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Arbutin Analysis In Leaves, Fruit And Branches Of Pyrus Amygdaliformis Vill. Var. Amygdaliformis Method Optimization

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ABSTRACT
Arbutin is a derivative of hydroquinone that develops naturally. It is produced in numerous plant species belonging to various families, such as *Lamiaceae*, *Ericaceae*, *Saxifragaceae* and *Rosaceae.* It is a tyrosinase inhibitor and one of its uses is as a cosmetic skin whitening agent. *Pyrus amygdaliformis* Vill. var. *amygdaliformis*, also known as the almond-leaved pear, is a species of plant in the Rosaceae family. It is native to southern Europe, the Mediterranean, and west Asia. In this study, Arbutin was analyzed in leaves, fruits and branches of *Pyrus amygdaliformis* Vill. var. *amygdaliformis* and analytical method was optimized. A modeling of the ultrasound assisted extraction of arbutin from leaves, fruits and branches of *Pyrus amygdaliformis* Vill. var. *amygdaliformis* was achieved using response surface methodology. A three-level-three-factor Box–Behnken design was implemented with the aim of optimizing three extraction variables, including extraction temperature (X1), extraction time (X2), and methanol concentration (X3), for the achievement of high extraction yield of the arbutin. The optimized conditions are extraction temperature of 43.76 ˚C, methanol concentration of 48.50 %, extraction time of 39.44 min. Under this optimized conditions, the experimental yield of arbutin is 8.13 %, which aligns well with the predicted yield of 8.05 %.
Keywords: Pyrus amygdaliformis Vill. var. amygdaliformis, Arbutin, Extraction, Optimization, RSM.

I. INTRODUCTION

*Pyrusamygdaliformis*Vill.var.*amygdaliformis*, also known as the almond-leaved pear, is a species of plant in the *Rosaceae* family. It is native to southern Europe, the Mediterranean, and west Asia. It grows to a height of 3 m. It has white flowers which bloom in April–May. The fruits are bitter and astringent [1]. It hybridizes well with Pyrus communis and Pyrus pyraster.The species was formally described by Dominique Villars in 1807 [2].

Arbutin(4-hydroxyphenyl-b-D-glucopyranoside) consists of a phenol molecule with a glucose moiety in the para-position and is a hydroquinone derivative. Arbutin may be found in various plant species, such as *Rosaceae* (*Pyrus communis* L. [3]), *Lamiaceae* (*Origanum majorana* L. [4]), *Myrothamnaceae* (*Myrothamnus flabellifolia* Welw. [5]) and *Ericaceae* (e.g. *Vaccinium spp*. [6] or *Arctostaphylos uva-ursi* L. [7]. Although in plants the amount of arbutin can reach considerable levels (up to 25% of the dry weight in M. Flabellifolia leaves [5,8] or up to 17% in the widely used *A. uva-ursi* [7, 9, 10]) what it brings for physiology and ecology is still under discussion. As it occurs in plant taxa with capability of withstanding extreme low temperatures or extended drought, scientists think arbutin plays an important role in resisting such environmental stress [11-13]. In *Pyrus spp*., it was found that hydroquinone formation from arbutin is involved in fire blight resistance [14,15].

In cosmetic preparations arbutin is widely used to lighten the skin [16, 17]. Arbutin is also well known for its diuretic and urinary anti-infective properties and the arbutin-rich leaves of *A. uva-ursi* (bearberry) are internally used for moderate inflammatory conditions of the urinary tract and bladder [18]. In both cases the active principle is hydroquinone, a metabolite of arbutin.

Extracting arbutin from pear has recently attracked considerable interest. *Pyrus pyrifolia* Nakai (fruit peel) [19] *P. pyrifolia* Niitaka (fruit peel), [20] *Pyrus biossieriana* Buhse (leaves) [21,22] four species of oriental pear (*Pyrus bretschnrideri*, *P. pyrifolia*, *Pyrus ussuriensis*, and *Pyrus sinkiangensis*), and one species of occidental pear (the flowers, buds, and young fruits of *P. anatolica* [23] are species and parts of pear arbutin has been extracted from.

There are many methods to determine the content of arbutin in plant extracts: spectrophotometric [24], capillary zone electrophoresis [25], densitometric [26], GC/MS [27]. Reversed-phase HPLC was found to be the more suitable chromatographic method for arbutin separation [28, 29] To our knowledge, the quantification of arbutin in various plant extracts cannot be achieved with a single validated HPLC method. There are no studies on the purification of arbutin in high purity from pear and other plants yet.

as Among others, solvent composition, extraction time, extraction temperature[30], solvent to solid ratio [31] and extraction pressure [32] are some of many factors which may significantly influence the extraction efficacy. In general, either empirical or statistical methods are used for optimization of a process; when it comes to complete optimization, the former have limitations. The traditional approach of one-factor-at-a-time is time consuming in process optimization. Moreover, the probability of approaching a true optimum is very low because of the chance that interactions among various factors may be ignored. Thus, one-factor-at-a-time procedure assumes there is no interaction among various parameters, that is, the process response is a direct function of the single varied parameter. However, it is the interactive influence of different variable which creates the actual response of the process. Unlike conventional optimization, it is possible to consider interactions among variables in the statistical optimization procedure [33].

Originally described by Box and Wilson [34], response surface methodology (RSM), makes evaluation of the effects of several process variables and their interactions on response variables possible. Thus, RSM has been successfully used for developing, improving and optimizing processes [35] as a collection of statistical and mathematical techniques. The main advantage of RSM is that it reduces the number of experimental trials while assessing multiple parameters and their interactions. Therefore, it requires less labor and time than other approaches in process optimization. Modeling and optimization of biochemical and biotechnological processes related to food systems [(36-41] including extraction of phenolic compounds from berries [31, 36] and evening primrose meal [30], anthocyanins from black currants [31] and sunflower hull [42] and vitamin E from wheat germ [43] among others, have successfully implemented response surface methodology.

Therefore, the aim of present investigation was to develop a simple, precise, accurate, and optimized method for arbutin analysis and apply such method for qualitative and quantitative analysis of arbutin in leaves, fruit and branches of *Pyrus amygdaliformis* Vill. var. *amygdaliformis*. An optimization study was conducted on experimental conditions to reach a result in the highest arbutin content of *Pyrus amygdaliformis* Vill. var. *amygdaliformis*. Figure 1 shows *Pyrus amygdaliformis* Vill. var. *amygdaliformis* and the molecular structure of arbutin.

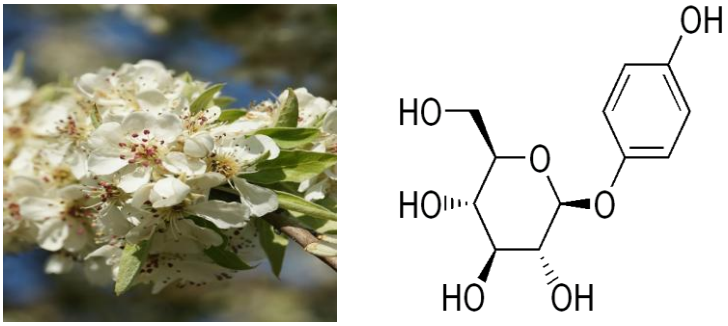

Fig. 1 *Pyrus amygdaliformis* Vill. var. *amygdaliformis* and the molecular structure of arbutin.

II. MATERIAL AND METHODS

1.1. Reagents and materials:

Pyrus amygdaliformis Vill. var. *amygdaliformis* used in this study has collected from 5 km north of New Erice Village, Sivaslı Town, Usak Province in October 2015. The collection and identification of the plant was performed by Mehtap Donmez Sahin. The plant sample was stored in Herbarium Material Warehouse of Usak University. Its leaves and branches were dried at room temperature in a dark room for fifteen days. Dried leaves and branches were ground to the size of 80–100 mesh before extraction. Its fruit was grated before extraction.

All chemicals used in all experiments were analytical grade and all solvents used for chromatographic purposes were of HPLC grade. 0.45µm membranes (Millipore, Bedford, MA, USA) were used for filtering the all solutions. Arbutin Standard was purchased from Sigma Chemical Co.

2.2 Ultrasound Assisted Extraction

Ultrasound assistant extraction was carried out using Bandelin Sonorex brand ultrasonic bath with 50 kHz frequency. For the standard ultrasonic conditions, erlenmeyer flasks were placed inside the ultrasonic bath. Solvent level in the erlenmeyer flask and water level in the ultrasonic bath were kept the same. The temperature and time value of the ultrasonic bath was set and extraction was carried out. After the extraction process had been completed, mixture was filtered with Whatman filter paper in order to prevent capillary blockage first and then filtered with 0.45 micron membrane filter (Millipore, Bedford, MA, USA).

1.2. HPLC Analysis

Identification and quantitative determination of arbutin was established by Agilent 1260 chromatographic system equipped with auto sampler, quaternary pump, column compartment and a UV-VIS detector. Final quantification was performed on a 250 mm × 4.6 mm id, 5 ìm particle size, ACE 5 C-18 column. The mobile phase was a solution of 7% methanol in water, The mobile phase filtered through 0.45 ìm Millipore filters. The flow rate was 1.2 ml/min and the injection volume was 10 ìL. The column temperature was maintained at 30 °C and detection was carried out at 280 nm. Chromatographic analysis was carried out using a single-column isocratic reverse phase method.

1.3. Analytical Method Validation

The method has been validated in terms of linearity, precision, accuracy and stability according to ICH guidelines, taking into account the recommendations of other appropriate guidelines. Results obtained from testing different parameters during validation of the analytical method were given in Table 1.

1.3.1. Standard Solution and Calibration Curves

Standard stock solution in water of arbutin was prepared at the final concentration of 1000 μg/ml for arbutin. Before calibration, the stock solution was diluted with water. The standard curve was prepared over a concentration range of 40-200 μg/ml for arbutin with five different concentration levels. Linearity for arbutin was plotted using linear regression of the peak area versus concentration. The coefficient of correlation (R^2) was used to judge the linearity. The dedection limits (LOD) and quantitation limits (LOQ) for tested compound were determined by the signal to noise (S/N) ratio (Table 1).

Table 1. Results obtained from testing different parameters during validation of the analytical method.

Parameters		Arbutin
Specifity	Peak Purity Ratio	0.0010
Linearity	Concentration Range (ppm)	40-200
	Correlation Coefficient	0.99987
	Intercept	1.81524
	Slope	1.60321
LOD (ppm)		0.891
LOQ (ppm)		2.972
Retention Time (min.)		4.580

1.4. Response Surface Methodology (RSM)

The RSM with the Box-Behnken design was then employed to design the experiment to investigate the influence of three independent parameters, temperature, time and methanol concentration on the extraction of arbutin. Optimal ranges of temperature (30-60 ^{0}C), time (20-60 min) and methanol concentration (25-75 %) were determined based on preliminary experiments. The independent variables and their code variable levels are shown in Table 2. To express the arbutin content as a function of the independent variables, a second order polynomial equation was used as follows and previously described by Vuong et al.

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} {X_i}^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j + e \qquad (1)$$

Where various X_i values are independent variables affecting the response Y: β_0, β_i, β_{ii} and β_{ij} are the regression coefficient for the intercept and the linear, quadratic and interaction terms, respectively and k is the number of variables.

Table 2. Treatment variables and their coded and actual values used for optimization of arbutin extraction from *Pyrus amygdaliformis* Vill. var. *amygdaliformis* by using Box-Behnken design.

Independent Parameters	**Units**	**Symbols of the parameters**	**Coded Levels**		
			-1	**0**	**1**

Extraction Temp.	^{0}C	(X1)	30	45	60
Extraction Time	min	(X2)	20	40	60
Methanol Conc.	%	(X3)	25	50	75

1.5. Statistical analysis

Statistical analysis on the means of triplicate experiments was carried out using the analysis of variance (ANOVA) procedure of the Instat® software version 3.0 (GraphPad, San Diego, CA, USA). Anova test was applied to identify the interaction between the variables and the response using Design-Expert program. Three replication analyses were carried out for each sample. ANOVA test was applied for identifying the interaction between the variables and the response by using Design-Expert program. The results of HPLC analysis were expressed as means of extraction efficiency.

III. RESULTS AND DISCUSSIONS

1.6. Effect of process variables on the UAE performance

Table 2 shows the experimental conditions of Box-Behnken design runs designed with Design Expert 9. Table 3 also displays the effects of methanol concentration, extraction time and extraction temperature on the extraction efficiency obtained by UAE.

Table 3. Box-Behnken Design of the independent variables (X1, X2, X3) and EY experimental results
*Data are expressed as the mean (n=3).

Run	Ext. Temperature ^{0}C	Ext. Time min	Methanol Concentration %	Arbutin Yield %
1	45	40	50	7.89
2	30	20	50	5.96
3	45	60	75	6.30
4	45	20	25	6.62
5	30	60	50	6.10
6	60	60	50	5.81
7	60	40	25	5.87
8	45	40	50	8.15
9	45	40	50	8.04
10	60	20	50	5.80
11	45	60	25	6.27
12	60	40	75	5.05
13	45	40	50	8.10
14	45	20	75	6.45
15	30	40	75	6.19
16	30	40	25	6.18
17	45	40	50	7.98

1.6.1. Effect of extraction time on the UAE performance

The extraction time influence on the extraction efficiency of arbutin was examined over a range of 20-60 min and Table 3 shows the results. The experiment results showed that 40 min is the optimum extraction time of the arbutin, as shown in Figure 2. When extraction time increased, the cell walls of the leaves of Pyrus

amygdaliformis Vill. var. amygdaliformis got fully fall apart and arbutin got into material liquid diffusion so that the extraction yield is relatively rapid. During long extraction time, *Pyrus amygdaliformis* Vill. var. *amygdaliformis* leaves overheating was prone to cause thermal decomposition of arbutin, because of the unstable chemical bonds of arbutin molecular, such as unsaturated bonds. And then the arbutin content was decreased. Therefore, 40 min is a favorable duration to extract the arbutin.

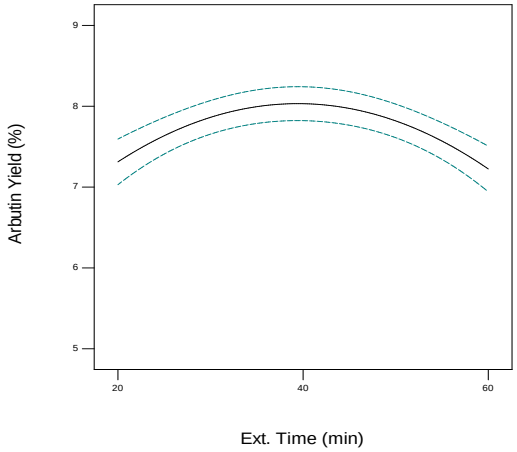

Fig. 2 The influence of extraction time on extraction performance

1.6.2. Effect of extraction temperature on the UAE performance

Extraction process was carried out using extraction temperature from 30 to 60 ˚C. As shown in Figure 3, extraction temperature has obvious effects on yield of arbutin. When extraction temperature increased, the extraction yield increased rapidly and reached a maximum at 44˚C. In general, higher temperatures in extractions are directly proportional to rates of mass transfer and extraction performance because of enhanced solute desorption from the active sites of plant matrix. When extraction temperature went above 45˚C, the extraction yield started to decrease. At initially, extraction yield increasing with the rising of temperature may be that elevated temperature accelerated the arbutin chemical bond rupture and speeded molecular motion, so that a large number of arbutin in cell dissolution into the solution. when heating temperature greater than 45˚C, high temperature caused the destruction of arbutin structure, accelerated the degradation reaction, and lost arbutin activity, and then arbutin content is rapidly reduced. Therefore, 44˚C is favorable for extracting the arbutin.

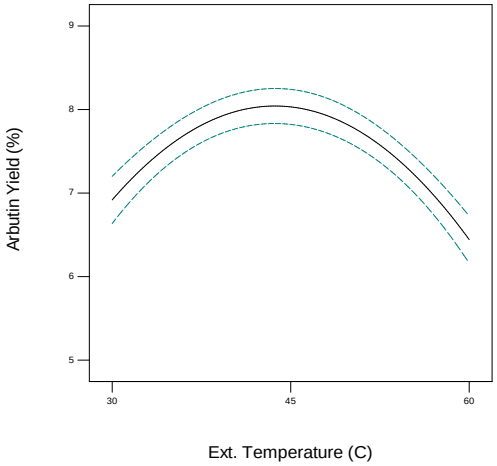

Fig. 3 The influence of extraction temperature on extraction performance

1.6.3. Effect of methanol concentration on the UAE performance

The utilized methanol concentration changed between 25% to 75% in the extraction process. The effect of methanol concentration on extraction yield of arbutin is shown in Figure 4. In the initial stage, along with the methanol concentration increased from 25% to 50%, the extraction yield of arbutin increased rapidly; while methanol concentration greater than 50% arbutin extraction yield was showing slow decreasing trend, and peak at 50% methanol concentration. This is because the increase of methanol concentration leads to enhanced mass transfer dynamics, solvents and *Pyrus amygdaliformis* Vill. var. *amygdaliformis* getting full access, and then the contents of arbutin dissolved increased. When the methanol concentration reached a certain level, some of

arbutin was difficult to be dissolved by high concentration of methanol, and also lead to the increase of the alcohol-soluble impurity content, resulting in a loss of arbutin content. Moreover, the greater of methanol concentration, the more difficult to refine arbutin and it will cause wasted and the cost of production increased. Therefore, the methanol concentration of 49 % is good for the arbutin extraction.

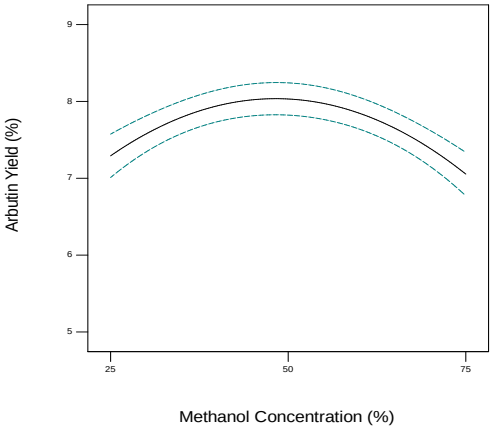

Fig. 4 The influence of methanol concentration on extraction performance

1.7. Optimisation of UAE by RSM

Individual effects of process variables, which is also known as one-factor at-atime approach was applied in previous section. This classical approach ignores the possible interactions of process variables with each other, which may result in misleading conclusions. Probable interactions between operation parameters are considered in response surface methodology (RSM). Table 2 shows the three parameters (methanol concentration, time and temperature) including minimum, centre, maximum points. Seventeen experiment were run and chosen randomly by the design expert software, and the responses were recorded (Table 3). Using response surface methodology owing to the software, a quadratic model applying with not only forward stepwise but also backward elimination regressions for EY were obtained. A quadratic model given below was derived using response surface methodology from the software:

$$A = -6.20500 + 0.32115X1 + 0.085888X2 + 0.090060X3 - 4.16667\ 10^{-5}X1X2 - 3.20000\ 10^{-4}X1X3 + 6.00000\ 10^{-5}X2X3 - 3.47667\ 10^{-3}X1^2 - 1.10563\ 10^{-3}X2^2 - 8.07600\ 10^{-4}X3^2 \quad (2)$$

In Table 4, X2, X3, X1X2, X1X3, X2X3, X3X4 are not significant effects for the model. After excluding their regression coefficients, new model may be given for better explanation of new condition.

$$A = -6.20500 + 0.32115X1 - 3.47667\ 10^{-3}X1^2 - 1.10563\ 10^{-3}X2^2 - 8.07600\ 10^{-4}X3^2 \quad (3)$$

Theoretical recovery values for arbutin calculated from this equation were plotted against practical ones. These relationships were shown in Figure 5. Figure 6, 7 and 8 shows three-dimensional contour and response surface plots for arbutin extraction showing the interactive effects of the extraction temperature, methanol concentration and extraction time.

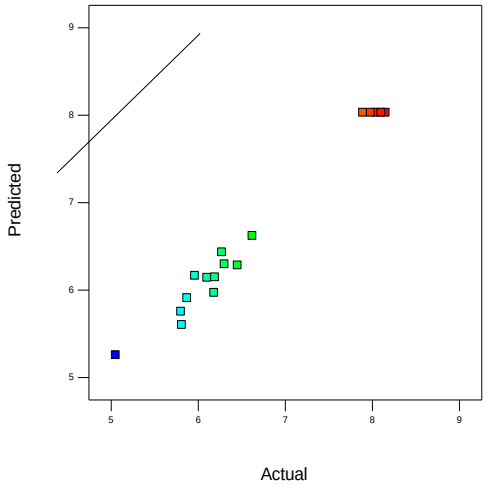

Fig. 5 The correlation between the values of the extraction yields that were experimentally obtained versus values calculated with the model equation.

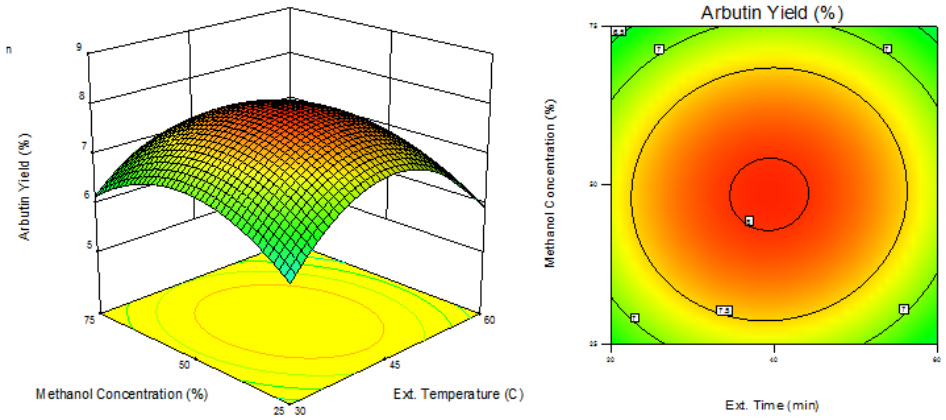

Fig. 6 Three-dimensional contour and response surface plots for arbutin extraction showing the interactive effects of the methanol concentration and extraction time.

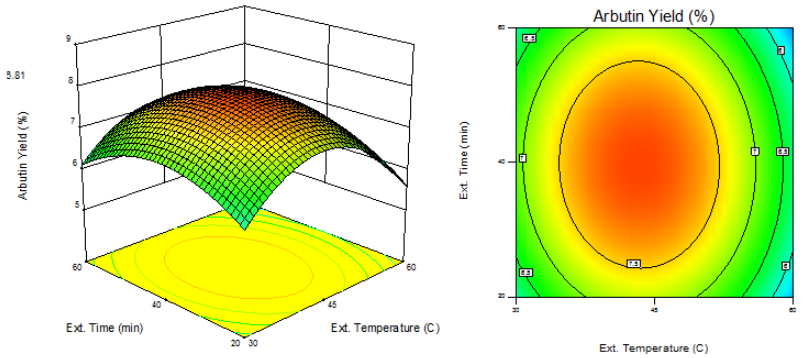

Fig. 7 Three-dimensional contour and response surface plots for arbutin extraction showing the interactive effects of the extraction time and extraction temperature.

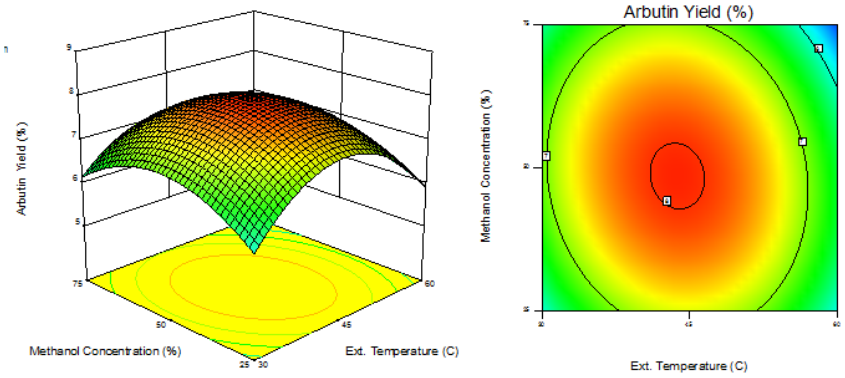

Fig. 8 Three-dimensional contour and response surface plots for arbutin extraction showing the interactive effects of the methanol concentration and extraction temperature.

We found the optimal extraction conditions to maximize the response by using optimization choice in design expert software. This value was measured at a methanol concentration of 48.50, an extraction time of 39.44 min, and an extraction temperature of 43.76 ^{0}C. The maximum response was found as (8.13 %) under these operating conditions.

After finding optimal conditions, real sample extraction experiments were repeated 6 times and then, average with relative standard deviation was calculated.

Average: 8.13 %
Standard Deviation: 0.04
Relative Standard Deviation: 0.45
Arbutin Yield (mg / 200 mg sample): 8.13 ± 0.04

3.3 Model fitting

The analysis of variance (ANOVA) for the quadratic equations of Design Expert 9 for the responses of EY are given in Table 4. Stepwise regression was used in order to have the most suitable set of variables. This process tests given variables and assesses them within the given alpha levels (0.1) using both backward and forward techniques. All the variables to estimate parameters are included in the backward techniques, and then variables with parameters that are not significant at alpha levels are removed from the equation. This process continues as long as there are significant variables and stops when there is none left. Like backward technique, the given variables within the given alpha levels are also assessed in forward tecnique. As opposed to the method in backward technique, when forward technique is starting, there are no variables included in the equation. The significant variable carrying the highest value of standardized beta (p<0.05) will be added onto the equation. Then an assessment on the next variable with the highest standardized beta value is made. If the variable is significant, it is added to the equation. This process continues as long as there are significant variables left. The same results were achieved in two of these regressions [35].

Table 4 shows the ANOVA for Design Expert 9's quadratic equations for the response. Regression analysis was done at 95% of confidence interval. F-value of the obtained model is 43.57 and p < 0,0001 shows the significance of the derived model. (X1), ($X1^2$), ($X2^2$), ($X3^2$) are significant model terms in the confidence interval (Table 4). Higher multiple coefficients that are closer (R-Squared, Adj R-Squared and Pred R-Squared) is an indicator of the higher accuracy of the model. Adj R-Squared also shows that a high degree of correlation between actual and predicted data. As seen in Table 4, on the response, the most significant variable is methanol concentration (X1). The 'F-value' of 'Lack of fit' (7.52) shows the significance of the lack of fit.

In our study, R-Squared (0.9825); Adj R-Squared (0.9599) and Pred R-Squared (0.7575) values for EY are indicative of good accuracy of the derived model. Thus, EY can be predicted from *Pyrus amygdaliformis* Vill. var. *amygdaliformis* with UAE with the sufficient implementation of the response surface modeling. Moreover, the coefficient value of variation (C.V. %) is found as 2.99 respectively. The lower value of the coefficient of variation is indication of a higher precision and reliability of the experimental results [17].

Table 4. The analysis of variance (ANOVA) for Response Surface Quadratic Model.

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	15.43	9	1.71	43.57	< 0.0001	significant
X1-Ext.	0.45	1	0.45	11.47	0.0117	significant

Temperature						
X2-Ext. Time	0.015	1	0.015	0.39	0.5525	
X3-Methanol Concentration	0.11	1	0.11	2.87	0.1342	
X1X2	$4.225\ 10^{-3}$	1	$4.225\ 10^{-3}$	0.11	0.7527	
X1X3	0.010	1	0.010	4.38	0.0747	
X2X3	7.69	1	7.69	0.25	0.6296	
$X1^2$	2.45	1	2.45	195.33	< 0.0001	significant
$X2^2$	3.10	1	3.10	62.39	< 0.0001	significant
$X3^2$	0.17	1	0.17	78.88	< 0.0001	significant
Residual	0.28	7	0.039			
Lack of Fit	0.23	3	0.078	7.52	0.0403	significant
Pure Error	0.041	4	0.010			

The regression equation coefficients were computed and a second-order polynomial equation was used to fit the data. The response, arbutin extraction from *Pyrus amygdaliformis* Vill. var. *amygdaliformis* dried leaves, is described in the following regression equation:

$$A = -10.81750 + 0.55657X1 - 6.00444\ 10^{-3}X1^2 - 1.90875\ 10^{-3}X2^2 - 1.37360\ 10^{-4}X3^2 \quad (3)$$

According to the regression equation obtained from the ANOVA, the R2 (multiple correlation coefficient) was 0.9825 (a value >0.75 is an indicator of the fitness of the model). This presents an estimate of the fraction of overall variation in the data computed by the model, and thus the model was able to explain 98.25% of the variation in response. The 'adjusted R2' is 0.9599 and the 'predicted R2' was 0.7575, which shows that the model was good (the R2 value should be in the range of 0–1.0 for a good statistical model, and as the value was nearer to 1.0, the model was deemed to be more fit). The present model's 'adequate precision value' was 43.57, and this also suggests the usability of the model to navigate the design space. The 'adequate precision value' was an index of the signal-to-noise ratio, and prerequisites for a model to be a good fit are values higher than 4. Simultaneously, a relatively lower value of the coefficient of variation (CV = 2.99 %) showed a better precision and reliability of the experiments carried out.

Thus, EY may be predicted from *Pyrus amygdaliformis* Vill. var. *amygdaliformis* with UAE by sufficiently achieving a response surface modelling. Higher precision and reliability of the experimental results are documented by the lower value of the coefficient of variation [18-19]. Our study found the coefficient value as 2.99. Figure 5 exhibits the corelation between the data of the experiments and the data predicted from Equation 2 concerning the EY of *Pyrus amygdaliformis* Vill. var. *amygdaliformis* leaves extracts obtained by UAE. It can be seen that the predicted data calculated in the model and the experimental data in the range of operating conditions are in good agreement. Figure 9 exhibits chromatogram of arbutin standard solution. Figure 10 exhibit chromatogram of *Pyrus amygdaliformis* Vill. var. *amygdaliformis* leaves extract.

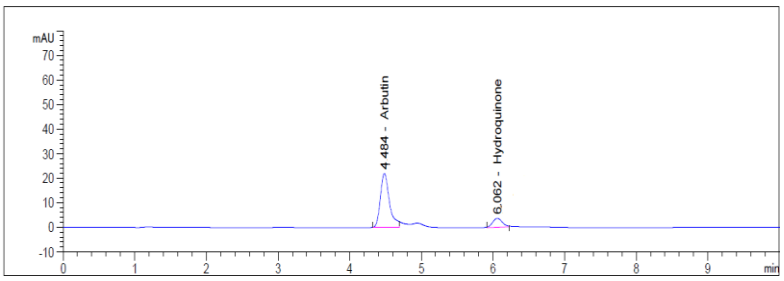

Fig. 9 Chromatogram of arbutin standard solution (Concentration: 150 ppm)

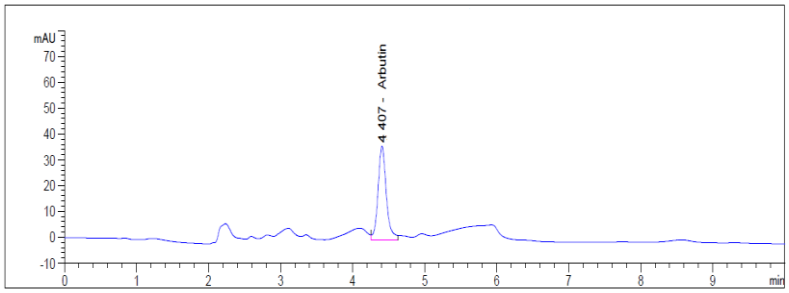

Fig. 10 Chromatogram of *Pyrus amygdaliformis* Vill. var. *amygdaliformis* leaves extract.

After completion of the method optimization, arbutin analyses were made in leaves, fruit and branches of *Pyrus amygdaliformis* Vill. var. *amygdaliformis*. The results are given in the following table.

Table 5. The results of arbutin analyses of leaves, fruit and branches of *Pyrus amygdaliformis*.

Source	**Arbutin %**
Leaves	8.13
Branches	4.10
Fruits	0.069

IV. CONCLUSIONS

In investigation of the optimum extraction parameters for extraction of arbutin from *Pyrus amygdaliformis* Vill. *var. amygdaliformis* leaves, response surface methodology was successfully utilised. For optimization of various parameters in extraction of arbutin from *Pyrus amygdaliformis* Vill. var. *amygdaliformis* leaves three parameters via temperature, time, temperature, solvent composition were tested by using Box-Behnken design criteria and on the extraction of arbutin, three parameters time, temperature solvent composition had significant effect. Applying Box-Behnken design optimized the extraction parameters and the parameters for best extraction of arbutin from *Pyrus amygdaliformis* Vill. var. *amygdaliformis* leaves was found to be extraction time (39.44 minutes), temperature (43.76 °C) and solvent composition (48.50 % methanol in methanol-water mixture). The second order polynomial model was satisfactorily descriptive of the experimental data. The maximum arbutin from *Pyrus amygdaliformis* Vill. var. *amygdaliformis* leaves was 8.13 % dry weight. Linear coefficient of methanol concentration and extraction temperature and square coefficient of extraction temperature, extraction time and methanol concentration have the most significant effect on the EY obtained by UAE. After finding optimal conditions, real sample extraction experiments were repeated 6 times and then, average with relative standard deviation was calculated. Arbutin (%): 8.13 ± 0.04. Results is appropriate for the statistical evaluation.

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