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Contributed Paper

Optimization of Ultrasound-assisted Extraction of Quercetin, Luteolin, Apigenin, Pinocembrin and Chrysin from *Flos populi* by Plackett-Burman Design Combined with Taguchi Method

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ABSTRACT

The objective of this study was to optimize for the extraction of five flavonoids: quercetin, luteolin, apigenin, pinocembrin and chrysin from *Flos populi*. A combination of the Plackett-Burman design (PBD) with Taguchi method was used to optimize the process parameters of ultrasound-assisted extraction (UAE). Based on the single-factor analysis, a Plackett-Burman design was initially employed and it was found that Temperature (°C), Particle diameter (mm), Ultrasonic time (min) and Ultrasonic intensity (W/cm^2) were key variables that effecting extraction yield. The optimal conditions were further determined using the Taguchi method. Results showed that the optimum temperature, ethanol concentration, particle diameter, ultrasonic time, liquid-solid ratio, ultrasonic intensity and experimental runs were 70 °C, 60%, 0.18 mm, 35 min, 25 mL/g, 3.3 W/cm^2, and two experimental runs respectively. Under these optimum conditions, approximately 16.26 mg of quercetin, 9.97 mg of luteolin, 8.57 mg of apigenin, 119.71 mg of pinocembrin, and 16.12 mg of chrysin could be obtained from 1 g of *Flos populi*, which well correlated with predicted values (15.91 mg of quercetin, 6.09 mg of luteolin, 7.49 mg of apigenin, 114.37 mg of pinocembrin, and 14.91 mg of chrysin).

Keywords: ultrasound-assisted extraction, flavonoids, *Flos populi*, Plackett-Burman design, Taguchi method

1. INTRODUCTION

Populus tomentosa Carr. is indigenous to China and is widely distributed because of its tolerance to a wide range of climatic conditions. *Flos populi* is prepared from the male inflorescence of *Populus tomentosa* Carr., which is used as a traditional medicine in

China (Salicaceae family) [1]. It contains flavonoids, cardiac glycoside and phenols and is used for the therapy of a variety of inflammatory diseases and as antidiarrheal agents in East Asian countries [2-4].

The medicinal use of *Flos populi* has attracted scientific interest in the screening of its bioactive constituents for pharmacological utilization. A range of flavonoid compounds have been identified in *Flos populi*, including quercetin, luteolin, apigenin, pinocembrin, and chrysin. These flavonoids exhibit anti-cancer, anti-diabetic, antiviral and anti-allergic activity [5-6]. Therefore, the optimization of the extraction conditions for these flavonoid compounds from *Flos populi* is worthy of further investigation.

Traditional extraction methods of flavonoids from *Flos populi* are very time-consuming and require relatively large quantities of solvents. However, ultrasound-assisted extraction (UAE) has increased in popularity due to its high efficiency and low energy requirement [7]; this increase in extraction yield is largely attributed to the disruption of the cell wall, reduction in particle size and the enhancement of the mass transfer of the cell content to the solvent caused by the collapse of the bubbles produced by cavitation [8]. Hence, UAE provides increased extraction yield, reduced extraction time and high processing throughput along with the advantage of reduced temperature and solvent volume [9].

To test the influence of different parameters on extraction, the Plackett-Burman design (PBD) was applied followed by the Taguchi method. PBD is powerful tool to identify key parameters from many variables [10]. The Taguchi method enables determination of the optimal process using an orthogonal matrix. So the joint application of these two methods can indicate the optimal extraction conditions as well as minimize the number, time, and cost of tests. Variables investigated were temperature, solvent composition, particle diameter, liquid-solid ratio, ultrasonic time, ultrasonic intensity and experimental runs.

UAE of flavonoids using different temperatures, ethanol concentrations, ultrasonic times and liquid to solid ratios have already been studied, however, UAE for the five flavonoid compounds (quercetin, luteolin, apigenin, pinocembrin and chrysin) from *Flos populi* have not been reported so far. In view of the above-mentioned facts, the objective of this study was to identify the key parameters using Plackett-Burman design and then apply the Taguchi method to develop the optimal conditions for extracting quercetin, luteolin, apigenin, pinocembrin and chrysin from *Flos populi* using UAE.

2. MATERIALS AND METHODS

2.1 Plant Material, Standards and Reagents

Flos populi (male inflorescence of *Populus tomentosa* Carr) was purchased from a medicinal herbs store (Anguo, Hebei Province, China) and authenticated by Associate Professor Junkai Wu (Heilongjiang University of Traditional Chinese Medicine, Harbin, China). The voucher specimen (Accession no. 1009015ch) has been deposited at Herbarium, in the College of Veterinary Medicine, Northeast Agricultural University.

The standards (quercetin, luteolin, apigenin, pinocembrin and chrysin) were obtained from the Chinese Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purities of standards exceeded 98% (w/w). Formic acid (HPLC grade), ethanol (analytical grade) and methanol (HPLC grade) were acquired from Hangzhou Reagent Company (Hangzhou, PR China). Distilled water was purified by a

Milli-Q academic water purification system (Millipore, Bedford, MA, USA).

2.2 Ultrasound-assisted Extraction

Flos populi was dried at 60 °C in a vacuum box (DHG-9023A, Shanghai Dute Scientific Instrument CO,. LTD) for 5 h and then pulverized using a mechanical grinder and stored at -20 °C until required. Ultrasonic-assisted extraction (UAE) was carried out using an ultrasonic device (BILON-S650CT) with a flat tip probe transducer. The ultrasound waves at a frequency of 50 kHz were kept throughout the extraction while the power levels at the probe was varied. The extraction cell was a brown glass tubes (4 cm diameter × 7 cm height) which was immersed in a thermostatic water bath to maintain the temperature. The solution can be treated with ultrasound given by a 1.2 cm probe which was located 1 cm from the top surface of the extraction cell. Off time for the ultrasound device regarded to burst of the bubbles, hence the duty cycle (on time/off time) and total time 1-2 s and 3 s, respectively.

The ultrasonic power was expressed as ultrasonic intensity (UI), calculated using Eq (1) [11].

$$UI = 4P/\pi D^2 \quad (1)$$

Where UI is the ultrasonic intensity (W/cm^2), D is the internal diameter (cm) of the ultrasonic reactor, and P is the value of power which is calculated according to the quation given by Chemat et al. [12-13].

In this single-factor experiment, the input power levels were adjusted to 100, 200, 300, 400, 500, and 600 W which were equal to 1.5, 3.3, 4.9, 6.7, 8.5, and 10.0 W/cm^2, respectively.

Powder (2.0 g) was moisturized for 10 min with solvent and then subjected to the extraction procedure. The extraction of materials was evaluated under different UAE conditions: temperatures (30 - 80 °C), ethanol concentration (0 - 95%), the range of particle diameters (0.15 - 2.00 mm) was determined by the prescription sieves (No. 1-9) which was standardized by Pharmacopoeia of People's Republic of China (2010) [1], ultrasonic time (5 - 45 min), liquid-solid ratio (5 - 30 mL/g), ultrasonic intensity (1.5 - 10.0 W/cm^2) and experimental runs (1 - 6). All tests were performed in triplicate. Extracts were centrifuged at 3000 rpm for 10 min using the table-type low speed centrifuge TD4 (Hu Nan Ke Cheng instrument equipment co., LTD). After centrifugation, the supernatant was filtered through a 0.45 μm syringe filter and stored at 4 °C until further analysed.

2.3 HPLC Analysis

Flavonoid compounds were identified using RP-HPLC. A SHIMADZU HPLC system equipped with LC-10ATVP binary pump, a SPD-10AVP detector, a CTO-10ASVP column oven and a N300 workstation was used for quantitative analysis. A C18 column (150 mm × 4.6 mm, 5.6 μm, Diamonsil, Dikma Technologies, China) was used for chromatographic separation. The mobile phase consisted of 0.1% formic acid (A) and methanol (B) at constant flow (1mL/min). The injection volume was 20 μL and the column temperature was kept at 25 °C. The solvent gradient elution schedule was as follows: 0 - 23 min, 65% B; the solvent gradient was increased to 90% B at 23 - 24 min and it was maintained at 90% B for 10 min; 34 - 35 min, 90% - 65% B; 35 - 50 min, 65% B. The detection was tested at a wavelength of 254 nm. All solutions were filtered with 0.45 μm membrane (Lab Instrument Co.Ltd) before injection. Quantification was performed using the external standard method with means of a

five-point calibration curve. The correlation coefficient and linear range of the compounds identified are listed in Table 1.

2.4 Screening of the Key Variables by Plackett-Burman Design (PBD)

The overall desirability function is a useful technique for the analysis of experiments in which several responses have to be optimized simultaneously [14]. This makes it possible to combine results obtained for properties measured on different scales. In this test, the evaluation index is the value of OD (overall desirability) of the flavonoid compounds, which is processed by Eq (2) and (3).

$$d_i = (Y_i - Y_{min})/(Y_{max} - Y_{min}) \quad (2)$$

$$\text{OD} = (d_1 \times d_2 \bullet\bullet\bullet \times d_n)^{1/n} \quad (3)$$

Where Y_i is the value of actual measurement; Y_{min} and Y_{man} are the minimum and maximum in the PB test, respectively; and n is the number of indicators.

Table 1. Regression parameters and precision of HPLC for the five compounds.

Compounds	Regression equation	Correlation coefficient	Linear range (μg/mL)
quercetin	$y = 6 \times 10^7 x - 328010$	0.9990	9.6~86.4
luteolin	$y = 6 \times 10^7 x + 32740$	0.9991	17.1~154.08
apigenin	$y = 5 \times 10^7 x + 62543$	0.9991	10~90.72
pinocembrin	$y = 5 \times 10^6 x - 17309$	0.9993	8~72
chrysin	$y = 6 \times 10^7 x - 229876$	0.9990	11.84~106.56

The Plackett-Burman design (PBD) can determine the significant parameters among some parameters that affect extraction yield. In this study, seven parameters including temperature (X_1), ethanol concentration (X_2), particle diameter (X_3), ultrasonic time (X_4), liquid-solid ratio (X_5), ultrasonic intensity (X_6) and experimental runs (X_7), with low levels setting, were considered to be the independent variables and are listed in Table 2. The seven assigned variables were screened in twelve experimental designs. The results for extractions performed under PBD conditions are shown in Table 3.

A first-order polynomial model was used to identify the main effect on the response which is presented in Eq (4)

$$Y = \beta_0 + \sum_{i=1}^{5} \beta_i X_i \quad (4)$$

Where the response variable is Y; β_0 is the constant (or model intercept); β_i is the linear coefficient; and X_i is the level of independent variable.

Table 2. Experimental factors and ranges in PBD.

Factors	Coded symbols	Level	
		-1	+1
Temperature (°C)	X_1	70	80
Ethanol concentration (%)	X_2	60	80
Particle diameter (mm)	X_3	0.18	0.25
Ultrasonic time (min)	X_4	35	45
Liquid-solid ratio (mL/g)	X_5	20	25
ultrasonic intensity (W/cm^2)	X_6	4.9	6.7
Experimental runs	X_7	2	3

Table 3. Plackett-Burman design matrix with coded variables and the responses measured.

Run No.	X_1	X_2	X_3	X_4	X_5	X_6	X_7	Extraction yield (mg/g)					
								quercetin	luteolin	apigenin	pinocembrin	chrysin	OD
1	1	-1	1	1	1	1	-1	12.74	4.97	8.21	67.28	6.70	0.543
2	-1	-1	-1	1	-1	1	1	28.40	5.18	7.13	64.69	5.40	0.437
3	1	1	-1	-1	-1	1	-1	8.86	6.26	2.81	45.47	7.45	0.342
4	1	-1	1	1	-1	1	1	12.64	5.08	5.51	52.81	5.72	0.343
5	1	1	1	-1	-1	-1	1	8.21	6.70	3.35	62.64	7.24	0.376
6	-1	1	1	-1	1	1	1	7.13	3.89	1.40	19.22	5.40	0
7	-1	1	1	1	-1	-1	-1	9.72	8.10	6.48	56.81	6.26	0.510
8	1	-1	-1	-1	1	-1	1	12.64	8.32	5.94	60.26	6.80	0.647
9	-1	-1	-1	-1	-1	-1	-1	11.45	9.07	6.16	54.86	5.40	0.420
10	1	1	-1	1	1	1	-1	11.56	5.40	3.02	58.75	11.88	0.503
11	-1	-1	1	-1	1	1	-1	8.96	4.43	1.73	19.87	5.18	0
12	-1	1	-1	1	1	-1	1	10.80	8.21	5.29	43.20	8.75	0.605

2.5 Optimization Based on Taguchi Method

The Taguchi methodology was used to optimize the operation conditions for UAE of five flavonoid compounds with the smallest number of possible runs [15]. In the test, four control factors (temperature [A], particle diameter [B], ultrasonic time [C], and ultrasonic intensity [D]) at three different levels were assessed with a $L_9(4^3)$ orthogonal array used to determine significant influence on the extraction yields as screened by Plackett-Burman design. The parameter level combinations for all of the tests are shown in Table 4. For designing the experiment and optimizing the process, Minitab 16 software was used.

Table 4. Factors and levels for Taguchi design.

Levels	Factors			
	Temperature (C)/A	Particle diameter (mm/B)	Ultrasonic time (min/C)	Ultrasonic intensity (W/cm^2/D)
1	60	0.15	25	3.3
2	70	0.18	35	4.9
3	80	0.25	45	6.7

The Taguchi method uses a signal-to-noise (S/N) ratio as the statistical measure of performance. The S/N ratio can be divided into three categories: the lower-the-better, the higher-the-better and the nominal-the-better. In this study, the experimental target was the yields of the five flavonoid compounds, therefore the higher-the-better option was selected and S/N ratio was calculated using following Eq (5):

$$S/N = -10 \log \frac{1}{n} \left(\sum \frac{1}{y^2} \right) \quad (5)$$

Where n is the number of measurements, S/N=signal-to-noise ratio and y is the value of the UAE yields.

2.6 Statistical Analysis

The Plackett-Burman Design was analyzed by Design Expert 8.0 software and the Taguchi design was optimized by Minitab 16 software. The results from the HPLC analysis were statistically analyzed by ANOVA. Tukey's test was then used to determine which mean values were different ($p < 0.05$). All analyses were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1 Single-factor Analyses

3.1.1 Effect of extraction temperature

Temperature can accelerate molecular movement and decrease solvent viscosity. Hence, the extraction temperature within the range 30-80 °C was tested. The other parameters were set constant at a determined level along the different tests (ethanol concentration 40%, particle size 0.25 mm, extraction time 30 min, liquid-solid ratio 20 mL/g, ultrasonic intensity 4.9 W/cm^2, experimental runs 1). The Figure 1A showed a constant increase in the extraction yield with the temperature increases. Pinocembrin, in particular, increased greatly with increasing temperature. As expected, the OD values were consistent with this trend. It could be explained that result under UAE is attributed to the combination of thermal and the cavitation effects. On the one hand, the increase of the temperature has a positive influence on extraction yield due to the fact that it accelerates the molecular movement. On the other hand, increasing temperature benefited to cavitation effect, for the decrease of the surface tension and the solvent viscosity could lead to the production of the ultrasonic wave [16]. Hence, the temperatures of 60 °C and 80 °C were tested in the PBD.

3.1.2 Effect of ethanol concentration

Cavitation involved in ultrasound assisted extraction is influenced by the solvent properties, including surface tension, viscosity and vapor pressure. In principle, if a solvent possesses favorable properties such as: a higher surface tension, lower vapor pressure and viscosity, a greater cavitation effect will be observed [8-9]. Moreover, an ideal mixed solvent benefited to the extraction of active ingredients from different natural products due to the principle "like dissolves like" [17]. For these reasons, the solvent composition was tested in the present study. A maximum yield was obtained for all compounds, excluding chrysin, at an ethanol concentration of 60% (Figure 1B). Moreover, the OD values showed that the highest yield could be obtained at this concentration. Other reports have also found that 60% ethanol was suitable to extract quercetin from maize samples using UAE [18]. This was probably due to the fact that the relative polarity of the 60% ethanol was suitable to extract the five active compounds from *Flos populi*. Accordingly, the ethanol concentration range of 40% - 60% was chosen for further optimization using PBD.

3.1.3 Effect of particle diameter

Extraction yield was significantly influenced by particle size. Figure 1C showed that extraction yield increased with decreasing mean particle size until the mean particle size of 0.18 mm; Indicating that a smaller particle size leads to better separation of the compounds from the plant powder. This is consistent with previous research [19]. However, the extraction yield was decreased slightly when the mean particle size from 0.18 to 0.15 mm. Thus, particle diameter of 0.18 mm and 0.25 mm were selected for further optimization using PBD.

3.1.4 Effect of ultrasonic time

The effect of ultrasonic time on extraction yield was analyzed at 5, 15, 25, 35 and 45 min. Other variables were maintained at; solvent concentration 60%, liquid-solid ratio 20 mL/g, temperature 80 °C, particle diameter 0.18 mm, ultrasonic intensity 4.9 W/cm^2 and experimental runs 1. Increasing the ultrasonic time was found to increase the extraction of 4 flavanoids (Figure 1D). However, the yield of chrysin decreased from 14.16 mg/g to 10.11 mg/g during the last 15 min. This phenomenon was likely the result of the decomposition of chrysin due to the extended extraction time [20]. The OD values also represented the total trend of the extraction yield, which indicated that extraction time of 35 and 45 min should be applied in the subsequent optimization.

3.1.5 Effect of liquid-solid ratio

Increasing the Liquid-solid ratio from 5 mL/g to 25 mL/g increased the extraction of the five flavonoids. This is likely due to the increase in the diffusivity of the solvent into the cells which enhances the dissolution of the compounds into the solvent. However, when the liquid-solid ratio was increased further to 30 mL/g, the extraction yield decreased (Figure 1E) because a high liquid-solid ratio prolonged the distance of diffusion toward the interior tissues [21]. Similar results have been previously observed found that a larger solvent volume did not lead to a higher yield [22]. Consequently, the liquid-solid ratio of 20 mL/g and 25 mL/g were adopted for further optimization using PBD.

3.1.6 Effect of ultrasonic intensity

Ultrasonic intensity is an important factor in the UAE. In this study, six levels of ultrasonic intensity (1.5-10.0 W/cm^2) were employed to test the effect on extraction yields. As it can be seen in Figure 1F, the extraction yields of the five flavonoids from *Flos populi* correspondingly enhanced with an increase in ultrasonic intensity from 1.5 to 4.9 W/cm^2. The results represented that the cavitation effect strengthened by increasing ultrasonic intensity facilitated the disruption of cell walls. However, the extraction yields declined when the ultrasonic intensity was increased beyond 4.9 W/cm^2, which be due to the degradations of these compounds. Li et al. and Jacotet-Navarro et al. reported that there is degradation when ultrasound is used for treatment of natural products [8-9]. Zhang et al. also found that the extraction yields of Luteolin and Apigenin from Celery rises up to threshold values and then decreases with the increases of ultrasonic intensity [23]. Statistical analysis showed that the extraction yields of the five flavonoids were significantly higher at 4.9 W/cm^2 of ultrasonic intensity than other levels (Figure 1F). Hence, the ultrasonic intensity range of 4.9 - 6.7 W/cm^2 was adopted for further optimization using PBD.

3.1.7 Effect of experimental runs

The experimental runs is directly related to the energy cost, extraction yield and efficiency of the extraction method. Under the above tests, six groups of test were investigated at varying experimental runs (1, 2, 3, 4, 5 and 6). Figure 1G showed that extraction yield increased greatly from extraction 1 to 2. However, the rate of increase was lowed after 3 extractions. It is due to that these active compounds have been almost extracted completely. Therefore, the experimental runs of 2 and 3 were chosen for further optimization using PBD.

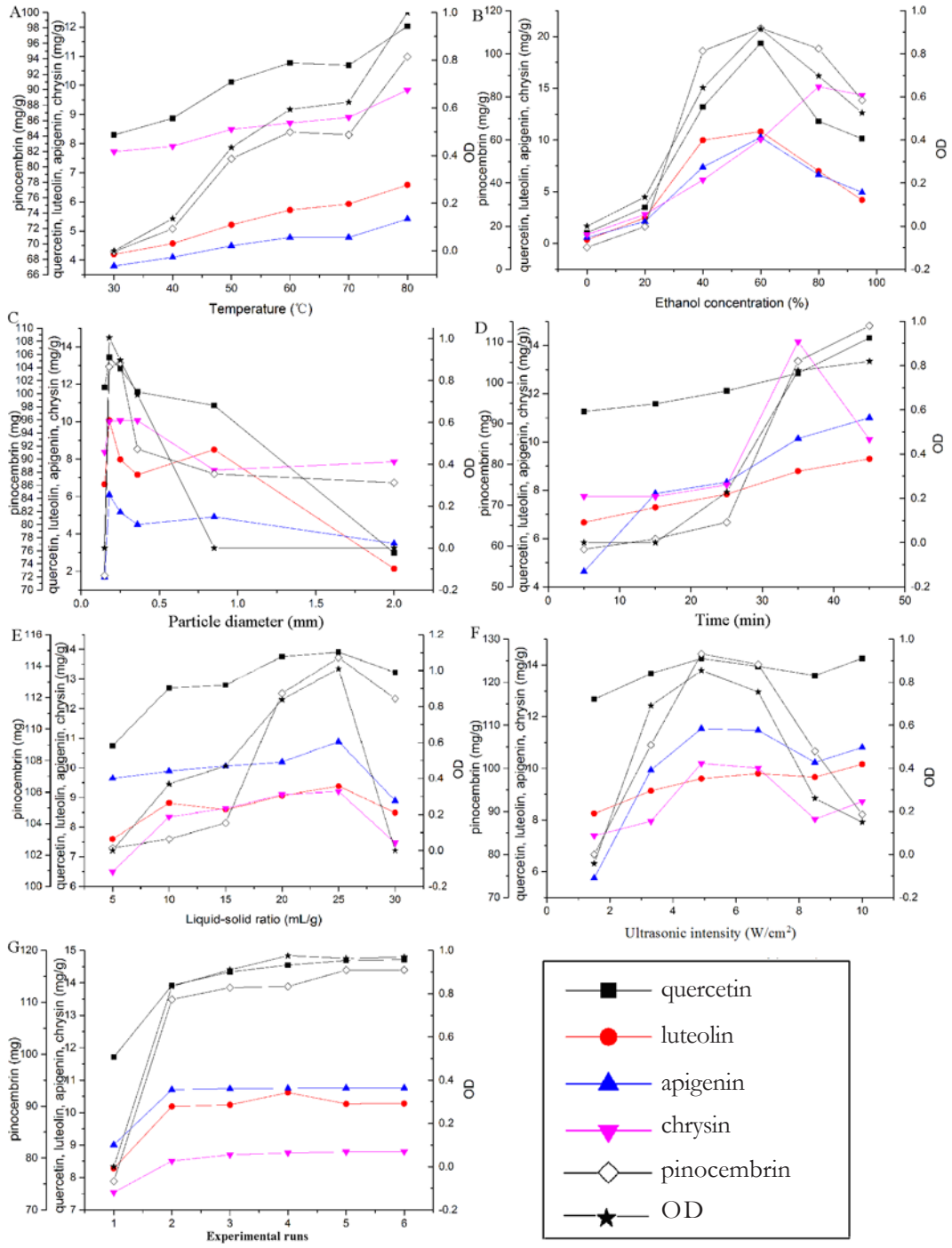

Figure 1. Effect of different extraction parameters (extraction temperature, °C; ethanol concentration, %; particle diameter, mm; extraction time, min; liquid-solid ratio, mL/g; ultrasonic intensity, W/cm^2 and experimental runs) on OD values and yields of five flavonoids.

3.2 Screening of Significant Parameters by Plackett-Burman Design

The experiment results showed that OD values were in accordance with the values of the five compounds (3.1). So a Plackett-Burman design consisting of twelve runs was applied to evaluate the effect of the selected variables using the OD values (Table 3). Statistical analysis of the responses was represented in Table 5.

Table 5. Results of the Plackett-Burman design.

Source	df	Sum of Squares	Mean Square	F Value	*p*-Value
Model	7	0.46	0.066	21.90	0.0049**
X_1	1	0.051	0.051	16.89	0.0147*
X_2	1	2.43×10^{-4}	2.43×10^{-4}	0.081	0.7907
X_3	1	0.12	0.12	38.58	0.0034**
X_4	1	0.11	0.11	36.90	0.0037**
X_5	1	1.41×10^{-3}	1.41×10^{-3}	0.47	0.5320
X_6	1	0.18	0.18	60.16	0.0015**
X_7	1	6.75×10^{-4}	6.75×10^{-4}	0.22	0.6609
Residual	4	0.012	3.02×10^{-3}		
Cor Total	11	0.47			

The *p* value is the probability that the magnitude of a contrast coefficient is due to stochastic process variability and is regarded as a tool for examining the significance of each of the coefficients. A low *p* value illustrates a real or significant effect [24]. A significance value of $p < 0.05$ was set; the *p value* of the model was 0.0049; indicating that the model is significant. The analysis of variance (ANOVA) for the experiment design showed that X_1, X_3, X_4 and X_6 were significant variables influencing the extraction yield of the flavonoid compounds. The R^2 and Adj.R^2 were both close to 1, which revealed that the regression model for the yields was satisfactory. A first order polynomial equation was derived representing the OD value as a function of the independent variables:

$$Y_{OD}=0.39+0.065X_1-4.5E\text{-}003X_2-0.098X_3+0.096X_4-0.011X_5-0.12X_6+7.5E\text{-}003X_7 \quad (6)$$

Thus, the four variables; extraction temperature (X_1), particle diameter (X_3), ultrasonic time (X_4) and ultrasonic intensity (X_6) were selected for further optimization using the Taguchi experimental design. Variables, that did not have a significant effect on extraction yield were not included in the subsequent optimization; the optimal values for which were chosen based on the single-factor analysis.

3.3 Further Optimization of the Ultrasonic-assisted Extraction Using the Taguchi Method

Based on the results of the Plackett-Burnman design single factor analysis, four variables including extraction temperature (X_1), particle diameter (X_3), ultrasonic time (X_4) and ultrasonic intensity (X_6) were chosen to investigate their optimal combination using the Taguchi orthogonal array design.

To analyze these data using the means and S/N function, a figure of responses was prepared to determine and isolate the effects of each factor on means and S/N. The main effects plot for means and S/N ratios are displayed in Figure 2. Parameters had varying influences on each compound; however, there is a consistency in the optimal combination for the maximum extraction yield. Therefore, the optimal conditions for the extraction of the flavonoid compounds was $A_2 B_2 C_2 D_1$ (temperature = 70, particle diameter = 0.18 mm, ultrasonic time = 35 min and ultrasonic intensity = 3.3 W/cm^2).

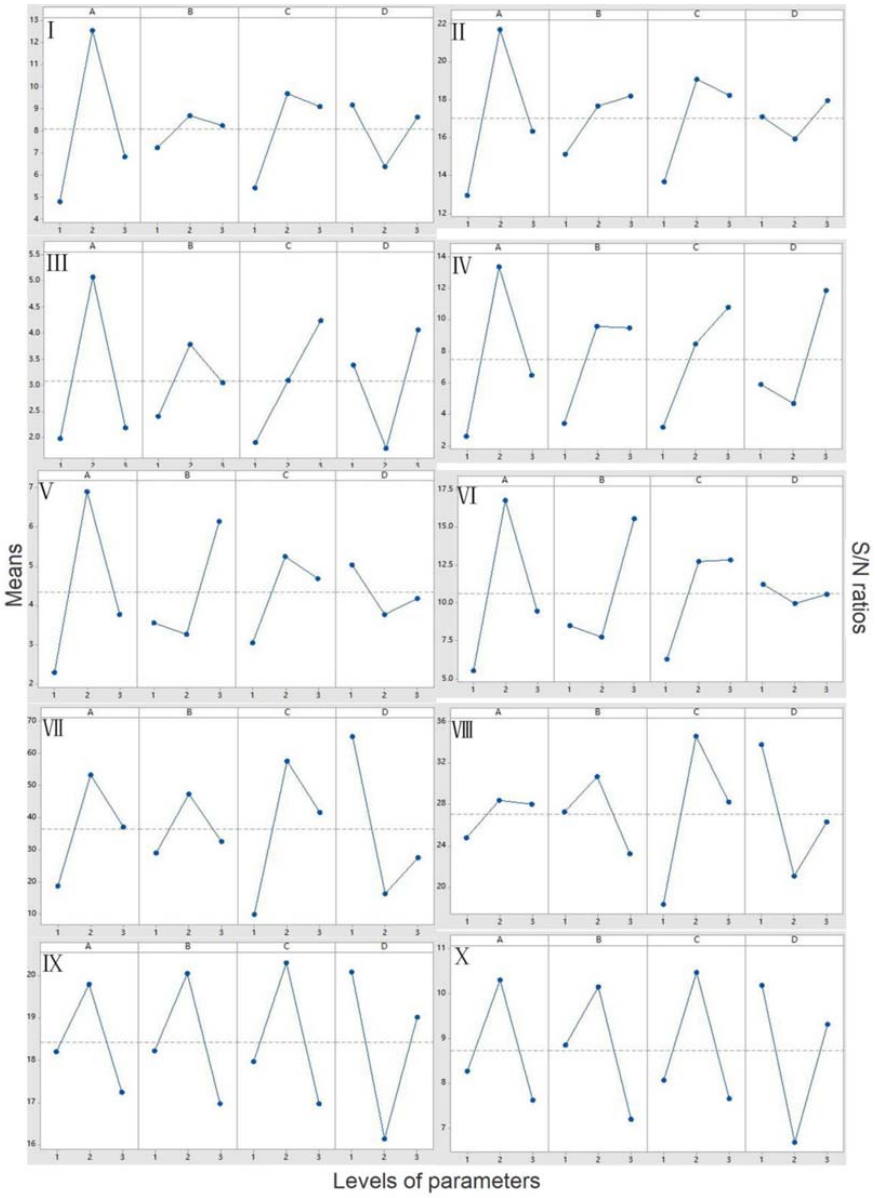

Figure 2. Effects of different levels of L9 orthogonal array for each parameter on the extraction yields of five kind flavonoids. Main effects plot for means (quercetin/I, luteolin/III, apigenin/V, pinocembrin/VII and chrysin/IX). Main effects plot for S/N ratios (quercetin/II, luteolin/IV, apigenin/VI, pinocembrin/VIII and chrysin/X).

The optimal combination of the parameters was not in the L9 array (see Table 6), which is an important property of the Taguchi method. However, the values obtained in the optimal configuration could be calculated using the optimal level of the design factors as:

$$[Y]predicated = Tm + \sum_{i=1}^{m}([Yji]_i - [Tm]) \quad (j = A, B, C, D, E) \quad (7)$$

where Tm is the total mean of yields and $[Yji]_i$ is the yields at optimal level.

Table 6. The results of orthogonal design L9 (4^3).

Test	A	B	C	D	Extraction yield (mg/g)					S/N ratio				
					Q	L	A	P	C	Q	L	A	P	C
1	60	0.15	25	40.8	2.48	0.43	0.97	14.15	9.18	7.89	-7.33	-0.26	23.01	19.26
2	60	0.18	35	61.1	5.4	1.4	1.62	31.1	9.4	14.65	2.92	4.19	29.86	19.46
3	60	0.25	45	81.5	6.59	4.1	4.32	11.6	6.26	16.38	12.26	12.71	21.33	15.93
4	70	0.15	35	81.5	13.93	5.4	6.91	58.43	12.74	22.88	14.65	16.79	35.33	22.1
5	70	0.18	45	40.8	15.34	7.24	6.91	98.39	12.1	23.71	17.19	16.79	39.86	21.66
6	70	0.25	25	61.1	8.42	2.59	6.91	3.13	6.05	18.51	8.27	16.79	9.91	15.64
7	80	0.15	45	61.1	5.4	1.4	2.81	15.01	4.64	14.65	2.92	8.97	25.53	13.33
8	80	0.18	25	81.5	5.4	2.7	1.3	12.85	8.96	14.65	8.63	2.28	22.18	19.05
9	80	0.25	35	40.8	9.72	2.48	7.24	83.48	9.29	19.75	7.89	17.19	38.43	19.36

The intention of these calculations is to validate the experiment. The validation tests were repeated three times under the optimized conditions. Under the modified conditions, the experimental yields of quercetin, luteolin, apigenin, pinocembrin and chrysin were 16.26 ± 0.58 mg/g (N=3), 9.97 ± 1.07 mg/g (N=3), 8.57 ± 0.46 mg/g (N=3), 119.71 ± 1.05 mg/g (N=3), 16.12 ± 0.53 mg/g (N=3) respectively, which were close to the predicted values, indicating that the model was adequate for the extraction process (Table 7).

Table 7. Experimental and predicted values of the target products at optimum conditions.

Target products	Optimum condition				Extraction yield (mg/g)	
	Temperature (°C)	Particle diameter (mm)	Ultrasonic time (min)	Ultrasonic intensity (W/cm²)	Experimental	Predicted
quercetin	70	0.18	35	3.3	16.26±0.58	15.91
luteolin					9.97±1.07	6.09
apigenin					8.57±0.46	7.49
pinocembrin					119.71±1.05	114.37
chrysin					16.12±0.53	14.91

Jacotet-Navarro et al. extracted active compounds from natural products using the pilot scale experiments based on the optimization results in Lab, which proved that ultrasound technique can be considered on an industrial scale [9]. The optimization results of UAE of quercetin, luteolin, apigenin, pinocembrin and chrysin from *Flos populi* will also provide some very favorable reference for UAE of these compounds from *Flos populi* on an industrial scale.

4. CONCLUSIONS

In this study, an effective ultrasonic-assisted extraction (UAE) technique was employed to extract flavonoid compounds quercetin, luteolin, apigenin, pinocembrin and chrysin from *Flos populi* by the Plackett-Burman design followed by the Taguchi method. The optimal conditions determined were: temperature, 70 °C; ethanol concentration, 60%; particle diameter, 0.18 mm; ultrasonic time, 35 min; liquid-solid ratio, 25 mL/g; ultrasonic intensity, 3.3 W/cm^2; and experimental runs, 2. Under these optimal conditions, the extract contained high levels of the five flavonoid compounds. It can be concluded that UAE method outlined is efficient and reliable for extracting compounds from *Flos populi*. Further studies may be carried out under the extraction conditions to enrich and purify these compounds from *Flos Populi* extracts.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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