

Antioxidant and antiproliferative properties of strawberry tree tissues

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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 fruit and leaf extracts enriched in polyphenols, but devoid of organic acids, vitamins, carotenoids and sugars, were prepared by solid phase extraction 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 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 [1]. 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 [2].

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 [1; 3]. In cancer, several studies point to the important role of the oxidative stress on the oncogenic stimulation. Various cancer cells have a cellular redox imbalance, i.e, a disruption in the balance 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 [4-6]. Free radical damage could influence DNA mutations and altered gene expression, lipid peroxidation due to malondialdehyde (MDA) and 4-hydroxynonenal (HNE) formation. [5; 7; 8], and protein oxidation [4]

Cancer cell invasion is a critical point for cancer metastasis and it is generally accepted that remodelling of the extracellular matrix (ECM) is required for cancer cell invasion [9] The primary response for the degradation of ECM components has been demonstrated to be the activation of zinc-dependent matrix metalloproteinases (MMPs) [10]. 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 [11]. 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 [12].

Strawberry tree (*Arbutus unedo* L.; *Ericaceae* family) is an evergreen shrub, a native Mediterranean species which is also cultivated in regions of Eastern Europe [13]. Its fruits are spherical, about 2 cm in diameter, dark red, and tasty only when fully ripe in autumn. *A. unedo* berries are rarely eaten as fresh fruits but are important in local agricultural communities who use them for the production of alcoholic beverages, jams, jellies and marmalades [14; 15]. 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 [16]. Recently, Carcache-Blanco and co-workers isolated ten compounds from *A. unedo* leaves exhibiting chemopreventive action through cyclooxygenase-2 inhibitory activity [17], which suggests that *A. unedo* could be a promising source of antiproliferative agents. Strawberry tree fruit has a high antioxidant capacity resulting from a range of antioxidants including phenolic compounds (e.g. anthocyanins, gallic acid derivatives, tannins and flavonoids), vitamin C, vitamin E and carotenoids [13-15; 18; 19].

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.

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 to what size? 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. Solid Phase Extraction

Hydroethanolic extracts were fractionated by Solid Phase Extraction (SPE) using a Giga tubes 2g/12mL, 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 vacuum and resuspended in 0.5% (v/v) glacial acetic acid in water, then they were applied to the columns and unbound material, which contained sugars, organic acids and 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 [20; 21].

2.4. Total phenolic measurement

Determination of total phenolic compounds was performed by the Folin-Ciocalteu method [22]. Briefly, to each well of a microplate, 235 μL water, 5 μL sample (or solvent in the control), 15 μL Folin-Ciocalteu'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 in 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 [23; 24]. 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 ($41.4 \text{ g}\cdot\text{L}^{-1}$). 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 1mM APMA in 100 mM Tris-HCl buffer (pH 7.5) containing 100 mM NaCl, 10 mM CaCl_2 , 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 by placing on 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 [25], 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 assays, tests using different concentrations of MMP-9 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 CaCl₂ 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 ($\lambda_{\text{emission}}=393$ nm, $\lambda_{\text{excitation}}=325$ 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 IC₅₀ values were determined.

2.7. HPLC-MS phenolic profile determination

Phenolic extracts were dried by rotary evaporation, resuspended in 5% (v/v) acetonitrile in water and was 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 x 150 mm, Phenomenex Ltd.) and eluted over a gradient of 95:5 solvent A:B at time=0 minutes to 60:40 A:B at time=60 minutes at a flow rate of 400 μ L/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 \pm SD. Differences among treatments were detected by analysis of variance [26] 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 in polyphenol-enriched fractions

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

After SPE fractionation, strawberry tree fruit and leaf extracts showed a decrease in phenolic content (18% and 62% respectively). These decreases observed are probably due to molecules detected by Folin-Ciocalteu method, such as vitamins or organic acids but that are eliminated by the SPE [27]. Indeed, components such niacin, ascorbic acid, β -carotene, L-malic and quinic acids (detected and quantified in strawberry tree fruits by Alarcão-E-Silva et al [14] or Ayaz et al [13]) 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 leaf extracts had an antioxidant capacity recovery of approximately 100%.

The IC_{50} values for MMP-9 inhibition for both fractions diminished 50%, which means that 50% of the amount of polyphenols was 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 phenolic compounds which diminished the IC_{50} value for MMP inhibition.

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 and were similar to the values obtained for blackberry and green tea extracts (1.68 ± 0.38 $\mu\text{g GAE. mL}^{-1}$

for fruits and $1.31 \pm 0.17 \mu\text{g GAE. mL}^{-1}$ for leaves) (Table 1). Since blackberry and green tea are recognized in the literature as highly effective antioxidants and anti-cancer agents [28-34], our results suggest that strawberry tree tissues are promising sources of effective antiproliferative extracts. To confirm these *in vitro* results, cell based assays should be performed as antiproliferative and intracellular antioxidant tests.

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 [15; 35] (Fig. 2; Table 2). The composition of fruits is relatively well-known compared to leaves [15; 35]. 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, cyanidin-3-galactoside, cyanidin-3-glucoside and cyanidin-3-arabinoside [15; 35]. The leaf extracts also yielded gallic acid derivatives but also contained (epi)catechin, tannins, myricetin derivatives and kaempferol derivatives. Some of these phytochemicals, such as tannins, quercetin derivatives and catechin gallate have already been reported as being responsible for anti-hypertensive and anti-aggregant effects using strawberry tree leaf extracts [36; 37].

4. Conclusion

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 [38]. Many naturally-occurring agents have shown chemoprotective potential in a variety of bioassay systems and animal models. In this work, polyphenol-enriched fractions of *Arbutus unedo* fruits and leaves were found to have high antioxidant and MMP-9 inhibitory activity which suggests that these tissues as promising sources of agents with pharmacological activities.

Gallic acid derivatives dominate the profile of both fruits and leaves of *Arbutus unedo*. Gallic acid, together with its derivatives, has been associated with a wide variety of biological actions, including: antioxidant, anti-fungal, anti-malarial, and anti-herpetic action [39-41]. However, the main interest in gallic acid and its derivatives has been related to its anti-tumoral activity, as it showed selective cytotoxicity toward a variety of tumor cells, being significantly more cytotoxic for tumor cells than for non-tumor cells [42]. Nevertheless, in studies using pure compounds, synergistic effects may be lost in determining anti-proliferative effects. Indeed, previous work found chemopreventive activity of pure compounds identified in *Arbutus unedo* leaves (17) However, the relatively high IC₅₀ values noted may have been influenced by the lack of synergistic positive effects between different polyphenols found in total tissue leaf extracts.

In this study, the use of polyphenol-enriched fractions, devoid of ascorbic acid and carotenoids, has demonstrated that polyphenols are responsible for the high antioxidant capacity and MMP-9 inhibitory activities of the extracts. Polyphenol-enriched fractions from fruits and leaves gave antioxidant capacity and IC₅₀ values for MMP-9 inhibition similar to those obtained for blackberry and green tea, samples with recognized high

antioxidant capacity and activity as anti-cancer agents. However, it is important to confirm the multi-target potential of these enriched fractions using cell-based assays such as proliferation and intracellular antioxidant tests.

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Table 1– Comparison of antioxidant capacity and MMP-9 inhibitory capacity (IC₅₀) 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. IC₅₀ 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. ns: 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 (IC₅₀) (µg GAE. mL⁻¹)
Fruits	Blackberry	118.75±17.15	1.52 ±0.16
	Strawberry tree	211.66±22.82***	1.68 ±0.38 nd
Leaves	Green tea	135.95±2.99	1.94 ±0.39
	Strawberry tree	308.56±26.74*	1.31 ±0.17 nd

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 [18]
4	14.04	255-300	331.1	169.1	Gallic acid 4-O-B-D-glucopyranoside or B-D-Glucog
5	16.29	255-300	325.0	169.0, 125.1	Galloyl shikimic acid
6	20.93	280	577.1	289.2	Proanthocyanidin dimer [18]
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 [15]
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.

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	Putative identity
A1	20.60	280, 525	579.1 , 465.1, 303.2	303.2	Delphinidin-3-galactoside [15]
A2	22.94	280, 515	49.0 , 287.2	287.2	Cyanidin 3-O-glucoside or cyanidin-3-galactoside [15]
A3	25.28	280, 515	419.1 , 287.2	287.2	Cyanidin 3-O-arabinoside [15]

The most abundant ions are shown in bold.

49.0 should be 449.0

We should also denote that figures in brackets within Tables 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 d
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/
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.

Figure 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), ▨- IC₅₀ of MMP-9 inhibitory capacity (mg GAE. mL⁻¹) and £ - antioxidant activity (x10 mmol TE) for peroxy radical were determined for crude extracts and polyphenols enriched fractions obtained from *A. unedo* fruits and leaves. Vertical bars represent ± SD.

Figure 2 – HPLC phenolic profile accessed 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.

Figure 1

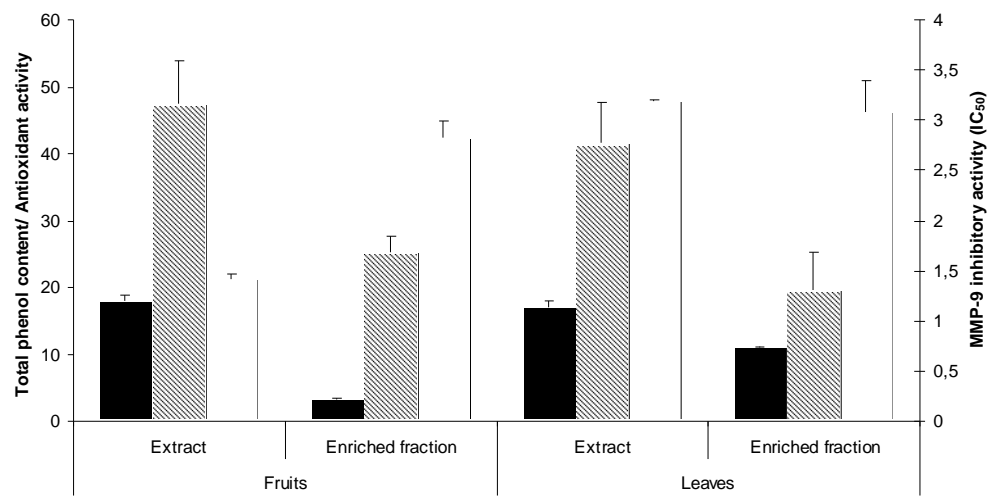


Figure 2

