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Seasonal Fluctuations of Crop Yield, Total Phenolic Content and Antioxidant Activity in Fresh or Cooked Borage (*Borago officinalis* L.), Mallow (*Malva sylvestris* L.) and Buck's-Horn Plantain (*Plantago coronopus* L.) Leaves

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Abstract: The interest for wild edible plants as functional food is increasing among consumers in the Mediterranean countries because of their high content of antioxidants. However, a critical point is the seasonality of wild edible species due to their spontaneity and the cultivation results necessary to satisfy market requests. Moreover, cooking may be necessary for most wild edible species to enhance their palatability. In the present experiment, the crop yield, total phenolic content (TPC) and antioxidant activity (AA) of leaves were determined in three wild edible species (*Borago officinalis* L., *Malva sylvestris* L. and *Plantago coronopus* L.), which were hydroponically cultivated in winter and in spring. Plants were recurrently harvested three times and the leaves were analyzed raw or after boiling in water for different times based on their palatability as evaluated by a hedonic test (2 min for *B. officinalis*, 2.5 min for *M. sylvestris* and 8 min for *P. coronopus*). The total crop yield was promising, especially for *P. coronopus*, with small differences between winter and spring (9.3 and 13.8 kg m^{-2}, respectively). The boiling treatment caused a loss of TPC and, in some cases, of the AA in *B. officinalis* and *M. sylvestris* due to the solubilization of phenolic and other antioxidant compounds in boiling water. Conversely, in *P. coronopus*, TPC and AA were higher in boiled leaves than in fresh leaves, likely due to the strong binding of phenolic compounds to the cell wall. This binding might lead to the inefficient extraction of these compounds through the boiling treatment.

Keywords: antioxidant activity; boiling; hydroponics; total phenolic content; wild edible plants

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1. Introduction

Leafy vegetables, among the most used foods in the Mediterranean diet, are considered a source of antioxidant compounds that play important roles in the prevention of chronic neurodegenerative diseases and inflammatory processes [1]. For this reason, there is increasing interest in potential new vegetable species rich in antioxidant compounds. Wild edible plants have been recently rediscovered to enrich the diet of antioxidant compounds [2]. Indeed, several studies have reported the high antioxidant activity (AA) of wild edible plants, which is mainly due to their high total phenolic content (TPC) [1,3,4]. Among wild edible plants, *Borago officinalis* L., *Malva sylvestris* L. and *Plantago coronopus* L. are known for their phenolic profile and AA, as well as for their use in traditional cooking recipes [5–7].

Borago officinalis, commonly named borage, belonging to the Boraginaceae family, is an herbaceous species with well-known medicinal and nutraceutical value. It is used in the pharmaceutical and food industry, and as forage [8]. The leaves are eaten either fresh or cooked as diuretic, emollient and expectorant ingredients [9]; leaf extracts have a strong

AA and contain several flavonoids, phenolic acids and their derivatives, secoiridoids and sterols [5,10], but also many volatile compounds classified as "green leaf volatiles" due to their characteristic "green" odor, such as hexanal, (E)-2-hexenal, hexanol, (Z)-3-hexenol, (E)-2-hexenol and nonanol, derivatives from the oxidative cleavage of polyunsaturated fatty acids such as linoleic acid and α-linolenic acid [11,12].

Malva sylvestris, commonly named mallow, in the Malvaceae family, is an annual herbaceous plant native to Asia, Europe and North Africa [13]. Its leaves are used for their medicinal properties [14] as they have potent anti-inflammatory, antioxidant (as 3.88 mM Trolox equivalents per g of fresh leaves; [15]), anticancer and skin tissue integrity activity [14]. These medicinal properties are conferred upon *M. sylvestris* leaves by their phytochemical profile, composed of terpenoids, hydroxycinnamic acids (e.g., 4-hydroxybenzoic acid, 4-methoxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 4-hydroxycinnamic acid, ferulic acid, methyl 2-hydroxydihydrocinnamate [16]), flavonoids (e.g., rutin, hyperoside and quercetin-3-D-glucoside, luteolin, apigenin and quercetin [17]), but also tannins and other phenolic compounds [18]. Young leaves of this species are consumed either fresh in salads or boiled [19].

Plantago coronopus, commonly named buck's-horn plantain, belonging to the Plantaginaceae family, is a facultative halophyte commonly consumed fresh in mixed salad [20]. The leaves of this species are appreciated for their salty taste and high nutritional value [20]; they have a high TPC and contain many bioactive compounds, especially flavonoids such as luteolin-7-O-glucoside, apigenin-7-O-glucoside, luteolin, apigenin, rutin and quercetin [21] and phenylpropanoids such as verbascoside and plantamajoside [22], essential amino acids and minerals, such as magnesium, calcium, potassium and sodium [20,23,24].

The market for fresh or processed leafy vegetables requires high and standardized organoleptic, technological and hygienic quality, which, in the case of wild edible plants such as those studied in this work, cannot be guaranteed by the spontaneous collection of plants (risks of contamination, prohibition of harvest from the wild, etc.). Therefore, these kinds of plants must be cultivated according to well-defined protocols and all year round. For these reasons, hydroponic cultivation under a greenhouse appears the most suitable productive technology for these species, as the level of bioactive compounds and AA in leaf tissues could be controlled and standardized through the regulation of climatic parameters and the composition of the nutrient solution [25–28]. Among closed-loop hydroponic techniques, the floating system is widely used for the cultivation of leafy vegetables [29,30].

The consumption of fresh leaves of many wild edible plants is usually hindered by their bitterness and/or other uncommon attributes (e.g., hairiness of borage leaves) and consequently their hard palatability [31,32] but also by some antinutrients present in wild leaves. For example, *M. sylvestris* leaves were found to contain mucilage, nitrates and tannins [33], while *B. officinalis* leaves were found to contain several sterols, mucilage, allantoin, potassium nitrate, resins, tannins and low amounts of toxic pyrrolizidine alkaloids that confer to these leaves antinutritive properties [34]. For these reasons, cooking (e.g., boiling) wild edible plants could reduce their undesirable organoleptic features and their antinutrient content. Nevertheless, in boiled leaves, high amounts of phenolics are leached into the cooking water since the high temperature leads to the disruption of the cellular structure [35–37]. In addition, boiling can reduce the content of flavonoids and phenolic acids due to diacylation and glycosylation, also reducing, consequently, the AA [38].

The goal of the present work is a preliminary step to investigate the effect of the growing season (winter and spring), repeated harvests and boiling treatment on the TPC and AA of *B. officinalis*, *M. sylvestris* and *P. coronopus* cultivated in a floating system under a greenhouse. Thus far, no studies have analyzed the biomass yield of *B. officinalis*, *M. sylvestris* and *P. coronopus* cultivated in a hydroponic system in different seasons and subjected to repeated cuttings (as with many common leafy vegetables). Very few studies have focused on the pattern of TPC and AA of boiled leaves of *B. officinalis* [39,40], while, to the best of our knowledge, no work has been conducted on these aspects on *M. sylvestris*

and *P. coronopus.* For all these reasons, a preliminary investigation on the TPC and AA of leaves of these species can be a first step to address further studies in more detailed ways.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Two experiments were carried out from December 2020 to March 2021 and from March to June 2021 (Table 1) in a greenhouse at the University of Pisa, Italy (lat. 42′42″48 N, long. 10°24′52″92 E). Seeds of *B. officinalis*, *M. sylvestris* and *P. coronopus* were purchased from Gargini Sementi (Lucca, Italy), sown in 240-cell rockwool trays and covered with vermiculite. In winter, the sowing was carried out on 14 December 2020, for all the species, while in spring, the sowing was carried out on 22 March 2021. In winter, seedlings with uniform height and leaf area were transplanted in a floating system 28, 32 and 49 days after sowing (DAS), respectively, for *B. officinalis*, *M. sylvestris* and *P. coronopus,* while in spring, the transplantation took place 21 DAS for *B. officinalis* and *M. sylvestris*, and 35 DAS for *P. coronopus* (Figure S1; Table 1).

Table 1. Environmental conditions during the experiment with *Borago officinalis, Malva sylvestris* and *Plantago coronopus* hydroponically grown under greenhouse in winter or in spring and subjected to three consecutive cuts (C1, C2, C3). The dates of sowing, transplantation and cuts are also shown.

Season	Species	Mean Air Temperature (°C)	Mean Air Relative Humidity (%)	Cumulative Solar Radiation (MJ m^{-2})	Sowing Date	Planting	C1	C2	C3
						Days from sowing			
Winter	*B. officinalis*	18.8	67.3	62.4	14/12/2020	28	49	63	77
	M. sylvestris	19.0	66.2	78.6		32	56	70	84
	P. coronopus	19.5	62.9	133.1		49	77	91	105
Spring	*B. officinalis*	24.0	54.3	77.7	22/03/2021	21	42	49	56
	M. sylvestris	24.0	54.3	77.7		21	42	49	56
	P. coronopus	25.6	48.3	216.9		35	56	70	84

Each species was grown in three separate 50 L plastic tanks on a polystyrene tray hosting 16 plants. All the plants were fed with a continuously aerated nutrient solution calculated for general leafy vegetables containing N-NO_3^- 16.0 mM, N-NH_4^+ 2.0 mM, P-PO_4^{3-} 2.0 mM, K^+ 10.0 mM, Ca^{2+} 4.5 mM, Mg^{2+} 1.0 mM, S-SO_4^{2-} 2.5 mM, Fe^{2+} 40.0 µM, BO_3^- 30.0 µM, Cu^{2+} 1.0 µM, Zn^{2+} 5.0 µM, Mn^{2+} 5.0 µM, Mo^{3+} 1.0 µM ([29] with minor modifications). Electrical conductivity (EC) and pH were 2.75 dS m^{-1} and 5.5, respectively; these parameters were checked every day and adjusted to keep them within 10% of the values measured in the fresh nutrient solution. During the experiment, the crop evapotranspiration was regularly compensated by refilling every three days the tank with fresh nutrient solution.

In both seasons, all species were cut three times after transplanting at 2 cm above the collar level (Figure S1), when leaf area approximated 60, 90 and 14 cm^2 $plant^{-1}$ in *B. officinalis*, *M. sylvestris* and *P. coronopus,* respectively, which is the suitable leaf area for fresh and cooked consumption of these species that have very different leaf morphology.

The cut timing depended on plant species and is reported in Table 1 and Figure S1. Since the growth and cut timing of each species under investigation were different to obtain the suitable leaves for both fresh and cooked consumption, greenhouse growth conditions were different. Table 1 reports the climatic conditions monitored by a weather station located inside the glasshouse, and the dates of sowing, transplantation and of the three consecutive cuts for each species under investigation.

2.2. Growth Analysis

On the occasion of each cut, all the leaves were harvested and the leaf fresh weight (FW) was measured on all the plants in each tank, and the dry weight (DW) was measured after drying in a ventilated oven (Memmert GmbH Co. KG Universal Oven UN30, Schwabach, Germany) at 105 °C until reaching a constant weight. The DW/FW ratio was determined.

The area of detached leaves was determined using ImageJ software (National Institute of Health, Bethesda, Rockville, MD, USA). Then, all the leaves were washed in distilled water and gently dried with paper towels.

2.3. Boiling Treatment

Immediately after the cut, a portion (50 g) of fresh leaves for each species was placed in 500 mL of boiling distilled H_2O for different times based on the palatability of the leaves: 2 min for *B. officinalis*, 2.5 min for *M. sylvestris* and 8 min for *P. coronopus*. This was the minimum cooking time to reach a similar softness, palatability and taste according to the Italian consumption habits. For each species, the best boiling conditions were determined in a preliminary experiment, in which the palatability of leaves was evaluated by 20 trained panelists (10 men and 10 women) through a hedonic test based on the texture, flavor and taste of leaves. After the boiling treatment, the boiling water was drained off for 60 s. An aliquot of boiled leaves was used to determine the DW content (data not shown) in order to express the TPC and the AA on a dry mass basis.

Samples of fresh and boiled leaves were frozen in liquid nitrogen and left at −80 °C until laboratory analyses.

2.4. Experimental Design

For each species, the growth and biochemical parameters were defined by a combination of two or three factors: the season, the cut timing and the boiling. The treatments were arranged following a completely randomized experimental design with three replicates, where each 50 L tank, for each species under investigation, was considered as a replicate.

2.5. Total Phenolic Content

The TPC was determined as reported by Dewanto et al. [41] with some modifications. For the extraction, 0.1 g of plant material (fresh or boiled) was homogenized in 1 mL 80% (*v*/*v*) methanol solution and centrifuged at 10,000 rpm for 10 min at 4 °C. For the assay, 62.5 µL extract aliquot was added to a solution composed of 250 µL of milliQ H_2O, 62.5 µL of Folin–Ciocalteu reagent. Then, after 6 min of reaction, 625 µL of 7% (*w*/*v*) Na_2CO_3 and 500 µL of milliQ H_2O were added. The consequent blue color due to the reduction of the metals present in the solution of phosphomolybdate/phosphotungstate of Folin–Ciocalteu reagent and the oxidation of phenolic compounds was measured at 760 nm with a spectrophotometer (Ultrospec 2100 Pro, GE Healthcare Ltd., Chalfont, RA, USA). Each measurement was compared with a standard curve of gallic acid (concentration range 30–600 µg mL^{-1}) and the TPC was expressed as mg gallic acid equivalents (GAE) per g DW.

2.6. Antioxidant Activity

The AA was measured as reported by Brand-Williams et al. [42] through the measurement of the DPPH scavenging activity of the plant material under investigation. An aliquot (10 µL) of phenolic extract was added to 990 µL of a methanolic solution of 3.12×10^{-5} M DPPH (*w*/*v*) and left for 30 min. The reduction of the DPPH radical by the antioxidant compounds was measured at 515 nm against a blank solution (with no extracts) with the same spectrophotometer reported above. Each measurement was compared with a standard curve of Trolox (concentration range 0–10 µM) and the AA was expressed as mg Trolox equivalents (TE) per g DW.

2.7. Statistical Analysis

All data were analyzed by 1-, 2- or 3-way analysis of variance (ANOVA) depending on the considered parameter, with plant species, growing season, cut timing and boiling treatment as sources of variation. All the means were separated by Tukey's HSD (honestly significant difference) post-hoc test ($p < 0.05$). The normality of data was tested using the Shapiro–Wilk test, whilst the homoscedasticity was tested using Bartlett's test. Pearson's correlation coefficient was calculated for the relationship between TPC and AA. All statistical analyses were conducted using GraphPad (GraphPad, La Jolla, CA, USA) or JMP software (SAS Institute Inc., Cary, NC, USA).

3. Results

The total crop yield has been analyzed by two-way ANOVA considering the species and the growing season as sources of variation, while the production of leaves harvested at different DAS and their TPC and AA have been analyzed by three-way ANOVA with the season, the cut timing and the boiling treatment among the independent variables.

3.1. Crop Yield

The highest crop yield was detected for *P. coronopus*, with a total production of 9.3 ± 2.1 kg m^{-2} and 13.8 ± 0.9 kg m^{-2} during winter and spring, respectively (Figure 1), while the lowest yield was observed in *M. sylvestris* (Figure 1).

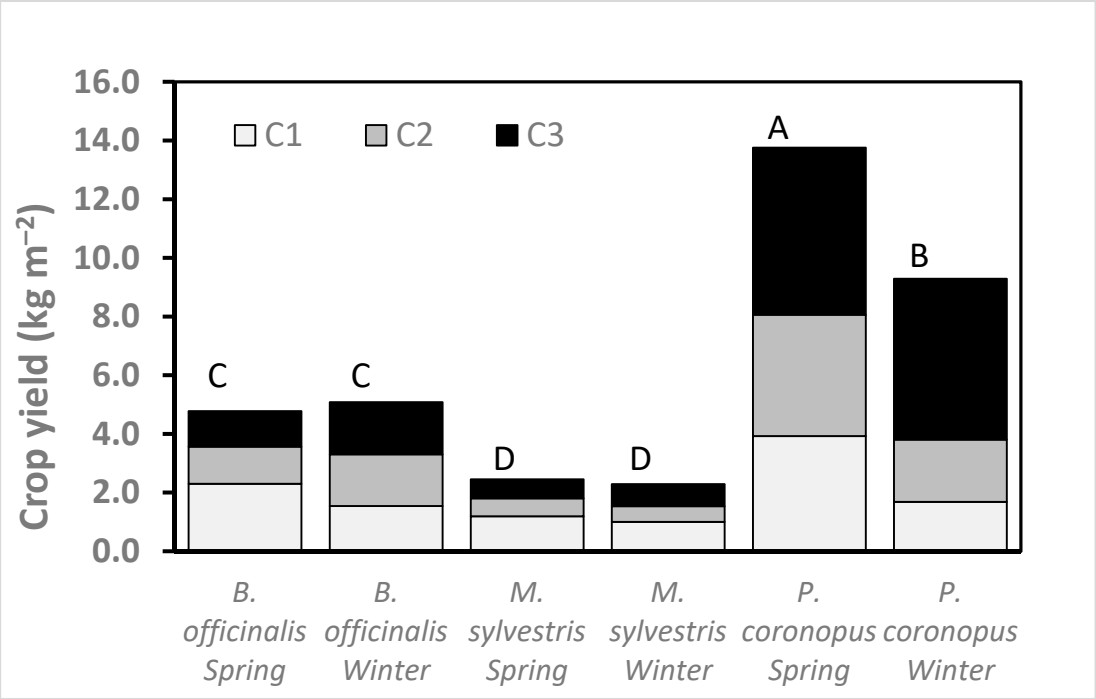

Figure 1. Crop yield (fresh leaves) of *Borago officinalis*, *Malva sylvestris* and *Plantago coronopus* hydroponically grown under greenhouse in winter or in spring and subjected to three consecutive cuts (C1, C2, C3). Data keyed with different letters are significantly different for $p < 0.05$ following two-way ANOVA and Tukey's HSD post-hoc test for the effect of plant species and growing season. Values are the mean (±S.E.) of three replicates.

The growing season significantly influenced the total leaf production in *P. coronopus*, where the production was higher (+48%) in spring than in winter (Figure 1), notwithstanding the shorter cultivation period and because of the higher air temperature and irradiance (Table 1). The effect of the cut on the crop depended on the growing season and the species. Indeed, leaf production was significantly higher on C1 than on the following cuts in *B. officinalis* during spring, and in *M. sylvestris* in both seasons (Table S1). Both

B. officinalis and *M. sylvestris* crop yield did not differ significantly on the C2 and the C3 (Table S1). Conversely, leaf production of *P. coronopus* during both seasons significantly increased on successive cuts (Table S1).

The DW/FW ratio (Table S2) was higher in spring than in winter in all the species under investigation. This value increased with the cut in *M. sylvestris*, whilst, in *B. officinalis* and *P. coronopus*, no significant differences were observed in successive cuts. The highest DW/FW values were reported in *M. sylvestris*.

3.2. TPC and AA

In *B. officinalis*, the interaction between season, cut and boiling was significant only as regards the AA analyzed through the DPPH assay, a generic analysis for a preliminary assessment of the DPPH scavenging activity of each species under investigation (Table 2). Both TPC and AA were significantly higher in spring than in winter, and tended to increase in successive cuts, although the rise in TPC was observed only in winter (Table 2). The boiling treatment decreased leaf TPC (−74.55%) and AA (−68.14%) only in winter and, to a greater extent, in the leaves of C2 (−83.01% for TPC and −84.63% for AA) and C3 (−70.36% for TPC and −48.51% for AA) (Table 2).

Table 2. Total phenolic content (TPC) and antioxidant capacity (AA) in raw or boiled leaves of *Borago officinalis* hydroponically grown under greenhouse in winter or in spring and subjected to three consecutive cuts (C1, C2, C3). Data keyed with different letters are significantly different for $p < 0.05$ following three-way ANOVA and Tukey's HSD post-hoc test for the effect of growing season, cut and boiling.

Cut (C)	Growing Season (GS)	Heat Treatment (HT)	TPC (mg GAE g^{-1} DW)	AA (mg TE g^{-1} DW)
C1	Winter	Raw	5.31	10.21 cd
		Boiled	0.99	4.43 d
	Spring	Raw	7.28	7.42 cd
		Boiled	7.91	12.98 bcd
C2	Winter	Raw	9.71	28.24 a
		Boiled	1.65	4.34 d
	Spring	Raw	13.85	7.91 cd
		Boiled	11.43	17.70 abc
C3	Winter	Raw	28.37	17.77 abc
		Boiled	8.41	9.15 cd
	Spring	Raw	17.00	22.66 ab
		Boiled	8.21	11.56 bcd
MEAN EFFECT				
C1			5.37 c	8.76 b
C2			9.16 b	14.55 a
C3			15.50 a	15.29 a
	Winter		9.07 b	12.36
	Spring		10.95 a	13.37
		Raw	13.59 a	15.70 a
		Boiled	6.43 b	10.03 b
C1	Winter		3.15 d	7.32
	Spring		7.60 c	10.20
C2	Winter		5.68 cd	16.29
	Spring		12.64 b	12.81
C3	Winter		18.39 a	13.46
	Spring		12.61 b	17.11
C1		Raw	6.30 cd	8.82 b
		Boiled	4.45 d	8.71 b

Table 2. *Cont.*

Cut (C)	Growing Season (GS)	Heat Treatment (HT)	TPC (mg GAE g^{-1} DW)	AA (mg TE g^{-1} DW)
C2		Raw	11.78 b	18.08 a
		Boiled	6.54 cd	11.02 b
C3		Raw	22.69 a	20.22 a
		Boiled	8.31 c	10.36 b
	Winter	Raw	14.46 a	18.74 a
		Boiled	3.68 c	5.97 c
	Spring	Raw	12.71 a	12.66 b
		Boiled	9.18 b	14.08 ab
ANOVA				
C			***	***
GS			**	ns
HT			***	***
C × GS			***	ns
C × HT			***	*
GS × HT			***	***
C × GS × HT			ns	***

Means (n = 3) flanked by the same letter are not statistically different for $p < 0.05$ after Tukey's HSD post-hoc test. Significance level: *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; ns = not significant.

In *M. sylvestris*, the interaction between season, cut and boiling was significant only as regards the TPC (Table 3). As found in *B. officinalis*, both the TPC and AA of *M. sylvestris* leaves were significantly higher in spring than in winter, and the highest values were measured after the last harvest (16.23 mg GAE g^{-1} DW and 20.26 mg TE g^{-1} DW for TPC and AA, respectively) (Table 3). Independently of the season, the boiling treatment reduced the TPC (−24.8%) to a greater extent in the C3 leaves, but it markedly increased the AA (Table 3).

Table 3. Total phenolic content (TPC) and antioxidant capacity (AA) in raw or boiled leaves of *Malva sylvestris* hydroponically grown under greenhouse in winter or in spring and subjected to three consecutive cuts (C1, C2, C3). Data keyed with different letters are significantly different for $p < 0.05$ following three-way ANOVA and Tukey's HSD post-hoc test for the effect of growing season, cut and boiling.

Cut (C)	Growing Season (GS)	Heat Treatment (HT)	TPC (mg GAE g^{-1} DW)	AA (mg TE g^{-1} DW)
C1	Winter	Raw	3.33 e	4.43
		Boiled	1.34 e	7.35
	Spring	Raw	9.75 bc	7.63
		Boiled	6.23 cde	15.36
C2	Winter	Raw	4.80 de	3.85
		Boiled	4.04 e	12.80
	Spring	Raw	7.30 bcde	6.70
		Boiled	8.58 bc	22.15
C3	Winter	Raw	9.89 bc	10.49
		Boiled	7.93 bcd	17.04
	Spring	Raw	16.23 a	20.26
		Boiled	10.46 b	19.09
MEAN EFFECT				
C1			5.16 b	8.69 c
C2			6.18 b	11.38 b
C3			11.13 a	16.72 a

Table 3. *Cont.*

Cut (C)	Growing Season (GS)	Heat Treatment (HT)	TPC (mg GAE g^{-1} DW)	AA (mg TE g^{-1} DW)
	Winter		5.22 b	9.33 b
	Spring		9.76 a	15.20 a
		Raw	8.55 a	8.89 b
		Boiled	6.43 b	15.63 a
C1	Winter		2.33	5.89 d
	Spring		7.99	11.50 b
C2	Winter		4.42	8.33 c
	Spring		7.94	14.43 a
C3	Winter		8.91	13.77 a
	Spring		13.35	19.68 a
C1		Raw	6.54 c	6.03 c
		Boiled	3.78 d	11.36 b
C2		Raw	6.05 c	5.28 c
		Boiled	6.31 c	17.48 a
C3		Raw	13.06 a	15.38 b
		Boiled	9.19 b	13.75 a
	Winter	Raw	6.01	6.26 d
		Boiled	4.44	12.40 b
	Spring	Raw	11.09	11.53 c
		Boiled	8.42	18.87 a
ANOVA				
C			***	***
GS			***	***
HT			***	***
C × GS			ns	***
C × HT			***	***
GS × HT			ns	***
C × GS × HT			*	ns

Means (n = 3) flanked by the same letter are not statistically different for $p < 0.05$ after Tukey's HSD post-hoc test. Significance level: *** $p \leq 0.001$; * $p \leq 0.05$; ns = not significant.

In *P. coronopus*, the interaction between season, cut and boiling was significant only as regards the TPC (Table 4). As found in *B. officinalis* and *M. sylvestris*, TPC and AA were higher in spring than in winter (+57.01% and +79.93% for TPC and AA, respectively) (Table 4). Conversely, these quantities tended to decrease in successive cuts in spring (Table 4). The TPC and AA were much higher in boiled leaves, with an average of 18.17 mg GAE g^{-1} DW and 8.55 mg TE g^{-1} DW, respectively), than in raw leaves (average of 9.18 mg GAE g^{-1} DW and 20.46 mg TE g^{-1} DW for TPC and AA, respectively), although the effect of boiling was significant only in spring and in the leaves of the first two cuts (Table 4).

In *B. officinalis* and *M. sylvestris*, the boiling treatment induced a decrease in TPC. Interestingly, in *P. coronopus*, significantly higher TPC and AA levels were detected in boiled than in raw leaves. An increase in AA content resulting from boiling was also observed in *M. sylvestris*.

Finally, the AA reflects the pattern of TPC in *B. officinalis* and *P. coronopus*, as, in these species, the correlation coefficient was positive and highly significant (Table 5). On the contrary, TPC and AA were not significantly correlated in *M. sylvestris* (Table 5).

Table 4. Total phenolic content (TPC) and antioxidant capacity (AA) in raw or boiled leaves of *Plantago coronopus* hydroponically grown under greenhouse in winter or in spring and subjected to three consecutive cuts (C1, C2, C3). Data keyed with different letters are significantly different for $p < 0.05$ following three-way ANOVA and Tukey's HSD post-hoc test for the effect of growing season, cut and boiling.

Cut (C)	Growing Season (GS)	Heat Treatment (HT)	TPC (mg GAE g^{-1} DW)	AA (mg TE g^{-1} DW)
C1	Winter	Raw	4.51 e	2.38
		Boiled	13.46 bcde	15.39
	Spring	Raw	12.73 bcde	28.44
		Boiled	37.24 a	51.44
C2	Winter	Raw	4.86 de	2.71
		Boiled	16.36 bc	16.80
	Spring	Raw	4.64 e	5.70
		Boiled	14.15 bcd	14.36
C3	Winter	Raw	7.20 cde	3.48
		Boiled	15.15 bc	13.86
	Spring	Raw	21.16 b	8.60
		Boiled	12.67 bcde	10.89
MEAN EFFECT				
C1			16.99 a	24.41 a
C2			10.00 b	9.89 b
C3			14.05 a	9.21 b
	Winter		10.26 b	9.10 b
	Spring		17.10 a	19.91 a
		Raw	9.18 b	8.55 b
		Boiled	18.17 a	20.46 a
C1	Winter		8.98 c	8.89 b
	Spring		24.99 a	39.94 a
C2	Winter		10.61 c	9.76 b
	Spring		9.40 c	10.03 b
C3	Winter		11.17 bc	8.67 b
	Spring		16.92 b	9.75 b
C1		Raw	8.62 cd	15.41 bc
		Boiled	25.35 a	33.42 a
C2		Raw	4.75 d	4.21 d
		Boiled	15.25 b	15.58 b
C3		Raw	14.18 bc	6.04 cd
		Boiled	13.91 bc	12.38 bcd
	Winter	Raw	5.52	2.86
		Boiled	14.99	15.35
	Spring	Raw	12.84	14.25
		Boiled	21.35	25.56
ANOVA				
C			***	***
GS			***	***
HT			***	***
C × GS			***	***
C × HT			***	*
GS × HT			ns	ns
C × GS × HT			***	ns

Means ($n = 3$) flanked by the same letter are not statistically different for $p < 0.05$ after Tukey's HSD post-hoc test. Significance level: *** $p \leq 0.001$; * $p \leq 0.05$; ns = not significant.

Table 5. Pearson's correlation between total phenolic content (TPC) and antioxidant activity (AA) in leaves of *Borago officinalis*, *Malva sylvestris* and *Plantago coronopus* hydroponically grown under greenhouse in winter or in spring and subjected to three consecutive cuts and analyzed fresh or boiled. Each analysis was performed in triplicate.

Species		AA		
		r	***p***	***N***
B. officinalis	TPC	0.49 **	0.002	36
M. sylvestris	TPC	0.30 ns	0.072	36
P. coronopus	TPC	0.87 ***	<0.001	36

Pearson's correlation coefficient: r; *p*-value: *p*; number of XY pairs: *N*. Significance level: *** $p \leq 0.001$; ** $p \leq 0.01$; ns = not significant.

4. Discussion

Independently of the season, the three species under investigation were found to be promising in terms of total leaf production, which was similar to or greater than that of other leafy vegetables, such as lettuce [29,43], lamb's lettuce [44] or chicory [45].

Puccinelli et al. [24] recently reported a higher crop yield of *P. coronopus* compared with two other wild edible species, *Rumex acetosa* and *Portulaca oleracea,* confirming the high productivity of *P. coronopus* leaves found in the present work. Likewise, Ceccanti et al. [4] reported higher productivity of *R. acetosa* than another wild species, namely *Sanguisorba minor*, even though the productivity was found to be lower compared to *P. coronopus* in the present experiment and more common leafy vegetables such as lamb's lettuce [46] and rocket [47].

A reduction in leaf biomass after two successive cuts, as found in the present study in *B. officinalis* during spring and in *M. sylvestris* in both seasons, has been reported in other crops, such as sweet basil [48] and lettuce [49]. Puccinelli et al. [29] observed an increase in the leaf production of hydroponically grown sweet basil on the second cut, in contrast to the findings of Corrado et al. [48].

An increase in leaf TPC after consecutive cuts was also reported in rocket and spinach by Bantis et al. [50], in basil by Ciriello et al. [51], in *R. acetosa* by Ceccanti et al. [52], in *S. minor* [31] and in other leafy vegetables [53], in agreement with our findings in *B. officinalis* and *M. sylvestris*. Indeed, the accumulation of bioactive compounds represents a means by which plants counteract the production of oxygen reactive species (ROS) induced by different stresses such as drought, excess light and wounding [54–56]. Another response to wounding is the production of quinones, isothiocyanates, melanoidins and other oxy-radicals due to the release from the vacuole of glycosylated phenolic compounds [57]. The newly synthetized compounds can react with Folin–Ciocalteau reagent, thus increasing the TPC values [58]. However, independently of the cut, our findings demonstrated agreement with results already reported by other authors in *M. sylvestris*. Indeed, TPC ranging between 1.42 and 24.12 mg GAE g^{-1} FW was found in *M. sylvestris* leaves [15,59], whilst lower TPC values were observed in borage and mallow leaves, if compared with our results (2.36 mg GAE g^{-1} FW and 9.25 mg g^{-1} DW, respectively) [39,60,61].

Conversely to the effect of successive cuts, a reduction in the TPC as a result of boiling has been reported by several other authors in different species [35–37,62]. For example, Giusti et al. [27] found a reduction in delphinidin 3-glucoside in boiled black beans with respect to raw seeds. Domínguez-Fernández et al. [37] reported a loss of TPC in boiled globe artichoke. The leaching of the phenolic compounds in boiling water is facilitated by the elevated temperature and by the destruction of the cellular structures, such as lignin and polysaccharides [35,37,62].

The loss of AA content analyzed through the DPPH assay, a generic but significant assay for the assessment of the scavenging activity of plant material, recorded in *B. officinalis* may be explained by the leaching of antioxidant compounds such as phenolics in the boiling water or as a consequence of the loss of plant antioxidants and formation of new compounds with pro-oxidant activity [62]. Indeed, different hypotheses have been

proposed in the scientific literature about this trend: (i) hydrolysis reactions of phenolic compounds; (ii) different cross-linking of bound phenolics in the plant cell wall; (iii) possible redistribution of antioxidant compounds; (iv) more efficient extraction of antioxidant compounds thanks to the high temperature [62,63]. The choice to use only the DPPH assay for the AA evaluation could be limiting and further AA assays might confirm our findings in future works.

Moreover, the increase in TPC and AA values detected in *P. coronopus* boiled leaves compared to raw leaves could be explained by the different microstructures and biochemical compositions of leaf tissues, which may result in differences in the solubility of phenolic compounds in boiling water [35]. Furthermore, phenolic compounds in fruits and vegetables can be in their free or bound form, associated with some plant structures such as proteins or polysaccharides by covalent or other chemical bounds [64]. Indeed, it is well known that phenolic compounds, such as rutin or chlorogenic acid, may be strongly bound to the cell wall molecules and, therefore, they could not be efficiently extracted by leaves [35].

Finally, the negative Pearson's correlation between TPC and AA in *M. sylvestris* leaves displayed agreement with the negative correlation between TPC and AA observed by Liu et al. [65] in lettuce. Indeed, these authors explained this negative correlation by hypothesizing that various phenolic compound classes might behave differently using the Folin–Ciocalteau method, and the different phenolic chemical structures might influence the molecular antioxidant response [65]. On the contrary, several other authors found a positive correlation between the TPC and AA of leafy vegetables, confirming our findings in *B. officinalis* and *P. coronopus* [66–68].

Thanks to their TPC, independently of the season of cultivation and the consecutive cuts, the species under investigation in the present experiment can be considered a source of compounds with high AA but also with high anti-inflammatory, anticarcinogenic, diuretic and emollient activity, as reported by several other authors [9,16–18,20–24].

5. Conclusions

In conclusion, the wild edible species *B. officinalis*, *M. sylvestris* and, especially, *P. coronopus*, grown in a floating system under a greenhouse, showed a promising crop yield, which was similar to or even greater than the yield of more popular leafy vegetables. However, leaf production was significantly affected by the season and multiple cuts. Indeed, leaf production was higher on C1 as compared to the following cuts in *B. officinalis* during spring, and in *M. sylvestris* in both seasons. On the contrary, the leaf production of *P. coronopus* during both seasons significantly increased after the first cut. The boiling treatment caused a loss of TPC and, at least in *B. officinalis* and *M. sylvestris*, of the AA, probably due to the solubilization of phenolic and other antioxidant compounds in boiling water. In contrast, boiling noticeably increased the TPC and AA in *P. coronopus* (also in *M. sylvestris*, at least AA), though further work is necessary for a better understanding of this phenomenon.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8030253/s1, Figure S1: Growth cycle of *Borago officinalis, Malva sylvestris* and *Plantago coronopus* hydroponically grown under greenhouse in winter or in spring and subjected to three consecutive cuts; Table S1: Total crop yield (fresh leaves) of *Borago officinalis*, *Malva sylvestris* and *Plantago coronopus* hydroponically grown under greenhouse in winter and in spring and subjected to three consecutive cuts (C1, C2, C3). Details of 2-way ANOVA and Tukey's HSD test for the effect of growing season and cut; Table S2: Dry matter content of fresh leaves (DW/FW) of *Borago officinalis*, *Malva sylvestris* and *Plantago coronopus* hydroponically grown under greenhouse in winter and in spring and subjected to three consecutive cuts (C1, C2, C3). Details of 2-way ANOVA and Tukey's HSD test for the effect of growing season and cut.

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