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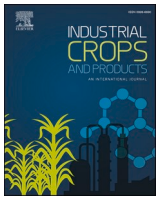

Optimization of the conditions for ultrasound-assisted extraction of phenolic compounds from *Opuntia ficus-indica* [L.] Mill. flowers and comparison with conventional procedures

Fatiha Brahmi [a,*], Federica Blando [b,*], Redha Sellami [a], Sabrina Mehdi [a], Luigi De Bellis [c], Carmine Negro [c], Hayate Haddadi-Guemghar [a], Khodir Madani [a,d], Lila Makhlouf-Boulekbache [a]

[a] *Laboratory of Biomathematics, Biochemistry, Biophysics and Scientometry, Faculty of Natural and Life Sciences, University of Bejaia,06000 Bejaia, Algeria*
[b] *Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Research Unit of Lecce, Via Prov. le Lecce-Monteroni, 73100 Lecce, Italy*
[c] *Department of Biological and Environmental Sciences and Technologies (DiSTeBA), Salento University,73100 Lecce, Italy*
[d] *Agri-Food Technologies Research Center, Targua Ouzemmour Road, 06000 Bejaia, Algeria*

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ABSTRACT

Opuntia ficus-indica [L.] Mill. (OFI) flower has been studied less than OFI fruit and cladode for the bioactive compounds occurrence. Also, ultrasound-assisted extraction (UAE) technique has been applied less than conventional techniques to extract bioactive compounds, generally. In this work, UAE was developed to extract phenolic compounds from OFI flowers, collected from wild population plants in the department of Bejaia (Algeria). Single-factor experiments and response surface methodology were designed to study the effect of ethanol concentration (20–100%), extraction time (10–60 min), and extraction temperature (30–70 °C) on the total phenolic content (TPC). Optimal UAE conditions were 36% ethanol, an extraction time of 60 min, and a temperature of 53 °C, which produced an extraction of 24.4 ± 0.82 mg of total phenolics expressed as mg Gallic Acid Equivalent (GAE)/g Dry Weight (DW). The bioactive compounds extraction efficiency was statistically higher using UAE than using conventional extraction methods (maceration and Soxhlet extraction). The Liquid Chromatography Time-of-Flight Mass Spectrometry (LC/MS–TOF) analysis of the optimized UAE extract detected mostly the occurrence of phenolic acids (piscidic and eucomic acids) and isorahmnetin derivatives. Interestingly, piscidic and eucomic acids have been identified and quantified for the first time in OFI flower extract. The antioxidant activity assessed with different assays were 13.8 ± 0.27μmol Trolox Equivalent (TE)/g DW for DPPH, 4078 ± 80.3 μmol TE/g DW for Ferric Reducing Antioxidant Power (FRAP), 3091 ± 139 μmol TE/g DW for total antioxidant activity (TAA) by phosphomolybdate assay, 80.8 ± 4.17μmol TE/g DW for Trolox Equivalent Antioxidant Capacity (TEAC) by ABTS assay. The UAE process deeply enhanced the yield and significantly reduced the extraction time in comparison with conventional methods (maceration and Soxhlet extraction), confirming that OFI flower is a valuable source of bioactive compounds that can be exploited in some industries.

1. Introduction

Opuntia ficus-indica [L.] Mill. (OFI) (cactus pear) is the most common *Opuntia* species throughout the world and the only one cultivated in the Mediterranean area, where is used mainly for fruit production, but also as forage in arid and semi-arid lands during drought period when shortage of herbaceous plants occurs. OFI is a fast growing plant, showing easy adaptation to poor soils and scarce water requirement (Aragona et al., 2018; El-Hawary et al., 2020). The potential of biomass production and carbon sequestering of OFI is an interesting trait as it grows where no other crops are able to grow. OFI can be considered a multipurpose dryland crop, which is supposed to become more important in view of an ever-increasing world population and water/land scarcity (Nefzaoui et al., 2014). In Algeria, OFI grows both as spontaneous plant and cultivated resource in the semi-arid areas of the east, especially (Neffar et al., 2011).

Due to the scientific interest and economic potential, in the last years *Opuntia* spp. and OFI in particular have been the object of a tremendous

* Corresponding authors.
E-mail addresses: fatiha.brahmi@univ-bejaia.dz (F. Brahmi), federica.blando@ispa.cnr.it (F. Blando).

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production of scientific literature, especially focusing on biological and functional properties (García et al., 2020; Silva et al., 2021; Das et al., 2021 and references therein).

Various parts of OFI such as cladode, fruit, and flower have been traditionally utilized as food and feed in humans and animals diet due to their nutritious characteristics (Pérez-Torrero et al., 2017). Moreover, all parts of the plant have been used in traditional folk medicine, especially in Mexico, which is the centre of domestication of this species (Feugang et al., 2006; Osuna-Martínez et al., 2014; Díaz et al., 2017).

Studies from the last few years have reported that both fruits and cladodes contain a range of pythochemicals (phenolics, betalains) with bioactive properties (Albano et al., 2015; Blando et al., 2019; Silva et al., 2021).

Flowers have been studied less, although they have been reported to contain polyphenols as fruits and cladodes, in particular phenolic acids and flavonols (De Leo et al., 2010; Yeddes et al., 2014; Benayad et al., 2014; Tsafantakis et al., 2019). It has been reported that cactus flowers can be considered the most important source of polyphenols and flavonoids, among the cactus organs and tissues (El-Mostafa et al., 2014).

Furthermore, they are a considerable source of other secondary metabolites including essential oils such as carboxylic acids and camphor (Ammar et al., 2015a; De Leo et al., 2010; Ennouri et al., 2014; Ouerghemmi et al., 2017). Likewise, they are rich in proanthocyanidin (condensed tannin) (Benayad et al., 2014; Ouerghemmi et al., 2017).

OFI flowers extract comprises substantial quantities of minerals (potassium and calcium) and sugars (Berrabah et al., 2019). The lipids of OFI flower contain essentially linoleic, oleic, and palmitic acids (Ouerghemmi et al., 2017).

Flowers, appearing on the top of the pads, are yellow in color and turn red when the plant is approaching senescence. Flowering takes place in April-May and fruiting around July-August. The flowers of OFI constitute a by-product in cactus industry, and can be therefore exploited in a circular economy approach.

OFI flowers have been extensively utilized in traditional folk medicines in different places of the world (Mexico, North Africa, Sicily in Italy), used mainly for renal ailments as depurative, diuretic and relaxant of renal excretory tract. El-Mostafa et al. (2014) reported that in the folk sub-Saharan medicine, cactus flowers were considered as anti-ulcerogenic and anti-hemorrhoid remedy.

Recent studies have attempted to determine the validity of these anecdotally reported properties. OFI flower extracts have been tested both in vitro and in vivo systems and have been shown to possess anti-ulcerogenic and diuretic activities as reported in folk medicine (Alimi et al., 2011; Galati et al., 2002). Moreover, antioxidant, antibacterial, anti-inflammatory and wound healing properties have been reported (Ammar et al., 2012, 2018; Aragona et al., 2018; El-Hawary et al., 2020). In addition, cytotoxic and genotoxic effects as well as protective effect against H_2O_2-induced genotoxicity have been evaluated on OFI flower extract, in addition to others plant part (Tsafantakis et al., 2019).

As extraction is the initial step for the evaluation of the chemical composition and biological activities of a plant matrix, the development of an adequate and effective technique for extracting phenolic compounds from OFI flowers is a pre-requisite step. Previous studies investigated the best conditions for polyphenolic extraction from OFI flowers, varying solvent and extraction method (maceration or Soxhlet) (Ammar et al., 2015b; Berrabah at al, 2019). However, ultrasound-assisted extraction (UAE) has been never tested in this context, although it is a methodology widely applied from laboratory to industry for extraction of bioactive compounds from plant material (Chemat et al., 2017).

Extraction assisted by sonication is a simple, effective, and inexpensive method. In a liquid medium the propagation of the waves generates successive cycles of compression and rarefaction. This pressure difference produces cavitation phenomena and the implosion of cavitation bubbles allows increasing mass transfer of extractants (Medina-Torres et al., 2017). Compared with conventional extraction, UAE offers several advantages, like a decrease of the extraction and processing time, of the amount of energy and solvents used, making UAE an 'environmental friendly' technique (Chemat et al., 2017).

When using UAE technique, many factors affect the extraction performance, such as the nature of the solvent and the physical parameters as treatment time and temperature, all these factors interacting each others (Chemat et al., 2017). Hence, it is important to test the interdependencies of the different variables to maximize the metabolites extraction.

The analysis of the simultaneous effect of the different variables can be performed by employing the Response Surface Methodology (RSM), a mathematical and statistical tool using a sequence of designed experiments to optimize the experimental conditions of a process (Chen et al., 2015).

The number of studies carried out to optimize the conditions of extraction of the OFI phenolic compounds using ultrasound are limited (Amrane-Abider et al., 2018). In addition, extracted tissues included cladodes (Tranquilino-Rodríguez et al., 2020), fruit skin (Jorge et al., 2013) and fruit seeds (Amrane-Abider et al., 2018), whereas no study dealt with the optimization of the extraction of phenolic compounds from OFI flowers. Moreover, studies carried out on OFI flowers of Algerian origin are very scarce; to authors' knowledge only one paper delt with the chemical composition and antioxidant activity of OFI flowers from the Algerian germplasm (Berrabah et al., 2019).

Hence, the aim of the present work was to evaluate the composition and properties of OFI flowers polyphenolic extract obtained by ultrasound-assisted extraction (UAE), as alternative technique compared with conventional methods (maceration and Soxhlet extraction). Furthermore, this UAE is expected to maximize the extraction yield and the biological activity of the extract.

2. Materials and methods

2.1. Chemicals

The used chemical reagents [ferric chloride ($FeCl_3$), potassium ferricyanide ($K_3[Fe(CN)_6]$), trichloroacetic acid (TCA), Folin–Ciocalteu's reagent, sodium carbonate (Na_2CO_3), aluminum chloride ($AlCl_3$), potassium persulfate, ammonim molybdate, sulfuric acid (H_2SO_4), sodium phosphate dibasic anhydrous ($Na_2H_2PO_4$)] were purchased from Biochem Chemopharma (Montreal, Canada). Quercetin, gallic acid, 2,2-diphenyl-2-picrylhydrazyl (DPPH), 2,20-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) as well as acetonitrile (HPLC grade), ethanol, methanol, formic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). In all experiments, Milli-Q (Merck Millipore, Darmstadt, Germany) water was used. All used reagents were of analytical grade.

2.2. Preparation of the sample

Flowers from *O. ficus-indica* [L.] Mill. (OFI) were collected from wild population plants (orange color), grown in the region of Barbacha, about thirty kilometers from the department of Bejaia (Algeria), during the period of flowering in April-May 2016.

The samples were dried in the open air and shade for 15 days and subsequently ground, using an electric grinder (Kika Labortechnik, Staufen, Germany), then the powder was sieved to obtain a fine powder with particle size < than or equal to 125 μm, and stored in paper bags in the absence of light at room temperature.

2.3. Extraction of antioxidants

2.3.1. Ultrasonic-assisted extraction

The samples powder (1 g) was mixed with 10 mL ethanol-water mixture (different concentrations) in a tubes, in triplicate, then immersed in a water bath sonication (300 W) (VWR, Leuven, Belgium)

Table 1
Ranges used for the factors in the preliminary study, coded and real values for Central Composite Design (CCD) of Ultrasound-Assisted Extraction (UAE).

Factors	Used ranges		
X_1 Ethanol concentration (%, *v/v*)	20 40 60 80 100		
X_2 Extraction time (min)	10 20 30 40 50 60		
X_3 Extraction temperature (°C)	30 40 50 60 70		
Factors	Coded levels		
	-1	0	+ 1
X_1 Ethanol concentration (%, v/v)	0	40	80
X_2 Extraction time (min)	10	35	60
X_3 Extraction temperature (°C)	20	45	70

1 (low), 0 (center point), and + 1 (high), were the codes of factor levels.

and irradiated at a power of 50 Hz testing different temperatures (°C) and extraction time (min) (Table 1). After extraction, the mixtures were centrifuged (5000 x *g*, 20 min, at 4 °C) and the supernatants were collected and stored at 4 °C until further use for a maximum of one week.

2.3.2. Maceration extraction

The samples powder (1 g) was mixed with 10 mL ethanol-water mixture (36%), in triplicate, then shaken on a stirring plate during a period of time (Table 1). After extraction, the mixtures were centrifuged (5000 x *g*, 20 min, 4 °C) and the supernatant was collected and stored at 4 °C until further use for a maximum of one week.

2.3.3. Soxhlet extraction

The samples powder (10 g) was put into a Soxhlet cartridge and extracted with 100 mL of aqueous ethanol solution (36%). After 4 h of extraction at 85 °C, the resulting solution was collected and stored at 4 °C for a maximum of one week for subsequent analysis.

2.4. Experimental model

2.4.1. One-factor experiments

One-factor experiments were conducted to evaluate the effects of the three experimental parameters on the extraction efficiency, namely ethanol concentration (20–100%), time (10–60 min) and temperature (30–70 °C) (Table 1).

2.4.2. Response surface methodology (RSM) experimental design

The optimization of the extraction conditions was carried out by varying one parameter and fixing the others, using Centered Composite Design (CCD) based RSM.The impact of the three independent variables (X_1: ethanol concentration; X_2: extraction time; X_3: extraction temperature) on the dependent variable (Total Phenol Content, TPC) was studied by CCD (Table 1). The level of the independent variables was chosen according to the results achievied in single-factor assays. Twenty associations of the independent variables were selected per experimental design for three parameters, with a centered test (0, 0, 0) repeated six times to verify the standard error and reproducibility of the extraction process (Table 2).

2.5. Phenolics and betalains quantification

The total phenolics, flavonoids, and betalains contents were assessed by spectrophotometric assays.

The Folin–Ciocalteu assay was evaluated as described in Albano et al. (2015). A rapid microplate methodology, using a microplate reader (Infinite M200, Tecan Trading AG, Switzerland) and 96-well plates (Costar, 96-well black round bottom plate, Corning) were used. Two independent assays were performed in triplicate. The results were expressed as mg Gallic Acid Equivalent (GAE)/g Dry Weight (mg GAE/g DW).

The assessment of total flavonoids content was carried out according to the method reported by Brahmi et al. (2015). The results were

Table 2
Plan and results (in terms of Total Phenolics) of the optimized conditions by CCD *Opuntia-ficus indica* [L.] Mill. flower extraction by Ultrasound-Assisted Extraction (UAE).

	Experiment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Independent variables	Ethanol (%) X_1	0(-)	0(-)	80 (+)	40(0)	0(-)	40(0)	40(0)	0(a)	80 (+)	80 (A)	40(0)	40(0)	40(0)	80 (+)	40(0)	40(0)	40(0)	0(-)	40(0)	80 (+)
	Time (min) X_2	60 (+)	10(-)	10(-)	35(0)	60 (+)	35(0)	35(0)	35(0)	10(-)	35(0)	10(a)	35(0)	35(0)	60 (+)	35(0)	35(0)	60 (A)	10(-)	35(0)	60 (+)
	Temperature (°C) X_3	20(-)	70 (+)	70 (+)	45(0)	70 (+)	45(0)	45(0)	45(0)	20(-)	45(0)	45(0)	45(0)	45(0)	20(-)	70 (A)	20(a)	45(0)	20(-)	45(0)	70 (+)
Response: Total phenolics (mg/g DW)	Experimental values	10.99	13.51	10.72	19.46	14.55	23.29	23.78	18.2	5.54	12.97	23.74	21.67	21.89	5.14	21.8	14.19	23.42	10.99	25.9	13.38
	Predicted Values	10.56	13.85	11.28	22.84	15.17	22.84	22.84	17.37	5.05	13.27	23.01	22.84	22.84	4.92	20.44	15.02	23.61	11.29	22.84	13.21

expresse as mg Quercetin Equivalent/g Dry Weight (mgQE/g DW).

The most appropriate method to quantify betalains is spectrophotometric technique. The ethanolic extracts resulted from different extraction methods (maceration, Soxhlet and UAE) were diluted to 1/5. The values of the extracts absorbance were obtained by a UV–Visible spectrophotometer (Spectro Scan 50 Shimadzu, Tokyo, Japan). A quartz cell of 1 cm was used. The content of betalains (BC) (betacyanins and betaxanthins) was evaluated by spectrophotometry following Stintzing et al. (2003) methodology, and quantified as:

$$BC = (A \times DF \times MW \times 1000)/(\varepsilon \times l)$$

where BC is betacyanins or betaxanthins content (mg/L), A is absorbance ($\lambda = 538$ nm for betacyanins and $\lambda = 483$ nm for betaxanthins), DF is a dilution factor at the moment of reading, MW is the molecular weight (550 g/mol for betanin and 308 g/mol for indicaxanthin), ε is the molar extinction coefficient (60,000 L/mol cm for betanin and 48,000 L/mol cm for indicaxanthin), and l is the length of the cell (1 cm) of the cuvette.

The results were expressed in mg/g of Dry Weight (DW) betacyanins, betaxanthins and total betalains (sum of betacyanins and betaxanthins content).

2.6. Evaluation of the antioxidant activity by different assays

The 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging capacity of samples was performed following the protocol already described (Brahmi et al., 2018). The degree of DPPH radical scavenging activity of the samples was estimated from a Trolox calibration curve run in parallel.

The Ferric Reducing Antioxidant Power (FRAP) was measured following the method already described (Brahmi et al., 2018; Debbabi et al., 2016).

The total antioxidant activity of the extract was determined by the phosphomolybdate test carried out according to the method described by Brahmi et al. (2012).

Trolox equivalent antioxidant capacity (TEAC) by ABTS assay was evaluated as described in Albano and collaborators (2015). The percentage (%) inhibition of absorbance at 734 nm was calculated and plotted as a function of the Trolox® reference standard.

The results were expressed as μmol Trolox® equivalents per gram of Dry Weight (μmol TE/g DW) in the all tests.

2.7. Identification of phenolic components

The identification and quantification of phenolic compounds in the optimized extract of OFI flower (by UAE) was performed using an Agilent 1200 Liquid Chromatography system (Agilent Technologies, Palo Alto, CA, USA) equipped with a standard autosampler, and the chromatographic conditions and column were the same already reported (Blando et al., 2019).

2.8. Statistical analyses

All the experiments were performed in triplicate and the results were expressed as mean value ± standard deviation (SD). Statistical analysis was performed using STATISTICA. 12. The optimization was done using the JMP.10 software.

3. Results and discussion

3.1. Single-factor experiment

The single-factor trial aimed at assessing the impact of each variable on the total phenolic content (TPC) of the extract from the OFI flower under ultrasound treating. The solid-to-liquid ratio of 1 g/10 mL was the same for all experiments.

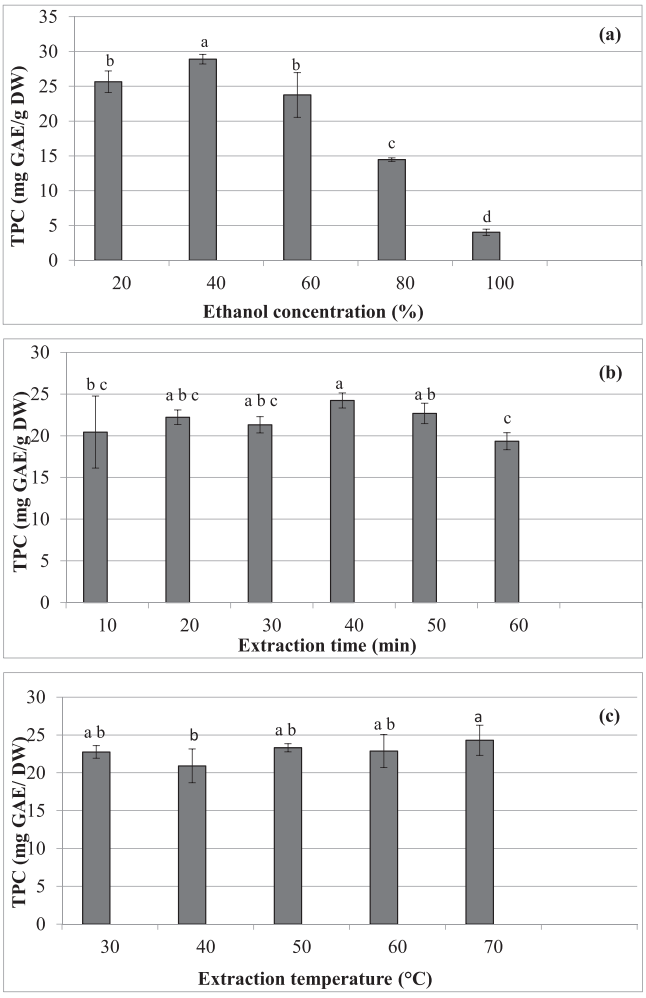

Fig. 1. Effects of: ethanol concentration (a); extraction time (b); extraction temperature (c) on the ultrasound-assisted extraction (UAE) effectiveness in terms of Total Phenolic Content (TPC) of *Opuntia ficus-indica* [L.] Mill. flower extract. Different letters on the bars mean that there is a significant difference among treatments ($p \leq 0.05$).

3.1.1. Impact of ethanol concentration

The effects of different ethanol concentrations (20%, 40%, 60%, 80% and 100%, *v/v*) on the extraction efficiency of phenolics from OFI flower with UAE extraction were studied. The TPC increased with ethanol concentration (from 20% to 40%), and then progressively decreased while ethanol concentration increased (from 40% to 100%) (Fig. 1a). Therefore, the highest phenolic content was obtained with 40% ethanol (with a significant difference at $p \leq 0.05$), while the lowest value was obtained with pure ethanol. This behavior was expected as phenols are more soluble in polar solvent, being water more polar than ethanol. Phenolic compounds are characterized by the presence of hydroxyl groups in their structure, making them easily extracted using polar solvents.

As the solvent is concerned, ethanol possess some benefits comparatively to other organic solvents. It is recognized by its green character and sustainability together with its low toxicity for humans (Hamid et al., 2020, 2022). So, ethanol or aqueous ethanol are safer and more tolerable for application in agrifood manufactures (Brahmi et al., 2012; Gerardi et al., 2015).

Higher extraction of phenolics using aqueous ethanol was also found in a work using UAE method from *Osmanthus fragrans* flowers (Li et al.,

Table 3
ANOVA for response surface models.

	Sum of		Mean	F	*p*-value
Source	Squares	df	Square	Value	Prob > F
Model	736.10	9	81.79	27.78	< 0.0001[a]
X_1-Ethanol concentration	*41.98*	*1*	*4.98*	*14.26*	*0.0036*[a]
X_2-Extraction time	*0.89*	*1*	*0.89*	*0.30*	*0.5949*[b]
X_3-Extraction temperature	*73.50*	*1*	*73.50*	*24.96*	*0.0005*[a]
X_1*X_2	*0.19*	*1*	*0.19*	*0.063*	*0.8066*[b]
X_1*X_3	*6.73*	*1*	*6.73*	*2.29*	*0.1614*[b]
X_2*X_3	*2.10*	*1*	*2.10*	*0.71*	*0.4180*[b]
X_1^2	*155.78*	*1*	*155.78*	*52.90*	*< 0.0001*[a]
X_2^2	*0.60*	*1*	*0.60*	*0.21*	*0.6603*[b]
X_3^2	*71.99*	*1*	*71.99*	*24.45*	*0.0006*[a]
Residual	29.45	10	2.94		
Lack of Fit	*5.48*	*5*	*1.10*	*0.23*	*0.9343*[b]
Pure Error	*23.96*	*5*	*4.79*		
Cor Total	765.55	19			
r^2	0.9615				
r^2_{adj}	0.9269				
r^2_{pred}	0.8968				
Adeq Precision	15.395				
C.V.%	10.24				

ANOVA = analysis of variance;
C.V.% coefficient of variation;
df = degrees of freedom
[a] Significant at $p < 0.05$.
[b] Not significant.

2016). A similar trend was observed in other study where 40% ethanol concentration was the most efficient for antioxidants extraction from *Limonium sinuatum* flowers by UAE (Xu et al., 2017). Since 40% ethanol was appropriate to maximize TPC from OFI flowers, the following analysis were performed using that ethanol concentration.

3.1.2. Impact of extraction time

In order to show the impact of extraction time on UAE efficiency, the extraction time was varied between 10 and 60 min. The TPC increased extending the extraction time up to 40 min, even if extraction times from 20 to 50 min were not statistically different (Fig. 1b).

Results from these experiments are in accordance with those of Irakli et al. (2018) who revealed that TPC using UAE was some what prominent over the first 30 min and then reduced gradually.

During the extraction of secondary metabolites by UAE, the sonication time follows two principal phases. In the beginning 'washing step' (20–30 min), the maximum of metabolites is recovered. Then, comes the 'slow extraction phase' (that lasts between 60 and 100 min), in which metabolites are transferred from the matrix to the solvent by diffusion mechanism (Medina-Torres et al., 2017). Therefore, the 'washing step' can be short and sufficient to release the bioactive compounds; moreover, a longer period of time can lead to the degradation of these compounds, due to radiations generated by ultrasound.

3.1.3. Impact of extraction temperature

The impact of temperature on the extraction of phenolic compounds by UAE was evaluated in the range of 30–70 °C. The highest TPC was recorded at 70 °C, even if results for extraction temperature were not statistically different, except for 40 °C, which was the lowest (Fig. 1c).

Katsampa et al. (2015) found that increasing temperatures from 50° to 80°C had a favorable impact on the phenolics extraction from onion solid wastes by UAE, inducing a rise by 2.49 times. An elevated temperature promotes phenolics propagation and enhance solubilization. In general, the increase in this variable correlates with improvements in phenol extraction due to the breakdown induction of matrix bonds, the phenolics propagation with their increased solubility and solvent diffusion rate (Celli et al., 2015). However, temperature above certain level (i.e. 80 °C) promote degradation of extracted compounds and reduction of the extraction rate constant (Medina-Torres et al., 2017).

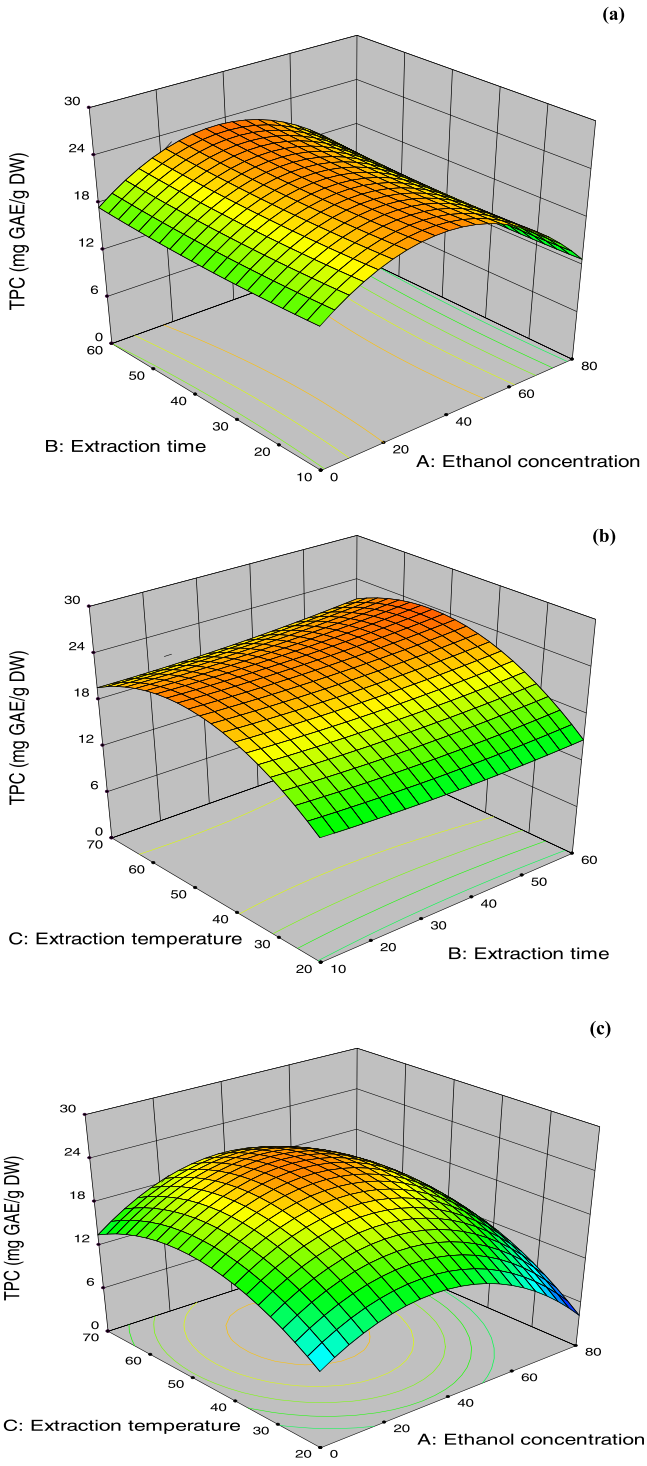

Fig. 2. Response surfaces for the combined effects of: ethanol concentration and extraction time (a); extraction time and extraction temperature (b); ethanol concentration and extraction temperature (c), on the ultrasound-assisted extraction (UAE) effectiveness in terms of Total Phenolic Content (TPC) of *Opuntia. ficus-indica* [L.] Mill. flower extract.

3.2. Optimization by RSM

The three independent variables (X_1: ethanol concentration; X_2: extraction time; X_3: extraction temperature), studied by CCD,impacted on the dependent variable (TPC), which varied from 5.14 to 25.9 mg GAE/ g DW (Table 2). The highest value (25.9) resulted from experiment 19, where Ethanol concentration = 40%; Extraction time = 35 min;

Table 4
Comparison of maceration, Soxhlet and Ultrasound-Assisted Extraction (UAE), in terms of bioactive compounds content and antioxidant activity.

Extraction method	*Ethanol [C] (%)*	*Time (h)*	*T (°C)*	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	Betacyanin (mg betanin eq/g DW)	Betaxanthin (mg indicaxanthineq/g DW)	Betalain (mg/ g DW)	Antioxidant activity (µmol Trolox Equivalent/g DW)		
									FRAP	TAA	DPPH
Maceration	*35.70*	*24*	*25*	5.66 ± 0.37^{b}	1.96 ± 0.01^{b}	0.30 ± 0.01^{c}	0.33 ± 0.01^{c}	0.63 ± 0.01^{c}	5614 ± 673^{a}	3373 ± 94.4^{a}	34.78 ± 0.87^{a}
Soxhlet	*35.70*	*4*	*85*	1.96 ± 0.59^{c}	1.05 ± 0.01^{c}	0.62 ± 0.04^{b}	0.59 ± 0.04^{b}	1.21 ± 0.08^{b}	521 ± 62.5^{c}	318.2 ± 28^{b}	20.3 ± 1.36^{b}
UAE	*35.70*	*1*	*52.6*	24.38 ± 0.82^{a}	9.70 ± 0.10^{a}	0.86 ± 0.01^{a}	0.93 ± 0.02^{a}	1.79 ± 0.01^{a}	4078 ± 80.3^{b}	3091 ± 139^{a}	13.76 ± 0.27^{c}

TPC: total phenolic content;
TFC: total flavonoid content;
GAE: Gallic Acid Equivalent;
QE: Quercetin Equivalent;
FRAP: Ferric Reducing Antioxidant Power;
TAA: Total Antioxidant Activity;
DPPH: 2,2-DiPhenyl-2-Picryl Hydrazyl.
Values are mean (n = 3) ± standard deviation (SD).
For each extraction method, values in the same column for each test followed by a different letter (^{a}, ^{b} and ^{c}) are significantly different ($p \leq 0.05$).

Extraction temperature = 45 °C were evaluated (Table 2). The results of the analysis of variance (ANOVA) of each factor and their interactions, the goodness of fit, and the adequacy of the models are summarized in Table 3.

The Model F-value of 27.78 implies that the procured mathematical model was appropriate and highly significant ($p < 0.01$) with an elevated coefficient of determination ($r^2 = 0.9615$). In addition, the 'r^2_{Pred}' of 0.8968 is in reasonable agreement with the 'r^2_{Adj}' of 0.9269; i.e. the difference is less than 0.2. The 'Lack of Fit F-value' of 0.23 implies the Lack of Fit is not significant which indicates good predictability.'Adeq Precision' measures the signal to noise ratio and a ratio greater than 4 is desirable. The ratio of 15.395 indicates an adequate signal. So, this model can be used to navigate the design space.Values of 'Prob > F' less than 0.05 indicate model terms are significant, in this case X_1, X_3, X_1^2, X_3^2 are significant model terms but values greater than 0.1 indicate the model terms are not significant (as for X_2, $X_1{*}X_2$, $X_1{*}X_3$, $X_2{*}X_3$ and X_2^2). If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model.

The procured mathematical model was employed to assess a predictive equation for the TPC in OFI flower extracts taking into account the significant variables. The following relation depicted the expected modeling within the meaning of coded values:

$$TP\ (mg\ GAE/g\ DW) = 22{\cdot}84 - 2{\cdot}04X_1 + 2{\cdot}71X_3 - 7{\cdot}52X_1^2 - 5{\cdot}11X_3^2 \quad (1)$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as + 1 and the low levels of the factors are coded as − 1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

The factors that showed a significant effect on the response (TPC) were the independent variables of solvent concentration (X_1), UAE temperature (X_3) and their quadratic terms (X_1^2, X_3^2), as was mentioned in Eq. (1).

The linked impact of the different factors on the extraction of phenolic compounds from OFI flowers was depicted in Fig. 2.

The response surface diagram was produced by combining first solvent (ethanol) concentration (0–80%) and extraction time (0–60 min) but with a fixed extraction temperature of 60 °C (Fig. 2a). The ethanol concentration showed a favorable effect on the effectiveness of phenolics extraction from OFI flowers. TPC augmented with ethanol concentration from 0% to 50% as water polarity made phenols more extractable, after that diminished.

Combining extraction temperature and extraction time, with a constant ethanol concentration (40%), TPC increased with temperature up to around 60 °C then decreased by an additional increasing of the temperature. The extraction time did not improve the extraction capability, only the temperature showed a significant effect (Fig. 2b).

Combining ethanol concentration and extraction temperature, at a fixed extraction time (40 min), TPC increased to reach its maximum at around 60 °C with an ethanol concentration of around 40%, then decreased (Fig. 2c).

In conclusion, the optimal conditions for the extraction of OFI flowers phenolics (as generated by the software used) were: an ethanol concentration of 36%, an extraction time of 60 min and an extraction temperature of 53 °C. In accordance with these optimal conditions, the predicted TPC was 24.2 ± 2.6 mg GAE/g DW. With the aim of verifying the model capacity to precisely project the effective value, the result of the TPC using the same conditions was 24.38 mg GAE/g DW, which was in accordance with the expected value.

3.3. Comparison of ultrasound-assisted extraction with other methods

After the determination of the optimal conditions for the extraction of phenolic compounds from OFI flowers using the UAE method, the effectiveness of the latter was compared with conventional extraction methods (Soxhlet and maceration).

Total phenolics, flavonoids and betalains content and the antioxidant activities were compared to define the technique producing an extract with maximum content and activity. The UAE maximized total phenolics and flavonoids content (24.38 mg GAE/g DW and 9.7 mg QE/g DW, respectively) compared with the other techniques (Soxhlet extraction and maceration) which gave significantly lower values (5.66 mg GAE/g DW; 1.96 mg GAE /g DW for total phenolics and 1.96 and 1.05 mg QE/g of DW for flavonoids, respectively) (Table 4).

By using maceration and methanol as solvent, Tunisian OFI flowers freeze-dried extract was found to contain 159.7 mg GAE/g extract of TPC and 79.5 mg Rutin Equivalent/g extract of flavonoids (Alimi et al., 2011). According to Ammar et al. (2012), the TPC in ethanol extracts of OFI flowers obtained from various developing phases and originating also from Tunisia fluctuated from 13 to 49 mg of GAE/g of extract. It is not possible to compare with our results as those authors reported the phenolics and flavonoids values on extract basis, without reporting the yield (%DW).

The same authors (Ammar et al., 2015b) by comparing the efficiency of two extraction methods (Soxhlet and maceration), proved that the TPC of OFI flowers varies from 7.5 (hexane extract) to 270.9 mg GAE/g (methanol extract) for Soxhlet extraction and from 14.7 (dichloromethane extract) to 227.8 mg GAE/g (methanol extract) for maceration. From % yield, it can be found that Soxhlet extraction produced ~

Table 5
The Liquid Chromatography Time-of-Flight Mass Spectrometry (LC/MS–TOF) $(M-H)^-$ characterization of optimized *Opuntia-ficus indica* [L.] Mill. flower extract.

N	RT	$[M-H]^-$	*m/z* exp	*m/z* calc	Δ (ppm)	Name	Reference
1	2.261	$C_{11}H_{11}O_7$	255.0519	255.0510	-3.49	Piscidic acid	(Ginestra et al., 2009)
2	7.471	$C_{11}H_{11}O_6$	239.0564	239.0561	-1.00	Eucomic acid	(Ginestra et al., 2009)
3	12.102	$C_{21}H_{19}O_{12}$	463.0859	463.0882	5	Quercetin hexoside	
4	12.461	$C_{27}H_{29}O_{16}$	609.1437	609.1461	4	Rutin	(Santos-Zea et al., 2011)
5	12.726	$C_{34}H_{41}O_{20}$	769.6771	769.6773	-0.39	Isorhamnetin 3-rhamnosyl rutinoside	(Santos-Zea et al., 2011)
4	12.799	$C_{33}H_{39}O_{20}$	755.6513	755.6508	0.53	Isorhamnetin glucosil rhamnosylpentoside	(Santos-Zea et al., 2011)
7	13.426	$C_{27}H_{29}O_{16}$	609.1430	609.1461	5.06	Isorhamnetin sambubioside	(Ginestra et al., 2009)
8	13.860	$C_{27}H_{29}O_{15}$	593.1479	593.1512	5.49	Kampherol glucosil rhamnoside	(Santos-Zea et al., 2011)
9	14.333	$C_{28}H_{31}O_{16}$	623.1606	623.1618	1.93	Isorhamnetin rutinoside	(Santos-Zea et al., 2011)
10	14.522	$C_{22}H_{21}O_{12}$	477.1020	477.1038	3.86	Isorhamnetin glucoside	(Ginestra et al., 2009)

40.1 mg GAE/g DW, and maceration extracted ~ 23.7 mg GAE/g DW, and those values are much higher then the value recorded in this current study and the other values reported in literature, and also in contrast (Soxhlet more efficient than maceration). The total flavonoid content varied from 1.7 to 60.8 mg RE/g extract for Soxhlet and from 3.2 to 27.4 mg RE/g extract when using maceration, with the maximum value associated with methanol extract. From % yield, it was ~ 6.3 mg RE/g DW (Soxhlet method) and 2.8 mg RE/g DW (maceration method).

Experiments on Morrocan OFI flowers extracted with accelerated solvent extraction (ASE) or maceration, revealed ASE more efficient than maceration to extract phenolics. TPC ranged between 2.34 and 3.18 (mg GAE/g) and total flavonoids between 0.78 and 1.58 (mg CE/g) (Benayad et al., 2014).

Extraction of phenolics by maceration of cactus flower from Tunisia using six solvents revealed that TPC varied from 0.01 (petroleum ether and chloroform extracts) to 3.89 mg GAE/g (methanol extract) and the TFC from 0.01 (petroleum ether) to 2.44 (methanol) mg CE/g DW (Ouerghemmi et al., 2017).

By evaluating the aqueous extract prepared by maceration of OFI flowers coming from six Algerian localities, TPC fluctuated from 7.52 mg GAE/g (sample from Ain Defla' region) to 10.89 mg GAE/g (sample from Tizi-Ouzou region), whereas total flavonoids content was lowest in the sample from Tiaret locality (0.51 mg QE/g) and highest in the sample from Msila (0.96 mg QE/g) (Berrabah et al., 2019). Those values are in accordance with the recorded results on Algerian OFI flowers, from a different area, extracted with maceration.

With regard to literature, to best of authors' knowledge no studies have been focused on the extraction of phenolic compounds from cactus flowers using UAE.

In addition to polyphenols, the other bioactive compounds found in OFI flower are betalains. Using UAE, Soxhlet and maceration methods, total betalains content (betacyanins plus betaxanthin) were 1.79, 1.21 and 0.63 mg/g DW, respectively (Table 4). To the best of the authors' knowledge, betalains content of OFI flower has not been determined in the literature. On the contrary, betalains have been extensively reported in OFI fruit (Castellanos-Santiago and Yahia, 2008), but also in cladode (De Wit et al., 2019), interestingly at the same level as reported in our experiment on flowers.

The results of the antioxidant assays on the OFI flower extract by UAE showed that the ultrasound technique was similar or lower as effectiveness to the other tested methodologies (Table 4). However, extracting bioactive compounds from flowers with UAE is efficient and uses a reduced extraction time compared with conventional extraction techniques (Xu et al., 2017). Thus, in comparison to the conventional extraction methods (Soxhlet and maceration extraction), UAE was a more efficient extraction method for the recovery of antioxidants from OFI flowers with shorter time, higher phenolic yield and reduced temperature.

Previous research on OFI flower extracts obtained by conventional methods revealed an interesting ability to reduce $DPPH^•$ radical and iron (by DPPH and FRAP tests) (Alimi et al., 2011; Ammar et al., 2015b; Ouerghemmi et al., 2017; Berrabah et al., 2019), to reduce Mo (VI) to Mo (V) (Ouerghemmi et al., 2017) and to reduce $ABTS^•$ radical (Ammar et al., 2015a). The results reported in the present work are expressed as μmol Trolox Equivalent (TE) per g of dry weight (DW) (Table 4), which is not consisted with previous reports from those authors, referred as g of flower extract.

Table 6
Phenolic acids, flavonols, total phenolic content (TPC), and antioxidant capacity (by Trolox® Equivalent Antioxidant Capacity) of optimized *Opuntia ficus-indica* [L.] Mill. flower extract (by ultrasound-assisted extraction).

(Peak number) Compound	*mg/g DW*
(1) Piscidic acid	6.65 ± 0.56
(2) Eucomic acid	13.4 ± 0.85
(3) Quercetin hexoside	0.22 ± 0.02
(4) Rutin	0.40 ± 0.08
(9) Isorhamnetin rutinoside	3.34 ± 0.02
(10) Isorhamnetin glucoside	1.26 ± 0.17
TPC (mg GAE/g DW)	23.66 ± 2.8
TEAC (μmol TE/g DW)	80.84 ± 4.17

Peak number as per Table 5.
Phenolic acids content was quantified using calibration curves of *p*-hydroxybenzoic acid.
Flavonols content was quantified using calibration curves with the closest appropriate standard (rutin or isorhamnetin rutinoside).
TPC was quantified by Folin-Ciocalteu assay.
Values are the mean (n = 3) ± standard deviation (SD)

Antioxidant capacity of the extracts was conducted using the DPPH, FRAP and phosphomolybdate assays (Table 4). It is important to evaluate the antioxidant capacity of an extract by different assays.When compared to the antioxidant capacity of other species of flowers, by the DPPH methodology and maceration extraction, OFI flower presented values very similar to previous studies on edible flowers (Chen et al., 2015; Negro et al., 2021). Interestingly, DPPH value of OFI flower was much closer to that one of *Gomphrena globosa* L., a plant species belonging to the Amaranthaceae family, rich of betalains (Chen et al., 2015). However, when using UAE, the value was significantly lower than reported in other flowers ultrasound-extracted (Barros et al., 2020).

Following the validated conditions for UAE, additional extraction experiments produced OFI flower extracts with the aim to analytical characterize them by HPLC-MS. Additional Folin-Ciocalteu and antioxidant activity assays (ABTS) were performed on the replicated extracts. Folin-Ciocalteu assay confirmed the high TPC as previously detected (Table 4 and Table 6). TEAC value of the optimized UAE (Table 6) was as high as *Calendula officinale* L. and other flower species (Chen et al., 2015) and interestingly higher than OFI fruit and cladode extracts (Albano et al., 2015; Blando et al., 2019).

3.4. Phenolic composition of OFI flowers optimized extract

A Liquid Chromatography Time-of-Flight Mass Spectrometry (LC/MS–TOF) analysis was done on the flower extract obtained by UAE, to characterize the phenolic compounds profile. Main components were

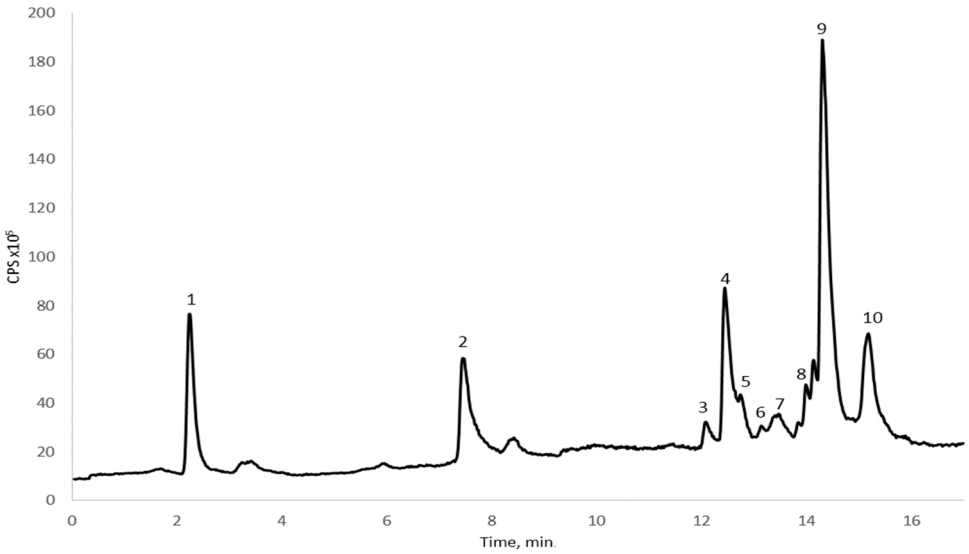

Fig. 3. High performance liquid chromatography time-of-flight mass spectrometry (HPLC/MSTOF) chromatogram of the ultrasound-assisted extraction (UAE) optimized extract of *Opuntia ficus-indica* [L.] Mill. flower. The peaks numbers relate to the identified phenolic compounds mentioned in Table 5.

phenolic acids (eucomic and piscidic acids), followed by isorhamnetin (differently glycosilated), and flavonols (quercetin derivatives) (Table 5). These phenolic compounds were identified with the aid of their retention times, UV-Vis spectra and fragmentation patterns, in addition to comparison of their mass spectral profiling with the accessible literature (Ginestra et al., 2009; Santos-Zea et al., 2011) (Fig. 3).

Piscidic and eucomic acids have already been identified and quantified in OFI cladode extract (Ginestra et al., 2009; Blando et al., 2019). It is the first time they have been identified and quantified in flower extract (Table 6). The amount of these phenolic acids was exceptionally high, with value very closed to the amount found in immature cladode (Blando et al., 2019). Previous studies on OFI flower reported the presence of hydroxycinnamic acid derivatives and quinic acid derivatives (Benayad et al., 2014; Ammar et al., 2018).

Flavonols (isorhamnetin and quercetin derivatives) were identified and quantified (Table 6). Also the amount of isorhamnetin rutinoside, the major flavonol, was similar to that one of immature cladode (Blando et al., 2019) and to the values reported by other studies on OFI flower (Yeddes et al., 2014; Benayad et al., 2014).

Isorhamnetin derivatives are endowed with several interesting biological activities (antiatherogenic, anti-hypercholoesterolemia, antioxidant, anticancer, and antimicrobial) (Ressaissi et al., 2017; Skalski et al., 2019; Blando et al., 2019). Therefore, OFI flower can be a valuable and sustainable source (being a by-product) of bioactive flavonols.

The optimized ultrasonic-assisted extract showed a total phenolic content (TPC) of ~ 24 mg GAE/g DW, which is associated to a high TEAC value (Table 6).

4. Conclusion

In this work, a green ultrasound-assisted extraction (UAE) procedure was established for the recovery of antioxidant polyphenols from the flowers of OFI, by employing single-factor experiments and response surface methodology to optimize the extraction factors.

The optimal conditions (ethanol concentration 36%, extraction time 60 min, temperature 53 °C) allowed maximizing the extraction of total polyphenols.

The extracted phenolic compounds (mostly piscidic and eucomic acids, and isorhamnetin derivatives) may be responsible for the antioxidant capacity of OFI flowers. Moreover, the optimized process was more effective than the traditional techniques (maceration and Soxhlet extraction) in extracting the antioxidants from OFI flowers. Due to the biological activities associated with those polyphenolic compounds, OFI flower extract can be considered as a valuable ingredient for food supplement or nutraceutical/cosmeceutical ingredients.

CRediT authorship contribution statement

Fatiha Brahmi: Conceptualization, Investigation, Methodology, Writing – original draft. **Federica Blando:** Investigation, Writing – review & editing. **Redha Sellami:** Methodology. **Sabrina Mehdi:** Methodology. **Luigi De Bellis:** Writing – review & editing. **Carmine Negro:** Investigation, Methodology, Data analysis. **Hayate Haddadi-Guemghar**: Data analysis. **Khodir Madani**: Supervision. **Lila Makhlouf-Boulekbache**: Conceptualization, Supervision, All authors have read and agreed to the submitted version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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