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Research Article

Ultrasound-Assisted Extraction: Effect of Extraction Time and Solvent Power on the Levels of Polyphenols and Antioxidant Activity of *Mesembryanthemum edule L. Aizoaceae* Shoots

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Abstract

Purpose: *To investigate the influence of extraction conditions assisted by ultrasound on the quality of extracts obtained from Mesembryanthemum edule shoots.*
Methods: *The extraction procedure was carried out in an ultrasonic bath. The effect of two solvents (methanol and ethanol) and two extraction times (5 and 10 min) were evaluated on the basis of the phenolic content and antioxidant activity of the plant extract.*
Results: *Significant variability in phenolic content and antioxidant activity, depending on the solvent (S), and to a lesser degree, extraction duration (D) and their interaction (S/D) were found. Methanol extract was significantly richer in total polyphenols than ethanol extract, their levels being 104.7 and 74.2 mg GAE.g^{-1}DW, respectively. For the two solvents used, longer extraction duration yielded higher polyphenol content. The ethanol extract exhibited higher antioxidant activity than the methanol extract. Moreover, Also, the longer sonication duration (10 min) yielded extracts with higher antioxidant activities.*
Conclusion: *The antioxidant capacity of M. edule is strongly influenced by the nature of the extracting solvent and the duration of sonication extraction.*

Keywords: *Antioxidant activity, Extraction duration, Mesembryanthemum edule, Polyphenols, Sonication*

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INTRODUCTION

Phenolic compounds are secondary metabolites characterized by the presence of an aromatic ring bearing free hydroxyl groups or engaged with a carbohydrate [1]. These molecules are present in all plant parts and are involved in many physiological processes such as cell growth, root formation, seed germination and fruit ripening [1]. Moreover, one of the most important properties attributed to these compounds is their antioxidant capacity which is mainly due to the redox properties of polyphenols which enable them to act as reducing agents, hydrogen donors and scavengers of reactive oxygen species [2]. Furthermore, polyphenols exhibit various biological activities such as antifungal, antibacterial, antiviral, anti-inflammatory, antiallergic, vasodilator and cardio-protective activities [3].

However, the extraction processes of phenolic compounds are a major variability factor in the antioxidant properties of extracts. Indeed, temperature, solvent extracting power, extraction time and the method adopted for extraction influence significantly the composition of the extract [4]. This variability is due to the different affinities of these compounds for solvent extraction and specifically to the polarity of the molecules constituting the solvent [5]. In addition, extraction of molecules from biological materials by conventional techniques, such as simple maceration, is time- and solvent-consuming. The development of modern techniques such as extraction assisted by microwave or ultrasound is intended to overcome these difficulties by increasing extraction efficiency, selectivity and kinetics [6]. In this context, the use of ultrasound or sonication to break the cell membranes has the advantage of reducing considerably the extraction time and increasing the extract yield. The application of ultrasound disrupts the cell wall structure and accelerates diffusion through membranes; thus, the cell lyses and hence facilitates the release of cell contents [6].

Due to their biologically active molecules, such as phenolic compounds, many halophyte species are used traditionally as a remedy against certain diseases [7]. Among them, *Mesembryanthemum edule* L. (Aizoaceae) is a facultative and spontaneous halophyte which is widespread in areas with harsh climatic conditions (high salinity, excessive luminosity, drought, etc). This halophyte has many economic and ecological interests [8]. In addition, *M. edule* stem is used in traditional medicine as a remedy against microbial infections, as well as treatment for sinusitis, diarrhea, childhood eczema and tuberculosis [9-10]. Similarly, their leaves are used as an antiseptic on wounds and burns and can treat mouth infections and sore throat [11].

The objective of this study was to optimize the extraction conditions for *M. edule* shoots in order to achieve the highest polyphenols levels and antioxidant activities based on the choice of the most suitable solvent and duration of sonication extraction.

EXPERIMENTAL

Plant sampling and extraction procedure

M. edule shoots were collected from Djerba region, Tunisia (an arid bioclimatic zone) in late 2009. The harvested plants were identified by a taxonomist, Dr A Smaoui, of the Biotechnology Center, Borj-Cédria, Tunisia, and a voucher specimen [AME27] was deposited at the herbarium of the Laboratory of Plant Extremophiles at the Biotechnology Center (Technopark of Borj-Cédria). The samples were freeze-dried and then ground into fine powder. For the polyphenol extraction, 0.5 g of the powder was placed in Kimax glass tubes and separated into two groups. The first batch was treated with methanol while the second was extracted with ethanol. The mixture was homogenized by vortexing for 2 min, placed in an ultrasonic bath (Turner Designs 88 155) at room temperature and sonicated for two periods: 5 and 10 min continuously. At

the end of sonication, the suspension was cooled to room temperature and then filtered through ashless filter paper (Whatman no. 4) to remove solid debris.

Determination of phenolics content

Total phenolic compounds in *M. edule* extracts were determined with Folin-Ciocalteu reagent using the method described by Falleh *et al* [7]. To 125 µl of the suitably diluted (20-fold) extract obtained above, 500 µl of Folin-Ciocalteu reagent and 125 µl of distilled water were added. The mixture was shaken, mixed with 1250 µl of Na_2CO_3 and adjusted with distilled water to a final volume of 3 mL. After incubation for 90 min at 23 °C in the dark, the absorbance was read spectrophotometer (Anthelie Advanced 2, Secoman) at 760 nm. Total phenolic content was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW) using a calibration curve constructed with gallic acid (0 - 400 µg mL^{-1}). All samples were analyzed in triplicate.

Evaluation of 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulphonic acid) (ABTS) scavenging activity

Antioxidant activity of the extract was measured using an improved ABTS method [7]. ABTS solution containing the cation, $ABTS^{\circ+}$,was prepared by the reaction of 7 mM ABTS with 2.45 mM potassium persulfate, and then incubating the resultant mixture in the dark for 16 h. The $ABTS^{\circ+}$ solution was then diluted with absolute ethanol to obtain an absorbance of 0.700 ± 0.005 at 734 nm, 950 µl was added to 50 µl of the test sample at varied concentration and mixed vigorously. The reaction mixture was allowed to stand at 23 °C for 6 min and the absorbance at 734 nm was immediately recorded spectrophotometrically. The inhibition of $ABTS^{\circ+}$ radical was calculated as in Eq 1.

$ABTS^{\circ+}$ scavenging effect (%) = [(A0-A1)/A0]*100 …… (1)

where A0 and A1 are the absorbance values of control and test sample, respectively. Radical scanvenging activity was expressed as IC_{50} (µg.ml^{-1}), *i.e*., the extract dose required to cause 50 % decrease in absorbance. A lower IC_{50} value corresponds to higher antioxidant activity. All samples were analyzed in triplicate.

Evaluation of iron reducing power

The reducing power of the extract was determined *via* the transformation of Fe^{3+} to Fe^{2+} using a previously described method [7]. The test extract solution, ranging from 50 - 500 µg.mL^{-1}, was mixed with 2.5 mL of 0.2M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1 %w/v). The mixture was incubated at 50 °C for 20 min, 2.5 mL of 10 % trichloroacetic acid (TCA) was added and the mixture centrifuged for 10 min at 1000 g. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL of ferric chloride (0.1 % w/v), and the absorbance was read spectrophotometrically at 700 nm. EC_{50} value (µg.mL^{-1}), *i.e*, the effective concentration of the extract at which the absorbance was 0.5.

***β*-Carotene bleaching test (BCBT)**

Two mg of *β*-carotene was dissolved in 20 mL chloroform and to 4 mL of this solution, linoleic acid (40 mg) and Tween 40 (400 mg) were added. The chloroform was evaporated under vacuum at 40 °C and 100 ml of oxygenated water was added and vigorously shaken to yield fresh an emulsion. An aliquot (150 µl) of the *β*-carotene/linoleic acid emulsion was distributed in 96-well microtitre plates and the extract (10 µl) added at different concentrations, ranging from 100 - 800 µg.mL^{-1}. Three replicates per concentration were prepared. The microtitre plates were incubated at 50 °C for 120 min, and the absorbance was measured using a model EAR 400 microtitre reader (Labsystems Multiskan MS) at 470 nm. Readings of the samples were performed immediately (t = 0 min) and after 120 min of

incubation [7]. The antioxidant activity of the extracts was evaluated in terms of blanching inhibition of the β-carotene using Eq 2.

β-carotene bleaching inhibition (%) = [(S-C_{120})/(C_0-C_{120})]*100 ……(2)

where C_0 and C_{120} are the absorbance values of the control at 0 and 120 min, respectively, and S the sample absorbance at 120 min. The results are expressed as IC_{50} values (µg mL^{-1}).

Statistical analysis

The results are expressed as mean ± standard deviation of three replicates. Multiple sample comparison was performed using Statgraphics Plus program version 5.1 for windows. Analysis of variance (ANOVA) followed by Duncan's multiple comparison test were applied to the data as appropriate. Where ANOVA could not be used, Kruskal-Wallis test was applied after checking for normal distribution of the groups and homogeneity of variances. The level of significance was $p < 0.05$.

RESULTS

Table I shows that solvent extracting power (S), extraction duration (D) and, to a lesser extent, their interaction (S/D), exerted a significant effect [$p < 0.05$] on the total polyphenol levels and antioxidant activity of the plant extracts.

Table I: Effect of extraction solvent and duration on the levels of total polyphenols and antioxidant activity of *Mesembryanthemum edule* shoot extracts.

Parameter	S	D	S/D
Total polyphenols	2977***	537**	121*
Antiradical activity against ABTS	6114***	313**	33*
Reducing power	7972***	583**	17*
β-Carotene bleaching inhibition activity	31***	7**	6*

*** $p < 0.001$; ** $p < 0.05$; * $p < 0.01$

Total polyphenolic content of *M. edule* shoots

As Figure 1 shows, there was a significant variation in the phenolic levels of the extracts. Regardless of the sonication duration, methanol extract was significantly richer in total polyphenols (105 mg GAE.g^{-1}DW) than ethanol extract (75 mg GAE.g^{-1}DW). Furthermore, increase in the duration of sonication from 5 to 10 min enhanced the polyphenol contents of ethanol and methanol extracts by 32 and 8 %, respectively.

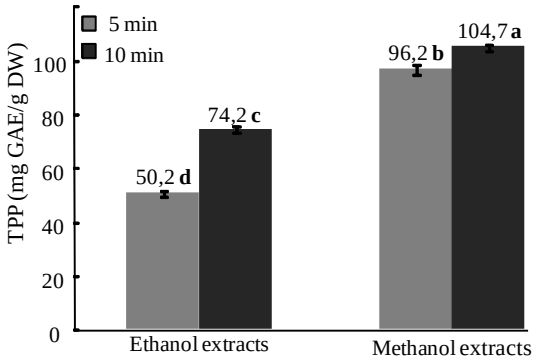

Figure 1: Total polyphenol contents (TPP) of methanol and ethanol extracts of *Mesembryanthemum edule* shoots after 5 and 10 min of sonication; n = 3, $p < 0.05$]; TPP (total polyphenol content) is expressed in milligram of gallic acid equivalent per gram of dry weight.

Antioxidant activities of *M. edule* shoot extract

Scavenging activity

Fig 2 indicates that the ethanol extract exhibited higher scavenging activity than the methanol extract against ABTS. This superiority is reflected in the values of the inhibition concentration at 50 % (IC_{50}) which are statistically lower ($p < 0.05$, 500 and 573 µg.ml^{-1} at 5 and 10 min, respectively) for the ethanol extract than for methanol extract (> 700 µg.ml^{-1} for both 5 and 10 min sonication). The results also show that the extract obtained by longer sonication duration exhibited greater scavenging activity, and this effect was more pronounced for the ethanol

extract. Furthermore, solvent extracting power was the more important factor in enhancing extract scavenging activity than sonication duration.

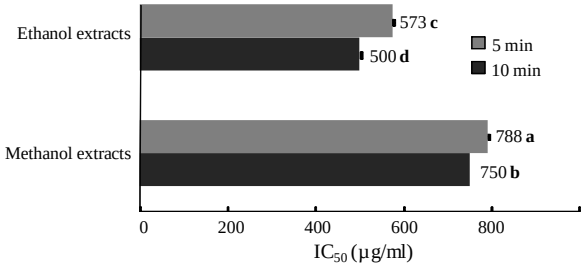

Figure 2 Scavenging activity of methanol and ethanol extracts of *Mesembryanthemum edule* shoots after 5 and 10 min of sonication, against ABTS (n = 3, p< 0.05)

Reducing power of iron

The results for the iron reducing capacity of the extracts are shown in Fig 3. The ethanol extract had a significantly higher reducing power than the methanol extract with 50 % effective concentration (EC_{50}) of 164 and 127 $\mu g.ml^{-1}$ (5 and 10 min sonication, respectively) for the former, and 234 and 255 $\mu g.ml^{-1}$ (5 and 10 min, respectively) for the latter. Here again, the solvent extracting power had a greater influence on reducing power than change in sonication duration.

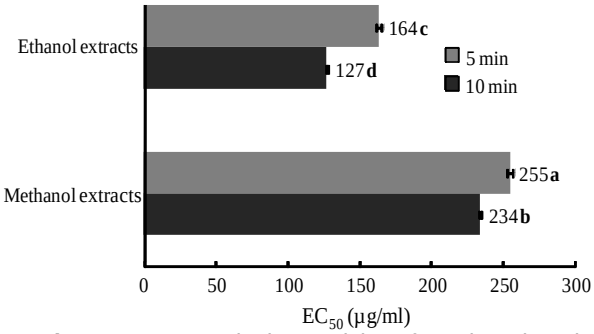

Figure 3: Iron reducing activity of methanol and ethanol extracts of *Mesembryanthemum edule* shoot extracts after 5 and 10 min of sonication (n = 3, p< 0.05)

Inhibition of β-carotene bleaching activity

The results for the inhibitory activity of the extracts against *β*-carotene bleaching are shown Figure 4. Again, the ethanol extract has a lower IC_{50} value (475 $\mu g.ml^{-1}$), and hence a stronger inhibitory activity than the methanol extract (IC_{50} = 690 $\mu g.ml^{-1}$) against lipid peroxidation. In addition, Figure 4 also shows that sonication extraction time had a significant ($p < 0.05$) effect on the inhibitory activity of the ethanol extract (IC_{50} = 691 and 475 $\mu g.ml^{-1}$, after 5 and 10 min sonication, respectively) but sonication time had no significant effect ($p < 0.05$) on the activity of the methanol extract. Here too, solvent extracting power was more important than sonication duration in enhancing inhibitory activity.

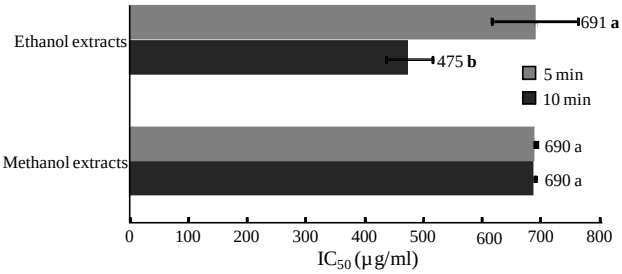

Figure 4: Inhibition of *β*-carotene bleaching activity by methanol and ethanol extracts of *Mesembryanthemum edule* shoots after 5 and 10 min of sonication (n = 3, p< 0.05)

DISCUSSION

Phenolic content and antioxidant capacities of plant extracts largely depend on extraction conditions and compositions [12]. Antioxidant molecules and capacities are influenced by several factors that cannot be fully evaluated by a single method. Therefore, it is necessary to examine more than one extraction condition. Overall, the results indicate that the most pronounced effect on the quality of the extracts was exerted by solvent extracting power (S), followed by the duration of extraction (D) and then by their interaction (S/D).

Furthermore, there was a significant difference in total polyphenol content obtained between the two extracting solvents (ethanol and methanol) and to a lesser extent between the two sonication extraction times. Although methanol is one of the most frequently used extraction solvents, its use is often questioned because of its high toxicity for humans [13]. This thereforemakes ethanol a more attractive solvent for the extraction of phenolic compounds since it has similar chemical properties to those of methanol while being much less toxic [13]. Moreover, the methanol has a polarity 6.6 compared to 5.2 for ethanol [6], and. this difference in polarity might have influenced in the amount of phenolic compounds in the extract. Several studies have demonstrated that the solubility of phenolics in the extraction solvent is dependent on solvent polarity and that methanol is one of the most suitable solvents for the extraction of phenolics from plants [14,15]. In this regard, Trabelsi *et al* [16] found that the ethanol leaf extract of the halophyte, *Limoniastrum monopetalum*, exhibited a much lower content of polyphenols (1 mg GAE.g DW^{-1}) than the methanol extract which had a corresponding content of 15.8 mg GAE.g DW^{-1}.

The levels of total polyphenols found for methanol extracts of *M. edule* shoots in the present study vary between 96.2 and 104.7 mg GAE.g DW^{-1}; these levels are generally higher than those reported in the literature for other halophytic species of medicinal interest [12,17, 18]. The high content of these secondary metabolites is probably due to the harsh climatic conditions that usually cause various abiotic stresses in halophytes biotopes. These abiotic stresses (drought, salinity, high temperature and sunlight) are the main cause of the biosynthesis stimulation of phenolic compounds that also called 'stress metabolites' [14].

The findings for the ethanol extract are interesting in that the extract, despite its lower phenolic content, showed higher anti-oxidant activity than the methanol extract. This may be due to the different qualities of the polyphenols present in both types of extracts. Although phenolic compounds are known for their strong antioxidant activity [18,19], two key criteria often influence their effectiveness. Firstly, the structure of each phenolic compound is a key element in determining the effectiveness of its activity. Although these molecules share a common structure in the aromatic ring with at least one hydroxyl group, they are most often substituted by different kinds of chemical groups such as hydroxyl, methoxyl, sulfate and others [18]. The position and nature of these substituents affect greatly the effectiveness of phenolic compounds activity. Secondly, it is recognized that these molecules interact with each other through synergism and/or antagonistic processes. With regard to antagonism mechanism, the mixture of phenolic compounds has a relatively low antioxidant activity [20]. Thus, the combination of different structures as well as their relative proportions and interactions (synergism or antagonism) are the source of the variability of their antioxidant activity [20]. These phenomena may explain why the methanol extract, though richer in polyphenols, exhibited a lower antioxidant activity than the ethanol extract which contained lower levels of these molecules.

CONCLUSION

This study shows that the total polyphenol levels of *M. edule* extract as well as its antioxidant activities are significantly influenced by the nature of the extraction solvent, sonication extraction time and the interaction of these two factors. However, solvent extracting power is the most important factor affecting the antioxidant capacity of *M. edule*. Thus, for this halophyte, the use of ethanol and 10 min sonication extraction time are recommended for ultrasound-assisted extraction.

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