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Antioxidant and antiproliferative properties of strawberry tree tissues

L. Tavares[a], S. Fortalezas[a], C. Carrilho[a], G.J. McDougall[b], D. Stewart[b], R.B. Ferreira[a,c] and C.N. Santos[a,*]

[a]*Disease & Stress Biology Laboratory, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal*
[b]*Plant Products and Food Quality Programme, Scottish Crop Research Institute, Dundee, Scotland, UK*
[c]*Departamento de Botânica e Engenharia Biológica – Instituto Superior de Agronomia, Lisboa, Portugal*

Abstract. Strawberry tree (*Arbutus unedo*) belongs to the *Ericaceae* family and is endemic to the Mediterranean area. Its fruits are edible and its fruits and leaves are used in folk medicine for diverse purposes. Previous studies have shown that the fruits are rich in flavonoids, responsible for their antioxidant properties and compounds isolated from the entire plant were promising in cancer chemopreventive therapy.

Strawberry tree fruits and leaves extracts enriched in polyphenols, but devoid of organic acids, carotenoids and sugars, were prepared by solid phase extraction (SPE) and tested for their antioxidant activities and their ability to inhibit metalloproteinases: attributes that could be related with initiation and proliferation of cancer cells.

After fractionation by SPE, the apparent polyphenol yield was reduced for both leaf and fruit samples by the elimination of vitamins and organic acids, but the antioxidant and metalloproteinases inhibitory activities were potentiated.

The antioxidant activity and the MMP-9 inhibitory activity of the polyphenol-enriched fractions of *A. unedo* tissues were similar or higher than those of blackberry and green tea, which have been recognized in the literature as highly effective.

The phenolic profile of the fruit was dominated by gallic acid and quercetin derivatives with smaller amounts of proanthocyanidins and anthocyanins. The phenolic profile of the leaves was also dominated by gallic acid derivatives, flavonol derivatives and some tannins but lacked anthocyanins.

The fractions obtained from both strawberry tree tissues seem to be quite promising as antioxidants and antiproliferative agents. Further cell-based assays are underway to study these possible outcomes.

Keywords: *Arbutus unedo*, polyphenols, antioxidant activity, MMP-9, HPLC-MS

1. Introduction

There is considerable epidemiological evidence that insufficient intake of fruits and vegetables may predispose the human body to a range of chronic health disorders, including cancer and cardiovascular disease [9]. Phytochemicals, such as polyphenols, present in many fruits and vegetables may participate in disease prevention and this has contributed to the growing interest in identifying components in edible plants responsible for anticancer effects [19].

Polyphenols demonstrate diverse biological activities attributed to their general free radical trapping capacity, or antioxidant activity *per se*, iron chelation, activation of survival genes, cell signalling pathways and regulation of mitochondrial function [9, 23]. In cancer, several studies point to the important role of the oxidative stress on the

*Corresponding author: C.N. Santos, Disease & Stress Biology Laboratory, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, Apartado 127, 2781-901 Oeiras, Portugal. Tel.: +351 214469651; Fax: +351 214433644; E-mail: csantos@itqb.unl.pt.

oncogenic stimulation. Various cancer cells have a cellular redox imbalance, i.e, a disruption between endogenous antioxidants and reactive oxygen and nitrogen species (ROS and RNS) balance when compared with normal cells, which could modify permanently the cell biology. This redox imbalance could lead to damage in cellular biomolecules that contribute to induction of mutagenesis and carcinogenesis [37–39]. Free radical damage could influence DNA mutations and altered gene expression, lipid peroxidation due to malondialdehyde (MDA) and 4-hydroxynonenal (HNE) formation [16, 25, 38], and protein oxidation [39].

Cancer cell invasion is a critical point for cancer metastasis. It is generally accepted that remodelling of the extracellular matrix (ECM) is required for cancer cell invasion [41]. The primary response for the degradation of ECM components was demonstrated to be the activation of zinc-dependent matrix metalloproteinases (MMPs) [27]. Although MMPs are expressed in normal tissue in remodelling conditions, such as during embryonic development, wound healing, uterine and mammary involution, cartilage-to-bone transition in ossification, and placenta development, the aberrant expression of various MMPs has been correlated with pathological conditions, such as rheumatoid arthritis, tumour cell invasion and metastasis [12]. Matrix metalloproteinase inhibitors (MMPIs) have been shown to inhibit angiogenesis in various models. However the synthetic inhibitors have demonstrated some disadvantages, such as the lack of selectivity and the secondary effects [24].

Strawberry tree (*Arbutus unedo* L.; *Ericaceae* family) is an evergreen shrub, a native Mediterranean species which is also cultivated in other regions of Eastern Europe [3]. Its fruits are spherical, about 2 cm in diameter, dark red, and tasty only when fully ripen in autumn. *A. unedo* berries are rarely eaten as fresh fruits but have some importance in local agricultural communities which use them for the production of alcoholic beverages, jams, jellies and marmalades [2, 28]. The fruits are also used in folk medicine as antiseptics, diuretics and laxatives, while the leaves have long been employed as an astringent, diuretic, urinary anti-septic agent and, more recently, in the therapy of hypertension and diabetes [4]. Recently, Carcache-Blanco and co-workers isolated ten compounds from *A. unedo* leaves exhibiting chemopreventive action through cyclooxygenase-2 inhibitory activity [6]. This work reinforces *A. unedo* as promising source of antiproliferative agents. Strawberry tree fruit contains several classes of naturally occurring antioxidants such as phenolic compounds (e.g. anthocyanins, gallic acid derivatives, tannins and flavonoids), vitamin C, vitamin E and carotenoids [2, 3, 22, 28, 29], and a high antioxidant capacity [28].

The aim of this work was to assess an *in vitro* potential of polyphenols from *A. unedo* fruits and leaves to provide an antiproliferative effect. The extracts prepared were enriched in polyphenols, but devoid of organic acids (vitamin C), carotenoids and sugars. Using this approach, we expect to maximize the activities of the compounds through positive synergisms between the different polyphenols or by eliminating antagonisms, in contrast to the Carcache-Blanco approach that tested pure compounds [6]. Synergistic positive effects are well described between polyphenols [1, 33, 35]. For example, Seeram et al. detected for cranberry extracts [33], after removing sugars and organic acids, a higher antiproliferative activity of polyphenols enriched fraction, when compared against the individual purified phytochemicals.

2. Material and methodology

2.1. Biological material

Arbutus unedo L. fruits and leaves were collected in November 2007, in Arrábida Natural Park (southern region of Portugal) and frozen. For comparison purposes, the blackberry cv. Apache produced in Fataca experimental field (Odemira, Portugal) and commercial tea leaves (Lipton Green Tea Pure, Lipton) were used as control samples for fruits and leaves, respectively. The samples were freeze-dried, ground and stored at –80°C prior to extraction.

2.2. Extract preparation

To each 1 g of lyophilized powder, 12 mL of hydroethanolic solvent (50% (v/v) ethanol/water) was added and the mixture was shaken for 30 min at room temperature in the dark. The mixture was then centrifuged at 12400 *g* for 10 min at room temperature. The supernatant was filtered through paper filter and then through 0.2 μm cellulose acetate membrane filters. The resulting extracts were stored frozen at –80°C.

2.3. Fractionation by solid phase extraction

Hydroethanolic extracts were fractionated by Solid Phase Extraction (SPE) using a Giga tubes 2 g/12 mL, C18-E units (Phenomenex®). The columns were pre-washed in 0.5% (v/v) glacial acetic acid in acetonitrile and then pre-equilibrated in 0.5% (v/v) glacial acetic acid in water. The extracts were dried under vaccum and ressuspended in 0.5% (v/v) glacial acetic acid in water, then they were applied to the columns and unbound material, which contained the free sugars, organic acids or vitamin C, was discarded. The columns were washed with 0.5% (v/v) aqueous acetic acid and then polyphenol-enriched bound fractions were eluted with 0.5% (v/v) glacial acetic acid in acetonitrile [30, 32].

2.4. Total phenolic measurement

Determination of total phenolic compounds was performed by the Folin-Ciocalteau method [34]. Briefly, to each well of a microplate, 235 μL water, 5 μL sample (or solvent, in the blank), 15 μL Folin-Ciocalteau's reagent (Fluka®) and 45 μL saturated Na_2CO_3 were added. The microplate was incubated for 30 min at 40°C and the absorbance at 765 nm measured. Gallic acid was used as the standard and the results were expressed as mg of gallic acid equivalents (mg GAE).

2.5. Peroxyl radical scavenging capacity assay

Peroxyl radical scavenging capacity was determined by the ORAC (Oxygen Radical Absorbance Capacity) method [5, 40]. Briefly, the reaction mixture contained 150 μL of sodium fluorescein (0.2 nM) (Uranine, Fluorescein Sodium Salt® TCI Europe), 25 μL sample and 25 μL of 2,2′-azobis(2-amidopropane) dihydrochloride (41.4 $g.L^{-1}$). All solutions are prepared in 75 mM phosphate buffer (pH 7.4). The blank contained 25 μL 75 mM phosphate buffer (pH 7.4) instead of sample, whereas the standards contained 25 μL of 10 to 50 μM 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) in place of the sample. The fluorescent emission at 515 nm was then monitored kinetically during 30 min at 37°C, after excitation at 493 nm using a FLx800 Fluorescence Microplate Reader (Biotek). The final results were calculated using the area differences under the fluorescence decay curves between the blank and the sample, and were expressed as mM Trolox equivalents (mM TE).

2.6. MMP-9 inhibitory activity assay

MMP-9 pro-enzyme was activated using 4-aminophenylmercuric acetate (APMA). The enzyme was incubated with 1 mM APMA in 100 mM Tris-HCl buffer (pH 7.5) containing 100 mM NaCl, 10 mM $CaCl_2$ and 0.05% (v/v) Brij 35 and 2% (v/v) DMSO for 22 h at 37°C in the dark. Then the reaction was stopped with ice and the active MMP-9 was kept at 4°C. MMP-9 activity was measured by the direct hydrolysis of MCA-Pro-Leu-Gly-Leu-β-(Dnpa)-Ala-Ala-Arg-amida substrate [18], which has a fluorescent group 7-metoxicumarin-4-acetil (MCA) and a quenching group 2,4-dinitrofenilamine (Dnpa). Therefore, an increase in fluorescence intensity occurs as a result of substrate hydrolysis, namely the cleavage of peptide Gly-Leu. Before inhibition tests, enzymatic activity using different concentrations of MMP-9 was measured, which revealed a linear relationship between enzyme activity and enzyme concentration (data not shown). The highest concentration was chosen for inhibition assays. The assays were performed on 96 well plates measuring a residual enzymatic activity. The enzyme (3.2 nM) was pre-incubated with 100 mM Tris-HCl buffer (pH 7.5) containing 100 mM NaCl, 10 mM $CaCl_2$ and 0.05% (v/v) Brij 35 and different concentrations (0–25 μg GAE. mL^{-1}) of test fractions at 37°C for 30 min. The reaction was initiated by adding the substrate (12 μM in the same buffer as the enzyme) in a final volume of 100 μL. Fluorescence ($\lambda_{emission} = 393$ nm, $\lambda_{excitation} = 325$ nm) was measured for 10 min at room temperature. Results were transformed using a non-linear regression with Origin Pro 6.1 software (OriginLab©, USA) and the IC_{50} values were determined.

2.7. HPLC-MS phenolic profile determination

Phenolic extracts were dried by rotary evaporation, ressuspended in 5% (v/v) acetonitrile in water and were analyzed on a LCQ-DECA system controlled by the XCALIBUR software (2.0, ThermoFinnigan). The LCQ-Deca system

comprised a Surveyor autosampler, pump and photo diode array detector (PDAD) and a Thermo Finnigan mass spectrometer iontrap. The PDA collected spectral data from 200–600 nm and scanned three discrete channels (at 280, 365 and 510 nm). The samples were applied to a C-18 column (Synergi Hydro C18 column with polar end capping, 4.6 mm × 150 mm, Phenomonex Ltd) and eluted over a gradient of 95:5 solvent A:B at time = 0 min to 60:40 A:B at time = 60 min at a flow rate of 400 μL/min. Solvent A was 0.1% (v/v) formic acid in ultra pure water and solvent B 0.1% (v/v) formic acid in acetonitrile. The LCQ-Deca LC-MS was fitted with an ESI (electrospray ionization) interface and analyzed the samples in positive and negative-ion mode. Two scan events, full scan analysis in mass range 80–2000 m/z followed by data dependent MS/MS of the most intense ions, were used for compounds detection and identification. The data-dependent MS/MS used collision energies (source voltage) of 45%. The capillary temperature was set at 275°C with sheath gas at 60 psi and auxiliary gas at 10 psi. Before the analysis, the system was tuned by using known concentrations of cyanidin-3-glucoside (positive mode) and quercetin-3-glucoside (negative mode) in ultrapure water.

2.8. Statistical analysis

The results reported in this work are the averages of at least three independent experiments and are represented as the mean ± SD. Differences among treatments were detected by analysis of variance [31] with Tukey HSD (Honestly Significant Difference) multiple comparison test ($\alpha = 0.05$) using SigmaStat 3.10 (Systat).

3. Results and discussion

3.1. Assessment of bioactivities recovery in polyphenols enriched fractions

After the hydroethanolic extraction, crude extracts were fractionated by SPE column, to obtain polyphenol-enriched fractions. The crude extracts and enriched fractions were assessed for total phenolic content, antioxidant activity for peroxyl radical and the MMP-9 inhibitory activity (Fig. 1).

After fractionation by the SPE column, strawberry tree fruit and leaf extracts showed a decrease in phenolic content (18% and 62% respectively). The decreases observed are probably due to molecules detected by Folin-Ciocalteu method, such as vitamins or organic acids that are eliminated by the SPE [13]. Indeed, components such

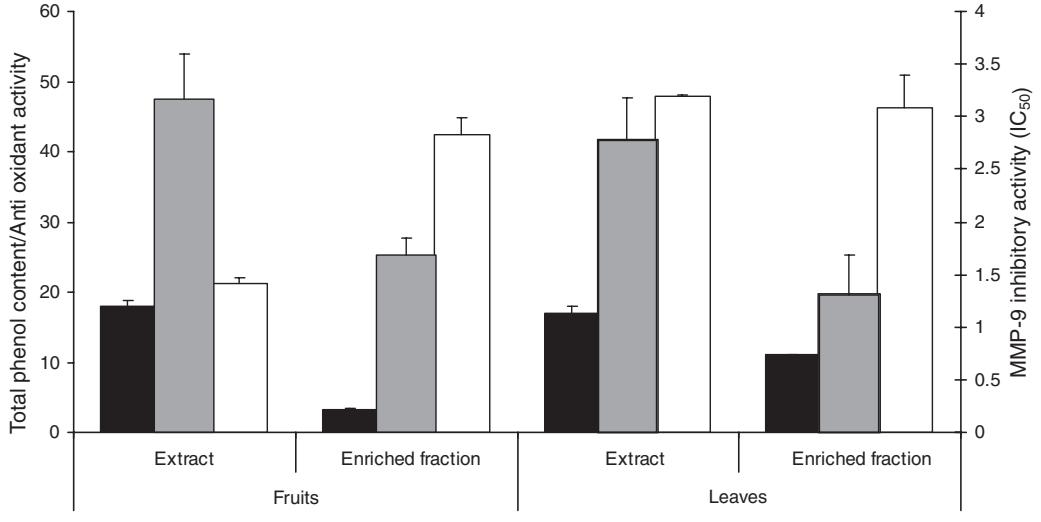

Fig. 1. Comparison of total phenol content, antioxidant capacity and metalloproteinases inhibitory activity between crude extracts and polyphenol-enriched fractions obtained from *A. unedo* fruits and leaves. ■-total phenol content (mg GAE), ▒- IC_{50} of MMP-9 inhibitory capacity (mg GAE. mL^{-1}) and □ antioxidant activity (×10 mmol TE) for peroxyl radical were determined for crude extracts and polyphenols enriched fractions obtained from *A. unedo* fruits and leaves. Vertical bars represent ± SD.

as niacin, ascorbic acid, β-carotene, L-malic and quinic acids (detected and quantified in strawberry tree fruits by Alarcão-E-Silva et al. [2] or Ayaz et al. [3]) could account for the apparent loss in "total phenol content" after SPE. The greater decrease in the fruit fraction could be due to the higher contents in vitamins and organic acids in fruits.

The strawberry tree fruit polyphenol-enriched fraction presented an increase of 100% of antioxidant activity, suggesting that the SPE fractionation eliminated some compounds with antagonistic effects. Strawberry tree leaves had an antioxidant capacity recovery of approximately 100%.

Concerning the MMP-9 inhibitory capacity, the IC_{50} values for both fractions diminished 50%, which means that 50% of the amount of polyphenols is required to produce the same effect as the crude extract.

Therefore, fractionation of strawberry tree tissues using SPE simultaneously eliminated molecules that could exert some antagonistic effects (e.g. in the antioxidant activity) and enriched the phenolic compounds (which diminished the IC_{50} value for MMP inhibition). However, further fractioning could not be so beneficial, since could eliminate positive synergistic effects, already detected by others [33]. In fact Carcache-Blanco have tested *A. unedo* isolated compounds and detected lower efficacies (high IC_{50}) than their positive controls (13-*cis*-Retinoic acid and *trans*-Resveratrol) in chemopreventive assays [6].

3.2. Antioxidant and MMP-9 inhibitory activities

The strawberry tree fruit and leaf extracts gave significantly higher antioxidant capacities (211.66 ± 22.82 mM TE and 308.56 ± 26.74 mM TE, respectively) than blackberry and green tea, respectively (Table 1). The IC_{50} values of MMP-9 inhibitory activity for both strawberry tree tissues were not significantly different of the amount required for the respective control samples (1.68 ± 0.38 μg GAE. mL^{-1} for strawberry tree fruits and 1.31 ± 0.17 μg GAE. mL^{-1} for leaves) (Table 1).

Control tissues used in this work, blackberry and green tea leaves, are recognized in the literature as highly antioxidants and anti-cancer agents [7, 8, 15, 36, 42–44], Blackberries are a rich source of polyphenols and present a recognized *in vitro* antioxidant activity (6221 μmol TE. 100 g^{-1} determined by ORAC method by Wolfe et al.) [42] as well as a good cellular antioxidant activity detected by a decrease in ROS in cells [42]. These fruits also have shown to inhibit MMP-2 and MMP-9 activities [36]. Moreover, they also could inhibit HT-29 colon tumor cell growth in a concentration-dependent manner [8]. Green tea leaves have been studied for many years and it has been shown their capacity to inhibit carcinogen-induced DNA damage in a number of cell line studies as reviewed by Yang et al. due to their direct radical scavenging and metal chelation [43, 44]. Tea and tea constituents have been shown to inhibit the development of several cancer in animal models like oral, esophageal, forestomach, stomach, intestinal, colon, skin, liver, bladder, prostate, and breast cancer [7, 15, 44].

In this work results for the *A. unedo* tissues, in comparison with the positive controls, revealed to be promising sources of effective antiproliferative extracts. To confirm these *in vitro* results, cell based assays should be performed as antiproliferative and intracellular antioxidant tests.

Table 1

Comparison of antioxidant capacity and MMP-9 inhibitory capacity (IC_{50}) values for polyphenol-enriched fractions from strawberry tree fruit and leaves with the respective positive controls (blackberry and green tea). Antioxidant capacity was determined by ORAC method and expressed as mM TE. IC_{50} values (μg GAE. mL^{-1}) were obtained by OriginPro 6.1 software. The values presented are the mean value of triplicates ± SD. The values of each tissue were statistically compared with the value of respective control. nd: not significantly different for $p < 0.05$; *: significantly different for $p < 0.05$; ***: significantly different for $p < 0.001$

Tissue	Sample	Antioxidant capacity (mM TE)	MMP-9 inhibitory capacity (IC_{50}) (μg GAE. mL^{-1})
Fruits	Blackberry	118.75 ± 17.15	1.52 ± 0.16
	Strawberry tree	211.66 ± 22.82***	1.68 ± 0.38nd
Leaves	Green tea	135.95 ± 2.99	1.94 ± 0.39
	Strawberry tree	308.56 ± 26.74*	1.31 ± 0.17nd

3.3. HPLC-MS phenolic profile determination

To identify the main compounds present in fruits and leaves of strawberry tree, phenolic extracts were analysed by HPLC-ESI-PDA-MS. The compounds were tentatively identified in the bibliography by their PDA and MS fragmentation patterns [28, 29] (Fig. 2; Table 2). The fruits composition is relatively well-known compared to leaves [28, 29]. The fruits yielded mainly gallic acid derivatives (as glucosides, galloylquinic acid, galloylshikimic acid and gallotannins) but also some proanthocyanidins, quercetin derivatives and ellagic acid derivatives. The anthocyanins are not abundant in these fruits, but were identified in accordance with the bibliography as delphinidin-3-galactoside,

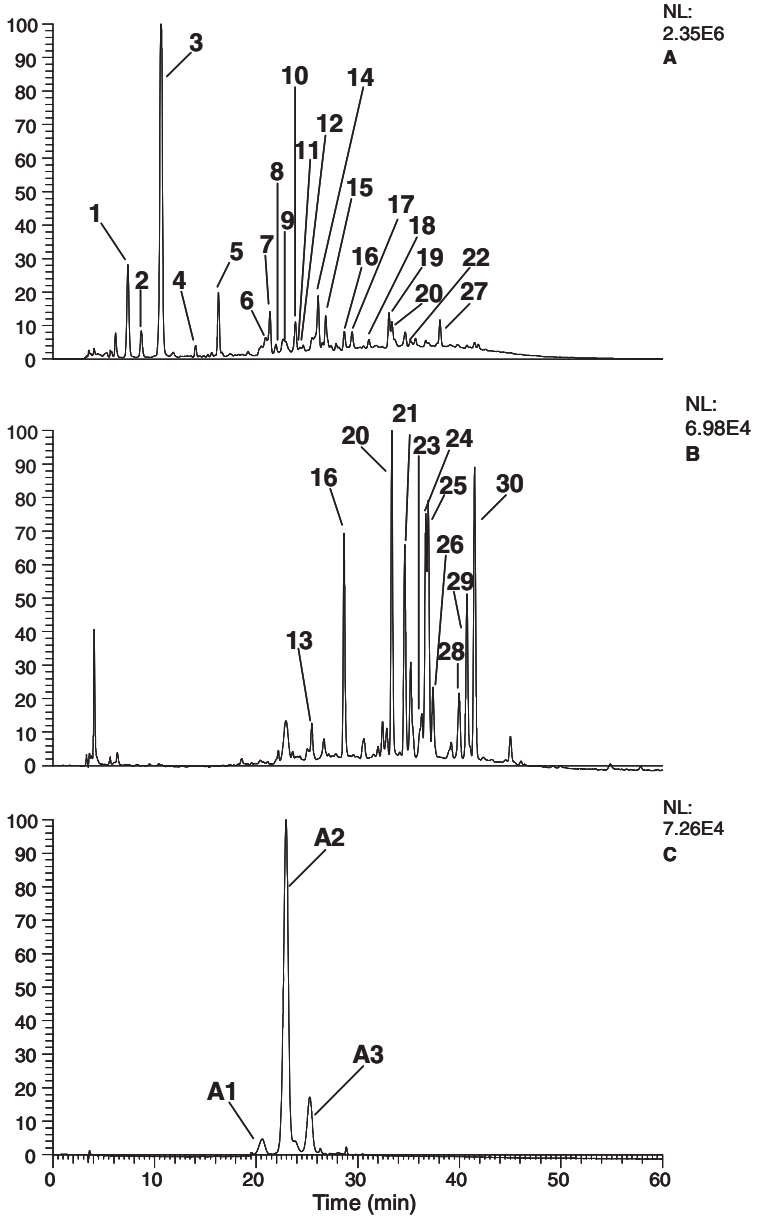

Fig. 2. HPLC phenolic profile assessed for *A. unedo* fruit extract. The chromatogram in (A) shows the absorbance of peaks at 280 nm, (B) peaks at 365 nm and (C) peaks at 560 nm. The peaks are numbered and assignments are given in Table 2 for phenols and Table 3 for anthocyanins.

Table 2
Peaks assignments, retention times and mass spectral data of phenols present in *A. unedo* fruits extract

Peak no.	RT	PDA	M/Z [M-H]	MS2	Putative identity
1	7.37	280	331.1	**271.0**, 211.1, **169.0**	Gallic acid glucoside
2	8.70	265	331.1	**169.0**, 125.0	Galloyl glucoside
3	10.64	270	343.0	191.2, 169.0	3-O- or 5-O-galloylquinic acid [29]
4	14.04	255–300	331.1	**169.1**	Gallic acid 4-O-B-D-glucopyranoside or B-D-Glucogalline [29]
5	16.29	255–300	325.0	169.0, 125.1	Galloyl shikimic acid
6	20.93	280	577.1	289.2	Proanthocyanidin dimer [29]
7	21.36	270–290	495.0, 465.0, 343.0, 191.2	**343.0**, 191.0	Digalloylquinic acid
8	21.95	295	495.0, 465.0, 343.0, 191.2	**343.0**, 191.0	Isomer of digalloylquinic acid
9	22.66	280	577.0, 423.2, 407.2, 289.2	425.0, 407.2, 289.2	Procyanidin dimer B2 [28]
10	23.86	280	**289.1**	261.0, 175.0	Catechin
11	24.31	320	865.1, 453.2, 325.1	577.1	Procyanidin trimer
12	24.6	285	541.1, 483.1, 467.3, 321.0, 301.2	453.1, 301.4, 169.2	Gallic acid derivative
13	25.50	280, 525	**477.0**, 325.1	325.0, 169.0	Digalloyl shikimic acid
14	26.10	275	**477.0**, 325.1	325.0, 169.0	Digalloyl shikimic acid
15	26.84	270	**633.1**, 463.1, 301.2, 275.2	463.0, 301.1	Strictinin ellagitannin
16	28.68	360	**463.2**, 301.3	301.2	Quercetin-3-glucoside
17	29.44	275	**1109.0**, 972.9, 647.0, 635.1, 588.1, 441.0, 301.3	783.1, 492.8	Gallotannin derivative
18	31.09	280	**366.2**, 186.0	**204.1**, 186.1, 142.0	Unknown
19	33.08	275	953.0	633.0, 463.2, 301.2	Tannin
20	33.36	360	433.1, 301.2	301.0	Quercetin-3-xyloside
21	34.67	260–355	615.2, 463.2, 433.1, 301.1	463.0, 301.1	Quercetin hexose galloyl derivative
22	35.21	260–355	615.2, 463.2, 433.1, 301.1	463.0, 301.1	Quercetin hexose galloyl derivative
23	36.30	260–355	615.2, 463.2, 433.1, 301.1	463.0, 301.1	Quercetin hexose galloyl derivative
24	36.69	255, 370	**301.2**	301.2, 257.2	Ellagic acid
25	36.96	345	463.1, 301.2	301.2	Quercetin 3-galactoside
26	37.40	275–355	**463.1**, 301.2	301.2	Quercetin 3-glucoside
27	38.09	280	939.1, 769.1, 729.0, 617.1, 544.2, 480.2, 469.2	769.0, 617.2	Gallotannin
28	39.81	355	**599.0**, 301.0	463.1, 301.1	Ellagic acid-hexose derivative
29	40.75	355	**433.0**, 301.1	301.1	Ellagic acid arabinoside/xyloside
30	41.49	355	**447.0**, 301.1	301.1	Ellagic acid rhamnoside

The most abundant ions are shown in bold. Numbers in brackets are references.

cyanidin-3-galactoside, cyanidin-3-glucoside and cyanidin-3-arabinoside [28, 29]. The leaf extracts also yielded gallic acid derivatives common to fruits, (epi)catechin, tannins, myricetin derivatives and kaempferol derivatives. Some of these phytocompounds, such as tannins, quercetin derivatives and catechin gallate have already been described as responsible for anti-hypertensive and anti-aggregant effects using strawberry tree leaf extracts [20, 26].

4. Conclusion

In this work the polyphenol-enriched fractions of *Arbutus unedo* fruits and leaves were characterized for antioxidant and MMP-9 inhibitory activity, and the results obtained suggest these tissues as promising sources of agents with pharmacological activities. Many naturally occurring agents have shown chemoprotective potential in a variety of

Table 3
Peaks assignments, retention times and mass spectral data of anthocyanins present in *A. unedo* fruits extract

Peak no.	RT	PDA	M/Z [M+H]	MS2	Pututative identity
A1	20.60	280, 525	**579.1**, 465.1, 303.2	303.2	Delphinidin-3-galactoside [28]
A2	22.94	280, 515	**449.0**, 287.2	287.2	Cyanidin 3-O-glucoside or cyanidin-3-galactoside [28]
A3	25.28	280, 515	**419.1**, 287.2	287.2	Cyanidin 3-O-arabinoside [28]

The most abundant ions are shown in bold. Numbers in brackets are references.

Table 4
Peaks assignments, retention times and mass spectral data of phenols present in *A. unedo* leaves extract

Peak No.	RT	PDA	M/Z [M-H]	MS2	Putative identity
1	9.57	280	**1111.2**, 899.3, 839.3, 817.3, 567.3, 317.0	**839.5**, 729.2, 676.1, 567.2, 270.9, 160.9, 109.2	Unknown
2	10.98	280	**331.2**, 271.1, 169.1	**271.0**, 211.1, 169.1	Gallic acid glucose derivative
3	12.80	260	**331.2**, 271.2, 169.1	271.1, 169.1, 125.1	B-D-Glucogalline or gallic acid 4-O-B-D-glucopyranoside
4	13.54	270	**363.2**, 169.2	169.1	B-D-Glucogalline or gallic acid 4-O-B-D-glucopyranoside derivative
5	14.80	275	**343.1**, 191.2	191.2, 169.1	Galloylquinic acid derivative
6	20.57	275	**325.2**, 169.1, 125.2	169.1, 125.2	Galloylshikimic acid
7	26.48	275	**745.1**, 577.2, 495.1, 343.1, 289.1	593.0, 575.1, 425.0, 407.2	Proanthocyanidin derivative
8	26.94	275	423.3, 313.2	423.2, **313.1**, 169.1	Galloyl derivative
9	27.92	280	**289.2**	245.2, 231.2, 205.2, 179.1	Catechin
10	30.82	270	**477.1**, 325.2, 169.2	325.1	Digalloylshikimic acid derivative
11	33.26	275	**951.1**, 933.1, 729.2, 477.1, 301.3, 273.2	914.9, 896.9, 765.0, 613.1, 461.1, 445.1, 301.3	Tannin
12	36.91	355	1105.0, 1085.0, 953.2, 935.2, 785.1, 633.2, 479.2, 316.2, 301.3	909.1, 801.0, 633.1, 463.2, 301.3	Tannin
13	38.57	280	629.0, 477.1, 325.2	325.1	Galloylshikimic acid derivative
14	41.26	360	**463.2**, 449.2, 316.2	316.2	Myricetin-3-rhamnose
15	43.04	280, 355	**939.2**, 769.2, 617.2, 469.3, 301.3, 169.1	787.1, **769.1**, 617.2, 601.2	Gallotannin
16	46.51	255, 350	447.2, 300.2	301.1	Quercetin 3-rhamnoside
17	50.25	350	583.2, **417.2**, 300.2, 284.2	417.2, 327.1, **284.1**, 255.3	Kaempferol derivative (Arabinoside/Xyloside)
18	51.17	265, 340	476.8, **431.2**, 285.2, 227.3	**285.2**	Kaempferol derivative (Rhamnoside)
19	38.03	355	**479.2**, 317.2	317.1	Myricetin-Hexoside
20	42.56	350	463.0, 300.1	301.1	Quercetin-Hexoside

The most abundant ions are shown in bold.

bioassay systems and animal models. Phenolic compounds are reported to have antioxidant, antimutagenic and anticarcinogenic activity and are expected to reduce the risk of disease and to bring health benefits with daily intake [11].

Gallic acid derivatives dominate the profile of both fruits and leaves of *Arbutus unedo*. Gallic acid is an intermediate component of plant metabolism and, together with its analogs, has been associated with a wide variety of biological actions, including: antioxidant, antifungal, antimalarial, and antiherpetic action [10, 14, 17]. However, the main interest in gallic acid and its derivatives is related to its antitumoral activity, showing selective cytotoxicity toward a variety of tumor cells, more cytotoxic for tumor cells than for non-tumor cells [21]. Nevertheless, in studies using

pure coumpounds, synergistic effects may be lost in determining anti-proliferative effects. In this study, the use of polyphenols-enriched fraction, devoid of ascorbic acid and carotenoids, allows interactions between polyphenols and the detected bioactivity could be directly associated to them and their interactions. Fruits and leaves polyphenols-enriched fractions presented IC_{50} values similar to the ones obtained for positive control tissues with recognized activity as anti-cancer and antioxidant agents. Therefore, it is important to confirm the potential of these enriched fractions as multitarget medicines with antiproliferative efficacy, using cell based assays such as proliferation and intracellular antioxidant tests.

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