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ORIGINAL ARTICLE

Response Surface Methodology (RSM) for Optimization of *Euphorbia resinifera* and *Euphorbia officinarum* Extracts with Antioxidant and Anti-Diabetic Activities

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ABSTRACT

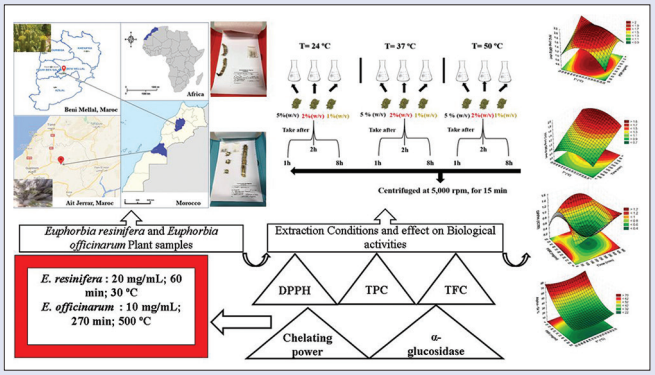
Background: *Euphorbia* is one of the plants most used by herbalists and therapists in Morocco. **Objectives:** The aerial part of two plant samples (*Euphorbia resinifera* and *Euphorbia officinarum*) collected in Morocco was examined for the solvent effect, extraction time, and plant concentration in order to determine the best extraction conditions. **Materials and Methods:** To achieve this goal, a response surface methodology (RSM) using a full three-level factorial design was used to optimize the conditions for the extraction of antioxidants and α-glucosidase inhibitors. Temperature, time, and plant-to-solvent ratio (PSR) and their linear and quadratic interactions on TPC (total phenol concentration), TFC (total flavonoid concentration), DPPH (2,2-diphenyl-1-picrylhydrazyl) trapping activity, and α-glucosidase inhibiting activities were studied. **Results:** According to desirability functions, the optimum operating conditions to achieve a higher extraction yield of phenols and higher antioxidant and anti-diabetic activity were found by using extraction during 60 min at 30°C using a PSR of 20 mg/mL, whereas a longer extraction time (270 min) was needed for *E. resinifera* and a higher extraction temperature (50°C), with a lower PSR (10 mg/mL) for *E. officinarum*. **Conclusion:** In order to find the best conditions to extract secondary metabolites with biological activity and application in phytotherapy, the appropriate solvent generally used by populations, water in this case, should be used, but the best extraction conditions have to be found in order to enhance the pharmacological actions.

Key words: α-glucosidase inhibitory activity, factorial design, response surface methodology, total phenolic compounds, total flavonoids

SUMMARY

- In Morocco, since ancient times that *Euphorbia resinifera* and *E. officinarum* are used in folk medicine.
- Aqueous extractions of *Euphorbia resinifera* and *E. officinarum* revealed that extraction time, extraction temperature and plant-to-solvent ratio are determinants on extraction yield of the secondary metabolites and *in vitro* biological properties.

Abbreviations used: ANOVA: One-way analysis of variance; BHT: Butylated hydroxytoluene; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; Dw: Dry weight; EDTA: Ethylenediaminetetraacetic acid; ET: Extraction time; ET°: Extraction temperature; GAE: Gallic acid equivalents; IC_{50}: Sample concentration providing 50% inhibition; L and Q: Linear and quadratic; PCA: Principal component analysis; PGI: Protected geographical indication; PNPG: *p*-Nitrophenyl-β-D-glucopyranoside; PSR: Plant solvent ratio; QE: Quercetin equivalents; RSM: Response surface methodology; R2: Coefficient of determination; SD: Standard deviation; TFC: Total flavonoid concentration; TPC: Total phenol concentration.



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INTRODUCTION

Euphorbia resinifera O. Berg, with the local name "Zaggoume, Takiout", is an endemic melliferous species of Morocco well adapted to drought. Currently, it is considered as a "terroir" product with a Protected Geographical Indication (PGI).[1] In habitat, this plant forms densely branched, compact shrubs of some meters in diameter and until 1.50 m height. Since ancient times, the North-African Berber tribes have used this species as a remedy.[2] In Morocco, where the use of traditional medicine is a widespread practice, it is one of the oldest plant drugs in folk medicine much used by Moroccan herbalists and therapists. The leaf stem's decoction[3] or one drop of latex in a

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glass of water once a day[4] has been used orally to treat diabetes in Morocco.

The second most important species in Morocco, belonging to the family of Euphorbiaceae, is *Euphorbia officinarum* L.; "Daghmouss" is the local name. This endemic plant is generously found over the north of the Souss river until Western Sahara reaching the region of Zemmour.[5] In Morocco, this species has been largely used in folk medicine over the years for the treatment of various diseases such as diabetes because of the presence of several secondary metabolites such as phenolic compounds.[6] According to Idm'hand *et al.*,[7] prolonged uncontrolled diabetes leads to an increase in the activation of oxidative stress.

The extraction of phenolics or other secondary metabolites from plant sources is the first step involved in their analysis. In spite of several works on the analysis of the plant and phenolics, there is still no available standardized procedure for sample preparation and extraction.[8] It is necessary to optimize the solid–liquid extraction process to obtain the highest biological activities of the extracts, which are generally associated with the polyphenol yield.[9] Response surface methodology (RSM) is an important tool in the experimental design of analytical chemistry procedures offering a broad range of information ranging from the significance of independent variables to the interaction between them.[10]

The objective of this study was the optimization of the best conditions, namely, solute/solvent (water) ratio (10–50 mg/mL), extraction temperature (24–50°C), and extraction time (60–480 min), in order to obtain aqueous extracts with higher antioxidant and anti-α-glucosidase activities obtained from the aerial part of *E. resinifera* and *E. officinarum*.

MATERIALS AND METHODS

Plant samples

E. resinifera and *E. officinarum* aerial parts were collected directly from the fields of Beni Mellal, Morocco, and Ait Jerrar, Tiznit, Morocco, respectively [Figure 1]. Dried plant material was deposited as authenticated vouchers in the Herbarium of the Universidade do Algarve (acronym ALGU), with the accession numbers 15745/ALGU and 15746/ALGU, respectively.

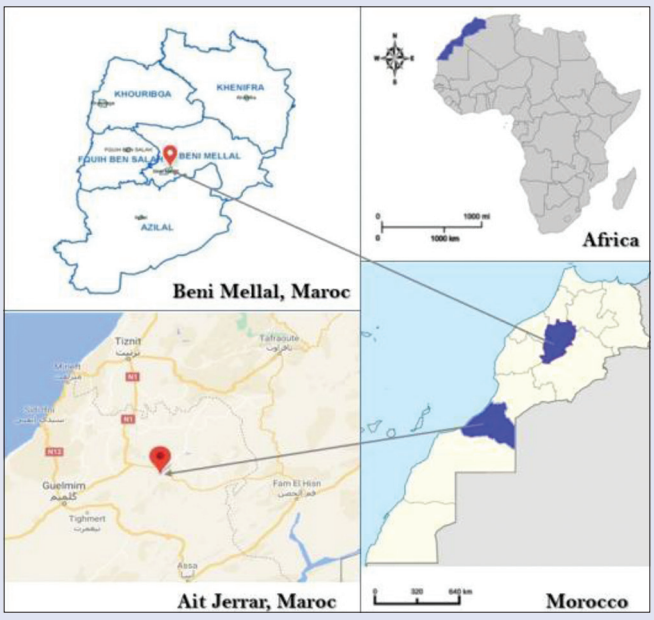

Figure 1: Moroccan map showing the harvesting locations of *E. resinifera* and *E. officinarum*

Sample preparation

E. resinifera and *E. officinarum* were dried at room temperature in the dark and grinded. Then, one gram of the aerial parts was extracted by maceration with 20 mL, 50 mL, and 100 mL of distilled water (w/v) in three different temperatures (24°C, 37°C, and 50°C) during 1 hr, 2 hr, and 8 hr to yield 27 extracts for each plant. Each extract was centrifuged at 5,000 rpm for 15 min. The supernatant was recovered and kept at −20°C until further analysis.

Total phenol content (TPC)

The total phenolic content of the extracts was evaluated using Folin-Ciocalteu reagent according to the method previously described by Singleton and Rossi.[11] The extracts (50 µL) mixed with 125 µL of Folin-Ciocalteu's phenol reagent (0.2 N) and 100 µL of 7.5% Na_2CO_3 were left incubated for 1 h at room temperature. After this period, the absorbance was measured at 765 nm and the total phenol content was expressed as mg gallic acid equivalents (GAE) per g of dried plant after a calibration curve was obtained with diverse concentrations of gallic acid (0.001 – 1 mg/mL). Tests were carried out in triplicate.

Total flavonoid content (TFC)

The amounts of flavones and flavonols in extracts were determined according to the method described by Miguel *et al.*[12] A solution made by 100 µL of $AlCl_3$ 20% and 100 µL of each extract was left to stand for 1 h at room temperature; after that, the absorbance was read at 420 nm. The total flavonoid content was expressed as mg quercetin equivalents (QE) per g of dried plant after a calibration curve was obtained with diverse concentrations of quercetin (0.002–1 mg/mL). Tests were carried out in triplicate.

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals' scavenging ability

DPPH free-radical scavenging activity was assessed as described by Miguel *et al.*[13] The extracts (25 µL) were mixed with 250 µL of a solution of DPPH 63.4 µM and left to stand 30 min at room temperature. After this incubation time, the absorbance was read at a wavelength of 517 nm. Diverse extract concentrations were submitted to this procedure, and a graph of inhibition percentages versus extract concentration was made and the IC_{50} values were determined; these values are defined as the sample concentration providing 50% inhibition. For the evaluation of inhibition percentage, the following formula was used: Inhibition = $[(A_0\text{-}A_1)/A_0 \times 100]$, where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. The same procedure was performed for the positive control, which was butylated hydroxytoluene (BHT) in a concentration range of 0.03–1 mg/mL.

α-Glucosidase inhibition activity

α-Glucosidase inhibition assay was carried out according to El-Guendouz *et al.*[14] The plant extracts (70 µL) mixed with 50 µL of yeast α-glucosidase (2.4 U/mL) prepared in phosphate buffer (100 mM; pH = 6.8) were incubated for 10 min. After this period, 100 µL of a solution of *p*-nitrophenyl-β-D-glucopyranoside (PNPG) 5 mM in the same phosphate buffer was added. The reaction solution was incubated at room temperature for 30 min, and after this period, 80 µL of sodium carbonate solution (0.4 mM) was added to stop the reaction. The absorbance reading was performed at 405 nm. The activities were presented as IC_{50} values and determined as reported for the antioxidant activity. Acarbose was used as a positive control in the range of 0.002–1 mg/mL and submitted to the same experimental conditions of the plant extracts.

Chelating metal activity

The degree of chelating ferrous ions of aqueous extracts was assayed as reported by El-Guendouz *et al.*[14] Two hundred microliters of different

concentrations of the extracts, 25 µL of $FeCl_2.4H_2O_2$ mM, 50 µL of distilled water, and 25 µL of ferrozine (5 mM) were mixed. Immediately, the absorbances were read at 562 nm. The results were calculated as aforementioned. Ethylenediaminetetraacetic acid (EDTA) was used as a positive control in the following range: 0.001–1 mg/mL.

Experimental design

Three levels three factor full factorial design was used to evaluate the effect of the combinations of three independent variables, namely, temperature, time, and plant-to-solvent ratio (PSR), on the extraction of phenolic compounds (TFC and TPC), antioxidant activity, and α-glucosidase inhibition activity from *E. resinifera* and *E. officinarum* aqueous extracts. The experimental design consists of 81 runs, including 27 experiments [Table 1] with three replicates for each.

A second-order polynomial equation (Equation 1) was used to fit the experimental data. The general form of the mathematical quadratic response equation is given as below:

$$Y = \beta 0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j + \varepsilon \qquad \text{Equation 1}$$

where Y indicates the predicted response; $\beta 0$, βi, βii, and βij, are the regression coefficients for intercept, linear, quadratic, and interactive effects, respectively; Xi, $Xi2$, and Xij are the coded independent variables; and k is equal to the number of the tested factors (k = 3 in this study).

Statistical analysis

All experiments were performed in three replicates. The results of analyses were reported as mean ± standard deviation (SD). The factorial design was designed and analyzed using the free version StatSoft, Inc. (2011), STATISTICA (data analysis software system), version 10. A total of 27 combinations were used [Table 1]. The results were analyzed using ANOVA. Significant differences were determined by Tukey's test, with $P < 0.05$ as the significance criterion.

RESULTS

Statistical analysis and model fitting

The identification of the main factors that affect the experimental response must be initiated by the determination of the optimal conditions in the extraction of phenol compounds. In this study, the three-level full factorial design was conducted to identify the main and interaction effects of process variables, namely, extraction temperature, extraction time, plant percent, and solvent-to-plant material ratio, PSR (expressed as the concentration of the plant material in the extraction solvent in mg/mL), on the extraction of phenolic compounds [total phenol content (TPC) and total flavonoid content (TFC)], the anti-oxidant activity, and the α-glucosidase inhibition activity of the yielded aqueous extracts from *E. resinifera* and *E. officinarum*. Water was chosen as solvent extraction because the ethnobotanical studies reported the use of aqueous extracts and because it is generally safer for the potential applications in food or pharmaceutical fields. Analysis of variance (ANOVA) was performed with a confidence level of 95% (P = 0.05) to evaluate the robustness of the empirical model and verify the adequacy of the model generated by the factorial experiment. The probability value (p values) was used as a tool to verify the significance of each coefficient.

The test of reliability for predicting the equation had been carried out by Fisher's variance ratio test, known as the F-test. The fit of the model has also been expressed by the determination coefficient (R^2) and the adjusted coefficients of determination (Adj-R^2), both indicating how well the polynomial equation predicts the data. The linear and quadratic effect of the independent variables as well as their interaction on the response variables were analyzed, and the results are summarized in Table 2.

The determination coefficient value of the quadratic regression model coefficient (R^2), respectively, for TPC and TFC, was 0.97 and 0.94, indicating that only about 3% and 6% of the total variations were not explained by the model for the two responses, and can be explained by the residues. Meanwhile, the model explained 98% of the total variation in TPC and TFC values for *E. officinarum*. Furthermore, the adjusted (R^2) value was obtained from R^2 after the elimination of the unnecessary model terms. The adjusted (R^2 adj.) presented by the two species were 0.94 and 0.98 for TPC and 0.93 and 0.97 for TFC for *E. resinifera* and *E. officinarum*, respectively. Those values were very high and very close to the value of R^2 supporting the high correlation between the observed and predicted values for TPC and TFC. Concerning the DPPH antioxidant activity, the values of R^2 were, respectively, 0.79 and 0.93 for *E. resinifera* and *E. officinarum*. Closer values of 0.73 and 0.91 were presented, respectively, by adjusted R^2.

Regarding the α-glucosidase inhibitory activity, the values of R^2 were 0.92 and 0.98, respectively, for *E. resinifera* and *E. officinarum*, which imply that more than 92% and 98% of experimental data can be, respectively, explained by the model for the two responses. Additionally, the high correlation between the observed and the predicted values was also observed because R^2 and adjusted R^2 (R^2 adj.) were in rational agreement. Concurrently, the chelating power also presented high R^2 values of 0.89 and 0.91 for *E. resinifera* and *E. officinarum*, respectively, which were in their turn strongly correlated to the adjusted R^2.

Figure 2 (a, b) display the normal probability distribution of residuals of ANOVA for all response variables for *E. resinifera* [Figure 2a] and *E. officinarum* [Figure 2b]. The plots prove the adequacy of the model as the former is approximately a line in which the values of residuals fit on a straight line to a major extent.

Effect of the process variables on the total phenolic content (TPC)

Data regarding the effect of extraction parameters on the amount of total phenolic contents are presented in Table 1. Among the 27 extracts obtained from *E. resinifera*, the amount of TPC extracted ranged from 1.25 to 7.02 mg/g of dry plant material, measured as gallic acid equivalent (GAE). The mean value of TPC extraction was 4.64 mg GAE/g dw depending on the extraction conditions. Run 19 [T 50°C; time 60 min and PSR (10 mg/mL)] exhibited the highest TPC, whereas run 16 [T 37°C; time 480 min and PSR (10 mg/mL)] showed the lowest TPC. Likewise, the amount of TPC in *E. officinarum* ranged from 2.80 to 8.65 mg GAE/g dw, found in run 12 [T 37°C; time 60 min and PSR (50 mg/mL)] and run 4 [T 24°C; time 120 min and PSR (10 mg/mL)], respectively.

The results depicted in Table 2 from ANOVA analysis indicate that time (L) and PSR (L and Q) were the most significant factors in determining the optimum TPC recovery for *E. resinifera*, with a P value of 0.000000, followed by the interaction temperature–PSR, temperature linear, and the interaction temperature–time with P values of 0.0004, 0.02, and 0.03, respectively, Whereas temperature (Q) and time (Q) were without significant effects ($p < 0.05$). Meanwhile, the factors having the greatest impact in the case of *E. officinarum* were temperature (Q), PSR (L and Q), and the interaction temperature–PSR ($p = 0.000000$), unlike time (L) and its interaction with PSR, which were without significant effects.

Multiple regression analysis was used to determine the correlation of the three process variables and TPC levels in the extracts. The second-order polynomial equation yielded after elimination of the non-significant coefficients is given by the following equations:

TPC(mg GAE/g dw) *E. resinifera*=7.2+5.2*10^{-2}(ET°) + 7.2* 10^{-2}(ET)–1.6*10^{-4}(ET)^2–4.8*10^{-1}(PSR)–4.4 *10^{-3}(ET°)(ET) + 7.2*10^{-6}(ET°)(ET)^2+5.5*10^{-5}(ET°)^2(ET)–9.03*10^{-8}(ET°)^2(ET)^2 (Equation 2)

Table 1: Experimental design

	Run			TPC		TFC		DPPH		Inhibition of α-Glucosidase		Chelating Power	
	Temperature (°C)	Time (min)	PSR (mg/mL)	*E. resinifera*	*E. officinarum*	*E. resinifera*	*E. officinarum*	*E. resinifera*	*E. officinarum*	*E. resinifera*	*E. officinarum*	*E. resinifera*	*E. officinarum*
1	24	60	10	6.90±0.38	8.05±0.57	1.28±0.02	0.54±0.01	0.69±0.04	0.57±0.02	71.83±1.34	83.23±0.41	6.47±0.10	0.73±0.00
2	24	60	20	5.42±0.39	4.85±0.24	1.59±0.06	0.28±0.01	1.22±0.04	1.02±0.01	66.54±0.82	39.46±1.00	11.15±0.45	5.86±0.00
3	24	60	50	3.86±0.12	3.04±0.11	1.19 ± ± 0.01	0.19±0.01	0.89±0.07	1.88±0.02	30.93±0.46	17.52±1.02	15.49±0.21	26.82±0.40
4	24	120	10	6.74±0.45	8.65±0.32	1.46±0.09	0.62±0.01	0.59±0.01	0.49±0.07	66.02±1.13	92.09±0.99	6.27±0.13	1.02±0.01
5	24	120	20	5.73±0.22	4.88±0.25	1.38±0.03	0.35±0.02	0.66±0.03	0.92±0.05	50.66±0.37	45.90±0.86	9.01±0.27	0.93±0.01
6	24	120	50	4.05±0.09	3.11±0.11	1.35±0.03	0.18±0.00	0.86±0.03	1.42±0.03	29.69±0.30	22.25±0.35	0.44±0.01	9.67±0.43
7	24	480	10	1.80±0.01	8.07±0.33	0.39±0.03	0.44±0.02	0.98±0.07	0.58±0.01	75.50±0.75	75.01±1.85	ND	0.89±0.01
8	24	480	20	4.31±0.28	5.09±0.21	0.78±0.00	0.24±0.02	0.92±0.06	1.21±0.00	42.33±0.83	45.45±0.48	ND	3.73±0.01
9	24	480	50	3.58±0.09	3.21±0.07	0.97±0.03	0.16±0.00	0.99±0.03	1.59±0.07	25.60±0.09	18.58±0.36	1.28±0.02	11.28±0.04
10	37	60	10	6.92±0.29	6.05±0.27	1.64±0.10	0.49±0.03	0.74±0.04	0.62±0.02	68.80±1.20	94.99±0.23	ND	0.94±0.00
11	37	60	20	6.12±0.10	4.55±0.22	1.83±0.04	0.29±0.00	0.87±0.03	1.05±0.07	56.51±0.53	39.84±0.35	ND	4.56±0.02
12	37	60	50	4.09±0.15	2.80±0.06	1.74±0.04	0.21±0.01	0.98±0.08	1.56±0.02	27.93±0.19	19.45±0.16	23.78±1.66	11.73±0.26
13	37	120	10	6.26±0.46	7.04±0.41	1.47±0.07	0.60±0.01	0.63±0.02	0.62±0.01	65.25±1.36	77.42±0.83	ND	1.30±0.01
14	37	120	20	5.76±0.35	4.68±0.21	1.76±0.03	0.22±0.02	0.69±0.04	1.02±0.02	48.38±0.34	45.33±0.93	ND	0.67±0.01
15	37	120	50	3.87±0.38	2.95±0.11	1.47±0.04	0.20±0.01	1.03±0.07	1.48±0.02	29.83±0.41	21.27±0.04	15.95±2.45	8.54±0.01
16	37	480	10	1.25±0.01	7.19±0.03	0.39±0.03	0.38±0.03	1.31±0.04	0.79±0.03	37.06±0.76	69.43±0.75	ND	0.85±0.01
17	37	480	20	3.25±0.29	4.37±0.32	0.81±0.05	0.16±0.01	1.06±0.03	1.59±0.11	45.98±0.53	35.95±0.74	ND	3.64±0.00
18	37	480	50	4.09±0.08	2.89±0.15	1.42±0.05	0.11±0.00	0.74±0.04	1.54±0.05	32.58±0.23	13.80±0.15	3.89±0.03	6.28±0.16
19	50	60	10	7.02±0.31	7.52±0.10	1.72±0.05	0.43±0.01	0.54±0.03	0.69±0.02	97.50±1.75	61.26±1.57	ND	1.54±0.00
20	50	60	20	5.76±0.23	5.31±0.04	1.63±0.04	0.32±0.02	0.64±0.01	1.11±0.01	50.67±0.96	40.73±0.35	ND	1.37±0.08
21	50	60	50	3.94±0.08	3.12±0.04	1.28±0.05	0.19±0.00	0.82±0.03	1.79±0.05	31.94±0.21	29.06±0.27	6.00±0.58	2.15±0.12
22	50	120	10	6.69±0.45	7.10±0.15	1.67±0.03	0.45±0.04	0.53±0.03	0.71±0.01	82.02±1.11	73.22±0.70	ND	2.36±0.06
23	50	120	20	5.62±0.16	5.22±0.08	1.06±0.05	0.33±0.01	0.61±0.00	0.93±0.04	47.24±1.97	44.18±0.65	8.77±0.03	1.20±0.00
24	50	120	50	3.63±0.06	3.06±0.04	1.30±0.02	0.19±0.00	0.95±0.07	1.57±0.03	28.88±0.21	22.23±0.25	6.92±0.15	7.96±0.05
25	50	480	10	2.23±0.06	6.65±0.25	0.61±0.01	0.25±0.00	0.88±0.02	1.08±0.03	58.92±1.25	57.17±1.92	ND	2.49±0.01
26	50	480	20	3.71±0.50	5.00±0.24	0.80±0.02	0.30±0.01	1.17±0.03	1.26±0.02	48.66±0.59	43.08±0.97	9.28±0.09	1.69±0.01
27	50	480	50	2.66±0.16	2.95±0.08	0.59±0.01	0.11±0.01	1.52±0.10	2.93±0.11	23.04±0.29	15.35±0.38	10.04±0.16	1.27±0.05
All Runs				4.64±1.65	5.09±1.86	1.24±0.43	0.30±0.14	0.87±0.25	1.18±0.53	49.86±19.9	83.23±0.41	6.47±0.10	4.49±5.55

ND: not determined

Table 2: Analysis of variance for the response surface quadratic model

	E. resinifera		*E. officinarum*	
	F	***P***	***F***	***P***
TPC				
(1) Temperature (L)	5.76	0.019328	14.92	0.00027
Temperature (Q)	0.00	0.96522	36.93	0.00000
(2) Time (min)(L)	516.17	0.000000	0.01	0.92193
Time (min)(Q)	2.73	0.103503	2.97	0.08955
(3) PSR (mg/mL) (L)	63.87	0.000000	2985.93	0.00000
PSR (mg/mL) (Q)	39.86	0.000000	386.37	0.00000
1*2	2.80	0.033211	5.40	0.00085
1*3	5.96	0.000391	20.14	0.00000
2*3	5.76	0.019328	1.28	0.28600
	R-sqr: 0.97; R-Adj: 0.95		R-sqr=0.98; R^2-Adj: 0.97	
TFC				
(1) Temperature (L)	0.80	0.374588	32.18	0.000000
Temperature (Q)	70.68	0.000000	10.72	0.001740
(2) Time (min)(L)	513.65	0.000000	140.57	0.000000
Time (min)(Q)	0.03	0.863763	19.41	0.000043
(3) Plant %(mg/mL) (L)	21.33	0.000020	1686.63	0.000000
PSR (mg/mL) (Q)	10.47	0.001950	298.86	0.000000
1*2	3.77	0.008333	3.28	0.016738
1*3	19.39	0.000000	50.10	0.000000
2*3	0.80	0.374588	23.57	0.000000
	R-sqr: 0.94; R-Adj: 0.93		R-sqr=0.98; Adj: 0.97	
DPPH				
(1) Temperature (L)	3.44	0.068435	56.1248	0.000000
Temperature (Q)	0.00	0.989553	6.0009	0.017137
(2) Time (min)(L)	40.10	0.000000	33.4127	0.000000
Time (min)(Q)	12.54	0.000763	18.7680	0.000055
(3) PSR (mg/mL) (L)	26.58	0.000003	552.8890	0.000000
PSR (mg/mL) (Q)	0.80	0.374274	19.3065	0.000044
1*2	9.44	0.000005	9.6806	0.000004
1*3	7.89	0.000033	10.6834	0.000001
2*3	6.79	0.000133	2.5006	0.051426
	R-sqr: 0.79; R-Adj: 0.73		R-sqr=0.93; R-Adj: 0.91	
Chelating Power (IC_{50})				
(1) Temperature (L)	1.4829	0.227932	72.0713	0.000000
Temperature (Q)	44.8097	0.000000	0.3672	0.546761
(2) Time (min)(L)	0.5262	0.470956	37.0449	0.000000
Time (min)(Q)	9.6883	0.002804	23.4855	0.000009
(3) PSR (mg/mL) (L)	236.3545	0.000000	176.9106	0.000000
PSR (mg/mL) (Q)	1.0483	0.309872	13.8364	0.000000
1*2	17.8756	0.000000	29.8266	0.000000
1*3	16.8026	0.000000	11.8049	0.000000
2*3	5.6204	0.000632		
	R-sqr=0.89; R-Adj:.85911		R-sqr=0.91; R-Adj: 0.88	
α - Glucosidase				
(1) Temperature (L)	0.04	0.848560	19.41	0.000043
Temperature (Q)	6.98	0.010444	2.23	0.140751
(2) Time (min)(L)	42.19	0.000000	26.66	0.000003
Time (min)(Q)	6.80	0.011390	8.10	0.006006
(3) PSR (mg/mL)(L)	455.04	0.000000	2886.67	0.000000
PSR (mg/mL)(Q)	24.68	0.000006	388.45	0.000000
1*2	2.12	0.088456	6.68	0.000155
1*3	13.08	0.000000	31.57	0.000000
2*3			9.69	0.000004
	R-sqr: 0.92; R-Adj: 0.90		R-sqr=0.98; Adj:.98	

TPC(mg GAE/g dw) *E. officinarum*=29.420–1.086(ET°) + 1.3410^{-2}(ET°)^2–1.077(PSR)+ 1.3510^{-2}(PSR)^2+3.8110^{-3}(ET°)(ET)–5.6610^{-5}(ET°)^2(ET) + 9.1710^{-8}(ET°)^2(ET)^2+3.6010^{-2}(ET°)(PSR)–4.5210^{-4}(ET°)(PSR)^2 (Equation 3)

where ET° is the extraction temperature, ET is the extraction time (min), and PSR is the plant/solvent ratio (mg/mL).

All the presented factors in the equations are significant ($p < 0.05$). The negative sign in the equation represents an antagonistic effect of the variables, whereas the positive sign represents a synergistic effect.

According to Equation 2, the extraction temperature and time had a linear positive effect on TPC recovery, in contrast to the linear effect of the PSR, which was negative, indicating that more plant material in the extraction medium results in less TPC extraction efficiency. In terms of interactions, we noticed that the process variable interactions had a negative effect on TPC extraction, except the interaction (ET°) $(ET)^2$. With regard to *E. officinarum* (Equation 3), temperature (L) and PSR (L) had a significant negative effect on TPC recovery unlike their quadratic

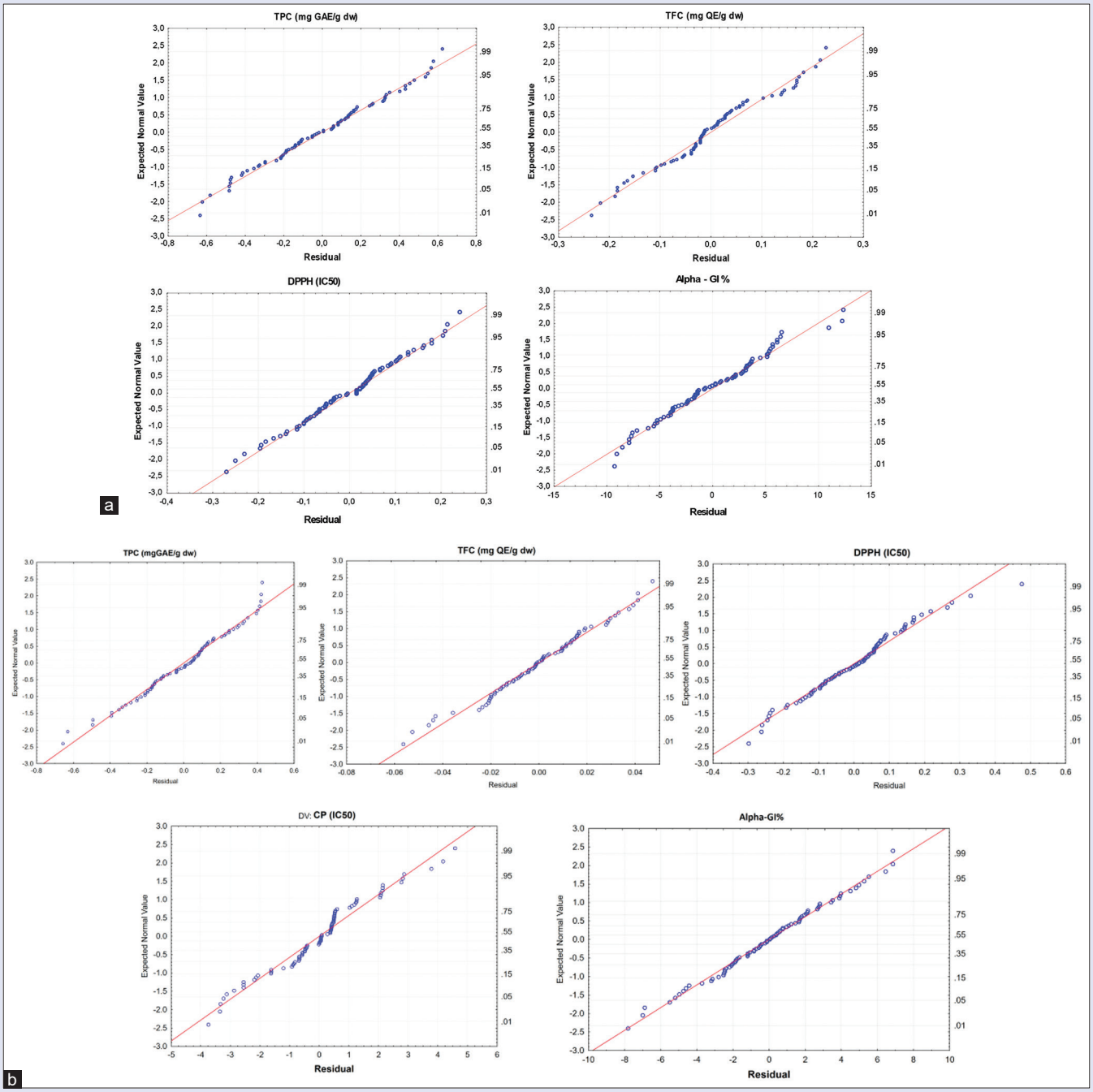

Figure 2: (a) Normal probability plot of the residuals (TPC, TFC, DPPH, alpha-GI%) for *E. resinifera* and (b) (TPC, TFC, DPPH, chelating power CP, alpha-GI%) for *E. officinarum*

terms, which had a positive effect along with the linear and quadratic interactions temperature–time and temperature–PSR (L), whereas the rest of the coefficients did not show any significant effect.

To investigate the linear, quadratic, and interactive effects of the process variables on the total phenolic compound's extraction from the two species, the three-dimensional surface plots [Figure 3: a, b, c, d, e, and f] were constructed according to Equations 2 and 3. The color changing from green to red indicates an increase in the response.

The plot exhibiting the function of time versus PSR effect on the extract TPCs at a fixed temperature is depicted in Figure 3(a). It was noticed that TPC levels decrease sharply with the increase of PSR as well as with the increase of extraction time. This effect is more pronounced when the two factors increase simultaneously. The best levels of TPC (>7 mg GAE/g dry plant) can be achieved using a lower PSR at the shortest extraction time, during a maximum period of 1 hour. At high PSR, the extraction time did not have a significant effect and vice versa. As for *E. officinarum*, the increase in PSR during extraction decreased highly TPC recovery, without the noteworthy effect of temperature, Figure 3d.

Regarding the time and temperature of the extraction effect on *E. resinifera*, as shown in Figure 3b, the TPC values decrease with increasing extraction time (from >7 mg/g dry plant at T = 0 min to <1.25 g of a dry plant at T = 500 min) without any remarkable effect of temperature.

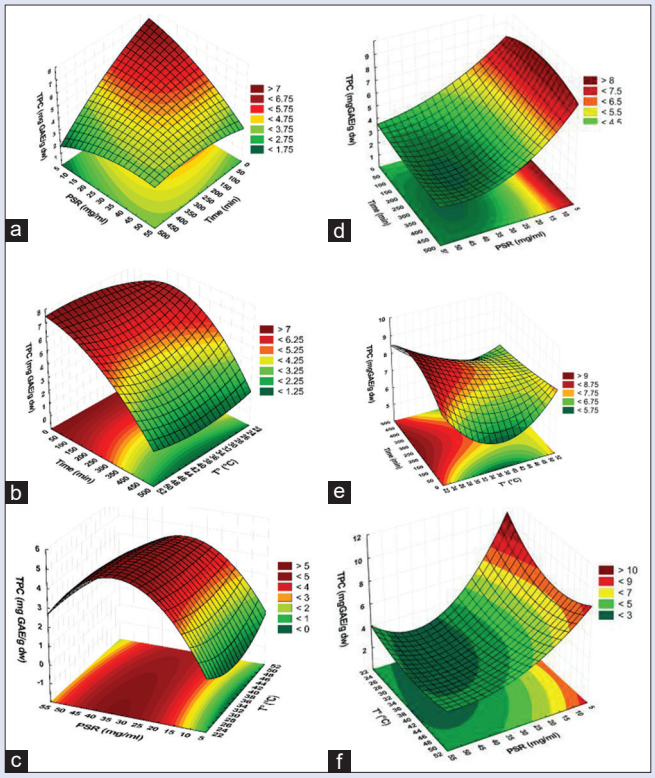

Figure 3: Three-dimensional response surface plots showing the combined effects of time (min), temperature T° (°C), and plant-to-solvent ratio (PSR) on total phenol content (TPC) in the *E. resinifera* (a-c) and *E. officinarum* (d-f) extracts

The plot presenting the interaction effect between extraction T° and PSR for *E. resinifera* [Figure 3c] revealed that the maximum of phenol extracted (>5 mg GAE/g dw) was found using PSR between 30 to 35 mg/mL and a temperature between 22 and 28° C. Regardless of the extraction temperature, TPC recovery first increases with the rise of PSR and starts to decrease at PSR higher than 35 mg/100 mL. However, the TPC recovery for *E. officinarum* was found to increase along with the decrease in PSR values [Figure 3e].

Effect of the process variables on the total flavonoids content (TFC)

The TFCs of the aqueous extracts from the two species are listed in Table 1. The maximum TFC yield (1.83 ± 0.04 mg QE/g dw) was obtained in run n° 11, with the following extraction conditions: temperature 37°C, plant-to-solvent ratio 20 mg/mL, 60 min [Table 1]. According to Table 2, the most significant factors presenting the lowest *P* values, influencing TFC extraction for *E. resinifera*, were temperature (Q), time (L), and the interaction of PSR with both temperature and time (P-value < 0.000001), followed by the linear and quadratic effects of PSR and the interaction time–temperature, whereas the linear effect of temperature and the quadratic effect of time in addition to the interaction time–PSR were not significant (P > 0.05). With respect to *E. officinarum*, we noticed that all factors and their interactions were significant, emphasizing that time (Q), temperature (Q), and PSR (L + Q), besides the interactions of PSR with temperature and time, were highly significant ($p < 0.000001$) [Table 2].

Applying RSM, the regression equation (Equation 4 and Equation 5) for TFC, after elimination of the non-significant coefficients, is presented as

TFC(mg QE/g dw) *E. resinifera* $= -1.2 + 5.110^{-2}(ET°) + 4.710^{-2}(ET) - 8.610^{-5}(ET)^2 - 1.810^{-3}(ET°)(ET) + 3.010^{-6}(ET°)(ET)^2 + 2.110^{-5}(ET°)^2(ET) - 3.510^{-8}(ET°)^2(ET)^2 + 1.210^{-2}(ET°)(PSR) - 1.410^{-4}(ET°)(PSR)^2 - 2.010^{-4}(ET°)^2(PSR) + 2.310^{-6}(ET°)^2(PSR)^2 - 1.410^{-3}(ET)(PSR) + 2.2410^{-5}(ET)(PSR)^2 + 2.6410^{-6}(ET)^2(PSR) - 4.1710^{-8}(ET)^2(PSR)^2$ (Equation 4)

TFC(mg QE/g dw) *E. officinarum* $= -0.975 + 0.104(ET°) - 1.5910^{-3}(ET°)^2 + 0.009(ET) - 1.6410^{-5}(ET)^2 + 0.105(PSR) - 1.8010^{-3}(PSR)^2 - 8.4910^{-3}(ET°)(PSR) + 1.3710^{-4}(ET°)(PSR)^2 + 1.3210^{-4}(ET°)^2(PSR) - 2.1010^{-6}(ET°)^2(PSR)^2 - 3.1510^{-4}(ET)(PSR) + 4.4510^{-6}(ET)(PSR)^2 + 6.3610^{-7}(ET)^2(PSR) - 9.0010^{-9}(ET)^2(PSR)^2$ (Equation. 5)

where ET° is the extraction temperature, ET is the extraction time (min), and PSR is the plant/solvent ratio (mg/mL).

According to Equation 4, the linear effect of temperature (ET°) and time (ET) of extraction in addition to the interactions $(ET°)^2$, $(ET°)(ET)^2$, $(ET°)^2(ET)$, $(ET°)(PSR)$, $(ET°)^2(PSR)^2$, and $(ET)(PSR)^2$ had a significant positive effect on the yield of flavonoids from *E. resinifera*, indicating that their increase increases the TFC recovery. However, the rest of the interaction had an antagonistic effect. The linear effect of time was positive, whereas its quadratic effect was negative, indicating that the increase of extraction time leads to TFC increase to reach an optimum in the first hour of extraction, after which the recovery of TFC starts to decline.

Concerning *E. officinarum* (Equation 5), we noticed that the linear effect of the three extraction factors exhibited the highest positive influence on TFC extraction, whereas their quadratic effects had a negative effect. Correspondingly, time was reported to have a negative quadratic effect because the TFC yield increased for the first 76 min and then decreased.

The three-dimensional surface plots were designed to ascertain the effect of temperature and the percentage of the plant (PSR) on TFC recovery [Figure 4a and b]. The results indicate that the content of flavonoids found in the aqueous extracts of *E. resinifera* increased slightly with the temperature rise from 0.9 mg QE/g in T = 24°C to >1.5 mg QE/g in T = 37°C; after that, the rate starts to decrease slightly until <1.1 5 mg QE/g for T = 50°C. On the other hand, we can see that PSR equal to 30 mg/mL of a plant used in the extraction records the greatest content on flavonoids (>1.5 mg QE/g), and thereafter, the rate begins to decrease with the increase in the PSR (<1 mg QE/g), Figure 3a.

The combined effect of time and temperature of extraction revealed a significant negative effect of extending extraction time over 60 min, which results in a decrease of TFC level with the continuous increase of extraction time, regardless of the temperature used for extraction, and this was more pronounced at the low PSR. As shown by the same figures, the highest TFC levels are obtained at 38°C using a PSR ranging from 25 to 35 mg/mL, whereas their contents decreased at higher and lower temperatures. Likewise, increasing PSR from 10 to 30 mg/mL improved TFC recovery, but over 35 mg/mL, the recovery starts to diminish.

As for *E. officinarum*, the yield of TFC increased with the extent of extraction time to reach its maximum after 3 hr; thereafter, it starts to decrease [Figure 4c]. However, their yield decreased with the increase of extraction temperature and PSR [Figure 4d].

DPPH scavenging activity

The total phenolic content along with the antioxidant capacity is a useful tool in determining the potential of an extract for its application in functional foods, cosmetics, nutraceuticals, or any other field. The free-radical scavenging capacity from all *E. resinifera* and *E. officinarum* extracts increased in a concentration-dependent manner (data not shown), and the IC_{50} values are depicted in Table 1. The results show that *E. resinifera* extracts exhibited good antioxidant activity (IC_{50} = <0.3 mg/mL).

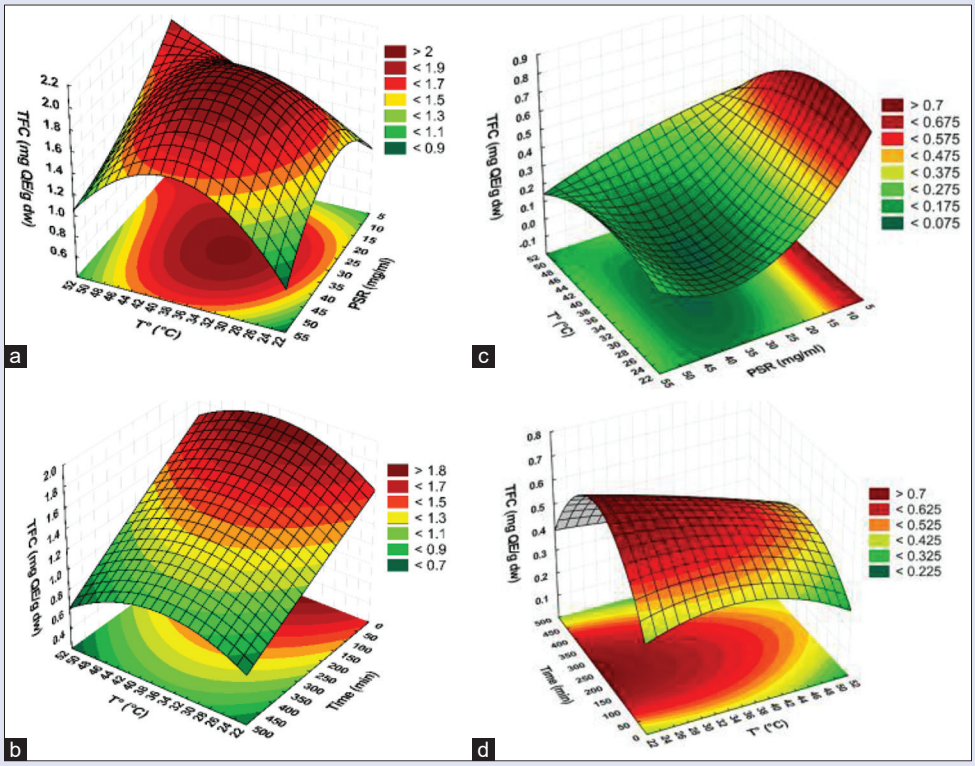

Figure 4: Three-dimensional response surface plots showing the combined effects of time (min), temperature T° (°C), and plant-to-solvent ratio (PSR) on total flavonoid concentration (TFC) in the (a and b) *E. resinifera* and (c and d) *E. officinarum* extracts

According to ANOVA [Table 2], the linear effect of temperature, besides the quadratic effect of both temperature and PSR, did not show any significant effect ($p < 0.05$), although the rest of the parameters and their interactions had a significant effect, noting that time (L) was the most significant in terms of antioxidant recovery from *E. resinifera* (P < 0.000001). In the case of *E. officinarum*, all the coefficients were significant, drawing attention that the linear effects and the interaction time–PSR were the most significant ($p < 0.000001$), followed by the interaction temperature–time and the quadratic effects of the three extraction factors.

The second-order equations for DPPH scavenging activity after elimination of the non-significant coefficients are shown in Equations 6 and 7.

DPPH scavenging activity (IC_{50}) *E. resinifera* = - 2.3 10-2 (ET°) – 2.7 10^{-2} (ET) + 2.68 10-5 $(ET)^2$ + 1.7 10^{-1} (PSR) + 1.6 10^{-3} (ET°) (ET) – 3.0 10^{-6} (ET°) $(ET)^2$ – 1.9 10^{-5} $(ET°)^2(ET)$ + 3.5 10^{-8} $(ET°)^2(ET)^2$-9.3 10^{-4} (ET)(PSR) + 1.7 10^{-5} (ET) $(PSR)^2$ + 1.5 10^{-6} $(ET)^2(PSR)$ – 2.8 10-8 $(ET)^2(PSR)^2$ (Equation 6)

DPPH scavenging activity (IC_{50}) *E. officinarum* = 3.18 10^{-3} (ET°) – 3.09 10^{-4} (ET°) $(PSR)^2$ – 2.31 10^{-4} $(ET°)^2(PSR)$ + 4.65 10^{-6} $(ET°)^2(PSR)^2$ (Equation 7)

where ET° is the extraction temperature, ET is the extraction time (min), and PSR is the plant/solvent ratio (mg/mL).

Related to Equation 6, expressing the relationship between antioxidant activity, expressed as IC_{50} values, and the extraction conditions, we found that the IC_{50} values were negatively influenced by the linear and quadratic effects of extraction temperature, indicating that lower extraction temperatures resulted in decreased IC_{50} values and thus higher antioxidant activity. Concerning the extraction time, the linear effect was negative, whereas the quadratic effect was positive, which implies that the increase of extraction time till certain levels increases the antioxidant activity, but increasing it too much has an inverse effect, by lowering the recovery of molecules endowed antioxidant activity in the yielded extracts. Furthermore, PSR has a positive effect and increases the IC_{50} of the extracts, indicating that a lower PSR is suitable for extraction of antioxidants from this plant. It is noteworthy to state that the most important effect on increasing antioxidant extraction was the linear effect of extraction time, whereas the linear effect of PSR mostly decreased their extraction. As for *E. officinarum*, the quadratic effect of temperature and its interaction with the quadratic effect of PSR affected positively the antioxidant activity of the yielded extracts, unlike the interactions T° (L) – PSR (Q) and T° (Q) – PSR (L), which had a negative impact, pointing out that the rest of factors and their interactions did not have any significant effect.

The surface plots [Figure 5a and b] indicate that both the extraction temperature and PSR along with extraction time influence the extraction of antioxidant compounds from *E. resinifera*. At lower levels of PSR (below 30 mg/mL) and extraction times (below 300 min), the ability to eliminate free radicals by *E. resinifera* aqueous extracts increases with the increase of the two factors; conversely, these factors start to have a negative impact on extracting antioxidants when they exceed these levels [Figure 5a], whereas extraction temperature had a negative effect regardless of the other factors because over time and with increasing temperature, the antioxidant activity decreases (IC_{50} = >1.8 mg/mL) [Figure 5b]. The best antioxidant activity of *E. resinifera* extract has been recorded for extraction times ranging from 200 to 350 min using a PSR ranging from 25 to 35 mg/mL at low temperatures. According to previously reported results, the amount of the sample added in the solvent exerted the important effects on the extraction of antioxidants and beyond the optimal value of the sample to a solvent ratio of 28 mg/mL; the total antioxidant activity of the extracts decreased progressively.

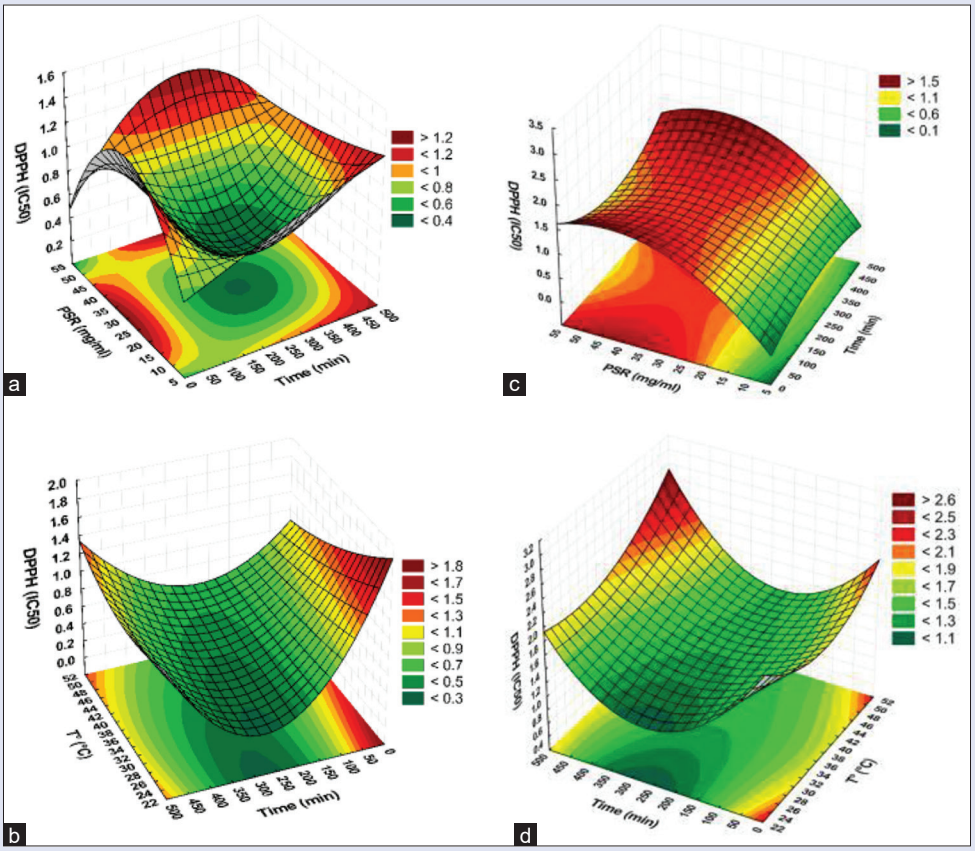

Figure 5: (a and b) Three-dimensional response surface plots showing the combined effects of time (min), temperature T° (°C), and plant-to-solvent ratio (PSR) on the ability for scavenging DPPH free radicals measured through IC_{50} values of the extracts of *E. resinifera* and (c and d) *E. officinarum*

In relation to *E. officinarum*, the IC_{50} values increase with the increase of PSR [Figure 5c], indicating that high PSR results in low anti-oxidants yields. The extraction time exerted a positive effect during the first 3 hr of extraction; afterward, the antioxidant activity starts to diminish [Figure 5d].

α-Glucosidase inhibition activity

The α-glucosidase inhibitory activity results of water extracts are depicted in Table 1. Run 19 (T° 50°C; time 60 min and plant ratio 10%) showed the greatest α-glucosidase inhibitory activity. The most efficient extracts to inhibit this enzyme were obtained using a PSR concentration below 15 mg/mL regardless of the temperature and the time of extraction.

The second-order polynomial equation stating the relationship between extraction conditions and α-glucosidase inhibitor recovery is given by the Equations 8 and 9.

α-Glucosidase inhibition(%) *E. resinifera*=482.93–2.29(ET°) + $0.32(ET°)^2$-23.18(PSR)-$0.30(PSR)^2$+$1.29(ET°)(PSR)$–$1.7910^{-2}(ET°)(PSR)^2$-$1.8810^{-2}(ET°)^2(PSR)$ + $2.6210^{-4}(ET°)^2(PSR)^2$ (Equation 8)

α-Glucosidase inhibition(%) *E. officinarum*=$15.020(ET°)–0.223(ET°)^2$+$1.466(ET)$-$0.002(ET)^2$-$0.088(ET°)(ET)$+ $1.4610^{-4}(ET°)(ET)^2$+$1.1510^{-3}(ET°)^2(ET)$–$1.8910^{-6}(ET°)^2(ET)^2$–$0.623(ET°)(PSR)$+$9.0010^{-3}(ET°)(PSR)^2$+$1.0110^{-2}(ET°)^2(PSR)$–$1.4410^{-4}(ET°)^2(PSR)^2$ (Equation 9)

where ET° is the extraction temperature, ET is the extraction time (min), and PSR is the plant/solvent ratio (mg/mL).

All the presented coefficients had a significant effect ($p < 0.05$) on extracting molecules with α-glucosidase inhibitory activity. Those molecules in the yielded extracts were negatively influenced by extraction temperature and PSR, whereas their linear and quadratic interaction resulted in a positive effect, indicating a synergetic effect between the two factors. The highest negative effect was expressed by PSR. Thus, increasing PSR resulted in a great decrease in extraction efficiency of molecules inhibiting the α-glucosidase enzyme [Figures 6a-c]. However, the linear effect of temperature and time increased the yield of anti-diabetic molecules in the case of *E. officinarum* (Equation 9). The α-glucosidase inhibition capacity decreased strongly with the enhancement of the PSR in *E. officinarum* extracts despite the time and temperature of extraction [Figure 6c and d].

With regard to the plot [Figure 6a], depicting the combined effect of PSR and extraction temperature, the anti-diabetic activity of the *E. resinifera* aqueous extracts strongly decreased from >80 (%) to <20 (%) as the PSR used during extraction increased from 10 to 50 mg/mL. The temperature of extraction had a weak significant effect, whereas the extraction time did not have any significant effect ($p < 0.05$).

Chelating power

This assay was performed to evaluate the chelating capacity of the yielded extracts from the two *Euphorbia* species, and the second-order polynomial equation stating the relationship between extraction conditions and α-glucosidase inhibitor recovery is given by Equations 10 and 11.

Chelating power(IC_{50}) *E. resinifera* = $+2.4(ET°)(PSR)–4.510^{-2}(ET°)(PSR)^2–3.810^{-2}(ET°)^2(PSR)+6.910^{-2}(ET°)^2(PSR)$ $^2–0.15(ET)(PSR)$ + $2.310^{-3}(ET)(PSR)^2+2.510^{-4}(ET)^2(PSR)–4.110^{-6}(ET)^2(PSR)^2$ (Equation 10)

Chelating power (IC_{50}) *E. officinarum* =– 1.41 10^{-2} (ET)(PSR) +

$2.58\ 10^{-5}\ (ET)^2(PSR) - 3.47\ 10^{-7}\ (ET)^2(PSR)^2$ (Equation 11)

According to Equation 10, the linear and the quadratic interactions between extraction time and PSR as well as the interactions (ET) $(PSR)^2$ and $(ET)^2$ (PSR) had a significant positive consequence on the extraction of molecules endowed chelating power, whereas the rest of the interactions decreased their extraction from *E. resinifera*. With respect to *E. officinarum* (Equation 11), the linear and the quadratic interactions between extraction time and PSR decreased the chelating power of the yielded extract, whereas the interaction $(ET)^2(PSR)$ enhanced their chelating capacity.

Figure 7a shows the surface plots with the chelating power from *E. resinifera* as a function of time and PSR. The chelating power of the extracts increased with extraction time to reach its maximum after 3 hr; after that, it begins to decrease. The same behavior has been observed with the PSR, in which an increase is observed when the concentration goes from 5 to 10 mg/mL; after that, it begins to decrease gradually. With respect to *E. officinarum* [Figure 7b], the increase in temperature extraction corresponds to a reduced chelating power of the extracts, whereas the best PSR concentration was found to be between 15 and 30 mg/mL.

Desirability

Optimization using the desirability function was carried out for maximizing the total phenolic compounds, the antioxidant capacity (DPPH), and α-glucosidase inhibitory activity of the extracts. The results are presented in Figure 8 (a) and (b) for *E. resinifera* and *E. officinarum*, respectively. The result for the simultaneous optimization including all responses using the desirability function suggested that aqueous extraction of *E. resinifera* dried aerial part powder during 60 min at 30°C using a plant-to-solvent ratio of 2% extracts yielded extracts with the high TPC and TFC contents along with high antioxidant and α-glucosidase inhibitory activities. However, extraction during 270 min at 50°C using a PSR concentration of 10 mg/mL (1%) was the best condition to yield the extract with the optimum values for all the studied responses from *E. officinarum*.

PCA analysis

Plotting principal component analysis (PCA) scores in two or three dimensions is an easy way to notice the data distribution. The first three PCs explained 88.07% of the variance in the data for *E. resinifera* and 90.4 for *E. officinarum*, which was high enough to represent all variables. In the PC1-PC2 plot [Figure 9a], the first two PCs contain about 73.75% (PC1: 49.78%, PC2: 23.97%) information of the raw data, whereas the third component (PC3) explained 14.32% of the total variance [Figure 9b]. However, the PC1-PC2 plot [Figure 9c] of *E. officinarum* explained 77.6% of the total variance.

Related to Figure 9 (a) and (b) corresponding to *E. resinifera*, in PC1, TPC has a heavy positive loading, whereas DPPH has negative loading. However, time, PSR, TFC, and α-glucosidase have a load in both axes. Meanwhile, extraction temperature did not present any load for the

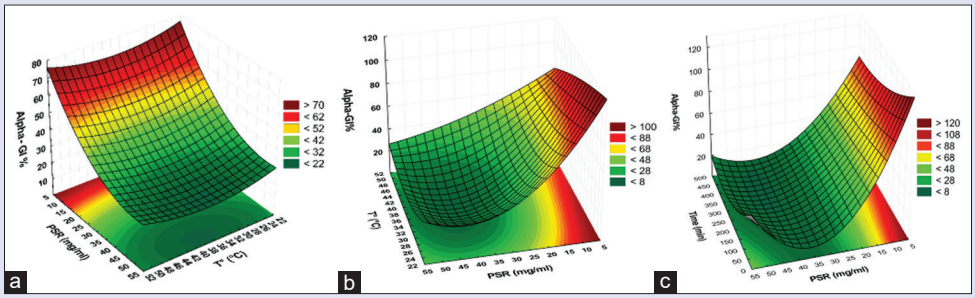

Figure 6: (a) Three-dimensional response surface plots showing the combined effects of time (min), temperature T° (°C), and plant-to-solvent ratio (PSR) on the α-glucosidase inhibition percentage (alpha-Gl%) by the extracts of *E. resinifera* and (b and c) *E. officinarum*

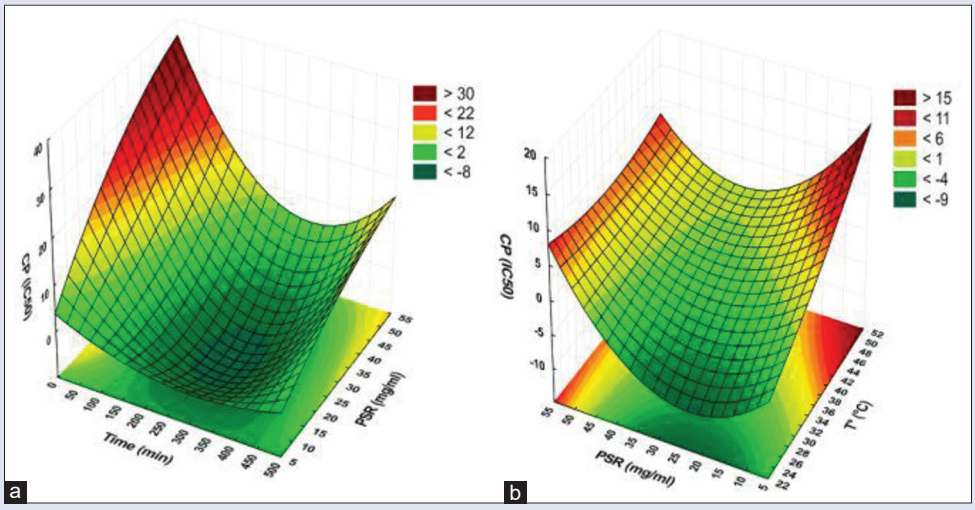

Figure 7: (a) Three-dimensional response surface plots showing the effect of time (min) and plant-to-solvent ratio (PSR) on the chelating power activity for *E. officinarum*; Effect of temperature T° (°C) and PSR on the chelating power activity for (b) *E. officinarum*

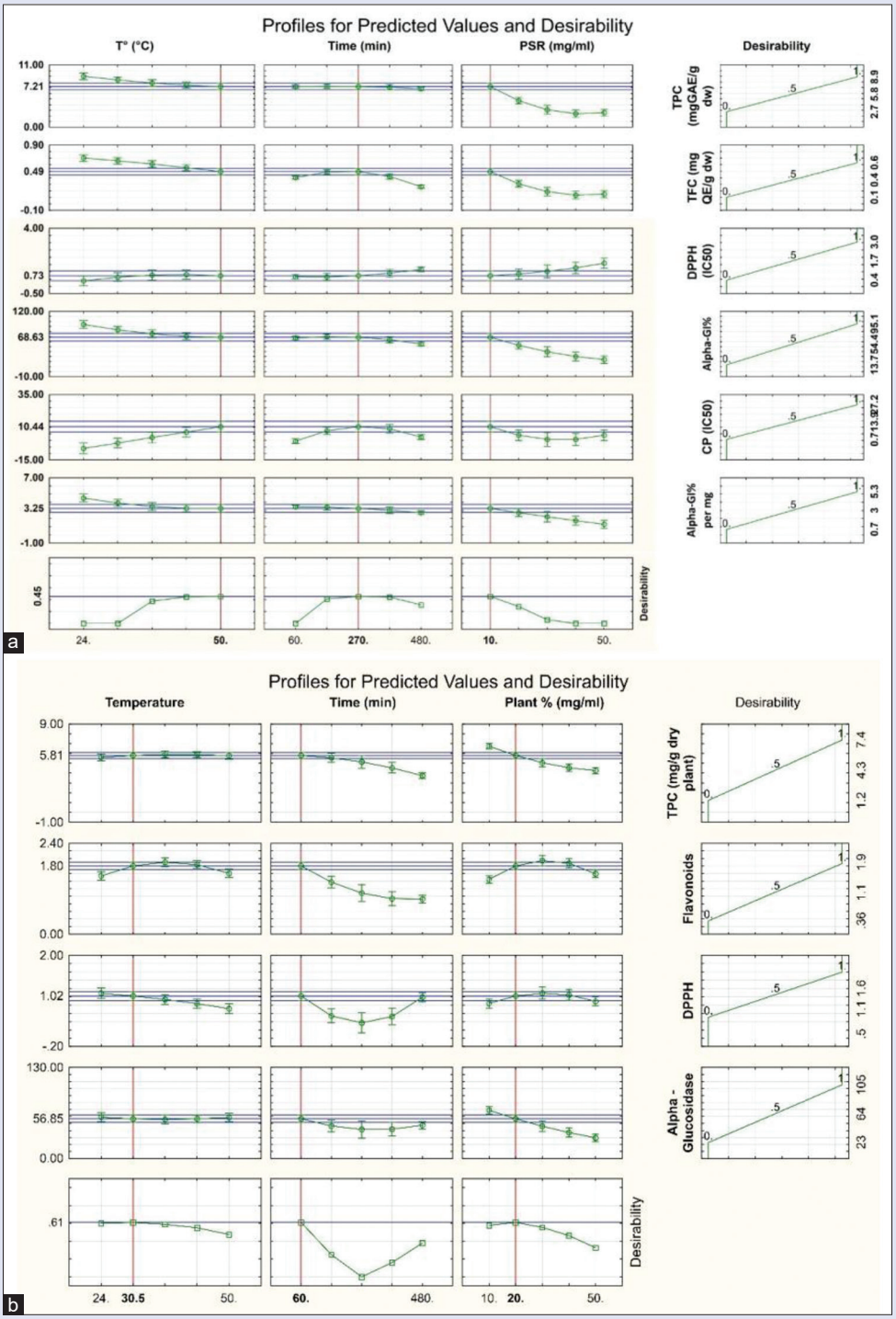

Figure 8: Best experimental parameters [temperature, time, and plant (%)] using the desirability function that maximize phenol and flavonoid contents and anti-oxidant and alpha-glucosidase inhibitory activities of (a) *Euphorbia resinifera* and (b) *Euphorbia officinarum*

two axes. This plot displays a strong correlation between DPPH and TPC, indicating their high contribution to the antioxidant activity of the extracts. TFC recovery was negatively affected by extraction time, whereas α-glucosidase inhibition activity was negatively affected by PSR.

According to Figure 9b, showing the PC1-PC3 plot, all the factors were strongly loaded on the PC1 except extraction temperature, which was highly loaded on PC3. The antioxidant and α-glucosidase inhibition activities along with TPC and TFC recovery were negatively influenced by both time and PSR. As for *E. officinarum* [Figure 9c and d], TPC, TFC, and α-glucosidase inhibition and DPPH scavenging activity were positively correlated and simultaneously were negatively correlated to PSR.

DISCUSSION

Secondary metabolites with antioxidant activity can also act as anti-inflammatory agents because the inflammatory response is an oxidative burst that can occur in monocytes, neutrophils, eosinophils, and macrophages.[15] For this reason, the search of these bioactive natural compounds can be considered so important. Excess metal ions can

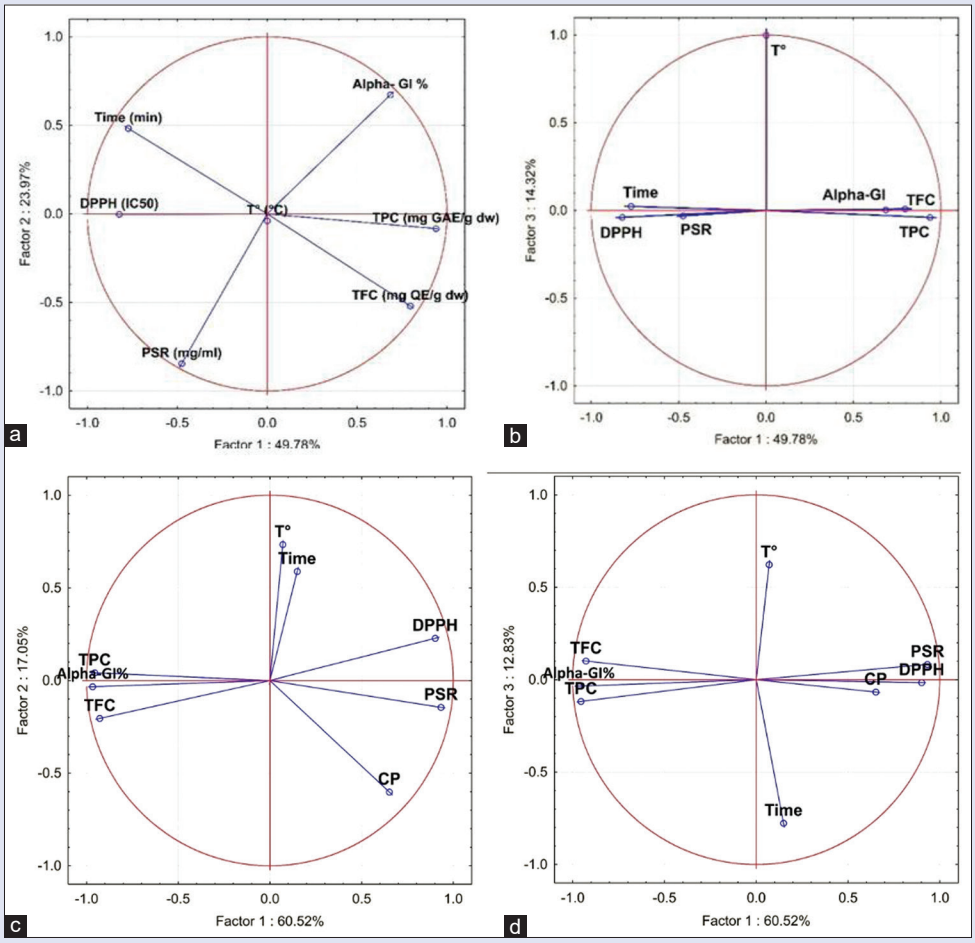

Figure 9: Principal component analysis: (a and b) represent *Euphorbia resinifera*; (c and d) represent *Euphorbia officinarum*. (a) and (c) Plot using the first and second components. (b) and (d) Plot using the first and third components

contribute to oxidative damage in some neuro-degenerative disorders such as Alzheimer's and Parkinson's diseases. Moreover, the formation of ROS can be triggered by the presence of metal ions; thereby, the reduction of their formation can be achieved by using adequate chelating agents.[16]

The development of the α-glucosidase inhibitors can be a new approach in the handling of diabetes.[17] Inhibition of intestinal α-glucosidase delays the digestion of starch and sucrose, therefore reducing the post-prandial blood glucose, and consequently mimics the effects of dieting on hyperglycemia.[18] *E. resinifera* leaf stem decoction[3] or one drop of latex in a glass of water once a day[4] has been used orally to treat diabetes in Morocco. However, this species is among the medicinal plants from Morocco that have not been explored experimentally for anti-diabetic activity.[7]

The determination coefficient (R^2), which was defined as the ratio of the explained variation to the total variation, was a measure of the degree of fit.[19] The empirical model fits the actual data in a better way when the R^2 value is closer to unity. This coefficient was used to check the robustness of the fit of the model. Normal probability plots of the residuals were used for checking the adequacy of the model, and the results are presented in Table 2. ANOVA must statistically satisfy the fundamental assumption of the experience, in which the legitimacy of the model was diagnosed using residual plots. Thus, the adequacy of the model was also evaluated with the help of the residuals. The straight line in the normal plot of the residuals means a normal distribution of the errors and adequacy of the constructed model.[20]

According to Equation 2, the extraction temperature and time had a linear positive effect on TPC recovery, in contrast to the linear effect of the PSR, which was negative, indicating that more plant material in the extraction medium results in less TPC extraction efficiency. Likewise, Pinelo *et al.*[21] obtained the highest phenolic concentration and anti-radical activity by increasing the solvent-to-solid ratio, that is, a lower PSR. A higher PSR leads to a decrease of the phenol extraction as well as of the biological activities, which is expected because it is consistent with mass-transfer principles. According to those authors, the concentration gradient between the solid and the bulk of the liquid is the driving force during mass tranfer, which is greater when a higher solvent-to-solid ratio is used. Similarly, Cheok *et al.*[22] noticed an increase in the TPC yield as the solid-to-solvent ratio decreases.

The significant influence of extraction time on total polyphenol content was reported previously for black tea.[23] Upadhya *et al.*[24] reported that 5 min was found as being the most adequate for TPC and antioxidant activities in continuous shaking extraction from different parts of *Achyranthes aspera*, whereas 1 hour of extraction time was sufficient for the extraction of phenolic compounds from mangosteen hull powder.[22]

Divergent results related to the temperature effect during extraction have been reported, even though the increase of extraction temperature tends to improve extraction.[21] In fact, the temperature has a positive effect on the extraction of phenols and, consequently, on the antioxidant activity. According to the results obtained, temperature and time are two factors essential to have better phenol yields and antioxidant activity through

scavenging the DPPH free radicals or to inhibit the α-glucosidase activity. The temperature improved the extraction at short periods, but for relatively long periods, the effect was the opposite. Similar results were reported by Yim *et al.*[25] in the aqueous extracts of *Schizophyllum commune*. According to these authors, better diffusion coefficients of polyphenols were observed with increased temperatures allowing higher extraction yields; nevertheless, above a limit, the inverse occurs, which was explained by the decomposition of thermo-sensistive compounds. This decomposition is also responsible for the loss of antioxidant activity for higher-temperature extraction, which is accentuated when the time of extraction is prolonged.

Flavonoids' extraction has been reported to be influenced by many factors including time, temperature, solid–liquid ratio, and extraction cycle.[26] Concerning *E. officinarum*, we noticed that the linear effect of the three extraction factors exhibited the highest positive influence on TFC extraction, whereas their quadratic effects had a negative effect. Time was reported to have a negative quadratic effect because the TFC yield increased for the first 76 min and then decreased. A possible explanation can include a decomposition phenomenon with a relatively extended extraction time[27] and is already reported for TPC. Because total phenols including flavonoids have an important role in the antioxidant activity and inhibition of α-glucosidase, the parameters that influence the extraction also have repercussions on the biological activities found.

CONCLUSION

The present study settled that RSM is a powerful tool for optimizing the extraction conditions of *E. resinifera* and *E. officinarum* aerial parts. This tool also allowed realizing the relationship between independent variables and response variables. The results reported a decrease in extraction efficiency with the increase of the plant ratio. The best extraction temperature was between 30°C and 35°C. Extraction using PSR of 20 mg/mL during 1 hour at 30°C yielded extracts with an optimal phenolic content and optimal values of the studied activities for *E. resinifera*. However, extraction during 270 min at 50°C using PSR of 10 mg/mL was the best extraction condition to yield extracts with optimum values of the studied responses for *E. officinarum*.

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Conflicts of interest

There are no conflicts of interest.

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