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Research Article

Polyphenolic profile, total phenolic content and antioxidant activity of Tunisian cultivated sage (*Salvia officinalis* L.) extracts

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Abstract

Medicinal and aromatic plants have always been the major source of bioactive molecules that have many biological activities and health benefits. Sage (*Salvia officinalis* L.) is known to be an effective potential source of natural antioxidants which confer benefits to human health. Their bioactive properties are mainly due to the polyphenolic compounds. The aim of the present work was to determine the polyphenolic profile, to estimate the total phenolic content (TPC), and to evaluate the antioxidant activity of Tunisian cultivated sage hydroethanolic extract. The qualitative and quantitative identification performed by HPLC analysis allowed for identifying and quantifying fourteen polyphenolic compounds, including four phenolic acids, two phenolic diterpenes, and eight flavonoids. This analysis revealed the predominance of carnosol and rosmarinic acid (2920.55 and 1371.33 µg/gDW, respectively), known to be responsible for the main antioxidant activity of sage. The TPC was 41.65 mg gallic acid equivalent/g dry extract (mg GAE/g DE). Furthermore, the sage extract showed potential antioxidant activity and the IC_{50} value reached 13.21 µg/mL. Overall, the results reveal that sage aerial parts hydrohythanolic extract have proven to be an effective potential source of polyphenols, as natural antioxidants which is beneficial to human health, and could be useful in replacing or even decreasing synthetic antioxidants in foods, cosmetics and pharmaceutical products.

1. Introduction

During the last decade there has been a growing interest in the formulation of new cosmetic, food, and pharmaceutical products containing natural bioactive molecules. In fact, the use of aromatic and medicinal plants has been of great interest, as they have been the sources of natural products, commonly named as bioactive compounds with antioxidant potential and other beneficial properties [1]. Specifically, the natural compounds from the *Lamiaceae* family (thyme, sage and rosemary) have been reported in several studies for their antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anti-aging and anti-carcinogenic activities [2-6].

The genus Salvia is one of the most cultivated worldwide due to its use as a natural food conservative and flavoring agent. Several Salvia

species are the most economically important aromatic and medicinal plants since they have been used as spices, flavoring agents and phermaceutical herbs [7, 8]. Furthermore, the species of this genus are used in traditional medicines all around the world and showed to be promising for their reputed beneficial effects on memory disorders and depression [9, 10]. In particular, *Salvia officinalis* L. is known to be an effective potential source of natural antioxidants and which confer benefits to human health. Their bioactive properties are mainly due to their polyphenolic compounds [7, 11-14]. The aerial parts of the *S. officinalis* plant has a long history of use in food and traditional medicine. Because of its flavoring and seasoning properties, this plant has been widely used in the preparation of many foods and for the treatment of different kinds of disorders, including rheumatism, inflammation, dizziness, tremor, paralysis, diarrhea, and hyperglycemia [7]. Furthermore, the "green extracts" macerates of sage can be used as effective natural antioxidant additives for oils and for other food products [15].

In this context, our study has been undertaken with the aim to determine the polyphenolic profile, to estimate the total phenolic content (TPC), and to evaluate the antioxidant activity of Tunisian cultivated hydroethanolic macerate of sage as a "natural and safe extract". This work constitutes a proof of concept of the revalorizing process of this plant by recovering polyphenols as a source of bioactive molecules with benefits to human health.

2. Materials and methods

2.1. Plant material

Aerial parts of cultivated sage were randomly collected from Gafsa (35°14' N, 9°08' E) at the bloom phenological stage. After collecting, the leaves samples were brought to the laboratory, washed and remove excess of water from the leaves by using tissue paper. Fresh aerial parts were then dried at room temperature for ten days, then dried in a forced-air drier at 35 °C for 48 h, until they reached a constant weight. The samples were ground to powder and stored in glass cans at 4°C until use.

2.2. Preparation of the plant extracts

Dried samples (2g) were macerated in 20 mL of ethanolic solvent (EtOH 75%, v/v) for 48 hours at room temperature. The sage extract was filtered and dried in an oven at 37°C. The residue was redissolved in hydroethanol solvent and made up to 5 mL [16]. The final extract was kept in vials at 4°C until the corresponding analyses were conducted. The yield of the extract was expressed in terms of milligrams of dry hydroethanolic extract per gram of dry plant weight (mg DE/g DPW).

2.3. Identification and quantification of polyphenolic compounds by HPLC-DAD analysis

Polyphenolic compounds were identified and quantified by HPLC analysis based on the method adapted from Zheng and Wang [1]. Chromatographic analyses were performed on a reverse phase high-performance liquid chromatography (RP-HPLC) system using an Agilent1260 Series HPLC (Agilent Technologies, Germany) coupled to a diode array detector (HPLC-DAD). A 4.6 mm x 100 mm, 3.5 µm Hypersil ODS C18 reversed-phase column was used to separate individual phenolic compounds at ambient temperature. The mobile phase was methanol (A) and acidified water containing 0.1% formic acid (B). The gradient program was as follows: 35% A/65% B, 0–6 min; 60% A/40% B, 6–9 min; 80% A/20% B, 9–14 min; 100% A/0% B, 14–25 min and 35% A/65% B, 25–30 min. The injection volume was 2 µL, the flow rate was 0.4 mL/min and the wavelengths of detection were set at 280 and 340 nm. The identification of the polyphenolic compounds was made through the comparison of retention times and spectra with those of commercially available standard compounds. For quantification, linear regression models were determined using standard dilution techniques. The results were expressed as µg of compound per gram of dry weight (µg/g DW).

2.4. Determination of the total phenolic content

The total phenolic content (TPC) was determined by the Folin-Ciocalteu reagent method [17]. A reaction mixture of 20 µL of the sage extract, 1155 µL of distilled water and 100 µL of 10% Folin-Ciocalteu reagent was prepared. A vigorous stirring was performed and 225 µL of a sodium carbonate (20%) solution was added. After 30 min of incubation at 25 °C, the absorbance of the resulting blue-colored solution was measured at 765 nm. A standard curve

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was prepared by using different concentrations ranging from 0.1 to 1 mg/mL of gallic acid. TPC was expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/g DE). All experiments were performed in triplicate.

2.5. Antioxidant activity or DPPH• radical-scavenging activity

The scavenging activity of the hydroethanolic sage extract was measured according to the method described by Brand-Williams et al., [18]. 500µL of sage extract, at different concentrations (5–20 µL) were added to 1mL of DPPH• solution (0.1mM). Estimated time of reaction (30 min) was determined by considering the reduction of the absorbance at 517 nm. The absorbance was measured at room temperature in the dark against a blank (500µL of sample plus 1mL of methanol). The absorbance of the control (500µL of ethanol in 1mL of DPPH• solution) was measured daily. All the assays were conducted in triplicate. The percent activity for the DPPH• technique was calculated according to:

$$\% I = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

The results were expressed as the inhibitory concentration of the extract needed to decrease DPPH• absorbance by 50% (IC_{50}). Concentrations are expressed in micrograms of dry extract per milliliter of hydroethanol (IC_{50}, µg/mL).

2.6. Statistical analysis

All experiments were performed in triplicate (n = 3) and data were reported as means ± standard deviation (SD). A General Linear Model procedure was carried out to assess for significant differences (significant model was accepted for a p-value < 0.05) using the IBM SPSS Statistic Program (v. 20).

3. Results and discussion

3.1. Polyphenolic profile of Salvia officinalis L.

Fourteen phenolic compounds were identified in the hydroethanolic extract of sage, including four phenolic acids, two phenolic diterpenes, and eight flavonoids (Table 1). As expected, among the mentioned phenolic compounds, carnosol and rosmarinic acid were the major components quantified in sage extract (Fig. 1). Indeed, carnosol was present in the high amount followed by rosmarinic acid at 1371.33 and 1371.33 µg/g, respectively. Much lower contents were detected for epigallocatechin (446.98 µg/g) and luteolin (116.52 µg/g).

Previous studies conducted by Ben Farhat et al., [2] showed similar results regarding the major polyphenolic profile of sage plants harvested from populations located at different geographic origins in Tunisia. Furthermore, several phenolic compounds were earlier reported for the Sage extracts with different amounts [4, 7-10, 12, 19]. In fact, the phytochemical composition of *S. officinalis* could be varied depending on the environmental conditions such as climate, water availability, and altitude [20]. This statement is also corroborated by Almela et al., [21] and Ben Farhat et al., [9] who reported that the drying and/or distillation treatments of plant material strongly affected the content of rosmarinic acid and carnosic acid (two compounds of strong antioxidant activity).

3.2. Extract yield and total phenolic content

Sage extract yield, expressed as milligrams of dry hydroethanolic extract weight per gram of dry plant weight extend the value of 142.3. The total phenolic content (TPC) in sage extract reached value of 41 mg gallic acid equivalent/g dry extract (mg GAE/g DE) (Table 2). This value proves that hydroethanolic sage extract is rich in polyphenolic compounds. In comparison with a previous study, *S. offiinalis* plants growing in various Tunisian habitats revealed higher total phenolic content (112.93-161.37 mg GAE/g DW) [12].

Later on, similar results were obtained by Khiya et al., [13] who found that the TPC concentration values of the leaves of *S. officinalis* harvested in two regions of Morrocco (Khenifra and Boulemane) varied from 70.5 to 176.5 mg GAE/g DW. A recent investigation including several Salvia species revealed lower amounts (11.8-68.9 mg GAE/g DW) of total phenolic content [22]. Furthermore, ethanol concentration and extraction temperature have been shown to have a significant effect on the evolution of total polyphenols in sage extract [23]. Considering the variability found in the total polyphenol content produced by this aromatic species, it is important to mention that the contents of phenolic compounds depend on several

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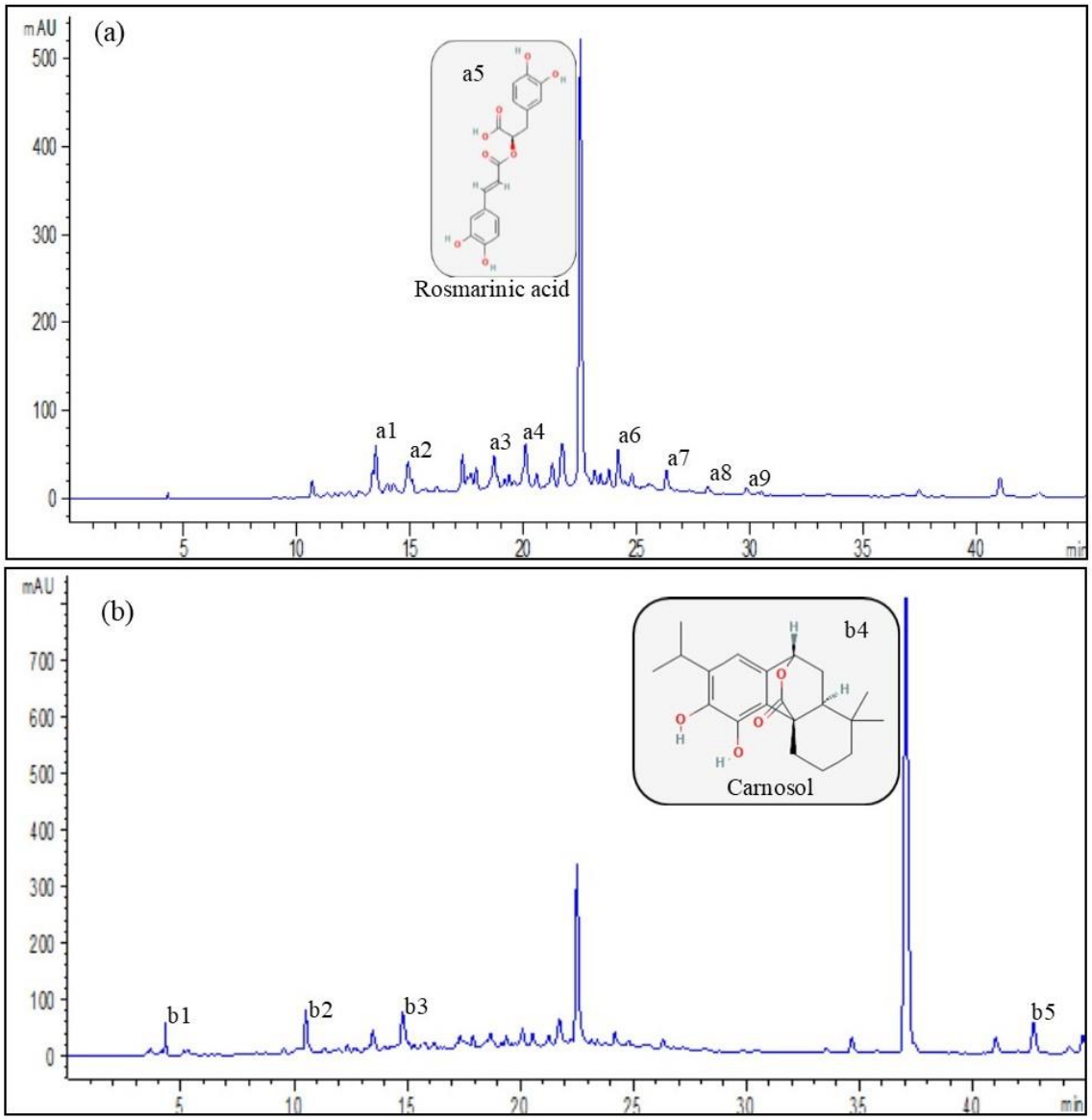

Figure 1. HPLC Chromatogram and structure of the main identified compounds of sage hydroethanolic extract at **λ** = 330nm (a): a1, Caffeic acid; a2, Ferulic acid; a3, Myrecitin-3-O-galactoside; a4, Rutin; a5, Rosmarinic; acid; a6, Luteolin; a7, Quercetin a8; Apigenin; a9, Kaempferol-3-rutinoside and **λ** = 280nm (b): b1, Gallic acid; b2, Epigallocatechin; b3, Catechin; b4, Carnosol ; b5, Carnosic acid.

factors, such as the species, the part of the plant, the harvest season, the geographical origin, as well as the extraction methods, and consequently their bioactive properties.

3.3. Antioxidant activity

The ability to scavenge the DPPH free radical reached the value of 13.21 micrograms of dry plant hydroethanol extract per milliliter of ethanol (µg/ mL) (Table 2). This plant proves a strong antioxidant activity. This result shows that plants with high antioxidant capacity are characterized by high levels of total phenolic content. In this case, the anti-radical activity is due to the quality of the extract, not to the quantity. Wojdyło et al., [24] reported a highly significant positive correlation between the antioxidant activity of Lamiaceae family and total polyphenols demonstrating the importance of these antioxidant compounds in spices and their significant contribution to the total antioxidant activity. Indeed, the results obtained in different tests of antioxidant activity showed that it is evident that the interaction of an antioxidant with DPPH depends not only on the

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Table 1. Content of phenolic compounds in *Salvia officinalis* L. hydroethanolic extract.

Phenolic compounds (µg/g DW)	λ (nm)	R. T.	Molecular Formula	*S. officinalis* Gafsa
Phenolic acids				
Gallic acid	280	4.31	$C_7H_6O_5$	24.26
Caffeic acid	340	13.49	$C_9H_8O_4$	13.36
Ferulic acid	340	18.37	$C_{10}H_{10}O_4$	50.51
Rosmarinic acid	340	22.54	$C_{18}H_{16}O_8$	1371.33
Flavonoids				
Epigallocatechin	280	10.6	$C_{15}H_{14}O_7$	446.98
Catechin	280	14.8	$C_{15}H_{14}O_6$	73.96
Myrecitin-3-O-galactoside	340	14.91	$C_{21}H_{20}O_{13}$	54.88
Rutin	330	20.10	$C_{27}H_{30}O_{16}$	50.36
Luteolin	340	24.18	$C_{15}H_{10}O_6$	116.52
Quercetin	340	26.16	$C_{15}H_{10}O_7$	66.74
Apigenin	340	28.31	$C_{15}H_{10}O_5$	6.31
Kaempferol-3-rutinoside	340	29.84	$C_{27}H_{30}O_{15}$	29.48
Phenolic diterpenes				
Carnosol	280	37.06	$C_{20}H_{26}O_4$	2920.55
Carnosic acid	280	42.70	$C_{20}H_{28}O_4$	189.53

Table 2. Extract yield, total phenolic content (TPC) and radical scavenging activity of sage hydroethanolic extract.

Sample	**Extract yield** (mg DE/g DPW)	**Total phenolic content** (TPC, mg GAE/g DE)	**DPPH** (IC_{50}, µg/mL)
Sage extract	142.3	41.65	13.21

concentration but also on the structure and nature of the antioxidants [13].

Previous studies revealed significant correlations between various phenolic compounds and the antioxidant activity, proving the significance of these compounds and their contribution to the antioxidant power of the plant extract [4, 6, 9, 25]. The interaction or synergistic effect among the polyphenolic compounds contained in sage extract may also contribute to their antioxidant capacity. The polyphenols appear to be effective donors of hydrogen to the DPPH radical because of their ideal structural chemistry [7]. The most effective antioxidant constituents of *S. officinalis* are carnosol, rosmarinic acid, and carnosic acid, followed by caffeic acid, rosmanol, rosmadial, genkwanin, and cirsimaritin. Other minor phenolic compounds should not be neglected because the synergy between different chemicals should be taken into account in biological activity.

4. Conclusions

The present study has investigated the polyphenolic profile, total phenolic content, and antioxidant activity of sage aerial parts hydrethanolic extract. The qualitative and quantitative identification performed by HPLC analysis allowed for identifying and quantifying fourteen polyphenolic compounds including four phenolic acids, two phenolic diterpenes, and eight flavonoids. This analysis revealed the predominance of carnosol and rosmarinic acid (2920.55 and 1371.33 µg/gDW, respectively), known to be responsible for the main antioxidant activity of sage. Furthermore, the sage extract showed a high amount of total phenolic content and a strong antioxidant activity (41.65 mg GAE/g DE and 13.21 µg/mL, respectively). In fact, these results proved that the plants with high levels of total polyphenolic content were characterized by high antioxidant capacity. This highlights confirmed that sage extract has proven to be an effective potential

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source of polyphenols, as natural antioxidants with beneficial properties to human health, and could be useful in replacing or even decreasing synthetic antioxidants in foods, cosmetics and pharmaceutical products.

Authors' contributions

Conceptualization, K.H.; Methodology, K.H.; M.B.F., M.BZ., S.K; Formal analyses, K.H., M.B.F.; Investigation, K.H., M.B.F., S.K.; Resources, K.H., M.B.F.; Writing-original draft preparation, K.H., M.B.F.; Writing-review, editing and Supervision, S.SE.

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Availability of data and materials

All data will be made available on request according to the journal policy

Conflicts of interest

The authors declare no conflict of interest.

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