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ENHANCED PRODUCTION OF PHENOLIC COMPOUNDS FROM *JASMINUM GRANDIFLORUM* LEAVES BY RESPONSE SURFACE METHODOLOGY

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The main objectives of this study were to optimize the extraction parameters of phenolics compounds and antioxidants activities from *Jasminum grandiflorum* Linn. leaves using ultrasound assisted extraction method including four parameters : extraction time (min), temperature (°C), liquid /solid ratio (ml/g) and pH. The highest values of the TPC (27.53 mg GAE/g DW), TFC (18.60 mg QE/g DW), DPPH (24.27 µg/ml) and FRAP (890 µM of BHT/g DW) were observed after 32 min at 53 °C in a liquid-to-solid ratio 33 ml/g and in pH (5.5). The obtained results could be useful for further exploitation and application of sustainable resources.

INTRODUCTION

Jasmine (*Jasminum grandiflorum* Linn.), belongs to the *Oleaceae* family, is an important flowering plant found in many regions of the world including East and South Asia, the Arabian Peninsula, and Northeast Africa (Figure 1). It is used as antiseptic, aphrodisiac, anti-inflammatory, antioxidant, antihelmintic, diuretic and for treatment of toothache, ringworm infection, ulcer, stomatitis, skin diseases, and wounds. Iridoids, secoiridoids, lignans, flavonoids and triterpenes are the classes of compounds previously reported in this genus.[1,2] Therefore, traditional extraction of the previous compounds comprises solid-liquid techniques involving organic solvents that have some drawbacks such as unhealthy solvents, high temperature, long extraction time and toxicwastes.[3] Nowadays, it has been proposed new extraction methods (microwave, ultrasound...) present several advantages in terms of shorter extraction time, less concentration of organic solvent, lower environmental pollution and have increased the consumption of energy. Among all new methods, Ultrasound Assisted Extraction (UAE) takes simple and more economical method. The cavitation generated during the propagation of the ultrasound waves enhanced the solvent permeability into plant cells and the extraction efficiency.[4] The aim of this study was to obtain rich phenolic compounds extract with high antioxidants properties from *Jasminum grandiflorum* L. leaves'. For this purpose, the effects of independent variables of time (15–45 min), ultrasonic

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temperature (40-60°C), liquid-to-solid ratio (20–40 ml/g), and pH (2-6) using water as the solvent were investigated and optimized on the dependent variables of the total phenolic and flavonoids contents as well as the antioxidants activities of the extracts using mathematical and statistical method: response surface methodology (RSM) which is regarded to be a significant tool and has been applied in various pharmaceutics fiels.[5-7]

Fig. 1 – *Jasminum grandiflorum* L. plant.

MATERIALS AND METHODS

Sample Collection and Preparation

The collection of *Jasminum grandiflorum* L leaves has been carried out from Sfax (Tunisia) in September 2019. After collection of plant material, the leaves were separated manually from the steams and dried for 10 days at room temperature and then powdered with a mechanical grinder. The plant samples were identified by Dr. Maher Boukhris from University of Sfax (Tunisia) and the voucher specimens of *Jasminum grandiflorum* L. (JGHR2019) was preserved at Faculty of Sciences of of Sfax, University of Sfax, Tunisia.

Ultrasound-assisted extraction

Ultrasound-assisted extraction was performed with anultrasonic apparatus (BANDELIN HD3200) equipped with a BANDELIN SONPULS Noise protection box LS: frequency, 20 kHz; Temperature monitoring and measurement varied between 0-120°C; Pulsation: ON cycles 0.2-600 s, OFF cycles 0.3-600 s; Amplitude control: 10-100 %. The Jasmine leaves powders was placed in a Beaker (100 ml) and mixed with an appropriate amount of the extraction solution in order to get liquid-to-solid ratio varied between 20 and 40 ml/g. The extraction time varied between 15–45 min. while, ultrasonic temperature ranged from 40 to 60°C and pH=2-6 which was adjusted using acetic acid (≥99 %).The resulting extracts were evaporated at 35°C to dryness then stored at 4°C until use. The coded and encoded levels were presented in Table 1. A central composite design was applied to determine the effects of the above parameters on extraction TPC, TFC and antioxidant activities. The four parameters with 3 replicates as the centre point produce 27 extractions (coefficient α =1.4141) were listed in Table 2. To evaluate the relationship between variables (independent and dependent), a second order polynomial equation (Equation 1) and coefficient determinant (R^2) were calculated.

$$\widehat{Y} = b_0 + \sum_{j=1}^{k} b_j X_j + \sum_{u,j=1}^{k} b_{uj} X_u X_j + \sum_{j=1}^{k} b_{jj} X_j^2$$

Here, Y is the response variable (Y_{TPC}, Y_{TFC}, Y_{DPPH}, Y_{FRAP}), b_0 was fixed coefficient ; b_j is the linear coefficient; b_{jjis} the square coefficient; b_{uj} is the interaction coefficient; X_u and X_j present the coded independent variables, term of $X_u X_j$ and X_j two are the interaction and quadratic terms, respectively.

Table 1

Independent variables and their levels in RSM

	Independent variables			
Levels	t (min)	T (°C)	R (mL/g)	pH
- α	9	36	16	1.2
-1	15	40	20	2
0	30	50	30	4
1	45	60	40	6
+ α	51	64	44	6.8

Table 2

The experimental design and response values of *Jasminum grandiflorum* Linn. using CCD

Run	Extraction condition (Decoded variables)				Response variables			
	t (min)	T (°C)	R (mL/g)	pH	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	DPPH (µg/mL)	FRAP (µM of BHT/g DW)
1.	30	64.14	30	4	23.66	15.83	25.97	875
2.	45	40	40	2	14.75	7.43	35.87	819
3.	15	60	20	2	14.03	7.63	35.21	818
4.	30	50	44.14	4	24.56	15.77	26.77	875
5.	15	60	40	6	22.64	14.88	26.96	869
6.	45	40	40	6	22.70	14.00	27.51	862
7.	30	35.86	30	4	21.30	12.23	28.87	851
8.	51.21	50	30	4	22.95	14.67	27.19	854
9.	15	40	20	2	12.24	05.63	37.17	794
10.	30	50	15.86	4	21.99	13.97	27.93	864
11.	45	60	20	2	15.06	8.65	33.77	830
12.	30	50	30	1.172	14.96	8.29	35.99	820
13.	30	50	30	4	25.86	16.89	26.01	879
14.	30	50	30	4	25.86	16.89	26.01	879
15.	8.79	50	30	4	20.90	11.81	29.60	843
16.	45	40	20	6	21.06	12.56	28.00	853
17.	45	60	20	6	23.39	15.31	25.29	870
18.	30	50	30	6.828	25.86	17.11	26.34	880
19.	30	50	30	4	25.91	16.88	25.86	879
20.	15	40	40	6	20.94	11.66	29.27	852
21.	15	60	40	2	15.33	8.44	33.28	825
22.	15	40	40	2	14.11	6.19	36.33	809
23.	15	40	20	6	19.26	10.47	30.01	837
24.	45	60	40	6	25.03	17.29	26.00	868
25.	15	60	20	6	21.06	13.65	28.91	862
26.	45	60	40	2	16.40	9.86	32.88	831
27.	45	40	20	2	13.00	6.77	35.91	809

Total phenolic determination

The total phenolic content (TPC) of all samples was determined using the method of Folin–Ciocalteau reagent described by Kallel and co-workers in 2020 with some modification.[8] It was expressed as milligrams gallic acid equivalents (mg GAE) per g of plant dry weight (DW). For gallic acid, the curve of absorbance versus concentration is described by the equation y=2.265x (r^2=0.914). In addition, total flavonoids content (TFC) was estimated as reported previously by Ben Hmed *et al.*[9] The total flavonoids content was quantified using quercetin standard curve (r^2=0.996) and expressed as mg quercetin equivalent/g DW (mg QE/g DW). The absorbance was measured at 765 nm and 510 for TPC and TFC, respectively, using a UV-Visible spectrophotometer (BECKMAN DU 800). Three determinations were performed on each sample.

Antioxidant activities

DPPH assay

The antioxidant activity of *Jasminum grandiflorum* L. extracts towards DPPH radical was determined according to the method described by Rigane *et al.*[10-12] with some modification. A fresly DPPH solution was prepared by mixing 6 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl) with 100 mL of ethanol. For each concentration, one milliliter was added to 250 µL of ethanolic solution of DPPH. The mixture was stirred vigorously and then incubated at room temperature for 30 min in the dark, the absorbance at 515 nm using the UV spectrophotometer. The test was carried out in triplicate.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was estimated following the procedure originally described by Rigane *et al.*[10,11] Results are expressed as millimolar of BHT per gram of dry weight (y=13.134x + 0.0351; R^2=0.9805).

Software

Minitab 16 statistical software has been used in order to built the experimental designs and regression analysis of the experimental data.

RESULTS AND DISCUSSION

Optimization of single factors tests on TPC, TFC and antioxidant activities

The influence of time

The extraction time was an important effect on the extract of bioactive compounds. The effects of different treated time were tested during 15 min and 75 min with other extraction conditions as: T = 50°C, R = 30 ml/g and pH = 4 (Fig. 2A). The TPC increased with an increasing time (10 min – 30 min). The best suitable choice for the extraction time parameter was 30 min. The results of our study were in accordance with several previous studies in which authors mentioned that a long ultrasonic irradiation exceeding 30 min caused the degradation of some bioactive compounds.[13-17] The same tendency was observed in the TFC as well as antioxidant activities (Data not Shown).

The influence of ultrasonic temperature

Temperature is an important parameter in the extraction process of phenolic compounds. As illustrated in Fig. 2B, the TPC were significantly increased with the ultrasonic temperature (20-50°C) to be more than 27 mg GAE/g DW. The late results were in agreement with those reported by Zhang *et al.*[18] and Cui *et al.* [19] who mentioned that the increase in temperature can lead to the enhancement of the diffusion rate of solvent and mass transfer, which can improve the dissolution of target compounds. Moreover, TPC decreased when the temperature was higher than 50°C. The minimum of TPC was obtained at T = 70°C (18.86 mg GAE/g DW). These results could be explained as reported before in two scientific reports made by Dzah et *al.*[20]and Yusof et *al.*[21] who mentioned that extraction at high temperatures increased the rate of phenol oxidation and decreased TPC yields'. In addition, the last two teams declared that temperatures more than 70°C have been shown to lead to rapid polyphenol degradation, hence the need to select efficient extraction temperatures that maintain the stability of phenolic compounds.

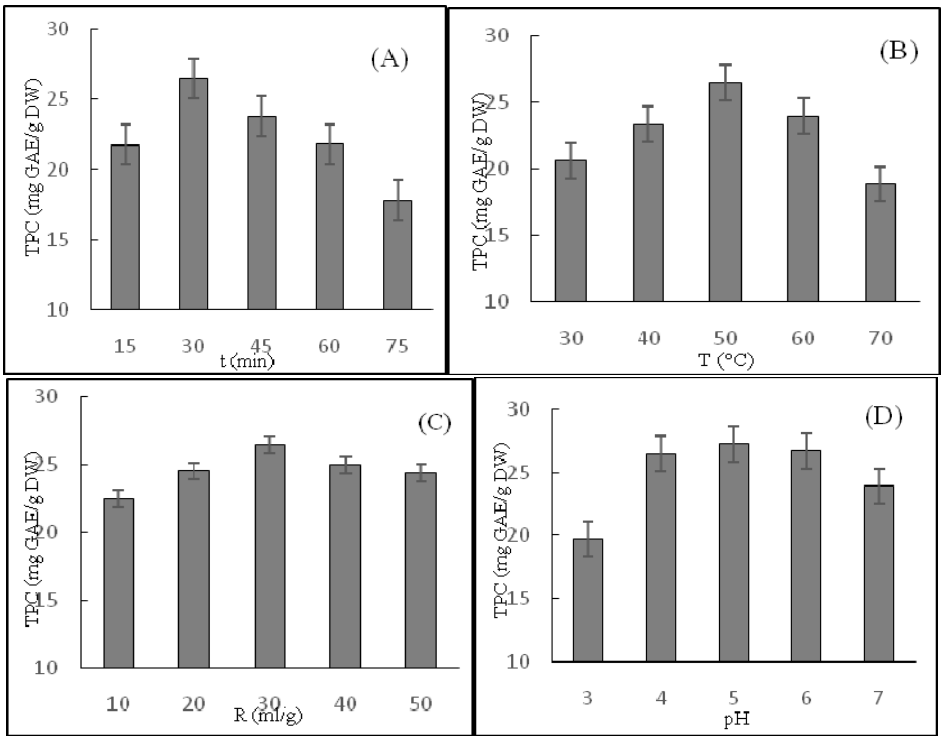


Fig. 2 – Effect of extraction time (A), ultrasonic temperature (B), liquid-solid ratio (C) and solvent pH (D) on the total phenolic from *Jasminum grandiflorum* Linn.

The influence of the liquid-solid ratio

In this study, different liquid-solid ratios (10-50 ml/g) were tested to evaluate their influence on TPC. Therefore, to attempt our objective, our research team has fixed the following parameters: t = 30 min, T = 50°C and pH = 4 (Fig. 2C). As it can be seen from Fig. 2C, there was a positive correlation between liquid-solid ratio (10 to 30 ml/g) and TPC (22.53 mg GAE/g DW to 26.47 mg GAE/g DW). On the other hand, increasing the liquid-solid ratio from 40 ml/g to 50 ml/g produced no significant change in the efficacy of phenolic compounds extraction (p<0.05). These results were in accordance with those reported by Ince et *al.*[22] who mentioned that the effect of the liquid-solid ratio was not significant, when the liquid-solid ratio was insufficient or excessive. Furthermore, the TPC showed a linear relationship with the TFC, radical-scavenging activity and Ferric reducing antioxidant power results (data not shown).

The influence of pH

In order to explore the influence of pH on TPC, different pH values were selected while other factors has been fixed (t = 30 min, T = 50°C and R = 30 ml/g) (Fig. 2D). The results in Fig. 2D showed that when the solvent pH increased from 3 to 5, the total phenolic content increased from 20 to 26.47 mg GAE/ g DW. On the other hand, when pH> 5, we observed that TPC decreased significantly (p<0.05) to be 23.98 mg GAE/g DW at solvent pH=7. These results could be explained such as reported by Friedman and Jurgens [23] who mentioned that pH values could affect the stability of phenolic compounds present in medicinal plants. Among this study, they demonstrated that phenolic compounds could be damaged when exposed to high pH. The same tendency was observed in the content of flavonoids as well as antioxidant activities (Data not Shown).

Response Surface Optimization

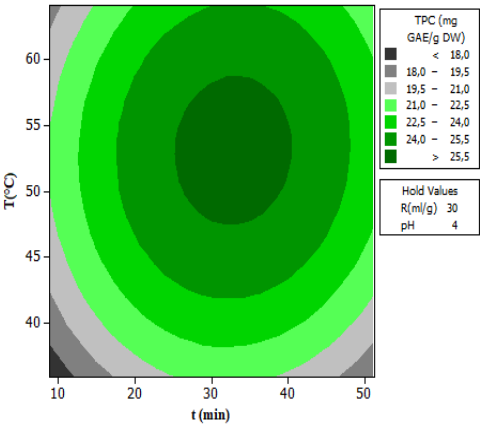
Model fitting

Using the central composite design, the TPC, TFC, DPPH and FRAP of *Jasminum grandiflorum* Linn extract by UAE were the responses. The independent variables were: extraction time, ultrasonic temperature, liquid-to-solid ratio and pH. The analyses of variance (ANOVA) were summarized in Table 3: the coefficients of determination values (R^2) as well as the coefficients of determination predict (R^2 predict) and the lack of the fit of the proposed model were illustrated.

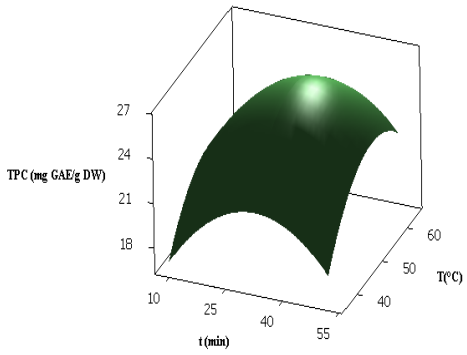
Table 3

ANOVA for design by the total phenolic content, total flavonoid content DPPH and FRAP method from *Jasminum grandiflorum* Linn

Source	Y1 : TPC (mg GAE/g DW)		Y2 : TFC (mg QE/g DW)		Y3 : DPPH (µg/mL)		Y4 : FRAP (µM of BHT/g DW)	
	***F*-value**	***p*-value**	***F*-value**	***p*-value**	***F*-value**	***p*-value**	***F*-value**	***p*-value**
Regression	4005.45	<0.001	1505.12	<0.001	607.51	<0.001	550.00	<0.001
t	1378.01	<0.001	712.97	<0.001	187.60	<0.001	647.11	<0.001
T	2610.24	<0.001	940.12	<0.001	138.96	<0.001	331.85	<0.001
R	1345.76	<0.001	266.63	<0.001	74.75	<0.001	148.34	<0.001
pH	4616.01	<0.001	1101.65	<0.001	1097.19	<0.001	711.46	<0.001
t^2	3599.84	<0.001	1567.00	<0.001	312.25	<0.001	874.08	<0.001
T^2	2657.75	<0.001	948.60	<0.001	122.90	<0.001	231.35	<0.001
R^2	1556.77	<0.001	460.42	<0.001	112.65	<0.001	77.00	<0.001
pH^2	6897.68	<0.001	2078.54	<0.001	1323.27	<0.001	788.48	<0.001
t x T	25.21	<0.001	0.32	0.585	0.01	0.938	18.78	0.001
t x R	0.17	0.689	7.89	0.016	18.17	0.001	18.78	0.001
t x pH	143.72	<0.001	47.47	<0.001	18.56	0.001	2.78	0.121
T x R	6.30	0.027	6.68	0.024	11.25	0.006	36.00	<0.001
T x pH	15.56	0.002	52.77	<0.001	15.92	0.002	1.78	0.207
R x pH	0.21	0.653	23.70	<0.001	0.07	0.798	0.44	0.518
Lack of fit	16.13	0.067	11.06	0.086	8.15	0.112	15.83	0.071
R^2	99.98%		99.94%		99.52%		99.94%	
R^2 (predict)	99.87%		99.67%		97.08%		97.23%	



3a (2D)



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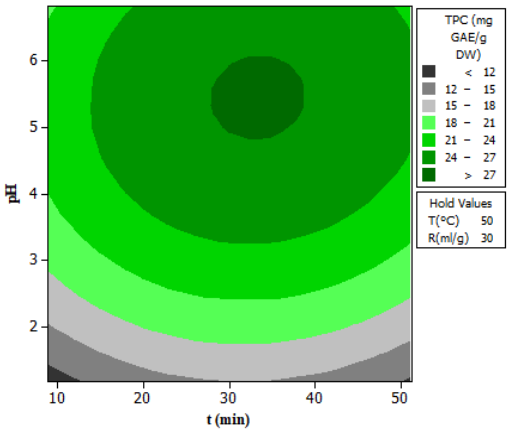

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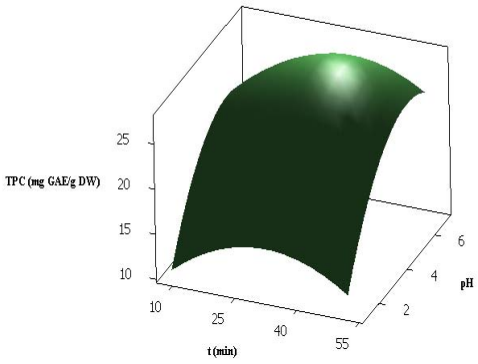

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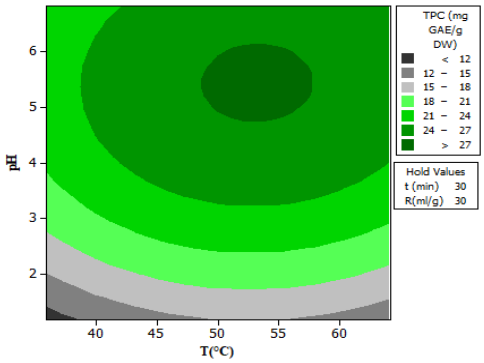
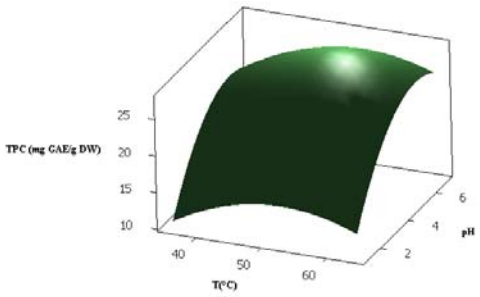


3d (2D)



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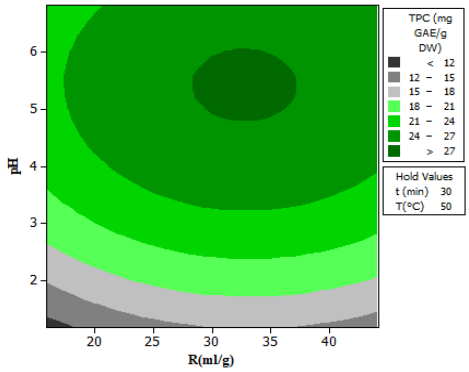

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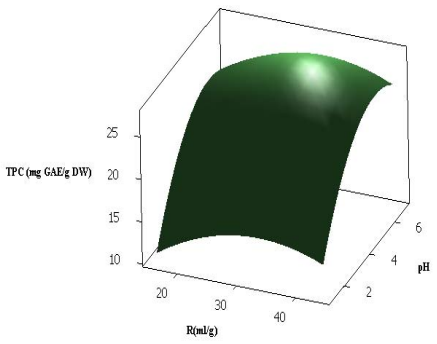


3e (3D)

Fig. 3 – Response surface contour plots (2D and 3D) of extraction time, temperature, liquid to solid ratio and pH on the TPC.

The effects of the variables on TPC and analysis of responses surface plots

The analysis of variance (ANOVA) of TPC with a low *p*-value of the model (p <0.001) and the non-significance of the lack of fit (p= 0.067) demonstrated that the Y_{TPC} model was remarkably significant. The model coefficient of determination R^2 was 0.9998, which indicated that this model was adequate. Therefore, our results were in accordance with those reported by Uysal *et al.*[6] On the other hand, our research team concluded that t, T, pH, R, t^2, T^2, R^2, pH^2,t x T and t x pH, influenced more significantly on the TPC (p<0.001) than T x R and T x pH (p<0.05) (Table 3). In addition, Fig. 3 indicated respectively the relationship between TPC and the four factors by two (2D) and three dimensional (3D) response surfaces. In the beginning, an increase in the time and temperature produced an increase in the TPC with liquid to solid ratio and pH set at 30 ml/g and 4, respectively. By contrast, an extension of time and temperature were not significant (p>0.05). These results demonstrated that a long duration of extraction time caused the degradation of phenolic compounds (30min) as reported by Belwal *et al.*[24] and Iftikhar *et al.*[16] who declared that increasing temperature reduce the extraction time to obtain optimum phenolic compounds extraction. On the other hand, Ezzoubi *et al.*[25] demonstrated that the optimum conditions for the extraction of the polyphenols from *Lavandula stoechas* using ultrasound were an ethanol concentration of 40%, a liquid/solid ratio of 30 ml/g, and a time processing of 32.62 min.

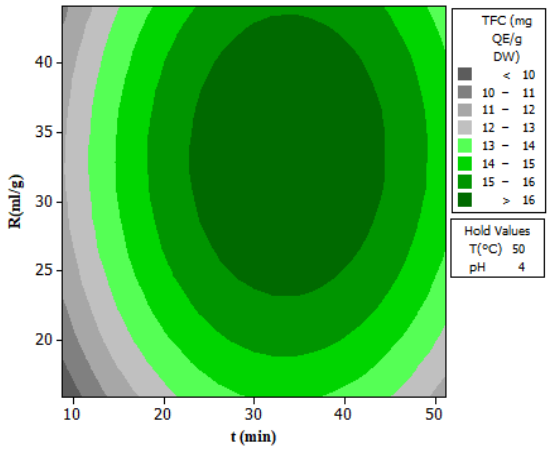
The effects of the variables on TFC and analysis of responses surface plots

As seen in Table 3, the ANOVA of TFC suggests the signification of this model: a low *p*-value of the model (p <0.001) and a high *F*-value (1505.5). In detail, the TFC has been influenced significantly not only by the linear term but also by the square term (p <0.001) with the R^2 value equal to 0.9994. All that demonstrated that this model was suitable. t x R, R x pH and T x pH are the most significant interaction at p< 0.001. But t x R, T x R are significant at p< 0.05. Fig. 4.b (3D and 2D) showed an increased extraction of TFC which was recorded with increasing time up to 33 min and the liquid to solid ratio up to 32 mL/g when the temperature and the pH were set at 50 and 4, respectively. Many studies were similar to our results such as reported by Styaningsih *et al.*[26] and Sendi *et al.*[27]: major flavonoids remained stable below 55°C and above 60°C, temperature affected negatively the TFC.

The effects of the variables on antioxidant activity and analysis of responses surface plots

To evaluate the significance of this model, the ANOVA of DPPH was analyzed: a very low probability value (p<0.001) and a high *F*-value (607.51) with R^2 =0.9952. The obtained results indicated that an acceptable correlation between experimental and calculated value ($R^2_{predict}$ = 0.9752). Linear term (p<0.001), square term (p<0.001), t x pH (p<0.05), t x R (p<0.05), T x pH (p<0.001) and T x R (p<0.001) affected the DPPH significantly (Table 3). The Fig. 5d (3D and 2D) clearly showed that when the temperature was beyond 55°C and pH equal to 5.3, the DPPH decreased. The interaction of extraction time and liquid to solid ratio showed a significant impact of both parameters (p<0.05). At the same time, an increase in time up to 32 min and an increase in liquid to solid ratio up to 33 mL/g. The DPPH was influenced by cross product of time and pH (p<0.05).

On the other hand, the significance of this model were generally related to the *p*-value of the model (p <0.001)and the *F*-value (550). It can be seen that the R^2 of this model was 0.9994 (Table 3). Fig. 6 was shown the 2D and the 3D dimensional response surfaces based on CCD-RSM. The 2D and 3D plot (Fig.6.a) presented the effect of the extraction time, the temperature and keeping the ratio and pH at a constant level on the FRAP. It can be seen a maximum point extraction time =32.5 min, ultrasonic temperature equal to 56.5°C and keeping liquid-to-solid ratio at 30 mL/g with a pH = 4. It means that an increasing on FRAP was effected by the combination of time and temperature. It is clear from figure 6b, 6d and the analysis of the ANOVA that also the independent variables time and liquid-to-solid ratio, temperature and pH show significant interactions (p<0.05).



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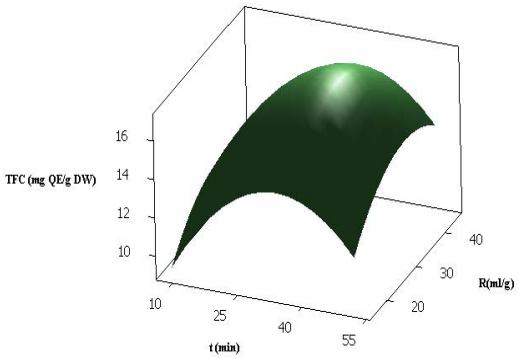
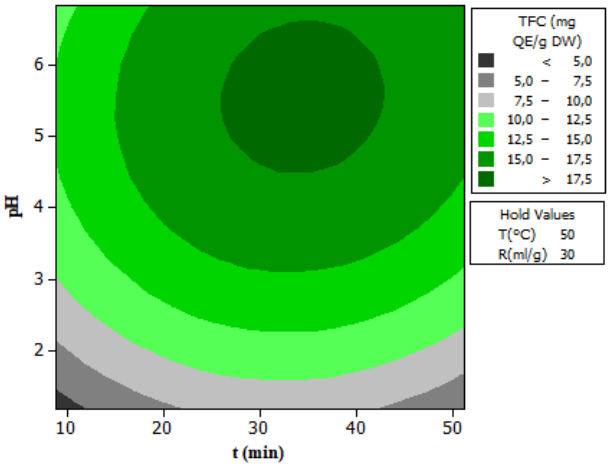
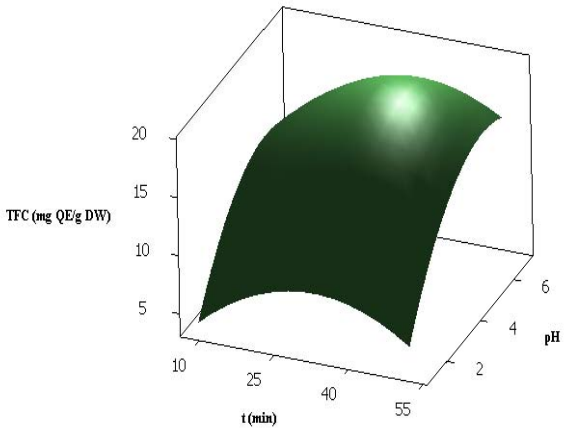


4b (3D)



4c (2D)



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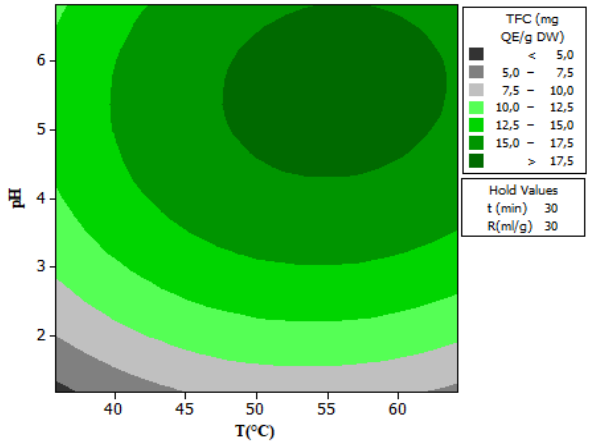
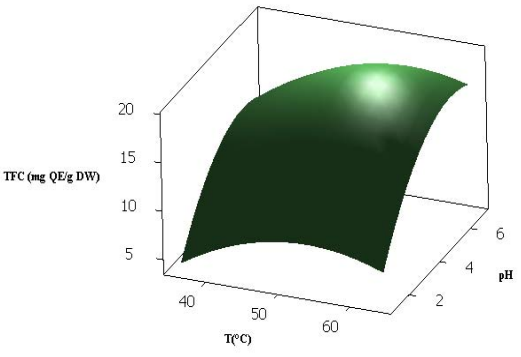
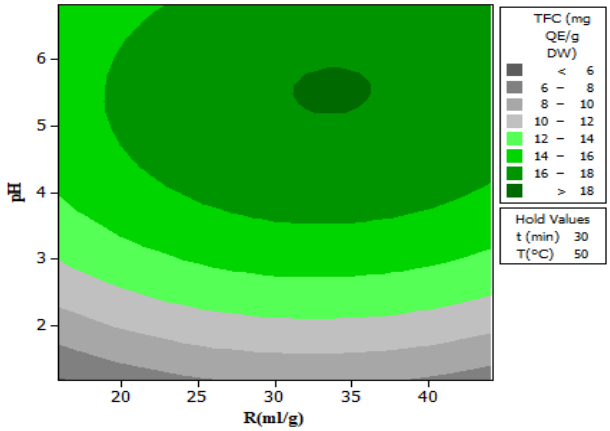


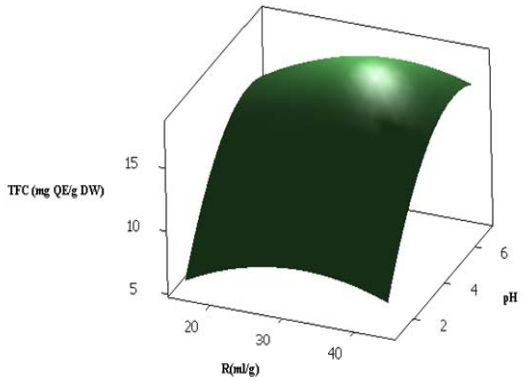
4d (2D)



4d (3D)



4e (2D)



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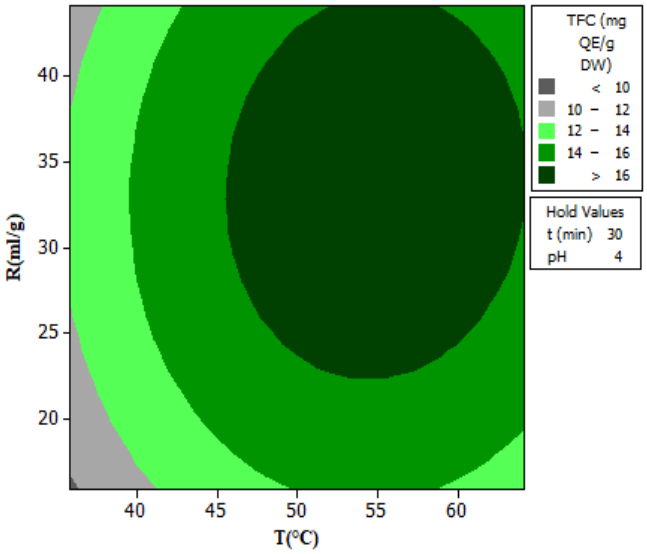
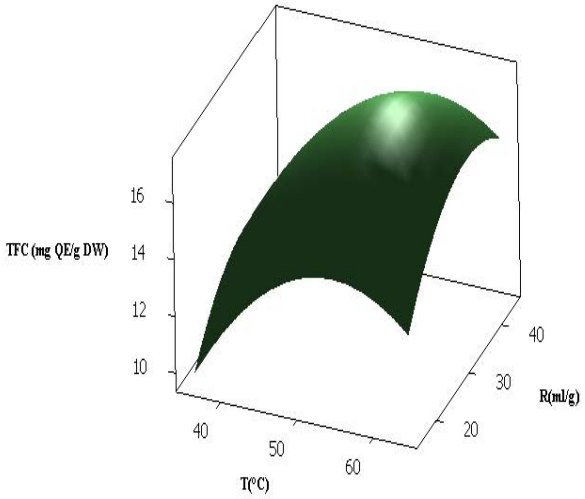
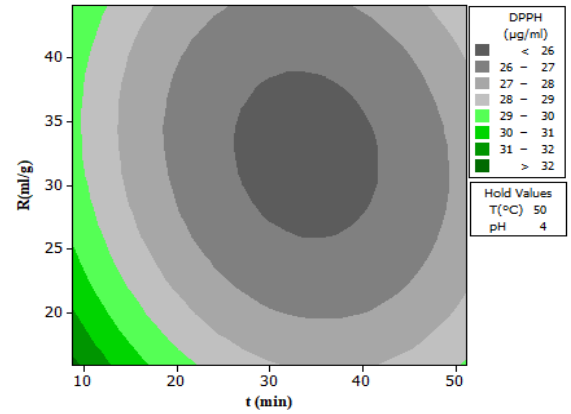


4f (2D)

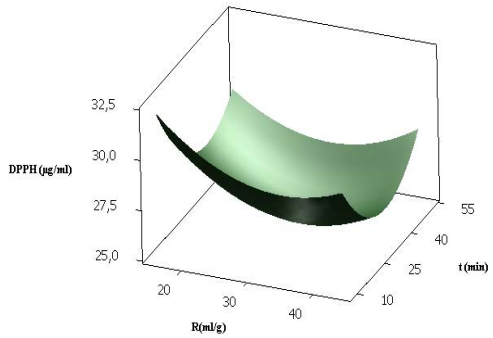


4f (3D)

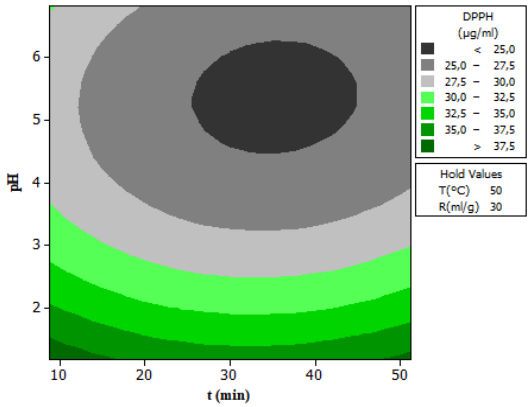
Fig. 4 – Response surface contour plots (2D and 3D) of extraction time, temperature, liquid to solid ratio and pH on the TFC.



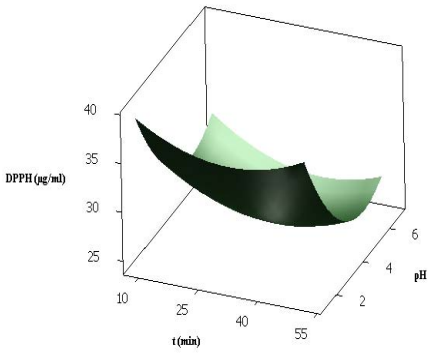
5b (2D)



5b (3D)



5c (2D)



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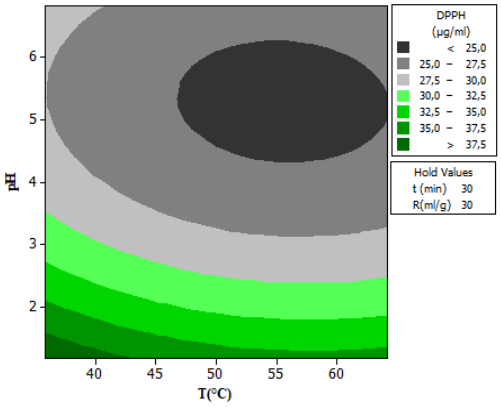
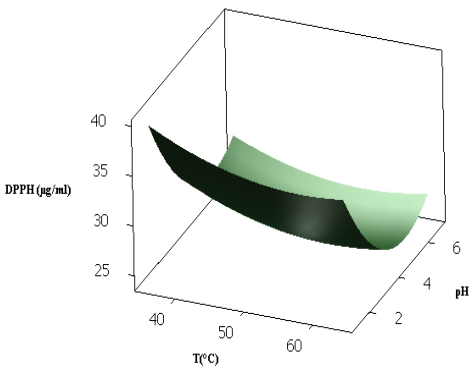


5d (2D)



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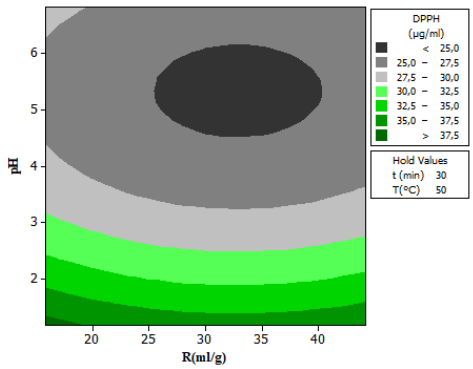
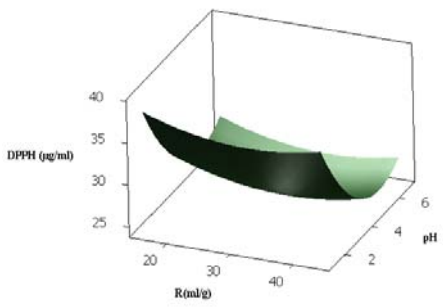
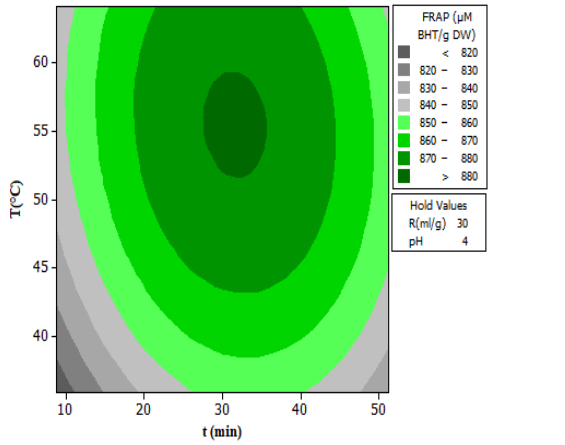


5e (2D)

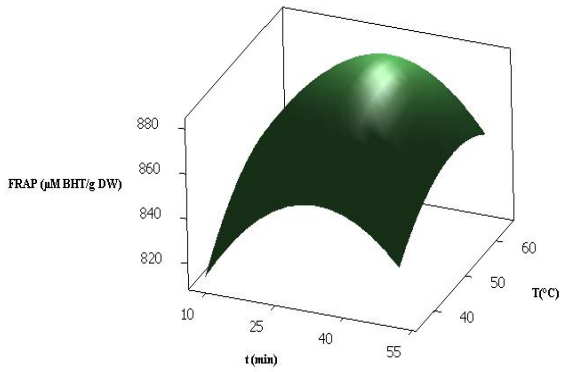


5e (3D)

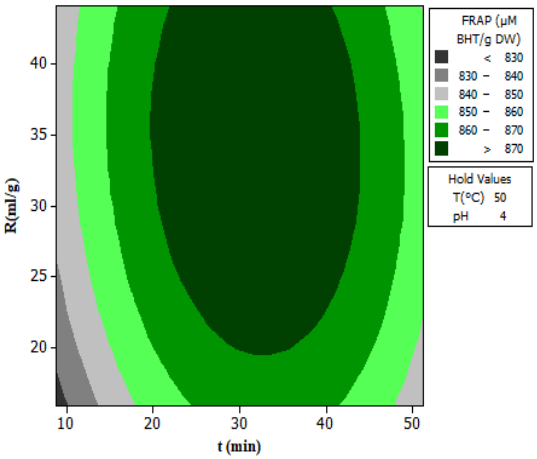
Fig. 5 – Response surface contour plots (2D and 3D) of extraction time, temperature, liquid to solid ratio and pH on the DPPH.



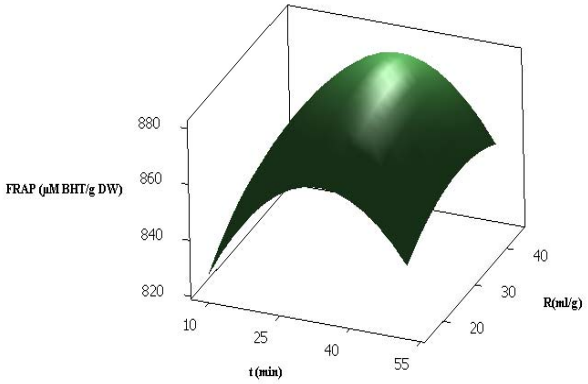
6a (2D)



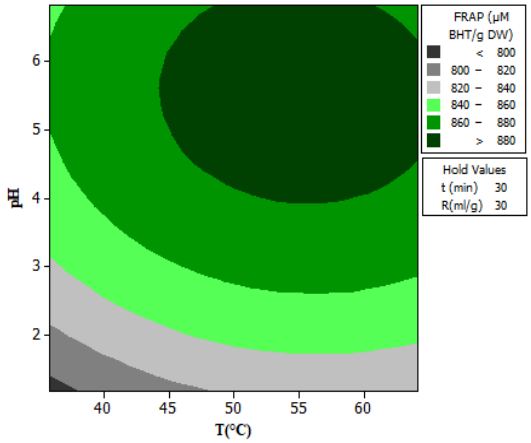
6a (3D)



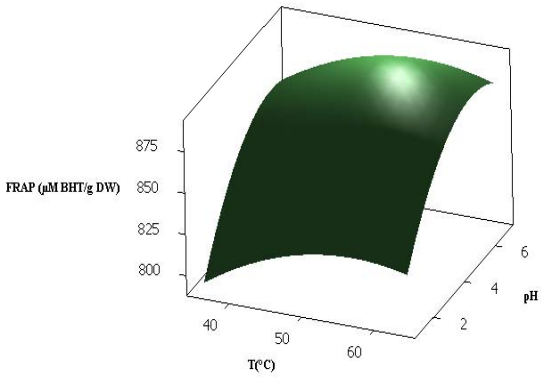
6b (2D)



6b (3D)



6d (2D)



6d (3D)

Fig. 6 – Response surface contour plots (2D and 3D) of extraction time, temperature, liquid to solid ratio and pH on the FRAP.

RSM optimization

The aim of this study was to optimize the phenolic compounds extraction. The optimal UAE conditions were: extraction time (31.99 min), ultrasonic temperature (54.42°C), liquid-to-solid ratio (32.99 ml/g), and pH (5.45). The optimal conditions were adjusted as flows: extraction time (32 min), ultrasonic temperature (53°C), liquid-to-solid ratio (33 mL/g) and pH (5.5) (Table 5).

Table 4

Optimum extraction conditions, Total phenolic and flavonoid contents, DPPH and FRAP from *Jasminum grandiflorum* Linn. obtained by optimization condition of ultrasonic assisted extraction

Method extraction	Optimum extraction conditions				Optimum responses			
	t (min)	T (°C)	R(mL/g)	pH	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	DPPH (µg/mL)	FRAP (µM of BHT/g DW)
UAE	32	53	33	5.5	27.53	18.60	24.27	890

UAE : Ultrasound assisted extraction; t = time ; T : Temperature; R : liquid-solid ratio.

The TPC, TFC, DPPH and FRAP under these optimal conditions were respectively 27.53 mg GAE/g DW, 18.60 mg QE/g DW, 24.27 µg/ml and 890µM of BHT/g DW (Table 4). These results were more better to those reported by Arun *et al.*[28] who demonstrated that the successive extracts of *J. grandiflorum* have total phenolic content was equal to 21.94±0.74 mg GAE/mg, a DPPH 33.62±0.52 µg/mL. The highest antioxidant activities found in extract obtained by UAE method could be explain according to Jerman *et al.*[29] who declared that both, high and low power sonications have shown to be an efficient extraction tool providing higher phenol recoveries in comparison to conventional extraction methods and consequently a positive correlation between phenolic content and antioxidant activities.[30] On the other hand, comparing with the maceration extraction methods (ME), UAE reduce the extraction time of the phenolic compounds from plant materials.[28] For that reason, UAE considered as an economic and green extraction method which demonstrated that the use this method for the extraction of the bioactive components was an efficient. The results indicated that the UAE-RSM approach was effective for maximizing the TPC, TFC and antioxidant activities, and the knowledge gained from this study should be useful for further exploitation and application of the phenolic compounds.

CONCLUSIONS

Ultrasound assisted extraction was used to optimize the extraction of the phenolic compounds from jasmine based on RSM model. The best conditions were obtained as extraction time (32 min), ultrasonic temperature (53°C), liquid-to-solid ratio (33 ml/g) and pH (5.5). Under these conditions, the maximal values of the TPC, TFC, DPPH and FRAP were respectively 27.53 mg GAE/g DW, 18.60 mg QE/g DW, 24.27 µg/ml and 890 µM of BHT/g DW.

Abbreviation list

TPC: Total Phenolic content
TFC: Total Flavonoid Content
GAE: Gallic Acid Equivalent
DW: Dry Weight
QE: Quercetin Equivalent
FRAP: Ferric Reducing Antioxidant Power
BHT: Butylated Hydroxytoluene
RSM: Response Surface Methodology
UAE: Ultrasound Assisted Extraction
ME: maceration extraction
CCD: Central Composite Design
DPPH: 2,2-diphenyl-1- picrylhydrazyl
t: time
T: Temperature
R : liquid-solid ratio.

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