RICH EXTRACT ON TOTAL POLYPHENOLS AND ANTIOXIDANT ACTIVITY OBTAINED BY CONVENTIONAL AND NON-CONVENTIONAL METHODS FROM AHMEUR BOUAMER GRAPE SEED

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ABSTRACT: Conventional and non-conventional extraction methods were applied in the first time for the determination of total polyphenols from Ahmeur Bouamer, an anthococnous variety grape seeds. Independently of the time and temperature, a positive effect of particle size parameter on maceration has been noted and relativly high concentrations in polyphenols for 500 µm (67.78 ± 0.46 mg GAE/gdm), 500-355 µm (90.95 ± 0.24 mg GAE/gdm) and 355-180 µm (114.82 ± 1.24 mg GAE/gdm) have been obtained. The higher value of yield was reached by the microwave extraction (37.74±0.32%), followed by the maceration (14.16±0.15%) and the ultrasound (6.70±0.25%). The appropriate extraction method to obtain a relatively strong antioxidant activity from seeds was the microwave with inhibition of 69.67±1.76 % and 78.05±2.05 % of DPPH and ABTS⁺ free radicals, respectively.

Keywords: Ahmeur Bouamer, extraction, grape seeds, total polyphenols, antioxidant activity.

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1. INTRODUCTION

The interest carried to the active components like polyphenols has greatly increased in recent years. These compounds are known as good natural antioxidant agents arising from natural sources (fruits, vegetables, plants etc.) and can be an alternative to synthetic antioxidants used in food and pharmaceutical industry like BHA and BHT, which their undesirable effect on health was often revealed [1]. Among the widely consumed fruits which are considered as an excellent source of polyphenols, the grapevine is the favorite. All the parts of grape contain polyphenols but 60-70% of extractible polyphenols are concentrated in seeds [2], as monomeric, dimeric, trimeric and tetrameric phenolic compounds [3]. To achieve these rich extracts in polyphenols, it is important to develop efficient methods of extraction leading to quite best yields. Conventional method such as maceration, used for a long time [4], is still relevant even if it is often criticized for its extraction time (few hours) and high temperature (over 80 °C). Some authors improve continually this technique by varying the extraction parameters (type of solvent, liquid / solid ratio, etc.) in order to reduce the extraction time and the temperature [2, 5, 6]. The introduction of non-conventional methods such as ultrasound and microwave has revolutionized the extraction by reducing significantly the extraction time and increasing the yield [7]. The use of ultrasound waves to heat the matrix increases the contact surface between solid and liquid phases; this is mainly due to the dispersion of the particle [8]. The heating of the matrix internally and externally without thermal gradient gives numerous advantages to microwave extraction, including a shorter time (few seconds), less solvent used, a higher extraction rate and less polarity limitation for the extractant [9]. In this present work and for the first time, the autochthonous variety Ahmeur Bouamer, targeted by the development of grape planting in Algeria has been chosen to extract total polyphenols from seeds. This variety of the species Vitis vinifera L from Medea region is one of the varieties that have the advantage of seniority and local attributes "mountain-Piedmonts." [10,11]. Total polyphenols were extracted by maceration, ultrasound and microwave, after the optimization of the extraction conditions by focusing on the positive role played by the particle size on the extraction efficiency. A tentative explanation has been advanced for the understanding of the structural changes inherent in the extraction of polyphenols from secreting cells by scanning electron
microscopy images of different particle sizes, before and after extraction. The comparison of these extraction methods was performed and the obtained results were also compared with those cited by previous works. The quality of the different extracts was assessed by evaluation of radical scavenging activities (% inhibition of DPPH• and ABTS•+ free radicals).

2. MATERIALS AND METHODS

2.1. Chemicals

All aqueous solutions used for extraction were prepared by using ultra-pure water by Milli-Q System (Millipore). Chemicals have analytical grade, Folin & Ciocalteu reagent F9252 (Sigma-Aldrich), gallic acid for analysis (UCB, Belgium), ethanol absolute (CHEM-LAB), extra pure sodium carbonate decahydrated (Na₂CO₃ 10H₂O) (Merck), 2-2´Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) di.ammonium salt (ABTS•−), 1,1-diphenyl-2-picrylhydrazyl (DPPH•) persulfate (K₂S₂O₈), butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA) (Sigma-Aldrich).

2.2 Preparation of grape seed extracts

2.2.1 Plant material

Ahmeur Bouamer grapes were harvested in September 2010 at Benchica in Medea hills (80Km South of Algiers) with the collaboration of the Technological Institute of Fruit Arboriculture and Vine. The storage and transport conditions have been respected (at 8°C in coolers). Once in laboratory, the clusters were washed with distilled and de-ionized water, then dried and stored at -20 °C in the freezer. Grape seeds were removed from berries, weighted, washed and dried between two filter papers for 24 hours and weighted again. The dry seeds were ground into a fine powder and stored hermetically at 4°C prior to use.

2.2.2. Sieve analysis and dry matter content

Grape seeds were ground in a grinder ultracentrifuge (ZM 200, Retsch) with ring sieve of 500 µm. The powder was placed in a sieve shaker (A200 Digit Retsch) in a decreasing aperture size order and the sieves (seed powder) were shaken for 10 min. Different portions size were selected. Dry matter content of grape seeds was determined by drying at 105°C to constant mass in triplicate (Table 1).
2.3. Extraction methods

2.3.1 Maceration

In the first time, the milled powder given by sieve analysis was used to optimize various parameters of maceration extraction (Table 1). In the second time the grape seeds were milled with ring sieve of 250 µm and used to compare the three studied extraction methods in the same conditions. Ethanol is a good solvent for the extraction of polyphenols from grape seeds and gives a lower toxicity relative to methanol, which provides nearly the same results [12]. The use of aqueous ethanol improves the solubility of the bioactive components, the optimum mixture composition being 50/50 v/v, which is the most cited composition in previous works [13, 5, 6]. All the extractions were done in triplicate.

2.3.1.1. Determination of ethanol / water composition

In test tubes, 0.5g of milled grape seeds was mixed with 10 mL of various ethanol / water solvent compositions (25/75, 50/50, 75/25 and 90/10 v/v, respectively) (test tubes were covered with aluminum foil to avoid light) at 25°C during 120 min with 20s shaking each test tubes at 15 min intervals by Vortex. The ethanolic extract was centrifuged for 20 min on 3500 g at 20°C and then filtered on Whatman filter paper #4 (9.0 cm diameter). The extract volume was measured, brought up to 25 mL with aqueous ethanol solution of different composition and stored at -20°C.

2.3.1.2. Determination of ratio Liquid/Solid (L/S)

In the test tubes, 0.5 g of powder grape seeds (size 500-355 µm) was mixed with 5, 10, 15 and 20 mL of 50% aqueous ethanol to obtain L/S ratios of 10, 20, 30 and 40 mL/g, respectively. Test tubes were conserved at 25°C during 120 min with 20s shaking by Vortex of each test tube at 15 min intervals. The ethanolic extracts were prepared as described previously.

2.3.1.3. Optimization of time, temperature and particle size

The higher total polyphenol content was obtained with the ratio 20 mL/g (Fig.1B). This ratio was thus used for this study. Three particle sizes were used: > 500 µm, 500-355µm and 355-180µm. For each particle size, the extraction was carried out for 30, 60, 120 and 180 min at different temperatures (25°C, 50°C and 80°C). The extraction was done as described previously. SEM analysis of the milled grape seeds of different particle sizes were achieved.
before and after the maceration extraction method. The powder was mounted on SEM stubs. The specimens were metalized on high vacuum evaporator MED020 (Leica). The observations were performed with an ESEM Quanta 200 FEG (Tecnai) microscope. Images were analyzed and processed by AnalySIS software.

2.3.2. Ultrasound

In the ultrasonic bath (BRANSON 3210, work frequency of 47 KHz ± 6 %, power supply of 335 W and output power of 105 W), test tubes of 0.5 g of powder seeds (<250 µm) mixed with ethanol/water 50/50 in liquid solid ratio of 20 mL/g were immersed at 25°C. The extraction was performed for a time of 5, 15, 30 and 60 min. Extracts were prepared in the same way as described previously.

2.3.3. Microwave

Extractions were performed on an analytical microwave (CEM Discover, Co, USA) with a regulator of temperature and pressure and a cooling system. Time and temperature were optimized. 0.5 g of grape seed powder (<250 µm) was put into a 100 mL flask connected to a refrigerant and mixed with ethanol/water 50/50 in liquid solid ratio of 20 mL/g. The mixture was stirred under an open system. Extraction was performed at times of 2.5, 5, 15, 30 and 60 min and at different temperatures (25°C, 50°C and 80°C). The preparation of extracts was similar to that used previously.

2.4. Determination of total polyphenols

Total polyphenols were determined by the Folin-Ciocalteu method [13]. In this study, the micro method cited by Waterhouse was applied [14]. 20 µL of the extract was mixed with 100 µL of the Folin-Ciocalteu reagent and 1580 µL of water. After 8 min, 300 µL of 7.5% of sodium carbonate solution were added and the mixture was shaken a few seconds on the Vortex. The solution was kept at 25°C for 2 h and then the absorbance was measured at 767 nm (UV/Vis LAMBDA 25/35/45 Perkin-Elmer Spectrophotometer). Calibration solutions of gallic acid in the range of 0 to 24 mg/L were prepared and 100 µL of each gallic acid solution (0, 2.4, 4.8, 7.2, 12 and 24 mg/L) was used for the Folin-Ciocalteu test in the same way that cited previously. The absorbance was measured at 767 nm on each concentration of these calibration solutions. The total polyphenol content was calculated by using the standard curve.
equation \( (A= 0.0624 C+0.0701, \text{ with } r^2 \text{ value of } 9.9996) \) and expressed in milligram of gallic acid equivalent per dry matter of grape seeds (mg GAE/g dm).

**2.5. Radical scavenging activity of extracts**

The Ahmeur Bouamer grape seed extracts obtained under the three extraction methods were subjected to antioxidant activity measurement analysis. 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2-2’Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS\(^{++}\)) radical scavenging assays were used to determine the radical scavenging activity of extracts. Percentage of inhibition was calculated using the following equation:

\[
\% \text{ inhibition} = \left(1 - \frac{A_{\text{Extract}}}{A_{\text{Blank}}} \right) \times 100
\]

With \( A_{\text{Blank}} \) absorbance of blank at initial time reaction and \( A_{\text{Extract}} \) absorbance of extract at reaction time.

**2.5.1. DPPH radical scavenging assay**

Antiradical activities were determined using the procedure as described by Brandwilliams et al. [15] with minor modifications. An aliquot of 25 µL of extract was added to 975 µL of DPPH solution (60 µM in ethanol) and vortexed for few seconds. The decrease absorbance at 517 nm was read continuously every 5 min by spectrophotometer until reaction reached the steady state. The kinetic reaction of each extract was determined and the antioxidant activity was calculated after an appropriate dilution.

**2.5.2. ABTS radical scavenging assay**

ABTS assay described by Francisco and Resurreccion [16] with some modifications was based on the relative ability of antioxidant to scavenge the radical ABTS\(^{++}\) which was resulted by the reaction of ABTS (7mM) with potassium persulphate (2.45mM). The mixture was left to stand in the dark at room temperature for 12-16 hrs before use and the ABTS\(^{++}\) solution was diluted with ethanol to a maximum absorbance of 0.80 ± 0.02 at 748 nm. In the 6th minute after adding the sample to the ABTS\(^{++}\) radical, the extract ability to scavenge the radical was determined by an absorbance measurement, at a wave length of 748 nm. For the two assays, a standard curve drawn up for solutions of the synthetic vitamin E (Trolox) was used to calculate antioxidant capacity of each extract and the result was expressed as µmol TE per
gram of sample (dried matter).

2.6. Statistical analysis

The analyses were done in triplicate. Means and standard deviations of data were calculated. All figures were represented with error bars corresponding to the ratio of standard deviation (RSD).

3. RESULTS AND DISCUSSION

3.1. Sieve analysis and dry matter content

Different size portions collected after sieving and the average of 93.40 ±0.24 % of dry matter content obtained from the grape seed powder have been illustrated on table 1.

| Size (μm) | Size Portion (%) |
|-----------|------------------|
| >500      | 36.65            |
| 500-355   | 20.04            |
| 355-180   | 36.80            |
| ≤180      | 4.19             |

Dry matter content (%) 93.40±0.24

3.2. Maceration

3.2.1. Determination of mixture solvent composition

The change in composition of ethanol/water ratio provides a concentration of total polyphenols ranging from 36.83 ± 0.47 to 50.87 ± 0.81 mg GAE/g.dm and the maximum obtained concentration of 50.87 ± 0.81 mg GAE/g.dm is provided by the ratio 50/50 v/v ethanol/water (Fig.1A). This result is in agreement with the fact that the penetration in the hydrophobic areas of the seed matrix is facilitated and can help to precipitate soluble seed proteins as found in previous works [13].

3.2.2. Determination of liquid/solid ratio

For a fixed mixture of ethanol / water 50/50 v/v, the ratio L/S was varied from 10 to 40 mL/g. The obtained concentration of total polyphenols was ranged from 28.98 ± 0.66 to 78.20 ± 0.48 mg GAE/g.dm. The maximum concentration of total polyphenols was obtained with the ratio
20 mL/g (Fig.1B). This concentration represents 7.82% (w/w) of the total grape seeds and can be considered as a good yield compared to other works (6.68% for Bucic-Kojik and 3.92% for Shi) [5,17], with the same solvent and the same mixture composition.

Fig.1. (A) Determination of mixture composition after 120 min at 25°C, (B) Evaluation of liquid-solid ratio for particle size of 500-355 μm after 120 min at 25°C.

3.2.3. Determination of particle size, temperature and time

The results illustrate on Fig.2 revealed that whatever the time and the temperature, the amount of extracted polyphenols depends on the particle size of powder seeds. For the size 500 μm, the maximum concentration was 67.78 ± 0.46 mg GAE/gdm (Fig.2c), the size 500-355 μm gave a maximum concentration of 90.95 ± 0.24 mg GAE/gdm (Fig.2c) while the highest concentration was obtained for the size 355-180 μm (114.82 ± 1.24 mg GAE/gdm) (Fig.2a).
These results corroborate those already found in previous works [13, 18].

**Fig. 2.** Effect of particle sizes on total polyphenols extraction, (a) at 25°C, (b) at 50°C, (c) at 80°C.
To understand the structural changes inherent in the extraction of polyphenols from secreting cells, SEM images (FEI quanta 200 feg) were recorded on the milled grape seeds of different particle sizes before and after extraction (Fig.3). Before extraction, at 500 μm, the grinding is superficial. Some blocks have an alveolar appearance, while others remain intact (fig.3 pictures 1). For 500-355 and 355-180 μm, the alveolar aspect is clearly present (fig 3 picture 2 and 3), the secretory cells (as cavities) are different and depend on the ground material size. The variation in the extracted polyphenols quantity is directly related to the accessibility of polyphenol secretory cells. After extraction, the structural changes related to the extraction are mainly materialized by the presence of large cavities. Among the different particle sizes, it can distinguish changes in the depth of the pores (Fig. 3, pictures 4, 5 and 6). Moreover, by looking more closely to the cavities, it can observe that for the larger particles, some are closed (Fig.3, picture 4), while for the two other sizes (Fig. 3 picture 5 and 6), nearly all the cavities are open. The release of polyphenols present in the secretory cells depends on the accessibility to these cells, which is shallow for the size 500 μm (Fig.3 picture 4) and deep for the two other sizes (Fig.3 pictures 5 and 6). The highest concentration of total polyphenols obtained for size 355-180 μm (114.82 ± 1.24 mg GAE/gdm , (Fig.2a)) can be explained by the fact that some cavities have been subjected to a cellular bursting which has released more polyphenols (Fig.3, picture 6c). A small difference has been noted between the concentrations found at 25°C and 120 min (114.82 ± 1.24 mg GAE/gdm) and that found at 80°C and 180 min (114.29 ± 0.11 mg GAE/gdm) (Fig 2). The time and the temperature parameters have no important effect on extraction with the variation of the particle size parameter. These findings were in agreement with those reported by Bucic - Kojic et al.[5].
3.5. Effect of three extraction methods on the total polyphenols yield

After optimised extraction parameters for ultrasound and microwave, the three methods (maceration, ultrasound and microwave) were compared under optimal conditions (powder seeds at 250 µm, T at 25°C, extraction time between 5-60min).

3.5.1. Ultrasound

As illustrated in Fig.4, ultrasound extraction gives at 25°C the lowest amount of extracted polyphenols (29.86±0.36 mg GAE/gdm) after 5min. The maximum amount is obtained at 15 min and reaches 70.50 ± 0.81mg GAE/gdm of total polyphenols. The concentration obtained at 30 min (50.95±0.89 mg GAE/gdm) is similar to that obtained by Ghafoor et al, (54.1±1.5 mg GAE/gdm) but at a temperature of 56.03°C [19]. According to Usaquen-Castro et al., it is better to work at ambient temperature in order to preserve polyphenol compound from eventual degradation if a long exposure time is applied at high temperature [20, 21]. These results corroborate this observation since a concentration of 7.05% (w/w) of the total grape seeds is reached in only 15 min at 25°C.

3.5.2. Microwave

The results found by microwave extraction are significantly important. So, after 2.5 min, the
polyphenol concentration reached 183.36 ± 0.53 mg GAE/g\textit{dm}\textsubscript{m} (Fig.4), which is higher than those found by both maceration and ultrasound in their optimum time and temperature. The maximum value of 193.11 ± 1.08 mg GAE/g\textit{dm}\textsubscript{m} is obtained after 5min and the lowest (119.11 ± 1.92 mg GAE/g\textit{dm}\textsubscript{m}) after 60 min (Fig.4 microwave extraction). This decrease of total polyphenol concentration with the increase of the extraction time is in perfect agreement with the advantages of the microwave extraction reported by many previous research works [22]. The amount of total polyphenols decreases when the temperature increases from 193.11 ± 1.08 mg GAE/g\textit{dm}\textsubscript{m} at 25°C to 139.13 ± 1.59 mg GAE/g\textit{dm}\textsubscript{m} at 50°C and finally 133.76 ±1.51 mg GAE / g\textit{dm}\textsubscript{m} at 80°C. In this case, the use of microwave heating in open vessels has a little influence on the extraction when the temperature increases from 50°C to 80°C (139 to 133 mg GAE/g\textit{dm}\textsubscript{m}). This may be explained by the fact that a part of the solvent (ethanol/water) was directly heated, which reduces the extraction efficiency by the solvent evaporation if the temperature increases above its boiling point [23]. This is not the case with closed vessels [22].

![Comparison between the three extraction methods at 25°C.](image)

**Fig. 4.** Comparison between the three extraction methods at 25°C.

### 3.5.3. Comparison between the three extraction methods

The amount of total polyphenols extracted by the microwave method was higher than that extracted by both maceration and ultrasound methods at each extraction time (Fig.4). For microwave extraction, the maximum amount of 193.11 ± 1.08 mg GAE/g\textit{dm}\textsubscript{m} was reached after 5min while the maximum amounts of 139.93 ± 1.51 mg GAE /g\textit{dm}\textsubscript{m} and 70.50 ± 0.89 mg
GAE/g$_{dm}$ were obtained after 15min, for maceration and ultrasound methods respectively. These results were supported by the yields of the three extraction methods reported in Table 2.

Table 2. Yields, Total polyphenols concentration and Antioxidant Activity of Grape seed extracts.

| Extracts | Yields % | TPC (mg GAE/g$_{dm}$) | DPPH (µmol TE/g$_{dm}$) | ABTS (µmol TE/gdm) |
|----------|----------|------------------------|--------------------------|-------------------|
| Mw 5min  | 37.74±0.32 | 193.11 ± 1.08          | 23.69 ±1.05              | 4218.46 ±48.90    |
| Mac 15min | 14.16±0.15    | 139.93±1.51           | 17.84±2.02              | 3902.27 ± 32.66   |
| Us 15 min | 6.70±0.25      | 70.50±0.89            | ND                      | ND                |

All extractions were done in the same conditions at 25°C; Results were the average of three analysis and expressed as mean ±SD. Microwave extract (Mw 5 min); Maceration extract (Mac 15 min); Ultrasound extract (Us 15 min); ND no determined.

Higher concentrations of total polyphenols were obtained in this work for the three extraction methods, generally with a shorter time and a lower temperature, in comparison with those reported in literature (Table 3). For the extraction by maceration, Bucic-Kojic et al. found a concentration of 66.81 ± 0.9 mg GAE/g$_{dm}$ after 200 min at 80°C with particle size less than 630µm [5]. The same authors found 129.59 ± 7.7 mg GAE/g$_{dm}$ in the same condition of time and temperature but with particle size less than 560µm [6]. With the same particle size, a concentration of 130 mg GAE/g$_{dm}$ has been recently found by Bucic-Kojic et al. after improving the mathematical model of the extraction kinetics [24]. Boussetta et al., reached a maximum concentration of 86 ± 0.2 mg GAE/g$_{dm}$ with particle size less than 400µm in only 15 min and at 50°C [25]. In this work, the grinding less than 250µm gives a concentration of 139.93 ± 1.51 mg GAE/g$_{dm}$ in only 15min with a temperature of 25°C. These are probably the good conditions to obtain a rich extract on polyphenols. So, it is important to note that a long heating at a high temperature can denature the phenolic compounds as cited by Usaquén-Castro et al. [20]. Regarding the difference between the results found by Ghafour et al. [19], for the ultrasound extraction (54.1 ± 1.5 mg GAE/g$_{dm}$ after 29.03 min and at 56.30°C) and those obtained in this study (70.50 ± 0.89 mg GAE/g$_{dm}$ after 15 min and at 25°C), it can
be deduced that for the same reasons [20], the best extraction conditions are achieved with shorter time and less heating.

**Table 3.** Comparison of the best conditions for extraction of total polyphenols from grape seeds by different extraction methods.

| Extraction method | Best extraction condition | *TPC (mgGAE/g dm) | Reference |
|-------------------|---------------------------|-------------------|-----------|
| Ultrasound        | Size (<500 µm) | L/S (50 mL/g) | Ethanol/water (53.15%) | Temperature (56.03°C) | Time (29.03 min) | 54.1±1.5 | Ghafoor et al. 2009 |
| Ultrasound        | <250 | 20 | 50 | 25 | 15 | 70.50±0.89 | Present work |
| Maceration        | <630 | 40 | 50 | 80 | 200 | 66.81±0.9 | Present work |
| Maceration        | <560 | 40 | 50 | 80 | 200 | 129.59±7.7 | Present work |
| Maceration        | <630 | 40 | 50 | 80 | 200 | 130.2±1.4 | Present work |
| Maceration        | <400 | 5 | 50 | 60 | 15 | 90.0±0.2 | Boussetta et al. 2012 |
| Maceration        | <250 | 20 | 50 | 25 | 15 | 139.93±1.51 | Present work |
| Microwave         | unknown | 45.3 | 47.2 | 60 | 4.6 | 96.3±1.5 | Li et al. 2011 |
| Microwave         | <250 | 20 | 50 | 25 | 5 | 193.11±1.08 | Present work |

* Total polyphenols concentration (TP) expressed by mg GAE/g dm, results were the average of three analysis and expressed as mean ±SD

Concerning the microwave extraction, the value of 96.3 ± 1.5 mg GAE/g dm recently found by Li et al. [22], represents 50% of the concentration obtained in this study nearly in the same time of 4.6 min. These authors have worked in closed vessels at 60°C and this parameter was known to increase the efficiency of extraction compared to the open vessels [26]. However, in this case, extraction was performed in both closed and open vessels in 5 min at 25°C. The obtained concentrations in closed and open vessels were respectively 163.66 ± 1.02 mg GAE/g dm and 159.35 ± 1.24 GAE mg /g dm. This small difference of concentration suggests that the
use of open /closed vessels has little influence on the microwave extraction at 25°C.

3.6. Radical scavenging activity of extracts

All the grape seed powder extracts have exhibited an appreciable scavenging activity for the both methods (DPPH and ABTS\(^+\)). For the DPPH test, the results of the kinetic reaction illustrated in Fig.5A indicate that the extracts present a reaction time (maximum of inhibition) of 30 min. Moreover, the microwave and the maceration extracts exhibit more than 50% of DPPH inhibition, whereas for ultrasound extract, only 38% of DPPH inhibition was reached. Several dilutions were applied to the extracts (from 2 to 1000- fold dilutions), except for the extract obtained by microwave technique which exhibits more than 50% of DPPH inhibition after 10 dilutions, the rest of extracts present lower inhibition from 2 dilutions (Fig.5B). This result may be explained by the yield of microwave extraction (37.74 ± 0.32%) which is nearly three times higher than maceration yield (14.16 ± 0.15%) and five times higher than ultrasound (6.70 ± 0.25%). By ABTS\(^+\) test (Fig. 5C), the same result was obtained. The extracts obtained by microwave and maceration exhibit strong radical scavenging activities (78.05% and 72.30%, respectively) whereas the ultrasound extract exhibit only 41.65% of ABTS inhibition. The comparison of the two tests indicates that higher inhibitions were obtained by ABTS test for the three extraction methods. These results corroborate those found by Floegela et al. [27] which reports that, for vegetables, fruits and beverages, ABTS inhibition is higher than DPPH inhibition. Regarding the reported results in Fig 5C, it can denote that all the extracts possess antioxidant activity higher than BHA and BHT at the concentration of 1 ppm. Both synthetic antioxidants used in food processing which the allowable daily intake (ADI) should not exceed 0.5 and 0.05 ppm (BHA and BHT respectively) [28], present a risk for health and can be replaced by those naturally produced.
Fig. 5. (A). Kinetics of DPPH’ reduction of extracts obtained by different extraction methods, (B). Radical scavenging activity at different extract dilution (from 2 to d1000-fold dilution) by DPPH method, (C). Comparison of radical scavenging activity (DPPH and ABTS+ assays) of extracts with BHA and BHT.
4. CONCLUSION

In conclusion, this present work has permitted to introduce for the first time the Algerian autochthonous variety Ahmeur Bouamer for the determination of total polyphenols from grape seeds. This study has allowed us to optimize the conditions and extraction methods to obtain important extraction yields of total polyphenols in comparison with previous works [5, 6, 19, 22, 24, and 25]. It’s also important to note the positive influence of particle size parameter on the extraction yield, whatever the time and temperature of extraction. The extract which exhibit high radical scavenging activity is that obtained by microwave. This extraction method is a promising environment friendly technique for the obtaining of bioactive compounds from grape seeds which can be considered as a good source of natural and healthy compounds.

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