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Optimization of ethanol-based extraction of phenolic compounds from edible flowers of *Cassia auriculata*

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- ✓ Response surface method.

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Abstract

Edible flowers of *Cassia auriculata* have been reported to have various health benefits owing to its phenolic composition. However, appropriate extraction of phenolic compounds is required for the effective utilization of these flowers. This study was aimed to make use of response surface methodology, to simultaneously optimize the process parameters to obtain maximum yield of phenolics and anthocyanins along with maximum DPPH radical scavenging activity from *C.auriculata* flowers. Central composite design was employed with four independent variables; solid:liquid ratio (X_A), ethanol concentration (X_B), temperature (X_C) and time (X_D). The results showed that the polynomial models for all responses were significant ($p<0.05$) and did not show lack of fit and presented determination of coefficients above 95%. This indicates the suitability of the models for prediction purposes. The optimum process parameters obtained were X_A; 1:33 (W/V), X_B; 40.3%, X_C; 25°C and X_D; 60 minutes. The experimental values obtained based on optimum extraction parameters agreed with the predicted values. The findings suggests the application of optimized parameters to acquire functionally active phenolics and anthocyanins from *C.auriculata* flowers using ethanol and make use of them in food and pharmaceutical industry

1. Introduction

Awareness on exploring novel natural dietary sources of antioxidants has increased among food researchers, due to the increasing risk of chronic disease development effected by oxidative stress. Various plant sources have been identified as rich sources of antioxidants especially phenolic compounds which could combat oxidative stress through diverse mechanisms and exert various pharmacological properties. As documented by various herbalists, a large group of plant species belonging to *Cassia* family has been used extensively in indigenous medicine [1] due to their phenolic composition and bioactive properties. Among them, *Cassia auriculata* is an economically prominent plant species, which has been used in age old Ayurvedic and Siddha medicinal formulations as a remedy for different diseases conditions.

C.auriculata, commonly known as Tanner's Cassia belonging to the family Caesalpiniaceae is an ethnomedicinally important herbal shrub [2] which is widely grown in Asian regions. This plant bears alternatively arranged leaflets, large bright yellow flowers and short legume fruits which has been reported with several phenolic constituents having therapeutic properties [3]. Among the various

parts employed for medicinal uses, edible flowers of *C.auriculata* has been of much interest of researchers recently, owing to their identified unique phenolic constituents and their expanding dynamic capacity to protect against various disorders. Traditionally, *C.auriculata* flowers have been used to treat diabetes, rheumatic disorders, liver disorders, urinary discharges, nocturnal emissions, skin disorders and throat irritations [4]. Previous investigations have documented that *C.auriculata* flowers posses bioactive properties such as anti-helminthic, antioxidative, anti-hyperlipidemic, anti-inflammatory and antimicrobial properties [5]. These properties can be attributed to the phenolic acids, flavonoids, anthracene derivatives, carotenoids, alkaloids and tannins detected in various extracts of *C.auriculata* flowers [6, 7, 8]. Thus, for effective application of these flowers in functional food development and nutraceuticals, it is very much important to appropriately extract these bioactive compounds [9].

Various extraction methods such as solid-liquid extraction, microwave assisted extraction, enzyme assisted extraction, ultra sound assisted extraction and supercritical fluid extraction have been reported for the recovery of phenolic compounds from different plant matrix. However, solid-liquid extraction is a widely employed simple and reliable, technique to extract phenolics from different plant matrixes [10, 11, 12]. Extraction efficiency and the quality of the extract depends on the technique applied and the process parameters influencing the particular technique. Solid-liquid extraction is commonly a function of process parameters such as extraction temperature, pH, time, solvent composition, solid to solvent ratio, particle size, number of extraction cycles etc [13]. Therefore, it is necessary to optimize the process parameters which facilitates to yield maximum quantity of phenolics with good quality.

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 [14]. RSM estimates the effects of individual factors as well as multiple factors and their interactions on the responses, based on the fit of experimental data to the empirical models generated in relation to experimental design. This approach is beneficial over other traditional methods, due to its lower number of required experiments, a statistical basis to interpret the data, identification of the interactive effects between variables, less labor requirement and time-consuming than traditional optimization methods [15]. The present study aims to apply RSM to simultaneously optimize the process parameters in order to maximize the yield of total phenolics and total anthocyanin content along with maximum antioxidant activity from *Cassia auriculata* flowers.

2. Methodology

2.1 Sample preparation and extraction

Fresh *C.auriculata* 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.

Varying volumes (20-40 mL) of aqueous ethanol of different concentrations (40% to 100%) were mixed with 1 g of freeze-dried samples. The homogenized samples were exposed to different temperatures (30 to 60°C) for time period ranging from 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 selected as defined by the RSM design generated by Minitab statistical software version 17.

2.2 Experimental Design and Model validation

Response surface optimization of process parameters was achieved through the central composite design (CCD). The study was designed to evaluate the individual effects as well as interactive effects of four independent variables (X_A= solid to liquid 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 CCD, with 31 experimental runs including 7 replicates at the center points was performed and the obtained results are summarized in **Table 1**. Response surface analysis and Analysis of variance (ANOVA) was used to determine the regression coefficients and the statistical significance of the model terms. Second order polynomial equation was used to express the investigated responses as a function of the independent variables as shown in the **Eqn. 1**

$$\mathbf{Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\beta_{ij} X_{ij}} \qquad \textbf{Eqn. 1}$$

Y indicates the response variable, X_i and X_j represents the independent variables, β_0, β_i, β_{ii} and β_{ij} represents the coefficients of the constant, linear effects, quadratic effects and interactive effects of independent variables respectively.

The adequacy of the model was evaluated by the coefficient of determination (R^2), and p values for the model and lack-of-fit test. The appropriateness of models was evaluated by comparing the experimental values with response values predicted by the mathematical models. The composite desirability index was evaluated setting weight at 1. Desirability index was assessed for the maximum target values to maximize all responses together.

2.3 Determination of Total Phenolic Content (TPC)

The TPC of the flower extracts were assayed using the Folin-Ciocalteu method described in Janarny et al. [16]. Briefly, 0.1 mL of 0.5 N Folin–Ciocalteu reagent was mixed with 0.5 mL of ethanolic extracts and the homogenized mixture was incubated in dark for 15 minutes. Then 2.5 mL of sodium carbonate (7.5%,W/V) 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.

2.4 Determination of total anthocyanin content (TAC)

Procedure of pH differential method described in Janarny and Gunathilake, [17] was adopted to determine the TAC of the flower samples. Approximately, 0.5 mL of ethanolic flower extracts 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 minutes. The absorbance of resulting mixture was measured at 510 and 700 nm. The difference in the absorbance was calculated as follows: A =[(A510 − A700)pH 1.0 − (A510 − A700)pH 4.5]. The concentration of monomeric anthocyanin extracted from HS flowers, was calculated using the formula, absorbance × MW × dilution factor ×1000)/(ε × 1, where the molar absorptivity (ε) and molecular weights (MW) of cyanidin-3-glucoside was ε = 26900; MW = 449.2 respectively. TAC was expressed as milligrams of cyanidin 3-glucoside equivalents (cy-3-glu) per gram of DW of flowers.

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

Runs	Process variables				Response variables		
	Solid : Liquid ratio (X_A-W/V)	Ethanol concentration (X_B-%)	Temperature (X_C-°C)	Time (X_D-minutes)	TPC (mg GAE/g DW)	TAC (mg Cy-3-Glu/g DW)	DPPH (% scavenging/g DW
1	40	40	30	30	101.49	0.32	50.65
2	30	130	45	45	147.77	3.53	63.69
3	30	70	45	45	135.09	1.92	70.08
4	20	40	60	60	127.31	0.01	60.96
5	40	40	60	30	115.09	1.92	65.87
6	20	100	30	60	129.77	1.28	61.36
7	30	70	45	45	151.31	0.64	64.81
8	40	100	30	60	128.91	1.28	59.29
9	30	70	45	45	140.80	2.78	71.43
10	20	40	30	30	138.74	0.01	58.55
11	40	100	60	30	130.74	1.50	58.70
12	40	100	60	60	144.34	1.28	64.81
13	30	70	45	45	102.51	3.63	65.35
14	20	100	60	60	152.06	0.48	63.58
15	30	70	45	45	130.63	0.32	65.03
16	30	70	45	45	148.63	1.44	61.36
17	30	70	75	45	139.89	1.92	60.28
18	40	100	30	30	183.31	1.28	62.87
19	40	40	60	60	131.66	1.82	70.47
20	20	40	60	30	100.11	0.21	75.74
21	10	70	45	45	111.66	2.89	64.92
22	20	100	60	30	138.34	1.60	64.81
23	30	70	15	45	126.51	1.39	69.84
24	20	40	30	60	134.91	1.28	63.92
25	30	10	45	45	147.77	1.92	62.62
26	30	70	45	15	174.86	0.27	57.46
27	50	70	45	45	187.89	2.14	63.69
28	40	40	30	60	136.63	2.56	61.22
29	30	70	45	75	133.20	2.56	62.87
30	30	70	45	45	107.66	1.92	66.27
31	20	100	30	30	101.49	0.32	50.65

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

The procedure reported previously [18] was adopted to determine the DPPH radical scavenging activity of the extracts. Flower extracts (0.4 mL) were mixed with 3.6 mL of DPPH (0.1 mM) solution and homogenized. The homogenized mixture was incubated in the dark at 37°C for 30 minutes. Absorbance of the incubated mixture was measured at 517 nm using a UV–Visible spectrophotometer. Percentage of DPPH radical scavenging was calculated using the formula;

$\%\ \text{Scavenging} = (A_{control} - A_{sample})/A_{control} * 100$

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

3. Results and Discussion

3.1 Model fitting

CCD was used to evaluate the individual as well as interactive effects of ethanol percentage, extraction time, temperature and solid to liquid ratio on the TPC, TAC and DPPH radical scavenging activity of *C.auriculata* flowers. The defined combinations of uncoded process parameters and the respective experimental values of response variables are shown in **Table 1**. Second order polynomial equation was used to model the independent variables. The regression coefficients for the investigated responses indicate the significance of the models with a p-value less than 0.05 for the three responses (**Table 2**). In other hand, results showed that the models could be applied to predict the studied responses reflected by p values for lack of fit ($p > 0.05$). The reduced models generated after the elimination of all insignificant variables could be applied to predict the studied responses. Also, the three-dimensional (3D) response surface plots obtained, can be used to visualize the interaction effects between process parameters towards the model responses.

3.2 Effect of extraction variables on TPC

Based on the obtained responses, after the elimination of all insignificant effects of extraction variables, the final model generated for TPC by fitting the second order polynomial, could be expressed as follows,

$$\text{TPC (mg GAE / g DW)} = 126.1 + 0.64\ X_B + 1.37\ X_A - 0.005\ X_B^2 + 0.007\ X_C^2 - 0.007\ X_{BD} + 0.03 X_{AD}$$

From the analysis it was observed that the model p value was 0.001 and lack of fit was insignificant with the p value of 0.214, indicating that the proposed model is well fitted. Also, the model displayed a good model prediction with $R^2 = 0.83$ and $Adj.R^2 = 0.80$. The linear effect of solid to liquid ratio and ethanol concentration showed a significant positive effect ($p<0.05$) for the yield of phenolics. As noted in the quadratic model, it could be seen that ethanol concentration showed a negative effect on the yield whereas, temperature showed a significant positive effect on TPC and the values are expressed in **Table 2**. Considering the interaction effect, solid to liquid ratio and time expressed a significant ($p<0.05$) positive output. Interactive effects of ethanol concentration extraction time displayed negative effects on TPC. The response surface plots obtained for the interactive effects of different variables is shown in **Figure 1**.

Based on the results, the TPC of the flower extracts varied from 100.11 to 187.89 mg GAE/g DW. As observed in **Figure 1 (f)** the TPC of the extracts were high at low temperature and with increasing extraction temperature at a fixed extraction time the TPC has decreased. This observation indicates that temperature plays an important role in the extraction of phenolics. Basically, at moderate temperature, the yield shows an increasing trend which is achieved by activating the phenolic compounds and increasing the diffusion of molecules from the flower matrix into the solvent. Also high temperatures weaken the intercellular interactions and soften the plant tissues facilitating efficient extraction [19]. However, since phenolics are thermolabile compounds, after reaching a particular temperature, degradation and transformation of phenolics leads to a lower TPC [20]. Also as observed from **Figure 1 (b)**, at fixed levels of solid:liquid ratio (1:30) and extraction time of 45 minutes, the TPC has slightly increased at temperature range of 45°C to 65°C. This may be due to the availability of phenolic compounds which require higher energy of activation and molecular movement.

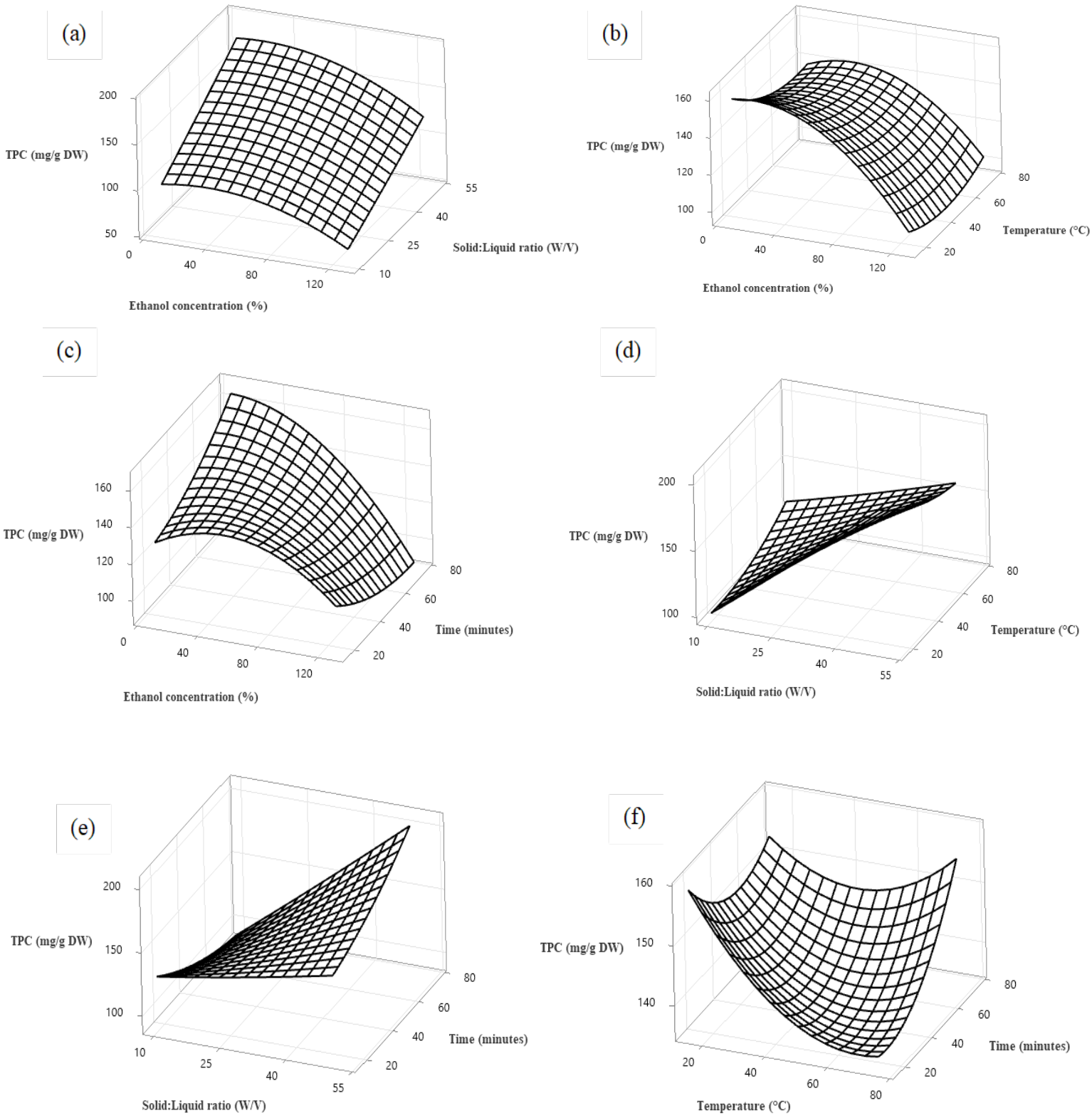

Figure 1. Response surface plots of phenolic extraction (mg GAE /g DW) extraction from *Cassia auriculata* flower as a function of solid to liquid ratio, ethanol concentration, extraction temperature and time. Values were kept constant as follows, Temperature 45°C, ethanol concentration 70%, Extraction time 45 minutes and solid to liquid ratio 1:30.

As illustrated in **Figure 1 (a),** increasing solid to liquid ratio increases the yield of the TPC at fixed ethanol concentration, time and temperature. This is primarily because of the increasing gradient of mass transfer between the solid and liquid which facilitates the diffusion of phenolic compounds [21]. Also, when increasing the solid:liquid ratio it dilutes the concentration of the phenolics present in the surface and establishes higher concentration gradient between the external and internal surface leading to higher leaching out rate [22].

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 *C.auriculata* flowers and model adequacy

Factor	TPC	TAC	DPPH
Intercept	126.1	4.56	95.9
Linear	1.37	0.15	-1.59
X_A	0.64	0.01	0.06
X_B	-0.58	-0.06	0.01
X_C	1.28	-0.18	-0.12
X_D			
Quadratic	0.002	-0.002	0.01
X_A	-0.005	-0.001	-0.001
X_B	0.007	0.003	0.001
X_C	0.006	0.001	0.001
X_D			
Cross product	-0.003	-0.004	0.003
X_{AB}	-0.02	-0.001	-0.001
X_{AC}	0.03	0.001	0.005
X_{AD}			
X_{BC}	0.003	0.004	-0.001
	-0.007	-0.001	-0.001
X_{BD}	0.006	0.001	-0.001
X_{CD}			
R^2	0.83	0.78	0.86
Adjusted R^2	0.80	0.72	0.84
p value (model)	0.001	0.04	0.00
p value (Lack of fit)	0.21	0.78	0.26

It was also noted that when increasing the concentration of ethanol from 10% to 50% the TPC has increased and gradually a reduction in the TPC was noted when increasing the ethanol concentration further and the lowest yield was reported when using absolute ethanol. Depending on the composition of phenolics present in *C.auriculata* flowers, the proportion of water mixed to the organic solvent could influence the extraction yield. According to the reports of Ćujić et al., [23] TPC

from dried chokeberry was maximized at 50% ethanol and significantly reduced upon increasing the ethanol concentration. Also authors have documented that, extraction efficiency of phenolics from apple pomace and olive leaves were maximized at 50% ethanol followed by a drop with further increase in ethanol concentration [24,25]. As reported by Yang et al., [26] penetration of ethanol into the plant cells is easier at lower concentration and this facilitates extraction of phenolics. At higher concentrations, ethanol can cause protein denaturation and prevent dissolution of phenolics from the matrix and reduce the yield of TPC. Combination of water and ethanol facilitates efficient extraction of polyphenols, where water acts as a swelling agent and ethanol breaks down the bonds between the solutes and the flower matrix, thus absolute ethanol shows the poorest yield of TPC [27].

Considering the effect of extraction time on TPC of the flowers, it was identified that increasing extraction time yielded higher TPC. However, when interacted with ethanol concentration, extraction time did not significantly influence the yield. In the current study, under fixed levels of temperature, solid to liquid ratio and ethanol concentration, when varying the time from 40 minutes to 60 minutes a rapid increase in the yield from 140.80 to 187.89 mg GAE/g DW was noted. Investigations by [28] has identified 50 minutes as the optimum time for the extraction of oleanolic and ursolic acid from pomegranate flowers which is consistent with findings of the current work. Extraction time is a crucial fact in solvent extraction of phenolics. Basically, appropriate time is required for the equilibrium to be reached between the solution in the flower matrix and extraction solvent. The equilibrium concentration is important for the efficient diffusion of phenolics from the flower matrix and the time required to reach the equilibrium concentration varies depending on the phenolic composition of the matrix [29].

3.3 Effect of extraction variables on TAC

The proposed model for TAC shown below, had a p value of 0.045 and the lack of fit was insignificant (p=0.78) with the predication of terms (R^2=78.07 and Adj.R^2=72.51) indicating that the model is well-fitted and reliable to explore further. Considering the linear effects, solid:liquid ratio had a significant ($p<0.05$) positive and extraction temperature had a significant ($p<0.05$) negative effect on TAC, whereas the linear effects of other variables was insignificant. Based on the quadratic model, a significant ($p<0.05$) negative effect of solid to liquid ratio was observed for the yield of TAC.

$$\text{TAC (mg cy-3-glucoside / g DW)} = 4.56 + 0.157X_A - 0.061\ X_C - 0.002X_A^2 - 0.001\ X_{AC} + 0.001X_{AD}$$

The response surface plots obtained for the interactive effects of different variables is shown in **Figure 2.** The interactive effect between solid:liquid ratio and temperature influenced the yield negatively whereas, the interactive effect between solid:liquid ratio and time positively influenced the yield of anthocyanins.

Data from the present study shows that TAC of the flower sample varied from 0.01 to 3.53 mg cy-3-glu/g DW. Considering the effect of ethanol concentration, similar to that of TPC, increasing ethanol concentration increased the yield and then a steady reduction on TAC was observed with increasing ethanol concentration. In the case of anthocyanins, high ethanol concentrations, probably decrease the yield due to the lower dielectric constant of the ethanol and protein denaturation which prevents the dissolution of phenolics [30]. A similar result was reported previously by various authors. For example, [31] has documented that increasing ethanol concentration more than 50% has resulted in lower content of anthocyanins from red radish. Lee et al. [32] 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 [33], has observed that increasing solvent concentration has led to lower monomeric anthocyanin recovery from the peels of egg plant.

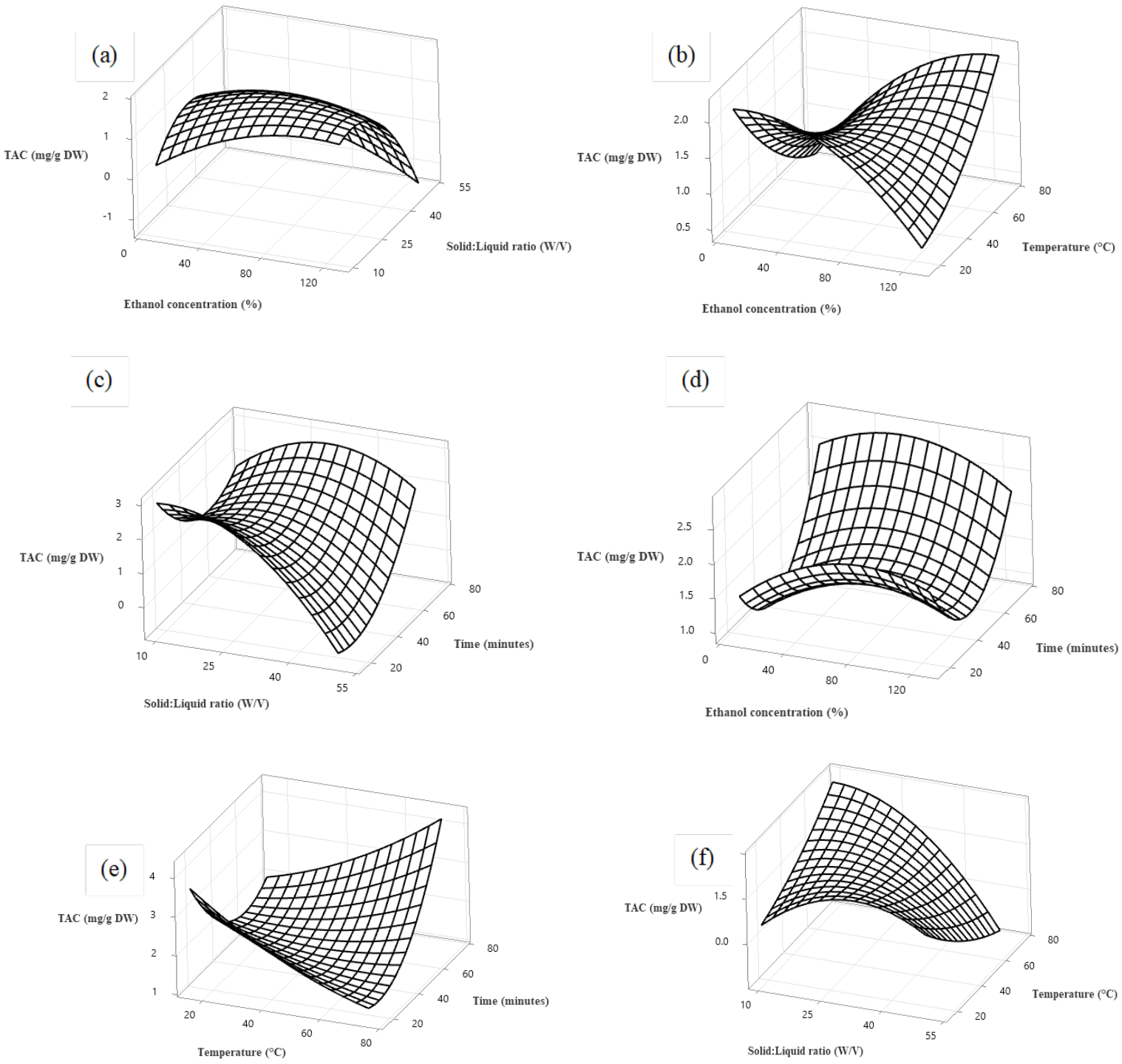

Figure 2. Response surface plots of anthocyanin (mg cyanidin-3-glucoside /g DW) extraction from *Cassia auriculata* flowers as a function of solid to liquid ratio, ethanol concentration, extraction temperature and time. Values were kept constant as follows, Temperature 45°C, ethanol concentration 70%,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

Considering the impact of temperature on the yield of anthocyanins, it was noted that within the temperature range of 30°C to 60°C, TAC has decreased from 3.53 to 0.21 mg cy-3-glu / g DW,

indicating that lower temperature favors the extraction of anthocyanins. As evidenced by literature, anthocyanins have been found to be denatured by chemical or enzymatic reactions beyond a certain range of temperature in different food matrix [34]. Elevated temperatures trigger competing processes such as decomposition and polymerization leading to the conversion of anthocyanins to colorless chalcone form during heating. Moreover, high temperature might affect TAC, indirectly by favoring more tannin extraction and protein–anthocyanin complexations. Ryu and Koh [35], have reported that yield of anthocyanins from black soya beans have increased while increasing the temperature from 20°C to 40°C and a further increase of temperature did not show an improvement in the yield. Xue et al. [36] has also documented a significant decline in the yield of anthocyanins from raspberries at temperatures more than 50°C

From Figure 2, it was noted that, increasing extraction time increased the extractable content of anthocyanins. Considering the effect of solid:liquid, increasing solid to liquid ratio upto 1:30 has shown increase in the of yield of TAC from 0.01 to 3.53 mg cy-3-glu / g DW. A lower solid to liquid ratio will not yield higher TAC since, lower volume of solvent is insufficient to fill up the flower matrix and the hypertonic environment will not be created. This holds up the color in the vacuole of the material and when the solvent reaches a certain volume, the cell rapidly absorbs water swells and bursts out releasing the anthocyanins within the vacuole [37].

3.4 Effect of extraction variables on DPPH radical scavenging activity

As per the results obtained from ANOVA, the model for DPPH scavenging activity was significant and the model could be used to predict the responses. The generated model with insignificant lack of fit (p=0.26) is represented as shown below, the model performance was evaluated using the determination coefficients which were as follows, R^2=0.86 and Adj.R^2=0.84:

DPPH scavenging activity (%) = $95.9 + 0.064\ X_B + 0.012\ X_C - 0.001\ X_B^2 + 0.001X_C^2 - 0.001X_{BC} + 0.005\ X_{AD}$

DPPH radical scavenging activity varied from 50.65 % to 75.74 % and was mainly affected by the ethanol concentration and temperature in the linear model as well as in the quadratic model. Considering the interactive effect, interaction between solid to liquid ratio and time showed a significant positive effect on the DPPH radical scavenging activity ($p<0.05$) whereas interactive effect between the ethanol concentration and temperature showed a significant negative impact on the radical scavenging activity, and the response surface plots obtained for the interactive effects of different variables is shown in **Figure 3**.

As illustrated in **Figure 3 (b)**, ethanol concentration more than 50°C has shown a reduction in the radical scavenging activity. This was similar to the trend displayed by TPC. Interestingly radical scavenging activity was observed to be high at lower solid:liquid ratio and then started to decline gradually with increasing solvent proportion. When considering the impact of temperature, maximum activity of 63% was observed at a temperature of 30°C, when interacted with ethanol concentration. A similar result was reported by Hernández-Carranza et al. [38] where the DPPH radical scavenging activity of banana peel extracts were maximized at 45°C.

3.5 Optimization of process variables and model validation

The determination of optimum conditions for the simultaneous extraction of phenolics and anthocyanins and optimum DPPH radical scavenging activity was carried out using Minitab version 17. The optimal conditions were obtained using the desirability function in the scale of 0-1. The desirability value of 1 represents the ideal case and 0 indicates that one or more responses fall outside the desirable range. The generated optimum conditions for maximum TPC and TAC with maximum

radical scavenging activity was 40.30% ethanol, 1:33 solid:liquid ratio, 25°C and 60 minutes of extraction time. An experimental run was conducted with the recommended optimum conditions and the obtained responses for TPC, TAC and radical scavenging activity were compared with the predicted values to study the appropriateness of the response models.

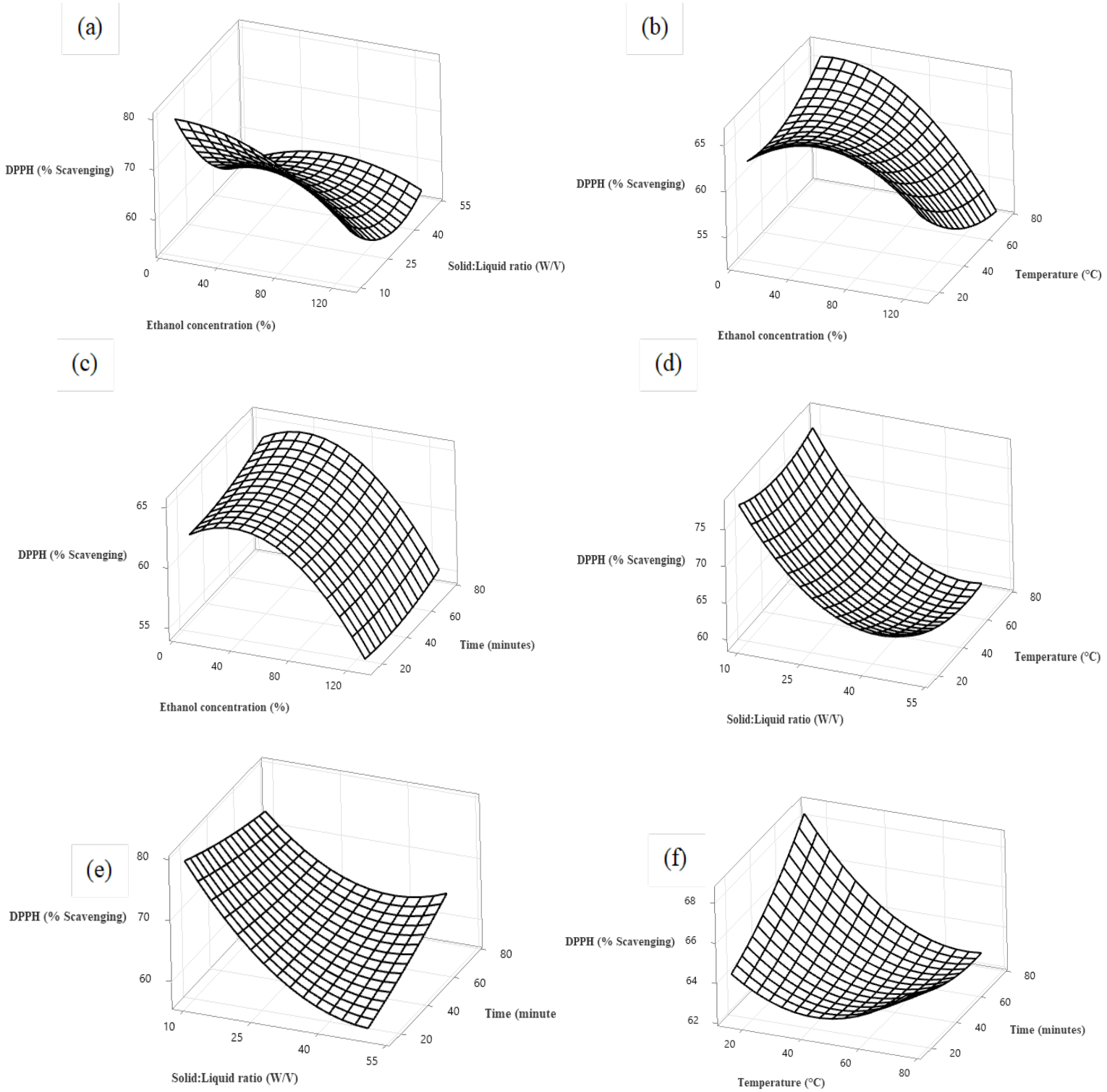

Figure 3. Response surface plots of DPPH radical scavenging activity (% scavenging) of *Cassia auriculata* flowers as a function of solid to liquid ratio, ethanol concentration, extraction temperature and time. Values were kept constant as follows, Temperature 45°C, ethanol concentration 70%, Extraction time 45 minutes and solid to liquid ratio 1:30.

The obtained values are presented in **Table 3.** The was no statistically significant ($p>0.05$) difference between the predicted and experimental values at 95% confidence interval. The outcomes indicate the reliability of the parameters for the extraction of phenolics and anthocyanins with maximum antioxidant activity from *C.auriculata* flowers.

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)	189.12	188.19±1.96
TAC (mg cy-3-glucoside)	2.89	2.88±1.64
DPPH (% scavenging)	68.08	67.45±1.32

Experimental values are expressed as mean±standard deviation

Conclusion

The current study investigated the optimized conditions to enhance the recovery of phenolics and anthocyanins from *C.auriuclata* flowers along with optimum radical scavenging activity. The RSM based second order polynomial models were adequate to optimize the ethanol-based extraction of antioxidant compounds due to satisfactory ANOVA and descriptive statistics parameters. The optimum conditions obtained were: 40.30% ethanol, 1:33 solid:liquid ratio, 25°C and 60 minutes of extraction time. The findings of the current study may be useful for the food industry and pharmaceutical analysis to directly apply the optimized conditions so that it can reduce the cost and labor-intensive process with efficient extraction of antioxidant compounds.

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References

[1] R. W. Mwangi, J. M. Macharia, I. N. Wagara, R. L. Bence, The medicinal properties of Cassia fistula L: A review. *Biomedicine and Pharmacotherapy*, 144 (2021) 112240. https://doi.org/10.1016/j.biopha.2021.112240

[2] K.D.P.P. Gunathilake, K.K.D.S. Ranaweera, H.P.V. Rupasinghe, Optimization of Polyphenols and Carotenoids Extraction from Leaves of Cassia auriculata for Natural Health Products. *Asian Plant Research Journal*, 6(1) (2020) 14-25. https://doi.org/10.9734/aprj/2020/v6i130118

[3] G.Sharmila, V.S.Nikitha, S.Ilaiyarasi, K. Dhivya, V. Rajasekar, N.M. Kumar, C. Muthukumaran, Ultrasound assisted extraction of total phenolics from Cassia auriculata leaves and evaluation of its antioxidant activities. *Industrial crops and Products*, 84 (2016) 13-21. https://doi.org/10.1016/j.indcrop.2016.01.010

[4] M.Juan-Badaturuge, S.Habtemariam, M.J.K. Thomas, Antioxidant compounds from a South Asian beverage and medicinal plant, Cassia auriculata. *Food Chemistry* 125 (2011) 221–225. https://doi.org/10.1016/j.foodchem.2010.08.065

[5] G.C.Nille, K.R.Reddy, A phytopharmacological review of plant–Cassia auriculata. *International Journal of Pharmaceutical and Biological Science Archive*, 6 (2015) 1-9.

[6] S. Nakamura, F. Xu, K. Ninomiya, S. Nakashima, Y.Oda, T. Morikawa, H.Matsuda, Chemical structures and hepatoprotective effects of constituents from Cassia auriculata leaves. *Chemical and Pharmaceutical Bulletin*, 62 (10) (2014) 1026-1031. https://doi.org/10.1248/cpb.c14-00420

[7] G.Janarny, K.D.P.P. Gunathilake, K.K.D.S. Ranaweera, Nutraceutical potential of dietary phytochemicals in edible flowers—A review. *Journal of Food Biochemistry*, 45(4) (2021) p.e13642. https://doi.org/10.1111/jfbc.13642

[8] G.Janarny, K.K.D.S. Ranaweera, K.D.P.P.Gunathilake, Digestive recovery of polyphenols, antioxidant activity, and anti-inflammatory activity of selected edible flowers from the family Fabaceae. *Journal of Food Biochemistry*, 46(2) (2022) p.e14052. https://doi.org/10.1111/jfbc.14052

[9] K.D.P.P.Gunathilake, Emerging technologies available for the enhancement of bioactives concentration in functional beverages. In Biotechnological Progress and Beverage Consumption, 1st ed.; Grumezescu, A., Holban, A.M., Eds.; Academic Press: New York, NY, USA, 2020; pp. 39–69.

[10] K.D.P.P. Gunathilake, K.K.D.S. Ranaweera, H.P.V. Rupasinghe, O.D.A.N. Perera, H.P.S. Jayaweera, Response surface optimization of extraction of polyphenols and carotenoids from Sesbania grandiflora leaves with ethanol-water system. *Asian Journal of Biotechnology and Bioresource Technology*, 2 (2017) 1–10. https://doi.org/10.9734/AJB2T/2017/38282

[11] K.D.P.P. Gunathilake, K.K.D.S. Ranaweera, H.P.V. Rupasinghe, Optimization of phenolics and carotenoids extraction from leaves of Passiflora edulis using response surface methods. *Asian Journal of Biotechnology and Bioresource Technology*, 1 (2017) 1–11. https://doi.org/10.9734/AJB2T/2017/33480

[12] P. Ongkowijoyo, D.A. Luna-Vital, E.G.de Mejia, Extraction techniques and analysis of anthocyanins from food sources by mass spectrometry: An update. *Food chemistry*, 250 (2018) 113-126. https://doi.org/10.1016/j.foodchem.2018.01.055

[13] M. Radojković, Z.Zeković, S. Jokić, S.Vidović, Z. Lepojević, S. Milošević, Optimization of solid-liquid extraction of antioxidants from black mulberry leaves by response surface methodology. *Food Technology and Biotechnology*, 50(2) (2012) 167-176.

[14] M. A. Bezerra, R.E. Santelli, E.P.Oliveira, L.S. Villar, L.A. Escaleira, Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*, 76(5) (2008), 965-977. https://doi.org/10.1016/j.talanta.2008.05.019

[15] H.S. Arruda, G.A. Pereira, G.M. Pastore, Optimization of extraction parameters of total phenolics from Annona crassiflora Mart.(Araticum) fruits using response surface methodology. *Food Analytical Methods*, 10(1) (2017) 100-110. https://doi.org/10.1007/s12161-016-0554-y

[16] G. Janarny, K.K.D.S. Ranaweera, K.D.P.P. Gunathilake, Antioxidant activities of hydro-methanolic extracts of Sri Lankan edible flowers. *Biocatalysis and Agricultural Biotechnology*, 35 (2021) 102081. https://doi.org/10.1016/j.bcab.2021.102081

[17] G. Janarny, K.D.P.P. Gunathilake, Changes in rice bran bioactives, their bioactivity, bioaccessibility and bioavailability with solid-state fermentation by Rhizopus oryzae. *Biocatalysis and Agricultural Biotechnology*, 23 (2020) 101510. https://doi.org/10.1016/j.bcab.2020.101510

[18] H. Oztürk, U. Kolak, C. Meric, Antioxidant, anticholinesterase and antibacterial activities of *Jurinea consanguinea* DC. *Record Natural Products*. 5 (1) (2011) 43.

[19] I.S.C. Sulaiman, M. Basri, H.R.F. Masoumi, W.J. Chee, S.E. Ashari, M. Ismail, Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of *Clinacanthus nutans Lindau* leaves by response surface methodology. *Chemistry Central Journal*, 11(1) (2017) 1-11. https://doi.org/10.1186/s13065-017-0285-1

[20] A.A. Kamarudin, N.M. Esa, N. Saad, N.H. Sayuti, N.A.A, Razak, Heat assisted extraction of phenolic compounds from *Eleutherine bulbosa* (Mill.) bulb and its bioactive profiles using response surface methodology. *Industrial Crops and Products*, 144 (2020) 112064. https://doi.org/10.1016/j.indcrop.2019.112064

[21] T. Belwal, P. Dhyani, I.D. Bhatt, R.S. Rawal, V. Pande, Optimization extraction conditions for improving phenolic content and antioxidant activity in Berberis asiatica fruits using response surface methodology (RSM). *Food chemistry*, 207 (2016) 115-124. https://doi.org/10.1016/j.foodchem.2016.03.081

[22] P.W. Tan, C.P. Tan, C.W. Ho, Antioxidant properties: Effects of solid-to-solvent ratio on antioxidant compounds and capacities of pegaga (Centella asiatica). *International Food Research Journal* 18 (2011) 557-562. https://doi.org/10.1016/j.foodchem.2016.03.081

[23] N. Ćujić, K. Šavikin, T. Janković, D. Pljevljakušić, G.Zdunić, S. Ibrić, Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. *Food chemistry*, 194 (2016) 135-142. https://doi.org/10.1016/j.foodchem.2015.08.008

[24] L. Wang, N. Boussetta, N. Lebovka, E. Vorobiev, Effects of ultrasound treatment and concentration of ethanol on selectivity of phenolic extraction from apple pomace. *International Journal of Food Science & Technology*, 53(9) (2018) 2104-2109. https://doi.org/10.1111/ijfs.13835

[25] M. Irakli, P.Chatzopoulou, L. Ekateriniadou, Optimization of ultrasound-assisted extraction of phenolic compounds: Oleuropein, phenolic acids, phenolic alcohols and flavonoids from olive leaves and evaluation of its antioxidant activities. *Industrial Crops and Products*, 124 (2018) 382-388. https://doi.org/10.1016/j.indcrop.2018.07.070

[26] Y.C. Yang, J. Li, Y.G. Zu, Y.J. Fu, M. Luo, N. Wu, X.L. Liu, Optimisation of microwave-assisted enzymatic extraction of corilagin and geraniin from *Geranium sibiricum* Linne and evaluation of antioxidant activity. *Food Chemistry*, 122(1) (2010) 373-380. https://doi.org/10.1016/j.foodchem.2010.02.061

[27] K.D.P.P. Gunathilake, K.K.D.S. Ranaweera, H.P.V. Rupasinghe, Response surface optimization for recovery of polyphenols and carotenoids from leaves of *Centella asiatica* using an ethanol-based solvent system. *Food science & nutrition*, 7(2) (2019) 528-536. https://doi.org/10.1002/fsn3.832

[28] Q. Fu, L. Zhang, N.Cheng, M. Jia, Y.Zhang, Extraction optimization of oleanolic and ursolic acids from pomegranate (Punica granatum L.) flowers. *Food and Bioproducts Processing*, 92(3) (2014) 321-327. https://doi.org/10.1016/j.fbp.2012.12.006

[29] Y.Y. Thoo, S.K. Ho, J.Y. Liang, C.W. Ho, C.P, Tan, Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (*Morinda citrifolia*). *Food Chemistry*, 120(1) (2010) 290-295. https://doi.org/10.1016/j.foodchem.2009.09.064

[30] F. Dahmoune, B. Nayak, K. Moussi, H. Remini, K. Madani, Optimization of microwave-assisted extraction of polyphenols from Myrtus communis L. leaves. *Food chemistry*, 166 (2015) 585-595. https://doi.org/10.1016/j.foodchem.2014.06.066

[31] G. Patil, M. C. Madhusudhan, B. R. Babu, K. S. M. S. Raghavarao, Extraction, dealcoholization and concentration of anthocyanin from red radish. *Chemical Engineering and Processing: Process Intensification*, 48(1) (2009) 364-369. https://doi.org/10.1016/j.cep.2008.05.006

[32] X. T. Le, M. T., Huynh, T. N. Pham, V. T. Than, T. Q. Toan, L. G. Bach, N. Q. Trung, Optimization of total anthocyanin content, stability and antioxidant evaluation of the anthocyanin extract from Vietnamese *Carissa carandas* L. fruits. *Processes*, 7(7) (2019) 468. https://doi.org/10.3390/pr7070468

[33] F. Dranca, M.Oroian, Optimization of ultrasound-assisted extraction of total monomeric anthocyanin (TMA) and total phenolic content (TPC) from eggplant (*Solanum melongena* L.) peel. *Ultrasonics sonochemistry*, 31 (2016) 637-646. https://doi.org/10.1016/j.ultsonch.2015.11.008

[34] W. Quan, W. He, M. Lu, B. Yuan, M. Zeng, D. Gao, Z. He, Anthocyanin composition and storage degradation kinetics of anthocyanins-based natural food colorant from purple-fleshed sweet potato. *International Journal of Food Science and Technology*. 54 (2019) 2529–2539. https://doi.org/10.1111/ijfs.14163

[35] D. Ryu, E. Koh, Application of response surface methodology to acidified water extraction of black soybeans for improving anthocyanin content, total phenols content and antioxidant activity. *Food Chemistry*, 261 (2018) 260-266.

[36] H. Xue, J. Tan, Q. Li, J. Tang, X. Cai, Optimization ultrasound-assisted deep eutectic solvent extraction of anthocyanins from raspberry using response surface methodology coupled with genetic algorithm. *Foods*, 9(10) (2020) 1409. https://doi.org/10.3390/foods9101409

[37] T.N. Pham, T.D. Lam, M.T. Nguyen, X.T. Le, D.V.N. Vo, T.Q. Toan, T.S. Vo, Effect of various factors on extraction efficiency of total anthocyanins from Butterfly pea (*Clitoria ternatea* L. Flowers) in Southern Vietnam. *In IOP Conference Series: Materials Science and Engineering* 544 (2019) 012013. IOP Publishing.

[38] P. Hernández-Carranza, R. Ávila-Sosa, J.A. Guerrero-Beltrán, A.R. Navarro-Cruz, E. Corona-Jiménez, C.E. Ochoa-Velasco, Optimization of antioxidant compounds extraction from fruit by-products: Apple pomace, orange and banana peel, *Journal of food processing and preservation*, 40(1) (2016) 103-115. https://doi.org/10.1111/jfpp.12588

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