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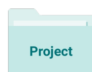

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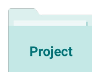

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# Optimization of Phenolics and Carotenoids Extraction from Leaves of *Passiflora edulis* Using Response Surface Methods

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***Authors' contributions***

*This work was carried out in collaboration between 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

The optimum extraction conditions for the highest recovery of total phenolics and carotenoids contents for leaves of *Passiflora edulis* were developed using response surface methodology. The effects of solvent concentration (30-100%), extraction temperature (30-60℃) and extraction time (30-90 min) on the recovery of total phenolics and carotenoids were investigated. A second order polynomial model produced a satisfactory fitting of the experimental data with regard to total phenolics ($R^2$ = 84.75%, $p < 0.004$) and carotenoid ($R^2$ = 78.74, $p < 0.019$) contents. The optimum extraction conditions of ethanol concentration, extraction temperature and extraction time for phenolics, were 6.1%, 70.2℃, and 110.5 min and for ca rotenoids, the optimum parameters were

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100%, 70.2℃ and 110.5 min, respectively. The optimal predicted contents for total phenolics (9.03 mg Gallic Acid Equivalent (GAE)/ g DW) and carotenoids (8.74 mg/g DW) values in the extracts were agreed with the experimental values obtained with optimum extraction conditions for each response.

*Keywords: Passiflora edulis leaves; phenolics; carotenoids; response surface methodology.*

## 1. INTRODUCTION

Phenolics and carotenoids are dietary bioactive compounds commonly found in fruits and vegetables. Various epidemiological studies have reported that a diet rich in these bioactives may have protective effects against various degenerative diseases, including cancers and cardiovascular diseases [1,2]. Most of these preventive effects of phenolic and carotenoid compounds are associated with their antioxidant activity, protecting cells and tissues from oxidative damage by various free radicals and reactive oxygen species (ROS) [3,4]. Currently, research and development activities that are aimed at bioactives rich dietary sources have become a global interest.

*P. edulis* is a tropical plant which is popular not only because of its delicious fruit but also because of the infusions made with the leaves [5]. In many countries, leaves of *P. edulis* are consumed as a leafy vegetable as well as have been used in many pharmaceutical preparations [6]. Leaves of *P. edulis* reported to be rich sources of polyphenols (9.23 mg GAE/g dry weight-DW) and carotenoids (4.17 mg/g DW) [7]. Coleta and co-authors [8] have reported that the leaves of *Passiflora* species contain polyphenols such as orientin 2″-*O*-rhamnoside and luteolin 7-*O*-(2-rhamnosylglucoside. In another study, it has been mentioned about several apigenin and luteolin derivatives from leaves of *P. edulis* [9]. Changes in oxygen utilization in the body and the excess formation of ROS such as hydroxyl radicals, nitric oxide radicals and singlet oxygen, can damage cellular macro molecules such as lipids, proteins and DNA by oxidative action [10]. Polyphenols and carotenoids have antioxidant properties as they could neutralize or quench free radicals or ROS which are responsible for the initiation of many chronic diseases such as cardiovascular diseases and cancers [11]. Carotenoids are much effective antioxidant in scavenging singlet molecular oxygen and peroxyl radicals [1]. Gunathilake & Ranaweera [7] have reported that *P. edulis* leaves posses antioxidant activities such as free radical scavenging, lipid peroxidation inhibition and reducing potential. Beninca et al. [12] have reported an aqueous extract of *P. edulis* leaves exhibited potent anti-inflammatory action in the experimental model *in vivo*. These properties could be due to the presence of bioactives such as polyphenols and carotenoids in these leaves. Previous findings revealed that the leaves of *P. edulis* have potent antioxidant properties and might be considered as possible dietary sources of natural antioxidants [13]. Further studies are needed to optimize the bioactive extraction methods and explore the potential use of *P. edulis* leaf extract in the prevention of specific chronic diseases.

There is a current trend in investigating natural dietary sources of antioxidants. Therefore, the exploration of antioxidant-rich natural sources such as leaves of *P. edulis* will be interested in the functional foods and nutraceutical industry. Extraction is the initial and most vital step in the recovery and purification of bioactive compounds from plant sources [14]. Many factors such as solvent concentration, extraction temperature, solvent-to-solid ratio and extraction duration may significantly influence the extraction efficiency and bioactive concentration [15]. Therefore, it is necessary to optimize the extraction conditions to obtain the highest bioactives recovery. Response surface methodology (RSM) was introduced and widely used nowadays as a useful tool to evaluate effects of multiple factors and their interactions in one or more response variables because the traditional one-factor-at-a-time approach has several drawbacks. RSM is one of the most popular optimisation techniques in the area of food science and technology and has been applied for extraction of antioxidant bioactives from a number of dietary sources such as *Zingiber officinale* [16], *Parkia speciosa* [15], *Mangifera pajang* [14], kinema [17] and *Brassica napus* [18]. However, there are no studies reported to optimize the extraction conditions for polyphenols and carotenoids from leaves of *P. edulis*. Hence, the objective of the present study was to optimize the extraction conditions for *P. edulis* leaves to obtain the highest polyphenols and carotenoids content. The findings would be much helpful for the functional foods and nutraceuticals formulations.

# 2. MATERIALS AND METHODS

## 2.1 Plant Materials

*P. edulis* leaves were collected from home gardens in Makandura area of Sri Lanka. The leaves samples were taxonomically identified by a botanist and the 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. Edible portions of cleaned leaves were oven dried at 48℃ for 48 h, and ground into powder using a blender and were store at -18℃ until use.

## 2.2 Reagents

Gallic acid and ethanol were purchased from Sigma-Aldrich, St. Louis, MO, the USA through Analytical Instrument Pvt Ltd, Colombo, Sri Lanka. All other chemicals used were of analytical grade.

## 2.3 Preparation of Extracts

One gram of air dried powder of leaf sample was placed in a conical flask with aqueous ethanol at desired concentrations and extraction was carried out for using a rotary shaker (Unimax 1010, Heidolph, Kelheim, Germany) at 400 rpm, at specified temperature as dictated by the experimental design. The optimization procedure was designed based on a three-factor inscribed central composite design (CCD) consisting of ethanol concentration (30–100%), temperature (30–60℃) and extraction time (30-90 min) as shown in Table 1. The extracts were then filtered through a filter paper (Whatman No. 42; Whatman Paper Ltd, Maidstone, UK) and the filtrates were used to determine the total phenolic content and carotenoid contents.

## 2.4 Determination of Total Phenolic Content

The total phenolic content was determined using Folin–Ciocalteu assay [19] with some modification, as described by Gunathilake and Ranaweera [7]. About 0.5 mL of ethonolic extract and 0.1 mL of Folin–Ciocalteu reagent (0.5N) were mixed and incubated at room temperature for 15 minutes at dark. Then 2.5 mL 7.5% sodium carbonate was added to the mixture and further incubated for 2 hours at dark at room temperature and then the absorbance was measured at 760 nm using UV/VIS spectrometer (Optima, SP-3000, and Tokyo, Japan). The concentration of total phenols was expressed as mg of gallic acid equivalents (GAE) per gram DW. Gallic acid was used in the construction of standard curve.

## 2.5 Total Carotenoids Content

The carotenoid content was analyzed according to the method described by [20] with slight modifications and carotenoid contents were reported as mg/g DW. According to this method, total carotenoids can be determined after having subtracted the concentration of chlorophyll A and B, using wavelengths 661.6 and 644.8 nm, respectively, and corresponding absorption coefficients at which carotenoids do not absorb.

## 2.6 Experimental Design

RSM was used for investigating the influence of three independent variables, ethanol concentration, extraction temperature, and extraction time; and the response variables were total phenolic and total carotenoid contents. A three-factor inscribed central composite design (CCD) was used to identify the relationship existing between the response functions and the process variables, as well as to determine those conditions that optimized the extraction process of total phenolics and carotenoids contents of the extracts. The independent variables and the range studied were ethanol concentration (30–100%), temperature (30–60℃) and extraction time (30-90 min). The selection and range of these three factors were based on previous studies. Each variable to be optimized was coded at three levels 1, 0, +1 (Table 1). 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 measured total phenolic content and carotenoid content, are given in Table 2.

## 2.7 Statistical Design

Results for the contents of total phenolics and carotenoids were presented as mean ± standard deviations (SD). 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. The assumptions of normality and constant variance

**Table 1. Levels of extraction variables for experimental designs**

| Independent variables | Level total phenol content/ carotene content | | | | |
|---|---|---|---|---|---|
| | **+1** | **0** | **-1** | **+1.682** | **-1.682** |
| X1: Ethanol (%) | 100 | 65 | 30 | 123.86 | 6.13 |
| X2: Temperature (°C) | 60 | 45 | 30 | 70.23 | 19.77 |
| X3: Time (min) | 90 | 60 | 30 | 110.45 | 9.55 |

**Table 2. Central composite design arrangement for extraction of phenolics and carotene from *Passiflora edulis***

| Run order | Ethanol % | Temperature (°C) | Time (min) | Phenolics | Carotenoids |
|---|---|---|---|---|---|
| 1 | 65 | 70.2 | 60 | 4.45 | 4.35 |
| 2 | 100 | 60 | 30 | 2.976 | 0.86 |
| 3 | 100 | 30 | 30 | 4.43 | 5.25 |
| 4 | 65 | 45 | 60 | 3.84 | 3.71 |
| 5 | 65 | 45 | 60 | 2.74 | 3.01 |
| 6 | 6.137 | 45 | 60 | 4.93 | 3.14 |
| 7 | 30 | 30 | 90 | 5.2 | 0.95 |
| 8 | 65 | 45 | 9.5 | 4.01 | 3.02 |
| 9 | 65 | 45 | 60 | 3.59 | 2.33 |
| 10 | 65 | 45 | 60 | 3.41 | 2.59 |
| 11 | 65 | 19.8 | 60 | 4.77 | 2.68 |
| 12 | 100 | 45 | 60 | 3.814 | 4.47 |
| 13 | 100 | 60 | 90 | 3.68 | 5.67 |
| 14 | 30 | 60 | 30 | 4.15 | 4.41 |
| 15 | 65 | 45 | 60 | 3.69 | 3.45 |
| 16 | 65 | 45 | 110.5 | 4.74 | 3.79 |
| 17 | 30 | 30 | 30 | 4.75 | 2.93 |
| 18 | 65 | 45 | 60 | 3.2 | 1.73 |
| 19 | 100 | 30 | 90 | 2.99 | 4.36 |
| 20 | 30 | 60 | 90 | 5.44 | 3.22 |

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. A second-order polynomial model was applied to predict the response variables as given below:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_1^2X_1^2 + \beta_2^2X_2^2 + \beta_3^2X_3^2 + \beta_1\beta_2X_1X_2 + \beta_1\beta_3X_1X_3 + \beta_2\beta_3X_2X_3$$

where Y is the predicted dependent variable; $\beta_0$ is a constant that fixes the response at the central point of the experiment; $\beta_1$, $\beta_2$ and $\beta_3$ are the regression coefficients for the linear effect terms; $\beta_1^2$, $\beta_2^2$ and $\beta_3^2$ 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 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 (Phenolic and carotenoids contents) 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 polyphenols and carotenoids. For the verification of predicted extraction conditions that would give higher levels of phenolics and carotenoids, experimental data for the contents of phenolics and carotenoids in *P. edulis* leaf samples were determined based on the best extractions conditions obtained with RSM.

## 3. RESULTS AND DISCUSSION

Leaves of *P. edulis* exhibits high levels of total phenolics and carotenoid contents and antioxidant activity [7] and the extract of this leaves could be an option to enhance the supply of antioxidants to safeguard against oxidative

stress [5]. Further, flavonoids present in *P. edulis* leaves have been characterized and sixteen apigenin or luteolin derivatives including vitexin, isovitexin, orientin and isoorientin have been reported [9]. Carotenoids are bioactive isoprenoid molecules which play a role in the protection of plants against photo-oxidative processes and they are known for antioxidant activity of scavenging singlet oxygen and peroxyl radicals [1]. Extraction is one of the most important steps in the recovery and purification of bioactives from potential dietary sources. The efficiency and effectiveness of the phenolics and carotenoids extraction process are generally manipulated by multiple variables, such as extraction time, temperature and solvent composition [21]. The uncoded coefficient values for the experimental designs for total phenolics and carotenoids of *P. edulis* leaves are given in Table 2. The obtained data were used for the prediction of an optimum set of extraction parameters from leaf extract with high phenolics and carotenoids contents. A number of phenolics and carotenoids in the extracts were employed in a multiple regression analysis, performed using RSM to fit the second-order polynomial equations are given in Tables 3 and 4 for phenolics and carotenoids, respectively. The "fitness" of the model was studied through the lack-of-fit test ($p > 0.05$), which indicated the adequacy of models to accurately predict the variation [22]. The quality of fit to the second-order polynomial models for leaf extracts of *P. edulis* 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. The software generated the quadratic equations from estimated regressions coefficients for RSM as appeared in Tables 3 and 4 and they demonstrate the empirical relationship between extraction parameters (solvent concentration, extraction temperature and extraction time) and response variables (phenolics and carotenoids).

### 3.1 Model Fitting of Parameters Based on Total Phenolic and Carotenoid Content

The responses, phenolics and carotenoids yields, of each run of the experimental design, were presented in Table 2. Total phenolics content of leaf extracts varied from 2.74 to 5.44 mg GAE/g dry sample. Total carotenoids contents varied from 0.86 to 5.67 mg/g DW. The ANOVA of the second order polynomial models for the phenolics extractions from *P. edulis* leaves show that the models were significant ($p < 0.05$) with $R^2$ and p-values of 0.85 and 0.004, respectively (Table 3). There was no significance in the lack of fit ($p = 0.41$) in the model indicating that the model could be used to predict the responses. The quadratic regression models for carotenoids extraction showed that the models were significant ($p < 0.05$) with $R^2$ and p-values of 0.79 and 0.019, respectively (Table 4). The lack of fit ($p = 0.34$) in the model was not significance ($p<0.05$) and this indicated that the model could be used to predict responses. The software generated the estimated regression coefficients of the second-order polynomial equations for RSM analysis of total phenolics and carotenoids extraction as shown in Tables 3 and 4 and they are demonstrating the empirical relationship between ethanol concentration (A), extraction temperature (B), extraction time (C), and phenolics and carotenoids in terms of uncoded units.

### 3.2 Effect of Extraction Parameters on Total Phenolic Content

The responses demonstrated that the ethanol concentration, extraction temperature and the duration of the extraction greatly affect the recovery of phenolics from *P. edulis* leaves (Fig. 1). The type of solvent plays an important role in the extraction of phenolic and other antioxidant compounds from complex biological materials. Many researchers have used aqueous alcohols particularly ethanol for the extraction of various bioactive antioxidants from plants sources when used for food uses [23,4]. Based on the results, ethanol concentration had a slightly curved relationship with phenolic extraction. Phenolic extraction from *P. edulis* prefers ethanol-water solvent combinations than use of pure ethanol. Generally, higher recovery of phenolics was observed at lower ethonolic concentration in the range used (Fig. 1). As the extraction and separation of phenolics depend greatly on the polarity of the extraction solvent, use of a pure solvent may not be effective for the separation of phenolics from plant materials [14]. Therefore, a combination of alcohol with water is more effective in extracting phenolics. This is consistent with several earlier findings which convey that phenolics are more extractable in polar solvents as compared to non-polar ones [14,4]. Contradictory to our results, Saha et al. [17] obtained highest phenolic content when 100% methanol was used for kinema, a bacillus-fermented soybean food. However, kinema is a processed product and the phenolics

extractability may differ compared with unprocessed plant materials.

Extraction temperature showed great influence on the recovery of phenolics from *P. edulis* leaves. When ethanol-water combinations were employed, with the increasing extraction temperature and time within the selected range, enhanced the recovery of phenolic extraction when compared to 100% ethanol use. Recovery of phenolics was increased considerably when the extraction temperature was increased to 60 ℃, while the % ethanol maintained at a low level (Fig. 1a and b). Results showed that at lower solvent concentration (30%), the use of higher extraction temperature (60 ℃) and extraction time (90 min), increased the extractable phenolics from 4.75 to 5.44 mg GAE/g DW, compared with the use of lower extraction temperature (30 ℃) and extraction time. This might be due to the increase in phenolic solubility, diffusion rate, mass transfer rate, extraction rate and reduced solvent viscosity and surface tension at higher temperatures and solvent polarities which could improve the phenolic extractability [24]. However, temperatures above a certain level, depending on the type of solvent used, could lead to solvent evaporation and degradation of phenolics [14,25]. Cacace & Mazza [26] reported that extraction temperature affected the extraction of anthocyanins from blackcurrant and increasing the temperature beyond 30–35 ℃ resulted in the degradation of anthocyanins. Similarly, Juntahote et al. [27] have reported that extraction temperature influenced the yield of phenolics from lemon grass. It was reported that, at higher extraction temperatures, plant tissues become soft and weaken the phenol–other macro molecules linkages of bound phenolics and migration of the phenolics into the extraction solvent can be occurred [28].

The extraction time was another important parameter in the extraction of bioactoives. However, the results showed that extraction time did not have a significant effect on the phenolics extraction from *P. edulis* leaves at $P < 0.05$ level. These results were in agreement with reports that extraction time had no significant effect on the extraction yield of ginseng components [29] and lemon grass phenolics [27]. Furthermore, there were significant interaction effects between solvent concentrations, between extraction temperatures and between extraction temperature and extraction time on the extraction yield of phenolics from passion fruit leaves at $P < 0.05$ level in a second-order relationship (Table 3).

### 3.3 Effect of Extraction Parameters on Carotenoids Content

As the food industry and health conscious consumers are interested in food containing bioactive carotenoids, demand for natural dietary sources of carotenoids increases. Among 34 leafy vegetables tested, leaves of *P. edulis* contained relatively higher amounts of carotenoids [7]. Many methods have been employed for carotenoids extraction and among them, solvent extraction method is universally acceptable [30,31,18]. Ethanol is also a good solvent that can be used for carotenoids extraction and the extraction is highly influenced by procedural variables including solvent concentration, extraction temperature and time [18]. However, many researchers have used non polar solvent for carotenoid extraction, hexane/acetone/alcohol (2/1/1) for lycopene [32]

**Table 3. Estimated regression coefficients of the second-order polynomial equations for RSM analysis of total phenolics extraction**

| Terms | Regression coefficients | Regression P-value | $R^2$ | Lack of fit |
|---|---|---|---|---|
| Constant | 10.5238 | 0.004 | 84.75% | 0.413 |
| Ethanol % | -0.0223 | | | |
| Temperature (℃) | -0.1946 | | | |
| Time (min) | -0.04928 | | | |
| Ethanol %*Ethanol % | 0.0002 | | | |
| Temperature (℃)*Temperature (℃) | 0.0016 | | | |
| Time (min)*Time (min) | 0.0003 | | | |
| Ethanol %*Temperature (℃) | -9.61905E-05 | | | |
| Ethanol %*Time (min) | -2.94762E-04 | | | |
| Temperature (℃)*Time (min) | 0.0008 | | | |

**Table 4. Estimated regression coefficients of the second-order polynomial equations for RSM analysis of total carotenoids extraction**

| Terms | Regression coefficients | Regression P-value | $R^2$ | Lack of fit |
|---|---|---|---|---|
| Constant | 7.9761 | 0.019 | 78.74% | 0.355 |
| Ethanol % | 0.00303 | | | |
| Temperature (℃) | -0.0722 | | | |
| Time (min) | -0.1547 | | | |
| Ethanol %*Ethanol % | 0.0003 | | | |
| Temperature (℃)*Temperature (℃) | 0.0010 | | | |
| Time (min)*Time (min) | 0.0002 | | | |
| Ethanol %*Temperature (℃) | -0.0016 | | | |
| Ethanol %*Time (min) | 0.0008 | | | |
| Temperature (℃)*Time (min) | 0.0018 | | | |

and petroleum ether/acetone (1/1) for rapeseed [18]. Influence of three extraction conditions towards total carotenoids extraction was reported through the significant ($p < 0.05$) coefficient of the second-order polynomial regression equation in Table 4. Extraction of carotenoids had a greater influence of ethanol concentration (30–100%) and was significant ($p < 0.05$). As there are polar carotenoids (e.g. Lutein) as well as non-polar carotenoids (e.g. β-carotenoids), the extraction and separation of carotenoids depend largely on the nature of the polarity of the solvents [18]. However, for *P. edulis*, higher carotenoids extractions were observed when 100% ethanol was used (Fig. 2). When ethanol concentration increased from 30% to 100% while keeping extraction temperature and time at 30℃ and 30 min, respectively, increase in the carotenoids content from 2.93 to 5.25 mg /g DW was observed (Table 2). This may be due to the presence of more non-polar carotenoids in *P. edulis* and hence could extract more carotenoids towards decreasing polarity as the solvent polarity is decreased with increasing solvent concentration. Our results are in agreement with Rahman et al. [33] where the total carotenoid content of *Centella asiatica* leaves increased when ethanol concentration increased. They have reported higher carotenoids content (1.1 mg/g) from *Centella asiatica* when 100% ethanol was used compared to the 50% ethanol used (0.70 mg/g).

Extraction temperature and extraction duration showed some influence on carotenoids extraction from *P. edulis* leaves (Fig. 2). Higher carotenoids extractions were observed when 100% ethanol and low temperature is used (Fig. 2). However, when extraction temperature increased from 30℃ to 60℃, while keeping the solvent concentration and extraction time at 100% and 30 min respectively, a decrease in the carotenoids content from 5.25 to 0.86 mg /g DW was observed (Figs. 2c and 2d). This may be due to the degradation of carotenoids at higher temperatures. Meléndez-Martínez et al. [34] have reported that carotenoids are degraded at elevated temperatures and therefore, this study corresponds to the findings by Gu et al. [35] who also reported an optimum temperature of 30℃ for carotenoid extraction. A similar observation has also been reported for oil palm fronds [36]; for rapeseed [18]; and for microalgae [34]. Further, a reduction in extractable carotenoids content from 5.25 to 4.36 mg/g DW when the extraction time increased from 30 min to 90 min while keeping solvent concentration and extraction temperature at 100% and 30℃, respectively. Moreover, extraction of carotenoids was found to be positively influenced by the synergism between ethanol concentration and extraction temperature ($p < 0.05$). Similar to the phenolic extraction, the extraction time did not have a significant effect on the carotenoids extraction from *P. edulis* leaves at $P > 0.05$ level. However, there were significant interaction effects between solvent concentrations, between solvent concentration and extraction temperature, and between extraction temperature and extraction time on the extraction yield of carotenoids from passion fruit leaves at $P < 0.05$ level in a second-order relationship (Table 4).

### 3.4 Optimization of Phenolics and Carotenoids and Verification of the Model

Multiple graphical and numerical optimizations were run for determining the optimum levels of studied extraction conditions with desirable levels of phenolics and carotenoids contents. Optimum

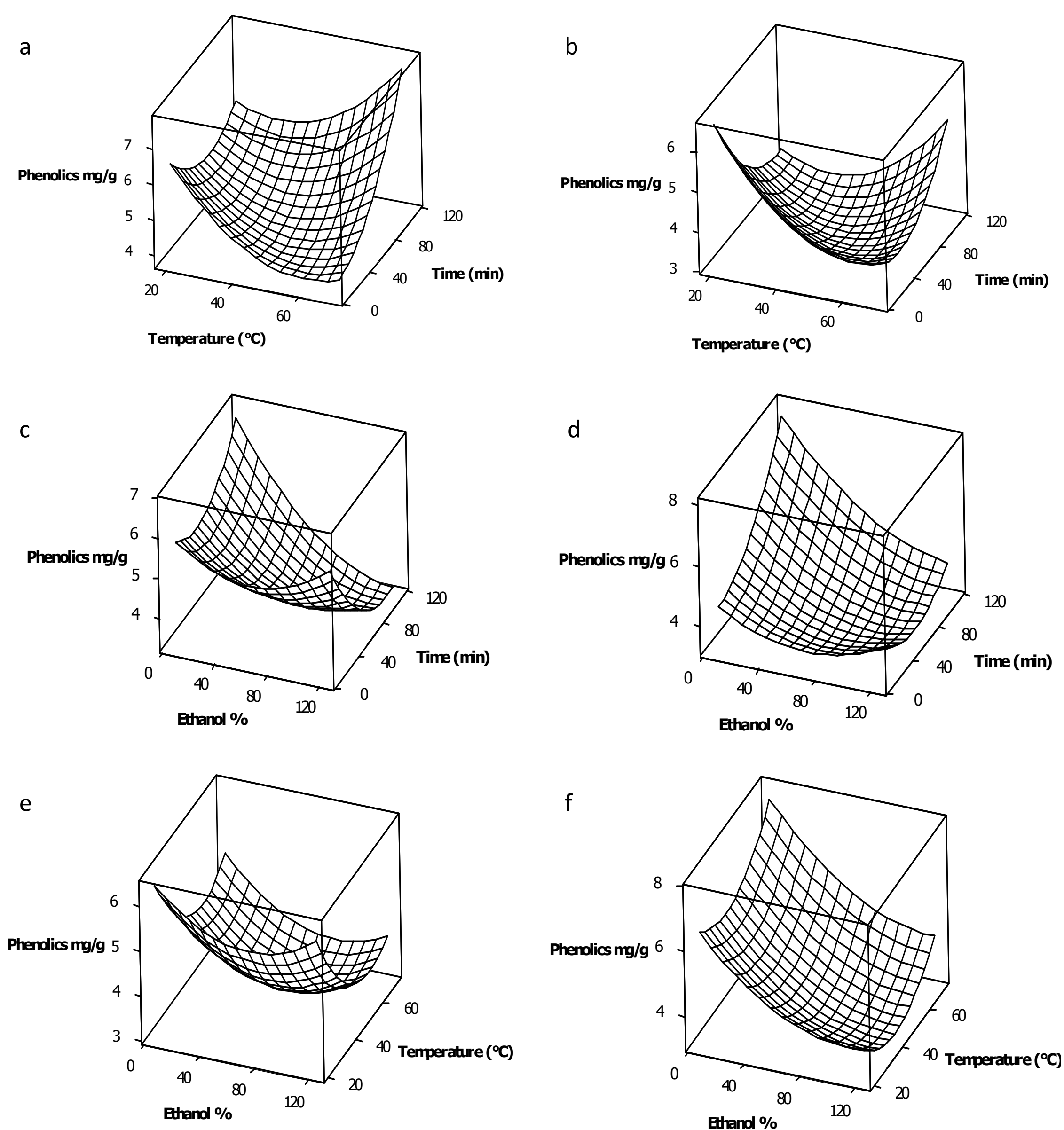


**Fig. 1. Pair wise response surface plots of the phenolics (mg GAE/g DW) extraction from *Passiflora edulis* leaves as a function of ethanol %, extraction temperature and time: ethanol % was kept constant at 30% (a) and 100% (b); temperature of extraction was kept constant at 30℃ (c) and 60℃ (d); the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

ethanol concentration, extraction temperature, extraction time were developed for the two responses and they were 6.14%, 70.20℃ and 110.45 min for phenolics and 100%, 19.77℃ and 110.45 min for carotenoids, respectively. For these optimum extraction conditions, the corresponding predicted response values for phenolics and carotenoids were 9.03 mg GAE/g DW and 8.74 mg/g DW, respectively. An experiment was run in accordance with the recommended optimum conditions for two responses, phenolics and carotenoids. More interestingly, in this study, the values obtained experimentally for both response variables are near to the predicted values, indicating a satisfactory model. The experimental values for total phenolics were 9.12 ± 1.84 mg GAE g extract and 9.04 ± 3.02 mg/g DW carotenoids

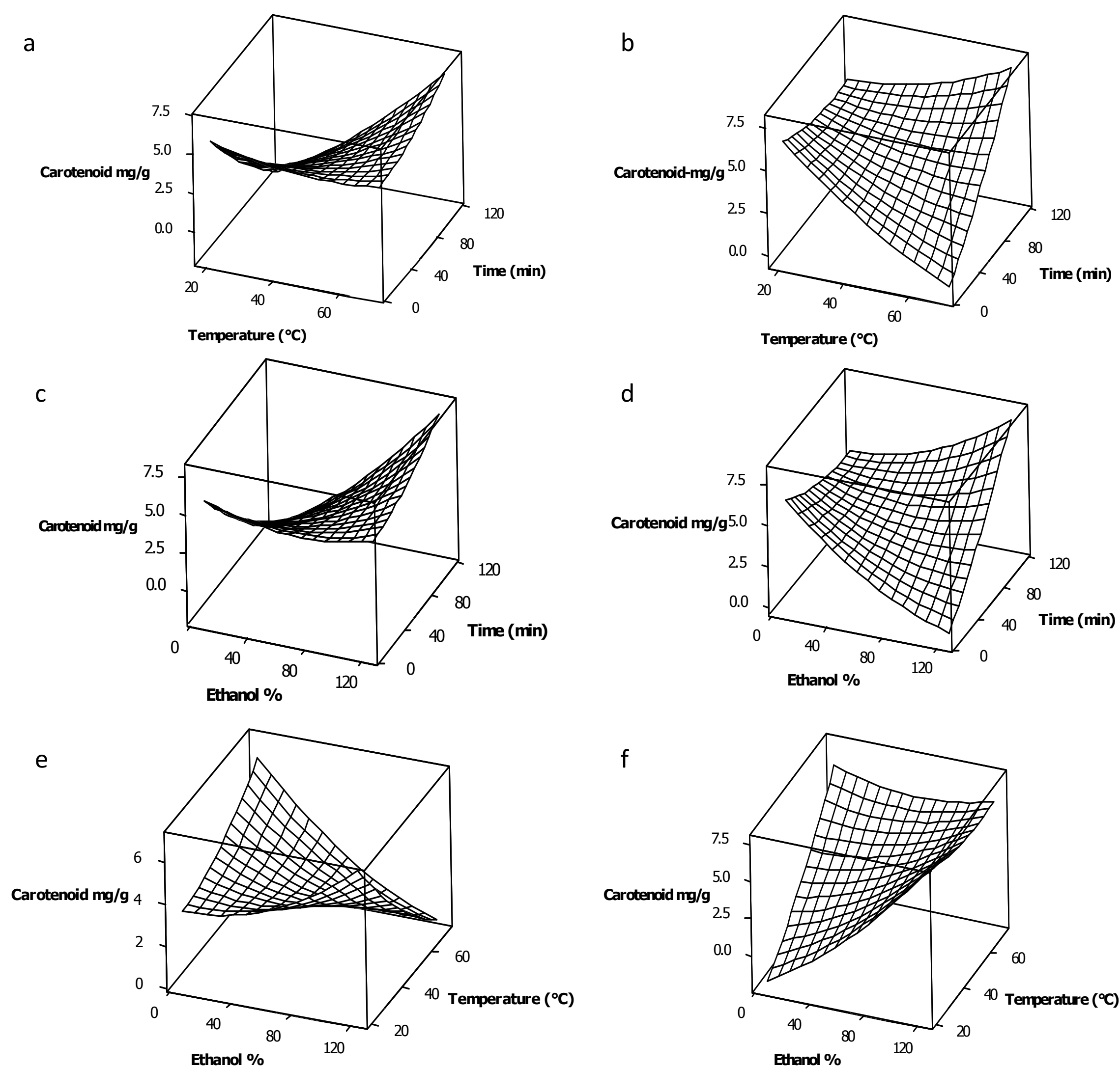


**Fig. 2. Pair wise response surface plots of the carotenoids (mg/g DW) extraction from *Passiflora edulis* leaves as a function of ethanol %, extraction temperature, and time: ethanol % was kept constant at 30% (a) and 100% (b); temperature of extraction was kept constant at 30℃ (c) and 60℃ (d); the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

and no significant difference ($p < 0.05$) was found between the experimental and predicted values of the extractable phenolics and carotenoids from leaves of *P. edulis* extract. Therefore, the data confirm the validity of the optimized model.

## 4. CONCLUSIONS

In our study, RSM was successfully implemented for optimization of total phenolics and carotenoid extraction from leaves of *P. edulis*. Overall, phenolic extraction prefers low ethanol concentration, higher temperature and longer extraction time, whereas higher carotenoid recovery was observed at higher ethanol concentrations and low temperatures. The most efficient extraction conditions were at ethanol concentration of 6.14%, 70.20℃, and 110.45 min for phenolics, while for carotenoids, it was 100%, 19.77℃ and 110.45 min respectively. This research will renew interest in utilizing leaves of *P. edulis* as an inexpensive source of natural antioxidants.

This research finding will support functional and nutraceutical industries for the isolation of phenolics and carotenoids from this leaves.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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