Investigation of Microwave-assisted Extraction Conditions of Quercetin from *Cinnamonum zeylaniccum* with Response Surface Methodology

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Abstract: Increase in cancer diseases has led to the focus on the availability of using controlled-drug-releasing capsules for their treatments. The economics of the production process of the cancer cell-focusing capsules depends on the yield of the extraction (the initial operation) of drug material. The aim of this study was to optimize the extraction of raw-material from *Cinnamonum zeylaniccum* with methanol by using Response Surface Methodology. Microwave-assisted extraction experiments had been carried out with the parameters of temperature, solid/liquid ratio and microwave power, by fixing the microwave application time and mixing rate of the extraction medium as constants. Multi-parameter optimization was carried out with three-parameter, three-level, three-centered Box-Behnken design considering the optimum values obtained in single-parameter optimization. As a result of the statistical analysis, the function expressing the effect of the parameters on the resulting amount of quercetin production in industry had been derived. With this function, the maximum quercetin amount found to be extracted in 74 different conditions. The highest amount (21mg/100g) was produced within the verification experiments made at the selected optimum condition (50°C, 1g/30ml, 0.5 kW). Studies considering the effectiveness of aqueous solutions of the extract loaded capsules on cancer treatment are continuing.

Keywords: *Cinnamonum zeylaniccum*, Extraction, Flavonoid, Response surface methodology.

I. INTRODUCTION

Currently the most widespread disease in the world is cancers of different types. Due to this fact there are several researches investigating the reasons, the metabolism and also their treatments. Cancer focusing drug delivery systems are gaining importance in that studies and loaded drug material usage is an important subject for this area.

Herbal plants are popular remedies for diseases used by the most of the world's population. Phytochemicals exert positive effects on human health and thus they are important compounds found in medicinal plants. Cinnamon, a member of the Laurel family, native to the South and Southeast Asia, is a genus of evergreen aromatic fragrant tree. Substantially dried bark of the cinnamon tree is used in general consumption and as a spice in daily life. There are about 100 kinds of cinnamons present. Countries performing the most of cinnamon production in the World are China, Indonesia and Sri Lanka. This plant was known with its special property of releasing the ureteral and kidney stones [1].

Flavonoids are the most effective antioxidants having more than 4000 different types [2], [3]. Capability of binding free radicals and specificity of interacting with the cancer cells without damaging the normal ones make them attractive for curing those diseases [4]. These compounds, especially quercetin, are found in fruits like apples, grapes and strawberries, and also in spices [5], [6].

One of the most important properties of the quercetin is formation of the chelates with heavy metals [3], [7], [8], [9], [10]. It reacts with titanium-based anions in 1:2 ratios [7]. This chelate formation characteristic is especially preferred in curing of food poisoning and an adsorption of radiation [7], [9]. In some of the researches, quercetin was found to be effective on cancer treatments and curing.

The type and the amount of the flavonoids obtained from the herbs are strongly affected by the extraction. Today there are several different types of extraction techniques present; ultrasonic, microwave, and supercritical. These new methods especially some advantages over classical extractions: short extraction times, less solvent requirement, less amount of waste production etc. In nearly all of the researches, the scientists focus on the type of flavonoid and the amount found in the plant material, they do not deal with the optimization of the process considering the economics.

Today's the most approved model for the optimization, namely response surface method is a combination of statistical and mathematical techniques used for analyzing several independent variables and also interactive effects among the variables on the response. This method has been used in several optimizations including adsorption [11], extraction [12], fermentation [13], and production processes [14]. Thus, the aim of the study was determined as to optimize for the microwave-assisted extraction of raw material (quercetin) from cinnamon controlled drug releasing purposes. In order to investigate the combinational effects of the parameters, a software program called Design-Expert used with Response Surface Methodology. This method has an advantage of prediction of the effect of several parameters simultaneously without doing any more experiment [11],

[12], [13], [14]. Due to this property, it has mostly used in several different industries. Thus, it was also aimed that to derive an industrially applicable equation representing the extraction surface of the quercetin.

II. MATERIALS AND METHODS

Cinnamonum zeylaniccum and the analytical grade chemicals (aluminum chloride, sodium acetate, and acetic acid) were purchased from Sigma Co. The experimental design optimization was applied in two steps; firstly single-parameter optimization was used by changing the range of the selected parameter while keeping all the others at their respective value, and then multiple-parameter was achieved with three-parameter-and-three-level Box-Behnken Design by using the results of the single-parameter optimization.

In order to study the effects of temperature, solid-to-liquid ratio, and microwave power on the extracted amount of quercetin, independent variables were coded according to (1) in multiple-parameter optimization studies.

$$x_i = \frac{x_i - x_0}{\Delta x} \tag{1}$$

where x_i is the dimensionless coded value of ith independent variable, x_0 is the value of x_i at the center point, and Δx is the step change value. The parameters of the study were summarized in Table 1. The center points (coded as "0") of the parameters were obtained from the single-parameter optimization results.

Table 1 Box-Behnken Design parameters used in the study

Parameters	**-1**	**0**	**+1**
x_1: Temperature (^{0}C)	30	40	50
x_2: Solid/liquid (g/ml)	1/30	1/40	1/50
x_3: Microwave power (kW)	0.3	0.6	0.9

The surface of the system is explained by the second-order polynomial model given in (2).

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k-1}\sum_{j=2}^{k} \beta_{ij} x_i x_j + \varepsilon \tag{2}$$

where Y is the predicted response, x_i, x_j,...,x_k are input variables, which affect the response Y, x_i^2, x_j^2,..., x_k^2 are the square effects, β_0 is the intercept term, x_ix_j, x_jx_k and x_ix_k are the interaction effects, β_i (i = 1, 2, . . . , k) is the linear effect, β_{ii} (i = 1, 2, . . . , k) is the squared effect, β_{ij} (i = 1, 2, . . . , k) is the interaction effect, and ε is the random error [15], [16].

The Design-Expert 9.0 (Stat-Ease Inc., Minneapolis, MN, USA) software was used for regression and graphical analysis of the experimental data to fit the equations developed and evaluation of their statistical significance. BBD is frequently used under response surface method due to its suitability to fit quadratic surface that usually works well for process optimization. The optimum values of the selected variables were obtained by solving the regression equation at desired values of the process responses was fixed at the optimization criteria.

Microwave was applied to 1g of *Cinnamonnum zeylaniccum* during 3 minutes before starting to the each of the extraction since it was found as the best microwave power for extraction of quercetin in single-parameter optimization. Classical extraction was realized batch-wise in a 250 mL Erlenmeyer flask with methanol as a solvent according to the Box-Behnken design conditions. Mixing rate of the extraction medium was set at constant at its maximum value. At the end of the extraction, the content of the flask was filtered through 110 mm filters (FilterLab) and filtered samples were used for quercetin analysis. Quercetin was measured by colorimetric method of aluminum chloride [17]. 1ml of sample was mixed with acetic acid-sodium acetate buffer solution of pH-4 and 2 ml of $AlCl_3$ solution. The absorbance of the resultant solution was read at 415 nm by UV- Spectrophotometer (LANGF, DR 5000). In the analysis, methanol-buffer containing blank was used. The amount of quercetin extracted were calculated with the (3) (R^2= 0.9997) derived from the absorbance values of solutions prepared by solving different amounts of pure quercetin in a 200 ml of methanol.

$$Absorbance@415nm = (0.0025).Concentration\ (mg/g) + 0.0192 \tag{3}$$

III. RESULTS AND DISCUSSION

In the study, after entering the coded parameters and their respective yields into the software as it was summarized in Table 2, statistical tests were applied in each of the suggested functions. The model having the highest regression coefficient and the lowest lack of fit value was chosen as the predicted best function for representation of the extraction surface. Those values for the suggested quadratic model of software were 0.9963 and 0.78, respectively. The predicted R-squared was found reasonably in agreement with the adjusted R-squared value of 0.9895. Thus, the fitness in between the experimental data (actual) and their respective calculated values of the function (predicted) (Fig. 1) was approved the statistical conclusions. As a result, the model function was chosen as quadratic.

Table 2 Box-Behnken Design

No	x_1	x_2	x_3	Abs	Quercetin (mg)
1	-1	-1	0	1.0649	12.55
2	+1	-1	0	1.1108	21.832
3	-1	+1	0	0.6165	7.17
4	+1	+1	0	0.6695	13.006
5	-1	0	-1	0.7775	9.0996
6	+1	0	-1	0.8008	15.632
7	-1	0	+1	0.7160	8.36
8	+1	0	+1	0.8150	15.916
9	0	-1	-1	1.0621	16.6864
10	0	+1	-1	0.6430	9.9808

11	0	-1	+1	1.0729	16.8592
12	0	+1	+1	0.6564	10.1952
13	0	0	0	0.7756	12.1024
14	0	0	0	0.7760	12.1088
15	0	0	0	0.7713	12.0336

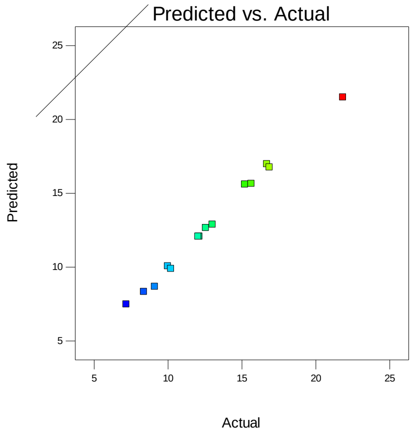

Figure 1. Statistical approval of the quadratic model

The Analysis of Variance (ANOVA) for the quadratic model was constructed with software (Table 3). In that, A demonstrates the temperature; B and C were representing solid-to-liquid ratio and microwave power, respectively. Since the larger the magnitude of the F-value and the smaller the p-value, the more significant the corresponding coefficient. The most effective parameters on the extraction of quercetin from *Cinnamonnum zeylaniccum* were found as temperature and solid-to-liquid ratio. In addition, the results showed that these parameters were mostly interrelated parameters on this operation.

Table 3 Analysis of variance (ANOVA) for quadratic function

ANOVA for Response Surface Quadratic model					
Analysis of variance table [Partial sum of squares - Type III]					
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**
Model	207,59	9	23,07	148,07	< 0.0001
A	*101,43*	*1*	*101,43*	*651,18*	*< 0.0001*
B	*95,05*	*1*	*95,05*	*610,20*	*< 0.0001*
C	*0,078*	*1*	*0,078*	*0,50*	*0,5116*
AB	*2,97*	*1*	*2,97*	*19,06*	*0,0073*
AC	*0,023*	*1*	*0,023*	*0,15*	*0,7163*
BC	$4{,}3.10^{-4}$	*1*	$4{,}3.\ 10^{-4}$	$2{,}8.\ 10^{-3}$	*0,9600*
A^2	*0,037*	*1*	*0,037*	*0,24*	*0,6479*
B^2	*7,85*	*1*	*7,85*	*50,40*	*0,0009*
C^2	*0,044*	*1*	*0,044*	*0,28*	*0,6171*

In order to investigate the interactive effects of the parameters in detail, three-dimensional surface graphs were constructed. The interactive effects of the parameters were shown in Fig. 2-4. In those, red regions shows the highest amount of quercetin extracted, yellow and blue parts represent the lower and much lower extraction yields than those. As it can be seen from figures, the combination of the lowest solid-to-liquid ratio and the highest the temperature was required for the highest quercetin extraction (Fig.2).

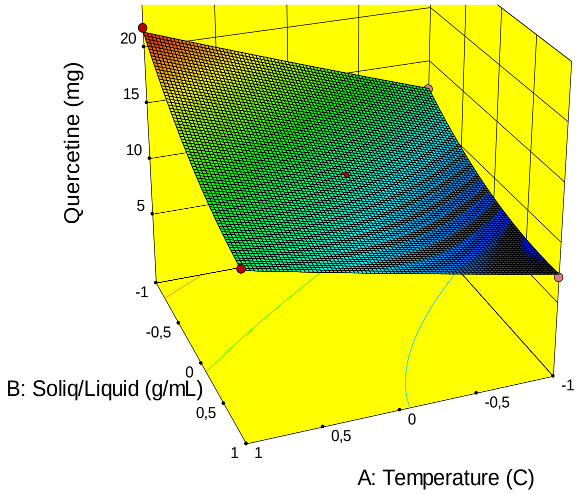

Figure 2. Three-dimensional response surface of solid-to-liquid ratio and temperature

Investigation of the response surface of solid-to-liquid ratio and microwave power (Fig. 3), it was concluded that all of the microwave power was found applicable if the solid/liquid ratio was set at [-1;0] coded range. Red region was not seen in this surface because those parameters were not so effective on the extraction. In addition, it can be concluded that solid-to-liquid ratio was not so interrelated with the microwave power used.

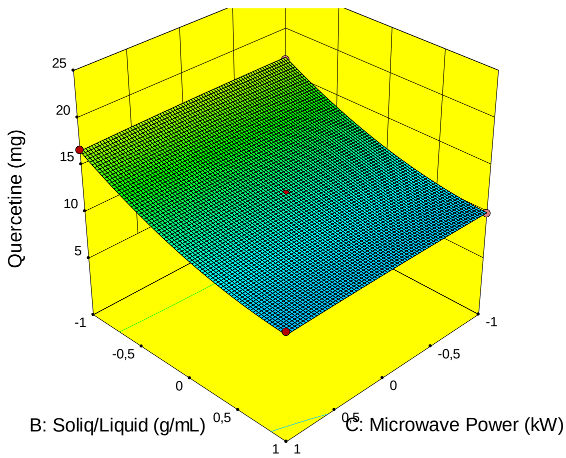

Figure 3. Three-dimensional response surface of solid-to-liquid ratio and microwave power

The same behavior was observed in the temperature and microwave power relation (Fig. 4). The increase in temperature was increasing the extraction yield whereas the increase in microwave power did not cause any improvement. The temperature cannot be increased more in the study because of the boiling temperature of the solvent. Thus, there was not any more condition for increasing the yield of extraction and the investigations were stopped here.

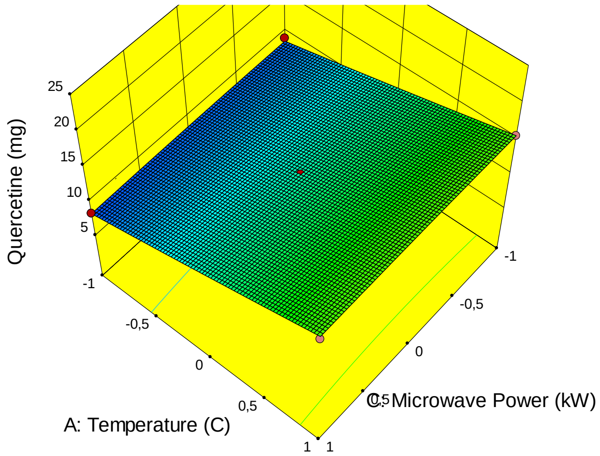

Figure 4. Three-dimensional response surface of temperature and microwave power

IV. CONCLUSION

In this study our aim was to optimize the microwave-assisted extraction conditions of quercetin from *Cinnamonnum zeylaniccum* by investigating the effect of parameters for each and also in combination. In addition, in order to use the results of the study in an industrial production processes, the modeling equation was needed. Due to the results of statistical analysis, quadratic model function was chosen as the resultant equation (Eqn. 4) for the industry.

$$Quercetin = 12.08 + (3.56)A - (3.45)B - (0.099)C - (0.86)AB + (0.076)AC + (0.01)BC + (0.1)A^2 + (1.46)B^2 - (0.11)C^2 \quad (4)$$

By using the numerical solutions section of the software, there were 74 different conditions determined as applicable for the industry. It was calculated that the highest amount of quercetin (21.511mg/100g) could be extracted at 50°C, 1g-plant/30mL-metanol, and 0.5 kW microwave power usage. These results were verified with the experiments.

In this research, we found that the selected range of microwave power were to be applicable but ineffective in the extraction yield. However our prediction was it would be effective on the other phenolic compounds present in the plant that may have symbiotic effect of reducing free radicals in cancer cells. Nowadays, unpurified aqueous solutions of the extracts obtained were loaded to drug-releasing capsules in order to examine their effectiveness.

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