Leaf parts from Greek artichoke genotypes as a good source of bioactive compounds and antioxidants

Spyridon A. Petropoulos, Carla Pereira, Lillian Barros and Isabel C. F. R. Ferreira

Globe artichoke is an important vegetable in the Mediterranean diet. However, the marketable part of the plant constitutes a small portion of its total crop biomass and large amounts of waste are produced during its cultivation. In this study, the phenolic compound content and composition and the antioxidant activity of leaf parts [blades (SLB), petioles and midribs (SPM)] from various Greek artichoke genotypes were evaluated. The results showed significant variation in antioxidant activities and bioactive compound contents between the studied genotypes, with the leaf blades of most of the genotypes having a high content of flavonoids (mainly luteolin glycoside derivatives), which ranged between 49 and 78% of total phenolic compounds (TPC). In contrast, in petioles and midribs phenolic acids (mainly hydroxycinnamic acid derivatives) were the main phenolic compounds, ranging between 64 and 76% of TPC. The antioxidant activity of blades was significantly higher than that of petiole and midribs for all the genotypes and the tested assays. In conclusion, artichoke leaves (blades, petioles and midribs) of the studied genotypes showed different bioactive compound profiles and significant antioxidant properties, and could be further used in the food and nutraceuticals industries as a cheap source of phenolic compounds and antioxidants.

Introduction

Globe artichoke is considered a rich source of bioactive compounds and antioxidants, where the edible parts (artichoke heads) are ranked within the thirty richest plant sources. Leaves and stems are the main crop residuals which also include the outer bracts and any non-edible parts of the heads that are discarded after processing, as well as offshoots and leaves that are removed during cultivation. Crop by-products represent about 80% of total biomass, they are also rich sources of phenolic compounds and are usually used as herbal medicines, either as infusions and herbal preparations or as ingredients in dietary supplements for hepatic and other diseases. Leaves and stems of artichoke present significant antioxidant activity, and for this purpose they are the main ingredients in many dietary supplements and drugs. In particular, leaves are the most common plant part used for therapeutic purposes, since they have numerous medicinal properties including antitumor, antioxidant, antibacterial, antifungal, and hepatoprotective effects and so forth, mostly attributed to their high content of phenolic compounds. Moreover, leaf extracts, infusions and decoctions have shown significant scavenging properties against reactive oxygen species.

The phenolic compound content and composition of artichoke is affected by the genotype and the plant part (edible and non-edible parts), as well as by the growth conditions, cultivation practices and harvest stage. In particular, according to Lombardo et al., total phenolic content and phenolic compound composition differed significantly among seventeen Italian globe artichoke cultivars, while they also observed significant differences among the studied plant parts (receptacle, floral stem, inner and outer bracts). The main phenolic compounds reported for artichoke heads so far include mainly caffeoylquinic acids, such as chlorogenic acid, 1,5-dicaffeoylquinic acid and cyanarin, as well as apigenin and luteolin derivatives, such as apigenin-7-O-glucuronide and flavone glycosides.
Bearing in mind the great genetic diversity in artichoke germplasm throughout the Mediterranean, the aim of the present study was to evaluate the antioxidant activity of leaf parts (blades and midribs) of various artichoke landraces and cultivars that are mainly cultivated in Greece. Moreover, although the phenolic compound composition of artichoke by-products and plant parts has been reported, to the best of our knowledge no studies regarding the individual leaf parts have been reported so far. Considering the portion of total biomass that artichoke leaves constitute and their large size, knowing the chemical composition of different leaf parts (blades, midribs and petioles) is of special interest. Therefore, the phenolic compound composition of individual leaf parts was also evaluated in order to provide information regarding their phenolics profile and evaluate the possibility of using specific by-products as alternative sources of phenolic compounds in the food and pharmaceutical industries.

Materials and methods

Plant materials and sampling

Field experiments were carried out at the experimental farm of the University of Thessaly in Velestino. Samples of four globe artichoke [Cynara cardunculus L. ssp. scolymus (L.) Fiori] and two wild artichoke [Cynara cardunculus L. subsp. sylvestris (L.) Fiori] genotypes were assessed for their phenolic compound composition and antioxidant activity.

The studied genotypes have been previously described by the authors. More specifically, the following genotypes were evaluated: (a) local landrace with dark purple round heads (S1), (b) Greek cultivar “Purple of Attika” with purple round heads (S2), (c) wild globe artichoke with green small flat round heads and bracts with small spines (S3), (d) commercial cultivar with green round heads (Geniki Fytochniki S.A.; S4), (e) commercial cultivar with dark purple oblong heads and bracts with small spines (Geniki Fytochniki S.A.; S5), and (f) wild globe artichoke with green small round heads and bracts with big spines (S6).

Samples of leaves were collected prior to anthesis from the latest fully developed leaves, and further separated into blades (SLB) and petioles and midribs (SPM). All samples were taken from plants at the third year after crop establishment, the growth stage when artichoke plants are considered to have reached their full production potential. After leaf separation, all samples were chopped, placed in air sealed bags and under deep freezing conditions (−80 °C), and lyophilized prior to further analyses.

Phenolic compound characterization

For phenolic compound analyses, extracts were prepared by stirring the dry sample (1 g) and 30 mL of methanol/water (80:20 v/v, at 25 °C at 150 rpm) for 1 h and afterwards filtered using Whatman paper no. 4. The residue was then extracted with an additional portion of methanol/water and the combined extracts were evaporated under reduced pressure (Büchi R-210 rotary evaporator; Flawil, Switzerland) until the complete removal of methanol. The aqueous phase was frozen and lyophilized (FeeZone 4.5, Labconco, Kansas City, MO, USA).

The hydroalcoholic extracts were re-dissolved in methanol/water (80:20 v/v) to a final concentration of 2 mg mL−1 for phenolic compound identification and quantification. LC-DAD–ESI/MSn analyses were performed for phenolic compound identification and quantification, using a Dionex Ultimate 3000 UPLC instrument (Thermo Scientific, San Jose, CA, USA) equipped with a diode-array detector and coupled to a mass detector, using a procedure previously reported by Bessada et al. The chromatogram was recorded at several wavelengths, characteristic of different classes of polyphenols, such as 280 nm for hydroxybenzoic acids and flavonones, 330 nm for hydroxycinnamic acids, and 370 nm for flavones. For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV signal. For the identified phenolic compounds for which a commercial standard was not available, quantification was performed through the calibration curve of the most similar available standard. The results were expressed as mg per g of extract.

Antioxidant activity

Antioxidant activity was evaluated with four different assays (DPPH radical-scavenging activity, reducing power, inhibition of β-carotene bleaching in the presence of linoleic acid radicals and inhibition of lipid peroxidation using TBARS in brain homogenates) as has been previously described by the authors.

The same extracts from the phenolic characterization were re-dissolved in methanol/water (80:20, v/v) in order to be subjected to distinct in vitro antioxidant activity assays, at a final concentration of 20 mg mL−1 and further diluted to different concentrations.

DPPH radical-scavenging activity was evaluated using an ELX800 microplate reader (Bio-Tek Instruments, Inc.; Winooski, VT, USA), and calculated as a percentage of DPPH discoloration using the formula: [(A_{DPPH} − A_{S})/A_{DPPH}] × 100, where A_{S} is the absorbance of the solution containing the sample at 515 nm, and A_{DPPH} is the absorbance of the DPPH solution. Reducing power was evaluated by the capacity to convert Fe³⁺ to Fe²⁺, measuring the absorbance at 690 nm in the microplate reader mentioned above. Inhibition of β-carotene bleaching was evaluated through the β-carotene/linoleate assay; the neutralization of linoleate free radicals avoids β-carotene bleaching, which was measured using the formula: ([β-carotene absorbance after 2 h of assay/initial absorbance] × 100). Lipid peroxidation inhibition in porcine brain homogenates was evaluated by the decrease in thiobarbituric acid reactive substances (TBARS); the colour intensity of malondialdehyde–thiobarbituric acid (MDA–TBA) was measured by its absorbance at 532 nm; the inhibition ratio (%) was calculated using the following formula: ([A − B]/A) × 100%, where A and B are the absorbances of the control and the sample solution, respectively. The results were expressed as EC₅₀ values (the sample concentration providing 50% antioxidant activity).
activity or 0.5 absorbance in the reducing power assay) for anti-
oxidant activity, and Trolox was used as a positive control.

Statistical analysis
For chemical composition analyses and antioxidant activity
assays, three samples were analyzed for each treatment,
whereas all the assays were carried out in triplicate. The results
were expressed as the mean values and standard devi-
tions (SD), and analyzed using one-way analysis of variance
(ANOVA) followed by Tukey’s HSD test with \( p = 0.05 \). This ana-
lysis was carried out using the SPSS v. 23.0 program (IBM
Corp., Armonk, NY, USA).

Results and discussion
The phenolic compound profile of hydromethanolic extracts of
globe artichoke leaf blades is presented in Table 1. The
samples of the studied genotypes were characterized by the
presence of eleven phenolic compounds, from which six were
classified as phenolic acids and five as flavonoid glycoside
derivatives (Table 1). The phenolic acids were all identified as
hydroxycinnamoyl derivatives (compounds with peak numbers
1–5 and 10), being distinguished and identified by the typical
fragmentation patterns as described by Clifford et al.,28,29 with
the exception of compounds with peak numbers 2 and 3 that
were identified as caffeic acid hexoside ([M – H\(^-\)] at m/z 341)
and p-coumaric acid hexoside ([M – H\(^-\)] at m/z 325). All data
for acetylquinic acids in this study used the recommended
IUPAC numbering system.10 The identified flavonoids were all
flavones (compounds with peak numbers 6–9 and 11), being
all luteolin glycoside derivatives (\( \lambda_{\text{max}} \) around 348 nm and an
MS\(^2\) fragment at m/z 285). Compounds with peak numbers
1–10 have been previously reported in artichoke leaves,31 leaf
extracts,11 by-products,32,33 dietary supplements,34 cultivated
artichoke, Madeira cardoon and artichoke-based dietary sup-
plements (juices and dragées).25,35 Compound with peak number
11 presented a pseudomolecular ion [M – H\(^-\)] at m/z 533, releasing MS\(^2\) fragments at m/z 285 ([M – H – 86 – 162]\(^-\),
loss of a malonylhexoside moiety). A compound with a similar
pseudomolecular ion has been identified by Gouveia and
Castilho35 in artichoke-based dietary supplements (dragées)
as a luteolin-7-O-malonylhexoside. However, the fragmentation
pattern presented matched better with luteolin-7-O-malonyl-
hexoside, also identified by Pandino et al.36 in leaves of globe
artichoke. Therefore, this compound was tentatively assigned as
luteolin-7-O-malonylglucoside.

Flavonoids were the most abundant phenolic compounds
in leaf blades for most of the studied genotypes (57–78% of
total phenolic compounds, TPC), except for genotype SLB4
where phenolic acids prevailed (75.8% of TPC) and genotype
SLB6 where equal amounts of total flavonoids (TF) and total
phenolic acids (TPA) were detected (48.9 and 51.1%, respect-
ively) (Table 2). Flavonoid content ranged from 6.9 (genotype
SLB6) to 25.9 mg per g of extract (genotype SLB5). Phenolic
compound composition varied between the studied genotypes,
with 5-O-cafeoylquinic acid and luteolin-7-O-rutinoside being
among the most abundant phenolics in genotype SLB4, and
luteolin-7-O-glucoside, pinoresinol-4-O-hexoside, 3,5-O-
dicafeoylquinic acid and luteolin-7-O-malonylglucoside in
genotype SLB5.

The phenolic compound profile of hydromethanolic
extracts of globe artichoke leaf petioles and midribs is pre-
sented in Table 3. Samples of the studied genotypes were
categorized by the presence of thirteen phenolic compounds,
of which nine were classified as phenolic acids (compounds
with peak numbers 1–4, 7 and 9–12) and four as flavonoid
glycoside derivatives (compounds with peak numbers 5, 6, 8 and
13) (Table 3). Compounds with peak numbers 2–8, 10, 11 and
13 have been previously reported in the literature in different
artichoke samples.3,5,11,33,35,36 Compounds with peak numbers
1 and 9 were identified as the cis form of hydroxycinnamoyl
derivatives, since these would be expected to elute before the
the corresponding trans ones, as observed after UV irradiation
(366 nm, 24 h), of hydroxycinnamic acids in our laboratory.37
 cis and trans isomers of 3-O-cafeoylquinic acid (compounds
with peak numbers 1 and 2) and 3,5-O-dicafeoylquinic acid
(compound with peak numbers 9 and 10), as all the hydroxyc-
namoyl derivatives identified, were distinguished and
identified by typical fragmentation patterns as described by

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### Table 1

Retention time \( (R_t) \), wavelengths of maximum absorption in the visible region \( (\lambda_{\text{max}}) \), mass spectral data and tentative identification of phenolic compounds in globe artichoke leaf blades

<table>
<thead>
<tr>
<th>Peak</th>
<th>( R_t ) (min)</th>
<th>( \lambda_{\text{max}} ) (nm)</th>
<th>Molecular ion</th>
<th>Main MS(^2) fragments (m/z)</th>
<th>Tentative identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.59</td>
<td>324</td>
<td>353</td>
<td>191(100), 179(46), 173(3), 161(5), 135(6)</td>
<td>3-O-Caffeoylquinic acid</td>
</tr>
<tr>
<td>2</td>
<td>6.14</td>
<td>326</td>
<td>341</td>
<td>179(100)</td>
<td>Caffeic acid hexoside</td>
</tr>
<tr>
<td>3</td>
<td>6.83</td>
<td>310</td>
<td>325</td>
<td>163(100)</td>
<td>p-Coumaric acid hexoside</td>
</tr>
<tr>
<td>4</td>
<td>7.46</td>
<td>326</td>
<td>353</td>
<td>191(100), 179(10), 161(3), 135(3)</td>
<td>5-O-Caffeoylquinic acid</td>
</tr>
<tr>
<td>5</td>
<td>14.03</td>
<td>325</td>
<td>367</td>
<td>193(12), 191(100), 173(6), 143(3), 134(5)</td>
<td>5-O-Feruloylquinic acid</td>
</tr>
<tr>
<td>6</td>
<td>18.57</td>
<td>348</td>
<td>593</td>
<td>285(100)</td>
<td>Luteolin-7-O-rutinoside</td>
</tr>
<tr>
<td>7</td>
<td>19.80</td>
<td>346</td>
<td>461</td>
<td>285(100)</td>
<td>Luteolin-7-O-glucuronide</td>
</tr>
<tr>
<td>8</td>
<td>19.54</td>
<td>347</td>
<td>447</td>
<td>283(100)</td>
<td>Luteolin-7-O-glucoside</td>
</tr>
<tr>
<td>9</td>
<td>20.11</td>
<td>227, 279</td>
<td>519</td>
<td>357(100), 343(9), 151(50), 136(4)</td>
<td>Pinoresinol-4-O-hexoside</td>
</tr>
<tr>
<td>10</td>
<td>21.15</td>
<td>328</td>
<td>515</td>
<td>353(94), 335(5), 191(100), 179(12), 173(3), 161(3), 135(3)</td>
<td>3,5-O-Dicafeoylquinic acid</td>
</tr>
<tr>
<td>11</td>
<td>24.42</td>
<td>347</td>
<td>533</td>
<td>489(100), 285(60)</td>
<td>Luteolin-7-O-malonylglucoside</td>
</tr>
</tbody>
</table>
Phenolic acid content ranged from 4.4 (genotype SPM6) to 12.8 mg per g of extract (genotype SPM4), while the most abundant phenolic compounds were 5-O-cafeoylquinic acid (genotype SPM4), luteolin-7-O-glucoside and luteolin-7-O-malonylglicoside (genotype SPM6), and trans-3,5-O-dicafeoylquinic acid (genotypes SPM3 and 5).

Reports regarding the phenolic compound composition of leaves show contradictory results. In particular, similarly to our study, Pandino et al. have detected significantly higher flavonoid content than phenolic acids in the leaves of clones of two Sicilian artichoke varieties, whereas Pistón et al., Sihem et al., and Pereira et al. have reported a higher content of phenolic acids than flavonoids in leaf extracts and dietary supplements, respectively. However, even in the study by Pandino et al., significant variation between the studied clones was observed, a finding that is similar to the results of the present study. Moreover, in our study, the contribution of
Blades DPPH (EC50 value, µg mL\(^{-1}\)) TPB (EC50 value, µg mL\(^{-1}\)) β-Carotene (EC50 value, µg mL\(^{-1}\)) TBARS (EC50 value, µg mL\(^{-1}\))
SLB1 1364 ± 44a 682 ± 4c 664 ± 29c 1575 ± 61a
SLB2 850 ± 13d 1039 ± 9b 398 ± 17e 1047 ± 37b
SLB3 913 ± 41c 1111 ± 2a 428 ± 21e 801 ± 23c
SLB4 369 ± 9f 2087 ± 0.5e 515 ± 27d 532 ± 25de
SLB5 537 ± 8e 292 ± 3d 1449 ± 30a 502 ± 16e
SLB6 957 ± 35b 685 ± 8c 743 ± 37b 555 ± 27d

Petioles + midribs DPPH (EC50 value, µg mL\(^{-1}\)) TPB (EC50 value, µg mL\(^{-1}\)) β-Carotene (EC50 value, µg mL\(^{-1}\)) TBARS (EC50 value, µg mL\(^{-1}\))
SPM1 2706 ± 112b 1200 ± 30c 4172 ± 163b 1791 ± 76d
SPM2 3766 ± 77a 1275 ± 9b 4013 ± 69e 3837 ± 22a
SPM3 1367 ± 64c 889 ± 12f 3048 ± 85e 1215 ± 37e
SPM4 2416 ± 84c 1079 ± 5d 6171 ± 93a 2634 ± 92b
SPM5 1952 ± 33d 943 ± 25e 3401 ± 154d 599 ± 23f
SPM6 2329 ± 82c 1785 ± 5a 1588 ± 17f 2030 ± 99c

The antioxidant activity was expressed as EC50 values, which means that higher values correspond to lower reducing power or antioxidant potential. EC50 is extract concentration corresponding to 50% antioxidant activity or 0.5 absorbance in the reducing power assay. Trolox EC50 values: 41 µg mL\(^{-1}\) (reducing power), 42 µg mL\(^{-1}\) (DPPH scavenging activity), 18 µg mL\(^{-1}\) (β-carotene bleaching inhibition) and 23 µg mL\(^{-1}\) (TBARS inhibition). In each column, different letters mean significant differences between samples (p < 0.05). nd: not detected; tr: traces; TPA: total phenolic acids; TF: total flavonoids; TPC: total phenolic compounds. For peak names consult Table 3.

Antioxidant activity differed significantly between the studied genotypes and the various assays (Table 5). Regarding leaf blades, genotype SLB4 showed the highest antioxidant activity in DPPH and reducing power (RP) assays, while it did not differ significantly with genotype SLB5 in TBARS. Considering the highest content of TPA and TPC in genotype SLB4 compared to the other genotypes, antioxidant activity of leaf blades, as indicated by the results of DPPH, RP and TBARS assays, could be attributed to total phenolic compound content. The same applies for the antioxidant activity of petioles and midribs, where genotypes SPM3 and SPM5 had the highest content of TPC.

Table 5 Antioxidant properties of the studied globe artichoke genotypes

<table>
<thead>
<tr>
<th>Blades</th>
<th>DPPH (EC50 value, µg mL(^{-1}))</th>
<th>TPB (EC50 value, µg mL(^{-1}))</th>
<th>β-Carotene (EC50 value, µg mL(^{-1}))</th>
<th>TBARS (EC50 value, µg mL(^{-1}))</th>
</tr>
</thead>
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<tr>
<td>SLB1</td>
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<td>682 ± 4c</td>
<td>664 ± 29c</td>
<td>1575 ± 61a</td>
</tr>
<tr>
<td>SLB2</td>
<td>850 ± 13d</td>
<td>1039 ± 9b</td>
<td>398 ± 17e</td>
<td>1047 ± 37b</td>
</tr>
<tr>
<td>SLB3</td>
<td>913 ± 41c</td>
<td>1111 ± 2a</td>
<td>428 ± 21e</td>
<td>801 ± 23c</td>
</tr>
<tr>
<td>SLB4</td>
<td>369 ± 9f</td>
<td>2087 ± 0.5e</td>
<td>515 ± 27d</td>
<td>532 ± 25de</td>
</tr>
<tr>
<td>SLB5</td>
<td>537 ± 8e</td>
<td>292 ± 3d</td>
<td>1449 ± 30a</td>
<td>502 ± 16e</td>
</tr>
<tr>
<td>SLB6</td>
<td>957 ± 35b</td>
<td>685 ± 8c</td>
<td>743 ± 37b</td>
<td>555 ± 27d</td>
</tr>
</tbody>
</table>

Petioles + midribs

<table>
<thead>
<tr>
<th>Blades</th>
<th>DPPH (EC50 value, µg mL(^{-1}))</th>
<th>TPB (EC50 value, µg mL(^{-1}))</th>
<th>β-Carotene (EC50 value, µg mL(^{-1}))</th>
<th>TBARS (EC50 value, µg mL(^{-1}))</th>
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<tr>
<td>SPM1</td>
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<tr>
<td>SPM2</td>
<td>3766 ± 77a</td>
<td>1275 ± 9b</td>
<td>4013 ± 69e</td>
<td>3837 ± 22a</td>
</tr>
<tr>
<td>SPM3</td>
<td>1367 ± 64c</td>
<td>889 ± 12f</td>
<td>3048 ± 85e</td>
<td>1215 ± 37e</td>
</tr>
<tr>
<td>SPM4</td>
<td>2416 ± 84c</td>
<td>1079 ± 5d</td>
<td>6171 ± 93a</td>
<td>2634 ± 92b</td>
</tr>
<tr>
<td>SPM5</td>
<td>1952 ± 33d</td>
<td>943 ± 25e</td>
<td>3401 ± 154d</td>
<td>599 ± 23f</td>
</tr>
<tr>
<td>SPM6</td>
<td>2329 ± 82c</td>
<td>1785 ± 5a</td>
<td>1588 ± 17f</td>
<td>2030 ± 99c</td>
</tr>
</tbody>
</table>

The antioxidant activity was expressed as EC50 values, which means that higher values correspond to lower reducing power or antioxidant potential. EC50 is extract concentration corresponding to 50% antioxidant activity or 0.5 absorbance in the reducing power assay. Trolox EC50 values: 41 µg mL\(^{-1}\) (reducing power), 42 µg mL\(^{-1}\) (DPPH scavenging activity), 18 µg mL\(^{-1}\) (β-carotene bleaching inhibition) and 23 µg mL\(^{-1}\) (TBARS inhibition). In each column, different letters mean significant differences between samples (p < 0.05).
the highest TPC content, which resulted in better antioxidant properties for the DPPH and RP (genotype SPM3) and TBARS (genotype SPM5) assays. However, the great differences in antioxidant activity between leaf blades, and petioles and midribs for all the assays do not conform to the differences in TPC between the leaf parts. Therefore, although phenolic compounds contribute to antioxidant properties, they cannot be considered as the only bioactive compounds responsible for such properties. Similar results have been reported by Sihem et al.,40 who also suggested that the free scavenging activity of artichoke leaf tissues is not exclusively due to phenolic compound content. Moreover, in the same study, leaf extracts showed higher antioxidant activity in the reducing power assay compared to other tested assays (ABTS, DPPH and phosphomolybdenum assay), which is also the case in our study for the petioles and midribs of most of the genotypes.

In contrast, Pagano et al.52 have reported that the cellular antioxidant activity (CAA) of artichoke by-products (leaves and bracts) was significantly correlated with dicafeoylquinic acids and total phenolics content. However, according to Kollia et al.,8 the extraction method also has a significant impact on the antioxidant properties of artichoke leaf extracts, while the contribution of total phenolics content to antioxidant properties depends on the tested assay. Similar findings have been reported by Coinu et al.,7 Pistón et al.,11 and Vamanu et al.,41 who suggested that antioxidant potency depends on the extraction method and solvents and is not related to total phenolics or individual phenolic subclass content.

Conclusions

In conclusion, significant variation in phenolic compound composition and total phenolic contents, as well as in antioxidant activities, was observed not only between the genotypes, but also, most importantly, between the leaf parts. The most abundant phenolics in leaf blades were 5-O-cafeoylquinic acid, luteolin-7-O-rutinoside, luteolin-7-O-glucoside, pinoresinol-4-O-hexoside, 3,5-O-dicafeoylquinic acid and luteolin-7-O-malonylglicoside, while in leaf petioles and midribs prevailed 5-O-cafeoylquinic acid, luteolin-7-O-glucoside, luteolin-7-O-malonylglicoside and trans 3,5-O-dicafeoylquinic acid. Genotype S4 has the highest phenolic compound content in the leaf blades (74.9 mg per g of extract), mostly due to the abundance of 5-O-cafeoylquinic acid, followed by genotype S5 (45.3 mg per g of extract) with similar amounts of flavonoids and phenolic acids being detected in its blades. The fact that in most of the studied genotypes, flavonoids prevailed in the leaf blades and phenolic acids in the petioles and midribs could be of major importance for the food and nutraceutical industries towards the design of new products, especially food additives that could increase the health benefits of various food products. Moreover, the great variation between the studied genotypes in terms of bioactive compounds highlights the importance of genetic conservation for breeding and therapeutic purposes.

Author contributions

S. Petropoulos and I. C. F. R. Ferreira conceived the study. C. Perreira and L. Barros carried out the experiments. L. Barros and I. C. F. R. Ferreira performed data organization and analysis of the results. S. Petropoulos and L. Barros wrote the manuscript. I. C. F. R. Ferreira revised the manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest.

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