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Antioxidant and anti-inflammatory activities of *Arbutus unedo* aqueous extract

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ABSTRACT

Objective: To evaluate the antioxidant and anti-inflammatory activities of aqueous extract of *Arbutus unedo* (*A. unedo*) leaves.

Methods: In this context, the *in vitro* antioxidant activity was demonstrated by 2,2-diphenyl-1-picrylhydrazyl, hydroxyl radical and H_2O_2 radical scavenging, ferrous ion chelating, ferric reducing power, total antioxidant capacity and by the protection against peroxidation of β-carotene-linoleic acid in emulsion. The anti-inflammatory activity was evaluated first by studying the membrane of human red blood cells against different hypotonic concentrations of NaCl and against heat, inhibiting the denaturation of albumin.

Results: Total phenolic and flavonoid content were found respectively [(207.84 ± 15.03) mg gallic acid equivalent/g, and (13.070 ± 0.096) mg quercetin equivalent/g]. The extract displayed significant scavenging activity of some radicals such as 2,2-diphenyl-1-picrylhydrazyl [IC_{50} at (7.956 ± 0.278) μg/mL], $^{\bullet}OH$ [IC_{50} = (1 015.74 ± 46.35 μg/mL)], H_2O_2 [IC_{50} = (114.77 ± 16.86) μg/mL] and showed a good antioxidant activity through ferrous ion chelating activity [IC_{50} = (1 014.30 ± 36.21) μg/mL], ferric reducing power [IC_{50} = (156.55 ± 17.40) μg/mL], total antioxidant capacity [IC_{50} = (461.67 ± 4.16) μg/mL] and β-carotene-linoleic acid protection against peroxidation [I % = (87.04 ± 1.21)% at 1 000 μg/mL].

Conclusions: *A. unedo* showed *in vitro* anti-inflammatory activity by inhibiting the heat induced albumin denaturation and red blood cells membrane stabilization. Our results show that aqueous leaf extract of *A. unedo* has good antioxidant activity and interesting anti-inflammatory properties. *A. unedo* aqueous extract can be used to prevent oxidative and inflammatory processes.

1. Introduction

Medicinal plants are considered as an important source of new molecules with high antioxidant potential. Polyphenols, commonly referred to as antioxidant compounds, play a major role in safeguarding health, and a protection against diseases like cancer, has recently been shown [1].

Free radicals are generated by various exogenous chemicals and several endogenous metabolic processes oxidize the biomolecules leading to cell death and tissue damage. The organism must keep free radicals at relatively low concentrations using various defense systems and antioxidant molecules such as glutathione [2].

Poorly absorbed and extensively metabolized polyphenols cannot act a direct antioxidant activity, but they have the ability to interact with target proteins to regulate various cellular and molecular processes, giving them biological activity [3].

Drug that plays a role in the stabilization of erythrocyte membrane against osmotic lysis and heat indicates their potential to maintain the integrity of biological membrane. Knowing that erythrocyte membrane resembles to lysosomal membrane, the stabilization of erythrocyte can be extrapolated to the stabilization of lysosomal membrane.

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The study protocol was performed according to the Helsinki declaration and approved by Scientific Committee of the Faculty of Biology (CSFB). Informed written consent was obtained from Hospital Department of Hematology (University Hospital Nedir Mohamed of Tizi-Ouzou).

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Arbutus unedo L. (Ericaceae family) (*A. unedo*), commonly called strawberry tree, is an evergreen shrub endemic to Mediterranean region [4]. It is widely used in traditional medicine, and nowadays many of its virtues have been scientifically proven as antioxidant, antihypertensive, anti-hyperglycemic and anti-inflammatory [5–7].

The objective of this work is to deepen the knowledge of antioxidant and anti-inflammatory capacities of *A. unedo* leaves.

2. Materials and methods

2.1. Plant collection

A. unedo leaves were collected in mid-December 2014 from Tizi-Ouzou, Algeria. The plant was identified by Doctor Mahmoud Laribi, botanist at Mouloud Mammeri University of Tizi-Ouzou department of vegetal biology, where a voucher specimen was deposited (FSBSA/MK/2105).

The sample was dried and then ground to obtain a powder that was stored at room temperature and in the dark until extraction.

2.2. Extract preparation

Twenty grams of powder were dissolved in 200 mL of distilled water. After 24 h of maceration at room temperature, the filtrate was lyophilized.

2.3. Determination of total phenolic content

The concentration of phenolic compounds in plant extract was determined using the Folin–Ciocalteu spectrophotometric method [8].

The reaction mixture was prepared by mixing 200 μL of extract (40 μg/mL) with 1 mL of Folin–Ciocalteu reagent (diluted ten times) and 800 μL of sodium carbonate (75 mg/mL). The mixture was incubated for 45 min at room temperature and the absorbance was measured against a blank at 760 nm.

The same procedure was repeated for the standard solution of gallic acid and the calibration curve was constructed. The results are expressed in mg of gallic acid equivalent per gram of extract.

2.4. Determination of total flavonoid content

The total content of flavonoids in leaves extract was determined using the aluminum chloride spectrophotometric assay [9,10]. The plant extract (1 mL) was mixed with the same volume of a methanolic solution containing 2% of aluminum trichloride. After incubation for 10 min, the absorbance of the reaction mixture was measured at 430 nm against a methanol blank.

A standard curve of quercetin was drawn and the results were expressed as mg of quercetin equivalent per gram of extract.

2.5. Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging activity

The free radical scavenging activity of the extract was measured using the stable free radical DPPH test according to the method described before [11,12]. A total of 250 μL of 0.8 mmol/L DPPH in ethanol was mixed with 3.75 mL of the extract. The reaction was carried out in triplicate and the absorbance was measured at 517 nm after 30 min in dark. L-Ascorbic acid was used as reference standard.

The percent of scavenging activity was calculated using the following equation.

$$\text{Scavenging activity } (\%) = [(\text{Ac} - \text{As})/\text{Ac}] \times 100$$

where, Ac stands for the absorbance of the control and As stands for absorbance of the sample.

2.6. β-Carotene bleaching assay

Antioxidant activity of the extract of leaves of the strawberry tree and level of butylated hydroxytoluene (BHT) were measured according to the published method [13,14]. The emulsion mixture was prepared in 50 mL round-bottom flask containing 1 mL of chloroform (high performance liquid chromatography grade), 0.5 mg β-carotene, 25 μL linoleic acid and 200 mg of Tween 40. Chloroform was completely evaporated using a vacuum evaporator at 40 °C for 10 min.

After evaporation, the mixture was immediately diluted in 100 mL of distilled water saturated with oxygen. The ethanolic stock solution of the extract (350 μL, concentrations were 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL) was mixed with 2.5 mL of emulsion. The same procedure was repeated with positive control BHT.

Absorbance of the mixtures was measured at 470 nm immediately after their preparation ($t = 0$ min) and at incubation time $t = 120$ min against the blank.

The percentage of inhibition was calculated with the following equation:

$$\text{Inhibition } (\%) = [(\text{Aa}_{120} - \text{Ac}_{120})/(\text{Ac}_0 - \text{Ac}_{120})] \times 100$$

where, Aa_{120} is the absorbance of the antioxidant at $t = 120$ min, Ac_{120} is the absorbance of the control at $t = 120$ min and Ac_0 is the absorbance of the control at $t = 0$ min.

2.7. Hydroxyl radical scavenging assay

Scavenging activity of hydroxyl radical of the extract was measured according to the method of Rajamanikandan *et al.* [15]. Three milliliters of the final reaction solution consisted of aliquots (500 μL) of various concentrations of the extract, 1 mL $FeSO_4$ (1.5 mmol/L), 0.7 mL hydrogen peroxide (6 mmol/L) and 0.3 mL sodium salicylate (20 mmol/L). The reaction mixture was incubated for 1 h at 37 °C. L-Ascorbic acid was used as the standard.

The color development was measured at 560 nm against a blank.

2.8. Hydrogen peroxide radical scavenging activity

The scavenging ability of the water extract of *A. unedo* on hydrogen peroxide was determined according to the method of Serteser *et al.* [16]. A solution of hydrogen peroxide (40 mmol/L) was prepared in phosphate buffer (pH 7.4). The extract in distilled water (3.4 mL) was added to a hydrogen peroxide solution (0.6 mL, 40 mmol/L). Absorbance of hydrogen peroxide at 230 nm was measured 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide.

The percentage of hydrogen peroxide scavenging by the extract and standard (L-ascorbic acid) was calculated using the following equation:

$$\text{Scavenged } H_2O_2\ (\%) = (1 - As/Ac) \times 100$$

where, Ac is the absorbance of the control (without the extract) and As is absorbance in the presence of the extract. The experiment was repeated in triplicate.

2.9. Ferrous ion chelating activity

Ferrous ion chelating activity was determined by inhibition of the formation of iron(II)–ferrozine complex, following the previous published method [17,18]. Briefly, 100 μL of 0.6 mmol/L $FeCl_2$ was added to 500 μL of different concentrations of the extract or ethylenediamine tetraacetic acid (EDTA) (positive control). The reaction mixture was adjusted to a final volume of 1.5 mL with methanol, and then 100 μL of 5 mmol/L ferrozine solution were added.

The mixture was shaken vigorously and left to stand at room temperature for 5 min. Absorbance was determined at 562 nm and percent of chelation was calculated using the following equation:

$$\text{Chelation}\ (\%) = \left(A_{sample}/A_{control}\right) \times 100$$

where, A_{sample} is the absorbance of the sample and $A_{control}$ is the absorbance of the control.

2.10. Ferric reducing power assay

Reducing power was determined by the method described by Oyaizu and Hazra *et al.* [19,20]. Different concentrations of the extract were mixed with 1.25 mL of 0.2 mol/L, pH 6.6 sodium phosphate buffer and 1.25 mL of potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 min.

After incubation, the reaction mixture was acidified with 1.25 mL of trichloroacetic acid (10%) and centrifuged at 3 000 r/min for 10 min. Finally, 0.5 mL of freshly prepared $FeCl_3$ (0.1%) was added to this solution, and the absorbance was measured at 700 nm. Ascorbic acid at various concentrations was used as standard.

2.11. Total antioxidant capacity

Total antioxidant capacity was estimated by phosphomolybdenum assay [21,22]. The tubes containing the extract and reagent solution (0.6 mol/L sulfuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate) were incubated at 95 °C for 90 min. Then the solution was cooled to room temperature and the absorbance was read at 695 nm. Ascorbic acid was used as standard.

2.12. Antihemolytic activity

2.12.1. Red blood cell suspension

Blood was obtained by venipuncture from healthy volunteers collected in heparinized tubes and centrifuged at 2 000 r/min for 10 min at 4 °C. After removing the plasma, red blood cells (RBCs) were washed for three successive times using phosphate buffer saline (PBS) (0.9% NaCl). The study protocol was performed according to the Helsinki declaration and approved by Scientific Committee of the Faculty of Biology (CSFB). Informed written consent was obtained from Hospital Department of Hematology (University Hospital Nedir Mohamed of Tizi-Ouzou).

2.12.2. Hypotonic solution induced hemolysis

Membrane stabilizing activity of the extract was assessed using hypotonic solution induced hemolysis, and the method was described by de Freitas *et al.* [23]. In hypotonic solution, the test sample consisted of washed stock erythrocyte (RBC) suspension (40 μL) with 1 mL of hypotonic solution (0.1%, 0.3%, 0.5%, 0.7%, 0.9% NaCl) in sodium PBS (pH 7.4) containing either of the different concentrations of aqueous extract.

The mixture was incubated for 30 min at 37 °C under gentle stirring, centrifuged for 10 min at 2 000 r/min and the absorbance of the supernate was measured at 540 nm.

$$\text{Inhibition of hemolysis}\ (\%) = [(OD1 - OD2)/OD1] \times 100$$

where, OD1 is the optical density of hypotonic-buffered saline solution alone (control) and OD2 is the optical density of test sample in hypotonic solution.

2.12.3. Heat induced hemolysis

Different concentrations of the extract (μg/mL) or aspirin dissolved in isotonic PBS (pH 7.4) was mixed with 1 mL of 2% RBCs suspension. The reaction mixture was incubated in a water bath at 56 °C for 30 min. After incubation, the tubes were cooled under running tap water, then centrifuged at 2 000 r/min for 10 min and the absorbance of the supernatants was estimated at 560 nm [24].

The percentage of protection against heat induced hemolysis was calculated by using the following equation:

$$\text{Production}\ (\%) = \left(1 - OD_{sample}/OD_{control}\right) \times 100$$

2.12.4. Oxidant induced hemolysis

One milliliter of RBC suspension (5%) in PBS (pH 7.4) was incubated for 15 nm at 37 °C with 1 mL of the extract at different concentrations. After pre-incubation, the mixture was centrifuged at 2 000 r/min for 10 min at 4 °C, the supernatant was removed and packed RBCs were resuspended with 0.5 mmol/L HOCl in PBS. After this, the incubation was performed as previously described. The absorbance was determined at 540 nm [25,26].

$$\text{Production}\ (\%) = \left(1 - OD_{sample}/OD_{control}\right) \times 100$$

2.12.5. Inhibition of albumin denaturation

A solution of 0.2% (w/v) of egg albumin was prepared in a PBS (pH 6.4). A volume of 50 μL of the extract or standard at different concentrations was added to 5 mL of this stock solution.

The test tubes were heated at 72 °C for 5 min and then cooled. The absorbance of these solutions was determined at 660 nm [27].

3. Results

3.1. Phenolic content

Many studies report that phenolic compounds play an important role in human health due to their antioxidant activity.

The total phenols of *A. unedo* aqueous extract [(207.84 ± 15.03) mg gallic acid equivalent/g of extract] was calculated according to the equation $y = 0.006x + 0.027$ ($R^2 = 0.990$).

3.2. Flavonoid content

After construction of the calibration curve [$y = 0.022x + 0.182$ ($R^2 = 0.994$)], collected data clearly showed a good amount of flavonoid content in the aqueous extract [(13.070 ± 0.096) mg quercetin equivalent/g of extract].

3.3. DPPH scavenging activity

The DPPH radical scavenging activity was recorded in terms of inhibition percent as shown in Figure 1.

The parameter used to compare the radical scavenging activity of the extract and ascorbic acid is IC_{50} value, defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals. The IC_{50} value for ascorbic acid was (2.359 ± 0.091) μg/mL, which was comparatively lower than the IC_{50} [(7.956 ± 0.278) μg/mL] of aqueous extract.

3.4. β-Carotene bleaching

In the evaluation of the antioxidant activity, β-carotene bleaching method was used to measure the ability of the extract to inhibit lipid peroxidation. The antioxidant activity was expressed as percent inhibition. Figure 2 shows that the antioxidant activity increases with the increasing concentrations of the extract used.

Almost similar results were obtained for BHT [% Inhibition = 96.88 ± 0.34 (positive control)] and *A. unedo* (% Inhibition = 87.04 ± 1.21) at 1 000 μg/mL, which indicates a high potential antioxidant activity of the extract.

3.5. Hydroxyl radical scavenging

The hydroxyl scavenging activity of *A. unedo* aqueous extract was evaluated by its ability to compete with salicylic acid for hydroxyl radicals.

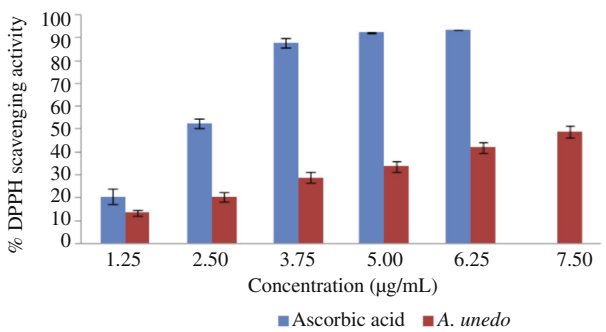

Figure 1. DPPH radical scavenging activity of ascorbic acid and aqueous leaf extract of *A. unedo*.

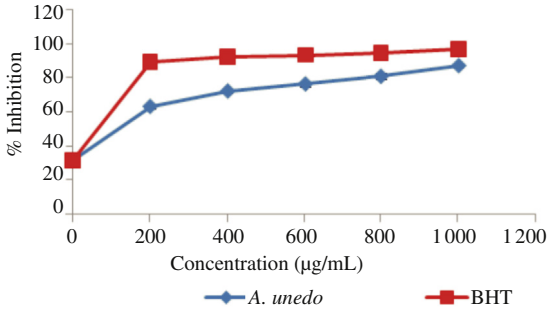

Figure 2. β-Carotene bleaching inhibition by BHT and aqueous extract of *A. unedo*.

As shown in Figure 3, hydroxyl radical scavenging increased with increase in concentrations. The ascorbic acid [IC_{50} = (758.83 ± 7.40) μg/mL] showed more effective scavenging ability when compared to that of aqueous extract [IC_{50} = (1 015.74 ± 46.35) μg/mL].

The maximum scavenging activity was found to be (97.80 ± 0.18)% for ascorbic acid and (79.23 ± 1.14)% for aqueous extract at 2 mg/mL.

3.6. Hydrogen peroxide radical scavenging activity

Scavenging activity of the extract and ascorbic acid as reference compound against hydrogen peroxide in terms of effective concentration was remarkably different and they were shown to be 83.14% (600 μg) and 96.3% (90 μg), respectively (Figure 4).

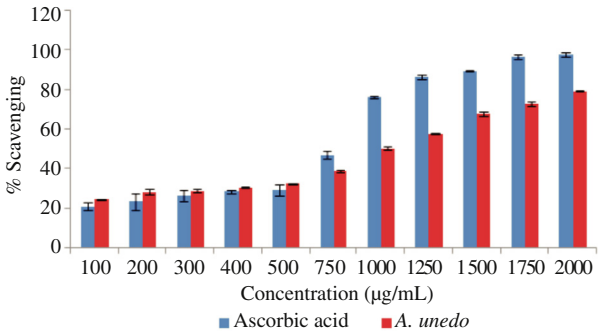

Figure 3. Hydroxyl radical scavenging activity of ascorbic acid and aqueous extract of *A. unedo*.

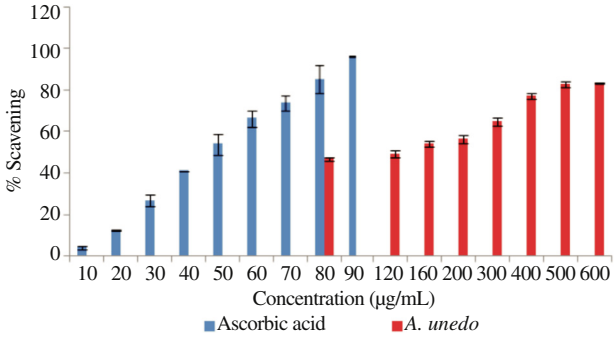

Figure 4. Hydrogen peroxide radical scavenging activity of ascorbic acid and aqueous extract of *A. unedo*.

According to the results, *A. unedo* showed an activity dependent on the concentration and the H_2O_2 scavenging IC_{50} was (114.77 ± 16.86) μg/mL, which indicates a less effective scavenging potential referring to ascorbic acid [IC_{50} = (49.19 ± 2.70) μg/mL].

3.7. Ferrous ion chelating activity

Antioxidant action may be of secondary type. One of the most important mechanisms is the chelating of pro-oxidant metals such as iron. Ferrozine forms a complex with Fe^{2+} with a characteristic red color, but in the presence of chelating agent, the complex formation is disrupted and the red color is decreased. Measurement of color reduction, therefore, allows the estimation of the chelating activity of the plant extract.

The metal chelating effect of investigated extract and EDTA were dependent on concentrations (Figure 5).

EDTA [IC_{50} = (57.21 ± 0.44) μg/mL] in this assay demonstrated relatively high activity in comparison to the extract [IC_{50} = (1 014.30 ± 36.21) μg/mL].

3.8. Ferric reducing power

The extract showed concentration-dependent reducing power. However, its reducing power [IC_{50} = (156.55 ± 17.40) μg/mL] was lower than that of ascorbic acid [IC_{50} = (56.72 ± 2.79) μg/mL] (Figure 6).

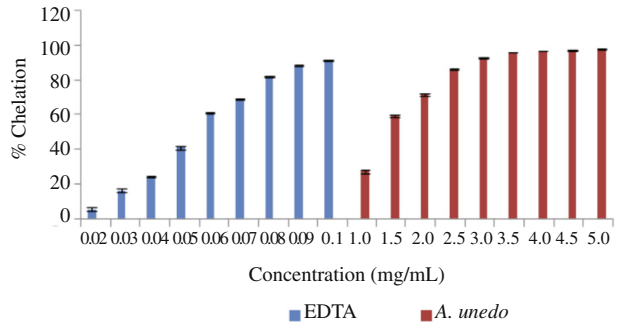

Figure 5. Ferrous ion chelating activity of EDTA and aqueous extract of *A. unedo*.

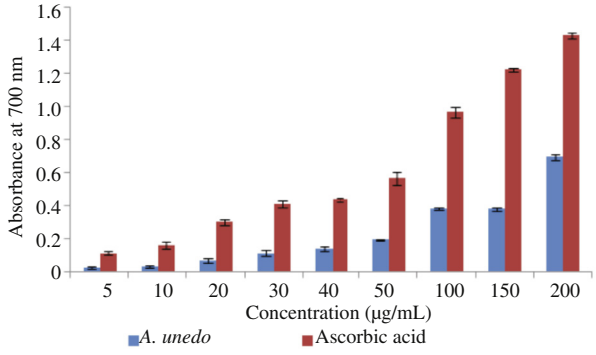

Figure 6. Ferric reducing power of ascorbic acid and aqueous extract of *A. unedo*.

3.9. Total antioxidant capacity

This test was based on the reduction of Mo(VI) to Mo(V) by the extract and formation at acid pH of green phosphate/Mo(V) complex.

Results showed the antioxidant activity of the extract and ascorbic acid in a dose dependent manner at concentrations 100–500 μg/mL. The IC_{50} value of antioxidant capacity for the ascorbic acid [(292 ± 7.54) μg/mL] was greater than the extract IC_{50} [(461.67 ± 4.16) μg/mL) (Figure 7).

3.10. Membrane stabilizing activity

As shown in Figures 8 and 9, the extract prevented the erythrocyte membrane against lysis induced either by hypotonic solution and heat.

For hypotonic solution induced hemolysis, at concentration range of 0.250–1.500 mg/mL, the extract showed significant inhibitory effect against RBCs hemolysis [(46.15 ± 0.70)%, (79.53 ± 0.43)%, (70.78 ± 1.38)%, (71.95 ± 2.26)% and (76.46 ± 1.80)% respectively for 0.1%, 0.3%, 0.5%, 0.7% and 0.9% of NaCl when the concentration of the extract was 1.500 mg/mL].

In heat induced hemolysis, the extract inhibited lysis of erythrocyte membrane in the range of (45.70 ± 1.30)%–(79.66 ± 1.92)% at concentration range of 0.025–0.500 mg/mL. Aspirin demonstrated protection in the range of (25.65 ± 1.57)%–(58.49 ± 1.23)%.

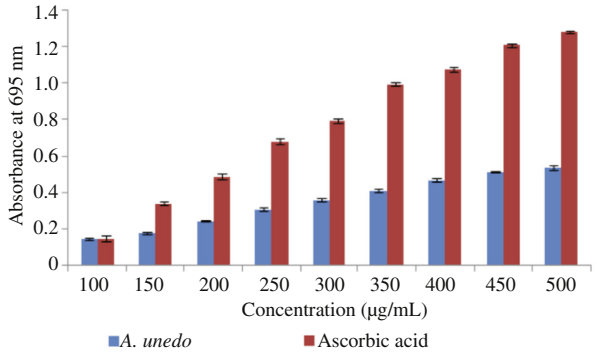

Figure 7. Total antioxidant capacity of ascorbic acid and aqueous extract of *A. unedo*.

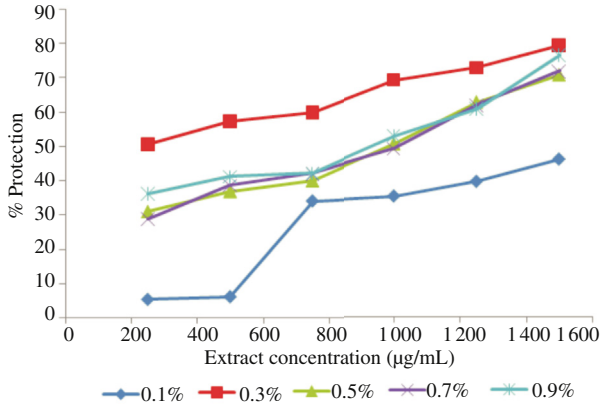

Figure 8. Effect of *A. unedo* aqueous extract on hypotonicity-induced hemolysis.

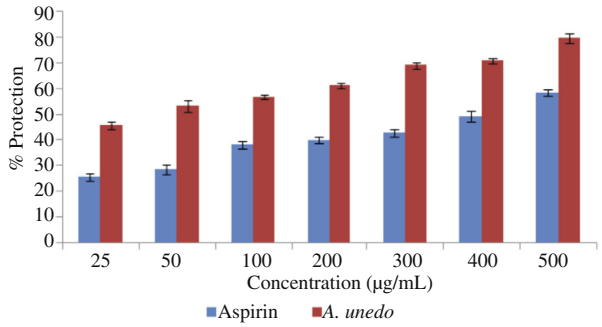

Figure 9. Effect of *A. unedo* aqueous extract on heat-induced hemolysis.

3.11. Oxidant induced hemolysis

As shown in Figure 10, protective effect of the extract against HOCl induced hemolysis was dose dependent. In fact, the hemolysis ratio gradually decreased with the increasing dose of the extract. Protection was already evident at 1 mg/mL of the extract with (73.90 ± 2.08)% protection.

3.12. Inhibition of albumin denaturation

Protein denaturation is involved in inflammation and plant extracts showing inhibition of denaturation are often tested for anti-inflammatory activity.

For inhibiting thermally induced denaturation of albumin, the extract showed an astonishingly effect at different concentrations as shown in Figure 11.

A maximum inhibition of (74.28 ± 0.86)% was observed at 500 µg/mL for the extract, and (92.23 ± 0.32)% at 500 µg/mL for aspirin, the anti-inflammatory standard.

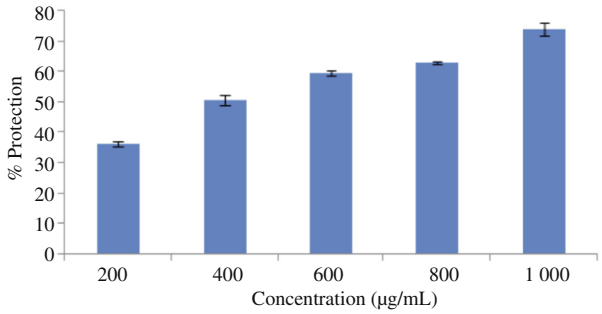

Figure 10. Effect of *A. unedo* aqueous extract on HOCl induced hemolysis.

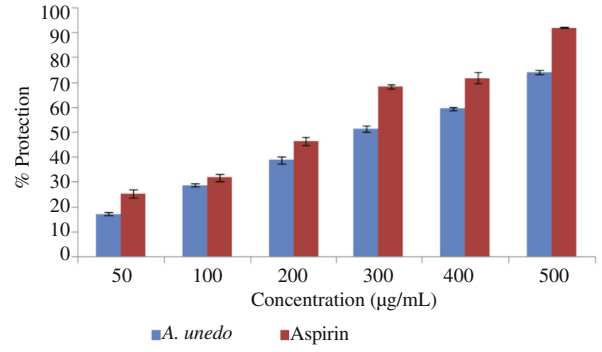

Figure 11. Effect of *A. unedo* aqueous extract on albumin denaturation.

4. Discussion

Antioxidants are substances that prevent various pathologic changes in living cell by protecting oxidation of its major constituents (proteins, lipids, carbohydrates and DNA) [28]. In plant extracts, the antioxidant activity is performed by polyphenols and is correlated positively with their concentration. From the obtained results, we can see a great antioxidant potential of the studied extract when compared to other species described in the literature.

So, the phenolic content of the studied extract exceed that reported by Mendes *et al.* [29] with (170.3 ± 1.4) mg GAE/g and Oliveira *et al.* [30] with (172.21 ± 6.29) mg GAE/g, that of *Malva parviflora* L. with (0.83 ± 0.063) mg/g [31], *Anacardium excelsum* with (1.49 ± 0.03) mg/g and *Piper putumayoense* with (10.20 ± 0.03) mg/g [32].

In the first part of our study, we focused on the antioxidant activity of the aqueous extract of *A. unedo*.

The large amount of polyphenols in this plant can explain the scavenging and antioxidant activity, due to their loss of proton properties, chelate formation, dismutation of radicals and giving up hydrogen atoms from their hydroxyl groups with radicals to form stable phenoxyl radicals [33].

Monitored in our study by several tests, this potential was confirmed in comparison to other works.

So, DPPH scavenging activity, in comparison to other works on the aqueous extract of *A. unedo* [487.2 µg/mL [34], (73.7 ± 6.3) µg/mL [30] and (87 ± 7) µg/mL [29]], our sample presented a lower IC_{50} which revealed a higher scavenging activity.

The hydroxyl radical, the representative reactive oxygen species generated in biological systems, can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions.

Considered as the most reactive free radical, hydroxyl radical is most often implicated in the pathology of free radical because of its ability to interact with intracellular targets such as DNA, thus causing significant damage.

The extract was found to be a less effective scavenger of hydroxyl radical compared to reported results [(80.160 ± 0.015)% at 1 mg/mL] for *Quisqualis indica* [35].

Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because it can give rise to hydroxyl radical in the cells [36,37]. Thus, the removal of H_2O_2 is very important for antioxidant defense in cell systems or food [38].

Compared to the scavenging effect reported by Kumar and Pandey [39] with (26.02 ± 1.91)% at 250 µg/mL, our extract showed a better scavenging activity with (60.53 ± 1.18)% at the same concentration.

The ability of the extract to slow down the β-carotene bleaching is used in the evaluation of its antioxidant activity and its ability to inhibit lipid peroxidation.

The inhibition percentage for *A. unedo* extract at 1 mg/mL, was significantly important compared to those reported by Norhaiza *et al.* [40] for *Labisia pumila* var. *alata* with (89.72 ± 0.95)% and *Labisia pumila* var. *pumila* with (59.09 ± 2.24)% at 40 mg/mL.

These data lead us to believe the great biological activity of the extract such as anti-cancer activity, for which one of the causes of its occurrence is lipid peroxidation.

A. unedo showed a stronger chelating activity ferrous ion chelating activity (IC_{50}) compared to reported data for aqueous extract of *Smilax excelsa* with (1.55 ± 0.06) mg/mL] [41].

This chelating potential indicated a significant protective activity of the extract against oxidative damage by sequestering iron (II) ions that may turn into catalyst for Fenton-type reactions or participate in metal-catalyzed hydroperoxide decomposition reactions [42].

Ferric reducing power is a simple test of antioxidant capacity, and often used as indicator of antioxidant potential for a plant extract. In this test, antioxidant electron donation leads to the neutralization of the free radical [43].

An increase in absorbance corresponds to an increase of the reducing power of the extract tested [44,45].

A. unedo water extract was characterized by higher ferric reducing power than other data reported for the same water extract of the plant [IC_{50} = (287.7 ± 15.6) μg/mL] [30].

Electron donating capacity and antioxidant activity are two related concepts and reflecting reducing power of a plant extract. The antioxidant molecules present in the extract play a reductant role and cause the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form.

A. unedo leaves extract showed greater total antioxidant capacity compared to *Pistacia lentiscus* [IC_{50} = (500.0 ± 22.3) μg/mL] [46]. This result suggests an important electron donating ability of the extract and so a great antioxidant capacity.

In the second part of our study, we explored anti-inflammatory activity of *A. unedo* aqueous extract through the study of its ability to stabilize RBCs membrane.

For hypotonic solution induced hemolysis, compared to 24.5% produced by *Momordica charantia* aqueous extract at 2.0 mg/mL [47], the studied extract presented stronger protection.

In heat induced hemolysis, compared to *Murraya paniculata* [(33.49 ± 0.51)% at 2 mg/mL] [48], the plant extract showed greater protection.

While the protection percentage against HOCl induced hemolysis was lower than that recorded for the aqueous extract of *Rhus typhina* [(61.06 ± 2.53)% at the concentration of 20 μg/mL] [49].

These results provide evidence of anti-inflammatory activity of the extract which showed a good protective effect of RBCs against heat, oxidant and hypotonic solution induced hemolysis. This feature can be explained by the ability of the extract to edit the calcium influx in erythrocytes [50].

Knowing that the erythrocyte membrane resembles to lysosomal membrane and as such, the effect of extracts on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane [51]. The anti-inflammatory activity can also be explained by the inhibition of release of lysosomal content at the site of inflammation [52].

During the investigation of the activity of the plant extract on albumin denaturation we observed that *A. unedo* extract showed a greater protection comparatively to data observed for *Erythrina indica* [(65.21 ± 1.77)% at 800 μg/mL] [53].

According to the fact that proteins denaturation is the cause of inflammation and rheumatoid arthritis, the protection of albumin denaturation confirms and contributes to anti-inflammatory activity of *A. unedo* extract.

This study demonstrated *in vitro* antioxidant and anti-inflammatory activities of *A. unedo*. Aqueous leaves extract, through scavenging, chelating and reducing activities indicated in the performed tests, showed a good antioxidant activity. Furthermore, the protection of RBCs indicated a membrane stabilizing effect of the extract.

These results lead to the conclusion that *A. unedo* aqueous extract has antioxidant and anti-inflammatory potential.

Conflict of interest statement

We declare that we have no conflict of interest.

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