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OPTIMIZATION OF PHENOLIC COMPOUNDS EXTRACTION FROM *BELLIS PERENNIS* FLOWERS AND ASSESSMENT FOR ANTIOXIDANT PROPERTIES

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Abstract

Bellis perennis is an interesting daisy not for its ornamental usage but also for its bioactive compounds, particularly phenolics, and related biological effects that explain traditional application for many disorders. The objective of this study was the optimization of phenolic compounds extraction from *B. perennis* flower with microwave-assisted extraction (MAE) using response surface methodology (RSM) and assessing optimal extract for bioactive contents and *in vitro* biological properties. The optimization of phenolic extraction was performed by studying four parameters (solvent concentration, time, microwave power, and solvent to solid ratio). Furthermore, the optimal extract was analyzed for flavonoid content, antioxidant capacity, anti-inflammatory activity, and FTIR analysis. Data fitting to the secondary polynomial model revealed that optimal conditions allowing the best phenolic extraction regarding solvent concentration, time, microwave power, and solvent to solid ratio were 56%, 83s, 200 W, and 68 mL/g, respectively. The phenolic yield obtained using this optimal combination was 135.67 mg GAE/g dw. The obtained optimal extract demonstrated an interesting flavonoids content (27.68 mg EQ/g dw) and expressed a good antioxidant activity measured with DPPH free radical scavenging activity (46.4mg GAE/g dw), ABTS free radical scavenging activity (59.6mg TE/g dw), reducing power (288.0mg GAE/g dw), iron chelating activity (32.7mg EDTAE/g dw), and anti-inflammatory activity (7.9mg IbuE/g dw). FTIR analysis revealed the presence of characteristic functional groups of phenolic compounds in the studied extract. This investigation allowed the development of a validated mathematic model for phenolic

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compounds extraction from *B. perennis* flower using MAE and optimal extract expressed good bioactive contents with the best antioxidant properties.

Keywords: *Bellis perennis* flower, phenolic compounds, extraction optimization, antioxidant properties, anti-inflammatory activity, FTIR analysis

Introduction

The recourse to natural resources and the exploitation of plant heritage for the research of bioactive substances is the current preoccupation of many researchers, manufacturers, and consumers. This is the consequence of the increase in chronic diseases (cardiovascular, diabetes, neurodegenerative, etc.) which are often linked to oxidative stress. Consuming products rich in bioactive compounds such as phenolic *Bellis perennis* L. (common daisy) is one of the botanical species used in traditional medicine with distinguished biological potential. This perennial herbaceous plant of the Asteraceae family is widely distributed in Europe, North America, and North Africa (Al-Snafi, 2015). The aerial parts of *B. perennis* are also consumed as a vegetable in Antakya in southern Turkey and as a condiment or additive used in various food preparations in some Mediterranean countries (Kavalcıoğlu *et al*., 2010). The interest in this species has increased in recent years, due to its broad spectrum of bioactive components and associated activities. *B. perennis* contains many secondary metabolites including terpenes, polyphenols, several anthocyanins and flavonoids, essential oil, saponins, and tannins (Al-Snafi, 2015).

B. perennis has been used for the treatment of wounds, eczema, rheumatism, eye diseases, and respiratory tract infections in folk medicine (Karakas *et al*., 2017). Also for the treatment of colds, bronchitis, and respiratory and inflammation problems (Al-Snafi, 2015). This daisy expresses antidiabetic (Haselgrübler *et al*., 2018), antioxidant (Siatka and Kašparová, 2010), anti-degreasing properties as well as cytotoxic effects against certain human cancer cell lines (Karakas *et al*., 2017).

From all these methods, microwave-assisted extraction gained more attention and demonstrated high efficiency in the research field as well as industrial scale. This method required short treatment times using reduced solvent volumes while offering high extraction yields and extracts with the required quality (Aourach *et al*., 2021).

The objectives of this investigation were, firstly, the optimization of phenolic compounds recovery using microwave-assisted extraction following response surface methodology (Box-Behnken design) from *B. perennis* flower with study ethanol concentration, irradiation power, time, and solvent to solid ratio. Secondly, the optimal extract was assessed for bioactive content, antioxidant activity (free radical scavenging activity, ferric reducing power, and metal chelating ability), anti-inflammatory activity estimation, and Fourier-transform infrared spectroscopy (FTIR) analysis.

Materials and methods

Plant material

B. perennis flowers were harvested during the flowering phase in Chemini (Bejaia, eastern Algeria), and the flowers were dried in a dark ventilated area at room temperature (20±3°C) for about 20 days until weight stabilization. Once dried, the flowers were milled to a fine powder using an electric grinder (IKA, A11model, Staufen, Baden, Germany). The powder passed through a laboratory sieve with a mesh diameter of 125 µm and the collected powder was stored at 4°C in airtight containers until use.

Optimization of phenolic compounds extraction

Experimental design

Optimization of phenolic compounds extraction from *B. perennis* flowers was performed using Response Surface Methodology (RSM) following the quadratic Box-Behnken design (BBD). The independent quantitative parameters considered were ethanol concentration (A), irradiation time (B), power (C), and solvent to solid ratio (D). Three values were chosen for each factor set at the lower, medium, and higher levels. Twenty-seven experiments were performed for the optimization of the four chosen parameters in order to maximize total phenolic content (TPC) and the central point was repeated three times for evaluating the pure error (Table 1).

Microwave-assisted extraction

The extraction of phenolic compounds from *B. perennis* flowers was performed using a microwave system (MAXMOS23S, Maxi power model, 2450MHZ, China). An aliquot of flower powder was placed in a 250 mL round flask containing the appropriate extraction solvent according to the experimental design in which the influence of each parameter was studied. The parameters evaluated were ethanol concentration (40-80%), irradiation time (60-120s), microwave power (200-400W), and solvent to solid ratio (40-80 mL/g). After irradiation, the extracts were recovered by filtration through a Whatman N°1 filter.

Determination of total phenolic content

TPC was determined according to the method of Taga *et al*. (1984). Briefly, a volume of 100 µL of each extract was mixed with 2.0 mL of 2% Na_2CO_3, after 4min, 100 µL of 50% Folin-Ciocalteu reagent was added, and this mixture was incubated in the dark at room temperature for 30 min. The absorbance was measured at 750 nm using a UV/VIS spectrophotometer (Shimadzu UV spectrophotometer). The experiment was performed in triplicate, the polyphenol contents were calculated from a calibration curve performed with gallic acid, and the results were expressed in milligrams of gallic acid equivalent per gram of sample dry weight (mg GAE/g dw).

Characterization of optimal extract

The obtained optimal extract was assessed for total flavonoid content (TFC), diphenyl picrylhydrazyl (DPPH) and azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABST) free radical scavenging assays, ferric reducing power (FRP), anti-inflammatory activity, and FTIR spectral analysis.

Determination of total flavonoid content

The total flavonoid content (TFC) of the daisy extract was determined following the aluminum chloride method as described by Quettier-Deleu *et al.* (2000). A volume of 1 mL of 2% $AlCl_3$ methanolic solution was added to 1 mL of extract, after 15 minutes of incubation in the dark, the absorbance was read at 430 nm using a spectrophotometer. The results were reported as milligrams of quercetin equivalent per gram dry weight (mg QE/g dw).

Table 1. Box–Behnken design with measured and predicted results for MRS matrix on TPC of daisy flowers.

Run	Variables				TPC (mg GAE/g dw)	
	A (%)	B (s)	C (W)	D (mL/g)	Experimental	Predicted
1	60	90	200	80	126.50	125.16
2	40	90	300	80	131.26	131.57
3	60	120	300	80	103.00	100.71
4	80	90	300	40	109.69	106.29
5	60	120	200	60	116.94	113.74
6	60	90	400	80	105.13	106.63
7	40	120	300	60	110.50	114.80
8	60	90	300	60	133.79	131.68
9	60	120	300	40	98.76	99.63
10	60	60	300	40	73.60	79.17
11	60	90	200	40	105.94	104.26
12	60	120	400	60	124.78	120.57
13	60	90	400	40	100.09	101.24
14	60	60	300	80	101.98	104.39
15	60	90	300	60	131.59	131.68
16	40	90	200	60	119.08	121.29
17	80	120	300	60	100.08	104.60
18	80	90	300	80	85.91	85.32
19	40	90	400	60	128.17	128.55
20	40	60	300	60	113.05	108.35
21	40	90	300	40	86.81	84.31
22	60	90	300	60	129.65	131.68
23	60	60	200	60	121.84	122.95
24	80	60	300	60	98.75	94.27
25	60	60	400	60	94.46	94.57
26	80	90	200	60	124.29	127.19
27	80	90	400	60	97.32	98.39

A, ethanol concentration (%); B, extraction time (s); C, microwave power (W); D, solvent to solid ratio (mL/g); TPC, total phenolic content (mg GAE/g dw).

Antioxidant activity

DPPH free radical scavenging assay

The free radical scavenging capacities of ethanolic extract of *B. perennis* flowers were determined using the DPPH assay according to the method described by Alam *et al.*

(2013). Briefly, 0.2 mL of flower extract was mixed with 2 mL of DPPH (0.2mM) dissolved in methanol. The mixture was incubated for 30 minutes at room temperature and the absorbance was recorded at 517 nm against a blank (methanol) using a spectrophotometer. The results were expressed in milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw) using gallic acid as a standard.

ABTS free radical scavenging assay

The scavenging activity against ABTS radicals is based on the ability of bioactive substances to reduce $ABTS^{\bullet+}$ radicals. The percentage of inhibition of $ABTS^{\bullet+}$ was determined by the method of Re *et al*. (1999). The radical solution of $ABTS^{\bullet+}$ was prepared by incubating a mixture of 7 mM ABTS and 2.45 mM potassium persulfate in dark at room temperature for 14 hours. This solution was then diluted with ethanol to an absorbance of 0.7 ± 0.02 at 734 nm. For the test, 100 µL of the extract was added to 2 mL of radical solution. The absorbance was measured at 734 nm after 7min of incubation. The results were reported in milligrams of Trolox equivalent per gram of sample dry weight (mg TE/g DW) using Trolox as a reference.

Ferric reducing power

The ferric reducing power (FRP) of daisy flower extract was evaluated following the method of Oyaizu (1986). In brief, a mixture containing 1 mL of extract, 2.5 mL of phosphate buffer (0.2M, pH 6.6), and 2.5 mL of 1% potassium ferricyanide was incubated at 50°C for 30 min. Then, 2.5 mL of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride after allowing the solution to stand for 10 minutes. The absorbance was measured at 700 nm and the results were expressed in milligrams of gallic acid equivalent per gram of sample dry weight (mg GAE/g dw) using gallic acid as a standard.

Iron chelating activity

Metal chelating activity was evaluated by the ferrozine method according to Abbou *et al*. (2019). For this purpose, a volume of 250 µL of the extract was added to 50 µL of ferrozine (5 mM) and 25 µL of Iron (II) chloride ($FeCl_2$, 2 mM). The mixture was homogenized and incubated for 10 min at room temperature and the absorbance was measured at 562 nm. The inhibition activity was expressed as mg EDTA equivalent/g dry weight (mg EDTAE/g dw) using EDTA as a reference.

Anti-inflammatory activity

The anti-inflammatory capacity *in vitro* of flower extracts was studied using the protein BSA (Albumin Serum Albumin) denaturation test according to the procedure described by Lekouaghet *et al*. (2020). BSA solution (0.5 mL, 0.2%) prepared in Tris buffer (pH 6.8) was added to 0.5 mL of extract. The mixture was incubated at 37°C for 15 min and then immersed in a water bath at 72°C for 5 min. The absorbance was measured at 660 nm after cooling. The results were expressed in milligrams of Ibuprofen equivalent per gram of sample dry weight (mg IbuE/g dw) using Ibuprofen as a standard.

FTIR spectral analysis

FTIR analysis of *B. perennis* dried flower extract obtained under optimal conditions was performed using a Shimadzu FTRI 8400 FT-IR spectrophotometer (IRAffinity-1S,

Shimadzu, Japan) equipped with IR solution software in the scanning range of 500-4000 cm^{-1} with KBr (potassium bromide) pellets and 4 cm^{-1} resolution. The obtained peaks and bounds of functional groups were compared with the spectra reported in the literature.

Statistical analysis

The results were given as means from triplicate independent analyses. The construction of the experimental design, response surface methodology results analysis, and construction of three-dimensional plots were performed by JMP software (Version 13.0). Values were considered statistically significant at $p < 0.05$. ANOVA (Analysis of Variance) was used to estimate the statistical parameters, the significance of the regression coefficients was determined by Student's t-test, and the model was fitted as a second-order polynomial equation.

Results and discussion

Results analysis and construction of optimized extraction model

In order to reach the best conditions allowing maximizing phenolic compounds extraction from *B. perennis* flowers using MAE, four factors with significant influence were studied, namely solvent concentration, microwave power, extraction time, and solvent to solid ratio. The levels chosen for each parameter were analyzed in a preliminary study according to a single-factor methodology (data not shown).

The polyphenol concentrations ranging from 73.6 to 133.8 mg GAE/g dw indicate a significant influence by extraction conditions (Table 1). The experimental data were very close to predicted values through the quadratic TPC model. This was supported by a high level of the correlation coefficient (R^2) showing a value of 0.97 indicating that 97% of experimental data were explained by the developed model.

The analysis of variance regarding the developed TPC model, as shown in Table 2, indicated a Fisher ratio of 27.13 which corresponds to the probability of less than 0.0001, indicating a high degree of significance of the model.

Table 2. Adjustment and analysis of variance (ANOVA) of TPC model.

Source	DF[a]	Sum of Squares	Square of means	*F* Ratio	Prob. > F
Model	14	6420.69	458.62	27.13	<0.0001
Error	12	202.87	16.91		
Total model	26	6623.56			
Lack of Fit	10	194.30	19.43	4.54	0.194
Pure error	2	8.57	4.28		
Total error	12	202.87			
R^2	0.969				
Adj-R^2	0.934				

[a] Degrees of freedom; * Value statistically significant at $p < 0.05$.

In addition, the model presented a lack of fit with an F-value of 4.54 giving a probability of 0.19, higher than the significance threshold set ($p<0.05$), indicating that the error on the fitted model was not significant. Therefore, these findings demonstrated the suitability and adequacy of the TPC model in the prediction of the response.

The total polyphenol content was significantly influenced ($p < 0.05$) by all linear parameters (A, B, C, and D), with a positive linear effect for time and solvent to sample ratio indicating that the increase of these two parameters allowed to better TPC extraction, but with negative linear influence when the ethanol concentration and microwave power increased (Table 3).

Table 3. Regression coefficient, standard error, and Student's t-test results of response surface for TPC model.

Source	Estimate	Standard error	*t* ratio	Prob.>|*t*|
Intercept	131.68	2.37	55.47	<0.0001*
A	-6.07	1.19	-5.11	0.0003*
B	4.20	1.19	3.54	0.0041*
C	-5.39	1.19	-4.54	0.0007*
D	6.57	1.19	5.54	0.0001*
AB	0.97	2.06	0.47	0.6454
AC	-9.01	2.06	-4.38	0.0009*
BC	8.80	2.06	4.28	0.0011*
AD	-17.06	2.06	-8.30	<0.0001*
BD	-6.03	2.06	-2.94	0.0125*
CD	-3.88	2.06	-1.89	0.0837*
A^2	-10.14	1.78	-5.69	0.0001*
B^2	-16.03	1.78	-9.01	<0.0001*
C^2	-2.69	1.78	-1.51	0.1572
D^2	-19.67	1.78	-11.05	<0.0001*

A, ethanol concentration (%); B, extraction time (s); C, microwave power (W); D, solvent to solid ratio (mL/g); TPC, total phenolic content (mg GAE/g dw); * Value statistically significant at $p < 0.05$.

Four interaction effects were noticed for the studied factors of which three AC, AD, and BD were negative, meaning that the simultaneous increase of both parameters induced a decrease in TPC. The response displayed negative significant quadratic effects concerning ethanol concentration, time, and solvent to sample ratio. This indicated that at certain levels of these parameters, TPC was rapidly decreased indicating that the conditions become not suitable for phenolic extraction.

Therefore, the predictive mathematical equation given below showed the relationship between the TPC response and the four factors studied (Eq. 1).

$$Y_{TPC} = -6.07A + 4.20B - 5.39C + 6.57D - 9.01AC + 8.80BC - 17.06AD - 6.03BD - 3.88CD - 10.14A^2 - 16.03B^2 - 19.67D^2 \quad (1)$$

where Y_{TPC}, Predicted TPC (mg GAE/g dw); A, ethanol concentration (%); B, Microwave power (W); C, extraction time (s); D, solvent to solid ratio (mL/g).

The relationships of independent variables (factors) and their interactions with the dependent variable (TPC) were plotted on three-dimensional response surfaces (Figure 1). Total phenolic content was increased with ethanol concentration and extraction time increase (Figure 1a); the highest TPC value was reached at 56% and 83 s, respectively. Exceeding these optimal values, the phenolic yield was significantly reduced with a 27% loss by using the highest levels of the two parameters, 80% for solvent concentration and 120 s for extraction time. The same results were observed by Jaafar *et al.* (2020) who found that ethanol concentration at 51% was the best solvent for phenolic extraction from *Clitorea ternatea* flowers.

The increase in ethanol portion decreased polarity which improved the extraction of phenolics until reaching 56% ethanol. Nevertheless, when ethanol becomes higher than optimal concentration the adequacy of the polarity of resulted solvent will be reduced and the phenolics yield was decreased.

Time was an important parameter in phytochemicals extraction. The desired compounds were accumulated during the extraction process taking the time necessary for solvent osmosis inside particles and the outside solute diffusion until reaching an equilibrium of concentration between the matrix and extraction medium (solvent). Our results were consistent with those other studies that suggested that treatments with short extraction times might be more effective for TPC using MAE and that longer extraction times tended to decrease the yield of phenolic compounds (Sanchez-Reinoso *et al*., 2020; Pengdee *et al*., 2020). Beyond optimal time (83 s), the TPC decrease was observed and this could be related to the degradation of thermolabile compounds already extracted. Such degradation was linked to a possible increase of temperature under the microwave radiation increasing thus oxide-reduction reactions and thereby degradation of bioactive compounds, particularly with prolonging time and exposure to dissolved oxygen in extraction solvent (Bachir bey *et al*., 2013; Ismail-Suhaimy *et al*., 2021).

The effects of solvent concentration and microwave power were shown in Figure 1b. The linear negative significant influence and no significance of the quadratic effect of microwave power on TPC indicated the reduction of phenolics yield with the increase of this parameter. The use of lower microwave power (200 W) was more adequate for phenolic extraction from *B. perennis* flowers but the increase of this parameter resulted in a gradual TPC decrease. This could explain by the sensitivity of phenolic compounds of *B. perennis* flowers to microwave effects. Therefore, 200 W was retained as the best microwave power for phenolic extraction.

Similarly, it was observed that the optimization of microwave-assisted extraction for antioxidants from waste *Achillea millefolium* dust revealed that the lower microwave power (170 W) was the best for polyphenols and flavonoids extraction and the increase of microwave power caused the rising in temperature and enhanced the extraction of components other than polyphenols (Milutinović *et al*., 2015).

The solvent to solid ratio presented a significant influence on both linear and quadratic effects (Figure 1c). TPC increased gradually with the ratio rising from 105 mL/g (ratio 40) until reaching 68 mL/g but decreased for higher ratios. This is consistent with the mass transfer principle that is based on the gradient of

concentration between solid (plant matrix) and liquid (extraction solvent), when a high solvent to solid ratio is used, the solute extraction was better (Bachir bey *et al*., 2013). The high volume of extraction solvent may provide more dissolved oxygen causing an increase in phenolic oxidation particularly with a long extraction time under high temperatures (Saci *et al*., 2017).

The RSM 3D plots showing influences of time and microwave power, time and solvent to solid ratio as well as microwave power and solvent to solid ratio were illustrated in Figure 1d, e, and f. The quadratic effect of time and the negative linear influence of microwave power were observed in Figure 1d. From Figure 1e and f the quadratic effects of time and solvent to solid ratio on phenolics extraction were seen by the increase then decrease of the response indicating the sensibility of phenolic compounds recovery to these two factors.

Determination of optimal conditions for phenolic extraction and model validation

In order to select the optimal phenolic compounds extraction from *B. perennis* flowers, the mathematic equation was resolved using the prediction profiler of JMP software. The predicted best extraction conditions were 56%, 83 s, 200 W, and 68 mL/g for solvent concentration, time, microwave power, and solvent to solid ratio, respectively.

The last step that must be performed was the validation of the polyphenols extraction model, for this additional experiments were performed under the optimal conditions obtained by RSM and were compared to those obtained by the prediction equation. The measured TPC value was 135.67±6.89 mg/g which was very close to the predicted value of 136.60 mg/g indicating that the model was adequate and reliable for the extraction process of phenolic compounds from *B. perennis* flowers.

Characterization of optimal extract

Total phenolic and flavonoid contents

The phenolic content of daisy flowers was evaluated from the extract obtained under the determined optimal conditions. The TPC result revealed a yield of 135.67±6.89 mg GAE/g dw. The content of phenolic compounds of analyzed *B. perennis* flowers was higher than that obtained by conventional methods. Indeed, TPC obtained from daisy flowers, collected at three different regions for eight months and extraction performed twice with 60% ethanol using a shaker water bath at 60 °C for 10 min, ranged from 28.1 to 35.7 mg/g (Siatka and Kašparová, 2010). TPC obtained using soxhlet was 4, 43, 52, and 11 mg/g using hexane, dichloromethane, methanol, and water as extraction solvents, respectively (Karakas *et al*., 2017). MAE is based on localized and rapid heating which may promote the extraction of phenolic compounds giving a high yield in a reduced time (Mandal and Mandal, 2010).

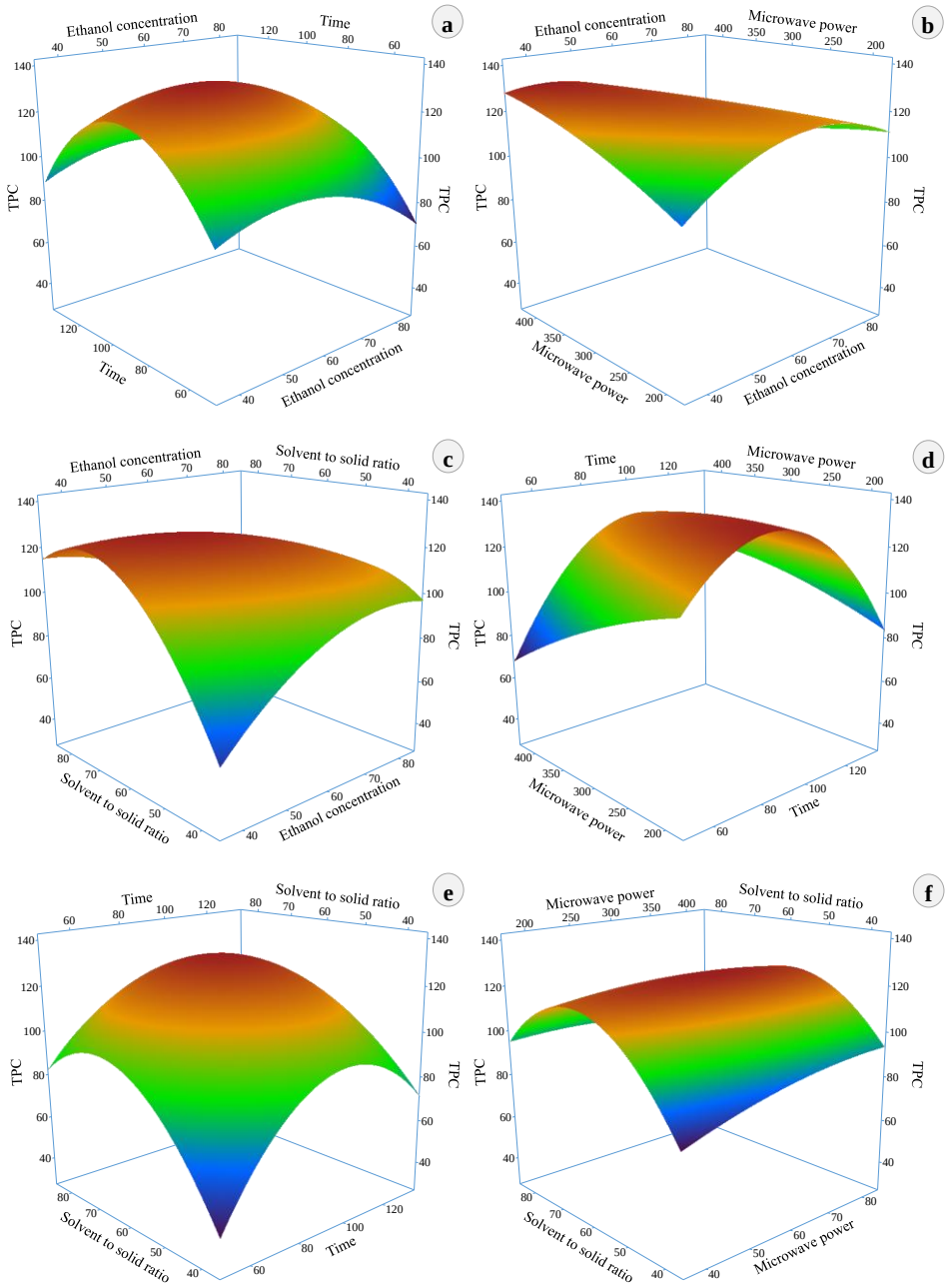

Figure 1. Three-dimensional surface plots illustrating the impacts of MAE extraction on TPC. (a): ethanol concentration and time, (b): ethanol concentration and microwave power, (c): ethanol concentration and solvent to solid ratio, (d): microwave power and time, (e): solvent to solid ratio and time, (f): solvent to solid ratio and microwave power.

Flavonoids are other phenolic interesting groups commonly found in plants particularly flowers (de Morais *et al*., 2020). Flavonoids content of *B. perennis* flowers obtained under optimal extraction conditions was 27.68 mg EQ/g dw. The obtained content was relatively higher than that found by Siatka and Kašparová (2010) who revealed concentrations from 22.0 to 13.7 mg/g. Total flavonoid content obtained by drying methods using hot-air dried and microwave as well as fresh flowers were 11.90, 15.86, and 16.45 mg/g, respectively, but that obtained by freeze-drying (25.62 mg/g) agree with that obtained in the present study (Dorozko *et al*., 2019).

Antioxidant activity

DPPH radical scavenging assay

The DPPH free radical scavenging activity of *B. perennis* flowers of optimal extract presented 46.4 mg GAE/g dw (Table 4). These results suggested that daisy flower extract was a good DPPH free radical scavenging agent as suggested by Siatka and Kašparová (2010).

ABTS radical scavenging assay

The ABTS test was also used to evaluate the ability of substances to scavenge free radicals. The flower extract obtained under optimal conditions showed an interesting free radical scavenging activity with the value of 59.6 mg TE/g dw. Some reporters suggested that flower extracts expressed a good ABTS antioxidant activity such as arabica and conilon coffee flowers (de Abreu Pinheiro *et al*., 2021) and *Chromolaena scabra* flowers (Villamil *et al*., 2020).

Ferric reducing power

The reducing power methods were common techniques that measure the potential of metals' reducing ability; the model usually used was the reduction of ferric ions (Fe^{3+}) to ferrous (Fe^{2+}) (Shahidi and Zhong, 2015). The extract obtained under optimal conditions showed an excellent antioxidant activity with 288.0mg GAE/g dw (Table 4). This interesting reducing potential confirmed the work performed by Kavalcıoğlu *et al*. (2010).

Iron chelating activity

The metal chelating ability of ethanolic flower extract was measured by the formation of a ferrous ion ferrozine complex. Phenolic compounds were considered secondary antioxidants because they compete with ferrozine to form a metal complex, thus reducing the redox potential of ferrous iron by stabilizing the oxidized form of metal ions (Jamuna *et al*., 2012). According to the obtained result, optimal extract expressed chelating activity of 32.7 mg EDTAE/g dw. This activity was comparable to that of male date palm flowers (Karra *et al*., 2020).

Anti-inflammatory activity

From the results, it was found that the concentration that allows the inhibition of denaturation at 50% of albumin obtained for optimal extract was 7.9 mg IbuE/g dw (Table 4). The *in vitro* anti-inflammatory activity measured by LPS-induced NO from different *B. perennis* flower extracts obtained by hexane, dichloromethane,

methanol, and water were studied by Karakas *et al*. (2017). The authors found that methanolic extract had a stronger inhibitory effect (96.3% inhibition) than dichloromethane (86.4% inhibition), hexane (25.3% inhibition), and water (14.2% inhibition) extracts at a concentration of 20 µg/mL. The best anti-inflammatory property of *B. perennis* flowers may be attributed to particularly phenolic and flavonoid constituents. These also support the traditional uses of this plant in inflammatory-related diseases such as rheumatism, tonsillitis, and eczema, eye disorders.

Table 4. Optimal extraction conditions and maximal predicted and experimental TPC, and optimal extract antioxidant characteristics of daisy flowers.

Optimal results		Characterization of optimal extract	
Parameter	Optimal values	Antioxidant parameter	Antioxidant values
A (%,v/v)	56	TFC	27.68±2.71
B (s)	83	DPPH-FRSA	46.4±0.77
C (W)	200	ABTS-FRSA	59.6±1.59
D (mL/g)	67:1	FRP	288±5.02
Experimental TPC	135.67±6.89	ICA	32.7±5.61
Predicted TPC	136.60	AIA	7.9±0.23

A - ethanol concentration (%); B - extraction time (s); C - microwave power (W); D - solvent to solid ratio (mL/g); TPC - total phenolic content (mg GAE/g dw); TFC - total flavonoid content (mg QE/g dw); DPPH-FRSA - DPPH free radical scavenging activity (mg GAE/g dw); ABTS-FRSA - ABTS free radical scavenging activity (mg TE/g dw); FRP - ferric reducing power (mg GAE/g dw); ICA - iron chelating activity(mg EDTAE/g dw); AIA - Anti-inflammatory activity (mg IbuE/g dw).

FTIR analysis

The extract of *B. perennis* flowers obtained under optimal conditions by MAE was characterized by FTIR spectroscopy and several functional groups were identified (Figure 2). The broad band observed at about 3450 cm^{-1} was attributed to O-H bonds of hydroxyl groups, which indicated the presence of phenolic compounds in the extract (Rahmani *et al*., 2020). The spectra also display an intense peak at about 1645cm^{-1}, due to aromatic C=C bonds. Oliveira *et al*. (2016) also observed a band at 1640 cm^{-1} from FTIR analysis of polyphenol extracts and reported that the presence of a strong absorption peak in the interval 1640-1670 cm^{-1} corresponds to the presence of flavonoids. The third broad peak at about 500 cm^{-1} was associated with the vibrations of aromatic C-H. The association of all these absorption bands indicated the presence of phenolic compounds in the prepared extract from *B. perennis* flowers.

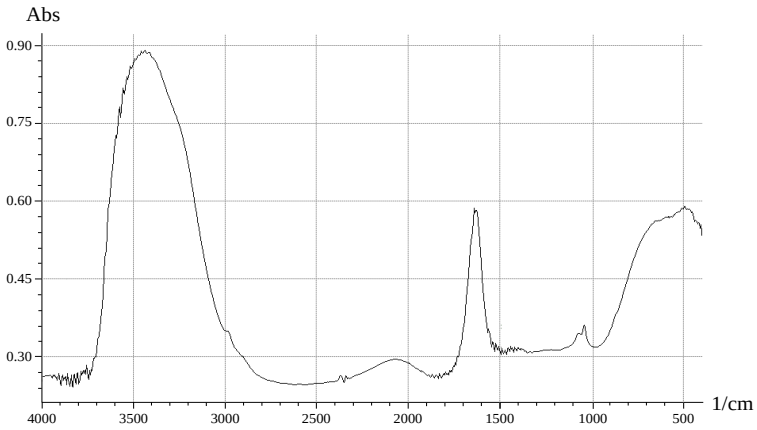

Figure 2. FTIR spectrum of *B. perennis* extract obtained using optimal conditions.

Conclusion

From the present study, it can be retained that extraction of phenolic compounds from *B. perennis* by MAE was influenced significantly by the four tested parameters, and the optimization investigation through the Box-Behnken design allowed fitting the extraction to the quadratic model. This last expressed a high significativity and explained adequately the phenolics recovery. The optimal values obtained by the mathematic model were experimentally confirmed indicating its accuracy and validity. The optimal extract expressed a good antioxidant activity measured by four methods and expressed an interesting anti-inflammatory activity. *B. perennis* represented an attractive source of phenolic antioxidants that justify at least a part of its use in traditional medicine, therefore, furher investigations and applications are required.

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