Food Research International 126 (2019) 108651

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Valorisation of the green waste parts from turnip, radish and wild cardoon: Nutritional value, phenolic profile and bioactivity evaluation

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ARTICLE INFO

Keywords:
Brassica rapa L.
Raphanus sativus L.
Cynara cardunculus L. var. *sylvestris*
Chemical/nutritional characterization
Polyphenols
Antioxidant/antibacterial/hepatotoxic properties

ABSTRACT

The recovery of bio-wastes to obtain high added value compounds is of great interest for the pharmaceutical, medicinal and food industries. Therefore, the aerial parts of turnip (*Brassica rapa* L.), radish (*Raphanus sativus* L.) and leaf blade of wild cardoon (*Cynara cardunculus* L. var. *sylvestris* (Lamk) Fiori) were characterized regarding their nutritional composition, as also their content in soluble sugars, organic acids, fatty acids, and tocopherols. Furthermore, their hydroethanolic extracts and infusion preparations, were profiled regarding individual phenolic compounds by HPLC-DAD/ESI-MS and their antioxidant, antibacterial and hepatotoxic activities were evaluated. Regarding the nutritional content, wild cardoon revealed the best results, however it was radish and turnip that showed higher values for organic acids and phenolic compounds. The hydroethanolic extract and infusion preparation of wild cardoon stood out for its antioxidant and antibacterial activity. Overall, the hydroethanolic extracts seemed more effective (regarding antioxidant and antibacterial activity) than the infusions. Total phenolic acids proved to be strongly correlated with the antioxidant and antibacterial (against *Morganella morganii*) activities. This study showed that the discarded parts of these plants can be used as an important natural source of valuable nutrient content and new and safe bioactive compounds, beneficial for human health. Moreover, the extraction of those compounds from underused parts of turnip, radish and cardoon could be used to preserve foods, avoiding artificial additives and thus, contributing to the development of new natural ingredients.

1. Introduction

Consumption of vegetables can reduce the incidence of many diseases, which may be due to their high content in bioactive phenolic compounds and nutritional properties (Sun, Simon, & Tanumihardjo, 2009). To meet the consumers expectations, researchers and industry are increasingly searching for new recovery techniques of high added-value compounds from food wastes, applying also conventional and emerging technologies to overcome the technological and scale-up boundaries for the commercialization of these type of compounds (Galanakis, 2012, 2013). Many *Brassicacea* crops (crucifers) are widely recognized for their contribution to human nutrition and other health benefits (Singh, Upadhyay, Prasad, Bahadur, & Rai, 2007), representing also one of the most economically important vegetables in the global agriculture and markets, since The Organization for Food and Agriculture of the United Nations (FAO) reported that 92 million tons of brassica plants are being grown in > 150 countries, occupying 5.4 million hectares of land (70% only in Asia, China) (Francisco et al., 2017). *Brassica rapa* L., commonly known as turnip, is a very popular crop that has been used all over the world for human consumption since ancient times (Liang et al., 2006). Its edible or useful parts are found in large quantities, which are normally consumed as a boiled vegetable and in the preparation of soups and stews (Takuno, Kawahara, & Ohnishi, 2007). *B. rapa* leaves are also eaten in some countries; however, their use is comparatively less common compared to the root bulbs (Azam, Khan, Mahmood, & Hameed, 2013). *Raphanus sativus* L., radish, is grown worldwide, being mostly eaten raw in salads and in a less usual way they can be also cooked, dried and even pickled. Many studies proved the richness of the aerial parts of radish in bioactive compounds, such as phenolic compounds (Goyeneche, Fanovich, Rodriguez Rodrigues, Nicolao, & Di Scala, 2018), which could be exploited for further uses. The wild cardoon (*Cynara cardunculus* L. var.

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https://doi.org/10.1016/j.foodres.2019.108651
Received 24 March 2019; Received in revised form 10 August 2019; Accepted 31 August 2019
Available online 03 September 2019
0963-9969/

sylvestris (Lamk) Fiori) is another highly perishable vegetable (Foti et al., 1999; Ierna & Mauromicale, 2010; Portis, Barchi, Acquadro, Macua, & Lanteri, 2005), and has been used in food preparations and for its folklore therapeutical uses, due to its diuretic and choleretic properties, and also antioxidant and antimicrobial activities (Durazzo et al., 2013; Falleh et al., 2008; Pandino, Lombardo, Mauromicale, & Williamson, 2011b). Wild cardoon is widely recognized for its richness in phenolic compounds (Falleh et al., 2008; Pandino et al., 2011b; Pinelli et al., 2007; Venere, Linsalata, Calabrese, Cardinali, & Sergio, 2015), and also for its nutritional value (Venere et al., 2015). However, the leaves and floral stems are usually considered as waste (Pandino et al., 2011b), and therefore could be considered a valuable residue resource.

There are few studies regarding the nutritional value, as also the phenolic compounds characterization of the hydroethanolic extracts and infusion preparations of the aerial parts of *B. rapa* and *R. sativus* and the leaf blade of *C. cardunculus* var. *sylvestris*. Therefore, this study will contribute in adding a higher knowledge dealing with the nutritional and chemical composition in terms of sugars, fatty acids, tocopherols, organic acids and phenolic compounds of bio-wastes of the three mentioned plant species, and also evaluate their antioxidant, antibacterial and cytotoxic potential, in their hydroethanolic and infusions extracts. Furthermore, a Pearson's correlation was performed to understand the possible interaction between the presence of some phenolic compounds and the bioactivities studied. The results obtained could add value to these by-products, with the potential to be applied in different industrial sectors.

2. Material and methods

2.1. Samples

The aerial tops (leaves and stems) of *Brassica rapa* L. (turnip) and *Raphanus sativus* L. (radish), and the leaf blade of *Cynara cardunculus* L. var. *sylvestris* (Lamk) Fiori (wild cardoon) were purchased (1 kg of each plant) from farmers after the root harvest in January of 2017 in Monastir (centre eastern coastline of Tunisia). Their botanical identification was confirmed by Professor Fathia Harzallah-Shkiri and voucher specimens (N° 130–132) were deposited in the herbarium of the laboratory of Botany, High Institute of Biotechnology of Monastir. The fresh material was oven dried at 40 °C for 48 h (performed immediately after harvesting and until obtaining constant weight) and then reduced to a fine powder (~ 40 mesh).

2.2. Chemical composition

2.2.1. Macronutrients

Proteins, fat, carbohydrates, and ash were determined using described AOAC analytical methods (AOAC, 2016). The macro-Kjeldahl method was used to determine the nitrogen content (AOAC 978.04), the protein content was calculated as N × 6.25. Crude fat was determined using a Soxhlet apparatus with petroleum ether (AOAC 920.85). Ash content was determined by incineration at 600 °C until a constant mass was achieved (AOAC 923.03). Total carbohydrates were calculated by difference and energy was calculated following the equation: Energy (kcal) = 4 × (g protein) + 4 × (g carbohydrate) + 9 × (g fat) (Regulation (EC) No 1169/2011, 2011).

2.2.2. Sugars

Free sugars were determined by high performance liquid chromatography coupled to a refractive index detector (HPLC-RI, Knauer, Smartline system 1000, Berlin, Germany) as previously described by the authors Barros et al. (2013). Sugar identification was made by comparing the relative retention times of sample peaks with authentic standards and quantification was performed by the internal normalization of the chromatographic peak area using melezitose. The results were expressed in g per 100 g of plant dry weight.

2.2.3. Organic acids

The organic acids were analysed by ultra-fast liquid chromatography coupled to photodiode array detector programmed to record at 215 nm as the preferred wavelength (UFLC-PDA; Shimadzu Coperation, Kyoto, Japan) as previously described procedure Barros et al. (2013). The identification and quantification of the individual organic acids was performed by comparison to authentic standards, by comparison of the peak area in the programmed wavelength. The results were expressed in g per 100 g of plant dry weight.

2.2.4. Fatty acids

The fatty acids characterization was performed by gas–liquid chromatography with flame ionization detection (GC-FID, DANI model GC 1000 instrument, Milan, Italy) as previously described (Barros et al., 2013). The results were recorded and processed using Clarity Software (DataApex, Prague, The Czech Republic) and expressed in g/100 g dw.

2.2.5. Tocopherols

The extraction procedure and chromatographic characterization was performed following a procedure described by Barros et al. (2013). A high performance liquid chromatography coupled to a fluorescence detector (Knauer, Smartline system 1000, Berlin, Germany) was used, and the compounds were identified by comparisons with authentic standards. Identification and quantification was based on the fluorescence signal response of each standard, using the internal standard method (tocol) and by using calibration curves obtained from commercial standards of each compound. The results were expressed as mg per 100 g of dry weight.

2.3. Bioactivities and phenolic profile of the hydroethanolic extract and infusion

2.3.1. Extracts preparation

The hydroethanolic extracts (ethanol:water, 80:20, *v/v*) were prepared by maceration using 1 g of the dried samples in 30 mL of solvent for 1 h. Thereafter, filtered using Whatman no.4 filter paper and re-extracted using the same volume of solvent and the same time. The combined extracts were evaporated to dryness of the ethanol under vacuum at 35 °C by using a rotary evaporator (Buchi, 3000 series, Switzerland), and the aqueous phase was frozen and further lyophilized.

For infusion preparation 1 g of dried samples were added to 100 mL of boiling distilled water, left to stand for 5 min, filtered, and then frozen and lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA). The lyophilized hydroethanolic and aqueous extracts were stored in a sealed plastic container at room temperature (25 °C) under vacuum until further assays.

2.3.2. Antioxidant activity

The lyophilized hydroethanolic and aqueous extracts were re-dissolved in ethanol:water (80:20, *v/v*) mixture and water, respectively, to obtain stock solutions of 5 mg/mL, which were further diluted to obtain a range of six concentrations below the stock solution. The antioxidant activity was evaluated through the DPPH radical-scavenging activity, reducing power, inhibition of *β*-carotene bleaching and lipid peroxidation inhibition in porcine brain homogenates by using the TBARS assay (Sarmento, Barros, Fernandes, Carvalho, & Ferreira, 2015). The final results were expressed as EC_{50} values (μg/mL), Trolox was used as positive control.

2.3.3. Antibacterial activity

The lyophilized hydroethanolic and aqueous extracts were re-dissolved in ethanol:water (80:20, *v/v*) mixture and water, respectively, to obtain stock solutions of 100 mg/mL, which were further diluted to

obtain a range of 7 concentrations below the stock solutions. The antibacterial activity was evaluated using six Gram-negative bacteria: *Escherichia coli*, *Escherichia coli ESBL* (broad-spectrum enterobacteria producing beta-lactamases), *Klebsiella pneumoniae*, *Klebsiella pneumoniae ESBL*, *Morganella morganii*, and *Pseudomonas aeruginosa*; and four Gram-positive bacteria: *Enterococcus faecalis*, *Listeria monocytogenes*, MRSA (Methicillin resistant *Staphylococcus aureus*), and MSSA (Methicillin susceptible *Staphylococcus aureus*). The Minimal Inhibitory Concentration (MIC) determination was performed following the method previously described by Svobodova et al. (2017), using the colorimetric assay of *p*-iodonitrotetrazolium. Ampicillin and imipenem were used as positive controls for the Gram-negative bacteria, while ampicillin and vancomycin were used for Gram-positive bacteria. Three negative controls were prepared: (i) with the Mueller-Hinton Broth (MHB); (ii) with the extract, and (iii) with medium and antibiotic. One positive control was prepared for each inoculum with MHB.

2.3.4. Hepatotoxic activity

The lyophilized hydroethanolic and aqueous extracts were re-dissolved in ethanol:water (80:20, *v/v*) mixture and water, respectively, to obtain a stock solution of 4 mg/mL, which were further diluted to obtain a range of six concentrations below the stock solution. Non-tumour cells were also tested; a cell culture (named as PLP2, porcine liver primary cells) was prepared from a freshly harvested porcine liver obtained from a local slaughterhouse, according to a procedure established by the authors (Abreu et al., 2011). Sulforhodamine B assay was performed according to a procedure previously described by the authors (Barros et al., 2013). Ellipticine was used as a positive control and the results were expressed in GI_{50} values (correspond to the sample concentration achieving 50% of growth inhibition in liver primary culture PLP2).

2.4. Phenolic compounds profile

The lyophilized extracts and infusions were redissolved in ethanol:water (80:20, *v/v*) and pure water, respectively, to determine the phenolic profiles by chromatographic analysis using a Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA), as previously described by Bessada, Barreira, Barros, Ferreira, and Oliveira (2016). Detection was carried out with a diode array detector (DAD) using 280 nm and 370 nm as the preferred wavelengths and connected in line with a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an ESI source and working in negative mode. Data acquisition was carried out with Xcalibur® data system (Thermo Finnigan, San Jose, CA, USA). The phenolic compounds were identified through the available standard compounds and by using literature information regarding the fragmentation pattern. Quantification was performed using 5-level calibration curves obtained from commercial standard compounds. The results were expressed in mg per g of extract.

2.5. Statistical analysis

Three replicates of each plant part and all the assays described above were independently analysed in triplicate ($n = 9$). The results were expressed as mean values and standard deviation (SD) and analysed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $p = .05$. For phenolic compounds quantification and comparison between hydroethanolic extract and infusion preparation, a Student's *t*-test was used to determine the significant difference between the two different samples, with $p = .05$. Furthermore, a Pearson's correlation analysis between the bioactivities and all the sum contents of the analysed compounds (total phenolic acids, total flavonoids, and total phenolic compounds) was carried out, with a 95% confidence level. The analyses were carried out using IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp., Armonk, New York, USA).

3. Results and discussion

Prior to the presentation and discussion of the obtained results, it is important to underline the importance of bio-waste revalorization, justifying the importance of such studies and also some of the applied methodologies in this study. Firstly, we must address the problem of bio-waste in today's society. Tons of bio-waste are produced every year, and the vast majority of them are not exploited for food and non-food purposes (Mahro & Timm, 2007). In addition to the obvious future use of these bio-wastes as energy sources and for the production of biomaterials, it is also obvious that they could be used as non-conventional food resources due to food scarcities. As these bio-residues are sources of compounds with high nutritional and bioactive value, as described below, it should be highlight their potential use for the food sector, not only for direct consumption, but also after processing in the development of new functional foods and as a source of natural ingredients. Therefore, it is very important to study the proximate composition, free sugars and fatty acids (nutritional sources and with high beneficial effects on health) content of bio-waste material, as well as tocopherols, organic acids, and phenolic compounds (molecules recognized for their high antioxidant and antibacterial properties, among others). Following an exhaustive discussion of the results obtained for the chemical composition and bioactive activity of three wildly appreciated plant species, turnip, radish and wild cardoon, will be performed.

3.1. Chemical composition of turnip, radish and wild cardoon

The chemical composition regarding macronutrients, free sugars and organic acids of the aerial parts of turnip, radish, and wild cardoon are presented in Table 1. As expected, carbohydrates were the major macronutrient found in all three samples. Wild cardoon revealed higher amounts of fat (1.5 ± 0.1 g/100 g dw) and carbohydrates (71.0 ± 0.5 g/100 g dw), while for proteins and ash it was turnip

Table 1
Proximate composition, soluble sugars and organic acids composition in turnip, wild cardoon, and radish (mean ± SD results expressed on dry weight basis).

	Turnip	Wild cardoon	Radish
Humidity (%)	86.43^{b}	84.64^{c}	88.54^{a}
Nutritional values (g/100 g dw)			
Fat	0.67 ± 0.03^{c}	1.5 ± 0.1^{a}	0.95 ± 0.01^{b}
Proteins	21.9 ± 0.2^{a}	15.7 ± 0.1^{c}	21.6 ± 0.4^{b}
Ash	19.03 ± 0.04^{b}	11.8 ± 0.5^{c}	27.7 ± 0.2^{a}
Total available carbohydrates	58.4 ± 0.1^{b}	71.0 ± 0.5^{a}	49.75 ± 0.04^{c}
Energy contribution (kcal/100 g dw)	327.24 ± 0.01^{b}	360.03 ± 1.09^{a}	293.21 ± 0.02^{c}
Soluble sugars (g/100 g dw)			
Fructose	0.22 ± 0.01^{c}	2.4 ± 0.2^{a}	0.79 ± 0.01^{b}
Glucose	0.45 ± 0.03^{b}	1.14 ± 0.10^{a}	nd
Sucrose	0.9 ± 0.1^{b}	4.7 ± 0.2^{a}	nd
Trehalose	1.4 ± 0.1^{b}	2.57 ± 0.02^{a}	0.57 ± 0.03^{c}
Sum	2.9 ± 0.1^{b}	10.8 ± 0.5^{a}	1.36 ± 0.04^{c}
Organic acids (g/100 g dw)			
Oxalic acid	4.38 ± 0.03^{a}	2.32 ± 0.07^{c}	3.38 ± 0.04^{b}
Quinic acid	nd	0.45 ± 0.05	nd
Shikimic acid	0.04 ± 0.01^{a}	0.010 ± 0.001^{c}	0.02 ± 0.001^{b}
Citric acid	1.31 ± 0.03^{b}	nd	6.57 ± 0.33^{a}
Fumaric acid	0.04 ± 0.01^{a}	tr	0.002 ± 0.0004^{b}
Sum	5.8 ± 0.1^{b}	2.78 ± 0.03^{c}	9.9 ± 0.4^{a}

dw – dry weight basis; nd – not detected; Calibration curves for organic acids: Oxalic acid ($y = 9 \times 10^{6}x + 45{,}973$, $R^2 = 0.9901$); Quinic acid ($y = 610607x + 46{,}061$, $R^2 = 0.9995$); Shikimic acid ($y = 7 \times 10^{7}x + 175{,}156$, $R^2 = 0.9999$); Citric acid ($y = 1 \times 10^{6}x + 45{,}682$, $R^2 = 0.9997$); and Fumaric acid ($y = 154{,}862x + 1 \times 10^{6}$, $R^2 = 0.9977$). In each row different letters mean significant differences between species ($p < 0.05$)

Table 2
Fatty acids and tocopherols composition of turnip, wild cardoon, and radish (mean ± SD).

	Turnip	Wild cardoon	Radish
Fatty acids (g/100 g dw)			
Caproic acid (C6:0)	0.0003 ± 0.0001^{b}	0.0010 ± 0.0001^{a}	0.00030 ± 0.00001^{c}
Caprylic acid (C8:0)	nd	0.00040 ± 0.00001^{b}	0.00030 ± 0.00001^{a}
Capric acid (C10:0)	0.0007 ± 0.0001^{a}	0.00040 ± 0.00001^{c}	0.00040 ± 0.00001^{b}
Lauric acid (C12:0)	0.0020 ± 0.0001^{a}	0.0020 ± 0.0001^{c}	0.0011 ± 0.0001^{b}
Myristic acid (C14:0)	0.0068 ± 0.0003^{a}	0.0060 ± 0.0002^{c}	0.0043 ± 0.0001^{b}
Myristoleic acid (C14:1)	0.0005 ± 0.0001^{a}	nd	0.00030 ± 0.00001^{b}
Pentadecanoic acid (C15:0)	0.011 ± 0.004^{a}	0.0089 ± 0.0004^{b}	0.0032 ± 0.0001^{c}
Palmitic acid (C16:0)	0.136 ± 0.001^{a}	0.19 ± 0.01^{b}	0.120 ± 0.002^{c}
Palmitoleic acid (C16:1)	0.0098 ± 0.0001^{a}	0.0079 ± 0.0001^{b}	0.0025 ± 0.0001^{c}
Heptadecanoic acid (C17:0)	0.0047 ± 0.0001^{a}	0.0045 ± 0.0003^{c}	0.0032 ± 0.0001^{b}
Stearic acid (C18:0)	0.034 ± 0.001^{a}	0.0282 ± 0.0001^{b}	0.0145 ± 0.0003^{c}
Oleic acid (C18:1n9)	0.031 ± 0.001^{a}	0.053 ± 0.005^{b}	0.0273 ± 0.0002^{c}
Linoleic acid (C18:2n6)	0.0600 ± 0.0002^{c}	0.265 ± 0.006^{a}	0.1128 ± 0.0001^{b}
α-Linolenic acid (C18:3n3)	0.33 ± 0.01^{c}	0.843 ± 0.001^{b}	0.638 ± 0.003^{a}
Arachidic acid (C20:0)	0.0072 ± 0.0001^{a}	0.0132 ± 0.0002^{b}	0.0040 ± 0.0001^{c}
cis-11-Eicosenoic acid (C20:1)	nd	0.0013 ± 0.0001	nd
cis-11,14,17-Eicosatrienoic acid and Heneicosanoic acid (C20:3n3)	0.0020 ± 0.0001^{a}	0.0016 ± 0.0001^{c}	0.0018 ± 0.0001^{b}
Eicosapentaenoic acid (C20:5n3)	0.0023 ± 0.0001	nd	nd
Behenic acid (C22:0)	0.0120 ± 0.0001^{a}	0.023 ± 0.001^{b}	0.0096 ± 0.0001^{c}
Erucic acid (C22:1n9)	nd	nd	0.0014 ± 0.0001
Lignoceric acid (C24:0)	0.0050 ± 0.0001^{a}	0.0079 ± 0.0003^{b}	0.0048 ± 0.0002^{c}
Nervonic acid (C24:1)	0.009 ± 0.001^{a}	0.0056 ± 0.0003^{c}	0.0104 ± 0.0001^{b}
SFA	0.379 ± 0.003^{a}	0.29 ± 0.01^{b}	0.166 ± 0.003^{c}
MUFA	0.087 ± 0.002^{a}	0.068 ± 0.004^{b}	0.042 ± 0.001^{c}
PUFA	0.684 ± 0.001^{c}	1.11 ± 0.01^{b}	0.752 ± 0.003^{a}
Tocopherols (mg/100 g dw)			
α-Tocopherol	0.10 ± 0.01^{c}	2.16 ± 0.04^{b}	2.85 ± 0.03^{a}
β-Tocopherol	nd	2.31 ± 0.01	nd
γ-Tocopherol	2.13 ± 0.13^{b}	2.36 ± 0.01^{a}	0.48 ± 0.01^{c}
δ-Tocopherol	nd	2.8 ± 0.1	nd
Sum	2.22 ± 0.14^{c}	9.6 ± 0.1^{a}	3.33 ± 0.03^{b}

nd – not detected; dw – dry weight. SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. In each row different letters mean significant differences between species ($p < .05$).

(21.9 ± 0.2 g/100 g dw) and radish (27.7 ± 0.2 g/100 g dw) that revealed the highest contents, respectively. In a similar study, performed with radish from Argentina, its leaves showed higher concentration of proteins (36.3 ± 0.32 g/100 g dw) in comparison to our results, but a lower concentration in carbohydrates (38.5 g/100 g dw) and ash (16.2 ± 0.06 g/100 g dw) (Goyeneche et al., 2015). Comparing to the roots, also studied by the same author, radish leaves showed higher contents in proteins, ash, carbohydrates, which emphasizes their valorisation (Goyeneche et al., 2015; Lu et al., 2008). The results obtained for radish were also considerably higher than those reported by Azam et al. (2013) and Lu et al. (2008) in radish root tuber. Regarding fat content, the aerial parts studied by Goyeneche et al. (2015) contained 3.52 ± 0.07 g/100 g dw, which represents a lipid fraction of 73% higher in comparison with the herein studied sample of radish. These differences between the studied samples, and taking into account literature data reported by other authors, can be explained by the geographic difference of the samples and with the biotic and abiotic difference inherent in its growth and production (Aires et al., 2011).

For turnip samples the protein content reported (21.95 ± 0.2 g/100 g dw) was considerable higher than those reported on turnip from Pakistan (5.54 ± 0.88 g/100 g dw) (Azam et al., 2013); however, the lipid content reported by the same author for turnip was higher (1.56 ± 0.21 g/100 g dw). These differences could be also explained by the geographical differences and the inherent edaphoclimatic conditions of its grow and production, but also to the differences in the cultivar of *B. rapa* studied (Aires et al., 2011).

For wild cardoon samples the results for protein content were in the same range of the ones reported in the flower heads of *C. cardunculus* L. var. *altilis* DC cv. Biango Avorio from Italy (15.95 ± 0.02 g/100 g dw; Petropoulos, Pereira, Tzortzakis, Barros, & Ferreira, 2018), and considerably higher than the ones reported in the biomass of wild cardoon *C. cardunculus* from Sicily (8.0 g/100 g dw; Foti et al., 1999). Furthermore, the lipid fraction was also lower that the ones reported by Petropoulos et al. (2018) in the flower heads of *C. cardunculus*. As far as the authors knowledge, no previous studies have reported the energy contribution of the three studied species., however wild cardoon gave the highest energetic contribution (360.03 ± 1.09 kcal/100 g dw), preceded by turnip and radish (327.24 ± 0.01 and 293.21 ± 0.02 kcal/100 g dw, respectively).

Regarding the content in soluble sugars (Table 1), turnip and wild cardoon presented a similar profile, with the presence of fructose, glucose, sucrose, and trehalose. Wild cardoon revealed the highest amount of total soluble sugars (10.8 ± 0.5 g/100 g dw) among the three studied samples. On the other hand, radish sample only presented fructose and treahalose, and as expected the lowest amount of total soluble sugars (1.36 ± 0.04 g/100 g dw). The results reported by Venere et al. (2015) in leaves of wild cardoon from Italy showed lower amounts in fructose (~ 3 g/100 g dw), glucose (~ 1.40 g/100 g dw), and sucrose (~ 6 g/100 g dw). However, for radish and turnip, Azam et al. (2013) reported higher amounts in total sugars (19.6 g/100 g dw and 25.7 g/100 g dw, radish and turnip, respectively); Lu et al. (2008) also reported higher amounts of total soluble sugars in different cultivars of radish ranging from 2.233 to 15.457 g/100 g dw. The different edaphoclimatic conditions in which the plants were grown could explained the substantial differences between the studied plants and the reported values by other authors (Roshani, A. Sahari, Amirkaveei, & G. Ardabili, 2016).

Unlike the soluble sugars, for organic acids the profile between samples was very different. Overall five organic acids were identified (oxalic, quinic, shikimic, citric, and fumaric acid). Radish leaves presented the highest content in total organic acids (9.9 ± 0.4 g/100 g dw) followed by turnip (5.8 ± 0.1 g/100 g dw), and cardoon (2.78 ± 0.03 g/100 g dw). Oxalic acid was present as the major organic acid found in turnip (4.38 ± 0.03 g/100 g dw) and wild cardoon

Table 3
Bioactive properties of the hydroethanolic extracts and infusions preparations of turnip, wild cardoon, and radish (mean ± SD).

	Hydroethanolic			Infusion		
	Turnip	Wild cardoon	Radish	Turnip	Wild cardoon	Radish
Antioxidant activity (EC_{50} values, mg/mL)						
DPPH scavenging activity	1.57 ± 0.06^{b}	0.07 ± 0.00^{f}	0.14 ± 0.01^{e}	1.90 ± 0.06^{a}	0.22 ± 0.03^{d}	0.64 ± 0.06^{c}
Reducing power	1.07 ± 0.29^{b}	0.27 ± 0.09^{e}	0.58 ± 0.07^{c}	1.21 ± 0.07^{a}	0.22 ± 0.00^{e}	0.50 ± 0.00^{d}
β-carotene bleaching inhibition	0.67 ± 0.15b^{c}	0.19 ± 0.05^{c}	0.48 ± 0.02^{c}	1.63 ± 0.31^{a}	0.30 ± 0.04^{ab}	1.21 ± 0.74^{ab}
TBARS inhibition	0.60 ± 0.12^{a}	0.05 ± 0.01^{c}	0.43 ± 0.07^{b}	0.43 ± 0.05^{b}	0.40 ± 0.04^{b}	0.69 ± 0.13^{a}
Antibacterial activity (MIC values, mg/mL)						
Gram-negative bacteria						
Escherichia coli	20	2.5	20	> 20	2.5	20
Escherichia coli ESBL	20	10	20	> 20	5	> 20
Klebsiella pneumoniae	> 20	20	> 20	> 20	20	> 20
Klebsiella pneumoniae ESBL	> 20	20	> 20	> 20	20	> 20
Morganella morganii	20	10	10	20	2.5	20
Pseudomonas aeruginosa	20	10	20	> 20	20	> 20
Gram-positive bacteria						
Enterococcus faecalis	20	5	20	20	10	> 20
Listeria monocytogenes	> 20	10	20	10	10	10
MRSA	> 20	5	20	20	5	20
MSSA	> 20	5	> 20	20	5	> 20
Hepatotoxicity						
PLP2 (GI_{50} values, μg/mL)	> 400	> 400	> 400	> 400	> 400	> 400

EC_{50} values correspond to the extract concentration achieving 50% of antioxidant activity or 0.5 of absorbance in reducing power assay. Trolox EC_{50} values: 43.03 ± 1.71 μg/mL (DDPH), 29.62 ± 3.15 μg/mL (reducing power), 2.63 ± 0.14 μg/mL (β-carotene bleaching inhibition) and 3.73 ± 1.9 μg/mL (TBARS inhibition). MIC values correspond to the minimal extract concentration that inhibited the bacterial growth. ESBL – extended spectrum β-lactamases. MRSA – Methicillin-resistant *Staphylococcus aureus*. MSSA – Methicillin-susceptible *Staphylococcus aureus*. GI_{50} values correspond to the sample concentration achieving 50% of growth inhibition in liver primary culture PLP2. Ellipticine GI_{50} values: 2.29 mg/mL. In each row different letters mean significant differences ($p < .005$).

(2.32 ± 0.07 g/100 g dw). However, in radish sample the major organic acid found was citric acid (6.57 ± 0.33 g/100 g dw). Quinic acid was only detected in cardoon, and fumaric acid was found in trace amounts in wild cardoon samples. Despite the important role played by these organic acids in human health (Olthof, Hollman, & Katan, 2001), as far as the authors knowledge they have not been previously identified in the studied species.

Regarding the lipid fraction of the three studied samples, data on the individual fatty acid profile and tocopherol composition are described in Table 2. Up to twenty-three fatty acids were identified in the samples, which presented a very similar profile among them, apart from some minor compounds that were not detected (*cis*-11-eicosenoic acid (C20:1) in turnip and radish; eicosapentaenoic acid (C20:5n3) in wild cardoon and radish; and erucic acid (C22:1n9) in turnip and wild cardoon). In all samples, α-linolenic acid (C18:3n-3, PUFA) was the major fatty acid, followed by palmitic acid (C16:0, SFA) in turnip samples, and linoleic acid (C18:2n-6c, PUFA) in wild cardoon and radish samples. Wild cardoon gave the highest levels of PUFA (1.11 ± 0.01 g/100 g dw) with α-linolenic acid (0.843 ± 0.001 g/100 g dw) as the major compound. On the other hand, turnip presented the lowest amounts of PUFA, but still represented 59.5% of the fatty acid fraction, and showed the highest levels of SFA (0.379 ± 0.003 g/100 g dw), with the important contribution of palmitic acid (0.136 ± 0.001 g/100 g dw), and MUFA (0.087 ± 0.002 g/100 g dw), mainly due to oleic acid (C18:1n9, 0.031 ± 0.001 g/100 g dw). The results obtained for wild cardoon were very different to those mentioned by Petropoulos et al. (2018), in the immature capitule (edible part) of cultivated cardoon, in which saturated fatty acid were the most abundant group found (palmitic, behenic, stearic and myristic acids). The results published by Azam et al. (2013) also showed differences in fatty acids profile, since it presented palmitic acid as the main compound identified in the roots of radish, and linoleic acid as the main compound in turnip. The differences found regarding the fatty acid profile can be explained by the different environmental conditions of the region during harvest, such as edaphic and climatic conditions, and also due to the different cultivars and plants harvest time (Roshani et al., 2016).

All the samples presented PUFA/SFA ratio much higher than 0.45 (1.08, 3.80 and 4.54 for turnip, wild cardoon and radish, respectively), which is essential for a high nutritional value of food products (Petropoulos et al., 2018), and confirms the beneficial use of bio-residues of the studied species for human health. In addition, epidemiologic studies and clinical trials have provided consistent evidence that replacing saturated fat with polyunsaturated fat is beneficial for coronary heart disease (Siri-Tarino, Sun, Hu, & Krauss, 2010). In particular, in all samples α-linolenic acid represented > 49% of the total fatty acids fraction, being this compound an essential omega-3 polyunsaturated fatty acid. This fatty acid is defined as a fundamental factor for brain and visual function in humans (Sakayori et al., 2016), and its deficiency could led to many health problems, because it cannot be synthesized in humans, therefore it must be supplied through the diet (Schettino et al., 2017).

For tocopherols composition, wild cardoon presented all the different isoforms of tocopherol (α-, β-, γ- and δ) and, as expected, the highest amount of total tocopherol (9.6 ± 0.1 mg/100 g dw), being δ-tocopherol the most abundant isoform followed by γ-tocopherol (2.8 ± 0.1 and 2.36 ± 0.01 mg/100 g dw, respectively). However, in radish and turnip, only α- and γ- tocopherols were detected. Tocopherols, with different isoforms, presents high antioxidant potential and specific biological activities. Until now, and as far as the authors knowledge, there is no previous reports regarding tocopherols composition in the studied samples. Tocopherols play a very important role for human health, showing several very important biological activities, such as anti-inflammatory (Reiter, Jiang, & Christen, 2007).

3.2. Bioactivity of the hydroethanolic and infusion extracts

The results for the antioxidant properties of the hydroethanolic extract and infusion preparations of turnip, wild cardoon and radish, are shown in Table 3. In general, wild cardoon hydroethanolic extracts and infusion preparations revealed the lowest EC_{50} values (higher antioxidant activity) for all the assays performed, in comparison with turnip and radish. Regarding the type of extract, the hydroethanolic extracts also showed lower EC_{50} values than the corresponding infusions. Moreover, the hydroethanolic extracts of wild cardoon presented

the lowest EC_{50} values for DPPH scavenging activity (0.07 ± 0.01 mg/mL), β-carotene bleaching inhibition (0.19 ± 0.05 mg/mL), and TBARS inhibition (0.05 ± 0.01 mg/mL). For reducing power assay, the hydromethanolic extract and infusion preparation of wild cardoon revealed similar EC_{50} value (0.27 ± 0.09 and 0.22 ± 0.01 mg/mL, respectively) without statistically significant differences between them.

For turnip leaves, our results were consistent with those presented by Aires et al. (2011) with EC_{50} values of 1.32 mg/mL in water extracts from turnip leaves and roots from Portugal. Regarding wild cardoon, our results showed better antioxidant activity for DPPH scavenging activity assay, than the ethanolic extracts of (96% *v/v*) of the leaf blade of cardoon from Slovenia, reported by Kukić et al. (2008), that presented higher EC_{50} values (EC_{50} values = 0.157 mg/mL), and therefore a poorer antioxidant activity. However, the opposite was observed for the infusion preparations were the EC_{50} values from the Slovenian plants were lower for DPPH scavenging activity (EC_{50} values = 0.173 mg/mL). The results reported by Petropoulos et al. (2018) in the leaf blade of cultivated cardoon from Greece, showed that the hydromethanolic extracts presented lower antioxidant potential for DPPH and TBARS assays (EC_{50} = 0.2 and 0.11 mg/mL, respectively), and similar results for reducing power and β-carotene bleaching inhibition (EC_{50} = 0.27 and 0.114 mg/mL, respectively). For radish aerial parts, the methanolic and aqueous extracts of Indian radish leaves and stems, presented lower EC_{50} values for DPPH scavenging activity (EC_{50} = 0.031 and 0.216 mg/mL, respectively), than the ones reported herein (Beevi, Narasu, & Gowda, 2010).

The effects of the hydroethanolic extracts and infusions on the Gram-negative bacteria (*Escherichia coli*, *Escherichia coli ESBL*, *Klebsiella pneumonia*, *Klebsiella pneumonia* ESBL, *Morganella morganii*, *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Enterococcus faecalis*, *Listeria monocytogenes*, *MRSA* and *MSSA*) were also evaluated and reported in Table 3. Overall, the hydroethanolic extracts and infusion preparations of wild cardoon revealed the lowest MIC values for all tested bacteria; except for *Listeria monocytogenes*, were the infusions of turnip and radish presented equal MIC values than the two preparations of wild cardoon (10 mg/mL). The lowest MIC values (2.5 mg/mL) were observed against the Gram-negative bacteria, *E. coli* (hydroethanolic and infusion of wild cardoon) and *M. morganii* (infusion of wild cardoon). Regarding the Gram-positive bacteria, the lowest MIC values (5 mg/mL) were observed for MRSA, MSSA (hydroethanolic and infusion of wild cardoon), and *E. faecalis* (hydroethanolic of wild cardoon).

As far as the author's knowledge, the antibacterial activity of the aerial parts of turnip and radish have not been reported. However for wild cardoon, previous studies reported good antibacterial activity in other parts of wild cardoon, thus also bio-wastes (Dias et al., 2018). Kukić et al. (2008) reported lower MIC values in the hydroethanolic and aqueous extracts of fresh involucre bracts of cardoon from Bratislava against *E. coli* and *Staphylococcus aureus* (1.0 mg/mL and 1.5 mg/mL, respectively); however is important to state that the bacteria strains used were ATCC, which present a lower resistance profile when compared to the ones studied herein (clinical isolates with high resistance profile).

The hepatoxicity was also evaluated for all the samples (Table 3), and a concentration higher than 400 μg/mL was observed in all hydroethanolic extracts and infusion preparations, which means that no sample reveal toxicity against PLP2 cells, being also important to mention that this is the first hepatoxicity study is these samples, as far as the author's knowledge.

3.3. Phenolic profile of the hydroethanolic extract and infusion

Chromatographic data, including retention time, $\lambda_{máx}$, pseudomolecular ion, and fragmentation pattern, was used to tentatively identify the phenolic compounds present in the hydroethanolic extracts and infusions preparations of turnip, wild cardoon and radish (Table 4). Quantification of the phenolic compounds was also performed (Table 4). Overall, twenty-two phenolic compounds were tentatively identified in turnip (eight phenolic acids and fourteen flavonoids), twelve in wild cardoon (nine phenolic acids, two flavonoids, and one lignan), and thirteen in radish (five phenolic acids and eight flavonoids).

Peaks 1^{CC}, 3^{CC}, 4^{CC}, and 2^{BR} ($[M-H]^-$ at *m/z* 353) and 9^{CC}/10^{CC} ($[M-H]^-$ at *m/z* 515) were identified as caffeoylquinic and dicaffeoylquinic acid derivatives, respectively. Peaks 1^{CC} and 2^{BR} were assigned as 3-*O*-caffeoylquinic acid, yielding the base peak at *m/z* 191 and *m/z* 179, as reported by Clifford, Johnston, Knight, and Kuhnert (2003) and Clifford, Knight, and Kuhnert (2005). Peak 3^{CC} was distinguished from other isomers by its base peak at *m/z* 173 [quinic acid-H-H2O]$^-$, accompanied by a secondary fragment ion at *m/z* 191 with approximately 80% abundance of base peak, which allowed assigning it as 4-*O*-caffeoylquinic acid according to the fragmentation pattern previously described (Clifford et al., 2003, 2005). 5-*O*-caffeoylquinic acid (peak 4^{CC}) was positively identified according to its UV spectra ($\lambda_{máx}$ 322 nm) and pseudomolecular ion in comparision with the commercial standard. Peaks 9^{CC} and 10^{CC} ($[M-H]^-$ at *m/z* 515) were assigned to 3,4-*O*– and 3,5-*O*– dicaffeoylquinic acids, respectively, taking into account the hierarchical fragmentation pattern previously reported by Clifford et al. (2003) and Clifford et al. (2005). Three compounds (1^{BR}, 2^{RS}, and 3^{RS}) were tentatively identified as caffeic acid derivatives, peak 1^{BR} showed a UV spectrum similar to caffeic acid with $\lambda_{máx}$ at 326 nm, but eluted at a different retention time. It presented a molecular ion $[M-H]^-$ at *m/z* 323 corresponding to caffeic acid; this represents a loss of −132 u, that could be attributed to a pentosyl moiety, and thus the compound was tentatively identified as a caffeic acid pentoside, which was coherent with its earlier elution (greater polarity) with regard to caffeic acid. Peaks 2^{RS} and 3^{RS} ($[M-H]^-$ at *m/z* 295) were tentatively identified as caffeoyl malate, revealing fragments characteristic of a caffeic acid (*m/z* 179 and at *m/z* 135), with the loss of 116 u (malate moiety), as previously described by Afzan et al. (2012). Another large group of identified phenolic acids were ferulic acid (peak 13^{BR}, positively identified with the standard compound) and its derivatives (peaks 5^{RS}, 2^{CC}, 5^{CC}, and 6^{CC}). Peak 5^{RS} ($[M-H]^-$ at *m/z* 309) was tentatively identified as feruloyl malate, yielding a base peak at *m/z* 193 [ferulic acid-H]$^-$ and a fragment at *m/z* 133, corresponding to the malic acid fragment (Harbaum et al., 2007). Peaks 2^{CC} ($[M-H]^-$ at *m/z* 371) presented MS^2 fragments at *m/z* 209 (yielding 50% of the base peak) and at *m/z* 193 (yielding 100% of the base peak), corresponding to the loss of a glucosyl unit and the ferulic acid fragment, respectively, being tentatively identified as 5-hydroxyferuloylglycoside (Harbaum et al., 2007). Peaks 5^{CC} and 6^{CC} ($[M-H]^-$ at *m/z* 367) were tentatively identified as 3-*O*-feruloylquinic acid and 5-*O*-feruloylquinic acid, taking into account the hierarchical keys previously reported by Clifford et al. (2003, 2005) allowing to distinguish the two oxygen position in the two peaks.

Sinapic acid derivatives also represented one of the main phenolic acids group identified, however they were only found in turnip tops (peaks 14^{BR}, 20^{BR}, 21^{BR}, and 22^{BR}). These compounds were characterized as synapoylmalic acid (14^{BR}), sinapoyl-feruloylgentiobiose (21^{BR}), di-sinapoyl-gentiobiose (20^{BR}), and tri-sinapoyl-gentiobiose (22^{BR}), being from the same type of those previously described in shoots of *Brassica oleracea* L. (Ferreres et al., 2009). Two coumaric acid derivatives were tentatively identified (peaks 4^{RS} and 3^{BR}), peak 4^{RS} was assigned as *p*-coumaric acid by comparison with its UV–visible spectra and MS^2 fragmentation pattern to the standard compound, while, peak 3^{BR} ($[M–H]^-$ at *m/z* 337) showed a MS^2 fragment at *m/z* 191, which represents quinic acid molecule after the neutral loss of a coumaroyl moiety (−146 u), and was tentatively identified as 3-*p*-coumaroylquinic acid. Citric acid was only found in radish (peak 1^{RS}), and the tentatively identification was based on the comparison of the ESI–MS/MS data with standards and literature (Elsadig Karar & Kuhnert, 2016), this is also in agreement with its high content found in this study, in the dry sample of radish.

Table 4
Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, tentative identification and quantification (mg/g of extract) of the phenolic compounds present in the hydroethanolic extracts and infusions preparations of *B. rapa*, *C. cardunculus* and *R. sativus*.

Peak	Rt (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	Hydroethanolic	Infusion	*t*-Students test *p*-value
Turnip								
1^{BR}	4.43	326	323	191(10), 179(63),173(5),161(39),149(8)	Caffeic acid pentoside	0.47 ± 0.01	0.351 ± 0.003	< .001
2^{BR}	4.68	320	353	191(100),179(45),161(15),135(10)	3-*O*-Caffeolyquinic acid	0.69 ± 0.01	1.30 ± 0.02	< .001
3^{BR}	6.01	312	337	191(5), 163(100), 119(10)	3-*p*-Coumaroylquinic acid	1.30 ± 0.06	0.57 ± 0.03	< .001
4^{BR}	6.58	328	933	771(100),609(5),447(5),429(6),285(5)	Kaempherol-3-*O*-caffeoyl-di-glucoside-7-*O*-glucoside	1.237 ± 0.003	1.08 ± 0.01	< .001
5^{BR}	6.92	350	625	463(32),301(95)	Quercetin-3-*O*-sophoroside	1.11 ± 0.01	1.05 ± 0.01	.039
6^{BR}	7.27	316	963	801(100),787(30),625(15)	Kaempherol-3-*O*-hydroxyferuloylsophoroside-7-*O*-glucoside	1.01 ± 0.02	0.98 ± 0.01	.588
7^{BR}	8.49	332	977	815(100),623(100),609(80)	Kaempherol-3-*O*-sinapoylsophoroside-7-*O*-glucoside	1.9 ± 0.1	1.49 ± 0.01	< .001
8^{BR}	9.16	344	609	447(100),285(15)	Kaempherol-*O*-dihexoside	1.85 ± 0.04	1.50 ± 0.03	< .001
9^{BR}	10.09	319	917	755(100),609(100),591(23),429(5),285(5)	Kaempherol-3-*p*-coumaroylsophoroside-7-*O*-glucoside	1.06 ± 0.01	1.01 ± 0.02	.135
10^{BR}	10.57	353	639	477(100),315(10)	Isorhametin-*O*-dihexoside	2.8 ± 0.1	2.11 ± 0.01	< .001
11^{BR}	13.61	350	625	463(32),301(95)	Quercetin-*O*-sophoroside	0.99 ± 0.01	1.01 ± 0.01	.554
12^{BR}	15.29	325	801	609(100),429(5),285(5)	Kaempherol-3-*O*-hydroxyferuloylsophoroside	1.05 ± 0.01	1.18 ± 0.01	.001
13^{BR}	15.53	323	193	179(100),149(5),133(5)	Ferulic acid	0.48 ± 0.01	0.50 ± 0.01	.928
14^{BR}	15.99	329	339	223(100),208(5),179(5),164(6)	Synapoylmalic acid	2.8 ± 0.1	1.29 ± 0.03	< .001
15^{BR}	17.59	331	815	623(100),609(95),591(36)	Kaempherol-3-*O*-(synapoyl)-sophoroside	1.29 ± 0.03	1.01 ± 0.01	< .001
16^{BR}	18.31	356	463	301(100)	Quercetin-3-*O*-glucoside	0.99 ± 0.01	nd	–
17^{BR}	19.17	326	785	623(100),591(98),461(17),443(22),285(39)	Kaempherol-*O*-feruloylhexoside-*O*-hexoside	1.09 ± 0.01	nd	–
18^{BR}	21.68	340	447	285(100)	Kaempherol-3-*O*-glucoside	1.068 ± 0.004	0.99 ± 0.02	.011
19^{BR}	22.61	352	447	315(100)	Isorhamnetin-*O*-pentoside	1.34 ± 0.04	1.02 ± 0.02	< .001
20^{BR}	25.06	328	753	529(100),205(70)	Di-sinapoyl-gentiobiose	tr	tr	–
21^{BR}	25.69	326	723	529(17),499(100),223(5)	Sinapoyl-feruloylgentiobiose	tr	tr	–
22^{BR}	29.55	324	959	735(100),529(11),511(17)	Tri-sinapoyl-gentiobiose	tr	tr	–
					Total phenolic acids	5.8 ± 0.2	4.01 ± 0.03	< .001
					Total flavonoids	18.8 ± 0.4	14.44 ± 0.01	< .001
					Total phenolic compounds	25 ± 1	18.45 ± 0.03	< .001
Wild cardoon								
1^{CC}	4.41	327	353	191(100),179(10),161(5),135(5)	3-*O*-Caffeoylquinic acid	0.48 ± 0.01	0.66 ± 0.01	< .001
2^{CC}	6.37	266	371	209(50),193(100),191(36),179(11)	5-Hydroxyferuloylglycoside	1.3 ± 0.1	0.95 ± 0.01	< .001
3^{CC}	6.81	320	353	191(80),179(12),173(100),161(5),135(5)	4-*O*-Caffeoylquinic acid	13.6 ± 0.1	10.2 ± 0.1	< .001
4^{CC}	7.99	322	353	191(100),179(10),161(5),135(5)	5-*O*-Caffeoylquinic acid	0.179 ± 0.001	0.185 ± 0.003	.027
5^{CC}	12.74	323	367	193(5),191(100),173(4),135(5)	3-*O*-Feruloylquinic acid	0.32 ± 0.01	0.25 ± 0.1	< .001
6^{CC}	13.07	320	367	193(15),191(100),173(9),135(5)	5-*O*-Feruloylquinic acid	0.53 ± 0.01	0.39 ± 0.01	< .001
7^{CC}	12.45	347	447	285(100)	Luteolin-*O*-hexoside	5.5 ± 0.1	1.6 ± 0.1	< .001
8^{CC}	18.78	270,345	519	357(50),151(100),135(35)	Pinoresinol-*O*-hexoside	0.37 ± 0.01	0.21 ± 0.01	–
9^{CC}	19.85	327	515	353(100),191(8),179(7),161(5),135(5)	3,4-*O*-Dicaffeoylquinic acid	6.492 ± 0.002	3.5 ± 0.2	< .001
10^{CC}	22.05	326	515	353(100),191(10),179(8),161(5),135(5)	3,5-*O*-Dicaffeoylquinic acid	0.331 ± 0.001	0.319 ± 0.001	< .001
11^{CC}	22.99	347	533	489(10),285(100)	Luteolin-*O*-malonylhexoside	7.39 ± 0.02	5.8 ± 0.2	< .001
12^{CC}	27.53	335	473	269(100)	Acetylapigenin-*O*-hexoside	1.85 ± 0.01	4.73 ± 0.04	< .001
					Total phenolic acids	23.6 ± 0.2	16. ± 0.3	< .001
					Total flavonoids	14.7 ± 0.1	12.1 ± 0.2	< .001
					Total phenolic compounds	38.3 ± 0.3	29 ± 1	< .001
Radish								
1^{RS}	4.79	206	133	115(100)	Citric acid	1.4 ± 0.1	1.2 ± 0.1	< .001
2^{RS}	9.11	327	295	179(100),135(20)	Caffeoyl malate	4.00 ± 0.02	3.7 ± 0.1	< .001
3^{RS}	9.4	327	295	179(100),135(20)	Caffeoyl malate	1.5 ± 0.1	0.514 ± 0.004	< .001
4^{RS}	13.87	313	163	133(100)	*p*-Coumaric acid	16.5 ± 0.1	3.2 ± 0.1	< .001
5^{RS}	15.64	327	309	193(100),133(5)	Feruloyl malate	2.48 ± 0.03	0.968 ± 0.004	< .001
6^{RS}	16.22	346	593	447(100),285(15)	Kaempherol-*O*-rutinoside	7.1 ± 0.1	3.9 ± 0.1	< .001
7^{RS}	17.13	346	593	447(100),285(25)	Kaempherol-3-*O*-rutinoside	7.26 ± 0.03	3.9 ± 0.2	< .001
8^{RS}	18.13	346	563	417(100),285(12)	Kaempherol-*O*-rhamnoside-*O*-pentoside	5.85 ± 0.03	3.3 ± 0.1	< .001
9^{RS}	20.89	343	577	431(100),285(20)	Kaempherol-*O*-dirhamnoside	5.74 ± 0.03	3.1 ± 0.1	< .001
10^{RS}	21.47	330	769	285(100)	Kaempherol-*O*-glucuronyl-rutinoside	1.24 ± 0.01	1.056 ± 0.001	< .001
11^{RS}	22.48	330	901	755(100),285(55)	Kaempferol-3-*O*-(2,6-di-*O*-rhamnosyl-glucoside)-7-*O*-rhamnoside	1.74 ± 0.03	1.289 ± 0.002	< .001
12^{RS}	23.94	328	901	285(100)	Kaempherol-*O*-hydroxyferuloylglucoronide-*O*-malonylhexoside	1.37 ± 0.01	1.159 ± 0.001	< .001
13^{RS}	25.49	329	915	769(100),285(28)	Kampferol-*O*-deoxyhexoside-*O*-feruloylrutinoside	1.09 ± 0.01	1.02 ± 0.01	< .001
					Total phenolic acids	26.2 ± 0.2	9.5 ± 0.3	< .001

(*continued on next page*)

Table 4 (*continued*)

Peak	Rt (min)	λ_{max} (nm)	[M-H]$^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	Hydroethanolic	Infusion	*t*-Students test *p*-value
					Total flavonoids	31.4 ± 0.1	18.8 ± 0.4	< .001
					Total phenolic compounds	57.6 ± 0.2	28 ± 1	< .001

tr – traces, nd – not detected. Standard calibration curves: Apigenin-7-*O*-glucoside (y = 10,683x – 45,794, R^2 = 0.9906, LOD (136.95 μg/mL) and LOQ (414.98 μg/mL), peak 12^{CC}); Caffeic acid (y = 388,345x + 406,369, R^2 = 0.9939, LOD (8.57 μg/mL) and LOQ (25.57 μg/mL), peaks 1^{BR}, 20^{BR}, 21^{BR}, 22^{BR}, 2^{BR}, 3^{BR}, 2^{RS}, 3^{RS}, 1^{CC}, 3^{CC}, 4^{CC}, 9^{CC}, and 10^{CC}); Chlorogenic acid (y = 168,823x – 161,172, R^2 = 0.9999, LOD (0.83 μg/mL) and LOQ (2.50 μg/mL), peaks 2^{BR} and 1^{RS}); Ferulic acid (y = 633,126x – 185,462, R^2 = 0.999, LOD (1.85 μg/mL) and LOQ (5.61 μg/mL), 13^{BR}, 5^{RS}, 2^{CC}, 5^{CC}, and 6^{CC}); *p*-coumaric acid (y = 301,950x + 6966.7, R^2 = 0.9999, LOD (1.10 μg/mL) and LOQ (3.32 μg/mL), peak 3^{BR}, 4^{RS}); Quercetine-3-*O*-glucoside (y = 34,843x – 160,173, R^2 = 0.9998, LOD (17.01 μg/mL) and LOQ (51.54 μg/mL), peaks 4^{BR}, 5^{BR}, 6^{BR}, 7^{BR}, 8^{BR}, 9^{BR}, 10^{BR}, 11^{BR}, 12^{BR}, 15^{BR}, 16^{BR}, 17^{BR}, 18^{BR}, 19^{BR}, 6^{RS}, 7^{RS}, 8^{RS}, 9^{RS}, 10^{RS}, 11^{RS}, 12^{RS}, 13^{RS}, 7^{CC}, and 11^{CC}); Sinapic acid (y = 197,337x + 30,036, R^2 = 0.9997, LOD (1.91 μg/mL) and LOQ (6.01 μg/mL), peak 14^{BR}).

The group of flavonoids is undoubtedly the one with the highest number of compounds identified, the majority of them (seventeen compounds) were assigned as kaempferol derivatives (peaks 18^{BR}, 8^{RS}, 9^{RS}, 6^{RS}, 7^{RS}, 8^{BR}, 10^{RS}, 17^{BR}, 12^{BR}, 15^{BR}, 11^{RS}, 12^{RS}, 13^{RS}, 9^{BR}, 4^{BR}, 6^{BR}, and 7^{BR}) according to their UV–visible spectra and MS^2 fragmentation pattern (Table 4). Peak 18^{BR} was positively identified as kaempferol-3-*O*-glycoside by comparison of its UV spectrum ($\lambda_{máx}$ 340 nm) and retention time with the commercial standard. Peaks 8^{RS}, 9^{RS}, 6^{RS}, 7^{RS}, 8^{BR}, 10^{RS}, and 11^{RS} were tentatively identified as kaempferol linked to different sugar moieties, rhamnoside-pentoside ([M–H]$^-$ at *m/z* 563), dirhamnoside ([M–H]$^-$ at *m/z* 577), rutinoside ([M–H]$^-$ at *m/z* 593), dihexoside ([M–H]$^-$ at *m/z* 609), glucoronyl-rutinoside ([M–H]$^-$ at *m/z* 769), and rhamonosyl-glucosisde-rhamnoside ([M–H]$^-$ at *m/z* 901), respectively (Lillian Barros, Dueñas, Ferreira, Carvalho, & Santos-Buelga, 2011; Kachlicki, Piasecka, Stobiecki, & Marczak, 2016). Peaks 17^{BR}, 12^{BR}, 15^{BR}, 12^{RS}, 13^{RS}, 9^{BR}, 4^{BR}, 6^{BR}, 7^{BR} were tentatively identified as kaempferol linked to sugar moieties and phenolic acids (ferulic, malonic, sinapic, and *p*-coumaric acids). In some cases, the connection to the phenolic acid decrease the polarity of the molecule, which increased the retention time. Peaks 17^{BR}, 12^{BR}, 12^{RS}, 13^{RS}, and 6^{BR} were linked to ferulic acid moieties (except for peak 12^{RS} that also presented a malonyl moiety), ands were tentatively identified as kaempherol-*O*-feruloylhexoside-*O*-hexoside ([M–H]$^-$ at *m/z* 785), kaempherol-3-*O*-hydroxyferuloylsophoroside ([M–H]$^-$ at *m/z* 801), kaempferol-*O*-deoxyhexoside-*O*-feruloylrutinoside ([M–H]$^-$ at *m/z* 915), kaempherol-3-*O*-hydroxyferuloylsophoroside-7-*O*-glucoside ([M–H]$^-$ at *m/z* 963), and kaempherol-*O*-hydroxyferuloylglucoronide-*O*-malonylhexoside ([M–H]$^-$ at *m/z* 901), respectively (Pinela et al., 2018; Xiao et al., 2013). Peak13^{RS} was described for the first time, as far as the author's knowledge, and presented a pseudomolecular ion [M–H]$^-$ at *m/z* 915 and two main MS^2 fragments at *m/z* 769 ([M-H-146]$^-$) and at *m/z* 285 ([M-H-176-308]$^-$), that corresponded to the loss of a deoxyhexoside moiety and the subsequent loss of a ferulic and rutinoside moieties, respectively. Peaks 15^{BR} and 7^{BR} were linked to sinapic acid, previously reported by Xiao et al. (2013), and tentatively identified as kaempherol-3-*O*-(synapoyl)-sophoroside ([M–H]$^-$ at *m/z* 815) and kaempherol-3-*O*-sinapoylsophoroside-7-*O*-glucoside ([M–H]$^-$ at *m/z* 977), respectively. Xiao et al. (2013) also reported peaks 9^{BR} and 4^{BR}, linked to coumaric and caffeic acids, respectively, being tentatively identified as kaempherol-3-*p*-coumaroylsophoroside-7-*O*-glucoside ([M–H]$^-$ at *m/z* 917) and kaempherol-3-*O*-caffeoyl-di-glucoside-7-*O*-glucoside ([M–H]$^-$ at *m/z* 933), respectively.

Quercetin (peaks 16^{BR}, 5^{BR}, and 11^{BR}) and isorhamnetin (peaks 10^{BR} and 19^{BR}) derivatives were also detected, but only on turnip tops. Peak 16^{BR} was identified as quercetin-3-*O*-glucoside in comparison with the commercial standard. Peaks 5^{BR} and 11^{BR} presented a pseudomolecular ion [M–H]$^-$ at *m/z* 625, and MS^2 fragments at *m/z* 463 and at *m/z* 301, that correspond to the subsequent loss of two hexosyl units, being tentatively identified as quercetin-3-*O*-sophoroside, previously identified in turnip tops by Romani, Vignolini, Isolani, Ieri, and Heimler (2006). Peaks 10^{BR} presented a pseudomolecular ion [M–H]$^-$ at *m/z* 639 two MS^2 fragments at *m/z* 447 and at *m/z* 315, that correspond to the subsequent loss of two hexosyl units, being tentatively identified as isorhametin-*O*-dihexoside (Romani et al., 2006). Peak 19^{BR} presented a pseudomolecular ion [M–H]$^-$ at *m/z* 639 and a unique MS^2 fragment at *m/z* 315 (132 mu), being tentatively identified as isorhamnetin-*O*-pentoside.

Finally, luteolin (peaks 7^{CC} and 11^{CC}) and apigenin (peaks 12^{CC}) derivatives were only found in wild cardoon leaves. Peak 7^{CC}, tentatively identified as luteolin-*O*-hexoside, presented a pseudomolecular ion [M–H]$^-$ at *m/z* 447 and a unique MS^2 fragment at *m/z* 285 (162 mu); while peak 11^{CC} |([M–H]$^-$ at *m/z* 533) was tentatively identified as luteolin-*O*-malonylhexoside, presenting MS^2 fragments at *m/z* 489 ([M-H-44]$^-$) and at *m/z* 285 ([M-H-42-162]$^-$), that corresponded to the loss of the malonyl moiety (86 mu) and the hexosyl moiety (Dias et al., 2018). Peak 12^{CC} ([M–H]$^-$ at *m/z* 473) was tentatively identified as acetylapigenin-*O*-hexoside presenting a unique MS^2 fragment at *m/z* 269, corresponding to the loss acetyl and hexosyl moieties, respectively. This compounds was previously identified in cardoon samples (Dias et al., 2018).

Finally, the lignin derivative found in cardoon samples, previously reported by Petropoulos et al. (2018) in the leaf blead of cardoon, was tentatively as pinoresinol-*O*-hexoside ([M–H]$^-$ at *m/z* 519).

For turnip tops, the most abundant class of phenolic compounds were flavonoids, in both hydroethanolic extracts and infusion preparation (18.8 ± 0.4 and 14.44 ± 0.01 mg/g of extract, respectively), being glycosylated derivatives of kaempferol, quercetin and isorhamnetin the most abundant, especially isorhamnetin-*O*-dihexoside both in hydroethanolic extract and infusion preparation (2.8 ± 0.1 and 2.11 ± 0.01 mg/g of extract, respectively). These results are in accordance with the ones reported by Romani et al. (2006) in the hydroethanolic extract of different cultivars of turnip tops from Italy, where isorhamnetin derivatives were the highest flavonols, especially isorhamnetin-*O*-dihexoside in the hydroethanolic extract (2.8 ± 0.1 mg/g of extract) and infusions (2.11 ± 0.01 mg/g of extract). It has been suggested, that isorhamnetin glycosides may be the primary flavonoids in the *B. rapa* group that plays an important role as a nectar guide (Romani et al., 2006; Sasaki & Takahashi, 2002). In addition, hydroxycinnamic acid derivatives were also detected in the studied samples, being synapoylmalic acid the major one in the class of phenolic acids (2.8 ± 0.1 and 1.29 ± 0.03 mg/g in the hydroethanolic extract and infusion preparation, respectively), being described for the first time in turnip samples. This compound has many biological activities namely antioxidant, antimicrobial, anti-inflammatory, anticancer and anti-anxiety, suggesting the potential use of this plant in food processing, cosmetics, and in the pharmaceutical industry (Nićiforović & Abramovič, 2014), giving a high add value to this bio-residue from turnip.

The leaf blade of wild cardoon showed higher contents in phenolic acids (23.6 ± 0.2 and 16 ± 0.3 mg/g in the hydroethanolic extract and infusion preparations, respectively), then in flavonoids (14.7 ± 0.1 and 12.1 ± 0.2, mg/g in the hydroethanolic extract and

infusion preparations, respectively); and revealed better results than the ones reported for the inflorescence parts of wild cardoon (Dias et al., 2018). These incongruities are notable especially regarding caffeoylquinic acid derivatives, namely 4-*O*-caffeoylquinic acid and 3,4-*O*-dicaffeoylquinic acid. This same inconsistency was also observed in the results reported by Pandino, Lombardo, Mauromicale, and Williamson (2011a); Pandino et al. (2011b) where it reports smaller amounts of these type of compounds in the leaves of wild cardoon from Italy. It is also important to underline the abundance of glycosylated luteolin derivatives in wild cardoon, both in hydroethanolic extracts and infusion preparation, especially luteolin-*O*-malonylhexoside (7.39 ± 0.02 and 5.81 ± 0.24 mg/g of extract, respectively), followed by glycosylated apigenin derivatives. Likewise, many authors reported apigenin derivatives as the major flavonoids present in the leaves of wild cardoon (Pandino et al., 2011a) and luteolin derivatives were only detected in var. *scolymus* (Pandino et al., 2011b). These differences could be explained by the different geographical origin, as also due to different geo-climatic conditions and the different harvest times (Dias et al., 2018).

Regarding the phenolic profile of radish aerial parts, this sample presented the highest content in total phenolic compounds (57.6 ± 0.2 and 28 ± 1 mg/g in the hydroethanolic extract and infusion preparations, respectively). The most abundant phenolic compound in this sample was *p*-coumaric acid, especially in the hydroethanolic extract (16.5 ± 0.1 mg/g of extract). In contrast, Beevi et al. (2010) showed that the most abundant phenolic compounds in the methanolic extract of radish leaves was vanillic acid, followed by catechin and sinapic acid. However, Goyeneche, Di Scala, and Roura (2013) found that epicatechin was the most abundant phenolic compound found, followed by coumaric acid in the hydromethanolic extract of radish.

Finally, a Pearson's correlation analysis between the bioactivities and the sum of total phenolic acids, total flavonoids, and total phenolic compounds (results not shown) was performed in order to prove the relationship between the structure of the compounds and their activity. The results with a confidence level higher than 90% were classified as very strong; between 70% and 90% strong, 50%–70% as moderate, 50%–30% as weak and negligible correlations between 30% and 0%.

Regarding antioxidant activity, it was possible to observe that the phenolic acids were the molecules that had the highest correlations (strong and moderate negative correlations), with the DPPH scavenging activity, reducing power, and TBARS inhibition ($r^2 = -0.899$, -0.736, and -0.572, respectively). The negative correlation is an indicator of the phenolic/bioactivity ratio, since the higher the concentration of these bioactive compounds, lower the EC_{50} value concentration to achieve 50% of the antioxidant activity. Total phenolic compounds also presented a strong correlation with the DPPH scavenging activity assay ($r^2 = -0.724$). As previously stated, chlorogenic acid derivatives were, among the phenolic acid derivatives group, presenting the highest amounts. The *in vitro* and *in vivo* antioxidant effects of chlorogenic acid derivatives have been extensively studied and reported by several authors (Kweon, Hwang, & Sung, 2001; Sato et al., 2011; Xiang & Ning, 2008), all reporting the high capacity of this type of compounds in chelating reactive oxygen species in both water soluble and fat soluble systems.

Regarding the antibacterial activity, only *Morganella morganii* presented promising results, showing a negative strong correlation with the group of total phenolic acids ($r^2 = -0.740$). Feruloylquinic acid derivatives are also an important fraction of the phenolic compounds found in all samples, and recent studies shown that the presence of ferulic acid has an inhibitory effect on the growth of these bacteria. Thus, the results presented herein are in accordance with the previously described in literature by Chatterjee et al. (2015).

The results for Pearson's correlation have fallen short of what would be expected in a natural extract, as the phenolic compound-bioactivities relationship has been intensively studied and proven by several authors. However, it is noteworthy that while studying un-purified natural extracts, we may have other types of compounds, also bioactive, that may have some influence on the bioactivity of these plant samples and therefore further studies to determine this relationship have to be performed.

4. Conclusion

The leaf blade of wild cardoon gave higher contents of fat, total available carbohydrates, energetic value, total soluble sugars (sucrose, fructose, trehalose and glucose), polyunsaturated fatty acids (mainly *α*-linolenic acid), tocopherols (*α*-, *β*-, *δ*-, and γ-isoforms), and phenolic acids (mainly 4-*O*-caffeoylquinic acid). However, both turnip and radish were richer in protein, ash, total organic acids (including oxalic, shikimic, citric, fumaric and quinic acids), and flavonoids. For instance, radish top revealed higher levels of total phenolic compounds (mainly *p*-coumaric acid and kaempferol glycoside derivatives), while, turnip tops gave higher content of saturated and monounsaturated fatty acids (palmitic and oleic acids, respectively). The hydroethanolic extract and infusion preparation of wild cardoon stood out in comparison to the other two samples in relation to its antioxidant and antibacterial activity. None of the samples showed hepatotoxicity effect against PLP2 cells.

For phenolic compounds, the hydroethanolic extracts were richer than infusion preparations and the results of the biological activities of the different samples showed that the hydroethanolic extracts seemed more effective (regarding antioxidant and antibacterial activity) than the infusions. The antioxidant activities of DPPH scavenging activity, reducing power and TBARS inhibition, as also the antibacterial effect against *Morganella morganii* have proven to be strongly correlated with the presence of phenolic acids. However, further studies need to be conducted to better understand this correlation, and clarify and identify the specific compounds responsible for the distinct bioactivities in the samples.

This study showed that the discarded parts of these plants, can be used as an important natural source of valuable nutrient contents and new and safer bioactive compound, beneficial to human health.

Acknowledgements

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programmer PT2020 for financial support to CIMO (UID/AGR/00690/2019). National funding by FCT, P.I., through the institutional scientific employment program-contract for M.I. Dias and L. Barros contracts. The authors are also grateful to FEDER-Interreg España-Portugal programme for financial support through the project 0377_Iberphenol_6_E.

Declaration of competing interests

The authors declare that they do not have any conflict of interest.

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