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Algerian strawberry tree fruit (lyophilized powder): Chemical properties and antioxidant activity.

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Abstract

The physicochemical characterization of the lyophilized powder (LP) from Arbutus berries was undertaken. LP is essentially constituted from organic matter, including carbon (~46%), hydrogen (~6%), oxygen (~46%) and nitrogen (~1%.). Potassium (16841.92 mg/kg), Calcium (3800.00 mg/kg) and Sodium (2542.75 mg/kg) are the most abundant elements and the antioxidant properties of LP were studied. Extraction experiments were carried out by maceration, using water and of methanol; Ethyl Acetate (EA) and acetone in water were used as solvent. The antioxidant activity of the extracts was evaluated by using two methods DPHH and reducing power. Flavonoids and polyphenols were found in all the produced extracts and were also quantified. These findings are of interest since antioxidant phenol compounds have an outstanding role in the health area, and wide applications in food and pharmaceutical products. Currently, studies are in progress to isolate and to identify the active principle components.

Keywords: Arbutus berries, Powder, Antioxidant, Favonoids, and DPPH.

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Introduction

Wild edible fruits constitute an important contribution in the nutritional cultures like minerals, polyphenols; vitamins etc. and are used in folk medicine as potential sources of healthy compounds. Therefore, wild fruits are often considered healthy foods by Zhang et al. [1]. The wild strawberry tree is a Mediterranean typical tree whose fruit is generally not consumed in fresh form by Ayaz et al. [2] but after processing by Simonetti et al. [3]. Like other plants which are fitted with wonderful defense system assured by various biopharmaceuticals by Allicin et al. [4] the berries are also known to be used in folk medicine as antiseptic, diuretic and laxative Pallauf et al. [5]. Therefore, a number of researches are carried out on the polyphenol content by Saral [6] and antioxidant capacity by Fadda et al. [7] of berries and continue to attract a great deal of interest. Within the anthocyanins, cyanidin has been identified as the main contributor to the characteristic red color of the fruit by Proloiac A, Ruiz-Rodriguez [8,9]having earlier supported that the higher antioxidant potential of the arbutus berries may be due to the activity of various bioactive components including vitamin C. Phenolic compounds in A. Unedo fruits by Isbilir et al. [10-15], leaves, flowers are well documented. The lyophilized powder (LP) arbutus wild berries (*Arbutus unedo* L.) has been used previously in the elaboration of tablets by Aksil et al. [16,17] and then studied their moisture sorption characteristics under various environmental conditions However, there are no reports on the phenolic content and we are interested by phenolic compound to the overall antioxidant activities of Algerian *Arbutus unedo* L. fruits grown in Kabylian region (north Algeria). The objective of the present work was to investigate, for the first time the effects of solvents on the extraction of polyphenol from LP. The antioxidant activity of the extracts by the method of DPPH free radical scavenging and the reduction of is also investigated this because the antioxidants are vital substances which possess the ability to protect the body from possible harms caused by the free radical by Percival et al. [18].

Materials and Methods

Fruit and fruit powder

Fully ripe arbutus berries were collected during the winter 2019, from the region of Kabylia in the North of Algeria. The fruit was submitted to freeze drying at 64°C under vacuum (0.045 mbar) during 48 h, using lyophilize (Type Christ Alpha 1-4LD) provided with vacuum pump (RZ 6 max pressure=0.04 Pa). The dried product was then ground and sieved (sieve of type Euromatest-Sintoo, NFX11-501) to obtain lyophilized powder (LP) ($200 \leq size \leq 400$ µm) which is kept in closed glass flask at 4°C.

Chemicals

Methanol, ethyl acetate and sodium hydrogen carbonate were purchasedfrom Merck, Darmstadt, Germany. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, Gallic acid, Folin-Ciocalteu (FC), Butyrate hydroxytoluene (BHT),quercetine reagents were purchased from Sigma (Sigma, France), aluminium chloride ($AlCl_3$) trichloroaceticacide (CCl_3COOH) and potassium ferricyanide (K_3[Fe (CN)6]) were from Fluka Chemie AG, Buchs, Switzerland. All other solvents and chemicals were of analytical grade.

Détermination of Qualité Parameters

Analytical methods: The elementary analysis was performed on LP sample (2 mg) using microanalyses of type CHNS-932. LECO.

Moisture content was determined by treating 0.5 g of powder by means of laser-apparatus (type thermo control Sartorius) during 17 min.

- The mineral elements were determined according to AFNOR V 05-133, 1972. The residue of incineration was dissolved in 1 mL of HCl (6 N). The mixture was shaken vigorously and the total volume was adjusted to 100 mL with distilled water. All measurements were carried out using an atomic absorption spectrophotometer (SOLAR Thermo Elemental).

- Measurement of other parameters was conducted by preparing, first a powder aqueous extract at different ways: Crude fiber wasevaluated following to AFNOR.V. 03040. 1977 procedure; pH was monitored by potentiometer(type ERWEKA) over an homogenized sample 1/10 (w/v) in distilled water by Hurwitz W [19] the conductivity was measured in the same solution. Titrable acidity (TA) was determined by titration with 0.1 N NaOH until pH of 8.1 was reached; Pectin was determined according to by Multon [20].

Scanning Electron Microscopy (SEM): Microstructure of LP powder was analyzed using Scanning Electron Microscopy (The Quanta 250/450/650). Samples were dehydrated by putting them in a critical point drying equipment and fastened with a special glue to stub (samples holder).

Fourier Transform Infrared (FTIR) spectroscopy: First, the LP was subjected to drying at 50°C during three days in order to eliminate traces of water. Pastilles were prepared by mixing dry powder with KBr (spectroscopic quality 1/100). Thereafter, the infrared spectra were plotted over the range (500-4000 cm^{-1}) using FTIR Spectra 2000 (Perkin Elmer) at room temperature.

Antioxidant properties

Preparation of extracts and yield: Extraction of antioxidant was prepared with a slightly modified method described by Isbilir [21] The LP fruit (0, 4 g) was mixed with the solvent (21 mL) in the dark at room temperature; after 24 h, the infusions were filtered. The residue was re-extracted with the same solvent. The supernatant obtained was concentrated under vacuum at 37°C and the freeze-dried extract was accurately weighed to determine the extraction yield. All freeze-dried extracts were kept at 4°C.

Determination of total polyphenol content: Total phenolic content (TPC) in the LP was determined using the Folin-Ciocalteu colorimetric method by Singleton V L [22] with minor modifications. Aliquots (0.1 mL) of the extracts were diluted with 2 mL of distilled water, oxidized by 1 mL of Folin-Ciocalteau's phenol reagent and neutralized with 0.9 mL of 20% (w/v) $NaHCO_3$. The mixture was kept in the dark at room temperature for 90 min. The absorbance of the solution was determined at 725 nm using a Thermo Electron Corporation Genesis

10 UV spectrophotometer. TPC was expressed as milligrams Gallic acid equivalents per gram dry weight (mg GAE/g). The calibration curve was established using Gallic acid (0-100 mg/mL).

Determination of total flavonoid content

TFC of each extract was investigated using $AlCl_3$ colorimetric method described by Kumazawa S, Hamasaka T [23] with however some modifications. The extract sample was mixed with 1.5 mL of 2% (w/v) methanolic $AlCl_3$. After 10 min incubation at room temperature, the absorbance was read measured at 420 nm. Quercetine (QE) was used as standard and the concentrations of flavonoid compounds expressed as milligrams QE equivalents per gram of dry weight (mg QE/g). The calibration curve was established using Gallic acid (0-60 mg/mL).

Antioxidant activity

DPPH radical-scavenging activity: The antioxidant activity of the extract was measured with the DPPH method. The extracts (0.3 mL) were mixed with 2.7 mL of methanol solution containing DPPH radicals (6×10^{-5} mol/L). The mixture was vigorously shaken and left to stand for 30 min in the dark (until stable absorption values were obtained). The reduction of DPPH radical was determined by measuring the absorbance at 517 nm. The percentage inhibition of DPPH radical was calculated by the relation:

$$\%Inhibition=[(A_{control}-A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of DPPH solution without extract and A_{sample} the absorbance of sample with DPPH solution. All tests were performed at least in triplicate, and graphs were plotted using the average of three determination.

Ferric-reducing power

The reducing power was evaluated according to the Oyaizu M [24]. This reducing power was investigated by observing the reduction of Fe^{+3} to Fe^{+2}. Aliquots (2.5 mL) ofthe extracts were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% centrifuged (1000 rpm, 8 min) in a refrigerated centrifuge (Centrinon K24 OR-2003), after addition of 10% CCl_3COOH (w/v). The upper layer was mixed with 2.5 mL of deionized water and 0.5 ml of 0.1% $FeCl_3$ and the absorbance was measured at 700 nm. The reducing power was estimated from a standard curve using Butyrate hydroxytoluene (BHT) as standard.

Statistical analysis

As mentioned above, all the determinations performed in TPC and antioxidant activity were replicated three times. Each data presented as means ± standard deviation. Data were analyzed statistically (ANOVA) using XLSTAT2012 logical, analysis of variance and differences among the average values were determined for significance at p 0.05.

Results and Discussion

Determination of quality parameters of lyophilized powder from strawberry tree fruits

The quality parameters of the LP are summarized in Table 1 by comparison with other powder fruits. The water content of the obtained powder is similar to that found by Osorio C [25]

Table 1. *Physicochemical of powder from freeze-dried strawberry tree fruits.*

Parameter	Value[a]
C%	46.46 ± 0.22
H%	6.08 ± 0.10
N%	1.29 ± 0.02
O%	46.17 ± 0.07
pH	3.83 ± 0.08
Treatable acidity(%)	0.21 ± 0.01
Ash content (%)	3.01 ± 0.01
Crude fiber (%)	2.4 ± 0.03
Mineral composition	**Value (mg/kg)**
K	16841.92
Ca	3800.00
Na	2542.75
Zn	466.70
Fe	122.00
Cu	1.80

[a]Values expressed are mean ± SD. of three experimental.

for Guava (*Psidium guajava* L.) lyophilized powder (2.75%). This is also consistent with findings from Liaotrakoon et al. [26] who found that dry matter of two dragon fruit varieties increased from about 10% to 95% after freeze-drying. The equivalent concentration of carbon and oxygen in LP must be noticed, in opposite to those of Tinospora crispa which found that the first element is higher (52%) and the second one is less higher (33.77%) Amom [27] Concerning pH, value is slightly higher than that communicated by Ruiz-Rodríguez [28,29] for fresh Hispanic and Turquoise strawberry tree fruits (3.4 and 4.6 respectively). On the other hand, the ash content (3.01%) is twofold higher than that of fresh strawberry tree fruits from Portugal investigated by Barros et al. [30] (1.71 g/100 g of dry weight) but comparable to that reported by Özcan [29] from Turkish samples (2.72% and 2.82% respectively). The mineral content of LP revealed that K is the major macro-element of powder, followed by Ca and Na. Nevertheless, taking into account the dry state of the studied material where the potassium content is strangely higher than that reported for various fresh fruit species (in mg/100 g) by Valvi et al. [31] *Grewia tillifolia* (1302), *Ficus racemosa* (1922), *Flacourtia Indica* (1184.3), *Elaeagnus confetti* (1338.6), *Meyna laxiflora* (1278), *Cordia dichotoma* (1561.3), *Ziziphus rugosa* (1502.3) and *Glycosmis pentaphylla* (1432.3). A part from specie, climatic and growing conditions should be the causes of the differences observed. Concerning microelements, Cu, Zn, Mg and Fe are detected with high concentrations, exceeding those found (1.65, 8.09, 1315.57 and 12.15 mg/kg respectively) by Özcan et al. [29] for the same fruit in fresh state. It is known that the presence of these chemical species is highly desirable. In fact, most of enzymes contain in their structure Mg, Fe and Zn as prosthetic group. Moreover, their deficiencies can lead to growth inhibition in children and changes in their weight loss Black et al. [32] Cu has already been postulated as an essential micro-nutrient for the hematologic and neurologic systems, and its deficiency can impair the function of the nervous system Tan [33] Crude fiber of LP is comparable to that reported by Ruiz et al. [28] and it is less than that reported by Özcan [29] for fresh strawberry tree fruits (6.4 g/100 g of cellulose, 2.93 g/100 g soluble fibers respectively).

FTIR analysis

The FTIR spectra (Figure 1) revealed various characteristic signals corresponding to various broad and intense bands. Thus, five specific peaks can be mentioned. The intense band in the range (3400-3500 cm^{-1}) can be assigned to stretching (υ) vibrations of hydroxyl group (-OH), whereas those around 2900 cm^{-1} fit to C-H absorption. These include CH, CH_2, and CH_3 stretching and bending vibrations. The peaks in the region (1750-1340 cm^{-1}) express the presence of carboxylic groups Niimura et al.[34] on the strawberry refer absorbance in this region in the presence of remaining hemicellulose and pectin. The peaks at 1743 cm^{-1} indicates the stretching group C=O of non-ionized carboxylic acid (methylated or protonated). The same peak was identified in the spectrum of pictine by Sato et al. [35]. Also, the spectrum shows two another specific peaks; at 1025 and ~600 cm^{-1} which could be assigned to -CH-OH bending (alcohol) and stretching (υ) of CH_2 and CH groups respectively. The latter has been suggested by Kamil [36] for various tomato products. The IR-spectrum also reveals the presence of different groups of alcohols (1000-1700 cm^{-1}).

SEM studies

The SEM analysis (Figure 2) showed that LP has irregular shaped particles where some of them are smooth indicating more flow-ability (no presented here).

Caparino et al. [37] reported similar structure for freeze-dried mango powder; this occurs because the ice in the material during freeze drying prevents shrinkage and collapse of the structure.

Effects of solvent on extraction yield and polyphenol content

Extraction yield: The yields of the extracts obtained per 100 g of LP fruit with water and different concentrations of aqueous methanol, ethyl acetate and acetone (50% and 100%) are given in Table 2.

The extraction yield of LP varied from 88.34 to 6.3%. From these results it can be seen that the highest extract yields (88.34; 78.14%) were obtained from extraction with acetone while the ethyl acetate (100; 50%) extracts give the lower yields (6.3; 26.05% respectively). It can also be found that the yield of the water extract (47.85%) is slightly less than that of pure methanol extract (48.15%). The result also showed higher extraction yield with aqueous solvent compared to pure

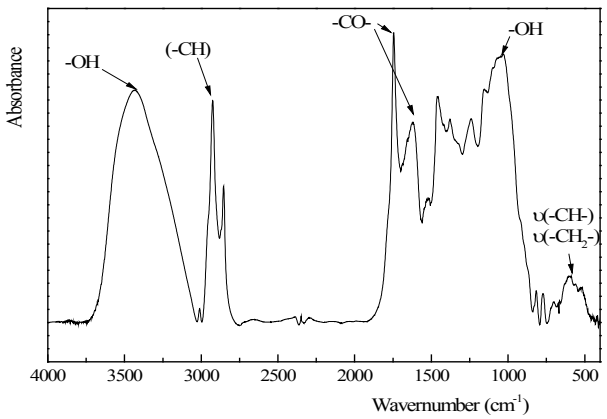

Figure 1. *FT-IR spectrum of powder from freeze-dried arbutus-berries.*

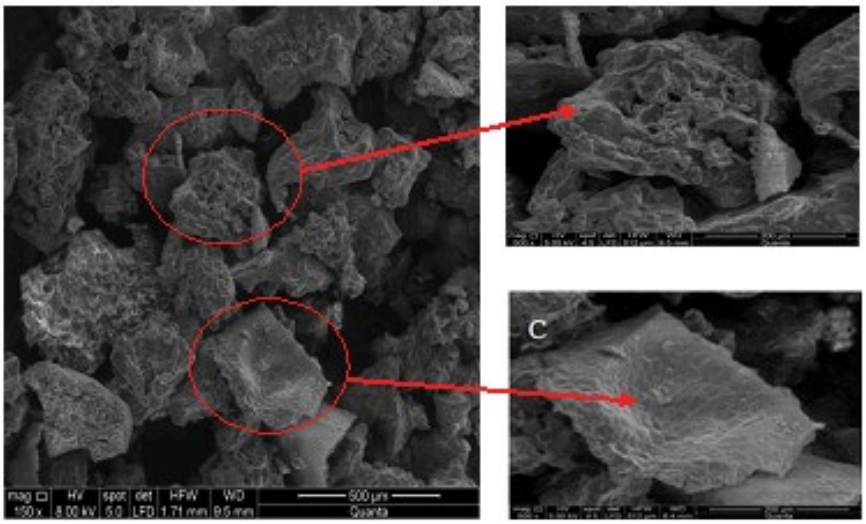

Figure 3. *DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity of LP extracts in different solvents.*

Table 2*. Extraction yield, TPC, TFC of freeze-dried arbutus-berries extracts.*

Solvents[a]	Extraction yield (%)[b]	TPC (mg GAE/g)	TFC (mg QCE/g)
Water	47.85 ± 3.90	52.34 ± 1.45	2.04 ± 0.98
Methanol (M)			
100% (M)	48.15 ± 1.94	145.03 ± 2.04	2.78 ± 0.02
50% aqueous methanol (aM)	65.92 ± 0.55	60.68 ± 1.50	3.56 ± 0.57
Ethyl acetate			
100% (Ea)	6.30 ± 0.99	35.45 ± 1.42	14.54 ± 0.60
50% aqueous ethyl acetate (aEa)	26.05 ±1.24	25.34 ± 1.07	10.49 ± 0.20
Acetone			
100% (Ac)	78.14 ± 1.30	225.13 ± 1.99	14.34 ± 0.68
50% aqueous acetone (aAc)	88.34 ± 1.09	200.01 ± 1.90	11.46 ± 0.53

[a]Values expressed are mean ± SD of three experiments.
[b]Expressed as (g dry extract/g dry berries).
GAE: Gallic Acid Equivalent; QCE: Quercetine; TFC: Total Flavonoid Content; TPC: Total Phenol Content.

solvent. Thismaybeduetoextractionof both polar and nonpolar substances with aqueous organic solvents. Moreover, water can extract proteins and carbohydrates in addition to polar secondary metabolites Dhar et al. [38].

Total Phenolic Content (TPC)

After plotting the calibration curve (y=0.145 × 0.015 with R^2=0.998), TPC ofLP extracts (Table 1) showed that acetone extract gave the highest TPC followed by aqueous acetone and methanol with 225.13; 145.03 mg GAE/g dry matter respectively. The lowest content of TPC was obtained in water and ethyl acetate extracts (25.34; 35.45 GAE/g dry matter resp.), whereas the content obtained with aqueous ethyl acetate was much smaller (25.34 mg Gallic acid equivalent/g). Such results are very similar to those of medicinal plants Pourmorad et al. [39, 40].

The TPC in methanol extracts is smaller than that reported by Salem et al. [41] from Strawberry tree Fruit Tunisian (180.75 mg GAE/g) but is comparable to that found by Bouyahya et al. [42] in methanol extract (141.72 mg GAE/g). In aqueous extracts, it is less that found by Oliveira I [43] (172.21 mg GAE/g) and more that found by Mendes et al. [44,45] in fruits from Portugal (16.7 mg GAE/g) but it is comparable to that evaluated by Tawaha et al., 2007 of Arbutu andrachne L. Wild (58.6 mg GAE/g). In this work the al extracts of LP is higher than that determined by Vidrih et al. [46] in the mixture of fruits/ met phosphoric acid (0.59 mg GAE/g).

Total Flavonoid Contents (TFC)

The content of total flavonoids (Figure 3) expressed as Quercetine equivalents was calculated according to the equation y=3.847 × 3.962 (R^2=0.999), gives a better extraction with ethyl acetate and acetone (14.54; 14.34 mg QE/gresp.) compared to water, methanol and aqueous solvents extracts. TFC of 100% ethyl acetate extract is not significantly higher than that of the 100% acetone extract, whereas TPC of the water extract is significantly less than that of other solvents p<0.01). These amounts are comparable with those reported for other extracts of plant products Xie et al. [47,48]. The arbutus berries have a higher amount of these compounds Pascual-Teresa S [49] ten compounds were identified and quantified by Pallauf et al. [50]. TFC aqueous extracts are comparable to those reported by Bouzid K [51] from *A. unedo* fruit extracts of *Arbutus unedo* L. from Tiaret Area (2.18 mg QE/g). The value in ace tonic extract (14.34 mg/g) is higher that observed by Turker et al. [52] who reported 3.05 mg rutin equivalent/g of fresh fruit. On eight medicinal plants of Algeria (Laghouat region) analyzed by Bakchiche and the hydroethanolic extract of arbutus occupies the second position after *Cytisus monspessulanus* L., in terms of

TPC (104.98 mg EAG/g) and flavonoids (17.46 mg EQ/g). It is useful to note that have shown that the only flavonoid identified in the Croatia's arbutus is the isoquercitrin, with a content ranging from 0.10 to 0.29% in the methanolic extract Males et al. [53].

The rich-polyphenol and flavonoid plants could be a good source of antioxidants that can delay the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions Emmons et al. [54].

Solvent effects on antioxidant activities

DPPH radical scavenging activity: Antioxidant activities of the extracts were investigated using DPPH and Ferric-reducing power. DPPH is a stable radical that has been widely utilized to assess the antioxidant activity of various natural by Hu et al. [55]. Figure 3 shows the DPPH scavenging activities of the extracts in a concentration-dependent manner. The extract obtained by 100% methanol yielded the highest DPPH radical scavenging activity at concentrations ranging from 50 to 180 µg /mL.

However, at concentrations ranging from 180 to 220 µg /mL, its DPPH radical scavenging. Activity is not significantly different from those of other extracts. All extracts obtained by using pure and aqueous organic solvents gave stronger scavenging capacity than that of water extract. Phenolic are the main antioxidant components, and their total contents are directly proportional to their antioxidant activity by Liu et al. [56] According to the results (Figure 3) we noted a correlation between TPC and antioxidant activity of LP extracts. With increasing water content in the solvent, the yield augments whereas TPC and antioxidant activity decreases. Such result is in agreement with previous studies on freeze-dried *Limnophila aromatic* extract DoQ et al. [57].

Reducing Power (RP): The reducing capacity of a compound may serve as a significant indicator of its antioxidant potential. RP was assayed in extracts using BHT (y=3.489x+0.0083, R^2=0.998) as standard and the presence of antioxidants in the sample causes the reduction of Fe^{3+}/ferricyanide complex to the Fe^{2+} form, which is monitored by measuring the formation of the Perl's Prussian blue at 700 nm Yang et al. [58] The relative reducing power of different extracts of LP presented in Figure 4 express marked variations.

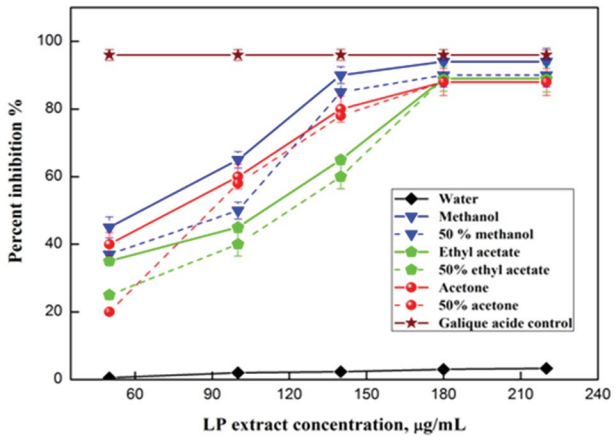

Figure 3. *DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity of LP extracts in different solvents.*

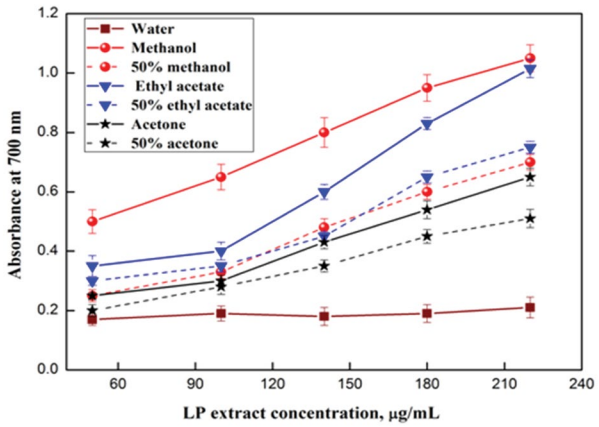

Figure 4. *Reducing power of LP extracts indifferent solvents.*

50% aqueous methanol extract gave the highest reducing power and is significantly higher ($p<0.05$) than that of other extracts followed by 50% aqueous ethyl acetate and ethyl acetate extracts. The reducing power of 100% ethyl acetate extract at concentrations ranging from 50 to 100µg/mL is insignificantly higher than that of other extracts. On the other hand, at concentrations higher than 100 µg/mL the reducing power of 100% ethyl acetate extract is significantly higher ($p<0.05$) than those of 100% acetone, aqueous methanol, aqueous acetone, and aqueous ethyl acetate. The lowest reducing power was observed in the water extract; its value is also significantly lower than that of the other extracts.

Conclusion

Results studies of Algerian lyophilized *Arbutus unedo* L. fruits showed that the powder is rich in organic matter (96.988%). This result is in agreement with the elemental analysis which gave respectively 46.46, 6.08, 1.29 and 46.17% of carbon, hydrogen, nitrogen and oxygen. Macro and micro elements which are indispensable for the activity of various enzymes are also present. We have observed that the extraction yield increases in aqueous solvents (ethyl acetate, acetone, and methanol system). This shows that the addition of the water to organic solvent facilitates the extraction of all compounds from PL. On the other hand this study indicated that the extracts obtained from the fruit of A. *Unedo* has excellent antioxidant properties. The data suggested that the powder from Kabiley area is a potential source of natural antioxidants, the high nutritional quality of this edible fruit is likely to be lost if it is not managed and conserved in the harsh environment.

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