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ORIGINAL ARTICLE

Wild strawberry (*Arbutus unedo*): Phytochemical screening and antioxidant properties of fruits collected in northern Morocco

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Abstract The aim of this study was to determine the polyphenolic compounds and the antioxidant ability of *Arbutus unedo* fruits, collected from three regions of northern Morocco, using high-performance liquid chromatography coupled to diode array and electrospray ionization mass spectrometry detection. The proper extraction method has been selected to achieve this objective. After delipidation, the three harvests were extracted by sonication using two solvents with increased

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DPPH;
HPLC-MS

polarity ethyl acetate and MeOH:water, 80:20 (*v*/*v*). Total polyphenols, flavonoids, tannins and anthocyanins were respectively: 108.41 ± 9.29 mg GAE/g (*w*/*w*) dry weight (DW), 101.07 ± 5.6 mg QE/g (*w*/*w*) (DW), 0.45 ± 0.48 mg EC/g (*w*/*w*) (DW) and 0.35 ± 0.48 mg Pg-3-glu/g (*w*/*w*) (DW). EC50 values for reducing power and DPPH radical scavenging activities were between 1.37 ± 0.2 and 17.82 ± 0.12 µg/mL (*w*/*v*). A total of 75 compounds were tentatively identified and some of these had never been found until nowadays in *Arbutus unedo*. The average amount of antioxidant compounds obtained by semi-quantitative analyses was 120.35 ± 32.05 mg/100 g (*w*/*w*) (DW). The attained results clearly highlight the potential of *A. unedo* as a source of healthy compounds, which could be advantageously added to the daily diet, making it a potential candidate for the cure for many emerging diseases.

1. Introduction

The human body is exposed to several diseases, mostly due to malnutrition and this appears to be related to reactive oxygen species (ROS). The integration of natural substances with antioxidant activity in our diet is considered as the main solution to reduce the manifestation of many health problems. For this reason, an ever-increased interest is now poured on not-yet explored plants characterized by bioactive molecules with potential beneficial effects.

A. unedo L. (Ericaceae family) is a wild Mediterranean species, commonly known as a strawberry tree. It grows in the Mediterranean forest including the Moroccan ones, due to its tolerance to dryness and its ability to regenerate and recolonize forest fire. This species can also withstand hot summers and mild rainy winters (Celikel et al., 2008).

This fruit is not widely consumed by the Moroccan population and its consumption remains seasonal also because of the lack of knowledge concerning its potential benefits. However, in other Mediterranean regions, *A.unedo L.* is already known as a good source of sugars, organic acids, antioxidants including phenolic compounds, vitamins C and E, and carotenoids (Alarcao-E-Silva et al., 2001; Ayaz et al., 2000; Fortalezas et al., 2010; Tavares et al., 2010; Pallauf et al., 2008; Pawlowska et al., 2006). Many studies showed that the extract of this species does possess vasorelaxant, diuretic, natriuretic, antihyperglycemic, astringent, urinary antiseptic, antidiarrheal properties, and more recently, it was used in the therapy of hypertension and inflammation without displaying any acute toxic effect (Bnouham et al., 2010; Mariotto et al., 2008; El Amine Dib et al., 2013; Ziyyat et al., 2002; Legssyer et al., 2004; Mekhfi et al., 2006). Based on its high yield of antioxidants compounds *A. unedo* could be used as a source of health-promoting compounds for both the food industry or the pharmaceutical and chemical sectors.

Therefore, it is challenging to ameliorate the knowledge about the nutritional and production of the strawberry-tree fostering the consumption of this fruit for food products such as yogurts, pie and pastry fillings, cereal or meat products as recently described (Alarcao-E-Silva et al., 2001; Ganhão et al., 2010).

However, there are only a few studies on the chemical composition of *A. unedo* fruits from Morocco. These studies have a limited identification and quantification of the occurring compounds. Recently, a study was focused on increasing the extraction process from strawberry-tree fruits with the identification of 30 phenolics compounds including 8 anthocyanins (Alexandre et al. 2020). It is worth mentioning that the variation of ecophysiological factors may have an influence on the nutritional parameters of fruits (Fang et al., 2008; Reddy and Sathyanarayana, 2017). In particular, climatic differences may affect production rates and nutritional composition (harvest in late autumn and flowering in autumn of the preceding year). However, no data have been found about the geographical impact on the variation of antioxidants compound content in *A. unedo*.

Thus, the aim of this work was first to a) obtain the highest yield in terms of polyphenolic compounds and evaluate their antioxidant properties using fractionation by two solvents with increasing polarity b) determination of the phytochemical content *A. unedo* fruits, influenced by the geographical variations of the year 2017 in Morocco, by high-performance liquid chromatography coupled to diode array and electrospray ionization mass spectrometry detection (HPLC-DAD-ESI/MS) in order to evaluate their potential value.

2. Materials and methods

2.1. Samples

The fruits of *Arbutus unedo L.* were collected fully ripened in October 2017 from three different forests: Achakar (AA), Qsar Kbir (AQ), and Chaoun-Qalaa (AC) (northern region of Morocco). Mature berries with seeds were freeze-dried, ground, and stored at –20 °C prior to extraction.

2.2. Chemicals

2,20-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azobis (2-amidinopropane), gallic acid dihydrochloride (AAPH), L-ascorbic acid, trichloroacetic acid (TCA), 1,1,3,3- tetraethoxypropane (TEP), thiobarbituric acid (TBA), and butylated hydroxytoluene (BHT) were purchased from Sigma (St. Lois, MO). Folin-Ciocalteu phenol reagent was obtained from Fluka. Standards (gallic acid, caffeic acid, rutin, catechin, coumaric acid, kaempferol, isorhamnetin, cinnamic acid, apigenin, and vanillic acid) were obtained from Merck Life Science (Merck KGaA, Darmstadt, Germany). LC-MS grade methanol, acetonitrile, acetic acid, acetone, and water were purchased from Merck Life Science (Merck KGaA, Darmstadt, Germany). All other chemicals were of analytical grade and obtained from Sigma (St. Louis, MO).

2.3. Extraction

5 g of lyophilized powder was defatted three times with 50 mL of n-hexane, dried, and were homogenized with 50 mL of two solvents with increased polarity (EtOAc and MeOH:water, 80:20 *v*/*v*). Each fraction was extracted by sonication in an ultrasound bath (130 kHz) for 45 min. After centrifugation at 5000 g for 5 min, the supernatant filtered through a paper filter, dried, reconstituted with MeOH:water, 80:20 (*v*/*v*) and then filter through 0.45 μm Acrodisc nylon membrane (Merck Life Science, Merck KGaA, Darmstadt, Germany) prior to HPLC-DAD-ESI/MS analysis. The resulting extracts were stored at 4 °C until use.

2.4. Phytochemical screening

Phytochemical screening was made according to the method of Trease and Evans (1989) in order to detect the presence of starch, saponins, flavonoids, tannins, catechic tannins, gallic tannins, anthocyanins, and alkaloids. The tests were based on visual observation of the color change or the formation of a precipitate after the addition of specific reagents.

2.5. Determination of total polyphenols

Total polyphenols content was determined with the Folin-Ciocalteu reagent by spectrophotometry according to the method of Singleton and Rossi. (1965) with some modifications. Gallic acid was used as standard (10, 25, 50, 100, 200 ppm). The total phenolic content was measured at 755 nm and was expressed in mg of gallic acid (GAE) / g dry weight (DW).

2.6. Determination of the total flavonoids

Quantification of flavonoids was made by the method of Zhishen et al., (1999), using $AlCl_3$ to 10% (*w*/*v*), NaOH 4% and $NaNO_2$ to 5%. The absorbance was determined at 510 nm. A curve of catechin (12.5, 25, 50, 100, 200 ppm) was also carried out. The total flavonoids content was expressed in mg of quercetin (QE)/g dry weight (DW).

2.7. Determination of anthocyanins

Determination of the total anthocyanin content was based on the differential pH (pH = 1 and pH = 4.5) by the method of Giusti and Wrolstad, (2001) with some modifications. Measurement was conducted at 510–700 nm in the UV–Vis spectrophotometer. The absorbance was calculated by the following formula:

$$A = [(A_{510} - A_{700})\ \text{to pH 1.0}] - [(A_{510} - A_{700})\ \text{at pH 4.5}]$$

The total anthocyanin content was calculated by the molecular weight of pelargonidin-3-glucoside by the following formula:

$$[\text{mgPg} - 3 - \text{glu/gMS}] = \frac{A * M * F * V * 1000}{\varepsilon * d * Q}$$

2.8. Determination of tannins

The content of condensed tannins has been determined by the vanillin method described by Julkunen-Tiitto (1985). A volume of 50 μL of each extract was added to 1500 μL of the 4% vanillin (*w*/*v*) in MeOH: water, 80:20 (*v*/*v*) then mix vigorously. Immediately after that 750 μL of hydrochloric acid concentrated (HCl) was added. The absorbance of the resulting mixture was measured at 550 nm after being allowed to react for 20 min at room temperature. The results were plotted after a (+)-catechin standard made in the same manner.

2.9. Determination of antioxidant activity

DPPH radical scavenging activity of entire fruit extracts was measured according to the method described by Braca et al., (2002) with a few modifications. Briefly, different concentration of extracts (25 μL) were added to 2 mL of 6.25–$10-5$ M DPPH MeOH:water, 80:20 (*v*/*v*) solution. After gentle mixing and 30 min of standing at room temperature, the absorbance of the resulting solutions was measured at 517 nm. The antiradical activity is estimated according to this equation: % of antiradical activity = [(Abs control –Abs sample) / Abs control] × 100. IC50 values of the extract i.e., the concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

2.10. HPLC-DAD-ESI/MS

LC analyses were performed on a Nexera-e liquid chromatograph (Shimadzu, Kyoto, Japan), consisting of a CBM-20A controller, two LC-30AD dual-plunger parallel-flow pumps, a DGU-20A5R degasser, a CTO-20AC column oven, a SIL-30AC autosampler, an SPD-M30A photodiode array detector, and an LCMS-8050 triple quadrupole mass spectrometer, through an ESI source (Shimadzu, Kyoto, Japan), operating in both negative and positive ionization modes.

As chromatographic column, an Ascentis Express RP C18 column (150 × 4.6 mm; 2.7 μm) (Merck Life Science, Merck KGaA, Darmstadt, Germany) was employed. The mobile phase was composed of water/acetic acid (99.85/0.15 *v*/*v*, solvent A) and acetonitrile/acetic acid (99.85/0.15 *v*/*v*, solvent B), The flow rate was set at 1 mL/min under gradient elution: 0–5 min, 5% B, 5–15 min, 10% B, 15–30 min, 20% B, 30–60 min, 50% B, 60 min, 100% B. DAD detection was applied in the range of λ = 200–400 nm and two different wavelengths were monitored at λ = 280 nm and λ = 350 nm (sampling frequency: 40.0 Hz, time constant: 0.08 s). MS conditions were as follows: scan range and the scan speed were set at *m*/*z* 100–800 and 2500 u/sec, respectively, event time: 0.3 sec, nebulizing gas (N_2) flow rate: 1.5 L/min, drying gas (N_2) flow rate: 15 L/min, interface temperature: 350 °C, heat block temperature: 300 °C, DL (desolvation line) temperature: 300 °C, DL voltage: 1 V, interface voltage: −4.5 kV. Calibration curves ($R^2 \geq 0.997$) of eleven polyphenolic standards used for the semi-quantification in sample extracts were obtained using triplicate injections with different concentrations in the range of (1, 5, 10, 50, 100 ppm), and according to the area of peaks acquired at following wavelengths of 270 nm, 277 nm, 278 nm, 280 nm,

Table 1 Phytochemicals detected in the extracts of *A. unedo* fruits from three different regions.

Regions		AA		AC		AQ		All
Compounds groups/ solvent of extraction		EtOAc	MeOH: water, 80:20 (*v*/*v*)	EtOAc	MeOH: water, 80:20 (*v*/*v*)	EtOAc	MeOH: water 80:20 (*v*/*v*)	H_2O
Alcaloids		–	–	–	–	–	–	–
Polyphenols	Flavonoids	B	D + +	A +	D + +	A +	C + +	D + +
	Tannins	+	+	+	+	+	+	+
	Anthocyanins	+	+	+	+	+	+	±
	Catechic tannins	+	–	+	–	+	–	+
	Gallic tannins	+	–	+	–	+	–	+
	Coumarins	+	–	+	–	+	–	–
	Quinones	–	–	–	+	–	+	+
	Anthraquinones	+	+	+	+	+	+	+ +
Steroids	Saponosides	–	–	–	–	–	–	–
	Insaturated sterols/Terpenes	–	±	–	±	–	±	±
	Sterols Steroides	+ +	+ +	+ +	+ +	+ +	+ +	+
Sugars	Deoxy sugars	+	+	+	+	+	+	+
	Glycosides	+	+	+	+	+	+	+
	Mucilage	–	–	–	–	–	+	+

A: Flavones; B: Isoflavones; C: Flavonols; D: Flavonones + +: Abundant; + Presence of metabolite; ± trace; – Absence of metabolite.

Table 2 Extraction yields, total phenolic, flavonoids, anthocyanins, tannins contents, and antioxidant activity EC50 values of the wild fruits. The results are expressed as means ± SD (n = 3). In each column different letters mean significant differences ($p < 0.05$).

	N%		Total Phenolic content (mg GAE/g) (*w*/*w*) (DW)		Flavonoid content (mg QE/g) (*w*/*w*) (DW)		Anthocyanines content (mg Pg-3-glu)/g (*w*/*w*) (DW)		Tannin content (mg EC/g) (*w*/*w*) (DW)		DPPH scavenging activity EC50 (µg/mL) (*w*/*v*)	
Sample	EtOAc	MeOH: water, 80:20 (*v*/*v*)	EtOAc	MeOH: water, 80:20 (*v*/*v*)	EtOAc	MeOH: water, 80:20 (*v*/*v*)	EtOAc	MeOH: water, 80:20 (*v*/*v*)	EtOAc	MeOH: water, 80:20 (*v*/*v*)	EtOAc	MeOH: water, 80:20 (*v*/*v*)
AA	$1.28^{a} \pm 0.21$	$51.11^{c} \pm 6.61$	$34.8^{c} \pm 0.3$	$73.62^{b} \pm 3.28$	$34.08^{c} \pm 1.24$	$66.99^{a} \pm 1.55$	$0.25^{b} \pm 0.01$	$0.1^{b} \pm 0.012$	$0.4^{a} \pm 0.004$	$0.82^{a} \pm 0.05$	$17.82^{a} \pm 0.12$	$1.64^{a} \pm 0.14$
AC	$1.1^{a} \pm 0.23$	$67.23^{a} \pm 6.43$	$51.61^{a} \pm 0.98$	$75.88^{a} \pm 3.1$	$41.51^{a} \pm 0.04$	$64.4^{b} \pm 0.08$	$0.13^{c} \pm 0.006$	$0.14^{a} \pm 0.015$	$0.14^{c} \pm 0.005$	$0.5^{b} \pm 0.04$	$11.42^{b} \pm 0.13$	$1.37^{c} \pm 0.2$
AQ	$0.71^{b} \pm 0.18$	$59.17^{b} \pm 6.58$	$45.84^{b} \pm 0.61$	$61.37^{c} \pm 0.43$	$37.43^{b} \pm 0.22$	$54.9^{c} \pm 2.8$	$1.42^{a} \pm 0.09$	$0.06^{c} \pm 0.002$	$0.21^{d} \pm 0.007$	$0.9^{a} \pm 0.1$	$11.52^{b} \pm 0.09$	$1.51^{b} \pm 0.1$

321 nm, 330 nm, 336 nm, 365 nm, 370 nm, 355 nm. All protocatechuic acid, gallic acid, ellagic acid, methylellagic acid, ellagitannins, and their derivatives were quantified as gallic acid. Caffeoylquinic acid, caffeic acid and its derivatives, feruloylquinic acid, and syringic acid were quantified as caffeic acid while cinnamic acid derivatives and p-coumaroylquinic acid were quantified as cinnamic acid. Dihydroxyflavone and apigenin derivatives were quantified as apigenin. Epigallocatechin, catechin, and procyanidin were quantified as catechin. Myricetin and kaempferol derivatives were quantified as kaempferol while all quercetin derivatives were quantified as rutin. For the rest of compounds, calibration curves of vanillic acid, coumaric acid and isorhamnetin, were employed. (Mosele et al., (2016).

2.11. Statistical analysis

For each one of the samples, three replicates were analyzed. The results of these assays are expressed as mean values and standard deviation (SD). Differences among treatments were detected by analysis of variance ANOVA ($P < 0.05$).

3. Results and discussions

3.1. Phytochemical screening

To obtain a general vision of the existing compounds occurring in the extracts, general phytochemical screening of all extracts was carried out. The employed colorimetric method showed the presence of flavonoids, tannins, anthocyanins, anthraquinones, sterols, steroids, deoxysugars, and glycosides, while alkaloids, and saponosides were not detected (Table 1). This finding, except for the presence of alkaloids, was in agreement with a previous work (Dib et al., 2013).

3.2. Polyphenolic content

The determination of the content of phenolic acids, flavonoids, carotenoids, vitamins, and minerals occurring in fruits and vegetables is mandatory for the evaluation of their health-promoting properties and immunity-boosting effects. Table 2 shows the extraction yields, total phenolic, flavonoid, anthocyanins, tannin contents, and antioxidant activity EC50 values of the wild fruits. The higher extract yields for total phenolic, flavonoids, and tannins were attained for the MeOH:water, 80:20 (*v*/*v*) extract with respect to EtOAc. On the other hand, the highest concentration of anthocyanins contents was observed for the EtOAc extract. The average of the total contents of phenolic acid, flavonoids, anthocyanins and tannins were respectively 108.41 ± 9.29 mg GAE/g (*w*/*w*) (DW), 101.07 ± 5.6 mg QE/g (*w*/*w*) (DW), 0.35 ± 0.48 mg Pg-3-glu/g (*w*/*w*) (DW) and 0.45 ± 0.48 mg EC/g (*w*/*w*) (DW). The results concerning the polyphenolic contents are 5-fold higher than another study which found it between 9.51 and 19.73 mg GAE/g (*w*/*w*) (DW) (Ruiz-Rodríguez et al., 2011). Also, this concentration is higher than those obtained in the fruits collected from Portugal (16.7 ± 0.4 mg GAE/g (*w*/*w*) (DW); (Mendes et al., 2011). The fruit extract from Chaoun-Qalaa (AC) presented the highest yield (68.33%) and the highest quantity of polyphenols and flavonoids (127.5 ± 7.14 mg GAE/g (*w*/*w*) (DW) and 105.9 ± 3.2 mg QE /g (*w*/*w*) (DW)). It was higher than the ones found by Barros et al., 2010 (126.83 ± 6.66 mg GAE/g (*w*/*w*) (DW) and 34.99 ± 1.55 mg CE/g (*w*/*w*) (DW)).

The highest quantity of total anthocyanins and tannins content was obtained from AQ fruit extract by EtOAc (1.42 ± 0.09 mg Pg-3-glu/g (*w*/*w*) (DW) and 0.9 ± 0.1 mg EC/g (*w*/*w*) (DW) respectively). In another study 0.76 ± 9.85 mg cy-3-glu/g (*w*/*w*) (DW) was found as the total amount of anthocyanin (Fortalezas et al., 2010). All extract obtained from EtOAc and MeOH:water, 80:20 (*v*/*v*) showed a significant amount of phenolic compounds. Another research realized on aqueous methanol extracts (methanol:water, 80:20 *v*/*v*) using sonication exposed an amont of 34.3 ± 1.9 mg GAE/g and 2.1 ± 0.1 mg RE/g respectively for total phenolic acids and total flavonoids (Asma et al., 2019).

In general, these differences could be due to environmental characteristics, the period of harvesting, cultivar variability, or fruit maturity (Ancos et al., 2000). For testing the antiradical activity of a large variety of the existing compounds in all extracts, the scavenging activity on DPPH was used as screening method. The results presented in Fig. 1 show that all *A. unedo* samples do have antiradical ability (lowest EC50 values). This efficiency was 10-fold higher in samples extracted by MeOH:water, 80:20 (*v*/*v*) than other extracted by EtOAc. AC fruits presented the highest phenolic content which is compatible to its lower EC50 (11.42 ± 0.13 and 1.37 ± 0.2 µg/mL (*w*/*v*) for MeOH:water, 80:20 (*v*/*v*) and EtOAc extracts). The value of EC50 indicates that the investigated sample extracts

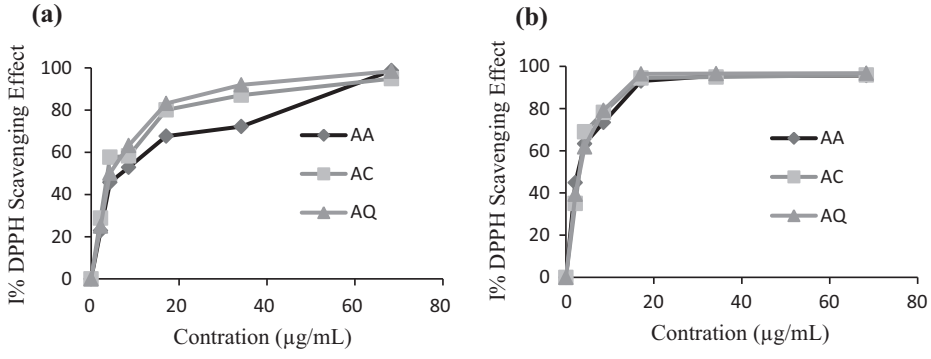

Fig. 1 Scavenging activity on DPPH radicals of EtOAc (a) and MeOH:water, 80:20 (*v*/*v*) (b) extracts of *A. unedo* fruits from three different regions.

Table 3 Compounds detected in the ethyl acetate extract of *A. unedo* from three regions by HPLC-DAD-ESI/MS.

Peak	Tentative Identification	t_R (min)	Identification type	λ_{MAX} (nm)	$[M-H]^-$	Major fragments	A. A	A. C	A. Q
Organic acid									
2	Citric acid	1.83	(Ayaz et al., 2000) DAD/MS	254	191	–	–	+	+
5	Malic acid	2.27	(Ayaz et al., 2000) DAD/MS	206	133	–	+	+	+
44	Malic acid derivative	2.45	DAD/MS	–	133	–	+	–	–
Phenolic acid and derivatives									
1	Protocatechuic acid	9.71	(Ayaz et al., 2000; Mosele et al., 2016) DAD/MS	295	153	–	–	–	
3a	Quinic acid derivative	1.65	DAD/MS	–	383	191	+	–	–
3b	Quinic acid derivative	1.77	DAD/MS	–	287	191	–	–	+
6	Cinnamic acid derivative	3.67	DAD/MS	277	267	147	+	+	+
7	Galloylquinic acid	5.59	(Mendes et al., 2011; Tavares et al., 2010) DAD/MS	208–273	343	169,191	+	+	+
8a	Ellagitannin N.I	6.79	DAD/MS	273	687	343	–	–	+
9	Gallic acid glucoside	8.63	(Mendes et al., 2011; Tavares et al., 2010) DAD/MS	255–294	331	169,191	+	+	+
11	Galloyl shikimic acid	10.69	(Mendes et al., 2011; Tavares et al., 2010) DAD/MS	259–272	325	169	+	+	+
12	Ellagic acid glucoside	10.61	(Mendes et al., 2011) DAD/MS	273–275	463	303+	–	–	+
13a	Caffeic acid derivative	1.58	DAD/MS	276–321	357	179	–	–	+
13b	Caffeic acid derivative	11.10	DAD/MS	280–350	359	179	–	+	–
15	Protocatechuic acid glucoside	12.65	DAD/MS	270–357	315	153	+	–	+
19	Methylellagic acid rhamnoside	15.98	(Pallauf et al., 2008) DAD/MS	278	477	303+	–	–	+
20	Methylellagic acid rhamnopyranoside	16.60	DAD/MS	278	461	315	–	–	+
21	Digalloyl shikimic acid	19.75	(Mendes et al., 2011; Mosele et al., 2016) DAD/MS	272–275	477	325	+	+	+
24	Digalloyl glucose	20.07	DAD/MS	274	483	325,191	–	–	+
25	Strictinin ellagitannin	20.50	(Mendes et al., 2011; Tavares et al., 2010) DAD/MS	280	633	–	–	–	+
27	Gallic acid gallate	22.15	DAD/MS	281	321	169	–	–	+
29	Methylellagic acid derivative	25.67	DAD/MS	272	477	301	–	–	+
30	Galloyl derivatives	25.95	DAD/MS	267	357	169	–	–	+
32	Caffeoylquinic acid	26.56	(Guimarães et al., 2013) DAD/MS	282–364	353	191	–	–	+
40	Ellagic acid arabinoside/ xyloside	31.08	(Mosele et al., 2016; Tavares et al., 2010) DAD/MS	265–347	599	–	–	–	+
45	Syringic acid	21.69	DAD/MS	270	197	–	+	–	–
49	Feruloylquinic acid	1.63	(Guimarães et al., 2013) DAD/MS	280–350	367	193,191	–	+	–
50	Quinic acid	2.09	DAD/MS	242	191	–	–	+	–
51	Cinnamic acid derivative	4.24	DAD/MS	277	343	267,147	–	+	–
52	Galloyl hexoside	6.83	DAD/MS	275	331	169, 191	–	+	–
53	4-hydroxycoumarin	9.77	DAD/MS	275–350	161	–	–	+	–
54	Galloyl derivative	25.95	(Mosele et al., 2016) DAD/MS	273	463	327, 169	–	+	–
Flavone									
4	Dihydroxyflavone	2.36		287	253	–	–	–	+
Flavan-3-ols									
10	Epigallocatechin	8.99	(Mosele et al., 2016) DAD/MS	270	325	305	–	–	+
14	Procyanidin dimer type B	11.58	(Mendes et al., 2011) DAD/MS	274	577	289	+	+	+
16	(-)-Catechin	13.69	(Mosele et al., 2016) DAD/MS	275–285	289	–	+	–	+
17	(+)-Catechin	14.80	(Mosele et al., 2016) DAD/MS	275–285	291+	289	+		+
18	(+)-Epicatechin	15.30	(Mosele et al., 2016) DAD/MS	270	289	–	+	+	+
22	(-)-Epicatechin gallate	18.62	(Mosele et al., 2016) DAD/MS	278	441	289	–	+	+
28	Catechin glucose	23.26	DAD/MS	276	451	289	–	–	+
34	Prodelphinidin dimer	27.43	(Mendes et al., 2011) DAD/MS	265–360	593	441, 289	–	–	+
43	Procyanidin gallate	36.97	(Mendes et al., 2011) DAD/MS	278–320	729	503	–	–	+
Flavonols									
23	Isorhamnetin glucoside	19.41	DAD/MS	278–385	477	315	–	–	+
26	Myricetin	21.04	DAD/MS	267–356	317	–	–	–	+
31	Quercetin galloyl hexoside derivative	26.41	(Mendes et al., 2011; Guimarães et al., 2013) DAD/MS	264–354	615	433	+	+	+

Table 3 (*continued*)

Peak	Tentative Identification	t_R (min)	Identification type	λ_{MAX} (nm)	$[M-H]^-$	Major fragments	A. A	A. C	A. Q
33	Quercetin pentoside	31.55	(Guimarães et al., 2013) DAD/MS	253–361	433	301	+	+	+
35	Quercetin glucoside	28.37	(Mendes et al., 2011; Guimarães et al., 2013) DAD/MS	353–354	463	301	+	+	+
36	Quercetin arabinose/ xyloside	27.88	(Mendes et al., 2011) DAD/MS	252–347	433	301	–	+	+
37	Quercetin rhamnoside-glucoside	28.26	(Mendes et al., 2011) DAD/MS	252–353	609	463, 301	–	–	+
38	Quercetin hexoside	31.50	(Guimarães et al., 2013) DAD/MS	254–351	433	303 +	–	–	+
39	Kaempferol galloyl glucoside	30.84	(Mendes et al., 2011) DAD/MS	276	599	447, 285	–	–	+
41	Quercetin rhamnoside	32.05	(Guimarães et al., 2013) DAD/MS	255–347	447	303 +	+	+	+
42	Kaempferol xyloside	34.68	(Mendes et al., 2011; Guimarães et al., 2013) DAD/MS	264–350	417	285	+	+	+
45	Apigenin pentoside	20.14	(Guimarães et al., 2013) DAD/MS	282–366	401	–	+	+	–
47	Quercetin hexose protocatechuic acid	29.91	DAD/MS	350	551	301	+	–	–
48	Dihydroquercetin rhamnoside	27.74	DAD/MS	349	565	301	+	+	–
Others									
Q	Ascorbic acid derivative	21.32	DAD/MS	210–279	366	175	+	+	+
Z	Benzyl alcohol hexose pentose	19.18	DAD/MS	203–282	401	–	–	–	+
P	Hydroxyphenylvaleric acid	10.97	(Mosele et al., 2016)	207	193		+	–	–
Non-identified									
N.1	Unknown	11.20	–	208–260	357	–	+	–	–
N.2	Unknown	24.89	–	207–275	581	–	+	–	+
N.3	Unknown	24.52	–	241–283	387	–	–	–	+
N.4	Unknown	24.73	–	271	581	319	–	–	+
N.5	Unknown	36.52	–	264–363	263	–	–	–	+

show significantly higher antioxidant capacity. The results are superior than the ones obtained in other studies (EC50 447.92 ± 0.81, Barros et al., 2010 and 790 ± 0.016 μg/mL (*w*/*v*), Mendes et al., 2011).

3.3. Phytochemical profile by HPLC-DAD-ESI/MS

Fruits of strawberry tree were partitioned with two different solvents in increasing order of polarity with the aim to identify the maximum number of compounds with different solubility. Concerning the qualitative composition of *A. unedo* fruits, all extracts were analyzed by - HPLC-DAD-ESI/MS (Tables 3 and 4) and a large variety of compounds were tentatively identified based on their retention times, MS data and comparison with available references and literature survey. The main compounds present in the strawberry tree were represented by organic acids (malic acid and citric acid), phenolic acids (quinic acid, protocatechuic acid, gallic acid, caffeic acid, ferulic acid, cinnamic acid, ellagic acid, syringic acid, and hydroxycoumarin), flavones (dihydroxyflavone), flavanols (catechins, epicatechins, procyanidin dimer with respective gallate and prodelphinidin), flavonols (hexoside of isorhamnetin, myricetin, quercetin, kaempferol and apigenin) and other compounds (ascorbic acid, benzyl alcohol pentose, and hydroxyphenylvaleric). Most of these compounds were already described in *A. unedo* by Pawlowska et al., (2006), Fortalezas et al., (2010), Mendes et al., (2011), Guimarães et al., (2013) and Mosele et al., (2016). The number of determined compounds is the highest one compared to previously published data reporting many new compounds for the first time.

AQ fruits presented the highest phytochemical profile (34 compounds) followed by AC (28 compounds) and the last was AA (24 compounds) without considering the unknown compounds. EtOAc extract was the only one that presented two organic acids, namely citric acid (peak 2) and malic acid (peak 5) identified according to their UV spectra (λ_{max} = 254 nm) and pseudo molecular ion (*m*/*z* at 191 and 133, respectively).

Peak 1 (*m*/*z* 153) showed a λ_{max} at 295 nm in the extract of AQ-EtOAc and AA- MeOH: water, 80:20 (*v*/*v*), respectively and was identified as protocatechuic acid. Peak 15 (λ_{max} at 357 and 364) presented *m*/*z* of 315 and gave a fragment at *m*/*z* 153; it was identified as protocatechuic glucoside. It was presented in all the extracts of AA, in AQ- MeOH: water 80:20 (*v*/*v*) extract and AA-EtOAc extract. Peaks 3a,3b,3c and 3d showed the same $[M-H]^-$ fragment ions at *m*/*z* 191 (quinic acid) and different $[M-H]^-$ parent ion at *m*/*z* 383, 287,371 and 533 respectively, with maximum UV spectra at 325 nm typical of quinic acid derivative. Except for AQ-MeOH: water, 80:20 (*v*/*v*), all extracts presented peak 6 that showed the same parent ion at *m*/*z* 267, MS fragment ion at *m*/*z* 147 and similar λ_{max} at 277 nm, characteristics of cinnamic acid derivatives. Similarly, peak 51 (AC-EtOAc) had the parent ion at *m*/*z* 343, MS fragment ions at *m*/*z* 267, 147, and UV spectra identical to peak 6 identified as cinnamic acid

Table 4 Compounds detected in the methanol: water, 80:20 (*v*/*v*) extract of *A. unedo* from three regions by HPLC-DAD-ESI/MS

Peak	Tentative Identification	t_R(min)	Identification type	λ_{MAX}(nm)	$[M-H]^-$	Major fragments	A. A	A. C	A. Q
Phenolic acid and derivatives									
50	Quinic acid	2.08	DAD/MS	243	191	–	+	+	+
3c	Quinic acid derivative	1.47	DAD/MS	272	371	191	+	–	–
3d	Quinic acid derivative	1.58	DAD/MS	325	533	191	+	+	+
32	Caffeoylquinic acid	26.56	(Guimarães et al., 2013) DAD/MS	278–349	353	179	–	+	+
70	Dicaffeoylquinic acid	8.97	DAD/MS	278–371	515	179	–	+	–
71	Caffeoyl dihexoside	2.50	DAD/MS	286–292	503	179	–	+	–
13b	Caffeic acid derivative	1.46	DAD/MS	277–380	359	179	–	–	+
13c	Caffeic acid derivative	4.38	DAD/MS	288–342	665	179	–	–	+
13d	Caffeic acid derivative	11.69	DAD/MS	274–356	601	179	–	–	+
15	Protocatechuic acid glucoside	8.21	DAD/MS	364	315	153	+	+	
1	Protocatechuic acid	9.71	(Ayaz et al., 2000; Mosele et al., 2016) DAD/MS	295	153	–	+	–	–
9	Gallic acid glucoside	6.73	(Mendes et al., 2011) DAD/MS	254–278	331	169, 191	+	+	+
7	Galloylquinic acid	6.84	(Mendes et al., 2011) DAD/MS	273	343	169,191	+	+	–
63	Digalloylquinic acid	14.10	(Mendes et al., 2011) DAD/MS	272–371	495	191	+	+	–
11	Galloyl shikimic acid	10.14	(Mendes et al., 2011) DAD/MS	272–331	325	169	+	+	+
21	Digalloyl shikimic acid	10.37	(Mendes et al., 2011) DAD/MS	273–279	477	325	+	+	+
24	Digalloyl glucose	17.08	DAD/MS	274	483	325,191	+	+	+
58	Gallotannin	25.19	DAD/MS	274	629	407	–	–	+
64	Trimethyl gallic acid glucuronide	19.66	DAD/MS	223–277	387	169	+	+	–
30b	Galloyl derivatives	9.44	DAD/MS	254–365	663	331,169	+	–	–
30c	Galloyl derivatives	16.34	DAD/MS	276	423	169, 191	+	–	–
8a	Ellagitannin N.I	7.67	DAD/MS	273	687	343	+	–	+
8b	Ellagitannin N.I	21.12	DAD/MS	273	725	343	–	+	–
25	Strictinin ellagitannin	21.02	(Mendes et al., 2011) DAD/MS	296–361	633	–	+	+	+
6	Cinnamic acid derivative	4.54	DAD/MS	288–365	267	147	+	+	–
53	4-hydroxycoumarin	4.87	DAD/MS	275–350	161	–	–	–	+
60	p-coumaroylquinic acid	2.71	(Guimarães et al., 2013) DAD/MS	292	337	191	+	–	–
65	Vanillic acid hexoside	20.38	DAD/MS	277	329	167	+	–	–
Flavan-3-ols									
10	Epigallocatechin	9.51	(Mosele et al., 2016) DAD/MS	270	325	305	–	+	–
61	Gallocatechin + catechin	11.02	(Mosele et al., 2016) DAD/MS	279	593	289	+	+	+
14	Procyanidin dimer type B	13.72	(Mendes et al., 2011) DAD/MS	274–371	577	495,289	+	+	+
16	(-)-Catechin	15.61	(Mosele et al., 2016) DAD/MS	275–285	289	–	+	+	+
17	(+)-Catechin	15.96	(Mosele et al., 2016) DAD/MS	275–285	291+	289	–	–	+
18	(+)-Epicatechin	16.14	(Mosele et al., 2016) DAD/MS	270	289	–	+	+	+
59	(-)-Epicatechin dimethyl gallate	31.30	DAD/MS	214–279	469	289	–	–	+
Flavonols									
26	Myricetin	22.25	DAD/MS	267–356	317	–	+	–	+
31	Quercetin galloyl hexoside derivatives	24.91	(Mendes et al., 2011; Guimarães et al., 2013) DAD/MS	269–355	615	433	+	+	+
33	Quercetin pentoside	31.89	(Guimarães et al., 2013) DAD/MS	252–357	433	301	+	+	+
35	Quercetin glucoside	23.19	(Mendes et al., 2011) DAD/MS	370–356	463	301	+	+	+
37	Quercetin rhamnoside-glucoside	28.55	DAD/MS	252–353	609	463, 301	+	+	–
41	Quercetin rhamnoside	32.41	(Guimarães et al., 2013) DAD/MS	254–348	447	303+	+	+	+
45	Apigenin pentoside	20.24	(Guimarães et al., 2013) DAD/MS	282–366	401	–	–	+	–
47	Quercetin hexose protocatechuic acid	31.30	DAD/MS	350	551	301	–	+	–
48	Dihydroquercetin rhamnoside	27.86	DAD/MS	349	565	301	+	+	–
55	Quercetin glucoronide	29.31	(Guimarães et al., 2013) DAD/MS	235–355	477	301	+	+	+
56	Isorhamnetin rutinoside	20.35	(Guimarães et al., 2013) DAD/MS	277–350	461	315	–	–	+
57	Dihydromyricetin rhamnoside	27.86	DAD/MS	252–360	465	317	+	–	+
62	Apigenin glucuronide	12.36	DAD/MS	277–371	445	–	+	–	–
63	Apigenin glucoside	12.82	DAD/MS	284–371	419	269	+	–	–

Table 4 (*continued*)

Peak	Tentative Identification	t_R(min)	Identification type	λ_{MAX}(nm)	$[M-H]^-$	Major fragments	A. A	A. C	A. Q
66	Myricetin rhamnoside	23.42	(Mendes et al., 2011; Guimarães et al., 2013) DAD/MS	277–378	463	317	+	–	–
67	Quercetin galloyl glucoside	24.91	DAD/MS	271–361	585	301	+	+	+
68	Quercetin galloyl hexuronide	25.23	DAD/MS	277–365	629	477,325	+		
69	Quercetin galloyl pentoside	28.36	DAD/MS	254–351	585	433,301	–	+	+
Others									
A	Ascorbic acid	2.14	(Pallauf et al., 2008) DAD/MS	244	175	–	+	–	–
Q	Ascorbic acid derivative	21.44	DAD/MS	279–370	366	175	+	–	–
Non-identified									
N.6	Unknown	11.46	–	209–274	645	381	+	+	–
N.7	Unknown	4.99	–	373–365	553	161	+	–	–
N.8	Unknown	21.61	–	280–379	465	–	+	–	–
N.9	Unknown	21.47	–	–	487	–	+	–	–
N.11	Unknown	29.93	–	234–277	597	–	+	–	
N.12	Unknown	31.35	–	279–354	469	–	+	–	
N.13	Unknown	19.70	–	275–341	387	–	–	–	+
N.14	Unknown	24.52	–	274	581	–	+	+	+
N.15	Unknown	19.89	–	214–279	496	387	–	–	
N.16	Unknown	21.47	–	217–279	599	366	–	+	–

derivative. All peaks 7,9,11,21,24,27,30(a,b,c),52,54,63 yielded a fragment ion at *m*/*z* 169 typical of gallic acid. Gallic acid and its derivatives (such as glucosides, gallic acid gallate, galloylquinic acid, galloyl shikimic acid, trimethyl gallic acid glucuronide, galloyl quercetin and gallotannins) were the dominating peaks in all extracts. The isomers identification was carried out by their comparison to the following references: Mosele et al., 2016; Tavares et al., 2010; Mendes et al., 2011. According to MS fragment at *m*/*z* 179 and UV spectra, all peaks 13a, 13b, 13c;13d (λ_{max} at 321,350,380 and 356 nm respectively) were related to caffeic acid and showed different parent ions at *m*/*z* 357, 359, 665 and 601 respectively. Caffeic acid isomers were identified thanks to their comparison to the analytical standards. Ellagic acid, is a dimeric derivative of gallic acid, and is generated by the hydrolysis of the ellagitannins. Its derivatives (ellagic acid glucoside, methylellagic acid rhamnoside, methylellagic acid rhamnopyranoside, strictinin ellagitannin, and ellagic acid arabinoside/xyloside) were distributed differently in the fruits on the regions studied. The content of gallic acid and its derivatives could have scientific value in various fields of medicine and biotechnology. They have also been implicated as anticarcinogenic, antimicrobial, antimutagenic, antiangiogenic and anti-inflammatory agents besides their use in treating critical diseases like depression, cancer, microbial infections, lipid-related diseases, etc (Choubey et al., 2015). Peak 53 showed UV spectra at 275–350 nm and molecular ion at *m*/*z* 161, characteristic of 4-hydroxycoumarin. AA- MeOH: water 80:20 (*v*/*v*) fruits showed peaks 60 and 65 (*m*/*z* 337 and 329; λ_{max} at 292 nm and 277 nm and MS fragments *m*/*z* 191 and 167 respectively) were identified as p-coumaroylquinic acid vanillic acid hexoside, respectively by comparison of their mass fragment and UV spectra profiles with previously published data Stanoeva et al., (2017), Mena et al., (2012).

Only AQ-EtOAc extract showed a peak 4 (*m*/*z* 253; λ_{max} at 287 nm) that was identified as dihydroxyflavone. Flavan-3-ols presented in this sample were catechin and its derivatives (epigallocatechin, procyanidin dimer type B, (-)-epicatechin gallate, catechin glucose, prodelphinidin dimer, procyanidin gallate). These compounds were abundant only in the fruits of AQ regions.

Also, 24 flavonols were found in this species and were distributed differently depending on the extraction solvent (14 and 15 compounds in EtOAc and MeOH: water, 80:20 (*v*/*v*) respectively) and the regions of harvest (22, 19 and 22 compounds in AA, AC and AQ respectively). Their UV spectra are in fact characteristic of the flavonols structure, with two absorption bands. The first band, determined by the benzene moiety, in 250–270 nm range, the second one in the 340–350 nm range, with intensities and relative positions reflecting hydroxylation pattern and degree of substitution (Dugo et al. 2009). The presence of MS fragments at *m*/*z* 301, 285, 315, 317, and 269 indicate the presence of aglycones as quercetin, kaempferol, isorhamnetin, myricetin, and apigenin. Accordingly, to these MS fragments ions, these compounds were identified as results from glycosylation of the flavonols at various positions. All studied samples were rich in quercetin derivatives. Peaks 33, 35, and 41 respectively for quercetin pentoside, quercetin glucoside, quercetin rhamnoside ($[M-H]^-$ at *m*/*z* 433, 463 and 447, respectively) were found in all the studied fruits. In ethyl acetate extract (AC and AQ), two compounds were tentatively identified as quercetin arabinose/xyloside (peak 36) and quercetin hexoside (peak 38) detected a similar $[M-H]^-$ ion at *m*/*z* 433 also similar UV spectra (252–347 nm and 254–351 nm, respectively). Also, all methanolic samples presented quercetin glucuronide (*m*/*z* 477; 235–355 nm). Other quercetin derivatives were assigned according to pseudo molecular ions, MS fragments, UV spectra, and by comparison with literature data. Peak 31 ($[M-H]^-$ at *m*/*z* 609) detected in all samples was identified as quercetin galloyl hexoside derivative. Peak 37 assigned to quercetin rhamnoside-glucoside ($[M-H]^-$ at *m*/*z* 609) was present in AQ-EtcOH and

Table 5 Semi-quantification of polyphenols detected in lyophilized *A. unedo* fruit from three different regions mg/100 g (*w*/*w*).

Compounds	A. A		A. C		A. Q		Standard used for semi-quantification
	EtOAc	MeOH: water, 80:20 (*v*/*v*)	EtOAc	MeOH: water, 80:20 (*v*/*v*)	EtOAc	MeOH: water, 80:20 (*v*/*v*)	
Phenolic acid and derivatives							
Protocatechuic acid	–	0.15 ± 0.008	–	–	0.02 ± 0.06	–	Gallic acid
Protocatechuic acid glucoside	0.05 ± 0.06	0.61 ± 0.004	–	0.1 ± 0.05	0.4 ± 0.09	–	Gallic acid
Gallic acid glucoside	0.44 ± 0.9	9.82 ± 3.34	0.59 ± 0.25	7.93 ± 1.43	4.18 ± 0.03	20.27 ± 8.3	Gallic acid
Gallic acid gallate	–	–	–	–	0.13 ± 0.03	–	Gallic acid
Galloyl hexoside	–	–	0.30 ± 0.05	–	–	–	Gallic acid
Galloylquinic acid	3.37 ± 1.9	–	4.26 ± 1.19	26.38 ± 3.2	6.24 ± 0.09	–	Gallic acid
Digalloylquinic acid	–	3.19 ± 0.005	–	1.70 ± 0.02	–	–	Gallic acid
Galloyl shikimic acid	0.59 ± 0.4	0.19 ± 0.006	1.26 ± 0.7	2.03 ± 0.03	3.48 ± 0.05	1.10 ± 0.03	Gallic acid
Digalloyl shikimic acid	0.35 ± 0.05	4.22 ± 0.005	1.47 ± 0.3	1.53 ± 0.36	3.4 ± 1.8	1.55 ± 0.03	Gallic acid
Digalloyl glucose	–	0.66 ± 0.003	–	0.52 ± 0.12	0.7 ± 0.03	0.41 ± 0.03	Gallic acid
Galloyl derivatives	–	–	–	–	–	–	Gallic acid
Galloyl derivatives	–	–	–	–	0.48 ± 0.03	–	Gallic acid
Galloyl derivatives	–	1.46 ± 0.008	–	–	–	–	Gallic acid
Galloyl derivatives	–	3.63 ± 0.008	–	–	–	–	Gallic acid
Gallotannin	–	–	–	–	–	0.25 ± 0.02	Gallic acid
Ellagic acid glucoside	–	–	–	–	0.75 ± 0.06	–	Gallic acid
Ellagic acid arabinoside/xyloside	–	–	–	–	0.49 ± 0.02	–	Gallic acid
Methylellagic acid methyl pentose	–	–	–	–	–	–	Gallic acid
Methylellagic acid rhamnoside	–	–	–	–	0.40 ± 0.03	–	Gallic acid
Methylellagic acid rhamnopyranoside	–	–	–	–	0.53 ± 0.06	–	Gallic acid
Methylellagic derivative	–	–	–	–	0.76 ± 0.02	–	Gallic acid
Trimethyl gallic acid glucoronide	–	0.64 ± 0.01	–	1.27 ± 0.3	–	–	Gallic acid
Strictinin ellagitannin	–	–	–	–	0.7 ± 0.05	–	Gallic acid
Ellagitannin N.I	–	46.69 ± 0.1	–	–	19.98 ± 0.56	5.56 ± 0.5	Gallic acid
Ellagitannin N.I	–	–	0.07 ± 0.007	–	–	–	Gallic acid
Total of Hydroxybenzoic acids	4.8 ± 0.1	71.22 ± 1.5	7.95 ± 0.01	41.46 ± 1.3	42.64 ± 0.6	29.14 ± 0.8	
Caffeoylquinic acid	–	–	–	–	0.11 ± 0.03	–	Caffeic acid
Dicaffeoylquinic acid	–	–	–	0.5 ± 0.006	–	–	Caffeic acid
Caffeic acid dihexoside	–	–	–	0.32 ± 0.08	–	–	Caffeic acid
Caffeic acid derivative	–	–	–	–	0.01 ± 0.001	–	Caffeic acid
Caffeic acid derivative	–	–	0.01 ± 0.001	–	–	0.24 ± 0.001	Caffeic acid
Caffeic acid derivative	–	–	–	–	–	0.06 ± 0.002	Caffeic acid
Caffeic acid derivative	–	–	–	–	–	0.01 ± 0.002	Caffeic acid
Feruloylquinic acid	–	–	0.02 ± 0.006	–	–	–	Caffeic acid
Syringic acid	1.82 ± 0.0014	–	–	–	–	–	Caffeic acid
Cinnamic acid derivative	0.003 ± 0.001	–	0.2 ± 0.02	0.13 ± 0.004	–	–	Cinnamic acid
Cinnamic acid derivative	–	0.35 ± 0.007	–	–	–	–	Cinnamic acid
p-coumaroylquinic acid	–	0.75 ± 0.002	–	–	–	–	Cinnamic acid
Vanillic acid hexoside	–	0.65 ± 0.01	–	–	–	–	Vanillic acid
Total of Hydroxycinnamic acids	1.82 ± 0.6	0.75 ± 0.021	0.23 ± 0.02	0.95 ± 0.04	0.12 ± 0.05	0.31 ± 0.02	
4-hydroxycoumarin	–	–	0.067 ± 0.03	–	–	0.03 ± 0.001	Coumaric acid

Table 5 (*continued*)

Compounds	A. A		A. C		A. Q		Standard used for semi-quantification
	EtOAc	MeOH: water, 80:20 (*v*/*v*)	EtOAc	MeOH: water, 80:20 (*v*/*v*)	EtOAc	MeOH: water, 80:20 (*v*/*v*)	
Flavone							
Dihydroxyflavone	–	–	–	–	0.03 ± 0.008	–	Apigenin
Flavan-3-ols							
Epigallocatechin	–	–	–	–	1.26 ± 0.12	–	Catechin
Gallocatechin + catechin	–	1.66 ± 0.1	–	0.81 ± 0.01	–	–	Catechin
Procyanidin dimer type B	4.62 ± 0.07	12.69 ± 0.6	5.17 ± 2.37	9.01 ± 0.83	13.26 ± 0.85	8.62 ± 0.86	Catechin
Procyanidin gallate	–	–	–	–	0.69 ± 0.06	–	Catechin
Prodelphinidin dimer	–	–	–	–	2.07 ± 0.48	–	Catechin
(-)-Catechin	1.06 ± 0.7	9.25 ± 0.006	0.53 ± 0.03	7.77 ± 0.1	25.33 ± 5.6	6.35 ± 1.85	Catechin
(-)-Epicatechin	–	–	–	–	2.83 ± 0.05	–	Catechin
(-)-Epicatechin gallate	–	–	0.70 ± 0.05	–	1.68 ± 0.1	–	Catechin
(-)-Epicatechin dimethyl gallate	–	–	–		–	3.35 ± 0.85	Catechin
Catechin glucose	–	–	–	–	1.69 ± 0.22	–	Catechin
Total of flavan-3-ols	5.68 ± 1.2	23.6 ± 0.8	6.47 ± 1.4	17.6 ± 0.4	48.84 ± 0.65	18.35 ± 1.12	
Flavonols							
Isorhamnetin glucoside	–	–	–	–	0.03 ± 0.05	–	Isorhamnetin
Isorhamnetin rutinoside	–	–	–	–	–	–	Isorhamnetin
Myricetin	–	0.01 ± 0.005	–	–	0.80 ± 0.03	–	Kaempferol
Myricetin rhamnoside	–	0.01 ± 0.0041	–	–	–	–	Kaempferol
Quercetin glucoside	0.3 ± 0.01	1.55 ± 0.1	0.22 ± 0.07	0.84 ± 0.06	0.3 ± 0.0.01	0.73 ± 0.03	Rutin
Quercetin arabinose/xyloside	–	–	–	–	0.46 ± 0.02	–	Rutin
Quercetin pentoside	1.11 ± 0.07	1.70 ± 0.17	1.92 ± 0.07	2.02 ± 0.36	0.69 ± 0.06	1.2 ± 0.02	Rutin
Quercetin rhamnosyl-glucoside	–	0.39 ± 0.01	–	0.42 ± 0.02	1.34 ± 0.03	–	Rutin
Quercetin hexoside	–	–	–	–	5.7 ± 2.54	–	Rutin
Quercetin rhamnoside	1.36 ± 0.07	0.65 ± 0.01	1.26 ± 0.8	0.57 ± 0.01	6.9 ± 0.3	0.74 ± 0.02	Rutin
Quercetin glucoronide		0.62 ± 0.4	–	–	–	–	Rutin
Quercetin galloyl glucoside	–	0.14 ± 0.01	–	0.32 ± 0.02	–	–	Rutin
Quercetin galloyl pentoside	–	–	–	0.2 ± 0.01	–	–	Rutin
Quercetin galloyl hexose derivative	0.15 ± 0.04	–	0.17 ± 0.06	–	3.52 ± 0.52	–	Rutin
Quercetin galloyl hexuronide	–	0.36 ± 0.04	–	–	–	–	Rutin
Quercetin hexose protocatechuic acid -		–	–	–	–	–	Rutin
Quercetin derivative	0.92 ± 0.04	–	–	–	–	–	Rutin
Kaempferol galloylglucoside	–	–	–	–	0.5 ± 0.05	–	Kaempferol
Kaempferol xyloside	0.52 ± 0.03	–	1.18 ± 0.001	–	0.43 ± 0.03	–	Kaempferol
Apigenin pentoside	0.17 ± 0.003	–	0.28 ± 0.01	–	–	–	Apigenin
Apigenin glucoside	–	0.02 ± 0.008	–	–	–	–	Apigenin
Apigenin glucuronide	–	0.15 ± 0.009	–	–	–	–	Apigenin
Total of flavonols	5.53 ± 0.4	5.6 ± 0.002	5.03 ± 0.005	4.37 ± 0.01	20.67 ± 1.43	2.67 ± 0.02	
Dihydromyricetin rhamnoside		LOQ >	–	–	–	–	Kaempferol
Dihydroquercetin rhamnoside	0.27 ± 0.03	0.08 ± 0.006	0.37 ± 0.07	–	–	–	Rutin
Total of dihydroflavonols	0.27 ± 0.03	0.08 ± 0.006	0.37 ± 0.07	–	–	–	

(AA/AC)- MeOH:water, 80:20 (*v*/*v*). Only the samples of AA-EtcOH presented peak 47 ($[M-H]^-$ at *m*/*z* 551) which was identified as quercetin hexose protocatechuic acid. Peak 48 in ethyl acetate samples of AA and AC was tentatively identified as dihydroquercetin rhamnoside. Other detected peaks 67, 68, and 69 were identified as quercetin galloyl glucoside, quercetin galloyl hexuronide ($[M-H]^-$ at *m*/*z* 629), quercetin galloyl pentoside ($[M-H]^-$ at *m*/*z* 585) respectively.

In AQ samples, two molecules resulted from glycosylation of isorhamnetin: peak 23 (isorhamnetin glucoside; *m*/*z* 477) and peak 56 (isorhamnetin rutinoside; *m*/*z* 461). These two compounds showed an identical pattern fragment at *m*/*z* 315, but two different range of UV spectra at 278–385 nm and 277–350 nm respectively. Myricetin and its derivatives present in fruits of AA and AQ. Peaks 26, 57, and 66 were tentatively identified as myricetin, dihydromyricetin rhamnoside, and myricetin rhamnoside. Other detected flavonols corresponded to apigenin and kaempferol derivatives. Apigenin pentoside (peak 45 in AA and AC), apigenin glucuronide (peak 62 in AA), and apigenin glucoside (peak 63 in AA) were identified according to their mass spectra and UV range. Peak 39 identified as kaempferol galloyl glucoside in accordance with $[M-H]^-$ at *m*/*z* 599, MS fragment at *m*/*z* 447 and by comparison with results reported by Mendes et al., (2011). Peak 42 detected in all ethyl acetate extract was tentatively identified as kaempferol xyloside ($[M-H]^-$ at *m*/*z* 417).

The investigated samples showed other compounds that were tentatively identified according to their mass spectra, MS fragments, and λ_{max}. These compounds were detected as ascorbic acid and its derivatives, benzyl alcohol hexose pentose, and hydroxyphenylvaleric acid (Mosele et al., 2016).

The content of phenolic acids, flavones, flavan-3-ols and flavonols in the *A. unedo* fruit is presented in Table 5. Regarding the profile composition, the most abundant group was represented by hydroxybenzoic acids with 76.02 ± 1.3 mg/100 g (*w*/*w*) (DW) founded in AA fruit followed by AQ fruit 71.78 ± 1.5 mg/100 g (*w*/*w*) (DW) and in the last AC fruit 49.41 ± 1.01 mg/100 g (*w*/*w*) (DW) caffeic acid and its derivatives were abundant in AA (3.57 mg/100 g (*w*/*w*) (DW)) and AC (1.18 mg/100 g) (*w*/*w*) (DW). The highest content of flavan-3-ols was in AQ (67.19 mg/100 g (*w*/*w*) (DW)), instead. These results do not match other studies which showed that proanthocyanidins present the highest content (De Pascual al., 2000; Pallauf et al., 2008). However, in agreement with Pallauf et al., (2008) quercetin derivatives are abundant flavonols in all samples. On the other hand, flavonol derivates are 10-fold higher than one found in the fruits of Portugal (10.86 ± 0.24 mg/100 g (*w*/*w*) (DW); Guimarães et al., 2013). Semi-quantification by HPLC-DAD analysis gave the higher content of antioxidant compounds for the AQ fruits (162.74 ± 27.41 mg/100 g (*w*/*w*) (DW)) followed by AA fruits (120.43 ± 27.41 mg/100 g (*w*/*w*) (DW)).

4. Conclusions

This study demonstrates that *A. unedo* contains a large variety of polyphenols including a high level of antioxidant capacity. All samples showed a good phytochemical profile. AC fruits presented the highest total polyphenols and flavonoids content (127.5 ± 7.14 mg GAE/g (*w*/*w*) (DW) and 105.9 ± 3.2 mg QE/g (*w*/*w*) (DW)). AQ fruit showed the highest content of anthocyanins and tannins (1.48 ± 0.09 mg Pg-3-glu/g (*w*/*w*) (DW) and 1.22 ± 0.1 mg EC/g (*w*/*w*) (DW) respectively). HPLC-DAD-ESI/MS analysis revealed 75 compounds tentatively identified as hydroxybenzoic acids, hydroxycinnamic acids, flavone, flavan-3-ols, flavonols, and dihydroflavonols. The most abundant group was represented by hydroxybenzoic acids. According to the obtained results, this fruit can be considered a potential application for pharmacological research aiming to find the right treatment for many diseases emerged recently.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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