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ORIGINAL PAPER

Effect of in vitro gastrointestinal digestion on the total phenolic contents and antioxidant activity of wild Mediterranean edible plant extracts

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Abstract
The recent interest in wild edible plants is associated with their health benefits, which are mainly due to their richness in antioxidant compounds, particularly phenolics. Nevertheless, some of these compounds are metabolized after ingestion, being transformed into metabolites frequently with lower antioxidant activity. The aim of the present study was to evaluate the influence of the digestive process on the total phenolic contents and antioxidant activity of extracts from four wild edible plants used in the Mediterranean diet (*Beta maritima* L., *Plantago major* L., *Oxalis pes-caprae* L. and *Scolymus hispanicus* L.). HPLC-DAD analysis revealed that *S. hispanicus* is characterized by the presence of caffeoylquinic acids, dicaffeoylquinic acids and flavonol derivatives, *P. major* by high amounts of verbascoside, *B. maritima* possesses 2,4-dihydroxybenzoic acid, 5-*O*-caffeoylquinic acid, quercetin derivatives and kaempferol-3-*O*-rutinoside, and *O. pes-caprae* extract contains hydroxycinnamic acids and flavone derivatives. Total phenolic contents were determined by Folin–Ciocalteu assay, and antioxidant activity by the ABTS, DPPH, ORAC and FRAP assays. Phenolic contents of *P. major* and *S. hispanicus* extracts were not affected by digestion, but they significantly decreased in *B. maritima* after both phases of digestion process and in *O. pes-caprae* after the gastric phase. The antioxidant activity results varied with the extract and the method used to evaluate the activity. Results showed that *P. major* extract has the highest total phenolic contents and antioxidant activity, with considerable values even after digestion, reinforcing the health benefits of this species.

Keywords *Beta maritima* L. · Gastric digestion · Intestinal digestion · *Plantago major* L. · *Oxalis pes-caprae* L. · *Scolymus hispanicus* L

Introduction

Wild edible plants have represented an important food source for the communities of the Mediterranean basin, providing a relevant role in Mediterranean diet [1–3]. The habit of eating spontaneous plants is increasing nowadays because they are considered a healthy way of diversifying and enriching the modern diet with distinct colours and flavours [4–7]. Indeed, it is well recognized that the diversification of food habits with wild resources contributes to improve nutrition, health, livelihoods and also ecological sustainability [8]. Wild vegetables have been highly appreciated raw in salads or cooked in traditional recipes and the basis of human diets for centuries [5]. The knowledge about the bioactive properties of underutilized plants could provide feedback about their value and agro-industrial potential, and could also be used by gastronomic companies interested in the exploitation of these plants as additives or natural ingredients [9, 10].

Some wild edible plants have been recently considered as interesting functional foods since they provide health benefits [11]. These plants are recognized as valuable sources of bioactive compounds such as antioxidants [3, 7, 11]. The intake of food rich in antioxidants is correlated with the reduction of some chronic diseases in which oxidative stress may play a role, namely diabetes, cancer, cardiovascular diseases, etc. [12]. Antioxidants scavenge reactive

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oxygen species (ROS) and other reactive species involved in the progression of such diseases and, therefore, there is particular interest in the potential health benefits of plants with the greatest ROS scavenging activity [13].

Among plant bioactive compounds, phenolics are probably the most important candidates contributing to the claimed antioxidant properties of plants. Phenolics have strong antioxidant activity associated with their ability to scavenge free radicals, break radical chain reactions, and chelate metals [14, 15]. However, phenolics, particularly flavonoids and phenolic acids, are metabolized after ingestion and gastrointestinal absorption, usually being transformed into plasma metabolites with lower antioxidant activity than the precursor molecules [16]. In this sense, the comparison of antioxidant activity of food products before and after in vitro digestion is important to evaluate their real therapeutic capabilities [17]. Although there has been extensive investigation on the evaluation of antioxidant activity of plant extracts and foods, research studying the effect of digestion on the activity is scarce. In vitro methods of simulated gastrointestinal digestion have proven to be useful in determining the stability of bioactive compounds under gastrointestinal conditions and the results can be well correlated with those from human studies and animal models [18, 19]. The present study focused on four wild edible plants (*Beta maritima* L., *Plantago major* L., *Oxalis pes-caprae* L. and *Scolymus hispanicus* L.) used in Mediterranean diet (Fig. 1; Table 1) with nutritional and health benefits [8, 20–27]. The aim of the present study was to assess, for the first time, if the

Fig. 1 Aspect of the plants studied in their natural habitat. **a** *B. maritima*; **b** *O. pes-caprae*; **c** *P. major* and **d** *S. hispanicus*

Table 1 Edible and medicinal uses of the studied species

Plant species	Family	English common name	Edible part	Food use	Medicinal use
Beta maritima L.	Chenopodiaceae	Wild beet; sea beet	Basal leaves	Stewed	Digestive disorders, burns and throat pains and anaemia
Plantago major L.	Plantaginaceae	Common plantain	Leaves and seeds	The leaves in salads and soups; the seeds in snacks, cakes and breads	Skin infections and other infectious diseases, digestive and respiratory disorders, to enhance the circulation and reproduction, for pain and fever relief, and to prevent cancer
Oxalis pes-caprae L.	Oxalidaceae	Bermuda buttercup; cape sorrel	Leaves and peduncle of inflorescence	Salads	Not found
Scolymus hispanicus L.	Asteraceae	Golden thistle	Peeled basal leaves; roots and flowers	Peeled leaves boiled and fried in olive oil with garlic or raw in salads; roots employed as a coffee substitute and flowers as a colouring alternative to saffron	Diuretic, depurative, digestive, choleretic and lithiuretic

total phenolic contents and antioxidant activity of extracts from these species are affected by simulated gastrointestinal digestion.

Materials and methods

Chemicals and reagents

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) tablets, ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, pepsin from porcine gastric mucosa (CAS: 9001-75-6), pancreatin from porcine pancreas (CAS: 8049-47-6), bovine bile extract, porcine bile extract, trichloroacetic acid (TCA) and $K_2S_2O_8$ were purchased from Sigma-Aldrich (Steinheim, Germany). Folin–Ciocalteu reagent (F–C reagent), gallic acid, Na_2CO_3, CH_3COONa and $FeCl_3$ were acquired from VWR (Leuven, Belgium). $K_3[Fe(CN)_6]$, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) were purchased from Acros Organics (Geel, Germany). Fluorescein was acquired from Panreac (Barcelona, Spain). Formic acid was obtained from Merck (Darmstadt, Germany). 3-*O*-Caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid were from Chengdu Biopurity Phytochemicals Ltd. (Schuan, China), 2,4-dihydroxybenzoic acid was obtained from Sigma-Aldrich (St. Louis, MO, USA) and 5-*O*-caffeoylquinic acid, verbascoside, luteolin-8-*C*-glucoside, luteolin-6-*C*-glucoside, apigenin-7-O-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-rutinoside were purchased from Extrasynthèse (Genay, France).

Plant material and extraction procedure

Leaves of *B. maritima, O. pes-caprae, P. major* and *S. hispanicus* were collected from plants growing wild in the Algarve region (south Portugal). A representative sample of each plant was authenticated by JM Rosa Pinto from the herbarium of the University of Algarve (Faro, Portugal). The plant material was dried at 40 °C until constant weight and powdered in a blender (< 2 mm particle size). Dried plant material was extracted twice by maceration with 80% methanol (1:20, *w*/*v*) during 24 h at room temperature. After filtration, the extracts were concentrated to dryness in a rotary evaporator at 40 °C and under reduced pressure, and stored at − 20 °C.

High-performance liquid chromatography-diode array detection (HPLC-DAD) analysis

The extracts were analyzed on an analytical HPLC unit (Gilson), using a Spherisorb ODS2 column (4.6 × 250 mm, 5 µm, particle size). The solvent system used was a gradient of water–formic acid (19:1) (A) and methanol (B), starting with 5% methanol and installing a gradient to obtain 15% B at 3 min, 25% B at 13 min, 30% B at 25 min, 35% B at 35 min, 45% B at 39 min, 45% B at 42 min, 55% B at 47 min, 75% B at 56 min, and 100% B at 60 min, at a solvent flow rate of 0.9 ml/min. Detection was achieved with a Gilson diode array detector (DAD). Spectral data from all peaks were accumulated in the range 200–400 nm. Chromatograms were recorded at 280 nm for hydroxybenzoic acids, at 320 nm for hydroxycinnamic acids and at 350 nm for flavonoids. The data were processed on an Unipoint® System software (Gilson Medical Electronics, Villiers le Bel, France). The compounds in each extract were identified by comparing their retention times and UV–Vis spectra in the 200–400 nm range with authentic standards injected in the same conditions. Phenolic compound quantification was achieved by the absorbance recorded in the chromatograms relative to external standards, using the following equation:

$$C(c) = \frac{A\,(c)}{A\,(\mathrm{st})} \times C(\mathrm{st}),$$

where C(c) and A(c) are the concentration and the area of the compound in the sample, respectively, and C(st) and A(st) are the concentration and the area of the standard, respectively.

In vitro digestion procedure

The in vitro digestion model was preformed as described by Ryan et al. [28] with some modifications. The extracts were mixed with saline solution in a final volume of 20 ml. The resulting solutions were acidified to pH 2.0 with 1 ml of porcine pepsin preparation (0.04 g/ml in 0.1 M HCl) and were incubated for 1 h at 37 °C in a shaking bath. After gastric digestion, the pH was increased to 5.3 with 0.9 M sodium bicarbonate solution, followed by the addition of 200 µL of bovine and porcine bile extract solution (0.1 g/ml in saline), and 100 µl of pancreatin solution (0.08 g/ml in saline). The pH was increased to 7.4 with 1 M NaOH and then the samples were incubated again at 37 °C for 2.5 h to complete the intestinal phase of the in vitro digestion process. Samples were stored at − 20 °C and were analyzed within 2 weeks.

Determination of the total phenolic contents

The total phenolic contents of undigested extracts, and after gastric and pancreatic digestion were measured using a colorimetric method [29]. Briefly, 200 µl of 10% (v/v) F–C reagent was mixed with 100 µl of each extract in phosphate buffer (75 mM, pH 7.0) and 800 µl of 700 mM Na_2CO_3. After an incubation period of 2 h at room temperature, 200 µl of each reaction mixture was transferred to a clear 96-well microplate and the absorbance was measured at 765 nm. Instead of the plant extracts, gallic acid was used as a positive control and phosphate buffer as a negative control. A standard curve was calculated using several gallic acid dilutions and the results were expressed as gallic acid equivalents per gram of extract ($\mu mol_{GAE}/g_{extract}$).

Antioxidant activity

The ABTS, DPPH and peroxyl radical scavenging capacity, and ferric reducing antioxidant power (FRAP) of the extracts from the four plant species were evaluated before, and after gastric and intestinal digestion.

$ABTS^{\cdot+}$ radical cation decoloration assay

The ABTS free radical scavenging activity of each sample was determined as described by Re et al. [30]. A stock solution of 7 mM $ABTS^{\cdot+}$ prepared using potassium persulfate as the oxidizing agent was diluted to an absorbance of 0.700 at 734 nm to form the test reagent. Then, 190 µl of this reagent was mixed with 10 µl of each extract and the absorbance was determined at 734 nm. The extract dilution that achieved 20–80% inhibition of the blank was used for the calculations and the results were expressed as trolox equivalents per gram of extract ($\mu mol_{TE}/g_{extract}$).

DPPH free radical scavenging assay

The ability of the extracts to scavenge DPPH radicals was determined using the procedure described by Soler-Rivas et al. [31] with some modifications. One hundred microliters of 90 µM DPPH methanolic solution was added to 10 µl of extract at different concentrations, and the mixture was diluted with 190 µl of methanol in a clear 96-well microplate. Methanol was used as negative control and Trolox as positive control. After 30 min, the reduction of DPPH radicals was measured at 515 nm. The extract dilution that achieved 20–80% inhibition of the blank was used for the calculations and the results were expressed as trolox equivalents per gram of extract ($\mu mol_{TE}/g_{extract}$).

Oxygen radical absorbance capacity (ORAC) assay

ORAC assay was performed as described by Gillespie et al. [32] using fluorescein as the fluorescent probe and AAPH as peroxyl radical generator. A black microplate was loaded with 150 µl of 0.08 µM fluorescein and 25 µl of plant extract. Trolox was used as standard and phosphate buffer (75 mM, pH 7) as a negative control. The reaction was initiated with the addition of 25 µl 150 mM AAPH to each well after incubating for 10 min at 37 °C. The reduction in fluorescence was determined by reading fluorescein excitation at 485 nm and emission at 530 nm every minute for 90 min. The ORAC values were calculated using the area under the curve (AUC) and the regression equation between the TE and the net AUC, and the results were expressed as TE per gram of extract.

Ferric reducing antioxidant power (FRAP)

The reducing properties of the extracts were determined as described by Pulido et al. [33] with some modifications using $FeCl_3$. In brief, 100 µl of plant extract was mixed with 250 µl sodium phosphate buffer (200 mM, pH 6.6) and 250 µl 1% $K_3[Fe(CN)_6]$. The mixture was incubated at 50 °C for 20 min followed by the addition of 250 µl 10% TCA. After centrifugation at 650 rpm for 10 min, 100 µl of the supernatant was mixed with 100 µl of water and 20 µl 0.1% $FeCl_3$ in a 96-well microplate. Instead of the plant extracts, ascorbic acid was used as a positive control and phosphate buffer as a negative control. Reducing activity was measured by determining the absorbance at 700 nm and the results were expressed as ascorbic acid equivalents ($\mu mol_{AAE}/g_{extract}$).

Statistical analysis

The data were presented as the mean ± standard error of three replicates of each experiment. Data were analyzed by one-way analysis of variance (ANOVA) ($p < 0.05$). Statistical analysis was carried out using the SPSS statistical package for Windows (release 18.0; SPSS Inc., Chicago, IL, USA).

Results and discussion

Phenolic composition of the plant extracts

Phenolic compounds are considered the major contributors to the antioxidant capacity of many plants and an important part of human diet [34, 35]. Hence, the phenolic composition of the extracts studied in this work was analyzed by HPLC-DAD (Table 2; Fig. 2). *Scolymus hispanicus* extract

Table 2 Phenolic compounds identified in the extracts (mg/g of dry extract)

Peak	Compounds	RT (min)	*B. maritima*	*O. pes-caprae*	*P. major*	*S. hispanicus*
1	3-*O*-Caffeoylquinic acid	9.95	–	4.25 ± 0.34	–	–
2	4-*O*-Caffeoylquinic acid	15.43	–	0.75 ± 0.18	–	2.28 ± 0.13
3	2,4-Dihydroxybenzoic acid	16.51	1.53 ± 0.19	–	–	–
4	5-*O*-Caffeoylquinic acid	17.38	0.73 ± 0.02	–	–	33.30 ± 1.09
5	Luteolin-8-*C*-glucoside	30.49	–	0.74 ± 0.02	–	–
6	Verbascoside	31.87	–	–	32.37 ± 0.50	–
7	Luteolin-6-*C*-glucoside	34.88	–	2.92 ± 0.12	–	–
8	3,4-Di-*O*-caffeoylquinic acid	36.00	–	–	–	0.24 ± 0.02
9	3,5-Di-*O*-caffeoylquinic acid	38.58	–	–	–	2.05 ± 0.19
10	Quercetin 3-*O*-galactoside	41.51	3.62 ± 0.29	–	–	2.68 ± 0.19
11	Quercetin 3-*O*-rutinoside	42.84	5.47 ± 0.76	–	–	2.09 ± 0.22
12	4,5-Di-*O*-caffeoylquinic acid	43.51	–	–	–	0.80 ± 0.05
13	Apigenin-7-*O*-glucoside	44.5	–	0.21 ± 0.01	–	–
14	Kaempferol-3-*O*-glucoside	45.98	–	–	–	29.34 ± 3.22
15	Kaempferol-3-*O*-rutinoside	46.72	3.85 ± 0.54	–	–	2.27 ± 0.27
16	Isorhamnetin-3-*O*-rutinoside	47.56	–	–	–	0.28 ± 0.03
	Σ		15.20	8.87	32.38	75.33

- Not detected

[a]Values are expressed as mean ± SD ($n = 3$)

contained the highest amount of the identified phenolic compounds, 75.33 mg/g of dry extract. Among the identified compounds, the most abundant in this extract was 5-*O*-caffeoylquinic acid (chlorogenic acid) (33.30 mg/g of dry extract) and kaempferol-3-*O*-glucoside (29.34 mg/g of dry extract). Another caffeoylquinic acid derivative (4-*O*-caffeoylquinic acid) and three dicaffeoylquinic acids (3,4-, 3,5- and 4,5-di-*O*-caffeoylquinic acids) were also identified (Table 2). Caffeoylquinic and dicaffeoylquinic acids are frequent in several Asteraceae species [36, 37]. In addition, *S. hispanicus* extract also contained four other flavonoids: quercetin 3-*O*-galactoside, quercetin 3-*O*-rutinoside, kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-rutinoside. In a chemotaxonomic study with several Asteraceae species, Sareedenchai and Zidorn [38] also identified several flavonoids in *S. hispanicus*, namely quercetin, kaempferol and isorhamnetin derivatives.

Phenylethanoid glycosides are key metabolites in *Plantago* species [39]; therefore, it is not surprising that *Plantago major* extract contains a large amount of verbascoside (32.37 mg/g of dry extract). This compound was previously identified in *P. major*, as well as in several other *Plantago* species [24, 39, 40].

Five compounds were identified in *Beta maritima* extract: 2,4-dihydroxybenzoic acid (1.53 mg/g of dry extract), 5-*O*-caffeoylquinic acid (0.73 mg/g of dry extract), quercetin 3-*O*-galactoside (3.62 mg/g of dry extract), quercetin 3-*O*-rutinoside (5.47 mg/g of dry extract) and kaempferol-3-*O*-rutinoside (3.85 mg/g of dry extract). *Oxalis pes-caprae* extract contained 3-*O*-caffeoylquinic acid (4.25 mg/g of dry extract), 4-*O*-caffeoylquinic acid (0.75 mg/g of dry extract), luteolin-8-*C*-glucoside (0.74 mg/g of dry extract), luteolin-6-*C*-glucoside (2.92 mg/g of dry extract) and apigenin-7-*O*-glucoside (0.21 mg/g of dry extract). Recently, three luteolin derivatives and three apigenin derivatives were identified in an extract from this species [20]. Additionally, Güçlütürk et al. [21] reported the presence of chlorogenic acid in an *O. pes-caprae* methanol extract. In our study, we did not find chlorogenic acid (5-*O*-caffeoylquinic acid), but 3-*O*-caffeoylquinic and 4-*O*-caffeoylquinic acids. This incoherency may be due to the confusion in the literature about the nomenclature of caffeoylquinic acids. According to IUPAC recommendations (IUPAC Commission on the Nomenclature of Organic Chemistry and IUPAC-IUB Commission in Biochemical Nomenclature, 1976) [41], 3-*O*-caffeoylquinic acid is now designated as 5-*O*-caffeoylquinic acid [42]; however, there are still papers where 3-*O*-caffeoylquinic acid isomer is called chlorogenic acid.

Total phenolic contents and antioxidant activity

The total phenolic contents of the extracts from the four species studied before and after in vitro digestion are shown in Fig. 2. Total phenolic contents of undigested extracts varied between 121.66 ± 2.71 $\mu mol_{GAE}/g_{extract}$ in *O. pes-caprae* and 431.89 ± 14.54 $\mu mol_{GAE}/g_{extract}$ in *P. major*. The total phenolic content of a methanol extract from *B. maritima* (61.91 $mg_{GAE}/g_{extract}$) was previously evaluated by Morales et al. [25] and the results were similar to the obtained in

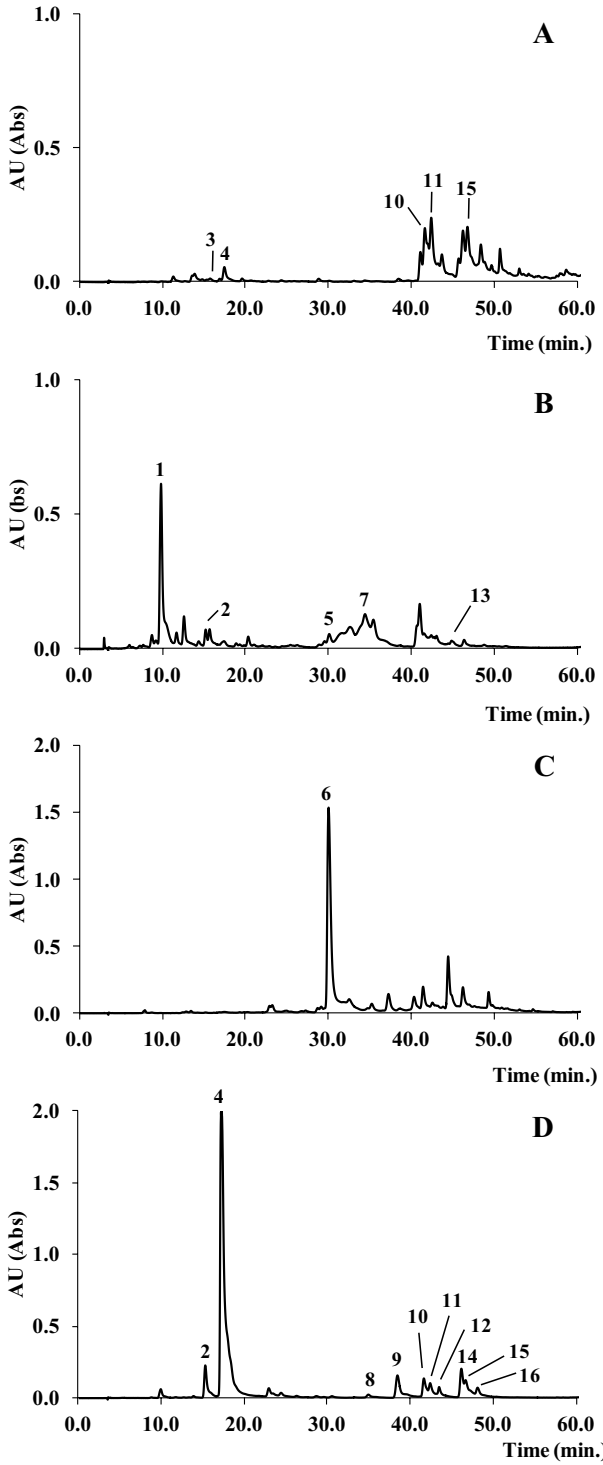

Fig. 2 HPLC-DAD chromatograms of *B. maritima* (**a**), *O. pes-caprae* (**b**), *P. major* (**c**) and *S. hispanicus* (**d**). Detection at 320 nm; identity of compounds as in Table 2

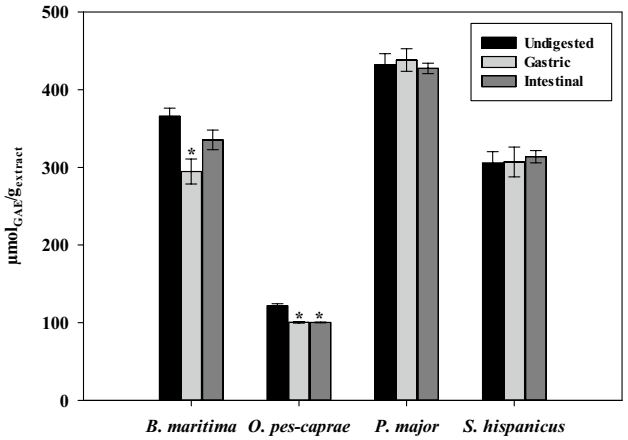

Fig. 3 Total phenolic content of extracts from the four plant species studied before (undigested samples) and after in vitro digestion (gastric and intestinal digests). Values are expressed as mean ± SE ($n = 3$). Asterisk denotes significantly different ($p < 0.05$) in comparison with undigested extract

this study (53.70 $mg_{GAE}/g_{extract}$). The total phenolic contents in different *O. pes-caprae* extracts were also previously reported; however, since those results are expressed in a fresh weight basis it is difficult to compare them with the obtained in this work [8, 21]. Recently, Mazzutti et al. [24, 43] reported the total phenolic contents in *P. major* extracts obtained by different extraction techniques, solvents and extraction conditions. The values obtained ranged from 2.1 to 132.20 $mg_{GAE}/g_{extract}$ and the value found in this work was 68.81 $mg_{GAE}/g_{extract}$. The total phenolic content found in *S. hispanicus* (52.52 $mg_{GAE}/g_{extract}$) was higher than that found in an ethanol extract of the same species obtained by Soxhlet extraction (18.24 $mg_{GAE}/g_{extract}$) [23].

The benefits of phenolic compounds for human health are incontestable; however, most of these compounds are considered as xenobiotics by the human body and their bioavailability is relatively low in comparison with other nutrients [44]. Thus, studies reporting the effect of in vitro digestion on the bioactivity of phenolic extracts are important. In this study, the effect of in vitro digestion on total phenolic contents and antioxidant activity of extracts from the four species studied was evaluated using an in vitro digestion protocol to simulate digestion. The total phenolic content of *P. major* and *S. hispanicus* was not affected by in vitro digestion. On the other hand, the total phenolic content of *B. maritima* and *O. pes-caprae* extracts obtained after the two phases of the process and after the gastric phase, respectively, significantly decreased ($p < 0.05$) (Fig. 3). Significant decreases in the total phenolic content of extracts from various plants after gastrointestinal simulation were also reported by several authors [45–47]. In the literature, different results can be found concerning the effect of gastrointestinal digestion on total phenolic contents. Jayawardena et al. [48] observed no decreases on total phenolic contents of extracts from ten edible plants after digestion process. Chen et al. [49] studied the effect of digestion on total phenolic contents of extracts from 23 edible flowers and observed that

the results varied considerably between species. Phenolics are sensitive to various factors, such as pH and enzymatic reactions, and different changes in total phenolic contents after digestion could be due to the stability of each type of phenolic compound present in the food matrix [18, 50]. Thus, the differences in the phenolic behaviour observed between extracts can be related to the different qualitative and quantitative phenolic profiles of extracts from the different plants.

The antioxidant activity of the extracts studied was measured before and after in vitro simulated digestion using DPPH, ABTS, ORAC and FRAP assays (Fig. 4). All the extracts were capable of scavenging DPPH radicals with values ranging from 24.70 ± 0.44 to 404.47 ± 10.35 $\mu mol_{TE}/g_{extract}$ before digestion. Although *P. major* extract showed a significant decrease in the activity after both phases of digestion, it showed the strongest scavenging capacity of DPPH radicals (404.47, 239.06 and 345.98 $\mu mol_{TE}/g_{extract}$, before digestion, after gastric phase and intestinal phase, respectively). The DPPH scavenging capacity also significantly decreased after both phases of digestion of *B. maritima* extract and after gastric digestion of *S. hispanicus* extract. On the other hand, the DPPH scavenging capacity of *O. pes-caprae* extract significantly increased after intestinal digestion. The ABTS scavenging capacities of undigested extracts varied between 88.45 ± 33.36 $\mu mol_{TE}/g_{extract}$ for *O. pes-caprae* and 355.47 ± 29.19 $\mu mol_{TE}/g_{extract}$ for *S. hispanicus*. After digestion process, values significantly decreased after the gastric phase for all the extracts and after intestinal phase only for *P. major* extract. The capacities of the extracts to neutralize peroxyl radicals was evaluated by the ORAC assay, an hydrogen atom transfer-based method that uses a fluorescent probe to compete with antioxidants for peroxyl radicals generated by the decomposition of AAPH. The ORAC values of undigested extracts varied between 354.75 ± 4.85 $\mu mol_{TE}/g_{extract}$ for *O. pes-caprae* and 1344.87 ± 18.15 $\mu mol_{TE}/g_{extract}$ for *P. major*. The digestion process did not significantly affect the ORAC values in *B. maritima* and *O. pes-caprae* extracts ($p \geq 0.05$). Otherwise, the ORAC values significantly decreased after both phases of digestion process in *P. major* and *S. hispanicus* (Fig. 4). Similarly to the observed in the other assays, the initial FRAP values of the tested extracts varied considerably among species, between 78.22 ± 1.91 for *O. pes-caprae* and 497.67 ± 10.74 $\mu mol_{AAE}/g_{extract}$ for

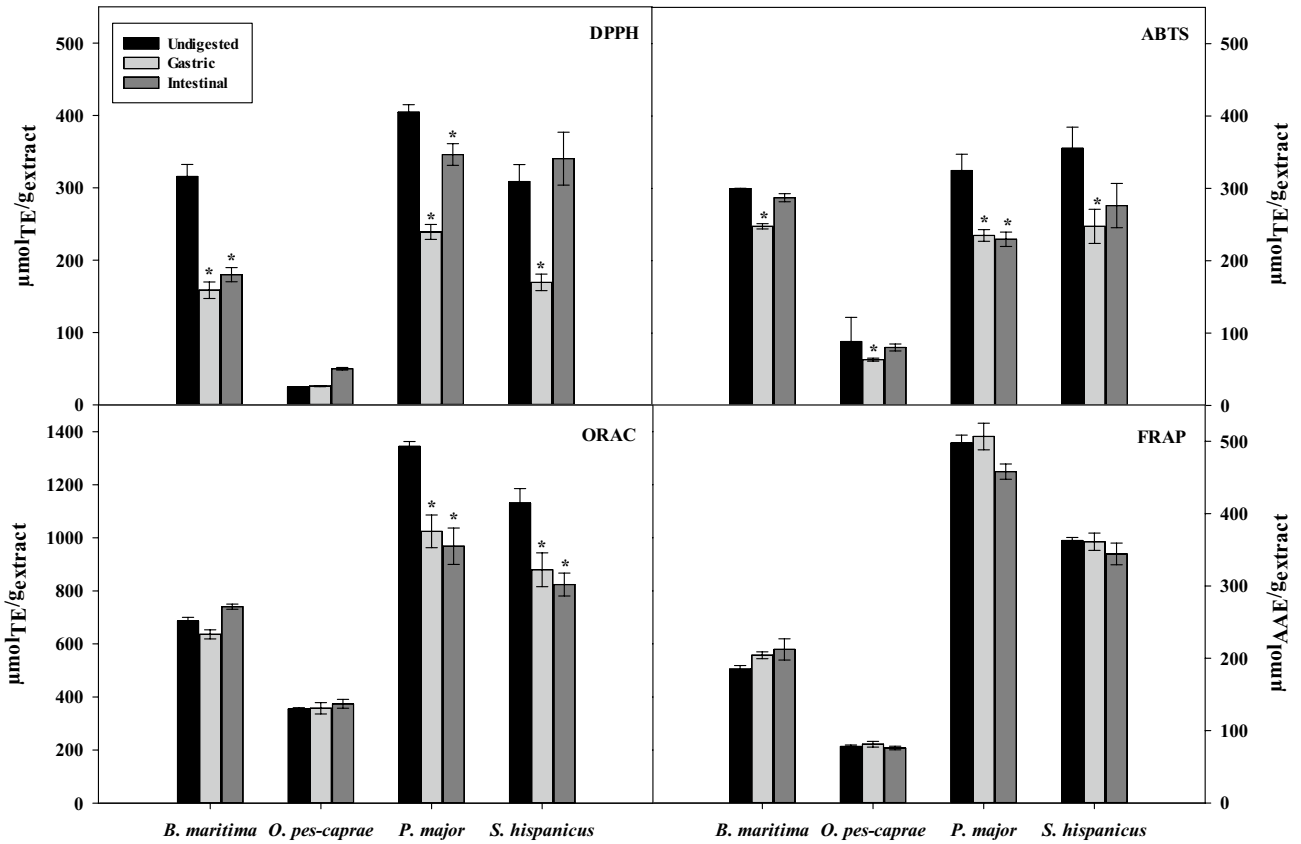

Fig. 4 Antioxidant activity of extracts from the four plant species studied before (undigested samples) and after in vitro digestion (gastric and intestinal digests). Values are expressed as mean ± SE ($n = 3$). Asterisk denotes significantly different ($p < 0.05$) in comparison with undigested extract

P. major. In this method, the values obtained after both phases of the digestion process of all the extracts did not differ significantly ($p \geq 0.05$) from the initial (Fig. 4).

There are some contradictory data in literature about the effect of gastrointestinal digestion on the antioxidant activity of plant matrices. Interactions with other components of the extract and pH variations also cause changes in antioxidant activity [51]. For example, pH affects the racemization of molecules, leading to two chiral enantiomers with different bioavailability, and, as a result, different bioactivity [52]. Phenolic compounds can interact with other dietary components released during digestion (e.g., minerals, proteins, dietary fibres, volatile compounds), which also play an important role in bioactivity [18, 53, 54]. The assay employed could also affect the assessment of antioxidant activity since pH modifications may alter the structure of phenolic compounds and, consequently, the antioxidant activity [55]. For instance, assays carried out at pH 7, such as ABTS and ORAC, are proposed as more appropriate to evaluate the activity of intestinal digests [18, 56].

Despite some exceptions, in this study it was noticed a trend for the radical scavenging capacity to be more affected by the gastric conditions than by the intestinal ones (Fig. 4). Results from Jayawardena and co-authors [48, 57] showed significant increases in the antioxidant activity, particularly when measured with ORAC and FRAP assays, of extracts from some edible plants and fruit juices after the intestinal phase. According to Bouayed et al. [18], the change from acidic to alkaline environment improves the antioxidant activity of phenolics by causing deprotonation of the hydroxyl moieties present on their aromatic rings. Furthermore, the results vary with the extract and the method used to analyze the antioxidant activity. The activity measured by DPPH assay increased after intestinal phase for *O. pes-caprae* extract, although total phenolic content decreased ($p < 0.05$). In other cases, no differences in the total phenolic contents were observed after in vitro digestion but the antioxidant activity decreased ($p < 0.05$). These results suggest that the studied extracts probably also contain non-phenolic substances, such as peptides, that could be involved in this activity [50].

Comparing the results of the different antioxidant assays, *P. major* extract appears to be the most potent among the studied extracts. The antioxidant activity of this extract was probably related with the high content in verbascoside (Table 2). This compound is a phenylethanoid glycoside present in several *Plantago* species, which possesses beneficial activities for human health, namely antioxidant, anti-inflammatory, antimicrobial, wound-healing and neuroprotective properties [58]. Some investigations suggest that the four hydroxyls at the *ortho* position in the two aromatic rings of verbascoside contribute to its remarkable antioxidant activity [59]. In addition to its food uses (Table 1), *P. major* is certainly one of the most commonly used medicinal herb in the world [27]. The leaves are employed in many countries for the treatment of skin infections and other infectious diseases, digestive and respiratory disorders, to enhance the circulation and reproduction, for pain and fever relief, and to prevent cancer [27].

Overall, the results of the present study demonstrate the importance of evaluating the bioactivity of plant extracts after digestion. Moreover, this study highlights the importance of analyzing the antioxidant activity by different methods with distinct mechanisms. Although antioxidant activity is affected by digestion conditions, in some cases the results obtained indicate the wild plants studied as sources of natural antioxidants. In future studies, it is important to study the phenolic profile of the extracts after gastrointestinal digestion, and to analyze the bioaccessibility, bioavailability and bioactivity of the extracts in other systems, to accurately assess the health promoting benefits of these species. Knowledge about the biological potential of these spontaneous plants makes them especially attractive, given the increasing awareness of people to consume natural healthy products, as well as interest in rediscover local traditions and food habits.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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