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Productive and Morphometric Traits, Mineral Composition and Secondary Metabolome Components of Borage and Purslane as Underutilized Species for Microgreens Production

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Abstract: Neglected and underutilized species (NUS) offer largely unexplored opportunities for providing nutritious plant food, while making agro-ecosystems more diverse and resilient to climate change. The aim of this work was to explore the potential of two typical Mediterranean underutilized species, purslane and borage, as novel vegetable product (microgreens). Micro-scale production of edible plants is spreading due to the simplicity of their management, rapid cycle, harvest index, and phytochemical value of the edible product. Microgreens, therefore, represent an opportunity to link NUS, nutrition, and agricultural and dietary diversification. By analyzing yield, antioxidants activities, mineral composition, and main phenolic acids and flavonoids, our work indicated that the two species provide interesting results when compared with those reported for crops and horticultural species. Specifically, purslane should be considered highly nutritional due to the amount of phenolic compounds and ascorbic acid, and to potential good β-carotene bioavailability. Borage microgreens have a very high fresh yield and a more composite and balanced phenolic profile. In conclusion, our work provided evidence for implementing new ways to expand the NUS market-chains and for developing added-value food products.

Keywords: *Portulaca oleracea*; *Borago officinalis*; yield; antioxidants; phenolics; flavonoids

1. Introduction

Neglected and underutilized endogenous plants are an appealing option to increase food variety [1]. The Mediterranean basin has a plenty of wild and semi-domesticated plants that have been exploited by rural communities for centuries and even during food crises, thus earning the name of "famine food" [2]. A declining consumption of non-cultivated edible plants is occurring due to social factors and the diffusion of the so-called "Western-style diet" [3,4]. Nonetheless, NUS are receiving increased scientific attention due to nutritional benefits, richness of bioactive components, and suitable micronutrient contents [3,5]. Numerous non-cultivated species are considered weeds in intensive crop systems and therefore, are largely ignored by researchers, farmers, and consumers [2]. These non-commodity plants offer the opportunity to enrich urban-style diets at an affordable cost [2,5]. For instance, several wild and semi-domesticated plants are consumed raw in salads, such as purslane and borage [6]. These halophytes are commonly richer

in bioactive compounds than typical salad crops and can provide nutritious food while ensuring a more diversified food basket and a sustainable diet [2].

Purslane (*Portulaca oleracea* L.) is generally seen as a common species among summer crops [7,8] but it was already considered a medicinal and food source by the Ancient Egyptian civilization [9]. In Southern Italy, this herbaceous plant is typically harvested in the wild, although some family farms leave an area for this species to grow, with the harvest typically sold in small markets serving a specific community or area. Purslane is potentially suitable for hydroponics due to the ease of harvest (i.e., stems and leaves are edible), mechanical properties (i.e., the succulent leaves and stems are suitable for motorized harvest), and amenability to the cut-and-come again strategy (i.e., ability to regrow and to produce roots). In floating systems, the cultivation cycle can last from a minimum of 13 days in a summer cycle [10] to approximately 3 months from sowing to the final harvest [7,8]. Purslane is consumed in the Mediterranean basin fresh, cooked or as a dried vegetable [8]. In Southern Italy, it is employed mainly as fresh salad, often with wild rocket. It is rich in molecules with antioxidant potentials such as alfa-tocopherol, beta-carotene, and ascorbic acid [7–9,11]. This species has a high content of proteins, carbohydrates, and minerals (iron, phosphorus, magnesium, calcium, and potassium) [1,7]. On the other hand, mature leaves of purslane frequently contain a high amount of oxalates (between spinach and tea) [12] and nitrates (similar to spinach and celery) [13], which make this species more suitable for occasional consumption. Nonetheless, it has been pointed out that these traits have sufficient intraspecific variability for the selection of improved lines for ready-to-eat products [14].

Borage (*Borrago officinalis* L.) is an annual herb [15–17], probably native to Syria [15], naturalized in the Mediterranean basin and common in Asia Minor, Europe, North Africa, and America [15,18]. In several countries, this herb is cultivated in open-field conditions mainly to extract oil from seeds. Especially in Europe, *B. officinalis* is also grown for culinary and medicinal uses and often harvested in the wild [17,18]. Nonetheless, a recent study indicated the suitability of borage to produce ready-to-eat fresh cut leaves [19]. To our knowledge, information on the cultivation of borage in soilless systems is limited to the study of the effect of salinity on yield and seed characteristics [20]. Borage smells as cucumber, while leaves, stems, and sometimes flowers, are eaten (cooked or raw) in soups and salads, as well as in vegetable and meat dishes [17]. The same authors mentioned that in Northern Spain, borage leaves, petioles, and stems are eaten fresh or moderately fried in salads, whereas in Italy, borage flowers and leaves are eaten in omelets, stews, soups, condiments or pickled and in oil. Borage is rich in fatty acids and is consumed under the belief that it is a treatment for various diseases such as diabetes, arthritis, multiple sclerosis, and eczema [16]. In addition, borage is characterized by tannins, saponins, flavonoids (kaempferol, quercetin, and isorhamnetin), and phenolic acids (p-coumaric, vanilic, chlorogenic, rosmarinic, and caffeic) [17].

In recent years, the production of microgreens to complement that of mature plants has become a trendy market opportunity for novel foods, due to their rich phytochemical content and sensory value [21]. These young leafy greens enhance the human diet by representing not only a different source of bioactive compounds but also by combining vivid colors and tastes. Currently, several horticultural species have been evaluated and exploited to produce microgreens [22].

There is a wide consensus that we need to reverse the abandonment of NUS by changing their reputation (e.g., old-fashioned and associated with the rural lifestyle) and microgreens offer interesting nutritional and social benefits. Producing and selling NUS microgreens to populations who already are accustomed to specific plant species is a practical way to actively encourage people to improve the nutritional value of their diet by harnessing traditional biodiversity. Moreover, microgreens also represent a way to promote NUS in an urban cultural context. The exploitation of the NUS qualities as microgreens is strongly limited by insufficient awareness of their nutritional value. The current study aimed to characterize two underutilized species, purslane and borage, grown

as microgreens, with the goal of promoting their value as a novel and sustainable food complement. Specifically, we assessed yield, macro- and micronutrients using an inductively coupled plasma mass spectrometer (ICP-OES), carotenoids by a high-performance liquid chromatographic method with diode-array detection (HPLC-DAD), and polyphenols by ultra-high-performance liquid chromatography coupled to quadrupole orbitrap high-resolution mass spectrometry (UHPLC-Q-Orbitrap HRMS).

2. Materials and Methods

2.1. Plant Material and Growth Condition

Seeds of borage (*Borrago officinalis* L.), also known as starflower, and of purslane (*Portulaca oleracea* L.), also known as duckweed, were obtained from "Pagano Costantino & F.lli S.R.L" (Scafati, Italy) and "Nehme Establishment for Trade & Agriculture" (Batroun, Lebanon), respectively. Sowing density was 40,000 seeds m^{-2} for borage and 80,000 seeds m^{-2} for purslane. Plants were sown and grew in a climatic chamber (KBP-6395F, Termaks, Bergen, Norway) in 204 cm^2 plastic trays filled with 650 cm^3 of peat-based substrate (Special Mixture, Floragard, Vertriebs-GmbH, Oldenburg, Germany). The macronutrient supply of the substrate (electric conductivity (EC): 282 μS cm^{-1}; pH: 5.48) is described elsewhere [23]. Fertigation was applied daily using a modified (quarter-strength) Hoagland's nutrient solution (NS) prepared with osmotic water (EC: 100 ± 25 μS cm^{-1}). The NS had an EC of 500 ± 50 μS cm^{-1}, a pH of 6 ± 0.2, and the following mineral composition: 2.0 mM NO_3^--N, 0.25 mM S, 0.20 mM P, 0.62 mM K, 0.75 mM Ca, 0.17 mM Mg, 0.25 mM NH_4^+-N, 20 μM Fe, 9 μM Mg, 0.3 μM Cu, 1.6 μM Zn, 20 μM B, and 0.3 μM Mo. Light was provided by light-emitting diode (LED) panels (K5 XL 750, Kind LED Grow Light, Santa Rosa CA, USA) with a 12 h photoperiod. The photosynthetic photon flux density was 300 ± 15 μmol m^{-2} s^{-1} measured at the tray level. The temperature and relative humidity were set at 24 °C day and 18 °C night (± 2 °C) and 65% day and 75% night (±5%), respectively. Each species was replicated three times and randomly placed on the shelf of the climate chamber. Trays were relocated daily across the shelf to avoid time-invariant position effects among experimental units.

2.2. Sampling and Morphometric Measurements

At the emergence of the first true leaf, 23 days after sowing (DAS), borage and purslane microgreens were harvested with scissors by cutting at the substrate level (Supplementary Figure S1). Fresh weight (fw) was measured, and yield expressed in kg fw m^{-2}. Each replicate was divided into homogeneous sub-samples for the destructive analyses. A pool was immediately frozen and stored at −80 °C for the determination of total ascorbic acid and chlorophylls. Another pool was weighed, placed in a forced-air oven (65 °C) until constant weight, for dry weight (dw) assessment and the subsequent analysis of the mineral composition. An additional pool was first snap-frozen in liquid nitrogen and then cold-lyophilized (Christ, Alpha 1–4, Osterode, Germany) for the quantification of phenolic compounds, carotenoids (lutein and β-carotene), and antioxidant activities.

2.3. Antioxidant Activity Measurements

The free radical scavenging activity was quantified with a 2,2-diphenyl-1-picrylhydrazyl (DPPH)-based method using a previously described procedure with few modifications [24]. The DPPH radicals were formed by dissolving 4 mg in 10 mL of methanol. Samples were diluted with the same solvent to obtain a DPPH radical working solution (DRWS) with an absorbance of 0.90 (±0.02) at 517 nm. A mixture of 1 mL of DRWS and 200 μL of sample was incubated for 10 min at room temperature, and absorbance was spectrophotometrically read at 517 nm. The activity was expressed as TEAC (mmol Trolox equivalents kg^{-1} dw of sample).

The ferric reducing antioxidant activity was measured using a FRAP assay [25] with few modifications. Briefly, the FRAP working solution was prepared by mixing 1.25 mL

of 10 mM 2,4,6- tripyridyl-striazine (TPTZ) in HCl (40 mM), 1.25 mL of $FeCl_3$ (20 mmol) in water, and 12.5 mL of 0.3 M sodium acetate buffer (pH 3.6). The reaction of the FRAP solution (2.850 mL) and samples (150 µL) was incubated at room temperature for 4 min and then, absorbance was read at 593 nm. The results were expressed as TEAC (mmol Trolox equivalents kg^{-1} dw of sample).

The ABTS-scavenging activity was evaluated according to the previously published procedures with minor modifications [26]. The 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$) radicals were generated by mixing 5 mL of 7 mM ABTS aqueous stock solution with 88 µL of 2.45 mM aqueous potassium persulfate, diluted with ethanol to a working solution with an absorbance of 0.700 ± 0.002 at 734 nm. Subsequently, 100 µL of sample and 1 mL of the above resulting solution were mixed and incubated for 3 min at room temperature. Absorbance was then read at 734 nm. The results were expressed as TEAC (mmol Trolox equivalents kg^{-1} dw of sample).

2.4. Quantification of Chlorophylls, Catotenoids, and Total Ascorbic Acid

Chlorophylls were spectrophotometrically quantified according to a previously published procedure [27]. Briefly, samples were weighed, and pigments extracted in 90% acetone. Aliquots were read at 662 and 645 nm using a Hach DR 4000 spectrophotometer (Hach Co., Loveland, CO, USA). Total chlorophyll was estimated as the sum of chlorophyll a and b and expressed in mg kg^{-1} fw. Total ascorbic acid was determined as previously described [28] and expressed as mg ascorbate equivalents 100 g^{-1} fw.

Carotenoids (β-carotene and lutein) were quantified by HPLC-DAD essentially as reported [29]. The apparatus comprised a 1200 Series quaternary pump and a 1260 Diode Array Detector Separation (Agilent Technologies, Santa Clara, CA, USA), equipped with Gemini C18 (Phenomenex, Torrance, CA, USA) reverse phase columns (250 × 4.6 mm, 5 µm). Calibration curves were built with using β-carotene and lutein commercial standards (Sigma-Aldrich, Milan, Italy) in the 5 to 100 µg mL^{-1} range. Results were expressed in µg g^{-1} dw.

2.5. Analysis of Macro- and Micro-Minerals by ICP-OES

Minerals (P, K, Ca, Mg, Na, Mn, Fe, Zn, Cu, Se, B, Cr, Mo, Ni, Al, Ba, Cd, and Pb) were quantified by inductively coupled plasma-optical emission spectrometry (Spectroblue, Spectro Ametek, Berwyn, PA, USA) [30]. Briefly, 1 g of dried plant tissue was processed by microwave-assisted digestion (MLS-1200, Microwave Laboratory Systems, Milestone, Shelton, CT, USA) in 10 mL of a 3:1 (*v/v*) solution of nitric acid and fuming hydrochloric acid. The slurry was brought to a final volume of 50 mL with ultra-pure water (Merck Millipore, Darmstadt, Germany). For non-alkaline elements (Fe, Mn, Mo, Se, and Zn), the calibration curve was built in the 1.0 to 100 µg L^{-1} interval and the quantity of the minerals expressed in µg g^{-1} dw. For alkaline elements (P, K, Ca, Mg, and Na), the calibration curve was built in the 100 µg L^{-1} to 10 mg L^{-1} range and the quantity of the minerals expressed in mg g^{-1} dw. For the determination of the accuracy, we used standard reference material (BCR CRM 142R-Commission of the European Communities, 1994). The recovery range was in the 86% to 98% interval.

2.6. Analysis of Polyphenols by UHPLC-Q-Orbitrap HRMS

Lyophilized plant tissue (100 mg) was extracted using 5 mL of a methanol/water (60:40, *v/v*) solution by sonication for half an hour. The mixture was centrifuged (4000 rpm, 15 min), the supernatant filtered through Whatman paper, and then aliquots (10 µL) were used for anthocyanins and polyphenols quantification with a UHPLC system (Dionex UltiMate 3000, Thermo Fisher Scientific, Waltham, MA, USA) coupled to a Q-Exactive Orbitrap mass spectrometer (UHPLC, Thermo Fisher Scientific, Waltham, MA, USA) essentially as described [29,31]. Chromatographic separation was carried out in a Luna Omega PS 1.6 µm column (50 × 2.1 mm, Phenomenex, Torrance, CA, USA) and identification was performed with a Q-Exactive Quadrupole-Orbitrap mass spectrome-

ter (Thermo Fisher Scientific, Waltham, MA, USA) operating in fast negative/positive ion switching mode. Two scan events (full scan MS and all ion fragmentation, AIF) were set for the compounds. Data processing was carried out with the Xcalibur software 3 (Xcalibur, Thermo Fisher Scientific, Waltham, MA, USA). Polyphenols were expressed in μg g^{-1} dw. The individual phenolic compounds were identified and quantified by comparison with the available standards as described [29,31].

2.7. Statistical Analysis

Morphometric measurements were independently carried out on the three replications. All instrumental determinations for each of the three biological replications were performed in two technical replicates. For all variables, the equality of the means between the two species was evaluated with an unpaired two-tailed Student's *t*-test.

3. Results

3.1. Morphometric and Yield Characteristics

Microgreens were harvested 23 days after sowing. Borage provided the highest amount of fresh product per cultivated surface and a largely higher dry biomass (Table 1). In our controlled conditions, yield was highly correlated with the harvested dry mass (r = 0.99; $p < 0.001$; Pearson two-tails). On the other hand, the percentage of dry matter was higher for purslane.

Table 1. Fresh yield, dry weight, dry matter, and hypocotyl length of the two microgreens growing in controlled conditions. Means were statistically separated using a two-tailed Student's *t*-test. **: $p < 0.01$; ***: $p < 0.001$.

Species	Yield (kg fw m^{-2})	Dry Weight (g m^{-2})	Dry Matter (%)	Hypocotyl Length (cm)
Borage	6.44 ± 0.09	307.4 ± 5.93	4.77 ± 0.05	5.32 ± 0.11
Purslane	1.19 ± 0.01	74.26 ± 0.68	6.23 ± 0.04	4.35 ± 0.07
t-test	***	***	***	**

3.2. Mineral Content

Regardless of the higher percentage of dry matter, purslane had a higher amount of 13 mineral elements out of the 18 analyzed, with only Se and Cu (not detected in purslane) present in higher percentage in borage (Table 2). In relative terms, the largest difference was observed for Mo (+461%), followed by Zn (+297%) for the microelements, and for K (+127%) and Mg (+98%) among the macro-elements. B, Al, and Pb did not display a significant variation between the species. As expected, K was the most abundant mineral element for both species. In addition, the Na/K ratio was not significantly different between purslane and borage (not shown), being K approximately 10 × higher than Na.

Table 2. Mineral composition of the microgreens growing in controlled conditions. Means were statistically separated using a two-tailed Student's *t*-test. ns: Not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; nd: Not detected.

Species	P (mg g^{-1} dw)	K (mg g^{-1} dw)	Ca (mg g^{-1} dw)	Mg (mg g^{-1} dw)	Na (mg g^{-1} dw)	Mn (μg g^{-1} dw)	Fe (μg g^{-1} dw)	Zn (μg g^{-1} dw)	Cu (μg g^{-1} dw)
Borage	11.95 ± 0.26	16.03 ± 0.15	11.51 ± 0.26	2.70 ± 0.07	1.72 ± 0.11	32.57 ± 0.06	27.42 ± 0.44	20.62 ± 0.49	3.95 ± 0.52
Purslane	18.93 ± 0.60	36.43 ± 1.12	17.44 ± 0.49	5.34 ± 0.08	3.63 ± 0.11	76.03 ± 1.76	32.07 ± 0.88	81.86 ± 2.32	nd
t-test	***	***	***	***	***	***	**	***	-
Species	**Se (μg g^{-1} dw)**	**B (μg g^{-1} dw)**	**Cr (μg g^{-1} dw)**	**Mo (μg g^{-1} dw)**	**Ni (μg g^{-1} dw)**	**Al (μg g^{-1} dw)**	**Ba (μg g^{-1} dw)**	**Cd (μg g^{-1} dw)**	**Pb (μg g^{-1} dw)**
Borage	0.89 ± 0.05	35.06 ± 0.31	0.08 ± 0.01	0.13 ± 0.03	0.48 ± 0.04	2.52 ± 0.08	23.23 ± 1.10	0.07 ± 0.002	0.10 ± 0.02
Purslane	0.68 ± 0.04	32.16 ± 1.32	0.15 ± 0.02	0.73 ± 0.03	0.72 ± 0.03	2.78 ± 0.17	67.28 ± 0.64	0.15 ± 0.001	0.10 ± 0.03
t-test	*	ns	*	***	**	ns	***	***	ns

3.3. Radical Scavenging Activity

Purslane microgreens had significantly higher antioxidant properties as indicated by the activities measured in the plant extracts with the three different assays (Table 3). Specifically, the different methodologies gave a comparable ranking of antioxidant activities, consistently higher (around 40%) in purslane.

Table 3. Antioxidant activities in microgreen extracts. Means were statistically separated using a two-tailed Student's *t*-test. **: $p < 0.01$; ***: $p < 0.001$.

Species	DPPH (mmol Trolox eq. kg^{-1} dw)	ABTS (mmol Trolox eq. kg^{-1} dw)	FRAP (mmol Trolox eq. kg^{-1} dw)
Borage	31.09 ± 1.59	62.60 ± 1.17	31.09 ± 1.59
Purslane	45.40 ± 1.70	82.83 ± 0.82	45.40 ± 1.70
t-test	**	***	**

3.4. Ascorbic Acid, Chlorophyll, Lutein, and β-Carotene

The total ascorbic acid differed significantly between the two microgreen genotypes studied (Table 4). Interestingly, purslane had significantly more total ascorbic acid (more than 3 times higher than that of borage), while determinants of the leaf color (i.e., chlorophylls and carotenoids) were not different between the two species (Table 4). The data implied that the different colors of the microgreens (Supplementary Figure S1) are mainly due to other components, such as chromogenic phenolic compounds (see below). In absolute terms, β-carotene was more abundant than lutein for both species (Table 4). Nonetheless, the β-carotene/lutein ratio was significantly higher for purslane ($p < 0.01$; *t*-test).

Table 4. Total ascorbic acid, total chlorophylls, and carotenoids (lutein and β-carotene) in microgreen extracts. Means were statistically separated using a two-tailed Student's *t*-test. ns: Not significant; ***: $p < 0.001$.

Species	Total Ascorbic Acid (mg Ascorbate 100 g^{-1} fw)	Total Chlorophyll (mg kg^{-1} fw)	Lutein (μg g^{-1} dw)	β-Carotene (μg g^{-1} dw)
Borage	85.27 ± 3.97	612.0 ± 7.11	23.67 ± 1.22	132.0 ± 15.6
Purslane	276.9 ± 19.6	635.9 ± 17.5	25.99 ± 2.83	197.4 ± 26.4
t-test	***	ns	ns	ns

3.5. Polyphenols

The phenolic profiling of the microgreens indicated the presence of qualitative and quantitative differences (Table 5). Purslane had more than double the amount of total phenols per dry matter compared to borage, with a larger difference present for the flavonoid class (25.6 × higher) compared to phenolic acids (2.2 × higher). These strong dissimilarities are likely to reflect the species-specific biochemical profiles of the microgreens, considering the short cycle and the controlled environmental conditions. Specifically, purslane presented one largely predominant flavonoid (rutin), while other members of this chemical class were essentially present in a much lower amount. On the other hand, borage had a more balanced flavonoid profile, with kaempferols and luteolins being the two equally represented major flavonoid forms in microgreens. Species-specific differences were more complex for phenolic acids.

Seven of the 11 of the detected compounds were present in only one species. Moreover, of the four shared compounds, two (ferulic and caffeic acids) were present in a higher quantity in borage and two (Caffeoyl quinic acid and sinapinic acid hexose) in purslane.

Table 5. Phenolic profiles and phenolic acids and flavonoid composition of the microgreen species grown in controlled conditions. Means were statistically separated using a two-tailed Student's *t*-test. ***: $p < 0.001$; nd: Not detected.

Species	Phenolic Acids										
	Caffeic Acid ($\mu g\ g^{-1}$ dw)	**Caffeoyl Feruloyl Tartaric Acid ($\mu g\ g^{-1}$ dw)**	**Caffeoyl Quinic Acid ($\mu g\ g^{-1}$ dw)**	**Dihydroferulic Acid ($\mu g\ g^{-1}$ dw)**	**Ferulic Acid ($\mu g\ g^{-1}$ dw)**	**Feruloyl Hexoside ($\mu g\ g^{-1}$ dw)**	**Feruloyl Quinic Acid ($\mu g\ g^{-1}$ dw)**	**Rosmarinic Acid ($\mu g\ g^{-1}$ dw)**	**Salicylic Acid Glucoside ($\mu g\ g^{-1}$ dw)**	**Sinapinic Acid Hexose ($\mu g\ g^{-1}$ dw)**	**Vanillic Acid ($\mu g\ g^{-1}$ dw)**
Borage	313.1 ± 14.6	nd	4.43 ± 0.42	5.27 ± 0.81	1124 ± 95.9	nd	nd	257.3 ± 12.2	1218 ± 73.4	1.06 ± 0.06	102.4 ± 11.3
Purslane	2.26 ± 0.17	701.7 ± 26.5	4926 ± 43.6	nd	284.4 ± 8.69	862.0 ± 23.4	17.98 ± 0.85	nd	nd	16.85 ± 0.21	nd
t-test	***	-	***	-	***	-	-	-	-	***	-
Species	**Flavonoids**								**SUMS**		
	Catechin-Glucoside ($\mu g\ g^{-1}$ dw)	**Kaempferol Trimethyl Ether ($\mu g\ g^{-1}$ dw)**	**Kaempferol-3-glucoside ($\mu g\ g^{-1}$ dw)**	**Luteolin trimethyl eher ($\mu g\ g^{-1}$ dw)**	**Luteolin-7-*O*-glucoside ($\mu g\ g^{-1}$ dw)**	**Quercetin rhamnoside ($\mu g\ g^{-1}$ dw)**	**Rutin ($\mu g\ g^{-1}$ dw)**		**Total Phenolic Acids ($\mu g\ g^{-1}$ dw)**	**Total Flavonoids ($\mu g\ g^{-1}$ dw)**	**Total Phenols ($\mu g\ g^{-1}$ dw)**
Borage	2.47 ± 0.11	7.66 ± 0.16	3.27 ± 0.17	7.50 ± 0.16	3.67 ± 0.16	3.72 ± 0.17	nd		3026 ± 183	28.28 ± 0.39	3054 ± 183
Purslane	nd	nd	0.25 ± 0.02	nd	0.28 ± 0.02	0.31 ± 0.03	721.2 ± 29.9		6812 ± 49.6	722.1 ± 29.9	7534 ± 36.6
t-test	-	-	***	-	***	***	-		***	***	***

4. Discussion

The two species provided very different yields that, according to the literature, place borage among the most productive species for microgreens (grown in a peat-based substrate) and purslane among the less productive [32,33]. Specifically, the fresh yield of borage was higher compared to plant species that were sown at a similar density (e.g., purple and green basil, tatsoi, mibuna) or that were harvested after a similar time (e.g., jute, kohlrabi, basil) [32,34]. If fresh yield is standardized considering the plant density (the number of seeds per unit of surface), borage still ranked among the most productive species, following radish and Swiss chard [32,34]. The fresh weight and hypocotyl length of borage was also higher than in another study on the same species, most likely due to an almost double growing cycle (23 vs. 12 days) [35]. On the other hand, purslane's yield was low, only higher than chicory [33], slightly below jute, basil, and rocket [32,34,36,37]. NUS are often disregarded due to their modest yield, but this is also related to the cultivation in marginal and poor soils, and to low or absent agricultural input [38]. The good performance of purslane, which deserves further consideration, is likely to be related to the succulent nature of the plant, as also implied by the high percentage of dry matter of the edible product [31,39].

Our evaluation also explored the mineral composition of the microgreen since nutritional parameters are considered one of the most interesting assets of this class of horticultural products, thus essential for promoting their commercial value [40]. There were significant differences between the two species, with the low-yield purslane providing the highest accumulation for the mineral elements of major dietary importance, such as K, Ca, Mg, Mn, Fe, and Zn. In absolute terms, both species presented a predominant presence of K and Ca, as also previously seen in purslane [41] and other microgreens [39]. It is significant that P was present in amounts similar to K, to an extent higher than previously observed in other species [32,39]. In addition, both species presented a low Na/K ratio, an index of dietary importance [42]. The observed ratio is close to the lowest level recorded in microgreens [32,39]. Purslane demonstrated a higher accumulation efficiency (on dry matter basis) also of the non-essential plant elements. This was shown for some (i.e., Ba and Cd) of the non-essential elements for humans (i.e., Al, Ba, Cd, and Pb). Consistent with the literature on other species, Mn, Zn, and Fe were among the most abundant micro-elements [39,43].

The high antioxidant activity is thought to be one the potential dietary advantages provided by microgreens [44]. Purslane resulted as the most valuable species also having a higher ascorbate content. Ascorbates are usually the most abundant water-soluble antioxidant molecules in plant cells [45] and it has been previously noted that faster growing microgreens have a higher quantity of ascorbic acid than sprouts [32]. The total ascorbate concentration of borage and purslane was within the range of a study on 12 species of microgreens (between 25.1 and 147.0 mg 100 g^{-1} fw) [46]. Differences between borage and purslane were not present in the main pigments of the plants, but purslane had a higher β-carotene/lutein ratio. This represents a useful feature since it is important that vitamins are present in plants in a form that can be easily absorbed. Specifically, it has been reported that lutein has an inhibitory effect on β-carotene absorption [47].

The antioxidant activity measured with the three methods consistently indicated the better performance of purslane compared to borage, coherent with the higher content of total polyphenols. The analysis of the phenolic compounds revealed the large diversity that exists between the two analyzed species. Regarding the absolute content, while the amount of total phenols in borage was comparable to that of several microgreens, purslane can be ranked among the top-producing microgreen species of phenolic compounds [32,39]. The polyphenols in borage and purslane were higher than in rocket, lettuce, mustard, and tatsoi [37,43], and lower than in basil and coriander [43]. Purslane extracts were characterized by a predominant amount of rutin [48]. This flavonol glucoside is common in the plant kingdom, especially in the Polygonaceae and Fabaceae families [49]. Rutin was not detected in several Brassica species, while it was highly concentrated in coriander

microgreens [32]. A major source of rutin in the Mediterranean diet is represented by capers, olives, and asparagus. The amount detected in purslane microgreens is higher than in most vegetables and fruits, except for some berries, such as red raspberry and black currant [50,51]. Rutin has several beneficial properties, and is used in pharmacology for its vasoprotective and capillary stabilizing activities [52]. On the other hand, borage had a more composite phenolics' profile evident for both the phenolic acids and flavonoids. Among phenolic acids, ferulic acid for borage and caffeoyl quinic acid for purslane were confirmed as being major compounds in these species also at the microgreen stage [53]. Considering the literature relative to the adult plants, borage had a significant amount of salicylic acid glucoside. Salicilates (e.g., salts or esters of salicylic acids) are known to be involved in the plant response to biotic stress. Specifically, inactive forms of the plant hormone salicylic acid are made through conjugation with glucose (or other small organic molecules), to be stored in plant vacuoles [54]. Moreover, the borage flavonoid profile was characterized by a more balanced amount of kaempferols and luteolins, among the most widely distributed flavones in the plant kingdom [55].

5. Conclusions

Our work characterized main features and demonstrated the potential of two underutilized species for microgreens production. These species are expected to provide yield and phytochemical compositions comparable or higher to that of microgreens from largely cultivated and genetically improved horticultural varieties [29,32,39]. Compared with other microgreens, purslane should be considered highly nutritional due to the amount phenolic compounds with known beneficial impacts on human health, the high amount of ascorbic acid, and possibly, a good β-carotene bioavailability. Although some mineral-specific differences were present, purslane also displayed a higher mineral utilization efficiency. Even so, the difference with borage, and more generally with other microgreen species reported in the literature, were limited and unlikely to have a significant impact on the human diet. On the other hand, borage microgreens provided the benefit of a much higher fresh yield and a more composite and balanced phenolic profile, which may possibly increase the commercial interest for this underutilized species.

Supplementary Materials: The following is available online at https://www.mdpi.com/article/10.3390/horticulturae7080211/s1. Figure S1: Microgreens at harvest. A: *Portulaca oleracea*; B: *Borago officinalis*. Not to scale.

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References

1. Srivastava, R.; Srivastava, V.; Singh, A. Multipurpose benefits of an underexplored species purslane (*Portulaca oleracea* L.): A critical review. *Environ. Manag.* **2021**, 1–12. [CrossRef]
2. Petropoulos, S.; Karkanis, A.; Martins, N.; Ferreira, I.C. Edible halophytes of the mediterranean basin: Potential candidates for novel food products. *Trends Food Sci. Technol.* **2018**, *74*, 69–84. [CrossRef]
3. Morales, P.; Ferreira, I.C.; Carvalho, A.M.; Sánchez-Mata, M.C.; Cámara, M.; Fernández-Ruiz, V.; Pardo-de-Santayana, M.; Tardío, J. Mediterranean non-cultivated vegetables as dietary sources of compounds with antioxidant and biological activity. *LWT Food Sci. Tech.* **2014**, *55*, 389–396. [CrossRef]
4. Adlercreutz, H. Western diet and Western diseases: Some hormonal and biochemical mechanisms and associations. *Scand. J. Clin. Lab. Investig.* **1990**, *50*, 3–23. [CrossRef]
5. Guarrera, P.; Savo, V. Wild food plants used in traditional vegetable mixtures in Italy. *J. Ethnopharmacol.* **2016**, *185*, 202–234. [CrossRef]
6. Torija-Isasa, M.E.; Matallana-González, M.C. A historical perspective of wild plant foods in the mediterranean area. In *Mediterranean Wild Edible Plants*; Springer: New York, NY, USA, 2016; pp. 3–13.
7. Gonnella, M.; Charfeddine, M.; Conversa, G.; Santamaria, P. Purslane: A review of its potential for health and agricultural aspects. *Eur. J. Plant Sci. Biotech.* **2010**, *4*, 131–136.
8. Dabbou, S.; Lahbib, K.; Pandino, G.; Dabbou, S.; Lombardo, S. Evaluation of pigments, phenolic and volatile compounds, and antioxidant activity of a spontaneous population of *Portulaca oleracea* L. grown in Tunisia. *Agriculture* **2020**, *10*, 353. [CrossRef]
9. Sdouga, D.; Branca, F.; Kabtni, S.; Di Bella, M.C.; Trifi-Farah, N.; Marghali, S. Morphological traits and phenolic compounds in tunisian wild populations and cultivated varieties of *Portulaca oleracea* L. *Agronomy* **2020**, *10*, 948. [CrossRef]
10. Ochoa, J.; Niñirola, D.; Conesa, E.; Lara, L.; López-Marín, J.; Fernández, J. Predicting Purslane (*Portulaca oleracea* L.) Harvest in a Hydroponic Floating System. In *Proceedings of the V International Symposium on Seed, Transplant and Stand Establishment of Horticultural Crops 898, Murcia and Almería, Spain, 27 September–1 October 2009*; ISHS: Leuven, Belgium, 2011; pp. 205–209.
11. Alam, M.; Juraimi, A.S.; Rafii, M.; Hamid, A.A.; Aslani, F.; Hasan, M.; Zainudin, M.A.M.; Uddin, M. Evaluation of antioxidant compounds, antioxidant activities, and mineral composition of 13 collected purslane (*Portulaca oleracea* L.) accessions. *BioMed. Res. Int.* **2014**, *2014*, 296063. [CrossRef]
12. Noonan, S.; Savage, G. Oxalate content of foods and its effect on humans. *Asia Pac. J. Clin. Nutr.* **1999**, *8*, 64–74. [PubMed]
13. Corré, W.J.; Breimer, T. *Nitrate and Nitrite in Vegetables*; Pudoc: Wageningen, The Netherlands, 1979.
14. Egea-Gilabert, C.; Ruiz-Hernández, M.V.; Parra, M.Á.; Fernández, J.A. Characterization of purslane (*Portulaca oleracea* L.) accessions: Suitability as ready-to-eat product. *Sci. Hortic.* **2014**, *172*, 73–81. [CrossRef]
15. Gupta, M.; Singh, S. *Borago officinalis* linn. An important medicinal plant of mediterranean region: A review. *Int. J. Pharm. Sci. Rev. Res.* **2010**, *5*, 27–34.
16. Asadi-Samani, M.; Bahmani, M.; Rafieian-Kopaei, M. The chemical composition, botanical characteristic and biological activities of *Borago officinalis*: A review. *Asia Pac. J. Trop. Med.* **2014**, *7*, S22–S28. [CrossRef]
17. Borowy, A.; Chwil, M.; Kapłan, M. Biologically active compounds and antioxidant activity of borage (*Borago officinalis* L.) flowers and leaves. *Acta Sci. Pol. Hortorum Cultus* **2017**, *16*, 169–180. [CrossRef]
18. Abu-Qaoud, H.; Shawarb, N.; Hussen, F.; Jaradat, N.; Shtaya, M. Comparison of qualitative, quantitative analysis and antioxidant potential between wild and cultivated *Borago officinalis* leaves from palestine. *Pak. J. Pharm. Sci.* **2018**, *31*, 953–959.
19. Miceli, C.; Moncada, A.; Vetrano, F.; D'Anna, F.; Miceli, A. Suitability of *Borago officinalis* for minimal processing as fresh-cut produce. *Horticulturae* **2019**, *5*, 66. [CrossRef]
20. Urrestarazu, M.; Gallegos-Cedillo, V.M.; Ferrón-Carrillo, F.; Guil-Guerrero, J.L.; Lao, M.T.; Álvaro, J.E. Effects of the electrical conductivity of a soilless culture system on gamma linolenic acid levels in borage seed oil. *PLoS ONE* **2019**, *14*, e0207106. [CrossRef]
21. El-Nakhel, C.; Pannico, A.; Graziani, G.; Kyriacou, M.C.; Giordano, M.; Ritieni, A.; De Pascale, S.; Rouphael, Y. Variation in macronutrient content, phytochemical constitution and in vitro antioxidant capacity of green and red butterhead lettuce dictated by different developmental stages of harvest maturity. *Antioxidants* **2020**, *9*, 300. [CrossRef]
22. Caracciolo, F.; El-Nakhel, C.; Raimondo, M.; Kyriacou, M.C.; Cembalo, L.; De Pascale, S.; Rouphael, Y. Sensory attributes and consumer acceptability of 12 microgreens species. *Agronomy* **2020**, *10*, 1043. [CrossRef]
23. Pannico, A.; Graziani, G.; El-Nakhel, C.; Giordano, M.; Ritieni, A.; Kyriacou, M.C.; Rouphael, Y. Nutritional stress suppresses nitrate content and positively impacts ascorbic acid concentration and phenolic acids profile of lettuce microgreens. *Italus Hortus* **2020**, *27*, 41–52. [CrossRef]
24. Brand-Williams, W.; Cuvelier, M.-E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci. Tech.* **1995**, *28*, 25–30. [CrossRef]
25. Benzie, I.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The frap assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef]
26. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved abts radical cation decolorization assay. *Free. Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
27. Lichtenthaler, H.K.; Wellburn, A.R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591–592. [CrossRef]

28. Minutolo, M.; Chiaiese, P.; Di Matteo, A.; Errico, A.; Corrado, G. Accumulation of ascorbic acid in tomato cell culture: Influence of the genotype, source explant and time of in vitro cultivation. *Antioxidants* **2020**, *9*, 222. [CrossRef]
29. Kyriacou, M.C.; El-Nakhel, C.; Pannico, A.; Graziani, G.; Soteriou, G.A.; Giordano, M.; Palladino, M.; Ritieni, A.; De Pascale, S.; Rouphael, Y.; et al. Phenolic constitution, phytochemical and macronutrient content in three species of microgreens as modulated by natural fiber and synthetic substrates. *Antioxidants* **2020**, *9*, 252. [CrossRef] [PubMed]
30. Volpe, M.G.; Nazzaro, M.; Di Stasio, M.; Siano, F.; Coppola, R.; De Marco, A. Content of micronutrients, mineral and trace elements in some mediterranean spontaneous edible herbs. *Chem. Cent. J.* **2015**, *9*, 1–9. [CrossRef] [PubMed]
31. El-Nakhel, C.; Pannico, A.; Graziani, G.; Kyriacou, M.C.; Gaspari, A.; Ritieni, A.; De Pascale, S.; Rouphael, Y. Nutrient supplementation configures the bioactive profile and production characteristics of three brassica microgreens species grown in peat-based media. *Agronomy* **2021**, *11*, 346. [CrossRef]
32. Kyriacou, M.C.; El-Nakhel, C.; Graziani, G.; Pannico, A.; Soteriou, G.A.; Giordano, M.; Ritieni, A.; De Pascale, S.; Rouphael, Y. Functional quality in novel food sources: Genotypic variation in the nutritive and phytochemical composition of thirteen microgreens species. *Food Chem.* **2019**, *277*, 107–118. [CrossRef] [PubMed]
33. Paradiso, V.M.; Castellino, M.; Renna, M.; Gattullo, C.E.; Calasso, M.; Terzano, R.; Allegretta, I.; Leoni, B.; Caponio, F.; Santamaria, P. Nutritional characterization and shelf-life of packaged microgreens. *Food Funct.* **2018**, *9*, 5629–5640. [CrossRef]
34. Bulgari, R.; Baldi, A.; Ferrante, A.; Lenzi, A. Yield and quality of basil, swiss chard, and rocket microgreens grown in a hydroponic system. *N. Z. J. Crop. Hortic. Sci.* **2017**, *45*, 119–129. [CrossRef]
35. Viršilė, A.; Sirtautas, R. Light irradiance level for optimal growth and nutrient contents in borage microgreens. *Rural. Dev.* **2013**, *8*, 1241–1249.
36. Di Gioia, F.; De Bellis, P.; Mininni, C.; Santamaria, P.; Serio, F. Physicochemical, agronomical and microbiological evaluation of alternative growing media for the production of rapini (brassica rapa l.) microgreens. *J. Sci. Food Agric.* **2017**, *97*, 1212–1219. [CrossRef]
37. Kyriacou, M.C.; El-Nakhel, C.; Soteriou, G.A.; Graziani, G.; Kyratzis, A.; Antoniou, C.; Ritieni, A.; De Pascale, S.; Rouphael, Y. Preharvest nutrient deprivation reconfigures nitrate, mineral, and phytochemical content of microgreens. *Foods* **2021**, *10*, 1333. [CrossRef]
38. Chivenge, P.; Mabhaudhi, T.; Modi, A.T.; Mafongoya, P. The potential role of neglected and underutilised crop species as future crops under water scarce conditions in sub-saharan africa. *Int. J. Environ. Res. Public Heal.* **2015**, *12*, 5685–5711. [CrossRef]
39. Xiao, Z.; Codling, E.E.; Luo, Y.; Nou, X.; Lester, G.E.; Wang, Q. Microgreens of brassicaceae: Mineral composition and content of 30 varieties. *J. Food Compos. Anal.* **2016**, *49*, 87–93. [CrossRef]
40. Kyriacou, M.C.; Rouphael, Y.; Di Gioia, F.; Kyratzis, A.; Serio, F.; Renna, M.; De Pascale, S.; Santamaria, P. Micro-scale vegetable production and the rise of microgreens. *Trends Food Sci. Technol.* **2016**, *57*, 103–115. [CrossRef]
41. Giménez, A.; Martínez-Ballesta, M.d.C.; Egea-Gilabert, C.; Gómez, P.A.; Artés-Hernández, F.; Pennisi, G.; Orsini, F.; Crepaldi, A.; Fernández, J.A. Combined effect of salinity and led lights on the yield and quality of purslane (*Portulaca oleracea* L.) microgreens. *Horticulturae* **2021**, *7*, 180. [CrossRef]
42. Drewnowski, A.; Maillot, M.; Rehm, C. Reducing the sodium-potassium ratio in the us diet: A challenge for public health. *Am. J. Clin. Nutr.* **2012**, *96*, 439–444. [CrossRef] [PubMed]
43. Pannico, A.; El-Nakhel, C.; Graziani, G.; Kyriacou, M.C.; Giordano, M.; Soteriou, G.A.; Zarrelli, A.; Ritieni, A.; De Pascale, S.; Rouphael, Y. Selenium biofortification impacts the nutritive value, polyphenolic content, and bioactive constitution of variable microgreens genotypes. *Antioxidants* **2020**, *9*, 272. [CrossRef]
44. Choe, U.; Yu, L.L.; Wang, T.T. The science behind microgreens as an exciting new food for the 21st century. *J. Agric. Food Chem.* **2018**, *66*, 11519–11530. [CrossRef] [PubMed]
45. Paciolla, C.; Fortunato, S.; Dipierro, N.; Paradiso, A.; De Leonardis, S.; Mastropasqua, L.; de Pinto, M.C. Vitamin c in plants: From functions to biofortification. *Antioxidants* **2019**, *8*, 519. [CrossRef] [PubMed]
46. Di Gioia, F.; Renna, M.; Santamaria, P. Sprouts, microgreens and "baby leaf" vegetables. In *Minimally Processed Refrigerated Fruits and Vegetables*; Springer: New York, NY, USA, 2017; pp. 403–432.
47. Van den Berg, H. Effect of lutein on beta-carotene absorption and cleavage. *Int. J. Vitam. Nutr. Res.* **1998**, *68*, 360–365. [PubMed]
48. Uddin, M.; Juraimi, A.S.; Ali, M.; Ismail, M.R. Evaluation of antioxidant properties and mineral composition of purslane (*Portulaca oleracea* L.) at different growth stages. *Int. J. Mol. Sci.* **2012**, *13*, 10257–10267. [CrossRef]
49. Frutos, M.J.; Rincón-Frutos, L.; Valero-Cases, E. Rutin. In *Nonvitamin and Nonmineral Nutritional Supplements*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 111–117.
50. Atanassova, M.; Bagdassarian, V. Rutin content in plant products. *J. Univ. Chem. Techn. Metall.* **2009**, *44*, 201–203.
51. Azimova, S.S.; Vinogradova, V.I. *Natural Compounds: Flavonoids*; Springer: New York, NY, USA, 2013.
52. Ganeshpurkar, A.; Saluja, A.K. The pharmacological potential of rutin. *Saudi Pharm. J.* **2017**, *25*, 149–164. [CrossRef] [PubMed]
53. Zadernowski, R.; Naczk, M.; Nowak-Polakowska, H. Phenolic acids of borage (*Borago officinalis* L.) and evening primrose (oenothera biennis l.). *J. Am. Oil Chem. Soc.* **2002**, *79*, 335–338. [CrossRef]
54. Thompson, A.M.G.; Iancu, C.V.; Neet, K.E.; Dean, J.V.; Choe, J.-y. Differences in salicylic acid glucose conjugations by ugt74f1 and ugt74f2 from arabidopsis thaliana. *Sci. Rep.* **2017**, *7*, srep46629. [CrossRef] [PubMed]
55. Calderon-Montano, J.; Burgos-Morón, E.; Pérez-Guerrero, C.; López-Lázaro, M. A review on the dietary flavonoid kaempferol. *Mini-Rev. Med. Chem.* **2011**, *11*, 298–344. [CrossRef]