



Antioxidants of *Salvia aegyptiaca* L. residues depending on geographical origin



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ABSTRACT

Variations in polyphenolic contents and antioxidant capacities depending on the collection sites of *Salvia aegyptiaca*, were examined. Total polyphenolics assessed by the Folin-Ciocalteu procedure reached the values of 79.67 and 77.91 mg GAE/g DW in post-distilled *Salvia aegyptiaca* from Enfida and Chott Meriem. HPLC analysis permitted the identification of 14 phenolic components. Methyl carnosate (416.29–4052.24 µg/g DW), rosmarinic acid (562.46–1172.24 µg/g DW) and apigenin-7-glucoside (512.96–774.02 µg/g DW) were detected as the main phenolics. Samples of Chott Meriem were characterized by the best antioxidant performance as evaluated by DPPH (IC₅₀, 29.62 µg/mL), ABTS (198.48 µM TE/mg) and FRAP (155.34 mM Fe(II)/mg) assays. Also, significant ($p < 0.05$) correlations were established between several identified phenolics and antioxidant tests. Residues of *S. aegyptiaca* might be valuable natural antioxidants and seemed to be useful in both pharmaceutical and food industries with appreciable health-promoting properties.

1. Introduction

External chemicals and internal metabolic mechanisms in the human body or in the food system could generate highly reactive free radicals, in particular oxygen derived radicals, which can oxidize biomolecules, resulting in cell death and tissue damage (Halliwell and Gutteridge, 2003). Several degenerative disorders, such as cardiovascular and brain diseases, arthritis, diabetes, cancer and immune system decline involve cellular damage potentially associated to free radicals (Rauter et al., 2012). Antioxidants could reduce oxidative damages of biomolecules by modulating reactive free radicals. Thus, the requirement of antioxidants in food and cosmetics is evident (Balboa et al., 2013).

Synthetic antioxidants such as butylhydroxyanisole (BHA) and butylhydroxytoluène (BHT) are mainly used as food additives to extend their shelf life. However, because of the concerns of consumers about the safety of such compounds and the harmful effects of synthetic antioxidants (liver damage and carcinogenesis), the search for natural antioxidants in plants and their by-products have attracted major interest nowadays.

Essential oil industries produce large quantities of solid residues that could lead to serious environmental issues worldwide (De Elguea-Culebras et al., 2016). However, organic residues of essential oils

manufacturing could constitute a readily available source of functional compounds. Thus, wastes of the hydro-distillation process of aromatic plants oils had been studied for their contents of a diversity of biologically active compounds including antioxidants such as phenolic acids and flavonoids. Wastes of distillation process could be employed to increase the shelf life of food (Nieto et al., 2011) in food industries.

It should be pointed out that many antioxidative phenolic compounds in plants usually occurred as a covalently bound form. Accordingly, some processing methods were employed to liberate them so as to enhance the antioxidant capacity (Seok-Moon et al., 2004; Xu et al., 2007). Specifically, the heat treatment may liberate some low molecular weight phenolic compounds and hence increase the antioxidant capacity such as for *Citrus* peel (Seok-Moon et al., 2004). Similarly, the distilled plant material of several Mediterranean herbs and aromatic plants was found to exhibit a higher phenolic content as well as a stronger antioxidant and radical scavenging activities than the non-distilled material (Parejo et al., 2002).

The genus *Salvia* is one of the most widespread members of the Lamiaceae family. Several *Salvia* species are of considerable economic importance since they have been used as spices, flavoring agents and pharmaceutical herbs. Members of the genus are used in traditional medicines all around the world and showed to be promising for their reputed beneficial effects on memory disorders, depression and cerebral

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ischaemia (Howes et al., 2003). Polyphenolics constitute a typical group of secondary metabolites that are largely represented in species of the genus *Salvia*. The major part of these compounds occur as phenolic acids, in particular those based on caffeic acid building block, anthocyanins and as flavones, flavonols and their glycosides (Lu and Foo, 2002) along with phenolic abietane diterpenes and their derivatives (Cuvelier et al., 1996) in *Salvia* species. Being natural antioxidants, these compounds play a major role as functional food ingredients and/or as food supplements.

The current study focuses on residues (wastes of hydro-distillation of essential oils) of *S. aegyptiaca* L. of different collection sites with the aim of investigating their antioxidant properties and polyphenolic contents.

2. Materials and methods

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH[•]), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt [ABTS (NH₄)₂], 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium persulfate, the Folin-Ciocalteu reagent, gallic acid 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, FeCl₃·6H₂O and sodium acetate 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.

2.2. Plant material

At the flowering season, aerial parts of randomly collected individual plants of *Salvia aegyptiaca* L. were considered as homogenous samples from three different collection sites (Table 1). Data about voucher specimen was previously reported by Ben Farhat et al. (2013).

2.3. Polyphenol extraction

Hydro-distillation of air-dried plant material was undertaken for 3 h by using a Clevenger-type apparatus. The resulting solid residue was oven-dried at 35 °C until its weight was constant and then grounded. Powdered samples (0.5 g) were stirred with 30 mL of petroleum ether for 5 min and dried at room temperature. Polyphenol extraction was made with a Soxhlet extractor (B-811) (Büchi, Flawil, Switzerland) using methanol as solvent (150 mL). Methanol extracts were dried at 40 °C in an evaporator system (Syncore Polyvap R-96) (Büchi, Flawil, Switzerland). The residue was re-dissolved in 5 mL of methanol. The extracts were kept in vials at – 80 °C until analysis.

2.4. Determination of total phenolic content

Total phenolic contents were determined according to the Folin–Ciocalteu colorimetric procedure (Singleton and Rossi, 1965). Briefly, a mixture of 15 µL of methanol extracts, 1185 µL of distilled water and 75 µL of Folin–Ciocalteu reagent was prepared. A vigorous stirring was applied and 225 µL of a solution of sodium carbonate

(20%) were added. After 2 h of incubation, the absorbance of the blue-coloured solution was measured at 765 nm and 25 °C with a Shimadzu (UV-2401PC, Japan) spectrophotometer. Quantitative measurements were performed, based on a standard calibration curve of concentrations ranging from 25 to 300 mg/L of gallic acid.

2.5. HPLC analysis

HPLC analysis was executed on a reversed-phase Zorbax SB-C18 column (4.6 mm x 250 mm, 5 µm pore size, Hewlett Packard, USA) using a guard column (Zorbax SB-C18 4.6 mm x 125 mm, 5 µm pore size, Hewlett Packard, USA) at ambient temperature, based on the method adapted from Zheng and Wang (2001). Extracts of 20 µL were injected in a Hewlett Packard (Germany) 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% of 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 and then held for 10 min before returning to the initial conditions. The flow rate was 1.0 mL/min and the detection wavelengths were set at 280 and 330 nm. The identification of the phenolic constituents was made by comparison of retention times and spectra with those of commercially available standard compounds. To quantify, linear regression models were determined using standard dilution techniques.

2.6. DPPH[•] radical-scavenging activity

The method described by Brand-Williams et al. (1995) was applied to evaluate the ability of methanol extracts to scavenge DPPH[•] free radicals. Briefly, 500 µL of methanol extracts at different concentrations (2.5–40 µL/mL) were added to 1 mL of DPPH[•] methanol solution (0.1 mM). Decolorations were measured using a Shimadzu (UV-2401PC, Japan) 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 and 1 mL of methanol. The absorbance of the control of 500 µL of methanol and 1 mL of DPPH[•] solution was measured daily against a blank of 1.5 mL of methanol. The percentage activity for the DPPH[•] was calculated as follows:

$$\% \text{Decoloration} = [1 - (\text{Absorbance sample} / \text{Absorbance control})] \times 100$$

2.7. ABTS^{•+} radical cation decoloration assay

The method described by Re et al. (1999) was employed to assess the ABTS free radical-scavenging activity of samples. ABTS^{•+} radical cation was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate. The resulting mixture was 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 at 734 nm and 30 °C. An aliquot of 15 µL of samples or Trolox standard was mixed with 1.5 mL of the working solution of ABTS^{•+} and the decrease of absorbance was measured after 6 min at 734 nm using a Shimadzu (UV-2401PC, Japan) spectrophotometer.

Table 1

Geo-geographical characteristics of *S. aegyptiaca* three collection sites.

| N° | Collection sites | Bioclimatic stage | Soil pH | Longitude (N) | Latitude (E) | Altitude (m) |
|----|------------------|-------------------|---------|---------------|--------------|--------------|
| 1 | Enfida | Lower semi-arid | 7.96 | 36°02' | 10°24' | 10 |
| 2 | Chott Meriem | Lower semi-arid | 8.05 | 35°53' | 10°35' | 8 |
| 3 | Ghraba | Higher arid | 7.50 | 34°59' | 10°44' | 98 |

2.8. Ferric Reducing Antioxidant Power (FRAP)

The ability to reduce ferric ions was measured using the method described by Benzie and Strain (1996). The FRAP reagent was freshly prepared from 300 mM acetate buffer, pH 3.6, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) made up in 40 mM HCl and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution. The mixture was made in the ratio of 10:1:1 (v/v/v). An aliquot of 40 μL of each sample was added to 1.2 mL of FRAP reagent. The absorption was measured at 593 nm after 2 min of incubation at 37 °C. Fresh working solutions of known Fe (II) concentrations ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) of (0–2 mM) were used for calibration curve.

2.9. Statistical analysis

Statistical analysis was processed by using Excel and STATISTICA software version 5.1. Results were reported as mean \pm standard deviation of three experiments. The significance of the differences between various experiments was analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range tests. Pearson's correlation coefficients were calculated. A p value less than 0.05 was considered to be statistically significant.

3. Results and discussion

3.1. Determination of methanol extract yields and total phenolic contents

S. aegyptiaca extract yields expressed as milligrams of dry methanol extract weight per gram of dry plant weight and their total phenolic contents determined as milligrams of gallic acid equivalents (GAE) per gram of dry plant weight are shown in Fig. 1. Residues of *S. aegyptiaca* from Enfida were characterized by the highest extract yield (100.68 mg/g DW) and differed significantly ($p < 0.05$) from the remaining collection sites. Post-distilled plants of Enfida (79.67 mg GAE/g DW) and Chott Meriem (77.91 mg GAE/g DW) displayed the largest phenolic amounts. In comparison with a previous study (Ben Farhat et al., 2013), non-treated *S. aegyptiaca* plants revealed higher total phenolic contents (94.10–106.86 mg GAE/g DW). A recent investigation including several *Salvia* species revealed lower amounts (11.8–68.9 mg GAE/g DW) of total phenolics of non-distilled plant material (Gezek et al., 2019).

Alternatively, results reported by Parejo et al. (2002) showed that plant material submitted to hydro-distillation has been found to contain a higher amount of phenolic substances than the non-distilled plant

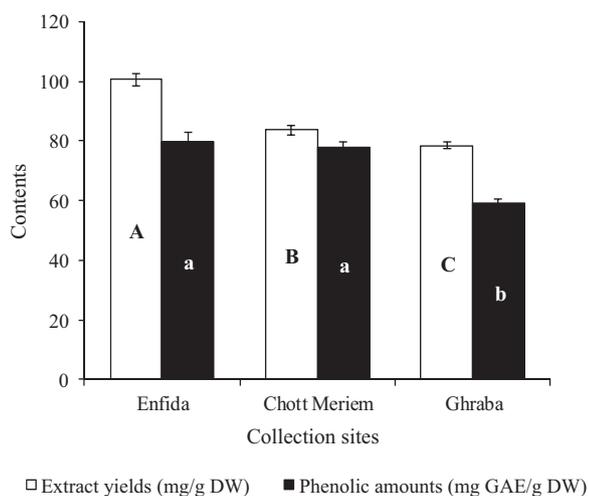


Fig. 1. Methanol extract yields and phenolic contents (mg GAE/g DW) of *S. aegyptiaca* post-distilled plants collected in different habitats; Bars sharing the same small letter did not share significant differences at $p < 0.05$ (Duncan test).

material, in particular in the case of lavender and tarragon. In certain cases, cell wall phenolics or bound phenolics could be released consequently to heat exposure, thus generating more phenolics to be extracted.

3.2. HPLC identification and quantification of phenolic compounds

Qualitative and quantitative determinations of *S. aegyptiaca* residues polyphenols were performed using RP-HPLC coupled with an UV-visible multi-wavelength detector. Based on the retention times of calibration standards, methanol extracts of *S. aegyptiaca* showed a phenolic profile composed of 14 identified phenolic compounds (Table 2). Polyphenolics of *S. aegyptiaca* by-products are composed of four phenolic acids (*p*-hydroxybenzoic acid, caffeic acid, ferulic acid and rosmarinic acid), three phenolic diterpenes (carnosic acid, carnosol, methyl carnosate), five flavones (luteolin, cirsimaritin, salvigenin, apigenin, genkwanin) and two flavone glycosides (luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside). In agreement with previous investigations on non-treated *S. aegyptiaca*, polyphenolics identified in the current study were reported by Ben Farhat et al. (2015) and El-Missiry et al. (1994). Nevertheless, *S. aegyptiaca* collected from Qatar showed the presence of a phenolic acid, namely herniarin (El-Missiry et al., 1994) and those harvested from Tunisia revealed the occurrence of naringin (Ben Farhat et al., 2013).

Phenolic amounts were expressed in micrograms per gram of dry plant weight. Residues of Chott Meriem displayed the highest total phenolics identified by HPLC with a content of 7287.85 $\mu\text{g/g}$ DW, approximately 3.5 times higher than the lowest amount (2026.20 $\mu\text{g/g}$ DW) attributed to Ghraba collection site. Methyl carnosate was found to be the major phenolic compound with a value of 4052.24 $\mu\text{g/g}$ DW in *S. aegyptiaca* collected in Chott Meriem. Samples of Ghraba were characterized by the prevalence of rosmarinic acid (562.46 $\mu\text{g/g}$ DW) followed by apigenin-7-*O*-glucoside (512.96 $\mu\text{g/g}$ DW) and methyl carnosate (416.29 $\mu\text{g/g}$ DW) and those of Enfida revealed rosmarinic acid (1109.91 $\mu\text{g/g}$ DW) followed by methyl carnosate (1000.64 $\mu\text{g/g}$ DW) and apigenin-7-*O*-glucoside (703.81 $\mu\text{g/g}$ DW) as the most abundant polyphenolics. Ben Farhat et al. (2013) reported the predominance of rosmarinic acid (5843.58–6060.01 $\mu\text{g/g}$ DW) followed by methyl carnosate (1394.75–5215.99 $\mu\text{g/g}$ DW) in non-distilled *S. aegyptiaca*. Interesting proportions of luteolin-7-*O*-glucoside (379.41–401.20 $\mu\text{g/g}$ DW), *p*-hydroxybenzoic acid (229.74–280.61 $\mu\text{g/g}$ DW) and ferulic acid (187.12–191.08 $\mu\text{g/g}$ DW) characterized *S. aegyptiaca* residues, particularly samples collected in Chott Meriem and Enfida.

Many *Salvia* species showed to be rich in phenolic compounds, in particular rosmarinic acid was highly represented in the genus (Tepe, 2008; Ben Farhat et al., 2009, 2013; Uysal, 2018; Zengin et al., 2018). A large spectrum of biological properties including antioxidant, antitumor, antibacterial, anti-inflammatory and antiviral activities was correlated to the bioactive compounds of *Salvia* species, such as polyphenolics. As previously described by Ben Farhat et al. (2009, 2013, 2015), Uysal (2018) Zengin et al. (2018) the best antioxidant activity was linked to the highest rosmarinic acid contents. Also, Tepe (2008) reported several biological activities of rosmarinic acid including inhibiting the HIV-1, antitumor, antihepatitis and protecting the liver, inhibiting the blood clots and anti-inflammation. In addition, Nowicka et al. (2018) revealed a positive correlation between the contents of phenolic acids and the inhibition of digestive enzymes (pancreatic lipase and α -glucosidase) in relation with obesity and type 2 diabetes.

It should be noted that post-distilled *S. aegyptiaca* showed lower concentrations of phenolics compared to non-treated plants (Ben Farhat et al., 2013). Differences observed in composition of the plant extracts of distilled and non-distilled material could derive from the extraction conditions applied, including exposure to high temperature in the course of the hydro-distillation process. As advanced by Ahmad-Qasem et al. (2013), heat treatments can improve extraction kinetics, however, they reduce both the phenolic content and antioxidant capacity. Solely,

Table 2
Variations of phenolic composition according to *S. aegyptiaca* collection sites.

| Identified compounds | Chott Meriem | Ghraba | Enfida |
|---------------------------------|-------------------|-------------------|-------------------|
| Phenolic acids | | | |
| <i>p</i> -Hydroxybenzoic acid | 280.61 ± 10.09 a | 126.01 ± 4.15 c | 229.74 ± 4.96 b |
| Caffeic acid | 62.53 ± 3.62 a | 51.33 ± 3.14 b | 60.21 ± 2.47 a |
| Ferulic acid | 187.12 ± 10.90 a | 136.67 ± 0.36 b | 191.08 ± 8.52 a |
| Rosmarinic acid | 1172.24 ± 63.69 a | 562.46 ± 25.30 b | 1109.91 ± 66.59 a |
| Phenolic diterpenes | | | |
| Carnosic acid | 58.12 ± 0.06 a | 58.51 ± 0.71 a | 53.93 ± 0.39 b |
| Carnosol | 35.00 ± 0.47 a | 23.35 ± 2.74 b | 33.66 ± 0.88 a |
| Methyl carnosate | 4052.24 ± 89.44 a | 416.29 ± 5.47 c | 1000.64 ± 23.79 b |
| Flavonoids | | | |
| Luteolin-7- <i>O</i> -glucoside | 379.41 ± 18.29 b | 61.51 ± 3.10 c | 401.20 ± 3.12 a |
| Apigenin-7-glucoside | 774.02 ± 25.79 a | 512.96 ± 27.96 c | 703.81 ± 12.24 b |
| Luteolin | 12.35 ± 0.53 b | 25.31 ± 1.45 a | 11.78 ± 0.21 b |
| Cirsimaritin | 18.30 ± 0.20 b | 15.04 ± 0.24 c | 19.49 ± 0.33 a |
| Salvigenin | 231.09 ± 9.15 a | 6.78 ± 0.66 b | 8.02 ± 0.45 b |
| Apigenin | 19.35 ± 1.20 b | 23.04 ± 0.55 a | 18.34 ± 0.94 b |
| Genkwanin | 5.47 ± 0.26 b | 6.95 ± 0.19 a | 5.58 ± 0.09 b |
| Total | 7287.85 ± 58.12 a | 2026.20 ± 28.60 c | 3847.40 ± 59.07 b |

Contents of phenolic compounds are expressed as µg/g of dry plant material weight; Values followed by the same letter did not share significant differences at 5% (Duncan test); *n* = 3.

carosol was apparently not affected by the distillation process since approximately similar ranges were detected in distilled (23.35–35.00 µg/g DW) and non-treated (35.01–36.69 µg/g DW) *S. aegyptiaca* plants (Ben Farhat et al., 2013). Alternatively, carnosol level showed to be higher in distilled *Rosmarinus officinalis* compared to non-distilled plant material, according to Almela et al. (2006).

Published literature data reported the influence of the distillation process on polyphenolic contents in several species of the Lamiaceae family, namely *S. officinalis* (Ben Farhat et al., 2009), *Rosmarinus officinalis* (Almela et al., 2006) and *Thymus zygis* ssp. *gracilis* (Jordán et al., 2009). The decrease of hydrophilic components in post-distilled plant material could explain the changes of polyphenolic concentrations according to Jordán et al. (2009) and Nieto et al. (2011).

During the distillation process, the plant material is exposed to temperature close to 100 °C which can lead to changes in thermolabile components such as hydrolysis of esters, polymerization of aldehydes or decomposition of other components. Nevertheless, it should be pointed out that the steam saturation provides a partial protection of phenolic compounds (Almela et al., 2006).

The drying and/or distillation treatments used with the plant material strongly affected the content of two compounds of strong antioxidant activity, namely rosmarinic acid and carnosic acid, as reported by Almela et al. (2006). Especially, the proportion of rosmarinic acid fell markedly in distilled *Rosmarinus officinalis*, which is in coherence with results of the current study. Previous investigation on non-distilled *S. aegyptiaca* showed contents of rosmarinic acid approximately five times higher (Ben Farhat et al., 2013) than distilled plant material.

Additionally, Almela et al. (2006) reported a larger number of compounds in distilled rosemary extracts. Most of them derived from carnosic acid alteration that generate γ -lactone (rosmanol, epirosmanol, epirosmanol-methyl ether) or δ -lactone (carosol, epirosmanol, epirosmanol-methyl ether) compounds (Almela et al., 2006; Schwarz et al., 1992).

Phenolics occur in plants mainly as aglycones, glycosides, or esters or are bound to the cell wall. Xu et al. (2007) reported that the heat treatment changed the distribution of phenolic acids due to the cleaving of esterified bond and glycosylated bond, etc. In short, the free fraction increased, whereas the ester, glycoside, and ester-bound fractions decreased. Moreover, in agreement with the current study showing lower amount of phenolic acid fraction (876.47–1722.50 µg/g DW) in distilled *S. aegyptiaca* compared with non-treated material (6767.19–6904.92 µg/g DW; Ben Farhat et al., 2013), there was a decrease of total phenolic acid content according to Xu et al. (2007) in huyou peel.

The influence of collection site on *S. aegyptiaca* phenolic composition was significant (*p* < 0.05). Variations could be attributed to several factors, whether genetic (Lamien-Meda et al., 2010) or related to the geographic origin, the harvesting time and the culture techniques (Liu et al., 2010). Climate factors such as season, sunlight duration, UV radiation and temperature are known to influence the plant metabolism since some compounds may be accumulated at a particular period to respond to environmental changes (Generalić et al., 2011; Koenen, 2001).

3.3. Antioxidant capacity

The antioxidant activity was estimated using three complementary tests namely the DPPH and ABTS radical scavenging assays and the FRAP reducing power test. As can be seen in Table 3, results of DPPH were determined as the inhibitory concentration of the extract necessary to decrease 50% (IC₅₀) of the DPPH[•] absorbance and concentrations were expressed in micrograms (µg) of dry plant methanol extract per milliliter (mL) of methanol. The ABTS^{•+} scavenging rate was calculated in terms of Trolox equivalent antioxidant capacity (TEAC, µM of Trolox equivalents per mg of dry plant methanol extract) and the FRAP values were expressed as millimolar (mM) of FeSO₄ equivalents per

Table 3
Antioxidant capacity of *S. aegyptiaca* methanol extracts.

| Collection site | DPPH (IC ₅₀ , µg/mL) | ABTS (µM TE/mg) | FRAP (mM Fe(II)/mg) |
|-----------------|---------------------------------|------------------|---------------------|
| Chott Meriem | 29.62 ± 3.66 c | 198.48 ± 10.02 a | 155.34 ± 0.23 a |
| Enfida | 47.54 ± 0.76 b | 188.33 ± 6.19 a | 93.22 ± 1.36 b |
| Ghraba | 62.21 ± 0.63 a | 122.28 ± 3.02 b | 89.80 ± 11.83 b |

Values are means ± SD of three independent replicates from three different samples of each collection site; Values followed by the same small letter did not share significant differences at 5% (Duncan test).

Table 4

Linear correlation coefficients established between phenolics and the antioxidant activity.

| | DPPH | ABTS | FRAP |
|---|--------|--------|-------|
| <i>p</i> -Hydroxybenzoic acid | 0.99* | 0.96* | 0.77* |
| Caffeic acid | 0.81* | 0.90* | 0.64 |
| Ferulic acid | 0.82* | 0.95* | 0.46 |
| Rosmarinic acid | 0.90* | 0.98* | 0.61 |
| Carnosic acid | −0.14 | −0.44 | 0.38 |
| Carnosol | 0.92* | 0.96* | 0.55 |
| Methyl carnosate | 0.90* | 0.71* | 0.98* |
| Luteolin-7- <i>O</i> -glucoside | 0.86* | 0.97* | 0.48 |
| Apigenin-7- <i>O</i> -glucoside | 0.97* | 0.96* | 0.72* |
| Luteolin | −0.85* | −0.98* | −0.51 |
| Cirsimaritin | 0.73* | 0.92* | 0.31 |
| Salvigenin | 0.83* | 0.60 | 0.98* |
| Apigenin | −0.73* | −0.87* | −0.34 |
| Genkwanin | −0.89* | −0.96* | −0.58 |
| Total phenolics identified by HPLC | 0.97* | 0.83* | 0.94* |
| Total phenolics identified spectrophotometrically | 0.83* | 0.94* | 0.46 |

* Significant correlation at $p < 0.05$.

milligram of dry plant methanol extract. The three antioxidant tests (IC₅₀, 29.62 µg/mL; TEAC, 198.48 µM TE/mg; 155.34 mM Fe(II)/mg) showed the best activity for *S. aegyptiaca* residues of Chott Meriem. Earlier study conducted by Kamatou et al. (2010) on DPPH radical scavenging activity of several South African *Salvia* species considered an extract with IC₅₀ < 30 µg/mL as having good antioxidant performance and characterized by moderate antioxidant capacity with an IC₅₀ comprised between the values of 30 and 80 µg/mL. According to Kamatou et al. (2010), samples of Chott Meriem were marked by good antioxidant activity and those of Ghraba and Enfida by moderate capacity. Compared to results advanced on non-treated *S. aegyptiaca* (Kamatou et al., 2010), our post-distilled plants showed lower antioxidant activity except for residues of Chott Meriem. These latter demonstrated a FRAP value in the same range of non-distilled plants (149.24–164.09 mM Fe(II)/mg). In addition, our samples of post-distilled *S. aegyptiaca* of Chott Meriem revealed higher antioxidant capacity than non-treated *S. argentea* (Ben Farhat et al., 2013).

These activities may be directly linked to phenolic amounts, and consequently to their free radical scavenging properties (Huang et al., 2006), since polyphenolics were associated with good antioxidant activity (Sreelatha and Padma, 2009). Several studies on *Salvias* antioxidants attributed their activities to phenolic abietane diterpenes (carnosic acid and its derivatives), rosmarinic acid and flavonoids such as genkwanin and cirsimaritin (Cuvelier et al., 1996; Kontogianni et al., 2013; Tepe, 2008). Whereas, it should be taken into consideration that the complexity of plant extracts emphasized the potential cumulative or synergistic effects of the diversity of major and minor phenolic components (Ben Farhat et al., 2013) along with the contribution of several non-phenolic compounds on the resulting antioxidant properties. In this context, decrease of distilled *S. aegyptiaca* material antioxidant power, in comparison with non-distilled samples (Ben Farhat et al., 2013) could be related to the lower content of polyphenolic compounds.

Table 4 showed results of linear correlation coefficients established between polyphenolics and antioxidant capacity in an attempt to estimate the contribution of these compounds to the total antioxidant activity. *p*-Hydroxybenzoic acid ($r = 0.96$; $r = 0.77$), methyl carnosate ($r = 0.71$; $r = 0.98$), apigenin-7-glucoside ($r = 0.96$; $r = 0.72$) and total phenolics estimated by HPLC ($r = 0.83$; $r = 0.94$) demonstrated significant ($p < 0.05$) positive correlation with ABTS and FRAP assays. Similarly, significant ($p < 0.05$) positive correlations associated the ABTS test to caffeic acid ($r = 0.90$), ferulic acid ($r = 0.95$), rosmarinic acid ($r = 0.98$), carnosol ($r = 0.96$), luteolin-7-*O*-glucoside ($r = 0.97$), cirsimaritin ($r = 0.92$) and total phenolics assessed by the Folin-Ciocalteu procedure ($r = 0.94$) and the FRAP test to salvigenin ($r = 0.98$). Also, the DPPH assay revealed significant ($p < 0.05$) negative

correlations with three flavonoids, namely luteolin ($r = -0.85$), apigenin ($r = -0.73$) and genkwanin ($r = -0.89$).

As previously described by Cuvelier et al. (1996), Generalić et al. (2011) and Kamatou et al. (2010), the use of Pearson's correlation coefficients revealed significant correlations between several phenolics and the antioxidant tests proving the significance of these compounds and their contribution to the antioxidant power of the plant extract.

4. Conclusions

The current study highlighted the effectiveness of *S. aegyptiaca* residues as natural antioxidants that might be useful in the pharmaceutical field and as potential functional food ingredients contributing to some health protection. Methyl carnosate, rosmarinic acid and apigenin-7-glucoside were found to be the main phenolics of *S. aegyptiaca* methanol extracts. This investigation demonstrated the significant effect of the collection site on the total phenolic contents, the concentrations of the individual phenolic compounds and the antioxidant activities and thus provided guidance on samples characterized by the adequate contents of polyphenolics and the best antioxidant performance.

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References

- Ahmad-Qasem, M.H., Cánovas, J., Barrajón-Catalán, E., Micol, V., Cárcel, J.A., García-Pérez, J.V., 2013. Kinetic and compositional study of phenolic extraction from olive leaves (var. Serrana) by using power ultrasound. *Innov. Food Sci. Emerg. Technol.* 17, 120–129. <https://doi.org/10.1016/j.ifset.2012.11.008>.
- Almela, L., Sánchez-Muñoz, B., Fernández-López, J.A., Roca, M.J., Rabe, V., 2006. Liquid chromatography-mass spectrometry analysis of phenolics and free radical scavenging activity of rosemary extract from different raw material. *J. Chromatogr. A* 1120, 221–229. <https://doi.org/10.1016/j.chroma.2006.02.056>.
- Balboa, E.M., Conde, E., Moure, A., Falque, E., Dominguez, H., 2013. *In vitro* antioxidant properties of crude extracts and compounds from brown algae. *Food Chem.* 138, 1764–1785. <https://doi.org/10.1016/j.foodchem.2012.11.026>.
- Ben Farhat, M., Jordán, M.J., Chaouech-Hamada, R., Landoulsi, A., Sotomayor, J.A., 2009. Variations in essential oil, phenolic compounds and antioxidant activity of Tunisian cultivated *Salvia officinalis* L. *J. Agric. Food Chem.* 57, 10349–10356. <https://doi.org/10.1021/jf901877x>.
- Ben Farhat, M., Landoulsi, A., Chaouech-Hamada, R., Sotomayor, J.A., Jordán, M.J., 2013. Characterization and quantification of phenolic compounds and antioxidant properties of *Salvia* species growing in different habitats. *Ind. Crops Prod.* 49, 904–914. <https://doi.org/10.1016/j.indcrop.2013.06.047>.
- Ben Farhat, M., Jordán, M.J., Chaouech-Hamada, R., Landoulsi, A., Sotomayor, J.A., 2015. Changes in phenolic profiling and antioxidant capacity of *Salvia aegyptiaca* L. by-products during three phenological stages. *LWT-Food Sci. Technol.* 63, 791–797. <https://doi.org/10.1016/j.lwt.2015.03.015>.
- Benzie, I.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *J. Anal. Biochem.* 239, 70–76. <https://doi.org/10.1006/abio.1996.0292>.
- Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol.* 28, 25–30.
- Cuvelier, M.E., Richard, H., Berset, C., 1996. Antioxidant activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. *J. Am. Oil Chem. Soc.* 73, 645–652. <https://doi.org/10.1007/BF02518121>.
- De Elguea-Culebras, G.O., Sánchez-Vioque, R., Santana-Méridas, O., Herraiz-Peñalver, D., Carmona, M., Berruga, M.L., 2016. *In vitro* antifungal activity of residues from essential oil industry against *Penicillium verrucosum*, a common contaminant of ripening cheeses. *LWT-Food Sci. Technol.* 73, 226–232. <https://doi.org/10.1016/j.lwt.2016.06.008>.
- El-Missiry, M.M., Hussiney, H.A., Ismail, S.I., Radwan, H.M., Rizk, A.M., 1994. Constituents of plants growing in Qatar XXIV phytochemical investigation of *Salvia aegyptiaca* L. *Qatar Univ. Sci. J.* 14, 249–251.
- Generalić, I., Skroza, D., Ljubenkov, I., Katalinić, A., Burčul, F., Katalinić, V., 2011. Influence of the phenophase on the phenolic profile and antioxidant properties of Dalmatian sage. *Food Chem.* 127, 427–433. <https://doi.org/10.1016/j.foodchem>.

- 2011.01.013.
- Gezek, G., Hashemi, P., Kalaycıoğlu, Z., Kaygusuz, H., Sarıoğlu, G., Döker, S., Dirmenci, T., Erim, F.B., 2019. Evaluation of some Turkish *Salvia* species by principal component analysis based on their vitamin B2, mineral composition, and antioxidant properties. *LWT-Food Sci. Technol.* 100, 287–293. <https://doi.org/10.1016/j.lwt.2018.10.066>.
- Halliwel, B., Gutteridge, J.M.C., 2003. *Free Radicals in Biology and Medicine*. Oxford University Press, Oxford, UK.
- Howes, M.J.R., Perry, N.S.L., Houghton, P.J., 2003. Plants with traditional uses and activities relevant to the management of Alzheimer's disease and other cognitive disorders. *Phytother. Res.* 17, 1–8. <https://doi.org/10.1002/ptr.1280>.
- Huang, Y.C., Chang, Y.H., Shao, Y.Y., 2006. Effects of genotype and treatment on the antioxidant activity of sweet potato in Taiwan. *Food Chem.* 98, 529–538. <https://doi.org/10.1016/j.foodchem.2005.05.083>.
- Jordán, M.J., Martínez, R.M., Martínez, C., Moñino, I., Sotomayor, J.A., 2009. Polyphenolic extract and essential oil quality of *Thymus zygis* ssp. *gracilis* shrubs cultivated under different watering levels. *Ind. Crops Prod.* 29, 145–153. <https://doi.org/10.1016/j.indcrop.2008.04.021>.
- Kamatou, G.P.P., Viljoen, A.M., Steenkamp, P., 2010. Antioxidant, anti-inflammatory activities and HPLC analysis of South African *Salvia* species. *Food Chem.* 119, 684–688. <https://doi.org/10.1016/j.foodchem.2009.07.010>.
- Koenen, E.V., 2001. *Medicinal Poisonous and Edible Plants in Namibia*. Klaus Hess, Berlin.
- Kontogianni, V.G., Tomic, G., Nikolic, I., Nerantzaki, A.A., Sayyad, N., Stosic-Grujicic, S., Stojanovic, I., Gerathanassis, I.P., Tzakos, A.G., 2013. Phytochemical profile of *Rosmarinus officinalis* and *Salvia officinalis* extracts and correlation to their antioxidant and anti-proliferative activity. *Food Chem.* 136, 120–129. <https://doi.org/10.1016/j.foodchem.2012.07.091>.
- Lamien-Meda, A., Nell, M., Lohwasser, U., Börner, A., Franz, C., Novak, J., 2010. Investigation of antioxidant and rosmarinic acid variation in the sage collection of the genebank in Gatersleben. *J. Agric. Food Chem.* 58, 3813–3819. <https://doi.org/10.1021/jf903993f>.
- Liu, W., Zu, Y.G., Fu, Y.J., Kong, Y., Ma, W., Yang, M., Li, J., Wu, N., 2010. Variation in contents of phenolic compounds during growth and post-harvest storage of pigeon pea seedlings. *Food Chem.* 121, 732–739. <https://doi.org/10.1016/j.foodchem.2010.01.023>.
- Lu, F., Foo, L.Y., 2002. Polyphenolics of *Salvia*—a review. *Phytochemistry* 59, 117–140. [https://doi.org/10.1016/S0031-9422\(01\)00415-0](https://doi.org/10.1016/S0031-9422(01)00415-0).
- Nieto, G., Huvaere, K., Skibsted, L.H., 2011. Antioxidant activity of rosemary and thyme by-products and synergism with added antioxidant in a liposome system. *Eur. Food Res. Technol.* 233, 11–18. <https://doi.org/10.1007/s00217-011-1486-9>.
- Nowicka, P., Wojdyło, A., Laskowski, P., 2018. Inhibitory potential against digestive enzymes linked to obesity and type 2 diabetes and content of bioactive compounds in 20 cultivars of the peach fruit grown in Poland. *Plant Foods Hum. Nutr.* 73, 314–320. <https://doi.org/10.1007/s11130-018-0688-8>.
- Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Flerlage, N., Burillo, J., Codina, C., 2002. Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilled mediterranean herbs and aromatic plants. *J. Agric. Food Chem.* 50, 6882–6890. <https://doi.org/10.1021/jf020540a>.
- Rauter, A.P., Dias, C., Martins, A., Branco, I., Neng, N.R., Nogueira, J.M., Goulart, M., Silva, F.V.M., Justino, J., Trevitt, C., Waltho, J.P., 2012. Non-toxic *Salvia sclareoides* Brot. extracts as a source of functional food ingredients: phenolic profile, antioxidant activity and prion binding properties. *Food Chem.* 132, 1930–1935. <https://doi.org/10.1016/j.foodchem.2011.12.028>.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* 26, 1231–1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3).
- Schwarz, K., Ternes, W., Schmauderer, E., 1992. Antioxidative constituents of *Rosmarinus officinalis* and *Salvia officinalis*. III. Stability of phenolic diterpenes of rosemary extracts under thermal stress as required for technological processes. *Z. Lebensm. Unters. Forsch.* 195, 104–107.
- Seok-Moon, J., So-Young, K., Dong-Ryul, K., Seong-Chun, J., Nam, K.C., Ahn, D.U., Seung-Cheol, L., 2004. Effect of heat treatment on the antioxidant activity of extracts from citrus peels. *J. Agric. Food Chem.* 52, 3389–3393. <https://doi.org/10.1021/jf04899k>.
- Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16, 144–158.
- Sreelatha, S., Padma, P., 2009. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant Foods Hum. Nutr.* 64, 303–311. <https://doi.org/10.1007/s11130-009-0141-0>.
- Tepe, B., 2008. Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of *Salvia virgata* (Jacq), *Salvia staminea* (Montbret & Aucher ex Benth) and *Salvia verbenaca* (L.) from Turkey. *Bioresour. Technol.* 99, 1584–1588. <https://doi.org/10.1016/j.biortech.2007.04.008>.
- Uysal, S., 2018. A comparative study of three drying methods on the phenolic profile and biological activities of *Salvia absconditiflora*. *J. Food Meas. Charact.* 1–7. <https://doi.org/10.1007/s11694-018-9929-7>.
- Xu, G., Ye, X., Che, J., Liu, D., 2007. Effect of heat treatment on the phenolic compounds and antioxidant capacity of *Citrus* peel extract. *J. Agric. Food Chem.* 55, 330–335. <https://doi.org/10.1021/jf062517l>.
- Zheng, W., Wang, S.Y., 2001. Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.* 49, 5165–5170. <https://doi.org/10.1021/jf010697n>.
- Zengin, G., Llorent-Martínez, E.J., Fernández-de Córdova, M.L., Bahadori, M.B., Mocan, A., Locatelli, M., Aktumsek, A., 2018. Chemical composition and biological activities of extracts from three *Salvia* species: *S. blepharochlaena*, *S. euphratica* var. *leiocalycina*, and *S. verticillata* subsp. *amasiaca*. *Ind. Crops Prod.* 111, 11–21. <https://doi.org/10.1016/j.indcrop.2017.09.065>.