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Evaluation of Pigments, Phenolic and Volatile Compounds, and Antioxidant Activity of a Spontaneous Population of *Portulaca oleracea* L. Grown in Tunisia

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Abstract: *Portulaca oleracea* L., commonly known as purslane, is a weed with worldwide distribution and considerable medicinal uses due to its high levels of phytochemical compounds. However, until now, few studies have been conducted on the biochemical characterization of *P. oleracea* grown in Tunisia, a possible area of its origin as other North African countries. Therefore, the aim of the present study was to determine the phytochemical composition and antioxidant potential of leaves and stems from a Tunisian spontaneous population of purslane. Particularly, samples were analyzed for their proximate composition, pigments, and volatiles, whereas ethanolic and aqueous extracts were evaluated for their composition in phenolic compounds and in vitro antioxidant activities. Stems showed higher content of moisture (89.9%) and anthocyanins (4.61 $\mu\text{g g}^{-1}$ dry matter, DM), whereas leaves revealed higher chlorophyll concentrations (7.42 mg g^{-1} DM). Significantly higher levels of phenolic compounds and antioxidant capacities ($p < 0.05$) were obtained in ethanolic extracts, compared with water extracts, irrespective of the analyzed plant part. A high antioxidant activity of stems was obtained, especially when extracted with ethanol. Headspace gas chromatography-mass spectrometry (GC-MS) analyses revealed six volatile classes with monoterpene hydrocarbons, oxygenated monoterpenes, and non-terpene derivatives as the highly represented compounds. Limonene (17.3–32.2%), carvone (38–46%), 2,6-dimethylcyclohexanol (2.2–6.4%), and nonanal (3.4–3.8%) were the most abundant volatiles. Based on the results of the present study, Tunisian purslane should deserve major consideration as an edible vegetable due to its richness in phytochemical compounds and, hence, for its potential health effects.

Keywords: *Portulaca oleracea* L.; plant parts; pigments; phenols; antioxidant activity; volatile compounds

1. Introduction

Common purslane (*Portulaca oleracea* L.) is best known as an invasive weed in the Mediterranean Basin [1,2], although its succulent leaves and stems are also completely edible. Purslane belongs to the Portulacaceae family containing more than 120 species of succulent herbs and shrubs, and it is ranked among the top eight common plants in the world [3]. It is well known as a cosmopolitan species under the term of ‘Global Panacea’ [4] and listed by the World Health Organization as one of the most used medicinal plants. The aerial parts of the plant are used as a folk medicine [5] in different countries to treat different ailments in humans for its diuretic, febrifuge, antiseptic, anti-ascorbic, antipyretic, anti-asthmatic, antispasmodic, vermifuge, and antitussive effects [6–9].

A wide range of pharmacological effects were shown for *P. oleracea* extracts, including antibacterial [10], hypolipidemic, anti-aging, anti-inflammatory [11,12], skeletal muscle relaxant [11,13], analgesic, wound healing [14], hypoglycemic [15], neuropharmacological [16], bronchodilatory [14,17], hypocholesterolemic [18], and antioxidant activities [15,19,20]. Other studies showed the importance of purslane consumption in the reduction of the occurrence of many diseases such as cancer [21,22] and heart disease [23]. *P. oleracea* is also consumed as a vegetable, especially in the Mediterranean Basin, where stems and leaves are similar to spinach in its salty and slightly acidic taste. In fact, it can be consumed fresh [12,14,24], cooked [25], or dried in tea or soup [19]. Purslane is characterized by its higher nutritional value compared with other cultivated vegetables; it is a rich source of α -tocopherol, β -carotene, ascorbic acid [3,26], polysaccharides, cardiac glycosides, anthraquinone glycosides [27], ω 3 fatty acids, and antioxidants [26,28–32]. It was reported that the major phenolic compounds found in *P. oleracea* are flavonoids (such as kaempferol, apigenin, myricetin, quercetin, and luteolin) [6,24,33], monoterpene glycosides [34], and alkaloids [8]. Recent research has also shown that *P. oleracea* contains considerable amounts of omega-3 fatty acids, such as α -linolenic acid known for its role in preventing cardiovascular diseases and reinforcing the immune system, as well as dietary glutathione [28] and gallotannins [35]. Purslane is a great source of fatty acids, and 27 compounds were detected in the plant. Three fatty acids (linolenic, palmitic, and oleic acids) have been shown to be the most abundant. The organic acids including citric, aconitic, fumaric, malic, and oxalic acids were also found in the plant [36]. However, the accumulation of oxalic acid in large amounts may limit the acceptance of purslane as a green leafy vegetable [37]. Purslane powder supplementation to broiler chickens’ diets with triiodothyronine-induced ascites causes an improvement of the oxidative status and a reduction of ascites incidence without affecting the growth performance of broiler chickens [38]. No alteration of growth performance was also observed in Moghani lambs after diet supplementation with purslane [39].

Purslane is consumed in Tunisia, especially in the region of Mahdia, where it is known as “bondleka” and “delbicha”. As reported by Salah and Chemli [40], the two botanical forms of *P. oleracea* are present in Tunisia: the subsp. *oleracea*, which spontaneously grows as an invasive weed both in irrigated crops and rural inhabited areas, and the subsp. *sativa* (Haw.) Celak, which is the cultivated form. Despite extensive studies on the chemical and bioactive properties of this species throughout the world [31–33,41–45], less information is currently available on the phytochemical characterization of this species grown in Tunisia [46], a possible area of its origin as other North African countries. Therefore, in the present study, we aimed to characterize the phytochemical compounds present in leaves and stems of a spontaneous population of *P. oleracea* grown in Tunisia, as well as to evaluate the antioxidant capacity of their ethanolic and aqueous extracts. In particular, for the first time, both proximate composition as well as phenolic and volatile compounds were simultaneously evaluated in order to provide a comprehensive view of the health-promoting phytochemicals present in the aerial parts of Tunisian purslane.

2. Materials and Methods

2.1. Experimental Site, Plant Material Collection, and Sample Preparation for Phytochemical Analyses

Plant tissue samples (whole aerial parts) of *P. oleracea* were collected during April–May 2012 from a natural spontaneous population in Mahdia, Tunisia (35° 31' N, 10° 58' E, 7 m a.s.l.). The local climate of the region is semiarid Mediterranean, with long, hot, dry summers and mild, wet winters. The mean annual rainfall is 348 mm, and the average temperature of 19.8 °C is mostly concentrated from autumn to early spring. The area of plant collection is characterized by coarse-textured limestone soils.

The authenticity of plants was confirmed by Pr. Fethia Skhiri, Senior Scientist, Department of Ethnobotany, Higher Institute of Biotechnology, Monastir, Tunisia. The collected plant material from at least 10 mature plants per replicate was thoroughly washed with tap water and then rinsed with distilled water. The fully grown leaves and stems were removed by knife, then dried in an oven (Binder, Milan, Italy) at 37 °C, with the aim to preserve phytochemicals from degradation at high temperatures. The dehydrated leaves and stems were separately ground, passed through a 1 mm sieve, and kept in air-tight glass bottles until the following chemical analyses. The latter were all performed in triplicate. All the reagents, solvents and products adopted in the present research were purchased from Sigma-Aldrich (Tunis, Tunisia) and were of analytical or high-performance liquid chromatography (HPLC) grade. Bi-distilled water was used throughout this research.

2.2. Moisture and Ash Determination

The moisture content was determined by drying the fresh ground sample in an oven at atmospheric pressure and at a temperature of 105 ± 2 °C [47]. Results were expressed as mg g^{-1} . Concerning the ash content, it was estimated from dried samples obtained previously (1.0 g) by incineration at 550 ± 2 °C for 24 h [48]. The resulting ash content was expressed as mg g^{-1} dry matter (DM).

2.3. Chlorophylls and Carotenoids

The pigments were extracted following the procedure adopted by Abugri et al. [49]. In brief, powdered samples (0.21 g) and 25 mL of acetone (99.9%) were agitated thoroughly in the dark in a water bath at 30 °C for 20 min. The resulting solution was then filtered using Whatman No. 1 filter paper in the presence of anhydrous sodium sulphate (Na_2SO_4) in order to extract all water molecules. Afterward, the absorbance of the solution was read at 470, 645, and 662 nm. The contents of chlorophyll (a and b), total chlorophylls, and carotenoids were calculated in accordance to formulas proposed previously [50–53]. Chlorophylls and carotenoid contents were expressed as mg g^{-1} DM.

2.4. Total Anthocyanins

Total anthocyanins were determined from acidified methanol (1%) extracts at 530 and 657 nm, and results were expressed as μg cyanidin 3-glucoside equivalents (CyE) g^{-1} DM [54].

2.5. Solvent Extraction Procedures for Antioxidant Compound Determination

Two solvents were used for extraction: water and ethanol. Concerning the ethanolic extraction, the powdered leaves and stems (50 g) were separately macerated with 95% ethanol (500 mL) under stirring at room temperature for 7 days [55]. The crude extracts were then filtered and concentrated to dryness under reduced pressure using a Rotary Evaporator. Yield of the extracts was about 39% (*w/w*) of dried plant material for leaves and 48% for stems. Water extraction was done by infusion by boiling 10 g of powder for 5 min in 100 mL distilled water. Then, the solution was cooled at room temperature and finally filtered to obtain a clear infusion. Each obtained extract was then transferred to vials and stored in the dark at 4 °C until further analysis.

2.6. Determination of Total Phenols and *o*-Diphenols

Total phenols content was determined using the Folin–Ciocalteu method [56,57]. Aliquots (100 μL) of each extract were diluted with deionized water and mixed with diluted Folin–Ciocalteu reagent (2.5 mL). Sodium carbonate solution (2 mL of 75% *m/v* anhydrous sodium carbonate in deionized water) was added after 1 min. After standing at room temperature for a duration of 2 h, the absorbance was taken at 765 nm. Hydroxytyrosol was used as a standard, and the results obtained were expressed as mg hydroxytyrosol equivalent (HE) g^{-1} of extract, on a DM basis.

The same reference was adopted for *o*-diphenol quantification [56,58]. In detail, 100 μL of the extract was mixed with 1 mL of each of three aqueous solutions (HCl (0.5 N), a mixture of NaNO_2 (1.45 N) and $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ (0.4 N), and NaOH (1 N) and incubated for 30 min at room temperature. The *o*-diphenols were determined at 500 nm, and contents were expressed on a DM basis as mg HE g^{-1} of extract.

2.7. Determination of Total Flavonoids

Total flavonoid content of the extracts was determined by the aluminium chloride spectrophotometric procedure [59]. Each extract (250 μL) or standard solution of catechin was mixed with distilled water (1.25 mL) and 5% sodium nitrite (75 μL). Five minutes later, 10% aluminum trichloride (150 μL) was added, and the solution was incubated for six minutes. Finally, 1 M sodium hydroxide (0.5 mL) and distilled water were added to a final volume of 2.5 mL. The absorbance of the mixed solution was determined against a blank at 510 nm. Total flavonoid contents were expressed as mg (+)-catechin equivalents (CEQ) g^{-1} of extract on a DM basis.

2.8. Determination of Condensed Tannins

The tannin contents of *P. oleracea* leaves and stems were determined following the methods reported previously [55,60]. Briefly, the mix of each extract or the standard solution (50 μL) with 4% vanillin in methanol (1.5 mL) and 12 M HCl (750 μL) were incubated for 20 min in darkness at room temperature. The absorbance at 500 nm was then read against a blank. The contents of condensed tannins were expressed as mg (+)-catechin (CEQ) g^{-1} extract on a DM basis.

2.9. Volatile Compound Analyses

Volatile compounds were analyzed as reported by Dabbou et al. [61]. The headspace of dried *P. oleracea* leaves and stems was sampled using Supelco (Bellefonte, PA, USA) SPME devices coated with polydimethylsiloxane (PDMS, 100 μm) inserted into a 5 mL vial and allowed to equilibrate for 30 min. The fiber was then exposed to the headspace for 50 min at room temperature. After sampling, the fiber was withdrawn into the needle and transferred to the injection port of the GC-MS system. A Varian (Palo Alto, CA, USA) CP 3800 gas chromatograph, a DB-5 capillary column (30 m \times 0.25 mm \times 0.25 μm ; Agilent, Santa Clara, CA, USA), a Varian Saturn 2000 ion trap mass detector, a splitless injector (250 $^\circ\text{C}$), transfer line (240 $^\circ\text{C}$), a helium gas carrier (1 mL min^{-1}), and an oven temperature program (60 to 240 $^\circ\text{C}$ at 3 $^\circ\text{C} \text{ min}^{-1}$) were used for GC-EIMS analyses. The constituents were identified after comparing their retention times with those of authentic samples. The comparison was based on a computer matching of the linear retention indices (LRIs) of a series of n-hydrocarbons against commercial (NIST 98 and Adams 95) and homemade library mass spectra (obtained from pure substances and MS literature data) [62–67]. In addition, all molecular weight substances identified were checked by GC-CIMS, using methanol as the ionizing gas.

2.10. Antioxidant Activity

Three assays were used to evaluate the antioxidant activity of extracts: 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), reducing power, and phosphomolybdenum assays. Influences of antioxidant concentration on the inhibition of radical cation absorption were considered when determining the antioxidant capacity.

2.10.1. ABTS⁺ Radical Cation Scavenging Assay

The ABTS⁺ free radical decolorization assay was determined after generating the ABTS^{•+} radical cation [68,69] through the interaction of ABTS (7 mM) and K₂S₂O₈ (2.45 mM). The obtained ABTS^{•+} radical cation solution was diluted with ethanol until an absorbance of 0.7 ± 0.02 at 734 nm. This solution represented a reagent blank (ABTS^{•+} solution without test sample, A0). For the spectrophotometric assay, the ABTS^{•+} diluted solution (3.9 mL) was mixed with ethanolic extract (100 mL). The mix was incubated for 5 min in darkness at room temperature. After that, the absorbance (ABTS^{•+} solution plus compound, At) was determined at 734 nm. The % inhibition was calculated, and IC₅₀ was determined from the plot made by % inhibition and concentration. The IC₅₀ is the concentration of the sample required to scavenge 50% of the ABTS free radical. The experiments were performed in triplicate.

2.10.2. Reducing Power Assay

The capacity of extracts to reduce Fe³⁺ was assessed according to the method proposed by Oyaizu [70] slightly modified [55]. A mix of equal volumes (250 µL) of each sample, sodium phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide III (1%) was incubated at 50 °C for 20 min. Then, 250 µL of trichloroacetic acid (10%) was added, and the obtained solution was centrifuged (3750 g; 10 min). Subsequently, the supernatant (100 µL), methanol (100 µL), and 0.1% ferric chloride (25 µL) were mixed, and absorbance against the blank was determined after 10 min in darkness at 700 nm. The IC₅₀ providing 0.5 absorbance for each sample was determined by graphing the absorbance at 700 nm against extract concentration.

2.10.3. Phosphomolybdenum Assay

The procedure of Prieto et al. [71] was followed to estimate the total antioxidant activity of ethanolic and aqueous extracts of *P. oleracea* leaves and stems. Briefly, an aliquot of each extract sample (200 µL) and 2 mL of the phosphomolybdenum reagent composed of sulfuric acid (0.6 M), sodium phosphate (28 mM), and ammonium molybdate (4 mM) were mixed and left to react for 90 min at 95 °C. Then, the solution was cooled at room temperature, and the absorbance was read at 695 nm. The percentage of molybdenum reduced by extracts was estimated through a standard curve of ascorbic acid, and results were expressed as µg ascorbic acid mL⁻¹ extract. Interpolation from linear regression analysis was used to determine the IC₅₀ (mg·mL⁻¹), representing the effective concentration at which the total antioxidant activity was 50%.

2.11. Statistical Analyses

To verify the statistical significance, the SPSS program release 17.0 for Windows (SPSS, Chicago, IL, USA) was used. Data were represented as means ± SD of three independent measurements, and analysis of variance (ANOVA) was assessed to determine differences among means. Differences between aerial plant parts were tested by Student's *t*-test. *p*-values of <0.05 were considered significant.

3. Results and Discussion

3.1. Proximate Composition

Ash, moisture, chlorophyll a, chlorophyll b, total chlorophylls, total carotenoids, and anthocyanins contents of leaves and stems of *P. oleracea* are shown in Table 1. The ash content was about 34%

in both leaves and stems. These contents were higher than the values reported previously for *P. oleracea* leaves [44]. Consequently, leaves and stems are a good source of minerals contributing to the bio-functional activities in plant. However, growth stages, planting dates, and salinity levels contribute also to the mineral composition among different purslane accessions [27,41,72], and this aspect deserves major consideration in further research.

Table 1. Chemical composition from leaves and stems of *P. oleracea* grown in Tunisia.

	Leaves	Stems
Ash (mg g ⁻¹ DM)	33.83 ± 0.01	33.70 ± 0.16
Moisture (mg g ⁻¹)	86.37 ± 0.74	89.93 ± 0.29 **
Chlorophyll a (mg g ⁻¹ DM)	2.69 ± 0.05	0.33 ± 0.01 **
Chlorophyll b (mg g ⁻¹ DM)	4.73 ± 0.09	0.58 ± 0.01 **
Total chlorophylls (mg g ⁻¹ DM)	7.42 ± 0.13	0.91 ± 0.02 **
Total carotenoids (mg g ⁻¹ DM)	tr	tr
Anthocyanins (µg g ⁻¹ DM)	1.08 ± 0.49	4.61 ± 0.05 **

Mean composition of sampled *P. oleracea* from three replications ± standard deviation. Different symbols (**), for the same parameter, within columns indicate significant differences (** $p < 0.01$) between the two aerial parts of the plant. tr: trace (<0.01 mg g⁻¹ DM).

Stems were rich in moisture (89.93 mg g⁻¹) and anthocyanins (4.61 µg g⁻¹ DM), whereas leaves showed high amounts of chlorophyll a, chlorophyll b, and total chlorophylls. Thus, *P. oleracea* appears to be an efficient photosynthetic plant, which is confirmed by Bessrouer et al. [73] experimental trials where severe stress conditions revealed a slight decrease in chlorophyll amounts in this species.

Leaves were poor in anthocyanins, which is not in agreement with other results [74] finding comparable levels of anthocyanins in stems, leaves, and flowers of purslane cultivated at two locations. Carotenoids, such as β-carotene, were detected in various Malaysian purslane varieties [31]. By contrast, our results demonstrated trace contents of carotenoids in both parts of the plant stems and leaves. These findings did not corroborate with the results of Alam et al. [31], and this can be attributed to both the different accessions analyzed and the environmental conditions. Finally, we obtained lower values of total carotenoids as compared to those reported by Sdouga et al. [46] performed on spontaneous populations of purslane collected in other Tunisian areas. This confirmed the relevant role of environmental conditions on the biosynthesis of phytochemicals.

3.2. Phenolic Compounds

The amounts of total phenols, *o*-diphenols, flavonoids, and tannins in *P. oleracea* leaves and stem extracts are summarized in Table 2.

Table 2. Phenolic composition (mg g⁻¹ DM) of ethanolic and water extracts from leaves and stems of *P. oleracea* grown in Tunisia.

Phenolic Composition	Ethanolic Extract		Water Extract	
	Leaves	Stems	Leaves	Stems
Phenols	13.92 ± 0.04	11.80 ± 0.26 **	2.45 ± 0.05	0.50 ± 0.05 **
<i>o</i> -Diphenols	15.75 ± 0.22	14.59 ± 0.07 *	2.32 ± 0.06	0.64 ± 0.06 **
Flavonoids	16.26 ± 0.18	13.58 ± 0.11 **	2.83 ± 0.04	1.88 ± 0.01 *
Tannins	31.98 ± 0.64	11.59 ± 0.86 **	7.72 ± 0.17	6.52 ± 0.17 *

Mean composition of sampled *P. oleracea* from three replications ± standard deviation. Different symbols (*, **), for the same parameter, within columns indicate significant differences (* $p < 0.05$; ** $p < 0.01$) between the two aerial parts of the plant within the same extraction method.

3.2.1. Total Phenols and *o*-Diphenols

As highlighted in Table 2, on the whole, total phenol contents varied from 0.5 to 13.92 mg g⁻¹. Comparing the different extracts in both leaves and stems, a significantly higher content ($p < 0.05$) of total phenols was obtained in leaves in both ethanolic (13.92 mg g⁻¹) and aqueous extracts (2.45 mg g⁻¹). Furthermore, total phenol contents found in this study were higher than those found by Santiago-Saenz et al. [75] and Sdouga et al. [46]. Previously, Sicari et al. [15] measured total phenol contents in dried leaves of *P. oleracea* in both aqueous methanolic extracts and ethanolic ones, and they found the highest contents in the latter. Leaves showed a higher amount of total phenols than stems, which is in accordance with the results of Oliveira et al. [36], although contents were lower than those of the present study. Consequently, the metabolic reactions were more developed in leaves than in stems. These findings are proven by previous research on other vegetables [55,76]. In addition, total phenol amounts in purslane were dependent on other factors, such as plant maturity [45], with the highest levels recorded in mature plants.

Comparing contents from the two solvents of extraction, total phenols from ethanolic extraction were higher than those from aqueous extraction, which proved that the extraction method greatly influences the total phenols amounts. In the literature, extraction methods using methanol or a chloroform–methanol mixture have shown to be the most efficient and reliable to obtain the highest content of total phenol compounds [19,41]. However, our results revealed that ethanolic extraction was also efficient at obtaining a high amount of total phenols. This may explain why we obtained significantly higher amounts of total phenols than values reported by Sdouga et al. [46], who determined the level of these bioactive compounds in Tunisian purslane by adopting acetone as the solvent for extraction.

Similarly, *o*-diphenols were extracted in greater amounts by using ethanol as the solvent rather than water, whereas differences between plant parts were only significant by adopting the proposed aqueous extraction procedure (Table 2).

3.2.2. Flavonoids Content

The concentrations of flavonoids varied according to the plant part and the solvent used (Table 2). Independently from the solvent used for extraction, flavonoid contents were significantly higher in leaves than stems. These results are in accordance with those of Xu et al. [6], who reported kaempferol and apigenin compounds as the most abundant flavonoids, and also with findings of Sdouga et al. [46], who reported myricetin and rutin as the major compounds in leaves and stems, respectively. By contrast, Zhu et al. [7] found that total flavonoid contents were higher in stems than leaves.

Flavonoids were efficiently extracted in ethanol compared to water as solvent. Furthermore, total flavonoid contents found in the ethanolic extract of Tunisian *P. oleracea* leaves were higher than those reported by Santiago-Saenz et al. [75]. Overall, these findings proved that flavonoid contents varied with plant parts and solvents used for their extraction. Flavonoid contents could also change with other main factors such as the genetic variability and edaphoclimatic conditions [75].

3.2.3. Tannins Content

Tannins are natural polyphenols having a higher antioxidant activity than other phenols. Particularly, they are able to inhibit lipid peroxidation in liver mitochondria [33,77,78]. In the present study, tannin contents varied from 6.52 to 31.97 mg g⁻¹ DM (Table 2). Significant differences were shown between the two plant parts regardless of the solvent used for their extraction. This result was explained by the fact that these compounds could be synthesized differently in stems and leaves [79].

3.3. Volatile Compounds

A total of 41 volatile compounds were detected in leaves (97.40%) and stems (96.17%), and they represented monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, apocarotenoids, nitrogen derivatives, and non-terpene derivatives (Table 3).

Table 3. Volatile compounds (% DM) from leaves and stems of *P. oleracea* grown in Tunisia.

Volatile Compounds	LRI	Leaves	Stems
Hexanal	804	0.40 ± 0.01	-
Heptanal	902	0.50 ± 0.10	-
2,5-dimethylpyrazine	913	0.60 ± 0.01	-
2,3-dimethylpyrazine	916	1.10 ± 0.01	-
benzaldehyde	962	1.30 ± 0.10	-
6-methyl-5-hepten-2-one	987	0.90 ± 0.01	-
2-pentylfuran	994	1.05 ± 0.05	1.27 ± 0.06 *
2,3,5-trimethylpyrazine	1003	0.80 ± 0.10	-
limonene	1032	17.30 ± 1.20	32.20 ± 5.50 **
(E)-2-octenal	1062	-	1.30 ± 0.50
(E)-2-octen-1-ol	1071	-	1.03 ± 0.50
(E,Z)-3,5-octadien-2-one	1076	0.55 ± 0.05	-
(E,E)-3,5-octadien-2-one	1095	0.50 ± 0.01	-
<i>n</i> -undecane	1100	0.40 ± 0.01	1.30 ± 0.60 **
linalool	1101	1.20 ± 0.20	0.70 ± 0.10 **
nonanal	1104	3.85 ± 0.05	3.37 ± 0.45
2,6-dimethylcyclohexanol	1110	6.45 ± 0.15	2.17 ± 0.15 **
3-nonen-2-one	1142	0.65 ± 0.05	-
camphor	1145	1.35 ± 0.05	0.97 ± 0.06 **
menthone	1154	1.10 ± 0.10	-
menthol	1174	1.05 ± 0.05	-
<i>cis</i> -dihydrocarvone	1195	0.40 ± 0.01	0.60 ± 0.10 **
<i>n</i> -dodecane	1200	-	0.77 ± 0.25
safranal	1202	1.00 ± 0.01	-
decanal	1206	0.50 ± 0.01	0.70 ± 0.10 **
β -cyclocitral	1222	1.70 ± 0.21	0.50 ± 0.01 **
cumin aldehyde	1241	1.90 ± 0.01	2.20 ± 0.40
carvone	1244	45.80 ± 2.00	37.80 ± 6.20
<i>cis</i> -carvone oxide	1262	-	1.80 ± 0.30
1-decanol	1272	-	0.50 ± 0.01
<i>trans</i> -carvone oxide	1276	-	0.90 ± 0.30
menthyl acetate	1289	0.40 ± 0.01	-
<i>n</i> -tridecane	1300	-	0.70 ± 0.01
undecanal	1307	-	0.40 ± 0.01
β -caryophyllene	1419	1.90 ± 0.20	4.67 ± 1.15 **
(E)-geranylacetone	1454	0.40 ± 0.01	-
α -humulene	1455	-	0.57 ± 0.15
<i>ar</i> -curcumene	1484	-	0.57 ± 0.15
(E)- β -ionone	1487	3.05 ± 0.25	0.60 ± 0.10 **
<i>n</i> -pentadecane	1500	-	0.63 ± 0.06
dihydroactinolie	1536	0.80 ± 0.10	-
Monoterpene hydrocarbons	-	17.30 ± 1.20	32.20 ± 5.50 **
Oxygenated monoterpenes	-	52.80 ± 1.70	44.97 ± 7.35
Sesquiterpene hydrocarbons	-	1.90 ± 0.20	5.77 ± 1.45 **
Apocarotenoids	-	6.75 ± 0.15	0.87 ± 0.35 **
Nitrogen derivatives	-	2.50 ± 0.10	-
Non-terpene derivatives	-	16.15 ± 0.55	12.40 ± 2.60
Total identified volatiles	-	97.40 ± 0.40	96.17 ± 1.05

Mean composition of sampled *P. oleracea* from three replications ± standard deviation. Different symbols (*, **), for the same parameter, within columns indicate significant differences (* $p < 0.05$; ** $p < 0.01$) between the two aerial parts of the plant. LRI, linear retention index.

The identified compounds accounted for 1.9–52.8% and 0.9–45.0% of the total aroma in leaves and stems, respectively. Aromatic compounds differed according to the plant part. Quantitatively, oxygenated monoterpenes (45.0–52.8%), followed by monoterpene hydrocarbons (17.3–32.2%), were found to be the most important class of volatiles identified in leaves and stems of *P. oleracea*.

Therefore, these volatiles may be the main contributors to the general aroma in both the analyzed plant parts. Other volatile classes were also identified by other studies in purslane, such as terpenoids [42].

Oxygenated sesquiterpenes and nitrogen derivatives were either absent or detected in trace amounts in *P. oleracea* leaves and stems. These findings provided useful information on the main classes of volatile compounds, allowing us to distinguish purslane's plant parts and to define their further utilization as a natural herbicide on a large scale. In this framework, the main volatile compounds in both the leaves and stems were carvone and limonene, followed by 2,6-dimethylcyclohexanol and nonanal (in leaves) and β -caryophyllene (in stems) (Table 3). However, each plant part highlighted a specific volatile composition. Some compounds were not detected in the stems, such as hexanal and menthol, and others were not found in the leaves (e.g., 1-decanol and undecanal). Purslane leaves from Egypt showed only 21 volatiles, where (*E*)-2-hexenal, (*E*)-2-nonenol, hexanal, and ethyl linoleate were shown to be the main volatile constituents [43].

3.4. Antioxidant Activity

Regardless of the adopted method, significant differences were obtained between plant parts, with significantly higher values in the leaves (Table 4). Concerning the ABTS assay, our results were not in agreement with those present in the literature [33,80]. As previously stated in the present work, ethanol is more efficient at extracting phenols and provides a higher antioxidant capacity in the samples; however, better results were reported for the aqueous extraction than with organic solvents for many antiradical activity tests [33].

Table 4. Antioxidant activities (IC₅₀ expressed as mg mL⁻¹) of ethanolic and water extracts from leaves and stems of *P. oleracea* grown in Tunisia.

	Ethanolic Extract		Water Extract	
	Leaves	Stems	Leaves	Stems
ABTS assay	6.82 ± 0.06	5.31 ± 0.01 *	6.03 ± 0.01	5.08 ± 0.06 **
Reducing power assay	0.83 ± 0.10	1.55 ± 0.07 **	6.19 ± 0.03	10.51 ± 0.03 **
Phosphomolybdenum assay	0.30 ± 0.02	0.46 ± 0.01 **	1.83 ± 0.13	2.66 ± 0.04 **

Mean composition of sampled *P. oleracea* from three replications ± standard deviation. Different symbols (*, **), for the same parameter, within columns indicate significant differences (* $p < 0.05$; ** $p < 0.01$) between the two aerial parts of the plant within the same extraction method. ABTS: 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid

A significant indicator of the antioxidant activity of the extracts is represented by their reducing power capacity, which might be due to their hydrogen-donating ability. The reducing power values obtained in this study were very different between plant parts (leaves and stems) and solvents used for the extraction (ethanol and water) (Table 4). Particularly, leaves reported a significantly higher reducing power capacity than stems. These results indicated that extracts of purslane leaves may act as an electron donor. They could react with free radicals and convert them to more stable products. They could also block the radical chain reaction [81]. Similarly, leaves reported significantly higher antioxidant activities through the phosphomolybdenum assay (Table 4). Significant differences in antioxidant activities between plant parts may indicate the heterogeneity of antioxidant compounds and may depend on environmental conditions, as observed in other vegetables [82–84].

4. Conclusions

In general, the results obtained in the present work indicate that purslane is an important source of phytochemicals known for their protective healthy effects due to their relevant biological activities. However, a great variability in the phytochemical profile was observed in relation to plant parts. Particularly, leaves were a significant source of chlorophyll, phenols, and tannins, whereas stems presented a good nutritional value showing higher contents of anthocyanins and *o*-diphenols. Both aerial parts of purslane were rich in flavonoids. In conclusion, the present research proves that

spontaneous Tunisian purslane represents an interesting species to be incorporated in the human diet; however, further studies are still necessary to screen the Tunisian *P. oleracea* germplasm of interest for phytochemical extractions.

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