



Original research

Response surface optimization of polyphenol extraction from petals of *Hibiscus-rosa sinensis*

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ABSTRACT

Edible flowers of *Hibiscus rosa-sinensis* have been identified as a source of antioxidant phenolics. Thus, effective extraction of phenolics is important to efficiently utilize these flowers. The present study aims to make use of a central composite design, to investigate the effects of extraction variables on the three response variables; total phenolic content, total anthocyanin content and antioxidant activity. Central composite design with four independent variables including solid: liquid ratio, ethanol concentration, temperature and time were used for the simultaneous optimization of response variables. The optimum process parameters generated were 24.0% ethanol, 1:40 solid: liquid ratio, 44°C temperature and 41 minutes of extraction. The experimental values obtained for the response variables under the generated optimum conditions confirmed the validity of the proposed second-order polynomial model. The results from the simultaneous optimization demonstrated the application of feasible process parameters for the extraction of phenolics from *Hibiscus rosa-sinensis* flowers and the effective utilization of these flowers in food as well as the pharmaceutical industry.

Keywords: *Hibiscus rosa-sinensis*; Optimization; Phenolics; Response surface method

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1. Introduction

Hibiscus rosa-sinensis (HS) is a perennial ornamental shrub belonging to the family of Malvaceae. This plant is widely cultivated in China and countries around the Indian and Pacific oceans. Traditionally edible flowers of this plant have been consumed as infusions and decoctions by different communities. Pharmacologically various extracts of HS flowers have been reported to exhibit diverse biological properties such as antioxidant, anti-inflammatory, analgesic, antipyretic, anti-diabetic and anti-asthmatic activities (Missoum, 2018; Janarny et al., 2021(a); Janarny et al., 2021(b)). Owing to its established biological properties recently, demand for exploiting these flowers for consumption as well as for extensive research related to oxidative stress and chronic diseases have increased drastically.

It is a well-known fact that the biological properties of plant sources correspond to the bioactive compounds present in these sources (Gunathilake, 2020). Previous studies conducted on HS flowers have reported the presence of various active compounds in HS flowers and their related biological properties. For example, fractionated water-soluble fractions of HS methanolic extracts exhibited a strong anti-implantation activity and were detected with quercetin-7-O-galactoside, neochlorogenic acid, p-hydroxybenzoic acid, gallic acid and apigenin (Salib et al., 2011). Quercetin-3-O-sophoroside isolated from fractionated ethanolic extracts of HS flowers was reported to control scopolamine-induced amnesia in mice (Shen et al., 2021). Based on GC-MS analysis, 1, 2 Benzene dicarboxylic acid from methanolic extracts of HS flowers were able to exhibit anti-microbial activity against the growth of *Chlamydia trachomatis* and *Treponema pallidum* (Vijayakumar et al., 2018). Thus as evidenced, it is vital to efficiently extract appropriate bioactive compounds for the effective utilization of HS flowers.

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Various techniques such as solid-liquid extraction (SLE), microwave-assisted extraction and supercritical fluid extraction have been employed to extract the bioactive compounds from plant matrixes. However, each method has its limitations and developing a single efficient method to extract maximum bioactive compounds from the plant matrix is yet a challenge. SLE is a widely used technique due to its reliability and convenience (Ongkowijoyo et al., 2018; Gunathilake et al., 2019, Gunathilake et al., 2020). Thus, the present study employs SLE to extract bioactive compounds from HS flowers. The phenolic content and the efficacy of extraction by SLE can be influenced by various factors such as the extraction time, temperature, solid to liquid ratio, solvent concentration, etc. To obtain the maximum possible yield, it is important to optimize the process variables that influence the extraction. Optimization facilitates employing a simple, cost-effective, labor-intensive process to discover conditions to obtain maximum yield of bioactive compounds from plant matrix.

Response surface methodology (RSM) is an extensively used multivariate statistical technique to optimize experimental conditions in research-based analytical procedures. RSM is a collection of mathematical and statistical approaches which fits a polynomial equation to the experimental data, that could describe the behavior of a data set facilitating statistical interpretations (Bezerra et al., 2019). By establishing a model equation RSM evaluates the relationship as well as the interactive effects among the multiple independent variables on the response variables. It is very useful in maximizing or minimizing various response variables and optimizing several independent variables simultaneously. Also, it acts as a visual aid to identify the region

where the response variables are optimized (Azahar et al., 2017). Despite various studies conducted on HS flowers, to the best of our knowledge investigations on simultaneous optimization of process parameters for extraction of polyphenols from HS flowers is scarce. Thus, the present study aims to optimize four process parameters simultaneously to maximize the yield of total polyphenols, anthocyanins along with maximum antioxidant activity from HS flowers using an ethanol-based solvent system.

2. Material and Methods

2.1. Sample preparation and extraction

Red HS flowers were collected from Makandura area of Sri Lanka and the voucher specimen was deposited in the herbarium of Wayamba University of Sri Lanka. The stalks were removed and the flowers were cleaned and washed appropriately and then freeze-dried. The freeze-dried flowers were powdered and stored at -18°C until further analysis.

Different concentrations (40 to 100%) of ethanol were mixed with 1 g of freeze-dried samples at different volumes (20-40 mL). Then the samples were homogenized and exposed to different temperatures (30 to 60°C) for varying periods (30 to 60 minutes). The obtained crude extracts were filtered through Whatman filter paper # 1 and stored at -4°C until further analysis. The combinations of the process parameters were based on the experimental design generated by Minitab statistical software version 17.

Table 1. Central composite design for process variables and corresponding response variables.

Runs	Process variables				Response variables		
	Solid : Liquid ratio (X_A -w/v)	Ethanol concentration (X_B -%)	Temperature (X_C -°C)	Time (X_D -min)	TPC (mg GAE/g DW)	TAC (mg Cy-3-Glu/g DW)	DPPH (%/g DW)
1	40	40	30	30	159.31	0.43	39.53
2	30	130	45	45	66.34	0.16	65.98
3	30	70	45	45	81.77	1.82	61.34
4	20	40	60	60	91.09	1.07	36.97
5	40	40	60	30	114.74	0.43	41.24
6	20	100	30	60	36.23	0.64	59.38
7	30	70	45	45	110.32	0.85	44.68
8	40	100	30	60	8.46	1.12	71.23
9	30	70	45	45	101.49	0.64	60.75
10	20	40	30	30	92.23	0.96	56.12
11	40	100	60	30	38.17	0.64	35.00
12	40	100	60	60	18.74	2.14	57.02
13	30	70	45	45	77.49	2.24	61.19
14	20	100	60	60	30.51	0.27	60.46
15	30	70	45	45	65.21	0.85	50.24
16	30	70	45	45	72.43	0.64	66.45
17	30	70	75	45	84.34	1.50	60.61
18	40	100	30	30	6.40	1.76	71.66
19	40	40	60	60	127.31	0.64	54.19
20	20	40	60	30	97.94	1.07	57.02
21	10	70	45	45	53.83	1.71	56.85
22	20	100	60	30	32.80	0.48	72.19
23	30	70	15	45	63.77	1.44	58.73
24	20	40	30	60	68.80	0.96	44.39
25	30	10	45	45	103.20	0.43	39.53
26	30	70	45	15	70.63	0.16	65.98
27	50	70	45	45	114.86	1.82	61.34
28	40	40	30	60	113.60	1.07	36.97
29	30	70	45	75	80.91	0.43	41.24
30	30	70	45	45	84.34	0.64	59.38
31	20	100	30	30	31.66	0.85	44.68

Table 2. Regression coefficients and ANOVA results describing the effect of process variables on the total phenolic content, total anthocyanin content and DPPH radical scavenging activity of *Hibiscus rosa-sinensis* flowers and model adequacy.

Factor	TPC	TAC	DPPH
Intercept	34	4.25	20.2
Linear			
X _A	5.96	-0.021	1.14
X _B	0.08	-0.049	0.065
X _C	1.29	-0.028	0.797
X _D	0.79	-0.022	0.602
Quadratic			
X _A ²	-0.0239	0.0005	-0.024
X _B ²	-0.0025	0.0003	-0.001
X _C ²	-0.0221	0.0004	-0.007
X _D ²	-0.0202	0.0001	-0.005
Cross product			
X _{AB}	-0.0494	-0.0002	-0.004
X _{AC}	0.002	-0.0008	0.0043
X _{AD}	-0.004	0.00017	0.0047
X _{BC}	0.0074	-0.0006	-0.0006
X _{BD}	0.0085	-0.0008	0.0015
X _{CD}	0.0094	0.0001	-0.0049
R ²	0.73	0.75	0.71
Adjusted R ²	0.70	0.71	0.69
p value (model)	0.015	0.012	0.011
p value (Lack of fit)	0.59	0.465	0.91

2.2. Experimental design

Response surface optimization of process parameters was achieved through the central composite design. The study was designed to evaluate the individual effects as well as interactive effects of four independent variables (X_A= liquid to solid ratio, X_B= Ethanol concentration, X_C= Temperature, X_D= Time) on the response variables TPC, TAC and DPPH radical scavenging activity. A two-level four-factor central composite design, with 31 experimental runs including 16 corner points, 8 axial points and 7 center points was performed and the obtained results are summarized in Table 1. Response surface analysis and Analysis of variance (ANOVA) were used to determine the regression coefficients and the statistical significance of the model terms. The second-order polynomial equation was used to express the investigated responses as a function of the independent variables as shown in Eq. 1.

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_{i2} + \Sigma\beta_{ij} X_{ij} \quad (1)$$

Y indicates the response variable, X_i and X_j represents the independent variables, $\beta_0, \beta_i, \beta_{ii}$ and β_{ij} represents the coefficients of the constant, linear effects, quadratic effects and interactive effects of independent variables respectively.

2.3. Model validation

The predictive capacities of mathematical models were evaluated by comparing the experimental values with response values predicted by the mathematical models. To determine this, experiments were performed under the optimal conditions which were obtained by analyzing the individual response surface plots, targeting the maximal attainable response for each independent variable. The composite desirability index was evaluated setting

weight at 1. The desirability index was assessed for the maximum target values to maximize all responses together.

2.4. Determination of total phenolic content (TPC)

The TPC of the flower extracts was assayed using the Folin-Ciocalteu method described by Singleton et al. (1999) with some modifications as mentioned in Kumari and Gunathilake (2020). Folin-Ciocalteu reagent (0.5 N, 0.1 mL) was mixed with 0.5 mL of ethanolic extracts and the homogenized mixture was incubated in dark for 15 minutes at room temperature. Then 2.5 mL of 7.5% (w/v) sodium carbonate was added and the mixture was incubated for 2 hours in the dark. The absorbance of the resulting mixture was measured at 760 nm using a UV/VIS spectrometer (840-210800 Thermo Fisher Scientific, USA). TPC was expressed as mg gallic acid equivalents (GAE) per g dry weight (DW) of flowers.

2.5. Determination of total anthocyanin content (TAC)

As adopted from the procedure of Loizzo et al. (2016), TAC of the flower samples was determined using the pH differential method. Extracts of flowers samples (0.5 mL) were mixed with 3.5 mL of potassium chloride buffer (0.025 M, pH 1) or 3.5 mL of sodium acetate buffer (0.025 M, pH 4.5) separately and incubated for 15 min. The absorbance of each resulting mixture was measured at 510 and 700 nm. The difference in the absorbance was calculated as follows: $A = [(A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}]$. The concentration of monomeric anthocyanin extracted from HS flowers was calculated using the formula, $\text{absorbance} \times \text{MW} \times \text{dilution factor} \times 1000 / (\epsilon \times l)$, where the molar absorptivity (ϵ) and molecular weights (M_w) of cyanidin-3-glucoside was $\epsilon = 26900$; $M_w = 449.2$ respectively. TAC was expressed as milligrams of cyanidin 3-glucoside equivalents (cy-3-glu) per gram of DW of flowers.

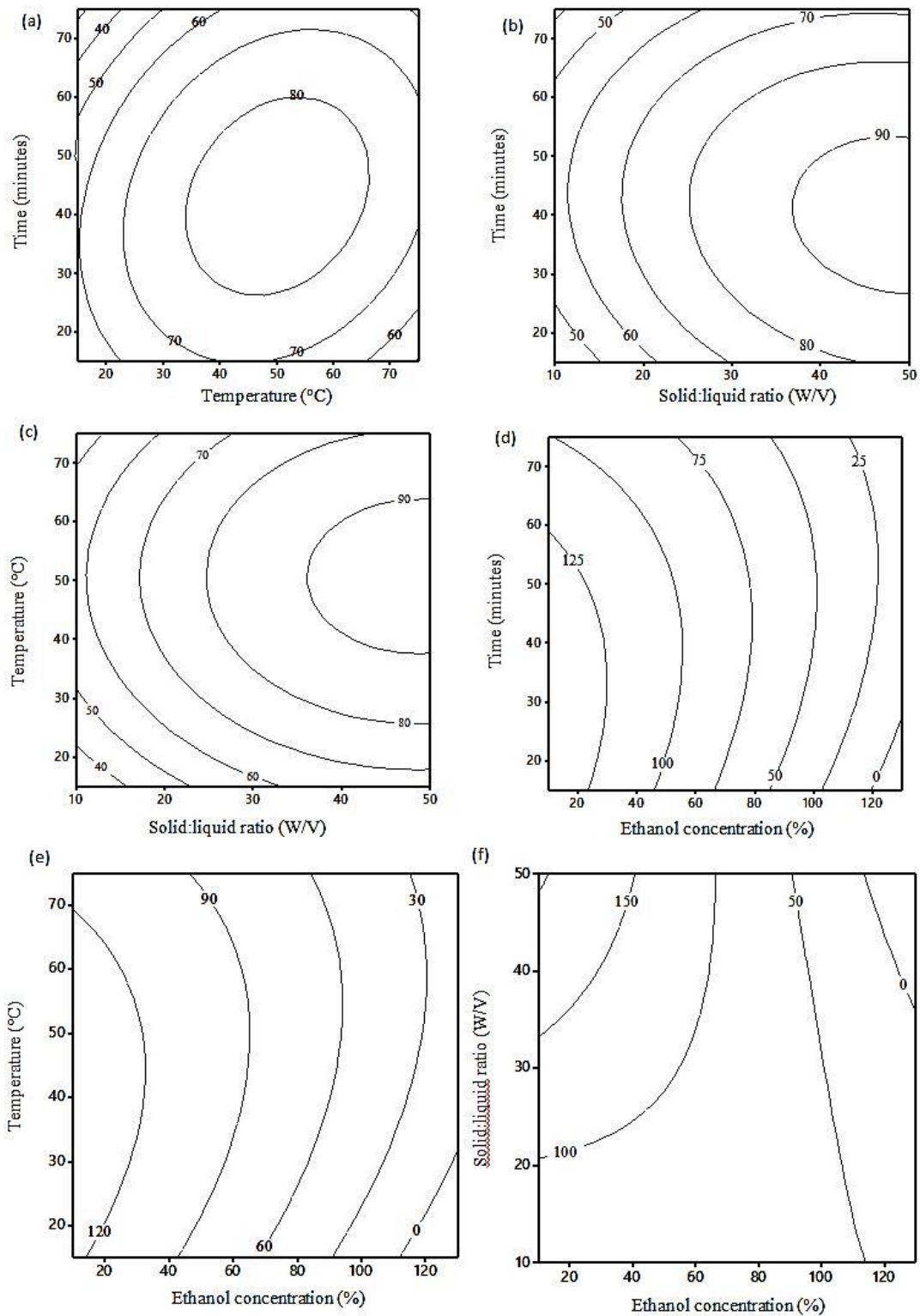


Fig. 1. Contour plots of phenolic extraction (mg GAE /g DW) extraction from *Hibiscus rosa-sinensis* flowers as a function of solid to liquid ratio, ethanol concentration, extraction temperature and time. Values were kept constant as follows, Temperature 45°C, ethanol concentration 70%, Extraction time 45 minutes and solid to liquid ratio 1:30.

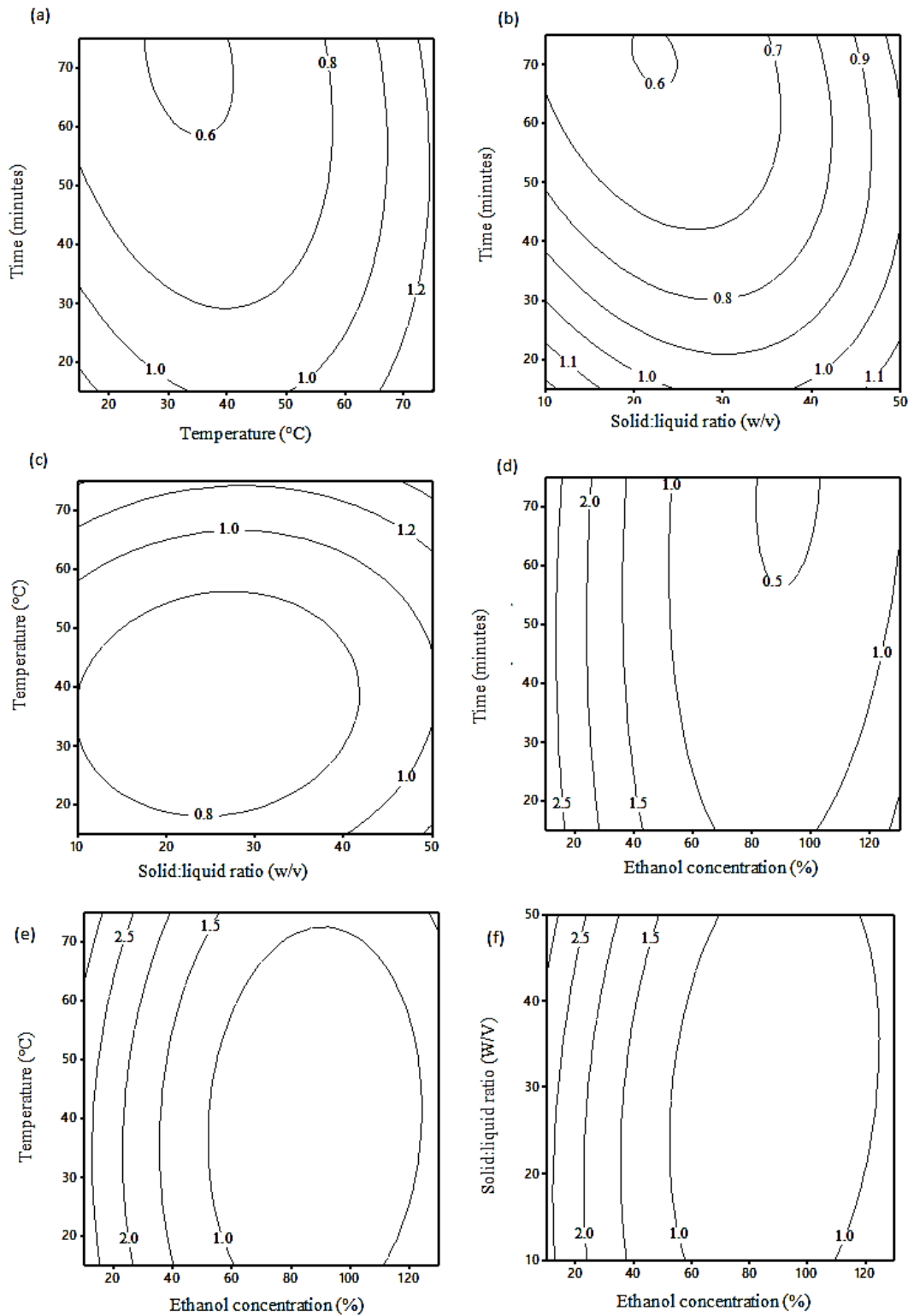


Fig. 2. Contour plots of anthocyanin (mg cyanidin-3-glucoside /g DW) extraction from *Hibiscus rosa-sinensis* flowers as a function of solid to liquid ratio, ethanol concentration, extraction temperature and time. Values were kept constant as follows, Temperature 45°C, ethanol concentration 70%, Extraction time 45 minutes and solid to liquid ratio 1:30.

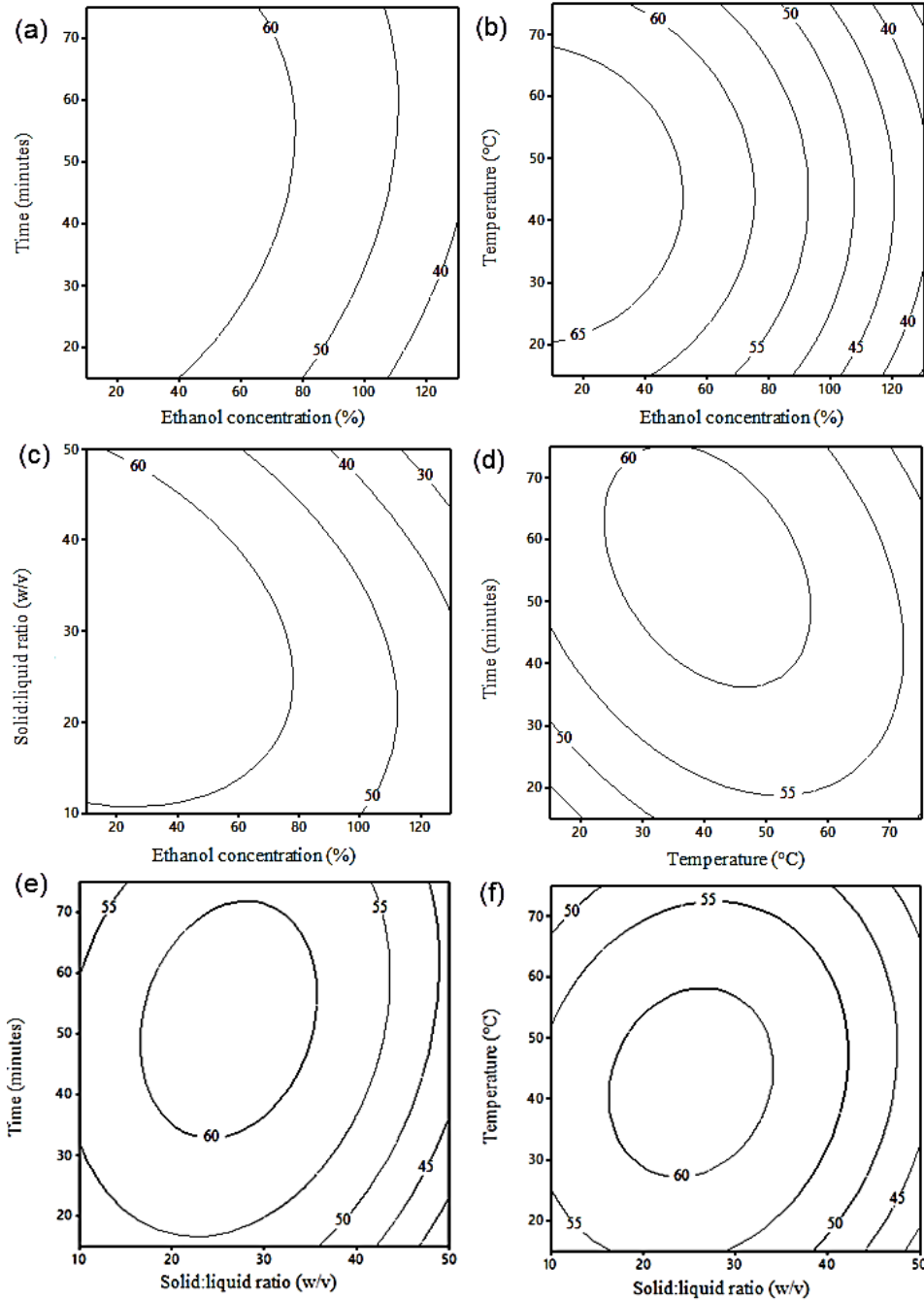


Fig. 3. Contour plots of DPPH radical scavenging activity (% scavenging) of *Hibiscus rosa-sinensis* flowers as a function of solid to liquid ratio, ethanol concentration, extraction temperature and time. Values were kept constant as follows, Temperature 45°C, ethanol concentration 70%, Extraction time 45 minutes and solid to liquid ratio 1:30.

Table 3. Predicted and experimental values of responses under optimum conditions for simultaneous optimization of responses.

Responses	Predicted values	Experimental values
TPC (mg GAE/ g DW)	157.98	155.42 ± 1.32
TAC (mg cy-3-glucoside)	2.18	2.21 ±0.54
DPPH (% scavenging)	65.75	62.86 ±0.87

*Experimental values are expressed as mean±standard deviation.

2.6. Determination of 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The procedure reported by Öztürk et al. (2011) with some modifications as described in Gunathilake and Ranaweera (2016) was followed to determine the DPPH radical scavenging activity of the extracts. Flower extracts (0.4 mL) were mixed with 3.6 mL of 0.1 mM ethanolic DPPH solution and homogenized. The homogenized mixture was incubated in the dark at 37°C for 30 minutes. The absorbance of the resulting mixture was measured at 517nm using a UV-Visible spectrophotometer. The percentage of DPPH radical scavenging was calculated using the formula.

$$\text{Scavenging (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (2)$$

A_{sample} and A_{control} denote the absorbance of the samples and control respectively.

3. Results and Discussion

To identify the effects of process parameters on the extraction yield of TPC, TAC and DPPH radical scavenging activity, a central composite design with two levels was conducted using four independent variables. The various combinations of uncoded process parameters with the respective experimental values for the response variables are tabulated in Table 1. The appropriate range for the process parameters (solid to liquid ratio, ethanol concentration, time and temperature) was selected based on literature. The Second-order polynomial equation was generated for the modeling of the process parameters. The significance of the models was determined based on the regression coefficients for the investigated responses. The models with p values less than 0.05 were considered statistically significant. Also, the ability of the models to predict the studied responses is reflected by p values for lack of fit ($p > 0.05$). Based on the ANOVA table it was identified that all model responses (TPC, TAC and DPPH) were significant ($p < 0.05$) while the lack of fit was insignificant ($p > 0.05$) (Table 2). The generated two-dimensional (2D) response surface plots displayed interaction effects between process parameters towards the model responses.

3.1. Effects of model parameters on TPC

The results from the ANOVA table indicate that the model generated for TPC after elimination of all insignificant variables is well fitted, with the model p-value 0.015 and p-value for lack of fit 0.59. Also, the model displayed a good prediction with $R^2 = 0.73$ and adjusted $R^2 = 0.70$.

$$\begin{aligned} \text{TPC (mg GAE/g DW)} \\ = 34 + 0.08X_B + 5.96X_A - 0.0221X_C^2 \\ - 0.0202X_D^2 - 0.0494X_{AC} \end{aligned} \quad (3)$$

Fig. 1 shows the contour map for the effect of independent variables on the yield of TPC based on the generated model. Considering the linear effects, ethanol concentration and solid to liquid ratio had a significant ($p < 0.05$) positive effect on the yield of TPC whereas the quadratic effects of extraction time and

temperature and interactive effects of solid to liquid ratio and temperature had a significant ($p < 0.05$) negative effect on the TPC. Though the interactive effects between solid to liquid ratio and ethanol concentration displayed a negative effect on TPC it was not significant.

Thus, the overall observed contour plot had a curvilinear effect. From Fig. 1a, it can be noted that approximately within the temperature range of 35 to 65°C higher yield of TPC was noted. A 10% increase in the TPC was observed while varying the TPC from 35 to 65°C. Initially, with lower temperatures and higher extraction times the yield of TPC was low. While gradually increasing the temperature and lowering the extraction times a curvilinear increase in the TPC can be noted. Increasing temperature increases molecular motion by weakening the bonds between the phenolics and plant matrix. Breakage of bonds facilitates desorption and accelerates the diffusion speed of phenolics. This increases mass transfer thereby enhancing the release of phenolics from HS petals (Dranca & Oroion, 2016). Temperature also increases the yield of TPC from the matrix by modifying solvent factors such as viscosity, diffusivity, surface tension and solubility (Boonkird et al., 2018). As noted in the present study increasing the temperature beyond 65°C, reduced the yield of TPC, this may be possibly due to the fragmentation and thermal degradation of phenolic compounds at high temperatures.

Considering the solid to liquid ratio, increasing the volume of ethanol from 10 mL to 50 mL has increased the TPC yield from 50-90 mg GAE /g DW while maintaining the temperature at 45°C, and ethanol concentration at 70%. A similar observation was reported by Dahmoune et al. (2015) where increasing solvent proportion has increased the yield of phenolics from the leaves of *Myrtus communis* using a microwave-assisted extraction process. Increasing ethanol concentration displayed a decreasing trend of TPC and the data suggest that ethanol concentration within 20% to 40% could yield the possible higher content of polyphenols from HS flowers. Depending on the composition of phenolics present in HS flowers, the proportion of water mixed to the organic solvent could influence the extraction yield. As reported by Yang et al. (2010) penetration of ethanol into the plant cells is easier at lower concentrations and this facilitates extraction of phenolics. At higher concentrations, ethanol can cause protein denaturation to prevent the dissolution of phenolics from the matrix and reduce the yield of TPC. The selection of suitable solvents for extraction plays a crucial role in the quantity and composition of phenolics extracted from the plant matrix. Generally, due to the polar nature of the majority of the phenolic compounds, polar organic solvents such as methanol and ethanol are used by various researchers for the extraction of bioactive compounds from various plant matrixes. Due to the eco-friendly nature and safety for human consumption, the non-toxic solvent approved by the FDA for a higher yield of phenolic compounds from plant matrix is ethanol (Izadiyan & Hemmateenejad, 2016). Thus, the current study has been conducted using an ethanol-based solvent system for the optimization of polyphenol extraction. As phenolic compounds are moderately polar, a combination of water with organic solvents has been used to extract phenolics. The mechanism that lies behind this is that organic solvent enhances the solubility of the solute while water increases the desorption of phenolics from the matrix. Also, to a certain extent, the organic solvent has been identified to inhibit the activity of polyphenol oxidase thus controlling the degradation of extracted phenolic compounds (Vázquez-Espinosa et al., 2019).

3.2. Effects of model parameters on TAC

Based on the proposed model, the linear effects of ethanol concentration and time as well as interactive effects between solid to liquid ratio and ethanol concentration had a significant ($p < 0.05$) adverse effect on the yield of TAC. Quadratic effects of ethanol concentration and the temperature had a significant positive effect on TAC. Thus, the generated second-order polynomial equation is as follows:

$$\begin{aligned} \text{TAC (mg cy-3-glu/g DW)} \\ = 4.25 - 0.0492X_B - 0.022X_D + 0.000345X_B^2 + 0.000399X_C^2 \\ - 0.00017X_{AB} \end{aligned} \quad (4)$$

From the analysis, it was observed that the model p-value was 0.012 and the lack of fit was insignificant with the p-value of 0.465 indicating that the proposed model is well fitted. Besides this, the proposed model displayed a good model prediction with $R^2 = 0.75$ and adj $R^2 = 0.70$.

As shown in the contour plots in Fig. 2, the combination of the linear and quadratic effects of the independent variables resulted in a curvilinear variation in the response variable TAC. The TAC of the samples was observed within the range of 0.16 – 2.24 mg cy-3-glu / g of DW. It was noted that at lower extraction times, higher yields of TAC were obtained (Fig. 2a and b). Within the extraction times of 20-40 minutes, more than 1mg cy-3-glu / g DW of anthocyanins were extracted. The decrease in anthocyanin content with prolonged extraction time could be justified with Fick's second law of diffusion as follows, when the solvent reaches the saturation level compared to the extracted compound after a particular period, the concentration gradient is nullified and movement of solute from the matrix decreases (Medouni-Adrar et al., 2015).

Similar to the yield of TPC, lower ethanol concentration and higher solid to liquid ratio yielded higher contents of anthocyanins. Considering the temperature, a comparatively higher yield of anthocyanins from HS flowers was obtained within the range of 30 to 65°C. Khazaei et al. (2016), has reported that at very high temperatures monomeric anthocyanins could have deteriorated and formed brown or colourless polymerized anthocyanins which cannot be estimated using the pH differential method. Thus, the overall data obtained indicates lower anthocyanin content at higher temperatures. Increasing content of anthocyanins with increasing solid to liquid ratio could be justified by the fact that with the rising solvent proportion, a higher concentration gradient and higher distribution coefficient is established facilitating the quick release of anthocyanins from the petals of HS, similar to the results from the current study, where higher ethanol concentration yielded lower anthocyanin content. Le et al. (2019) has reported that when increasing the ethanol concentration more than 50%, the TAC from *Carissa carandas* fruits have shown a steady decrease due to the high solubility of anthocyanins in moderate alcohol concentration. Also, Dranca and Oroian (2016) have observed that increasing solvent concentration has led to lower monomeric anthocyanin recovery from the peels of eggplant.

3.3. Effects of model parameters on DPPH radical scavenging activity

DPPH radical scavenging activity is one of the widely used and convenient approaches to evaluate the antioxidant activity of biological samples. It also can be used as an indicator to monitor

the phenolic content of samples (Jagadeesan et al., 2019). Based on the results of ANOVA it was noted that the model generated for DPPH radical scavenging activity was well fitted with a model p-value of 0.011 and insignificant lack of fit ($p = 0.91$). Also, the proposed model had a good prediction of terms with $R^2 = 0.71$ and adj $R^2 = 0.69$.

$$\begin{aligned} \text{DPPH (\% scavenging)} \\ = 20.2 + 0.065X_B + 1.14X_A - 0.0242X_A^2 \\ - 0.00794X_C^2 - 0.00446X_{AB} \end{aligned} \quad (5)$$

DPPH radical scavenging activity was significantly affected by the solid to liquid ratio and ethanol concentration in the linear model, while time and solid to liquid ratio were affected in the quadratic model. For interactive effects only solid to liquid ratio and ethanol concentration affected the radical scavenging activity.

Fig. 3 depicts the contour plots for the effects of independent variables on the DPPH radical scavenging activity of HS flowers. Similar to the results obtained for TPC, higher solid to liquid ratio, lower ethanol concentration and lower extraction time has facilitated to achieve higher radical scavenging activity. This indicates the relationship between the radical scavenging activity and phenolic compounds present in HS. It was noted that when increasing the ethanol concentration from 20% to 60% the scavenging activity was decreased by 10% while maintaining the extraction time at 45 minutes and solid to liquid ratio at 1:30.

3.4. Optimization of process parameters and model validation

The software Minitab version 17 was used to simultaneously optimize the process parameters to maximize the response variables TPC, TAC and DPPH radical scavenging activity. Optimal conditions were obtained using the desirability function on the scale of 0-1. The generated optimum conditions were 24.0% ethanol, 1:40 solid to liquid ratio at extraction temperature of 44°C and time 41 minutes. An experimental run was conducted with the identified optimum conditions and the experimental values for TPC, TAC and radical scavenging activity were compared with the predicted values to study the suitability of the response models. The obtained values are presented in Table 3. There was no statistically significant ($p > 0.05$) difference between the predicted and experimental values at a 95% confidence interval. The outcomes indicate the reliability of the parameters for the extraction of phenolics and anthocyanins with maximum antioxidant activity from HS flowers.

4. Conclusion

The present study investigated the optimized conditions to maximize the recovery of phenolics and anthocyanins from HS flowers along with maximum DPPH radical scavenging activity. The second-order polynomial models generated by RSM were adequate to optimize the process parameters for the extraction of antioxidant compounds based on the satisfactory ANOVA and descriptive statistics parameters. The optimum conditions obtained were: solid:liquid ratio 1:40, ethanol concentration 24.0%, extraction temperature 44°C and time 41 minutes. The findings of the current study are applicable, for the food industry as well as the

pharmaceutical industry, to develop industrial-scale processes in a cost-effective and less labor-intensive process for efficient extraction of antioxidant compounds.

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Conflict of interest

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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