Antihemolytic and radical scavenging activities of strawberry tree (*Arbutus unedo* L.) leaf and fruit: a comparative study

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Short title: Antioxidant activity of *Arbutus unedo* leaf and fruit

Abstract

This study aimed to assess the antioxidant properties of *Arbutus unedo* leaf and fruit. Aqueous extracts were prepared and their total phenolic contents determined by Folin-Ciocalteu method. Antioxidant potential was evaluated using different *in vitro* assays: reducing power, scavenging effect on DPPH free radicals, and inhibitory effect on AAPH-induced damage in human erythrocytes.

All models demonstrated antioxidant efficiency for *A. unedo* extracts, being invariably higher in the leaf. Accordingly, total phenolic content was significantly superior in leaf. EC_{50} values for reducing power and DPPH radical scavenging activities were, respectively, 0.318 and 0.087 mg/mL for leaf, and 2.894 and 0.790 mg/mL for fruit extracts. Under the oxidative action of AAPH, both extracts protected the erythrocyte membrane from hemolysis (IC_{50} of 0.062 and 0.377 mg/mL) and decreased the levels of malondialdehyde.

These results suggest *A. unedo* as a promising source of natural antioxidants with potential application in diseases mediated by free radicals.

Keywords: antioxidant activity; *Arbutus unedo*; fruit; hemolysis; leaf; phenolics

1. Introduction

Overwhelming evidence indicates that free radical-induced oxidative damage to cellular biomolecules (as proteins, carbohydrates, nucleic acids and lipids) is implicated in aging and in the pathogenesis of most major health problems of the industrialized world, including cardiovascular disease, atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders and cancer (Valko et al., 2007; Negre-Salvayre et al., 2008). The extent of damage caused by free radical species might be mitigated through supplementation with one or more antioxidants. Therefore, the consumption of plant foods rich in polyphenolic compounds, such as flavonoids, phenolic acids, anthocyanidins, and tannins, which possess remarkable antioxidant activities, may play an essential role in the prevention of these diseases. In the last decades, various medicinal plants have been screened and assessed in the context of therapeutic approaches to encounter and prevent diseases mediated by oxidative stress.

Arbutus unedo L., commonly known as strawberry tree, is an evergreen shrub or small tree (Ericaceae family) endemic to Mediterranean region, but also encountered in other regions with hot summers and mild rainy winters (Celikel et al., 2008). Its fruits (berries) are spherical, about 2-3 cm in diameter, red, and tasty only when fully ripe in autumn. Although the fruits are edible, they are seldom eaten fresh and generally processed before consumption. Processed products include highly appreciated alcoholic beverages such as wines, liquors, and brandies. Other food applications such as jams, marmalades and jellies can also be obtained from *A. unedo* fruits (Alarcão-E-Silva et al., 2001; Pallauf et al., 2008). It is also possible to incorporate the berries into yogurts, pie fillings, and cereal products. In traditional medicine, the fruits are recognized to have antiseptic, diuretic, and laxative effects, while the leaves are used as astringent, diuretic,

urinary antiseptic, antidiarrheal, depurative and, more recently, in the therapy of hypertension, diabetes, and inflammatory diseases (Afkir et al., 2008; Mariotto et al., 2008; Bnouham et al., 2010). Phytochemical studies showed that leaf extracts contain several phenolic compounds, like tannins, flavonoids, phenolic glycosides, among others (Males et al., 2006; Fiorentino et al., 2007; Pavlović et al., 2009), as well as α-tocopherol (Kivçak & Mert, 2001).The *A. unedo* berries are already known as a very good source of antioxidants, including phenolic compounds, vitamins C and E, and carotenoids (Ayaz et al., 2000; Alarcão-E-Silva et al., 2001; Males et al., 2006; Pawlowska et al., 2006; Pallauf et al., 2008). The most representative class of phenolic compounds is the flavonoid family and, within this group, proanthocyanidins are the most abundant, representing more than 80% of the total flavonoid content. Anthocyanins are also present as glycosides of cyanidin and delphinidin, being cyanidin-3-galactoside the most abundant. Other antioxidant phenolic compounds present in this fruit are ellagic acid and its diglucoside derivative (Pallauf et al., 2008).

Based on their chemical composition, it is reasonable to expect a high antioxidant activity for *A. unedo* fruit and leaf extracts. Therefore, in the present study, their antioxidant potential was assessed by the reducing power and DPPH radical scavenging activity assays. In addition, the biological significance of *A. unedo* antiradical activities was investigated using human cell-based model assays. The human erythrocyte was used as an *in vitro* model to study the oxidant/antioxidant interaction since its membrane is rich in polyunsaturated fatty acids, which are extremely susceptible to free radical-mediated peroxidation, and is considered to be representative of the plasma membrane in general (Shiva Shankar Reddy et al., 2007). Erythrocyte lipid peroxidation may be involved in normal cell aging and it has been associated with a variety of pathological events (Ko et al., 1997; Sivilotti, 2004). In this study, 2,2'-azobis(2-

amidinopropane) dihydrochloride (AAPH) was used as the free radical initiator to induce oxidative damage in erythrocytes. Thermal decomposition at physiological temperature of AAPH generates peroxyl radicals in the aqueous phase (Niki, 1990), which can attack the erythrocyte membrane to induce lipid peroxidation. Since peroxidation of membrane lipids is a free radical chain reaction, the erythrocyte membrane is quickly damaged, leading to hemolysis. The protective effects of *A. unedo* leaf and fruit extracts were evaluated by inhibition of erythrocytes hemolysis and lipid peroxidation mediated by peroxyl radicals. To the best of our knowledge, this is the first time that the antioxidant activity of *A. unedo* species is evaluated using human biological membranes.

2. Materials and methods

2.1. Reagents

2,2'-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), gallic acid, trichloroacetic acid (TCA), 1,1,3,3-tetraethoxypropane (TEP), thiobarbituric acid (TBA), and butylated hydroxytoluene (BHT) were purchased from Sigma (St Louis, MO). Folin–Ciocalteu's phenol reagent was obtained from Fluka. Phosphate buffer solution (PBS) was obtained from Lonza Laboratories (Verviers, Belgium). All other chemicals were of analytical grade and obtained from Sigma (St. Louis, MO).

2.2 Sample preparation and extraction

The leaves and fruits of *Arbutus unedo* L. were collected in January of 2009, in the Natural Park of Montesinho (Bragança, Northeast of Portugal). The samples were immediately frozen and freeze-dried (Ly-8-FM-ULE, Snijders) prior to extraction. Three powdered subsamples (approximately 5 g; 20 mesh) were extracted with 250 mL of boiling water for 45 min. The resulting extracts were then lyophilized and kept in a dessicator (in the dark), until analysis. The extraction yields were 39.3 ± 7.3% and 57.4 ± 1.2% for leaf and fruit, respectively.

2.3. Total Phenolic Content

The total phenolic content of leaf and fruit extracts was determined by using the Folin-Ciocalteu´s phenol reagent, according to a previously described procedure (Costa et al., 2009). Briefly, 100 µl of water extract solution was mixed with 1 ml of Folin-Ciocalteu's phenol reagent and 5 ml of 20% sodium carbonate solution and the mixture adjusted to 10 ml with water. The reaction was kept in the dark for 20 min, after which the absorbance was read at 735 nm. The content of total phenolics was determined from a standard curve using gallic acid as standard.

2.4. Reducing power assay

The reducing power of leaf and fruit extracts was determined according to the method of Oyaizu (1986). Various concentrations of sample extracts (1 mL) were mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50ºC for 20 min. At the end of incubation time, 2.5 mL of 10% tricloroacetic acid (w/v) were added and the mixture was centrifuged at 1000 rpm for 8 min in a refrigerated centrifuge (Centorion K24OR-2003). The upper layer (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL

of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm in a PG Instruments Ltd. T70 UV/VIS spectrometer. The assays were carried out in triplicate and the results expressed as mean values ± standard deviations (SD). The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance registered at 700 nm against extract concentration.

2.5. DPPH radical scavenging activity assay

The capacity to scavenge the DPPH free radical was monitored according to a method reported before Hatano et al., 1988). Various concentrations of sample extracts (0.3 mL) were mixed with 2.7 mL of methanolic solution containing DPPH radicals ($6x10^{-5}$ mol/L). The mixture was shaken vigorously and left to stand in the dark until stable absorption values were obtained. The reduction of the DPPH radical was measured by monitoring continuously the decrease of absorption at 517 nm. The radical scavenging activity was calculated as percentage of DPPH discoloration using the equation: % scavenging effect = $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. The assays were carried out in triplicate and the results expressed as mean ± SD. The extract concentration providing 50% inhibition (EC_{50}) was determined graphically by plotting the percentage of DPPH scavenging as a function of extract concentration.

2.6. Antioxidant assays using the human erythrocyte model

2.6.1. Preparation of human erythrocyte suspensions. Blood (5-10 ml) was obtained from healthy non-smoker adult individuals after informed consent. Human erythrocytes from citrated blood were immediately isolated by centrifugation at 1500 rpm for 10 min

at 4ºC. After removal of plasma and buffy coat, the erythrocytes were washed three times with PBS (pH 7.4), and centrifuged at 1500 rpm for 10 min at 4ºC. After the last washing, the erythrocytes were resuspended using the same buffer to the desired hematocrit level.

2.6.2. Oxidative hemolysis inhibition assay. In order to induce free-radical chain oxidation in erythrocytes, aqueous peroxyl radicals were generated by thermal decomposition of AAPH (dissolved in PBS; final concentration 50 mM). To study the protective effects of *A. unedo* aqueous extracts against AAPH-induced hemolysis, an erythrocyte suspension at 2% hematocrit was preincubated with the extracts of leaf (final concentrations of 50, 75, and 100 µg extract/mL diluted in PBS) and fruit (final concentrations of 400, 800 and 1600 µg extract/mL diluted in PBS) at 37ºC for 30 min, followed by incubation with and without 50 mM AAPH. This reaction mixture was shaken gently while being incubated at 37ºC for 4 hours. In all experiments, a negative control (erythrocytes in PBS), as well as extract controls (erythrocytes in PBS with each extract) were used.

The extent of hemolysis was determined spectrophotometrically according to a method reported before (Ko et al., 1997; Costa et al., 2009; Magalhães et al., 2009; Carvalho et al., 2010). Briefly, aliquots of the reaction mixture were taken out at each hour of the 4 hours of incubation, diluted with saline, and centrifuged at 4000 rpm for 10 min to separate the erythrocytes. The percentage of hemolysis was determined by measuring the absorbance of the supernatant (A) at 545 nm and compared with that of complete hemolysis (B) by treating an aliquot with the same volume of the reaction mixture with distilled water. The hemolysis percentage was calculated using the formula: $A/B \times 100$. The inhibitory concentration 50% (IC_{50}) at time 3 hours was calculated from the graph

plotted with hemolysis inhibition percentage against extract concentration. Five independent experiments were used for these calculations.

2.6.3. Lipid Peroxidation Inhibition Efficiency. To study the inhibitory effect of *A. unedo* extracts on AAPH-induced lipid peroxidation in human erythrocytes, we maintained the experimental conditions described for the hemolysis inhibition assay with the exception that erythrocyte suspensions at 5.2% hematocrit were used. The extention of lipid peroxidation in erythrocytes was estimated by HPLC-UV quantification of malondialdehyde (MDA), a well-known carbonyl product of lipid peroxidation (Soares et al., 2004). Briefly, 250 µL of erythrocyte suspensions were taken at time 3 hours and added to 25 µL of 0.2% BHT (in order to prevent further lipid peroxidation) and 1 mL of 1% TCA. The samples were then vortexed, centrifuged (10,000 rpm for 10 min, at 4ºC), and 500 µL of 1% TBA was added to equal volume of supernatant layer and allowed to stand at 95ºC during 45 min. After cooling at 4ºC, the samples were vortexed, centrifuged, and 50 µL of supernatants were injected on a HPLC system equipped with UV detector (Hewlett Packard 1100 series) set at 532 nm. Chromatographic separation was carried out using a Spherisorb C18 ODS2 column (5 µm; 4.6 x 250 mm) from Waters Corporation. The mobile phase consisted of methanol: 0.05 M ammonium acetate buffer (pH 5.5), at a solvent flow rate of 0.7 mL/min. TEP standards in a concentration range 0.25-10 µM were used to obtain the standard curve calibration. Results were calculated as µM MDA equivalent from the TEP standard calibration. The IC_{50} was calculated from dose-response curve obtained by plotting the percentage of lipid peroxidation inhibition against extract concentration. Inhibition percentage values were calculated considering as 100% the value of lipid peroxidation induced by AAPH in the absence of the extracts. Three independent experiments were used for these calculations.

2.7. Statistical analysis

Statistic analysis was performed using the Statistical Package for Social Sciences (SPSS, version 16.0) for Windows. Comparisons between two groups were performed by unpaired t-test. Multiple comparisons between more than two groups were performed by one-way ANOVA supplemented with Tukey´s HSD post hoc test. Significance was accepted at *P* lower than 0.05.

3. Results and discussion

3.1. Total phenolic content

The antioxidant capacity of plant materials has been reported to be highly correlated with the content in phenolic compounds Li et al., 2005; Kim et al., 2008). These phytochemicals are able to act as antioxidants in a number of ways, mainly as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelating agents (Rice-Evans et al., 1996; Cao et al., 1997). A few works were developed concerning the phenolic characterization of *A. unedo* leaves. Males et al. (2006) have studied the flavonoidic composition of samples collected in Croatia and identify quercitrin, isoquercitrin, hyperoside and rutin. More recently, Fiorentino et al. (2007) have referred the identification of twelve phenolic compounds in leaves collected in Central Italy, namely arbutin, ethyl gallate, *p*-hydroxybenzoyl arbutin, galloylarbutin, (+)-gallocatechin, catechin, kaempferol 3-*O*-α-L-ramnopyranoside, quercetin 3-*O*-α-L-ramnopyranoside, myricetin 3-*O*-α-L-ramnopyranoside, kaempferol 3-*O*-β-D-arabinofuranoside, quercetin 3-*O*-β-D-arabinofuranoside, and myricetin 3-*O*-β-D-

arabinofuranoside. As regards to the *A. unedo* fruits, phenolic compounds previously identified in this matrix were mainly gallic acid derivatives, anthocyanins and proanthocyanidins (Ayaz et al., 2000; Alarcão-E-Silva et al., 2001; Males et al., 2006; Pawlowska et al., 2006; Pallauf et al., 2008). Table 1 presents the total phenolic content of *A. unedo* leaf and fruit extracts. Leaf extract showed a great phenolic content (170.3 ± 1.4 mg/g) that was 10-fold higher than that found in the fruit extract (16.7 ± 0.4 mg/g). Our results are in the range of values described in the literature for *A. unedo* leaf (Oliveira et al., 2009) and fruit (Alarcão-E-Silva et al., 2001). The highest content of total phenols in *A. unedo* leaves might account for the better results found in their antioxidant activity.

3.2. Reducing power

The reducing capacity of a specific compound or extract may be a valuable indicator of its antioxidant potential. Fig. 1 shows the reducing power of *A. unedo* extracts as a function of their concentration. In this assay, the presence of reducing agents in the extracts causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous (Fe^{2+}) form, leading to a color change of the test solution. Fe^{2+} concentration is monitorized by measuring the formation of Perl's Prussian blue at 700 nm, with rising absorbances indicating an increase in reducing power. Leaf extract exhibited a reducing power clearly stronger than that of fruit. EC_{50} values calculated for *A. unedo* extracts were significantly different ($P < 0.05$) (Table 1). The lower EC_{50} value that corresponds to the highest reducing capacity was obtained by leaf extract (0.318 ± 0.007 and 2.894 ± 0.049 mg/mL, for leaf and fruit extracts, respectively). These results reveal that *A. unedo* extracts, specially the leaf, may act as an electron donor and therefore react with

free radicals, converting them to more stable products, and terminate the free radical chain reaction.

3.3. DPPH radical scavenging activity

The scavenging activity on DPPH radicals assay is widely used as a basic screening method for testing the antiradicalar activity of a large variety of compounds (Amarowicz et al., 2004). DPPH is a stable synthetic radical that possesses a characteristic absorption maximum between 515 and 517 nm (deep violet color), which decreases significantly on exposure to radical scavengers (turn into a yellow colored hydrazine). The degree of discoloration indicates the radical scavenging potential of the antioxidant. As shown in Fig. 2, both extracts showed considerable antiradical efficiency. As expected, considering the high phenolics contents of *A. unedo* leaf, this material is much more effective than fruit. As in the reducing power assay, significantly differences ($P < 0.05$) were observed in the EC_{50} values calculated for the extracts on DPPH assay and follows similar behavior: 0.087 ± 0.007 and 0.790 ± 0.016 mg/mL for leaf and fruit extracts, respectively (Table 1). The results obtained in this assay reveal that *A. unedo* extracts are free radical scavengers and able to react with the DPPH radical, which might be attributed to their electron donating ability.

3.4. Human erythrocyte protection against free radical damage

To elucidate the biological relevance of the antioxidant activities of *A. unedo* extracts, the human erythrocyte was used herein as a cell-based model system. The AAPH-induced oxidative damage on human erythrocytes has been extensively studied as model for the peroxidative injury in biological membranes (Zou et al., 2001; Costa et al., 2009; Magalhães et al., 2009; Carvalho et al., 2010). AAPH generates peroxyl radicals ($ROO^{\cdot}$)

that attack the erythrocytes to induce the chain oxidation of lipids and proteins, disturbing the membrane organization and eventually leading to hemolysis.

In this study, the protective effect of the *A. unedo* extracts on hemolysis by peroxyl radical scavenging activity was investigated. Fig. 3 shows the antihemolytic effects of *A. unedo* leaf (50–100 μg/ml) and fruit (400–1600 μg/ml) extracts on AAPH-induced hemolysis in human erythrocytes. Erythrocytes incubated at 37ºC as a 2% suspension in PBS were stable with little hemolysis observed within 4 hours (3.5 ± 0.7%). When AAPH was added to the suspension of erythrocytes, hemolysis induction was time-dependent. The hemolysis is lagged, indicating that endogenous antioxidants in the erythrocytes, mainly glutathione, α-tocopherol, L-ascorbate and enzymes, such as catalase and superoxide dismutase, can efficiently quench radicals to protect them against free radical-induced hemolysis (Zou et al., 2001). Both *A. unedo* extracts protected the erythrocyte membrane from hemolysis induced by AAPH in a time- and concentration-dependent manner. Once more, the leaf extract showed higher protective effect against erythrocytes hemolysis than the fruit. The IC_{50} values determined for *A. unedo* leaf and fruit after 3 hours of incubation were 0.062 ± 0.002 and 0.377 ± 0.047 mg of extract/ml, respectively (Table 1). When the cells were incubated with highest concentration tested for each extract alone, hemolysis was maintained at a background level similar to that in the control samples (data not shown). As far as we know, this is the first study evaluating the antioxidant potential of *A. unedo* species in this cell model.

Polyphenols are well-known effective scavengers of free radicals (Bors et al., 1990; Nanjo et al., 1996). Therefore, phenolic compounds of *A. unedo* present in the incubation medium most likely quench the peroxyl radicals formed in the aqueous phase before these radicals attack the biomolecules of the erythrocyte membrane to cause oxidative hemolysis. Our results are in agreement with other studies showing that

polyphenolics are able to protect erythrocytes from oxidative stress or increase their resistance to damage caused by oxidants (Youdim et al., 2000; Costa et al., 2009; Magalhães et al., 2009; Carvalho et al., 2010).

Erythrocyte membrane lipids, when subjected to considerable oxidative stress, lose a hydrogen atom from an unsaturated fatty acyl chain, thus initiating lipid peroxidation that propagates as a chain reaction. In this study, the extent of lipid peroxidation was assessed by measuring the formation of MDA, a well-known carbonyl product of oxidative lipid damage (Esterbauer et al., 1991). As shown in Fig. 4, the MDA levels in the erythrocytes of the control group was low (0.049 ± 0.014 μM) at 3 hours. When the erythrocytes were incubated with *A. unedo* extracts in the absence of AAPH, MDA formation was maintained at a background level similar to that of the control group (data not shown). As expected, MDA level significantly increased (by 1638%) after incubation with 50mM AAPH when compared to the respective control. Under the oxidative action of AAPH, human erythrocytes treated with *A. unedo* extracts significantly decreased the AAPH effect ($P < 0.05$) in a concentration-dependent manner. Treatment with leaf and fruit extracts at the highest concentration for 3 hours reduced MDA levels by 61% and 56%, respectively, over the AAPH-treated cells. The IC_{50} values, defined as the amount of extract that inhibits 50% of AAPH-induced lipid peroxidation, were respectively 0.075 ± 0.014 and 0.732 ± 0.452 mg of extract/mL for leaf and fruit extracts (Table 1).

Inhibition of lipid peroxidation by antioxidant compounds is a crucial property by which they can mitigate the induction and/or propagation of oxidative stress related diseases. Thus, it may be inferred that *A. unedo* extracts has potential as a preventive medicine against oxidative stress and damage to cellular macromolecules and membranes.

In conclusion, our results demonstrated antioxidant and free radical scavenging efficiency for *A. unedo* leaf and fruit aqueous extracts in both chemical and cell-based model assays. Their constituents scavenge different free radicals and exert protective effects against oxidative damage to biological macromolecules like lipids. *A. unedo* antioxidant capacity was found to be excellent in the leaf extract, which seems to be attributed to the higher levels of phenolic compounds.

As far as we know, this is the first work reporting the antioxidant properties of *A. unedo* species in human biological membranes. It is therefore suggested that *A. unedo*, particularly its leaf, may have prospective clinical use as preventive and/or therapeutic agents in oxidative stress-related diseases.

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Figure 1. Reducing power of *A. unedo* leaf and fruit extracts. Each value is expressed as mean ± SD of three independent assays.

Figure 2. Scavenging activity on DPPH radicals of *A. unedo* leaf and fruit extracts. Each value represents the mean ± SD of three independent assays.

Figure 3. Effect of *A. unedo* (A) leaf and (B) fruit extracts on AAPH-induced hemolysis in human erythrocytes. Erythrocyte suspension at 2% hematocrit was preincubated with extracts at the indicated concentrations for 30 min at 37ºC. The cell suspension was then incubated with 50 mM AAPH for 4 hours at 37ºC. In all experiments, control erythrocytes (incubated with PBS only) and AAPH-treated erythrocytes (incubated with 50 mM AAPH) were used. Values are expressed as mean ± SD of four independent experiments. *Represents significant results ($P < 0.05$) when the treated group was compared with the AAPH group, at the respective time; #Represents significant results ($P < 0.05$) when the treated group was compared with the control group, at the respective time.

Figure 4. Effects of *A. unedo* leaf and fruit extracts on AAPH-induced lipid peroxidation. Erythrocyte suspension at 5.2% hematocrit was incubated with PBS (control) or preincubated with the extracts at the indicated concentrations for 30 min at 37ºC. The cell suspension was then incubated with 50 mM AAPH for 3 hours at 37ºC. Values are expressed as mean ± SD of four independent experiments. *Represents significant results ($P < 0.05$) when the treated group was compared with the AAPH group.

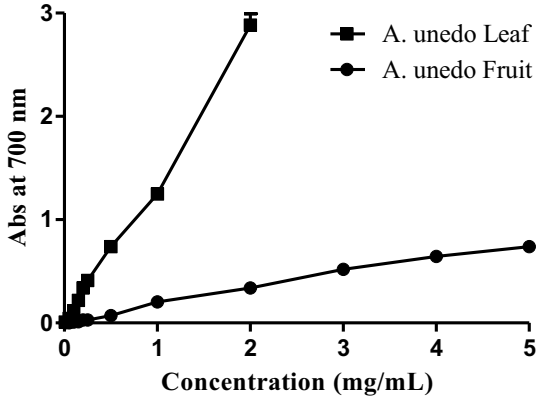

A. unedo Leaf
A. unedo Fruit
Abs at 700 nm
Concentration (mg/mL)
0
1
2
3
4
5

Figure 1

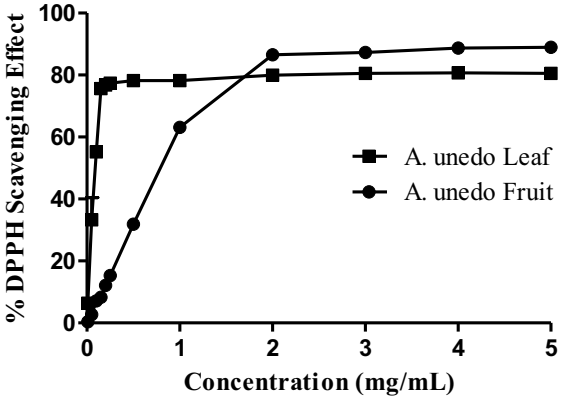
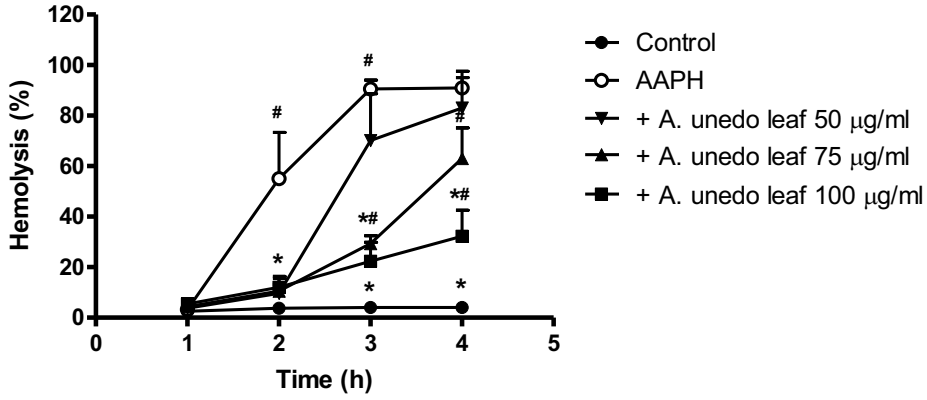

% DPPH Scavenging Effect
100
80
60
40
20
0
0
1
2
3
4
5
Concentration (mg/mL)
A. unedo Leaf
A. unedo Fruit

Figure 2

A


Hemolysis (%)
Time (h)
Control
AAPH
+ A. unedo leaf 50 μg/ml
+ A. unedo leaf 75 μg/ml
+ A. unedo leaf 100 μg/ml

B

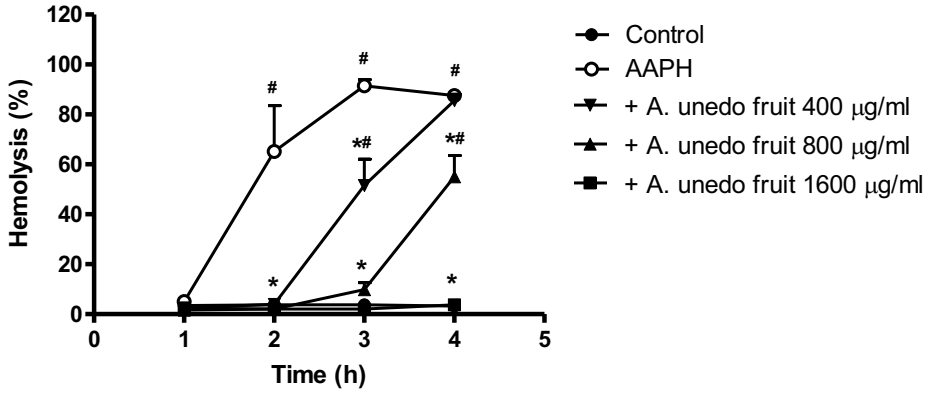

Hemolysis (%)
Time (h)
Control
AAPH
+ A. unedo fruit 400 μg/ml
+ A. unedo fruit 800 μg/ml
+ A. unedo fruit 1600 μg/ml

Figure 3

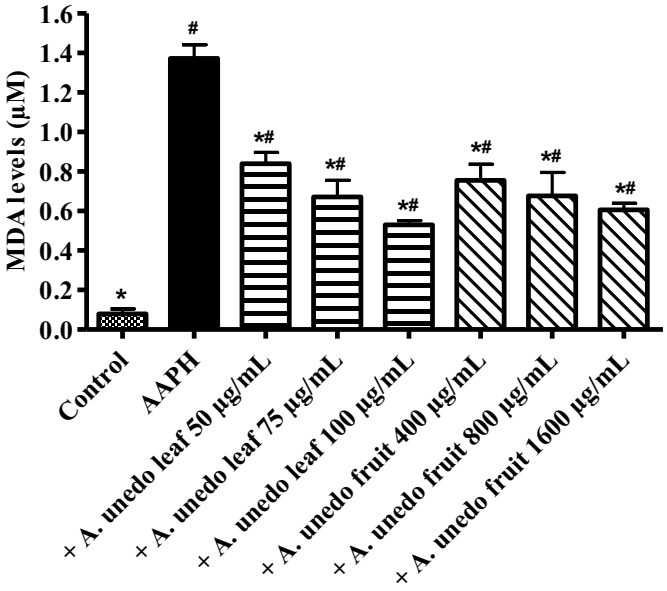

MDA levels (µM)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
#
*#
*#
*#
*#
*#
*#
Control
AAPH
+ A. unedo leaf 50 µg/mL
+ A. unedo leaf 75 µg/mL
+ A. unedo leaf 100 µg/mL
+ A. unedo fruit 400 µg/mL
+ A. unedo fruit 800 µg/mL
+ A. unedo fruit 1600 µg/mL

Figure 4

Table 1. Total phenolic content, EC_{50} values determined for the reducing power and DPPH radical scavenging activity, and IC_{50} values calculated for the antihemolytic and lipid peroxidation (LPO) inhibitory activities of *A. unedo* leaf and fruit extracts.

Sample	Phenolic content (mg/g)	Reducing power EC_{50} (mg/mL)	DPPH scavenging activity EC_{50} (mg/mL)	Antihemolytic activity IC_{50} (mg/mL)	LPO inhibitory activity IC_{50} (mg/mL)
Leaf	170.3 ± 1.4 a	0.318 ± 0.007 a	0.087 ± 0.007 a	0.062 ± 0.002 a	0.075 ± 0.014 a
Fruit	16.7 ± 0.4 b	2.894 ± 0.049 b	0.790 ± 0.016 b	0.377 ± 0.047 b	0.732 ± 0.452 b

Each value represents mean ± SD. Means marked with different letters, within each column, are significantly different ($P < 0.05$).