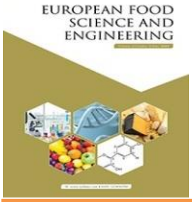

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An optimization study for processing variables in fabrication of cinnamon tincture by response surface methodology

Safa Karaman[1*]

[1] Food Engineering Department, Engineering Faculty, Niğde Ömer Halisdemir University, Niğde, Turkey

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ABSTRACT

In this study, effects of two processing variables namely liquid/solid ratio (3-10 mL/g) and ethanol concentration (25-75%) on total phenolic content and antiradical activity of cinnamon tincture were investigated using response surface methodology. Total phenolic content of the tincture samples ranged between 4667.9-14892.6 mg GAE/L while the IC50 values were in the range of 71.64-78.55. The effect of both liquid/solid ratio on total phenolic content of the cinnamon tincture samples was determined to be significant ($p<0.05$). Increase in the liquid level per gram of the cinnamon sample caused a significant decrease on the total phenolic content of the samples ($p<0.05$). Multiple response optimization of the processing variables showed that the maximum phenolic content could be obtained by the minimum liquid/solid ratio and for the best processing conditions of the high bioactive cinnamon tincture; liquid/solid ratio and ethanol concentration levels were determined to be 4.03 mL/g and 55.41% ethanol level. The results of the study would be an instructive for the tincture manufacturer.

1. Introduction

Tincture is produced from the plants with the hydroalcoholic extraction and it has a common application for the treatments of some diseases especially in phytotherapy. Tincture is a plant extract manufactured by especially hydroalcoholic extraction and it is more efficient compared to whole plant in the treatments of medicinal diseases because it is in liquid form and perform a better bioactive performance because of its bioactive compounds present in solution (Bone, 2000).

Medicinal plants are widely used for the treatment of many diseases in the worldwide because they are rich in many phytochemical substances bioactive performance. Seifried et al. (2007) informed the bioactive properties of phytochemicals in medicinal plants have an important role in the protecting the body against free radicals because these substances can easily reduce the oxidative stress due to their radical scavenging properties.

Cinnamon is a popular medicinal plant and it is widely used as a popular flavouring ingredient in some food formulations. It was reported that the cinnamon extracts showed some bio-functional properties such as antimicrobial activity, for controlling glucose intolerance and diabetes, inhibiting the proliferation of various cancer cell lines, and treating common cold (Anderson & Broadhurst, 2004; Murcia et al., 2004). Mancini-Filho & Van-Koiij (1998) reported that the cinnamon extracts were able to reduce lipid peroxidation in the β-carotene-linoleic acid system, and the inhibitory effect was comparable to the synthetic antioxidant standard (BHT).

The response surface methodology which is an important statistical modeling technique makes complex operations easier and widely used to optimize processing parameters (Sheng et al., 2013; Wang et al., 2013). It is used effectively due to need fewer attempts to evaluate multiple parameters and their interactions (Ma et al., 2010). It determines optimum working conditions depending on the variables by introducing functions that can predict responses conditions (Myers & Montgomery, 2002; Koç & Kaymak Ertekin, 2010; Cetin, 2022: Karaman & Sagdic, 2019).

In the current study, effect of two important processing parameters namely liquid/solid ratio and ethanol concentration on total phenolic content and antiradical activity of cinnamon tincture. To reveal the best processing variable levels to manufacture the most bioactive tincture, response surface methodology was used.

2. Materials and methods

2.1. Materials

Broken cinnamon was provided from Karakas Food Co (Kayseri, Turkey). The samples were ground and stored in a

*Corresponding author
E-mail address: safakaraman@ohu.edu.tr

plastic covered bag at room conditions until tincture preparations.

2.2. Methods

2.2.1. Preparation of tincture samples

For the production of tincture samples, two different processing parameters namely ethanol and liquid/solid level were used to determine the effect of these parameters on the bioactive properties of the final products. For this aim, ethanol concentration and liquid/solid ratio were selected as processing parameters and the levels of these factors were tabulated in Table 1. Central composite rotatable design having 11 runs was used to optimize the production parameters for the cinnamon tincture samples. Liquid/solid ratio was in the range of 3-10 g/mL while the ethanol concentration ranged between 25-75% (Table 1). To produce the tincture samples, the relevant parameters according to the Table 1 were applied and the final mixture was kept for 14 days at dark place in room conditions (25 ºC). At the end, the samples were centrifuged at 7500 *g* at +4 ºC for 5 min and the supernatant was used as tincture sample for the bioactivity tests.

2.2.2. Determination of total phenolic content (TPC)

To determine the total phenolics of the tincture samples, the method reported by Köprü et al. (2021) was used. For this aim, 0.2 mL tincture sample was mixed with 1.800 mL distilled water and then 1 mL of Folin reagent (1/10). After that, 2 mL of 2% of Na_2CO_3 was placed into the mixture and mixed well using vortex. The final mixtures were kept for 2 h in a dark place at 25 ºC. At the end, the sample absorbance was recorded using a spectrophotometer (Shimadzu, Japan) at 765 nm. A calibration curve prepared by gallic acid standard was used to calculate as mg gallic acid equivalent (mg GAE/L). The measurement was replicated with four repetitions.

2.2.3. Determination of antiradical activity

Radical scavenging performance of the tincture samples was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl), radical (Köprü et al., 2021). A 100 µL of tincture sample (1/40 v/v) was mixed with 3.9 mL of DPPH methanolic solution (0.1 mM) and mixed well by vortex. Then the samples were kept for 30 min in a dark place at 25 ºC and then, the sample absorbances were recorded at 550 nm using a spectrophotometer (Shimadzu UV-vis 1800, Japan). The antiradical activity (ARA) of the samples was calculated by Eq.1:

$$\text{ARA (\% Inh.)} = ((Abs_{control}-Abs_{sample})/Abs_{control}))*100 \quad (1)$$

where Abs is the absorbance. The measurement was replicated with four repetitions.

2.2.4. Data optimization

The effects of studied processing parameters were determined by response surface methodology approach. Table 1 shows the levels of processing variables by coded and uncoded terms. The regression models were fitted to the following polynomial equation (Eq. 2) and the model constants were calculated.

$$Y - \varepsilon = \beta_0 + \sum_{i=1}^{N} \beta_i x_i + \sum_{i=1}^{N} \beta_{ii} x_i^2 + \sum_{\substack{i=1 \\ i<j}} \sum_{j=i+1} \beta_{ij} x_i x_j \quad (2)$$

Design-Expert® Software Version 7.0 (Stat-Ease Inc., Minneapolis, USA) was used to calculate the regression coefficients of linear, quadratic and interaction terms for each output parameter. Also, the same software was used to perform variance analysis and optimization process.

Table 1. Experimental design for the production of cinnamon tinctures

	Coded Values		Actual Values	
Runs	**A**	**B**	**A**	**B**
1	0	0	6.50	50
2	-1	1	4.03	67.68
3	1	-1	8.97	32.32
4	0	0	6.50	50
5	0	1.414	6.50	75
6	1	1	8.97	67.68
7	0	0	6.50	50
8	-1	-1	4.03	32.32
9	0	-1.414	6.50	25
10	1.414	0	10.0	50
11	-1.414	0	3.00	50

A: Liquid/Solid Ratio (mL/g), B: EtOH Concentration (%)

3. Results and Discussion

3.1. Total phenolic content of tincture samples

Total phenolic content of the cinnamon tincture samples was in the range of 4667.9-14892.6 mg GAE/L (Table 2). The highest total phenolic content was determined for the tincture sample prepared by using 3 mL/g liquid/solid ratio and 50% ethanol concentration while the lowest total phenolic content was for the sample prepared by 6.5 mL/g liquid/solid ratio and 75% ethanol concentration.

Table 2. Total phenolic content and antiradical capacity of cinnamon tinctures

Runs	Liquid/Solid Ratio (mL/g)	EtOH Concentration (%)	TPC (mg GAE/L)	IC50
1	6.50	50	7745.6	75.74
2	4.03	67.68	9683.1	71.81
3	8.97	32.32	6173.0	72.24
4	6.50	50	6154.4	77.25
5	6.50	75	4667.9	78.55
6	8.97	67.68	5902.7	77.88
7	6.50	50	6525.0	71.64
8	4.03	32.32	10216.9	76.19
9	6.50	25	5194.9	76.12
10	10.0	50	8044.6	74.22
11	3.00	50	14892.6	70.96

TPC: Total phenolic content, ARA: Antiradical activity

As is seen in Table 3, linear effect of liquid/solid ratio was found to be very significant (p<0.05) while the linear effect of ethanol concentrations showed no significant effect (p>0.5). Additionally, quadratic effect of the ethanol concentration affected the total phenolic content of the cinnamon tincture. Figure 1 illustrates the change in total phenolic content of the tincture samples depending on the processing variables. As is seen, increase in the liquid level added per g of the cinnamon powder sample caused a significant decrease in the total phenolic content of the samples (p<0.05). However, an increment or decrement in ethanol concentration did not affect the total phenolic content of the tincture sample (Figure 1). To describe the effect of processing variable on total phenolic content of the cinnamon tincture, a regression model having quite high determination of coefficient was constructed (Table 4). Wijewardhana et al. (2019) compared the bioactive properties of cinnamon against garlic and black cumin and they reported that the total phenolic content of the cinnamon (18.94 mg GAE/100 g) is quite higher compared to garlic (5.46 mg GAE/100 g) and black cumin (8.45 mg GAE/100 g). Köprü et al (2020) investigated the effects of some processing variables on the total phenolic content of the clove tincture and they reported that the both liquid/solid ratio and ethanol concentration showed significant effect (p<0.05). Increase in liquid level in the extraction, caused a significant decrease in the total phenolic content of the tincture sample. Similar findings were also reported by Kaplan et al. (2018) for the dandelion tincture. They reported that the liquid/solid ratio and ethanol concentration showed a significant effect on the total phenolic content of the final tincture sample.

3.2. Antiradical activity of tincture samples

Antiradical performance of the tincture samples calculated as IC50 ranged between 71.64 78.55. The lowest IC50 showing the highest radical scavenging activity was determined for the tincture sample prepared by using 3 mL/g liquid/solid ratio and 50% ethanol concentration while the highest IC50 showing the lowest radical scavenging activity was for the sample prepared by 6.5 mL/g liquid/solid ratio and 75% ethanol concentration. As is seen in Table 3, both linear effects of liquid/solid ratio and ethanol concentration were found to be insignificant but the interactive effect of studied parameters was determined to be significant (p<0.05). IC50 was calculated using % inhibition values against total phenolic content of the tincture sample. So, the given IC50 values show the required total phenolic content of the tincture sample having the potential to scavenge the 50% of the DPPH radical. As is seen from the Figure 2, for 11 samples, the calibration curves used to calculate the IC50 values of the tincture samples were illustrated. Figure 3 also showed the change in the IC50 values of the samples and as is seen, there is an increase in IC50 values with the increase of liquid level added per g of the sample and also ethanol concentration (p>0.05). To describe the effect of processing variable on IC50 values of the cinnamon tincture, a regression model having an acceptable determination of coefficient was constructed (Table 4). Antiradical activity of cinnamon was determined to be significantly higher compared to garlic and black cumin (Wijewardhana et al., 2019). Kaplan et al. (2018) reported that the liquid/solid ratio and ethanol concentration showed a significant effect on the radical scavenging activity of the samples.

Table 3. ANOVA table for the effects of studied parameters on TPC and IC50 of cinnamon tincture

		TPC		IC50	
Source	**df**	**Mean Square**	**F Value**	**Mean Square**	**F Value**
Model	5	17002048	45.01[a]	11.21	3.01
A-Liquid/Solid Ratio (mL/g)	1	38327838	101.46[a]	5.67	1.52
B-EtOH concentration (%)	1	300075	0.79	2.76	0.74
AB	1	17360	0.05	25.10	6.75[a]
A^2	1	29329622	77.64[a]	8.84	2.38
B^2	1	5505482	14.57[b]	7.08	1.90
Residual	5	377774		3.72	
Lack of fit	3	167489	0.24	0.58	0.07
Pure error	2	693201		8,43	
Cor total	10				

[a] p<0.01; [b] p<0.05

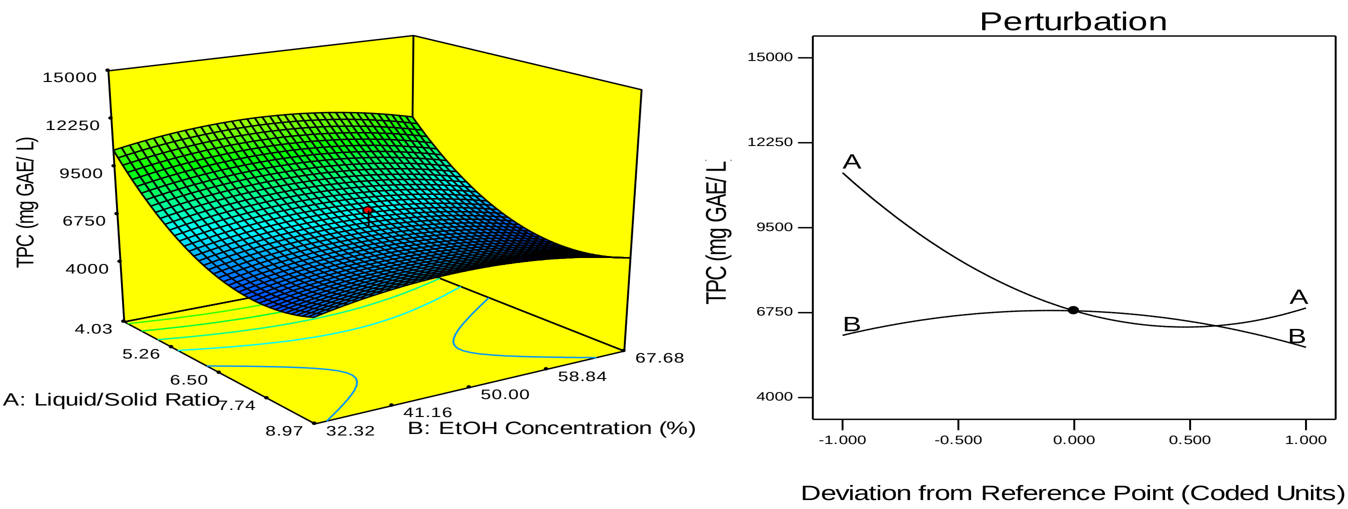

Figure 1. Change in total phenolic content of cinnamon tincture

Table 4. Regression equation for the studied parameters of cinnamon

Parameters	Regression model	R^2
TPC	Y_{TPC}=6808.1-2199.9A-194.1B+65.99AB+22812A^2-9876B^2	0.978
IC_{50}	Y_{IC50}=74.86+0.842A+0.587B+2.510AB-1.25A^{2+}1.12B^2	0.750

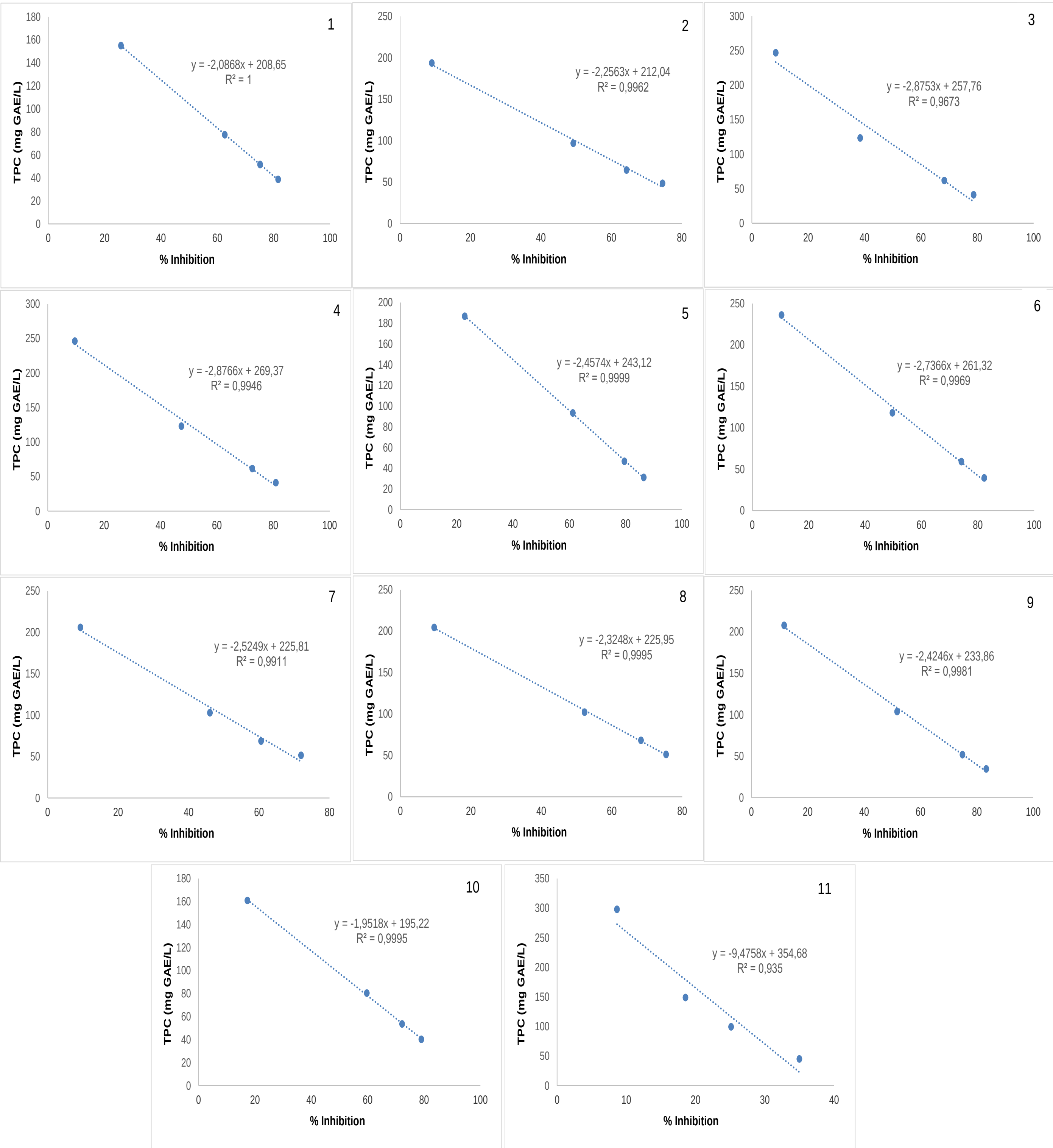

Figure 2. Change in antiradical activity of cinnamon tinctures by total phenolic content

3.3. Optimization of the processing variables

To determine the best processing conditions for the highest bioactivity, multiple response optimizations were conducted. For the maximization process, the highest total phenolic content (11108.42 mg GAE/L) and lowest IC50 value (72.29) were recorded for the sample which would be produced by 4.03 mL/g and 55.41% ethanol level (Table 5). In addition to that, the minimization process revealed that the minimum total phenolic content (5420.9 mg GAE/L) and maximum IC50 value (78.49) were recorded for the sample which would be produced by 8.53 mL/g and 67.68% ethanol level. Depending on the optimization results, to manufacture a high bioactive cinnamon tincture, the liquid level added per g of the sample should be low while the ethanol concentration levels ranged between 45-65%.

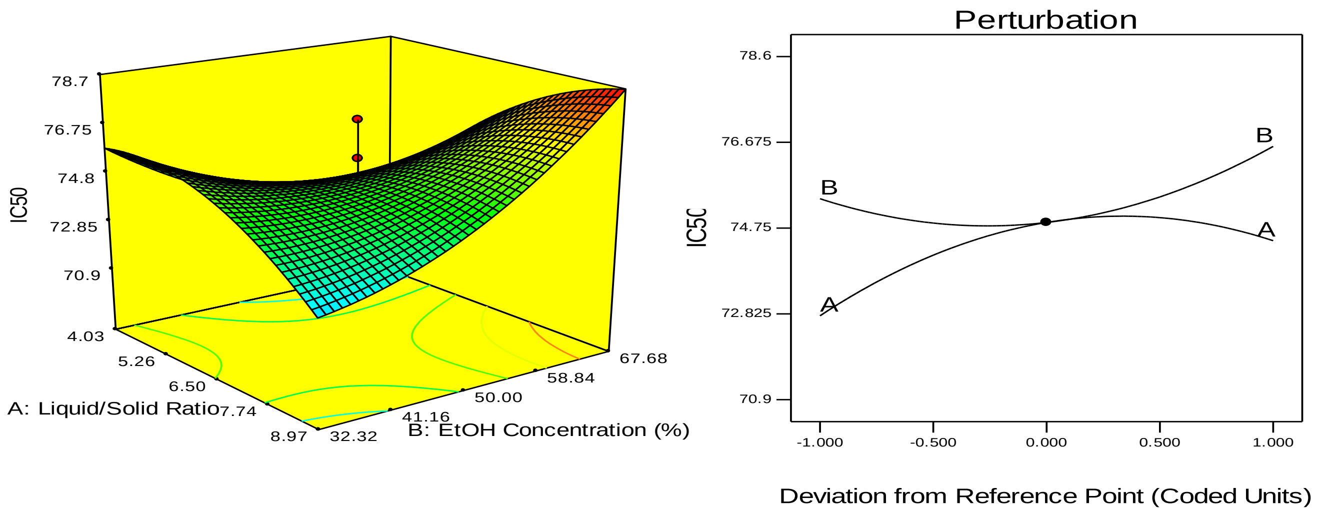

Figure 3. Change in antiradical activity of cinnamon tincture

Table 5. Multiple response optimization of the processing variables

Liquid/Solid Ratio (mL/g)	EtOH Concentration (%)	TPC (mg GAE/L)	IC_{50}	Desirability
Maximization				
4.03	55.41	11108.42	72.29	0.720
Minimization				
8.53	67.68	5420.972	78.49	0.958

4. Conclusions

Cinnamon tinctures which are the hydroalcoholic extract of the plants showed quite high total phenolic content and some important processing variables such as liquid/solid ratio and ethanol concentration affected the bioactive performance of the final product. Optimization results revealed that the highest total phenolic content and lowest IC50 of the samples could be obtained with the lowest liquid/solid ratio for the tincture production.

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