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OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM *VERBENA OFFICINALIS* L. LEAVES USING RESPONSE SURFACE METHODOLOGY AND EVALUATION OF ITS ANTIOXIDANT ACTIVITIES

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The ultrasound-assisted extraction of phenolic compounds from *Verbena officinalis* L. leaves parts was modeled using response surface methodology. Central Composite Design has been used to optimize four extraction parameters: extraction time (t), ultrasonic temperature (T), liquid-to-solid ratio (R) and pH (pH) in order to get a phenolic rich extract. The optimized conditions are t = 30.64 min, T = 52.70°C, R = 28.71 ml/g and pH = 5.22. Under these conditions, the experimental TPC= 87.089 mg GAE/g DW, TFC= 52.706 mg QE/g DW, IC_{50}= 27.975 µg/ml and FRAP assay= 992.566 µM BHT/g DW. Consequently, this optimized UAE method has demonstrated a potential application for efficient extraction of phenolic compounds from *Verbena officinalis* L. leaves in the nutraceutical industries.

INTRODUCTION

Verbena officinalis or *Verbana* belongs to the *verbenaceae* family which was cultivated in West Asia, North Africa and all over Europe. Traditionally, *Verbana* has been used in folk medicine as a diuretic, expectorant and anti-rheumatic while, in Navarra (Spain), it is used for its anti-inflammatory topical applications. In addition, the plant has been used also to treat acute dysentery, enteritis, amenorrhea, depression, antidepressant and anticonvulsant effect as well as for the treatment of jaundice, cough, cold and digestive problems, healing liver and gallbladder diseases and nervous exhaustion. The aerial part of *Verbena officinalis* has been effectively used to allevative conditions of anxiety, insomnia and nervous irritability. Various extracts of this plant have shown many biological activities including: antifungal, antibacterial, antioxidant, analgesic, anti-rheumatic and nerve growth factor-potentiating activities.[1,2] Phytochemical investigation found that *Verbana* contained many constituents such as flavonoids, iridoid glycosides, phenylpropanoid glycoside, sterols, triterpenes and glycoconjugate which were known for its contributing to the

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pharmacological efficacy of *Verbana*.[2] Therefore, extraction of phenolic compounds from medicinal plants can be carried out using classic ways (Soxhlet, maceration and heat reflux) and new technology such as microwave-assisted extraction. Unfortunately, these techniques could affect loss of active compounds due to the hydrolysis, oxidation, and thermal decomposition during the high temperature extraction. Actually, Ultrasonic-assisted extraction (UAE) is one of the most inexpensive and efficient green extraction techniques compared with conventional extraction and have been applied to extract bioactive compounds from different materials due to its high reproducibility at shorter time, simplified manipulation, significant reduction in solvent consumption, and temperature, in respect to other classic methods.[3] Furthermore, many reports used response surface methodology (RSM) for the optimization of bioactive compound extraction in order to reduced number of experimental trials needed to evaluate multiple parameters and their interactions.

In this work, and for the first time, the aims of the present study were to investigate the extraction variables including: extraction time (15-75 min), ultrasonic temperature (30-70°C), liquid-to-solid ratio (10-50 ml/g) and pH (3-7) using distillated water as solvent; optimize these variables values by RSM for the total phenolic and flavonoids contents as well as the antioxidants activities of the extracts using DPPH and FRAP assays.

MATERIALS AND METHODS

Plant material

Dried leaves of *Verbena officinalis* L. were purchased from a local market (Sfax, Tunisia) and botanically identified by Dr. Maher Boukhris (Departement of Sciences of Life, University of Sfax), where a voucher specimen was deposited (VHO2018). The plant material was ground using a Retsch blinder mill (Normandie-Labo, Normandy, France), sifted through 0.5 mm mesh screen to obtain a uniform particle size and subsequently essayed for their phenolic composition and antioxidant activities.

Extraction Process and *Box-Wilson central composite design*

Ultrasound-assisted extraction was performed with an ultrasonic 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%. Therefore, power amplitude has been adjusted to be equal to 25% while, a continuous mode has been chosen. The *Verbena officinalis* L. 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 10 and 50 mL/g using distillated water as a solvent. The extraction time varied between 15-75 min while, ultrasonic temperature ranged from 30 to 70°C and pH = 3-7 which was adjusted using acetic acid (≥ 99%). The resulting extracts were evaporated at 35°C to dryness then stored at 4°C until use.

Response Surface Methodology using Central Composite Design was used to optimize four independent parameters: extraction time (t, 15-75 min), Temperature (T, 30-70°C), liquid-to-solid ratio (R, 10-50 ml/g) and solvent pH (pH, 3-7) of four dependent variables: total phenolic content (Y_{TPC}), total flavonoid content (Y_{TFC}), DPPH scavenging activity (Y_{DPPH}), and FRAP activity (Y_{FRAP}). These independent parameters were selected due to their significant influence on the efficiency of UAE.[4] The independent variables were coded at three levels, and their actual values selected based on literature data and preliminary experimental results. The independent variables and their related codes and levels has been displayed in Table 1. A total of 27 experimental runs were performed randomly, which included three replicates at the center point (Table 2), and all the experiments were replicated three times to improve the analysis. Regression analysis for the experiment data was performed and was fitted into a second-order polynomial model:

$$Y = ß_0 + \sum ß_i X_i + \sum ß_{ii} X_i^2 + \sum ß_{ij} X_i X_j$$

where Y represents the estimated response; $ß_0$ the model constant, $ß_i$, $ß_{ii}$ and $ß_{ij}$ are the estimated coefficients of the model obtained by multiple regression.

Total Phenolic and Total flavonoids Content (TPC and TFC)

Total phenolics were determined with the Folin- Ciocalteu (F-C) assay according to Khedher *et al.*[5] with slight modification using a UV-Visible

spectrometer (BECKMAN DU 800). Gallic acid was used as the standard ($y = 2.265\ x$; $r^2 = 0.914$), and results were expressed as milligram of gallic acid equivalents per gram dry weight (mg GAE/g DW). On the other hand, flavonoid determination was estimated according to Yahyaoui *et al.*[6] with slight modification. The TFC was quantified using, quercetin standard curve ($y = 0.958\ x + 0.009$, $r^2 = 0.996$) and expressed as milligram of quercetin equivalent per gram of dry weight (mg QE/g DW). Each experiment was analyzed in triplicate.

Antioxydant activity

DPPH radical scavenging activity: The DPPH assay followed a reported method of Khedher *et al.*[5] with some modifications. The concentration of the test extract providing 50% inhibition (IC_{50}, expressed in $\mu g.mL^{-1}$) was calculated from the graph plotted with inhibition percentage against the extract concentration.

Ferric reducing antioxidant power (FRAP) assay: FRAP assay was estimated following the procedure described by Ben Hmed *et al.*[7] with some modification. Results were calculated as IC_{50} of reducing power expressed in $\mu g.mL^{-1}$.

All tests were carried out in triplicate.

Software

To build the experimental designs and regression analysis of the experimental data, we have used Minitab 2016 software.

RESULTS AND DISCUSSION

Single Factor Investigation

Effect of extraction time on Extraction Yield of Phenolic Compounds: Extraction process has been done using extraction time ranging from 15 to 75 min, while other parameters were as follows: T= 50°C, pH=4 and liquid-to-solid ratio has been equal to 30 mL/g. The effect of extraction time on phenolic content from *Verbena officinalis* leaves is shown in Figure 1. The obtained results revealed that if extraction time increases, the phenolic content reached a maximum at 30 min (more than 85 mg GAE/g DW) and then decreases as the extraction proceeds. The decrease in the phenolic content could be due to destruction and the decomposition of phenolic compounds during the prolonged extraction time.[8,9]

Effect of liquid-to-solid ratio on Extraction Yield of Phenolic Compounds: Extraction process of phenolic compounds from *Verbena officinalis* leaves was carried out using liquid–to-solid ratio ranged between 10 to 50 mL/g while extraction time, temperature and pH have been maintain constant at 30 min, 50°C, 30 mL/g and 4, respectively. From Figure 2, we can note that TPC increases significantly ($p<0.05$) and maximum TPC was achieved at 30 ml/g (TPC>80 mg GAE/g DW), and then slightly decreases after the liquid-to-solid ratio has been more than 40 mL/g. The obtained results were in accordance with those reported previously by Zhao and co-workers in 2014,[9] who explained this phenomenon as follow: using low liquid-to-solid ratio leads to higher diffusion and extraction yield, while the decrease of the distribution of ultrasonic energy density in the extraction solutions is dominant and has a negative effect on the total phenolic compounds content. All these results could be attributed to the mass transfer principle and the distribution of ultrasonic energy density in the extraction solutions.

Effect of Temperature on Extraction Yield of Phenolic Compounds: According to Alu'datt *et al.*[10] who mentioned that the solubility of phenolic compounds could be increased significantly with temperature used during extraction step. As presented in the Figure 3, the TPC were significantly ($p<0.05$) increased with the ultrasonic temperature (30-50°C) to be > 80 mg GAE/g DW using 50°C. Moreover, TPC decreased when the temperature was higher than 50°C. The minimum of TPC was obtained at T = 70°C (~65 mg GAE/g DW). The obtained results were in accordance with many previous researchers who mentioned that at temperature extraction; less than 50°C; strongly impacts the solvent's properties: An increase of temperature could result a decrease not only viscosity but also surface tension, and consequently an increase of vapor pressure. Furthermore, a rise in vapor pressure induces more solvent vapors to enter the bubble cavity and numerous cavitations bubbles, which will collapse less violently and reduce sonication effects.[4]

Table 1

Predicted and experimental values of TPC, TFC, DPPH and FRAP assays from *Verbana officinalis* extracts using ultrasound-assisted extraction

N°	$TP_{experimental}$ (mg GAE/g DW)	$TP_{predicted}$ (mg GAE/g DW)	$TF_{experimental}$ (mg QE/g DW)	$TF_{predicted}$ (mg QE/g DW)	DPPH experimentale (µg/ml)	DPPH predicted (µg/ml)	$FRAP_{experimental}$ (µM BHT/g DW)	$FRAP_{predicted}$ (µM BHT/g DW)
1.	57.11	56.46	20.24	20.11	34.11	33.83	963.77	964.13
2.	67.83	66.81	32.15	31.21	31.45	31.62	973.82	973.34
3.	50.33	49.92	20.69	19.35	33.33	33.60	986.14	958.72
4.	64.03	63.83	30.14	30.70	31.69	31.47	969.12	969.53
5.	62.86	62.85	28.11	27.65	32.24	32.31	968.13	967.71
6.	64.60	65.26	30.76	31.44	31.88	31.68	969.33	969.54
7.	62.97	63.01	28.88	28.87	32.03	31.96	968.05	968.73
8.	67.97	68.98	32.55	32.90	31.40	31.40	972.33	972.16
9.	74.34	72.98	38.55	37.04	30.93	31.02	980.14	979.23
10.	74.07	73.92	35.66	37.05	30.45	30.43	980.94	980.64
11.	77.01	76.24	42.33	43.03	29.84	29.94	982.88	983.05
12.	81.07	80.73	44.00	43.29	29.42	29.43	986.70	986.05
13.	70.05	70.14	35.00	35.83	30.60	30.73	975.33	975.30
14.	63.07	63.12	28.36	28.53	31.89	31.71	969.00	969.32
15.	79.43	80 .09	44.02	43.79	29.61	29.53	974.02	985.55
16.	76.10	76.64	35.22	36.70	30.40	30.59	981.14	981.10
17.	72.94	74.43	38.55	40.13	30.44	30.26	979.66	980.80
18.	79.91	79.31	45.07	43.00	29.31	29.45	984.15	984.28
19.	68.91	70.39	34.23	34.27	30.78	30.91	975.79	976.36
20.	75.91	75.32	40.07	39.54	30.12	29.95	980.14	980.91
21.	74.06	77.31	45.96	47.42	29.33	29.22	982.14	983.81
22.	81.29	78.94	50.08	48.13	28.89	28.97	983.22	982.89
23.	85.50	84.26	50.14	50.91	28.68	28.29	990.22	989.66
24.	68.32	68.51	33.22	34.19	30.97	31.13	974.02	974.27
25.	84.91	85.61	50.33	48.87	28.77	28.57	990.23	991.32
26.	85.01	84.26	51.11	50.91	27.81	28.29	991.29	989.66
27.	84.07	84.26	50.53	50.91	28.34	28.29	990.23	989.66

Table 2

ANOVA analysis for the response surface for optimization of TPC, TFC DPPH and FRAP on the extraction parameters from *Verbana officinalis* leaves'

Source	Y_{TPC} (mg GAE/g DW)		Y_{TFC} (mg QE/g DW)		Y_{DPPH} (µg/ml)		Y_{FRAP} (µM BHT/g DW)	
	***F*-valeur**	***p*- valeur**	***F*- valeur**	***p*- valeur**	***F*- valeur**	***p*- valeur**	***F*- valeur**	***p*- valeur**
Régression	59.54	<0.001	61.40	<0.001	52.96	<0.001	112.45	<0.001
,t	48.31	<0.001	90.43	<0.001	89.87	<0.001	100.87	<0.001
T	70.58	<0.001	148.89	<0.001	112,85	<0.001	142.55	<0.001
R	23.59	<0.001	15.49	0.002	28.31	<0.001	43.01	<0.001
,pH	36.82	<0.001	74.51	<0.001	65.57	<0.001	61.17	<0.001
t^2	47.74	<0.001	79.71	<0.001	68 .10	<0.001	90.85	<0.001
T^2	113.73	<0.001	179.03	<0.001	127.22	<0.001	219.97	<0.001
R^2	32.89	<0.001	8.97	0.011	17.85	0.001	72.06	<0.001
pH^2	45.34	<0.001	80.31	<0.001	67.66	<0.001	85.29	<0.001
t x T	5.01	0.045	0.02	0.880	0.08	0.788	41.17	0.173
t x R	25.03	<0.001	22.15	0.001	31.08	<0.001	44.72	<0.001
t x pH	35.16	<0.001	50.86	0.000	32.88	<0.001	50.08	<0.001
T x R	17.77	0.001	1.61	0.229	0.20	0.659	33.95	<0.001
T x.pH	38.02	<0.001	18.86	0.001	8.92	0.011	69.79	<0.001
R x.pH	33.80	<0.001	31.72	0.000	18.78	<0.001	46.37	<0.001
Lack of fit	5.53	0.163	11.97	0.080	0.30	0.924	4.14	0.210
R^2	98.58%		98.62%		98.41%		99.24%	
R^2 (predicted)	96.93%		97.02%		96.55%		98.36%	

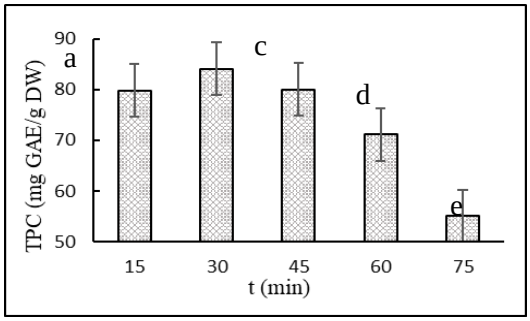


Fig. 1 – Effect of ultrasonic extraction time on total phenolic content (TPC) using UAE.

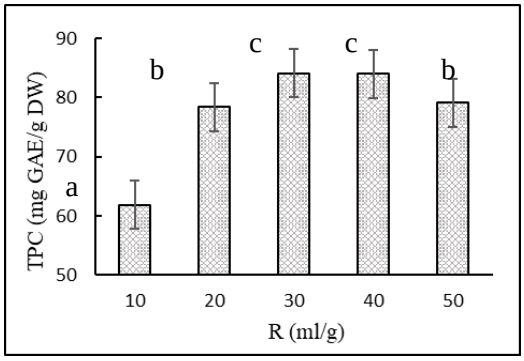


Fig. 2 – Effect of liquid-to-solid ratio on total phenolic content (TPC) using UAE.

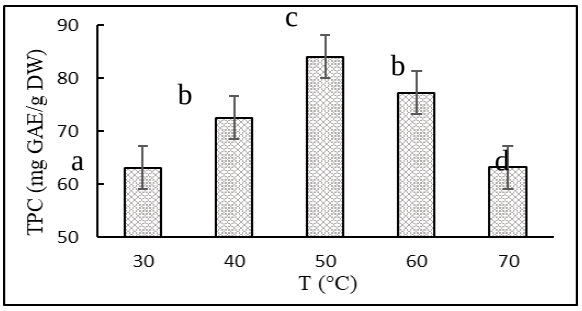


Fig. 3 – Effect of ultrasonic temperature on total phenolic content (TPC) using UAE.

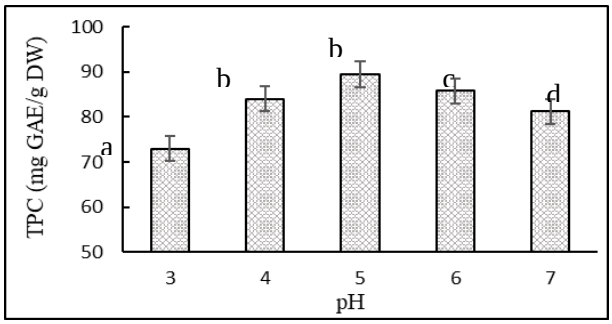


Fig. 4 – Effect of pH on total phenolic content (TPC) using UAE.

Influence of pH on Extraction Yield of Phenolic Compounds*:* 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). Figure 4 showed that when the solvent pH increased from 3 to 5, the TPC increased from ~75 to 90 mg GAE/ g DW. On the other hand, when pH more than 5, our research team mentioned that TPC decreased significantly ($p < 0.05$) to be ~80 mg GAE/g DW at solvent pH equal to 7. The obtained results were in accordance with a previous study done by

Friedman and Jurgens[11] who declared that phenolic compounds stabilities' could be affected by pH values used during extraction step, in addition, they mentioned also that at high pH phenolic compounds could be decomposed.

RSM Model Fitting

The effects and interactions of UAE factors including: t, T, R and pH ; on TPC, TFC, DPPH, and FRAP were optimized with the RSM approch using central composite design (CCD). Table 1 summurized the predicted and experimental values of TPC, TFC as well as DPPH and FRAP assays from *Verbana officinalis* extracts using ultrasound-assisted extraction. As shown in Table 1, TPC, TFC, DPPH and FRAP of *Verbana officinalis* extracts' ranged from 50.33 to 85.50 mg GAE/g DW, 20.24 to 50.53 mg QE/g DW, 27.81-34.11 µg/ml, 963.77 - 991.29 µM BHT/g DW, respectively.

Table 2 illustrated the ANOVA analysis of the complete design to evaluate the results for multiple regression analysis and response surface quadratic model of Y_{TPC}, Y_{TFC}, Y_{DPPH} and Y_{FRAP}. The ANOVA revealed that the four models extremely significant (p-value < 0.001). The coefficients $R^2_{experimental}$ and $R^2_{predicted}$ of the four responses were very close to 1 indicating a significant correlation. Moreover, fitting of the models were verified by in significant lack of fit ($p > 0.05$) values (Table 2).

Effect of the extraction variables on the phytochemicals (TPC and TFC)

Total Phenolic Content, TPC

CCD analysis produced a second-order polynomial equation (Y_{DPPH}), which represented the relationship between TPC and independent variables :

$Y_{TPC} = -117.868 + 1.516 \times t + 4.710 \times T + 1.920 \times R + 9.924 \times pH - 0.016 \times t^2 - 0.057 \times T^2 - 0.031 \times R^2 - 0.901 \times pH^2 + 0.006 \times t \times T - 0.013 \times t \times R - 0.079 \times t \times pH + 0.017 \times T \times R + 0.122 \times T \times pH - 0.115 \times R \times pH.$

Morovers, the linear and the quadratic effects showed a significant effect ($p < 0.05$). Furthermore, it was found that TPC affected principally by t x R, t x pH, T x pH and R x pH ($p < 0.01$) followed by T x R and t x T ($p < 0.05$).

Figure 5 revealed that the effect of the two independent facteurs on TPC when the third was keeped at the middle level. Figure 5A showed that the predicted response surface of the effect of temperature and time on TPC were at a constant liquid-to-solid ratio (30 ml/g) and pH equal to 4. The highest TPC was riched for the range of time at 20-32.5 min and temperature at 30–53°C. This may be due to the higher solubility and diffusion coefficient of polyphenols. However, an upper limit of time and temperature could be affected by degradation of these moleculars.[12] Based on Figure 5B, the increasing in extraction time (10-31 min) and in liquid-to-solid ratio (10- 29ml/g) enhanced the TPC of *Verbana officinalis* using UAE.

Total Flavonoid Content, TFC

$Y_{TFC} = -208.568 + 2.029 \times t + 6.694 \times T + 1.523 \times R + 13.814 \times pH - 0.021 \times t^2 - 0.070 \times T^2 - 0.016 \times R^2 - 1.173 \times pH^2 - 0.012 \times t \times R - 0.092 \times t \times pH + 0.005 \times T \times R + 0.084 \times T \times pH - 0.109 \times R \times pH.$

The linear and the quadratic effects showed a significant effect ($p < 0.05$). Furthermore, it was found that TPC effected principally by t x R, t x pH, T x pH and R x pH ($p < 0.01$) followed by T x R and t x T ($p < 0.05$).

Figure 6 illustrated the effect of the two independent factors on TFC when the third was keeped at the middle level. Based on Figure 6b, when the temperature and pH were fixed respectively at 50°C and 4, the maximum content of flavonoid was attained around 31 min of extraction time and 30 ml/g of liquid-to-solid ratio.

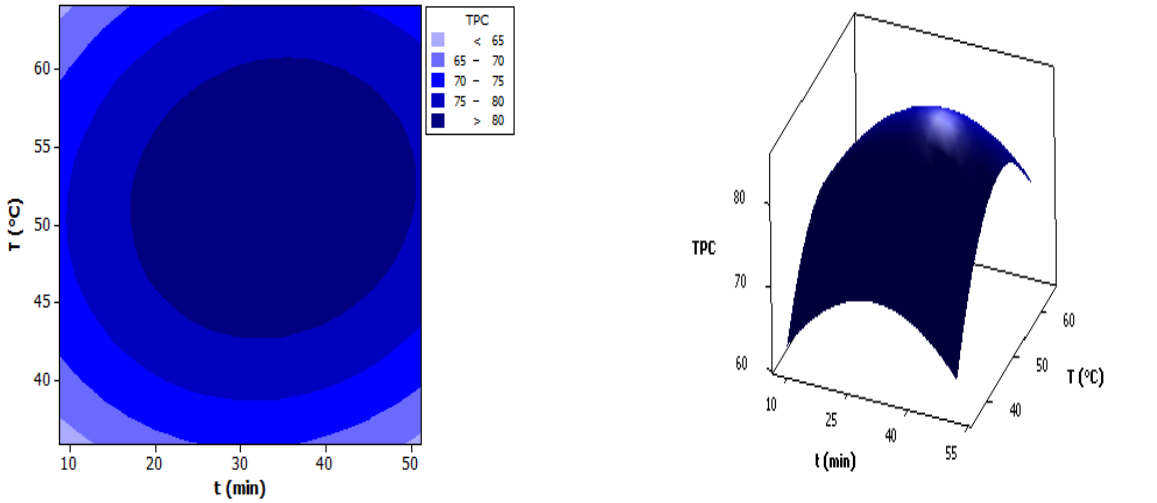
Effect of the extraction parameters on the Antioxidant capacity measured using DPPH and FRAP assays

The model was highly significant for all antioxidant activities (DPPH and FRAP) ($p < 0.001$). The linear and the quadratic effects had a significant effects on DPPH and FRAP. CCD analysis produced a second-order polynomial equation (Y_{DPPH} & Y_{FRAP}), which represented the relationship between the response and their independent variables:

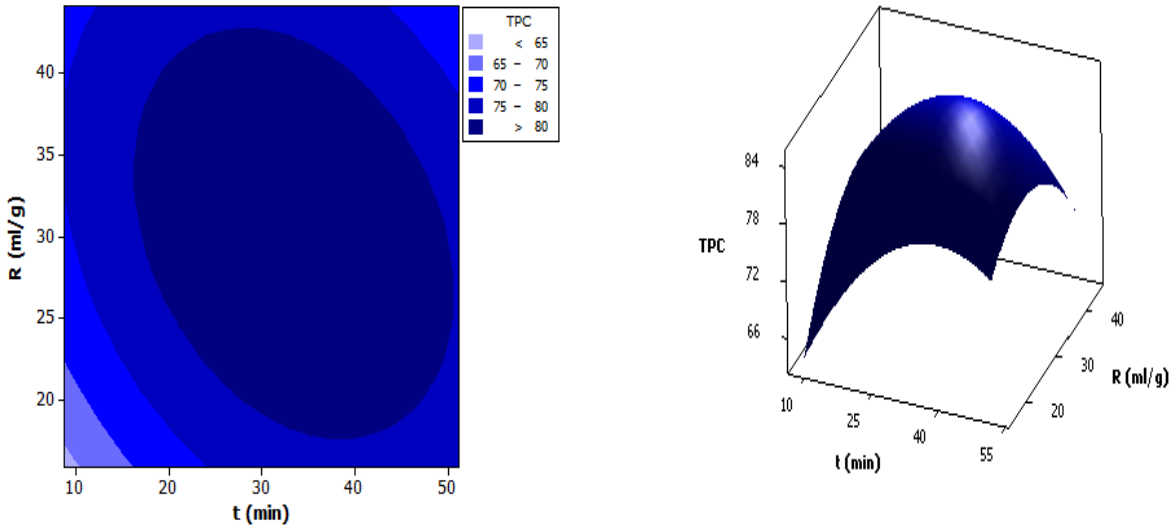
$Y_{DPPH} = 72.717 - 0.3671 \times t - 1.057 \times T\ 0.373 \times R - 2.351 \times pH + 0.003 \times t^2 + 0.010 \times T^2 + 0.004 \times R^2 + 0.195 \times pH^2 + 0.002 \times t \times R + 0.013 \times t \times pH - 0.010 \times T \times pH + 0.015 \times R \times pH.$

$Y_{FRAP} = 794.962 + 1.522 \times t + 4.652 \times T + 1.802 \times R + 8.889 \times pH - 0.016 \times t^2 - 0.055 \times T^2 - 0{,}055 \times R^2 - 0.858 \times pH^2 - 0.003 \times t \times T - 0.012 \times t \times R - 0.065 \times t \times pH + 0.016 \times T \times R + 0.115 \times T \times pH - 0.094 \times R \times pH.$

***Interaction t-T* (a)**



***Interaction t-R* (b)**



***Interaction t-.pH* (c)**

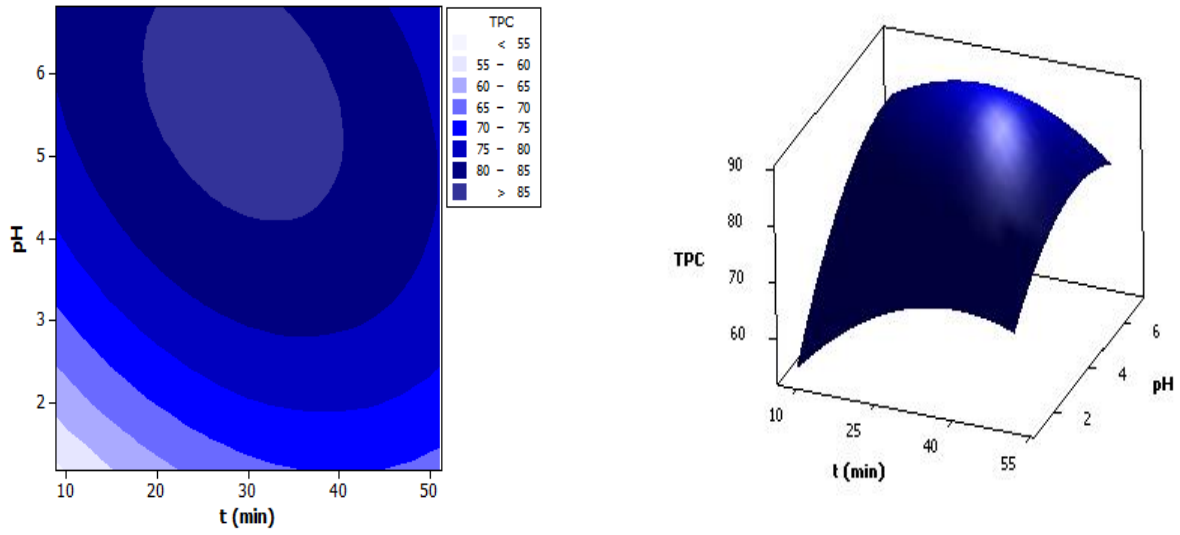
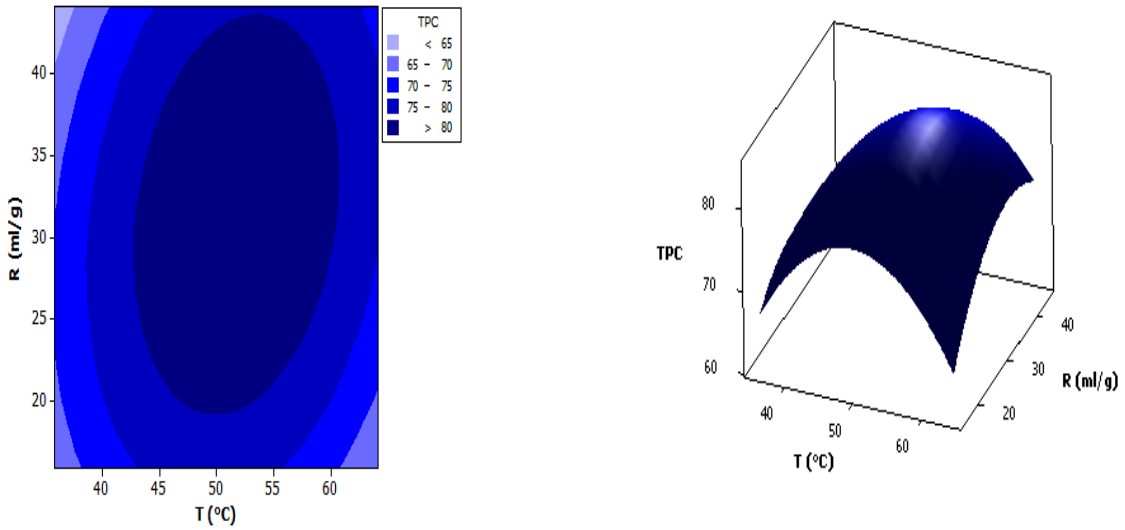
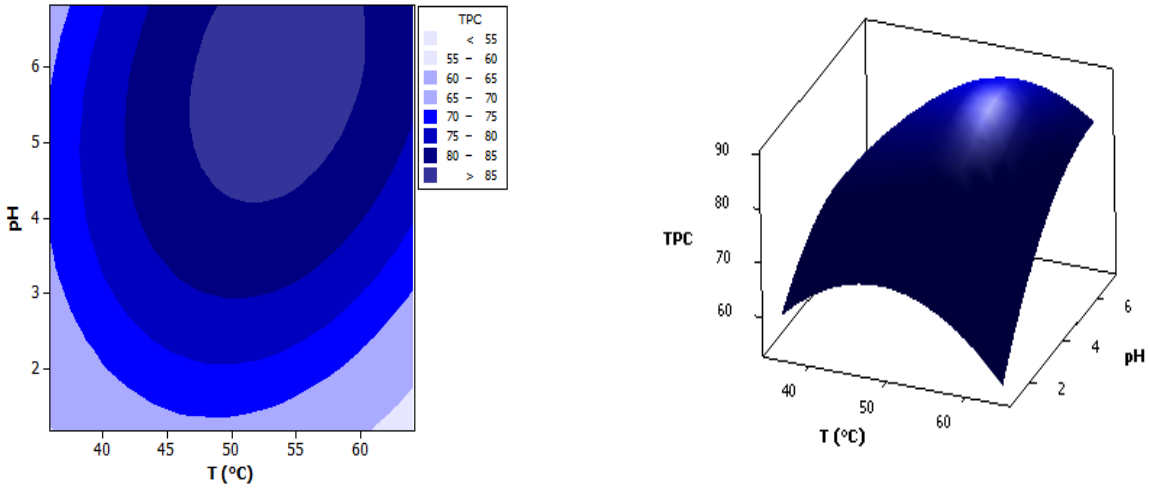


Fig. 5

***Interaction T- R* (d)**



***Interaction T- ipH* (e)**



***Interaction R- ipH* (f)**

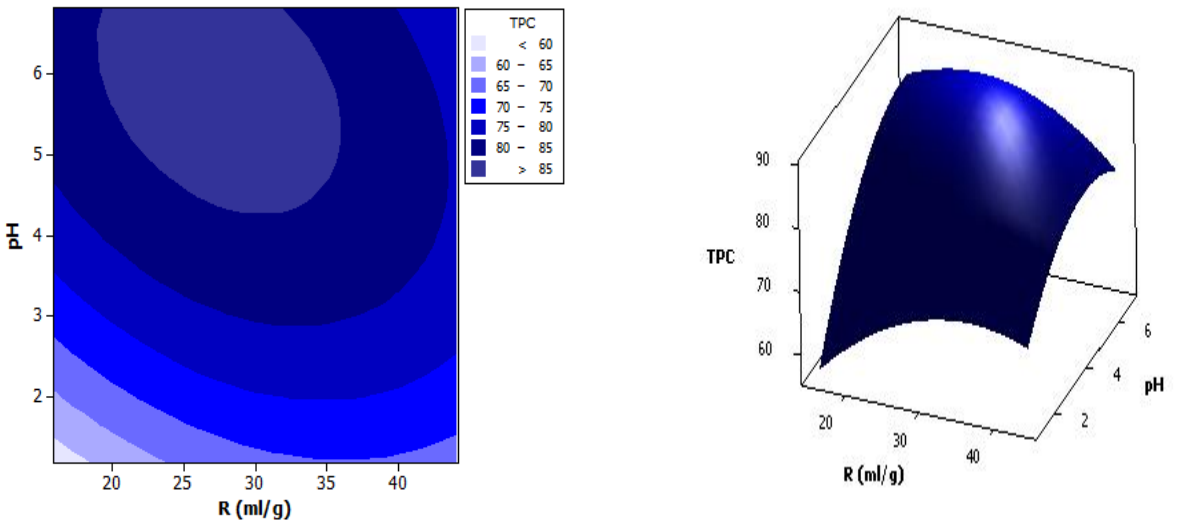
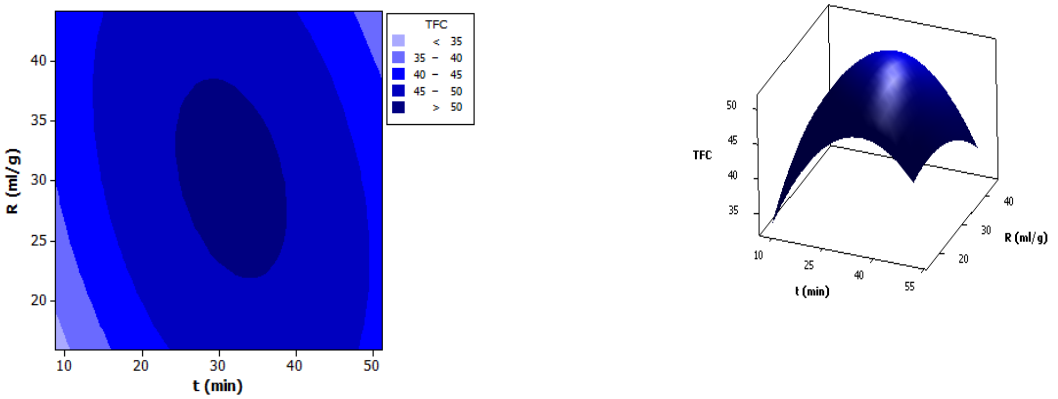
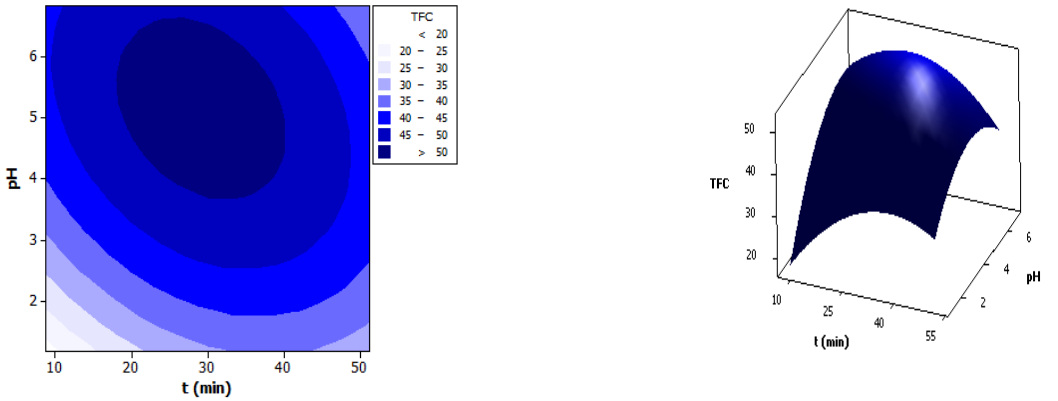


Fig. 5 – Response surface analysis (2D & 3D) on the effect of different factors on TPC of *Verbana officinalis.*

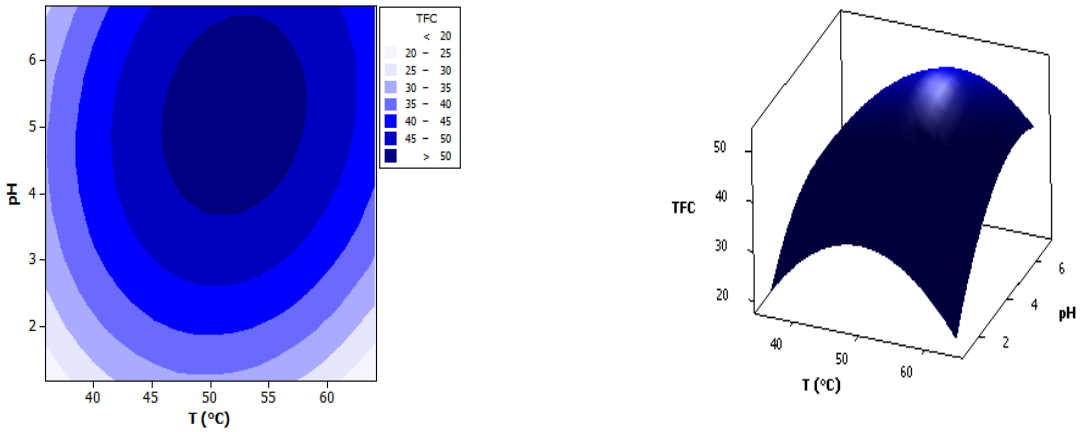
***Interaction t-iR* (b)**



***Interaction t-ipH* (c)**



***Interaction T- ipH* (e)**



***Interaction R- pH* (f)**

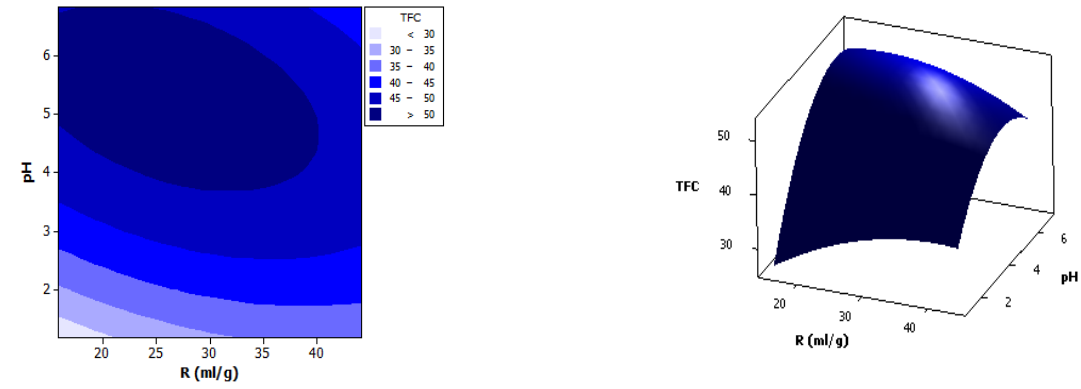
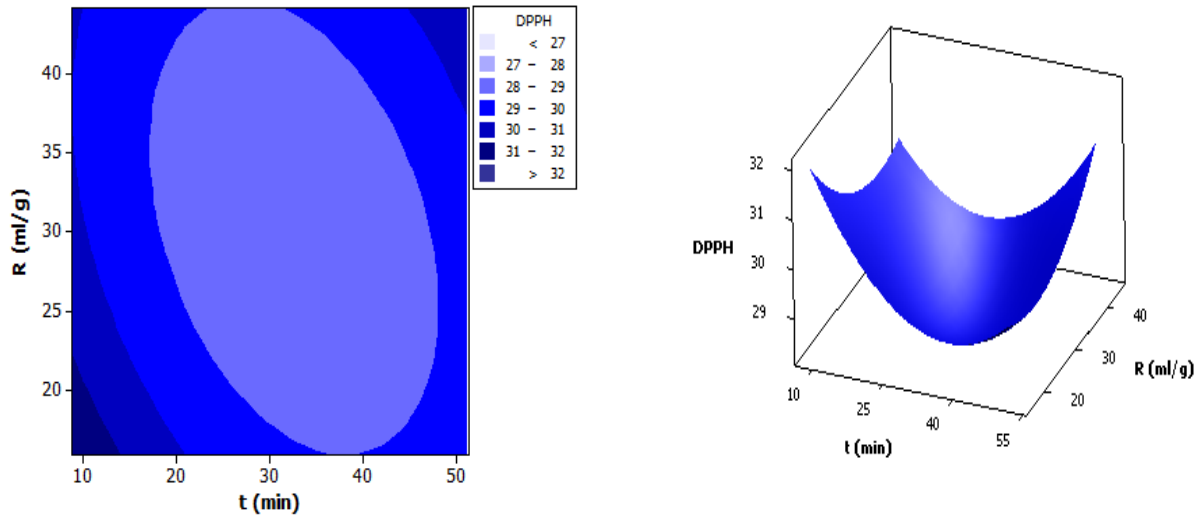
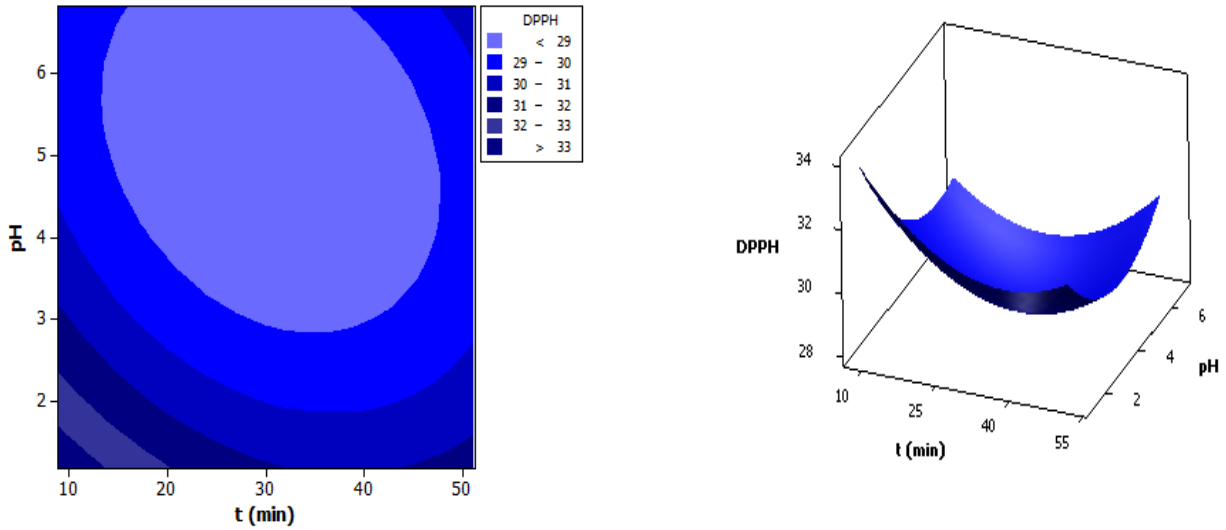


Fig. 6 – Response surface analysis (2D & 3D) on the effect of different factors on TFC of *Verbana officinalis*.

***Interaction t-R* (b)**



***Interaction t-pH* (c)**



***Interaction T- pH* (e)**

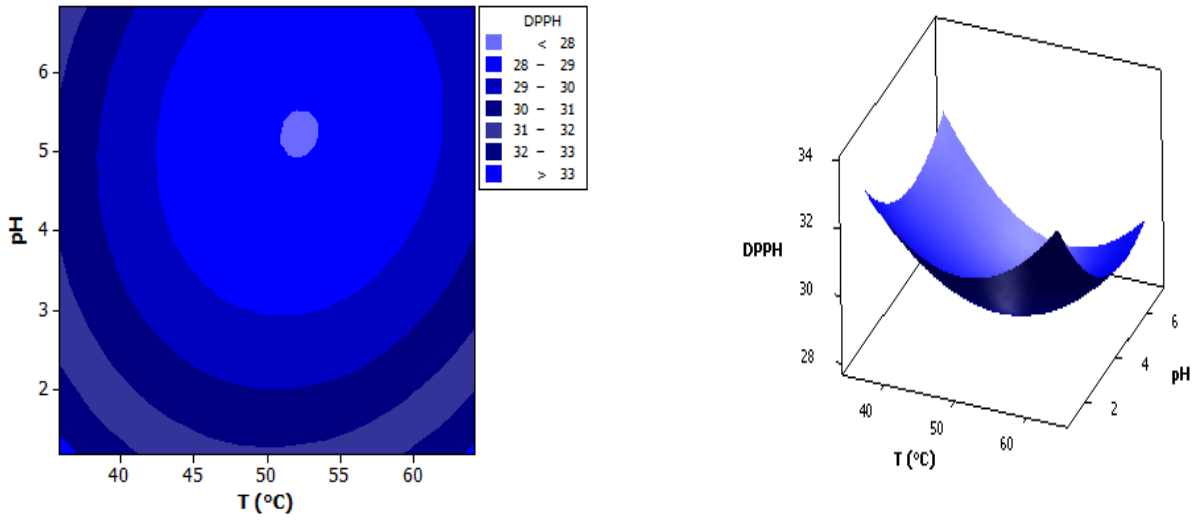


Fig. 7

***Interaction R- pH* (f)**

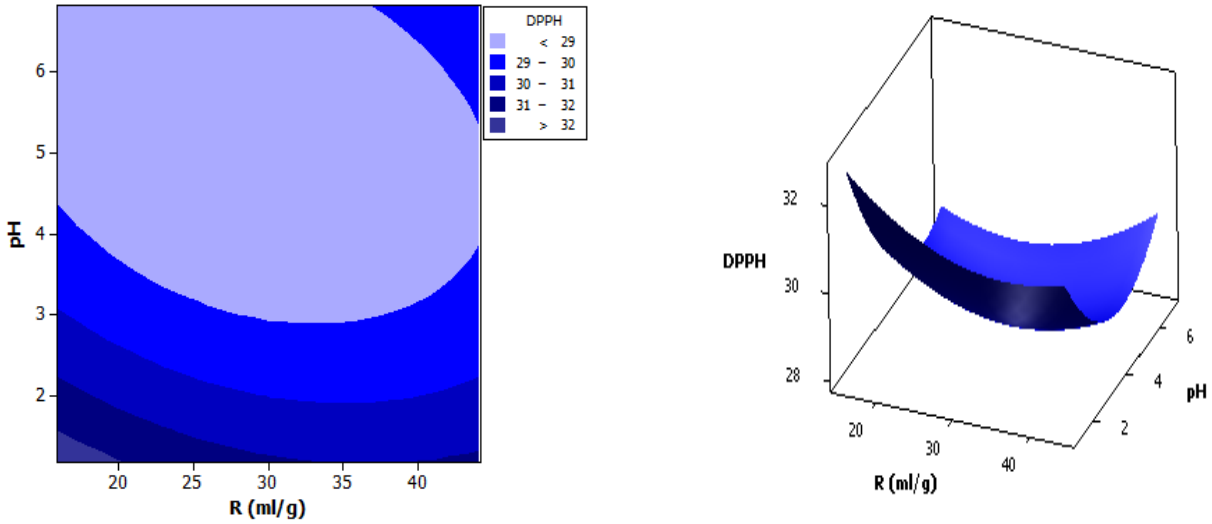
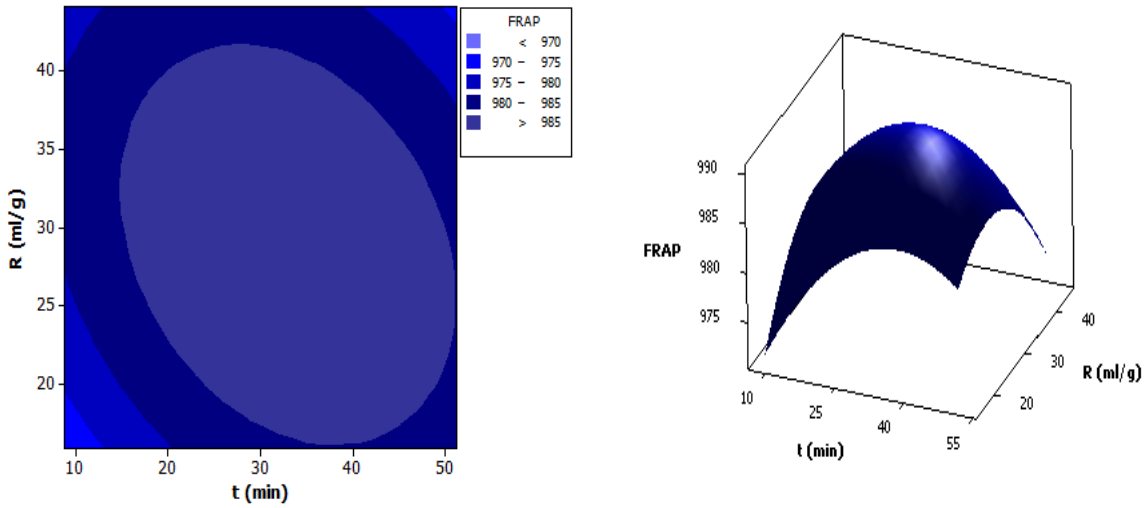


Fig. 7 – Response surface analysis (2D & 3D) on the effect of different factors on DPPH of *Verbana officinalis.*

***Interaction t-R* (b)**



***Interaction t-pH* (c)**

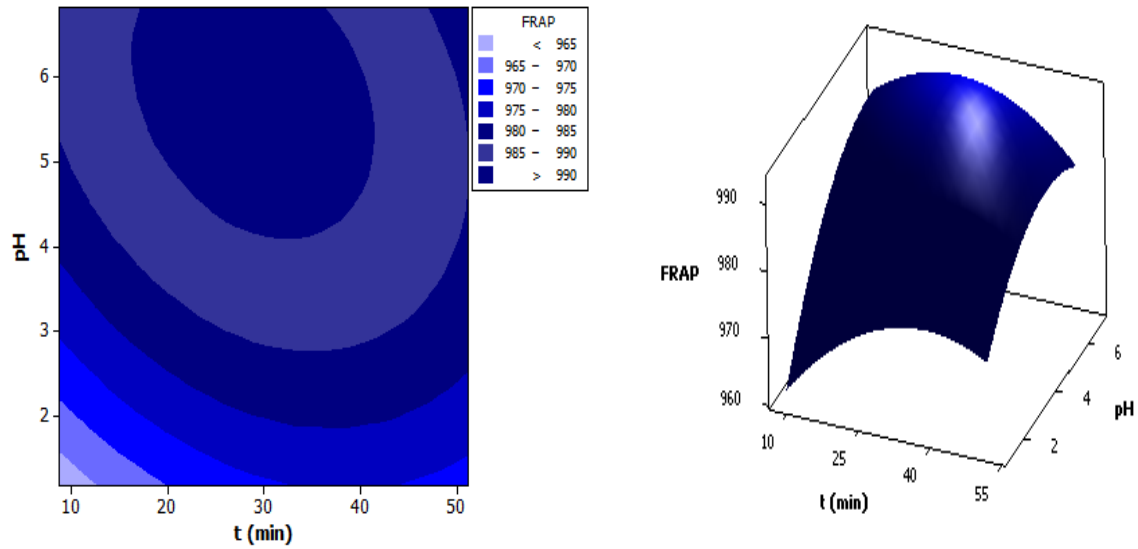
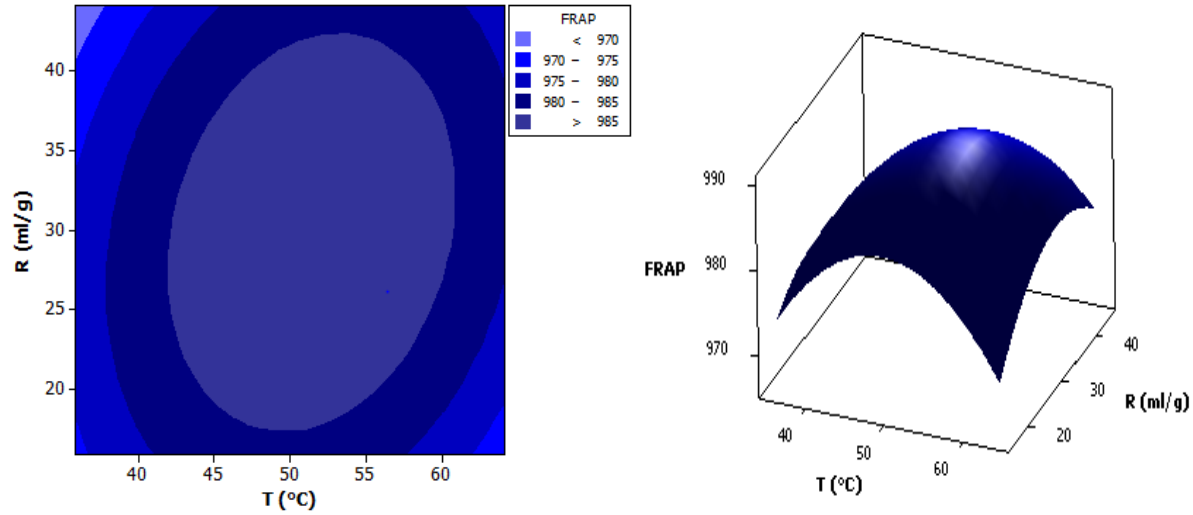
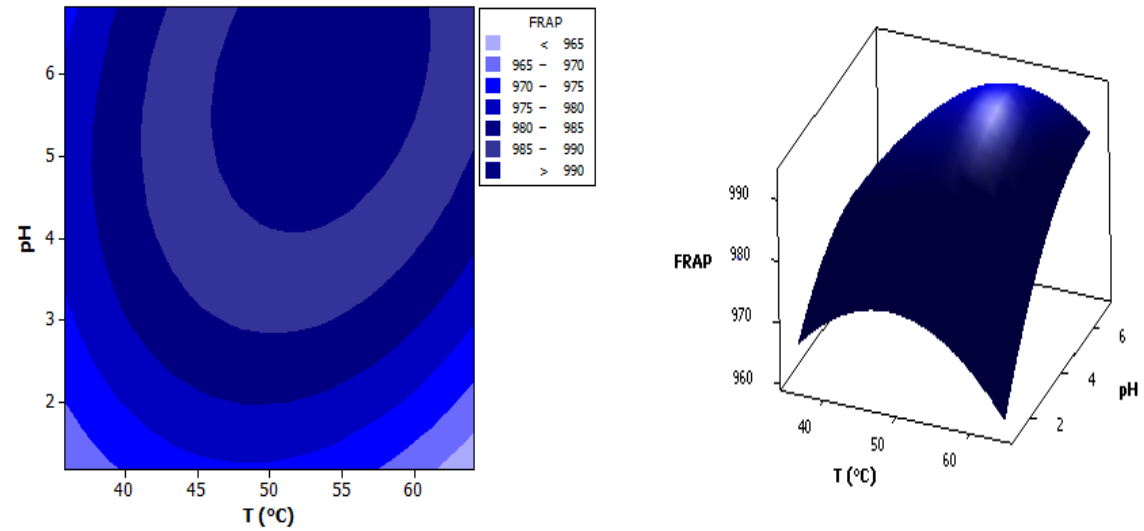


Fig. 8

***Interaction T- R* (d)**



***Interaction T- pH* (e)**



***Interaction R- pH* (f)**

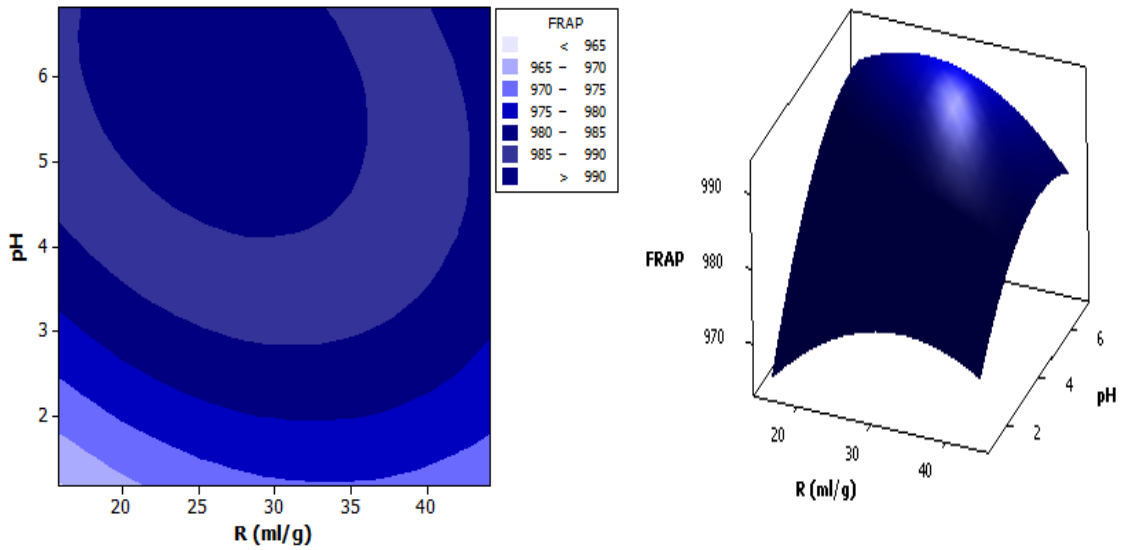


Fig. 8 – Response surface analysis (2D & 3D) on the effect of different factors on FRAP of *Verbana officinalis.*

Optimization and model verification

The optimal conditions were determinated to enhance the total phenolic content, total flavonoid content and the antioxidant activities from *Verbana officinalis* extracts using ultrasound-assisted extraction. The optimal UAE conditions were as shown: extraction time 30.64 min, temperature 52.70°C, liquid-to-solid ratio 28.71 ml/g and pH =5.22. Under these conditions, TPC, TFC, DPPH and FRAP were respectively 87.089 mg mg GAE/g DW, 52.706 mg QE/g DW; 27.975 µg/ml and 992.566 µM BHT/g DW. When these results were compared with the other plant (*Jasminum grandiflorum* L.) using ultrasound extraction[3] : 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 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 890 µM of BHT/g DW.

CONCLUSION

In this study, the conditions for optimized extraction of polyphenols and the antioxidant activity from *Verbena officinalis* maxim by UAE were determine with CCD of RSM. The optimal factors were extraction time 30.64 min, temperature 52.70°C, liquid-to-solid ratio 28.71 ml/g and pH 5.22. The TPC, TFC, DPPH and FRAP were significantly increased under these conditions. The present study shows the efficiency of polyphenol extraction by ultrasound. Other advantages of this method are rapid, simple, green and the obtained *Verbena officinalis* polyphenols can be utilized in food as a natural antioxidant.

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; **RSM:** Response Surface Methodology; **UAE :** Ultrasound Assisted Extraction; **CCD:** Central Composite Design; **DPPH:** 2,2-diphenyl-1- picrylhydrazyl; **t:** time, **T:** Temperature, **R:** liquid-to-solid ratio.

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