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Optimization of Polyphenols and Carotenoids Extraction from Leaves of *Cassia auriculata* for Natural Health Products

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Authors' contributions

This work was carried out in collaboration among all authors. Author KDPPG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KKDSR and HPVR managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *C. auriculata* leaves contains polyphenols and carotenoids and also it posses various antioxidant activities towards free radical scavenging, lipid peroxidation inhibition and reducing potential. The present study investigated the optimization of polyphenols and carotenoids extraction from *Cassia auriculata* leaves by response surface methodology (RSM).
Study Design: A three-factor, three-levels central composite design (CCD) was performed to determine the effect of solvent concentration (30-100%), extraction temperature (30-60°C) and extraction time (30-90 min) to obtain the best extraction parameters.
Place and Duration of Study: Fresh *C. auriculata* leaves were collected from home gardens in

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Makandura area of Sri Lanka and the experiments were conducted at the Department of Food Science and Technology of Wayamba University of Sri Lanka between June 2016 and August 2016.
Methodology: Total polyphenol and carotenoid contents of the ethanolic extracts of the *C. auriculata* leaves were determined. Total polyphenols and carotenoids content in the extracts were used as the response variables. According to the design used, twenty randomized experiments including six replicates as the center points were assigned based on the combinations of extraction variables used CCD and the values of independent process variables considered, as well as response variables. The optimal value of these factors was determined suing response surface methodology. Predicted values were compared with experimental values.
Results: The optimum extraction conditions for phenolics and carotenoids were 45.4% ethanol; 19.8°C; 110.5 min and 100% ethanol; 70.2°C; 9.5 min respectively. The optimal predicted contents for total polyphenols and carotenoids were 13.08 mg GAE/g-DW and 17.31 mg/g-DW respectively. Validation experiments results had good agreement with the predicted responses by RSM.
Conclusion: Ethanol concentration was the most significant factor affecting on total polyphenols and carotenoids extraction. Extraction temperatures and time did not significantly influence on carotenoids and polyphenols extraction from leaves of *C. auriculata.* The estimated optimum extraction conditions; were established and they were very close to the experimental values. These parameters can be used as the guidelines for scale-up extraction of bioactives from the leaves of *C. auriculata*.

Keywords: Cassia auriculata leaves; polyphenols; carotenoids; ethanol; response surface methodology.

1. INTRODUCTION

Chronic diseases such as, cancer, arthritis, cardiovascular diseases and neurodegenerative diseases are caused primarily due to oxidative stress triggered by reactive oxygen species (ROS) such as superoxide anion radical, hydrogen peroxide and hydroxyl radicals [1]. Although mammals have equipped with natural antioxidant defense mechanism, the endogenous system is not completely adequate to counteract the oxidative stress and therefore, adequate and regular intake of dietary antioxidants has been proposed [2]. A plethora of studies has indicated that dietary phytochemicals such as polyphenols and carotenoids from numerous fruits and vegetables exert several health-promoting functions, including reducing the risks of many chronic diseases such as certain cancers, cardiovascular and neurodegenerative diseases [3,4]. Most of these preventive effects of polyphenols and carotenoid compounds are associated with their antioxidant activity, protecting cells and tissues from oxidative damage mediated by various free radicals and ROS [5,6]. Therefore, there is a global trend of incorporating biologically active phytochemicals in functional foods, natural health products, and cosmetics.

Cassia auriculata Linn (Family: Caesalpiniaceae) commonly known as “Ranawara” in Sri Lanka, has been used extensively in indigenous medicine and as well as a leafy vegetable for hundreds of years. The plant parts such as leaves, flowers, barks and roots have been reported to possess many health promoting properties such as hepatoprotective [7], anticancer [8], antidiabetic [9], and antihyperglyceamic [10]. The major phytochemical constituents in the leaves of *C. auriculata* are reported as flavonoids, anthracene derivatives, alkaloids, and tannins [11,12]. Further, presence of benzocoumarin glycoside, avaraoside I, avaraol I, luteolin, kaempferol, quercetin, myricetin, 3-methoxyluteolin, kaempferol 3-*O*-glucopyranoside, epigallocatechin and emodin also reported [7]. Gunathilake & Ranaweera [13] have reported that *C. auriculata* leaves contains polyphenols and carotenoids and also it posses various antioxidant activities towards free radical scavenging, lipid peroxidation inhibition and reducing potential. Carotenoids are much effective antioxidant compound and capable of effective scavenging singlet molecular oxygen and peroxyl radicals [14]. Therapeutic properties of *C. auriculata* may possibly be attributed to these polyphenols and carotenoids. Therefore, determination of optimum conditions for the extraction of polyphenols and carotenoids from *C. auriculata* is important for future investigations and industrial applications.

For extraction, many factors such as solvent concentration, extraction temperature, solvent-to-

solid ratio and extraction time can significantly influence the extraction efficiency and bioactive recovery [15]. Therefore, it is necessary to optimize the extraction conditions to obtain the highest bioactives recovery for the use in functional food and natural health product applications. Extraction with food-compatible, low cost solvent system such as aqueous ethanol is one of the common method; however, there are only a few studies have been reported in the literature for leaves of *C. auriculata*. Further, bioactives such a polyphenols and carotenoids rich extracts can be used for the formulations of functional foods, ingredients and nutraceuticals as these commodities posses various functional properties such as antioxidants [16,17] and anti-inflammatory [18,19] etc. In an effort to examine the optimum extraction conditions for recovery of polyphenols and carotenoids using aqueous ethanol system, the present investigation was undertaken using response surface methodology (RSM). RSM is widely used tool to examine the effects of various extraction parameters and their interactions using one or more response variables.

2. MATERIALS AND METHODS

2.1 Plant Materials and Chemicals

Fresh *C. auriculata* leaves were collected from home gardens in Makandura area of Sri Lanka and washed with distilled water. The cleaned edible portions of this leaves were oven dried at 48°C for 48 h, and grounded into powder using a blender and were stored at -18°C until use. Voucher specimens of the samples have been deposited in the herbarium of the Department of Food Science and Technology of Wayamba University of Sri Lanka. All the chemicals used for this study were of analytical grade.

2.2 Preparation of Extracts

One gram of air dried and grounded leaf sample was placed in a conical flask with 20 mL aqueous ethanol (1:20 solid/liquid ratio) at selected concentrations and the extraction was carried out for using a rotary shaker (Unimax 1010, Heidolph, Kelheim, Germany) at 400 rpm, at specified temperature and time as according to the experimental design as shown in Table 1. The extracts were then filtered and the filtrates were stored at -18°C until used for the determination of total polyphenols and carotenoids in the extract.

2.3 Determination of Total Polyphenol Content

The total polyphenol content was determined using Folin–Ciocalteu assay [20] with some modification, as described by Gunathilake and Ranaweera [13]. Leaf extract (0.5 mL) and 1.5 mL of Folin–Ciocalteu reagent (0.2N) were mixed and incubated at room temperature (30°C) for 15 minutes at dark. Then 1.2 mL sodium carbonate (7.5%) was added and incubated for further 2 hours at dark. Absorbance was measured at 760 nm using UV/VIS spectrometer (Optima, SP-3000, and Tokyo, Japan) and the concentration of total polyphenols was expressed as mg of gallic acid equivalents (GAE) per gram DW.

2.4 Total Carotenoids Content

The carotenoid content was determined according to the method described by Sükran [21] with slight modifications as described in Gunathilake and Ranaweera [13] and the level of carotenoids was expressed as mg/g DW. The extracts were mixed for one minute using a vortex and the homogenate was filtered through a filter paper (No: 42 Whatman). Filtered extracts were centrifuged using the centrifuge (EBA20) for 10 min at 245g. The supernatant was separated and the absorbance was read at 470, 653, 666 nm on UV/VIS spectrometer (SP-3000).

The concentration of each pigment was calculated according to the following formulas and was reported as µg per g dry weight of sample.

$$\text{Carotenoid content} = 1000\ (A470) - 2.270\ (C_a) - 81.4\ (C_b)/227.$$

where C_a, Chlorophyll a and C_b, chlorophyll b;

$$\text{Chlorophyl a} = 11.75\ (A662) - 2.350(A645);$$
$$\text{Chlorophyl b} = 18.61\ (A645) - 3.960\ (A662).$$

2.5 Experimental Design

To explore the effects of independent variables on the responses within the range of investigation, a central composite design (CCD), with the three factors with three levels was conducted. These factors were, ethanol concentration (30–100%), extraction temperature (30–60°C) and extraction time (30-90 min) and the solid to liquid ratio were maintained at 1:20 at each experiment. Each variable to be optimized was coded at three levels 1, 0, +1 and the

Table 1. Levels of extraction variables for experimental designs

Independent variables	Level total polyphenol content/ carotene content				
	+1	0	-1	+1.682	-1.682
X1: Ethanol (%)	100	65	30	123.86	6.137
X2: Temperature (°C)	60	45	30	70.23	19.773
X3: Time (min)	90	60	30	110.45	9.546

variables and their levels for the CCD are shown in Table 1. Total polyphenols and carotenoids content in the extracts were used as the response variables. According to the design used, twenty randomized experiments including six replicates as the center points were assigned based on CCD and the values of independent process variables considered, as well as response variables, are given in Table 2. Acquired data were handled to calculate statistical values such as mean and standard deviation (SD) using Microsoft Excel (Microsoft Inc., Redmond, WA, USA). For data analysis, Minitab15 software was used and the assumptions of normality and constant variance were checked and confirmed. A response surface analysis and analysis of variance (ANOVA) were employed to determine the regression coefficients, the statistical significance of the model terms and to fit the mathematical models of the experimental data that aimed to optimize the overall region for both response variables (Tables 3 and 4). A second-order quadratic equation was then fitted to the data by multiple regression procedure. For a three-factor system, the model equation is given below:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_1^2 X_1^2 + \beta_2^2 X_2^2 + \beta_3^2 X_3^2 + \beta_1\beta_2 X_1 X_2 + \beta_1\beta_3 X_1 X_3 + \beta_2\beta_3 X_2 X_3$$

where Y is the predicted dependent variable; β_0 is a constant that fixes the response at the central point of the experiment; β_1, β_2 and β_3 are the regression coefficients for the linear effect terms; β_1^2, β_2^2 and β_3^2 are the quadratic effect terms; and $\beta_1\beta_2$, $\beta_1\beta_3$ and $\beta_2\beta_3$ are the interaction effect terms, respectively. X1, X2, and X3 are the independent variables (Table 1). The response model was expressed using the coded and uncoded variables. The optimal value of these factors was determined. The adequacy of the model was predicted through the regression analysis (R^2) and the ANOVA analysis. The relationship between the independent variables and the response variables was demonstrated by the response surface plots. Multiple graphical and numerical optimizations of the experimental data were done to identify the optimum extraction conditions to achieve the maximum recovery of response variable (Table 5). Predicted optimum extraction conditions that would give higher levels of responses were verified experimentally (Table 6).

3. RESULTS AND DISCUSSION

The recovery of bioactive constituents such as polyphenols and carotenoids is influenced by extraction conditions such as extraction solvent properties, extraction time, and extraction temperature [22]. The effects of a variety of extraction conditions on the extraction yield of total polyphenols and carotenoids from *C. auriculata* leaves were studied. RSM is accepted as a powerful tool in optimizing experimental conditions to maximize various responses [23]. Therefore, RSM was used to optimize the extraction conditions for total polyphenols and carotenoids.

The values of the independent process variables (solvent concentration, extraction temperature and extraction time) considered, as well as the measured values for total polyphenols and carotenoids are given analytically in Table 2 and 3. The ‘‘fitness’’ of the models was checked through the lack-of-fit test ($p < 0.05$), which indicated the adequacy of models to accurately predict the variation [24].The quality of fit to the second-order polynomial models for leaf extracts was established based on the coefficients of determination (70% $>R^2$), regression p-value ($p<0.1$) and lack of fit ($p>0.05$) indicating that the models could be used to predict the responses. Tables 2 and 3 shows the analysis of variance of the fitted quadratic polynomial model for total polyphenols and carotenoids. The regression coefficients of determination (R^2) for total polyphenols and carotenoids were 0.80 and 0.90, respectively. The p-values determined implied that the equation found for polyphenols and carotenoids (Table 5) can adequately predict the experimental results, as the fit was statistically significant ($p<0.1$). Amount of polyphenols and carotenoids in the extracts were employed in a multiple regression analysis, performed using RSM, to fit the second-order polynomial

equations and the software generated estimated regressions coefficients for quadratic equations as appeared in Tables 3 and 4 The coefficients for ethanol concentration were significant ($p<0.05$) for both responses, indicating that the ethanol concentrations affect the recovery of polyphenols and carotenoids from *C. auriculata*. Coefficients for extraction temperature and time for polyphenols and carotenoids recovery were insignificant ($p<0.05$) and the trends were easily recognized in three-dimensional response surface plots in Figs. 1 and 2.

Table 2. Central composite design arrangement for extraction of polyphenols and carotenoids from *Cassia auriculata*

Run Order	Ethanol %	Temperature (°C)	Time (min)	Polyphenols (mg/g)*	Carotenoids (mg/g)
1	100	30	90	5.71	11.13
2	100	60	30	4.94	11.69
3	65	45	10	7.39	6.09
4	65	45	60	5.68	3.33
5	65	45	60	7.02	5.73
6	65	45	60	8.22	6.52
7	65	45	60	8.23	6.34
8	65	20	60	11.44	8.57
9	100	60	90	4.85	11.67
10	30	30	90	9.33	2.89
11	65	45	110	8.86	6.74
12	100	30	30	3.00	11.13
13	6	45	60	5.58	2.65
14	30	60	90	9.69	2.89
15	65	70	60	8.96	7.81
16	30	60	30	9.62	2.97
17	30	30	30	8.40	2.28
18	65	45	60	7.79	6.34
19	65	45	60	5.68	3.33
20	124	45	60	2.90	11.69

** Values presented as mg GAE/g*

Table 3. ANOVA table for response surface for total polyphenols

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	80.478	80.478	8.9420	4.43	0.015
Linear	3	41.814	41.814	13.9381	6.91	0.008
Square	3	36.634	36.634	12.2113	6.05	0.013
Interaction	3	2.030	2.030	0.6766	0.34	0.800
Residual Error	10	20.173	20.173	2.0173		
Lack-of-Fit	5	13.105	13.105	2.6210	1.85	0.257
Pure Error	5	7.068	7.068			
Total	19	100.651	1.4136			

Table 4. ANOVA table for response surface for total carotenoids

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	199.913	199.913	22.2125	10.32	0.001
Linear	3	181.756	181.756	60.5853	28.14	0.000
Square	3	18.035	18.035	6.0116	2.79	0.095
Interaction	3	0.122	0.122	0.0406	0.02	0.996
Residual Error	10	21.527	21.527	2.1527		
Lack-of-Fit	5	9.936	9.936	1.9872	0.86	0.565
Pure Error	5	11.591	11.591	2.3182		
Total	19	221.440				

Table 5. Estimated regression coefficients for polyphenols and carotenoids using data in uncoded units

Terms	Polyphenols	P	Carotenoids	P
Constant	13.05	0.00*	9.876	0.0*
Ethanol %	0.067	0.01*	0.039	0.0*
Temperature (°C)	-0.325	0.78	-0.381	0.93
Time (min)	0.0169	0.28	-0.0287	0.77
Ethanol %*Ethanol %	-9.313E-04	0.12	-0.0005	0.15
Temperature *Temperature (°C)	0.0043	0.03*	0.0043	0.03*
Time (min)*Time (min)	-0.00026	0.55	0.0004	0.40
Ethanol %*Temperature (°C)	-1.191	0.90	9.762E-05	0.92
Ethanol %*Time (min)	0.00019	0.70	-6.548E-05	0.90
Temperature (°C)*Time (min	-0.00102	0.38	-1.972E-04	0.87
R^2	79.96		90.28%	
P values for regression	0.000		0.011	

**Significant at p<0.05*

3.1 Effect of Extraction Parameters on Total Polyphenol Content

To visualize the relationship between independent and dependent parameters for the total polyphenols and carotenoids extraction, three-dimensional surface plots were constructed according to the quadratic polynomial models generated by the Minitab software (Figs. 1-2). Response surfaces were used to illustrate the effects of solvent concentration, extraction time and the temperature on the responses. The responses demonstrated that the ethanol concentration greatly affect the recovery of polyphenols from *C. asiatica* leaves (Fig. 1). The extraction efficiency of the total polyphenols from plant matrices was highly influenced by the type of the extraction solvent used [25]. The use of ethanol is relatively cheap, reusable and nontoxic, could lend an environmentally friendly aspect to the low-cost preparation of potentially bioactive extracts for food uses. As shown in Fig. 1a, when ethanol concentration was fixed at 30%, total polyphenol yield was increased slightly by increasing extraction time to 120 min and reducing the extraction temperature and reached the maximum value at the lower extraction temperature and increasing the extraction duration. While lower ethanol % as extraction solvent were distinguished by the highest level of extract yield of polyphenols, pure ethanol (100% ethanol) showed the lowest level of all the other solvent systems used for *C. auriculata* (Fig. 1). Results showed that at lower solvent concentration (30%), while keeping the extraction temperature (30°C) and extraction time (30 min) at lower levels, higher the extractable phenolics (8.40 mg GAE/g DW) when compared with the extract from 100% ethanol (3.00 mg GAE/g DW). As the extraction and separation of polyphenols depend greatly on the polarity of the extraction solvent, use of a pure solvent may not be effective for the separation of polyphenols from plant materials [26]. This is indicating that the mixture of water and ethanol as solvent agent exhibited the best performance to extract polyphenols from various plant sources. Therefore, a combination of alcohol with water is more effective in extracting polyphenols and it was reported in many published literature that polyphenols are more extractable in polar solvents as compared to non-polar ones [27,26,6].

Regarding extraction temperature on total polyphenols, the recovery of polyphenols was increased considerably when the extraction temperature was at low level compared with that of at 60°C, while the % ethanol maintained at a low level (Figs. 1c and d). The highest extractable polyphenol content, 11.44 mg GAE /g, was observed at 65% ethanol concentration, 30°C temperature and 60 min extraction time. Results showed that the temperature increased to 70°C while keeping the ethanol concentration and extraction time at 65% and 60 min respectively, the extractable polyphenol content reduced from 11.44 to 8.96 mg GAE/g. This could be due to degradation and epimerization of some polyphenols when the extraction is conducted at too high temperatures, even though high extraction temperatures can increase the yield of polyphenols as the cell walls of leaves become more permeable to the solvent and to the constituents [28]. This is indicating that the extraction at low temperatures is desirable for *C. auriculata* leaves to avoid these changes. Extraction time was another important parameter

in the extraction procedure for bioactives in many previous studies [29,30]. Figs. 1e and f shows the effect of the interaction of ethanol concentration and extraction temperature on the total polyphenol contents at a fixed extraction time of 30 min and 90 min, respectively. Figs. c and d shows the interaction effect of ethanol and time on total polyphenols. Interestingly, this results showed that extraction time did not have a significant effect on the polyphenols extraction from *C. auriculata* leaves as similar polyphenol content were extractable at studied fixed times.

3.2 Effect of Extraction Parameters on Carotenoids Content

According to Ofori-Boateng and Lee [31], ethanol can be used for carotenoids extraction and this is highly influenced by various extraction variables such as solvent concentration, extraction temperature and time [32]. However, many researchers have used non-polar solvent for carotenoid extraction, for examples, petroleum ether/acetone (1/1) for rapeseed [32], ethanol/water for leaves of *Centella asiatica* [33],

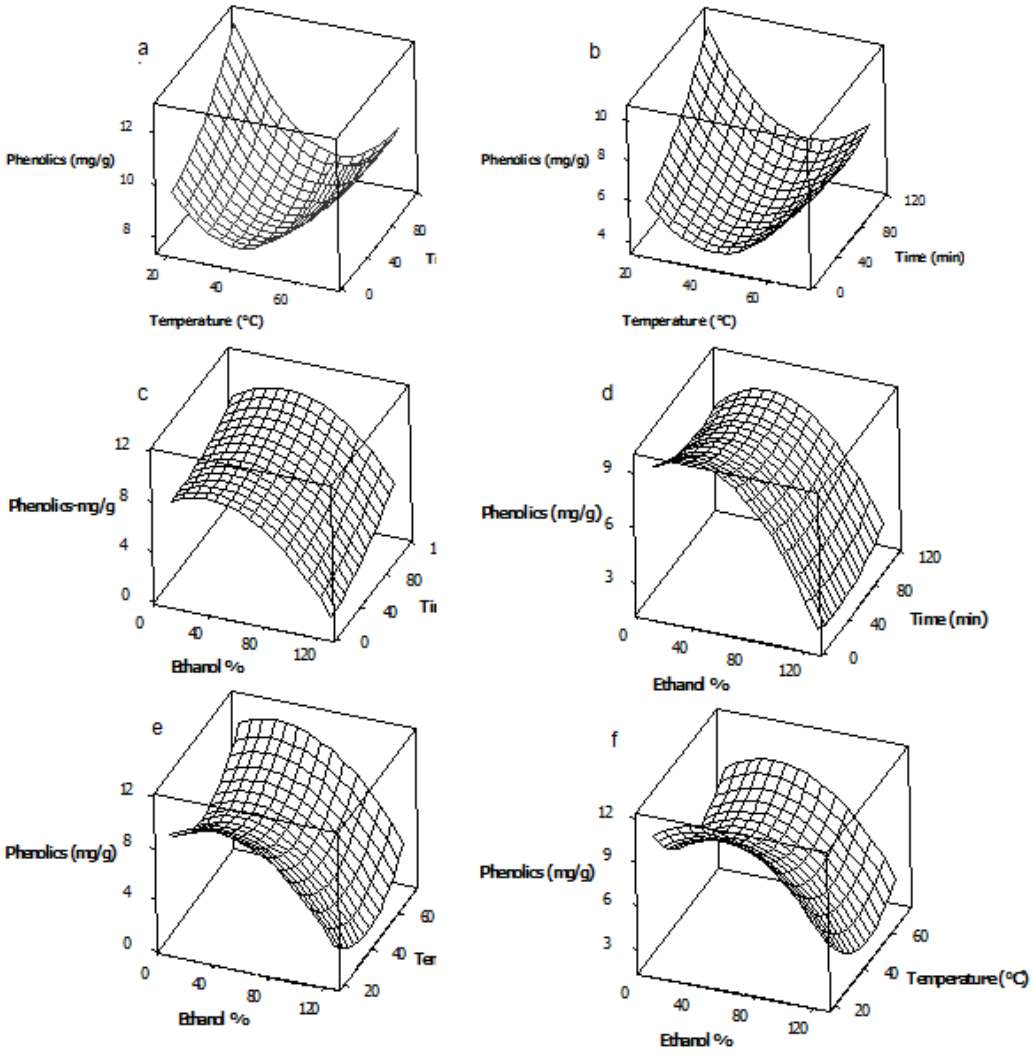

Fig. 1. Pair wise response surface plots of the polyphenols (mg GAE/g DW) extraction from *Cassia auriculata* leaves as a function of ethanol %, extraction temperature and time: ethanol % was kept constant at 30% (a) and 100% (b); temperature of extraction was kept constant at 30°C (c) and 60°C (d); the time of extraction was kept constant at 30 min (e) and 90 min (f)

water for ginger [3] and hexane/acetone/alcohol (2/1/1) for lycopene [34]. There are polar (eg; lutein) and non polar carotenoids (eg: β carotene) and these polar carotenoids can be easily extractable with ethanol [31]. Lutein is one of the common carotenoids in green leafy vegetables [16]. Influence of three extraction conditions towards total carotenoids extraction was reported with the coefficients of the second-order polynomial regression equation in Table 5. The effect of the variables and their interaction on the responses can be seen in Fig. 2. Figs. a and b shows the effect of the interaction of extraction temperature and time on the carotenoids at fixed ethanol concentration (30% and 100%). Figs. 2 c and d shows the effect of the interaction of ethanol concentration and extraction time on the carotenoid extraction at a fixed extraction temperature of 30 and 60°C, respectively. Similarly, Figs. 2 e and f show the effect of the interaction of solvent concentration and extraction temperature on carotenoid at 30 and 90 min. As shown in Fig. 2c-f, carotenoids extraction increased towards the higher ethanol concentration and the highest recovery was obtained at 100% ethanol concentration. As shown in Figs. 1a,b, when ethanol concentration increased from 30 to 100% while maintaining extraction temperature and time at 30°C and 30 min respectively, the extractable carotenoids content increased from 2.28 mg/g to 11.13 mg/g DW. The results indicating that the carotenoids can be extracted at higher ethanol concentration, towards non polarity. Results clearly showed that the extraction of carotenoids had a greater influence of ethanol concentration and was significant ($p < 0.05$). The extractability of carotenoids from plant sources depend largely on the nature of the polarity of the solvents [32]. *C. auriculata* prefers towards non polar conditions indicating that it may contained more non-polar carotenoids.

According to the Fig. 2, extraction temperature and extraction time have not significant effects ($p<0.05$) on carotenoid recovery though several published studies have reported the effect of extraction and time on carotenoids extraction [35,31]. Interaction effects of extraction temperature and time showed a curved relationship (Figs. 2 a and b). According to Guo et al. [36], for conventional maceration and soxhlet extraction requires high temperatures (over 70°C) for optimal carotenoid yields unless an advance technology such as ultrasonication is used to facilitate extraction of carotenoids from plant. Meléndez-Martínez et al. [35] have reported that carotenoids are degraded at elevated temperatures. Moreover, in our study, we have found that ethanol concentration was the most significant factor affecting the total carotenoids as well as total polyphenols.

3.3 Optimization of Polyphenols and Carotenoids and Verification of the Model

Optimum process parameters achieved by maximizing total polyphenols and carotenoid contents and the desirability function of the MINITAB statistical software is used to obtain the best compromise of the two responses with the weights of all 1.0. As shown in Table 6, the predicted optimal ethanol concentration, extraction temperature, extraction time were developed for maximizing the responses and they were 45.4%, 19.8°C and 110.5 min, respectively for polyphenols and under these condition could yield about 13.08 mg/g DW total polyphenols. For carotenoids, optimum extraction conditions were 100% ethanol, 70.20°C extraction temperature and 9.55 min extraction time and this could yield about 17.03 mg/g DW carotenoids. An experiment was run in accordance with the recommended optimum conditions for two responses and it was found that the values obtained for polyphenols and carotenoids were not significantly different ($p<0.05$) to that of the predicted values. This proves the validity of the optimized model used.

Table 6. Predicted values and experimental values of total polyphenols and carotenoids at the optimum extraction conditions for *Cassia auriculata*

Optimum extraction conditions		Predicted values (mg/g)		Experimental values (mg/g)	
Polyphenols	**Carotenoids**	**Polyphenols***	**Carotenoids**	**Polyphenols***	**Carotenoids**
ETOH:45.4%	ETOH:100%	13.1	17.0	11±3	14±5
Temp:19.8°C	Temp:70.2°C				
Time:110.5 min	Time:9.55 min				

** Values presented as mg GAE/g*

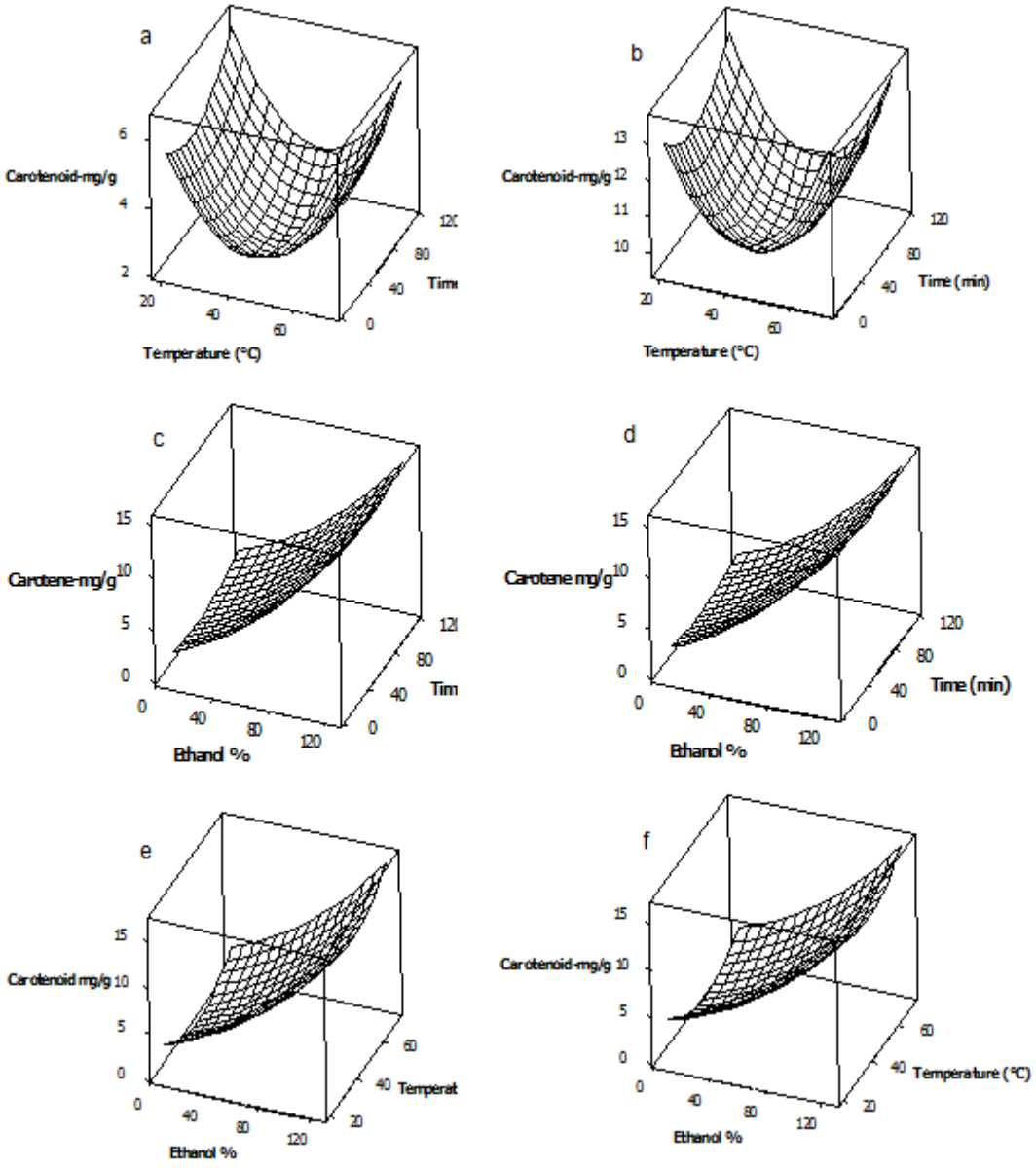

Fig. 2. Pair wise response surface plots of the carotenoids (mg/g DW) extraction from *Cassia auriculata* leaves as a function of ethanol %, extraction temperature and time: ethanol % was kept constant at 30% (a) and 100% (b); temperature of extraction was kept constant at 30°C (c) and 60°C (d); the time of extraction was kept constant at 30 min (e) and 90 min (f)

4. CONCLUSION

In the present study, RSM was used to optimize the extraction of total polyphenols and carotenoids contents from *C. auriculata* leaves. Among the extraction parameters studied, ethanol concentration was the most significant factor affecting total polyphenols and carotenoids extraction. Extraction temperatures and time did not significantly influence on carotenoids and polyphenols extraction from leaves of *C. auriculata.* Results showed that the estimated optimum extraction conditions for polyphenols and carotenoids were significantly different The estimated optimum extraction conditions; ethanol %, temperature and time; for polyphenols and carotenoids were 45.4%, 19.8°C, 110.5 min and 100%, 70.20°C, 9.55 min respectively. Under

optimized conditions the experimental values were very close to the predicted values and they were not significantly different. These parameters can be used as the guidelines for scale-up extraction of bioactives from the leaves of *C. auriculata*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tabaraki R, Nateghi A. Optimization of ultrasonic-assisted extraction of natural antioxidants from rice bran using response surface methodology. Ultrasonics Sonochemistry. 2011;18:1279–1286.
2. Kaliora AC, Dedoussis GVZ, Schmidt H. Dietary antioxidants in preventing atherogenesis – A review. Journal of Atherosclerosis. 2006;187:1–17.
3. Gunathilake KDPP. A fruit-based functional beverage designed to reduce the risk of cardiovascular disease. MSc Thesis.Dalhousie University; Halifax, NS, Canada; 2012.
4. Vita JA. Polyphenols and cardiovascular disease: Effects on endothelial and platelet function. American Journal of Clinical Nutrition. 2005;81:292−297.
5. Sies H, Stahl W. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. American Journal of Clinical Nutrition. 1995;62:1315–1321.
6. Hayouni EA, Abedrabba M, Bouix M, Hamdi M. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L and *Juniperus phoenicea* L. fruit extracts. Food Chemistry. 2007;105:1126–1134.
7. Nakamura S, Xu F, Ninomiya K, Nakashima S, Oda Y, Morikawa T, Muraoka O, Yoshikawa M, Matsuda H. Chemical structures and hepatoprotective effects of constituents from *cassia auriculata* leaves. Chemical and Pharmaceutical Bulletin. 2014;62(10): 1026–1031.
8. Prasanna R, Harish CC, Pichai R, Sakthisekaran D, Gunasekaran P. Anti-cancer effect of *Cassia auriculata* leaf extract in vitro through cell cycle arrest and induction of apoptosis in human breast and larynx cancer cell lines. Cell Biology International. 2009;33:127-134.
9. Kumar VG, Gokavarapu SD, Rajeswari A, Dhas TS, Karthick V, Kapadia Z, Shrestha Z, Barathy IA, Roy A, Sinha S. Facile green synthesis of gold nanoparticles using leaf extract of antidiabetic potent Cassia auriculata. Colloids and Surfaces B. Biointerfaces. 2011;87:159– 163.
10. Manickam P, Namasivaqyam N, Periyasamy V, Rajagopal SK. Effect of *Cassia auriculata* leaf extract on lipids in rats with alcoholic liver injury. Asia Pacific Journal of Clinical Nutrition. 2002;11:157-163.
11. Sharmila G, Nikitha VS, Ilaiyarasia S, Dhivya K, Rajasekar V, Kumar NM, Muthukumaran K, Muthukumaran, C Ultrasound assisted extraction of total phenolics from *Cassia auriculata* leaves and evaluation of its antioxidant activities. Industrial Crops and Products. 2016;84: 13–21.
12. Juan-Badaturuge M, Habtemariam S, Thomas MJ. Antioxidant compounds from a South Asian beverage and medicinal plant, *Cassia auriculata*. Food Chemistry. 2011;125(1):221–225.
13. Gunathilake KDPP, Ranaweera KKDS. Antioxidative properties of 34 green leafy vegetables. Journal of Functional Foods. 2016;26:176-186.
14. Stahl W, Sies H. Antioxidant activity of carotenoids. Molecular Aspects of Medicine. 2003;24:345–351.
15. Gan C, Latiff AA.. Optimisation of the solvent extraction of bioactive compounds from *Parkia speciosa* pod using response surface methodology. Food Chemistry. 2011;124:1277–1283.
16. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. Change of phenolics, carotenoids, and antioxidant capacity following simulated gastrointestinal digestion and dialysis of selected edible green leaves. Food Chemistry. 2018a; 245(15):371-379.

17. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. Analysis of rutin, β-carotene, and lutein content and evaluation of antioxidant activities of six edible leaves on free radicals and reactive oxygen species. Journal of Food Biochemistry. 2018b;42(5):p.e12579.
18. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. Influence of boiling, steaming and frying of selected leafy vegetables on the in vitro anti-inflammation associated biological activities. Plants. 2018c;7(1):22.
19. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. In vitro Anti-Inflammatory properties of selected Green Leafy vegetables. Biomedicines. 2018d; 6(4):107.
20. Singleton VL, Orthofer R, Lamuela-Raventos R. Analysis of total phenols and other oxidation substrates and antioxidants by means of FC reagent. Methods in Enzymology. 1999;29:152–178.
21. Sükran DERE, Gunes T, Sivaci R. Spectrophotometric determination of chlorophyll-A, B and total carotenoid contents of some algae species using different solvents. Turkish Journal of Botany. 1998;22(1):13-18.
22. Alothman M, Bhat R, Karim AA. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry. 2009;115:785–788.
23. Hajj YE, Louka N, Nguyen C, Maroun RG. Low cost process for phenolic compounds extraction from cabernet sauvignon grapes (*Vitis vinifera* L. cv. cabernet sauvignon) optimization by response surface methodology. Food and Nutrition Science. 2012;3:89–103.
24. Kong KW, Ismail AR, Tan ST, Prasad KMN, Ismail A. Response surface optimisation for the extraction of phenolics and flavonoids from a pink guava puree industrial by-product. International Journal of Food Science & Technology. 2010; 45:1739–1745.
25. Liyana-Pathirana C, Shahidi F. Optimization of extraction of phenoliccompounds from wheat using response surface methodology. Food Chemistry. 2005;93:47–56.
26. Prasad KN, Hassan FA, Yang B, Kong KW, Ramanan RN, Azlan A, Ismail A. Response surface optimisation for the extraction of phenolic compounds and antioxidant capacities of underutilised *Mangifera pajang* Kosterm peels. Food Chemistry. 2011;128(4):1121-1127.
27. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. Optimization of phenolics and carotenoids extraction from leaves of *Passiflora edulis* using response surface methods. Asian Journal of Biotechnology and Bioresource Technology. 2017;1(1):1-11.
28. Lee L, Lee N, Kim YH, Lee C, Hong SP, Jeon Y, Kim Y. Optimization of ultrasonic extraction of phenolic antioxidants from green tea using response surface methodology. Molecules. 2013;18:13530-13545.
29. Gunathilake KDPP, Rupasinghe HPV. Optimization of water based-extraction methods for the preparation of bioactive-rich ginger extract using response surface methodology. European Journal Medicinal Plants. 2014;4(8):893.
30. Sahin S, Samli R. Optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology. Ultrasonics Sonochemistry. 2013;20:595–602.
31. Ofori-Boateng C, Lee KT. Response surface optimization of ultrasonic-assisted extraction of carotenoids from oil palm (*Elaeis guineensis* Jacq.) fronds. Food Science & Nutrition. 2013;1(3):209–221.
32. Wang L, Liu Y. Optimization of solvent extraction conditions for total carotenoids in rapeseed using response surface methodology. Natural Science. 2009;1(01):23.
33. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. Response surface optimization for recovery of polyphenols and carotenoids from leaves of Centella asiatica using an ethanol-based solvent system. Food science & nutrition. 2019; 7(2):528-536.
34. Kaur D, Wani AA, Oberoi DPS, Sogi DS. Effect of extraction conditions on lycopene extractions from tomato processing waste skin using response surface methodology. Food Chemistry. 2008; 108(2):711-718.
35. Mele´ndez-Martı´nez JA, George B, Isabel MV. Relationship between the colour and the chemical structure of carotenoid pigments. Food Chemistry, 2007;101: 1145–1150.

36. Guo X, Zou X, Sun M. Optimization of extraction process by response surface methodology and preliminary characterization of polysaccharides from *Phellinus igniarius*. Carbohydrate Polymers. 2010;80:344–349.

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