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Usage of Different Plants in the Production of Polyphenols with Ultrasonic Extraction

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Optimization

Abstract: Antioxidants are compounds that neutralize and inactivate free radicals which are the main cause of diseases and aging in the bodies. There are a number of factors that influence the yield of extraction of these molecules. The purpose of this study was to optimize the ultrasonic extraction of polyphenols from *Cinnamonum zeylaniccum* and *Rhus coriaria* by response surface methodology. Experiments were performed using sweep mode ultrasound waves and methanol as solvent. The parameters were solid-per-liquid ratio, extraction temperature and duration. As a result of the statistical analysis done by Design Expert software, the function which expresses the effect of the parameters on the quantity to be obtained in industrial polyphenol production was found as a quadratic model. The optimum conditions were determined as 30°C for temperature, 100 g of plant /50 ml methanol for solid-to-liquid ratio, and 15 minutes for extraction time. At the optimum conditions, cinnamon's ultrasonic extraction on "sweep" mode produced 2726.4 mg/100 g of plant gallic acid equivalent of polyphenols, whereas sumac produced 1211 mg/100 g of plant polyphenols at the same conditions, except the temperature of 50°C.

Ultrasonik Ekstraksiyonla Polifenol Üretiminde Farklı Bitkilerin Kullanımı

Anahtar Kelimeler
Gallik asit,
Ekstraksiyon,
Cinnamonum zeylaniccum,
Rhus coriaria,
Optimizasyon

Özet: Antioksidanlar, vücutta yaşlanma ve hastalıkların temel sebebi olan serbest radikalleri nötralize eden ve etkisiz hale getiren bileşiklerdir. Bu moleküllerin ekstraksiyon verimini etkileyen çeşitli faktörler vardır. Bu çalışmanın amacı, cevap yüzey yöntemiyle, *Cinnamonum zeylaniccum* ve *Rhus coriaria*'dan ultrasonik ekstraksiyonla polifenol üretimini optimize etmektir. Deneyler süpürme modundaki ses dalgaları ve çözücü olarak metanol kullanılarak yapıldı. Parametreler, katı-sıvı oranı, ekstraksiyon sıcaklığı ve süresi olarak seçildi. Design Expert yazılımı tarafından yapılan istatistiksel analiz sonucunda, parametrelerin endüstriyel polifenol üretiminde elde edilecek miktar üzerindeki etkisini ifade eden fonksiyon kuadratik model olarak bulunmuştur. Optimum koşullar, sıcaklık için 30°C, katı-sıvı oranı için 100 g bitki /50 ml metanol ve ekstraksiyon süresi için 15 dakika olarak belirlenmiştir. Optimum koşullarda, sumağın "süpürme" modundaki ultrasonik ekstraksiyonu 2726,4 mg/100 g bitki gallik asit eşdeğeri polifenol üretirken, sıcaklık hariç (50°C) aynı koşullar altında, sumağın ekstraksiyonundan ise 1211 mg/100 g bitki polifenol elde edilmiştir.

1. Introduction

Polyphenols are substances that prevent damages of free radicals formed by various reasons in the human body; so called as antioxidants. Antioxidant substances either by inhibiting the formation of reactive oxygen or by neutralizing the reactive oxygen that is generated inhibit not only the formation of degenerative diseases but also the damage that promoted by oxidation reactions on the cellular basis [1, 2]. The common types of polyphenols found in plants (vegetables, fruits etc.) are catechin, gallic acid, kaempferol, and quercetin are the most common polyphenols in plants [3, 4]. Several plants, like cinnamon, have been declared to have these compounds. This plant is in the Laurel family of Asia region. It is always green and it has about 100 different kinds. The dried cinnamon tree is commonly used as a spice. It has been used for releasing the stones of urethra and kidney from the human body [5]. The most of cinnamon production in the World are realized in China. Sumac (*Rhus*

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coriaria L.), is a spice firstly recognized in Iran and spread to the Middle East. It contains different types of organic and phenolic acids, flavonoids, anthocyanins, tannins and terpenoids [6]. Because of the antioxidant properties of these plants, it is predicted that they can be used in the treatment of cancer. The types and the amounts of flavonoids obtained are affected on the process conditions, especially extraction conditions, rather than only the growth conditions [7]. Various extraction methods (ultrasonic, microwave, classical extraction, etc.) have been developed from the 19th century to nowadays. Less time and solvent usage, and high yield are the prominent advantages of the proposed extraction methods. Mathematical model equation based on extraction parameters is required for extraction to realize it with minimum time and energy consumption, and to optimize, design and control of it. The response surface method is known as the most suitable model for optimization. It combines the statistics and mathematical methods in analysis of the independent variables. The optimizations of adsorption [8], extraction [9], fermentation [10], and production processes [11] are realized by using this strategy successfully. The final equation of the response surface methodology is used in industrial production.

Considering those information, the optimization of ultrasonic extractions of polyphenols (as gallic acid equivalent) from cinnamon and sumac were aimed in this study. The extracts of these plants can be used in controlled drug release applications for cancer treatments. The investigation of parameters was done by Design-Expert software program. In that, Response Surface Methodology was applied to predict the effects of several parameters simultaneously, and with doing no more experiments [8, 11]. In the study, three-dimensional surface graphics expressing the extraction process behaviour and industrially applicable equations representing the extraction surface were derived by using Design Expert Program.

2. Material and Method

In the study, *Cinnamonum zeylaniccum* and *Rhus coriaria* L. were obtained from a regional herbalist, and chemicals (sodium carbonate, Folin-Ciocalteu etc.) was purchased from Sigma Co. Optimization of ultrasonic extraction for the total phenolics as gallic acid equivalent at the maximum amount per plant was performed in two steps. Firstly, in single parameter optimization, to determine its single-optimum value, extraction was performed at different values of a parameter while the other parameters were kept constant at a determined value. Then, by using the results obtained Box-Behnken design was constructed to realize multi-parameter optimization (Table 1). It was known that Box-Behnken design works well for representation of the surfaces of optimization processes. The same procedure of optimization was applied as in our previous studies [12, 13].

Table 1. Experimental design of extraction

Parameters	-1	0	+1
x_1: Temperature (^{0}C)	30	40	50
x_2: Time of extraction (min)	5	15	25
x_3: Solid-per-liquid (g/ml)	1/10	1/30	1/50

The regression and statistical analysis, and equation representing the extraction surface were determined different sections of Design-Expert 9.0. The optimum values of the variables of extraction were calculated by numerical optimization section of the software by fixing the desired values for any of the variables. Ultrasonic extraction was performed with methanol as batch wise at the specified conditions defined by Box-Behnken (Table 2). Elmasonic brand ultrasonication was fixed in sweep mode in all experiments. At the end of the extraction, the sample was filtered with a filter paper (FilterLab-110mm), and they were used in analysis. The total phenolic concentrations in the extracts from the filtered samples were determined using the Folin-Ciocalteu method. In the analysis, 0.4 ml of extract was diluted with 5.1 ml of water and then reacted with 0.5 ml of folin. Finally, 1.5 ml sodium carbonate solution (20% by weight) added this mixture was incubated during two hours at 25^{0}C. Colour formed was determined by spectrophotometer (LANGF, DR 5000 Brand) as absorbance at 765 nm. These absorbance values converted to concentrations by using the calibration curve (Absorbance = 0.01532 x concentration (μg/mL); R^2 = 0.9989).

3. Results

The concentrations obtained at the end of the extractions of 15 experimental conditions of Table 2 were supplied into the software the functions suggested by it were analyzed statistically. Three criteria were used for determination of the best model; firstly, the proposed model must be "significant", its "lack of fit" value must be "insignificant", and the regression values must be high and close to each other (given in Tables 3 and 4). Additionally, the low coefficient of variance value is required for acceptance of the model. Those CV values for the suggested quadratic model of software were as 6.59 and 6.92 for cinnamon and sumac, respectively. According to the criteria described above (Table 3 and 4), quadratic model was determined as the best model representing the extraction process. The statistical conclusions were approved by plot of predicted-actual values comparisons Figure 1.

Table 2.The results of experiments of Box-Behnken Design

				Cinnamonum zeylaniccum	*Rhus coriaria*
No	x_1	x_2	x_3	Total polyphenols (mg GAE/100g)	Total polyphenols (mg GAE/100g)
1	-1	-1	0	1582.4020	750.0000
2	+1	-1	0	2085.6922	826.4687
3	-1	+1	0	2258.5743	1055.8747
4	+1	+1	0	2025.2282	1167.1018
5	-1	0	-1	1019.2192	485.8682
6	+1	0	-1	1070.4438	533.3551
7	-1	0	+1	2726.3592	993.4726
8	+1	0	+1	2524.0469	1210.9987
9	0	-1	-1	975.6135	490.7637
10	0	+1	-1	1235.8363	484.3016
11	0	-1	+1	2365.7963	670.2024
12	0	+1	+1	2457.4719	1085.3459
13	0	0	0	2002.4715	970.9856
14	0	0	0	1932.9659	968.7337
15	0	0	0	2067.0517	965.1109

Analysis of variances of quadratic models were performed by creating ANOVA table (Table 3 and Table 4). In the tables, A represents the temperature, B is the duration, and C is the solid-liquid ratio. As the value of F increases and the value of p decreases, the effect on the concentration of polyphenol to be obtained as a result of the extraction of the parameters increases. The most effective single parameter for polyphenol extraction from *Cinnamonium zeylaniccum* was found to be the solid-per-liquid ratio (Table 3), while the most effective parameters for polyphenol extraction from *Rhus coriaria* were temperature and solid-per-liquid ratio (Table 4). When the F values of the binary interactions of the parameters were compared with the F values of those parameters alone, it was observed that the parameters did not interact with each other.

Table 3. ANOVA Table of quadratic function for *Cinnamonium zeylaniccum*

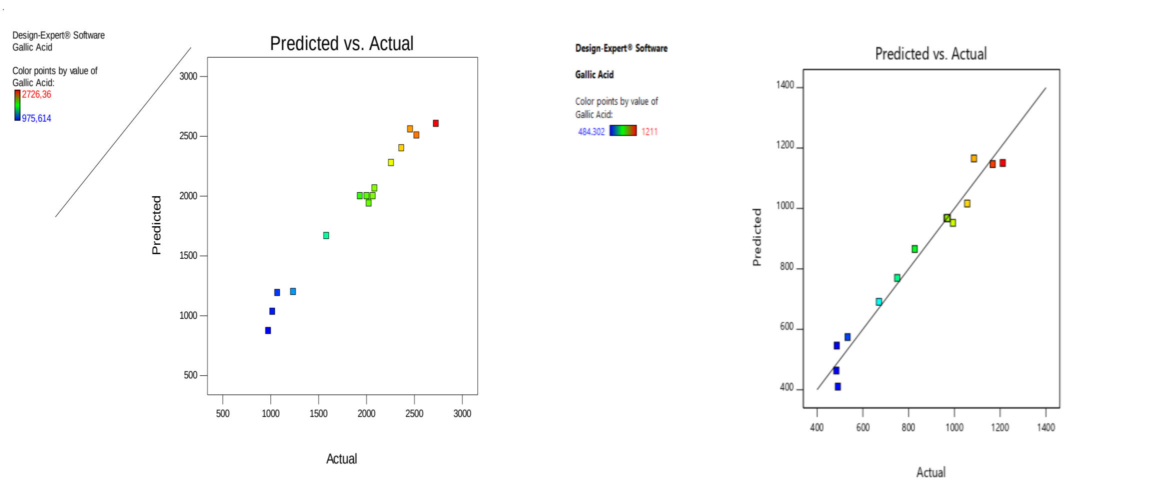
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	4.599E+06	9	5.110E+05	32.96	0.0006	significant
A-Temperature	1765.86	1	1765.86	0.1139	0.7495	
B-Time	1.170E+05	1	1.170E+05	7.55	0.0404	
C-S/L	4.165E+06	1	4.165E+06	268.67	< 0.0001	
AB	1.357E+05	1	1.357E+05	8.75	0.0316	
AC	16070.24	1	16070.24	1.04	0.3553	
BC	7102.04	1	7102.04	0.4581	0.5286	
A^2	3720.00	1	3720.00	0.2399	0.6450	
B^2	7343.49	1	7343.49	0.4737	0.5219	
C^2	1.441E+05	1	1.441E+05	9.29	0.0285	
Residual	77516.26	5	15503.25			
Lack of Fit	68522.72	3	22840.91	5.08	0.1689	not significant
Pure Error	8993.54	2	4496.77			
Cor Total	4.677E+06	14				

R^2: 0.9834; Adjusted-R^2: 0.9536; Predicted-R^2: 0.7612

Table 4. ANOVA Table of quadratic function for *Rhus coriaria*

Source	**Sum of Squares**	**Df**	**Mean Square**	**F-value**	**p-value**	
Model	9.009E+05	9	1.001E+05	217.68	0.0028	significant
A-Temperature	25618.16	1	25618.16	4.52	0.0867	
B-Time	1.392E+05	1	1.392E+05	24.58	0.0043	
C-S/L	4.830E+05	1	4.830E+05	85.29	0.0002	
AB	302.04	1	302.04	0.0533	0.8265	
AC	7228.33	1	7228.33	1.28	0.3098	
BC	44437.83	1	44437.83	7.85	0.0379	
A^2	10148.79	1	10148.79	1.79	0.2383	
B^2	18530.62	1	18530.62	3.27	0.1302	
C^2	1.703E+05	1	1.703E+05	30.08	0.0027	
Residual	28314.32	5	5662.86			
Lack of Fit	28296.75	3	9432.25	1073.75	0.0009	not significant
Pure Error	17.57	2	8.78			
Cor Total	9.292E+05	14				

R^2: 0.9695; Adjusted-R^2: 0.9147; Predicted-R^2: 0.7127



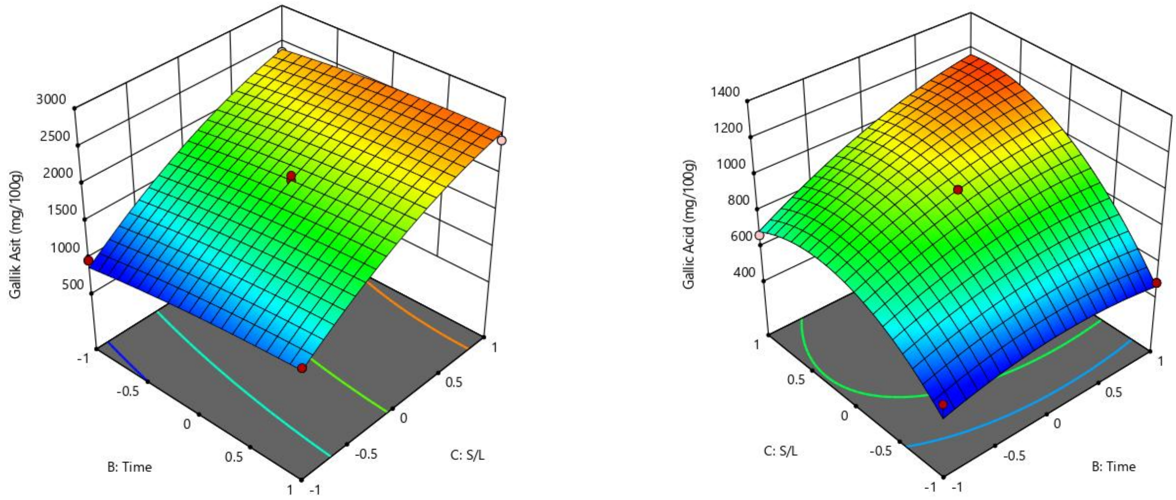
(a) (b)

Figure 1. Statistical analysis of the quadratic models suggested for *Cinnamonium zeylaniccum* (a) and (b) *Rhus coriaria*

4. Discussion and Conclusion

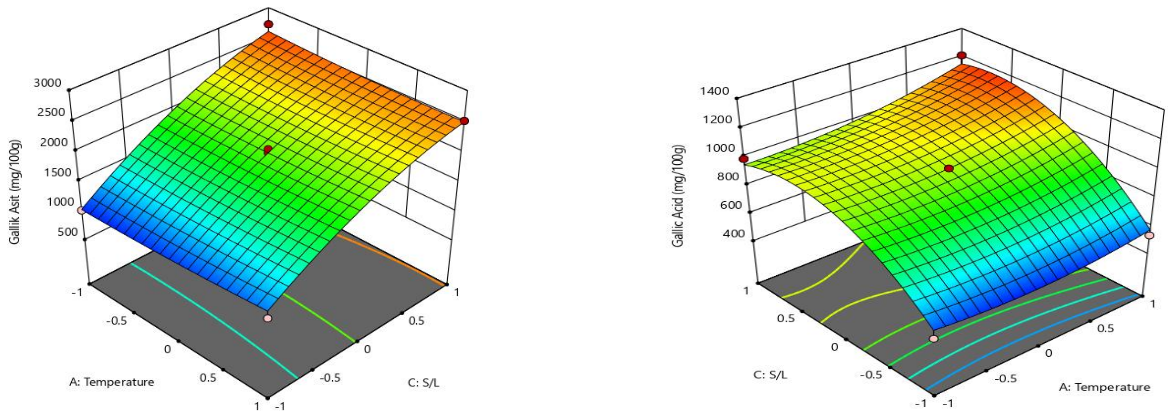
Three-dimensional extraction surface plots for both plants were generated at this stage of the study to examine the effect of binary interaction of the parameters on the polyphenol concentration to be obtained at the end of the extraction. The graphs for cinnamon were given in Figures 2(a)-3(a)-4(a), and their correspondences of sumac were represented in Figures 2(b)-3(b)-4(b). The red parts represent regions with the highest amount of polyphenols, while the yellow and blue regions represent decreasing polyphenol concentrations, respectively. As shown in Figure 2, it was determined that to obtain the maximum amount of polyphenol by ultrasonic extraction from cinnamon and sumac, conditions of maximum solid-per-liquid ratio and maximum extraction time should be satisfied.

When the binary effects of temperature and solid-per-liquid ratio parameters were examined by using a similar approach, ıt had been identified that both temperature and solid-per-liquid ratio should maximized for cinnamon to achieve high extraction efficiency (Figure 3-a). In the case of sumac, solid-per-liquid ratio that is in the range of [0.5: 1] will be enough to reach the highest yield (Figure 3-b). As can be seen in Figure 4, the extraction surface was flat and completely composed of yellow-green regions, the effect of temperature and time co-interaction on extraction yield was observed to be very low. Nevertheless, for sumac, it can be said that the maximum yield can be approached when the temperature and the time of extraction were maximized.



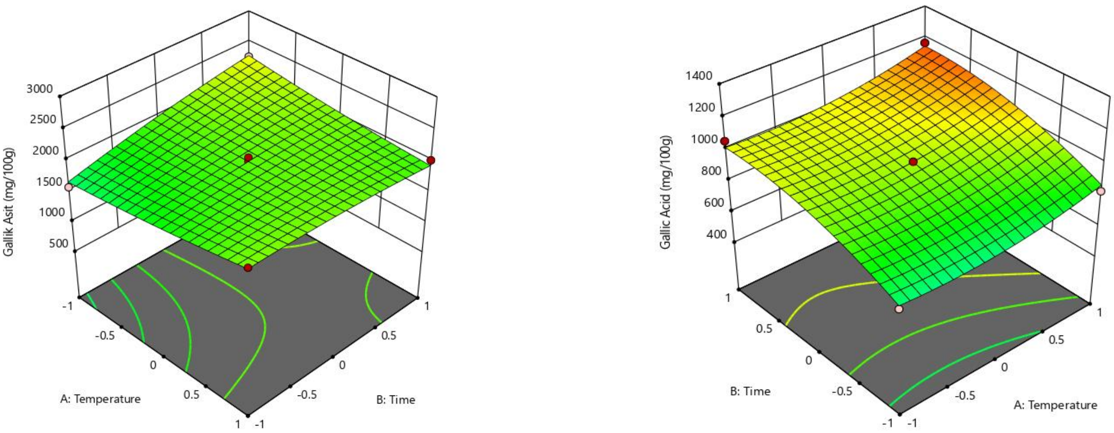
(x_2,x_3)

Figure 2. The effect of binary interactions of solid-per-liquid and time on polyphenol concentration (as gallic acid equivalent) *Cinnamonium zeylaniccum* and *Rhus coriaria*



(x_1,x_3)

Figure 3. The effect of binary interactions of solid-per-liquid and temperature on polyphenol concentration (as gallic acid equivalent) *Cinnamonium zeylaniccum* and *Rhus coriaria*



(x_1,x_2)

Figure 4. The effect of binary interactions of solid-per-liquid and time on polyphenol concentration (as gallic acid equivalent) *Cinnamonium zeylaniccum* and *Rhus coriaria*

In the study, the effects of parameters for total polyphenol extraction as gallic acid equivalent from *Cinnamonum zeylaniccum* and *Rhus coriaria* by ultrasonic extraction were investigated and optimizations of extractions were carried out. As a result of the statistical analyses in the study, the quadratic extraction equations to be used in the industrial production processes were obtained with the models determined. These equations are given in Equations 1 and 2 for cinnamon and sumac, respectively.

$$\begin{aligned} \textit{Gallic acid equivalent of total polyphenols} \\ &= +2000.83 + (14.86)A \\ &+ (120.95)B + (721.57)C \\ &- (184.16)AB - (63.38)AC \\ &- (42.14)BC + (31.74)A^2 \\ &- (44.60)B^2 - (197.55)C^2 \end{aligned} \tag{1}$$

$$\begin{aligned} \textit{Gallic acid equivalent of total polyphenols} \\ &= +968.28 + (56.59)A \\ &+ (131.90)B + (245.72)C \\ &+ (8.69)AB + (42.51)AC \\ &+ (105.40)BC + (52.43)A^2 \\ &- (70.84)B^2 - (214.78)C^2 \end{aligned} \tag{2}$$

Finally, the necessary extraction conditions (dots) were determined using the equations given above and the numerical analysis sections of software. The optimum conditions giving maximum yield of 2726.4 mg gallic acid equivalent of total phenolics/100 g of cinnamon as a result of multi-parameter optimization were calculated as 30^0C, 15 minutes, 1/50 g/mL. For the sumac (1211mg gallic acid equivalent of total phenolics/100 g of plant), the conditions was determined as 50°C, 15 minutes and 1/50 g/mL. These results have also been confirmed with the experimental study.

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