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EXTRACTION METHODS COMPARISON FOR POLYPHENOLS RECOVERY AND ANTIOXIDANT ACTIVITY FROM *OPUNTIA FICUS INDICA*'S SEEDS

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Abstract: The aim of this work is to develop an effective extraction route for biological compounds from *Opuntia ficus indica* seeds using two conventional methods: water bath shaker and agitation as well as a non-conventional one: microwave. The results indicate a significant effect of both temperature and time on the conventional extraction, with highest values for total phenolic content of 200 and 260 mg Gallic acid equivalent /100 g Dry matter (mg GAE/100 g DM) and for antioxidant activity of 22 and 24 mg GAE/100 g DM, obtained by water bath shaker and stirrer, respectively. However, the microwave extraction method under optimum conditions shows much better extraction efficacy with 300 and 43 mg GAE/100 g DM for total phenolic content and antioxidant activity, respectively. The efficiency of microwave technique could be a promising extraction tool of biological compounds from prickly pears seeds which could eventually be considered as an alternative source for synthetic antioxidants.

Keywords: prickly pear seeds, conventional method, non-conventional method, phenolic compounds, antioxidant activity.

INTRODUCTION

Opuntia ficus indica (*OFI*) also known as prickly pear is a tropical or subtropical plant from the *cactaceae* family, originally grown in South America (Özcan et al., 2011). This plant has a global distribution and grows wild mainly in arid and semi-arid regions. Nowadays, there are more than 250 species, distributed in Mediterranean Europe, India, and in American and African countries (Rocchetti et al., 2018).

The cactus pear has been used for a long time for human consumption or as a forage plant. Otherwise, it was used by numerous traditional medicines of different civilizations, particularly as treatment of diabetes, ulcer, burns, edema, wounds, diarrhea and indigestion (Kaur et al., 2012; Zeghbib et al., 2022). In Algeria, *OFI* was used principally for human food like fresh fruit, but also for livestock forage and planted as ornamentals or for fencing (Chougui et al., 2013).

The economic importance of *OFI* is growing due to its numerous nutritional properties and human health benefits due to the presence of some bioactive components like betalains, carotenoids, minerals, and phenolic compounds (Ammar et al., 2015). This latter represents a large group of secondary metabolites found in plants, and their consumption confer the main organoleptic characteristics of plant-derived food and beverage such as color and taste properties. Meanwhile, they present a high antioxidant potential by scavenging free radicals implicated in several human pathologies like cardiovascular diseases, neurodegenerative disorders, and cancers (Benayad et al., 2014). Nevertheless, it is important to highlight that the study of the biological effects of these molecules requires firstly an extraction stage defined as the separation of active therapeutic compounds, with removing the majority of insoluble materials using different solvents (Phuse et al., 2017).

In the literature, numerous extraction methods for biological compounds have been reported; however, these structures can be affected by various parameters like extraction time, temperature and solvent composition(Ilaiyaraja et al., 2015; Alara et al., 2021) . Thereby, the development of an appropriate extraction method is becoming necessary to promote a higher amount of active compounds. In this context, the aim of this research work consists on the development of an efficient approach for the extraction of bioactive compounds from *OFI* seeds through two conventional methods (water bath shaker and stirring) as well as non-conventional (microwave).

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu reagent, gallic acid, and sodium carbonate were purchased from Biochem, Chemopharma (France) and 1-1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma

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Chemical (Sigma-Aldrich GMBH, Sternheim, Germany).

Plant material

This study was conducted on cactus pear (*Opuntia ficus indica*) collected in Bejaia (Algeria). The fruits were chosen according to the same form, color and without externals injuries. They have been washed and manually peeled with a knife. The pulp was separated from the seeds after mixing the fruits in a blender then centrifuged at 1800×g for 15 min. The recovered seeds were washed with distilled water, lyophilized (Christ, alpha 1-4 LD plus, Germany), and then crushed using an electronic grinder (IKA Werke, Staufen, Germany). After sieving, the powder with a diameter ≤ 250 µm was stored for further analysis.

Preparation of extracts

An aliquot of 0.3 g *OFI* seeds powder was placed in a glass vial with 20 mL of distilled water. The extraction procedure was conducted with two conventional methods and one non-conventional method.

Conventional methods

In this experiment, the extraction procedure as illustrated in Figure 1, was carried out using water bath shaker (Memmert GmbH+co.KG, Germany) and a stirrer (Velp Scientifica, Europe) during different periods of time (5, 10, 15, 20, 25 and 30 min) at different temperatures (25, 35, 45, 55, 65 and 75 °C), then the obtained extracts were centrifuged (Nüve NF200, Turkey) and filtered with a standard paper filtered (Whatman, Germany).

Non-conventional method

The extraction procedure was conducted using a modified domestic microwave oven (Maxi power, China), equipped with a digital control system for irradiation time and power. The apparatus was modified by adding a refrigerating system in order to condense the vapors generated during the extraction and avoid any losses.

Two types of experiments were performed: (i) at first, the extraction time variation (5, 10, 15, 20, 25 and 30 min) was optimized at constant microwave power of 300 W; (ii) after that, an investigation of the power effect on antioxidants extraction was conducted at different levels (100, 300, 500 and 700 W) for the previously optimum extraction time. The obtained extracts were centrifuged and paper filtered. The experimental steps were summarized in Fig. 1.

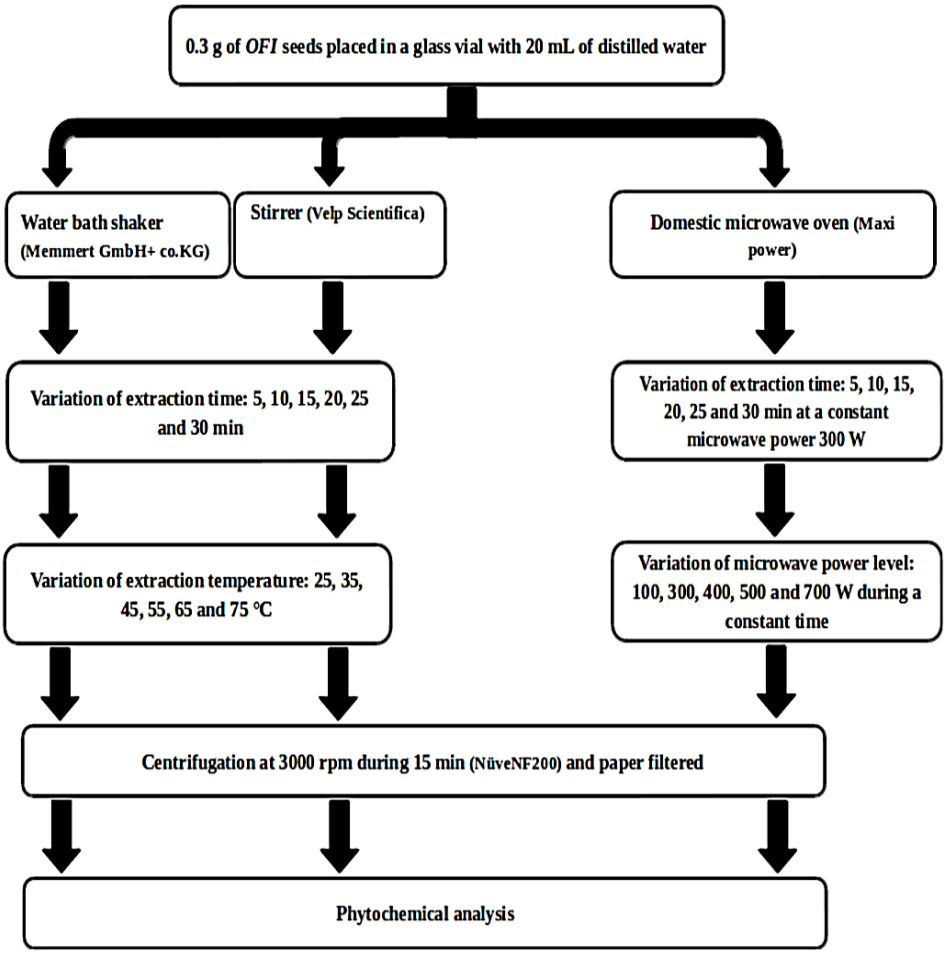

Fig 1. Flowchart of the extraction steps used for the conventional and non-conventional methods.

Total phenolic content (TPC)

TPC was determined according to the method described by Singleton et al. (1965), with slight modifications. An aliquot of the extract (150 µl) was mixed with 750 µl of Folin-Ciocalteu solution (ten-time diluted) and 600 µl sodium carbonate. After 1 hour of

incubation in darkness, the absorbance was measured at 750 nm with a spectrophotometer (Uviline 9400, France). TPC was expressed as mg gallic acid equivalent (GAE) per 100 g dry matter (DM).

Antioxidant activity

The DPPH radical scavenging test was used to evaluate the antioxidant activity of seed extracts as reported by a modified protocol of Brand-Williams et al. (1995). Briefly, 100 µl of extract was mixed with 1 ml of methanolic DPPH solution. After 30 min of incubation in darkness, the absorbance was measured at 515 nm. Gallic acid was used as standard and the antioxidant activity was expressed as mg gallic acid equivalent (GAE) per 100 g DM.

Statistical analyses

All data were reported as means of three replicates. Statistica 8 software was used to compare the results by the analysis of the variance (ANOVA / MANOVA) and the differences among means were determined using LSD (Least Significant Difference) test and the level of significance was taken at $p < 0.05$.

Figure edition

All figures were edited by the freeware GNU Image Manipulation Program GIMP 2.10.10.

RESULTS AND DISCUSSION

Polyphenols are molecules largely present in plants. These compounds are endowed with antioxidant properties; thus, they could be used as treatment of many diseases like inflammation and cancer. According to Benattia et al. (2018), the majority of these molecules are water-soluble; then, it is more suitable to use polar extracts rather than apolar ones for their recovery. Moreover, it is important to highlight that the range of extraction time is considerably variable; it can change from a few minutes to more than 24 hours, depending on bioactive compounds and plant material (Bouterfas et al., 2014). Thereby, in the current study, the choice of water and extraction time selected was to satisfy the practical and economic aspects.

Antioxidants extraction using conventional methods

Water bath shaker

The results of phenolic compounds extraction using water bath shaker from *OFI* seeds (Fig. 2A) indicated that TPC increased with the rise of temperature and the extraction time. The initial contact of powder with water as extraction solvent allowed recovering 126 mg GAE/100 g DM of phenolic compounds. At the extraction temperature of 25 °C, this content remained constant until 20 min, afterward; phenolic extraction increased significantly for the next five minutes then it stabilized around 138 mg GAE/100 g DM. At 35°C, the phenolic compound levels increased during the extraction time; the maximum concentration was achieved after 20 min reaching 156 mg GAE/100 g DM.

When varying temperatures (45, 55, 65, and 75 °C), the phenolic compounds yield increased during the first five minutes of extraction. The maximum content was obtained after 20 min for 45°C and after 25 min for 55°C with 183 and 188 mg GAE/ 100 g DM, respectively. For higher temperatures of 65 and 75°C, the phenolic extraction became steady at about 200 mg GAE/100 g DM after only 15 min.

The statistical analysis had shown globally that higher temperature promoted the extraction of more phenolic compounds. Also, it was shown that an efficient yield was obtained at 65°C after 25 min of extraction.

Alternatively, the evaluation of the antioxidant activity of *OFI* seeds extracts revealed a lower activity over time for the water bath shaker (Fig. 2B), it reached 19 mg GAE/100 g DM at 25ºC after 25 min. No significant improvement was achieved for high temperature as the same concentration was obtained at 35°C after 20 min.

At 45°C, the antiradical activity was found to be slightly higher reaching 20 mg GAE/100 g DM after 15 min, while the antioxidant activity was greater when increasing temperature with a mean value of 22 mg GAE/100 g DM after 15 min at 55, 65, and 75°C. Moreover, it is important to indicate that statistical analysis showed that temperature and extraction time had a significant effect on the DPPH antiradical activity and the optimum value was obtained at 65°C after 15 min of extraction time.

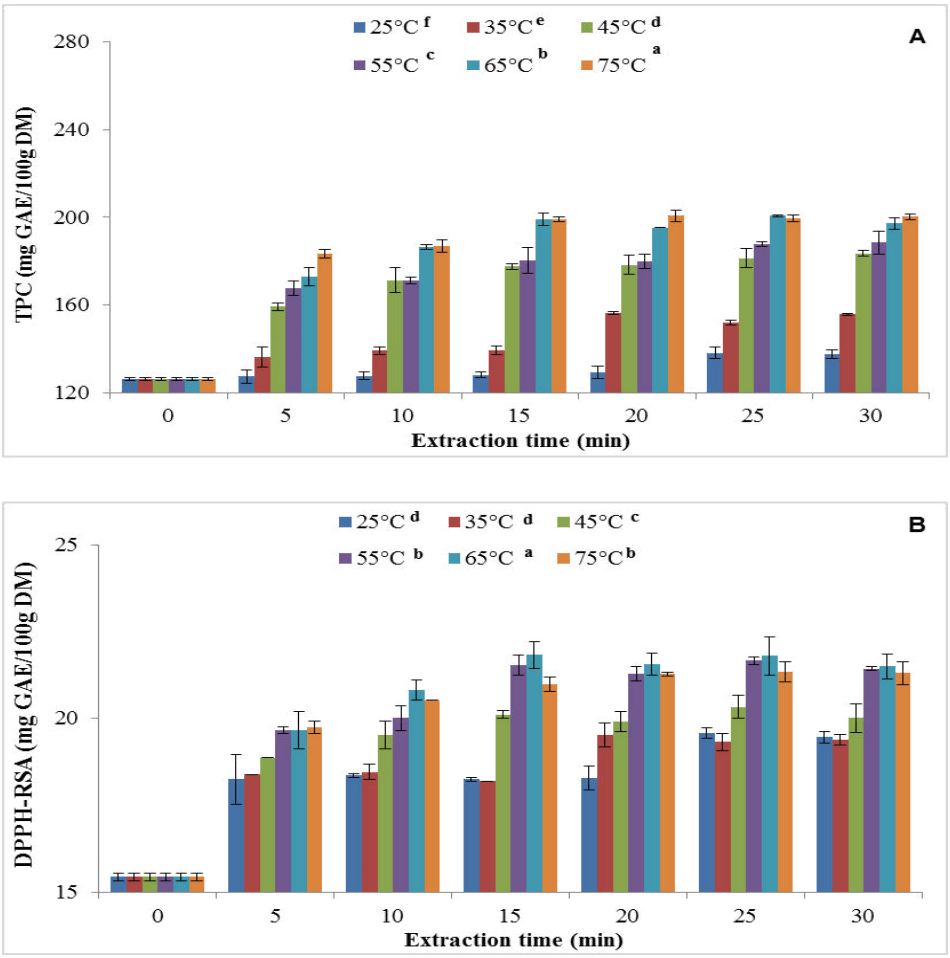

Fig. 2. Total phenolic contents (**A**) and DPPH radical scavenging activity (DPPH-RSA) (**B**) of prickly pear seeds obtained by water bath shaker extraction method. *The temperatures with different letters were significantly different ($p < 0.05$).*

Stirrer plate

Similarly, statistical analysis showed that temperature and time had a significant positive effect on phenolic compounds extraction of *OFI* seeds using a stirrer (Fig. 3A). Nevertheless, a small amount of phenolic compound extraction at 25°C was achieved, it enhanced from 10 to 20 min to finally reaching a steady state with a value around 190 mg GAE/100 g DM.

With increasing temperature (35, 45, and 55°C), the value of TPC increased similarly until 15 min, then increased gradually to reach a content of phenolic 214 mg GAE/100 g DM at 30 min, 233 mg GAE/100 g DM at 25 min, and 242 mg GAE/100 g DM at 30 min. For much higher temperatures (65 and 75°C), the extraction of phenols continued to increase during the first 10 min and the content thereafter stabilized at 264 and 260 mg GAE/100 g DM, respectively.

Both temperature and time showed a great influence on antioxidants extraction with the stirrer (Fig. 3B). Antioxidant activities obtained for the temperatures of 25 and 35°C increased similarly according to time, to reach finally an average value of 20 mg GAE/100 g DM after 20 min.

For temperatures 45, 55, and 65°C, there was a rapid increase of antioxidant activities during the first five minutes of extraction, which subsequently stabilized at an average activity of 24 mg GAE/100 g DM after 15, 20 and 10 min, respectively.

The extraction results using the temperature of 75°C indicated the increase of DPPH radical scavenging activity until 10 min, then remained steady during the next 5 min, after that it slightly decreased to reach a stable value of 22 mg GAE/100 g DM.

Overall, an efficient recovery of biological compounds with the stirrer was obtained at 55°C after 20 min. Thus, temperature increase did not only allow reducing the extraction time but also it led to improve the antioxidants recovery. However, using very high temperatures could lead to a decrease in antioxidant activity by oxidation of bioactive compounds.

Generally, for the conventional techniques, a prolongation of the extraction time resulted in a good yield recovery of phenolic and antioxidants compounds. Nevertheless, this latter stabilized after a certain time of extraction. The same finding was reported by Chaalal et al. (2012) for the extraction of phenolic compounds and *in-vitro* antioxidant capacity of prickly pear seeds. This result could be explained by Fick's second law of diffusion, which describes the transfer overtime of solute between two mediums separated by a membrane (Enderle, 2012), according to the following equation:

$$\frac{dq}{dt} = -DA\frac{dc}{dx}$$

(Where: q = quantity of solute; A = membrane surface area; c = concentration of the solute; D =

diffusion coefficient; dx = membrane thickness; $\frac{dc}{dx}$ = concentration gradient)

Thereby, it can be explained as follow: after a certain time, a final equilibrium will be reached between the solute concentration in the plant matrix and in the bulk solution (solvent), therefore; an increase in the extraction time would not necessarily mean the extraction of more phenolic antioxidants. On the other hand, an excessive prolongation of the extraction time may provoke phenolic oxidation due to light or/and oxygen exposure (Hismath et al., 2011).

Also, the rise of temperature in the range 25 - 75°C increased the extraction of phenolic compounds and improved the antiradical activity of prickly pear seeds. According to several studies in the literature, it was found that the increase of the temperature improved the extraction process, hence producing a better antioxidant activity. In fact, the rise in temperature softened plant tissues and break phenol-protein and phenol-polysaccharide complexes leading to an enhancement of the polyphenols migration outside the matrix by increasing the diffusion rate and the solubility of the extracted substances. Furthermore, a high temperature had a direct effect on the solvent by reducing its viscosity, favoring a better penetration within matrix particles, and therefore improved the extraction (Bouterfas et al., 2014 ; Mohamed Mahzir et al., 2018).

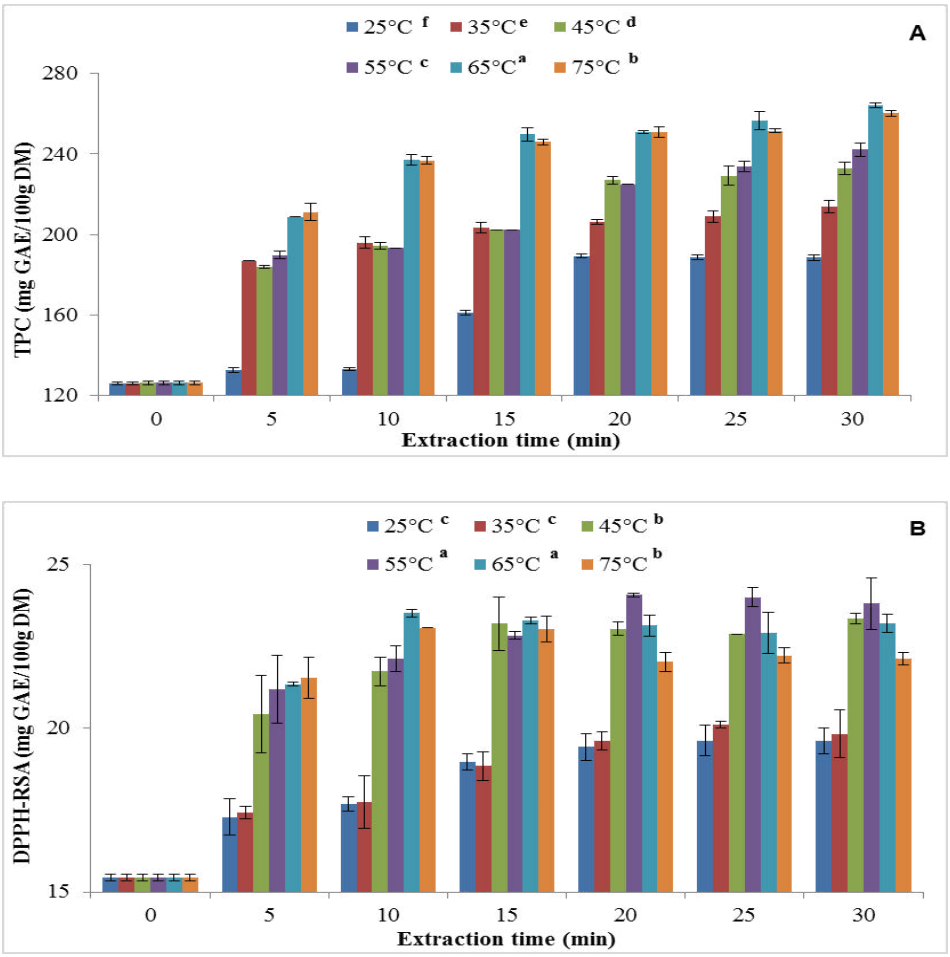

Fig. 3. Total phenolic contents (**A**) and DPPH radical scavenging activity (DPPH-RSA) (**B**) of prickly pear seeds obtained by magnetic stirring extraction method. *The temperatures with different letters were significantly different ($p < 0.05$).*

Antioxidants extraction using the non-conventional method

Microwave is considered as a recent extraction technique and the most used during the last decade compared to conventional methods. Its mechanism consists to irradiate directly the sample contained in the solvent. Then, the intense resulting heating will create a high vapor pression of the moisture inside the solids thereby creating breakage of the cells walls accompanied by the release of the trapped compounds (Pangarkar, 2008).

The statistical analysis of the microwave extraction (Fig. 4) showed a significant positive effect of time on TPC and antioxidant activity of bioactive compounds extraction from prickly pear seeds. Indeed, the phenolic compounds extraction by microwave showed an intense and rapid yield, particularly from 0 to 5 min and increased gradually during the rest of time to reach the highest content of 292 mg GAE/100 g DM at 30 min. The antiradical activity increased with the extraction time until 20 min, then became stable at a higher value corresponding to 32 mg GAE/100 g DM.

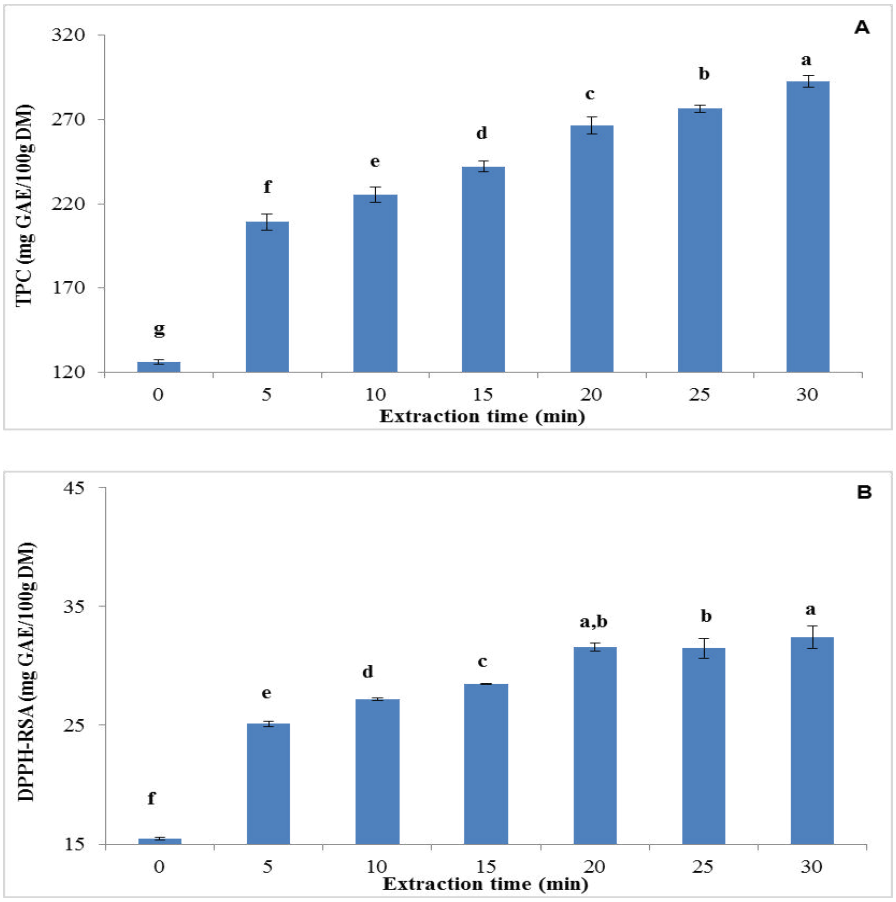

Fig. 4. Total phenolic contents (**A**) and DPPH radical scavenging activity (DPPH-RSA) (**B**) of prickly pear seeds obtained by microwave-assisted extraction method. *Results with different letters were significantly different ($p < 0.05$).*

In addition to the extraction time, the microwave power significantly influenced the phenolic compounds extraction and the antioxidant activity; it raised with increasing microwave power until reaching 500 W, then the maximum values became constant at corresponding values of 303 (TPC) and 43 mg GAE/100 g DM (antioxidant activity), as shown in Fig. 5. Moreover, Dahmoune et al. (2015) also observed that increasing of microwave power to 500 W led to a significant increase of TPC recovery from Myrtle leaves. However, this content decreased beyond 600 W, concluding that extraction at higher microwave power levels did not ameliorate phenolic compounds extraction.

The statistical comparison between the three methods, in terms of phenolic compound extraction and antioxidant activity, indicated that microwave technique could be considered as the best extraction method, followed by stirrer method and water bath shaker.

The results of *OFI* seeds showed a very high correlation ($p < 0.001$) between antioxidant activity and TPC for the two conventional methods with $R^2 = 0.79$ and the non-conventional method with $R^2 = 0.98$. This indicated that phenolic compounds contributed to antioxidant activity with 79% for the two first methods and with 98% for the microwave method.

In addition of the difference between the extraction mechanism of each method, the heat generated by the conventional used methods transferred from the heating medium to the interior of the sample and the mass transfer occurred from inside to the outside of the matrix. In comparison, the microwave method, favored a faster heating reaching rapidly a temperature of 73°C during the first five min which dissipated along the volume through the irradiated medium resulting in a reduced thermal gradient. This could explain the great efficiency of the microwave apparatus (Boggia et al., 2016).

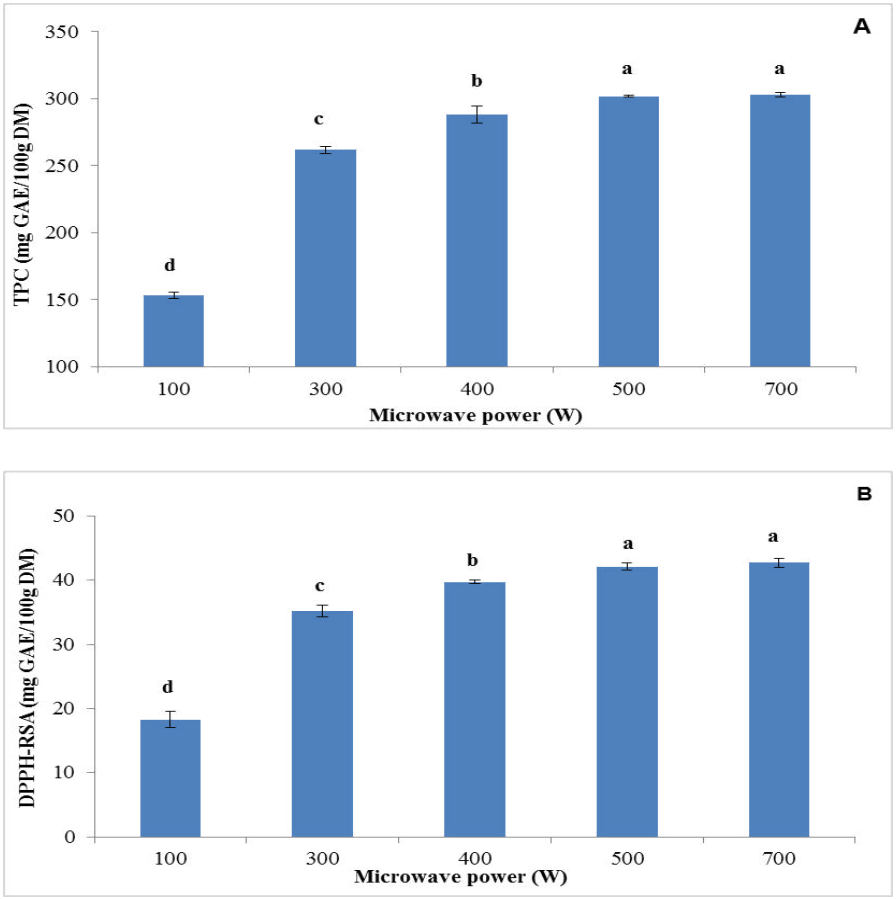

Fig. 5. Total phenolic contents (**A**) and DPPH radical scavenging activity (DPPH-RSA) (**B**) of prickly pear seeds as a function of microwave power. *For each parameter, results with different letters were significantly different ($p < 0.05$).*

Furthermore, many published studies reported that microwave extraction is cost-effective compared to conventional techniques because of its numerous advantages, including a reduced processing time, less amounts of solvent and lower energy consumption as well as high reproducibility. Hence, it was adopted as a powerful and potential alternative for the extraction of bioactive compounds from plants matrix (Boggia et al., 2016 ; Both et al., 2015).

CONCLUSION

The results of the comparative study showed a significant effect of the extraction methods for biological compounds from prickly pears seeds; however, the microwave method was revealed to be more efficient than the conventional ones, resulting a high recovery of phenolic compounds and a better antioxidant activity. Besides, *OFI* seeds could be considered as a promising source of phenolic antioxidant which could be a good alternative for synthetic antioxidants.

AUTHORS CONTRIBUTION

Conceptualization: Boudjouan F., Zeghbib W.; Methodology: Boudjouan F., Bachir Bey M.; Data collection: Zeghbib W., Djaouden O.; Data processing: Zeghbib W. ; Writing – original draft preparation: Boudjouan F., Zeghbib W. Bachir Bey M.; Writing – review and editing: Boudjouan F., Zeghbib W., Elothmani D., Bououdina M., Louaileche H.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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