Iranian Journal of Pharmaceutical Sciences
2023: 19 (3): 238- 249
https://journals.sbmu.ac.ir/IJPS

Original Article

Determination of Total Phenolic and Flavonoid Contents and Evaluation of Antioxidant Activity of an Algerian Medicinal Species, *Arbutus serratifolia* Salisb

Nadjah Belattar[a,b]*, Djamel Sarri[c], Fatima Ikhlef[a], Chawki Bensouici[d], Ramdane Seghiri[a], Samir Benayache[a], Fadila Benayache[a], Ratiba Mekkiou[a]*

[a]Research Unit of Valorisation of Natural Resources, Bioactive Molecules and Physicochemical and Biological Analyses (VARENBIOMOL), Mentouri Brothers University, Constantine-1, Algeria, [b]Pharmaceutical Sciences Research Center -Ali Mendjli- Constantine- 25000- Algeria, [c]Department of Biology, Faculty of sciences, Mohammed Boudiaf University, M'Sila, Algeria, [d]Biotechnology Research Center (CRBt), Ali Mendjli, Constantine-2, Algeria

Abstract

Arbutus serratifolia Salisb. is known as a strawberry tree belonging to the Ericaceae family and represents a Mediterranean evergreen shrub. The species distributed in the forest of El Milia region (Jijel) was chosen as a specimen for chemical investigation and biological evaluation for the first time from this region. The MeOH extract of the aerial parts underwent qualitative phytochemical screening using conventional techniques. While $CHCl_3$, EtOAc, *n*-BuOH, and even MeOH fractions were subjected to quantitatively determine the total phenolic and flavonoid contents using the Folin-Ciocalteu and Aluminium trichloride methods respectively. The antioxidant potential of these fractions was assessed using the DPPH scavenging activity, ABTS cation radical reduction, CUPRAC reducing capacity and Phenanthroline assays. This study revealed that *A. serratifolia* Salisb. fractions are endowed with potent antioxidant activity; thus, it is worth noting that this plant could be used as a rich food source for a wide range of phenolic compounds, particularly of new antioxidant agents.

Keywords: *Arbutus serratifolia* Salisb; Phytochemical screening; Phenolic compounds; Flavonoid contents; Scavenging power; Radical reduction.

Corresponding Authors: Belattar Nadjah AND Mekkiou Ratiba, Research Unit of Valorisation of Natural Resources, Bioactive Molecules and Physicochemical and Biological Analyses (VARENBIOMOL), Mentouri Brothers University, Constantine-1, Algeria. E-mails: nadjahorg@gmail.com AND mekkiou_ratiba@yahoo.fr.

Cite this article as: Belattar N, Sarri D, Ikhlef F, Bensouici Ch, Seghiri R, Benayache S, et al, *Determination of Total Phenolic and Flavonoid Contents and Evaluation of Antioxidant Activity of an Algerian Medicinal Species, Arbutus serratifolia* Salisb, Iran. J. Pharm. Sci., 2023, 19 (3): 238-249.

DOI: https://doi.org/10.22037/ijps.v19i3.43874

1. Introduction

Most species that characterize the biodiversity of the Algerian medicinal and aromatic plants occur naturally and in significant numbers throughout the country. However, phytochemical and pharmacological research has been deficient on the 3000 species of plants that make up the Algerian flora, 15% of which are endemic [1].

This diversity and this rarity confer a fundamental scientific interest in studying the Algerian flora, not only in terms of ethnobotany and traditional pharmacopeia, but also in the valorization of natural substances. *Arbutus serratifolia* Salisb, belonging to the Ericaceae family, is a native Mediterranean shrub widely used in folk medicine. This species is particularly widespread in the Mediterranean basin [2].

This species is prescribed in traditional medicine to regulate circulatory system disorders, especially blood deficiency, to reduce genital problems, and to cure broncho-pulmonary complications. Many ethnobotanical studies revealed that this plant can be used as a laxative, antiseptic, anti-cystitis, anti-lithiasis, and disinfectant agent, as well as it is endowed with antihypertensive, hypocholesterolemic, and anti-colitis properties [3]. Moreover, species' antioxidant and antibacterial activities have been extremely evaluated in recent years [4-8].

Due to the remarkable medicinal values of *A. serratifolia* Salisb., our research work was focused on, on the first hand, the screening of the main secondary metabolites classes and, on the second hand, the estimation of the total polyphenol and flavonoid contents, present in four fractions obtained from the aerial parts of *A. serratifolia* Salisb. collected for the first time in the El Milia (Jijel) region using Folin-Ciocalteu and Aluminium trichloride reagents, respectively, to assess its antioxidant potential using four different assays.

2. Materials and Methods

2.1. Chemicals and Reagents

All solvents and reagents used in phytochemical screening and total contents (Folin-Ciocalteu, Aluminium trichloride, gallic acid, quercetin) were purchased from Sigma Aldrich, as well as the reagents used in biological tests (2,2-diphenyl-1-picrylhydrazyl (DPPH); 2,20-azinobis-3-ethyl benzothiazoline-6-sulfonic acid (ABTS); potassium persulfate ($K_2S_2O_8$); 2,6-di-*tert*-butyl-4-hydroxyanisole (BHA); neocuproine; phenanthroline).

2.2. Plant material

The plant was harvested in the El Milia region in October 2017 (Jijel). It was dried in the shade for several weeks after being identified by Dr. Djamel Sarri (Biological Department, M'Sila University) and kept away from the light. A voucher specimen (EAS 10/17) was deposited at the Herbarium of the VARENBIOMOL unit at Mentouri Brothers University- Constantine 1.

2.2.1. Extraction of the secondary metabolites

A total of 477 g of the aerial parts (leaves, flowers and stems) of *A. serratifolia* Salisb. were macerated for 72 h three times in a hydroalcoholic system MeOH/H_2O (80/20, v/v) till covering the plant material; the methanol fraction was then filtered and dried by a rotary evaporator at 35 °C under reduced pressure and treated with 500 mL of distilled water. After the subsequent filtration, the resulting solution was extracted using solvents with different polarities on the separatory funnel starting with Petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH. The organic layers were dried with anhydrous Na_2SO_4, filtered, evaporated to dryness, and weighed.

2.2.2. Phytochemical screening

Phytochemical screening was performed to verify the presence of flavonoids, coumarins,

catechetical tannins, gallic tannins, saponins, anthocyanins, and alkaloids. These tests were based on the visual observation of the color change or precipitate formation after adding specific reagents to the tested fraction.

Identification of flavonoids (Shibata test) [9]: Mix 2 mL of the methanolic fraction and 1 mL of concentrated HCl. After stirring the solution, magnesium chips are added.

Characterization: an effervescence is envisaged when the metal chips are put in the solution, and the appearance of pink to red indicates the presence of flavonoids.

Identification of coumarins (Feigl-Frehden-Anger test) [10]: 0.5 mL of ammonia (25%) is added to 1 mL of methanolic fraction. After stirring, the fluorescence is observed under a UV lamp at 365 nm.

Characterization: intense fluorescence emitted from the solution indicates the presence of coumarins.

Identification of terpenes and sterols (Liebermann-Buchardad test) [11]: In two watch glasses, 1 mL of the methanolic extract is placed, one of which is taken up by two drops of acetic anhydride, and then one drop of concentrated sulfuric acid is poured into each watch glass.

Characterization: A-The purple color, turning green after treatment with two drops of acetic anhydride and a drop of concentrated sulfuric acid, indicates the presence of sterols.

B-The appearance of a brown ring between the two organic phases after treatment with acetic anhydride, chloroform and then concentrated sulfuric acid indicates the presence of triterpenes.

Identification of alkaloids (Mayer test) [12]: 1g of the methanolic fraction is dissolved in 10 mL of hydrochloric acid HCl (1.5%). After filtration, a few drops of Mayer's reagent previously prepared according to the following protocol (5 g of KI dissolved in 10 mL H_2O is added to 1.35 g of $HgCl_2$ is dissolved in 60 mL of distilled water, and the mixture is diluted to 100 mL), is then put into prepared solution.

Characterization: after treatment of acidified methanolic fraction with Mayer's reagent, a yellowish precipitate is formed, proving the presence of alkaloids.

Identification of tannins (Stiasny test): A- to 1 g of methanolic fraction, dissolved in 2 mL of boiling distilled water, a few drops of ferric chloride $FeCl_3$ (1%) is added.

Characterization: the appearance of a green-blackish color indicates the presence of catechetical tannins [13].

B- 1 mL of the infused methanolic fraction is saturated with sodium acetate, then a few drops of ferric chloride (1%) are added.

The appearance of a blue-blackish color indicates the presence of gallic tannins [14].

Identification of saponins [15]**:** 5 mg of methanolic fraction is soaked in 1 g of Na_2CO_3 dissolved in 1 mL of distilled water, and the solution is stirred for a few seconds.

Characterization: the formation of a honeycomb indicates the presence of the saponins.

2.2.3. Determination of total polyphenol content

The total phenolic contents of *A. serratifolia* Salisb. fractions were determined using the Folin-Ciocalteu method (FCR) on 96-well microplates, followed by a spectrophotometry reader [16]. In fact, in our experience, the calibration curve of gallic acid was carried out by preparing six dilutions (175-150-125-100-

50-25 µg/mL) from the main solution (200 µg/mL). 20 µL of each dilution were deposited on the microplate, and mixed with 100 µL of diluted (1/10) Folin-Ciocalteu reagent (2M), 5 µL volume of MeOH and 75 µL of Na_2CO_3 (7.5%) solution, the microplate was kept far from the light for 2 hours. All the fractions (1 mg dissolved in 1 mL of MeOH solvent) were prepared similarly. The absorbance values of gallic acid and the studied fractions were measured at 765 nm versus a blank prepared for control. The total phenolic content was calculated using the following equation Y= 0.0034 X + 0.1044, using Microsoft Office Excel 2013 software, and the results are expressed as microgram gallic acid equivalent per milligram of dry weight (µg GAE/mg of DW) [17].

2.2.4. Determination of total flavonoid content

The total flavonoid content was determined using the Topçu method [18]. The calibration curve was carried out after the preparation of the quercetin dilution series (175-150-125-100-50-25 µg/mL) from the principal solution of 200 µg/mL. In a 96-well microplate, 50 µL of each dilution was mixed with 130 µL of methanol, 10 µL of CH_3COOK (potassium acetate) (1M), and 10 µL of Aluminum Nitrate (10 %). The fractions (1 mg dissolved in 1 mL of MeOH) were also prepared similarly. After incubation of 40 min in the darkness, the absorbance values of quercetin and the studied fractions were measured at 415 nm versus a blank prepared for control. The total flavonoid content was calculated using the following equation Y= 0.0047 X + 0.0165, carried out in Microsoft Office Excel 2013, and the results are expressed as microgram quercetin equivalent per milligram of dry weight (µg QE/mg of DW) [18].

2.2.5. Determination of antioxidant activity

2.2.5.1. DPPH scavenging assay

The free radical scavenging activity was evaluated using the Kedare method [19]. In a 96-well microplate, 40 µL of the solution, prepared from 1 mg of each fraction dissolved in 1 mL of MeOH and then diluted to different concentrations (500-250-125-62.5-31.25-5.625 µg/mL), were added to 160 µL of DPPH (0.1 mM), whose absorbance is read at 517 nm. The microplate was kept in the darkness for 20 min. After that, the absorbance of the remaining DPPH was measured at 517 nm on the 96-well microplate reader (PerkinElmer Multimode Plate Reader EnSpire). BHA was used as an antioxidant standard for comparison. The inhibition percentage of DPPH radical is calculated using the following equation:

$$\textbf{\textit{DPPH scavenging capacity}} = [A_{control}-A_{extract}) / (A_{control})] \times 100 \quad (1)$$

$A_{control}$ is the absorbance of DPPH in the presence of MeOH and $A_{extract}$ is the absorbance in the presence of the sample.

The antioxidant activity of the fractions expressed as the 50% inhibitory concentration (IC_{50}) of the DPPH radical is calculated from the inhibition percent (% I) versus concentration plot using Microsoft Office Excel 2013. The IC_{50} value indicates the concentration (µg/mL) of the tested fraction required to scavenge 50 % DPPH free radicals.

2.2.5.2. ABTS radical scavenging activity

The trapping capacity of $ABTS^{\bullet+}$ cation, indicated by the discoloration of ABTS

solution, was determined according to the Re et *al.* method [20]. After preparation of $ABTS^{\bullet+}$ solution from dissolving ABTS (7mM) and potassium persulfate ($K_2S_2O_8$) (2.45 mM) in H_2O and adjusting the absorbance to 734 nm on the 96-well microplate reader (PerkinElmer Multimode Plate Reader EnSpire), the solution was protected in the darkness for 12–16 hours before use. 160 µL of the $ABTS^{\bullet+}$ solution was added to 40 µL of a solution prepared from 10 mg of each fraction dissolved in 1mL of MeOH and diluted to different concentrations (500-250-125-62.5-31.25-15.625 µg/mL). After 10 min, the absorbance of each dilution was further measured at 734 nm. Methanol was used as a negative control and BHA was used as a positive control for comparison. Moreover, the inhibition percentage of ABTS radicals is calculated using equation (1).

2.2.5.3. CUPRAC Reducing activity

The copper-reducing capacity was evaluated according to the CUPRAC (cupric-reducing antioxidant capacity) method [21]. In a 96-well microplate, 40 µL of each solution prepared from different fractions (1 mg of each fraction dissolved in 1mL of MeOH and diluted to different concentrations (500-250-125-62.5-31.25-15.625 µg/mL)) and 50 µL of copper (II) chloride solution, 50 µL of alcoholic neoprene solution, and 60 µL of aqueous buffer ammonium acetate, were mixed at pH = 7 to give a final volume of 200 µL. After 60 min, the absorbance of each dilution was measured at 450 nm on the 96-well microplate reader (Perkin Elmer Multimode Plate Reader EnSpire). The results were recorded regarding absorbance values compared with those of BHA used as an antioxidant standard.

2.2.5.4. Phenanthroline Reducing activity

The activity of phenanthroline was determined using the Szydlowska-Czerniaka method [22]. 10 µL of each dilution (500-250-125-62.5-31.25-15.625 µg/mL) prepared from different fractions, 50 µL of $FeCl_3$ (ferric chloride) (0.2%) solution and 30 µL of phenanthroline (0.5%) solution, and 110 µL MeOH were placed into 96 well microplate. The obtained solutions were mixed and left in the darkness at room temperature (30 °C). After 20 min, the absorbance of each dilution was measured at 510 nm against a blank control (50 µL of $FeCl_3$ (0.2%), 30 µL of phenanthroline (0.5%), and 120 µL of MeOH). BHA was used as standard.

2.2.6. Statistical analysis

The results are presented as the mean value ± SD of triplicates using XLSTAT software. The IC_{50} and $A_{0.50}$ values were determined by linear regression analysis, and one-way analysis of variance ANOVA to detect significant differences ($p<0.05$).

3. Results and Discussion

3.1. The yield of the fractions

After concentration and drying of the organic phases, the latter were weighed: 2.23 g of petroleum ether, 2.38 g of $CHCl_3$, 2.86 g of EtOAc, and 2.94 g of *n*-BuOH fractions. The total extract yield was found to be 26.89%. In summary, the highest yields were observed in the *n*-BuOH and EtOAc fractions (2.29% and 2.23% respectively), while the lowest yield was found in the $CHCl_3$ fraction (1.85%).

3.2. *Phytochemical screening*

Preliminary data revealed the presence of most chemical families in the crude methanol extract, indicating the richness of *A. serratifolia* Salisb in secondary metabolites, particularly in flavonoid, tannins, and terpenes components, reflecting a dark brown color throughout the studied fraction. **Table 1** summarizes the obtained results:

Table 1: The main secondary metabolites contained in *A. serratifolia* Salisb.

Secondary metabolites	The presence
Flavonoids	+
Sterols	+
Terpenes	+
Saponins	-
Alkaloids	+
Catechetical tannins	+
Gallic tannins	+
Coumarins	+

According to previous studies, El Cadi et al. reported that the colorimetric methods allowed the detection of different chemical groups such as flavonoids, tannins, anthocyanins, anthraquinones, sterols, and steroids, while alkaloids and saponosides were not detected [23]. Except for alkaloids, this finding was consistent with another work done by Dib et al. [24].

In the study of Belfekih et al. about *A. serratifolia* Salisb. species, collected in the El Harcha forest (Morocco) [25], the phytochemical screening and thin layer chromatography revealed that the three organs (fruit, stem and leaf) of *A. serratifolia* Salisb. contain essential secondary metabolites, such as flavonoids, tannins, saponins, alkaloids, coumarins, carotenoids, sterols, and triterpenes. Their investigation revealed the presence of alkaloids in all parts of the plant.

Furthermore, tannins were noted in high quantity in the leaf and stem compared to the fruit, particularly the presence of catechetical tannins found in high amounts in the leaves, unlike the fruits and the stems. Gallic tannins abundantly exist in the fruits rather than the stems and the leaves; similarly, the flavonoids are more available in the fruits, followed by the leaves and then the stems.

Coumarins are highly present in the fruits, but they are absent in the stems and the leaves. Carotenoids are moderately present in the fruits and slightly present in the stems, in contrast to the leaves. Saponins, characterized by foam appearance, were found in all the studied organs, with an exceptionally high concentration in the leaves [25].

The qualitative analyses revealed the presence of sterols and triterpenes in the different parts, particularly in the leaves than the fruits, followed by the stems. Another preliminary phytochemical investigation conducted by Mrabti et *al*. on the aqueous extract of *A. serratifolia* Salisb. from Beni Mellal region (Morocco) [26] revealed that the leaf and root parts contain high quantities of tannins, flavonoids, and anthraquinones while free quinones exist in moderate concentration. However, terpenoids are more abundant in the roots than leaves, with a total absence of alkaloids and saponins in both plant parts.

3.3. *Estimation of total phenolic and flavonoid contents*

The total phenolic and flavonoid contents of *A. serratifolia* Salisb. fractions are shown in **Table 2**. The highest total phenolic and flavonoid contents were found in the *n*-BuOH fraction (746.7350±0.6293µg GAE/mg; 87.0416±0.2947µg QE/ mg of fraction) followed by EtOAc (323.9767±1.1052µg GAE/mg; 73.1249±0.1886

µg QE/mg of fraction) and MeOH fraction (253.4100±0.8343µg GAE/mg; 28.6667±0.2357µg QE/mg of fraction) respectively, whereas $CHCl_3$ showed the lowest amounts in both phenolic and flavonoid contents.

Table 2: The total phenolic and flavonoid contents of the studied fractions.

Fractions	Total phenolic content (µg GAE/mg)	Total flavonoid content (µg QE/mg)
MeOH	253.41 ± 0.83	28.66 ± 0.23
$CHCl_3$	149.85 ± 1.29	13.81 ± 0.20
EtOAc	323.97 ± 1.10	73.12 ± 0.19
n-BuOH	746.73 ± 0.63	87.04 ± 0.29

The obtained results showed that the polyphenols in the EtOAc fraction are richer in flavonoids (22.57%) than the *n*-BuOH (11.66%) and MeOH (11.31%) fractions. Our results corresponding to the EtOAc fraction are in good agreement with those obtained by Boulanouar et *al***.** [6] (The rate of polyphenols and flavonoids compounds is 21.06 % in ethanolic fraction of aerial parts), and Guendouz et al. [7] (The rate of flavonoids versus the polyhenols compounds is 11.92% in methanolic fraction of the leaves). However, they are lower than those found by Asmaa et al. [8] (The rates of flavonoid versus polyphenol compounds were 37.17% in the maceration method and 66.37% in the sonication method for leaves, while the fruit's extraction gave the rates of 3.03% in the maceration method and 6.12% in the sonication method) and those reported by Didi et al**.** [5] whose phytochemical investigation demonstrated that the total phenolic and flavonoid contents are higher through the hot extraction than those of the cold extraction.

Polyphenols are the most abundant secondary metabolites identified in plants and are widely spread across the plant kingdom. The quantity and substitution patterns of the hydroxyl groups determine the differences in each flavonoid fraction. Due to their structural diversity, they can undertake various biological activities. Moreover, because the potent antioxidant activity of polyphenols and flavonoids is closely linked to their ability to scavenge hydroxyl and lipid peroxyl radicals, it seems obvious that the polarity of the solvent systems used in the extraction could affect the contents of polyphenols and flavonoids components [27]. As a result, the concentrations of these components in the different fractions significantly vary depending on the type of solvent used during the extraction.

Our study showed that the polar and semi-polar fractions have the highest proportions of polyphenols and flavonoids.

The results of phenolic content found by Mrabti et al. [26] showed that the aqueous extract of the roots of *A. serratifolia* Salisb. has relatively higher total polyphenol contents (47.55 ± 0.67 mg GAE/g) than that of the leaves (37.30 ± 0.19 mg GAE/ g). The flavonoids were also detected in higher concentrations in the aqueous extract of roots (49.66 ± 2.13 mg QE /g) than that of the leaves (25.73 ± 2.01 mg QE /g).

Mrabti et al. [26] reported that the aqueous extract of the roots has the highest amount, surpassing that reported by Fortalez et al. [28] who found that the $CHCl_3$ fraction of *A. serratifolia* Salisb. native to Spain, contains a total phenolic of 16.46 ± 3.66 mg GAE/g. This difference in the contents can be explained by the environmental conditions (climatic and collection periods), genetic factors, and experimental

methods, besides the polarity of the solvent, which plays an important role in increasing the solubility of phenolic compounds [29]. In addition, the results of total flavonoid content indicated the richness of root extract, compared with the results obtained by Doukani et al. [30].

These variations can be explained by ecological factors such as soil type, microclimatic conditions, geographic location, site, plant age, and vegetative stage [30].

3.4. *Evaluation of antioxidant activity*

Table 3 summarizes the IC_{50} values (the concentration corresponding to 50 % inhibition) and $A_{0.5}$values (the concentration corresponding to the absorbance 0.5) of *in vitro* tests corresponding to antioxidant potency: all the antioxidant methods used in this study revealed that the MeOH fraction is the most active than the other fractions, as well as this fraction is endowed with an important or moderate antioxidant efficiency, compared with those of BHA standard.

The results of DPPH scavenging capacity showed that the MeOH fraction has the highest antioxidant activity (IC_{50}=4.74±0.07 µg/mL), followed by EtOAc fraction (IC_{50}=8.87±0.33 µg/mL), then *n*-BuOH (IC_{50}=9.19 ±0.13 µg/mL) and $CHCl_3$ fractions (IC_{50}= 16.35±0.26 µg/mL). Furthermore, the MeOH fraction is more active than standard antioxidant BHA (IC_{50}=5.73±0.41 µg/mL), whereas EtOAc and *n*-BuOH fractions are less active than the BHA standard.

The ABTS radical scavenging activity displayed that the MeOH fraction is also the most active (IC_{50}=5.30±0.18 µg/mL), followed by EtOAc fraction (IC_{50}=10.02±0.26 µg/mL), then *n*-BuOH fraction (IC_{50}=10.37±0.52 µg/mL) and finally $CHCl_3$ fraction (IC_{50}=30.18±0.69 µg/mL). It is worth noting that all of these fractions have relatively lower activity, compared with that of the BHA standard (IC_{50}=1.81±0.10 µg/mL).

The results of CUPRIC reducing antioxidant capacity also exhibited the prevalence of MeOH fraction as the potent antioxidant agent ($A_{0.50}$=4.02±0.1 µg/mL), followed by EtOAc fraction ($A_{0.50}$=4.74±0.43 µg/mL), then *n*-BuOH fraction ($A_{0.50}$=4.79±0.36 µg/mL) and then $CHCl_3$ fraction ($A_{0.50}$=27.40±0.44 µg/mL).

These results indicate that MeOH, EtOAc and *n*-BuOH fractions are more active than standard antioxidant BHA ($A_{0.50}$=3.64±0.19 µg/mL). At the same time, the $CHCl_3$ fraction is less active than the tested fractions or the standard reference.

The results of Phenanthroline assay revealed that MeOH fraction has the best antioxidant activity ($A_{0.50}$=0.92±0.06 µg/mL), followed by EtOAc fraction ($A_{0.50}$=1.02±0.03 µg/mL), *n*-BuOH fraction ($A_{0.50}$=1.31±0.06 µg/mL) and then $CHCl_3$ fraction ($A_{0.50}$=8.11±0.3 µg/mL), thus the scavenging activity of MeOH is better than that of BHA standard ($A_{0.50}$=0.93±0.07 µg/mL) and the activities of EtOAc and *n*-BuOH fractions are very close to that of BHA standard. In contrast, the $CHCl_3$ fraction is less active (**Table 3**).

Table 3: The IC_{50} and $A_{0.5}$ values of the *in vitro* antioxidant activity of BHA and the studied fractions.

Antioxidant agent	IC_{50}(µg/mL) (Mean ± SD)		$A_{0.5}$(µg/mL) (Mean ± SD)	
	DPPH	ABTS	CUPRAC	Phenanthroline
BHA	5.73 ± 0.41	1.81 ± 0.10	3.64 ± 0.19	0.93 ± 0.07
MeOH	4.74 ± 0.07	5.30 ± 0.18	4.02 ± 0.10	0.92 ± 0.06
EtOAc	8.87 ± 0.33	10.02 ± 0.26	4.74 ± 0.43	1.02 ± 0.03
n-BuOH	9.19 ± 0.13	10.37 ± 0.52	4.79 ± 0.36	1.31 ± 0.06
$CHCl_3$	16.35 ± 0.26	30.18 ± 0.69	27.40 ± 0.44	8.11 ± 0.3

This quantitative evaluation of our results was consistent with those of antioxidant activity tests in so far as the total polyphenol and flavonoid concentrations were reported in the same fractions, indicating significant antioxidant activity against free radicals.

Considering the high efficiency of phenols in scavenging free radicals, the rate of inhibition of EtOAc and n-BuOH fractions is close to that of the standard antioxidant because polar fractions contain donor groups of hydrogen atoms [31].

Boulanouar et *al*. [6] reported that the ethanolic fraction is the most active for scavenging ABTS, DPPH, and superoxide anion radicals, as well as having the best reducing capacity. *A. serratifolia* Salisb. extract was the sole with relatively high reducing power and ability to scavenge the free radicals ABTS, DPPH, and superoxide, suggesting that its antioxidant capacity may be attributed to these mechanisms. Furthermore, the leaf extract of *A. serratifolia* Salisb. was found to have a lower amount of total polyphenols, determined using the Folin-Ciocalteu method, than the leaf fractions obtained in the experiments by other authors using ethanolic extraction [32] or boiling water extraction [33].

The radical scavenging assay (DPPH) of the leave and fruit fractions, examined by Asmaa et *al.* using maceration and sonication methods, gave IC_{50} = 2.5 ± 0.1 µg/mL in maceration and IC_{50} =52.4 ± 0.1 µg/mL in sonication for the leaves, whereas the fruit extract was found to be less active. However, the ABTS test showed a higher antioxidant activity of the fruit extract (IC_{50} = 0.10 ± 0.01 µg/mL in maceration and IC_{50} = 0.03 ± 0.01 µg/mL in sonication) compared to that of the leave extract (IC_{50} = 0.90 ± 0.01 µg/mL in maceration and IC_{50} = 0.03 ± 0.01 µg/mL in sonication).

The evaluation of the antioxidant activity of the methanolic leaf extract by Guendouz et *al.* (2015) [7] using DPPH and ABTS methods, revealed that the methanolic extract is the most active (IC_{50}=3.8 ± 0.2 µg/mL; IC_{50} = 4.2 ± 0.4 µg/mL), respectively, with an excellent correlation between the DPPH and ABTS results (r=0.96); since the two methods used to measure the ability to donate a hydrogen atom are quite comparable [34]. According to the findings of Didi et *al*. (2009) [5], methanolic flower extract's DPPH scavenging activity is superior to ascorbic acid. Moreover, the ethyl acetate fraction of the flowers (IC_{50} = 2 µg/mL in hot extraction and IC_{50} = 1.9 µg/mL in cold maceration) exhibited a scavenging power superior to that of ascorbic acid (IC_{50} = 3 µg/mL), then that of the stems (IC_{50} = 5.6 µg/mL in hot extraction and 3.5 µg/mL in cold maceration) and the leaves (IC_{50} =5.4 µg/mL in hot extraction and 27 µg/mL in cold maceration). Whereas, the butanolic fractions of flowers (IC_{50} = 4.4 µg/mL for hot extraction and 8.6 µg/mL for maceration in cold), stems (5.1 µg/mL for hot extraction and 9.4 µg/mL for maceration in cold), and then the leaves (5.3 µg/mL for hot extraction and 14 µg/mL for maceration in cold) are less active than ascorbic acid. These discrepancies could be related to the varied extraction processes. The authors conducted their extractions and obtained varying quantities of polyphenols depending on the method used during their extraction.

The results of the DPPH test found by Mrabti et *al.* (2017), showed that the aqueous root extract provides an antioxidant activity superior to that of the leaves, and this property is

compatible with its highest concentrations of polyphenol and flavonoid compounds. However, their antioxidant effect is comparable to ascorbic acid (3.12 ± 0.67 µg / mL) [26].

All previous studies have proven that phenolic compounds are the principal antioxidant ingredients in medicinal plants, vegetables, fruits, and spices [35], and that the activity prevalence of antioxidant agents depends on their solubility in either water or lipids [36].

Four potential interactions can explain the pharmacokinetic effects of different molecular connections characterizing the plant fractions: the first is the indifference interaction, in which the activity of each molecule is unaffected by the activity of the others; otherwise, the synergistic effect will significantly increase the interactive effect of the chemical connection when compared to each molecule reacting independently at the same concentration. Concerning the third condition in terms of addition, the effect of molecular association is equivalent to the total effects of each molecule responding in isolation at the same concentration as in their association. The antagonism results from the fact that combining molecules reduces the activity of each molecule separately, while the combined effect is smaller than the individual action of each molecule [37].

4. Conclusion

According to our bibliographic search, this phytochemical investigation and the biological evaluation were carried out using fractions of *A. serratifolia* Salisb. for the first time, collected in the El Milia region (Jijel). These plant fractions may help to produce novel antioxidant agents due to their high polyphenol content and remarkable antioxidant activity. However, more research is needed to understand the impact and significance of this antioxidant effect in both animals and humans; for this reason, *in-vivo* and clinical studies are requested to probe the anti-Alzheimer and anti-diabetic properties of the different fractions of this plant.

Acknowledgments

This investigation was supported by the Algerian Ministry of Higher Education and Scientific Research (MESRS) and the Algerian PRFU (B00L01UN250120180002) program.

Conflict of interest

The authors declare to have no conflict of interest.

References

[1] Quézel P, Santa S. Nouvelle flore de l'Algérie et des régions désertiques méridionales. Éditions du Centre National de la Recherche Scientifique : Paris (1963).

[2] Torres J A, Valle F, Pinto C, García-Fuentes A, Salazar C, Cano E. Arbutus unedo L. communities in southern Iberian Peninsula mountains. Plant Ecology (2002) 160:207-223.

[3] Bouzid A, Chadli R, Bouzid K. Étude ethnobotanique de la plante médicinale Arbutus unedo L. dans la région de Sidi Bel Abbés en Algérie occidentale. Phytothérapie .(2017) 15 : 373-378.

[4] Dib M A, Allali H, Tabti B, Bendiabdellah A, N. Djabou . A new proanthocyanidins from Arbutus unedo L. stems. Asian Journal of Chemistry (2008) 20(5):3926-3934.

[5] Didi A. Étude de l'activité antioxydante des flavonoïdes de l'Arbutus unedo et du Dapline gaidium L. de la région de Tlemcen. Mémoire de Magister, Université Abou-Bekr Belkaid: Tlemcen, January 2004.

[6] Boulanouar B, Abdelaziz G, Aazza S, Gago C, Miguel M G. Antioxidant activities of eight Algerian plant extracts and two essential oils. Industrial Crops and Products (2013) 46: 85-96.

[7] Guendouze-Bouchefa et al. Phenolic compounds, antioxidant and antibacterial activities of three Ericaceae from Algeria. Industrial Crops and Products (2015) 70 : 459-466.

[8] Asmaa N, Abdelaziz G, Boulanouar B, Carbonell-Barrachina A A, Cano-Lamadrid M, Noguera-Artiaga L. Chemical composition, antioxidant activity and mineral content of Arbutus unedo (Leaves And Fruits). Journal of microbiology, biotechnology and food sciences (2019) 8 (6): 1335-1339.

[9] Harborne J B, Baxter H. The handbook of natural flavonoids (Volume 1 and Volume 2): United Kingdom (1999).

[10] Koudougou K. Étude de la chimie et de l'activité antimycosique des extraits de Biophytum petersianum. Mémoire docteur en pharmacie, Université de Ouagadougou : Burkina Faso, 2000.

[11] Hopkins W G, Rambour S, Évrard C M. Physiologie végétale . Marie Evrard. Edition DEBOEK Université : Bruxelles (2003).

[12] Bruneton J. Pharmacognosie, phytochimie, plantes médicinales, 4ème ed., Lavoisier : Paris (2009).

[13] Kongo-Nzuzi Y. Evaluation in vitro des pouvoirs antifongiques des extraits des feuilles de papayer sur des souches de Candida albicans. Graduée en techniques de laboratoire, Institut Supérieur des Techniques Médicales: Kinshasa, 2009.

[14] Mibindzou Mouellet A. Screening phytochimique de deux espèces de plantes : crotalaria retusa L (Papilionaceae) et hallea ciliata Aubrev et Pellegr. (Rubiaceae) récoltées au Gabon. Docteur en pharmacie, Université de Bamako : Bamako, 2004.

[15] Dewick P M. Medicinal Natural Products: A Biosynthetic Approach, 3rd ed. John Wiley & Sons (2002): 551-552 .

[16] Al-Duais M, Müller L, Böhm V, Jetschke G. Antioxidant capacity and total phenolics of Cyphostemma digitatum before and after processing: use of different assays. European Food Research and Technology (2009) 228: 813-821.

[17] Singleton V L, Rossi J A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. The American Journal of Enology and Viticulture (1965) 16: 144-158.

[18] Topçu G, Ay M, Bilici A, Sarıkürkcü C, Öztürk M, Ulubelen A. A new flavone from antioxidant extracts of Pistacia terebinthus. Food Chemistry (2007) 103 (3): 816-822.

[19] Kedare S B ,Singh R P. Genesis and development of DPPH method of antioxidant assay. Journal of Food Science and Technology (2011) 48: 412-422.

[20] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine (1999) 26 (9-10): 1231-1237.

[21] Apak R, Güçlü K, Ozyürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. Journal of Agricultural and Food Chemistry (2004) 52 (26): 7970-7981.

[22] Szydłowska-Czerniak A, Dianoczki C, Recseg K, Karlovits G, Szlyk E. Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods. Talanta (2008) 76(4) : 899-905.

[23] El Cadi et al. Wild strawberry (Arbutus unedo): Phytochemical screening and antioxidant properties of fruits collected in northern Morocco. Arabian Journal of Chemistry (2020) 13: 6299-6311.

[24] Dib M E A, Allali H, Bendiabdellah A, Meliani N, Tabti B. Antimicrobial activity and phytochemical screening of Arbutus unedo L. Journal of Saudi Chemical Society (2013) 17: 381-85.

[25] Belfekih F, El Yahyaoui O, Chleh M, Ould Abdellahi L, Sammama A, Lrhorfi L A, Bengueddour R. Phytochemical screening of Arbutus unedo L. American Journal of Innovative Research and Applied Sciences (2017) 4 :237-245.

[26] Mrabti et al. Arbutus unedo L aqueous extract is associated with in vitro and in vivo antioxidant activity. Journal of materials and Environmental Sciences (2017) 8 (1): 217-224.

[27] Ghasemzadeh A, Hawa Z, Jaafar E, Rhmat A. Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (Zingiber officinale Roscoe.) extracts. Journal of Medicinal Plants Research (2011) 5(7): 1147-1154.

[28] Fortalezas S et al. Antioxidant properties and neuroprotective capacity of strawberry tree fruit (Arbutus unedo). Nutrients (2010) 2 : 214-229.

[29] Naczk M, Shahidi F. Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. Journal of Pharmaceutical and Biomedical Analysis (2006) 41(5):1523-1542.

[30] Doukani K, Tabak S. Profil Physico Chimique du fruit'Lendj' (Arbutus unedo L.). Nature & Technology (2015): 53-66.

[31] Miguel M G, Nunes S, Dandlen S A, Cavaco A M, Antunes M D. Phenols, flavonoids and antioxidant activity of aqueous and methanolic extracts of propolis (Apis mellifera L.) from Algarve, South Portugal. Food Science and Technology (2014) 34(1): 16-23.

[32] Andrade D, Gil C, Breitenfeld L, Domingues F, Duarte A P. Bioactive extracts from Cistus ladanifer and Arbutus unedo L. Industrial Crops and Products (2009) 30: 165-167.

[33] Mendes L, de Freitas V, Baptista P, Carvalho M. Comparative antihemolytic and radical scavenging activities of strawberry tree (Arbutus unedo L.) leaf and fruit. Food and Chemical Toxicology (2011) 49(9): 2285-2291.

[34] Huang D, Ou B, Prior R L. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry (2005) 53 : 1841-1856.

[35] Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sciences (2004) 74 (17): 2157-2184.

[36] Hosseinian et al. Proanthocyanidin profile and ORAC values of Manitoba berries, chokecherries, and seabuckthorn. Journal of Agricultural and Food Chemistry (2007) 55 (17) : 6970-6976.

[37] Pibiri M C. Assainissement microbiologique de l'air et des systèmes de ventilation au moyen d'huiles essentielles, thèse de doctorat. Institut des infrastructures, des ressources et de l'environnement : Lausane, décembre 2005.