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# Exploring the nutraceutical potential and biological activities of *Arbutus unedo* L. (Ericaceae) fruits

Issam Ben Salem[a], Souad Ouesleti[b], Yassine Mabrouk[a], Ahmed Landolsi[c], Mouldi Saidi[a], Abdennacer Boulilla[d,*]

[a] Laboratory of Biotechnology and Nuclear Technology, National Centre of Nuclear Science and Technology (CNSTN), Sidi Thabet Technopark, 2020 Ariana, Tunisia
[b] Laboratoire de Biochimie de l'Hôpital Farhat Hached, Sousse, Tunisia
[c] Faculty of Science of Bizerte, Laboratory of Biochemical Engineering, Faculty of Sciences of Bizerte, 7021 Zarzouna, Tunisia
[d] Laboratoire des Substances Naturelles, Institut National de Recherche et d'Analyse Physicochimiques, Biotechnopole de Sidi Thabet, Ariana 2020, Tunisia

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ABSTRACT

*Arbutus unedo* L. fruits have a great potential to serve as an important source of biomolecules known for panoply of applications in food and pharmaceutical industry. Therefore, the chemical composition, nutraceutical properties, antioxidant power, mineral content and biological properties of ripe *Arbutus unedo* L. fruits were investigated. Fruits contain 10.17% carbohydrates, 0.51% lipid, 1% protein, 48 mg/100 g vitamin C, 0.2 mg/100 g α-tocopherols, 0.117 mg/100 g β-tocopherols and 0.33 mg/100 g vitamin A. Fructose (57.96%) and glucose (33.87%) were the major sugars in fruits, followed by xylose (1.40%), maltose (0.51%) and sucrose (0.16%). The mineral content was 302.91 mg/100 g of Mg, 101.19 mg/100 g of Ca, 99.43 mg/100 g of K, 72.08 mg/100 g of Na, 5.11 mg/100 g of Fe, 3 mg/100 g of Zn, 0.82 mg/100 g of Cu and 0.9 mg/100 g of Mn. The liquid chromatography with photodiode array and electrospray ionisation mass spectrometric detection (LC/PDA/ESI-MS) demonstrates that the phenolic fraction was dominated by galloyl and quercetin derivatives. All extracts proved to have antioxidant activity (measured by ABTS and DPPH assays) being more significant for ethanolic one ($EC_{50}$ values lower than 324.06 ± 1.32 µg/mL). The ethanolic extract showed a strong antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa*, a moderate activity against *Salmonella typhimurium, Escherichia coli, Enterococcus feacium* and *Candida albicans*, and a poor activity against *Streptocoque* strain.

On the basis of these results, *A. unedo* fruits could serve as an excellent source of high added-value phytochemicals for industrial uses.

## 1. Introduction

Nowadays, recent studies have demonstrated that plant-derived foods were characterized by their richness in bioactive phytochemicals and their consumption has a protective effect for human health (Gasparetto et al., 2012). *Arbutus unedo* L, commonly known as strawberry tree, is an evergreen shrub belonging to the Ericaceae family and native to the Mediterranean region. It has been used as food and medicine making an important contribution to the health of local communities. In the literature, the strawberry tree fruits and leaves were characterized by the presence of bioactive substances such as polyphenols, aromatic acids, iridoids, monoterpenoids, phenylpropanoids, sterols, triterpenoids and flavonoids (Albuquerque et al., 2017a,b; Ayaz et al., 2000; Barros et al., 2010; Fonseca et al., 2015; Guimarães et al., 2013; Maleš et al., 2006; Pallauf et al., 2008; Pawlowska et al., 2006; Kähkönen et al., 2001) which may explain their nutraceutical and pharmacological properties. Mature strawberry tree berries can be classified as fruits with very high carotenoid content (Delgado-Pelayo et al., 2016). The attractive color, the energetic power due to the high sugar concentration and the presence of high contents in carbohydrates and precious contribution of polyunsaturated fatty acids of the wild *A. unedo* tree fruits, make them a distinctive food (Barros et al., 2010). The fruits were used in folk medicine for their antiseptic, diuretic, laxative and vascular properties (Pallauf et al., 2008). Nevertheless, fruits were consumed freshly or used traditionally for the fabrication of jam, marmalade, wine, alcohol and liqueur (Celikel et al., 2008). They are not used at industrial scale because of the heterogeneity of plant material as well as the difficulty of selection of

* Corresponding author.
*E-mail address:* abdennacer.boulila@inrap.rnrt.tn (A. Boulilla).

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cultivars/varieties with high fruit quality (Jaradat, 1995).

In Tunisia, *A. unedo* natural populations are severally destroyed by human activities (deforestation, over-collecting). Previous works indicate a low genetic diversity within a population together with a moderate genetic differentiation among *A. unedo* populations which may lead to the decreasing of the population's size and increasing of genetic drift (Tarkouni et al., 2012). An improved knowledge about the potential value of Tunisian *A. unedo* could contribute to preserve and to use this resource as a source of phytochemicals as well as to agronomic and economic advancement. In Tunisia, the fruits were consumed freshly and are not used for a high-value derived product fabrication like as other Mediterranean countries where the berries were used to make liqueurs and jams. To the best of our knowledge, so far no work refers to the characterization of Tunisian strawberry fruits, despite their high nutraceutical properties. Thus, the aim of the present report was to characterize the antioxidant and phytochemicals properties, the phenolic composition, vitamins content and the antimicrobial activities of ripe strawberry tree fruit.

## 2. Material and methods

### 2.1. Chemical and reagents

Polyphenolic standards, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid (Trolox) were from Sigma–Aldrich (St. Louis, MO). Folin–Ciocalteu reagent (100%), methanol and ethanol were from Fluka (St. Louis, MO). Vitamins were purchased from Merck. Phenolic standards were also purchased from Sigma–Aldrich (St. Louis, MO). Water was distilled and filtered through a Milli-Q apparatus before use.

### 2.2. Plant material

Fully mature wild *A. unedo* fruits were collected during november 2016 from trees at Aïn Drahem region (North West of Tunisia). Samples were taken at random from trees at a distance exceeding 100 m from each other to avoid sampling from the same parent. Samples were taken to the laboratory in the same day of harvesting, frozen at −80 °C and then lyophilized. The lyophilized samples were ground in a mortar and kept in −20 °C until the extraction and analysis. All experiments relative to *A. unedo* fruits characterization and their biological properties were repeated three times.

### 2.3. Extraction and chemical composition of polyphenols

10 g of powder of *A. unedo* fruits was macerated in the presence of 100 mL of aqueous ethanol or methanol solvent (80% v/v) at room temperature. After filtration with Whatman paper, the solvent was evaporated at 40 °C on a rotary evaporator. To prevent oxidation of the polyphenols, extraction was achieved rapidly and extracts were immediately used or conserved in darkness at −20 °C until further use.

#### 2.3.1. Total polyphenols content (TPC)

The TPC of *A. unedo* fruit extracts was estimated spectrometrically by the Folin–Ciocalteu method. Briefly, 100 µL of diluted sample were added to 400 µL of 1:10 diluted Folin–Ciocalteu reagent. After 5 min, 500 µL of 10% (w/v) sodium carbonate solution were added. Following 1 h of incubation at room temperature, the absorbance at 765 nm was measured in triplicate. TPC was calculated from the equation determined from linear regression after plotting known solutions of Gallic acid (10–100 ppm). Results are expressed in mg of gallic acid equivalent (GAE) per gram of fresh weight (fw) of plant material.

#### 2.3.2. Total flavonoids content (TFC)

The TFC in the extracts was determined by a spectrophotometric method based on the formation of complex flavonoid-aluminium with an absorptivity maximum between 420–430 nm (Dehpour and Fazel, 2009). Briefly, 500 µL of each extract were separately mixed with 1500 µL methanol (95%), 100 µL of $AlCl_3$ 10% (m/v), 100 µL of sodium acetate 1 M, and 2.8 mL of distilled water. The experiments were run in triplicate, and after incubation at room temperature for 30 min, the absorbance of the reaction mixtures was measured at 420 nm against a methanol standard. The TFC values were determined from a standard curve prepared with quercetin (ranging from 10 to 50 µg/mL) and expressed as mg quercetin equivalents (QE)/g fw.

#### 2.3.3. LC-PDA/ESI-MS analysis

The chemical composition of the *A. unedo* fruit extracts was performed by High-performance liquid chromatography-photo diode array/electrospray ionization mass spectrometry (LC-PDA/ESI-MS) using a Shimadzu LC–MS 2020 system equipped with an online degasser (DGU-20$A_3$R), two LC-20ADXR pumps, an autosampler (SIL-20AXR), a column heater (CTO-20AC), a diode array detector (SPD-M20 A, and a quadrupolar mass analyzer (LCMS-2020, Shimadzu, Japan), equipped with an electrospray ionization (ESI) interface, operated in the negative mode.

The chromatographic separation was performed using an AQUASIL $C_{18}$analytical column (150 mm x 3 mm x 3 µm particle size) with a flow rate of 0.4 mL/min at 40 °C. The mobile phase consisted of 0.1% formic acid in methanol (solvent B) and water with 0.1% formic acid in water (solvent A). The following multi-step linear solvent gradient was employed: 0–45 min 10% B, 45–55 min 100% B, 55–55.1 min 10% B, and 55.1–60 min 10% B. Photodiode array detector was set at 280 nm for acquiring chromatograms. The injection volume was 20 µL and peaks were monitored at 280 nm.

UV spectra were recorded from 210 to 550 nm and the mass spectra were recorded in negative ion mode, under the following operating conditions: capillary voltage, 4.5 kV; cone voltage, 60 V; probe temperature, 300 °C; ion source temperature, 120 °C. The spectra were acquired in the *m/z* range of 150–800 Da.

Peak identification was performed by comparing the retention time and the UV and MS spectra of the phenolic constituents with those of pure standards when available. Further identification was assessed by comparison of UV and MS spectra with literature (Pallauf et al., 2008; Tavares et al., 2010).

### 2.4. Assessment of antioxidant capacity

#### 2.4.1. DPPH scavenging activity

The antioxidant activity of the extracts was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical. A DPPH methanolic solution was prepared at a concentration of $4 \times 10^{-5}$ M. Then, 1 mL of the stock DPPH solution was added in each test tube followed by the addition of 25 µL of each polyphenolic extract. In parallel, the control was prepared containing all reagents except the polyphenolic extract and methanol was used as a blank solution. The antioxidant activity was compared with the positive control BHT. The mixture was shaken vigorously and left in the dark at room temperature. After 60 min, readings were taken using a spectrophotometer at a wavelength of 517 nm. Percent inhibition of the DPPH radical by the samples was calculated according to the formula: % inhibition = $((A_{C(o)} - A_{S(t)}) / A_{C(o)}) * 100$; where $A_{C(o)}$ is the absorbance of the control at t = 0 min and $A_{S(t)}$ is the absorbance of the sample at t = 60 min. The extract concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph of scavenging effect percentage against extract concentration in the solution.

#### 2.4.2. ABTS radical-scavenging assay

The radical scavenging capacity of antioxidant for the ABTS radical action was determined as described by Belkhir et al. (2013). The absorbance of the reaction mixture was measured at 734 nm and