# RESPONSE SURFACE OPTIMIZATION OF PHENOLICS AND CAROTENOIDS EXTRACTION FROM LEAVES OF *Olax zeylanica*

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***Abstract***
*Olax zeylanica* is a green leafy vegetable rich in phenolics and carotenoids and also posses good antioxidant activities. *The optimum extraction conditions for the higher recovery of total phenolics and carotenoids contents for leaves of Olax zeylanica were developed using response surface methodology. A three-factor inscribed central composite design (CCD) was used to identify the relationship existing between the response functions (total phenolics and carotenoids) 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 process variables used for the study were concentration (30-100%), extraction temperature (30-60 °C) and extraction time (30-90 min) on the recovery of total phenolics and carotenoids. Multiple graphical and numerical optimizations of the experimental data were done to identify the optimum extraction conditions to achieve the maximum recovery of total phenolics and carotenoids. Optimum extraction conditions of ethanol concentration, extraction temperature and extraction time for phenolics, were 6.14%, 19.8 °C, and 9.54 min and for carotenoids, the optimum parameters were 100%, 70.2°C and 110.5 min, respectively. The optimal predicted contents for total phenolics (16.7 mg Gallic Acid Equivalent (GAE)/ g DW) and carotenoids (6.03 mg/g DW) values in the extracts were agreed with the experimental values obtained with optimum extraction conditions for each response.*

Key words: *Olax zeylanica* leaves, phenolics, carotenoids, response surface methadology

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## 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 (Stahl & Sies, 2003). 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) (Sies & Stahl, 1995). Currently, research and development activities that are aimed at bioactives rich dietary sources have become a global interest. *Olax zealinica* is a tropical leafy vegetable and has been used in many pharmaceutical preparations. Leaves of *O. zeylanica* reported to be rich sources of polyphenols (6.91 mg GAE/g dry weight-DW) and carotenoids (2.15 mg/g DW) and it has potent antioxidant properties (Gunathilake & Ranaweera, 2016).

There is a current trend in investigating natural dietary sources of antioxidants. Therefore, the exploration of antioxidant-rich natural sources such as leaves of *O. zeylanica* 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 (Prasad et al., 2011). Many factors influence the extraction efficiency and bioactive concentration and therefore, it is necessary to optimize the extraction conditions to obtain the highest bioactives recovery. Response surface

methodology (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 However, there are no studies reported to optimize the extraction conditions for polyphenols and carotenoids from leaves of. *O. zeylanica.* Hence, the objective of the present study was to optimize the extraction conditions for *O. zeylanica* leaves to obtain the highest polyphenols and carotenoids content.

## 2. MATERIAL & METHODS

### 2.1. Plant materials and chemicals

*O. zeylanica* leaves were collected from home gardens in Negambo 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 °C for 48 h, and ground into powder using a blender, sieved and were stored at -18 °C until use. All other chemicals used were of analytical grade.

### 2.2. 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 described by the experimental design and the extracts were filtered and were stored at -18 °C until use. The optimization procedure was designed based on a three-factor inscribed central composite design (CCD) consisting of ethanol concentration (30–100%), temperature (30–60 °C) and extraction time (30-90 min) as shown in Table 1.

### 2.3.Determination of total phenolic content

The total phenolic content was determined using Folin–Ciocalteu assay (Singleton et al., 1999) with some modification, as described by Gunathilake and Ranaweera (2016). Gallic acid was used in the construction of standard curve.

### 2.4.Determination of Total carotene content

The carotenoid content was analyzed according to the method described by Türlerinde et al. (1998).

**Table 1: Central composite design arrangement for extraction of phenolics and carotene from *Olax zeylanica***

| Run order | Ethanol % | Temperature (°C) | Time (min) | Phenolics (mg/g) | Carotene mg/g) |
|---|---|---|---|---|---|
| 1 | 65.00 | 45.00 | 9.55 | 5.90 | 2.01 |
| 2 | 123.86 | 45.00 | 60.00 | 1.31 | 3.03 |
| 3 | 65.00 | 45.00 | 60.00 | 3.62 | 1.41 |
| 4 | 100.00 | 60.00 | 90.00 | 4.06 | 4.29 |
| 5 | 30.00 | 30.00 | 30.00 | 9.72 | 0.62 |
| 6 | 65.00 | 45.00 | 60.00 | 5.74 | 2.12 |
| 7 | 65.00 | 70.23 | 60.00 | 8.71 | 2.83 |
| 8 | 30.00 | 60.00 | 90.00 | 1.70 | 3.03 |
| 9 | 30.00 | 60.00 | 30.00 | 10.04 | 0.48 |
| 10 | 65.00 | 45.00 | 110.45 | 5.46 | 2.80 |
| 11 | 65.00 | 45.00 | 60.00 | 3.62 | 1.48 |
| 12 | 100.00 | 60.00 | 30.00 | 5.46 | 3.82 |
| 13 | 100.00 | 30.00 | 30.00 | 3.27 | 3.21 |
| 14 | 65.00 | 45.00 | 60.00 | 5.05 | 1.94 |
| 15 | 65.00 | 19.77 | 60.00 | 7.76 | 3.07 |
| 16 | 65.00 | 45.00 | 60.00 | 5.74 | 2.12 |
| 17 | 65.00 | 45.00 | 60.00 | 5.74 | 2.11 |
| 18 | 30.00 | 30.00 | 90.00 | 9.77 | 1.37 |
| 19 | 6.14 | 45.00 | 60.00 | 9.57 | 0.99 |
| 20 | 100.00 | 30.00 | 90.00 | 2.30 | 3.52 |

### 2.5.Experimental design

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 °C) 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. Twenty randomized experiments including six replicates as the center points were assigned based on CCD and

measured total phenolic content and carotenoid content as response variables are given in Table 1.

For data analysis, Minitab15 software was used. 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. A second-order polynomial model was applied to predict the response variables. A second-order polynomial model was applied to predict the response variables as 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; $\beta_0$ 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. 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 *O. zeylanica* leaf samples were determined based on the best extractions conditions obtained with RSM. 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 *O. zeylanica* leaf samples were determined based on the best extractions conditions obtained with RSM.

## 3. RESULTS AND DISCUSSION

Leaves of *O. zeylanica* exhibits high levels of total phenolics and carotenoid contents and antioxidant activity (Gunathilake & Ranaweera, 2016). Carotenoids play a role in the protection of plants against photo-oxidative processes and they are well known antioxidant (Stahl & Sies, 2003). Extraction is one of the most important steps in the recovery and purification of bioactives from potential dietary sources. The phenolics and carotenoids extraction process are generally manipulated by multiple variables, such as extraction time, temperature and solvent composition (Alothman et al., 2009). The uncoded coefficient values for the experimental designs for total phenolics and carotenoids of *O. zeylanica* leaves are given in Table 2 and the obtained data were used for the prediction of an optimum set of extraction parameters from leaf extract with high phenolics and carotenoids contents. 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 (Kong et al., 2010). The quality of fit to the second-order polynomial models for leaf extracts of *O. zeylanica* 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 Table 2 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 1.31 to 10.04 mg GAE/g dry sample. Total carotenoids contents varied from 0.48 to 4.29 mg/g DW. The ANOVA of the second order polynomial models for the phenolics and carotenoids extractions from *O. zeylanica* leaves show that the models were significant ($p < 0.05$) and there was no significance in the lack of fit ($p = 0.12$) in the model indicating that the model could be used to predict the responses.

**Table 2: Estimated regression coefficients of the second-order polynomial equations for RSM analysis of total phenol extraction**

| Terms | Estimated coefficients for phenolics uncoded | Estimated coefficients for carotenoids uncoded |
|---|---|---|
| Constant | 25.1089 | 3.56841 |
| Ethanol % | -0.233144 | 0.0395851 |
| Temperature | -0.450167 | -0.176362 |
| Time | 0.0219444 | -0.0187970 |
| Ethanol %*Ethanol % | 2.05564E-05 | 5.12637E-05 |
| Temperature*Temperature | 0.00450384 | 0.00175617 |
| Time*Time | 0.000122260 | 0.000224946 |
| Ethanol %*Temperature | 0.00278571 | -3.38095E-05 |
| Ethanol %*Time | 0.000704762 | -3.00238E-04 |
| Temperature*Time | -0.00245000 | 0.000545000 |
| Lack of fit | 0.117 | 0.074 |
| $R^2$ | 84.44% | 86.77% |

### 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 *O. zeylanica* leaves (Figure 1). Based on the results, ethanol concentration had a slightly curved relationship with phenolic extraction. Phenolic extraction from *O. zeylanica* prefers ethanol-water solvent combinations than pure ethanol. Higher recovery of phenolics was observed at lower ethonolic concentration in the range used (Figure 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 (Prasad et al., 2011).

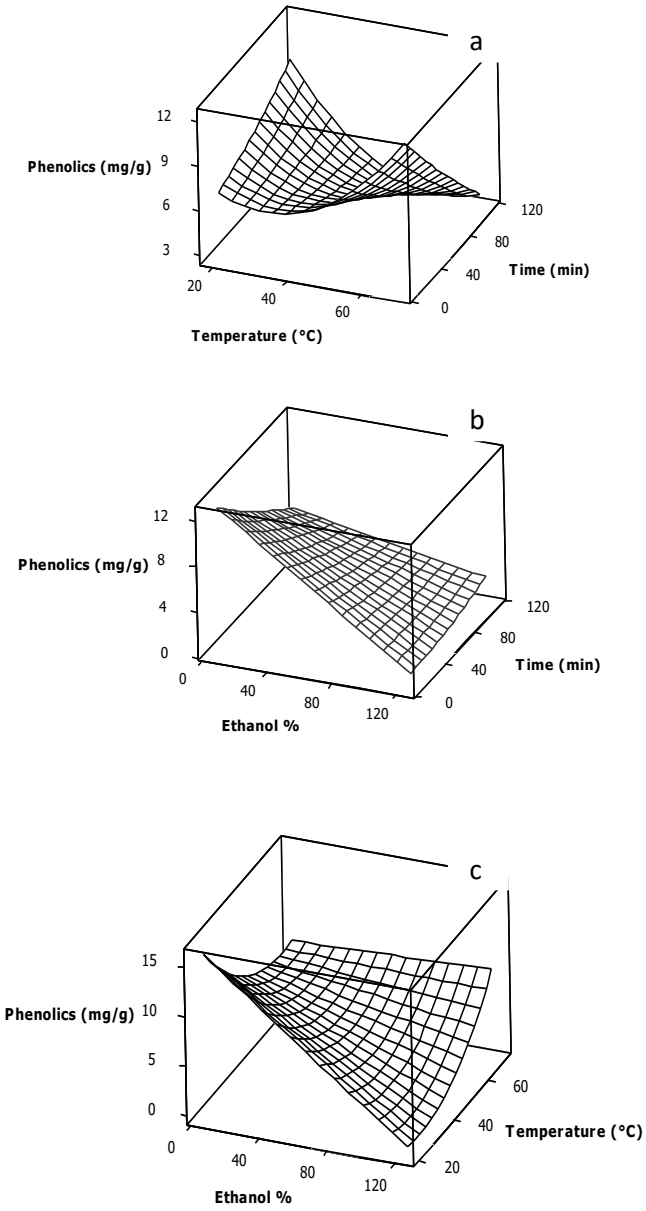


**Figure 1: Pair wise response surface plots of the phenolics (mg GAE/g DW) extraction from *Olax zeylanica* leaves as a function of ethanol %, extraction temperature and time: ethanol % was kept constant at 65% (a); temperature of extraction was kept constant at 45 °C (b) and the time of extraction was kept constant at 60 min (c).**

Therefore, a combination of alcohol with water is more effective in extracting phenolics. Extraction temperature showed great influence on the recovery of phenolics from *O. zeylanica* leaves. When ethanol-water combinations were employed, low extraction temperature prefers for more recovery of phenolic when compared to high extraction temperature. Recovery of phenolics was increased considerably when the extraction temperature decreased, while the % ethanol maintained at a low level. 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 *O. zeylanica* leaves at $P < 0.05$ level. 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.

### 3.3. Effect of extraction parameters on carotenoids content

Many methods have been employed for carotenoids extraction and among them, solvent extraction method is universally acceptable (Huang et al., 2008). Ethanol is a good solvent that can be used for carotenoids extraction and the extraction (Wang & Liu, 2009). 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. When ethanol concentration increased from 30% to 100% increase in the carotenoids content indicating carotenoid extraction prefers decreased polarity. This may be due to the presence of more non-polar carotenoids in *O. zeylanica*. Extraction temperature and extraction duration showed some influence on carotenoids extraction from *O. zeylanica* leaves (Figure 2). Higher carotenoids extractions were observed when higher temperature and extraction time increased while keeping ethanol concentration at 65% (Figure 2).

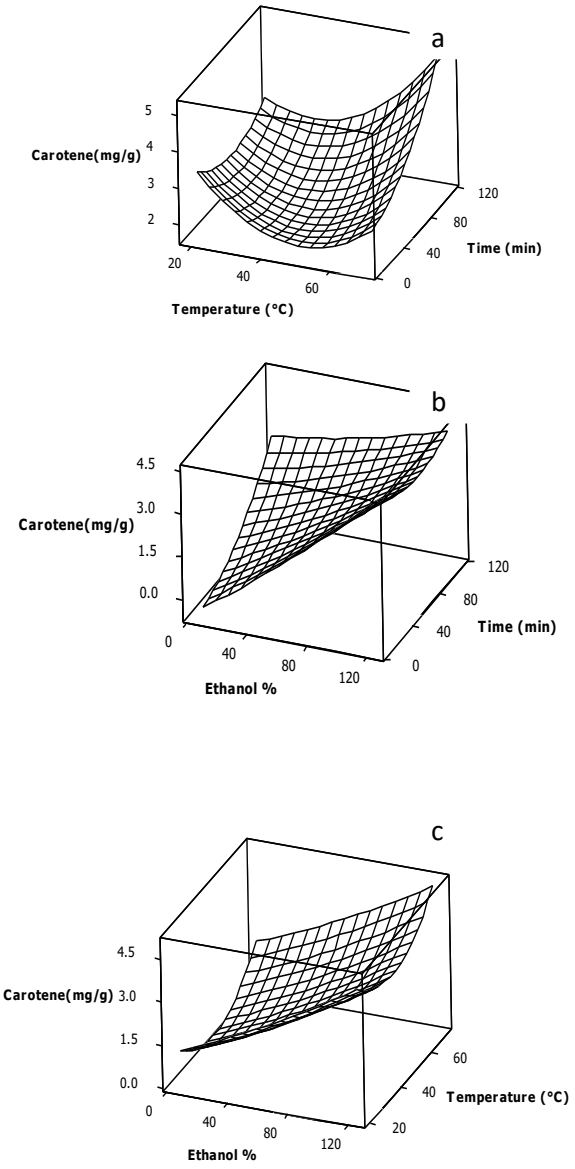


**Figure 2: Pair wise response surface plots of the carotenoids (mg/g DW) extraction from *Olax zeylanica* leaves as a function of ethanol %, extraction temperature and time: ethanol % was kept constant at 65% (a); temperature of extraction was kept constant at 45 °C (b) and the time of extraction was kept constant at 60 min (c).**

### 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 ethanol concentration, extraction temperature, extraction time were developed for the two responses and they were 6.14%, 19.8 °C and 9.54 min for phenolics and 100%, 70.23 °C and 110.45 for carotenoids, respectively. For these optimum extraction conditions, the corresponding predicted response values for phenolics and carotenoids were 16.68 mg GAE/g DW and 6.03 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 13.95 ± 4.37 mg GAE g extract and 6.54 ± 2.04 mg/g DW carotenoids and no significant difference ($p < 0.05$) was found between the experimental and predicted values of the extractable phenolics and carotenoids from leaves of *O. zeylanica* 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 *O. zeylanica*. Overall, phenolic extraction prefers low ethanol concentration, low temperature and shorter extraction time, whereas higher carotenoid recovery was observed at higher ethanol concentrations and high temperatures and longer extraction time. Experimental and predicted values of the extractable phenolics and carotenoids from leaves of *O. zeylanica* extract obtained from optimium extraction conditions were insignificant.

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