Available online on 15.06.2020 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Total Phenolic Contents and Antioxidant Properties of Algerian *Arbutus unedo* L. Extracts

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ABSTRACT

Arbutus unedo L. is a plant widely distributed in the Mediterranean basin and North Africa, frequently used in traditional folk medicine to treat diabetes and arterial hypertension. This study aims to evaluate the phenolic composition and antioxidant activity of ethyl acetate (EA.E) and *n*-butanolic (But.E) extracts prepared from leaves of *Arbutus unedo*. Total phenolic and flavonoids contents were determined spectrophotometrically. The antioxidant activity was evaluated using DPPH•, OH•, H_2O_2, $O^{\bullet-}_2$, ß-carotene bleaching and AAPH-induced erythrocyte oxidative hemolysis assays. The phytochemical analysis showed the presence of polyphenols and flavonoids in both extracts. The high amount was observed in EA.E which exerted the stronger antioxidant effect, with IC_{50} values of 3.43 µg/mL, 323.45 µg/mL, 38.40 µg/mL and 11.56 µg/mL, in DPPH•, OH•, $O^{\bullet-}_2$ and H_2O_2 tests, respectively. Both extracts inhibited β-carotene bleaching, but EA.E is always more potent (92%) than But.E (85%). Furthermore, the EA.E showed the highest protective effect on erythrocyte hemolysis induced by AAPH, with half time hemolysis (HT_{50}) of 122.02 min at 40 µg/mL. Taken together, this study showed that *Arbutus unedo* leaf extracts possess strong antioxidant potential, which may be attributed to the presence of a high amount of polyphenolic constituents. So, this plant might be exploited as a potential source of natural antioxidant agents for pharmaceutical and food applications.

Keywords: oxidative stress, antioxidant, phenolic compounds, *Arbutus unedo.*

Article Info: Received 28 March 2020; Review Completed 21 May 2020; Accepted 08 June 2020; Available online 15 June 2020

Cite this article as:

Laouicha S, Senator A , Kherbache A, Bouriche H, Total Phenolic Contents and Antioxidant Properties of Algerian *Arbutus unedo* L. Extracts, Journal of Drug Delivery and Therapeutics. 2020; 10(3-s):159-168
http://dx.doi.org/10.22270/jddt.v10i3-s.4182

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1. INTRODUCTION

Endogenous metabolic processes in the human body might produce strongly different forms of reactive oxygen species (ROS). These species can be free radicals such as superoxide anion, hydroxyl radicals and non-radical species such as hydrogen peroxide, singlet oxygen [1]. Lipids, DNA and proteins are the major targets of ROS in the body [2]. The oxidative stress caused by the excess of uncontrolled ROS is involved in several pathological situations including hypertension, hypercholesterolemia, diabetes, heart failure [3], atherosclerosis, ischemia-reperfusion, alzheimer's disease, rheumatic arthritis, cancer, aging process [1], immunological and chronic inflammation disorders [4].

Currently, many studies are focused on searching for medicinal plants with anti-antioxidant activities and their active principles in order to find new therapeutic agents. These natural products have little severe side effects at low cost. The use of natural antioxidants present in medicinal and dietary plants such as polyphenols has gained enormous importance. Polyphenols possess ideal structural chemistry for free radical scavenging activity [5]. Antioxidant properties of polyphenols arise from their high reactivity as hydrogen or electron donors, and from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain-breaking function), and from their ability to chelate transition metal ions and then inducing the termination of the Fenton reaction [6].

Arbutus unedo L. (Ericaceae) is widely distributed in the Mediterranean basin and North Africa, as well as in other regions with hot summers and mild rainy winters [7]. This plant is frequently used in traditional folk medicine as a natural remedy for diabetes and arterial hypertension. The leaves of *Arbutus unedo* (*A. unedo*) are used as a urinary antiseptic, antidiarrheal, astringent, depurative, against blenorrhagia [8]. In Turkey, the tea prepared from leaves is used as a diuretic [9]. The fruits of *A. unedo* are consumed

mainly as a processed product but maybe a good source of antioxidants if consumed as fresh fruit. Processed products include alcoholic beverages such as cider and an increasingly popular industrially produced aromatic distillate. Other non-alcoholic strawberry tree products are jams, marmalades and jellies [10].

Due to the ethnobotanical importance of *A. unedo* and the fewer studies that prove their antioxidant activity, this study was conducted to show the total phenolic content and to assess the potential antioxidant capacity of the ethyl acetate and *n*-butanolic extracts of this plant.

2. MATERIALS AND METHODS

2.1. Plant material

Arbutus unedo leaves were collected in June 2017 from Setif region (Algeria). The plant was identified and authenticated taxonomically by Pr. Laouar Hocine (Faculty of Natural and Life Sciences, University of Setif 1, Algeria). A voucher specimen (AU 06/17) is deposited in the herbarium of the same faculty.

2.2. Extraction

The aerial part of *A. unedo* was cleaned, shadow dried and pulverized to dry powder. Ethyl acetate and *n*-butanolic extracts were prepared according [11]. A sample of 100 g was treated three times with 70% methanol. The filtered methanol extract was evaporated to dryness and the residue was dissolved in boiling water and kept 24 h at room temperature. After filtration, the aqueous solution was treated with ethyl acetate and *n*-butanol successively, and then their extracts were concentrated to dryness (yields: 4% and 7.5%, respectively). Both extracts were then stored at -32°C until use.

2.3. Animals

Swiss Albinos mice (25-30 g) of either sex were purchased from Pasteur Institute of Algiers (Algeria). All animals were kept to acclimatize under the laboratory conditions for one week and had free access to a standard commercial diet and water ad libitum. All procedures were performed following European Union Guidelines for Animals Experimentation (2007/526/EC).

2.4. Determination of total phenolic content

Total phenolic content was determined by the Folin-Ciocalteu method [12]. Briefly, 100 µL of each extract (1 mg/mL) or gallic acid (used as standard) was mixed with 500 µL of Folin-Ciocalteu reagent for 4 min. Then, 400 µL of sodium carbonate (7.5%) was added. After incubation for 90 min at room temperature, the absorbance was measured at 765 nm. All tests were performed in triplicate, and the concentration of total phenolic compounds was expressed as µg of gallic acid equivalent per mg of extract (µg GAE/mg extract).

2.5. Determination of total flavonoid content

Total flavonoid content was evaluated by a colorimetric method based on the formation of a flavonoid-aluminum complex [13]. A volume of 1 mL of $AlCl_3$ (2% in ethanol) was added to 1 mL of each extract or quercetin at different concentrations. After 20 min of incubation, the absorbance was measured at 430 nm, and the total flavonoïd concentration was expressed as µg quercetin equivalent per mg of extract (µg QE/mg extract).

2.6. Antioxidant activities

2.6.1. DPPH radical scavenging assay

The free radical scavenging activity of samples was measured [14]. To 2 mL of ethanolic solution of DPPH• (0.1 mM), 2 mL of the extracts dissolved in ethanol was added to obtain different concentrations (100 – 500 µg/mL). An equal volume (2 mL) of ethanol was added to the control without the test sample. Butylated Hydroxytoluene (BHT) was used as a reference. After 30 min, the decrease in the absorbance of the test mixture was recorded at 517 nm, and the inhibition percentage was calculated as follows: Inhibition (%) = [(Absorbance of control- Absorbance of the sample)/ Absorbance of control] X 100.

2.6.2. OH• scavenging activity

The hydroxyl radical scavenging activity of extracts was assayed [15]. Briefly, 0.5 mL of $FeSO_4$ (1.5 mM) and 0.35 mL of hydrogen peroxide (6 mM) were mixed with 0.1 mL of extracts or vitamin C, used as reference, at varied concentrations (100-1400 µg/mL). Then, 0.15 mL of sodium salicylate (20 mM) was added. After incubation for 20 min at 37°C, the absorbance was measured at 562 nm. The hydroxyl radical scavenging activity was calculated as follow: Hydroxyl radical scavenging activity (%) = [1-(A_1-A_2) / A_0] x 100, where A_0: mean absorbance without extract, A_1: mean absorbance in the presence of the extract, A_2: mean absorbance without sodium salicylate.

2.6.3. H_2O_2 scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was determined [16]. Briefly, 20 µL of each extract or ascorbic acid (standard) at different concentrations (10-200 µg/mL) was mixed with 100 µL of phosphate buffer (0.1 M, pH 5) and 20 µL of hydrogen peroxide solution (10 mM) and incubated 5 min at 37 °C. Then, 30 µL of ABTS (1.25 mM) and 30 µL of peroxidase (1U/ml) were added, and the reaction mixture was incubated 10 min at 37°C. The absorbance was measured at 405 nm using a 96-well microplate reader (ELX 800 de Bio-TEK instruments). The ability of extracts to scavenge the hydrogen peroxide was calculated according to the following equation: Hydrogen peroxide scavenging activity (%) = [(Ac - As)/Ac] x 100, where Ac is the absorbance of the control and As is the absorbance in the presence of extracts or standard.

2.6.4. $O^{\bullet-}_2$ scavenging activity

The capacity of *A. unedo* extracts to scavenge superoxide anion radicals was measured [5]. Superoxide radicals are generated in the mixture containing 500 µL NBT (156 µM in 0.1 M phosphate buffer, pH 7.4), 500 µL NADH (468 µM NADH in 0.1 M phosphate buffer, pH 7.4) and 50 µL of each extract or gallic acid (standard) at different concentrations (5-500 µg/mL). The reaction was initiated by adding 50 µL of PMS (60 µM in 0.1M phosphate buffer, pH 7.4) to the mixture. After 5 min of incubation at 25°C, the absorbance was measured at 560 nm against a blank (without PMS). Decreased absorbance of the reaction mixture indicates the increase of superoxide anion scavenging activity. The superoxide anion scavenging activity was calculated using the following equation: Superoxide anion scavenging activity (%) = [(Ac – As)/Ac] x 100, where Ac is the absorbance of the control and As is the absorbance in the presence of extracts or standard.

2.6.5. Reducing power

The reducing power of the extracts was determined [17]. In this method, antioxidant compounds present in the extracts from a colored complex with potassium ferricyanide,

trichloroacetic acid and ferric chloride, which was measured at 700 nm. The increase in absorbance of the reaction mixture indicates the reducing power of the samples. Different concentrations (20-100µg/mL) of extracts or BHT (reference) were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferrocyanide (1%) and incubated at 50°C for 20 min. A volume of 2.5 mL of trichloroacetic acid (10%) was added to the mixture and then centrifuged at 800 rpm for 10 min. After that, the upper layer of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride ($FeCl_3$) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.

2.6.6. ß-carotene bleaching assay

The ability of extracts to prevent ß-carotene bleaching was determined [18]. A fresh emulsion of ß-carotene/linoleic acid was prepared by dissolving 0.5 mg of β-carotene, 25 mL of linoleic acid and 200 mL of Tween 40 in 1 mL of chloroform. The chloroform was completely evaporated under vacuum in a rotatory evaporator at 45°C. Next, 100 mL of bi-distilled water was added, and the obtained mixture was vigorously stirred. Then, 2.5 mL of prepared emulsion were mixed with 0.5 mL of each extract (2 mg/mL) or BHT (standard). After incubation for 2 h at 50°C, the absorbance was measured at 470 nm. The antioxidant activity was calculated using the following equation:

Antioxidant activity % = [1- (At0 – At120) test / (At0-At120) control] x 100, where At0 is the absorbance measured at time zero and At120 is absorbance measured after 2 h.

2.6.7. Anti-hemolytic activity

The anti-hemolytic activity of the extracts was evaluated by the inhibition of 2,2,-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced oxidative erythrocyte hemolysis [19]. Blood was obtained from mice and diluted to 1% with phosphate buffer saline (PBS, pH 7,4). A 120 µL of erythrocyte suspension was mixed with 60 µL of extracts (40, 20, 10, 5 µg/ml in PBS). The reaction was initiated by the addition of 120 µL of AAPH (120 mM), and the mixture was incubated at 37°C, in order to induce free radical chain oxidation in the erythrocytes by generating aqueous peroxyl radicals by thermal decomposition of AAPH. The decrease of the absorbance was measured at 630 nm every 10 min for 4 h using a 96-well microplate reader (ELX 800 de Bio-TEK instruments). Trolox and PBS were used as a standard and negative control, respectively. The blood resistance to free radical attack is expressed by the time necessary to hemolyse 50% of the initial erythrocytes (half-hemolysis time, HT_{50} in min). A high HT_{50} corresponds to a good resistance of erythrocytes.

Statistical analysis

The obtained results were expressed as means ± SD. The statistical analysis was performed using one way ANOVA followed by Tukey's multiple comparison tests. The differences were considered statistically significant at $p<0.05$.

3. RESULTS

3.1. Total phenols and flavonoids content

The values of total phenolic and flavonoid contents of both extracts of *A. unedo* (**Table 1)** showed that ethyl acetate extract was the richest. It contains a high amount of total phenolic compounds, which were two folds higher than those found in the *n*-butanolic extract.

Table 1: Polyphenol and flavonoid content of ethyl acetate (EA.E) and *n*-butanolic (Burt.E) extracts of *Arbutus unedo*.

Extract	Polyphenols (µg GAE/mg extract)	Flavonoids (µg QE/mg extract)
EA.E	836.51 ± 90.54	53.99 ± 5.33
But.E	559.33 ± 68.74	3.75 ± 0.19

Values are mean of triplicate determination (n=3) ± SD.

3.2. Antioxidant activities

3.2.1. DPPH radical scavenging activity

The free radical scavenging activity of *A. unedo* extracts was evaluated by measuring its capacity to scavenge the stable free radical formed in solution by donating a hydrogen atom or an electron. Results shown in figure 1 suggest that *A. unedo* exhibits a noticeable antiradical activity against free radical DPPH. Indeed, the ethyl acetate extract exerted a strong and concentration-dependent free radical scavenging activity with an IC_{50} = 3.43 µg/mL, which is better than that obtained with But.E (IC_{50} = 13.77 µg/ml) and BHT (IC_{50} = 8.21 µg/ml). These results are in agreement with Pabuçcuoğlu et al. [20], who demonstrated that the ethanol and methanol extract from the same plant leaf displayed potent antioxidant activity in ABTS free radical assay.

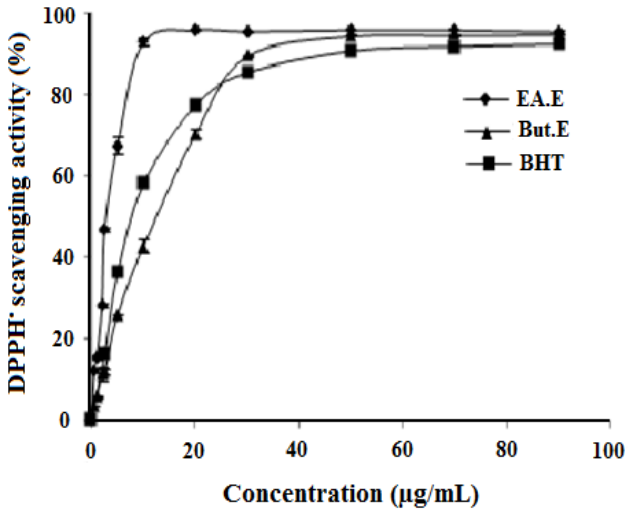

Figure 1: DPPH radical scavenging activity of ethyl acetate extract (EA.E), *n*-butanolic extract (But.E) of *Arbutus unedo* and BHT. Values are means ± SD (n=3).

3.2.2. OH· scavenging activity

Hydroxyl radical is the most reactive free radical, which can induce severe oxidative damages to biomolecules [5, 21]. It attacks proteins, DNA, the polyunsaturated fatty acid in membranes, and the most biological molecule it contacts [22]. It is known to be capable of abstracting hydrogen atoms from membrane lipids and leads to lipid peroxidation, which is a key process in many pathological conditions.

The obtained results suggest that *A. unedo* extracts are hydroxyl radical scavengers. This scavenging capacity may be due to their richness in phenolic compounds. Indeed, EA.E which is rich in this compounds exhibited the best effect (figure 2) with an IC_{50} = 323.45 µg/mL compared with But.E (IC_{50} = 611.19 µg/mL), but less important than ascorbic acid (IC_{50} = 290 µg/mL).

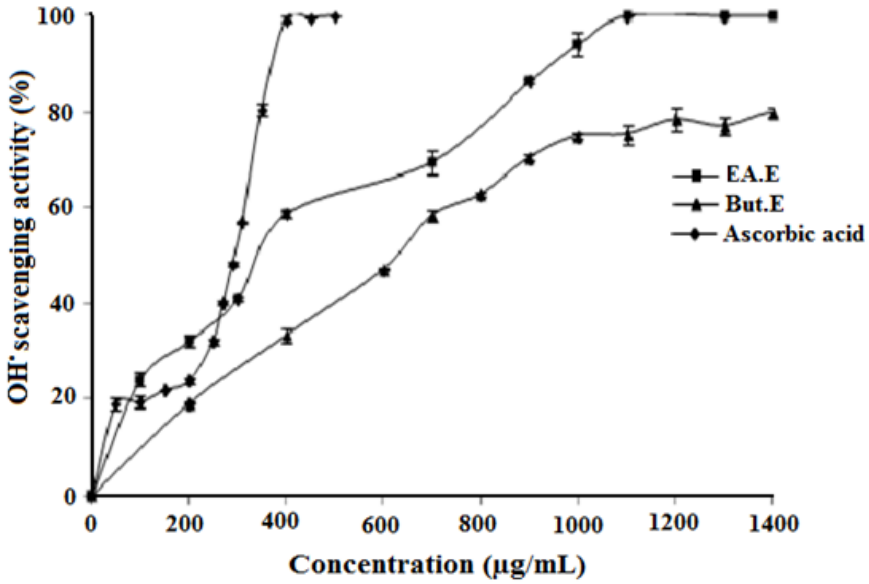

Figure 2: Hydroxyl radical scavenging activity of ethyl acetate extract (EA.E) and *n*-butanolic extract (But.E) of *Arbutus unedo* and ascorbic acid. Values are means ± SD (n=3).

3.2.3. H_2O_2 scavenging activity

Hydrogen peroxide can be toxic to cells because it may cross the cell membranes rapidly and produces many reactive hydroxyl radicals by the Fenton reaction [23] with Fe^{2+}, and possibly Cu^{2+} ions. Hydrogen peroxide can, therefore, inactivate a few enzymes directly by oxidation of essential thiol groups [22].

As shown in figure 3, *A. unedo* extracts had a strong H_2O_2 scavenging ability when compared to ascorbic acid used as standard, and this effect was concentration-dependent. At the concentration of 100 µg/mL, both extracts exerted the same percentage of H_2O_2 scavenging activity (98%). This effect was better than that obtained with ascorbic acid (87%).

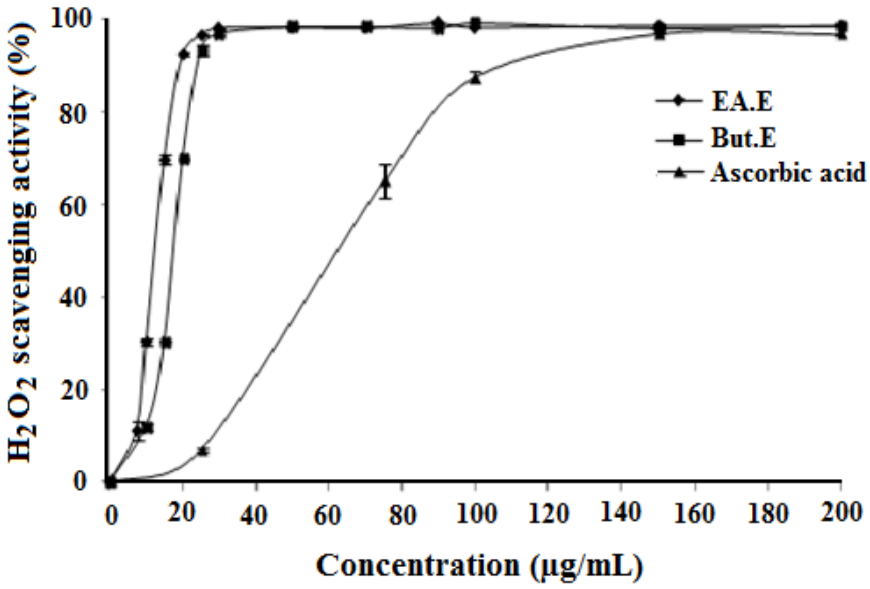

Figure 3: H_2O_2 scavenging activity of ethyl acetate extract (EA.E) and *n*-butanolic extract (But.E) of *Arbutus unedo* and ascorbic acid. Each value represents the means ± SD (n=3).

3.2.4. $O^{\bullet-}_2$ radical scavenging activity

It is well known that superoxide anions radicals are normally produced endogenously by several oxidative enzymes such as xanthine oxidase. In this study, superoxide anions radicals were derived in a non-enzymatic system (PMS-NADH-NBT) from dissolved oxygen by PMS-NADH coupling reaction reduces NBT [1, 24]. These radicals can directly initiate lipid peroxidation and damage, thereby biomolecules [22], and its effects may be magnified by producing more ROS contributing to tissue damage and various diseases [5]. The superoxide anion radical scavenging activity of *A. unedo* extracts, as shown in figure 4, is concentration-dependent. The ethyl acetate extract exhibited much higher activity (IC_{50} = 38.40 µg/mL) than gallic acid (IC_{50} = 52.23 µg/mL), used as reference and But.E which showed the smallest effect with an IC_{50} of 103.80 µg/mL. These differences are statistically significant ($p<0.05$). This capacity to scavenge superoxide anion may be attributed to the amount of phenolic compounds in the extracts, as ethyl acetate extract showed the best antioxidant activity.

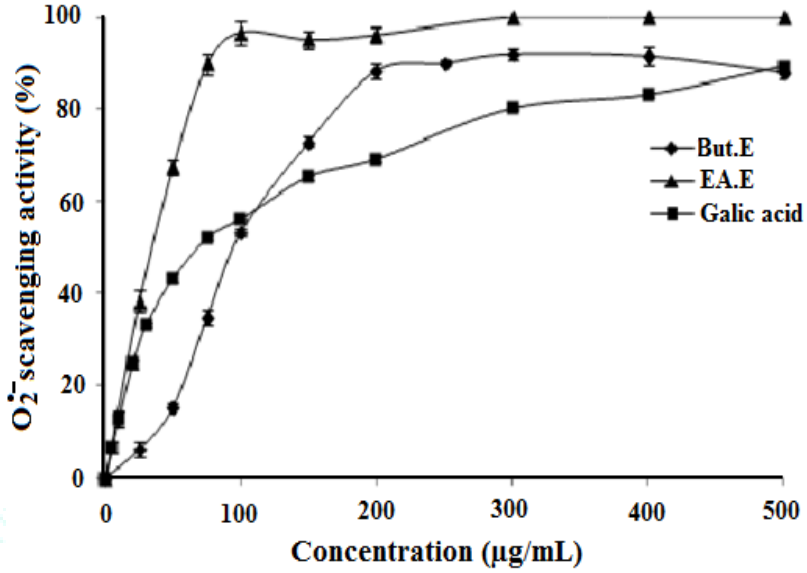

Figure 4: Anion superoxide radical scavenging activity of ethyl acetate extract (EA.E), *n*-butanolic extract (But.E) of *Arbutus unedo* and gallic acid. Values are means ± SD (n=3).

3.2.5. Total reducing capacity

The reduction of ferrous ion (Fe^{3+}) to ferric ion (Fe^{2+}) is measured by the strength of the green-blue color of the solution, which absorbs at 700 nm [17]. The obtained results suggest that both extracts exhibited a good concentration-dependent reducing power (figure 5). The EA.E exerted the stronger reducing power with IC_{50} = 12.25 µg/mL, followed by the But.E and BHT with IC_{50} of 21.47 µg/mL and 23.09 µg/mL, respectively.

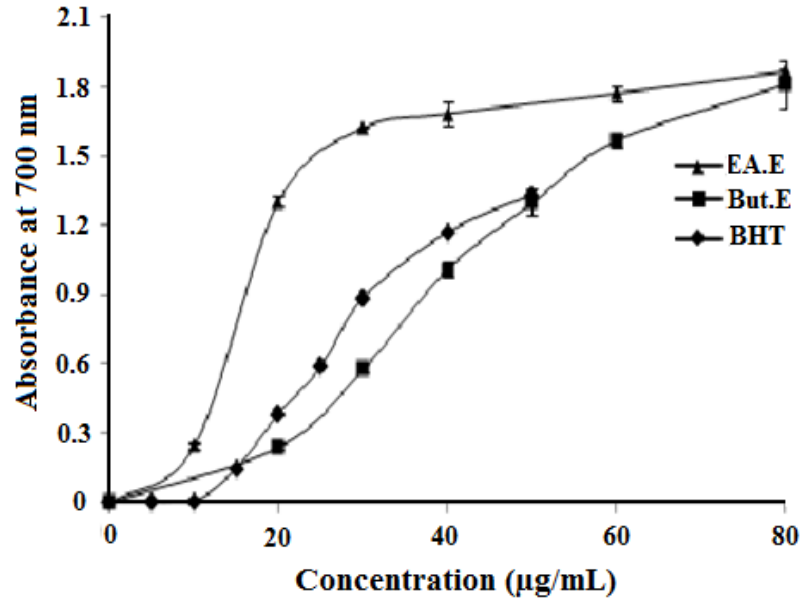

Figure 5: Reducing power of ethyl acetate extract (EA.E), *n*-butanolic extract (But.E) of *Arbutus unedo* and BHT. Values are means ± SD (n=3).

3.2.6. ß-Carotene bleaching

Lipid peroxidation is a chain reaction initiated by the hydrogen abstraction or the addition of oxygen radicals, resulting in the oxidative damage of polyunsaturated fatty acids of biological membranes [25], producing thereby derivatives such as hydroperoxides and malonaldehyde that can be used to quantify the degree of damage in the cell. These derivatives play a crucial role in the pathogenesis of many diseases [26]. In this study, the ability of *A. unedo* to inhibit lipid peroxidation was tested by the β-carotene bleaching method. Linoleic acid acts in this assay as a free radical generator that produces peroxyl radicals (hydroperoxides) under thermally induced oxidation. Peroxyl radicals react with β-carotene and lead to its rapid discoloration [27]. The presence of antioxidants can hinder the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system [28]. The results of the present study (figure 6) indicated that both extracts had acted as an effective antioxidant in the ß-carotene linoleic acid model system. The absorbance in the presence of both extracts of *A. unedo* was very low and remained stable during the 120 min of incubation. The activity of the *n*-butanolic extract is less (85%) than that obtained with EA.E, which showed the same effect (92%) as the standard (BHT).

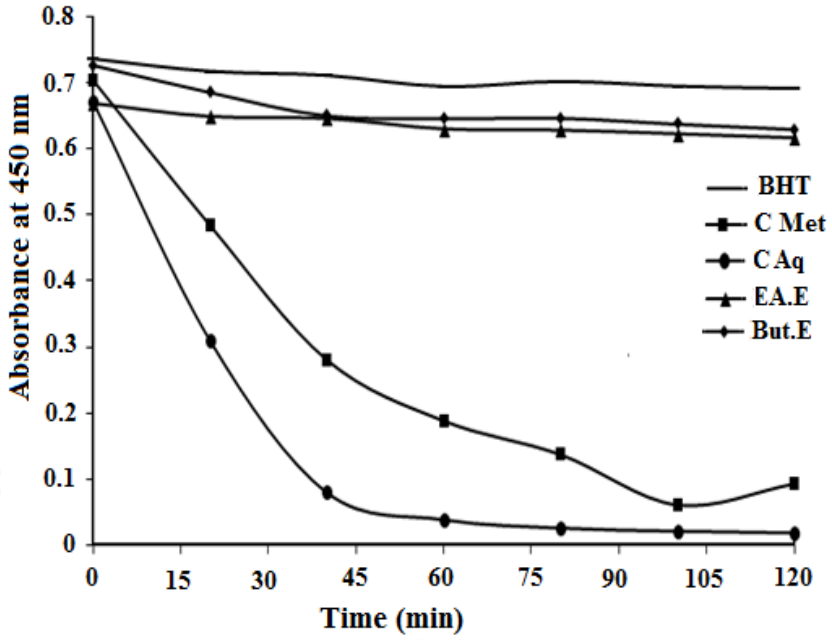

Figure 6: Kinetics of antioxidant activity of *Arbutus unedo* ethyl acetate extract (EA.E), *n*-butanolic extract (But.E) and BHT in a ß-carotene-linoleic acid system. C Met: methanolic control, C Aq: aqueous control. Values are expressed as means ± SD (n=3).

3.2.7. Anti-hemolytic effect of *Arbutus unedo* extracts

Results of the effect of *A. unedo* extracts and Trolox on erythrocyte hemolysis induced by AAPH were reported in figure 7 and table 2. Both extracts exhibit a dose-dependent effect, and the highest protective effect was obtained with EA.E, which possessed an approximate effect to that exerted by Trolox. At 40 µg/mL, the calculated half time hemolysis (HT_{50}) values are 122.02 min and 115.26 min, respectively. These values are statistically more important than that obtained with But.E at the same dose (HT_{50}= 73.48 min) and with the negative control (46.5 min).

Table 2: The antihemolytic activity of ethyl acetate (EA.E) and *n*-butanolic t (Burt.E) extracts of *Arbutus unedo* and Trolox.

	Hemolysis half-time (min)			
Samples	5µg/mL	10µg/mL	20µg/mL	40µg/mL
EA. E	66.67 ± 1.15**	81.04 ± 3.73***	109.42 ± 2.03***	122.02 ± 3.51***
But. E	49.15 ± 4.55 ns	56.68 ± 3.12**	62.86 ± 1.00***	73.48 ± 2.63***
Trolox	68.03 ± 8.10 **	79.95 ± 2.54***	92.59 ± 4.87***	115.26 ± 5.77***
Control	46.50 ± 0.56			

The values are means ± SD (n=3). **P<0.01; ***P<0.001; ns: Not significant.

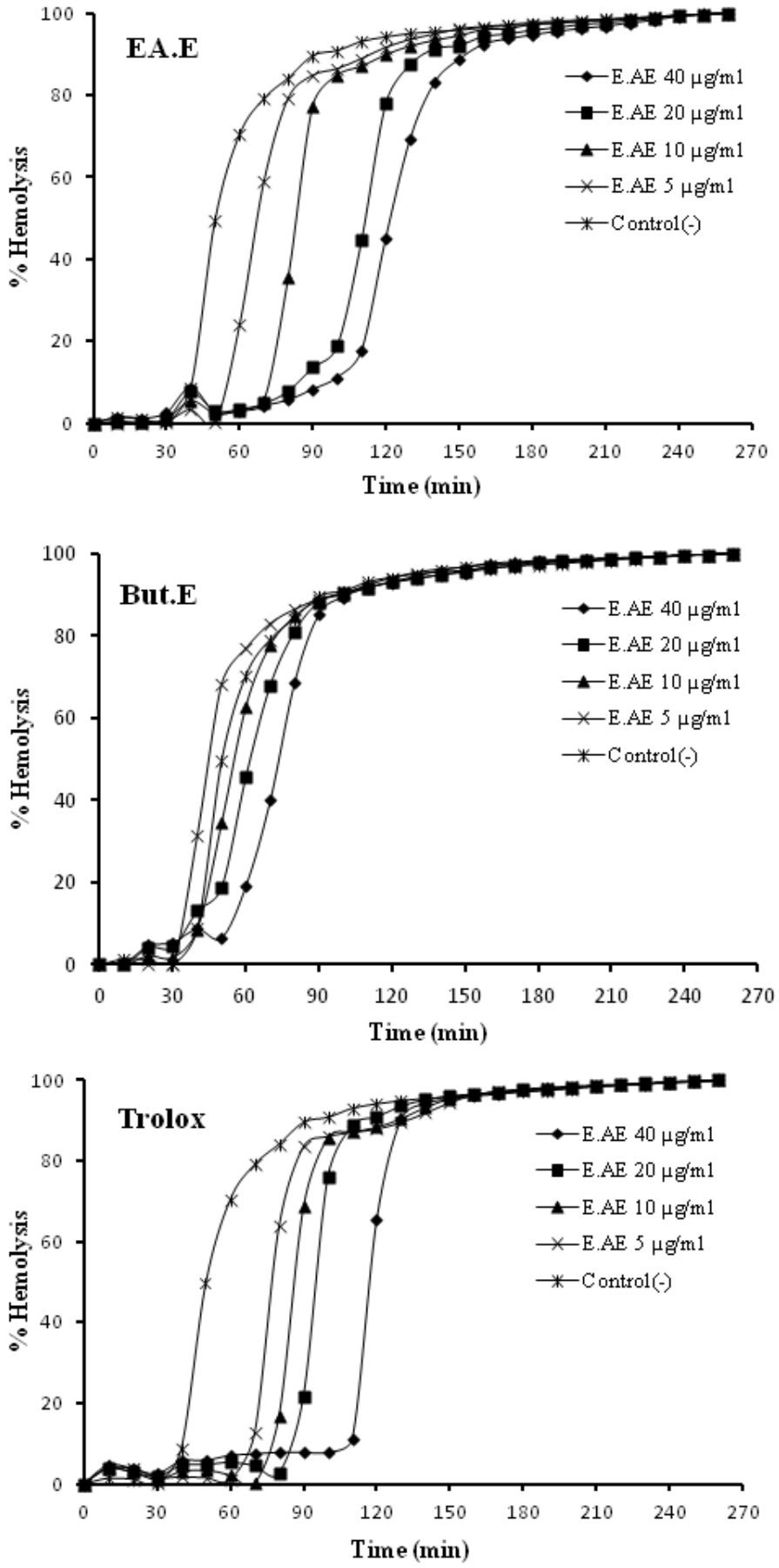

Figure 7: Protective effect of ethyl acetate extract (EA.E), *n*-butanolic extract (But.E) of *Arbutus unedo* and Trolox against AAPH induced erythrocyte hemolysis. The values are means ± SD (n=3).

4. DISCUSSION

Bioactive compounds from natural sources such as flavonoids and phenolic acids are well known for the prevention and/or treatment of various types of human diseases. There are close relationships and positive correlation between the phenolic content and antioxidant activity of plant extracts [29]. Several studies have focused on the biological activities of phenolic compounds and flavonoids, which are potential antioxidants agents [30]. These bioactive compounds can replace the synthetic antioxidants which are restricted for their side effects.

The ethyl acetate extract of *A. unedo* leaves was found to have a high quantity of polyphenols and flavonoids, and this could be due to different degrees of the polarity of the solvents used for the extraction of polyphenolic compounds. The content of flavonoids significantly differs from one plant sample to another, and this may be due to various factors, such as the type of soil, microclimatic conditions, geographic position, size, age and vegetational stage of plants and leaves[31].

Antioxidants can exert their protecting effects by various mechanisms, as prevention of radical chain reactions of oxidation and they act by inhibiting the initiation and propagation steps leading to the termination of the reaction and delay the oxidation process [30]. They also act by binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging or preventing the generation of ROS; subsequently, they retard the progress of many chronic diseases [24]. The ethyl acetate extract was found to possess the strong radical scavenging activity against DPPH•, OH•, H_2O_2 and $O^{\bullet-}_2$ and these results are correlated well with phenolic content, evidencing its ability to have extracted a considerable amount of polyphenols and flavonoids which are responsible for antioxidant activities in many plants species [30].

Flavonol glycosides and tannins might have been the active principles responsible for the antioxidant activity of *A. unedo* leaf [20]. The antioxidant activity of these compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals by their hydrogen donating ability [32], quenching singlet and triplet oxygen or decreasing peroxides [33]. These polyphenols contain conjugated ring structures and hydroxyl groups, which can act as an antioxidant by scavenging free radicals, lipid peroxy radicals and stabilizing free radicals involved in these oxidative processes [34]. Moreover, it has been reported that the antioxidant activities of phenolic acids and their derivatives depend on the number of hydroxy groups in the molecules [35].

The ferrous chelating capacity of *A. unedo* extracts is probably due to the presence of reductones and may serve as an indicator of its potent antioxidant activity. The antioxidant effect of reductones is based on the destruction of the free radical chain by donating a hydrogen atom [32]. Polyphenols may act in a similar way as reductones react with free radicals to turns them into more stable products and abort free radical chain reactions [36]. It has been reported that the ability of flavonoids to reduce depend on the standard redox potential of the metal and flavonoid structure [37].

The inhibitory effects on lipid peroxidation and autoxidation of linoleic acid have been attributed to the radical scavenging activity [38]. Both studied extracts inhibited the lipid peroxidation, probably by neutralizing free radicals formed in the system, and this ability could be attributed to their phenolic constituents that are potent antioxidants and inhibit strongly the lipid peroxidation [39, 40]. Phenolic compounds are electron donors that can quench and neutralize free radicals or decompose peroxides [41]. The antioxidant ability is related to the structural characteristics of the antioxidant agents and also to their ability to interact with and penetrate the lipid bilayer [42]. Also, it has been reported that the structure and the lipophilicity of antioxidant compounds are determinant factors of their antioxidant properties, most probably affecting the depth of incorporation of a compound in the lipid phase of membrane [43]. The non-polar antioxidants are concentrated within the lipid-water interface, preventing so radical formation of β-carotene and lipid oxidation. While, polar antioxidants are diluted in the aqueous phase, therefore, its ability to inhibit lipids peroxidation is lower than that of non-polar antioxidants [28].

Erythrocytes are susceptible to oxidation; this is why it has been used as a cellular model to investigate oxidative damage in biomembranes. These cells are considered as the first target for free radicals attacks due to the presence of two important and potent promoters of reactive oxygen species, which are: high membrane concentration of polyunsaturated fatty acids and O_2 transport associated with redox-active hemoglobin molecule [44]. Free radicals or oxidants are known to cause structural damage to erythrocyte membranes, by oxidation of lipid and protein composed [45]. AAPH is one of the peroxyl radical initiators that generates free radicals by its thermal decomposition and causes therefore hemolysis [19, 46].

The ability of *A. unedo* extracts to delay hemolysis might be attributed to its phenolic and flavonoids contents. Indeed, previous studies have reported that polyphenols and flavonoids can protect erythrocytes from free radical-induced oxidative hemolysis and increase their resistance to damage caused by oxidants [47, 48, 49, 50]. These studies are in agreement with those of [51] and [52], showing that flavonoids can penetrate in the lipid bilayers of erythrocytes and linked to the plasma membrane and thus inhibit the peroxidation of lipid and enhances the integrity of red blood cells against hemolysis.

5. CONCLUSION

On the basis of the overhead results, it can be concluded that the extracts of *Arbutus unedo* exhibit a good antioxidant activity. This activity may be due to phytochemical constituents present in these extracts. The amount of phenolic compounds in the extracts plays a significant role in their antioxidant capacity, and it can be concluded that ethyl acetate proved to be the best solvent for the extraction of the plant material. Furthermore, it can be concluded that these extracts can be used as a potent natural antioxidant source in the preparation of various herbal medicines for the prevention and/or treatment of oxidative stress-related diseases.

Conflict of Interest

Authors declare no conflict of interest.

Funding

This work was supported by the Algerian Ministry of Higher Education and Scientific Research (MESRS) [grant number CNEPRU/D01N01UN190120150001 Project].

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