*) may be responsible of high intraspecific variability within the essential

**Table 2.** Essential Oils Composition of *Salvia officinalis* L. Cultivated in Different Locations

| component[a] | RI[b,c] | RI[b,c] | OK[d] | OS[d] | OB[d] | OR[d] | identification |
|---|---|---|---|---|---|---|---|
| tricyclene | 927 | 1006 | tr | 0.04 ± 0.00 c | 0.04 ± 0.00 b | 0.05 ± 0.00 a | GC-MS |
| α-thujene | 930 | 1021 | 0.21 ± 0.05 b | 0.40 ± 0.19 ab | 0.44 ± 0.02 ab | 0.32 ± 0.00 ab | GC-MS |
| α-pinene | 939 | 1012 | 1.05 ± 0.02 a | 1.40 ± 0.58 a | 1.54 ± 0.09 a | 1.63 ± 0.00 a | GC-MS, Co-GC |
| fenchene | 953 | nd | tr | tr | tr | tr | GC-MS, Co-GC |
| camphene | 954 | 1056 | 0.43 ± 0.04 c | 1.19 ± 0.44 b | 1.92 ± 0.01 a | 2.22 ± 0.01 a | GC-MS, Co-GC |
| benzaldehyde | 963 | nd | tr | tr | tr | tr | GC-MS, Co-GC |
| sabinene | 976 | 1113 | 0.19 ± 0.02 b | 0.25 ± 0.10 ab | 0.35 ± 0.02 a | 0.27 ± 0.00 ab | GC-MS, Co-GC |
| β-pinene | 979 | 1097 | 1.81 ± 0.13 b | 3.58 ± 1.26 a | 3.80 ± 0.08 a | 3.32 ± 0.01 a | GC-MS |
| 1-octen-3-ol | 979 | 1453 | 0.03 ± 0.00 c | 0.06 ± 0.00 b | 0.08 ± 0.00 a | 0.06 ± 0.00 b | GC-MS, Co-GC |
| 3-octanone | 984 | nd | tr | 0.02 ± 0.00 a | 0.02 ± 0.00 a | 0.01 ± 0.00 b | GC-MS, Co-GC |
| myrcene | 991 | 1160 | 1.00 ± 0.08 a | 1.07 ± 0.25 a | 0.99 ± 0.02 a | 0.89 ± 0.01 a | GC-MS, Co-GC |
| 3-octanol | 995 | nd | 0.02 ± 0.00 a | 0.02 ± 0.00 a | 0.01 ± 0.00 b | 0.01 ± 0.00 c | GC-MS, Co-GC |
| α-phellandrene | 1003 | 1157 | 0.05 ± 0.00 a | 0.06 ± 0.02 a | 0.06 ± 0.00 a | 0.04 ± 0.00 a | GC-MS, Co-GC |
| δ3-carene | 1012 | 1142 | tr | tr | tr | tr | GC-MS, Co-GC |
| α-terpinene | 1017 | 1174 | 0.37 ± 0.03 ab | 0.44 ± 0.14 a | 0.45 ± 0.03 a | 0.29 ± 0.00 b | GC-MS, Co-GC |
| *p*-cymene | 1025 | 1263 | 0.27 ± 0.00 d | 0.43 ± 0.07 c | 0.63 ± 0.00 b | 1.39 ± 0.01 a | GC-MS, Co-GC |
| limonene | 1032 | 1191 | 0.51 ± 0.19 b | 0.61 ± 0.11 ab | 0.77 ± 0.08 ab | 0.53 ± 0.00 ab | GC-MS, Co-GC |
| 1,8-cineole | 1035 | 1207 | 8.85 ± 0.15 b | 12.65 ± 3.11 a | 14.60 ± 0.23 a | 15.60 ± 0.08 a | GC-MS, Co-GC |
| (*Z*)-β-ocimene | 1040 | 1232 | 0.28 ± 0.01 a | 0.30 ± 0.09 a | 0.22 ± 0.00 a | 0.08 ± 0.00 b | GC-MS |
| benzene acetadehyde | 1042 | nd | tr | 0.04 ± 0.00 a | 0.01 ± 0.00 b | tr | GC-MS |
| (*E*)-β-ocimene | 1050 | 1248 | 0.05 ± 0.00 a | 0.05 ± 0.02 a | 0.04 ± 0.00 a | 0.01 ± 0.00 b | GC-MS, Co-GC |
| γ-terpinene | 1060 | 1241 | 0.87 ± 0.05 a | 0.90 ± 0.19 a | 0.90 ± 0.06 a | 0.54 ± 0.01 b | GC-MS, Co-GC |
| (*E*)-sabinene hydrate | 1070 | nd | 0.27 ± 0.03 a | 0.22 ± 0.02 b | 0.29 ± 0.00 a | 0.18 ± 0.00 c | GC-MS |
| terpinolene | 1089 | 1278 | 0.18 ± 0.04 a | 0.18 ± 0.08 a | 0.20 ± 0.01 a | 0.14 ± 0.00 a | GC-MS, Co-GC |
| (*Z*)-sabinene hydrate | 1097 | nd | 0.20 ± 0.04 ab | 0.14 ± 0.06 b | 0.25 ± 0.00 a | 0.15 ± 0.00 b | GC-MS, Co-GC |
| linalool | 1098 | 1549 | 0.17 ± 0.06 a | 0.02 ± 0.00 b | 0.02 ± 0.00 b | 0.03 ± 0.00 b | GC-MS, Co-GC |
| α-thujone | 1117 | nd | 11.55 ± 0.59 c | 16.42 ± 2.65 b | 18.08 ± 0.02 ab | 19.23 ± 0.23 a | GC-MS, Co-GC |
| β-thujone | 1121 | nd | 5.45 ± 0.17 a | 5.98 ± 1.20 a | 5.91 ± 0.19 a | 6.17 ± 0.01 a | GC-MS, Co-GC |
| α-campholenal | 1127 | nd | 0.01 ± 0.00 c | 0.02 ± 0 0.00 b | 0.02 ± 0.01 b | 0.04 ± 0.00 a | GC-MS |
| camphor | 1146 | 1505 | 5.08 ± 0.44 c | 7.32 ± 1.51 b | 13.60 ± 0.55 a | 15.06 ± 0.05 a | GC-MS, Co-GC |
| pinocamphone | 1163 | 1576 | 0.29 ± 0.00 a | 0.26 ± 0.06 a | 0.28 ± 0.04 a | 0.21 ± 0.00 a | GC-MS |
| borneol | 1169 | 1705 | 1.35 ± 0.26 c | 2.22 ± 0.52 b | 2.87 ± 0.00 a | 2.27 ± 0.02 b | GC-MS, Co-GC |
| isocamphopinone | 1175 | nd | 0.12 ± 0.00 a | 0.11 ± 0.03 a | 0.10 ± 0.00 a | 0.07 ± 0.00 b | GC-MS |
| terpinen-4-ol | 1179 | 1606 | 0.42 ± 0.03 b | 0.59 ± 0.23 ab | 0.80 ± 0.04 a | 0.74 ± 0.01 a | GC-MS, Co-GC |
| *p*-cymen-8-ol | 1186 | 1850 | 0.01 ± 0.00 d | 0.04 ± 0.00 c | 0.07 ± 0.01 b | 0.10 ± 0.00 a | GC-MS, Co-GC |
| α-terpineol | 1189 | 1700 | 0.27 ± 0.06 b | 0.38 ± 0.15 b | 0.53 ± 0.01 a | 0.27 ± 0.00 b | GC-MS, Co-GC |
| myrtenal | 1193 | 1600 | 0.04 ± 0.00 b | 0.05 ± 0.00 a | 0.03 ± 0.00 c | nd | GC-MS, Co-GC |
| myrtenol | 1196 | 1626 | 0.12 ± 0.01 c | 0.11 ± 0.02 c | 0.15 ± 0.01 b | 0.20 ± 0.00 a | GC-MS |
| carveol | 1228 | nd | 0.02 ± 0.00 d | 0.03 ± 0.00 c | 0.04 ± 0.00 b | 0.04 ± 0.00 a | GC-MS |
| bornyl acetate | 1289 | 1575 | 0.09 ± 0.03 c | 0.09 ± 0.01 c | 0.27 ± 0.01 b | 0.38 ± 0.00 a | GC-MS, Co-GC |
| carvacrol | 1300 | nd | 0.04 ± 0.00 c | 0.03 ± 0.00 d | 0.04 ± 0.00 b | 0.13 ± 0.00 a | GC-MS |
| α-cubebene | 1351 | 1455 | 0.08 ± 0.00 a | 0.05 ± 0.00 b | 0.04 ± 0.00 c | 0.03 ± 0.00 d | GC-MS, Co-GC |
| eugenol | 1358 | nd | 0.01 ± 0.00 a | 0.01 ± 0.00 a | tr | nd | GC-MS, Co-GC |
| α-copaene | 1377 | 1480 | 0.14 ± 0.00 a | 0.09 ± 0.01 b | 0.06 ± 0.00 c | 0.05 ± 0.00 d | GC-MS |
| β-bourbonene | 1380 | nd | 0.04 ± 0.00 a | 0.03 ± 0.00 b | 0.03 ± 0.00 c | 0.02 ± 0.00 d | GC-MS |
| β-cubebene | 1401 | 1535 | 0.03 ± 0.00 a | 0.02 ± 0.00 c | 0.01 ± 0.00 d | 0.03 ± 0.00 b | GC-MS |
| β-caryophyllene | 1420 | 1654 | 9.24 ± 0.28 a | 6.49 ± 0.83 b | 4.69 ± 0.04 c | 2.63 ± 0.01 d | GC-MS, Co-GC |
| α-humulene | 1457 | 1667 | 8.94 ± 0.64 a | 3.83 ± 0.55 b | 2.15 ± 0.01 c | 1.93 ± 0.00 c | GC-MS, Co-GC |
| *allo*-aromadendrene | 1464 | nd | 0.16 ± 0.01 a | 0.12 ± 0.00 b | 0.09 ± 0.00 c | 0.09 ± 0.00 c | GC-MS, Co-GC |
| α-amorphene | 1481 | nd | 0.38 ± 0.03 a | 0.23 ± 0.01 b | 0.19 ± 0.00 c | 0.15 ± 0.00 d | GC-MS |
| α-muurolene | 1499 | nd | 0.24 ± 0.02 a | 0.15 ± 0.00 b | 0.12 ± 0.00 c | 0.10 ± 0.00 c | GC-MS |
| γ -cadinene | 1511 | nd | 0.25 ± 0.01 a | 0.14 ± 0.01 b | 0.11 ± 0.00 c | nd | GC-MS |
| calamenene | 1512 | 1853 | 0.09 ± 0.01 a | 0.06 ± 0.01 b | 0.05 ± 0.00 c | 0.04 ± 0.00 d | GC-MS |
| δ-cadinene | 1524 | 1760 | 0.55 ± 0.00 a | 0.31 ± 0.02 b | 0.24 ± 0.01 c | 0.16 ± 0.00 d | GC-MS, Co-GC |
| caryophyllene oxide | 1583 | 1987 | 0.81 ± 0.09 c | 0.87 ± 0.15 bc | 1.02 ± 0.06 b | 1.32 ± 0.00 a | GC-MS, Co-GC |
| viridiflorol | 1593 | 2099 | 19.46 ± 1.39 a | 14.88 ± 4.18 b | 11.13 ± 0.05 bc | 9.94 ± 0.05 c | GC-MS |
| manool | 1693 | nd | 13.06 ± 0.64 a | 11.82 ± 7.34 ab | 5.78 ± 0.00 b | 5.52 ± 0.00 b | GC-MS |
| chemical classes | | | | | | | |
| monoterpene hydrocarbons | | | 7.26 ± 0.54 b | 10.89 ± 3.31 a | 12.35 ± 0.38 a | 11.72 ± 0.02 a | |
| oxygenated monoterpenes | | | 33.89 ± 1.55 c | 46.20 ± 9.46 b | 57.47 ± 0.19 a | 60.45 ± 0.40 a | |
| sesquiterpene hydrocarbons | | | 20.14 ± 0.82 a | 11.52 ± 1.43 b | 7.76 ± 0.06 c | 5.22 ± 0.01 d | |
| oxygenated sesquiterpenes | | | 20.27 ± 1.30 a | 15.76 ± 4.33 b | 12.15 ± 0.12 b | 11.26 ± 0.05 b | |
| oxygenated diterpenes | | | 13.06 ± 0.64 a | 11.82 ± 7.34 ab | 5.78 ± 0.00 b | 5.52 ± 0.00 b | |
| others | | | 0.51 ± 0.00 a | 0.54 ± 0.14 a | 0.62 ± 0.06 a | 0.53 ± 0.01 a | |
| total identified | | | 95.13 ± 1.76 ab | 96.80 ± 0.19 a | 96.13 ± 0.08 ab | 94.68 ± 0.38 b | |
| oil yield[f] | | | 1.1 ± 0.1 ab | 1.2 ± 0.2 a | 1.1 ± 0.0 b | 1.1 ± 0.0 ab | |

[a] Components listed in order of elution in a HP-5 apolar column. [b,c] Retention indices calculated using, respectively, (*b*) an apolar column (HP-5) and (*c*) a polar column (DB-Wax); volatile compound proportions were calculated from the chromatograms obtained on the HP-5 column. [d] Plant codes are indicated in **Table 1**. Values followed by the same letter did not share significant differences at 5% (Duncan test). nd, not detected. tr, trace (<0.01%). Values are means ± SD of three independent replicates from two different samples of each collection site ($n = 6$). [f] v/w %, yield percentage was calculated as volume (mL) of essential oil per 100 g of plant dry matter.

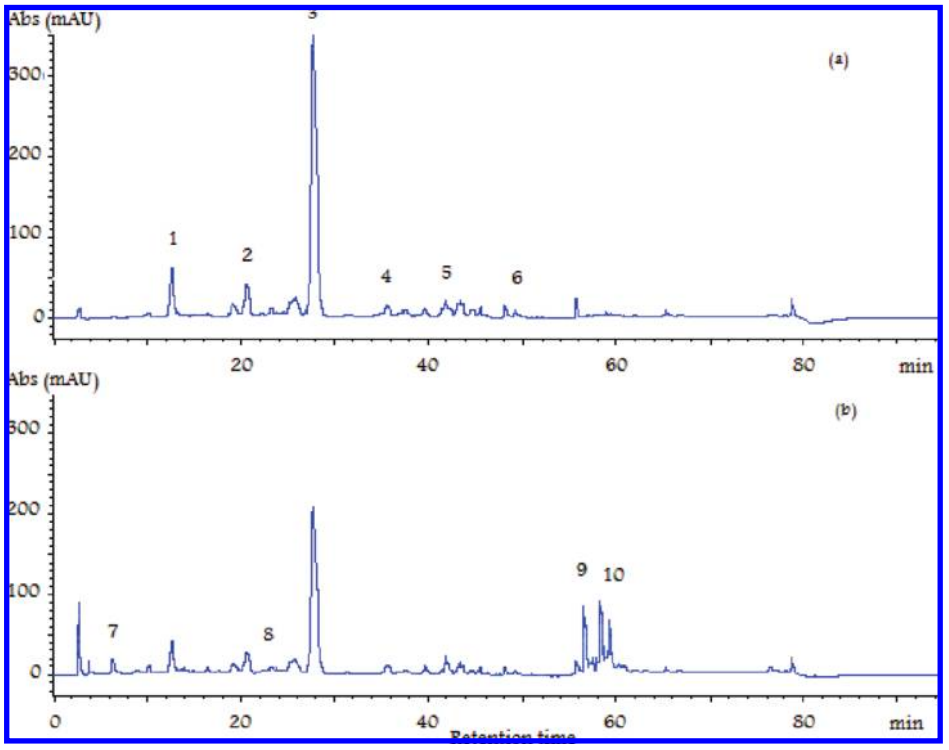


**Figure 1.** HPLC chromatograms of methanolic extracts of *Salvia officinalis* collected in Bou Arada with responses at 330 (**a**) and 280 nm (**b**) overlaid. Peaks: 1, caffeic acid; 2, ferulic acid; 3, rosmarinic acid; 4, luteolin; 5, apigenin; 6, genkwanin; 7, gallic acid; 8, naringin; 9, carnosol; 10, carnosic acid.

**Table 3.** Extract Yield and Content of Phenolic Compounds in *Salvia officinalis* Methanolic Extracts

| | content ($\mu$g/g of dry plant material weight) | | | |
|---|---|---|---|---|
| identified compound | OK[a] | OS[a] | OB[a] | OR[a] |
| phenolic acids | | | | |
| caffeic acid | 259.61 ± 5.11 b | 323.30 ± 1.61 a | 184.70 ± 6.15 d | 221.33 ± 3.52 c |
| ferulic acid | 244.10 ± 6.49 d | 490.33 ± 5.58 b | 524.37 ± 11.42 a | 411.40 ± 18.13 c |
| rosmarinic acid | 9731.15 ± 67.21 a | 9577.60 ± 177.35 a | 8781.47 ± 451.86 b | 8195.80 ± 323.61 c |
| gallic acid | 3.74 ± 0.44 c | 5.82 ± 0.70 a | 5.17 ± 0.12 ab | 4.80 ± 0.11 b |
| phenolic diterpenes | | | | |
| carnosic acid | 2915.05 ± 247.84 a | 3109.28 ± 221.62 a | 800.11 ± 5.70 b | 746.43 ± 4.16 b |
| carnosol | 5541.11 ± 276.41 a | 5283.01 ± 304.88 a | 2535.11 ± 0.29 c | 4116.22 ± 139.09 b |
| flavonoids | | | | |
| luteolin | 23.66 ± 3.99 a | 26.54 ± 1.29 a | 18.92 ± 0.14 b | 15.36 ± 0.03 b |
| apigenin | 22.90 ± 2.64 a | 22.99 ± 0.48 a | 24.19 ± 1.20 a | 23.54 ± 1.34 a |
| genkwanin | 17.70 ± 1.32 a | 18.45 ± 3.52 a | 15.66 ± 0.91 a | 14.97 ± 0.68 a |
| naringin | 171.95 ± 3.47 a | 169.23 ± 9.68 a | 142.23 ± 4.54 b | 125.97 ± 0.51 c |
| total | 18930.98 ± 160.93 a | 19026.57 ± 136.65 a | 13031.94 ± 323.93 c | 13875.83 ± 113.23 b |
| extract yield[b] (mg/g) | 138.83 ± 9.76 ab | 158.95 ± 34.61 a | 166.90 ± 1.92 a | 116.33 ± 1.97 b |

[a] Plant codes are indicated in **Table 1**. Values are means ± SD of three independent replicates from two different samples of each collection site ($n = 6$). Values followed by the same letter did not share significant differences at 5% (Duncan test). [b] Extract yield is expressed in milligrams of methanolic dry extract per gram of dry plant material weight.

oils of *S. officinalis*. In fact, these factors influence the plant's biosynthetic pathways and, consequently, the relative proportion of the main characteristic compounds (*38*).

**Contents of Polyphenolic Compounds.** Ten phenolic compounds were identified in the methanolic extracts of *S. officinalis* (**Figure 1**), including four phenolic acids (caffeic acid, ferulic acid, rosmarinic acid, and gallic acid), two phenolic diterpenes (carnosic acid and carnosol), and four flavonoids (luteolin, apigenin, genkwanin, and naringin). The results are shown in **Table 3**. The identified compounds were previously reported in *S. officinalis* extracts (*20*, *39*, *40*). Among the mentioned phenolic compounds, rosmarinic acid was present in the largest amounts ranging from 8195.80 to 9731.15 $\mu$g/g followed by carnosol and carnosic acid. Much lower contents were detected for luteolin, apigenin, and genkwanin, whereas the lowest rates were obtained for gallic acid (3.74−5.82 $\mu$g/g). Higher levels of caffeic acid, ferulic acid, luteolin, apigenin (*41*), gallic acid, genkwanin (*40*), carnosic acid (*42*), and rosmarinic acid (*43*) were obtained for sage extracts. Differences among phenolic compound levels, compared with our results, can be related to the distillation process, because according to Almela and co-workers (*44*), the drying and/or distillation treatments of *Rosmarinus officinalis* strongly affected the content of the two compounds of higher antioxidant activity: rosmarinic and carnosic acid. However, our samples seem to have higher concentrations of rosmarinic acid compared with previous studies (*40*, *44*).

It is worth noting that, commercially, the quality of a sage extract is highly dependent on the content of rosmarinic acid and

**Table 4.** Antioxidant Capacity of *Salvia officinalis* Methanolic Extracts

| collection site | DPPH[a] ($IC_{50}$, $\mu$g/mL) | ABTS[a] ($\mu$M TE/mg) | FRAP[a] (mM Fe(II)/mg) |
|---|---|---|---|
| OK[b] | 16.91 ± 0.44 c | 318.62 ± 14.40 a | 180.56 ± 19.30 a |
| OS[b] | 16.28 ± 1.92 c | 346.61 ± 2.36 a | 180.06 ± 4.98 a |
| OB[b] | 25.99 ± 3.01 a | 312.40 ± 5.91 a | 177.10 ± 24.34 a |
| OR[b] | 21.58 ± 0.88 b | 309.22 ± 2.69 a | 173.42 ± 0.07 a |

[a] Values are means ± SD of three independent replicates from two different samples of each collection site ($n = 6$). Values followed by the same small letter did not share significant differences at 5% (Duncan test). [b] Plant codes are indicated in **Table 1**.

diterpenoids (carnosol and carnosic acid) (*45*). Sage samples collected in the coastal regions Kelibia and Soliman showed the largest proportions of rosmarinic acid, carnosol, and carnosic acid, with no significant quantitative differences between the two collection sites. Previous studies also reported that the phenolic composition of the natural extracts and their antioxidative performance vary widely depending on environmental conditions (*20*, *22*). In agreement with these findings, our plants cultivated in different habitats showed significant differences in the quantitative composition of some phenolic compounds, namely, the phenolic acids and the diterpenes luteolin and naringin. On the other hand, Santos-Gomes and co-workers (*40*) and Başkan and co-workers (*42*) attributed the differences in sage extract composition to the instability of some of the most effective antioxidant compounds such as carnosol and carnosic acid depending on temperature, light, oxygen and solvent used in extraction.

**Antioxidant Capacity.** *DPPH*. The $IC_{50}$ values (the concentration reducing 50% of DPPH) obtained for scavenging activities on DPPH radical are shown in **Table 4**. The lower the $IC_{50}$ value is, the greater the free radical-scavenging activity is. The results illustrate a significant ($p < 0.05$) variation in the antioxidant activity between samples of *S. officinalis* from different locations. The samples collected in Kelibia and Soliman showed the highest antioxidant activities (16.91 and 16.28 $\mu$g/mL, respectively); a moderate antioxidant activity (21.58 $\mu$g/mL) was attributed to Sers, and the samples collected in Bou Arada were characterized by the weakest antioxidant activity (25.99 $\mu$g/mL). In earlier studies, Cuvelier and co-workers (*20*) reported that the main antioxidant activity of sage was attributed to carnosic acid, carnosol, and rosmarinic acid, whereas flavonoids such as luteolin and apigenin were less effective (*21*). These findings are in agreement with our results, which showed that samples cultivated in the coastal regions Kelibia and Soliman had the highest antioxidant activity and also the highest rosmarinic acid, carnosol, and carnosic acid levels. However, Grzegorczyk and co-workers (*43*) revealed the lack of direct association between the antioxidant activity measured using the DPPH assay upon sage methanolic extracts and chemical nature or content of compounds in the extracts, particularly rosmarinic acid, carnosol, and carnosic acid.

*ABTS*. The $ABTS^{\bullet+}$ assay measures the scavenging of free radicals as the discoloration of the $ABTS^{\bullet+}$ blue reactant. The decrease of $ABTS^{\bullet+}$ concentration is linearly dependent on the antioxidant concentration, including Trolox as a calibrating standard. The highest $ABTS^{\bullet+}$-scavenging rate was found for Soliman (346.61 $\mu$M TE/mg), and the lowest was obtained for Sers (309.22 $\mu$M TE/mg) (**Table 4**). However, the activities of sage growing in different habitats were not significantly different ($p > 0.05$).

*FRAP*. The FRAP assay is based on the ability of antioxidant to reduce ferric [Fe(III)] iron to ferrous [Fe(II)] iron in the presence of TPTZ, forming an intense blue $Fe^{2+}$−TPTZ complex with an absorption maximum at 593 nm. The absorbance increase is proportional to the antioxidant content (*27*). Although this assay was originally developed to measure plasma antioxidant capacity, it can be used to quantify the antioxidant capacity from a wide variety of foods and biological systems. FRAP antioxidant capacity values are expressed as millimolar ferrous iron(II) equivalents per milligram of sage extract (**Table 4**). The antioxidant activity ranged from 173.42 to 180.56 mM Fe(II)/mg of sage extract. The lowest value was found for Sers and the highest one for Kelibia; however, differences between samples collected in different localities were not significant ($p > 0.05$).

**Table 5.** Linear Correlation Coefficients of Phenolic Compounds and Total Identified Phenolics versus the Antioxidant Activity Determined by DPPH, ABTS, and FRAP

| | DPPH[a] | ABTS | FRAP |
|---|---|---|---|
| caffeic acid | −0.85** | 0.5 | 0.08 |
| ferulic acid | 0.5 | 0.1 | −0.05 |
| rosmarinic acid | −0.55* | 0.38 | 0.41 |
| gallic acid | 0.09 | 0.23 | −0.23 |
| carnosic acid | −0.81** | 0.33 | 0.2 |
| carnosol | −0.90** | 0.37 | 0.09 |
| luteolin | −0.62* | 0.28 | 0.26 |
| apigenin | 0.37 | −0.31 | 0.46 |
| genkwanin | −0.42 | 0.15 | 0.18 |
| naringin | −0.61* | 0.22 | 0.31 |
| total identified phenolics | −0.84** | 0.37 | 0.22 |

[a] Significant correlation at *, $p < 0.01$, or **, $p < 0.05$.

In agreement with DPPH and ABTS assays, the FRAP method showed that sage collected in the coastal regions Kelibia and Soliman revealed higher antioxidant activity than sage collected inland at Bou Arada and Sers. However, among the methods used to detect the antioxidant capacity, only the DPPH assay showed significant differences ($p < 0.05$) among the sages collected in different localities. This could be explained by the fact, reported by Prior and co-workers (*46*), that the redox potential of Fe(III)−TPTZ (0.7 V) is comparable with that of $ABTS^{\bullet+}$ (0.68 V); consequently, similar compounds react in both the ABTS and FRAP assays.

*Correlation between Phenolic Compounds, Total Identified Phenolics, and Antioxidant Activity*. Correlation coefficients are given in **Table 5**. Carnosol ($r = -0.90$), caffeic acid ($r = -0.85$), total identified phenolics ($r = -0.84$), and carnosic acid ($r = -0.81$) were negatively correlated to antioxidant activity measured by DPPH with highly significant correlation. Also, naringin ($r = -0.61$) and rosmarinic acid ($r = -0.55$) showed a negative significant correlation to DPPH antioxidant activity. However, no significant correlation was detected between the phenolic compounds and ABTS and FRAP methods. A similar result showing a lack of correlation to the widely distributed polyphenols was reported by Matkowski and Piotrowska (*47*). It should be noted that the synergistic effects of the diversity of major and minor phenolic components of the methanolic extracts of *S. officinalis* should be taken into consideration for their antioxidant activity.

In summary, the results revealed that the composition of essential oils and methanolic extracts and the antioxidant activity of *S. officinalis*, collected in different habitats, showed remarkable differences. Variations may be attributed to geographical origin and environmental conditions.

As shown, sage essential oils and methanolic extracts were characterized by the presence of biologically active compounds such as thujones, 1,8-cineole, camphor, rosmarinic acid, and phenolic diterpenes. The potency of these compounds could

provide a chemical basis of various applications in the cosmetic, pharmaceutical, and food industries.

As a continuation of this study, further analyses will be undertaken to select the optimal collection site and time of harvesting for the highest product quality and economical value.

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