



## ORIGINAL ARTICLE

# Microwave-assisted extraction optimization and conventional extraction of phenolic compounds from coriander leaves: UHPLC characterization and antioxidant activity

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## ABSTRACT

**Background:** Qualitative and quantitative investigations of bioactive compounds in plant materials are heavily based on the selection of an accurate extraction method. **Aims:** That's why; this work consists of a comparative study between Microwave Assisted Extraction (MAE) and Conventional Extraction (CE), based on the total phenolic compounds (TPC) yield, phenolic profile, and antioxidant activity of coriander leaves powder (*Coriandrum sativum* L.). **Material and Methods:** MAE was optimized and performed using the Response Surface Methodology (RSM), and was modeled by using a second-order regression equation. While CE was done using the classic water bath method. **Results:** Under the optimal conditions, the recovery of TPC yield obtained was  $37.94 \pm 2.06$  mg (MAE) vs  $44.47 \pm 0.57$  mg GAE/g DW (CE). The UHPLC characterization showed a close phenolic composition of the two extracts, mainly represented by quercetin glucosides and by dimethoxycinnamoyl hexoside. No significant difference ( $p > 0.05$ ) was recorded in terms of the antioxidant activity of both extracts, as estimated by Ferric reducing antioxidant power (FRAP), Nitric oxide (NO<sup>\*</sup>), and superoxide anion (O<sub>2</sub><sup>\*</sup>) scavenging tests. **Conclusions:** Hence, the exploitation of MAE has many valuable advantages, as the processing time is brief and the antioxidant activities and phenolic composition were not affected by the extraction process.

**Keywords:** *Coriandrum sativum* L., Microwave-Assisted Extraction, Conventional Extraction, Phenolic Compounds, UHPLC characterization, Antioxidant activity.

## ARTICLE INFORMATION

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## 1 Introduction

Herbs and spices are dietary supplements that improve sensory quality and introduce nutritional and medicinal value to food products <sup>1</sup>. They have also been indicated as an extremely good source of phenolic compounds and, consequently, have been regarded as potential antioxidant additives <sup>2</sup>. Coriander (*Coriandrum sativum* L.), belonging to the Apiaceae Family, is largely harvested in Mediterranean countries and it has recently received special attention because of its interesting chemical composition <sup>3</sup>. With the increasing

demand for herbal products that promote health benefits, the application of effective methods for extracting active ingredients from plants has turned into a very important research topic <sup>4</sup>. In general, methods are classified as conventional or new techniques. Each technique has its specific advantages and disadvantages <sup>5</sup>. Although, the ideal extraction process should be highly efficient, fast, easy to apply, durable, and non-deleterious <sup>6</sup>. Conventional extraction processes comprise maceration, soaking, Soxhlet extraction, water percolation, etc. These techniques usually require a long extraction time, which introduces the risk of

thermal degradation of thermolabile active compounds <sup>7</sup>. In turn, in recent years, many new extraction procedures have been instigated and explored, such as microwave-assisted extraction, ultrasound-assisted extraction, pressurized liquid extraction, and pressurized hot water extraction <sup>8</sup>. Among these modern extraction methods, MAE was classed as one of the leading methods in terms of time efficiency, higher level of automation, quantity, and non-destruction of the extracts obtained <sup>9</sup>. Furthermore, another important argument in favor of the MAE is its applicability in the laboratory as well as on a pilot and industrial scale <sup>10</sup>. Hence, previous studies on MAE of bioactive compounds from several plant matrices have been reported in the literature, including from avocado seeds <sup>11</sup>, *Nerium oleander* leaves <sup>12</sup>, brown macroalgae <sup>13</sup>, pomegranate peels <sup>14</sup>, lime peel waste <sup>15</sup>, saffron (*Crocus sativus* L.) <sup>16</sup> and peach byproducts <sup>17</sup>.

Response surface methodology (RSM) is the most commonly used for the development, improvement, and optimization of extraction processes <sup>18</sup>. It is applied as an effective statistical approach to optimize complex processes that minimize the number of experimental trials. It is widely used to assess the interactions amongst the multiple factors and response variables that affect outcomes <sup>19, 20</sup>. The central composite design (CCD) is the most commonly used fractional factorial design used in the RSM. In this design, the center points are augmented with a group of axial points called star points. With this design, quickly first-order and second-order terms can be estimated. It is also one of the most important experimental design methods used in the process of optimization studies <sup>21</sup>.

As far as we know, previous studies focusing on the extraction of bioactive compounds from coriander leaves have been tried by conventional methods using solvents of different polarity, while the optimization of the microwave process for the extraction of phenolic compounds from coriander has been limited to seeds <sup>22</sup>. In addition to this, it is worth noting that compared to seeds' extracts, those of leaf origin have been shown to exhibit superior antioxidant activity, a fact that researchers attributed to the greater richness of phenolic compounds <sup>23</sup>. Therefore, the present work aims to (i) optimize the parameters influencing the MAE process using a CCD-based RSM, in order to maximize the recovery of TPC from dried coriander leaves powders and (ii) to compare the microwave-optimized extract with the extract obtained by the conventional method based on their phenolic compound content, their phytochemical composition using HPLC-DAD-ESI-MS<sup>n</sup> analysis and their antioxidant activity.

## 2 Material and Methods

### 2.1 Plant material

Fresh coriander (*Coriandrum sativum* L.) was purchased from a local market (Bejaia-Algeria), stored at  $4 \pm 2$  °C, and used within 2 days of purchase. The leaves were separated from the

stems, cleaned thoroughly, and washed with fresh water to release foreign matter followed by a final rinse with distilled water. Subsequently, these were dried in a forced air oven at 40°C until a constant weight ( $\approx 12$  h) and then ground using an electric grinder. The powder obtained was sieved through standard sieves (125, 250, and 500  $\mu\text{m}$  for the particle size assay and 250  $\mu\text{m}$  for the remaining experiments), collected, and saved in shaded vials at ambient temperature and dry place until further use.

### 2.2 Reagents

Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) and Folin-Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>,  $\text{C}_{18}\text{H}_{12}\text{N}_5\text{O}_6$ ), sodium nitroprusside dehydrate (SNP,  $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}] \cdot 2\text{H}_2\text{O}$ ), butylated hydroxyanisole (BHA,  $\text{C}_{11}\text{H}_{16}\text{O}_2$ ) and nitroblue tetrazolium (NBT,  $\text{C}_{40}\text{H}_{30}\text{N}_{10}\text{O}_6$ ), ascorbic acid ( $\text{C}_6\text{H}_8\text{O}_6$ ) and phenazine methosulfate (PMS,  $\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}_4\text{S}$ ) were purchased from Sigma-Aldrich. Orthophosphoric acid ( $\text{H}_3\text{PO}_4$ ) and trichloroacetic acid (TCA,  $\text{C}_2\text{HCl}_3\text{O}_2$ ) (Reag. Ph. Eur.) were purchased from Panreac. Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ), sodium dihydrogen phosphate 1-hydrate (*H<sub>6</sub>NaO<sub>6</sub>P*), and potassium hexacyanoferrate III ( $\text{K}_3[\text{Fe}(\text{CN})_6]$ ) were purchased from Panreac AppliChem. Sulfanilamide ( $\text{C}_6\text{H}_8\text{N}_2\text{O}_2\text{S}$ ) was purchased from Merck KGaA (Germany). N-(1-Naphthyl) ethylenediamine dihydrochloride ( $\text{C}_{12}\text{H}_{16}\text{Cl}_2\text{N}_2$ ) (Analytical reagent) was purchased from VWR PROLABO. Iron (III) chloride ( $\text{FeCl}_3$ ) was purchased from Chem-Lab NV.  $\beta$ -Nicotinamide adenine dinucleotide ( $\beta$ -NADH,  $\text{C}_{21}\text{H}_{27}\text{N}_7\text{O}_{14}\text{P}_2$ ) (reduced form), disodium salt (*C<sub>10</sub>H<sub>18</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>10</sub>*) was purchased from VWR PROLABO. Trolox ( $\text{C}_{14}\text{H}_{18}\text{O}_4$ ) was purchased from ACROS ORGANICS. Sodium hydroxide-pellets (NaOH) was purchased from Fischer. Dimethyl sulfoxide (DMSO,  $\text{C}_2\text{H}_6\text{OS}$ ) (analytical reagent grade) was purchased from Fischer Chemical, UK. Gallic acid ( $\text{C}_7\text{H}_6\text{O}_5$ ) was purchased from Biochem Chemopharma. Standards phenolics used for HPLC quantitative analysis such as caffeic acid ( $\text{C}_9\text{H}_8\text{O}_4$ ), 5-CQA ( $\text{C}_{16}\text{H}_{18}\text{O}_9$ ), quercetin-7-O-galactoside ( $\text{C}_{21}\text{H}_{20}\text{O}_{12}$ ), cinnamic acid ( $\text{C}_9\text{H}_8\text{O}_2$ ) and coumaric acid ( $\text{C}_9\text{H}_8\text{O}_3$ ) were obtained from Extrasynthese. All the solvents used for HPLC analysis with high-performance chromatography (HPLC) purity were purchased from Lab-Scan (Lisbon, Portugal).

### 2.3 Microwave-assisted extraction

#### 2.3.1 Equipment and procedure of extraction

All extractions were carried out using a domestic microwave system (Maxipower, Model MASMO23S). The device was fitted with a digital control system for microwave power (linearly adjustable from 100 to 1000 W) and irradiation time. The oven has been adapted to condense the vapors generated

**Table 1.** Results from the preliminary study for MAE

Particle size		Solvent		Ethanol		Microwave power		Irradiation time		Solvent-to-solid ratio	
$\mu\text{m}$	TPC yield	Type	TPC yield	%v/v	TPC yield	W	TPC yield	min	TPC yield	mL/g	TPC yield
125	26.96 ± 0.67 <sup>a</sup>	Water	23.33 ± 0.07 <sup>c</sup>	20	27.51 ± 0.30 <sup>b</sup>	100	26.80 ± 0.35 <sup>b</sup>	1	28.69 ± 0.27 <sup>ab</sup>	40	29.51 ± 0.70 <sup>c</sup>
250	26.87 ± 0.52 <sup>a</sup>	MeOH 50 %	26.03 ± 0.64 <sup>b</sup>	40	28.67 ± 0.31 <sup>a</sup>	300	28.56 ± 0.50 <sup>a</sup>	2	29.67 ± 0.64 <sup>a</sup>	50	33.94 ± 0.59 <sup>d</sup>
500	22.70 ± 0.65 <sup>b</sup>	EtOH 50 %	29.18 ± 0.39 <sup>a</sup>	50	29.40 ± 0.58 <sup>a</sup>	500	28.69 ± 0.27 <sup>a</sup>	3	29.02 ± 0.83 <sup>a</sup>	60	38.94 ± 0.25 <sup>c</sup>
			Acetone 50 %	27.73 ± 0.61 <sup>a</sup>	60	26.84 ± 0.17 <sup>b</sup>	700	27.87 ± 0.57 <sup>a</sup>	4	28.51 ± 0.04 <sup>ab</sup>	70
				80	16.93 ± 0.20 <sup>c</sup>	900	27.67 ± 0.47 <sup>ab</sup>	5	27.16 ± 0.67 <sup>b</sup>	80	46.28 ± 0.59 <sup>a</sup>
				100	8.31 ± 0.43 <sup>d</sup>					90	38.33 ± 0.17 <sup>c</sup>

TPC, total phenolic compounds; Yield of TPC is expressed as mg of gallic acid equivalents (GAE) per gram of dry weight of coriander powder (mean ±SD).

a, b, c, d, e Means followed by different letters in the same column are significantly different according to ANOVA and Tukey's test.

**Table 2.** Central composite design for yield of total phenolic compounds (TPC) of *Coriandrum sativum* using MAE

Run	$X_1$ – Ethanol concentration (%) v/v)	$X_2$ – power (W)	$X_3$ – time (min)	$X_4$ – ratio (mL/g)	TPC yield (mg GAE/g DW)	
					Experimental	Predicted
1	50 (0)	500 (0)	3 (0)	60 (-2)	39.94 ± 1.11	40.25
2	50 (0)	500 (0)	5 (+2)	80 (0)	37.25 ± 0.12	37.44
3	40 (-1)	300 (-1)	2 (-1)	90 (+1)	39.67 ± 2.80	39.52
4	60 (+1)	300 (-1)	4 (+1)	90 (+1)	33.50 ± 0.17	33.57
5	50 (0)	500 (0)	3 (0)	80 (0)	37.00 ± 0.50	37.57
6	40 (-1)	700 (+1)	4 (+1)	90 (+1)	42.69 ± 1.59	42.84
7	60 (+1)	700 (+1)	2 (-1)	90 (+1)	34.67 ± 1.36	34.39
8	50 (0)	500 (0)	3 (0)	100 (+2)	41.89 ± 0.67	42.35
9	60 (+1)	300 (-1)	2 (-1)	90 (+1)	37.17 ± 1.74	36.71
10	50 (0)	100 (-2)	3 (0)	80 (0)	35.61 ± 1.11	35.66
11	40 (-1)	700 (+1)	2 (-1)	70 (-1)	36.50 ± 0.73	36.14
12	50 (0)	900 (+2)	3 (0)	80 (0)	35.72 ± 0.38	36.45
13	60 (+1)	700 (+1)	2 (-1)	70 (-1)	34.78 ± 1.57	34.27
14	60 (+1)	300 (-1)	2 (-1)	70 (-1)	38.25 ± 1.73	37.81
15	50 (0)	500 (0)	1 (-2)	80 (0)	36.50 ± 0.50	37.09
16	40 (-1)	300 (-1)	4 (+1)	90 (+1)	38.50 ± 1.17	38.52
17	30 (-2)	500 (0)	3 (0)	80 (0)	37.33 ± 0.60	37.02
18	50 (0)	500 (0)	3 (0)	80 (0)	38.00 ± 0.29	37.57
19	60 (+1)	700 (+1)	4 (+1)	90 (+1)	35.00 ± 0.69	34.11
20	40 (-1)	700 (+1)	4 (+1)	70 (-1)	39.67 ± 1.01	39.64
21	40 (-1)	300 (-1)	4 (+1)	70 (-1)	36.56 ± 2.55	36.54
22	40 (-1)	700 (+1)	2 (-1)	90 (+1)	41.17 ± 1.70	40.99
23	70 (+2)	500 (0)	3 (0)	80 (0)	29.11 ± 1.50	30.20
24	60 (+1)	300 (-1)	4 (+1)	70 (-1)	36.61 ± 0.10	36.31
25	50 (0)	500 (0)	3 (0)	80 (0)	37.72 ± 0.54	37.57
26	40 (-1)	300 (-1)	2 (-1)	70 (-1)	35.50 ± 1.45	35.90
27	60 (+1)	700 (+1)	4 (+1)	70 (-1)	35.78 ± 2.55	35.63

into the sample during extraction. The extraction procedure consisted of putting one gram of coriander powder into a 250 mL volumetric flask containing the extraction solvent. After the extraction process, the extract was filtered on Whatman N°1 filter paper and the total volume was adjusted to the initial extraction volume. The extract was preserved at 4 °C until subsequent analysis.

### 2.3.2 Experimental design

**Step I:** Preliminary studies were carried out to determine the individual influence of six parameters (particles size, type of solvents, concentration of solvents, power of microwave, time, and the liquid-solid ratio) on the extraction of polyphenols of the coriander powder. For all extractions, except for the particle size test, the coriander powder having a particle size <250  $\mu\text{m}$  was used. Each parameter (solvent,

concentration, power, time, and liquid-solid ratio) was optimized by fixing the other parameters (Table 1).

**Step II:** The main influencing factors were identified based on results from the preliminary study. Thereafter, a CCD-based RSM was established and designed to achieve maximum TPC recovery for the MAE.

Four variables were selected:  $X_1$ : ethanol concentration (%),  $X_2$ : microwave power (W),  $X_3$ : irradiation time (s), and  $X_4$ : solvent-to-solid ratio (mL/g), with five levels for each variable for the microwave extraction. All experimental data were obtained from a 27-run experiment (Table 2).

The output results, namely for the levels of TPC, were tuned to a second-order polynomial equation (quadratic model) which describes the interaction between the different experimental variables, according to the model in this Equation (Eq. (1)):

$$Y = B_0 + \sum_{i=1}^k B_i X_i + \sum_{i=1}^k B_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^k B_{ij} X_i X_j + E \quad (1)$$

Where  $Y$  represents the response function (TPC yield);  $B_0$  is a constant coefficient;  $B_i$ ,  $B_{ii}$  and  $B_{ij}$  are the linear, quadratic and interactive terms coefficients, respectively, and  $X_i$  and  $X_j$  represent the coded independent variables. The factor levels were coded as  $-2$ ,  $-1$ ,  $0$ ,  $+1$ , and  $+2$  respectively. The variables were coded in accordance with the subsequent equation (Eq. (2)):

$$x_i = \frac{X_i - X_0}{\Delta X} \quad (2)$$

Where  $x_i$  is the coded value of the variable  $X_i$ ;  $X_0$  is the value of  $X$  at the center point and  $\Delta X$  is the step change.

### 2.3.3 Conventional extraction

Conventional extraction of TPC from coriander was achieved using the modified procedure previously described by Dahmoune *et al.* (2013) <sup>24</sup>. Briefly, 1 g of coriander powder was placed in a 250 mL Erlenmeyer flask and 75 mL of 50 % ethanol (EtOH) (v/v) have been added. The mixture was then held in a thermostatic water bath (Memmert) at 60 °C for 2 hours. The extract was then recovered and subjected to the same analysis applied to the optimized extract.

## 2.4 Analytical determinations and identification of TPC

### 2.4.1 Determination of TPC

The TPC of coriander extracts was assayed by the Folin-Ciocalteu method as previously described by Bouaoudia-Madi *et al.* (2017) <sup>25</sup>. Briefly, 2.5 mL of Folin-Ciocalteu reagent diluted in water (1/10) was added to 0.5 mL of extract. The mixture was incubated at room temperature for 2 min, and 2 mL of sodium carbonate (75 g/L) was added, followed by

incubation at 50 °C for 15 min, and finally, cooled in a water-ice bath. The specific absorbance at 760 nm was immediately measured using a UV-Vis spectrophotometer (Shimadzu, Model: UV-1800, Germany). The TPC was provided in mg of gallic acid equivalents (GAE) per gram of coriander powder, on dry weight (DW) basis ( $y = 0.012 x$ ,  $R^2 = 0.99$ ).

### 2.4.2 Identification of the phenolic compounds HPLC–DAD–ESI/MS<sup>n</sup>

This was undertaken on an Ultimate 3000 (Dionex Co., USA) apparatus equipped with an ultimate 3000 Diode Array Detector (Dionex Co., USA) and paired with a mass spectrometer, as previously described by Beder-Belkhir *et al.* (2018) <sup>26</sup>. The chromatographic system was consisting of an autosampler a photodiode-array (PDA) detector, an automatic thermostatic column compartment, and a quaternary pump. The column dimension was a 100 mm length, 2.1 mm i.d., 1.9 μm particle diameter, end-capped Hypersil Gold C18 column (Thermo Scientific, USA) which was maintained at 30 °C. The Gradient elution was carried out with a mixture of 0.1% (v/v) of formic acid in water (solvent A) and acetonitrile (solvent B), which was degassed and filtered before use. The solvent gradient consisted of a series of linear gradients, starting with 15 – 28 % of solvent B over 5.6 min, increasing to 29 % at 8.8 min, reaching 100 % at 13.1 min and keeping up to 17 min, before returning to the initial conditions, with total running of 20 min. The flow rate used was 0.2 mL min<sup>-1</sup> and UV–vis spectral data for all peaks were accumulated in the range of 200 – 600 nm. The mass spectrometer used was a Thermo LTQ XL (Thermo Scientific, USA) ion trap MS equipped with an ESI source. Control and data acquisition was carried out with the Thermo X Calibur Qual Browser data system (Thermo Scientific, USA). Nitrogen above 99% purity was used and the gas pressure was 520 kPa (75 psi).

### 2.4.3 Quantitative analysis of phenolic compounds

For quantitative determination, the limits of detectability and quantification were calculated from the calibration curves parameters obtained by different standard compounds namely caffeic acid ( $y = 32516.95x - 23835.97$ ;  $R^2 = 0.99$ ), 5 – caffeoylquinic acid ( $y = 14621.02x - 13859.96$ ;  $R^2 = 0.99$ ), quercetin–7–*O*–galactoside ( $y = 10138.87x - 12806.02$ ;  $R^2 = 0.99$ ), cinnamic acid ( $y = 59407.85x - 7538.18$ ;  $R^2 = 1.00$ ) and coumaric acid ( $y = 48154.12x - 20233.87$ ;  $R^2 = 1.00$ ). Phenolic compounds, for which no commercial standard was available, were quantified using a compound of the same group. The results were expressed in mg per g of dried extract.

## 2.5 Antioxidant activity

### 2.5.1 DPPH<sup>•</sup> radical scavenging assay

The ability of the extracts of coriander to scavenge DPPH<sup>•</sup> free radical was estimated, according to the methodology previously



described by Neto *et al.* (2018) <sup>27</sup>. The test consists of mixing 50  $\mu\text{L}$  of the sample at different concentrations and 250  $\mu\text{L}$  of DPPH<sup>\*</sup> in each well. The microplate is then placed in the dark for 30 minutes followed by the absorbance reading at 517 nm. The IC<sub>50</sub> value was calculated and compared to the positive control (ascorbic acid,  $4113.70x - 0.71$ ,  $R^2 = 0.99$ ).

### 2.5.2 Ferric reducing antioxidant power (FRAP) assay

The reducing power of extracts was estimated according to the protocol described by Neto *et al.* (2018) <sup>27</sup>. The test consists of mixing 200  $\mu\text{L}$  of the various extracts at different concentrations with 200  $\mu\text{L}$  of a phosphate buffer solution (0.2 M and pH 6.6) and 200  $\mu\text{L}$  of potassium hexacyanoferrate (III) solution ( $\text{K}_3\text{Fe}(\text{CN})_6$ , 1%). The whole mixture was incubated in a water bath for 20 minutes at 50°C and then, 200  $\mu\text{L}$  of trichloroacetic acid (10%) was added. An aliquot (75  $\mu\text{L}$ ) of the mixture was added to 75  $\mu\text{L}$  of distilled water and 15  $\mu\text{L}$  of an aqueous solution of  $\text{FeCl}_3$  (0.1%). The absorbance was read at 700 nm. EC<sub>50</sub> value was determined and compared to the BHA used as a positive control ( $20.61x + 0.05$ ,  $R^2 = 0.99$ ).

### 2.5.3 Nitric Oxide (NO•) Assay

The method previously reported by Pereira *et al.* (2018) <sup>28</sup> was followed to determine the ability of coriander extracts to scavenge NO•. In brief, a portion of 100  $\mu\text{L}$  of different sample concentrations was added to 100  $\mu\text{L}$  of sodium nitroprusside solution (3.33 mM) in PBS 100 mM (pH = 7.4). The mixture was then incubated for 15 min under a fluorescent lamp. After incubation, 100  $\mu\text{L}$  of Griess reagent was added and the absorbance was measured spectrophotometrically at 562 nm after incubation in the dark for 10 min. The IC<sub>50</sub> value for this test was determined from the graphical plot of nitrite generation inhibition. Ascorbic acid was taken as the standard compound ( $1708x + 1.92$ ,  $R^2 = 0.98$ ).

### 2.5.4 Superoxide anion-radical scavenging activity

The sample's superoxide anion activity ( $\text{O}_2^{\bullet-}$ ) was determined following the same method as Pereira *et al.* (2018) <sup>28</sup>. The method comprised mixing 75  $\mu\text{L}$  of extracts dilutions with 75  $\mu\text{L}$  NBT (0.2 mM), 100  $\mu\text{L}$   $\beta$ -NADH (0.3 mM), and 50  $\mu\text{L}$  of PMS (0.015 mM). After incubation for 5 min at ambient temperature, the absorbance was read at 560 nm. Trolox was taken as the standard compound ( $111.7x + 12.03$ ,  $R^2 = 0.99$ ). The concentration of the extract/standard capable to scavenge 50 % of the radical (EC<sub>50</sub>) was determined from the linear regression plot of percent inhibition against extracts concentration.

## 2.6 Statistical analysis

Each extraction trial and all the analyses were carried out in triplicate and all the data in this study have been reported as means  $\pm$ SD. Influence of each factor on the TPC yield in the preliminary experiment and, the data generated from the CCD experiments for the MAE was statistically assessed by ANOVA and Tukey's post hoc test with 95% confidence level, using JMP software (Version 10.0, SAS). *P*-values were used to consider the significance of the influence of the parameters studied.

The comparison and study of the influence of the extraction technique (MAE or CE) on the extraction of TPC and the antioxidant activities were performed by univariate ANOVA and Fisher's test for the discrimination of the means (level 95% confidence) using the software Minitab 17.

## 3 Results and discussion

### 3.1 Microwave-assisted extraction

#### 3.1.1 Effect of extraction parameters

The extraction efficiency of any compound is influenced by many parameters that can present independent or interactive effects <sup>29</sup>. In order to establish the influence of the variables of extraction such as the diameter of the particles, the type of solvent and its concentration, the microwave power, the time, and the liquid-solid ratio were studied separately in the step I (preliminary study) in order to establish the appropriate experimental ranges to be considered during the optimization process. The results are illustrated in Table 1.

#### *Effect of particle size*

Based on data reported in Table 1, the maximum TPC yield was obtained with the smallest particulate dimension (125  $\mu\text{m}$ ), although this was not statistically different ( $p > 0.05$ ) from that of 250  $\mu\text{m}$ . Attending to this and the superior difficulties faced in filtrations step when extracting from the smallest-sized powder (clogging and agglomeration), the powder having a particles size below 250  $\mu\text{m}$  was fixed for the other experiments of preliminary studies. The same particle size was chosen by Bouaoudia-Madi *et al.* (2017) <sup>25</sup>, Bouaoudia-Madi *et al.* (2019) <sup>30</sup>, Guemghar *et al.* (2020) <sup>18</sup> and Himed-Idir *et al.* (2021) <sup>9</sup>, after a preliminary study for *Myrtus communis* plant, *Myrtus communis* L. pericarp, artichoke (*Cynara scolymus* L.) powder and rosemary (*Rosmarinus officinalis* L.) powder, respectively.

#### *Effect of solvents types*

The selection of solvent takes into consideration not only its affinity with the desired compound but also its ability to absorb microwave energy <sup>31</sup>. Aqueous organic solvents such as methanol, ethanol, and acetone are usually the most used for the extraction of phenolic substances <sup>32</sup>. To determine the most

appropriate solvent for extracting phenolic compounds from coriander leaves powder, the extraction was performed using four types of solvents, namely methanol, ethanol, acetone (each at 50 %), and water. As shown in Table 1, the maximum TPC recovery was obtained with aqueous ethanol, followed by aqueous acetone, which gave comparable results with no significant difference ( $p > 0.05$ ), while methanol 50% and water were less efficient. For this reason, ethanol 50% was fixed for the following preliminary assays, as well as for the experimental design. The preference of ethanol herein registered is consistent with that obtained by other authors for the extraction of phenolic compounds from different matrices, including wheat bran<sup>33</sup>, olive leaves<sup>34</sup>, and dried chokeberry<sup>5</sup>. This fact is quite important since ethanol is reported to have many advantages over other organic solvents, such as its safety and lower toxicity<sup>35</sup> as well as its large-scale use due to its low price, biodegradability, and availability in high purity<sup>36</sup>.

#### *Effect of ethanol concentration*

In order to establish the best proportion of the mixture (ethanol-water) to extract TPC, ethanol was tested in the range of 20 to 100 %. Our results (Table 1) showed that the increment of ethanol up to 50% increased the recovery of TPC, while the inverse tendency occurred above that concentration. This result can be explained, on the one hand, by the fact that the extractability of the phenolic compounds is increased with the addition of water to ethanol, which is attributed to the increase in the permeability of plant tissues and enables a better mass transfer by diffusion<sup>37</sup> and on the other hand, by the difference of the solubility of the phenolic compounds between the two phases water and ethanol, as well as their affinity. Previous studies also reported that 50% ethanol concentration was the best for extracting phenolic compounds from different matrices such as *Citrus limon* residues<sup>24</sup>, *Euryale ferox* seed<sup>38</sup>, sea buckthorn leaves<sup>36</sup>, chokeberries<sup>39</sup>, and dried chokeberry<sup>5</sup>. Attending that the maximum values of TPC were herein obtained for 40% - 50% EtOH (no statistical differences ( $p > 0.05$ ) between the two conditions), 40% EtOH was selected for further experiments in independent effects studies, while the range of concentrations between 40 and 60% was used in the RSM (Step II).

#### *Influence of microwave power*

Microwave power is one of the parameters that can strongly affect the yield of extraction of phenolic compounds from plant matrices. In general, up to a certain point, its rise causes a sudden temperature increase and a concomitant internal pressure inside the matrix that facilitates cell wall disruption and the release of bioactive compounds into the solvent<sup>7,34</sup>. As shown in Table 1, variation of MW power over the range of 300-900 W caused slight effects on the TPC yield (26.8 – 28.7 mg GAE/g DW), with maximum levels being tendentially achieved at 500 W. Naturally, the optimal MAE potency

depends on distinct factors, including the plant matrix and the nature of the phenolics. Still, this same power was found to be the best for extracting phenolic compounds from different matrices, namely *Pistacia lentiscus* leaves<sup>40</sup>, *Myrtus communis* L.<sup>41</sup> and *Citrus sinensis* peels<sup>42</sup>. Attending to the results of Table 1, the power of 500 W was retained for further preliminary experiments, and the range of 300 to 700 W was selected for RSM experiments.

#### *Influence of irradiation time*

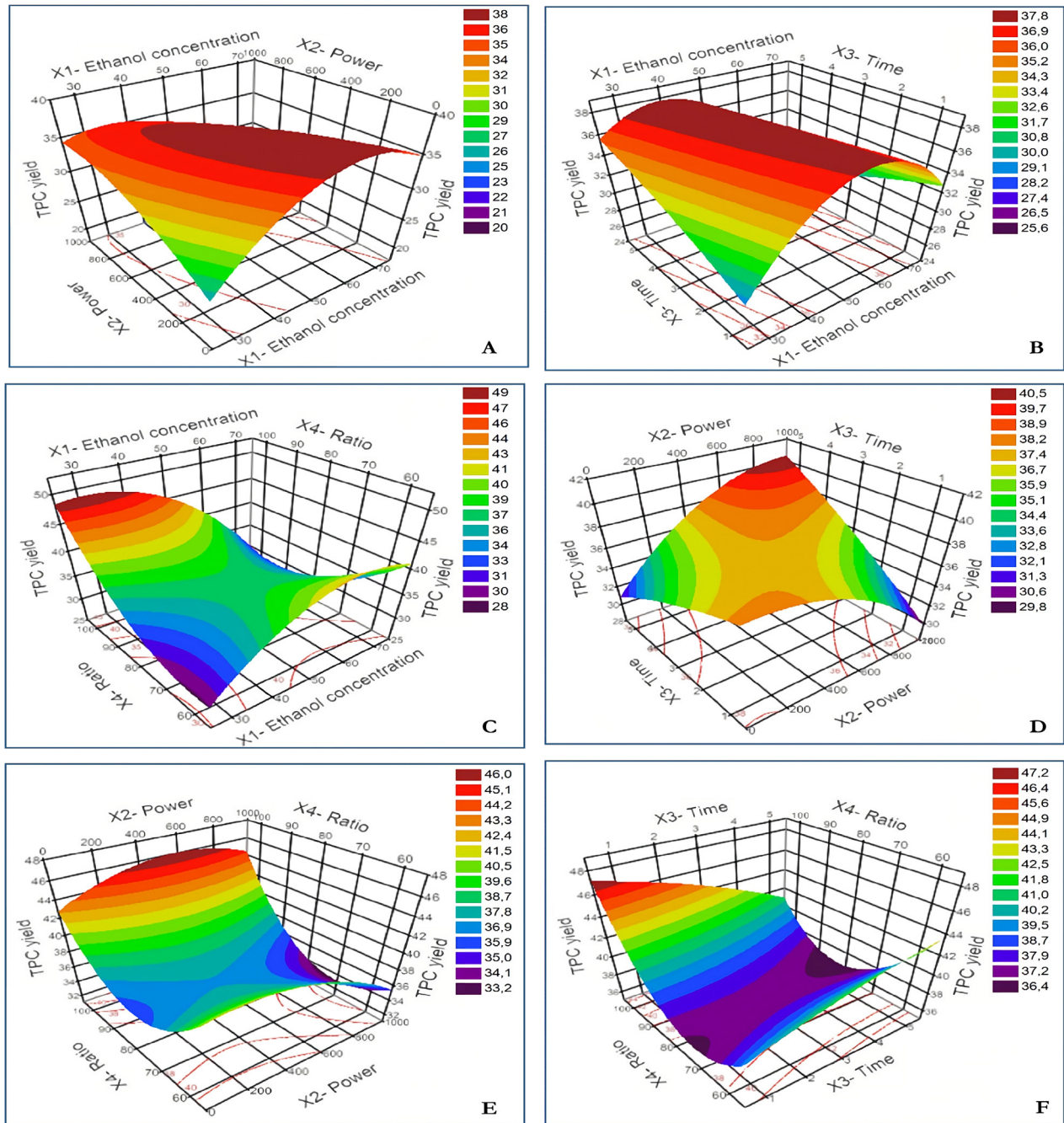
Extraction irradiation time in MAE is among the key variables impacting the yield of polyphenols<sup>12</sup>. It is a pertinent optimization parameter to minimize the energy cost of the process<sup>43,44</sup>. In this study, the influence of the extraction time has been evaluated between 1 and 5 min. According to the results shown in Table 1, TPC yields did not change significantly ( $p > 0.05$ ) for extractions occurring for 1 – 4 min, although a slight mean value was found for the time of 2 min. In turn, the prolongation of irradiation exposure over 4 min resulted in a reduced recovery of TPC, a fact that can be associated with the structural changes in the polyphenols (e.g. degradation and oxidation). Note that the decline in the levels of recovered TPC in MAE for long periods is a common observation. In this context, Spigno and De Faveri (2009)<sup>45</sup> demonstrated that at 900 W, MAE extraction was most efficient at very short durations (90 s than at 210 s). Dahmoune *et al.* (2013)<sup>24</sup> also reported that the best TPC yield of *Citrus limon* with MAE at 500 W was achieved with an irradiation time of 120 s. Based on these results, the irradiation time of 1 min was retained for preceding the preliminary experiments, and the range of 2 to 4 min was selected for RSM experiments.

#### *Influence of liquid-solid ratio*

The liquid-solid ratio is also one critical factor in MW-assisted extraction. In general, a larger dissolving volume improves the recovery of bioactive ingredients but this tendency is reversed at some point<sup>31</sup>. The latter behavior was observed in the present study, with a rise in the TPC yield being observed from 20:1 to 80:1 mL/g (Table 1). It is possible that the increment of extraction yield is associated with the increment of the solvent, to the solid, resulting in an increase in contact between the material and solvent as well as the solubility of the antioxidant components<sup>46</sup>, while the decline (ratios above 80:1) can be due to the solubility decline<sup>47</sup> as well as by the non-uniform distribution and exposure to microwave heating<sup>42</sup>. Based on these results, the range of 70:1 to 90:1 mL/g liquid-solid ratio was selected for RSM experiments.

### **3.1.2 Interactive effect of four variables**

The experiments in protocol I allowed us to delimit the range of influence for each variable;  $X_1$ : concentration of the solvent [40 – 60 %],  $X_2$ : microwave power [300 – 700 W],  $X_3$ : time [2 – 4 min] and  $X_4$ : liquid-solid ratio [70 – 90 mL/g].



**Figure 1.** Response surface plots of the effect of (A) Concentration and power, (B) Concentration and time, (C) Concentration and ratio, (D) Power and time, (E) Power and ratio, and (F) Time and ratio on TPC yield

An experimental design using the RSM was designed by introducing data into the JMP software and choosing the CCD. Overall, 27 combinations have been proposed and the corresponding results are summarized in Table 2. Mathematical equation (Eq. 3) governing was applied to provide the optimum conditions of the extraction of TPC from coriander powder. This was simplified by the elimination of effects of interactions considered insignificant in the analysis.

This approach facilitates calculations by reducing the expression but still maintains the quality of fitting<sup>39</sup>:

$$Y = 37.57 - 1.71X_1 + 0.53X_4 - 0.94X_1X_2 - 0.53X_1X_3 + 0.72X_2X_3 - 1.18X_1X_4 - 0.41X_3X_4 - 0.99X_1^2 - 0.38X_3^2 + 0.93X_4^2 \quad (3)$$

To verify the importance and adequacy of the chosen model, analysis of the variance (ANOVA) was used, the results are summarized in Table 3.

pronounced quadratic effect having a positive impact on the TPC extraction efficiency was represented by the effect of  $X_4^2$  (ratio-ratio) with a coefficient of 0.93 and a probability  $p < 0.0001$ .

**Table 3.** Analysis of variance (ANOVA) for the experimental results obtained using MAE

Model parameters	coefficients	Standard error	Df	Sum of squares	F-value	Prob > F
Intercept	37.57	0.37	14	193.76	33.04	<.0001*
<b>Linear</b>						
$X_1$ -Solvent concentration	-1.71	0.13	1	69.85	166.74	<.0001*
$X_2$ -Power	0.20	0.13	1	0.93	2.22	0.1622
$X_3$ -Time	0.09	0.13	1	0.19	0.44	0.5181
$X_4$ -Ratio	0.53	0.13	1	6.62	15.82	0.0018*
<b>Interaction</b>						
$X_1 X_2$	-0.94	0.16	1	14.27	34.07	<.0001*
$X_1 X_3$	-0.53	0.16	1	4.57	10.92	0.0063*
$X_2 X_3$	0.72	0.16	1	8.19	19.54	0.0008*
$X_1 X_4$	-1.18	0.16	1	22.30	53.23	<.0001*
$X_2 X_4$	0.31	0.16	1	1.49	3.57	0.0834
$X_3 X_4$	-0.41	0.16	1	2.69	6.41	0.0263*
<b>Quadratic</b>						
$X_1^2$	-0.99	0.14	1	20.96	50.04	<.0001*
$X_2^2$	-0.38	0.14	1	3.084	7.36	0.0188*
$X_3^2$	-0.08	0.14	1	0.13	0.31	0.5874
$X_4^2$	0.93	0.14	1	18.54	44.26	<.0001*
<b>Lack of fit</b>			10	4.49	1.69	0.4290
<b>Residual</b>			12	5.03		
<b>Pure error</b>			2	0.53		
$R^2$					0.97	
$R^2_{Adjusted}$					0.95	
RMSE	0.65					
<b>Corr total</b>			26	198.79		

\*: significance of the corresponding coefficient

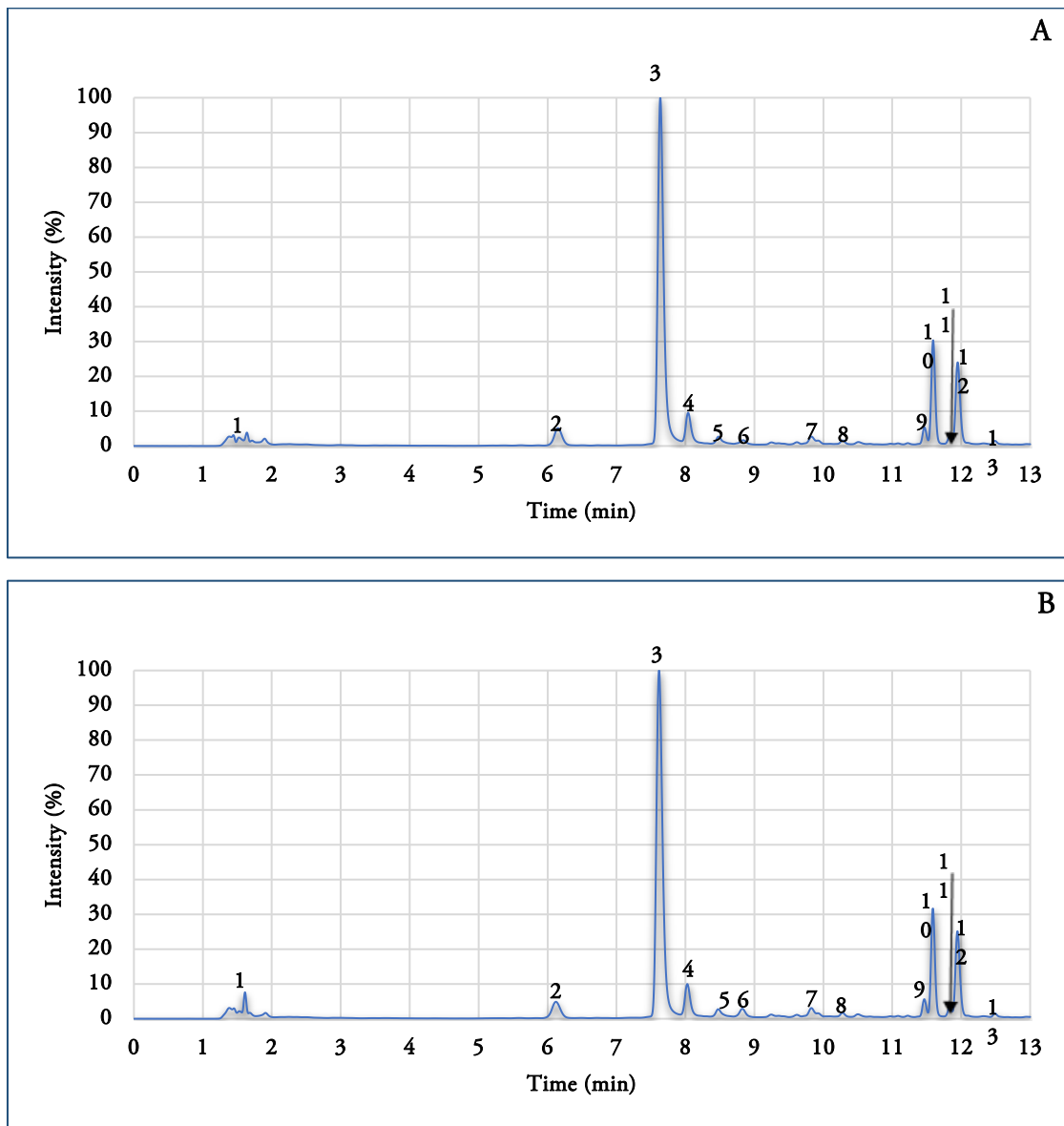
Overall, the values of the coefficients of determination ( $R^2$ ) and adjusted coefficient of determination ( $R^2_{Adj}$ ) of the model were in the order of 0.97 and 0.95, respectively, and thus enough to confirm the good significance of the model.

Furthermore, the adjustment defect value ( $p = 0.4290$ ) was not significant ( $p > 0.05$ ), which confirms the validation of the model. P-values are used as a tool to check the meaning of each coefficient and give the intensity of the interaction between the parameters. The smaller P-value, the greater the significance of the corresponding coefficient. The study of the effect of each variable (linear effect) showed that the liquid-solid ratio ( $X_4$ ) had a significant positive influence on the extraction of TPC from the coriander powder, showing a probability  $p = 0.0018$  and a coefficient of the order of 0.53. This can be explained by the fact that a higher liquid-solid ratio improves the mass transfer of the extracted compounds in the solvent<sup>48</sup>. In turn, the impact of other factors was either negative (solvent concentration ( $X_1$ )) or non-significant (power ( $X_2$ ) and extraction time ( $X_3$ )). Moreover, the most

Instead, the other effects, namely  $X_1^2$  (concentration-concentration),  $X_2^2$  (power-power), and  $X_1^2$  (time-time), regardless of their meaning, had a negative effect on the extraction yield of TPC.

The interaction effects between the four variables ( $X_1$ ,  $X_2$ ,  $X_3$ , and  $X_4$ ) on the TPC yield, represented on the 3D plane, are shown in Figure 1. Only the interaction between the parameters power and time ( $X_2 X_3$ ) had a positive effect (coefficient = 0.72) and was significant with a probability of 0.0008 (Figure 1.D). This Figure shows that on the time intervals of 2 – 4 min and powers of 500 – 700 W, the simultaneous increase of the MW power and the extraction time make it possible to improve the TPC recovery, albeit no significant difference was found by applying the low levels (2 min and 500 W) or the high levels (4 min and 700 W).





**Figure 2.** Chromatograms obtained by UHPLC-DAD-ESI/MS<sup>n</sup> analysis of optimized extract (A) and conventional extract (B) of *Coriandrum sativum* L. leaves powder at 280 nm

The interaction effects between the four variables ( $X_1$ ,  $X_2$ ,  $X_3$ , and  $X_4$ ) on the TPC yield, represented on the 3D plane, are shown in Figure 1. Only the interaction between the parameters power and time ( $X_2$   $X_3$ ) had a positive effect (coefficient = 0.72) and was significant with a probability of 0.0008 (Figure 1.D). This Figure shows that on the time intervals of 2 – 4 min and powers of 500 – 700 W, the simultaneous increase of the MW power and the extraction time make it possible to improve the TPC recovery, albeit no significant difference was found by applying the low levels (2 min and 500 W) or the high levels (4 min and 700 W). A positive and significant effect in a simultaneous variation of time and microwave power has been reported also in other

previous studies conducted on cultivars of *Sophora japonica* L., chokeberries, and defatted roselle seed<sup>39,49,50</sup>. It is possible that this result is associated with a temperature rise caused by the increment of such two factors, which in turn, might result in an increased solubility of the phenolic compounds and a decrease in the solvent viscosity, thereby accelerating the release and dissolution of TPC<sup>41</sup>. Indeed, it was reported that the increased absorption of microwave energy led to a higher temperature inside the sample leading to the rupture of cells and easier release of antioxidant compounds<sup>15</sup>.

Furthermore, the interactions between ethanol concentration and power ( $X_1$  $X_2$ ), ethanol concentration and time ( $X_1$  $X_3$ ), ethanol concentration and ratio ( $X_1$  $X_4$ ), and time and ratio



( $X_3X_4$ ), represented by Figures 1.A, 1B, 1.C, and 1.F, respectively, although they showed a significant interaction at values of  $p < 0.0001$ ,  $0.0063$ ,  $< 0.0001$ ,  $0.0263$ , respectively, this was negative. However, the power-ratio interaction ( $X_2X_4$ ) was not significant (Figure 1.E). The negative interaction between ethanol concentration and power ( $X_1X_2$ ), can be explained by the fact that by increasing the concentration of the solvent, its polarity decreases, which induces a negative response with the power of the microwave. This is also true for the other three negative interactions: ( $X_1X_3$ ), ( $X_1X_4$ ), and ( $X_3X_4$ ).

50% solvent concentration, 400W microwave power, 2.14 min of extraction time, and a 75 mL/g liquid-solid ratio. Under these conditions, the model predicted that TPC recovery would range from 36.72 to 38.13 mg GAE/g DW, and the experiments performed under the same optimal conditions resulted in a TPC content of  $37.94 \pm 2.06$  mg GAE/g DW (Table 5), which fits well in the proposed interval. This allows us to confirm the adequacy of the model for the intended optimization of phenolic compounds extraction from coriander leaves using a microwave-assisted method. It should be noted that previous works, also indicated the same results for each of the optimal conditions found in the present study,

**Table 4.** Identification of LC-DAD-ESI/MS<sup>n</sup> data of the most relevant fractions from the extract of *Coriandrum sativum* L.

Peak	Rt (min)	$\lambda_{max}$	[M-H] <sup>-</sup>	ESI MS/MS product ions	Probable compound	Phenolic Content ( $\mu\text{g}/\text{mg}$ of extract)	
						MAE	CE
1	1.5	266	133	MS <sup>2</sup> [133]: 115	Malic acid	NQ	NQ
				191	MS <sup>2</sup> [191]: 111, 173	Citric acid	NQ
2	6.1	290, 324	369	MS <sup>2</sup> [369]: 189, 207	Dimethoxycinnamoyl hexoside	$0.10 \pm 0.01$	$0.09 \pm 0.01$
3	7.6	296, 328	369	MS <sup>2</sup> [369]: 189, 207	Dimethoxycinnamoyl hexoside	$18.75 \pm 0.24$	$20.92 \pm 0.15$
4	8.1	296, 325	353	MS <sup>2</sup> [353]: 191, 179	5-O-caffeoylquinic acid	$7.01 \pm 0.31$	$7.61 \pm 0.13$
5	8.5	294	325	MS <sup>2</sup> [325]: 163, 119	Coumaroyl-hexoside	$0.40 \pm 0.02$	$0.42 \pm 0.01$
6	8.8	297sh, 326	179	MS <sup>2</sup> [179]: 135, 152, 151, 161, 179	Caffeic acid	$0.52 \pm 0.02$	$0.86 \pm 0.01$
7	9.9	297sh, 326	353	MS <sup>2</sup> [353]: 173, 191, 111	4-O-Caffeoylquinic acid	NQ	NQ
8	10.5	297sh, 326	367	MS <sup>2</sup> [367]: 191, 173	Feruloylquinic acid	NQ	NQ
9	11.5	256, 352	609	MS <sup>2</sup> [609]: 301	Quercetin-3-O-rutinoside	$3.46 \pm 0.05$	$3.80 \pm 0.03$
10	11.6	256, 354	609	MS <sup>2</sup> [609]: 301	Quercetin-3-O-rutinoside	$19.91 \pm 0.36$	$21.58 \pm 0.14$
11	11.8	256, 354	463	MS <sup>2</sup> [463]: 301	Quercetin-3-O-glucoside	NQ	NQ
12	12.0	256, 354	477	MS <sup>2</sup> [477]: 301	Quercetin-3-O-glucuronide	$19.44 \pm 0.45$	$21.16 \pm 0.16$
13	12.5	266, 350	593	MS <sup>2</sup> [593]: 285	Kaempferol-3-O-rutinoside	NQ	NQ

NQ: Not Quantified

**Table 5.** TPC yield and antioxidant activities of extracts obtained with MAE and CE methods and their comparison to standards

Sample	TPC yield (mg GAE/g DW)	DPPH <sup>*</sup> (IC <sub>50</sub> , mg/mL)	FRAP (EC <sub>50</sub> , mg/mL)	NO <sup>*</sup> (IC <sub>50</sub> , mg/mL)	O <sub>2</sub> <sup>-</sup> (IC <sub>50</sub> , mg/mL)
MAE	$37.94 \pm 2.06^b$	$0.08 \pm 0.00^a$	$0.18 \pm 0.01^a$	$0.19 \pm 0.02^a$	$0.52 \pm 0.06^a$
CE	$44.47 \pm 0.57^a$	$0.07 \pm 0.00^b$	$0.18 \pm 0.01^a$	$0.19 \pm 0.03^a$	$0.51 \pm 0.02^a$
Ascorbic acid	-	$0.01 \pm 0.00^c$	-	$0.03 \pm 0.00^b$	-
BHA	-	-	$0.02 \pm 0.00^b$	-	-
Trolox	-	-	-	-	$0.34 \pm 0.01^b$

a, b: Means followed by different letters in the same column are significantly different according to ANOVA and Tukey's test.

### 3.1.3 Experimental validation of MAE optimal extraction parameters predicted by RSM

To assess the adequacy and reliability of the model equation, the optimal conditions proposed by JMP software were tested:

as the best for the extraction of bioactive constituents from their vegetal matrices. In particular, a solvent concentration of 50%<sup>14, 20, 51</sup>, a microwave power of 400 W<sup>52-54</sup>, an optimal irradiation time of about 2 minutes<sup>9, 51</sup>, and a ratio close to 75 mL<sup>6, 8</sup>.

### 3.1.4 Comparison of MAE and Conventional extraction

#### *Yields of TPC and phenolic profile*

For comparative purposes, a conventional extraction was done in parallel with MAE. The concentration of the solvent and the liquid-solid ratio parameters adopted in the conventional extraction were the same as those applied in MAE (ethanol 50 % and 75 mL/g, respectively) to eliminate their influence on the yield of TPC. These parameters were applied for 2 hours at 60 °C. Under the extraction conditions applied, the extraction methods revealed TPC contents of  $44.47 \pm 0.57$  mg GAE/g and  $37.94 \pm 2.06$  mg GAE/g DW for conventional and MAE, respectively (Table 5). The superior recovery of TPC by the conventional method may in part be due to an insufficient microwave agitation of the solvent. Independently, it should be noted that the methodology that allows for the maximum extraction of phenols mainly depends on the plant material, and therefore different conclusions are expected in the literature<sup>47</sup>. The herein results are in line with those of Biswas *et al.* (2012)<sup>55</sup>, who reported a superior recovery of antioxidants from beans by 50% ethanol using the conventional method, as compared to MAE and those of Baiano *et al.* (2014)<sup>47</sup>, who found that conventional heat extraction was more efficient than MAE in extracting antioxidants from solid plant waste. In another study, Asofieci *et al.* (2016)<sup>36</sup> reported that the recovery of polyphenols by microwave and conventional techniques was similar, with reduced time of extraction pointed as the main advantage for MW. In contrast, higher yields of TPC recovery were obtained by Brahim, Gambier, and Brosse (2014)<sup>56</sup> for grape residues, or by Bouras *et al.* (2015)<sup>57</sup> for Quercus bark, when using MW extraction and compared to the conventional extraction method.

According to UHPLC-DAD-ESI/MS<sup>n</sup> analysis, the extracts obtained by conventional and MW-assisted methods had similar phenolic components (Figure 2 and Table 4), with slight differences in their content that tended to be higher than that obtained by the conventional method. The two extracts were particularly rich in quercetin derivatives, particularly of quercetin-3-*O*-rutinoside isomers (peaks 9 and 10) and quercetin-3-*O*-glucuronide (peak 12), which overall represented about 61-62% of the total quantified phenolic components. Besides, the extracts contained a considerable amount of dimethoxycinnamoyl hexoside, summing 18.85 and 21.01 µg/mg of extract in MAE and CE, respectively, and of 5-*O*-caffeoylquinic acid (7.01 and 7.61 µg/mg of extract in MAE and CE extracts, respectively). Such results are consistent with those reported by Barros *et al.* (2012)<sup>58</sup>, who reported that the main compounds in coriander leaves were flavonol derivatives (quercetin and kaempferol derivatives) and hydroxycinnamic acids derivatives.

#### *Antioxidant activity*

The MAE and CE extracts were also compared regarding their antioxidant abilities, by distinct methods, in order to evaluate their antiradical ability towards DPPH•, NO• and O<sub>2</sub>•- radicals, as also their ability to reduce iron ions. Except for DPPH• assay, the two extracts demonstrated similar antioxidant potential (Table 5). Moreover, it is also of note that the antiradical activity of both extracts towards DPPH•, NO•, and O<sub>2</sub>•- as well as their ability to reduce iron ions were much higher than that exerted by commercial standards. The promising antiradical activity of coriander extracts towards DPPH• has been previously reported, in particular, on various leaf and seed extracts (ethanol, diethyl ether, ethyl acetate, and butanol) by Wangenstein *et al.* (2004)<sup>22</sup>, on the seeds by Zeković *et al.* (2014)<sup>59</sup> as well as on three fruit varieties (Tunisian, Syrian and Egyptian varieties) by Msaada *et al.* (2017)<sup>60</sup>. The ability to reduce iron ions in coriander has also been previously reported, particularly in the study conducted by Msaada *et al.* (2017)<sup>60</sup>. However, NO• and O<sub>2</sub>•- radical scavenging effects have not been reported for coriander before.

### 4. Conclusion

The Response Surface Methodology (RSM) was used to investigate the individual and interactive effects of four variables (solvent concentration, microwave power, extraction time, and liquid-solid ratio) with the aim to optimize the MAE of phenolic compounds from coriander leaves. This methodology proved to be effective in predicting the effect of the tested parameters on the TPC yield. However, by comparison of the MAE method to the CE, experimental evidence has shown that higher extraction yield is obtained using the conventional system. The comparison of UHPLC profiles allowed us to conclude that the extracts obtained by the two techniques were similar to their individual phenolic species, which were present in similar quantities, although they tended to be higher in the extract obtained by the conventional method. Finally, among four tests used to evaluate the antioxidant activity of coriander extracts, only the DPPH• showed a small, although significant, difference between both extracts in favor of the conventional method.

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**Author Contribution:** All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

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Supplementary data: Pdf